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Training Course on Screening Technologies for Industrial Exploitation of Medicinal and Aromatic Plants

Panama, 30 November - 5 December 1998

CIFLORPAN
FACULTAD DE FARMACIA - UP

UNIVERSIDAD DE PANAMA

APPENDIX 6

HANDOUTS AND THE LITERATURE OF THE COURSE

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Curso de Entrenamiento sobre la Utilizacion Industrial de Plantas Medicinales y Aromaticas

Panamá, Noviembre 30 - Diciembre 5,1998

ACTO INAUGURAL

30 de Noviembre de 1998

Auditorio Bernardo Lombardo, Facultad de Farmacia, Universidad de Panamá.

9:00 h Palabras de bienvenida por el Dr. Mahabir P. Gupta, Coordinador Internacional del Subprograma X. Química Fina Farmacéutica, CYTED.

Palabras por el Dr. Ceferino Sánchez, Secretario Nacional de Ciencia y Tecnología e Innovación, Presidencia de la República de Panamá.

Palabras por Don Manuel Lorenzo, Embajador de España en Panamá

Palabras de apertura por el Dr. Gustavo García de Paredes, Rector de la Universidad de Panamá.

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FACULTAD DE FARMACIA - UP

SENACYT

Training Course on Screening Technologies for Industrial Exploitation of Medicinal and Aromatic Plants
Panama : 30.11. - 05.12.1998

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Department of Pharmaceutical Sciences
University of Antwerpen

Dr. P.M.L. Vanderheyden
Department of Molecular and Biochemical Pharmacology
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Training Course on Screening Technologies for Industrial Exploitation of Medicinal and Aromatic Plants

Panama : 30.11. - 05.12.1998

Monday 30 November
Auditorium Bernardo Lombardo

09.00-10.00h	Inauguration	
10.00-12.30h	Introductory Lecture	A. Vlietinck
14.30-16.00h	Sections 1.1 & 1.2	
16.30-18.00h	Sections 1.1 & 1.2 Contd.	

Tuesday 1 December
Auditorium Carmen de Herrera (Simón Bolívar Library)

09.00-12.30h	Section 1.3	P. Vanderheyden
14.30-18.00h	Section 1.3 Contd.	

Wednesday 2 December
Auditorium Bernardo Lombardo

09.00-12.30h	Sections 2.1 & 2.2	A. Vlietinck
14:30	Visit to Panama Canal and Touristic Places	

Thursday 3 December
Auditorium Bernardo Lombardo

09.00-12.30h	Sections 3.1 & 3.2	K. Odenthal
14.30-16.00h	Sections 3.1 & 3.2 Contd.	
16.30-18.00h	Presentation of CYTED Program	M. Gupta and A. Cáceres

Friday 4 December
Auditorium Bernardo Lombardo

09.00-12.30h	Sections 3.1 & 3.2 Contd.	K. Odenthal
14.30-18.00h	Strategies for Joint UNIDO Projects and Evaluation of Training Course Country Reports and Round Table Discussion	

Saturday 5 December

08.00 - 15.00h	Visit to a cultivationfarm of a medicinal plants in Chorrera

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SENACYT

Training Course on Screening Technologies for Industrial Exploitation of Medicinal and Aromatic Plants
Panama : 30.11. - 05.12.1998

Introductory Lecture

Medicinal plant preparations as health products and/or phyto-medicines

* Definitions: phytomedicines, pharmafoods, neutraceuticals, health products, phytopharmaceuticals
* Legal status in different countries: notification versus registration
* Economic importance of health products and phytomedicines
* Efforts for world-wide harmonisation

Section 1. Bioassay-guided isolation and identification of industrially useful phytoconstituents

1.1. Screening procedures for the evaluation of medicinal and aromatic plants

* Methods of selection of plants for screening
* Principles of bioassay-guided isolation
* Roles of bioassays
* Classification of bioassays
* Examples of bioassays
  - General screening bioassays: broad and primary screening bioassays
  - Specalised screening bioassays:
    Lower organisms, isolated subcellular systems, isolated cellular systems, isolated organs of vertebrates, whole animals
* Advantages and disadvantages of different bioassays

1.2. Isolation of industrially useful phytoconstituents

* Approach of bioassay-guided isolation
* Initial extraction and product capture= extraction schemes and the concept of dereplication
* Preparative separation methods for plant constituents•
  - Solid phase chromatography: preparative TLC (PTCL), centrifugal TLC (CTLC), overpressure-layer chromatography (OPCC), vacuum liquid

chromatography (VLC), pressure liquid chromatography (FC, LPLC, MPLC, HPLC): supercritical fluid chromatography (SFL)
- Liquid-liquid chromatography: Droplet counter current chromatography (DCCC), high speed counter current chromatography (HSCC)

• These methods will be illustrated by examples worked out at research group of Pharmacognosy and Phytochemistry of the University of Antwerp in the fields of Chemotherapy (antibacterial-antifungal, antiviral, antimutagenic, antiparasitic and insecticidal active plant products) and pharmacology (antioxidative-immunological, antihistaminic, antiplatelet aggregating, serotoninergic, anticomplement and cardiovascular plant products).

1.3 Introduction to Molecular Biology of screening methods: Ligand binding studies

* Molecular Pharmacology of Hormone-and neurotransmitter

Section 2. Production of quality medicines

2.1. Industrialisation of medicinal plants

* Plant material collected from the environment:
  Special problems: proper identification, adulteration and admixtures; quality assurance
* Plant material from cultured species:
  Special problems: microbial contamination, pesticides, agrochemicals, conformity with established specifications
* Guidelines for good agricultural practice (G.A.P.) of medicinal and aromatic plants

2.2. Quality control of starting materials, plant preparations and finished herbal medicinal products

* Analytical methods used for the quality control: spectroscopic methods, chromatographic methods, titrimetric methods and gravimetric methods; elaboration of Pharmacopoeia monographs of starting materials and plant preparations
* Control of starting materials including plants, excipients, primary packaging material and of intermediate plant preparations such as extracts and tinctures. Specification and routine test including charcteristics, identification tests such as macroscopic and microscopic description, qualitative chemical profile, chemical identity tests, detection of adulterants, determination of contamination by microorganisms, products of microorganisms, pesticides, toxic metals, radioactivity, fumigants, assays of the active ingredients and/or markers
* Overview of validation studies

Validation of the methods required for identity, tests and assays of starting materials and finished herbal medicinal products: analytical performance, parameters such as linearity, precision, accuracy, limit of detection, limit of quantitation, selectivity, range and ruggedness

* Control of finished herbal medicinal products and stability test on active substances or markers:
  Specification and routine testing including product specifications, control metods including identification, assay and other tests; pharmaceutical tests; identification and determination of excipients; scientific data including analytical validation and batch analysis; stability testing including normal test conditions and accelerated test conditions discussion; interpretations and conclusions; shelf-life and storage conditions

Section 3. Industrial utilisation of medicinal and aromatic plants

3.1. Applications for a marketing authorisation of finished herbal medicinal products as drugs

* Toxicological and pharmacological evaluation
  - Single dose and repeated dose toxicity; repeated dose tissue distribution studies, reproduction studies, testing for mutagenic and carcinogenic potential, specific aspects of regulatory genotoxicity tests
  - Pharmacokinetics and metabolic studies in the safety evaluation in animals, non-clinical local tolerance testing and preclinical biological safety testing
  - Abridged toxico-pharmacological dossier of phytomedicines: Bibliographical applications for well-established medicinal products: ESCOP and WHO monographs
* Clinical evaluation
  - Good clinical practice
  - Pharmacokinetic studies in men, dose-response information, clinical investigation for long term use, biostatistical methodology in clinical trials, fixed combination products, clinical testing of prolonged action, clinical requirements for locally acting drugs, clinical safety data management
  - Investigation of bioavailibity and bioequivalence
  - Abridged clinical dossier of phytomedicines: Bibliographic applications for well-established medicinal products: documentation on experience in the form of epidemiological studies
  - Post-marketing experience

3.2. Applications for a marketing authorization of finished herbal medicinal products as health products or foods

* Requirements in different countries

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PHYTOMEDICINES AND THE EUROPEAN PHARMACOPOEIA

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  N° 13 : PHYTOCHEMISTRY
  N° 13H : FATTY SUBSTANCES (Chairman, 1995)

- ALTERNATE MEMBER OF THE EUROPEAN PHARMACOPOEIA COMMISSION (1993)

PROCEDURES FOR THE ELABORATION OF MONOGRAPHS FOR THE EUROPEAN PHARMACOPOEIA (1) : PA/PH/SG(95)135,3R

1. PROCEDURE N° 1

ELABORATION BY A GROUP OF EXPERTS

1.1. ATTRIBUTION

* CONSENSUS IN THE COMMISSION
* PRIORITY SETTING
* ALLOCATION TO A GROUP OF EXPERTS
* ATTRIBUTION TO A RAPPORTEUR WITHIN THE GROUP
* STANDARD LETTER BY THE SECRETARIAT TO MANUFACTURERS/SUPPLIERS TO SUPPLY CURRENT PRODUCTION BATCHES (+ IMPURITIES) AND A BATCH AS POTENTIAL CHEMICAL REFERENCE SUBSTANCE (CRS)

1.2. PREPARATION OF THE DRAFT MONOGRAPH

* FIRST DRAFT BY THE RAPPORTEUR TAKING INTO ACCOUNT
  * STYLE GUIDE
  * GUIDE FOR TECHNICAL CONTENT OF MONOGRAPHS
* TRANSLATION AND EDITORIAL VERIFICATION OF THE TEXT BY THE SECRETARIAT

1.3. ADOPTION BY THE GROUP OF EXPERTS OF THE TEXT FOR PUBLICATION IN PHARMEUROPA

* MODIFICATIONS OF THE FIRST DRAFT
* CONSENSUS IN THE GROUP OF EXPERTS
* EDITORIAL AMENDMENTS MADE BY THE SECRETARIAT

1.4. PUBLIC SURVEY

* PUBLICATION IN PHARMEUROPA
* DISTRIBUTION FOR ACTION TO NATIONAL AUTHORITIES
* 3-MONTH DEADLINE FOR COMMENTS

1.5. EXAMINATION OF COMMENTS RECEIVED DURING PUBLIC SURVEY

* CONSIDERATION OF COMMENTS BY THE GROUP OF EXPERTS AND ADJUSTMENT, IF NECESSARY, OF THE DRAFT TEXT
* IN CASES OF IMPORTANT MODIFICATIONS, EITHER A SECOND PUBLICATION IS ENVISAGED OR NATIONAL AUTHORITIES ARE CONSULTED

1.6. ADOPTION BY THE COMMISSION

* REVISION OF THE TEXT APPROVED BY THE GROUP OF EXPERTS BY THE READER GROUP OF THE SECRETARIAT
* SUBMISSION OF 3/T COM DOCUMENTS TO THE COMMISSION FOR ADOPTION AS WELL AS TO THE MEMBERS OF GROUP 3 WHO ARE TO SEND THEIR COMMENTS BEFORE THE SESSION OF THE COMMISSION
* CONSENSUS : UNANIMOUS VOTE OF THE DELEGATIONS

1.7. PUBLICATION IN THE NEXT FASCICULE

PROCEDURES FOR THE ELABORATION OF MONOGRAPHS FOR THE EUROPEAN PHARMACOPOEIA (3) : PA/PH/SG(95)135,3R

2. PROCEDURE N° 2

ADAPTATION OF NATIONAL MONOGRAPHS

2.1. ATTRIBUTION

* PREPARATION BY THE SECRETARIAT OF A LIST OF PRODUCTS FOR WHICH THE COMMISSION HAS AUTHORISED THE ELABORATION AND AN EUROPEAN NATIONAL MONOGRAPH ALREADY EXISTS THAT COULD BE USED AS THE BASIS FOR AN EUROPEAN MONOGRAPH

* APPROVAL OF THE LIST BY THE "ADAPTATION OF NATIONAL MONOGRAPHS" (ANM) WORKING PARTY AND ALLOCATION TO THE APPROPIATE GROUP OF EXPERTS, IN CASE OF NEED IN THE FUTURE

* CIRCULAR LETTER BY THE SECRETARIAT TO THE NATIONAL AUTHORITIES REQUESTING ALL INFORMATION CONCERNING THE ELABORATION OF THE NATIONAL MONOGRAPH(S) AND THE MANUFACTURERS/SUPPLIERS

* STANDARD LETTER BY THE SECRETARIAT TO MANUFACTURERS/SUPPLIERS TO SUPPLY CURRENT PRODUCTION BATCHES (+ IMPURITIES) AND A BACTH AS POTENTIAL CHEMICAL REFERENCE SUBSTANCE (CRS)

2.2. PREPARATION OF THE DRAFT MONOGRAPH

* EXAMINATION OF THE BATCHES BY THE PH. EUR. LABORATORY USING THE METHOD(S) OF THE MONOGRAPH(S)

* PREPARATION OF AN ADAPTED MONOGRAPH, BASED ON THE NATIONAL MONOGRAPH TAKING INTO ACCOUNT

    * FINDINGS OF THE PH. EUR. LABORATORY

    * STYLE GUIDE

    * GUIDE FOR TECHNICAL CONTENT OF MONOGRAPHS

2.3. ADOPTION BY THE ANM WORKING PARTY OF THE TEXT FOR PUBLICATION IN PHARMEUROPA

* EXAMINATION OF THE ADAPTED MONOGRAPH BY THE ANM WORKING PARTY → PHARMEUROPA
* IN CASE OF A MAJOR PROBLEM WITH A TEST OR A LIMIT, SENDING OF THE COMPLETE FILE TO THE GROUP ORIGINALLY ALLOCATED → **STUDY OF THE MONOGRAPH ACCORDING TO PROCEDURE N° 1.**

2.4. PUBLIC SURVEY

* PUBLICATION IN PHARMEUROPA
* DISTRIBUTION FOR ACTION TO NATIONAL AUTHORITIES
* 3-MONTH DEADLINE FOR COMMENTS

2.5. EXAMINATION OF COMMENTS RECEIVED DURING PUBLIC SURVEY

* CONSIDERATION OF COMMENTS BY THE ANM WORKING PARTY
* CHOICE OF THE FOLLOWING COURSES OF ACTION :
    * PREPARATION BY THE SECRETARIAT OF THE COM DOCUMENT FOR SUBMISSION TO THE COMMISSION FOR ADOPTION
    * AS ABOVE, BUT WITH A RECOMMENDATION TO THE COMMISSION THAT A TEST WILL BE EXAMINED BY THE ALLOCATED GROUP FOR A FUTURE REVISION
    * DELEGATION OF AN EXPERT BY THE CHAIRMAN OF THE ALLOCATED GROUP TO COLLABORATE WITH THE PH. EUR. LABORATORY ON A MODIFICATION OF A TEXT → RESULTS SUBMITTED TO ANM WORKING PARTY FOR APPROVAL → REPUBLICATION IN PHARMEUROPA
    * SENDING BACK OF THE MONOGRAPH TO THE ALLOCATED GROUP OF EXPERTS TO STUDY THE TEST ON WHICH **A FUNDAMENTAL OBJECTION** HAS BEEN RAISED

2.6. ADOPTION BY THE COMMISSION

SEE PROCEDURE N° 1

2.7. PUBLICATION IN THE NEXT FASCICULE

3. PROCEDURE N° 3

NATIONAL SECRETARIAT ACTING AS A RAPPORTEUR

* ONLY FOR SUBSTANCES WHICH ARE PRODUCED BY ONLY ONE MANUFACTURER, WHICH ARE CLOSE TO THE PATENT EXPIRY DATE AND WHICH HAVE A LARGE POTENTIAL MARKET FOR GENERICS

REVISION PROCEDURE OF PUBLISHED EUROPEAN PHARMACOPOEIA

MONOGRAPHS (1)

* **REGULAR UP-DATING** OF EUROPEAN PHARMACOPOEIA MONOGRAPHS

* **PUBLICATION** OF ALL DRAFT REVISIONS IN **PHARMEUROPA** FOR PUBLIC SURVEY BEFORE THEIR FINAL ADOPTION

* **IMPLEMENTATION** OF REVISED TEXTS DEPENDING ON THE **URGENCY**

    * EITHER BY **PUBLICATION** IN THE **ANNUAL SUPPLEMENT** TO THE EDITION OF THE EUR. PH.

    * OR, IF URGENT, BY THE **RAPID IMPLEMENTATION PROCEDURE** WITH IMMEDIATE PUBLICATION OF THE CORRECTED TEXT AS A RESOLUTION OF THE PUBLIC HEALTH COMMITTEE OF THE COUNCIL OF EUROPE AND PUBLICATION IN PHARMEUROPA

* **SITUATIONS WHICH REQUIRE REVISION**

1. SELECTIVE REVISION FOR UP-DATING AND/OR CORRECTION OF AN ERROR

    * REQUESTED, WITH **JUSTIFICATION**, BY A NATIONAL PHARMACOPOEIA AUTHORITY OR A NATIONAL OR EUROPEAN LICENSING AUTHORITY

    * EXAMINATION OF THE REQUEST BY THE GROUP OF EXPERTS

2. REVISION RELATED TO CERTIFICATION OF SUITABILITY OF MONOGRAPHS OF THE EUROPEAN PHARMACOPOEIA

3. SYSTEMATIC REVISION OF A GENERAL METHOD OF ANALYSIS OR A SET OF MONOGRAPHS DUE TO THE IMPLEMENTATION OF A POLICY OF THE EUR. PH. COMMISSION

e.g. ELIMINATION OF REAGENTS CONSIDERED TO BE HAZARDOUS

REVISION PROCEDURE OF PUBLISHED EUROPEAN PHARMACOPOEIA

MONOGRAPHS (2)

4. REVISION WITH A VIEW TO INTERNATIONAL HARMONISATION WITH THE USP/JP

* GENERAL METHODS OF ANALYSIS
* MONOGRAPHS ON EXCIPIENTS
* MONOGRAPHS ON BIOLOGICALS

5. HARMONISATION OF MONOGRAPHS OF THE 2nd EDITION

* 2nd EDITION : FROM 1980-1996 : REVISION OF 367 MONOGRAPHS OF THE 1st EDITION, SUPPLEMENTED WITH MORE THAN 700 NEW MONOGRAPHS
* GENERAL REVISION OF THE PRESENTATION OF ALL THESE TEXTS FOR REASONS OF HARMONISATION AND INTERNAL CONSISTENCY AMONG SIMILAR SUBSTANCES THAT WERE PUBLISHED YEARS APART
* THIS WORK IS ENVISAGED FOR THE **4th EDITION**

PHARMACOPOEIAL MONOGRAPHS ON VEGETABLE DRUGS (2)

* NOMENCLATURE

ENGLISH (FRENCH) TITLE :

TRANSLATION OF THE LATIN TITLE, SOMETIMES A MORE COMMON NAME IS USED

LATIN TITLE :

USUALLY DERIVED FROM THE BOTANICAL NAME OF THE SOURCE-PLANT I.E. THE GENUS AND/OR SPECIES (GENITIVE) FOLLOWED BY : ...

1. VEGETABLE DRUGS

... THE NAME OF THE ORGAN USED (NOMINATIVE AND SINGULAR)

EX. BELLADONNAE FOLIUM - BELLADONA LEAF - RHAMNI PURSHIANAE CORTEX - CASCARA

2. PLANT RAW MATERIALS OBTAINED AFTER TREATMENT

2.1. VOLATILE OILS

... THE TERM : AETHEROLEUM

EX. MENTHAE PIPERITAE AETHEROLEUM - PEPPERMINT OIL

2.2. BALSAMS, RESINS, GUMS

THE TRADITIONAL NAME IS USUALLY GIVEN

EX. TRAGACANTHA - TRAGACANTH
BALSAMUM PERUVIANUM - PERU BALSAM

2.3. STARCHES

... THE TERM : AMYLUM

EX. TRITICI AMYLUM - WHEAT STARCH

PHARMACOPOEIAL MONOGRAPHS ON VEGETABLE DRUGS (3)

* NOMENCLATURE (Continued)

3. TINCTURES AND EXTRACTS

... THE TYPE OF PREPARATION (NOMINATIVE AND SINGULAR) : TINCTURA, EXTRACTUM SICCUM, FLUIDUM, SPICCUM

EX. BELLADONNAE TINCTURA - TINCTURE OF BELLADONNA - ALOES EXTRACTUM SICCUM NORMATUM - STANDARDISED ALOES DRY EXTRACT

* DEFINITION

1. VEGETABLE DRUGS

1. WHOLE DRUG, REDUCED DRUG OR DRUG IN POWDERED FORM

2. THE COMPLETE SCIENTIFIC LATIN NAME (GENUS, SPECIES, VARIETY, AUTHOR) OF THE PLANT OBTAINED FROM THE KEW INDEX AND ITS SUPPLEMENTS

3. THE PART OF THE PLANT USED

4. THE CONTENT OF THE ACTIVE CONSTITUENTS EXPRESSED AS LIMITS (UPPER AND/OR LOWER) OF ACCEPTABILITY

5. WHEN THE CONTENT OF ACTIVE INGREDIENTS IS CALCULATED WITH REFERENCE TO THE DRIED DRUG → TEST FOR LOSS ON DRYING UNDER ASSAY

EX. - "ANISEED CONSISTS OF THE DRY, WHOLE MESOCARP OF *PIMPINELLA ANISUM* L. IT CONTAINS NOT LESS THAN 2.0 PERCENT V/M OF ESSENTIAL OIL"

- "PREPARED BELLADONNA IS BELLADONNA LEAF POWDER (180) ADJUSTED IF NECESSARY BY ADDING POWDERED LACTOSE OR BELLADONNA LEAF POWDER WITH A LOWER ALKALOIDAL CONTENT TO CONTAIN 0.28 PERCENT TO 0.32 PERCENT OF TOTAL ALKALOIDS, CALCULATED AS HYOSCYAMINE (MR 289.4) WITH REFERENCE TO THE DRIED DRUG"

- CINCHONA BARK CONSISTS OF ... IT CONTAINS NOT LESS THAN 6.5 PERCENT OF TOTAL ALKALOIDS, OF WHICH NOT LESS THAN 30 PERCENT AND NOT MORE THAN 60 PERCENT CONSISTS OF QUININE-TYPE ALKALOIDS

PHARMACOPOEIAL MONOGRAPHS ON VEGETABLE DRUGS (4)

* DEFINITION (Continued)

2. PLANT RAW MATERIALS OBTAINED AFTER TREATMENT

1. THE COMPLETE SCIENTIFIC LATIN NAME (GENUS, SPECIES, VARIETY, AUTHOR) OF THE PLANT OBTAINED FROM THE KEW INDEX AND ITS SUPPLEMENTS

2. THE PART OF THE PLANT USED

2.1. VOLATILE OILS

3. THE STATE OF DRUG, FRESH OR DRY, AT THE TIME OF PREPARATION

4. THE METHOD OF EXTRACTION

5. THE CONTENT, IF NECESSARY, OF THE PRINCIPAL CONSTITUENTS EXPRESSED AS LIMITS (UPPER AND/OR LOWER)

6. A STATEMENT OF ANY TECHNOLOGICAL MODIFICATIONS MADE (DETERPINATION, RECTIFICATION ...)

EX. "BITTER ORANGE FLOWER OIL IS OBTAINED BY STEAM DISTILLATION FROM THE FRESH FLOWERS OF *CITRUS AURANTIUM* L. SUBSPEC. *AURANTIUM* (*C.AURANTIUM* L. SUBSPEC. *AMARA* ENGL.). IT CONTAINS NOT LESS THAN 3.0 PERCENT AND NOT MORE THAN 15.0 PERCENT LINALYL ACETATE ($C_{12}H_{20}O_2$; Mr 196.3)

2.2. BALSAMS, RESINS AND GUMS

3. THE MEANS BY WHICH IT WAS OBTAINED (INCISION, SCARIFICATION ...)

4. THE CONTENT OF THE PRINCIPAL CONSTITUENTS EXPRESSED AS LIMITS (UPPER AND/OR LOWER) OF ACCEPTABILITY

5. A STATEMENT OF ANY TECHNOLOGICAL MODIFICATIONS MADE

EX. "PERU BALSAM IS THE BALSAM OBTAINED FROM THE SCORCHED AND WOUNDED TRUNK OF *MYROXYLON BALSAMUM* (L) HARMS. VAR. PEREIRA (ROYLE) HARMS. IT CONTAINS NOT LESS THAN 45.0 PERCENT M/M AND NOT MORE THAN 70.0 PERCENT M/M OF ESTERS, MAINLY BENZYLBENZOATE AND BENZYLCINNAMATE"

2.3. STARCHES

EX. "WHEAT STARCH IS OBTAINED FROM THE CARYOPSIS OF *TRITICUM AESTIVUM* L. (T.VULGARE MILL)

PHARMACOPOEIAL MONOGRAPHS ON VEGETABLE DRUGS (5)

* DEFINITION (Continued)

3. TINCTURES AND EXTRACTS

1. THE NATURE OF THE RAW MATERIAL USED (VEGETABLE OR ANIMAL) AND ITS STATE (DRIED OR FRESH)

2. THE COMPLETE SCIENTIFIC LATIN NAME (GENUS, SPECIES, VARIETY, AUTHOR) OF THE PLANT OBTAINED FROM THE KEW INDEX AND ITS SUPPLEMENTS

3. ANY PRELIMINARY TREATMENT, SUCH AS INACTIVATION OF ENZYMES OR DEFATTING, IS MENTIONED

4. THE TECHNIQUE FOR PREPARATION, THE SOLVENTS USED AND THEIR PROPORTIONS MUST BE MENTIONED

5. THE TOTAL CONTENT OF ACTIVE INGREDIENTS IS DETERMINED AND EXPRESSED WITH REFERENCE TO THE MAJOR CONSTITUENTS

6. IN THE CASE OF STANDARDISED PREPARATIONS, A MINIMUM AND MAXIMUM CONTENT ARE PRESCRIBED

   EX. "FLOWERING TOPS OF *CRATAEGUS* 200 G
   60 PERCENT V/V ALCOHOL Q.S.
   PREPARE 1000 G OF TINCTURE BY PERCOLATING THE SUITABLY REDUCED DRUG WITH 60 PERCENT V/V ALCOHOL"

* CHARACTERS

* NOT TO BE REGARDED AS CONSTITUTING ANALYTICAL REQUIREMENTS

* IT DESCRIBES THE DIFFERENT CHARACTERISTICS AND DIFFERENTIATION CRITERIA WHICH MAY BE APPLIED SUBSEQUENTLY FOR PURPOSES OF IDENTIFICATION

* THE MAIN CRITERIA ARE :

1. ORGANOLEPTIC CHARACTERS

2. MACROSCOPIC AND MICROSCOPIC BOTANICAL CHARACTERS

   EX. "IT HAS AN ... ODOUR. IN ADDITION. IT HAS THE MACROSCOPIC AND MICROSCOPIC CHARACTERS DESCRIBED UNDER IDENTIFICATION A AND B

3. CERTAIN CRITERIA REGARDING CLARITY, SOLUBILITY OR MISCIBILITY, AND THE PHYSICAL STATE MAY ALSO BE INCLUDED

PHARMACOPOEIAL MONOGRAPHS ON VEGETABLE DRUGS (6)

* IDENTIFICATION

MANDATORY TESTS TO IDENTIFY THE DRUG

1. VEGETABLE DRUGS

A. MACROSCOPIC BOTANICAL CHARACTERS

IMPORTANT MACROSCOPIC BOTANICAL CHARACTERS

E.G. SIZE, COLOUR, SURFACE CHARACTERISTICS, TEXTURE, FRACTURE, ODOUR (TASTE)

B. MICROSCOPIC BOTANICAL CHARACTERS

B.1. MICROSCOPIC EXAMINATION OF A TRANSVERSE SECTION

* OBTAINED BY CUTTING WITH A RAZOR BLADE AT A RIGHT ANGLE TO THE LONGITUDINAL AXIS OF THE MATERIAL

* ONLY IF ESSENTIAL TO IDENTIFICATION

* MORPHOLOGICAL DESCRIPTION OF TISSUES

* SPECIFICATION OF HISTOLOGICAL REAGENTS

E.G. CELLULOSE CELL WALLS : I_2-$ZnCl_2$
LIGNIFIED CELL WALLS : PHLOROGLUCINOL
SUBERIZED OR CUTICULAR CELL WALLS : SUDAN RED

B.2. MICROSCOPIC EXAMINATION OF THE DRUG REDUCED TO A POWDER

* ONLY DOMINANT OR MOST SPECIFIC CHARACTERS

E.G. STOMATA AND STOMATAL INDEX (2.8.3)

* COLOUR OF THE POWDER, THE SIEVE NUMBER (USUALLY N° 355) (2.1.4)

* SPECIFICATION OF REAGENT

E.G. - WATER, WATER-ALCOHOL, GLYCEROL-ETHANOL
- CLARIFICATION REAGENTS : CHLORALHYDRATE (STARCH, CALCIUMOXALATE CRYSTALS, VOLATILE OILS)

PHARMACOPOEIAL MONOGRAPHS ON VEGETABLE DRUGS (7)

* IDENTIFICATION (Continued)

1. VEGETABLE DRUGS

C. THIN LAYER CHROMATOGRAPHY (TLC)

* DESCRIBED UNDER TESTS

* ALWAYS UNDER IDENTIFICATION EVEN IF GC OR LC ARE SUBSEQUENTLY USED

* AIMED AT IDENTIFYING THE CHROMATOGRAM OF THE DRUG WITH RESPECT TO CHEMICAL REFERENCE SUBSTANCES (CRS)

EX. "EXAMINE THE CHROMATOGRAMS OBTAINED IN THE TEST FOR CHROMATOGRAPHY IN DAYLIGHT/IN ULTRAVIOLET LIGHT AT 254 NM/365 NM. THE PRINCIPAL BAND IN THE CHROMATOGRAM OBTAINED WITH THE TEST SOLUTION IS SIMILAR IN POSITION, COLOUR, OR FLUORESCENCE AND SIZE TO THE PRINCIPAL BAND IN THE CHROMATOGRAM OBTAINED WITH THE REFERENCE SOLUTION (A)"

D. CHEMICAL REACTIONS FOR IDENTIFICATION

* SPECIFIC AS FAR AS POSSIBLE

* NOT TOO SENSITIVE, SO AS TO AVOID FALSE REACTIONS DUE TO THE PRESENCE OF TOLERATED IMPURITIES

* PERMIT VERY RAPID IDENTIFICATION (TEST TUBE) OF A CHARACTERISTIC CONSTITUENT OR FAMILY OF CONSTITUENTS PRESENT IN THE DRUG (ARE NAMED, IF POSSIBLE).
CERTAIN PURITY TESTS MAY BE SUITABLE FOR IDENTIFICATION PURPOSES, POSSIBLY IN MODIFIED FORM. A SYSTEM FOR REFERENCE TO THE TESTS SECTION IS USED, IF POSSIBLE.

EX. "TO 2 ML OF SOLUTION S (SEE TESTS) ADD 2 ML OF WATER AND 0.4 ML OF FERRIC CHLORIDE SOLUTION R_2 ; A BLACK PRECIPITATE DEVELOPS (TANNINS)"

* IDENTIFICATION (Continued)

2. PLANT RAW MATERIALS OBTAINED AFTER TREATMENT

2.1. VOLATILE OILS

A. TLC

B. GC

C. CHEMICAL IDENTIFICATION REACTIONS (IF NECESSARY)

2.2. BALSAMS, RESINS AND GUMS

A. MACROSCOPIC CHARACTERS

B. MICROSCOPIC CHARACTERS

C. TLC

D. CHEMICAL IDENTIFICATION REACTIONS (IF NECESSARY)

2.3. STARCHES

A. MICROSCOPIC CHARACTERS

- SHAPE OF GRANULES : POLYHEDRAL, ROUNDED OR OVOID, CRACKS OR IRREGULARITIES IN THE EDGE
- STATE OF THE GRANULES : SIMPLE OR COMPOUND GRANULES
- SIZE OF THE GRANULES OR GROUPS OF ELEMENTS ("X" µM TO "Y" µM)
- HILUM (CONCENTRIC OR ECCENTRIC, PUNCTIFORM, SLIT-SHAPED, BIFID OR STAR-SHAPED)
- CONCENTRIC STRIATIONS, PRESENT OR ABSENT
- OBSERVATION OF THE BLACK CROSS WHEN VIEWED UNDER POLARISED LIGHT

2.3. STARCHES (Continued)

B. CHEMICAL IDENTIFICATION REACTIONS

B.1. FORMULATION OF A MUCILAGE

B.2. REACTION WITH IODINE

* IDENTIFICATION (Continued)

3. TINCTURES AND EXTRACTS

A. TLC

B. CHEMICAL IDENTIFICATION REACTIONS

PHARMACOPOEIAL MONOGRAPHS ON VEGETABLE DRUGS (10)

* TESTS

1. VEGETABLE DRUGS

* DETECTION OF ADULTERATION WITH OTHER PLANT SP.

* EXCLUDE NATURALLY OCCURRING TOXIC SUBSTANCES
E.G. SOME ALKALOIDS, CARDIOTONIC HETEROSIDES, ANTHRONES

* LIMIT POSSIBLE DEGRADATION OF PLANT CONSTITUENTS

1.1. ADDITIONAL MICROSCOPIC AND/OR CHEMICAL REACTIONS

1.2. CHROMATOGRAPHIC TESTS

1.2.1. THIN LAYER CHROMATOGRAPHY (TCL) (2.2.27)

SPECIFICATION IS GIVEN CONCERNING :

(a) THE TYPE OF COATING SUBSTANCE

E.G. - SUPPORT DESCRIBED IN THE PHARMACOPOEIA UNDER REAGENTS VII.1.1. (E.G. SILICAGEL GR)

- PRECOATED PLATE (E.G. SUITABLE SILICAGEL) : CHECK OF THE SEPARATING POWER ON ADDITIONAL REFERENCE SOLUTION

(b) METHOD OF PREPARATION AND CONCENTRATION OF THE TEST AND REFERENCE SOLUTIONS

E.G. AT LEAST TWO REFERENCE SUBSTANCES OR MARKERS

(c) METHOD OF APPLICATION (BANDS OR SPOTS) AND THE VOLUME OF THE SOLUTIONS TO BE PLACED ON THE PLATE

(d) COMPOSITION AND VOLUME OF THE MOBILE PHASE (SATURATED CHAMBER OR NOT)

(e) DISTANCE OF DEVELOPMENT

(f) MODE OF DETECTION

(g) ASSESSMENT OF THE CHROMATOGRAMS
PRINCIPAL SPOTS OR BANDS IN RELATION TO THE POSITION OF THE REFERENCE SUBSTANCES OR MARKERS (SUBSTANCES ARE NAMED)

* TESTS

1. VEGETABLE DRUGS (Continued)

1.2.2. GAS-LIQUID CHROMATOGRAPHY (GC) (2.2.28)

* SPECIFICATION IS GIVEN CONCERNING :

(a) METHOD OF PREPARATION OF TEST AND REFERENCE SOLUTIONS

(b) NATURE OF THE STATIONARY PHASE I.E. COMPOSITION AND CONCENTRATION OF SUBSTANCE COVERING THE SUPPORT AND THE INERT SUPPORT ITSELF, ALSO ANY PRIOR TREATMENT

(c) TEMPERATURE PROGRAMME AS WELL AS ELUTION PROGRAMME, TEMPERATURES OF INJECTION PORT AND DETECTOR, DURATION OF THE CHROMATOGRAM

(d) METHOD OF INJECTION AND INJECTION VOLUME

(e) METHOD OF DETECTION

* SOME MONOGRAPHS (E.G. VOLATILE OILS) CONTAIN TYPICAL CHROMATOGRAMS : THEY SERVE AS INFORMATION E.G. ON SEQUENCE OF PEAKS AND ARE NO ANALYTICAL REQUIREMENTS

1.2.3. LIQUID CHROMATOGRAPHY (LC) (2.2.29)

* SUBTITLE : NAME OF IMPURITIES TO BE DETECTED

* SPECIFICATION IS GIVEN CONCERNING :

(a) IDEM AS UNDER GC

(b) NATURE OF COLUMN MATERIAL, DIMENSIONS, NATURE OF STATIONARY PHASE (ALSO PRETREATMENT), COMPOSITION AND FLOW RATE OF MOBILE PHASE INCLUDING ELUTION PROGRAMME, COLUMN TEMPERATURE, DURATION OF CHROMATOGRAPHY

(c) METHOD OF INJECTION, INJECTION VOLUME

(d) METHOD OF DETECTION

(e) DESCRIPTION OF RESOLUTION TEST

^ THE VALIDATION CONDITIONS ARE INDICATED WHENEVER NECESSARY

* TESTS

1. VEGETABLE DRUGS (Continued)

1.3. GENERAL TESTS

1.3.1. FOREIGN MATTER (2.8.2)

* VEGETABLE DRUGS SHOULD BE FREE FROM MOULDS, INSECTS AND OTHER ANIMAL CONTAMINATION

* FOREIGN MATTER IS MATERIAL CONSISTING OF :

  - MATTER COMING FROM THE SOURCE PLANT BUT NOT DEFINIED AS THE DRUG

  - MATTER NOT COMING FROM THE SOURCE PLANT AND EITHER OF VEGETABLE OR MINERAL ORIGIN

1.3.2 LOSS ON DRYING (2.2.32)

* DETERMINES THE AMOUNT OF WATER THAT MAY BE ELIMINATED UNDER THE STATED CONDITIONS

  E.G. HEATING TO 100-105°, DRYING IN A DESSICATOR OVER P_2O_5

* NOT MORE THAN 15.0 PERCENT DETERMINED ON ... (ONLY UPPER LIMIT)

* TO BE CARRIED OUT ON THE POWDERED DRUG INDICATING THE AMOUNT OF DRUG AND THE SIEVE NUMBER (2.1.4)

* DRUG WITH VOLATILE CONSTITUENTS : DETERMINATION OF WATER IS CARRIED OUT INSTEAD OF LOSS ON DRYING (2.2.13)

1.3.3. TOTAL ASH (2.4.16)

* MEASURES THE AMOUNT OF MATERIAL REMAINING AFTER IGNITION

* ON THE POWDERED DRUG ; IF NECESSARY, STATE THE SIEVE NUMBER

  E.G. NOT MORE THAN 16.0 PERCENT

1.3. GENERAL TESTS (Continued)

1.3.4. ASH INSOLUBLE IN HYDROCHLORIC ACID (2.8.1)

* MEASURES THE PRESENCE OF SILICA, ESPECIALLY SAND AND SILICEOUS EARTH

E.G. NOT MORE THAN 4.0 PERCENT

1.3.5. MICROBIAL CONTAMINATION

A. HERBAL REMEDIES TO WHICH BOILING WATER IS ADDED BEFORE USE

* TOTAL VIABLE COUNT (2.6.12) : NOT MORE THAN 10^7 AEROBIC BACTERIA AND NOT MORE THAN 10^5 FUNGI PER GR OR PER ML

* NOT MORE THAN 10^2 *E.COLI* PER GR OR PER ML (2.6.13), (USING SUITABLE DILUTIONS)

B. OTHER HERBAL REMEDIES

* TOTAL VIABLE AEROBIC COUNT (2.6.12) : NOT MORE THAN 10^5 AEROBIC BACTERIA AND NOT MORE THAN 10^4 FUNGI PER GR OR PER ML

* NOT MORE THAN 10^3 ENTEROBACTERIA AND CERTAIN OTHER GRAM NEGATIVE BACTERIA PER GR OR PER ML

* ABSENCE OF *E.COLI* (1.0 G), (2.6.13)

* ABSENCE OF *SALMONELLA* (10.0 G), (2.6.13)

1.3.6. MINERAL RESIDUES

* HEAVY METALS (2.4.8)
INCLUDING ALL THE NON-VOLATILE METALS : RESULTS EXPRESSED AS LEAD : NOT MORE THAN 10 PPM

* ARSENIC (2.4.2) : NOT MORE THAN 10 PPM

* PROPOSAL : Cd : MAX 0.3 PPM ; Hg : MAX 0.3 PPM

1.3. GENERAL TESTS (Continued)

1.3.7. PESTICIDE RESIDUES IN VEGETABLE DRUGS (2.8.13)

DEFINITION : FOR THE PURPOSES OF THE PHARMACOPOEIA, A PESTICIDE IS ANY SUBSTANCE OR MIXTURE OF SUBSTANCES INTENDED FOR PREVENTING, DESTROYING OR CONTROLLING ANY PEST, UNWANTED SPECIES OF PLANTS OR ANIMALS CAUSING HARM DURING OR OTHERWISE INTERFERING WITH THE PRODUCTION, PROCESSING, STORAGE, TRANSPORT OR MARKETING OF VEGETABLE DRUGS. THE ITEM INCLUDES SUBSTANCES INTENDED FOR USE AS GROWTH-REGULATORS, DEFOLIANTS, DESICCANTS OR ANY SUBSTANCE APPLIED TO CROPS EITHER BEFORE OR AFTER HARVEST TO PROTECT THE COMMODITY FROM DETERIORATION DURING STORAGE AND TRANSPORT

SUBSTANCE	LIMIT (in mg/kg)
ALACHLOR	0.02
ALDRIN AND DIELDRIN (SUM OF)	0.05
AZINPHOS-METHYL	1.0
BROMOPROPYLATE	3.0
CHLORDANE (SUM OF *CIS*, *TRANS*- AND OXYCHLORDANE)	0.05
CHLORFENVINPHOS	0.5
CHLORPYRIFOS	0.2
CHLORPYRIFOS-METHYL	0.1
CYPERMETHRIN (AND ISOMERS)	1.0
DDT (SUM OF *p,p'*-DDT, *o-p'*-DDE and *p,p'*-TDE)	1.0
DELTAMETHRIN	0.5
DIAZINON	0.5
DICHLORVOS	1.0
DITHIOCARBAMATES (as CS_2)	2.0
ENDOSULFAN (SUM OF ISOMERS AND ENDOSULFAN SULPHATE)	3.0
ENDRIN	0.01
ETHION	2.0
FENITROTHION	0.5
FENVALERATE	1.5
FONOFOS	0.05
HEPTACHLOR (SUM OF HEPTACHLOR AND HEPTACHLOR-EPOXIDE)	0.02
HEXACHLORBENZENE	0.1
HEXACHLORCYCLOHEXANE-ISOMERS (OTHER THAN γ)	0.3
LINDANE (γ-HEXACHLORCYCLOHEXANE)	0.6
MALATHION	1.0
METHIDATHION	0.2
PARATHION	0.5
PARATHION-METHYL	0.2
PERMETHRIN	1.0
PHOSALONE	0.01
PIPERONYL BUTOXIDE	3.0
PIRIMIPHOS-METHYL	4.0
PYRETHRINS (SUM OF)	3.0
QUINTOZENE (SUM OF QUINTOZENE, PENTACHLOROANALINE AND METHYLPENTACHLORPHENYLSULPHIDE)	1.0

* TESTS

1. VEGETABLE DRUGS (Continued)

1.3. GENERAL TESTS (Continued)

1.3.8. AFLATOXINS (PROPOSAL)

* LESS THAN 5 PPB AFLATOXIN B_1

* LESS THAN 10 PPB TOTAL AFLATOXINS

1.3.9. RADIOACTIVE CONTAMINATION (PROPOSAL)

* LESS THAN 600 Bq/Kg (16.2 nCi/Kg) Cs-134 AND Cs-137

1.4. SPECIFIC TESTS

- SWELLING INDEX (2.8.4)
- BITTERNESS VALUE
- HAEMOLYTICAL INDEX
- TEST FOR SPECIFIC IMPURITIES KNOWN TO BE USED TO ADULTERATE THE VEGETABLE DRUG ANALYSED

* TESTS

2. PLANT RAW MATERIALS OBTAINED AFTER TREATMENT

2.1. VOLATILE OILS

1. CHROMATOGRAPHIC METHODS

* GAS CHROMATOGRAPHY (GC) (2.2.28)
THE DESCRIPTION OF THE CHROMATOGRAM SPECIFIES THE COMPOSITION OF THE VOLATILE OIL AND INDICATES THE RELATIVE CONTENTS OF THE PRINCIPAL CONSTITUENTS

EX. ANISE OIL
DETERMINE FOR EACH OF THESE SIX COMPONENTS, USING THE CHROMATOGRAM OBTAINED WITH THE TEST SOLUTION, THE AREA OF THE PEAK AS A PERCENTAGE OF THE AREA OF ALL PEAKS EXCEPT THE PEAK CORRESPONDING TO HEXANE
THE PERCENTAGES RANGE BETWEEN THE FOLLOWING VALUES :

LINALOL	0.1 TO 1.5 PERCENT
ESTRAGOLE	0.5 TO 6.0 PERCENT
α-TERPINEOL	0.1 TO 1.5 PERCENT
CIS-ANETHOLE	LESS THAN 0.5 PERCENT
TRANS-ANETHOLE	84 TO 93 PERCENT
ANISIC ALDEHYDE	0.1 TO 3.5 PERCENT

Δ THE VALIDATION CONDITIONS ARE INDICATED WHENEVER NECESSARY

2. GENERAL TESTS

3. SPECIFIC TESTS

- FREEZING POINT (2.2.18)
- ANGLE OF OPTICAL ROTATION (2.2.7)
- RELATIVE DENSITY (2.2.5)
- REFRACTIVE INDEX (2.2.6)
- ACID VALUE (2.5.1)
- SOLUBILITY IN ALCOHOL (2.8.10)
- FOREIGN ESTERS (2.8.6)
- FATTY OILS AND RESINIFIED ESSENTIAL OILS (2.8.7)

TESTS

2. PLANT RAW MATERIALS OBTAINED AFTER TREATMENT (Continued)

2.2. BALSAMS, GUMS AND RESINS

1. CHROMATOGRAPHIC TESTS

2. GENERAL TESTS

3. SPECIFIC TESTS

- SWELLING INDEX (2.8.4)
- SAPONIFICATION VALUE (2.5.6)
- INSOLUBLE MATTER : SPECIFICATIONS ON THE QUANTITY OF THE DRUG, THE TYPE AND QUANTITY OF SOLVENT, THE EXTRACTION PROCEDURE, THE METHOD FOR DRYING OF THE EXTRACTS AND THE MAXIMUM VALUE OF THE MASS OF THE RESIDUE
- PH (2.9.17)
- FLOW TIME (2.2.8)
- VISCOSITY (2.2.5)
- RELATIVE DENSITY

2.3. STARCHES

1. GENERAL TESTS

2. SPECIFIC TESTS

- ACIDITY
- OXIDIZING SUBSTANCES
- SULFUR DIOXYDE
- GLUTEN

3. TINCTURES AND EXTRACTS

1. CHROMATOGRAPHIC TESTS

2. GENERAL TESTS

3. SPECIFIC TESTS

- RELATIVE DENSITY (2.2.5)
- ETHANOL CONTENT (2.9.10)
- METHANOL AND 2-PROPANOL CONTENTS (2.9.11)
- DRY RESIDUE

PHARMACOPOEIAL MONOGRAPHS ON VEGETABLE DRUGS (18)

* ASSAY

* ESSENTIAL PART OF ALL MONOGRAPHS UNLESS :

  - ALL THE FORESEEN IMPURITIES CAN BE DETECTED AND LIMITED WITH SUFFICIENT PRECISION
  - CERTAIN QUANTITATIVE TESTS, SIMILAR TO ASSAYS, ARE CARRIED OUT WITH SUFFICIENT PRECISION (SPECIFIC OPTICAL ROTATION, SPECIFIC ABSORBANCE, SWELLING INDEX, DETERMINATION OF ESSENTIAL OILS ...)

* EVERY ASSAY METHOD PROPOSED MUST BE VALIDATED. A VALIDATION DOCUMENT SHOULD BE PRODUCED IN WHICH THE FOLLOWING INFORMATION IS GIVEN :

  1. SELECTIVITY AND/OR SPECIFICITY OF THE METHOD

  2. EVIDENCE OF LINEARITY IN THE RANGE 70% TO 130% OF THE PRESCRIBED QUANTITY (EXCEPT FOR TITRATIONS)

  3. REPEATIBILITY WITH TABLE CONTAINING INDIVIDUAL RESULTS, MEAN, RELATIVE STANDARD DEVIATION AND RANGE

  4. REPRODUCIBILITY WITH TABLE CONTAINING INDIVIDUAL RESULTS, MEAN AND RANGE

  5. ACCURACY

* METHODS OF ASSAYS

  1. <u>SPECTROPHOTOMETRIC ASSAYS</u> IN THE UV OR VISIBLE RANGE :
     - DIRECT MEASUREMENT :
       E.G. *CINCHONA* ALKALOIDS
     - MEASUREMENT AFTER A COLOUR REACTION

  2. <u>VOLUMETRIC ASSAYS</u>
     - ACIDIMETRY AND ALKALIMETRY
       E.G. ALKALOIDS, AROMATIC ACIDS
     - NON-AQUEOUS TITRATIONS
       E.G. NEUTRAL ALKALOIDS

  3. <u>CHROMATOGRAPHIC ASSAYS</u>
     - GAS-LIQUID CHROMATOGRAPHY (GC)
       E.G. VOLATILE OILS
     - LIQUID CHROMATOGRAPHY (LC)
       E.G. OPIUM

  4. <u>DETERMINATION OF ESSENTIAL OILS IN VEGETABLE DRUGS</u> (2.8.12)
     STEAM DISTILLATION IN A SPECIAL APPARATUS IN STANDARDISED CONDITIONS

* STORAGE

* NO OFFICIAL REQUIREMENTS

* NECESSARY TO GUARANTEE, DURING STORAGE, THE QUALITY OF A DRUG INCLUDED IN THE PHARMACOPOEIA

1. VEGETABLE DRUGS

GENERALLY THE WORDING "STORE IN A WELL-CLOSED CONTAINER PROTECTED FROM LIGHT"

2. PLANT RAW MATERIALS OBTAINED AFTER TREATMENT

2.1. VOLATILE OILS

"STORE IN A WELL-FILLED, AIRTIGHT CONTAINER, PROTECTED FROM LIGHT AND HEAT"
DO NOT MIX VOLATILE OILS FROM DIFFERENT BATCHES

2.2. BALSAMS, RESINS, GUMS

"STORE IN A WELL-CLOSED CONTAINER"
"STORE IN A WELL-CLOSED CONTAINER PROTECTED FROM LIGHT" (BALSAMS)

2.3. STARCHES

"STORE IN A WELL-CLOSED CONTAINER"

3. TINCTURES AND EXTRACTS

"STORE IN AN AIRTIGHT CONTAINER, PROTECTED FROM LIGHT"

PHARMACOPOEIAL MONOGRAPHS ON VEGETABLE DRUGS (20)

* LABELLING

* NOT EXHAUSTIVE

* MANDATORY STATEMENTS OR RECOMMENDATIONS

TINCTURES AND EXTRACTS

THE LABEL ON THE CONTAINER STATES :

TINCTURES

- THE VEGETABLE OR ANIMAL MATTER USED

- WHERE APPLICABLE, THAT FRESH VEGETABLE OR ANIMAL MATTER WAS USED

- THE CONCENTRATION OF ETHANOL USED FOR THE PREPARATION

- THE CONCENTRATION OF ETHANOL IN THE FINAL TINCTURE

- THE CONTENT OF ACTIVE PRINCIPAL AND/OR THE RATIO OF THE STARTING MATERIAL TO EXTRACTION FLUID AND OF STARTING MATERIAL TO FINAL TINCTURE

EXTRACTS (LIQUID, SOFT, DRY)

- WHERE APPLICABLE, THE NAME AND AMOUNT OF THE INERT MATERIAL USED

- THE VEGETABLE OR ANIMAL MATTER USED

- WHERE APPLICABLE, THAT FRESH VEGETABLE OR ANIMAL MATTER WAS USED

- THE NAME AND CONCENTRATION OF THE SOLVENT USED FOR THE PREPARATION

- WHERE APPLICABLE, THE CONCENTRATION OF ETHANOL IN THE FINAL EXTRACT

- THE CONTENT OF ACTIVE PRINCIPLE AND/OR THE RATIO OF THE STARTING MATERIAL TO FINAL DRY EXTRACT

- WHERE APPLICABLE, THE NAME AND CONCENTRATION OF ANY ADDED PRESERVATIVE

ANALYTICAL VALIDATION

TEST PROCEDURE

THE TOTAL OPERATION NECESSARY TO PERFORM THE ANALYSIS OF AN ANALYTE : PREPARATION OF THE SAMPLE, OF THE REFERENCE MATERIAL, OF THE REAGENTS, USE OF THE APPARATUS, CALIBRATION CURVE, FORMULAE FOR THE CALCULATION, NUMBER OF REPLICATES AND OPERATING PROCEDURE FOR THE REPLICATES ETC ...

TYPE OF TEST PROCEDURES	IDENTIFICATION	TEST		ASSAY
VALIDATION CHARACTERISTICS		QUANTI-TATIVE	LIMIT TESTS	CONTENT POTENCY
SPECIFICITY	YES	YES	YES	YES
ACCURACY	NO	YES	YES/NO	YES
PRECISION	NO	YES	NO	YES
LIMIT OF DETECTION	NO	NO	YES	NO
LIMIT OF QUANTITATION	NO	YES	NO	NO
LINEARITY	NO	YES	NO	YES

VALIDATION IS NECESSARY NOT ONLY FOR FULFILLING GOVERNMENTAL REGULATIONS, INDEED IT IS A VITAL TASK IN SERIOUS ANALYTICAL LABORATORIES

SPECIFICTY

- IDENTIFICATION : TO ENSURE THE IDENTITY OF AN ANALYTE
- TESTS : TO ENSURE THAT ALL THE TEST PROCEDURES PERFORMED ALLOW AN EVALUATION OF THE CONTENT OF IMPURITIES OF AN ANALYTE
- ASSAY : TO ENSURE THAT THE SIGNAL MEASURED WITH THE TEST PROCEDURE COMES ONLY FROM THE SUBSTANCE BEING ANALYSED I.E. NO INTERFERENCES FROM DEGRADATION PRODUCTS AND/OR IMPURTIES

ANALYTICAL VALIDATION (Continued)

ACCURACY

THE CLOSENESS OF AGREEMENT BETWEEN THE "TRUE VALUE" AND THE MEASURED VALUE. IN PRACTICE IT EXPRESSED THE CLOSENESS OF AGREEMENT BETWEEN THE VALUE WHICH IS ACCEPTED E.G. IN-HOUSE STANDARD, INTERNATIONAL STANDARD AND THE VALUE FOUND (MEAN VALUE) OBTAINED BY APPLYING THE TEST PROCEDURE A NUMBER OF TIMES

(→ INDICATION OF SYSTEMATIC ERRORS)

PRECISION

THE CLOSENESS OF AGREEMENT BETWEEN A SERIES OF MEASUREMENTS OBTAINED FROM MULTIPLE SAMPLING OF THE SAME HOMOGENEOUS SAMPLE UNDER PRESCRIBED CONDITIONS

(→ INDICATION OF RANDOM ERROR)

REPEATIBILITY

EXPRESSES THE PRECISION UNDER IDENTICAL CONDITIONS

I.E. SAME ANALYST, SAME APPARATUS. SHORT INTERVAL OF TIME, IDENTICAL REAGENTS

REPRODUCIBILITY

EXPRESSES THE PRECISION UNDER DIFFERENT CONDITIONS

E.G. LABORATORIES, REAGENTS FROM DIFFERENT SOURCES, ANALYSTS, DAYS, APPARATUS FROM DIFFERENT MANUFACTURERS

THE RESULTS SHOULD BE EXPRESSED AS RESPECTIVELY REPEATIBILITY AND REPRODUCIBILITY STANDARD DEVIATION, COEFFICIENT OF VARIATION (RELATIVE STANDARD DEVIATION) AND THE CONFIDENCE INTERVAL OF THE MEAN VALUE ($n > 6$; $a = 0.05$ or $P = 95\%$)

ANALYTICAL VALIDATION (Continued)

LIMIT OF DETECTION (LOD)

THE LOWEST AMOUNT OF ANALYTE IN A SAMPLE WHICH CAN BE DETECTED BUT NOT QUANTITATED AS AN EXACT VALUE

→ MOSTLY A PARAMETER OF LIMIT TESTS

LIMIT OF QUANTITATION (LQD)

THE LOWEST AMOUNT OF AN ANALYTE IN A SAMPLE WHICH CAN BE QUANTITATIVELY DETERMINED WITH ACCEPTABLE PRECISION AND ACCURACY UNDER THE STATED EXPERIMENTAL CONDITIONS

LINEARITY

THE ABILITY (WITHIN A GIVEN RANGE) TO OBTAIN TEST RESULTS (AFTER CORRECTION FOR ANY BLANK VALUE) DIRECTLY PROPORTIONAL TO THE CONCENTRATION (AMOUNT) OF ANALYTE IN THE SAMPLE

RANGE

THE INTERVAL BETWEEN THE UPPER AND LOWER LEVELS OF ANALYTE (INCLUDING THESE LEVELS) FOR WHICH THE PROCEDURE HAS BEEN DEMONSTRATED AS SUITABLE WITH PRECISION, ACCURACY AND LINEARITY USING THE METHOD AS WRITTEN

SENSITIVITY

CAPACITY OF THE TEST PROCEDURE TO RECORD SMALL VARIATIONS

→ USUALLY EXPRESSED AS THE SLOPE OF THE CALIBRATION CURVE

The European Agency for the Evaluation of Medicinal Products
Human Medicines Evaluation Unit

17 September 1998
EMEA/adhocHMPWG/114/98

AD HOC WORKING GROUP ON HERBAL MEDICINAL PRODUCTS

- **Good Manufacturing Practice (GMP): Comments and proposals for revision**
- **Proposals for revision of Note for Guidance "Quality of Herbal Remedies"**
- **Proposal for new guidance: "Non-clinical testing of herbal drug preparations with long-term marketing experience" Guidance to facilitate mutual recognition and use of bibliographic data**
- **Notice to Applicants Volume 2A and Volume 2B Parts IB1, IC2, and III – Comments and proposals for revision**
- **Comments on Part 4 of Annex to Council Directive 75/318/EEC of 20 May 1975 "Clinical documentation"**
- **Proposal for a core-SPC for VALERIANAE RADIX**

RELEASE FOR CONSULTATION BY THE EMEA	January 1998
DEADLINE FOR COMMENTS	April 1998
RELEASE OF FINAL PROPOSALS	September 1998

NOTE:

At the request of the European Commission, the EMEA Executive Director asked for the support of the EMEA Management Board to set up in May 1997, an Ad Hoc Working Group on Herbal Medicinal Products.

The attached document has been prepared by the Ad Hoc Working Group on Herbal Medicinal Products and will be included in the Final Report from the Ad Hoc Working Group on Herbal Medicinal Products to the EMEA and to the European Commission.

Modifications from the original texts proposed by the group are in ***bold italic***.

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GOOD MANUFACTURING PRACTICE (GMP)
Comments and proposals for revision

The group reviewed existing provisions in the Good Manufacturing Practice guide, in particular Annex 7 on the Manufacture of Herbal Medicinal Products. It confirmed the appropriateness of this guidance, though a few minor changes are suggested as follows:

Principle

- The group indicated that a consistent quality of herbal drugs may need more detailed definitions of aspects of agricultural production. The selection of seeds, conditions of cultivation and harvesting represent an important aspect in producing a reproducible quality of herbal drugs. Ongoing discussions on Good Agricultural Practices (G.A.P) for medicinal plants should be monitored and cross-references should be included as soon as a consensus on G.A.P is obtained.

Documentation

- The group indicated that "starting material" could be a medicinal plant, a herbal drug or a herbal drug preparation. A clear distinction should be made between these starting materials. In the case where, for example, a herbal drug preparation is the starting material, there is a need to go back to the source material and to obtain the data on the source of the plant. In those cases where such data are lacking the applicant has to justify why these data cannot be obtained. It was indicated that in those cases additional specifications on possible contamination are required. Some Member States' representatives reported on the difficulties they encounter in obtaining information on the origin of plants and production conditions.

- The group agreed that ***water content*** should be included in the list of specifications to be provided for products such as medicinal plants and herbal drugs. Such a specification is not necessary for essential oils, fatty oils and resins.

- It was suggested to amend the seventh indent of the specifications for medicinal crude plants as follows: "assay of constituents of known therapeutic activity ***if identified***, or ***where appropriate*** of markers" (instead of "assay, where appropriate, of constituents of known therapeutic activity or of markers").

- The group examined the issue of pesticide contamination and the European Pharmacopoeia representative reminded participants of the general method to test pesticide residues which provides a list of pesticides and their maximum limits and information on the methods of testing. Therefore it was suggested to add in the text "***in accordance with European Pharmacopoeia methods***" after "methods suitable to determine possible pesticide contamination and limits accepted" (eighth indent). The group underlined the need for a general monograph on herbal drugs in the European Pharmacopoeia because harmonised criteria for limits for toxic metals and mycotoxins are still lacking.

Sampling

- It was indicated that a reference sample of the plant material will be necessary in those cases where the plant is not well known in Europe, for example, herbal drugs coming from Asia. Samples of plant material are particular necessary if powders are used.

PROPOSALS FOR REVISION OF NOTE FOR GUIDANCE "QUALITY OF HERBAL REMEDIES" (NOVEMBER 1988)

Note for Guidance "Quality of Herbal ***Medicinal Products***" (July 1998)

Note for Guidance concerning the application of Part *2* of the Annex to Directive 75/318/EEC, as amended. The special problems of herbal ***medicinal products*** and the differences between medicinal products containing chemically defined active ***substances*** are described in this Note for Guidance(*).

This Note for Guidance should be read in conjunction with the Annex 7 "Manufacture of Herbal Medicinal Products" of Good Manufacturing Practice (GMP) for medicinal products; GMP recommendations should be respected.

Consistent quality for products of ***herbal*** origin can only be assured if the starting materials are defined in a rigorous and detailed manner including especially the specific botanical identification of the plant material used. It is also important to know the geographical source and the conditions under which the ***herbal*** drug is obtained to ensure material of consistent quality.

Reference substances used in the control of all stages of the manufacturing process should be *specified*.

A. QUALITATIVE AND QUANTITATIVE PARTICULARS OF THE *ACTIVE SUBSTANCE(S) OF A HERBAL MEDICINAL PRODUCT*

1) In the case of a ***herbal*** drug ***or of a herbal drug preparation consisting of reduced, comminuted or powdered herbal drugs***

either (i) the ***native*** quantity of a ***herbal*** drug ***or of a herbal drug preparation shall*** be stated ***if constituents with known therapeutic activity are unknown***

or (ii) the ***native*** quantity of a ***herbal*** drug ***or of a herbal drug preparation shall*** be given as a range corresponding to a defined quantity of constituents with known therapeutic activity.

EXAMPLE

i) Active *substance*

Name	Quantity (**)
Valerianae radix	900 mg

Other substance(s)
Name
...

ii) Active *substance*

Name	Quantity
Sennae folium	415-500 mg, corresponding to 12.5 mg of hydroxyanthracene glycosides, calculated as Sennoside B.

Other *substance(s)*
Name
...

(*) In this Note for Guidance, the sequence used is designed to relate directly to Part *2* of the Annex to Directive 75/318/EEC, as amended.

(**) ***The quantity indicated refers to the specifications provided in the documentation.***

2) In the case of a *herbal* drug preparation ***produced by steps which exceed comminution and reduction, the nature and concentration of the solvent and the physical state of the extract have to be given. Furthermore the following has to be indicated:***

either (i) the equivalent quantity *x - y* (*), or the ratio *a - b: 1*(*) of the *herbal* drug to the *herbal* drug preparation ***shall*** be stated ***if constituents with known therapeutic activity are unknown*** (this does not apply to fatty or essential oils).

or (ii) ***if the constituents with known therapeutic activity are known***, the quantity of the *herbal* drug preparation may be given as a range corresponding to a defined quantity of ***these*** constituents (see example).

The composition of any solvent or solvent mixture and the physical state of the extract must be indicated.

If any other substance is added during the manufacture of the ***herbal*** drug preparation to adjust the ***herbal*** drug preparation to a ***defined content*** of constituents with known therapeutic activity, or for any other purpose, the added substance must be mentioned as an "other ***substance***" and the genuine extract as the "active ***substance***".

EXAMPLE

i) Active *substance*

Name	Quantity
Valerianae radix	125 mg
dry extract ethanolic 60% *(V/V)*	
(a - b : 1)	
or	
Valerianae radix	125 mg
dry extract ethanolic 60% *(V/V)*	***equivalent to x - y mg Valeriane radix***

Other substance(s)
Name
...

or

ii) Active *substance*

Name	Quantity
Sennae folium	50-65 mg, corresponding to 12.5 mg of
dry extract ethanolic 60% *(V/V)*	hydroxyanthracene glycosides, calculated as
(a - b: 1)	Sennoside B

Other *substance(s)*
Name
...

B. DESCRIPTION OF THE METHOD OF PREPARATION

The manufacturing process within the meaning of this section is the preparation of the finished product from ***herbal drug(s) or herbal drug preparation(s)***. The description should include details of ***the process together with the controls exercised. This section should be in accordance with the "Note for Guidance on Manufacture of the finished dosage form" (CPMP/QWP/486/95).*** If ***herbal***

(*) ***'a' and 'b' or 'x' and 'y' have to be justified by the applicant***

drug preparations are the starting material, the manufacture of the ***herbal*** drug preparations and their controls do not belong under this section but under section C.

C. CONTROL OF STARTING MATERIALS

1) Control of the ***herbal*** drug ***and of herbal drug preparations***

- ***Control of the herbal drug***

A ***comprehensive specification*** for each ***herbal*** drug must be submitted, even if the starting material is a ***herbal*** drug preparation. This also applies if the applicant is not the manufacturer of the preparation. In the case of fatty or essential oils ***used as active substances of herbal medicinal products,*** a ***specification*** for the ***herbal*** drug is ***required unless fully justified.*** The scientific name of the parent plant and its part(s) have to be stated.

If no monograph for the ***herbal*** drug is given in a Pharmacopoeia referred to in Directive 75/318/EEC, Annex 1, a ***comprehensive specification*** on the ***herbal*** drug must be supplied and should be set out in the same way where practicable, as the monographs on ***herbal*** drugs in the European Pharmacopoeia. This should include the botanical name and authority and the common name if used for labelling purposes. Information on the site of collection, the time of harvesting and stage of growth, treatment during growth with pesticides etc., and drying and storage conditions should be included if possible. The ***comprehensive specification*** should be established on the basis of recent scientific data. In the case of ***herbal*** drugs with constituents of known therapeutic activity, assays of their content (with test procedure) are required. The content must be included as a range, so as to ensure reproducibility of the quality of the finished product. ***In the case of herbal drugs where constituents of known therapeutic activity are not known, assays of marker substances (with test procedure) are required. The choice of markers should be justified.***

As a general rule, ***herbal*** drugs must be tested for microbiological quality and for residues of pesticides and fumigation agents, toxic metals, likely contaminants and adulterants, etc., unless otherwise justified. ***Radioactivity should be tested if there are reasons for concerns.*** Specifications and descriptions of the analytical procedures must be submitted, together with the limits applied. ***Analytical procedures not given in a Pharmacopoeia should be validated in accordance with the ICH guideline "Validation of analytical procedures: methodology" (CPMP/ICH/281/95).***

Reference samples of the ***herbal*** drugs must be available for use in comparative tests e.g. macro and microscopic examination, chromatography etc.

- ***Control of herbal drug preparations***

If the herbal ***medicinal product*** contains not the ***herbal*** drug itself but a preparation, the ***comprehensive specification*** on the ***herbal*** drug must be followed by a description and validation of the manufacturing process for the ***herbal*** drug preparation. ***The information may be supplied either as part of the marketing authorisation application or using the European Drug Master File procedure. If the latter is chosen the documentation should be submitted in accordance with the Note for Guidance "European Drug Master File Procedure for Active Substances" (Eudra/Q/93/014).***

For each ***herbal*** drug preparation, a ***comprehensive specification*** must be submitted. This must be established on the basis of recent scientific data and must give particulars of the characteristics, identification tests and purity tests. This has to be done e.g. by different appropriate chromatographic methods. If deemed necessary by the results of the analysis of the starting material, tests on microbiological quality, residues of pesticides, fumigation agents, solvents and toxic metals have to be carried out. ***Radioactivity should be tested if there are reasons for concerns.*** Quantitative determination (assay) of ***markers or of substances with known therapeutic activity*** is required. The content must be indicated with the lowest possible tolerance. The test methods must be described in detail.

If preparations from *herbal* drugs with constituents of known therapeutic activity are standardised (i.e. adjusted to a *defined content* of constituents with known therapeutic activity) it must be stated how such standardisation is achieved. If another *substance* is used for this purpose, it is necessary to specify as a range the quantity that can be added.

2) Control of excipients

Excipients including those added during the manufacture of the herbal drug preparations should be described according to the "Note for Guidance on Excipients in the dossier for application for marketing authorisation of a medicinal product" (Eudra/Q/91/015).

D. CONTROL TESTS CARRIED OUT AT AN INTERMEDIATE STAGE OF THE MANUFACTURING PROCESS OF THE FINISHED PRODUCT

Details of all control tests with details of test procedures and limits applied at any intermediate stages of the manufacturing processes are required, especially if these tests cannot be done in the finished product.

E. CONTROL TESTS ON THE FINISHED PRODUCT

This section should be in accordance with the "Note for Guidance on Specifications and control tests on the finished product" (Eudra/Q/91/020) and the analytical procedures should be validated according to the ICH guideline "Validation of analytical procedures: methodology" (CPMP/ICH/281/95).

The control tests on the finished product must be such as to allow the qualitative and quantitative determination of the composition of the active *substances* and a specification has to be given which may be done by using markers if constituents with known therapeutic activity are unknown. In the case of *herbal* drugs or *herbal* drug preparations with constituents of known therapeutic activity, these constituents must also be specified and quantitatively determined.

If a herbal *medicinal product* contains several *herbal* drugs or preparations of several *herbal* drugs and if it is not possible to perform a quantitative determination of each active *substance*, the determination may be carried out jointly for several active *substances*. The need for this procedure must be justified.

The criteria given by the European Pharmacopoeia to ensure the microbiological quality should be applied unless justified.

F. STABILITY TESTS

This section should be in accordance with the "Note for Guidance on Stability testing of new active substances and medicinal products" (Eudra/Q/92/021) and the "Note for Guidance on stability testing of existing active substances and related finished products" (CPMP/QWP/556/96).
Since the *herbal* drug or *herbal* drug preparation in its entirety is regarded as the active *substance*, a mere determination of the stability of the constituents with known therapeutic activity will not suffice. It must also be shown, as far as possible e.g. by means of appropriate fingerprint chromatograms, that other substances present in the *herbal* drug or in the *herbal* drug preparation are likewise stable and that their proportional content remains constant.

If a herbal *medicinal product* contains several *herbal* drugs or preparations of several *herbal* drugs and if it is not possible to determine the stability of each active *substance*, the stability of the medicinal product should be determined by appropriate fingerprint chromatograms, appropriate

overall methods of assay and physical and sensory tests or other appropriate tests. *The appropriateness of the tests shall be justified by the applicant.*

In the case of herbal drug preparations containing constituents with known therapeutic activity, the limit should be ± 5% of the initial assay value unless justified. In the case of constituents without known therapeutic activity, a limit of ±10% of the initial assay value can be accepted if justified by the applicant. These criteria shall apply to the stability testing of active substances in like manner.

ANNEX

GLOSSARY

Herbal *medicinal products* are medicinal products containing as active ***substances*** exclusively ***herbal drugs*** or ***herbal*** drug preparations.

Herbal drugs are ***plants***(*) ***or part of plants in an unprocessed state, which are*** used for a medicinal ***or pharmaceutical*** purpose. A ***herbal*** drug or a preparation thereof is regarded as one active ***substance*** in its entirety whether or not the constituents with therapeutic activity are known.

Herbal drug preparations are comminuted or powdered ***herbal*** drugs, extracts, tinctures, fatty or essential oils, expressed juices, ***processed resins or gums***, etc...prepared from ***herbal*** drugs, and preparations whose production involves a fractionation, purification or concentration process. However, chemically defined isolated constituents or their mixture are not ***herbal*** drug preparations. Other ***components*** such as solvents, diluents, preservatives may form part of ***herbal*** drug preparations. These ***components*** must be ***declared***.

Constituents with known therapeutic activity are chemically defined substances or groups of substances which are ***generally accepted*** to contribute ***substantially*** to the therapeutic activity of a ***herbal*** drug or of a preparation.

Markers are chemically defined constituents of a ***herbal*** drug which are of interest for control purposes ***independent of whether they have any therapeutic activity or not***. Markers may serve to calculate the quantity of ***herbal*** drug or preparation in the finished product if that marker has been quantitatively determined in the ***herbal*** drug or preparation when the starting materials were tested.

Standardisation(**) ***means adjusting the herbal drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity respectively by adding excipients or by mixing herbal drugs or herbal drug preparations (e.g. standardised extract from the European Pharmacopoeia).***

(*) ***Thallophytes, especially lichens, higher fungi and algae, are included in like manner***

(**) ***In some Member States the expression "standardisation" is used on a national level to describe all measures which are taken during the manufacturing process and quality control leading to a reproducible quality***

PROPOSAL FOR NEW GUIDANCE

"Non-clinical testing of herbal drug preparations with long-term marketing experience" Guidance to facilitate mutual recognition and use of bibliographic data

INTRODUCTION

Article 4 point 8 a) ii) of Council Directive 65/65/EEC makes it clear that the applicant shall not be required to provide the results of pharmacological and toxicological tests if he can demonstrate by detailed reference to published scientific literature presented in accordance with the second paragraph of Article 1 of Council Directive 75/318/EEC that the constituent(s) of the medicinal product have a well-established medicinal use, with recognised efficacy and an acceptable level of safety. This regulation in no way relaxes the requirements of proof of safety set out by the Annex to Council Directive 75/318/EEC. All aspects must be covered by appropriate bibliographic data and the expert report.
Published non-clinical tests for well-established herbal drug preparations are often incomplete or not in accordance with today's state of the art. Well-presented clinical experience (with regard to the time and extent of use in humans) as well as post-marketing experience gained by wide spread use in humans contribute to the avoidance of unnecessary tests in animals. Protection of animals should be taken into consideration when requesting non-clinical testing of well-established herbal drug preparations (86/609/EEC). Studies that do not agree with the current state of the art (e.g. GLP-conformity), should be judged for credibility; subsequent demands that could lead to a "blind" repetition of animal experiments should be avoided. In particular, it should be assessed whether the observed effects in animals studies would modify the benefit/risk assessment and would lead to a negative decision for the granting of a Marketing Authorisation.

In cases of reasonable suspicion, additional appropriate non-clinical tests can be requested.

NON-CLINICAL TESTING

Where there is sufficient experience available in humans, single dose and repeated dose toxicity, immunotoxicity as well as local tolerance testing of well-established herbal drug preparations is not necessary. Likewise, pharmacological tests including safety pharmacology and pharmacokinetics are not necessary. The expert report must address these aspects and give the grounds why the documented medical experience justifies a safe use of the herbal drug preparation.

Non-clinical testing of well-established herbal drug preparations should be directed towards the study of effects that are difficult, even impossible to detect clinically. These effects would include toxicity to reproduction, genotoxicity and carcinogenicity.

Reproductive toxicological investigations regarding fertility are generally not necessary, insofar as there are no grounds for suspicion that would necessitate testing.

The reproductive toxicological potential with regard to embryo-foetal and peri-post-natal development is to be clarified. Reproductive toxicity data are available for many old substances, however, these data are often not reliable. A repetition of the tests is only justified in cases in which the significance of the results is not clear and there are grounds for suspicion. Reproductive toxicological tests in animals are not necessary if one of the following criteria is fulfilled:

- Results from epidemiological data of adequate power or post-marketing safety studies are available.
- Results from investigations in pregnant women and neonates are present.
- The medicinal product is not intended to be used in women of child-bearing age or during pregnancy and lactation.

The clinical Expert Report should justify the distinction made between women of child-bearing age and pregnancy.

The genotoxic potential of herbal drug preparations should be clarified.

A repetition of the studies is only required in cases in which the significance of the results is unclear or they yield grounds for suspicion. Positive findings for one herbal drug preparation or for substances from one chemical class can frequently be extrapolated to another herbal drug preparation without necessitating further testing.

It is recommended to first perform *in vitro* tests for substances in which the genotoxicity tests are insufficient. Substances with negative results *in vitro* also exhibit negative results *in vivo* in the majority of cases. In cases in which positive results in vitro are present, these are to be clarified by way of appropriate investigations, mainly *in vivo*. (CPMP Note for Guidance on genotoxicity: a standard battery for genotoxicity testing of pharmaceuticals (CPMP/ICH/174/95), CPMP Note for Guidance on genotoxicity: guidance on specific aspects of regulatory genotoxicity tests for pharmaceuticals (CPMP/ICH/141/95) and OECD 1995).

It is appropriate to assess genotoxicity initially in a bacterial reverse mutation test using a test battery of different bacterial strains and metabolic activation (s. CPMP/ICH and OECD Guidelines). This test has been shown to detect relevant genetic changes and the majority of genotoxic rodent carcinogens. If positive results can not be clearly attributed to specific constituents with a well-established safety-profile (e.g. Quercetin) additional *in vitro* and, if necessary, *in vivo* studies should be performed. A co-operative approach is encouraged to investigate herbal drug preparations with the same specification.

Carcinogenicity studies are not needed in cases where there is no suspicion for a carcinogenic potential (Council Directive 75/318/EEC of 20 May 1975, Part 3, IIE. Carcinogenic Potential; CPMP Note for Guidance on the need for carcinogenicity studies of pharmaceuticals (CPMP/ICH/140/95), CPMP Note for Guidance on carcinogenicity: testing for carcinogenicity of pharmaceuticals (CPMP/ICH/299/95), Addendum to Note for Guidance on dose selection for carcinogenicity studies of pharmaceuticals: addition of a limit dose and related notes (CPMP/ICH/366/96)).

Even a positive suspicion of a carcinogenic effect of an well-established herbal drug preparation does not necessarily require a study to be performed. The following considerations should be included in the assessment:

- Is the suspicion based on positive results of genotoxicity studies and can it be clarified in further genotoxicity studies, mainly *in vivo?*

- Is there sufficient epidemiological experience in humans that could refute the suspicion?

EXPERT REPORT

The expert is obliged to point out the necessity or not of non-clinical testing for the herbal drug preparation. Plausible presentation of the facts contributes to the acceptance of the application for marketing authorisation and facilitates the evaluation performed by the authorities.
The expert should discuss available published toxicological data on closely related herbal drug preparations, different parts of the plant, data on related species of the same genus or plant family. If there are toxicological data on well-defined constituents of a herbal drugs preparation, the expert should discuss the relevance of these data for the safety-assessment of the herbal drug preparation.

- **NTA 2A**

It is suggested to include a paragraph on scientific monographs into the Draft Notice to Applicants
In Chapter I – 'Marketing Authorisations'
Section 4 – 'Stand Alone Applications for a Marketing Authorisation'.
This proposal was presented by the representative of the European Commission.

4.2 BIBLIOGRAPHICAL APPLICATIONS

1. Where the constituent or constituents of the medicinal product have a well established medicinal use, with recognised efficacy and an acceptable level of safety, demonstrated by detailed references to published literature presented in accordance with second paragraph of Article 1 of directive 75/318/EEC, an application (so called "bibliographical") for marketing authorisation may be submitted in accordance with Directive 65/65/EEC, article 4.8.(a)ii.

2. An applicant wishing to use Article 4.8 (a)ii) of Directive 65/65EEC must fully satisfy all the requirements of Article 1 of Directive 75/318/EEC as well as those of Directives 65/65/EEC and 75/319/EEC as amended, in effect, submit a "complete" application.

3. Directive 75/318/EEC Article 1 states that "where pursuant to point 8(a) of Article 4, second paragraph, of Directive 65/65/EEC, references to published data are submitted, the provisions of this Directive (i.e. Directive 75/318/EEC) shall apply in like manner." In such cases, the full article or reference should be supplied, with necessary translations. Moreover, the Expert Reports must clearly state the grounds for using published references under the conditions set out in Directive 75/318/EEC. This would include the completion of all of the tabular formats provided in "The rules governing medicinal products in the European Union, Volume 2B Notice to Applicants: Presentation and content of applications' where relevant, unless there is a justification that the study is no relevant for the medicinal product. The impurity/related substances profile and the decomposition products arising during storage must be clearly indicated in order to allow assessment of appropriate efficacy and safety.

4. In the event that neither detailed reference to published literature, nor appropriate justification is available to cover all the requirements, the applicant must supplement the missing data with appropriate additional studies.

   Scientific monographs on certain substances (e.g. those drafted by the European Scientific Co-operative on Phytotherapy (ESCOP) and the World Health Organisation (WHO) for herbal drugs) offer a valuable and updated overview on published scientific literature, which together may be used in support of the demonstration of the safety and efficacy of a medicinal product in a bibliographical application in accordance with Article 4.8 (a)ii. These monographs may help to avoid duplication of work and bring about gradual harmonisation in the evaluation of medicinal products, e.g. herbal medicinal products. Therefore the Commission and Member States recommend that both applicants and competent authorities should make use of these monographs. The use of these monographs should not preclude the future development or implementation of results of new clinical trials.

5. It should be noted that summary assessment reports such as the EPAR for Community marketing authorisations or evaluation reports on Maximum Residue Limits which are made publicly available by competent authorities for reasons of transparency would generally not be considered to supply sufficient information to meet the requirements of Directive 75/318/EEC.

- **NTA 2B, Part I B 1: Summary of Product Characteristics**

The requirements are found adequate for herbal medicinal products. It was clarified that marker substances will not be declared in section 2 of the SPC, Qualitative and Quantitative Composition.

- **NTA 2B, Part I C 2: Expert Report on toxico-pharmacological documentation**
  NTA 2B, Part III "Toxico-pharmacological Documentation"

These parts are found adequate for herbal medicinal products. It was confirmed that the Expert Report has to address all aspects of Part 3 of the Annex to Council Directive 75/318/EEC of 20 May 1975 'Toxicological and Pharmacological tests'.

COMMENTS ON PART 4 OF ANNEX TO COUNCIL DIRECTIVE 75/318/EEC OF 20 MAY 1975 "Clinical documentation"

The group made the following comments, including some suggestions for amendment.

The group found the terminology not well adapted to bibliographic applications. The group discussed the issue of the broader definition of a clinical trial as provided in the text with comparison to the definitions laid down in the Good Clinical Practice (GCP) Directive for "clinical trials" and "non-interventional studies". The group wondered whether epidemiological studies would be covered by the proposed definition. To clarify this matter, the group agreed to suggest the introduction of a new paragraph stating:
"***Documentation on experience in the form of epidemiological studies can be taken into account in bibliographical applications for well-established medicinal products***".

Section A. General requirements
It is suggested to modify the last sentence of the first paragraph as follows: "***Consequently, an essential requirement is that all clinical data should be communicated, both favourable and unfavourable***".

Section C. Presentation of results
In section C.1., the group agreed to introduce ***a statement on the need for identity or essential similarity between the product tested in the clinical trials and the product intended for marketing***. The precise composition and specification of the product investigated must be provided. For compounds with well-known therapeutic activity, the Expert Report must explain the relevance of any data submitted which concern a product different from the product intended for marketing. It is indeed up to the Expert to judge that the differences are small enough to consider that the product studied is similar to the product which will be granted a marketing authorisation.

In section C.2., the group also felt the need for ***a paragraph in the text which clearly mentions that the Expert Report must pay particular attention to any missing information in a bibliographical application*** for a well-established product. Justification must be given why demonstration of efficacy can be supported although some studies are lacking.

The group recognised that, beside the fact that there are mandatory requirements, which are to be met in principle, it is known that some of the data required are not available from bibliographic sources. It was stated that in the case of well-established herbal medicinal products the need for new clinical studies should not only derive from formal criteria. The expected benefit of additional studies for the safeguard of public health should be considered in each particular case. The group highlighted that this issue is linked to the claimed indication(s) and that more severe criteria for the acceptability of clinical data presented/missing data would be used in the case of "modern" indication, e.g. hypercholesterolemia .

Section D.1. Pharmacodynamics

The group reviewed this section and considered that the paragraph stating that "the demonstration of pharmacodynamic effects in human beings shall not in itself be sufficient to justify conclusions regarding any particular potential therapeutic effect" is an essential remark. This statement fully applies to herbal medicinal products. In some circumstances, where a well-established use is adequately documented, pharmacodynamic data may contribute to an acceptance of bibliographic applications for herbal medicinal products.
It was recognised that the demonstration of the mode of action of an herbal medicinal product often cannot be clarified by the applicant and that the documentation of efficacy, in those cases, should be a priority.

Section D.2. Pharmacokinetics

The issue of the request for pharmacokinetic data for herbal medicinal products, and in particular for complex mixtures with constituents of unknown therapeutic activity was discussed. *Ginkgo biloba* extract was mentioned as a relevant example of a complex active substances mixture in which case pharmacokinetic data should not be required. Requirements for pharmacokinetic data should always be realistic and relate to the Public Health and safety risks associated with the product. When there are safety concerns (example of photosensitisation in the case of Hypericum) pharmacokinetic data are useful to provide safety margins.

The group concluded its discussion on pharmacokinetic by stating that, for well-established medicinal products where the efficacy is based on bibliographical documentation, pharmacokinetic data would not be required unless there are ground for safety concerns from the presence of one or several constituents. If there is a constituent with known therapeutic activity and a narrow therapeutic range, pharmacokinetic data will be required. The Expert Report should carefully address all these issues.

Section E. Bioavailability/bioequivalence

The same principle as defined above for pharmacokinetic data shall apply for bioavailability/bioequivalence data. The group would also recommend the introduction of ***a statement on the need to provide bioavailability data for galenic preparations with modified release***, in view of the difficulty to use bibliographical data in such case.

Section F. Clinical efficacy and safety

The group reported some elements, which make it difficult for applicants to conduct proper comparative clinical trials. The blinding feature of a clinical trial cannot always be respected, for example in the case of essential oils with strong smell.

Section F.5 on vaccines and serums was considered irrelevant for herbal medicinal products. The group indicated that the principles of points 6 to 9 (safe use) apply to bibliographical applications and that these points should be underlined in the Expert Report for each individual product.

Section G. Documentation for applications in exceptional circumstances

This section was considered to be not relevant to well-established herbal medicinal products.

Section H. Post-marketing experience

This section was, on the contrary, felt to be of particular importance for herbal medicinal products. The group indicated that applicants should put a special emphasis to this issue for individual products and for all related products originating from the same herbal drug.

As a conclusion, the group recognised the validity of the framework provided by this document. The basic concepts and general rules should apply to bibliographical data, but taking into account the specific comments made by the group.

PROPOSAL FOR A CORE-SPC FOR VALERIANAE RADIX[1]
Proposal from Ad Hoc Working Group on Herbal Medicinal Products
(7 July 1998)

1. NAME OF THE MEDICINAL PRODUCT

To be specified for the individual finished product.

2. QUALITATIVE AND QUANTITATIVE COMPOSITION[2]

Valerian root
Extract prepared with water, ethanol/water (max 70%)
Tinctures (1:5, ethanol max 70% V/V)

3. PHARMACEUTICAL FORM

Herbal drug or herbal drug preparation in solid or liquid dosage forms (to be labelled according to the standard terms published by the European Pharmacopoeia)

4. CLINICAL PARTICULARS

4.1. Therapeutic indications

Herbal medicinal product for the relief of temporary mild nervous tension and temporary difficulty in falling asleep (see Section 4.4 Special warnings and special precautions for use).

4.2. Posology and method of administration

For oral use

Adults and children over 12 years
Single dose:
2 to 3 g of the drug (e.g. as herbal tea).
1 to 3 ml of tincture (1:5, ethanol max 70% V/V).
Extracts with water, ethanol/water (max 70%) equivalent to 2 to 3 g of the drug.
For nervous tension up to 3 time daily.
As an aid to sleep, a single dose half to one hour before bedtime with an earlier dose during the evening if necessary.

Elderly: As for adults.

4.3. Contra-indications

Because there is no experience available, the use is not recommended in children younger than 12 years of age.

4.4. Special warnings and special precautions for use

Please note that the Patient Leaflet invites the patient to seek medical advice if symptoms persist for more than 2 weeks or worsen.

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[1] The herbal drug complies with the European Pharmacopoeia

[2] The declaration of all active substances should be done in accordance to the Note for Guidance "Quality of Herbal Medicinal Products" (type A.1.i or type A.2.i.) as revised by the Ad Hoc Working Group on Herbal Medicinal Products

4.5. Interaction with other medicinal products and other forms of interaction

None reported.
If the patient is on other medication he/she is invited to seek medical advice.

4.6. Use during pregnancy and lactation

Because data on the use during pregnancy and lactation are not available, the use is not recommended as a general precaution. No adverse effects have been reported from the common use of Valerian roots as a medicinal product but experimental data are lacking.

4.7. Effects on ability to drive and use machines

Intake of valerian preparations immediately (up to two hours) before driving a car or operating machinery is not recommended. The effect of valerian preparations may be enhanced by consumption of alcohol.

4.8. Undesirable effects

No adverse effect known to date under the recommended conditions of short term use.

4.9. Overdose

Valerian root at a dose of approximately 20 g caused benign symptoms (fatigue, abdominal cramp, chest tightness, lightheadedness, hand tremor and mydriasis) which disappeared within 24 hours. If symptoms arise, treatment should be supportive.

5. PHARMACOLOGICAL PROPERTIES

5.1. Pharmacodynamic properties

Pharmacotherapeutic group: Hypnotics and sedatives, ATC code: N05C M09

The sedative effects of preparations of Valerian roots such as tea-infusions or tincture have long been recognised empirically but cannot be attributed with certainty to any known constituents. Orally administered dry extracts of valerian root prepared with water have been shown to improve sleep latency and sleep quality in man, although the statistical significance in these studies was more apparent from subjective evaluations than from objective measures of sleep.

5.2. Pharmacokinetic properties

No pharmacokinetic data available.

5.3. Preclinical safety data[1]

Extracts with ethanol and the essential oil of Valerian root have shown low toxicity in rodents during acute test and from repeated dose toxicity over periods of 4-8 weeks.

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[3] In case of Valerian root used as powder, the total exposure to valepotriates should not exceed the maximum exposure with herbal tea. Alkylating and cytotoxic properties of valepotriates are not relevant for finished products. This is because valepotriates decompose rapidly and only traces of valepotriates or their degradation products (in part, baldrinals) are found.

Chapter II

Principles and Guidelines of Good Manufacturing Practice

Article 6

Quality management

The manufacturer shall establish and implement an effective pharmaceutical quality assurance system, involving the active participation of the management and personnel of the different services involved.

Article 7

Personnel

1. At each manufacturing site, the manufacturer shall have competent and appropriately qualified personnel at his disposal in sufficient number to achieve the pharmaceutical quality assurance objective(*s*).

2. The duties of managerial and supervisory staff, including the qualified person(s), responsible for implementing and operating good manufacturing practice shall be defined in job descriptions. Their hierarchical relationships shall be defined in an organization(*al*) chart. Organization(*al*) charts and job descriptions shall be approved in accordance with the manufacturer's internal procedures.

3. Staff referred to in paragraph 2 shall be given sufficient authority to discharge (*carry out*) their responsibilities correctly.

4. Personnel shall receive initial and continuing training including the theory and application of the concept of quality assurance and good manufacturing practice.

5. Hygiene programmes adapted to the activities to be carried out shall be established and observed. These programmes include procedures relating to health, hygiene and clothing of personnel.

Article 8

Premises and equipment

1. Premises and manufacturing equipment shall be located, designed, constructed, adapted and maintained to suit the intended operations.

2. Lay out, design and operation must aim to minimize the risk of errors and permit effective cleaning and maintenance in order to avoid contamination, cross contamination and, in general, any adverse effect on the quality of the product.

3. Premises and equipment intended to be used for manufacturing operations which are critical for the quality of the products shall be subjected to appropriate qualification.

Article 9

Documentation

1. The manufacturer shall have a system of documentation based upon specifications, manufacturing formulae and processing and packaging instructions, procedures and

7. APPENDIX

7.1 Table 1: The major drug metabolising CYP450 enzymes, examples of substrates, inhibitors, inducers and markers.

P450 ENZYME	SUBSTRATES	INHIBITORS	INDUCERS	MARKERS
CYP1A2	Acetaminophen Aromatic amines Caffeine Phenacetin Theophylline	Fluvoxamine Furafylline	Charcoal-grilled beef Cigarette smoke Cruciferous vegetables	Caffeine
CYP2A6	Coumarin Butadien Nicotine	Diethyldithiocarbamate 8-methoxypsoralen Tranylcypromine	Barbiturates	Coumarin
CYP2C9	NSAID drugs Phenytoin Tolbutamide *S*-Warfarin	Sulfaphenazole Sulfinpyrazone	Rifampin Barbiturates	*S*-Warfarin Tolbutamide
CYP2C19	Citalopram Diazepam Hexobarbital Imipramine Omeprazole Proguanil Propranolol	Tranylcypromine	Rifampin Barbiturates	Mefenytoin Omeprazole
CYP2D6	Several anti-depressants Neuroleptics Beta-blockers Antiarrhytmics Codeine Dextromethorphan Etylmorphine Nicotine	Ajmalicine Chinidin Fluoxetine Paroxetine Quinidine Ritonavir	None known	Debrisoquine Dextromethorphane
CYP2E1	Acetaminophen Alcohols Caffeine Chlorozoxazone Dapsone Enflurane Theophylline	Diethyldithio-carbamate Dimethyl sulfoxide Disulfiram	Ethanol Isoniazid	Caffeine Chlorzoxazone
CYP3A4	Acetaminophen Carbamazepine Cyclosporin Digitoxin Diazepam Erythromycin Felodipne Fluoxetine Nifedipine Quinidine Saquinavir Steroids (e.g. cortisol) Terfenadine Triazolam Verapamil Warfarin	Clotrimazole Ketoconazole Ritonavir Troleandomycin	Dexamethasone Phenytoin Rifampin Troleandomycin	Dapsone Erythromycin Ketoconazole Lidocaine

inclusion of men in clinical trials. However, an assessment of male fertility by careful histopathological examination in the rodent 4 week repeated dose toxicity study has been found to be more sensitive in detecting effects on male reproductive organs than fertility studies (14.9), and is now recommended to be performed prior to the first clinical trial in Japan. In the EU and US, 2 week repeated dose studies are considered adequate for an overall assessment of the potential toxicity of a drug to support clinical trials for a short duration.

Note 3

A highly effective method of birth control is defined as those which result in a low failure rate (i.e. less than 1% per year) when used consistently and correctly such as implants, injectables, combined oral contraceptives, some IUDs, sexual abstinence or vasectomised partner. For subjects using a hormonal contraceptive method, information regarding the product under evaluation and its potential effect on the contraceptive should be addressed.

14. REFERENCES

1. ICH Guideline S6: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals (CPMP/ICH/302/95)
2. ICH Guideline E8: General Considerations for Clinical Trials (CPMP/ICH/291/95)
3. ICH Guideline S3A: Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies (CPMP/ICH/384/95)
4. United States DHHS Federal Register Notice August 26, 1996 (61 FR 43934)
5. ICH Guideline S2A: Genotoxicity: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (CPMP/ICH/141/95)
6. ICH Guideline S2B: Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals (CPMP/ICH/174/95)
7. ICH Guideline S1A: Guideline on the Need for Carcinogenicity Studies for Pharmaceuticals (CPMP/ICH/140/95)
8. ICH Guideline S5A: Reproductive Toxicology: Detection of Toxicity to Reproduction for Medicinal Products (CPMP/ICH/386/95)
9. ICH Guideline S5B: Reproductive Toxicology: Toxicity to Male Fertility (CPMP/ICH/136/95)
10. P.F. Arcy and D.W.G. Harron, "Proceeding of The First International Conference on Harmonisation, Brussels 1991, Queen's Univ. of Belfast, pp 183-184 (1992).
11. ICH Guideline S4: Duration of Chronic Toxicity Testing in Animals (Rodent and Non Rodent Toxicity Testing) (CPMP/ICH/300/95).

The European Agency for the Evaluation of Medicinal Products
Human Medicines Evaluation Unit

ICH Topic M 3
Non-Clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals

Step 4, Consensus guideline, 16 July 1997

NOTE FOR GUIDANCE ON NON-CLINICAL SAFETY STUDIES FOR THE CONDUCT OF HUMAN CLINICAL TRIALS FOR PHARMACEUTICALS
(CPMP/ICH/286/95)

TRANSMISSION TO CPMP	November 1996
TRANSMISSION TO INTERESTED PARTIES	November 1996
COMMENTS REQUESTED BEFORE	May 1997
FINAL APPROVAL BY CPMP	September 1997
DATE FOR COMING INTO OPERATION	March 1998

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The European Agency for the Evaluation of Medicinal Products
Human Medicines Evaluation Unit

ICH Topic E 6
Guideline for Good Clinical Practice

Step 4, Consolidated Guideline 1.5.96

NOTE FOR GUIDANCE ON GOOD CLINICAL PRACTICE
(CPMP/ICH/135/95)

TRANSMISSION TO CPMP	July 1996
FINAL APPROVAL BY CPMP	17 July 1996
PROPOSED DATE FOR COMING INTO OPERATION (STUDIES COMMENCING AFTER)	17 Ja..uary 1997

ANNEX 1

LIST OF RELEVANT ICH GUIDELINES AND TOPICS

Code	Topic
E1	The Extent of Population Exposure to Assess Clinical Safety for Drug Intended for Long-term Treatment of Non-Life-Threatening Conditions
E2A	Clinical Safety Data Management: Definitions and Standards for expedited Reporting
E2B	Clinical Safety Data Management: Data Elements for Transmission of Individual Case Safety Reports
E2C	Clinical Safety Data Management: Periodic Safety Update Reports for Marketed Drugs
E3	Structure and Content of Clinical Study Reports
E4	Dose-Response Information to Support Drug Registration
E5	Ethnic Factors in the Acceptability of Foreign Clinical Data
E6	Good Clinical Practice: Consolidated Guideline
E7	Studies in Support of Special Populations: Geriatrics
E8	General Considerations for Clinical Trials
E9	Statistical Considerations in the Design of Clinical Trials
E10	Choice of Control Group in Clinical Trials
M1	Medical Terminology (MEDDRA)
M2	Electronic Standards for the Transfer of Regulatory Information (ESTRI)
M3	Non-Clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals
S6	Safety Studies for Biotechnology-Derived Products

SCREENING METHODS FOR DETECTION AND EVALUATION OF BIOLOGICAL ACTIVITIES OF PLANT PREPARATIONS

1. INTRODUCTION

1.1. STRATEGIES AND TACTICS IN THE SEARCH FOR NEW DRUGS
1.2. ROLES OF SCREENING BIOASSAYS
1.3. CAPACITY OF SCREENING PROGRAMMES
1.4. REQUIREMENTS FOR SCREENING BIOASSAYS OF PLANT EXTRACTS

2. METHODS FOR DETECTION OF BIOLOGICAL ACTIVITY OF PLANT EXTRACTS

2.1. GENERAL SCREENING BIOASSAYS
2.1.1 PRIMARY SCREENING BIOASSAYS
2.1.2. BROAD SCREENING BIOASSAYS
2.2. SPECIALIZED SCREENING BIOASSAYS
2.2.1. LOWER ORGANISMS
2.2.2. ISOLATED SUBCELLULAR SYSTEMS
2.2.3. ISOLATED CELLULAR SYSTEMS
2.2.4. ISOLATED ORGANS OF VERTEBRATES
2.2.5. WHOLE ANIMALS

3. ON-LINE BIOLOGICAL DETECTION IN LIQUID CHROMATOGRAPHY

STRATEGY AND TACTICS IN THE SEARCH FOR NEW DRUGS

1. RATIONAL APPROACH

* SOPHISTICATED KNOWLEDGE OF THE TARGET STRUCTURE (ENZYME, RECEPTOR, MACROMOLECULE, REGULATORY PROTEIN)
* KNOWLEDGE OF THE STRUCTURAL, SPATIAL AND ELECTRONIC REQUIREMENTS FOR A COMPOUND TO INTERACT WITH THE TARGET

2. EMPIRICAL APPROACH

* SYSTEMATIC SCREENING OF PURE COMPOUNDS OR EXTRACTS
  $\Rightarrow$ "LEAD" COMPOUNDS (SERENDIPITY)
* SAR-STUDIES OF LEAD COMPOUNDS COMBINED WITH COMPUTER GRAPHIC MODEL BUILDING (RATIONAL SERENDIPITY)

  * OPTIMAL ACTIVITY
  * ACCEPTABLE THERAPEUTIC INDEX
  * OPTIMAL BIOAVAILABILITY
  * FEWER SIDE EFFECTS

APPROACHES TO DRUG DEVELOPMENT OF TRADITIONAL PLANT REMEDIES (2)

2. ISOLATION AND IDENTIFICATION OF BIOACTIVE COMPOUNDS POTENTIALLY USEFUL AS :

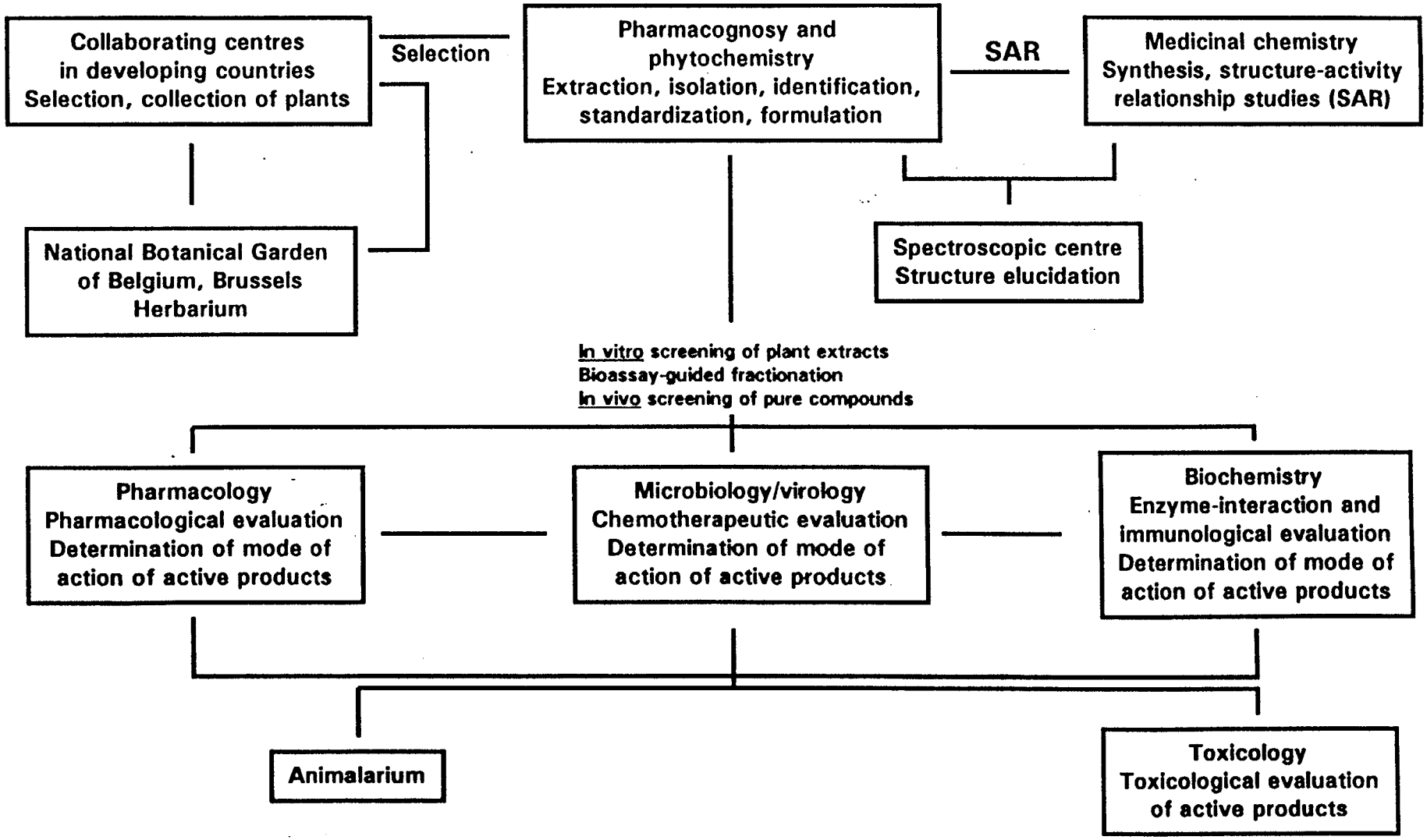
* NEW DRUGS

* UNIQUE TEMPLATES FOR SAR-STUDIES ("LEAD" OR PROTOTYPE COMPOUNDS)

* NEW BIOLOGICAL TOOLS

* NEW RAW MATERIALS FOR THE PRODUCTION OF DRUGS ("FEEDSTOCK" MOLECULES)

Organogram of the different laboratories of the multidisciplinary team, investigating medicinal agents from higher plants.

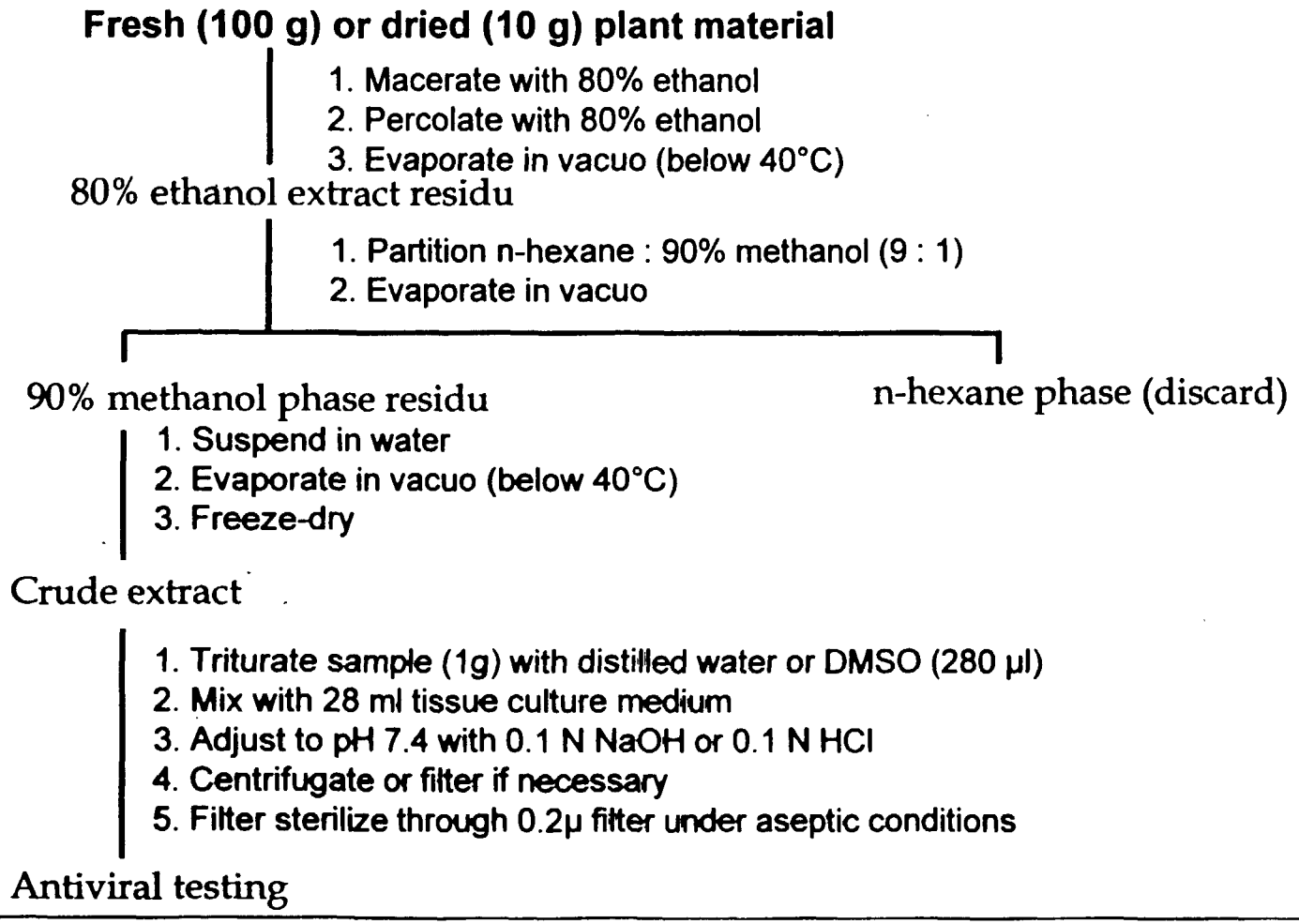


SELECTION OF PLANTS FOR ANTIVIRAL SCREENING

1. RANDOM COLLECTION
   * Plants readily available; best chance to find novel structures.
   * Large screening capacity required; low percentage of active leads.
2. ETHNOMEDICAL USE
   * Lower screening costs; high ratio of activity.
   * Difficulty of botanical identification; high procurement costs; lack of novel leads; leads will be missed; use of complex mixtures; use of rare plants; secrecy of primitive cultures; role of psychology in folk medicine.
3. BASED ON EXISTING LITERATURE
   * High ratio of activity.
   * High collection costs.
4. CHEMOTAXONOMY
   * High ratio of activity; discovery of useful analogues.
   * Re-isolation of known compounds.

After SUFFNESS and DOUROS, 1979.

Fractionation scheme of plant materials and preparation of samples for antiviral testing



REQUIREMENTS FOR SCREENING BIOASSAYS OF PLANT EXTRACTS

- **GENERAL REQUIREMENTS**
  - * VALIDITY
  - * LACK OF AMBIGUITY
  - * ACCURACY
  - * REPRODUCIBILITY
  - * SIMPLICITY
  - * REASONABLESS OF COST

- **SPECIAL CONSIDERATIONS**
  - * HIGH SELECTIVITY : TO LIMIT THE NUMBER OF LEADS FOR FOLLOW-UP EVALUATION
  - * HIGH SENSITIVITY : TO DETECT LOW CONCENTRATIONS OF ACTIVE COMPOUNDS
  - * HIGH SPECIFICITY : TO BE INSENSITIVE TO A WIDE VARIETY OF INACTIVE COMPOUNDS (ELIMINATION OF ALL FALSE POSITIVES)
  - * METHODOLOGY ADAPTABLE TO :
    - HIGHLY COLORED
    - TARRY
    - POORLY SOLUBLE IN WATER
    - CHEMICALLY COMPLEX MATERIALS

ROLES OF BIOASSAYS IN NATURAL PRODUCTS DRUG DISCOVERY (1)

* **PURPOSES AND APPROPIATE USAGE**

- PRESCREEN :

* ASSAY APPLIED TO LARGE NUMBERS OF INITIAL SAMPLES TO DETERMINE WHETHER OR NOT THEY HAVE ANY BIOACTIVITY OF THE DESIRED TYPE.

* HIGH CAPACITY, LOW COST, RAPID ANSWERS.

- SCREEN :

ASSAY USED TO SELECT MATERIALS FOR DETAILED INDIVIDUAL STUDY (SECONDARY TESTING).

ROLES OF BIOASSAYS IN NATURAL PRODUCTS DRUG DISCOVERY (2)

* **PURPOSES AND APPROPIATE USAGE**

- MONITOR :

* ASSAY USED TO GUIDE FRACTIONATION OF A CRUDE MATERIAL TOWARDS ISOLATION OF THE PURE BIOACTIVE COMPOUNDS.

* FAST, CHEEP, HIGH CAPACITY, READILY AVAILABLE TO THE PHYTOCHEMIST.

- SECONDARY TESTING :

* DETAILED TESTING OF LEAD COMPOUNDS IN MULTIPLE MODELS AND TEST CONDITIONS TO SELECT CANDIDATES FOR DEVELOPMENT TOWARDS CLINICAL TRIALS.

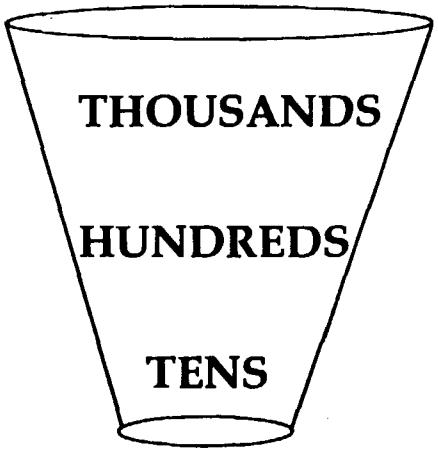
* LOW CAPACITY, EXPENSIVE, SLOW ASSAYS.

AFTER SUFFNESS AND PEZZUTO, 1991.

CAPACITY OF SCREENING PROGRAMMES

DRUG DISCOVERY FUNNEL

EVALUATION LEVEL	CAPACITY
PRESCREEN	THOUSANDS
SCREEN	HUNDREDS
SECONDARY EVALUATION	TENS
DEVELOPMENT CANDIDATES	ONES



AFTER SUFFNESS AND PEZZUTO, 1991.

METHODS FOR DETECTION OF BIOLOGICAL ACTIVITY OF PLANT EXTRACTS (1)

1. GENERAL SCREENING BIO-ASSAYS

1.1. PRIMARY SCREENING BIO-ASSAYS ("BENCH-TOP" BIO-ASSAYS)

* BRINE SHRIMP LETHALITY TEST
  - TARGET : CRUSTACEAN *ARTEMIA SALINA*
  - PREDICTION : CYTOTOXICITY AND PESTICIDAL ACTIVITY
* CROWN-GALL TUMOUR INHIBITION TEST (POTATO DISC)
  - TARGET : CELLS OF *SOLANUM TUBEROSUM* (POTATO TUBERS) TRANSFORMED BY *AGROBACTERIUM TUMEFACIENS*
  - PREDICTION : IN VIVO MURINE ANTILEUKEMIC ACTIVITY
* STARFISH OR SEA URCHIN ASSAY
  - TARGET : EGGS OF THE STARFISH, *ASTERINA PECTINIFERA* OR THE SEA URCHIN, *STRONGYLOCENTROTUS PURPURATUS* SP.
  - PREDICTION : ANTINEOPLASTIC ACTIVITY

FIGURE 1. Freshly hatched instar I nauplius showing antennulae (A), antennae (B), and mandibles (C), next to a hydrated cyst (D).

FIGURE 2. Adult male brine shrimp (8–10 mm long) with the antennae transformed into a pair of graspers and showing the stalked lateral eyes and 11 pairs of thoracic legs.

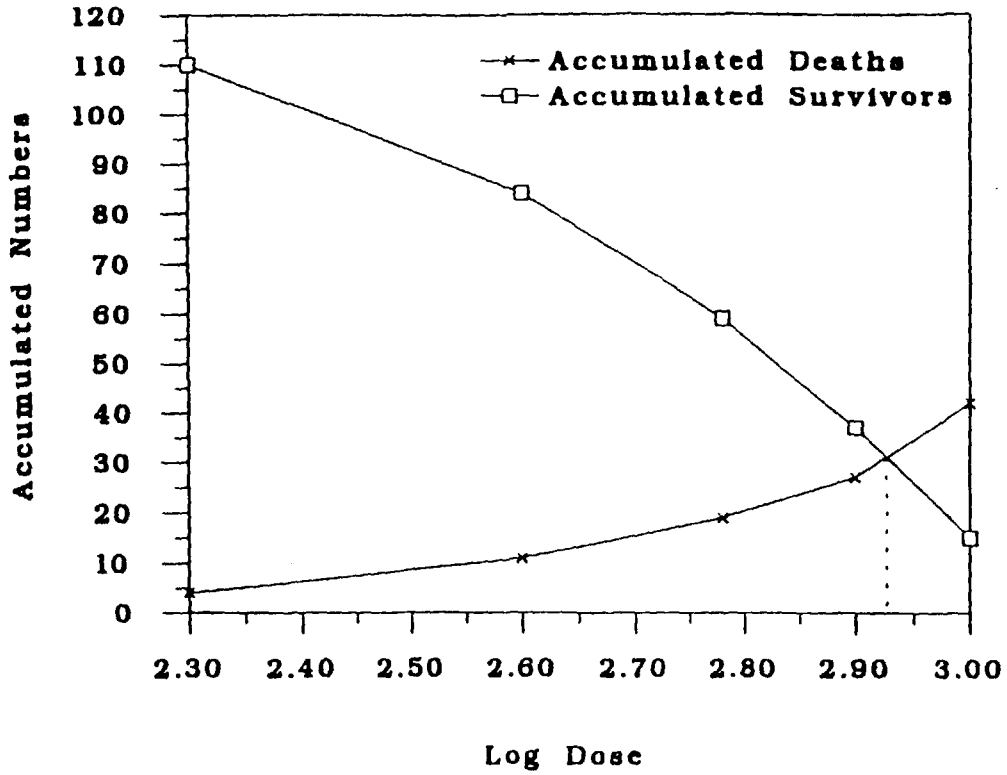


FIGURE 3. Estimation of LC_{50} by plotting the Reed-Muench accumulated deaths and survivors on the same set of axes. The two curves intersect at the 50% lethal dose required for the animal population.

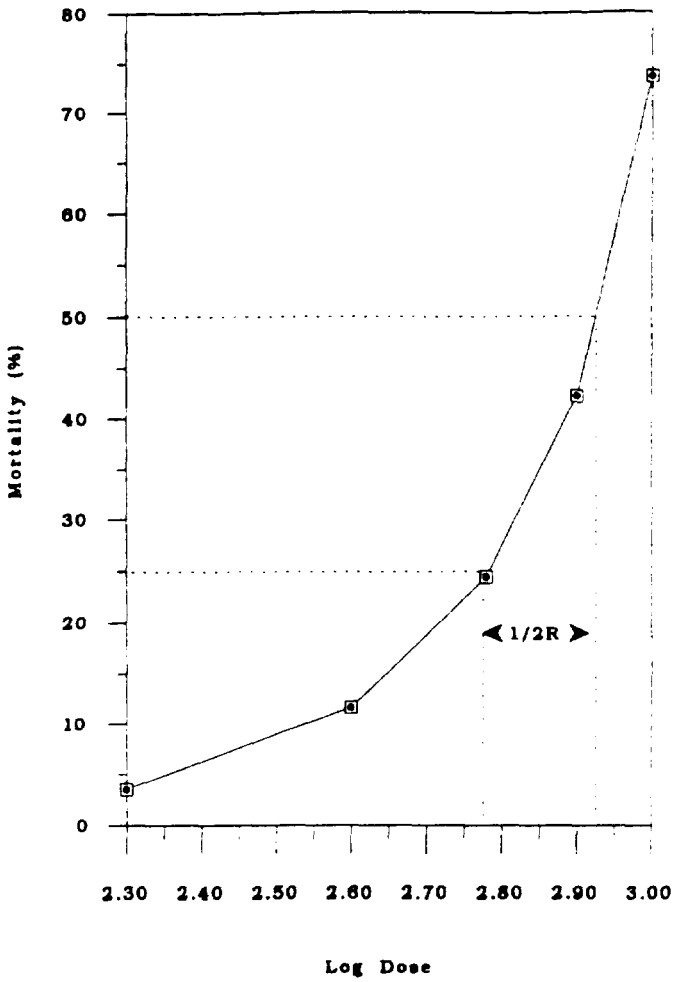


FIGURE 4. Estimation of LC_{50} and standard error by a plot of percent mortality against dosage. In this example half the interquartile range (1/2 R) is taken as Log LC_{50}–Log LC_{25}.

METHODS FOR DETECTION OF BIOLOGICAL ACTIVITY OF PLANT EXTRACTS (2)

1.1. PRIMARY SCREENING BIO-ASSAYS ("BENCH-TOP" BIO-ASSAYS)

* ANTIBIOTIC ACTIVITY
  - TARGET : LIMITED NUMBER OF HUMAN PATHOGENIC BACTERIA, YEASTS AND FUNGI E.G. *STAPHYLOCOCCUS AUREUS, ESCHERICHIA COLI, CANDIDA SP., DERMATOPHYTES, ASPERGILLUS* SP.
* PLANT GROWTH REGULATOR ACTIVITY
  - TARGET : ETIOLATED WHEAT COLEOPTILES
  - PREDICTION : MYCOTOXIC-, IMMUNOSUPPRESSANT- AND ANTIFUNGAL ACTIVITIES
* HERBICIDAL-, INSECT ANTIFEEDANT-, LARVICIDAL- AND MOLLUSCIDAL ACTIVITIES

THESE TESTS PROVIDE ONLY PRELIMINARY INFORMATION, BUT CAN BE PERFORMED IN NON-SPECIALIZED LABORATORIES.

METHODS FOR DETECTION OF BIOLOGICAL ACTIVITY OF PLANT EXTRACTS (3)

1. GENERAL SCREENING BIO-ASSAYS

1.2. BROAD SCREENING BIO-ASSAYS

* HIPPOCRATIC SCREENING
  - TARGET : INTACT RATS
  - PREDICTION : 63 SYMPTOMS AT 3 TO 5 DOSE LEVELS
  - COMMENTS : TIME-CONSUMING, EXPENSIVE, REQUIRES MUCH EXPERIENCE IN OBSERVATION AND LARGE AMOUNTS OF EXTRACTS, BUT SCREENING OF A WIDE VARIETY OF BIOLOGICAL ACTIVITIES POSSIBLE
* SMOOTH MUSCLE ASSAY
  - TARGET : ISOLATED ORGAN I.E. PIG ILEUM
  - PREDICTION : AGONIST-ANTAGONIST RELATIONS TO DIFFERENT AUTACOIDS SUCH AS ACETYLCHOLINE, ANGIOTENSIN, ARACHIDONIC ACID, BRADYKININE, HISTAMINE (H1-TYPE), PROSTAGLANDINS (E-TYPE), SEROTONIN AND SUBSTANCE P
  - COMMENTS : VERY SIMPLE, QUICK TO PERFORM, SMALL AMOUNTS OF EXTRACTS NEEDED, BUT RATHER NON-SPECIFIC

Date: 1980-07-14

Qualitative and Semi-Quantitative Screening and Toxicity Report of: TMP 7: H_2O-extract of leaves of Hoslundia oppasita

Vehicle for sample: H_2O Conc. 200 mg/ml

Sample dosage: 500 mg/kg

Test animal: Rat Fasted? No Sex: Female

Mark: III Color mark: Black Weight Gms. 210 Cage: B

ml injected: 0.53 Route inj.: I.P Time inj.: 8.30

Tested by: PAUA Evaluated by: G.S.

Parameter	C	RESPONSE AT TIME AFTER DOSAGE: min. 5	15	30	hours 1	2	4	6	days 2	3	4	5	6	7
CNS DEPRESSION														
Motor activity decr.	0	0	0	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	
Ataxia	0	0	0	0	0	0	0	0	0	0	0	0	0	
Loss rightning reflex	0	0	0	0	0	0	0	0	0	0	0	0	0	
Analgesia	0	0	0	0	0	0	0	0	0	0	0	0	0	
Anesthesia	0	0	0	0	0	0	0	0	0	0	0	0	0	
Resp. rate decr.	24	23	26	28	25	26	29	28	29	28	23	26	25	
Resp. depth decr.	0	0	0	0	0	0	0	0	0	0	0	0	0	
Loss corneal reflex	0	0	0	0	0	0	0	0	0	0	0	0	0	
Loss pinna reflex	0	0	0	0	0	0	0	0	0	0	0	0	0	
Paralysis: Forelegs	0	0	0	0	0	0	0	0	0	0	0	0	0	
Paralysis: Hind legs	0	0	0	0	0	0	0	0	0	0	0	0	0	
Paralysis: Head	0	0	0	0	0	0	0	0	0	0	0	0	0	
Screen grip: H.L. loss	0	0	0	0	0	0	0	0	0	0	0	0	0	
Screen grip: F.L. loss	0	0	0	0	0	0	0	0	0	0	0	0	0	
CNS STIMULATION														
Motor activity incr.	0	0	0	0	0	0	0.	0	0	0	0	0	0	
Fine body tremors	0	0	0	0	0	0	0	0	0	0	0	0	0	
Coarse body tremors	0	0	0	0	0	0	0	0	0	0	0	0	0	
Fasiculations	0	0	0	0	0	0	0	0	0	0	0	0	0	
Clonic Convulsions	0	0	0	0	0	0	0	0	0	0	0	0	0	
Tonic convulsions	0	0	0	0	0	0	0	0	0	0	0	0	0	
Mixed types convulsions	0	0	0	0	0	0	0	0	0	0	0	0	0	
Resp. rate incr.	24	23	26	28	25	26	29	28	29	28	23	25	25	
Resp. depth incr.	0	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	0	0	0	0	
EYES														
Enophthalmus	0	0	0	0	0	(+)	(+)	(+)	(+)	0	0	0	0	
Exophthalmus	0	0	0	0	0	0	0	0	0	0	0	0	0	
Palpebral ptosis	0	0	0	0	0	+1	+2	+2	+2	0	0	0	0	
Pupil size, mm.	1/4	1/4	1/4	1/4	1/4	1/4	1/4	1/4	1/4	1/4	1/4	1/4	1/4	
Pupil size, mm. (light)	0	0	0	0	0	0	0	0	0	0	0	0	0	
Nystagmus	0	0	0	0	0	0	0	0	0	0	0	0	0	
Lacrimation	0	0	0	0	0	0	0	0	0	0	0	0	0	
"Bloody" tears	0	0	0	0	0	0	0	0	0	0	0	0	0	

Parameter	C	RESPONSE AT TIME AFTER DOSAGE: min. 5	15	30	hours 1	2	4	6	days 2	3	4	5	6	7
BARS, ORAL MUCOSA														
Blanching	0	0	0	0	0	0	0	0	0	0	0	0	0	
Hyperemia	0	0	0	0	0	0	0	0	0	0	0	0	0	
Cyanosis	0	0	0	0	0	0	0	0	0	0	0	0	0	
Salivation	0	0	0	0	0	0	0	0	0	0	0	0	0	
GENERAL														
Tail erection	0	0	0	0	0	0	0	0	0	0	0	0	0	
Pilomotor erection	0	0	0	0	0	0	0	0	0	0	0	0	0	
Micturition	0	0	0	0	0	0	0	0	0	0	0	0	0	
Diarrhoea	0	0	0	0	0	0	0	0	0	0	0	0	0	
Colp	0	0	0	0	0	0	0	0	0	0	0	0	0	
Robichaud Test	0	0	0	0	0	0	0	0	0	0	0	0	0	
Circling motions	0	0	0	0	0	0	0	0	0	0	0	0	0	
Tail lashing	0	0	0	0	0	0	0	0	0	0	0	0	0	
Abdominal griping	0	0	0	0	0	0	0	0	0	0	0	0	0	
Rectal temp. °C.	37.6	/	/	/	37	37.4	37.2	37.2	38	38	38.2	37.2	37.4	
Body weight, Gms.	210	/	/	/	208	208	208	208	208	208	208	208	208	
Startle reaction	0	0	0	0	0	0	0	0	0	0	0	0	0	
SUBJECTIVE														
Head tap: Aggressive	0	0	0	0	0	0	0	0	0	0	0	0	0	
Passive	0	0	0	0	0	0	0	0	0	0	0	0	0	
Fearful	+	+	+	+	+	+	+	+	+	+	+	+	+	
Body touch: Aggress.	0	0	0	0	0	0	0	0	0	0	0	0	0	
Passive	0	0	0	0	0	0	0	0	0	0	0	0	0	
Fearful	+	+	+	+	+	+	+	+	+	+	+	+	+	
Statue positions	0	0	0	0	0	0	0	0	0	0	0	0	0	
Excess curiosity	0	0	0	0	0	0	0	0	0	0	0	0	0	

DEATH AND AUTOPSY NOTES (note: Resp. or Cardiac Arrest, systole or diastole, color: intest. wall and lungs, etc.

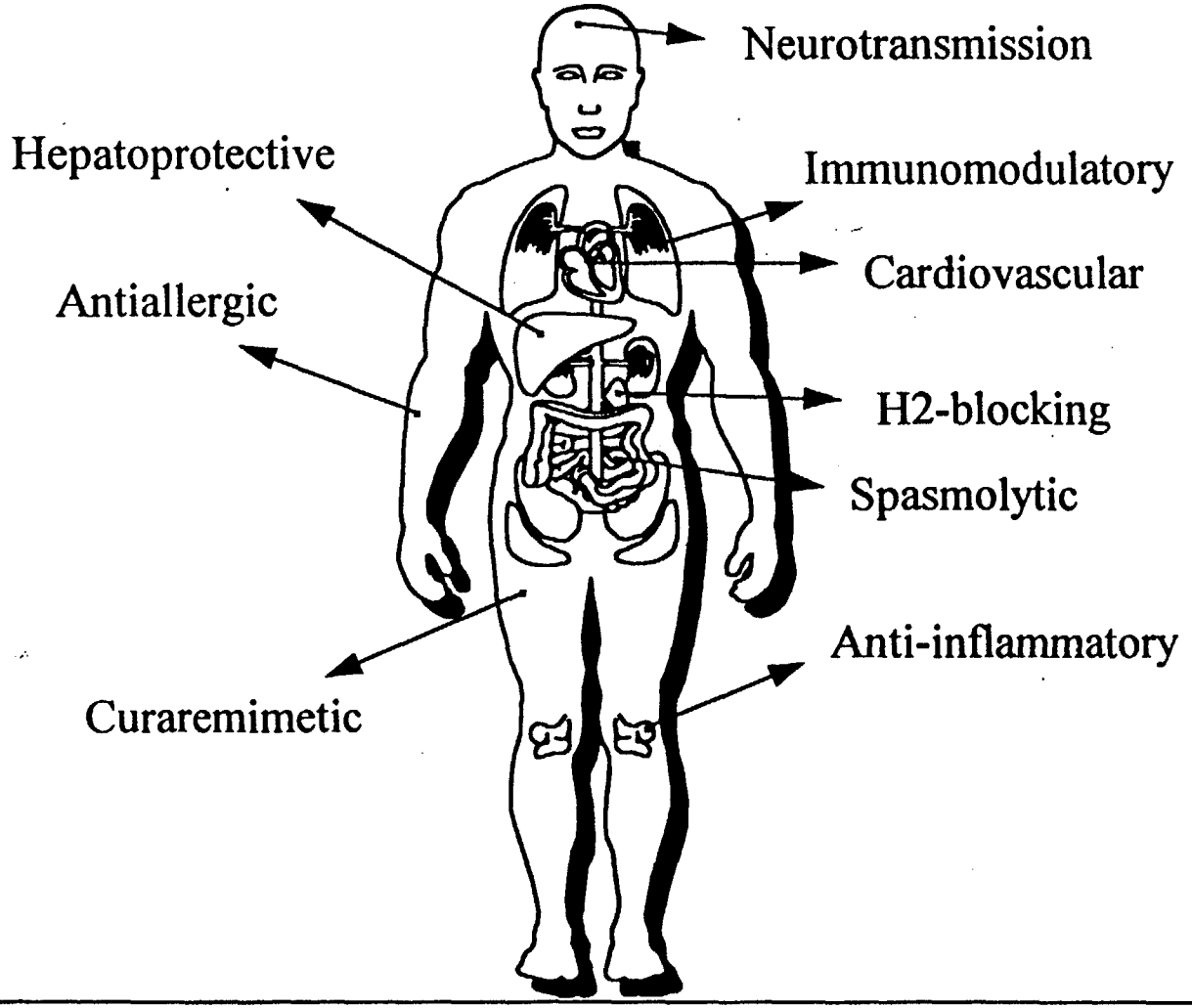
Screams when handled

Abdomen and all organs normal on autopsy

OTHER NOTES: Associate each symptom or observation with a specific time post-dosage. Note sterotypy.

FIG. 11.7. Worksheet for Hippocratic screening.

Pharmacological screening



PTEROTABERNA INCONSPICUA STAPH. (APOCYNACEAE)

* **FOLKLORIC USE**

LEAVES (CONGO)
- ACHES
- HYPERTENSION
- GASTROINTESTINAL UPSETS

* **PHARMACOLOGICAL EVALUATION**

METHUENINE (0.30%)
- NON-COMPETITIVE ANTAGONIST (1 µg/ML) (GUINEA PIG ILEUM)
  ACETYLCOLINE : $PD'_2 = 5.10 \pm 0.11$
  (PAPAVERINE : $PD'_2 = 5.08 \pm 0.18$)
  HISTAMINE : $PD'_2 = 5.13 \pm 0.14$

6-EPIMETHUENINE (0.16%)
- COMPETITIVE ANTAGONIST (1 µg/ML) (GUINEA PIG ILEUM)
  HISTAMINE: $pA_2 = 6.55 \pm 0.08$
  (DIPHENHYDRAMINE : $pA_2 = 7.7$; MEPYRAMINE : $pA_2 = 9.3$)

SPECIALIZED SCREENING BIOASSAYS

* **<u>CLASSIFICATION:</u> ACCORDING TO TARGET ORGANISM**
  1. LOWER ORGANISMS
  2. SUBCELLULAR SYSTEMS
  3. CELLULAR SYSTEMS
  4. ORGANS OF VERTEBRATES
  5. WHOLE ANIMALS
* **<u>CHARACTERISTICS:</u>**
  * THEY HAVE TO BE **RELEVANT** OR **CORRELATIONAL** I.E. PREDICT THE INTENDED THERAPEUTIC INDICATIONS
  * **BASIC CRITERIA**
    1. MUST BE **SENSITIVE** IN A **DOSE-DEPENDENT** FASHION TO STANDARD COMPOUNDS THAT ARE KNOWN TO POSSESS THE DESIRED THERAPEUTIC PROPERTY
    2. THE **RELATIVE POTENCY** OF KNOWN ACTIVE AGENTS IN THE BIOASSAY SHOULD BE **COMPARABLE** TO THEIR RELATIVE POTENCY IN CLINICAL USE
    3. SHOULD BE **SELECTIVE** I.E. THE EFFECTS OF KNOWN AGENTS IN THIS THERAPEUTIC INDICATION SHOULD BE DISTINGUISHABLE FROM EFFECTS OF DRUGS FOR OTHER INDICATIONS

AFTER VOGEL AND VOGEL, 1997.

METHODS FOR DETECTION OF BIOLOGICAL ACTIVITY OF PLANT EXTRACTS (4)

2. SPECIALIZED SCREENING BIOASSAYS

2.1. LOWER ORGANISMS

2.1.1. CELL CULTURE TEST SYSTEMS
BACTERIA, YEASTS, FUNGI, VIRUSES (LARGE BATTERIES)
(*E.G.* NCI AIDS ANTIVIRAL SCREEN, 1987)

2.1.2. *IN VITRO* OR *IN VIVO* SYSTEMS
(*E.G.* INSECTS, MOLLUSCS, PROTOZOA (*ENTAMOEBA HISTOLYTICA, LEISHMANIA, PLASMODIUM, TOXOPLASMA, TRYPANOSOMA*), HELMINTHS (NEMATODES, CESTODES, TREMATODES))

Prototype of a microbial battery for screening of plant extracts

Group of bacteria	Microorganism	Opitmal incubation conditions	Used media
Gram + cocci	Staphylococcus aureus	36°C, areobic	standard medium
	Streptococcus pyogenes	36°C, areobic	standard medium
Gram - cocci	Neisseria gonorrhoeae	36°C, aerobic (canophilic) (5-10% CO_2)	enriched medium
Gram + spore -forming rods	Bacillus cereus	36°C, aerobic	standard medium
Gram + spore -forming rods	Clostridium novyi	36°C, anaerobic	standard medium
Gram + asporogenous rods, acid fast	Mycobacterium fortuitum	36°C, aerobic	standard medium
Gram - rods	Escherichia coli	36°C, aerobic	standard medium
	Klebsiella pneumoniae (encapsulated)	facultatively anaerobic	standard medium
	Proteus vulgaris		standard medium
	Salmonella spp.		standard medium
Gram - rods	Pseudomonas aeruginosa	36°C, aerobic	standard medium
Gram - rods	Bacteroides fragilis	36°C, anaerobic	standard medium
Gram - curved rods	Campylobacter fetus	36°C, microaerophilic	enriched medium
Gram - obligate intracellular parasites	Chlamydia trachomatis	36°C, aerobic	tissue culture cells in virological medium
Yeasts	Candida albicans	36°C, aerobic	standard yeast medium

PROTOTYPE OF AN ANTIFUNGAL BATTERY FOR SCREENING OF PLANT EXTRACTS

GROUP OF FUNGI	MICROORGANISMS	OPTIMAL INCUBATION CONDITIONS	USED MEDIA
YEAST- LIKE FUNGI	*CANDIDA ALBICANS* *CANDIDA TROPICALIS*	36°C, AEROBIC	STANDARD YEAST MEDIUM
DERMATOPHYTES (DERMATOPHYTOSES)	*EPIDERMOPHYTON FLOCCOSUM* *MICROSPORUM CANIS* *TRICHOPHYTON MENTAGROPHYTES* *TRICHOPHYTON RUBRUM*	ROOM TEMPERATURE, AEROBIC	STANDARD FUNGAL MEDIUM
FUNGI (DEEP MYCOSES)	*ASPERGILLUS FLAVUS* *ASPERGILLUS FUMIGATUS* *ASPERGILLUS NIGER*	ROOM TEMPERATURE, AEROBIC	STANDARD FUNGAL MEDIUM

Different microorganisms

Extract dilution	1	2	3	4	5	6	7	8	9	10	11	12	
control	C	C	C	C	C	C	C	C	C	C	C	C	A
1/2													B
1/8													C
1/32													D
control	C	C	C	C	C	C	C	C	C	C	C	C	E
1/2													F
1/8													G
1/32													H

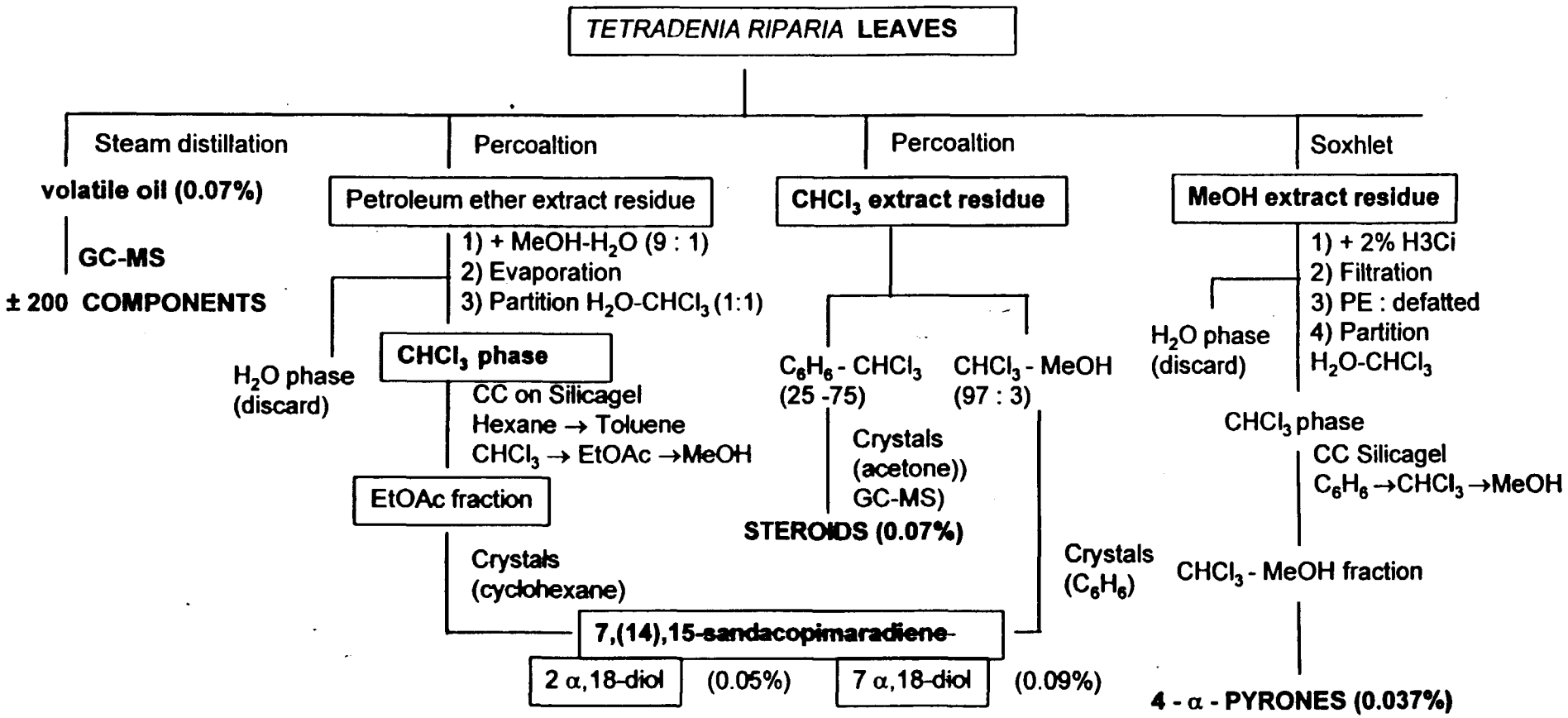
Properties and limitations of the available antimicrobial test methods of plant extracts

Method	Requirements for the plant extracts		Detectable antimicrobial activities	
	Sterility	Homogenous dispersion in water	Bacteriostatic and/or bactericidal evaluation	Detection of high and/or low potencies
Agar diffusion :				
- Discs, cylinders	NO	YES	bacteriostatic	high and low
- Holes	NO	NO	bacteriostatic	high and low
Agar dilution	NO	NO	bacteriostatic	high and low
Liquid dilution	YES	NO	bacteriostatic bactericidal	high and low
Bioauthography :				
- Contact	NO	NO	bacteriostatic	only high
- Direct	YES	NO	bacteriostatic	only high

TETRADENIA RIPARIA (HOCHST.) CODD. (LAMIACEAE)

FOLKLORIC USE

* RWANDA : *UMURAVUMBA*
* WHOLE PLANT
  - ACHES AND FEVERS E.G. HEADACHE
  - COUGHS AND RESPIRATORY TROUBLES
  - DENTAL ABSCESSES
  - DIARRHEA
  - INFECTIOUS DISEASES E.G. ANGINA, DENGUE, DYSENTERY, GONORRHEA, MALARIA, YAWS
  - INTESTINAL WORMS
  - GASTRO-ENTERITIS
  - RHEUMATISM
  - ULCERS
* USED AS
  - EMETIC AND VOMITORY
  - EXPECTORANT



Isolation of pharmacologically active components from the leaves of *Tetradenia riparia*

Van Puyvelde et al., Phytochemistry, **18**, 1295, (1979); **26**, 493, (1987); J. Org. Chem., **47**, 3628, 1982

TETRADENIA RIPARIA (HOCHST). CODD. (LAMIACEAE)

PHARMACOLOGICAL EVALUATION OF THE LEAVES

* ESSENTAIL OIL (0.07 %) (± 200 CONSTITUENTS)
  BROAD SPECTRUM ANTIMICROBIAL ACTIVITY : BACTERIA AND FUNGI : 10 µg/ml

* **8 (14), 15 - SANDARACOPIMARADIENE-7 α -18-DIOL** (0.9 %)
  - BROAD SPECTRUM ANTIMICROBIAL ACTIVITY
    GRAM + BACTERIA : MIC = 6.25 - 12.5 µg/ml
    GRAM - BACTERIA : MIC = 12.5 - 25 µg/ml
    YEASTS AND FUNGI : MIC = 25 - 100 µg/ml
    E.G. MYCOBACTERIUM AVIUM, M. SMEGMATIS, M. SIMIAE, M. TUBERCULOSIS :
    MIC = 12.5 - 100 µg/ml
    TRICHOMONAS VAGINALIS : MIC = 20 - 40 µg/ml
  - PHARMACOLOGICAL ACTIVITY : ANTISPASMODIC (10 - 25 µg/ml)
    NON-COMPETITIVE ANTAGONIST OF :
    ACETYLCHOLINE, HISTAMINE, BARIUM CHLORIDE (GUINEA PIG ILEUM)
    NORADRENALINE (RABBIT THORACIC AORTA)
  - ACUTE TOXICITY (MICE)
    LD_{50} > 600 MG/KG

* **α -PYRONES** (0.4%) : UMURAVUMBOLIDE, DEACETYLUMURAVUMBOLIDE,
  DEACETYLBORONOLIDE, 1',2'-DIDEACETYLBORONOLIDE
  POTENTIAL CARCINOGENS?

Definition of antivirals

1) Definition

Any product that is able to reduce *in vitro* or *in vivo,* directly or indirectly, the infectious virus in the host cells.

2) Approaches to the development of antivirals

a) Specific or non-specific stimulation of host defense mechanisms, which act indirectly on virus multiplication.

b) Limitation of the virulence of the invading virus by limiting virus replication in the infected host

- virucidals
- true antivirals.

Prototype of a virus battery for screening of plant extracts

Virus	Family	Morphology	Serotypes	Type of infection induced
Adenovirus	Adenoviridae	ds DNA, icosahedral, naked, spikes	34	respiratory, ophthalmic
Herpes simplex	Herpesviridae	ds DNA, icosahedral, enveloped	2	central nervous, skin oral, genital, ophthalmic upper respiratory
Poliomyelitis or Coxsackie	Picornaviridae	ss RNA, icosahedral, naked	Polio : 3 Coxsackie A : 23 Coxsackie B : 6	respiratory, cardiovascular, central nervous
Measles	Paramyxoviridae	ss RNA and segmented RNA, helical, enveloped	1	respiratory, skin, central nervous
Semliki forest	Togaviridae	ss RNA, polyhedral, enveloped	2	central nervous
Vesicular stomatitis	Rhabdoviridae	ss RNA, helical, enveloped, bullet shaped	1	respiratory

IN VITRO ANTIVIRAL SCREENING (1)

In vitro evaluation of :

ANTIVIRAL ACTIVITY (A)

Interference with dynamic processes during virus biosynthesis

OR

VIRUCIDAL ACTIVITY (V)

Extracellular action on virus infectiosity

SINGLE SUBSTANCES (S)

POSSIBLE CYTOTOXICITY IS DUE TO THE ANTIVIRAL AGENT ITSELF

DETERMINATION OF THERAPEUTIC INDEX IS ***POSSIBLE***

MIXTURES OF COMPOUNDS e.g. PLANT EXTRACTS (M)

POSSIBLE CYTOTOXICITY MIGHT BE DUE TO OTHER COMPOUNDS THAN THE ANTIVIRAL ONES

DETERMINATION OF THERAPEUTIC INDEX IS ***IMPOSSIBLE***

$$TI_X = \frac{C_Y D_{50}}{ED_X}$$

X = 50 , 90 or 99

TI_X = ratio of the maximum drug concentration at which 50 % of the growth of normal cells is inhibited ($C_Y D_{50}$) to the minimum drug concentration at which X % of the virus is inhibited (ED_X).

EPTT technique

TCD_{50}/cell	Dilution	titer	CYTOTOXIC 1/2		MNTD 1/4		1/8		1/16		1/32		titer
10	10^{-1}	+	T	T	+	+	+	+	+	+	+	+	+
1	10^{-2}	+			**0**	**0**	+	+	+	+	+	+	+
10^{-1}	10^{-3}	+					**0**	**0**	+	+	+	+	+
10^{-2}	10^{-4}	+							+	+	+	+	+
10^{-3}	10^{-5}	+							**0**	**0**	+	+	+
10^{-4}	10^{-6}	+									**0**	**0**	+
10^{-5}	10^{-7}	**0**	T	T									**0**
10^{-6}	10^{-8}												
RF =					100.000		10.000		100		10		

Virus control
+ : cell destruction
0 : normal cells

Cell control

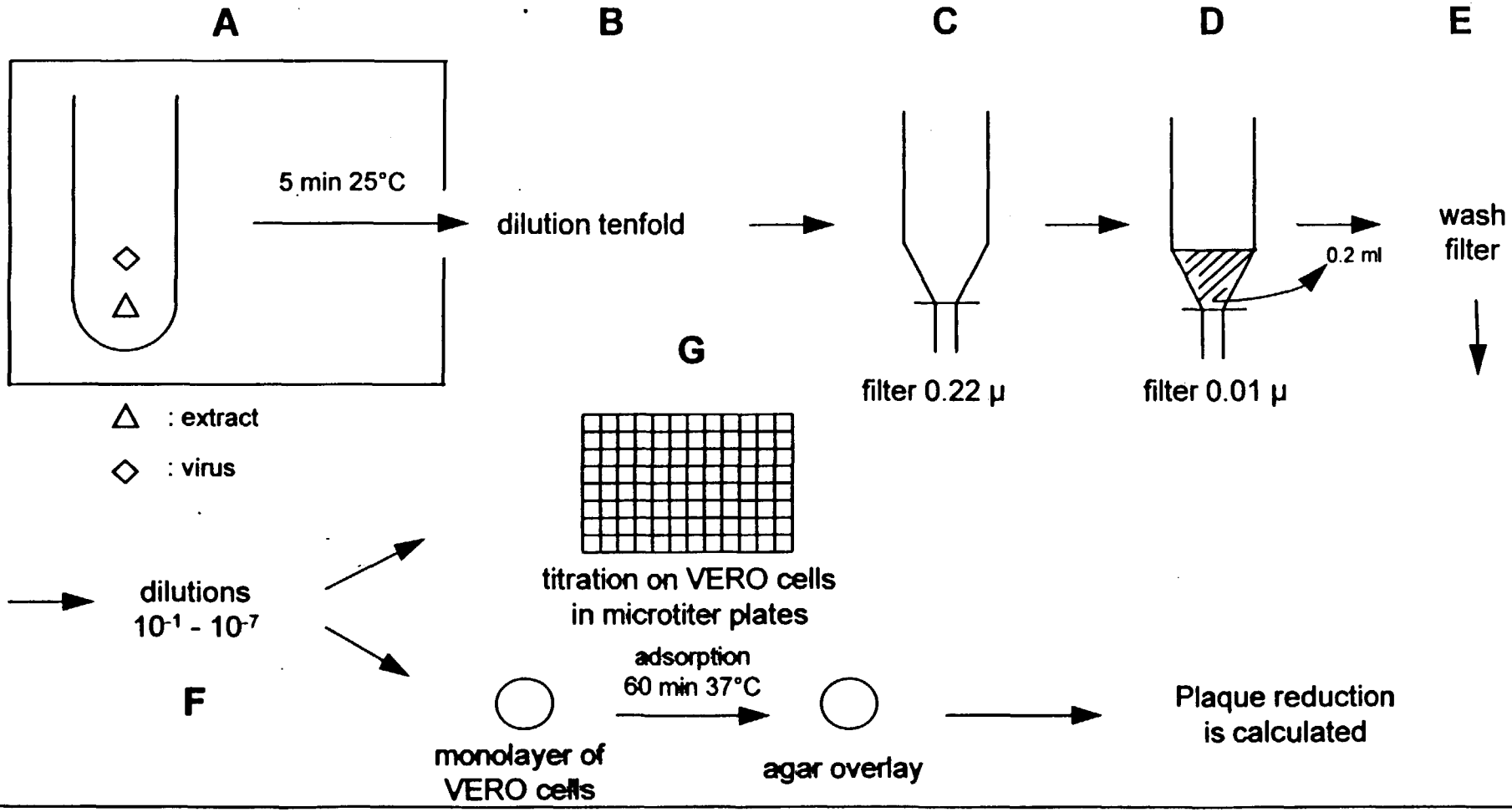
Extract control
cytotoxicity control
T : cytotoxic

Titration in the presence
of two-fold dilution
of extracts

ADVANTAGES AND DISADVANTAGES OF IN VITRO ANTIVIRAL SCREENING TESTS

- PIT
  - Inhibitors of first steps of virus multiplication cannot be determined
  - Problems of diffusion :
    - Precipitation of active compound
    - Faster diffusion of toxic substances
  - Accurate idea of the decrease of virus titer
- EPTT
  - Concentration of compounds remains constant
  - Exact duration of antiviral action can be determined
  - All possible steps of virus multiplication are included
  - Need of high virus titers (10^4 TCD_{50}/ml or more)
  - Correlation between extract toxicity and antiviral activity is possible :
    - Antiviral activity should be stable in at least two subsequent dilutions : otherwise toxic or virucidal
    - True antiviral should also protect cells infected with low virus dilutions (e.g. 0.1 PFU/ml)

Extracellular virucidal evaluation procedure



EUPHORBIA SPECIES (EUPHORBIACEAE)
FOLKLORIC USE

EUPHORBIA GRANTII OLIV. (RWANDA)

LEAVES

Venereal diseases
Childhood diseases e.g. poliomyelitis
Arrow and fish poisons
Leprosy (ashes)

LATEX

Rheumatism
Intestinal worms
Drastic purgative
Snake-bites (emetic)

EUPHORBIA BALSAMIFERA AIT (SENEGAL)

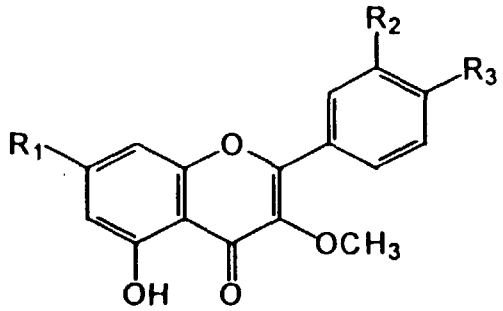
Oral antiseptic
Venereal diseases
Hemostatic
Purgative

EUPHORBIA HIRTA L. (ZAIRE)

LEAVES and/or LATEX

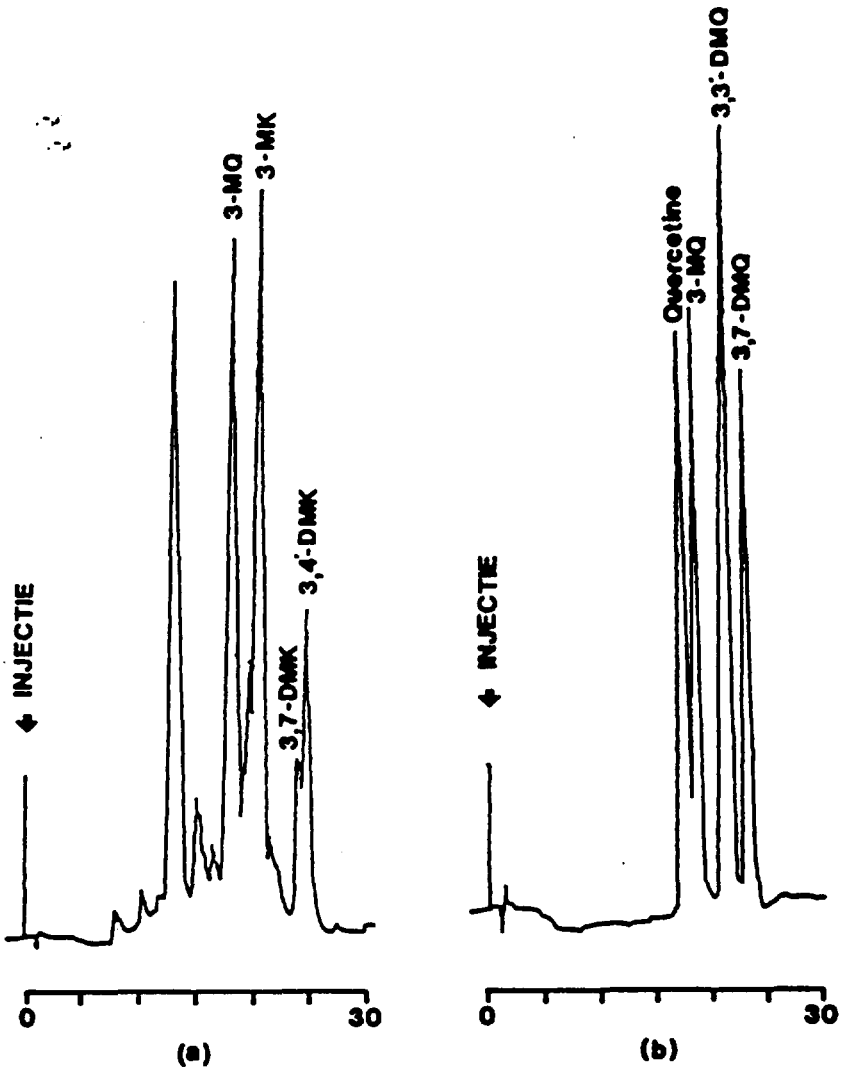
Infectious diseases
Bronchitis, conjunctivitis, dysentery, gonorrhea, oxyuris, diarrhea, stomachache, diuretic, mild purgative
Asthma remedy
Expectorant and cough remedy
Cathartic, gargle, poultice, insecticide
Snake-bites (roots)

Chemical structures of different flavonols with antiviral activity



R1	R2	R3	Name
OH	H	OH	3-O-methylkaempferol
OCH_3	H	OH	3,7-O-dimethylkaempferol
OH	H	OCH_3	3,4'-O-dimethylkaempferol
OH	OH	OH	3-O-methylquercetin
OH	OCH_3	OH	3,3'-O-dimethylquercetin
OCH_3	OH	OH	3,7-O-dimethylquercetin
OCH_3	OCH_3	OH	3,3',7-O-trimethylquercetin

HPLC analysis of the ethylacetate extract of Euphorbia grantii Oliv.



	retention time (min)		
references (b)	references (b)	EtAc extract (a)	EtAc extract (a)
quercetin	16.4	-	-
VVV2; 3-MQ	17.7	17.8	VVV2; 3-MQ
VVV1; 3,3'-DMQ	20.1	20.2	VVV1; 3,3'-DMQ
VVV3; 3,7-DMQ	22.1	22.2	VVV3; 3,7-DMQ
-	-	24.4	VVV4; 3,7,3'-TMQ

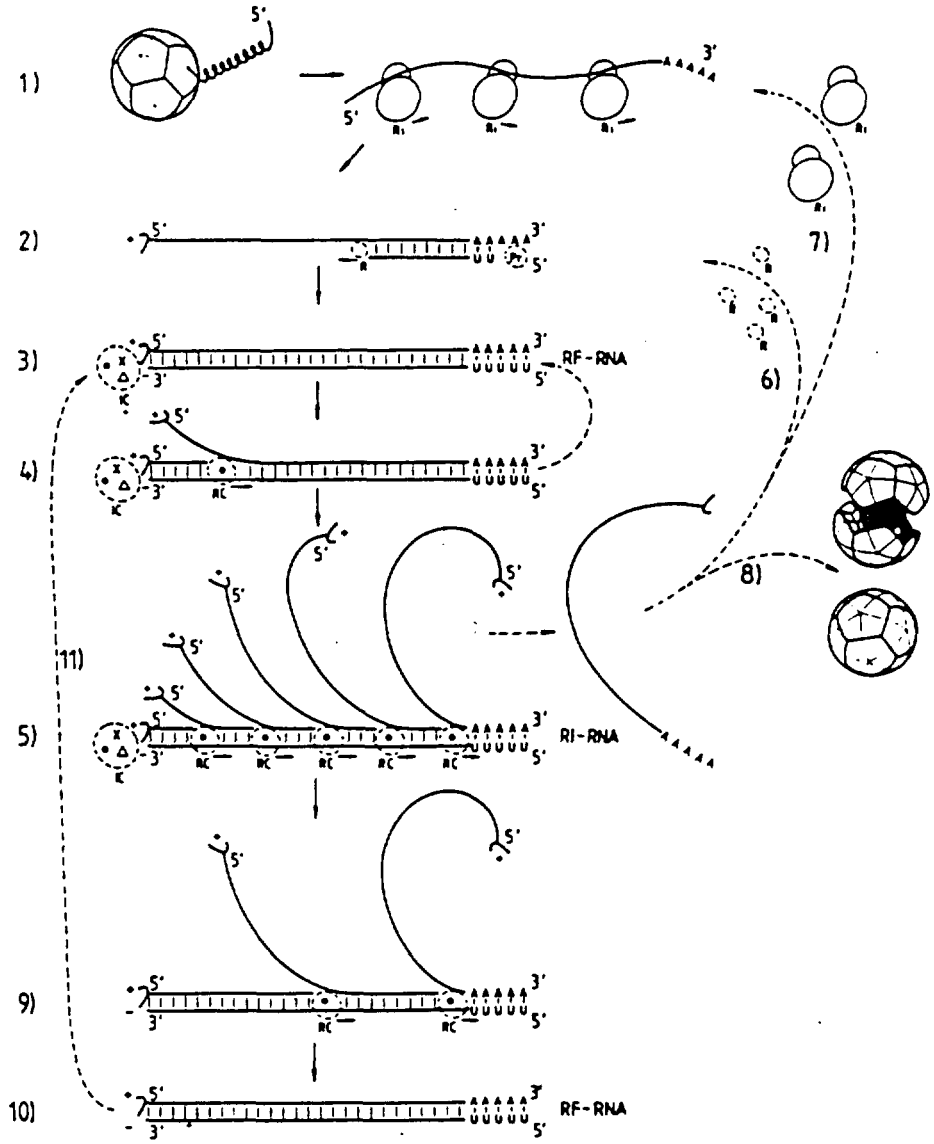
In vitro antiviral activity of 3-methoxyflavones

Virus	3,3'-DMQ		3-MQ	
	3D	7D	3D	7D
Polio (10^7)	10^6	10^6	10^7	10^7
Coxsackie B2 (10^7)	10^5	10^5	10^6	10^6
Rhino (10^3)	10	10	10	10
Mengo (10^4)	1	1	1	1
VSV (10^4)	10^3	1-10	10^3	1-10
Semliki forest (10^7)	1	1	1	1
Measles (10^5)	1	1	1	1
Herpes (10^7)	1	1	1	1
Adeno (10^3)	1	1	1	1
Bangin (10^5)	$10\text{-}10^2$	1	$10\text{-}10^2$	1
Bunyamwera ($10^{6.5}$)	10^3	10	10^3	10
Yellow fever (10^5)	10	1-10	10	1-10

In vivo activity of 3-methoxyflavones

Time course (days)	UNINFECTED SURVIVALS (%)		INFECTED SURVIVALS (%)	
	Control	Treated	Control	Treated
1	100	100	100	100
2	100	100	100	100
3	100	100	100	100
4	100	100	100	100
5	100	100	80	100
6	100	100	50	100
7	100	100	30	90
8	100	100	10	90
9	100	100	0	80
10	100	100	0	80
11	100	100	0	80
12	100	100	0	80
13	100	100	0	80
14	100	100	0	80

Mice survival after intracerebral infection with coxsackievirus B4 and intraperitoneal treatment with 3-O-methyl-quercetin (3-MQ) (20 mg/kg/day) during 9 consecutive days



ribosome

primer: ribosomal host factor or oligo-U

replicase (NCVP4)

replication complex

multicomponent initiation complex

VPg

RNA of virion polarity (mRNA, vRNA)

complementary RNA (cRNA)

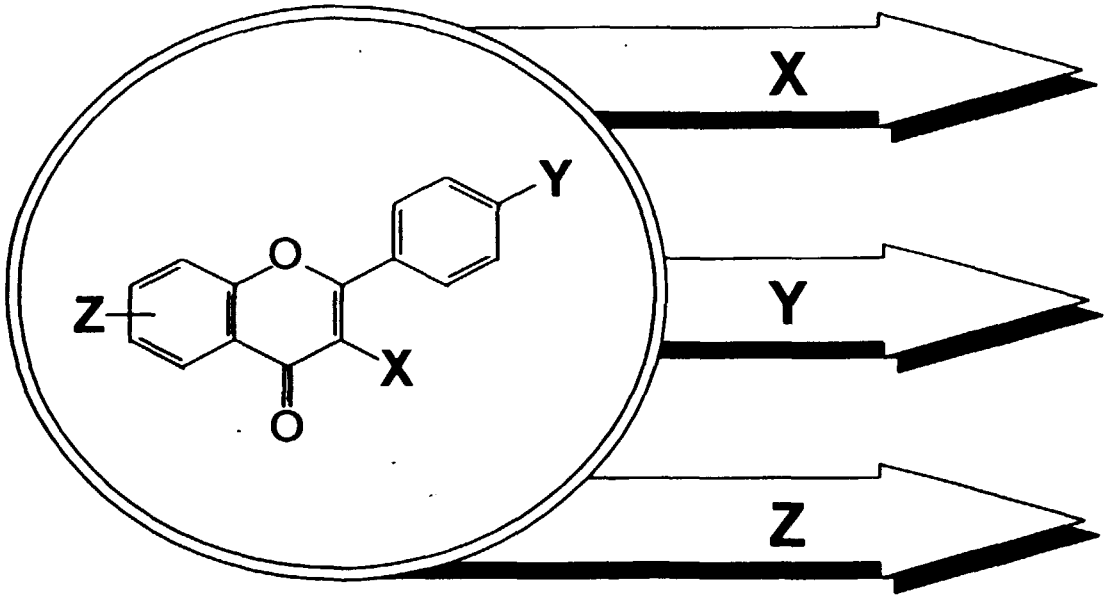
AAAAA 3' poly A

UUUUU 5' poly U

RF = replicative form

RI = replicative intermediate

Structure-activity relationship studies of antivirally active flavones



X: $-OCH_3$, $-OC_2H_5$, $-OCH(CH_3)_2$, $-CH_3$, $-NH_2$, $-OCOCH_3$, -Cl

Y: -OH, $-OCOCH_3$, $-OCH_2C_6H_5$, $-OCH_3$, -Cl

Z: -H, -OH, $-OCH_3$, $-CH_3$, $-OCH_2C_6H_5$, $-CH(CH_3)_2$, -I, -Br, -Cl, -F, $-NO_2$, $-NH_2$

Antiviral and cytotoxic activities of 3-methoxyflavones *in vitro* against polio- and rhinoviruses

		POLIO			RHINO	
Product	LD_{50}	ED_{99}	TI_{99}	LD_{50}	ED_{99}	TI_{99}
4',5,7-trihydroxy-3-methoxyflavone	10	0.2	50	nt	nt	-
4',5-dihydroxy-3,7-dimethoxyflavone	100	0.3	333.3	100	2.5	40
4',7-dihydroxy-3-methoxy-5-methylflavone	15	0.1	150	15	1	15
4'-hydroxy-3,7-dimethoxy-5-methylflavone	50	1	50	50	25	2
6-chloro-4'-hydroxy-9-methoxy-8-methylflavone	50	>50	< 1	nt	nt	-
4',7-dihydroxy-3-methoxy-5,6-dimethylflavone	>100	0.1	>1000	100	0.5	>200
* 4',5,7-trihydroxy-3,6-dimethoxyflavone	25	0.5	50	nt	nt	-
* 4',5-dihydroxy-3,6,7-trimethoxyflavone	100	0.2	500	10	10	1
* 4',5-dihydroxy-3,7,8-trimethoxyflavone	>5	0.2	25	>100	0.2	>200

* = natural product

nt = not tested
LD_{50} in µg/ml
ED_{99} in µg/ml

Table 2 : Activity of antirhinovirus products (MIC_{50} : ng/ml)

Rhinovirus serotype

Product	2	29	39	85	9	15	51	59	63	89	41	Med 1
142	44	78	88	76	156	88	104	16	74	500	38	88
140	2.400	175	12.000	6.000	200	400	800	3.000	2.500	200	3.750	3.750
Guanidine	$>64\cdot10^3$	$>64\cdot10^3$	$109\cdot10^3$	$750\cdot10^3$	$375\cdot10^3$	$438\cdot10^3$	$>64\cdot10^3$	$350\cdot10^3$	$500\cdot10^3$	$1000\cdot10^3$	$1000\cdot10^3$	$750\cdot10^3$
HBB	$>50\cdot10^3$	$>50\cdot10^3$	$>50\cdot10^3$	$>50\cdot10^3$	$>50\cdot10^3$	$>50\cdot10^3$	$>35\cdot10^3$	$>50\cdot10^3$	$>50\cdot10^3$	$>50\cdot10^3$	$>50\cdot10^3$	$>50\cdot10^3$

Product	42	45	14	70	72	86	Med 2
142	88	55	72	97	104	124	90
140	3.000	3.200	10.800	5.000	750	1.500	4.041
Guanidine	$>1000\cdot10^3$	$25\cdot10^3$	$7\cdot10^3$	$6\cdot10^3$	$5\cdot10^3$	$5\cdot10^3$	$7\cdot10^3$
HBB	$16\cdot10^3$	$18\cdot10^3$	$16\cdot10^3$	$50\cdot10^3$	$40\cdot10^3$	$17\cdot10^3$	$18\cdot10^3$

PRESENT STATUS OF THE MOST PROMISING ANTIRHINOVIRUS AGENTS (1993)

1. INHIBITORS of UNCOATING or ATTACHMENT TO THE CELL RECEPTOR, e.g. flavans, chalcones, pyridazines, thiazoles, furanylethanones and methylisoxazoles
   * Potent *in vitro* activity against most rhinoviruses
   * No effective prophylactic or therapeutic activity in clinical trials after oral or intranasal administration (flavans, chalcones, furanylethanones and thiazoles)
   * Positive clinical trials with antiviral pharmaceutical compositions containing cyclodextrins (pyridazines) : especially convenient for treating mucosal infections
   * De novo resistance due to the mechanism of action

2. INHIBITORS OF VIRAL RNA-SYNTHESIS, e.g. 3-methoxyflavones
   * Potent *in vitro* activity against all rhinoviruses tested → no induction of resistance
   * Broad-spectrum antipicornavirus activity except for mengovirus
   * No clinical trials up till now

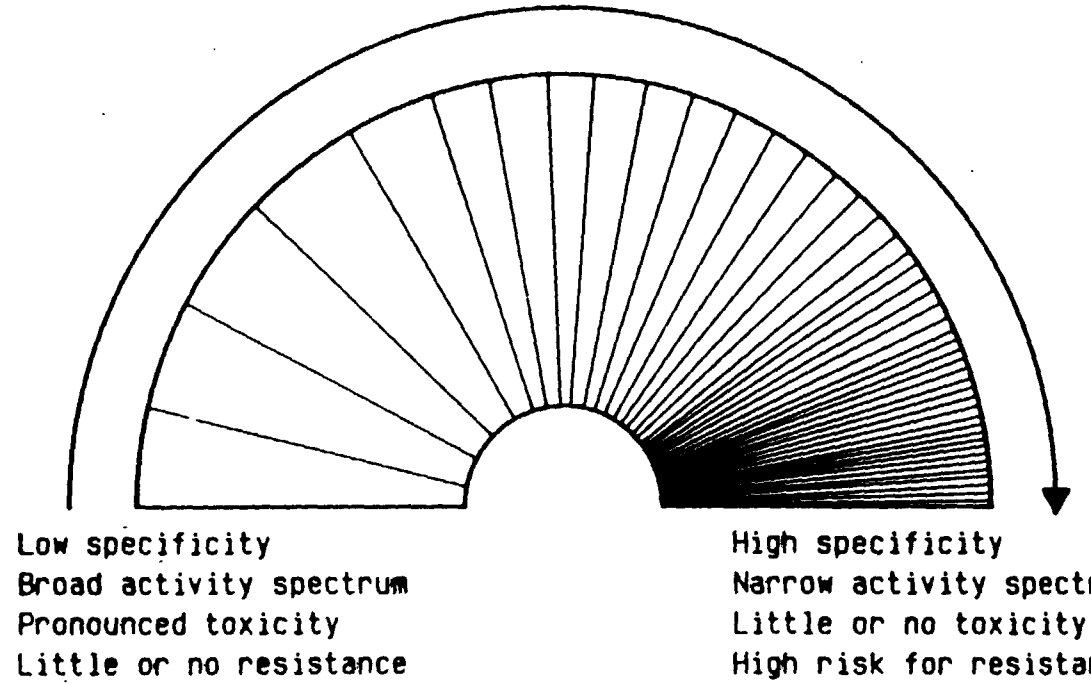


FIG. 20. Characteristics of antiviral agents, also extending to the antiretroviral agents.

Determination of schistosomicidal effects of plant extracts on mice infected with Schistosoma mansoni

Infection of female mice with
Schistosoma mansoni
(30 cercariae/mouse)

7 weeks ↓

Treatment with plant extract(s) = test group
or with vehicle = control group = untreated
or with praziquantel = reference group

1 week ↓ 1 ml daily/mouse by gavage

Feeding of treated and untreated mice

4 weeks ↓

Killing of treated or untreated mice

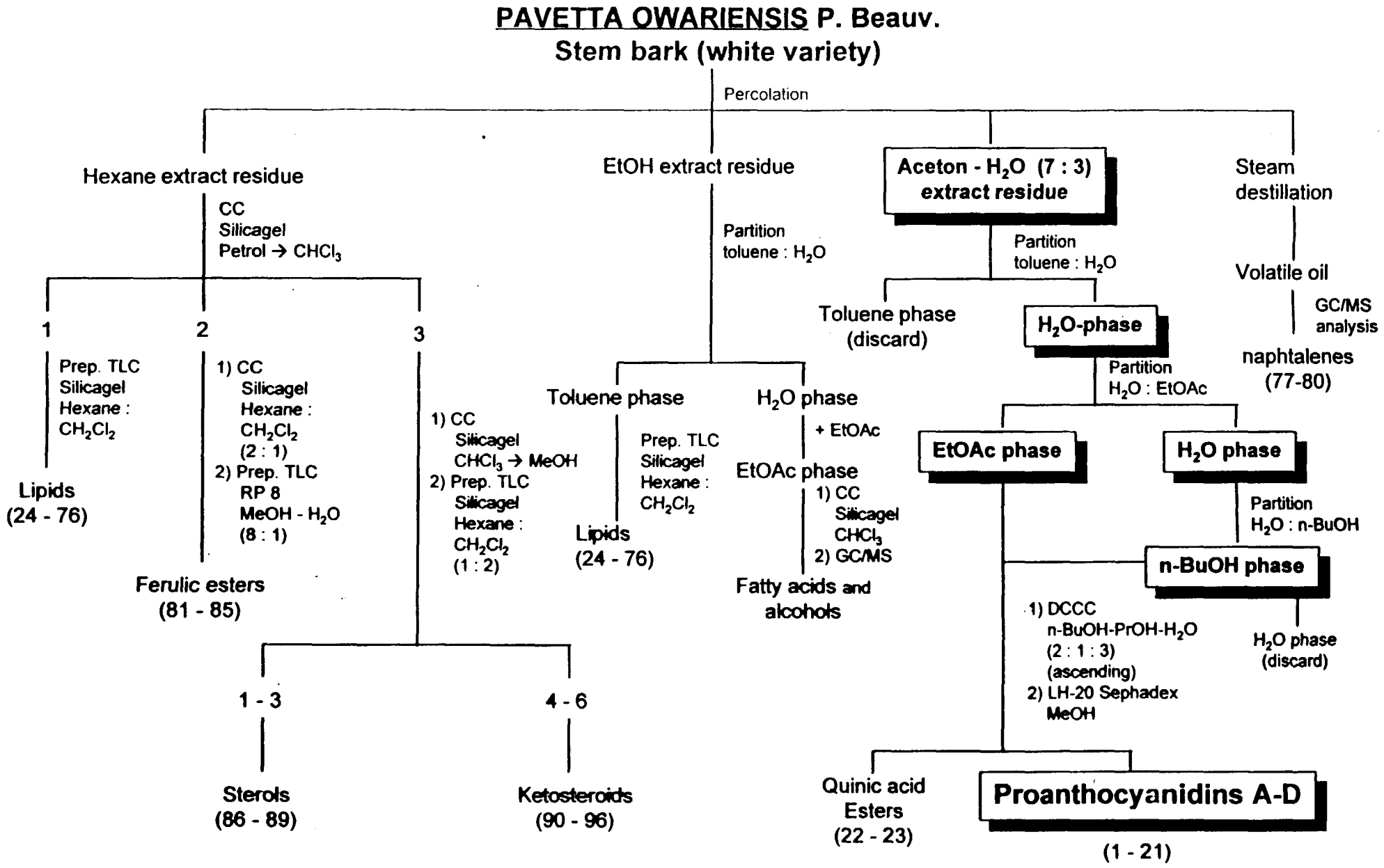
Experimental Procedure

Measurements :

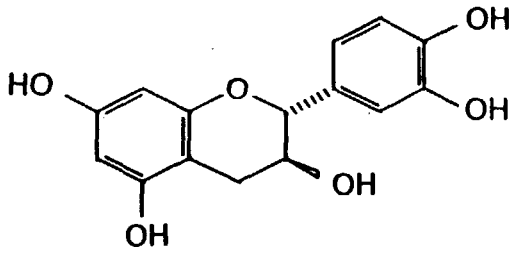
- counting of adult worms
- counting of eggs in liver and intestine
- measurement of the size of liver granulomas
- determination of the weight of liver and spleen
- statistical analysis of treated and untreated groups using the Mann-Whitney U-test and the Chi-square test

Pavetta owariensis P. Beauv. (RUBIACEAE)
Folkloric use

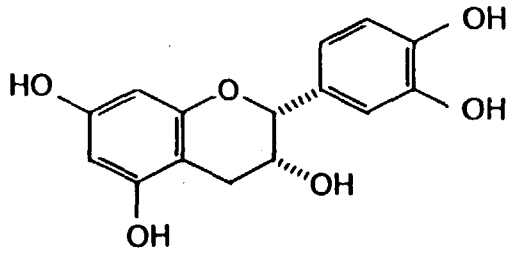
- **Guinea**
  Töma-ghenhoulaï (worm's tree)
  Guerzeg-kpeliwulu (worm's tree)
- **Stem bark**
  - Specific anthelminthic against Ascaris lumbricoides
  - Schistosomiasis
  - Stomach pain, sore throat
  - Correction of visceral obstructions
  - Indigestion
- **Leaves**
  - Antipyretic
  - Syphilis chancres (topically)
  - Against fleas (calves)
  - Snake bites (vomiting)
  - Obstinate itch
  - Schistosomiasis
  - Fracture
  - Aches
  - Liver dysfunction
  - Aphrodisiac


PAVETTA OWARIENSIS P. Beauv.
Stem bark (white variety)
Percolation
Hexane extract residue
CC
Silicagel
Petrol → CHCl3
1
2
3
Prep. TLC
Silicagel
Hexane :
CH2Cl2
Lipids
(24 - 76)
1) CC
Silicagel
Hexane :
CH2Cl2
(2 : 1)
2) Prep. TLC
RP 8
MeOH - H2O
(8 : 1)
Ferulic esters
(81 - 85)
1) CC
Silicagel
CHCl3 → MeOH
2) Prep. TLC
Silicagel
Hexane :
CH2Cl2
(1 : 2)
1 - 3
4 - 6
Sterols
(86 - 89)
Ketosteroids
(90 - 96)
EtOH extract residue
Partition
toluene : H2O
Toluene phase
Prep. TLC
Silicagel
Hexane :
CH2Cl2
Lipids
(24 - 76)
H2O phase
+ EtOAc
EtOAc phase
1) CC
Silicagel
CHCl3
2) GC/MS
Fatty acids and
alcohols
Aceton - H2O (7 : 3)
extract residue
Partition
toluene : H2O
Toluene phase
(discard)
H2O-phase
Partition
H2O : EtOAc
EtOAc phase
H2O phase
Partition
H2O : n-BuOH
n-BuOH phase
1) DCCC
n-BuOH-PrOH-H2O
(2 : 1 : 3)
(ascending)
2) LH-20 Sephadex
MeOH
H2O phase
(discard)
Quinic acid
Esters
(22 - 23)
Proanthocyanidins A-D
(1 - 21)
Steam
destillation
Volatile oil
GC/MS
analysis
naphtalenes
(77-80)

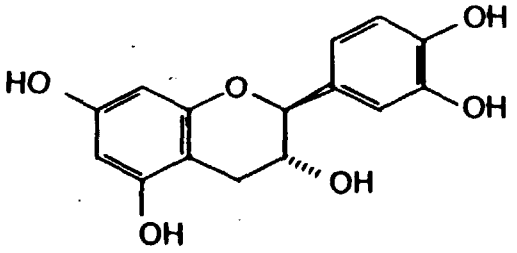
Monomers of condensed tannins



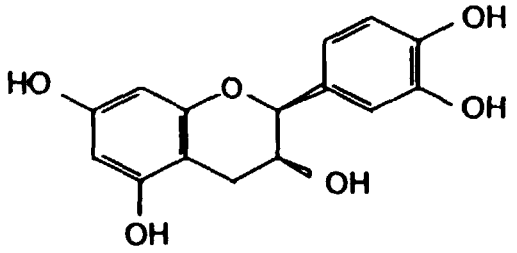
(+) catechin (2R, 3S)



(-) epicatechin (2R, 3R)

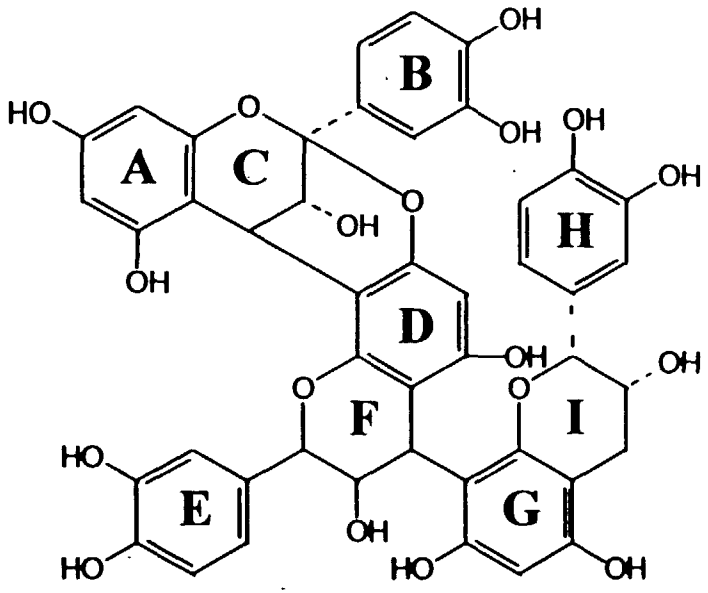


(-) ent-catechin (2S,3R)

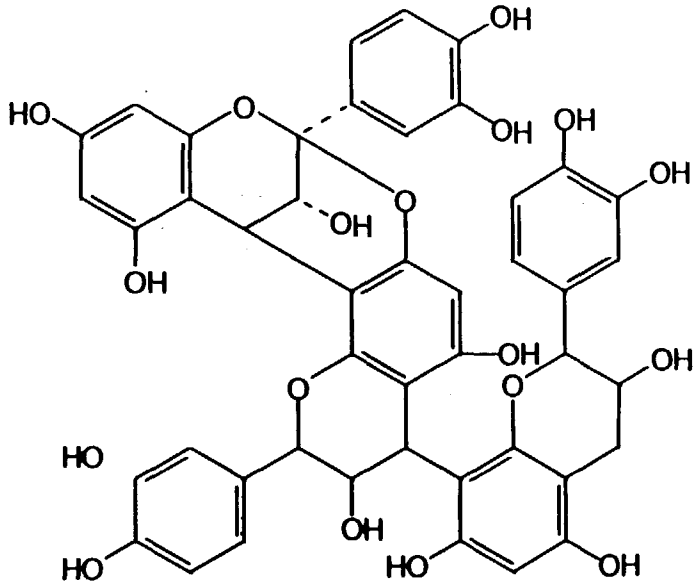


(+) ent-epicatechin (2S, 3S)

Condensed tannins isolated from <u>Pavetta owariensis</u> P. Beauv. (2)

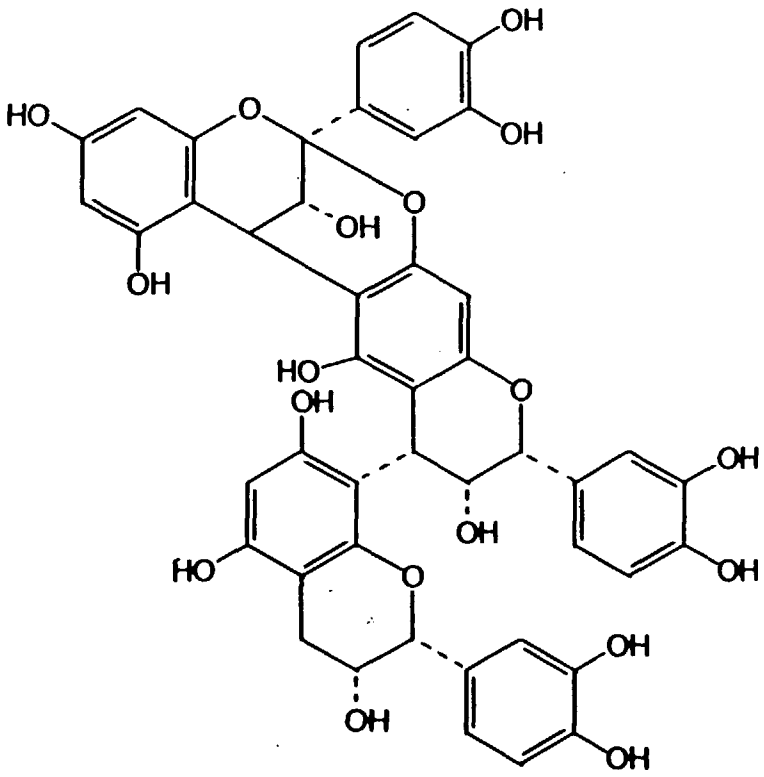


Cinnamtannin B_1

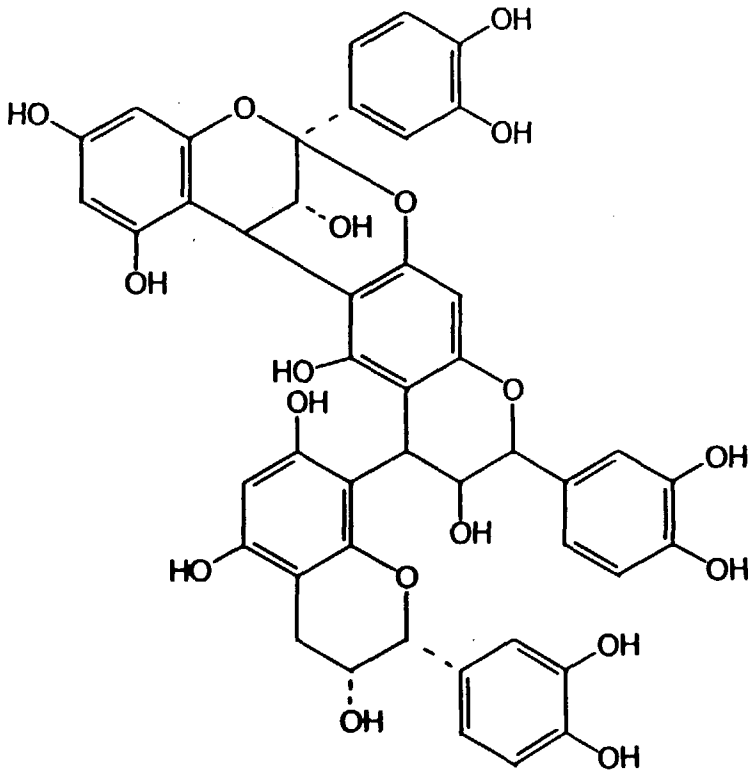


Pavetannin B_1

Condensed tannins isolated from <u>Pavetta owariensis</u> P. Beauv. (3)

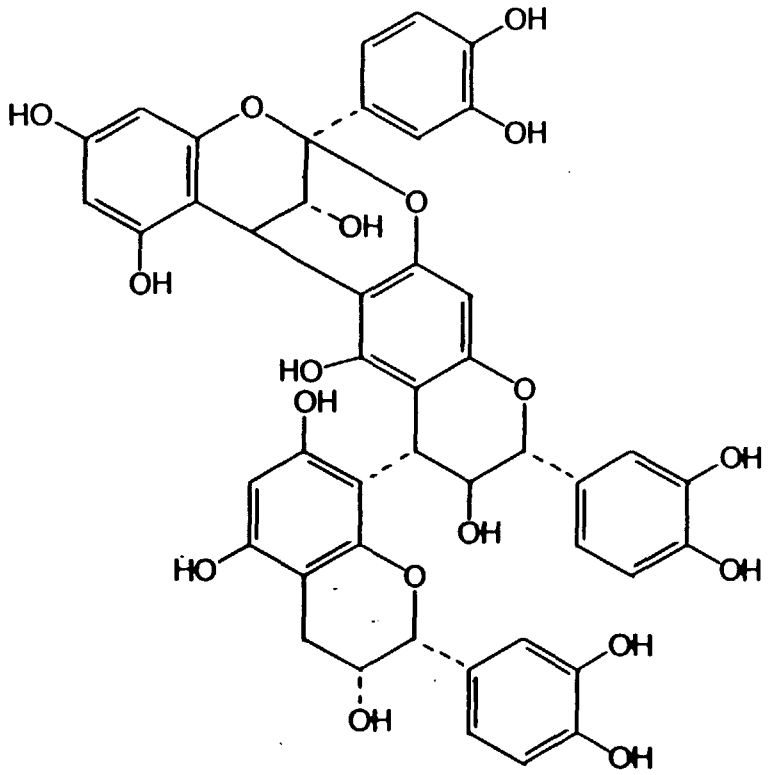


Pavetannin B_3

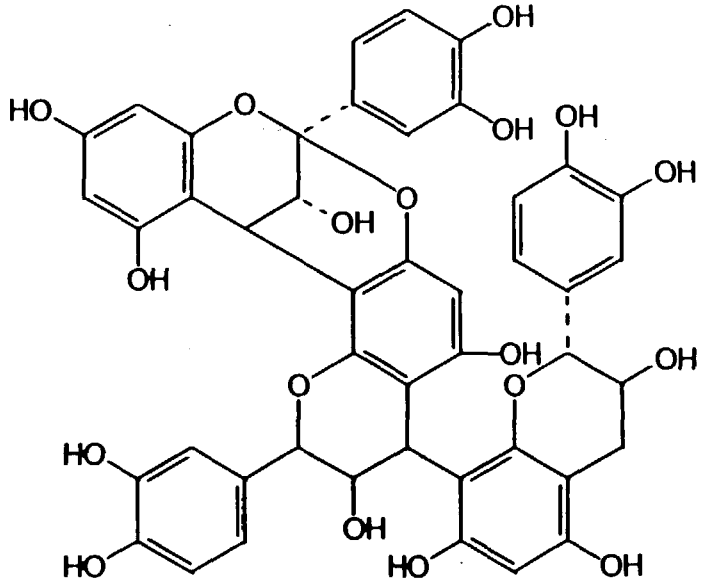


Pavetannin B_4

Condensed tannins isolated from Pavetta owariensis P. Beauv. (4)

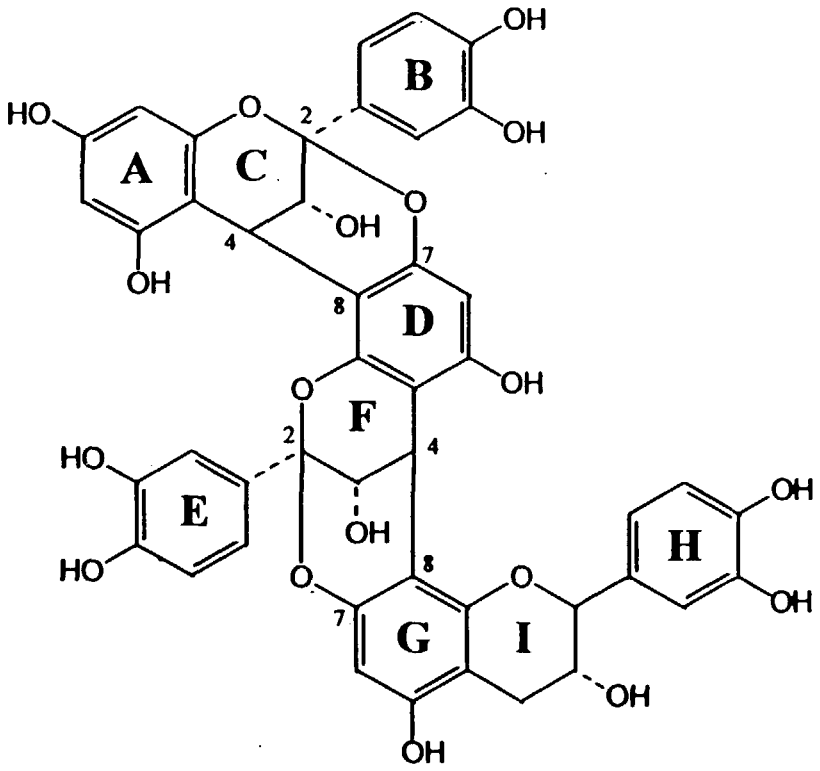


Pavetannin B_5

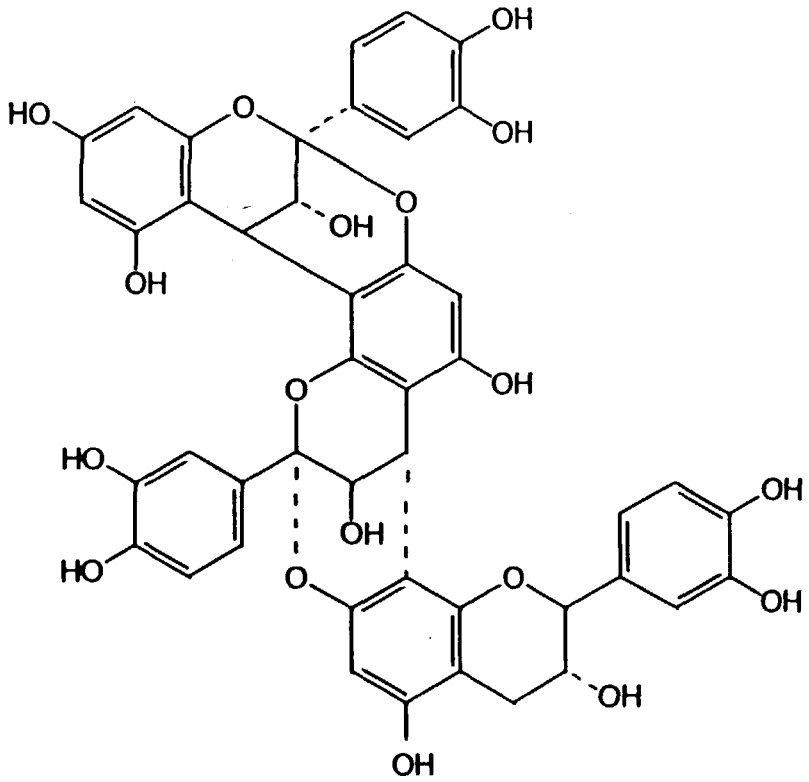


Pavetannin B_6

Condensed tannins isolated from <u>Pavetta owariensis</u> P. Beauv. (5)

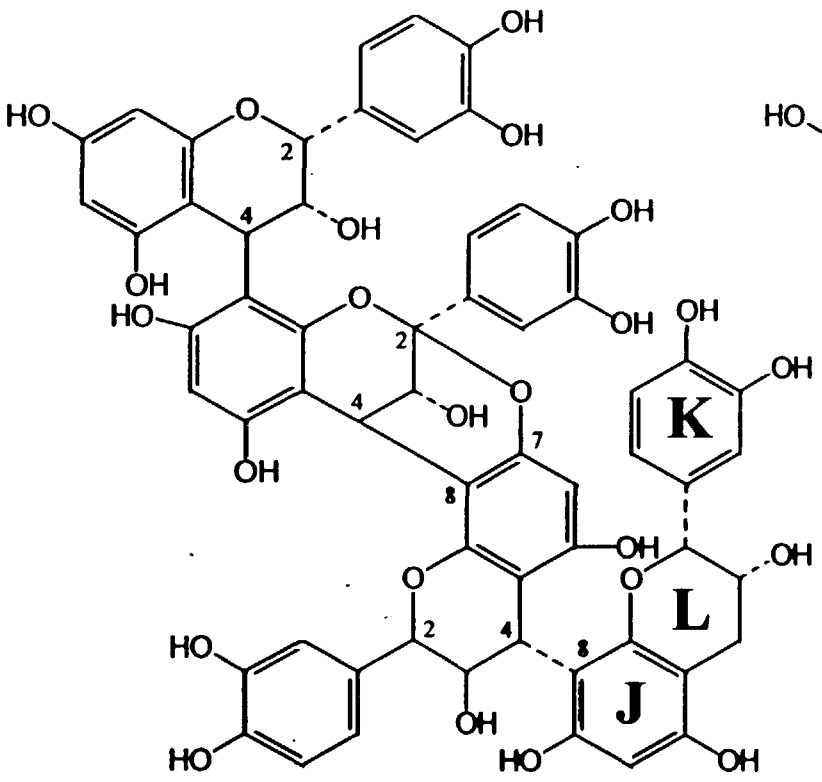


Pavetannin B_7

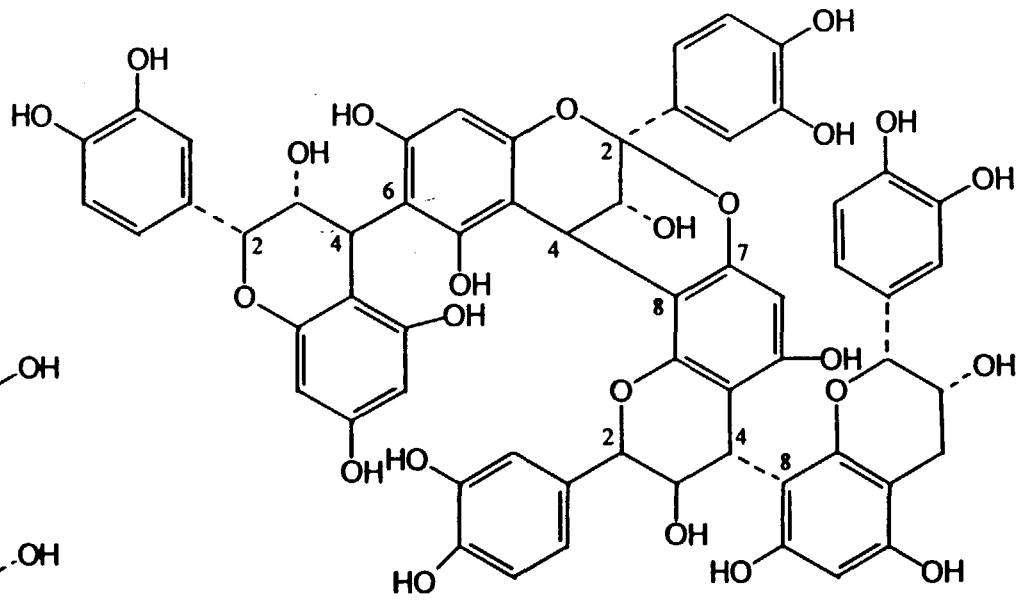


Pavetannin B_8

Condensed tannins isolated from Pavetta owariensis P. Beauv. (6)



Cinnamtannin B_2



Pavetannin C_1

Antiviral potency of proanthocyanidins
Table 1 :

Compound	Concentration µg/ml	*Herpes simplex*	*Coxsackie* B_2
Proanthocyanidin A-2 and pavetannin A-1	250	10^4 (T/4)	10^3 (T/3)
	125	10^3	10^2
	62.50	10^2	10
	31.25	10	1
Cinnamtannin B-1	125	T	10^3 (T/4)
	62.50	10^4 (T/4)	10^2
	31.25	10^2	10
Pavetannin B-1	125	T	10^3 (T/2)
	62.50	10^4 (T/4)	10^3 (T/4)
	31.25	10^2	10^2
	15.62	10	10
Acyclovir	0.1	10^4	1
3-O-methyl quercetin	5	1	10^6

The antiviral activity is expressed as the reduction factor of the viral titer.
T, T/2, T/4 : cytotoxicity scale

Antiviral potency of proanthocyanidins
Table 2 :

Compound	Concentration µg/ml	*Herpes simplex*	*Coxsackie* B_2
Pavetannin B-2, B-4, B-5	125	T	nt
	62.50	10^4 (T/2)	nt
	31.25	10^3 (T/4)	nt
	15.62	10	nt
Cinnamtannin B-2	62.50	T	nt
	31.25	10^2 (T/2)	nt
	15.62	10 (T/4)	nt
Pavetannin C-1	62.50	T	10^2 (T/4)
	31.25	10^2 (T/2)	10
	15.62	10 (T/4)	1
Pavetannin D-1	31.25	T	10^2 (T)
	15.62	10^2 (T/2)	10 (T/4)
Acyclovir	0.1	10^4	1
3-O-methyl quercetin	5	1	10^6

The antiviral activity is expressed as the reduction factor of the viral titer.
T, T/2, T/4 : cytotoxicity scale; nt = not tested

Harrisonia abyssinica Oliv. (SIMAROUBACEAE)
Folkloric use :

■ Roots

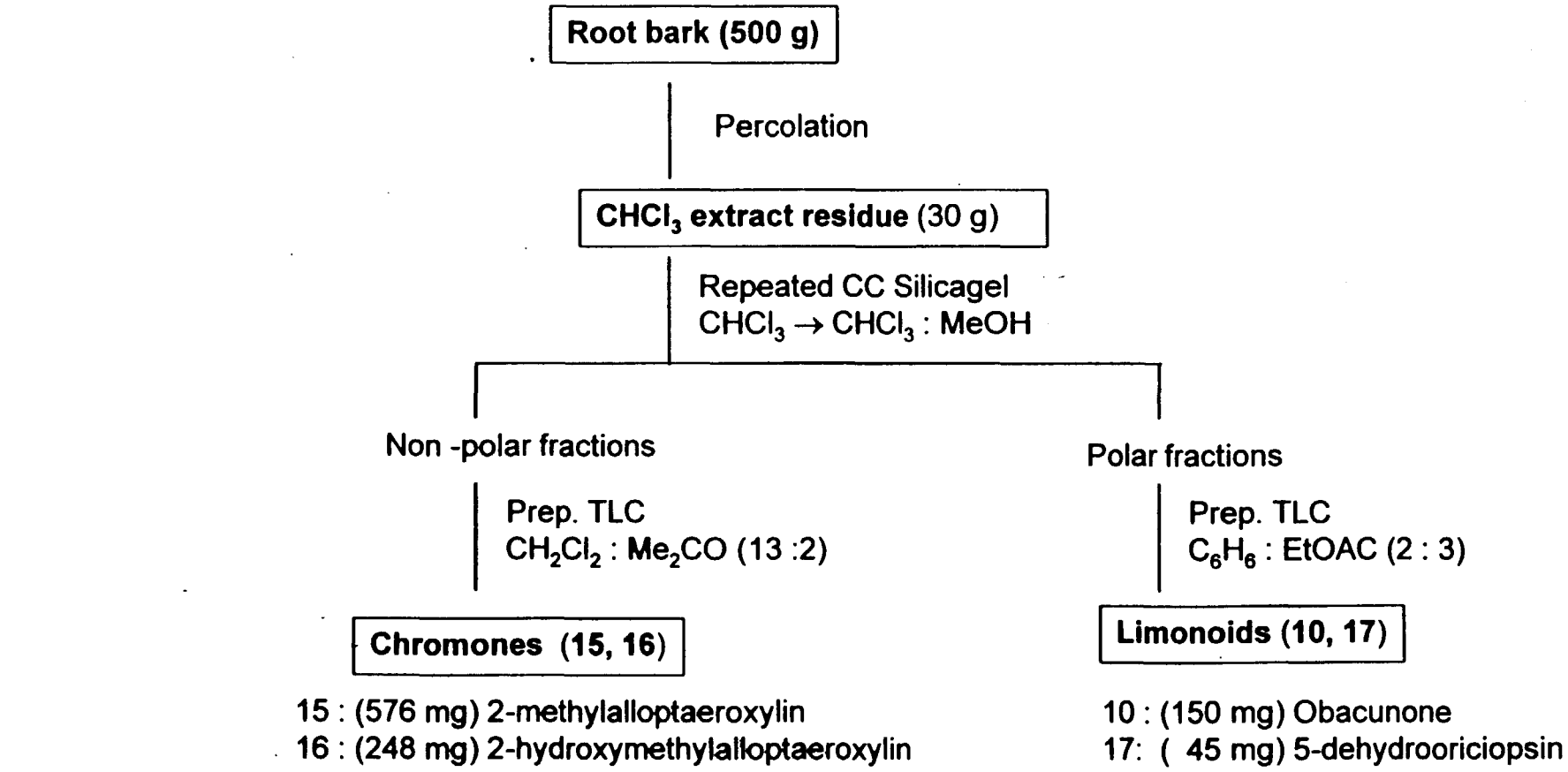
- **Guinea :**
  - Aphrodisiac (+ nut of a palm)
  - Gonorrhoea
  - Dysentery
- **Ivory-Coast :**
  - Gonorrhoea
  - All kinds of aches
- **Ghana :**
  - Laxative (+ palm wine)
- **East Africa :**

Fever	Insomnia	Nausea
Vomiting	Swelling of testicles	Tuberculosis
Blood in the faeces	Bilharzia infections	

■ Leaves

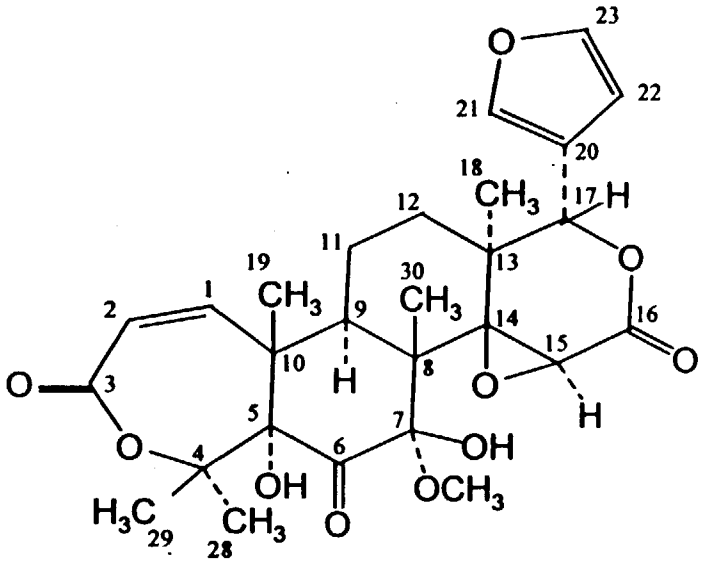
Scrofula	Cancerous tumours (+ butter)
Skin diseases e.g. Leprosy	
Abcesses	Carbuncles
Snake bites	Stomache and abdominal pains
Oxyuricide and ascaricide	Haemorrhoids

HARRISONIA ABYSSINICA OLIV (SIMAROUBACEAE)

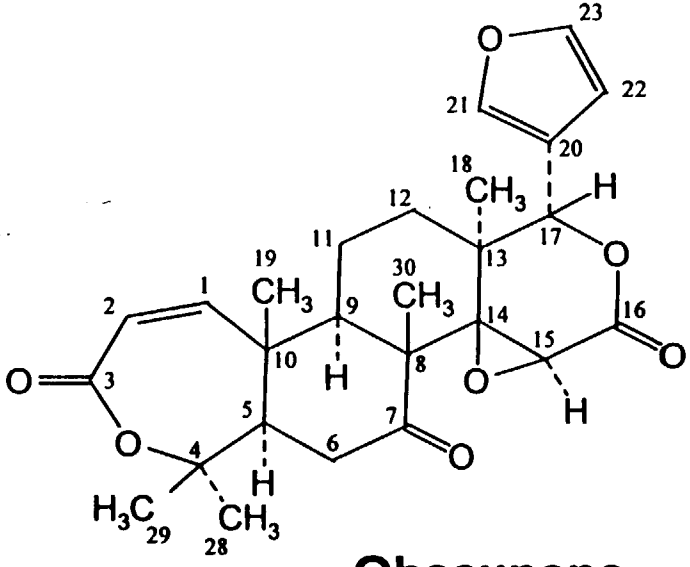


Isolation of active compounds from the root bark of Harrisonia abyssinica

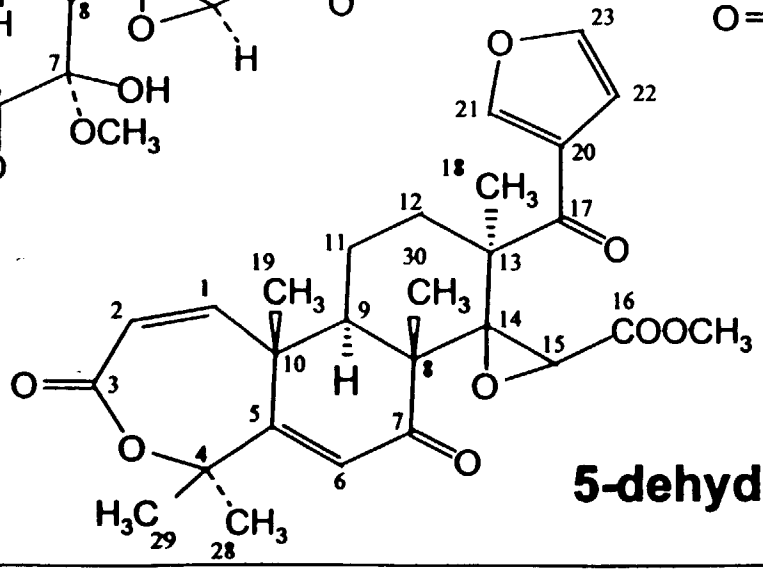
Harrisonia abyssinica Oliv. (SIMARUBACEAE)
Limonoids :



Harissonin

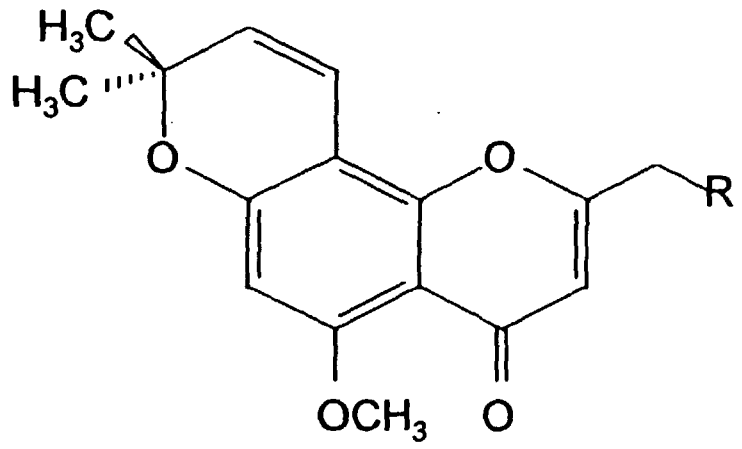


Obacunone



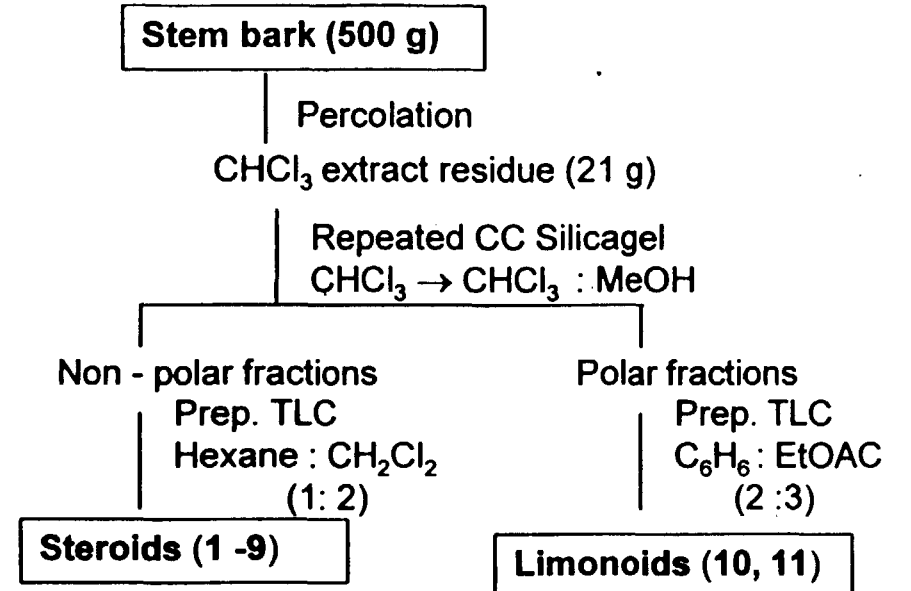
5-dehydro-oriciopsin

Harrisonia abyssinica Oliv. (SIMARUBACEAE)
Chromones :



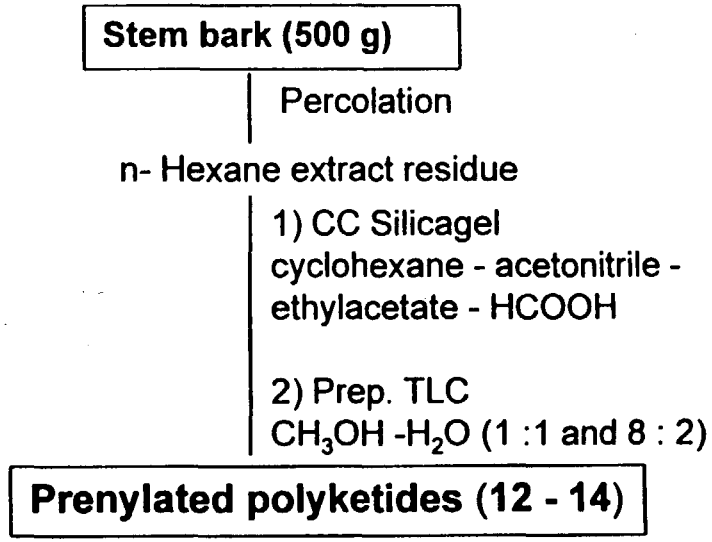
R = H : 2-methyl-alloptaeroxylin
R = OH : 2-hydroxymethyl-alloptaeroxylin

HARRISONIA ABYSSINICA OLIV. (SIMAROUBACEAE)



Group 1 (1 -3)
1 : (39 mg) β-Sitosterol
2 : (23 mg) Stigmasterol
3 : (10 mg) Campesterol
Group 2 (4 -6)
4 : (25 mg) β-Sistenone
5 : (10 mg) Stigmastenone
6 : (12 mg) Campestenone
Group 3 : Mixture : 28:13:9 (+ FAB-MS)
7 : Stigmasta - 3,5 dien - 3 - one
8 : Stigmasta - 3,5,22 trien - 7 - one
9 : Campesta - 3,5 - dien - 7 one

10 : (85 mg) Obacunone
11 : (100 mg) Harrisonine

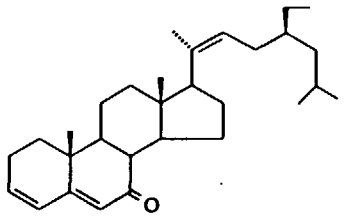


12 : (24 mg) Oumarone
13 : (49 mg) Bissaone
14 : (12 mg) Aissatone

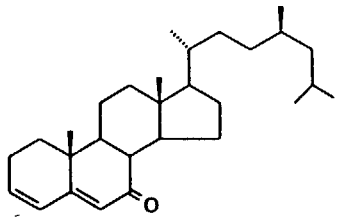
Isolation of active compounds from the stem bark of Harrisonia abyssinica

NEW ACTIVE COMPOUNDS ISOLATED FROM *HARRISONIA ABYSSINICA* OLIV (SIMAROUBACEAE)

*** Ketosteroids**

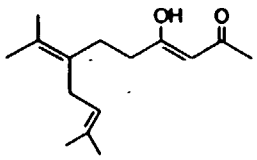


Stigmasta -or 24 – α –methylcholesta - 3, 5, 22 - trien - 7 - one

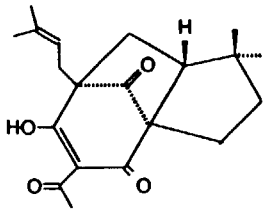


Campesta - or 24 - α - methyllcholesta - 3, 5 - dien - 7 - one

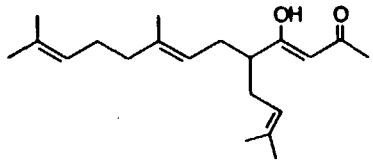
*** Prenylated polyketides**



Oumarone or 8 - methyl- 5 - (3-methyl - 2 - butenyl) - 7-nonen - 2, 4 - dione



Aissatone or (1S,5R,7R) -9-acetyl-4,4-dimethyl-7- (3-methyl-2-butenyl) tricyclo [5,3,1,0, ^{1,5}] undecan-8,10,11- trione

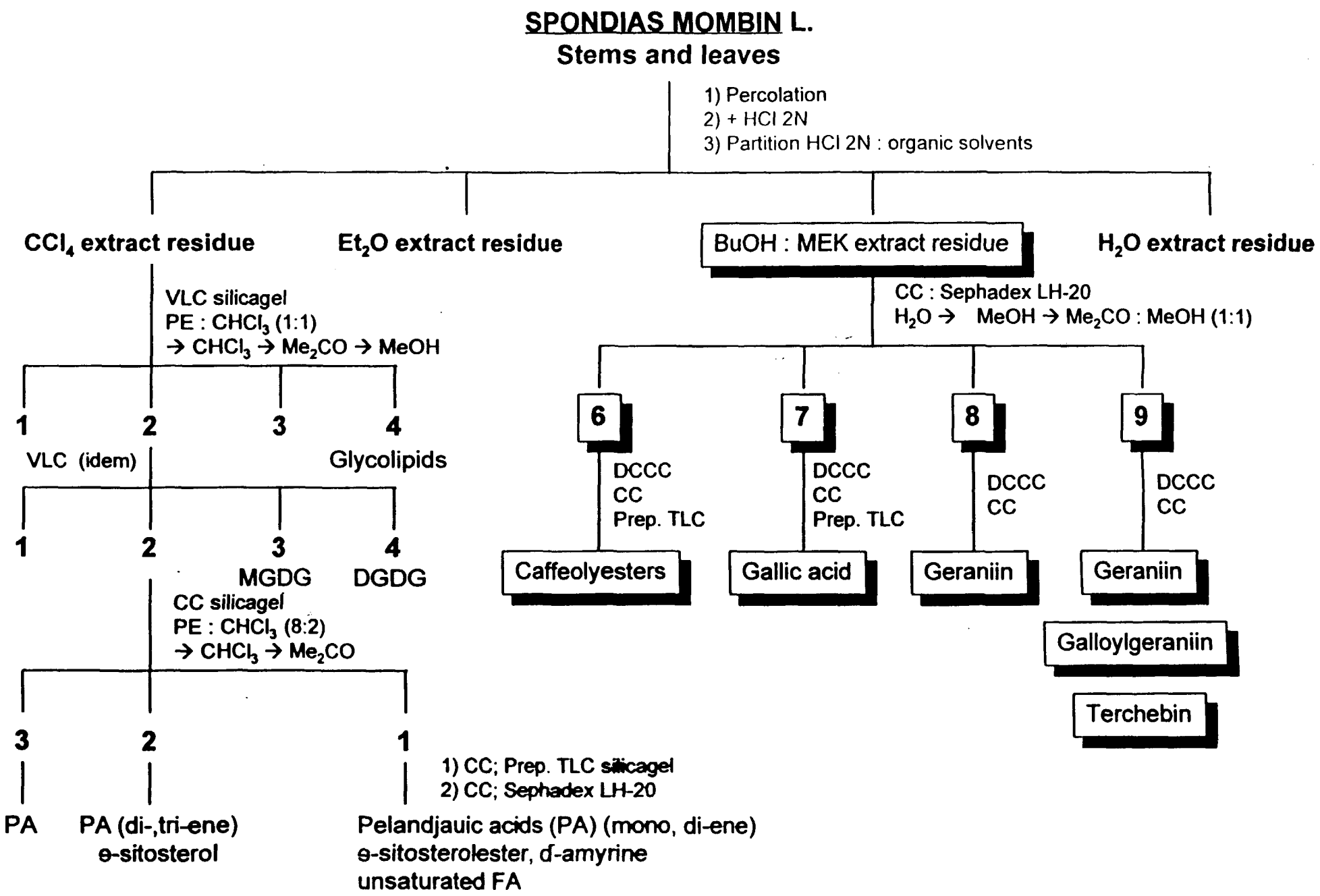


Bissaone or 8, 12 dimethyl - 5 -(3 -methyl -2 -butenyl) - 7, 11- tridecadien -2, 4 -dione)

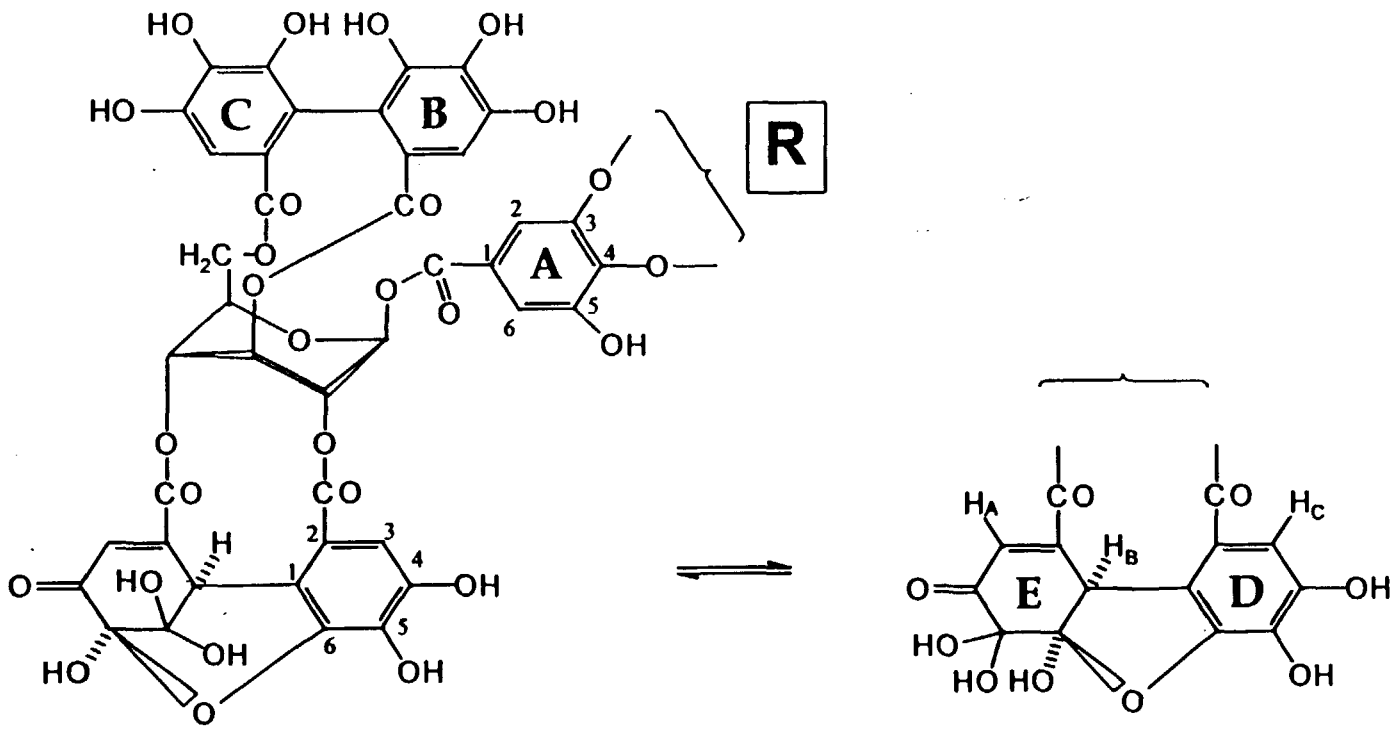
Spondias mombin L. (ANACARDIACEAE)

Folkloric use :

- **Leaves**
  - Oxytoxic properties
  - Antidiarrhoeal activity
  - Treatment of wounds
  - Astringent
- **Fruit**
  - Edible plum-fruit


SPONDIAS MOMBIN L.
Stems and leaves
1) Percolation
2) + HCl 2N
3) Partition HCl 2N : organic solvents
CCl4 extract residue
Et2O extract residue
BuOH : MEK extract residue
H2O extract residue
VLC silicagel
PE : CHCl3 (1:1)
→ CHCl3 → Me2CO → MeOH
1
2
3
4
VLC (idem)
Glycolipids
1
2
3
4
MGDG
DGDG
CC silicagel
PE : CHCl3 (8:2)
→ CHCl3 → Me2CO
3
2
1
PA
PA (di-,tri-ene)
ɵ-sitosterol
1) CC; Prep. TLC silicagel
2) CC; Sephadex LH-20
Pelandjauic acids (PA) (mono, di-ene)
ɵ-sitosterolester, ɗ-amyrine
unsaturated FA
CC : Sephadex LH-20
H2O → MeOH → Me2CO : MeOH (1:1)
6
7
8
9
DCCC
CC
Prep. TLC
DCCC
CC
Prep. TLC
DCCC
CC
DCCC
CC
Caffeolyesters
Gallic acid
Geraniin
Geraniin
Galloylgeraniin
Terchebin

Spondias mombin L. (ANACARDIACEAE)
Hydrolysable tannins :

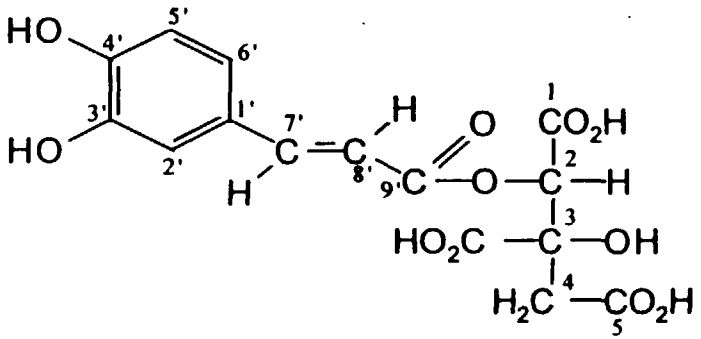


R = H, H : geraniin

R = H, galloyl : galloylgeraniin

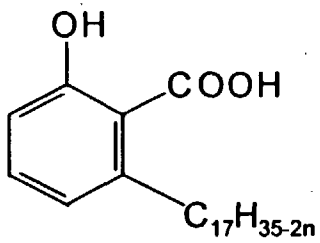
Spondias mombin L. (ANACARDIACEAE)

C_6C_3-compounds :

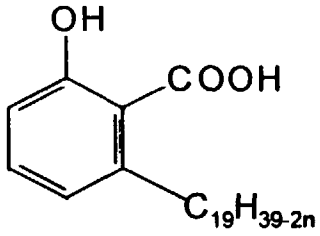


2-O-caffeoyl-(+)-allohydroxy-citric acid

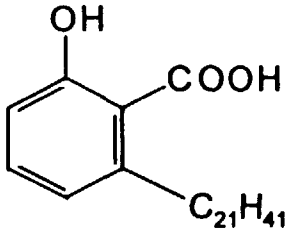
Aromatic acids :



n = 0 : (17:0) pelandjuaic acid
n = 1 : (17:1) pelandjuaic acid
n = 2 : (17:2) pelandjuaic acid
n = 3 : (17:3) pelandjuaic acid

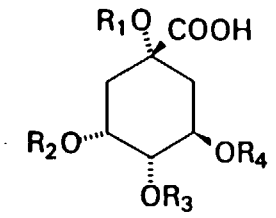


n = 0 : 6-nonadecyl salicylic acid
n = 1 : 6-(12'Z-nonadecenyl) - salicylic acid

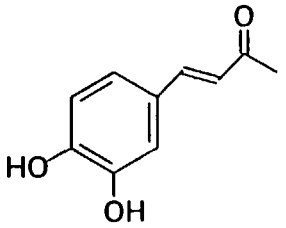


6-(15'Z-heneicosenyl) - salicylic acid

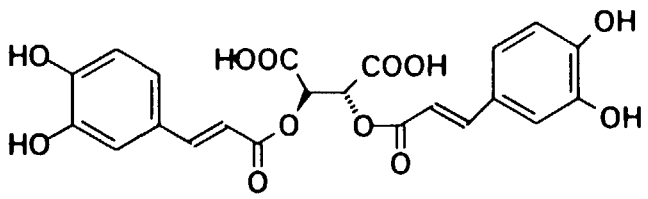
PLANT-DERIVED INHIBITORS OF HIV-1 INTEGRASE (2)



C =

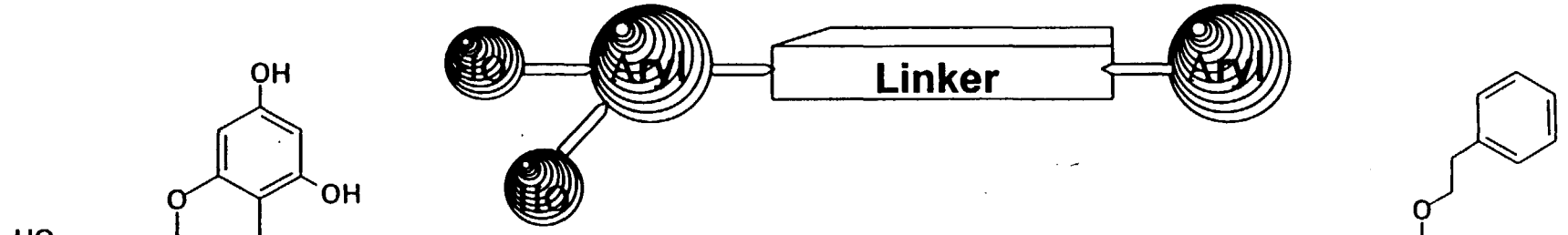


1,5 DCQA : $R_1 = R_4 = C$; $R_2 = R_3 = H$
4,5 DCQA : $R_3 = R_4 = C$; $R_1 = R_2 = H$
3,4 DCQA : $R_2 = R_3 = C$; $R_1 = R_4 = H$
3,5 DCQA : $R_2 = R_4 = C$; $R_1 = R_3 = H$
Chlorogenic acid : $R_4 = C$; $R_1 = R_2 = R_3 = H$

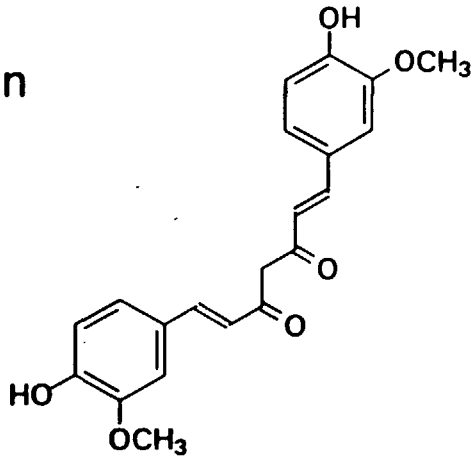


L- Chicoric acid

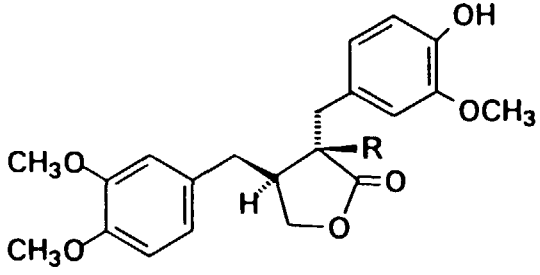
PLANT-DERIVED INHIBITORS OF HIV-1 INTEGRASE (1)



Quercetin



Curcumin

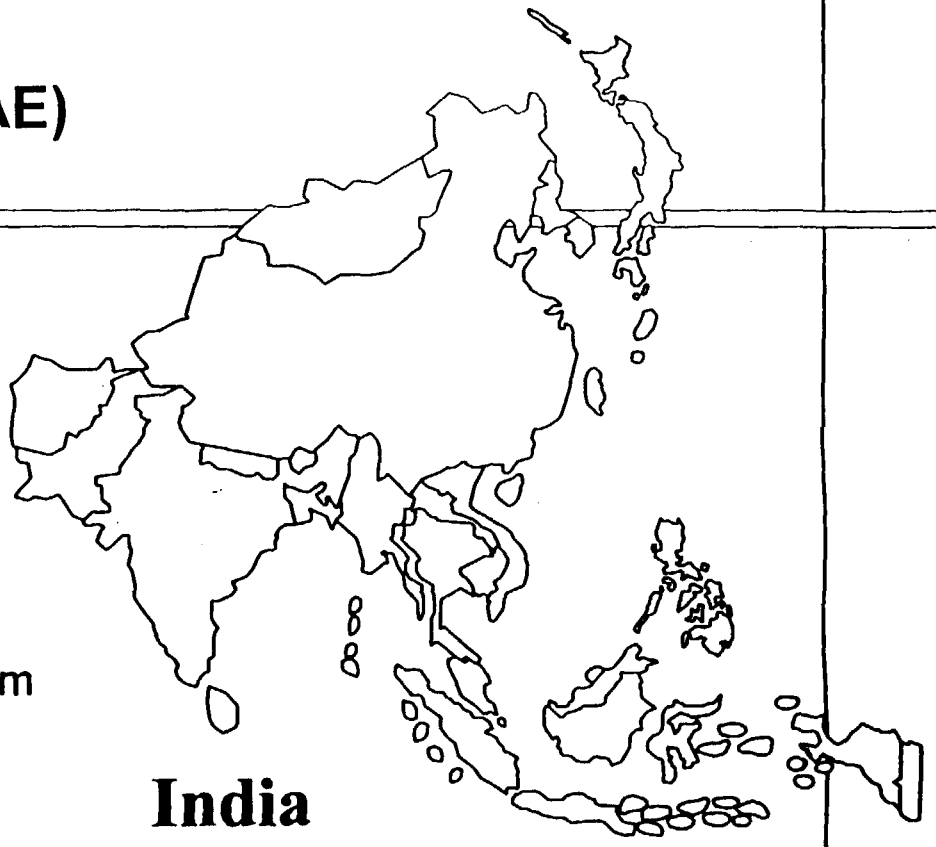


R = -H : (-) Arctigenin
R = -OH : (-)- Trachelogenin

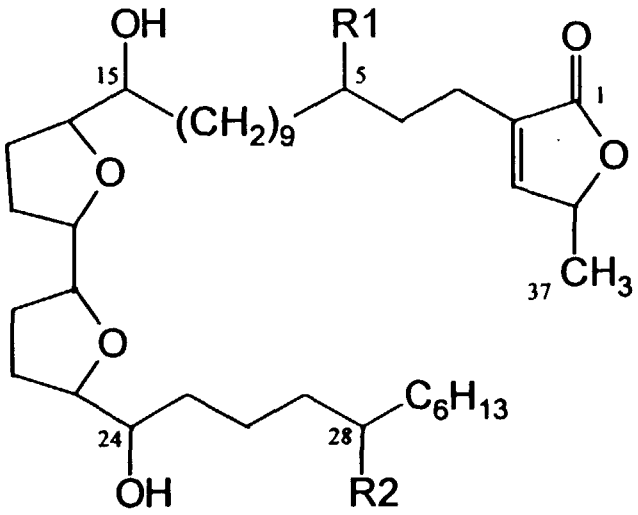
Caffeic acid phenylethylester (CAPE)

Uvaria narum Wall (ANNONACEAE)
Folkloric use :

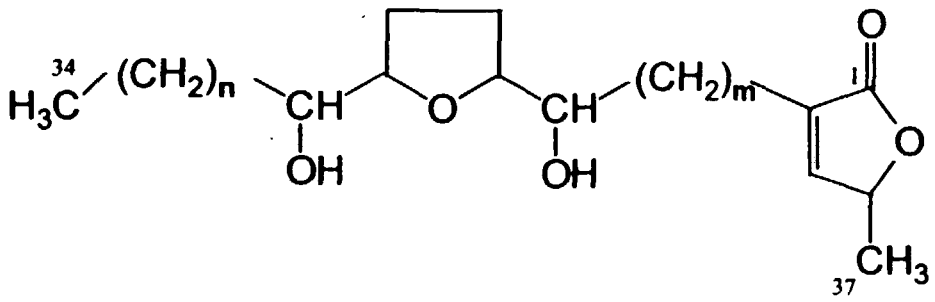
- **India : Narum panal**
- **Leaves**
  - Rheumatic swellings
  - Billiousness
  - Jaundice
  - Thyphoid
- **Root Bark (decoction)**
  - Control of fits at delivary
  - Bowels complaints (children)
  - Rheumatism
  - Eczema
- **Root oil**
  - Erysipelas
  - Eczema
  - Rheumatism
- **Whole plant**
  - Insecticidal properties



Uvaria narum Wall. (ANNONACEAE)
Acetogenins :

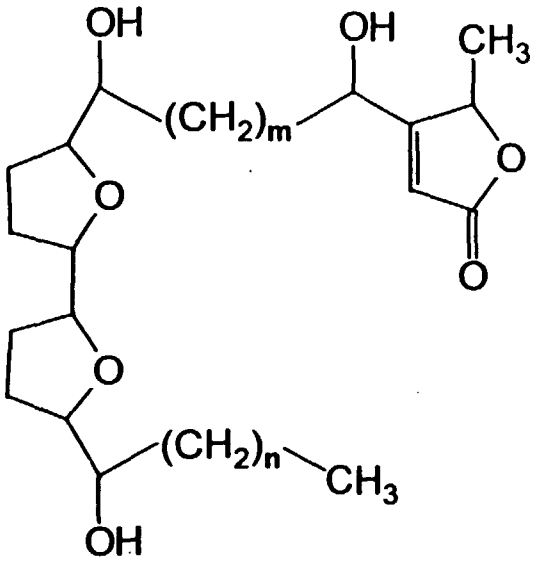


R1	R2	Name :
H	H	isodesacetyluvaricin
OH	H	narumicin I (threo-trans-threo-trans-threo)
OH	H	narumicin II (threo-trans-threo-trans-erythro)
H	OH	squamocin
H	O	squamocin-28-one
OH	OH	panalicin (threo-trans-threo-trans-erythro)



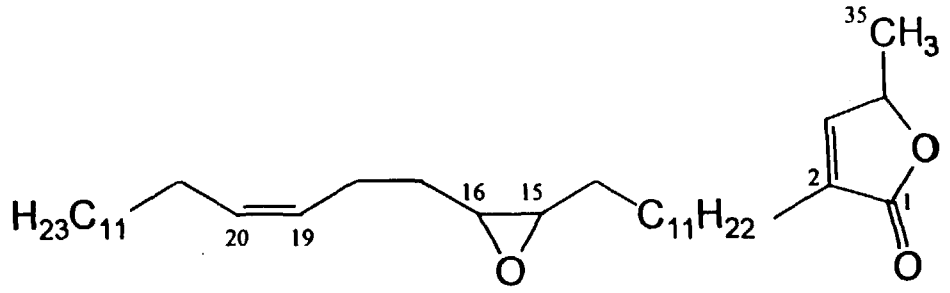
m = 12, **n** = 13 : uvariamicin I
m = 14, **n** = 11 : uvariamicin II
m = 16, **n** = 9 : uvariamicin III

Annona muricata L. (ANNONACEAE)
Acetogenins :

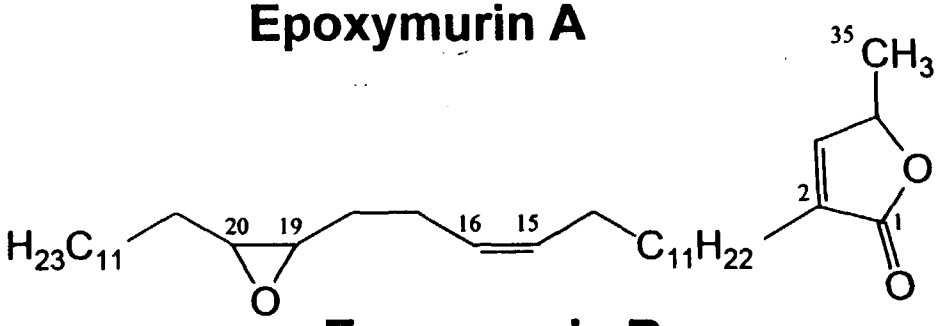


m = 10, n = 9 :
rolliniastatin-2
(= bullatacin)

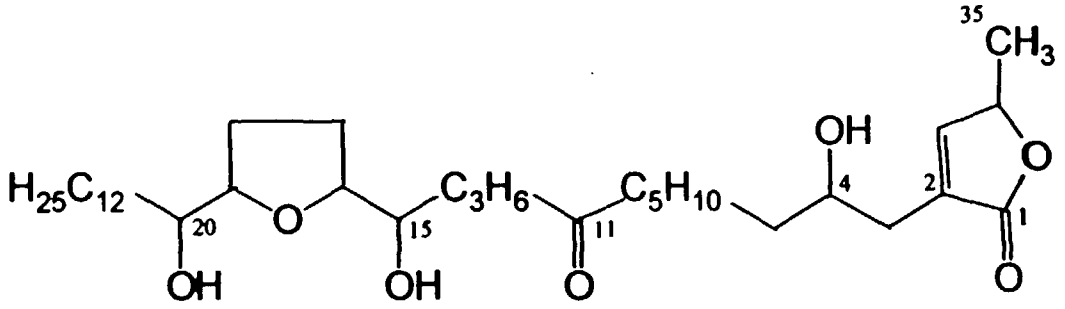
m = 8, n = 9 :
molvizarin



Epoxymurin A



Epoxymurin B



Reticulacinone

METHODS FOR DETECTION OF BIOLOGICAL ACTIVITY OF PLANT EXTRACTS (5)

2.2. ISOLATED SUBCELLULAR SYSTEMS

2.2.1. ENZYME INHIBITION OR ACTIVATION ASSAYS
MACROPHAGE ASSOCIATED CARBOXYPEPTIDASES (TRYPSIN, KALLIKREIN), HYPERTENSIVE PROTEOLYTIC CASCADES (RENIN, ANGIOTENSIN-CONVERTING ENZYME),
GENITAL TRACT PROTEOLYTIC SYSTEMS (ACROSIN),
ARACHIDONIC ACID PATHWAYS (CYCLO-OXYGENASE, LIPOXYGENASE, THROMBOXAN-SYNTHETASE)
CARDIOVASCULAR SYSTEM (ATP-ASE, HMG-COA-REDUCTASE, MAO, TYROSINE-HYDROXYLASE) ETC.

2.2.2. RECEPTOR BINDING ASSAYS
ACETYLCHOLINE-, ADRENALINE-, DOPAMINE-, SEROTONIN-, HISTAMINE-, EXCITATORY AMINO ACID-, GLYCINE-, GABA-, OPIOID-, TACHYNIN-, PROSTANOIDS-, PAF- AND ADENOSINE RECEPTORS

Screening battery of *in vitro* assays

Biochemical activities

Activation or **inhibition** of key-enzymes of important biochemical systems :

1) Macrophage associated carboxypeptidases
2) Hypertension associated proteolytic cascades
3) Genital tract proteolytic systems
4) Arachidonic acid pathways

ASSAY OF DIPEPTIDYL PEPTIDASE IV (DPPIV) (1)

* **AIM :**

IMMUNOMODULATORY ACTIVITY

* DECREASE OF T-CELL PROLIFERATION AND T-CELL CYTOTOXICITY

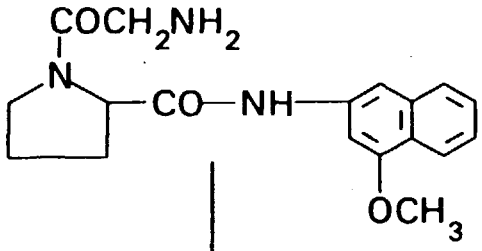
* DECREASE OF ACTIVATION OF B-CELL PROLIFERATION

* DECREASE OF ACTIVATION OF MACROPHAGE'S ACTIVITY

* DECREASE OF ACTIVATION OF NATURAL KILLER CELL'S ACTIVITY

ASSAY OF DIPEPTIDYL PEPTIDASE IV (DPPIV) (2)

*** TEST :**



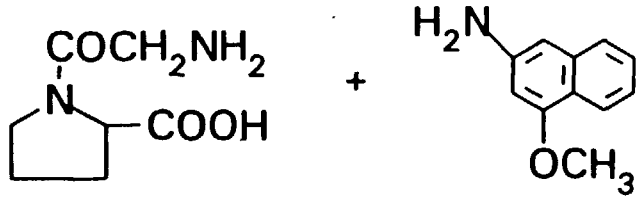
Gly - Pro - 4 - Me - 2 - NA

Tris Buffer
37° C ; 20 min

\+ DPPIV
\+ Test compound

* Fluorescence: 340 nm (excitation)
425 nm (emission)

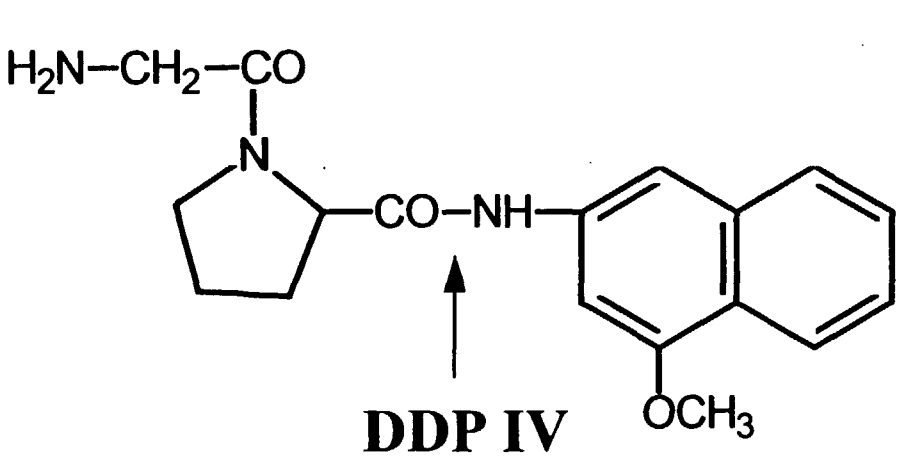
* 1 IU = 1µmol 4 - Me - 2 - NA / min



Gly - Pro

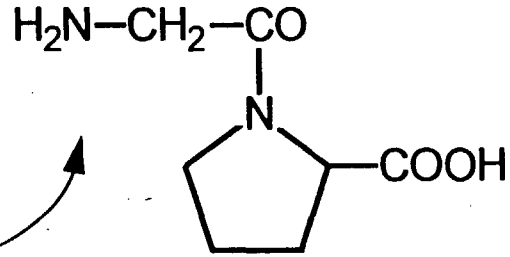
4 - Me - 2 -NA

Assay of dipeptidyl peptidase IV (DPPIV) by determination of the fluorescence of 4-Me-2-naphtylamine

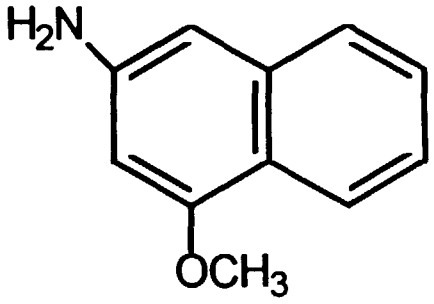


DDP IV

Gly-Pro-4-Me-2-NA



Gly-Pro



4-Me-2-Naphtylamine

ASSAY OF ANGIOTENSIN-CONVERTING ENZYME (ACE)

* **AIM : ANTIHYPERTENSIVE ACTIVITY**

* **TEST :**

$C_6H_5CONHCH_2CONHCH_2CONHCH_2COOH$ Hip-Gly-Gly

HEPES
NaCl ; Na_2SO_4
pH 8.0
37 ° C ; 30 min.

+ ACE
+ TEST COMPOUND

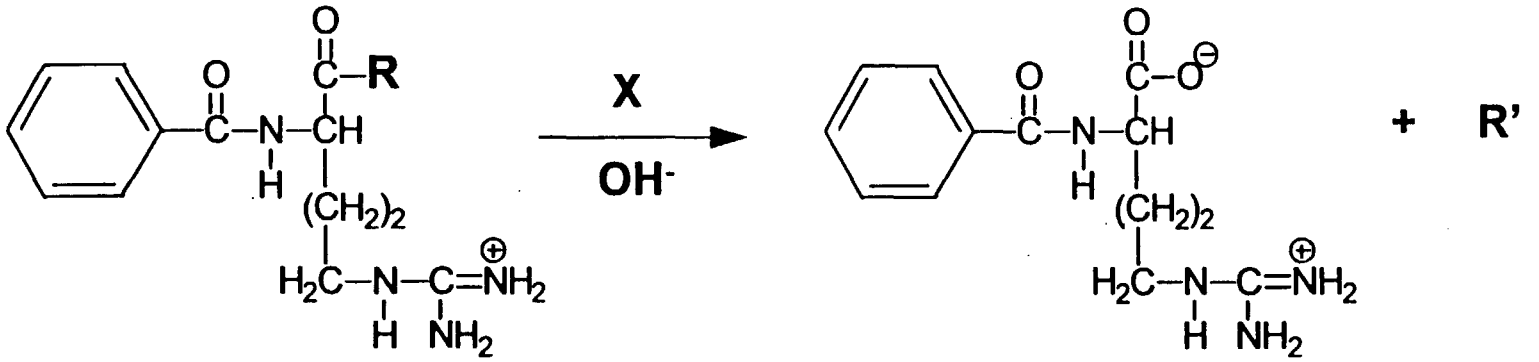
Hip $C_6H_5CONHCH_2COOH$ + $H_2NCH_2CONHCH_2COOH$ Gly-Gly

+ HCl + EtOAc

+ INTERNAL STANDARD : **0-Me-Hip**

HPLC of Hip and 0-Me-Hip

COLUMN : C_{18} (10 µm)
MOBILE PHASE : KH_2PO_4/CH_3CN (3/17) (pH 3.5)
DETECTION : UV AT 228 NM



Enzyme (X)	R	R'
Kallikreïne	Ethoxy	Ethanol
Trypsine	p-Nitroanilide	p-Nitroaniline

METHODS FOR DETECTION OF BIOLOGICAL ACTIVITY OF PLANT EXTRACTS (6)

2.3. ISOLATED CELLULAR SYSTEMS

2.3.1. CYTOTOXICITY (*IN VITRO*), ANTITUMOUR AND ANTINEOPLASTIC (*IN VIVO*) ACTIVITIES

* *IN VITRO* AND *IN VIVO* ACTIVITY AGAINST VARIOUS ANIMAL- OR HUMAN TUMOUR SYSTEMS

- *IN VITRO* PRESCREEN AGAINST 9 KB HUMAN NASOPHARYNX CARCINOMA AND MOUSE LYMPHOCYTIC P 388, FOLLOWED BY *IN VIVO* SCREEN AGAINST ADDITIONAL MOUSE TUMOURS SUCH AS P 388 MURINE LEUKEMIA, LEWIS LUNG CARCINOMA, COLON 38 AND CD 8f1 MAMMARY (NCI, 1956)

- HUMAN SOLID-TUMOUR-LINE BASED ANTICANCER SCREEN (NCI, 1989) BATTERY OF 60 CELL-LINES OBTAINED FROM 8 ORGAN SYSTEMS : *IN VITRO* PRESCREEN FOLLOWED BY *IN VIVO* SCREEN

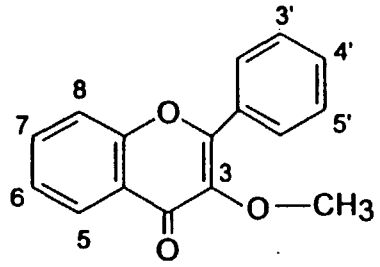
METHODS FOR DETECTION OF BIOLOGICAL ACTIVITY OF PLANT EXTRACTS (7)

2.3. ISOLATED CELLULAR SYSTEMS

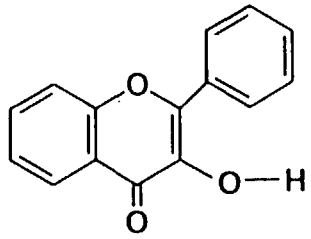
* MECHANISM BASED *IN VITRO* ASSAYS

- BASED ON CLINICALLY EFFECTIVE ANTITUMOUR COMPOUNDS
  E.G. TUBULIN DEPOLYMERIZATION (VINCRISTIN),
  TUBULIN STABILIZATION (TAXOL),
  TOPOISOMERASE I AND II INHIBITION
  (RESPECTIVELY CAMPOTHECIN AND ELLIPTICIN)
  AND DNA CLEAVAGE (BLEOMYCIN)
- TO DETECT CHEMOPREVENTIVE AGENTS
  E.G. ANTIMUTAGENIC ACTIVITY (AMES-TEST),
  BENZ(A)-PYREEN HYDROXYLASE INHIBITION,
  PROTEASE INHIBITION, RADICAL SCAVENGING ACTIVITY

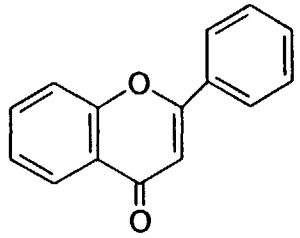
EFFECTS OF SELECTED FLAVONES ON *IN VITRO* TUBULIN POLYMERIZATION, [^{3}H] COLCHICINE BINDING AND CYTOTOXICITY DATA (GI_{50})



Comp. 1 - 26



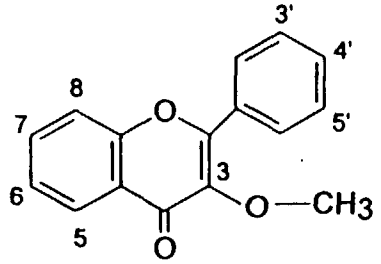
Comp. 27 - 41



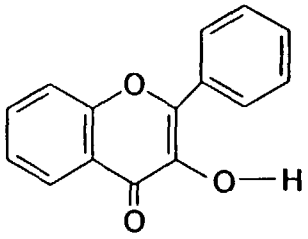
Comp. 42 - 79

Comp.	C5	C6	C7	C8	C3'	C4'	C5'	Mean GI_{50}	Tubulin polymerization IC_{50} (μM)	% Inhibiiton of [^{3}H] colchcine binding 5μM	50μM
1	HO	MeO	HO	H	HO	MeO	H	0.24	2.0 ± 0.5	35	76
2	HO	MeO	MeO	MeO	HO	MeO	H	0.13	0.83 ± 0.2	59	89
3	HO	H	HO	H	HO	MeO	H	1.7	3.0 ± 0.4	43	75
4	HO	MeO	MeO	MeO	MeO	MeO	H	7.1	> 40		4
5	HO	MeO	MeO	MeO	MeO	HO	H	7.6	> 40		0
7	HO	H	MeO	MeO	MeO	HO	H	9.5	> 40		0
15	HO	MeO	HO	MeO	MeO	HO	H	27	> 40		6
16	HO	H	MeO	H	HO	MeO	H	32	> 40		0
19	HO	MeO	HO	MeO	MeO	MeO	MeO	54	> 40		6
25	MeO	MeO	MeO	MeO	MeO	MeO	H	> 27	> 40		1

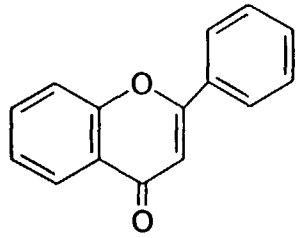
EFFECTS OF SELECTED FLAVONES ON *IN VITRO* TUBULIN POLYMERIZATION, [^{3}H] COLCHICINE BINDING AND CYTOTOXICITY DATA (GI_{50}) (CONTINUED)



Comp. 1 - 26



Comp. 27 - 41



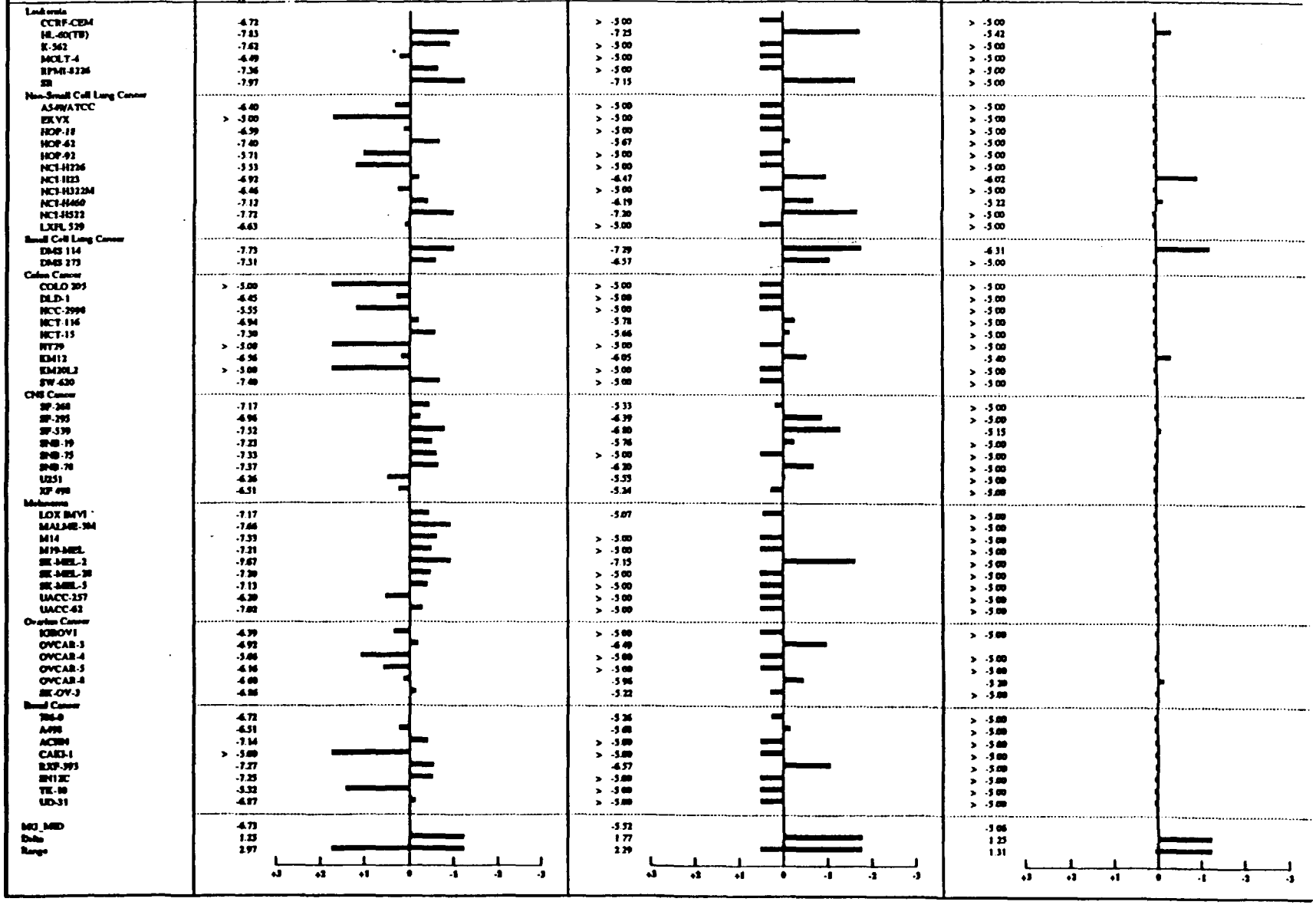
Comp. 42 - 79

Comp.	C5	C6	C7	C8	C3'	C4'	C5'	Mean GI_{50}	Tubulin polymerization IC_{50} (μM)	% Inhibiiton of [^{3}H] colchcine binding 5μM	50μM
27	HO	MeO	MeO	H	HO	MeO	H	4	> 40		9
30	H	H	MeO	H	MeO	MeO	H	5.5	> 40		0
31	HO	H	H	H	2', 4', 6' TRIONE			5.9	31 ± 5		22
32	MeO	MeO	MeO	H	MeO	MeO	H	6.6	> 40		0
34	MeO	MeO	MeO	H	HO	MeO	H	14	> 40		2
45	HO	MeO	HO	H	MeO	MeO	H	9.5	> 40		6
46	HO	MeO	MeO	MeO	H	MeO	MeO	17	> 40		5
47	H	H	H	H	MeO	MeO	MeO	18	> 40		0
48	HO	MeO	MeO	MeO	MeO	MeO	MeO	19	> 40		11
73	HO	MeO	HO	H	H	MeO	H	> 31	> 40		11
Colchicine									1.4 ± 0.3		
Podophyllotoxine											82

National Cancer Institute Developmental Therapeutics Program

Mean Graphs

NSC: V2302 /1	Units: Molar	SSPL:	Exp. ID: 9203NS82
Report Date: July 7, 1992			Test Date: March 23, 1992



be the ultimate
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leomycin, as de-
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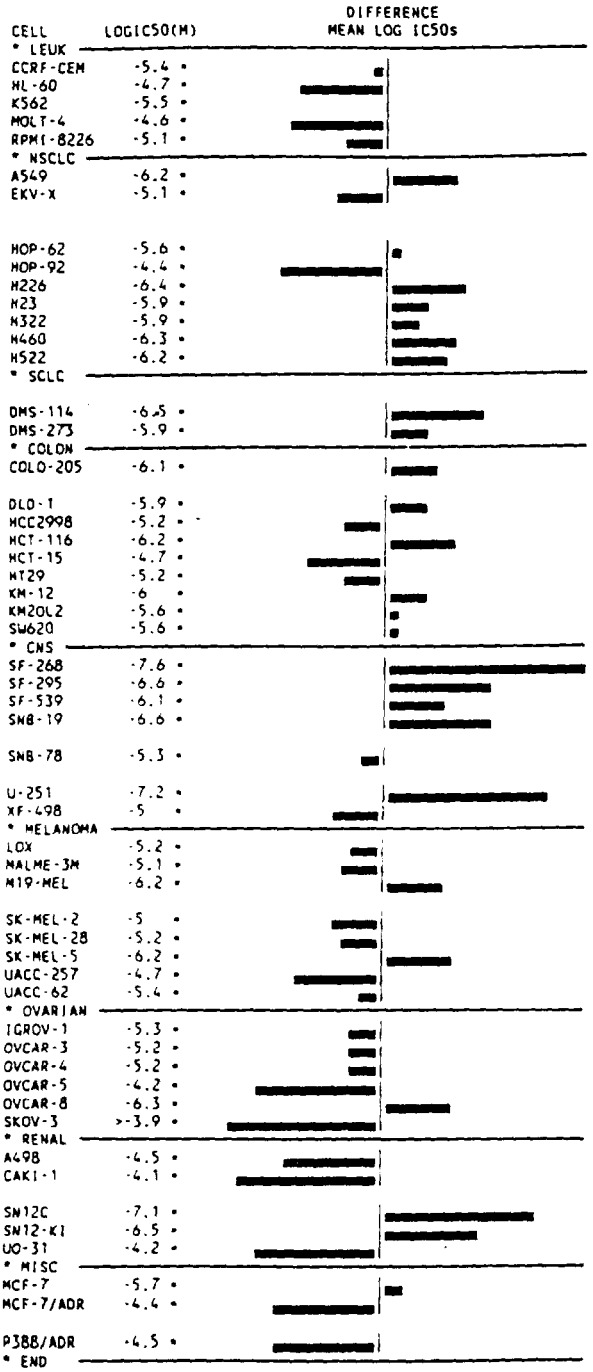


FIG. 7. Mean Graph obtained from screening of compound NSC 155693. The mean $\log_{10}$ IC_{50} for this compound was −5.5. Individual IC_{50} values for each cell line are shown.

ployed. Model systems that are being explored for *in vivo* investigations with panel lines include a microencapsulated tumor model, subcutaneous xenograft models, and orthotopic models.

THE MICROENCAPSULATED T

As one approach to development of
be useful with a wide diversity of tu
lated tumor assay (META) has been
ploys a proprietary microencapsulati
it possible to encapsulate human tu
sules (*e.g.*, see Figure 8) that can be
peritoneal cavity of nude mice. The
of the microcapsules are intended
tumor cells, but permit the flow of n
growth. Test drugs can be administe
sules recovered at the desired times
comparisons of viable tumor cell n
ered from treated vs. control animals

The general concept for this assay
attractive; however, our experience
desired *in vivo* drug screen applicati
ibility and other technical and prac
routine use in the NCI program. Any
likely be limited to specialized exp
rigidly controlled and defined resear
readily achievable in a busy *in vivo*

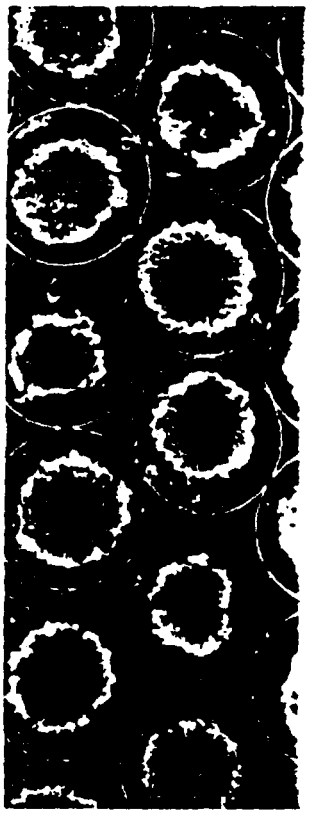
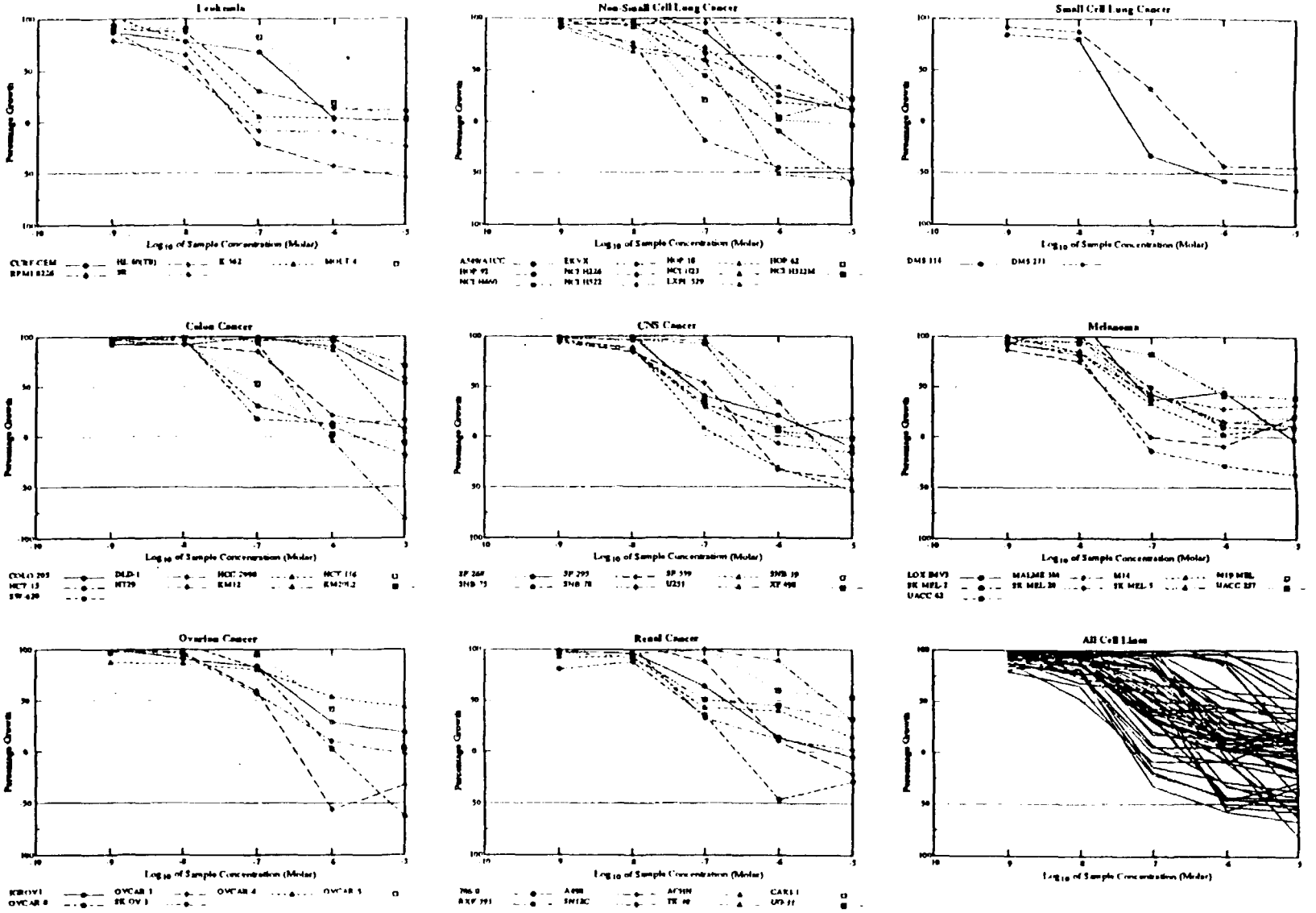


FIG. 8. Microencapsulated human
plantation. Individual microcapsul
mm in diameter. (Photomicrograp
James McMahon, NCI)

National Cancer Institute Developmental Therapeutics Program
Dose Response Curves

NSC: V2302 /1 Centaurecidin	SSPL:	Exp. ID: 9203NS82
Report Date: July 7, 1992	Test Date: March 23, 1992	



METHODS FOR DETECTION OF BIOLOGICAL ACTIVITY OF PLANT EXTRACTS (8)

2.3. ISOLATED CELLULAR SYSTEMS

2.3.2. IMMUNOMODULATORY ACTIVITY

AIM:
COMPOUNDS THAT STIMULATE THE NON-SPECIFIC IMMUNE SYSTEM *I.E.* THE EFFICIENCY OF GRANULOCYTES, MACROPHAGES, COMPLEMENT AND NATURAL KILLER CELLS

TESTS :

* *IN VITRO* GRANULOCYTE PHAGOCYTOSIS ASSAY (SMEAR EST)
* CHEMOLUMINESCENCE ASSAY
* CHEMOTAXIS ASSAY
* LYMPHOCYTE PROLIFERATION ASSAY
* ASSAY OF NATURAL KILLER CELL ACTIVITY
* ASSAY FOR TUMOUR NECROSIS FACTOR (TNF) PRODUCTION
* COMPLEMENT ACTIVATION TEST

2.3.3. INHIBITION OF PLATELET AGGREGATION

AIM: EVALUATION OF ANTITHROMBOTIC POTENTIAL
TEST: TURBIDIMETRIC ASSAY IN A PLATELET AGGREGOMETER

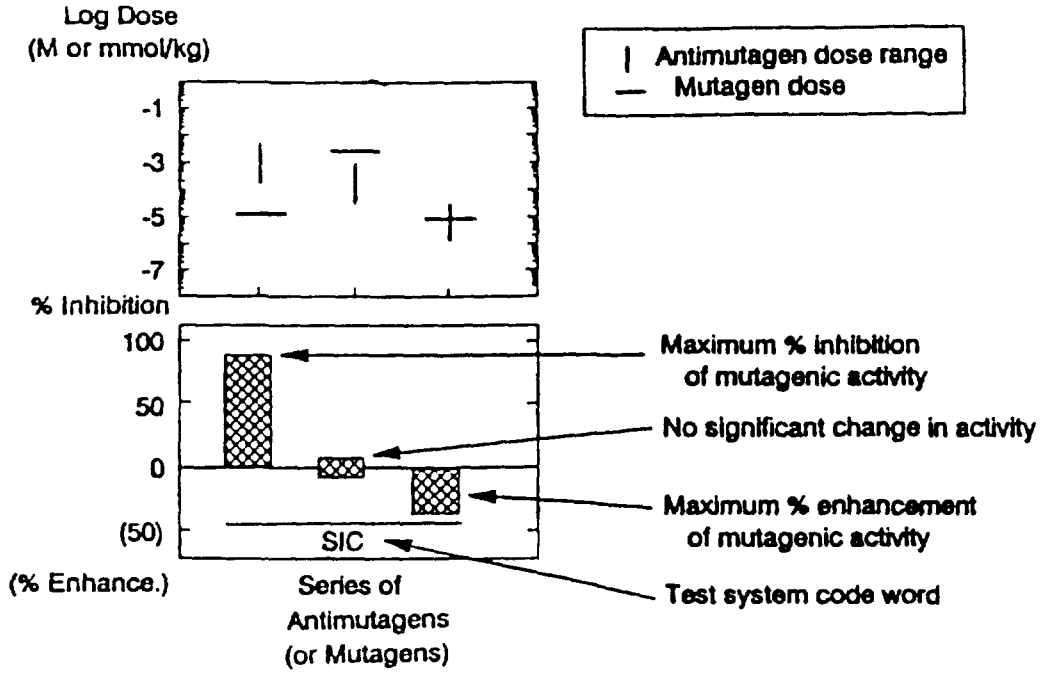
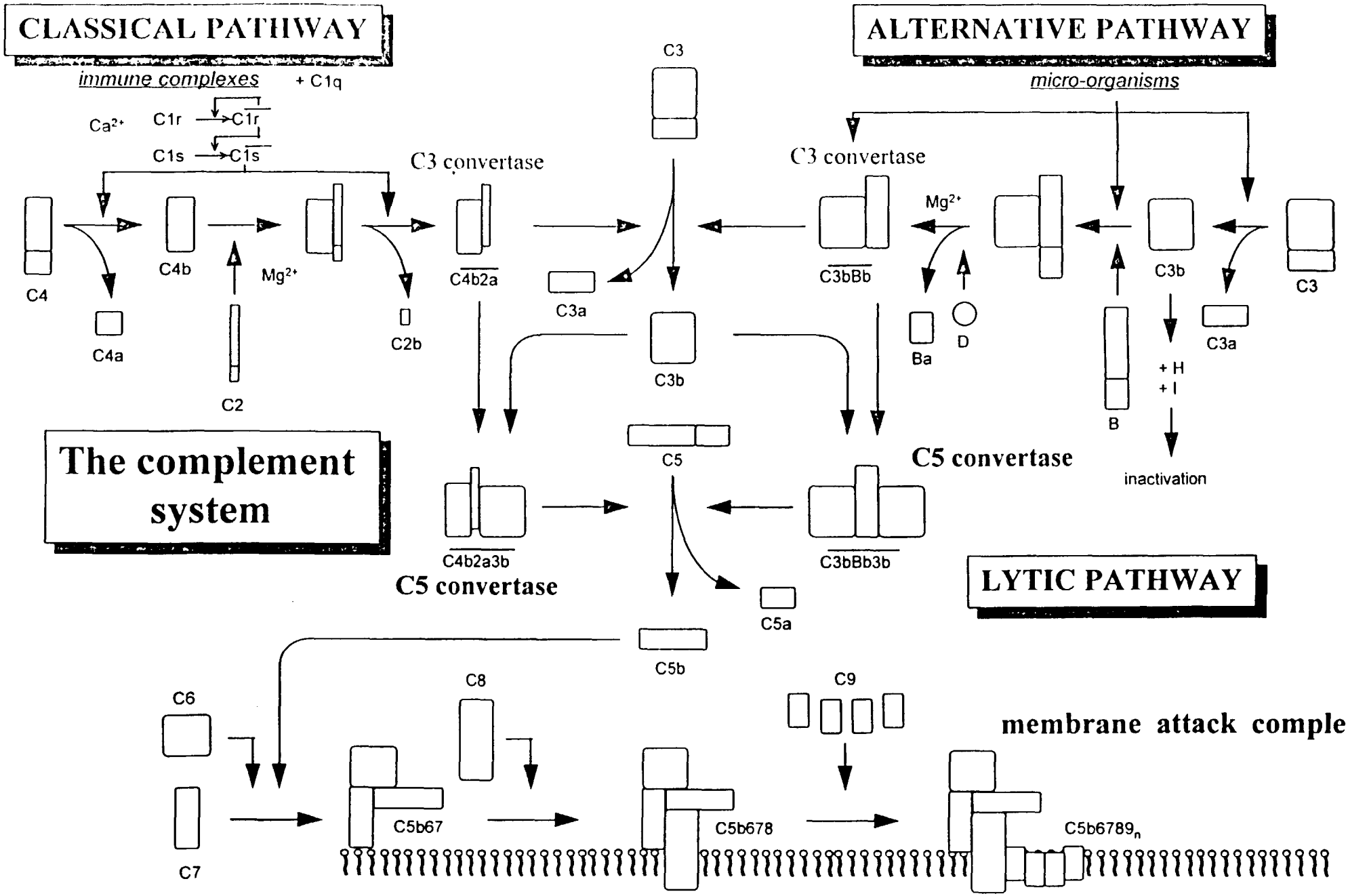


Fig. 1. Schematic diagram of an antimutagenicity profile. Profiles are organized to display either the antimutagenic activity of various antimutagens in combination with a single mutagen or the activity of a single antimutagen with various mutagens. The upper bar graph displays the mutagen concentration and the range of antimutagen concentration tested. The lower graph shows either the maximum percent inhibition represented by a bar directed upward from the origin or the maximum percent enhancement of the genotoxic response represented by a downward-directed bar. As illustrated in the lower graph, a bar across the origin indicates that no significant effect was detected (designated as 'negative data' in the text). Test codes are defined in the Appendix.


CLASSICAL PATHWAY
immune complexes
+ C1q
Ca2+
C1r
C1s
C3 convertase
C4
C4b
C4a
Mg2+
C2
C2b
C4b2a
C3
C3a
C3b
C4b2a3b
C5 convertase
C5
C5a
C5b
ALTERNATIVE PATHWAY
micro-organisms
C3 convertase
C3bBb
Mg2+
Ba
D
C3b
C3a
B
+ H
+ I
inactivation
C5 convertase
C3bBb3b
The complement system
LYTIC PATHWAY
C6
C7
C8
C5b67
C9
C5b678
C5b6789n
membrane attack comple

BRIDELIA FERRUGINEA BENTH. (EUPHORBIACEAE)

FOLKLORIC USE : CONGO

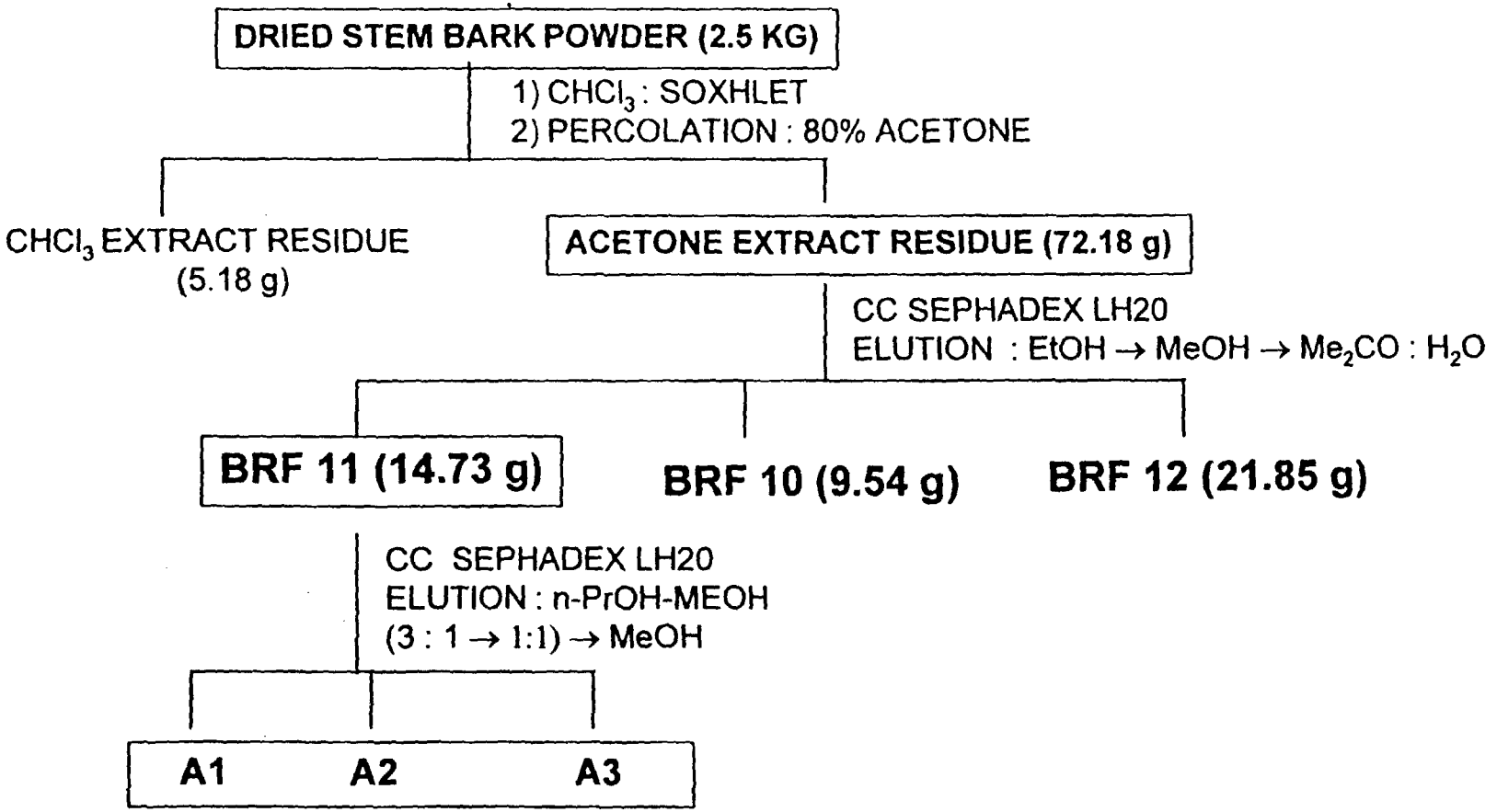
STEM BARK

* DIARRHEA
* DYSENTERY
* FEMALE STERILITY
* INTESTINAL DISORDERS
* INTESTINAL WORMS
* RHEUMATIC PAIN

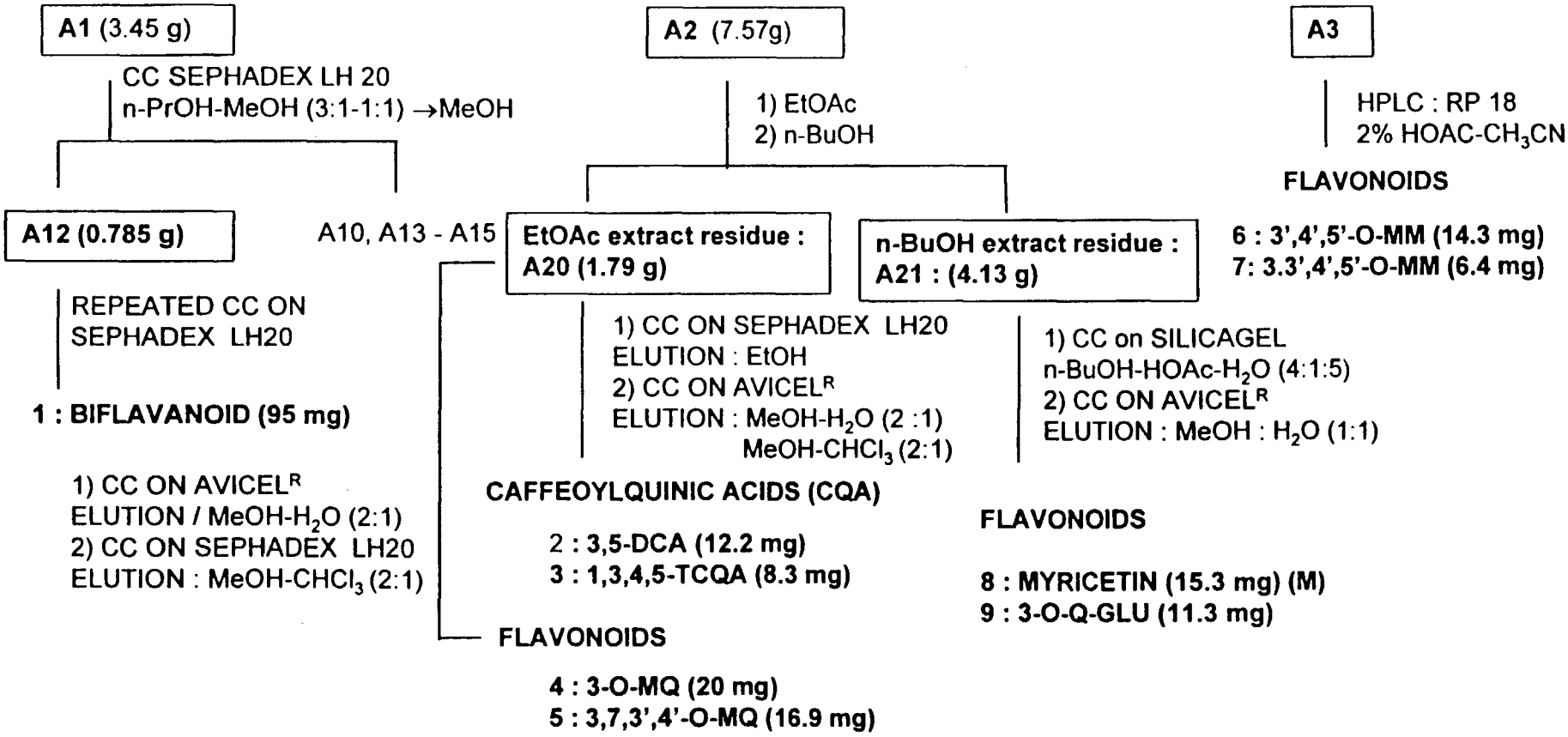
LEAVES

* DIABETES (HYPOGLYCAEMIC EFFECTS IN PATIENTS : ABSTINENCE OF ALCOHOL DURING TREATMENT)
* MOUTHWASH

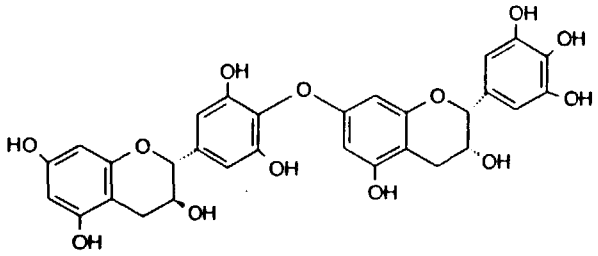
ISOLATION OF COMPLEMENT-INHIBITING CONSTITUENTS OF *BRIDELIA FERRUGINEA* BENTH. (EUPHORBIACEAE)



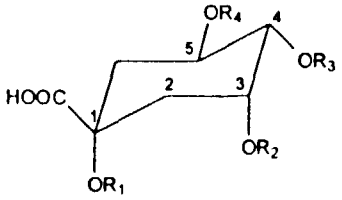
ISOLATION OF COMPLEMENT-INHIBITING CONSTITUENTS OF *BRIDELIA FERRUGINEA* BENTH. (EUPHORBIACEAE) (Continued)

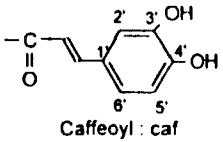


COMPLEMENT-INHIBITING CONSTITUENTS OF *BRIDELIA FERRUGINEA* BENTH (EUPHORBIACEAE)

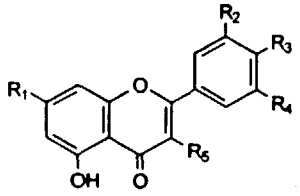


1 : Gallocatechin-4'-O-7-epigallocatechin
CP : 14.7 ± 3.0, AP : 86.2 ± 4.5





	R_1	R_2	R_3	R_4
2	H	caf	H	caf **3,4 DCQA** CP: 6.2 ± 1.4 AP : 272.5 ±4.0
3	caf	caf	caf	caf **1,3,4,5 TCQA** CP: 3.1 ± 0.1 AP : 60.6 ±1.0



	R_1	R_2	R_3	R_4	R_5	**CP**	**AP**
4	OH	OH	OH	H	OCH_3	215.2 ±11.7	**1531± 6.0**
5	OCH_3	OCH_3	OCH_3	H	OCH_3	147.6 ± 4.0	**223.0 ±11.0**
6	OH	OCH_3	OCH_3	OCH_3	OH	116.9 ± 10.9	>500
7	OH	OCH_3	OCH_3	OCH_3	OCH_3	84.5 ±12..8	128.6 ±7.2
8	OH	OH	OH	OH	OH	58.2 ± 10.7	137.4 ±12.9
9	OH	OH	OH	H	O-Glc	171.1 ± 5.8	>500

The anticomplementary activity of compounds **1 - 9** is expressed in IC_{50},µM ; rosmarinic acid : CP : 80.6 ± 4.7, AP : 408.3 ± 3.3

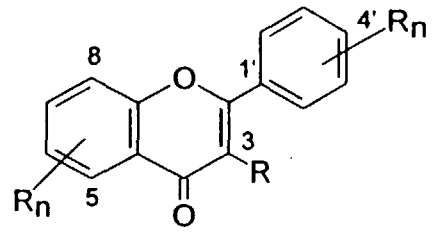
INHIBITION OF THE CLASSICAL ACTIVATION (CP) AND ALTERNATIVE (AP) PATHWAYS OF COMPLEMENT BY FLVAVONOIDS : IC_{50}-VALUES (µM)

COMPOUND	TYPE	CP	AP
QUERCETIN	FLAVONOL	526.0 ± 13.2	N.D.
QUERCITRIN	FLAVONOL	384.1 ± 8.8	N.D.
RUTIN	FLAVONOL	357.1 ± 2.9	N.D.
HYPEROSIDE	FLAVONOL	N.D	62.5 ± 5.
MYRICETIN	FLAVONOL	67.6 ± 1.3	153.8 ± 5.9
(±) - TAXIFOLIN	FLAVANONOL	402.2 ± 9.2	N.D.
PELARGONIDIN.CL	ANTHOCYANIDIN	167.2 ± 2.5	120.5 ± 9.4
CYANIDIN.CL	ANTHOCYANIDIN	89.7 ± 3.7	N.D.
BAICALEIN	FLAVON	N.D	942.2 ± 11.3
CATECHIN	FLAVANOL	647.2	N.D.
EPICATECHIN	FLAVANOL	655.5	N.D.
EPIGALLOCATECHIN	FLAVANOL	196	179.4
PROCYANIDIN B1		31.3	72.0
PROCYANIDIN B2		58.0	–
PROCYANIDIN B3		37.7	–
PROCYANIDIN B4		45.5	–
PROCYANIDIN B5		51.7	–
PROCYANIDIN B6		18.5	–
PROCYANIDIN B8		19.7	83.7
PROANTHOCYANIDIN A1		57.1	105.0
PROANTHOCYANIDIN A2		11.6	112.8
PROANTHOCYANIDIN C1		6.0	85.5
GALLOCATECHIN (4'-O-7)-EPIGALLOCATECHIIN	BIFLAVANOL	14.6	86.0

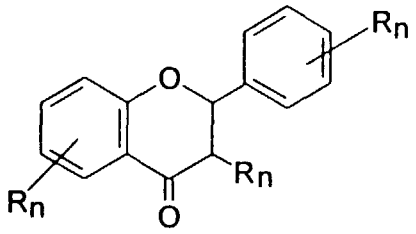
N.D. = NOT DETERMINED

LASURE ET AL., PHARM. PHARMACOL. LETT., **4**, 32, 1994

STRUCTURE-ACTIVITY RELATIONSHIP STUDIES OF FLAVONOIDS WITH COMPLEMENT ACTIVITY



(1)



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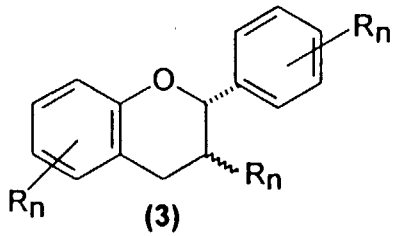
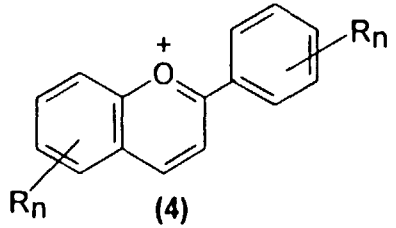


(3)

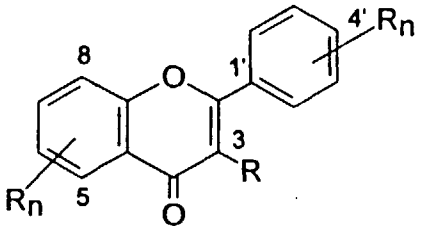


(4)

INHIBITORS OF THE CLASSICAL PATHWAY (CP)

* **DOUBLE BOND AT C-2** AND **CARBONYL GROUP AT C- 4** ARE NOT ESSENTIAL
* **HYDROXYLATION AT C-3** INCREASES INHIBITORY ACTIVITY
* **INCREASING** NUMBER OF **HYDROXYL GROUPS** IN THE **B-RING** ENCHANCES THE ACTIVITY,ESPECIALLY A **3',4',5'-TRIHYDROXYL** SUBSTITUTION (E.G. MYRICETIN)
* **METHOXYLATION** DECREASES THE INHIBITORY EFFECT
* **GLYCOSYLATION** HAS AN **AMBIGUOUS** EFFECT DEPENDING ON THE NATURE OF THE AGLYCONE AND ON THE TYPE OF LINKAGE TO THE AGLYGONE : **C-GLYCOSYLATION** DECREASES THE INHIBITORY ACTIVITY OF **FLAVONES**, **GLYCOSYLATION** DECREASES THE INHIBITORY ACTIVITY OF **ANTHOCYANIDINS**

STRUCTURE-ACTIVITY RELATIONSHIP STUDIES OF FLAVONOIDS WITH COMPLEMENT ACTIVITY (CONTINUED)



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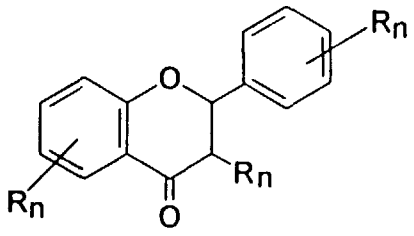

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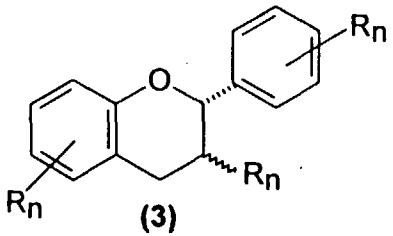

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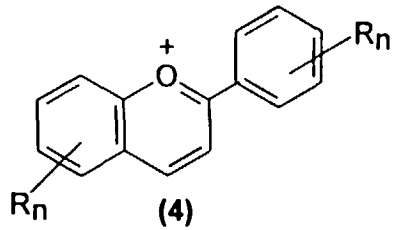


(4)

INHIBITORS OF THE ALTERNATIVE PATHWAY (AP)

ONLY SOME REMARKS

* **ANTHOCYANIDINS** SHOW AN IMPORTANT INHIBITION
* THE **CARBONYL** GROUP AT **C-4** IS IMPORTANT FOR A HIGH INHIBITORY ACTIVITY
* **METHOXYLATION** IN GENERAL CAUSES AN ACTIVATION OF THE AP, ESPECIALLY AT **C-4'**
* THE INHIBITION EFFECT INCREASES WITH THE **NUMBER OF HYDROXYL GROUPS IN RING B**

LASURE ET AL., PHARM. PHARMACOL. LETT., **4**, 32, 1994

VERNONIA AMYGDALINA DEL. (ASTERACEAE)

* **FOLKLORIC USE**
  - RWANDA : **UMUBIRIZI**
  - UPPER PARTS : LEAVES, TWIGS, STEMS, FRUITS

* - ACHES, ABDOMINAL PAIN, PAIN AFTER CHILDBIRTH
  - AMOEBIASIS
  - ASCARIASIS (CHILDREN)
  - CARDIOVASCULAR DISEASES
  - COLICS
  - ECZEMA
  - FEVER
  - GASTROINTESTINAL UPSETS
  - HEPATIC DISORDERS E.G. HEPATITIS
  - MALARIA
  - RHEUMATISM
  - SCHISTOSOMIASIS
  - SNAKE BITES

* USED
  - AS FOODSTUFF : BITTER TONIC
  - TO RENDER WOMEN SEXUALLY MORE ATTRACTIVE

VERNONIA AMIGDALINA DEL. (ASTERACEAE)

PHARMACOLOGICAL EVALUATION

*** FLAVONOIDS :**

- QUERCETIN (Q), KAEMPFEROL (K), RUTINE (R) (0.38%)
- 3-O-METHYLFLAVONES : 3-O-MQ , 3,3'-O-MQ (0.84%)

IN VITRO CARDIOVASCULAR EFFECTS (10 -100 µg/ml, Q, 3-O-MQ)

- INHIBITION OF PLATELET AGGREGATION
- VASORELAXATION (RABBIT EAR CENTRAL ARTERY)
- POSITIVE CHRONOTROPIC EFFECT (GUINEA-PIG RIGHT ATRIUM)
- ANTI-ARYTHMIC EFFECT (GUINEA-PIG LEFT ATRIUM)

ANTIPICORNAVIRUS ACTIVITY : POLIOMYELITIS, COXSACKIE, RHINOVIRUS : FROM 1 µg/ml 3-O-MQ on

*** SESQUITERPENE LACTONES**

VERNOLEPIN, VERNODALIN, VERNOLID (0.12%)

- SPASMOLYTIC ACTIVITY (GUINEA PIG ILEUM) (**VERNOLEPIN** : 5 - 10 µg/ml)
  COMPETITIVE ANTAGONIST OF HISTAMINE $pA_2 = 5.61$
  NON-COMPETITIVE ANTAGONIST OF ACETYLCHOLINE
- PLATELET ANTI-AGGREGATING ACTIVITY (**VERNOLEPIN** : 5 - 10 µg/ml)
  PREVENTS ADHESION OF PLATELETS, REVERSES STRONG PSEUDOPOD FORMATION AND REDUCES CENTRALISATION OF THE GRANULES DURING PLATELET AGGREGATION
- ANTHELMINTHIC, OXYURICIDAL AND AMOEBICIDAL ACTIVITIES (**VERNOLID**)
- CYTOTOXIC AND ANTITUMORAL ACTIVITY
  INHIBITION OF WALKER INTRAMUSCULAR CARCINOMA SARCOMA (RATS)

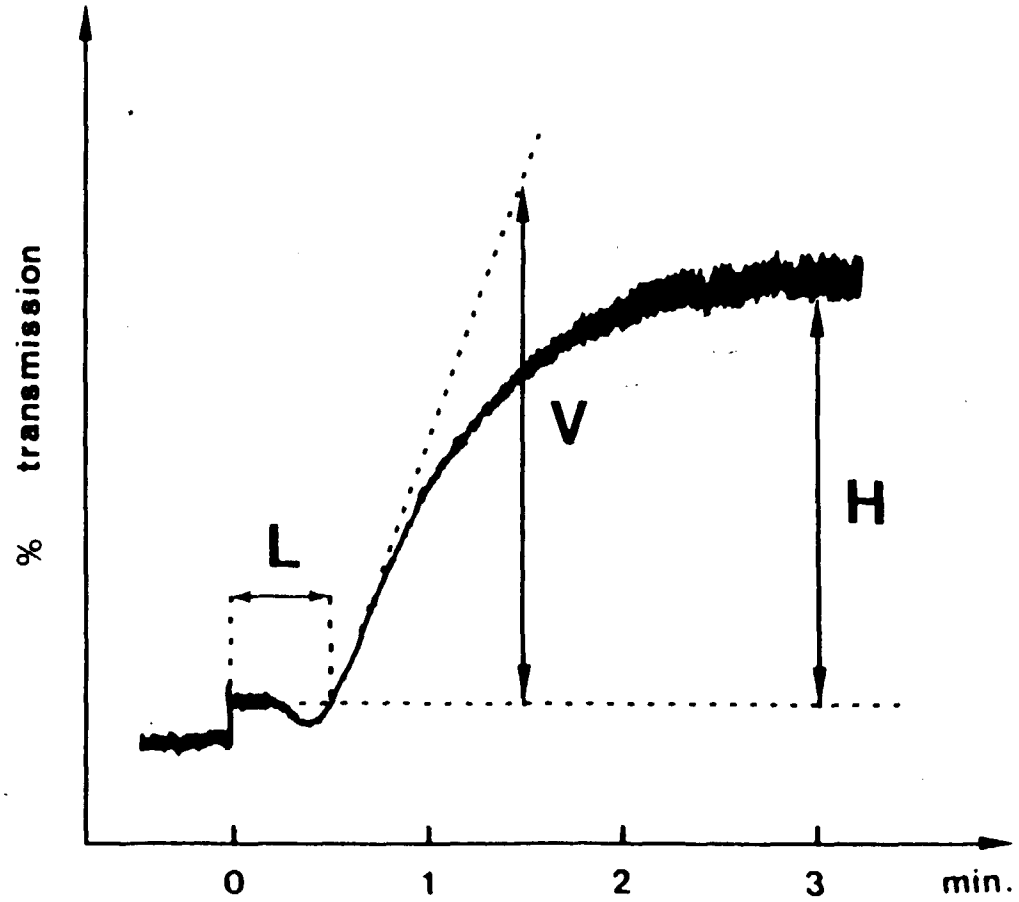


FIG. 10.1 Aggregation tracing after the addition of collagen to citrated PRP. L: lag phase, V: initial velocity of aggregation, H: height or amplitude of aggregation after 3 min.

METHODS FOR DETECTION OF BIOLOGICAL ACTIVITY OF PLANT EXTRACTS (9)

2.3. ISOLATED CELLULAR SYSTEMS

2.3.4. WOUND HEALING

AIM : STIMULATION OF WOUND REPAIR

TESTS :

* PROLIFERATION OF ENDOTHELIAL CELLS
* GROWTH FACTORS RELEASE BY CELL ACTIVATION
* REEPITHELIALIZATION

2.3.5. ANTIOXIDANT AND RADICAL SCAVENGING ACTIVITIES

AIM : PREVENTION AND/OR ELIMINATION OF OXIDATIVE STRESS

TESTS :

* ANTIOXIDATIVE ASSAY IN E.G. RAT LIVER MICROSOMES, RABBIT ERYTHROCYTE MEMBRANE GHOSTS
* RADICAL SCAVENGING ASSAY IN E.G. ACTIVATED POLYMORPHONUCLEAR NEUTROPHILIC LEUCOCYTES (PMNL)

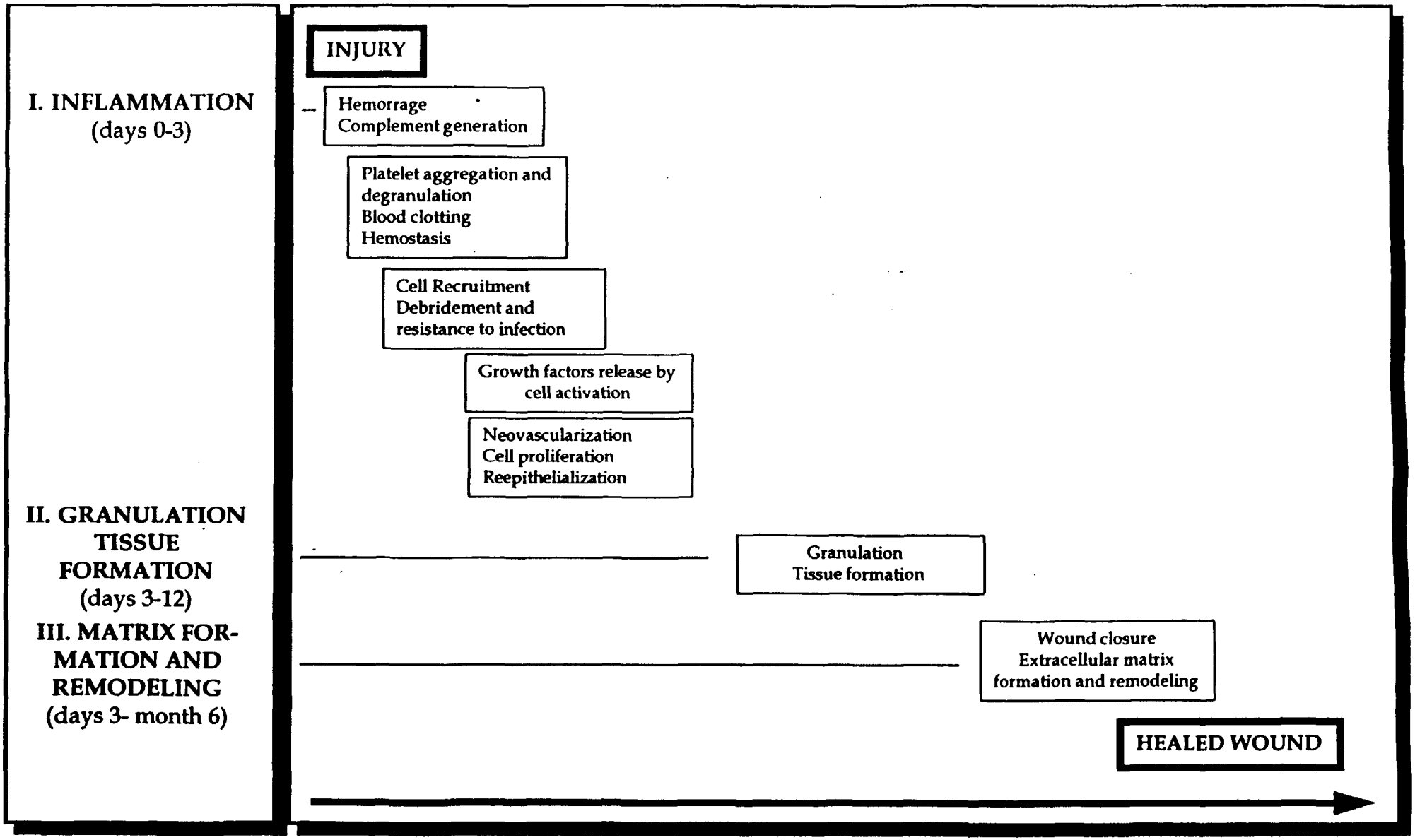
COMPARISON OF SUBCELLULAR VERSUS CELLULAR *IN VITRO* ASSAYS

* **CLASSIFICATION**
  * **SUBCELLULAR ASSAYS** : USE OF ISOLATED SYSTEMS FROM CELLS SUCH AS ENZYMES, RECEPTORS, DNA ... (MOLECULAR ASSAYS)
  * **CELLULAR ASSAYS** : USE OF INTACT CELLS

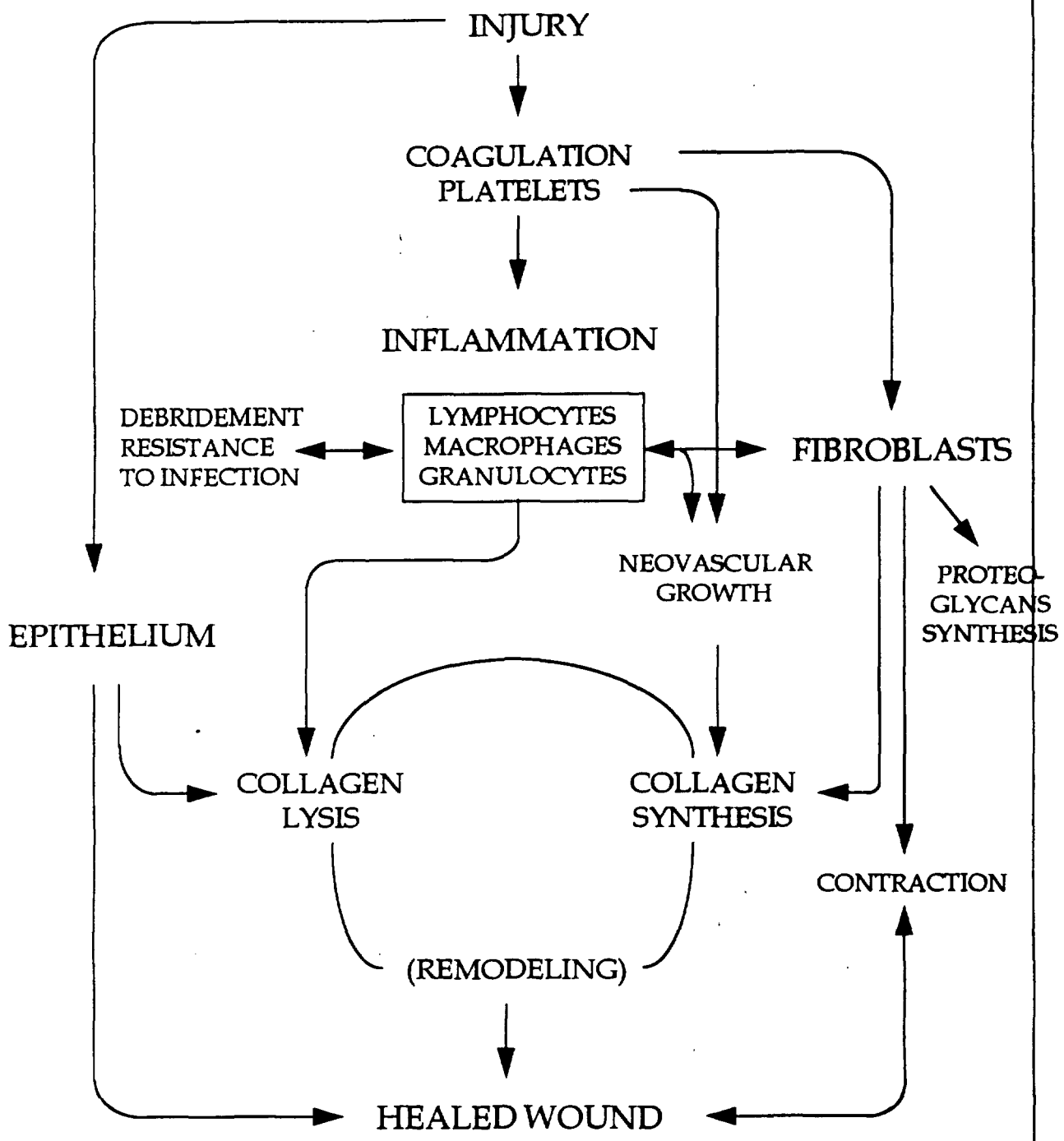
* **COMPARISON**

CHARACTERISTICS	SUBCELLULAR ASSAY	CELLULAR ASSAY
SPECIFICTY	HIGH	LOW
CAPACITY	VERY HIGH	MODERATE TO HIGH
HIT RATE	LOW BUT SPECIFIC	HIGH BUT MOST NOT OF INTEREST
FALSE POSITIVES	AGENTS THAT DON'T ENTER OR ARE RAPIDLY METABOLISED	WIDE VARIETY OF TOXINS
FALSE NEGATIVES	ALL COMPOUNDS WORKING BY OTHER MECHANISMS	FEW

Soft tissue repair pathways



Pathway of wound repair



Sangre de Drago

(Dragon's blood) (Sangre de Grado)

- Used in South American popular medicine for wound healing, ulcers and against cancer
- Red viscous latex extracted from the cortex of several Croton species (Euphorbiaceae)

  Croton lechleri L.

  Croton palanostigma (Klotzschs)

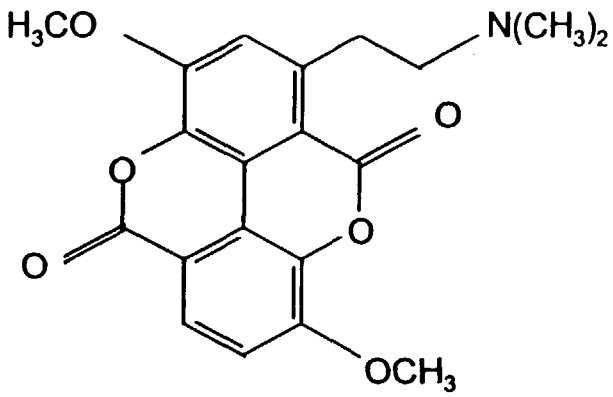
  Croton salutaris Casar

  Croton draconoides (Muell.) Arg.

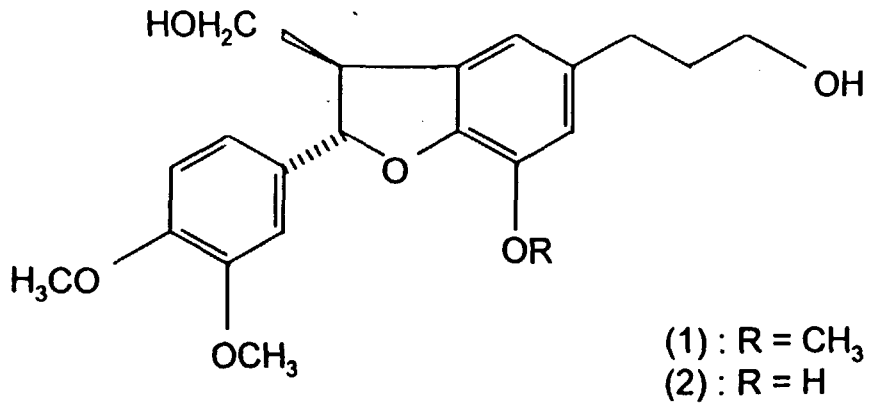
  Croton erythrochilus (Muell.) Arg.

- Pterocarpus amazonum (Benth.) (Sangre de Grado, Peru)
  The clear red bark exsudate is used for leishmaniasis treatment
- Pterocarpus draco L. (West Indian or American dragon's blood)
  Used against cancer
- Daemonorops draco (Willd.) B. (Calamus draco Willd., East Indian or Sumatra dragon's blood)
  Sanguis Draconis Asiaticus
  Used as an astrigent
- Dracaena draco L. (Canary Islands, Canarian or African dragon's blood)
  Sanguis Draconis Canariensis
- Other species of the same genera

Compounds isolated from Sangre de Drago



Taspine



(1) = 3',4-O-dimethylcedrusin
(2) = 4-O-methylcedrusin

In vivo wound healing activity. Legend for tables :

Macroscopic observations

a	contraction :	0 = no contraction + to +++ = increasing level of contraction
b	formation of a crust :	a first crust is formed on the wound after the given number of days
c	wound repair :	% of the wound volume filled with new tissue

Microscopic observations

d	new tissue :	1 to 3 = stage of formation of new tissue (see text) T = toxic
e	epithelial growth :	0 = no epithelial growth 0 to ++++ = increasing level of epithelial growth
f	new hair follicles :	0 = no hair follicles + = few introversions ++ = obvious introversions +++ = more obvious introversions ++++ = introversions with beginning differentiation

In vivo wound healing activity of Sangre de Drago (= SDD) and its constituents

Conclusions :

① Stimulation of wound contraction (polyphenols : ⊕)

② Formation of a crust (polyphenols : ⊕)

③ Stimulation of wound repair by stimulation of fibroblast and collagen formation (3',4-O-dimethylcedrusin : ⊕; polyphenols :⊖)

④ Stimulation of epithelial layer regeneration

⑤ Formation of new hair follicles

⑥ Wound healing without scars

South America

FLAVONOIDS AS INHIBITORS OF XANTHINE OXIDASE (XO) AND SUPEROXIDE SCAVENGERS (SS)

$$\text{Xanthine} + 2O_2 + H_2O \longrightarrow \text{Uric acid} + 2O_2^{\cdot -} + 2H^+$$

$$\text{Xanthine} + O_2 + H_2O \longrightarrow \text{Uric acid} + H_2O_2$$

Summary of the Classification of Flavonoids into Six Categories According to Their Inhibition of Xanthine Oxidase and Superoxide Scavenging Activity

Category	Inhibition of xanthine oxidase	Superoxide scavenging activity[a]	Example
A	0	+	(-)-epigallocatechin
B	+	0	baicalein
C	+	+	myricetin
D	+	-	galangin
E	0	-	7-hydroxyflavanone
F	0	o	naringenin

[a]Key: 0, no effect; +, effect; -, pro-oxidant effect

COS ET AL., J. NAT. PROD. **61**, 71, 1998

METHODS TO DETECT AND MEASURE BIOLOGICAL LIPID PEROXIDATION (1)

Method	What is measured
Analysis of fatty acids by GLC or HPLC	Loss of unsaturated fatty acids
Oxygen electrode	Uptake of oxygen by carbon-centered radicals and during peroxide decomposition reactions
Iodine liberation	Lipid peroxides
Heme degradation of peroxides (often first separated by HPLC)	Lipid peroxides
Glutathione peroxidase	Lipid peroxides
Cyclooxygenase	Lipid peroxides
GLC/mass spectrometry	Lipid peroxides/aldehydes
Spin trapping	Intermediate radicals
Hyrocarbon gases	Pentane and ethane

METHODS TO DETECT AND MEASURE BIOLOGICAL LIPID PEROXIDATION (2)

Method	What is measured
Light emission	Excited carbonyls, singlet oxygen
Fluorescence	Aldehydes and their reaction products
TBA test	TBA-reactive material
HPLC/antibody techniques	Cytotoxic aldehydes
Diene congugation	Diene-conjugated structures

METHODS FOR DETECTION OF BIOLOGICAL ACTIVITY OF PLANT EXTRACTS (10)

2.4. ISOLATED ORGANS OF VERTEBRATES

2.4.1. CLASSIC ORGAN BATH METHOD
E.G. GUINEA PIG ILEUM (NEUROTRANSMISSION):
WITH AND WITHOUT COAXIAL STIMULATION

2.4.2. ISOLATED ORGAN PERFUSION TECHNIQUE
E.G. RABBIT CENTRAL EAR ARTERY (VASODILATING EFFECT),
GUINEA PIG RIGHT ATRIUM AND LEFT ATRIUM
(CARDIOVASCULAR EFFECTS)

2.4.3. ISOLATED ORGAN SUPERFUSION TECHNIQUE
E.G. CASCADE OF RABBIT COELIAC AND MESENTERIC ARTERIES
AND A RAT STOMACH STRIP (PG-LIKE ACTIVITY),
CASCADE OF RABBIT COELIAC- AND MESENTERIC ARTERIES
AND RABBIT AORTIC TISSUE (SEROTONIN-LIKE ACTIVITY)

METHODS FOR DETECTION OF BIOLOGICAL ACTIVITY OF PLANT EXTRACTS (11)

2.5. WHOLE ANIMALS

2.5.1. CARDIOVASCULAR SYSTEM

* ANTI-ARRHYTHMIC ACTIVITY
* ANTIHYPERTENSIVE ACTIVITY
* DIRECT ACTION ON THE MYOCARDIUM
* ANTISCLEROTIC ACTIVITY : REDUCTION OF SERUM LIPIDS AND CHOLESTEROL

2.5.2. GASTRO-INTESTINAL TRACT

* ANTI-EMETIC ACTIVITY
* ANTI-ULCER ACTIVITY
* ANTIDIARRHOEAL ACTIVITY

2.5.3. LIVER AND BILIARY SYSTEM

* LIVER PROTECTIVE ACTIVITY
* CHOLERETIC AND ANTICHOLERETIC ACTIVITY

METHODS FOR DETECTION OF BIOLOGICAL ACTIVITY OF PLANT EXTRACTS (12)

2.5. WHOLE ANIMALS

2.5.4. RESPIRATORY SYSTEM

* ANTI-ASTHMATIC ACTIVITY
* ANTITUSSIVE ACTIVITY

2.5.5. RENAL ACTIVITY

* DIURETIC AND SALURETIC ACTIVITY
* URICOSURIC AND HYPOURICEMIC ACTIVITY

2.5.6. ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY

* ANTI-INFLAMMATORY ACTIVITY
* ANALGESIC AND ANTIPYRETIC ACTIVITY

METHODS FOR DETECTION OF BIOLOGICAL ACTIVITY OF PLANT EXTRACTS (13)

2.5. WHOLE ANIMALS

2.5.7. PSYCHOTROPIC AND NEUROTROPIC ACTIVITY

* EFFECTS ON BEHAVIOUR AND MUSCLE COORDINATION
* ANXIOLYTIC ACTIVITY
* ANTI-EPILEPTIC ACTIVITY
* HYPNOTIC ACTIVITY
* NEUROLEPTIC ACTIVITY
* ANTIDEPRESSANT ACTIVITY
* ANTIPARKINSON ACTIVITY

2.5.8. LEARNING AND MEMORY

2.5.9. IMMUNOMODULATING ACTIVITY

METHODS FOR DETECTION OF BIOLOGICAL ACTIVITY OF PLANT EXTRACTS (14)

2.5. WHOLE ANIMALS

2.5.10. ANTIDIABETIC ACTIVITY

2.5.11. ENDOCRINOLOGY

* ADRENAL STEROID HORMONES
* OVARIAN HORMONES
* TESTICULAR HORMONES
* THYROID HORMONES
* PARATHYROID HORMONE
* ANTERIOR PITUITARY HORMONES
* POSTERIOR PITUITARY HORMONES
* HYPOTHALAMIC HORMONES
* OTHER PEPTIDE HORMONES

In the UNIDO mandate

UNIDO ICS International Centre for Science and High Technology

at the AREA Science Park, Trieste, Italy

1

Objectives of ICS

- To foster and facilitate the transfer of technology in specific high-tech areas to developing countries
- To provide high-tech SMEs in developing countries with advanced tools and services for the enhancement of their sustainability and competitiveness

2

ICS core competence

- Chemistry
- Earth and Environment
- High-tech and New Materials
- Technology Services

3

ICS target beneficiaries and counterparts

- National/regional R&D institutions
- National policy and strategy decision-makers
- High-tech SMEs

4

Applied infosystems

- IT support tools (operational support, information sharing, development & calculation, validation)
- Software: mathematical modelling, simulation of industrial processes, image engineering
- Databases: best available technologies economically viable (BATEV)
- Networking, access to technology information
- Training packages
- Publications

5

Chemistry subprogrammes

- Catalysis & sustainable chemistry
- Bio-degradable plastics
- Remediation
- Combinatorial chemistry and technologies

6

Environment subprogrammes

Decision-support systems
Coastal zone management
Medicinal and aromatic plants

7

Coastal zone management

- Sustainable development
- Integration of scientific, economic, legislative aspects
- Application of decision-support systems for:
  - industrial siting
  - resource management and control
  - control and monitoring of pollution
  - marine navigation control

8

Industrial utilization of medicinal and aromatic plants

Promoting sustainable growth of industry based on medicinal plants in developing countries

Providing support through training courses, fellowships, workshops, publishing manuals and creating a network of databases

Creating a network of leading institutes from different countries for technical cooperation

Developing and working on demand based projects

9

High technology and new materials subprogrammes

Laser applications and optical technologies
Photovoltaic solar energy
New materials

10

Technology services

Technology Management
Business Alliances
Technology and Competitiveness

11

Networking

Identification, selection and evaluation of partner institutions in the world's various regions, willing to offer

co-operation and support

12

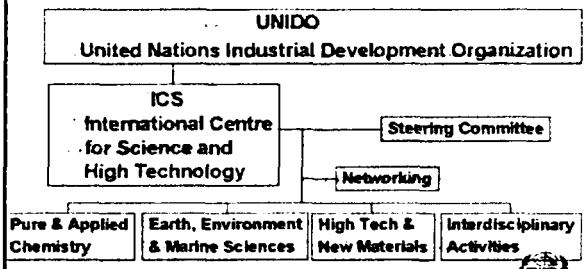
Project proposals

ICS is involving other international partners and donors in its activities.

These are identified through development and promotion of demand-oriented programmes and projects.

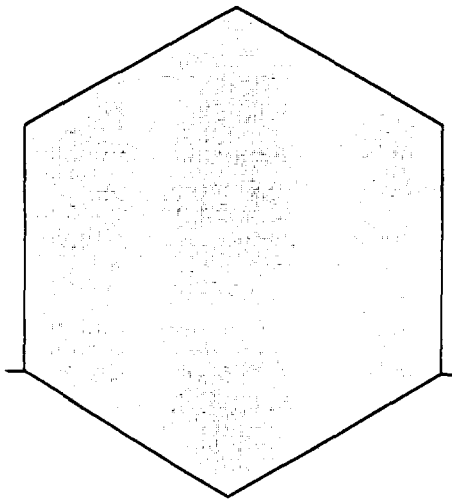
Some ten proposals are being selected each year.

Institutional Structure



Programa iberoamericano de ciencia y tecnología para el desarrollo CYTED

Subprograma X, Química fina farmacéutica: logros y perspectivas



MAHABIR P. GUPTA
Coordinador Internacional del Subprograma X. Centro de Investigaciones Farmacognósticas de la Flora Panameña. Facultad de Farmacia. Universidad de Panamá

1. ¿QUE ES EL PROGRAMA CYTED?

El Programa CYTED, creado en 1984, integra a los 21 países iberoamericanos y participan como Organismos Internacionales Observadores BID, CEPAL, OEA y UNESCO. Desde 1992, el CYTED se encuentra integrado entre los programas de cooperación de las Cumbres Iberoamericanas de jefes de Estado y de Gobierno, que se celebran anualmente. Es un instrumento de carácter internacional y multilateral que facilita la cooperación científica y tecnológica en Iberoamérica, mediante la coordinación e interacción entre Universidades, Centros de Investigación y Desarrollo Tecnológico y las empresas innovadoras de la región; así como, promueve la modernización productiva, la mejora de calidad de vida de los países iberoamericanos y sirve de puente para la cooperación entre América Latina y Europa.

Su objetivo primordial es el fomento de la investigación aplicada y el desarrollo tecnológico para la obtención de resultados transferibles a los sistemas productivos y a las políticas sociales de los países iberoamericanos que integran y participan en el Programa.

Su financiamiento está basado en los recursos que asignan los países a sus grupos de investigación nacionales participantes en las diversas Redes y Proyectos; el Programa consigue así un efecto de sinergia y de potenciación de los recursos existentes en Iberoamérica. Adicional a ello, recibe una financiación que proviene de la Comisión Interministerial de Ciencia y Tecnología de España, de la Agencia Española de Cooperación Internacional y de contribuciones de los diferentes países para financiar las actividades de gestión y cooperación que hacen posible el Programa: reuniones de coordinación, talleres, experimentos conjuntos, intercambios, movilidad de científicos y tecnólogos, publicaciones, etc. En general, el Programa CYTED es uno de los programas de cooperación que presenta un mejor balance coste/beneficio.

En el marco del CYTED se ejecutan los siguientes Subprogramas:

Temáticos

- Acuicultura.
- Biomasa como fuente de productos químicos y energía.
- Nuevas fuentes y conservación de la energía.
- Tecnología de materiales.
- Química fina farmacéutica.
- Diversidad biológica.
- Tecnología para viviendas de interés social.
- Biotecnología.
- Catálisis y adsorbentes.
- Electrónica e informática aplicadas.
- Microelectrónica.
- Tratamiento y conservación de alimentos.
- Tecnología mineral.
- Corrosión impacto ambiental sobre materiales.

Horizontales

- Gestión de la investigación y el desarrollo tecnológico

Las modalidades de cooperación que desarrolla el Programa CYTED son a través de:

- Redes temáticas. Facilitan la interacción, la cooperación y la transferencia de conocimientos y tecnologías entre grupos que trabajan en temas similares, a través de actividades de capacitación, movilidad de científicos e ingenieros y a la puesta en marcha de proyectos de investigación.

- Proyectos de investigación precompetitiva. Facilitan la ejecución de proyectos con objetivos aplicados y de naturaleza multidisciplinaria, a través de la colaboración y cooperación entre grupos de diferentes países que constituyen equipos internacionales.

- Proyectos de innovación Iberoeka. Facilitan la cooperación entre empresas de diferentes países, a través de proyectos conjuntos que están enfocados a aumentar la productividad y competitividad de la industria y economía; así como, la transferencia de los resultados de la investigación a los sectores productivos.

2. SUBPROGRAMA X. QUIMICA FINA FARMACEUTICA

2.1. Antecedentes y justificación

El consumo mundial de medicamentos en 1995 se estimó en una cifra de 250.000 millones de dólares. De esta cifra, la participación de los países latinoamericanos, España y Portugal se estima que es alrededor del 8,5%. Esta cifra, aunque es baja en relación con los países más desarrollados, representa un alto porcentaje del gasto total en salud. La producción de materias primas y productos auxiliares en la región iberoamericana es escasa y se calcula que un 75% de las necesidades en este rubro deben importarse. La débil situación económica de la mayoría de los países de América Latina, el oligopolio de las empresas internacionales y las leyes de protección de patentes impuestas a los países de la región, entre otras cosas, hacen prever que el acceso a los medicamentos de la población de América Latina seguirá empeorando.

Por otro lado, la biodiversidad de los países iberoamericanos es una de las más ricas en el mundo, y aún no ha sido estudiada de una manera sistemática dirigida a explotar su potencial médico y económico. En los países iberoamericanos, la medicina tradicional y popular aún juegan un papel muy importante en el cuidado de la salud.

El Subprograma X. Química Fina Farmacéutica se inició en 1989 bajo la coordinación internacional del Dr. Ceferino Sánchez. A través de las actividades realizadas durante los primeros cinco años se ha logrado imprimirle una dinámica muy especial. Hoy podemos ver con satisfacción y orgullo que el Subprograma ha logrado escalar una posición importante dentro del CYTED, así como en la comunidad científica iberoamericana. Desde su inicio el Subprograma se fijó como objetivo estratégico la "Búsqueda y Desarrollo de Medicamentos" y todas sus actividades han estado encaminadas hacia su cumplimiento. Se escogió como tema central los productos naturales pues representan un "nicho" de investigación y recoge el interés manifiesto de los centros e institutos de investigación de la región. A su vez, el Subprograma ha diseñado algunas estrategias con el fin de poder satisfacer las expectativas creadas a su alrededor, entre ellas las siguientes:

a) Buscar la participación de los 21 países iberoamericanos.
b) Promover una gran solidaridad entre los grupos participantes para alcanzar objetivos comunes.
c) Estimular la transferencia de conocimientos con un programa dinámico de movilidad de científicos.
d) Enfatizar el valor agregado de la cooperación en la obtención de resultados científicos.
e) Estimular la formación de redes nacionales.
f) Estimular la integración científico-técnico de la región.
g) Estabilizar y mantener grupos de investigación en el área.
h) Puesta al día en métodos y técnicas de laboratorio a través de talleres, reuniones de trabajo (*workshops*) de impacto en mejorar habilidades y conocimientos.

2.2. Situación actual

El Subprograma X actualmente tiene tres redes temáticas: Red X.A. Red Iberoamericana de Productos Naturales de Uso Medicinal (RIPRONAMED), Red X.B. Red Iberoamericana de Validación de Plantas Medicinales (RIVAPLAMED) y Red Iberoamericana de Productos Fitoterapéuticos X.C. (RIPROFITO), y cuatro proyectos de investigación X.1 Búsqueda de Principios Bioactivos de la Región, (concluido en 1996). X.2 Síntesis de Moléculas Bioactivas Análogas de Productos Naturales de Origen Iberoamericano. X.3. Evaluación de la Biodiversidad Vegetal de los países Iberoamericanos como fuente de agentes Inmunomoduladores y químio-terapéuticos y X.4. Obtención de Medicamentos Innovadores con Actividad Antihipertensiva y Vasodilatadora a través de Validación Orientada de Plantas Medicinales.

Las tres redes han sido muy importantes en la región iberoamericana ya que, por primera vez, han permitido la integración regional real de los grupos investigadores en productos naturales, han estimulado el espíritu de cooperación y colaboración y el trabajo interdisciplinario. Estas redes han organizado hasta la fecha 20 talleres en técnicas modernas de bioensayos, validación y plantas medicinales y métodos modernos de separación y caracterización de los principios bioactivos, desde los más sencillos a los más complejos, que han permitido la capacitación de unos 500 científicos iberoamericanos. Uno de los mayores logros del subprograma ha sido la efectiva participación de 600 científicos y técnicos iberoamericanos de 80 centros e institutos de investigaciones en 21 países miembros de CYTED. La movilidad de 41 científicos, con una duración total de 87 meses ha permitido establecer vínculos muy estrechos entre los grupos investigadores de la región y entrenar un grupo significativo de investigadores y tecnólogos. Hoy día, la investigación en productos naturales en Iberoamérica se hace con la metodología homologada del CYTED.

Otro logro del Subprograma ha sido el apoyo y el estímulo a los grupos nacionales de los países menos desarrollados, que sin la

)articipación del CYTED nunca ıubieran podido iniciar investiga- iones en este campo. En este entido la creación de las Subre- ies nacionales ha sido una moda- idad novedosa y única de nues- ro subprograma. Como guía de jemplo, sólo a través de la Su- red España-Portugal, por prime- a vez en la historia todos los gru- os investigadores de España y ortugal han podido reunirse año as año para discutir problemas omunes y acordar acciones con- retas a tomar. En la actualidad stán en funcionamiento las si- uientes redes nacionales: Espa- a-Portugal con 12 grupos, Co- ombia con 15 grupos, Ecuador on 6 grupos, Centroamérica con grupos, México con 12 grupos, erú con 9 grupos y Chile con 12 rupos.

.3. Proyectos de Investigación recompetitivos

royecto X.1 "Búsqueda de prin- ipios bioactivos en las plantas de a región".

l primer proyecto de investiga- ión precompetitivo concluyó en 996 y fue coordinado por el Dr. oberto Pinzón de la Universidad acional de Colombia. El objetivo inal de este proyecto ha sido la btención de pistas o cabezas de erie con actividad biológica, las uales, ellas o sus análogos, pue- an tener uso en terapéutica. Este royecto realizó un estudio siste- nático y ordenado de 238 espe- ies vegetales de Latinoamerica, spaña y Portugal, que tienen o e les adscriben propiedades me- icinales. En este proyecto partici- aron 13 grupos latinoamericanos e Argentina, Bolivia, Colombia, osta Rica, Chile, Ecuador, Guate- ala, México, Panamá y Perú, y cho grupos de España y uno de ortugal.

)urante la ejecución de este pro- ecto se han sometido a diferen- es bioensayos 568 extractos ve- etales. Los mismos extractos se nsayan en todos los centros par- cipantes que disponen de dife- entes pruebas. El efecto multipli- ador es obvio. Como resultado e este proyecto han aparecido 6 artículos científicos en revistas nternacionales de prestigio, una orción significativa de ellos con s firmas de dos o más investiga- ores de los centros participantes. e han presentado 56 trabajos en los congresos y se han realizado 20 tesis.

Proyecto X.2. Síntesis de moléculas bioactivas de productos naturales de origen iberoamericano

Este Proyecto se inició en 1995 bajo la coordinación del profesor Antonio Monge de la Universidad de Navarra. Este proyecto tiene los siguientes objetivos:

- Objetivos generales:

• Búsqueda de grupos de investigación en la preparación y manipulación en el laboratorio de compuestos con actividad medicinal de origen vegetal.
• Establecimiento de alternativas sintéticas a los productos activos de origen medicinal encontrados en plantas iberoamericanas.
• Mejora de las actividades de los productos de origen vegetal por manipulación molecular.
• Impulsar en la Región Iberoamericana la investigación de productos con actividad medicinal de origen sintético como alternativa a los productos de origen vegetal.
• La cooperación e integración de grupos de investigadores es tan importante tema.

- Son objetivos concretos iniciales de este proyecto:

• Compuestos anti-neoplásicos: análogos de a) 1,25 dibiotroxivitamina D3; b) CC-1065; c) taxol; d) retinoides; e) lignanos.
• Compuestos anti-sida: análogos de a) alcaloides de uña de gato; b) lignanos.
• Antimicrobianos y antiparásitarios: análogos de a) productos marinos antihelmínticos; b) epóxidos antimaláricos; c) quinonas y quinolinas como antimaláricos y tripanosomicidas; d) fungicidas por modificación de azúcares; e) cumarinas antimicrobianas.

Este proyecto tiene una participación de más de 130 científicos en 10 países. Se han organizado varios cursos y seminarios con la participación de industrias farmacéuticas de España. Se vislumbran decenas de publicaciones científicas como resultado de cooperación iberaomericana.

Proyecto X.3. Evaluación de la biodiversidad vegetal de países iberoamericanos como fuente de agentes inmunomoduladores y quimioterapéuticos

El Dr. Roberto Pinzón de la Universidad Nacional de Colombia es el Jefe de este proyecto, que se inició en 1997 y la primera reunión de iniciación de actividades se realizó durante los días comprendidos entre el 11 y el 14 de diciembre de 1997 con el apoyo de COLCIENCIAS, organismo signatario de Colombia.

Este proyecto, enmarcardo dentro del desarrollo sostenible de la biodiversidad de los países iberoamericanos, busca investigar el potencial de recursos naturales iberoamericanos como fuente de sustancias inmunomoduladoras y agentes quimioterapéuticos.

Se estudiarán especies que presentaron resultados de actividad biológica promisoria, en las clases terapéuticas mencionadas, en el proyecto X.1, así como especies a las cuales se encuentre asociado conocimiento etnomédico de las comunidades indígenas o campesinas que correlacionen directa o indirectamente con su actividad como inmunomoduladores, antimicóticos y antivirales y con base en consultas a bases de datos como Napralert ®.

El estudio estará orientado hacia la profundización de la actividad terapéutica mediante bioensayos específicos de los extractos, fracciones y compuestos aislados. Las plantas que representen actividad serán motivo de una evaluación química, farmacológica y toxicológica para descubrir agentes inmunomoduladores, antimicóticos y antivirales eficaces y seguros.

Con los compuestos aislados y caracterizados con potencial terapéutico, se continuarán las fases correspondientes al posible desarrollo de nuevos medicamentos.

Objetivos concretos:

- Evaluar plantas usadas en la medicina tradicional, sus extractos y compuestos puros derivados de ellas como fuentes de agentes inmunomoduladoras y quimiterapéuticos.
- Buscar y obtener, en la biodiversidad vegetal de los países iberoamericanos, compuestos con actividad inmunomoduladora y quimioterapéutica.
- Contribuir a la generación de conocimiento científico en áreas relacionadas con el tema del proyecto, y al mejor conocimiento de la biodiversidad vegetal de la región.

- Potenciar la capacidad investigadora y la infraestructura científica de las instituciones de I + D de la subregión que participan en la realización del proyecto.
- Promover la cooperación y solidaridad iberoamericana y el intercambio de conocimiento científico y tecnológico entre los países participantes.

En este proyecto participan 15 grupos de I+D en ocho países.

Proyecto X.4. Obtención de medicamentos innovadores con actividad antihipertensiva y vasodilatadora a través de validación orientada de plantas medicinales iberoamericanas

Este proyecto tiene como objetivo estudiar plantas medicinales utilizadas en la medicina tradicional para disminuir presión sanguínea. Los extractos liofilizados de plantas seleccionadas serán evaluadas en cuatro modelos de hipertensión. Los extractos activos serán purificados y estudiados en preparaciones aisladas para determinar sus mecanismos de acción. Una vez establecida la eficacia de la planta, su mecanismo de acción y los parámetros del control de calidad, se explorará la posibilidad de diseñar un proyecto Iberoeka con la participación de industrias iberoamericanas. En adición, los extractos activos serán probados según protocolos de estudios toxicológicos preclínicos para determinar seguridad antes de ensayos clínicos. Este proyecto dará oportunidades para integración científica iberoamericana, transferencia de tecnología y el desarrollo de centros en países iberoamericanos.

En este proyecto participan 10 grupos de I+D en nueve países.

3. REDES TEMATICAS

3.1. X.A. Red iberoamericana de productos naturales de uso medicinal (RIPRONAMED)

Esta es la Red Incubadora y Permanente del Subprograma X, que se inició en noviembre de 1990 y fue coordinado por la Dra. Olga Lock de Ugaz de la Pontificia Universidad Católica de Perú hasta el 1996. Desde 1997 el profesor Arturo San Feliciano de la Universidad de Salamanca es el coordinador internacional.

Se busca con la Red, lograr la ejecución de acciones, en los países iberoamericanos para el desarrollo de nuevos medicamentos de origen natural mediante: a) la sistematización de la investigación de los productos naturales con actividad medicinal y b) la transferencia de tecnología e información y la movilización de recursos humanos.

En esta Red participan unos 250 científicos en 21 países miembros del Programa CYTED.

3.2. X.B. Red iberoamericana de validación de plantas medicinales (RIVAPLAMED)

Esta Red inició sus actividades en abril de 1993, cuyo coordinador hasta 1996 fue el Dr. Antonio Lapa de la Escuela Paulista de Medicina, Brasil. Desde 1997 la Coordinadora de esta Red es la Dra. Thereza Lima de Nogueira de la Universidad de Santa Catarina, Florianápolis, Brasil.

Como objetivos científico-socioeconómicos complementarios a los del Subprograma X, RIVAPLAMED se propone adelantar acciones para validar pragmáticamente la efectividad y la toxicidad de las plantas medicinales de uso popular en Iberoamérica, con miras a su potencial uso terapéutico y al suministro de materias primas autóctonas a las industrias de la región.

La estrategia de RIVAPLAMED es la siguiente:

- Concentrar esfuerzos, integrando los grupos científicos de la región, para realizar los estudios necesarios para la validación científica de aquellas plantas medicinales con actividad analgésica/antiinflamatoria, cardiovascular, gastrointestinal, broncodilatadora/antiasmática y tranquilizante.
- Realizar el entrenamiento dentro de los grupos (cursos de validación) y entrenamiento complementario entre los diferentes grupos.

En esta Red participan 71 científicos de 16 países.

3.3. X.C. Red iberoamericana de productos fitoterapéuticos (RIPROFITO)

Esta Red inició sus actividades en 1996 bajo la Coordinación del Dr. Armando Cáceres de la Universidad de San Carlos en Guatemala.

Propósitos y objetivos

- El propósito de RIPROFITO es propiciar la cooperación internacional entre los sectores empresariales, académicos y gubernamentales para estimular la industrialización de las plantas medicinales, con el fin de optimizar la utilización de los recursos naturales autóctonos en el cuidado de la salud de Iberoamérica.
- RIPROFITO puede contribuir a identificar las necesidades de la

Tabla I
Proyectos y Redes Temáticas del Subprograma X

Proyecto/ Red	Coordinador Internacional de la Red/ Jefe del Proyecto	Correo electrónico	N° de países	N° de grupos	N° de científicos
X.A RIPRONAMED	Dr. Arturo San Feliciano	asf@gugu.usal.es	11	20	272
X.B RIVAPLAMED	Dra. Thereza C. de Lima Nogueira	thereza@farmaco.ufsc.br	16	12	71
X.C RIPROFITO	Dr. Armando Cáceres Estrada	farmaya@uvalle.edu.gt	18	64	335
X.2	Dr. Antonio Monge	cifa@unav.es	21	48	283
X.3	Dr. Roberto Pinzón	ropinzon@ciencias.ciencias.unal.edu.co	17	38	154
X.4	Dr. Antonio José Lapa	ajlapa.farm@infar.epm.br	15	30	150

región y las líneas de interés para facilitar el entrenamiento y transferencia tecnológica. Sus contenidos temáticos incluyen: diagnóstico de la situación de la región, fortalecimiento de la interacción multidisciplinaria en tecnología agronómica, industrial y farmacéutica, acercamiento a las autoridades gubernamentales y cooperación con otras instituciones.

Los objetivos específicos de RIPROFITO son:

- Detectar los elementos que limitan el desarrollo de los productos fitofarmacéuticos.
- Obtener información sobre la situación de la industria fitofarmacéutica.
- Hacer monografías de plantas medicinales nativas y sus preparaciones.
- Promover la armonización de la legislación sobre producción y distribución de productos fitofarmacéuticos.
- Estimular la transferencia tecnológica para la producción y aplicación de estos productos.
- Apoyar la investigación experimental y clínica sobre fitofármacos.

En esta Red participan 335 científicos de 18 países.

La Tabla I desglosa la participación de científicos en los proyectos de investigación precompetitivos y redes temáticas.

4. EXPERIENCIAS EN IBEROEKA

Los proyectos Iberoeka están orientados a empresas y centros de I+D de países iberoamericanos. El objetivo principal es, mediante estrecha colaboración entre empresas y centros de investiga-

Tabla II

Publicaciones del Subprograma X

- II Reunión Constitutiva de la Red Iberoamericana de Productos Naturales de Uso Medicinal RIPRONAMED. C. Sánchez, O. Lock de Ugaz, E. Ferro, Asunción, Paraguay, 24 de agosto de 1992.
- II Reunión Anual de la Red Iberoamericana de Productos Naturales de Uso Medicinal RIPRONAMED y Reunión Organizativa de Sub Red Centroamericana. C. Sánchez, O. Lock de Ugaz y A. Cáceres. CYTED. Guatemala, Julio 1993. 138 pp.
- Primera Reunión Nacional de Investigadores sobre Plantas Naturales de Uso Medicinal. E. Pérez Tuesta. CYTED, CONCYTED. Lima, Perú, 1993. 52 pp.
- Red Iberoamericana de Validação de Plantas Medicinais. RIVAPLAMED. Reunião de Constituçâo da RIVAPLAMED, Fortaleza-Ceará, Brasil. C. Sánchez, A. J. Lapa, G. S. Barrios. Viana. Septiembre, 1993. 111 pp.
- Directorio de la Subred Iberoamericana de Productos Naturales de Uso Medicinal. C. Sánchez y O. Lock de Ugaz. Lima, Perú. Diciembre de 1993. 82 pp.
- Proyecto X-1 Búsqueda de Principios Bioactivos en Plantas de la Región. CYTED. Marzo 1995. 50 pp.
- Manual de Técnicas de Investigación. Proyecto X-1. Marzo de 1995. 228 pp.
- Plantas Bajo Estudio por los Grupos de Investigadores. Proyecto X-1. CYTED. Marzo 1995. 190 pp.
- 270 Plantas Medicinales Iberoamericanas. M. Gupta (Editor). CYTED y SECAB. Editorial Presencia Ltda. Santafe, Bogotá, Colombia, agosto 1995. 617 pp. ISBN: 958-9206-50-6.
- 1ª Reunión de Coordinación Internacional. Proyecto X-2 Síntesis de Moléculas Bioactivas Análogos de Productos Naturales de Origen Iberoamericano. CYTED. Lima, Perú. Octubre 1995.
- Domesticación de Plantas Medicinales en Centroamérica. Colección Diversidad Biológica y Desarrollo Sustentable. 1. Especies Nativas (Memoria de la Reunión Técnica Centroamericana Domesticación de Plantas Medicinales de Centroamérica, 1994. Turrialba, Costa Rica. CYTED, OPS/OMS. 1994. 135 pp. Serie Técnica. Informe Técnico/CATIE, N_ 245.
- Situación de los Herbarios de Centroamérica y el Caribe 1996. Colección Diversidad Biológica y Desarrollo Sustentable. 4. Metodologías. Turrialba, Costa Rica. CYTED (Subprograma X. Química Fina Farmacéutica), CATIE Olafo, Universidad de Panamá y STRI. 96 pp. Serie Técnica. Informe Técnico Nº 280.
- Red Iberoamericana de Productos Naturales de Uso Medicinal, RIPRONAMED. Directorio. Febrero, 1997.
- Red Iberoamericana de Productos Fitofarmacéuticos (RIPROFITO). 1ª Reunión de Coordinación Internacional. CYTED. Antigua, Guatemala. Sept. 28-Oct. 01, 1995. 343 pp.
- Proyecto X-1: Búsqueda de Principios Bioactivos en Plantas de la Región. Informe Final. Roberto Pinzón. Jefe Internacional del Proyecto. 172 pp.
- Reunión de Coordinación 1996. Proyecto X-2 Síntesis de Moléculas Bioactivas Análogos de Productos Naturales de origen Iberoamericano.
- Reunión de Coordinación 1997. Proyecto X-2. Síntesis de Moléculas Bioactivas Análogas de Productos Naturales de Origen Iberoamericano. Taller de Trabajo: Objetivos Terapéuticos para el año 2000, Una Visión Latinoamericana. Sevilla, España. Septiembre 1997.
- Memoria del Curso sobre Producción de Fitomedicamentos en Panamá del 24 de noviembre al 5 de diciembre de 1997, con el coauspicio del Centro Internacional de Ciencia y Alta Tecnología (ICS/ONUDI), Trieste, SENACYT y la Universidad de Panamá. 670 pp.
- Taller Iberoamericano sobre Bioensayos Anticáncer y Antisida en el Descubrimiento de Nuevas Drogas Naturales, con el co-auspicio de la SENACYT, Instituto Nacional de Cáncer, Instituto Smithsonian de Investigaciones Tropicales y la Universidad de Panamá. Actividad conjunta con la RED CEN del Subprograma III. 9 - 13 de febrero de 1998. 450 pp.
- Memoria Curso Iberoamericano El descubrimiento del Medicamento. De la planta, la observación o la idea, al mercado. Universidad Nacional de Rosario, Argentina 9 -13 marzo 1998, 309 pp.

ción, aumentar la productividad y competitividad de las industrias y economías nacionales dentro de la comunidad iberoamericana, que consoliden las bases para una prosperidad duradera.

Proyecto Iberoeka IB-003 CRIFAPLAN "Cribado y Aislamiento de Principios Farmacológicamente activos de plantas iberoamericanas"

La agrupación de empresas alicantinas ASAC Pharmaceutical International, integrada por cinco laboratorios, desarrolla desde 1991 en colaboración con las empresas guatemaltecas DESHIDRAFARM y FARMAYA, un amplio programa de I+D junto a 11 centros de investigación españoles y extranjeros; así como con pueblos indígenas como Pastaza y otros de la Amazonia, el que tiene como principal objetivo, el rescate del uso de plantas medicinales de la región iberoamericana, seleccionadas previamente por estudios etnobotánicos.

Se trata de descubrir nuevos principios que incidan en las siguientes aplicaciones: antiinflamatoria, inmunomoduladora, antiviral, antiparasitaria y antitumoral. Adicionalmente, se pretende promover el cultivo y la producción agrícola de las plantas que revelen una utilidad farmacológica, contribuyendo de ese modo a la diversificación de las producciones agrícolas, haciendo rentables nuevos cultivos que sustituyan a otros más comunes o peligrosos.

Actualmente, ya se han obtenido los primeros resultados del Proyecto en su primera fase (1991-1994), materializados en diversos productos, patentes y registros farmacéuticos. Un ejemplo, es el dentrífico D-Bucal, que combina tres extractos de plantas, dos de las cuales tienen actividad microbiana y re-epitalizante en encías sangrantes; siendo el tercero, edulcorantes químicos utilizados habitualmente en las pastas de dientes.

Otro resultado del Proyecto es la solicitud de una patente para la obtención de extractos de Curcuma especie que ha demostrado una acción protectora frente al envejecimiento celular, mediante la técnica de extracción con fluidos en estado supercrítico, la menos contaminante conocida.

Se encuentra igualmente en el mercado una linea de productos de aceites vegetales ricos en ácidos grasos esenciales y otros poliinsaturados y un producto tópico con el mismo principio activo dirigido a pediatras, dermatólogos y ginecológos.

4.1. Publicaciones

La Tabla II indica las publicaciones del Subprograma: una de las publicaciones como resultado de cooperación internacional ha sido el libro titulado *270 Plantas Medicinales Iberoamericanas*, 1ª edición y consta de 617 páginas. Este libro incluye información sobre 270 plantas medicinales de 21 países iberoamericanos, pertenecientes a 119 géneros y 270 especies distribuidas en 82 familias. Para cada planta, la información fue actualizada con el uso de la base de datos NAPRALERT® hasta diciembre de 1993. Cada monografía presenta la siguiente información: familia botánica, nombre científico, sinónimos, nombres comunes en los países iberoamericanos, fotografía o dibujo, usos etnomédicos y modo de empleo, química, actividad biológica y farmacológica y sus referencias bibliográficas. Al final del libro se presentan los índices de familias botánicas, nombres científicos, comunes y de sinónimos, además de tablas y figuras. Dicha publicación fue posible gracias al apoyo de la Secretaría Ejecutiva del Convenio Andrés Bello y el Subprograma X.

4.2. Talleres y cursos

Desde el inicio del Subprograma se han organizado 29 cursos y talleres, la Tabla III desglosa los talleres y cursos iberoamericanos organizados.

4.3. Cooperación internacional

El Subprograma X ha podido lograr una colaboración efectiva con la OEA, UNESCO, OPS, UNIDO, IOCD, IFS y CATIE, organismos de cooperación internacional que también tienen programas en productos naturales. Especialmente con la celebración de eventos conjuntos, se han podido aprovechar al máximo los recursos del Subprograma. El Subprograma X tiene convenios vigentes con la Secretaría Ejecutiva del Convenio Andrés Bello y la Fundación Internacional para la Ciencia. Están en trámites los convenios de cooperación con el Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) y la Organización de las Naciones Unidas para el Desarrollo-Industrial (ONUDI). El Subprograma X ha estimulado la conformación y aprobación por la Unión Europea de una Red RELAPLAMED (Red Europea Latinoamericana de Plantas Medicinales) dentro del marco del Programa Alfa. Se ha aprobado un proyecto este año para la formación de 16 doctorados en la farmacología y química de productos naturales. Recientemente, se han establecido vínculos estrechos con el International Center for Science

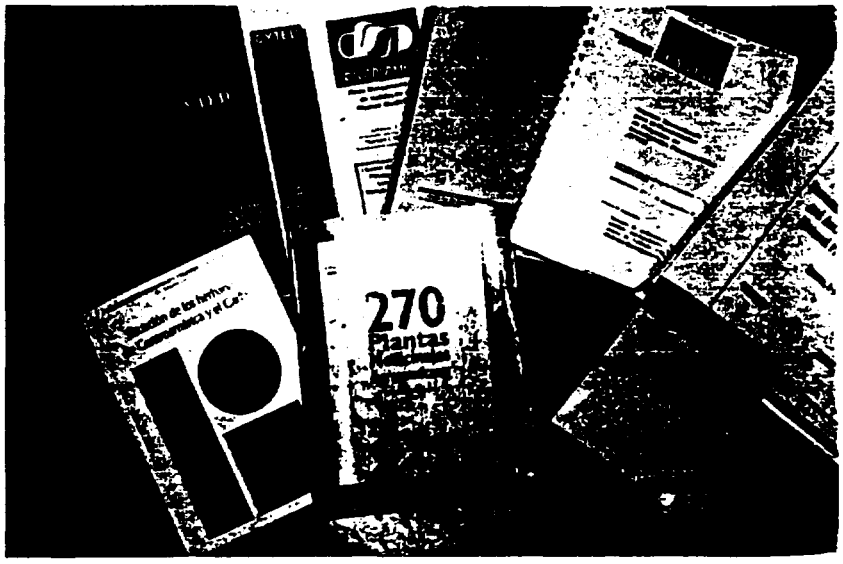


Tabla III

Cursos y talleres iberoamericanos ofrecidos por el Subprograma X

Nombre del Taller/Curso	N° Países	N° Participantes
Primer Curso-Taller sobre Bioensayos *in vitro* aplicados a Productos Naturales, agosto 1991. Panamá. Co-patrocinio de la O.E.A.	18	47
Curso-Taller "Bioensayos Quimioterapéuticos de Productos Naturales con Enfasis en actividad Antiviral". octubre,1992. Panamá.	12	27
Taller Centroamericano de Fitofarmacología. noviembre, 1992. El Salvador.	6	15
Curso sobre Biomodelos de Experimentación. Febrero, 1993, San José Costa Rica.	10	42
Taller de"Técnicas Cromatográficas Preparativas, Métodos Modernos de Separación y Aislamiento de Productos Naturales". marzo, 1993, Montevideo, Uruguay	17	38
Taller "Métodos Modernos Espectroscópicos Aplicados a Productos Naturales. Interpretación y Resolución de Problemas". junio, 1993, Valencia, España.	15	20
Taller "Técnicas de Estudios de Fármacos Activos a nivel de Inflamación y otros Procesos Mediados por Radicales Libres" julio,1993. Valencia, España.	12	12
Talleres sobre "Screening Antiviral y Antimicrobiano de Productos Naturales" octubre, 1993. Panamá.	16	30
Taller "Bioensayos Enzimáticos de Productos Naturales con Enfasis en Actividad Anticáncer", noviembre, 1993. Panamá.	18	37
Taller "Utilización de Productos Naturales en la Industria Farmacéutica" abril, 1994. La Habana, Cuba. Co-patrocinio de la ONUDI.	13	22
Taller "Resonancia Magnética Nuclear Aplicada a Productos Naturales", mayo, 1994. Lima, Perú.	15	16
Taller "Domesticación de Plantas Medicinales". junio, 1994. San José, Costa Rica. Co-patrocinio de CATIE.	6	15
Taller "Validación de Plantas Medicinales Utilizadas Popularmente en el Tratamiento de Disturbios Gastrointestinales". junio, 1994. Sao Paulo, Brasil.	12	12
Taller "Estrategias en el Aislamiento de Productos Naturales", agosto 1994. Panamá.	15	21
Taller "Usos y Cuidados del Animal de Laboratorio. Biomodelos", septiembre, 1994. La Habana, Cuba.	10	20
Curso Iberoamericano sobre Nuevos Avances en la Metodología de Screening Anticáncer e Informática en Productos Naturales, abril, 1995. Panamá.	17	20
Curso Iberoamericano sobre Quimioterapia Antiparasitaria: Malaria, Leishmania y Enfermedades de Chagas. Agosto, 1995. La Paz, Bolivia.	12	12
Taller Internacional sobre Ensayos Biológicos para Químicos de Productos Naturales., diciembre, 1995. Talca, Chile.	5	32
Curso Iberoamericano sobre Descubrimiento y Desarrollo de Fármacos basado en la Inmunomodulación de Productos Naturales, diciembre, 1995. Utrecht, Países Bajos.	15	15
Curso Iberoamericano de Validación de Plantas con Actividad Cardiovascular, febrero, 1996. Sao Paulo, Brasil.	12	12
Curso Iberoamericano de Validación de Plantas Medicinales con Actividad Sedativa Tranquilizante, julio, 1996. Coimbra, Portugal.	14	23
Curso sobre Inmunomodulación-Bioensayos. Diciembre, 1996. Madrid, España.	2	12
Simposio Internacional Conjunto IOCD-CYTED. Chemical, Biological and Pharmacological Properties of Medicinal Plants of the Americas, febrero, 1997.	62	202
Curso sobre la Química y Tecnología de Productos Naturales y Fitofármacos, agosto, 1997. Niteroi, Brasil.	15	28
Curso de Validación de Plantas Medicinales con Actividad en el Sistema Nervioso Central, octubre, 1997. Florianopolis, Brasil.	10	25
Seminario Taller sobre Producción de Fitomedicamentos. Noviembre de 1997. Panamá. Co-patrocinio del Centro Internacional de alta Tecnología ICS/UNIDO.	18	30
Curso Internacional sobre Farmacología Experimental, febrero, 1998. Panamá. Co-patrocinio de la Secretaría Nacional de Ciencia y Tecnología, SENACYT.	6	12
Seminario Taller Iberoamericano sobre Bioensayos Anticáncer y Anti-Sida en el Descubrimiento de Productos Naturales, febrero, 1998. Panamá. Con el apoyo del Instituto Nacional del Cáncer de Estados Unidos y la SENACYT.	15	37
Curso Iberoamericano "El descubrimiento del Medicamento, de la Planta, la Observación o la idea al Mercado", marzo, 1998. Rosario, Argentina.	11	51

and High Technology (ICS/ONUDI) Trieste para organizar talleres conjuntos.

Dentro del marco del convenio de cooperación entre la Secretaría Ejecutiva del Convenio Andrés Bello y el Subprograma X. Química Fina Farmacéutica, se financian proyectos de investigación en los países miembros del Convenio Andrés Bello y se apoyan las movilidades científicas. El componente de publicación de libros y manuales reviste una gran importancia.

4.4. Estrategias para el desarrollo futuro del Programa

El Subprograma X se ha consolidado y las actividades de sus diferentes redes y proyectos de investigación precompetitivos han permitido diagnosticar la situación y necesidades de investigación en el campo de la química fina farmacéutica y se han logrado resultados tangibles e intangibles importantes.

Para el futuro, el subprograma:

1. Se concentrará en proyectos de investigación precompetitivos en temas más puntuales y con grupos más pequeños y más especializados para lograr resultados más concretos y de alto nivel que tengan impacto en la región iberoamericana. Así por ejemplo se prevén los siguientes nuevos proyectos y redes:

- P.I.P. X.5 Búsqueda, obtención y evaluación de nuevos agentes antiparasitarios.

- P.I.P. X.6 Control de calidad de productos herbarios de venta en Iberoamérica.

- P.I.P. X.7 Búsqueda, obtención y evaluación de nuevos agentes antiinflamatorios.

- P.I.P. X.8 Obtención de agentes antituberculosos de origen natural.

- Red X.D. Red Iberoamericana de Productos Naturales de uso medicinal de Origen Marino

2. Se hará un mayor acercamiento a otros Subprogramas como por ejemplo el de la biodiversidad y de biotecnología para generar proyectos de investigación conjuntos.

3. Se intentará lograr mayores contactos con los científicos europeos para participar en los programas Alfa e INCO de la Unión Europea, para que el Programa CYTED sirva como puente para la cooperación con Europa.

4. Se dará un mayor énfasis en la generación de proyectos de innovación Iberoeka, y la participación del sector industrial se irá aumentando en las Redes y proyectos para que sea más fácil la transferencia de los resultados al sector productivo.

5. Se seguirá desarrollando el programa de la movilidad de científicos y técnicos como la base de la cooperación entre los grupos más avanzados con los relativamente más necesitados además de servir como el método más adecuado para preparar los recursos humanos que se necesitan. Se explorará la cooperación con otros Subprogramas.

6. Se priorizará la colaboración con organismos regionales e internacionales interesados en el tema del Subprograma para hacer más eficientes los presupuestos y los esfuerzos que uno y otros realizan en el campo de interés mutuo. En este sentido se logrará convenios de cooperación.

7. Se continuará con la organización de los talleres, que redunden en beneficio de los científicos de la región y los capaciten en las técnicas más avanzadas relevantes a los nuevos proyectos de investigación.

8. Se dará un énfasis especial en la publicación de libros especializados en diversos temas del Subproyecto que llenen el vacío existente. Ha sido muy exitosa por ejemplo la publicación del libro *270 Plantas Medicinales Iberoamericanas,* y se hará una versión más ampliada y en CD-ROM. Así mismo, se publicará un Manual de Técnicas de Cultivo e Industrialización de Plantas Medicinales Iberoamericanas y monografías especializadas

9. Se ha programado la publicación de la segunda edición del libro *500 Plantas Medicinales Iberoamericanas* y en CD-ROM. Asimismo, se publicará un libro sobre *Manual de Cultivo de Plantas Medicinales* y *Monografías específicas de Plantas Iberoamericanas* y *Manual de Tecnología Farmacéutica de Fitofármacos.*

4.5. Conclusiones

El gran éxito del CYTED, y especialmente del Subprograma X radica en haber logrado un mayor acercamiento entre los grupos iberoamericanos de investigación aprovechamiento de las sinergias estimuladas entre ellos y ha dado por resultado la capacitación de científicos de países menos desarrollados en una temática determinada por la generosidad de los países más desarrollados.

El Subprograma X se ha consolidado en el ámbito Iberoamericano. Se han establecidos vínculos muy fuertes entre los grupos que trabajan en cooperación sin egoismo con el objetivo común de buscar medicamentos de la flora iberoamericana o por modificación química que respondan a las necesidades de la región. El Subprograma es un equipo de investigadores que tienen la convicción y el convencimiento de que sólo través de la cooperación internacional e integración regional podremos lograr nuestros objetivos.

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de
CYTED
PROGRAMA IBEROAMERICANO
DE CIENCIA Y TECNOLOGÍA
PARA EL DESARROLLO
PROGRAMA DE LAS CUMBRES IBEROAMERICANAS
DE JEFES DE ESTADO Y DE GOBIERNO
Número 11
Junio 1998

LA XIV Asamblea General de CYTED, celebrada en Buenos Aires, Argentina, en Noviembre de 1997, aprobó la creación de dos Grupos de Trabajo: 1) *Areas de Actividades Temáticas* (coordinado por Venezuela e integrado por Brasil, Cuba, España, Honduras y Perú) y 2) *Modos de funcionamiento* (coordinado por México y conformado por Argentina, Chile, Colombia, España, Panamá y Uruguay), ambos bajo la Coordinación General de España, para preparar un documento a ser debatido por el Consejo Técnico Directivo y su aprobación en una Asamblea General Extraordinaria, en el primer semestre de 1998.

Los trabajos de ambos grupos han tenido su culminación en la XV Asamblea General Extraordinaria en la que se ha aprobado el documento de *«Reestructuración del Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo (CYTED)»* que marca las líneas futuras de desempeño con una *visión* clara y una nueva orientación en cuanto a los mecanismos, formas de actuación, procedimientos para la cofinanciación, etc., que permitirán un enfoque más adecuado a las necesidades actuales de la Región de las actividades de CYTED.

El documento aprobado por la XV Asamblea General Extraordinaria, en su versión final de 4 de Junio, contiene una serie de materias relevantes de las que, en esta ocasión, tan sólo se va a hacer una mención muy somera que permita una visión global.

La justificación primera de este giro en las actuaciones del Programa tienen su origen en la creciente globalización en el ámbito de la Ciencia y la Tecnología, que resulta ser un proceso en pleno desarrollo, mostrando entre sus principales rasgos el crecimiento acelerado de acuerdos de cooperación entre empresas, investigadores, grupos de investigación, instituciones de I+D y organismos nacionales de ciencia y tecnología de los diferentes países de la Región. La cooperación internacional en ciencia y tecnología es, entonces, un componente sustantivo de la relación entre los pueblos y los gobiernos, como medio complementario, pero significativo, de apoyo a los esfuerzos nacionales de desarrollo e instrumento apropiado para promover la solidaridad regional.

En el análisis de la problemática actual se ha tenido en cuenta la evolución de los Sistemas de Ciencia y Tecnología de los países iberoamericanos hacia un enfoque que tiende a valorizar las actividades en este campo por su impacto económico y social y no únicamente por las implicaciones científicas, sin por ello olvidar la necesidad de consolidar los aspectos positivos y contrarrestar las disfunciones existentes en el CYTED. Se ha hecho un énfasis especial en la debilidad de los instrumentos de gestión y seguimiento de las actividades; así como, en el proceso de evaluación, haciendo hincapié en la evaluación externa.

Por otra parte, se han analizado a fondo las posibilidades de agrupación de los Subprogramas en grandes Áreas Temáticas y las interrelaciones entre ellos, y se han definido procedimientos para la creación y supresión de Subprogramas, Redes Temáticas y Proyectos de Investigación.

También se ha procedido a una clarificación de la financiación del Programa, alcanzándose acuerdos importantes en relación con la cofinanciación de las actividades del mismo mediante las contribuciones de los Organismos Signatarios al CYTED y los procedimientos sobre cómo llevar a cabo esta aportación. La gestión financiera del Programa incluye los criterios que regirán para la elaboración del presupuesto.

Respecto a los órganos de decisión y gestión, se ha aprobado una redefinición de las áreas competentes y de responsabilidad de la Asamblea General, del Consejo Técnico Directivo, de los Organismos Signatarios y de la Secretaría General. Es de destacar que la Asamblea General adquiere una nueva dimensión al responsabilizarse del Plan de Actuación Estratégica a cinco años; así como, el plan operativo anual y la aprobación del presupuesto. En el Consejo Técnico Directivo estarán representados todos los países y sus funciones, además de las que ahora tenía, están muy relacionadas con la presentación de propuestas a la Asamblea General. Por su parte, a los Organismos Signatarios les corresponde una serie de actividades de cooperación al más alto nivel político y de difusión y apoyo al Programa, muy particularmente, en cuanto a las actividades que se desarrollan en cada país. La Secretaría General está integrada por un Secretario General, cuya sede permanente estará en España, y un Secretario Adjunto, a propuesta de un país miembro de CYTED. Tanto el uno como el otro deberán responder a unos determinados perfiles y tendrán unas funciones claramente establecidas, al igual que el resto de las personas integradas en la misma.

Los temas en cartera para la próxima Asamblea General son, entre otros, el proceso de evaluación, con énfasis en la evaluación externa y el análisis estratégico del Programa y la adecuada reorientación de los Proyectos de Innovación IBEROEKA.

Sin olvidar en ningún momento lo que el Programa CYTED ha supuesto para Iberoamérica en la colaboración en el ámbito de la Ciencia y la Tecnología en estos últimos catorce años, es preciso felicitarse ante la capacidad de autoanálisis y de generación de nuevas propuestas de reestructuración que, gracias al esfuerzo de todos cuantos han intervenido en la elaboración de las mismas, ha permitido a los miembros de la XV Asamblea la aprobación de un documento de futuro sobre el Programa más emblemático de las Cumbres de Jefes de Estado y de Gobierno y de mayor repercusión actual en la Región Iberoamericana.

XXVIII REUNIÓN DEL CONSEJO TÉCNICO DIRECTIVO DEL PROGRAMA CYTED

SE celebró en Santiago, Chile, el 1 de Abril de 1998 y participaron los delegados de Brasil, Chile, que la presidió, Cuba, España, Guatemala, México, Paraguay, Uruguay y Venezuela; la Secretaría General del Programa y como invitados los delegados de Argentina, Colombia, Honduras y Panamá. Fue inaugurada por D. Mauricio Sarrazin Arellana, Presidente de la Comisión Nacional de Investigaciones Científicas y Tecnológicas (CONICYT) del país sede.

Los temas tratados fueron: 1) *Presentación a cargo del Secretario General del documento* de reestructuración del Programa CYTED y posteriormente expuesto por el delegado de España, D. Gonzalo León de la Oficina de Ciencia y Tecnología de Presidencia del Gobierno; 2) Proceso hasta la Asamblea General Extraordinaria; 3) Próxima Reunión de Coordinadores Internacionales de Subprogramas; 4) Mecanismo de evaluación de las actividades de CYTED; 5) Situación de los Proyectos de Innovación IBEROEKA; 6) Redes Temáticas y Proyectos de Investigación aprobados en el XXVII CTD; 7) Agendas de la XV Asamblea General Extraordinaria y la IV Conferencia Iberoamérica-Unión Europea (Madrid, España, Junio de 1998); 8) Conferencia Científica de la VIII Cumbre Iberoamericana de Jefes de Estado y de Gobierno (Oporto, Portugal, Septiembre de 1998). El Secretario Adjunto 1998 CYTED, D. João Melo Borges, del Instituto de Cooperación Científica y Tecnológica Internacional de Portugal, cuya designación se reseña en el apartado correspondiente de esta edición, expuso sobre los preparativos y coordinación de los temas realizados hasta el momento en relación a este evento.

Participantes de la XXVIII Reunión del CTD.

XXIX REUNIÓN DEL CONSEJO TÉCNICO DIRECTIVO DEL PROGRAMA CYTED

SE efectuó en Madrid, España, el 1 de Junio de 1998, en la sede de Consejo Económico y Social. Participaron los delegados de: Brasil, Chile, Cuba, Guatemala, España, que la presidió, México, Paraguay, Uruguay y Venezuela; la Secretaría General del Programa y como invitados los delegados de Argentina, Costa Rica, el Coordinador del Subprograma XI Tecnología y Conservación de Alimentos, Doctor Efrén Parada (México). Presidió las sesiones de trabajo del CTD, D. Gonzalo León, de la Oficina de Ciencia y Tecnología de la Presidencia del Gobierno de España.

Los temas tratados fueron: 1) Análisis y aprobación de prórrogas de Redes Temáticas; 2) Ratificación de la aprobación por los Organismos Signatarios de las Redes Temáticas y Proyectos de Investigación Precompetitiva y sus Coordinadores Internacionales y Jefes, respectivamente, aprobados en la XXVII Reunión del CTD; 3) Informe de la situación presupuestaria; 4) Análisis de nuevas Redes Temáticas y Proyectos de Investigación Precompetitiva, propuestas de sus Coordinadores Internacionales de Redes y Jefes de Proyectos; 5) Certificación de Proyectos de Innovación IBEROEKA; 6) Presentación y discusión del Documento «Propuesta de Reestructuración del Programa CYTED».

Las actividades aprobadas por la XXIX Reunión del CTD, son:

1. Aprobación de **nuevas Redes Temáticas** y designación de los **Coordinadores Internacionales** respectivos:

RT VI.E	«Red Iberoamericana de Solarimetría (RISOL)». D. Hugo Grossi (Argentina)
RT VII.F	«Red Iberoamericana de Tecnologías Ultrasónicas (RITUL)» D. Lorenzo Leija Salas (México)
RT VII.G	«Red Iberoamericana de Automatización de los Procesos de Mecanizado (RIBAMEC)» D. Áureo Campos Ferreira (Brasil)
RT VIII.G	«Red Iberoamericana de óxidos semiconductores y materiales relacionados en aplicaciones ambientales ópticas (RIOMAO)» D. Miguel A. Blesa (Argentina)
RT VIII.H	«Red Iberoamericana de Aplicaciones Interdisciplinares de Materiales» (RAIMA) D. Manuel Torres Hernández (España)
RT X.D	«Red Iberoamericana de Búsqueda y Desarrollo de Nuevas Sustancias Bioactivas de Origen Marino (RIBUDESMAR)». D. Agustín Pérez Aranda (España)
RT XIV.C	«Red Iberoamericana de Transferencia y Capacitación Tecnológica para la Vivienda de Interés Social (RITYCVIS)». Por confirmar
RT XIV.E	«Red Iberoamericana para el Mejoramiento de la Calidad de Vida en los Asentamientos Rurales (RICVAR)» D. Jorge González Claveran (México)

La Coordinación Internacional de la Red VII.D «Red Iberoamericana Sistemas de Informática Industrial» ha sido asumida por D. Gerardo Fernández López (Venezuela) quien reemplaza en dicha coordinación a D. Luis Alonso Romero (España).

2. Aprobación de **nuevos Proyectos de Investigación Precompetitiva** y **Jefes de Proyectos**

PIP II.6	«Investigación y Desarrollo Tecnológico del cultivo de moluscos pectínidos en Iberoamérica». D. Alfonso N. Maeda Martínez (México)
PIP II.7	«Cultivo de Gasterópodos Tropicales y de Agua Fría». Dª Dalila Aldana Aranda (México)
PIP V.6	«Desarrollo de Adsorbentes para la remoción de metales pesados en efluentes industriales». D. Carmelo Bolívar González (Venezuela)
PIP VI.5	«Abastecimiento de agua en áreas rurales mediante bombeo fotovoltaico». D. Naum Fraidenraich (Brasil)
PIP VII.15	«Aplicaciones e implementaciones de Redes Neuronales en Reconocimiento de Patrones». D. Luis Alonso Romero (España)
PIP VIII.8	«Síntesis de Nuevos Materiales Poliméricos: hidrogeles biocompatibles y su aplicación en la liberación controlada de fármacos». D. Issa Katime (España)
PIP X.5	«Búsqueda, obtención y evaluación de nuevos agentes antiparasitarios». Por confirmar
PIP XII.2	«Conservación y Desarrollo de Reservas de Biósfera Iberoamericanas». D. Eduard Muller (Costa Rica)
PIP XIV.5	«Con techo: Soluciones de techos para viviendas de muy bajo costo». D. Pedro Lorenzo Galligo (España)

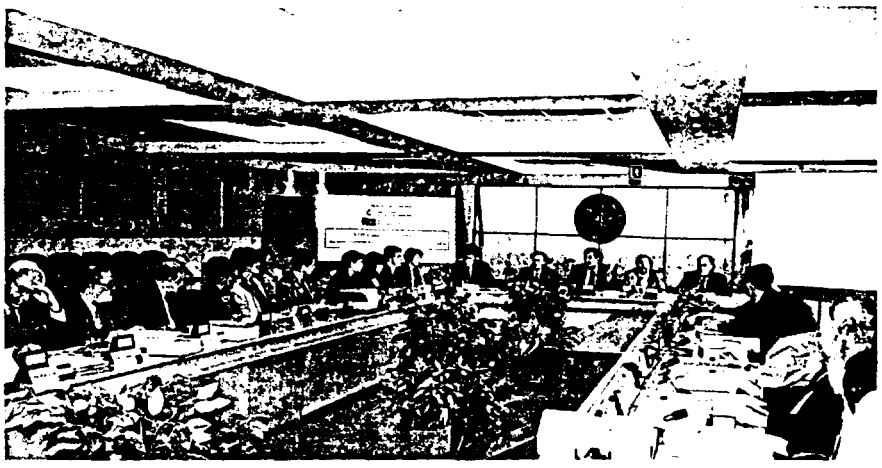
Se aprobó la certificación de 17 nuevos Proyectos de Innovación IBEROEKA que se reseñan en el apartado correspondiente de esta edición.

XV ASAMBLEA GENERAL EXTRAORDINARIA DEL PROGRAMA CYTED

SE celebró en Madrid, España, los días 3 y 4 de Junio de 1998, en la sede del Consejo Económico y Social, con la participación de los representantes de los Organismos Signatarios de los 21 países que integran el Programa y de la Secretaría General de CYTED. Fue presidida por D. Fernando Aldana, Director de la Oficina de Ciencia y Tecnología, de Presidencia del Gobierno de España.

La Asamblea General Extraordinaria examinó la marcha del Programa y los principales temas sobre su desarrollo y perspectiva. Entre los temas de Agenda que fueron tratados ampliamente y en los que se adoptaron los acuerdos respectivos, figuran: *1) Presentación y discusión del documento «Propuesta de Reestructuración del Programa CYTED»; 2) Papel de los Organismos Signatarios españoles en el futuro del Programa CYTED; 3) Presentación y discusión de los procedimientos para el compromiso formal de los Organismos Signatarios en el presupuesto CYTED 1999; 4) Presentación por España de la propuesta de Secretario General del Programa; 5) Presentación y discusión del contenido de la Conferencia Científica «Ciencia Global, Intereses Locales» de la VIII Cumbre Iberoamericana de Jefes de Estado y de Gobierno (Oporto, Portugal 21 y 22 de Septiembre de 1998), por D. Armando Trigo de Abreu, Presidente del Instituto de Cooperación Científica y Tecnológica Internacional de Portugal; 6) Presentación de la nueva organización española en Ciencia y Tecnología por D. Gonzalo León, Subdirector General de Planeamiento y Seguimiento de la Oficina de Ciencia y Tecnología; 7) Discusión preliminar sobre los Proyectos de Innovación IBEROEKA en el Programa CYTED y presentación por D. José Enrique Román, Subdirector General de Programas Internacionales del Centro para el Desarrollo Tecnológico Industrial de España, 8) Análisis de la problemática de la evaluación y seguimiento de las actividades del Programa, 9) Propuesta de temas para tratar en la próxima Asamblea General.*

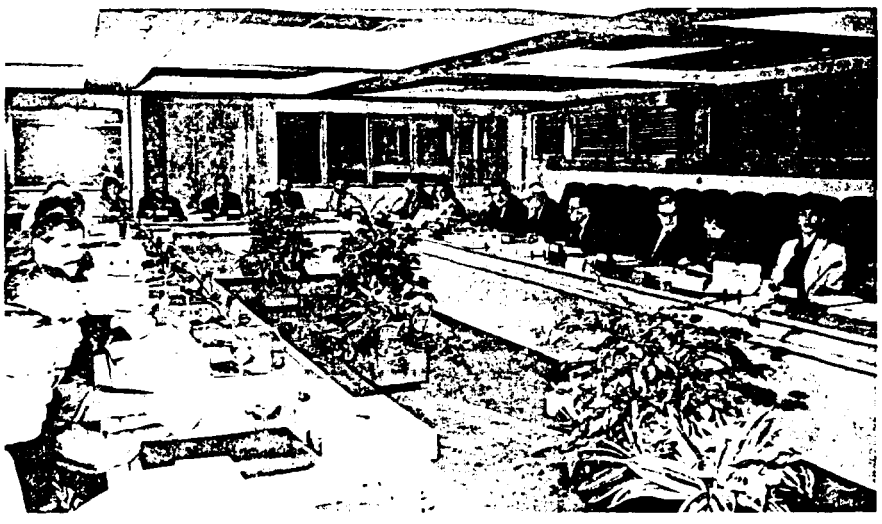
La propuesta del Doctor José Antonio Cordero, como Secretario General del Programa CYTED fue aceptada por unanimidad por los delegados de la Asamblea y durante su intervención expresó su profunda identificación con el Programa desde su experiencia y trayectoria de participación en el CYTED; presentó la nueva estructura de la Secretaría General; asumió compromisos ante la Asamblea General como los de realizar un análisis crítico anual sobre los procedimientos y métodos que se utilizan en CYTED, a fin de lograr una mejora continua de la metodología que facilite la cooperación en este marco; evaluar la metodología empleada en una reunión conjunta de Coordinadores Nacionales y Coordinadores Internacionales de Subprogramas para informar posteriormente a la Asamblea General; evaluar los resultados cuantificables obtenidos de dicha cooperación; buscar figuras novedosas que refuercen la transferencia de las tecnologías desarrolladas en las Redes y Proyectos hacia la industria y servicios de la sociedad de los países iberoamericanos.



Inauguración de la Asamblea, presidida por D. Fernando Aldana, Director General de la Oficina de Ciencia y Tecnología; D. Jesús Gracia Aldaz, Director General del Instituto de Cooperación Iberoamericana (ICI) de la Agencia Española de Cooperación Internacional; D. Luis Gómez-Acebo, Subdirector General del ICI; D. Gonzalo León, Subdirector General y D. Francisco Ferrándiz, Vocal Asesor de la Oficina de Ciencia y Tecnología.

Merecen destacarse la designación de D. Francisco Ferrándiz, como Coordinador Nacional CYTED de España, cuya elección fue ampliamente felicitada y aplaudida por la Asamblea; así como, el reconocimiento de la labor realizada por Dª. Alba Rodríguez, Jefa del Servicio del Programa CYTED y de otras personas que sistemáticamente han resultado ser el apoyo máximo al Programa.

La XXX Reunión del Consejo Técnico Directivo, la XVI Asamblea General del Programa CYTED y la Reunión del Grupo Ad-Hoc Iberoamérica-UE se celebrarán en Guatemala, en Noviembre de 1998.



Sesiones de trabajo de la Asamblea.

IV CONFERENCIA IBEROAMÉRICA-UNIÓN EUROPEA DE COOPERACIÓN CIENTÍFICA Y TECNOLÓGICA

LA IV Conferencia Iberoamérica-Unión Europea de Cooperación Científica y Tecnológica - IBERUE, se llevó a cabo en Madrid, España, el 2 de Junio de 1998 y fue presidida por D. Fernando Aldana, Director de la Oficina de Ciencia y Tecnología de Presidencia del Gobierno de España; D. Juan Carlos Del Bello, Secretario de Ciencia y Tecnología de Argentina, y D. Jorma Routti, Director General de Ciencia, Investigación y Desarrollo de la Comisión Europea. Participaron los delegados de Argentina, Bolivia, Brasil, Chile, Colombia, Costa Rica, Cuba, Ecuador, El Salvador, Guatemala, Honduras, México, Panamá. Paraguay, Perú, República Dominicana, Uruguay y Venezuela y como Observadores Europeos España y Portugal.

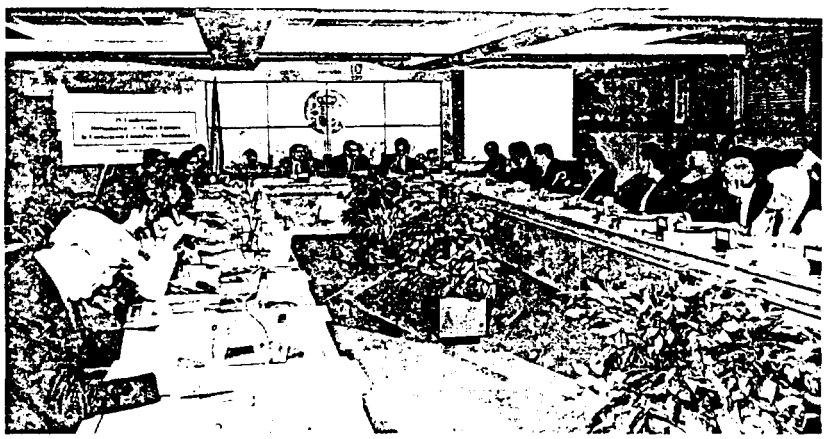
Los temas tratados versaron sobre la cooperación desarrollada en el IV Programa Marco; así como, los avances en la definición del V Programa Marco y las perspectivas de colaboración con América Latina. Durante el debate los países latinoamericanos manifestaron su interés por continuar el diálogo para el fortalecimiento de la colaboración en ciencia y tecnología y subrayaron una serie de aspectos positivos alcanzados, reafirmando los planteamientos formulados en las reuniones anteriores, en particular, la creación de un espacio específico para la cooperación con América Latina. Los representantes europeos manifestaron que para alcanzar el objetivo de construir dicho espacio en el V Programa Marco, será necesario contar con apoyo al más nivel político.

Las conclusiones de la IV IBERUE fueron las siguientes:

1. Ambas Partes coincidieron en la necesidad de crear un Comité de Coordinación en el cual se continuará el diálogo para, entre otros aspectos, definir áreas y mecanismos de cooperación, en cuyo seno participarán representantes de los países miembros de la Unión Europea y de los países de América Latina.
2. Como un posible modelo a seguir, las Partes mencionaron el Comité de Seguimiento que existe para la cooperación en ciencia y tecnología con los países terceros Mediterráneos.
3. A fin de poner en marcha dicho Comité, las Partes convinieron que éste podría iniciar su trabajo, en una primera fase, de manera informal. En este sentido, el representante de la Unión Europea mencionó la existencia de una experiencia similar con la República Popular China.

Sesiones de trabajo de la Conferencia.

4. Los países Latinoamericanos se comprometieron a sensibilizar sobre esta necesidad a sus contactos bilaterales en los Estados Miembros de la Unión Europea. Los representantes de España y Portugal en la Conferencia ofrecieron apoyar esta iniciativa y promoverla en los diversos foros comunitarios.
5. Las Partes coincidieron en la importancia de realizar talleres temáticos para definir las prioridades de la cooperación.
6. Dada la urgencia para la elaboración del Plan de Trabajo del Programa INCO, los representantes comunitarios solicitaron a los países Latinoamericanos el envío de temas de interés para la cooperación. Asimismo, solicitaron el envío de propuestas de expertos, que deberán tener conocimientos sobre el tema respectivo y su potencial utilización; así como, sobre las políticas nacionales y/o regionales en la materia.
7. Con el objetivo de promover la presentación de un mayor número de proyectos coordinados por científicos Latinoamericanos, los representantes comunitarios solicitaron a los Organismos Nacionales de Ciencia y Tecnología estimular esta opción. Asimismo, el representante comunitario mencionó que en la documentación de la Convocatoria se incluirá una redacción en este sentido, para reforzar esta posibilidad.
8. Respecto a la participación por parte de equipos latinoamericanos en otros programas del Programa Marco, también se planteó la necesidad de promover esta opción, por parte de los países Latinoamericanos, a través de sus contactos bilaterales con los Estados Miembros de la Unión Europea.
9. Los países Latinoamericanos manifestaron su interés en que se movilicen mecanismos complementarios para el fortalecimiento de la cooperación en ciencia y tecnología, en particular para financiar su participación en los programas comunitarios de I+DT abiertos a terceros países.



Sesiones de trabajo de la Conferencia.

NOTICIAS DE LOS ORGANISMOS SIGNATARIOS

EN este apartado se mencionan las actividades desarrolladas por los Organismos Signatarios del Programa CYTED durante el primer semestre de 1998, así como el anuncio de las próximas a efectuarse en el segundo semestre de 1998.

Secretaría de Ciencia y Tecnología, SECyT (Argentina)

• **Aprobación e incremento del 15% del Presupuesto 1998** para las actividades científico- tecnológicas. Puesta en funcionamiento del Programa de Crédito Fiscal, contenido en el Plan Nacional Plurianual de C y T, que permitirá asignar por convocatoria pública un cupo de crédito fiscal para proyectos I + D (Investigación Básica, Investigación Aplicada, Investigación Tecnológica Precompetitiva y Adaptaciones y Mejoras), solicitados por empresas que cuenten con un grupo de investigación propio o contratado, por Agrupaciones de Colaboración o por Unidades de Vinculación Tecnológica que dispongan del aval empresario. Además, se suscribió con el Banco de la Nación Argentina un contrato de fideicomiso por el cual se creó el Fondo Fiduciario para la Promoción Científica y Tecnológica.

• El Presidente de la República Argentina, Doctor Carlos Saúl Menem, junto con el Secretario de Ciencia y Tecnología, Licenciado Juan Carlos Del Bello, participaron en Febrero, en Egipto, de la inauguración oficial de un Reactor Nuclear fabricado e instalado por la empresa argentina INVAP.

• Creación de la **Comisión Interamericana de Ciencia y Tecnología**, aprobada en la Tercera Reunión Ordinaria del Consejo Interamericano para el Desarrollo Integral (CIDI/OEA), en Buenos Aires, los días 25 y 26 de Marzo. Esta iniciativa fue promovida por Argentina en la Reunión del MERCOCYT (Programa Mercado Común del Conocimiento CyT) celebrada en Washington D.C., USA, en Febrero. La Comisión permitirá jerarquizar y encuadrar institucionalmente el diálogo interamericano en materia de desarrollo científico e intercambio y transferencia de tecnología; se podrán identificar actividades y proyectos multilaterales de cooperación y fortalecer la presentación de propuestas de actividades y proyectos.

• Visita de una misión de expertos de la Comunidad Europea, el 13 de Marzo. En Noviembre se recibirá la visita de la Comisaria para la Ciencia, Investigación y el Desarrollo de la UE, Dª Edith Cresson, ocasión en la que se firmaría el acuerdo UE/Argentina dentro del 5º Programa Marco de la Unión Europea, a iniciarse en 1999.

• Inauguración del **Centro Regional de Investigaciones Científicas y Transferencia Tecnológica de La Rioja (CRILAR)**, perteneciente al CONICET, el 23 de Marzo, en Anillaco, provincia de La Rioja, con el fin de promover el desarrollo en las áreas de biociencias, geociencias, suelo y agua, sobre la base de proyectos multidisciplinarios.

• **XVI Reunión Especializada en Ciencia y Tecnología (RECyT) del Mercosur**, en Buenos Aires, con la participación de los países integrantes: Argentina, Brasil, Paraguay y Uruguay y por primera vez de delegaciones de Chile y Bolivia. Las distintas Comisiones Temáticas informaron sobre los avances en sus respectivas áreas, destacándose el consenso en la identificación de temas de capacitación y en líneas de investigación en Medio Ambiente y en Alimentos. Se aprobó el Proyecto INTERSUR, que implementará una red de alta velocidad que vincule en forma directa a los países miembros y asociados al Mercosur. Junto con la Red de Comunicaciones se desarrollarán aplicaciones sobre la educación, la salud, la ciencia y la tecnología que promuevan el desarrollo de la región. Se inició la elaboración de un Catálogo de Bases de Datos y se procedió al diseño de una Base de Datos sobre sistemas de información en medio ambiente en el ámbito del Subgrupo de Trabajo 6 Medio Ambiente -Grupo Mercado Común, Secretaría de Recursos Naturales y Desarrollo Sustentable. Se presentó ante la UNESCO un Proyecto Piloto para el Banco de Datos Terminológicos del Mercosur. Existe la posibilidad de que el IDRC -International Development Research Center/Canadá-, participe en el financiamiento de algunos de los proyectos de la RECyT.

• Visita de la Secretaria de Estado para la Cooperación Internacional del Ministerio de Ciencia y Tecnología de la República de Eslovenia, Dª. Verica Trstenjak, con una importante delegación científica. Se celebraron reuniones de trabajo con miembros de la SECyT y tomaron contacto con la comunidad científica local visitando centros y laboratorios a fin de promover la realización de proyectos de investigación en ámbitos de interés recíproco.

• Actividades de la **Agencia Nacional de Promoción Científica y Tecnológica:** 1) Convenio suscrito el 24 de Marzo, con la empresa SanCor, destinado a promover la investigación científica y tecnológica, mediante la apertura de convocatorias a concursos anuales orientados a áreas de la industria láctea. Los proyectos serán evaluados por expertos y los seleccionados recibirán un subsidio aportado en partes iguales por ambas instituciones; 2) Acuerdo suscrito el 1 de Abril, con la Fundación Green Cross Argentina para realizar un concurso público y abierto de proyectos de investigación científica y tecnológica, dirigidos a favorecer el desarrollo sustentable y la mejora del medio ambiente; 3) Asociación con la Comisión de Investigaciones Científicas de la Provincia de Buenos Aires para realizar un programa concertado de actividades definidas conjuntamente y financiar proyectos en las siguientes áreas: agua para consumo humano, alimentos orgánicos, forestación y madera, pescado fresco y congelado, rocas ornamentales y arcillas, prevención en salud humana y turismo cultural y ecológico; 4) Apertura del Primer Concurso de Consejerías Tecnológicas, programa incorporado en el Plan Nacional Plurianual de C y T 1998-2000, que será administrado por el Fondo Tecnológico Argentino -FONTAR-. Se seleccionaron 33 proyectos que involucran a 213 empresas y 201 consejeros tecnológicos. El programa apunta a fortalecer el desempeño de las pequeñas y medianas empresas brindándoles servicios especializados para diagnosticar problemas tecnológicos y colaborar en su solución. Estos servicios son prestados por equipos de profesionales, integrados por un Director Experto que coordina a un grupo de consejeros tecnológicos, quienes durante 10 meses trabajarán en empresas; 5) Inauguración el 15 de Mayo, del Centro de Investigación y Desarrollo para el Uso Racional de la Energía (CIPURE) del Instituto Nacional de Tecnología Industrial, que contribuirá al mejoramiento de la competitividad fabril, mediante la minimización y la reducción del impacto ambiental. Se concretó mediante un acuerdo entre los gobiernos de Japón y Argentina. En ese marco la SECyT, a través del Fondo Tecnológico Argentino (FONTAR), otorgó un crédito equivalente al 49% del total de la inversión; 6) Las evaluaciones de la convocatoria a Proyectos de Investigación Científica y Tecnológica (PICT 97) en las 14

áreas del conocimiento, aprobó la financiación de 703 proyectos, de las 2.588 presentaciones.

Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPQ (Brasil)

• **Entrega del título de «Doctor Honoris Causa» al Doctor José Galizia Tundisi,** Presidente del Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ), por la Universidad de Southampton, UK, el próximo 8 de Julio. Desde esta Secretaría General y por extensión de la comunidad científica y tecnológica iberoamericana, nuestras felicitaciones al Doctor Galizia Tundisi por esta condecoración que expresa el reconocimiento a su trayectoria profesional.

Ministerio de Ciencia, Tecnología y Medio Ambiente. (Cuba)

• **Seminario Internacional IBERGECYT 98,** celebrado los días 26 y 27 de Mayo, bajo el auspicio del Ministerio de Ciencia, Tecnología y Medio Ambiente de la República de Cuba, y con el apoyo del Subprograma XVI de CYTED.

• **VI Encuentro Nacional de Gestión Tecnológica,** celebrado los días 28 y 29 de Mayo y **Encuentro de Negocios para Nuevos Productos y Tecnologías,** el día 29 de Mayo, eventos que persiguen el objetivo de contribuir a incentivar la gestión tecnológica en Cuba.

Consejo Nacional de Ciencia y Tecnología, CONACYT (El Salvador)

• Designación del Ingeniero Carlos Roberto Ochoa Córdova, como Director Ejecutivo del Consejo Nacional de Ciencia y Tecnología, desde el mes de Mayo.

• **Creación de una Unidad de Vinculación Universidad-Empresa** en la Universidad de El Salvador, con apoyo del CONACYT. Está en elaboración un Programa de Apoyo a la Vinculación, coordinado por el CONACYT, a iniciarse en el tercer trimestre de este año. Se participa y apoya el Proyecto «Fundación de Universidad-Empresa» del Programa Nacional de Competitividad.

• Ejecución de la primera fase del Proyecto OEA-CTCAP **«Fortalecimiento del Desarrollo Científico y Tecnológico de Centro América y Panamá»**, que comprende las siguientes acciones: 1) Proyecto Subregional de indicadores de ciencia y tecnología y el proceso de estudio de la política científica y tecnológica; 2) Fortalecimiento de la capacidad de gestión tecnológica; 3) Fortalecimiento de la capacidad física operativa para interactuar entre los miembros de la CTCAP; 4) Fortalecimiento del sistema de centros de servicios tecnológicos para la micro y pequeña empresa.

• Ejecución del Proyecto **«Formación de Recursos Humanos de Centro América y Panamá»** CTCAP-Universidad de Chile. Su objetivo es concordar, de acuerdo a los intereses de la región, las áreas prioritarias de trabajo común, en materia de Postgrado y Postítulo, en: biología y biotecnología, ingeniería electrónica, telecomunicaciones y computación con especialidad en software, agricultura e ingeniería forestal, gestión de la tecnología, desarrollo de la capacidad de investigación, acuicultura y minería. Del 22 al 26 de Junio se celebrará en San Salvador, la Reunión de Coordinación del Sub Taller de Biología/Biotecnología y la Reunión de Coordinación de la Comisión CTCAP-Universidad de Chile.

• Inauguración del **Centro de Desarrollo de la Industria de Empaque y Embalaje de Centro América y Panamá,** en Julio, con apoyo del CONACYT y la Facultad de Ingeniería y Arquitectura, Escuela de Ingeniería Química de la Universidad de El Salvador. Permitirá desarrollar la capacidad de investigación y certificación de materiales para empaque y embalaje que faciliten la comercialización de los productos de la región. En este marco se impartirán el Curso «Laboratory Quality Management» (6/14 Julio) y «Specialized Packaging Personnel Training» (15/31 Julio).

• Adopción de las Normas de Calidad serie ISO 14000. Se ha equipado el Laboratorio Nacional Legal con patrones de mejor calidad, ampliándose el área de masas en otras magnitudes como densidad y presión.

• Capacitación de recursos humanos impartida por el Instituto Colombiano de Normalización, INCOTEC, a 50 empresas sobre **Aseguramiento de la Calidad ISO 9000,** en coordinación con el INSAFORP.

Oficina de Ciencia y Tecnología y Agencia Española de Cooperación Internacional (España)

• **Propuesta del nuevo Secretario General del Programa CYTED.** La Oficina de Ciencia y Tecnología propuso al Doctor José Antonio Cordero Martín, como nuevo Secretario General del Programa CYTED, durante la XV Asamblea General Extraordinaria, celebrada en Madrid, España, los días 3 y 4 de Junio de 1998. Ver reseña en el apartado correspondiente.

• **Nombramiento de nuevo Coordinador Nacional CYTED.** La Oficina de Ciencia y Tecnología designó como Coordinador Nacional CYTED al Doctor Francisco Ferrándiz García, durante la XV Asamblea General Extraordinaria, celebrada en Madrid, España, los días 3 y 4 de Junio de 1998. El Doctor Ferrándiz ocupa el cargo de Vocal Asesor en dicho organismo.

• Designación del Arquitecto Horacio Díaz del Barco, como **Representante del Organismo Signatario Agencia Española de Cooperación Internacional** en el Programa CYTED. El Señor Díaz del Barco, en su calidad de Coordinador de Área de Programas de Cooperación de la AECI ha asumido, además, la dirección del Programa de Cooperación Interuniversitaria (antiguo INTERCAMPUS).

Consejo Nacional de Ciencia y Tecnología, CONACYT. (México)

• **Creación del Programa MESURA,** apoyado por el CONACYT, con una inversión de más de 9 millones de pesos. Es interinstitucional y está destinado a promover la calidad y competitividad de la industria nacional. Con ese propósito firmaron un acuerdo el Licenciado Carlos Bazdresch, Director General del Consejo y el Doctor Héctor Nava Jaimes, Director del Centro Nacional de Metrología. El Programa contará con once y medio millones de pesos, ya que el CENAM aportará a su vez, dos millones.

• **Programa de Becas Crédito del CONACYT.** En 1998, se aplicará un presupuesto con un incremento de 25% en relación al de 1997, lo cual permitirá que el número total de becas administradas otorgadas por la institución se incremente en un 4%. Las becas nacionales administradas tendrán un crecimiento del 5%, en tanto que el acervo de becarios en el extranjero será el mismo que el año pasado. Este año, se recibió un número récord de solicitudes de posgrado en el extranjero: 2.667 aspirantes, de los cuales 40% se interesaron en realizar Doctorados, 56% Maestrías y 4% alguna especialización.

• **Apoyo económico del CONACYT a la Fundación México en Harvard A.C.** de 2.460.000 pesos para el financiamiento de las becas-crédito que esta asociación otorga a estudiantes mexicanos de posgrado en la mencionada universidad estadounidense. El Ingeniero Alfredo Elías Ayub, Vocal de la Comisión Ejecutiva de la Fundación y Director de Aeropuertos y Servicios Auxiliares, recibió este donativo entregado por el Director General del CONACYT, al término de una ceremonia que se llevó a cabo en la Sala Juárez del Consejo.

Instituto Nacional de Tecnología y Normalización, INTN. (Paraguay)

• **Décima Conferencia sobre Cooperación Económica entre la República de China y la República del Paraguay,** con la participación del Doctor Anthony Stanley, Director General del INTN, en Taipei, Taiwan, del 20 al 30 de Abril de 1998.

• Publicación de la Revista Cuatrimestral **«INTN Revista» Año II - Nº 2** (Noviembre/97-Febrero/98).

• **Curso sobre Gestión de la Calidad**, de carácter internacional, desarrollado por el INTN y la Asociación Paraguaya para la Calidad, con el patrocinio de la ALADI.

• **Curso sobre «Digestión Anaeróbica de Residuos con Producción de Biogas»**, del 8 al 11 de Junio de 1998, realizado en el marco del Fondo Argentino (FO-AR) de cooperación horizontal - Proyecto de Planificación y Aprovechamiento de las Energías no Convencionales.

Consejo Nacional de Investigaciones Científicas y Tecnológicas, CONICIT. (Venezuela)

• **Distinción con la Orden Francisco de Miranda en su Tercera Clase al Doctor Paulino Andreu,** otorgada por el Presidente de la República, en reconocimiento a su meritoria labor en pro de la construcción y puesta en marcha de la *«Planta Productora de Derivados Sanguineos»*. Ver reseña aparte.

• **CONICIT otorga cofinanciamiento de 1.600 millones de bolívares para 12 proyectos del sector olefinas-resinas-plástico en las áreas de desarrollo tecnológico, información técnica, ambiente y política comercial y mercado,** a través de la Agenda Industrial Cadena Olefinas- Plástico. Este cofinanciamiento al sector industrial privado del país se hará por la vía del crédito y la subvención, modalidades que cubrirán 65% de la inversión estimada para el total de propuestas. A fines de 1997, se realizó la convocatoria a empresas, consultores, centros de investigación y desarrollo y universidades nacionales a presentar propuestas para lograr mayor competitividad de la cadena, con miras a ampliar su mercado hacia el exterior. En cuanto al capital humano, los representantes de la cadena encargaron a una empresa especializada la evaluación y cuantificación de las necesidades en esta área. Fueron entrevistados gerentes generales, de plantas y de recursos humanos de 40 empresas del sector transformador del plástico (8 pequeñas, 13 medianas y 19 grandes), 5 empresas productoras de resinas y una empresa productora de olefinas (Pequiven). También fueron consultadas diez instituciones de capacitación técnica y artesanal en el área de plásticos. Los tres sectores coincidieron en que el capital humano es un factor clave para lograr la competitividad de la cadena. Entre los problemas de recursos humanos señalados se encuentran: falta de capacitación técnica, bajo nivel de preparación y conocimiento, rotación de personal, altos costos de mano de obra calificada, bajo nivel de educación básica y divorcio entre centros externos de capacitación y necesidades de las empresas.

• **Seminario Internacional «La Globalización, Modelos de Seguridad y la Función de las Fuerzas Armadas en el siglo XXI»**, organizado por la Comisión Permanente de Defensa de la Cámara de Diputados. CONICIT coordinó la mesa de trabajo correspondiente a ciencia, tecnología e industria militar. La participación en esta mesa y sus conclusiones son un primer paso para la creación de la Agenda Militar de CONICIT, en conjunto con las FF.AA. y el Congreso Nacional.

• **La Agenda Educativa del CONICIT aprobó tres proyectos de investigación en las áreas de educación superior, básica e innovaciones pedagógicas.** El primero de ellos busca establecer indicadores de gestión válidos y confiables para las universidades venezolanas. El segundo es la Consulta Educativa Nacional (CENA) y su objetivo es promover el debate nacional sobre las necesidades y prioridades de la educación básica, desde el nivel preescolar hasta el diversificado, incluyendo la educación técnica y para el trabajo, «Exploraciones para el cambio institucional, la consolidación de una innovación organizativa en un sistema local de educación básica». El tercer proyecto busca evaluar un nuevo modelo de organización y servicios en 105 escuelas de la región sucrense.

• **Primer Simposio del Estudio Paleoclimático Norte-Sur,** realizado en Mérida, en Marzo. Participaron científicos de todo el mundo para discutir y dar a conocer los resultados de los estudios sobre cambios climáticos ocurridos durante los últimos 2.000 años, desde el Polo Norte hasta la Patagonia, atravesando Estados Unidos, Canadá, Centro y Sur América. En Venezuela, con el apoyo de CONICIT, más de 70 científicos del país conforman el Comité Nacional para este estudio, cuyas conclusiones formarán parte del modelo predictivo y de funcionamiento del planeta. Las investigaciones venezolanas incluyen aspectos biosféricos del ciclo del agua, ecosistemas terrestres, química atmosférica, flujos oceánicos, interacciones terrestres y oceánicas de la zona costera, dinámica del ecosistema oceánico global, cambios del uso de la tierra, cambios globales en el pasado y sistemas de datos e información. En este programa participan más de cien países a nivel mundial, entre los que se cuentan, en Latinoamérica y el Caribe: Argentina, Brasil, Colombia, Chile, México, Jamaica y, recientemente, Venezuela.

•**Convenio de cooperación para promover y dar continuidad a las actividades de investigación, asesoría, consultoría, formación de recursos humanos, instalación de redes de información y capacitación técnica de la Agenda Salud en los Estados Frontera,** suscrito entre CONICIT, el Ministerio de Sanidad y Asistencia Social (MSAS) y las Fundacites Zulia, Guayana y Táchira, para trabajar en las siguientes áreas, que se corresponden con las líneas de investigación concertadas y convocadas públicamente por la Agenda Salud: 1) gestión de salud, epidemiología y participación comunitaria, 2) salud, ambiente, trabajo y calidad de vida, 3) biodiversidad, cultura y salud, 4) alimentación y nutrición, 5) prevención y control de enfermedades de alta prevalencia, 6) salud sexual y reproductiva.

•**Primera Reunión entre CONICIT y el Instituto Nacional de Deporte.** CONICIT, en su empeño por vincularse cada vez más con sectores no tradicionales de la sociedad, armó conjuntamente con el Instituto Nacional de Deporte una Agenda en esta área. En la primera reunión convocada, ambas instituciones lograron reunir a 256 personas de distintas disciplinas y sectores relacionados con el deporte venezolano y en representación de 60 organismos públicos y privados. Las áreas discutidas y sus conclusiones, que servirán de punto de partida para definir las líneas de investigación para proyectos, son las siguientes: *Educación, Planificación deportiva, Gerencia y administración, Infraestructura deportiva, Salud, Metodología y Social.*

• **Entrega de 2.400 millones de bolívares para financiar 19 proyectos de investigación,** a través del Programa de Apoyo a Grupos de Investigación del CONICIT, para fortalecer la investigación en grupos conformados por distintas universidades y centros de investigación públicos y privados del país. Más de mil millones se destinaran al área de biomedicina. El objetivo del programa es propiciar la integración funcional de investigadores y laboratorios del país que trabajen temáticas afines o complementarias, para potenciar las capacidades humanas y materiales y aprovecharlas de manera más eficiente en la búsqueda de soluciones a problemas de naturaleza compleja. En las áreas de química, física y matemáticas, agro e ingeniería se destinarán mil millones de bolívares para diez proyectos aprobados. Los resultados de estos proyectos se comenzarán a aplicar en cinco años, tiempo máximo de duración de cada uno de ellos, dependiendo de la complejidad y número de centros que conforman cada grupo.

• **CONICIT apoya el desarrollo de productos de la industria privada:** 1) Metalúrgica Chirica, ubicada en la región de Guayana, se está realizando conjuntamente con la Universidad Simón Bolívar, la fabricación de un equipo modular de camiones de volteo para la recolección de basura, que sustituirá a los costosos camiones que se importan actualmente. 2) La empresa Sanita recibirá 7.025.000 bolívares para la fabricación de máquinas de elaboración automática de toallas húmedas para bebés que permitirá aumentar la producción de 1.500 a 2.000 unidades diarias. Se expandirá su mercado hacia el oriente y sur del país y en el mediano plazo colocará el producto en las islas caribeñas. 3) Merlin de Venezuela contará con un

financiamiento por 148 millones de bolívares para desarrollar una herramienta híbrida hipermedia.

• **Actividad preparatoria para la conmemoración del Quinto Centenario de la llegada de Alonso de Ojeda, Américo Vespucio y Juan de la Cosa a las costas del Golfo de Venezuela.** La Comisión también participará en las actividades científicas preparatorias de la conmemoración, en 1999.

• **Propuesta presentada por Venezuela, a través del CONICIT, en torno a proyectos de investigación que llevan investigadores brasileños y venezolanos,** en la IV Reunión del Grupo VII Ciencia y Tecnología, fundamentada en la coincidencia de las líneas de actuación de ambos países para complementar esfuerzos y recursos, así como precisar metas y acciones a corto y mediano plazo. Las características de la propuesta giran en torno a proyectos de investigación que llevan investigadores brasileños y venezolanos, adscritos a laboratorios de investigación de instituciones de educación superior, los cuales servirán de base a las tesis de los estudiantes de doctorado. Las tesis estarían dirigidas bajo la modalidad de cotutela por expertos de los dos países y los títulos serían otorgados por las instituciones de acuerdo a convenios previos. Asimismo, se prevé la realización de pasantías cortas para estudiantes de posgrados; pasantías periódicas de expertos brasileños y venezolanos y paridad en el financiamiento de las actividades planificadas por ambas partes. Las áreas de interés común apoyadas desde la creación del Grupo VII son salud, tecnología de alimentos, recursos naturales amazónicos y redes de información.

• **VI Reunión de la Comisión Especial de Ciencia y Tecnología del Amazonia (Cecta),** organizada por CONICIT y la Secretaría Pro Tempore del Tratado de Cooperación Amazónica, los representantes de Bolivia, Brasil, Colombia, Ecuador, Guyana, Perú, Suriname y Venezuela disertaron sobre el futuro de la Comisión y las nuevas tareas que debe abordar en relación a la región. Acordaron la conformación de un comité de expertos en palmas, para la preparación y realización del Taller Regional sobre Palmeras en la Región Amazónica, apoyándose en los especialistas de estos países y en los estudios realizados.

• **Desarrollo del Proyecto «Análisis Geográfico de las Prioridades de Conservación de la Diversidad Biológica en Venezuela»,** a través del convenio suscrito entre CONICIT, Fundación Polar, Acoana (Asociación venezolana para la Conservación de Áreas Naturales) y la Organización No Gubernamental Wildlife Conservation Society. Su objetivo es actualizar la información básica sobre la distribución, el estado y las prioridades de conservación de la diversidad biológica, la definición de una estrategia nacional y desarrollar políticas nacionales e institucionales en el área.

• **Programa del Investigador Novel (PIN) del CONICIT, iniciado en 1991, ha formado a 216 nuevos investigadores,** de los cuales 66% forman parte del personal de planta de la Universidad Central de Venezuela, institución de mayor demanda en el programa. Las áreas en las que se desarrollan estos profesionales son farmacia, ingeniería, ciencias políticas, básicas y sociales.

• **Segundo Curso de Consultores Ambientales,** organizado por CONICIT, en Junio, dirigido a profesionales en el área ambiental, auditores, jefes de laboratorios de empresas venezolanas y consultores ambientales. Versará en temas como la evaluación de impacto ambiental y ciclo de vida, contaminación de aguas, suelos y aire, sistemas de gestión y auditorías ambientales, visitas a industrias y trabajo de auditoría ambiental en empresas. Se formarán 25 ecoconsultores y será dictado por instructores de la Escuela de Organización Industrial de España y de universidades venezolanas, coordinado por el CENDES y las Facultades de Ingeniería y de Ciencias de la UCV. En su primera edición realizada entre 1996 y 1997, el Curso de Ecoconsultores contó con el auspicio y apoyo financiero de la Agencia Española de Cooperación Internacional y la coordinación académica estuvo a cargo de la Escuela de Organización Industrial, EOI, institución española de trayectoria en la formación de gerentes, profesionales y técnicos para la industria. Se graduaron 23 ecoconsultores que cursaron los estudios teóricos en España y los trabajos prácticos en Venezuela. En sus informes finales generaron nueve auditorías ambientales de industrias y centros de salud del país.

• **Comisión Nacional de Seguridad y Prevención del Delito,** en cuyo proceso estuvo coordinado por el CONICIT, está integrada por representantes del gobierno nacional y de instituciones públicas y privadas. Su objetivo es planificar, coordinar y realizar proyectos y programas de investigación en las áreas de la información, seguridad y prevención del delito que contribuyan a orientar los poderes públicos en el diseño y formulación de políticas en la materia. Integran la Comisión los Ministerios de Relaciones Interiores, Defensa, Justicia y Secretaría de la Presidencia, PDVSA, Asociaciones de Gobernadores, Episcopal Venezolana, Cámara de Trabajadores de Venezuela, CONICIT y la Policía Técnica Judicial. La Comisión se encargará de determinar y aprobar programas y proyectos de investigación en materia de información, seguridad y prevención del delito, de designar las instituciones públicas o privadas que efectuarán la evaluación, seguimiento y control técnico-administrativo de los mismos y de fomentar y promover actividades destinadas a obtener recursos para financiarlos.

EXPERIENCIAS DE GESTIÓN DE LA I+D EN LA REGIÓN

CREACIÓN EN CUBA DE RED NACIONAL DE INDICADORES DE CIENCIA Y TECNOLOGÍA

CON la finalidad de contribuir al fortalecimiento y perfeccionamiento de los indicadores cubanos de ciencia y tecnología, el Ministerio de Ciencia, Tecnología y Medio Ambiente ha organizado la Red Nacional de Indicadores de Ciencia y Tecnología.

La Red está integrada por doce Ministerios y entidades gubernamentales cubanas y sus objetivos principales son los siguientes: 1) *Constituir un mecanismo de consulta y concertación nacional en materia de construcción y utilización de indicadores de ciencia y tecnología.* 2) *Coordinar y homologar metodológicamente la información estadística sobre ciencia y tecnología en Cuba para el consumo nacional y externo.* 3) *Actualizar y capacitar a los especialistas de diferentes entidades vinculados al trabajo con los indicadores de ciencia y tecnología.* 4) Actuar como contraparte nacional de la RICYT y potenciar la participación cubana en dicha red regional.

La Red Temática XVI.B «Red Iberoamericana de Indicadores de Ciencia y Tecnología (RICYT)», creada en 1995 en el marco del Subprograma XVI Gestión de la Investigación y el Desarrollo Tecnológico del Programa CYTED, se nutre de la información que proporcionan los organismos signatarios de los países que integran el CYTED, entre ellos el Ministerio de Ciencia, Tecnología y Medio Ambiente de Cuba, y hasta la fecha dentro de sus diversas actividades, ha publicado tres informes sobre esta materia.

NOTICIAS DE LOS SUBPROGRAMAS

En este apartado se menciona, en síntesis, algunas de las actividades efectuadas en los Subprogramas durante el primer semestre de 1998 y el anuncio de aquellas a efectuarse en el segundo semestre de 1998.

Subprograma III Biotecnología

• **Curso da Aplicaçâo das Técnicas de DNA Recombinante no Diagnóstico das Doenças Parasitárias,** em São Paulo, Brasil, del 15 al 27 de Setembro de 1997. Organizado por el Instituto de Medicina Tropical, FMUSP, Centro Brasileiro-Argentino de Biotecnologia (CBAB).

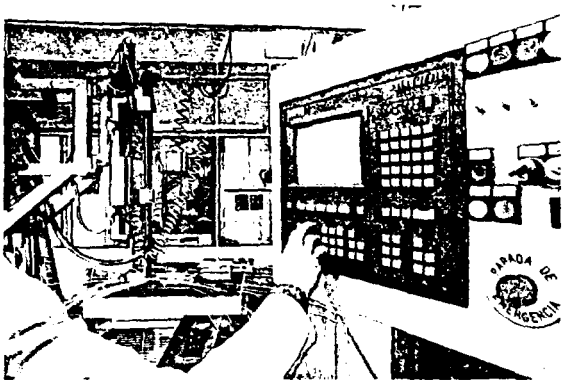
• **XXIV Annual Meeting on Basic Research in Chagas Disease , XIII Annual Meeting of Brazilian Society of Protozoology,** en Caxambu, MG, Brasil, del 11 al 14 de Noviembre de 1997, con apoyo de CNPq, CYTED, FAPEMIG, FAPESP, FAPERJ, FINEP, FIOCRUZ, FMRP/USP, CB/USP, UNIFESP/EPM. Se entatizaron aspectos de inmunología, biología celular y molecular, medicina tropical. Se entocaron recientes desarrollos en investigación en protozoología, vectores y enfermedades.

Subprograma IV Biomasa como Fuente de Productos Químicos y Energía

• **Red Aprovechamiento de Desechos Agroindustriales: Celebración del Curso Internacional Teórico-Práctico « Cultivo de Hongos Comestibles»**, en Cali, Colombia, del 31 de Marzo al 3 de Abril de 1998. Organizado por CYTED, Subprograma IV, Corporación Universitaria Autónoma de Occidente, Cali-Colombia, Universidad Nacional, Una, Heredia, Costa Rica, con la participación de Colombia, Costa Rica, Cuba, Mexico, Panamá, Perú, Portugal, Uruguay. Sus objetivos fueron: presentar los desarrollos metodológicos para el cultivo de algunos hongos comestibles cultivados (Pleurotus, Agaricus, Lentinus, Volvariella); transferir demostrativamente algunas técnicas de cultivo puro y de inoculación; establecer los acuerdos necesarios para el desarrollo del proyecto VII.D «Producción de hongos comestibles, bioabonos y vermicompost, a partir de sustratos agroindustriales».

Subprograma V Catálisis y Adsorbentes

Reunión de Coordinación General del Subprograma V, en Caraballeda, Venezuela, del 22 al 24 de Febrero.



• **Reunión de Puntos Focales de la Red Temática V.C**, en Caraballeda, Venezuela, los días 26 y 27 de Febrero.

• **Reunión del Proyecto V.3**, en Caraballeda, Venezuela, del 25 al 27 de Febrero.

• **Reunión del Proyecto V.5**, en Caraballeda, Venezuela, los días 25 y 26 de Febrero.

• **III Curso Iberoamericano de Tamices Moleculares y Reunión de Clausura de la Red V.A**, en Caracas, Venezuela, del 23 al 28 de Marzo.

• **Reunión para la definición del Proyecto de Tamices Moleculares y Catálisis Computacional**, en Caracas, Venezuela, los días 26 y 27 de Marzo.

• **Reunión inicial de la Red V.D «Catálisis Homogénea»**, en Caracas, Venezuela, los días 14 y 15 de Abril.

• **Asistencia del Coordinador Internacional del Subprograma V a la IX Conferencia de Coordinación del Programa CYTED**, en Santa Cruz de la Sierra, Bolivia, los días 22 y 23 de Mayo.

Actividades a desarrollarse en el segundo semestre de 1998

• **I Taller Iberoamericano de Catalisis Homogénea**, en Cartagena de Indias, Colombia, del 18 al 20 de Agosto.

• **Reunión de Seguimiento del Proyecto V.5: Desarrollo de Ctalizadores para Procesos de Química Fina**, en Cartagena de Indias, Colombia, los días 20 y 21 de Agosto.

• **III Taller Iberoamericano de Catalisis para la Protección Ambiental**, en Cartagena de Indias, Colombia, los días 21 y 22 de Agosto.

• **Presentación del Subprograma V al XVI Simposio Iberoamericano de Catalisis**, en Cartagena de Indias, Colombia, del 24 al 28 de Agosto.

• **Reunión de Cierre del Proyecto V.3: Separación de Gases Industriales por Adsorción y Definición de Nuevo Proyecto**, en Alicante, España, del 2 al 4 de Septiembre.

• **Curso-Taller de Catálisis Computacional**, en Rio de Janeiro, Brasil, del 1 al 4 de Diciembre.

Subprograma VII Electrónica e Informática Aplicadas

• Reunión de los grupos que participan en la creación de un nuevo **Proyecto de Investigación Precompetitiva sobre «Aplicaciones e Implementaciones de Redes Neuronales en Reconocimiento de Patrones» (AIRENE)**, en Braga, Portugal, del 26 al 30 de Enero.

• Reunión de los grupos del **Proyecto VII.10: «Integración de Sistemas y Actividades en Edificios Inteligentes» (SINTED)**, en La Habana, Cuba, del 2 al 6 de Febrero.

• **Curso sobre Informática Educativa,** en La Habana, Cuba, del 16 al 20 de Febrero, con la participación de grupos integrantes de la **Red Iberoamericana de Informática Educativa (RIBIE)**, coincidiendo con el Congreso Informatica '98 en La Habana, Cuba.

• **Primer Seminario Universitario sobre Programas Internacionales y Gestión de Proyectos,** con la participacion del Coordinador del Subprograma, celebrado en el Centro Hispano-Cubano de Postgrado, en La Habana, Cuba, del 17 al 19 de Febrero, organizado por la Conferencia de Rectores de Universidades Españolas y el Ministerio de Educación Superior de Cuba, coordinado por la Universidad de Barcelona, en colaboracion con la Comisión Europea (DGIB) y la Comision Interministerial de Ciencia y Tecnologia de España.

• **V Jornadas Iberoamericanas de Informática,** en Cartagena de Indias, Colombia, del 9 al 13 de Marzo, en el Centro Iberoamericano de Formacion, organizadas por la **Red Iberoamericana de Tecnologías del Software** y la **Agencia Española de Cooperación Internacional.** Se impartieron dos cursos sobre: ***Desarrollo de Software orientado a Objetos*** y ***Sistemas Colaborativos.*** Comprendió una introduccion y un Seminario.

• **I Jornadas Iberoamericanas de Ultrasonidos,** en Cartagena de Indias, Colombia, del 25 al 29 de Mayo, en el Centro Iberoamericano de Formación, organizadas por la **Red Iberoamericana de Informática Educativa (RIBIE)** y la

Agencia Española de Cooperación Internacional. Se impartieron dos cursos sobre: ***Procesamiento Digital de Señales Ultrasónicas (PDSUT) para aplicaciones médicas e industriales*** y ***Transductores Ultrasónicos para Medicina y Aplicaciones Industriales.*** Comprendió una introducción y un Seminario.

• **II Jornadas Iberoamericanas de Robótica,** en Antigua, Guatemala, del 8 al 12 de Junio, en el Centro Iberoamericano de Formación, organizadas por los Proyectos: **Diseño y Control de Robots de Aplicaciones Especiales (DYCRAE)** y **Sistema de Percepción Modular y Reconfigurable para Robótica (SISPER)** y la **Agencia Española de Cooperación Internacional.** Se impartieron dos cursos sobre: **Control y Programación de Robots y Percepción en Robótica.** Comprendió una introducción a dichos cursos y un Seminario.

• **II Jornadas Iberoamericanas de Informática Educativa**, en Santa Cruz de la Sierra, Bolivia, del 29 de Junio al 3 de Julio, en el Centro Iberoamericano de Formación, organizadas por la **Red Iberoamericana de Informática Educativa (RIBIE)** y la **Agencia Española de Cooperación Internacional.** Los cursos programados fueron: **Internet y Aprendizaje Cooperativo en Red y Sistemas de Autoría y Formación de Profesores,** comprendiendo una introducción y un Seminario

Actividades a desarrollar en el segundo semestre de1998

• **I Jornadas Iberoamericanas de Automatización del Proceso de Mecanizado,** en Santa Cruz de la Sierra, Bolivia, del 13 al 17 de Julio, en el Centro Iberoamericano de Formación, organizadas por la **Red Iberoamericana de Automatización de los Procesos de Mecanizado (RIBAMEC).** Constarán de dos cursos, con el temario: **La Integración de Herramientas de CAD-CAPP-CAM en el Desarrollo del Proceso de Mecanizado Automático y Sistemas de Control Numérico con Computador.** Cada curso comprenderá una introducción y un Seminario.

• **VI Jornadas Iberoamericanas de Informática,** en Santa Cruz de la Sierra, Bolivia, del 7 al 11 de Septiembre, en el Centro Iberoamericano de Formación, organizadas por la **Red Iberoamericana de Tecnologías de Software (RITOS).** Constarán de dos cursos por determinar. Cada curso comprenderá una introducción y un Seminario.

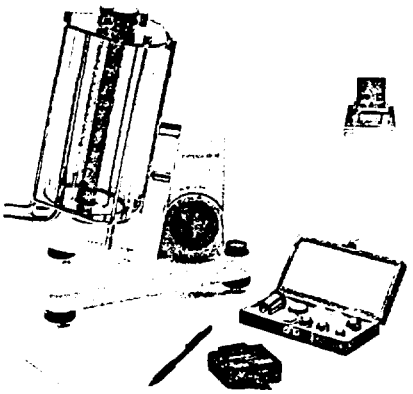
• **IV Jornadas Iberoamericanas de Automática,** en Antigua, Guatemala, del 21 al 25 de Setiembre, en el Centro Iberoamericano de Formación, organizadas por la **Red Iberoamericana de Informática Industrial.** Constarán de dos cursos con el temario: **Identificación de Sistemas en Base a Datos Reales y Sistemas Operativos de Tiempo Real.** Cada curso comprenderá una introducción y un Seminario.

Subprograma VIII Tecnología de Materiales

• Actividades del Coordinador Internacional, Doctor Miguel José Yacamán 1) Visita a la Universidade Federal do Rio Grande do Soul, Porto Alegre, Brasil, para establecer una Red en Aceleraciones; 2) Implementación y presentación de nuevos proyectos de investigación: a) «Desarrollo de Recubrimientos Vítreos Funcionales», b) «Nucleación de Fases Metaestables en Vidrios Bio-Vitrocerámicos».

• **Proyecto VIII.4 «Caracterización Morfológica Estructural y Mecánica de Materiales Compuestos de Matriz Polimérica»** a cargo del Dr. José María Pastor Barajas: 1) Presentación del Proyecto de Innovación IBEROEKA «Materiales Plásticos cargados reforzados con Fibras Agrovegetales» (Irausa Ingeniería, S. A., Repsol Química, S.A., CIDAUT e Industria e Comercio Marques, Ltd.); 2) Estancia en Mayo y Junio del Doctor Javier Santos en el Instituto de Capacitación e Investigación del Plástico y del Caucho (ICIPCC) en Medellín, Colombia. 3) Estancia durante Abril y Junio del Dr. Mauricio Ramírez en la Universidad de Valladolid y en el Centro de Investigación y Desarrollo de Automación (CIDAUT) en Valladolid, España. 4) Reunión de Coordinación en la Universidad Simón Bolívar, del 17 al 20 de Junio. 5) Conferencia del Jefe del Proyecto en la Universidad de La Habana el 23 de Junio con el título «Caracterización Estructural de Materiales Poliméricos y Composites mediante Espectroscopia Molecular».

• **Proyecto VIII.5 «Estudio Comparativo del Comportamiento Mecánico y Caracterización de Concretos Reforzados con Refuerzos no Convencionales»,** a cargo del Doctor Alejandro Manzano: 1) Curso Internacional sobre Materiales Compuestos Fibrorreforzados, en Cali, Colombia, del 11 al 13 de Marzo. 2) Publicación de las memorias del Curso Internacional. 3) Conformación de los siguientes proyectos: a) Sistemas de cubiertas de bajo costo: uso de residuos agroindustriales, b) Proyecto ATICA, c) Adiciones de cenizas volantes al hormigón.



• **Proyecto VIII.6 «Obtención y Caracterización de Biomateriales Compuestos con carga de Hidroxiapatito»** a cargo del Doctor Rafael Rodríguez Clemente. Organización del **2º Curso sobre Biomateriales para Aplicaciones Odontológicas y Traumatológicas.**

• Actividades del **Proyecto VIII.7 «Crecimiento y Caracterización de Recubrimientos Duros»** a cargo del Doctor Pedro Prieto Pulido: 1) Participación en la **25th International Conference on Metallurgical Coatings and Thin Films (ICMCTF 98)**, del 27 de Abril al 1º de Mayo, con dos ponencias orales: a) «Hardness and Morthological Characterization of Tungsten Carbide Thin Films» y b) «Sputter Deposition of TiN Thin Films using Optical Emission Spectroscopy (OES) as process monitor» ; 2) Realización del «Primer Taller de Técnicas Experimentales Aplicadas a Recubrimientos Duros», en el Centro de Ciencias de la Materia Condensada de la UNAM, Ensenada, Baja California, México, del 3 al 8 de Mayo.

• Actividades de la **Red VIII.B «Red Iberoamericana TEMACO»** a cargo del Doctor Antonio Madroñero de la Cal: 1) Reunión General de Coordinación, celebrada en el Centro Tecnológico para la Promoción de la Fibra de Vidrio, México, D.F., en Enero. 2) Puesta en marcha de los siguientes proyectos IBEROEKA: a) Megatube, b) Cerámicas para aplicaciones eléctricas, c) Depósitos sépticos hechos por filament winding.

• Actividades de la **Red VIII. C «Red Iberoamericana sobre Procesamiento de Materiales por Láser»**, coordinada por el Doctor Rui Vilar: 1) Participación en la 1ª Reunión ALFA «ESPERANZA» Ultra Hard Thin Films Deposited by Reactive Laser Ablation, en la Universidad de Lecce, Italia, del 14 al 19 de Mayo. 2) Tercera Reunión General de Coordinación, en Madrid, España, del 26 al 29 de Mayo. 3) Octavo Congreso de Ciencia y Tecnología Metalúrgica TRATERMAT '98, en Madrid, España, del 26 al 29 de Mayo. 4) Intercambio de profesores visitantes.

• Actividades de la **Red VIII.D «Red Iberoamericana de Adhesión y Adhesivos»**, coordinada por el Doctor Jose Miguel Martín Martínez: 1) 1ª Reuion General de Coordinacion, celebrada del 3 al 6 de Junio. 2) Organizacion del Curso «Fenómenos de Adhesion y Adhesivos», el día 7 de Junio.

• Actividades de la **Red VIII.F «Red Iberoamericana en Materiales Electrocerámicos»**, coordinada por el Doctor José Francisco Fernández Lozano: 1) 1ª Reunión General de

Coordinación de la Red, celebrada del 4 al 6 de Mayo; 2) Participación en el Primer Simposio de Electrocerámica, organizado por la Universidad Federal de Sao Carlos.

Actividades a desarrollar en el segundo semestre de 1998

• Actividades del Coordinador Internacional del Subprograma:1) Visita a las siguientes instituciones: Universidad del País Vasco, con el *Prof. Issa Katime; Universidad de Valladolid*, con el Dr. José María Pastor Barajas; Universidad de Santiago de Compostela, con el Dr. José Vásquez Tato; E.T.S.I.I. En Valladolid, con Jesús y Francisco Javier Martín Gil; Universidad Politécnica de Valencia, con el Dr. Avelino Corma Canos; Consejo Superior de Investigaciones Científicas, con el Dr. Manuel Torres; CIEMAT con el Dr. Félix Yndurain Muñoz; Centro de Investigaciones Biológicas, con la Dra. María del Carmen Risueño; Universidad Complutense de Madrid, con el Dr. Javier Piqueras; 2) Reunión General de Coordinación en Valladolid, España, en Julio; 3) Organización de un Simposium de Materiales, en Panamá, en Septiembre; 4) Organización de una «Escuela de Materiales», en Santa Cruz, Bolivia, del 12 al 16 de Octubre.

• **Proyecto VIII.2 «Estudio y Caracterización de Materiales Optoelectrónicos Puros y Dopados»** a cargo del Doctor Hector Murrieta, Instituto de Física, UNAM, México: 1) Reunión General de Coordinación, en Madrid, en Octubre. 2) 1ª Reunión sobre Materiales Opto-electrónicos, en Madrid, en Octubre, con la participación de Argentina, Brasil, Cuba, España y Mexico; 3) Intercambio de profesores visitantes entre Argentina, Cuba, España y México, de Julio a Noviembre.

• Actividades del **Proyecto VIII.7 «Crecimiento y Caracterización de Recubrimientos Duros»**: a cargo del Doctor Pedro Antonio Prieto Pulido: 1) Se iniciarán trabajos de recubrimientos superduros de BN usando nuevo equipo de sputtering que estará instalado en la Universidad del Valle. 2) Se ampliarán los contactos con empresas de la región interesadas en la utilización de los recubrimientos duros, a fin de configurar un proyecto IBEROEKA con el apoyo de COLCIENCIAS.

• Actividades de la **Red VIII.E «Red Iberoamericana sobre Ciencia y Tecnología de Materiales Vítreos»**, a cargo de la Doctora Alicia Duran: 1) *Participación en el XVIII Congreso* internacional de Vidrio (ICG) en San Francisco, del 5 al 10 de Julio, en el que se presentará un poster con las actividades y características de la Red, asimismo, un libro sobre la situación de la industria y la investigación en vidrios en Iberoamérica. 2) Segunda Reunión de la Red en Buenos Aires, Argentina, del 23 al 28 de Noviembre. 3) Seminario sobre Reciclado del vidrio en Iberoamérica. 4) Curso sobre «Gestión, Tratamiento y Utilización de Vidrio Reciclado en las Industrias de Vidrio Hueco».

• Actividades de la **Red VIII.F «Red Iberoamericana en Materiales Electrocerámicos»**, coordinada por el Doctor José Francisco Fernández Lozano: 1) Se efectuarán varios cursos: a) «Varistores basados en el sistema ZnO-Bi2O3», en Julio, en el IUTRC de Venezuela, a cargo del Dr. Amador Caballero del ICV-CSIC de España; b) «Materiales Electrocerámicos: procesado, propiedades y aplicaciones», en Agosto, en el ITCR de Costa Rica, por la *Doctora Marina Villegas del ICV-CSIC. de* España; c) «Procesos sol-gel para la obtención de nanopartículas» en Septiembre, en la UNAM de México, por el Doctor Jesus Tartaj del ICV-CSIC *de España*. 2) *Implementación de los* siguientes proyectos de investigación: a) Síntesis y caracterización de materiales electrocerámicos nanoestructurados; b) Aplicaciones *piezoeléctricas de bajo coste*; c) Gestión de un proyecto IBEROEKA sobre «Varistores».

Subprograma X Química Fina Farmacéutica

• **Nombramiento del Dr. Mahabir P. Gupta como Director Ejecutivo del Cómite Ejecutivo de la Asociación Interciencia.** Cargo que ostenta desde el 1º de Abril de 1998. Esta asociación internacional agrupa 16 Asociaciones para el Avance de la Ciencia en las Américas.

• **Proyecto X.4 «Obtención de medicamentos innovadores con actividad antihipertensiva y vasodilatadora a través de plantas medicinales»**, a cargo del Dr. Antonio José Lapa, de la Universidade Federal de Sao Paulo, Brasil. Se inició en 1997 y concluirá en el 2000. Sus principales objetivos son cooperar entre los científicos iberoamericanos para el estudio de las plantas medicinales que disminuyan la presión sanguínea y confirmar estas plantas medicinales como sustancias potenciales para la innovación de fitomedicamentos. Participan Argentina, Brasil, Costa Rica, Guatemala, Mexico, Paraguay, Perú, Portugal y España.

• **Curso Internacional sobre Producción de Fitomedicamentos,** en Panamá, del 24 de Noviembre al 5 de Diciembre de 1997, patrocinad por el International Center for Science *and High Technology (ICS), United Nations* Industrial Development Organization, Trieste, Italia, Secretaría Nacional de Ciencia y Tecnología e Innovación de Panamá, y la Universidad de Panamá. Trató sobre la situación actual de fitomedicamentos, principios y aplicaciones de evaluación etnobotánica y etnomedica, seguridad, eficacia y control de calidad, industrialización de plantas medicinales, problemas y limitaciones en la producción de fitomedicamentos en países en vía de desarrollo, cultivo y propagación de plantas tanto *in vitro* como en el campo, fabricación de productos herbarios, GMP y métodos de preparación de productos terminados, la gestión tecnológica, estabilidad y evaluación toxicológica y farmacologica de fitomedicinas. Participaron 30 científicos iberoamericanos de Bolivia, Brasil, Chile, Colombia, Costa Rica, Cuba, Ecuador, El Salvador, Guatemala, Honduras, Nicaragua, Panamá, Paraguay, Perú y Portugal. El Prof. Arnold Vlietinck de la Universidad de Antwerp fungió como coordinador técnico y el Prof. Enrico Feoli del Centro Internacional de Ciencia y Alta Tecno-

Participantes del International Training Course on Production of Phytomedicines.

Participantes del «Seminario Taller Iberoamericano sobre bioensayos *in vitro* Anticáncer y Antisida en el descubrimiento de droga de origen natural».

logía junto con otros 6 destacados profesores de Europa y América Latina colaboraron en la enseñanza del curso.

• **Primera Reunión del Proyecto de Investigación Precompetitiva X.3 «Evaluación de la biodiversidad iberoamericana como fuente de agentes inmunomoduladores y quimioterapéutico»,** en Cartagena de Indias, Colombia, del 11 al 14 de Diciembre de 1997. Participaron 16 científicos de Argentina, Colombia, Costa Rica, Cuba, Ecuador, España, Guatemala y Panamá.

• **Curso Internacional de Farmacología Experimental,** coauspiciado por la SENACYT y Universidad de Panamá, del 26 de Enero al 6 de Febrero. Participaron 15 científicos de Brasil, Costa Rica, El Salvador, Guatemala, Nicaragua y Panamá.

• **Seminario Taller Iberoamericano sobre los Bioensayos in vitro Anticáncer y Antisida en el Descubrimiento de Nuevas Drogas de origen Natural,** en Panamá, del 9 al 13 de Febrero. Coauspiciado por Secretaría Nacional de Ciencia y Tecnología (SENACYT), CYTED, National Cancer Institute, Smithsonian Tropical Research Institute y Universidad de Panamá. Actividad conjunta con la Red CEN del Suprograma III. Participaron 20 científicos de Argentina, Bolivia, Brasil, Colombia, Costa Rica, Cuba, España, Guatemala, Honduras, Nicaragua, Panamá y Portugal.

• **Curso Internacional sobre «El Descubrimiento del Medicamento. De la Planta, la Observación o la Idea, al Mercado»,** en Rosario, Argentina, del 9 al 13 de Marzo, coauspiciado por la Universidad de Rosario. Participaron 51 científicos de Argentina, Bolivia, Brasil, Colombia, Cuba, Chile, México, Panamá, Perú, uruguay, Venezuela y 15 profesores de Argentina, España, Inglaterra, Estados Unidos y Suecia.

• Actividades de la **Red X.C «Red Iberoamericana de Productos Fitofarmacéuticos (RIPROFITO)»:** 1) **I Taller sobre Cultivo y Manejo Postcosecha de Plantas Medicinales,** en Turrialba, Costa Rica, organizado por la Universidad de Costa Rica, sede del Atlántico bajo la dirección de M. Sc. Ana Cecilia Tapia, el apoyo técnico del Ing. Rafael Angel Ocampo de la Finca Bougainvillea S.A., del Dr. Armando Cáceres de la Universidad de San Carlos y de personal técnico de la UCR-Atlántico y la participación de más de 20 productores de Costa Rica. El evento se realizó en hizo en seguimiento al Taller organizado por el Ministerio de Salud para la presentación del «Reglamento para la industrialización, inscripción, comercialización y propaganda de preparaciones con base en productos naturales en forma farmacéutica y tisanas» realizado en San José en Noviembre de 1997. 2) **III Foro de Debates de Fitoterápicos,** organizado por el Sindicato de la Industria de Productos Farmacéuticos del Estado de Sao Paulo (SINDUSFARM), con el objetivo de discutir la situación legal de los productos fitoterápicos en el Brasil. Participaron conferencistas de Alemania, Brasil, Estados Unidos y Guatemala y 100 personas de más de 60 empresas de fitoterápicos; 3) Reuniones preparatorias: a) **Visita al Núcleo de Pesquisas en Productos Naturales (NPPN) y a la Facultad de Farmacia de la Universidad Federal de Río de Janeiro,** el 26 de Marzo, donde existe interés en ingresar a la Red X.A del Subprograma X. b) **Reuniones de trabajo y Coordinación en la Universidad Federal Fluminense,** para preparar un perfil de proyecto a presentar en la reuniones celebradas los días 27 y 28 de Marzo. c) **Reunión de Planificación del Proyecto QTROP-TB,** los días 30 y 31 de Marzo, en el que la Universidad Federal Fluminense (UFF) ejecuta un componente para establecer un núcleo gestor de la calidad de medicamentos para el tratamiento de tuberculosis con el apoyo de Financiadora de Estudos e Projetos (FINEP). La oportunidad fue aprovechada para buscar las posibilidades de internacionalizar el proyecto en vista de la creciente importancia de esta enfermedad reemergente, por lo que se decidió formular un proyecto precompetitivo para la búsqueda de nuevas drogas antituberculosas a partir de moléculas con actividad antimicobacteriana, el cual se presentaría al Subprograma X del CYTED bajo la coordinación del Doctor Nikolai Sharapin de la UFF.

• **II Curso Teórico-Práctico de Validación de Plantas Medicinas con actividad en tracto Gastrointestinal,** organizado por la **Red X.B «Red Iberoamericana de Validación de Plantas Medicinales (RIVAPLAMED)»** en el Sector de Productos Naturales del Departamento de Farmacología, Instituto de Farmacología, Escola Paulista de Medicina/Universidade Federal de Sao Paulo, en Sao Paulo, Brasil, del 25 de Abril al 8 de Mayo. Su objetivo fue capacitar grupos iberoamericanos asociados al Programa X en técnicas «in vitro» que permitan la comprobación de actividades de plantas medicinales sobre el aparato gastrointestinal. Participaron 18 científicos de Argentina, Bolivia, Brasil, Colombia, Ecuador, Guatemala, México, Paraguay, Perú y Venezuela.

• **Taller sobre Control de Calidad y Tecnología Farmacéutica de Productos Fitoterapéuticos,** en Niterói, Brasil, del 18 al 23 de Mayo, con el co-auspicio de la ONUDI, Viena y la Universidad Fluminense de Brasil. Participaron 26 científicos de Argentina, Bolivia, Brasil, Colombia, Costa Rica, Guatemala, Honduras, Panamá, Paraguay y Perú.

• **Curso sobre Validación de Plantas Medicinales, III Jornada Internacional de Umurama Paranaense (UNIPAR),** celebrada en Umuarama, Brasil, con el patrocinio de la Universidad de Paranaense, Brasil, los días 21 y 22 de Mayo.

• **Reunión de los Coordinadores Internacionales de CYTED. Consulta sobre Nuevos Modelos de Funcionamiento del Programa CYTED,** en Santa Cruz de la Sierra, Bolivia, los días 22 y 23 de Mayo.

• **Farmatox Meeting,** celebrada en la Escuela Paulista de Medicina de Sao Paulo, Brasil, del 29 al 31 de Mayo.

• Actividades de la **Red X.A «Red Iberoamericana de Productos Naturales de Uso Medicinal (RIPRONAMED)»:** 1) **Edición** (primera) **de un**

directorio informatizado e interactivo de RIPRONAMED. Contiene datos de los investigadores y grupos actualmente encuadrados en la Red. Esta primera edición reúne a 762 investigadores pertenecientes a 123 grupos de los 21 países de la Región CYTED, que investigan en el ámbito de las Plantas Medicinales y Productos Naturales Bioactivos. 2) **Reunión de Coordinación de la Red X.A,** en San José, Costa Rica, del 7 al 9 de Mayo, con participación de investigadores de 21 países de la Región CYTED, bajo la supervisión del Coordinador Internacional del Subprograma X. Se revisaron las actividades anteriores de la Red, se fijaron los criterios para la generación de nuevos PIP y el desarrollo de nuevas actividades, quedando definidos siete nuevos proyectos, seleccionando cinco cursos talleres responsables para tres publicaciones metodológicas y un compendio bibliográfico, que editará la Red. Se presentó la Base de Datos Interactiva de RIPRONAMED. 3) **Redacción de un Pre-proyecto de Investigación Precompetitiva sobre Agentes Terapéuticos Gastrointestinales,** en Sevilla, España, los días 28 y 29 de Mayo, y en coordinación con actividades de la Red ALFA coordinada por la Doctora María José Martín Calero; participaron especialistas de 5 países.

• **Primera Reunión del Proyecto X.4 Obtención de medicamentos innovadores con actividad antihipertensiva y vasodilatadora a través de plantas medicinales ,** en Recife, Brasil, del 17 al 19 de Junio, con el co-auspicio del CNPQ.

• **Publicaciones realizadas por el Subprograma X:** Informe de la Reunion de Coordinación del Proyecto X.3, Informe del Curso de Rosario, Argentina «El Descubrimiento del Medicamento. De la Planta, la Observación o la Idea al Mercado», Inicio de Monografías sobre Cultivos de Plantas Medicinales, Inicio de segunda edición de «500 Plantas Medicinales Iberoamericanas», Publicación del libro «Tecnología e Industrialización de Fitofarmacos» en prensa, Informe del Curso de Producción de Fitomedicamentos, Informe de Coordinación del Proyecto X.2, Sevilla, Publicación objetivos terapéuticos para el año 2000: Una visión latinoamericana 1997.

Actividades a desarrollar en el segundo semestre de 1998

• Actividades entre la Secretaría Ejecutiva del Convenio Andrés Bello (SECAB) y el Subprograma X: Nuevas convocatorias de movilidades de investigadores y subsidios a Proyectos de investigación en países miembros del Convenio Andrés Bello (Bolivia, Chile, Colombia, Cuba, Ecuador, Panamá, Perú, Venezuela). Fecha límite: 31 de Agosto de 1998.

• **Segunda Reunión Anual de la Red X.C RIPROFITO,** en Antigua, Guatemala, del 6 al 11 de Setiembre, con el co-auspicio del Consejo Nacional de Ciencia y Tecnología de dicho país, ASAC Pharmaceutical International y Centro Cultural Español (AECI).

• **Congreso Nacional de Ciencia y Tecnología,** en Panamá, del 10 al 12 de Septiembre, con el auspicio de SENACYT, APANAC y con la participación de los Subprogramasen Alicante, España, del 20 al 23 de Setiembre.

• **Reunión de la Sub Red España - Portugal de Productos Naturales de Uso Medicinal,** en Sevilla, España, los días 24 y 25 de Setiembre.

• **Seminario Taller sobre el Screening Químico de Plantas,** en Chile, del 28 al 30 de Septiembre, con el coauspicio de la International Organization of Chemistry for Development (IOCD) y la Universidad de Chile.

• **Training Course on Screening Technologies for Industrial Explotation of Medicinal and Aromatic Plants,** en Santiago, Chile, del 21 de Septiembre al 2 de Octubre. Patrocinado por el Centro Internacional de Ciencia y Alta Tecnología (ICS/ONUDI), Trieste y la Universidad de Chile.

• Actividades de la Red X.A RIPRONAMED: 1) **Reunión Anual de la Sub Red España - Portugal,** en Sevilla, España, los días 24 y 25 de Septiembre. 2) **Curso-Taller Iberoamericano de Técnicas** en Panamá, en el mes de Octubre.

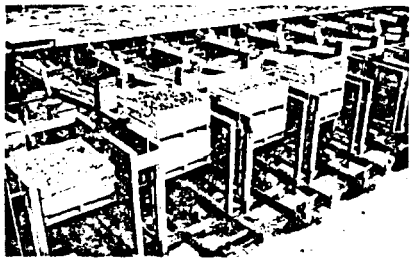
• **Simposio Satélite Subprograma X. Simposio Brasileiro de Plantas Medicinales,** en Sao Paulo, Brasil, del 13 al 18 de Octubre.

• **Curso sobre Resonancia Magnética Nuclear,** en Panamá, en Octubre.

• **Curso Iberoamericano sobre Control de Calidad de Productos Herbarios,** en La Plata, Argentina, del 16 al 22 de Noviembre, patrocinado por la Universidad de La Plata, la Universidad Complutense de Madrid y A.S.A.C. Pharmaceutical International.

Subprograma XI Tratamiento y Conservación de Alimentos

• **Symposium Internacional de la Red Iberoamericana de Alimentos sobre Regímenes Especiales,** en la Universidad Iberoamericana Plantel Santa Fe, Ciudad de México, México, del 18 al 20 de Mayo. Coauspiciado por el Instituto Politécnico Nacional, Universidad Iberoamericana, CYTED-CONACYT y el Instituto Nacional de la Nutricion Salvador Zubiran



• **Curso Biodisponibilidad de los Almidones.** en la Universidad Iberoamericana Plantel Santa Fe, Ciudad de Mexico, Mexico, del 18 al 21 de Mayo. Coauspiciado por el Instituto Politécnico Nacional, Universidad Iberoamericana, CYTED-CONACYT, Instituto Nacional de la Nutrición Salvador Zubirán y Kellogg's de Mexico. Impartido por el Dr. Juscelino Tovar Rodriguez.

• **Curso de Desarrollo de Alimentos para Regímenes Especiales,** en la Universidad Iberoamericana Plantel Santa Fe, Ciudad de Mexico, Mexico, del 18 al 21 de Mayo. Coauspiciado por el Instituto Politecnico Nacional, Universidad Iberoamericana, CYTED-CONACYT y el Instituto Nacional de la Nutricion Salvador Zubiran. Impartido por la Ing. Elisa Panades A. y la Ing. Lourdes Valdes. Sus objetivos fueron, establecer los conocimientos acerca de los diferentes tipos de alimentos para personas que requieren un regimen especial de alimentacion, así como los aspectos actuales con relacion a los alimentos funcionales o farma-alimentos; brindar los conocimientos necesarios para la elaboración y presentación de los alimentos régimene especial: en polvo y liquidos; establecer los pasos metodologicos para el diseño de alimentos para régimen especial; demostrar prácticamente la efectividad del «Formulador» en el diseño de alimentos para régimen especial.

• **Curso sobre Los Lípidos en la Nutrición,** en la Universidad Iberoamericana Plantel Santa Fe, Ciudad de México, Mexico, del 19 al 21 de Mayo. Coauspiciado por el Instituto Politecnico Nacional, Universidad Iberoamericana, CYTED-CONACYT y el Instituto Nacional de la Nutricion Salvador Zubiran. Impartido por la Dra. Monserrat Rivero Urgell.

Subprograma XIII Tecnología Mineral

• En la XIV Asamblea General del CYTED, los días 20 y 21 de Noviembre de 1997, se comunicó la elección del Coordinador Internacional, Doctor Roberto C. Villas Boas. Desde su inicio como tal, contacto con los Coordinadores de las Redes Temáticas XIII.C y XIII.B, respectivamente, Prof. Dr. Benjamín Calvo Pérez (Red Iberoamericana de rocas y minerales industriales) y Prof. Dr. Cesar Canepa Ianna-

cone (Red Iberoamericana sobre Materiales Precisos) con proposición de una reunión de Coordinadores prevista para los días 28 y 29 de Junio, en la Ciudad de Río de Janeiro, Brasil, en fecha próxima.

• Participación en la **IX Conferencia de Coordinación del Programa CYTED,** realizada en Santa Cruz de la Sierra, Bolivia, del 22 al 24 Mayo, donde se discutió propuesta de reestructuración del Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo-CYTED y agrupamientos en áreas temáticas; el Coordinador Internacional propuso la participación del XIII en las áreas temáticas de «Tecnología de Materiales», «Tecnologías de la Salud y de la Alimentación», «Tecnologías de la Información y de las Comunicaciones», «Recursos Energéticos» y «Medio Ambiente».

• **Consultas preliminares para acuerdo de cooperación entre el Subprograma XIII y el IMAAC de la UNIDO,** respecto al desarrollo sostenible de los minerales y metales.

• **Red Temática XIII.B Materiales Preciosos:** El Coordinador de la Red, Prof. Dr. Cesar Canepa, de la Universidad Mayor de San Marcos y de la North Cia. Minera S.A., del Perú, realizó reuniones en Moa, Cartago y Medellín, entre el 18 a 24 de Abril, con el objetivo de consolidar posiciones respecto al trabajo multidisciplinario de la Red, o sea, geologos, ingenieros de minas, metalurgicos, legisladores minerales, economistas, etc.

• **Red Temática XIII.C Rocas y Minerales Industriales:** El Coordinador de la Red, Prof. Dr. Benjamín Calvo Perez, de la Escuela Técnica Superior de Ingenieros de Minas, UNED, España, confeccionó Circulares RIMIN entre los participantes de la Red con excelentes resultados.

Actividades programadas para el segundo semestre de 1998

• Actividades a realizar por la **Red Temática XIII.B Materiales Preciosos:** 1) **Boletín Informativo de la Red XIII.B:** se encuentra en confección, con ampliación de las Circulares de la Red, ya tradicionales. 2) **Primera Reunión de Coordinación de la Red,** en Ouro Preto, Brasil, del 28 al 31 de Julio, con el objetivo de definir los tipos de actividades futuras que debe desarrollar la Red e integración. 3) **Cursillo-Taller de Mineralurgia de Metales Preciosos:** programado para el próximo Octubre, por determinar el lugar.

• Actividades a realizar por la **Red Temática XIII.C Rocas y Minerales Industriales:** 1) **Primera Reunión de Coordinación de la Red,** en Guayaquil, Ecuador, del 12 al 19 de Julio, con la participación del Curso de Doctorado y Postgrado sobre Minerales y Rocas Industriales de la ESPOL. 2) **Libro sobre Minerales y Rocas Industriales de Iberoamérica:** en confección, con un capítulo dedicado a cada país.

Subprograma XIV Tecnologías para la Vivienda de Interés Social

• **Proyecto XIV.4 MEJORHAB «Mejoramiento y Reordenamiento de Asentamientos Urbanos Precarios»:** 1)Constitución de la Asamblea del Proyecto , en Caracas, Venezuela, en Octubre de 1997, conjuntamente con el primer Seminario Internacional sobre «Mejoramiento y Reordenamiento de Asentamientos Urbanos Precarios», en torno a le experiencia del «Consorcio CATUCHE». El Proyecto MejorHab pretende aportar a la comunidad iberoamericana resultados en las areas de interés, cuya importancia será, en gran modo, consecuencia de la escala real sobre la que se propone actuar el Proyecto. 2) **Projeto Prorenda Urbano/Rs.** O Projeto PRORENDA Urbano no Rio Grande do Sul, no o Município de Caxias do Sul desenvolve-se através da parceria entre o governo do estado, através da Fundação de Planejamento Metropolitano e Regional - METROPLAN, a Sociedade Alemã de Cooperação Técnica - GTZ e prefeituras municipais. Os objetivos visam contribuir para a redução do desequilíbrio social e o fortalecimento da ciudadania, fazendo com que organizações comunitárias, governos municipais cooperem entre si e, em conjunto com outras entidades, promovam o desenvolvimento de áreas de subabitação.

• **V Asamblea General de la Red XIV.B. «Viviendo y Construyendo»,** en Lima, Perú, del 10 al 14 de Noviembre de 1997. Participantes de Argentina, Colombia, Costa Rica, Cuba, Chile, El Salvador, Honduras, México, Nicaragua, Perú, Venezuela. Se informó sobre el avance de los proyectos acordados en la IV Asamblea de México: Consolidación Asentamientos Precarios y Política Urbana; Perspectivas de la Autoconstrucción en el marco de la Globalización; Medición de la Sustentabilidad en los Asentamientos Precarios; Habitat en el centro de las ciudades;Acción Local (Municipios y Hábitat); Videoteca; Derecho al Habitat; Los Usuarios valoran su Hábitat; Análisis y Evaluación de Proyectos Autogestivos; Metodología y Experiencias de Diseño Participativo.

Actividades programadas para el segundo semestre de 1998

• **VI Asamblea de la Red XIV.B «Viviendo y Construyendo»,** en San Salvador, El Salvador, del 26 al 30 de Octubre. Durante los días 26, 27 y 28 se realizará la Asamblea propiamente dicha y los días 29 y 30 el Congreso Hábitat y Cambio Social en su IV versión, todo en el marco del 30º Aniversario de trabajo de FUNDASAL.

• **Publicaciones a lanzar en 1998 en el marco de la Red XIV.B «Viviendo y Construyendo»,** se preveé la edición y publicación de: 1) Centro Históricos (separatas de cada experiencia en Quito, Mexico, La Habana y San Salvador); 2) Postulados sobre los Centros Antiguos; 3) Publicación sobre Proyectos Autogestivos; 4) Ensayo sobre Gobiernos Locales (Acción Local); 5) Publicación sobre Diseño Participativo.

• **Proyectos para el año 1998 en torno a la Red XIV.B «Viviendo y Construyendo»: 1) Proyecto 1 «Hábitat en el Centro de las Ciudades»,** con el propósito de incluir la problemática en la Producción Social del Hábitat en los Centros de las Ciudades como un tema poco abordado en estos contextos, a nivel regional en la Red de Alcaldes de America Latina y a nivel nacional de las prácticas. **2) Proyecto 2 «Tecnología para la participación en el Planeamiento y Diseño del Hábitat de Producción Social»,** tiene como objetivos, abrir un espacio de relexión, informacion y difusión del tema, obtener experiencias, métodos y reflexiones, realizar un estudio del tema que avale la importancia de dichas tecnologías y de su implementacion en las PPSH. **3) Proyecto 4 «Acción Local»,** el trabajo contendrá una introduccion general y la exposición de casos, tomando como modelo las fichas de Polis (50 ideas para Municipios), fecha de entrega por e-mail: antes del 15 de Octubre de 1998; sus objetivos son: fortalecer desde la Red CYTED los postulados recogidos en el Proyecto, identificar y describir acciones ejemplificativas de aporte de centros CYTED a las áreas de Hábitat Popular de gobiernos locales, motivar la multiplicación de estos servicios de los Centros CYTED y su demanda por parte de entidades locales. **4) Proyecto 5 «Medición de la Precariedad en Asentamientos Humanos Urbanos»,** tiene como objetivos: a trave de un estudio comparativo conjunto, analizar las posibles consecuencias e impacto de sistemas de indicadores utilizados en Costa Rica, Chile; precisar costos de producción de indicadores, relacionar el análisis producido con los postulados de la Red. **5) Proyecto 6 «Consolidación de Asentamientos Precarios y Política Urbana: Nuevas lecturas sobre la forma de hacer ciudad»,** la elaboración de Ensayos Nacionales se hará en 1998 y la Síntesis subregionales y Edición de libro, en 1999. Los objetivos son, identificar, analizar y sistematizar las experiencias de autogestión en la producción del hábitat, comunicar y difundir las experiencias a nivel nacional y regional, comunicar metodologías de sistematización. **6) Proyecto 7 «Progresividad Residencial en sectores habitacionales: factibilidad de aplicación y desarrollo en Cuba y Chile»,** tiene como objetivos generales: avanzar en la busqueda de aplicabi-

lidad de modelos de progresividad residencial urbano, arquitectónico), utilizando programas y acciones en desarrollo en los diferentes países participantes; y como objetivos específicos, relevar modalidades de progresividad distinguiendo las particularidades de cada país, proponer alternativas posibles de diseñar e implementar en ambos países. 7) **Proyecto 8 «Publicación del libro de la Investigación sobre la Autogestión en la Producción Habitacional de la Zona Metropolitana de la Ciudad de México»**, tiene como objetivos, identificar, analizar y sistematizar las experiencias de autogestión en la producción del hábitat, comunicar y difundir las experiencias a niveles nacional y regional, comunicar metodologías de sistematización.

• **Proyecto XIV.4 MEJORHAB «Mejoramiento y Reordenamiento de Asentamientos Urbanos Precarios».** Actividades a realizar: 1) Realización de cursos intensivos en San Salvador, El Salvador, en Octubre, en torno a la experiencia del barrio «Las Palmas», con el soporte de FUNDASAL, y en 1999, sin precisar aún fecha y lugar para su desarrollo. 2) Desarrollo de asesorías, según criterios de factibilidad técnica y financiera, de impacto y replicabilidad.

• **Projetos PRORENDA Urbano/RS e MEJORHAB.** Actividades a desarrollar: 1) Realização da curso teórico prático «Melhorias e Reordenamentos de Assentamentos Urbanos Precários», em Novembro de 1998 en São Paulo, com promoção CYTED e IPT. 2) Desenvolvimento/prestação de assessoria, segundo critérios de viabilidade técnica e financeira que presentem alternativa inovadora possível de ser reproduzida/multiplicada; 3) Constituição da Assembléia do Projeto XIV.4 -MejorHab - CYTED em Caxias do Sul/RS - Brasil, em outubro/99, junto a realização do II Seminário Internacional sobre «Melhoramento e Reordenamento de Assentamentos Urbanos Precários» com base na experiência do Projeto PRORENDA Urbano/RS - Caxias do Sul. 4) Intervenções físicas e sociais nas areas CANYON - COOESP GARDELINO RAMOS.

Subprograma XV Corrosión /Impacto Ambiental sobre Materiales

• **International, Seminar and Workshop: The State of the Art of the Repair and Rehabilitation of Reinforced Concrete Structure»**, en Maracaibo, Venezuela, del 28 de Abril al 1° de Mayo de 1997. Fue auspiciado por la National Science Foundation (NSF) conjuntamente con el programa CYTED. Fue coordinado por CoHeSIVE y el Centro de Estudios de Corrosión de la Universidad de Zulia y la Asociación Venezolana de Corrosión (ASVENCOR). Contó con la colaboración de la Gobernación de Zulia, Obras Públicas del Estado y el Servicio Autonomo del Puente General Rafael Urdaneta, el Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICIT) y Petroleros de Venezuela (PDVSA), además de entidades privadas.

• **Red XV.B «Durabilidad de la Armadura (DURAR)»: Seminario-Taller «Inspección, Evaluación y Diagnóstico de Corrosión en Estructuras de Concreto Armado»,** en el Instituto de Corrosión y Protección de la Pontificia Universidad Católica del Perú, en Lima, Perú, del 25 al 27 de Noviembre de 1997.

• **Manual de Inspección, Evaluación y Diagnóstico de Corrosión en Estructuras de Hormigón Armado,** publicado por la **Red XV.B «Durabilidad de la Armadura (DURAR)».**

Subprograma XVI Gestión de la Investigación y el Desarrollo Tecnológico

• **Conferência International sobre «*Tecnologia, Comércio Internacional e Crescimento Econômico: Por que a Indústria Latino-Americana deve investir em Inovação*»**, em Santiago, Chile, de 21 a 23 de Abril, organizada pelo Council of Industrial Research Associations of the Americas/CIRAA, que reúne associações de P&D industrial de diferentes países latino-americanos, como ANPEI (Brasil), ADIAT (México), FUNPRECIT (Argentina), ACOLTEC (Colômbia) e CEBATEC (Costa Rica). Teve com objetivo principal, conscientizar as empresas que operam na América Latina, nacionais e multinacionais, sobre as necessidades e oportunidades para investimentos em inovação na região, fortalecendo assim, a base tecnológica do continente americano. Contou com a participação de empresários, autoridades governamentais responsáveis pela formulação de políticas e pesquisadores universitários interessados em fortalecer a inovação industrial através de vínculos com empresas. A interação desenvolvida durante a reunião certamente levará à realização de programas cooperativos e outras ações voltadas ao aumento da produtividade da pesquisa industrial. As conclusões da Conferência deverão ser editadas e disseminadas pelo CIRAA.

• **Cadernos de Gestão Tecnológica.** Com o propósito de ampliar a interação entre os pesquisadores, em particular entre estes e o setor empresarial o Sub Programa XVI/CYTED e o Núcleo de Política e Gestão Tecnológica da USP publicam desde 1993, os Cadernos de Gestão Tecnológica. A serie conta hoje com a edição de 38 títulos de diferentes autores ibero-americanos, dos quais em sequência aos já divulgados anteriormente, estão disponíveis em formato resumo na home page *http://www.usp.br/cyted/subXVI*.

• **Rede CYCOOP apóia programas de capacitação.** Em 1998, ano em que se comemora o 30º aniversário do conhecido *Triângulo de Sabato*, a Ibero-America está ampliando seus investimentos na formação de gestores da cooperação empresa -universidade/institutos de pesquisa-governo. A Rede CYCOOP apoiou no primeiro semestre os eventos seguintes: a) **PROTEU II,** desenvolvido em parceria com o PACTo/FIA/ Universidade de São Paulo sob o apoio da FINEP, com participantes do Brasil, Costa Rica, Cuba e Uruguai; b) **Seminario Internacional de Buenas Prácticas en Cooperación Universidad-Empresa,** organizado pela Universidad Politécnica de Valencia; c) **Programa de Formación de Gestores de Vinculación,** organizado pela Universidad Nacional Autonoma de Mexico; e d) **Programa Piloto para el Fortalecimiento de las Unidades de Transferencia de Tecnología en el Seno de las Universidades e Instituciones de Investigación y Desarrollo,** uma iniciativa conjunta da Comissão Latino-americana de Ciência e Tecnologia, CONICIT/Venezuela, Fundación Polar, AECI e CORDIPLAN. No segundo semestre estão previstas atividades de capacitação na Costa Rica, abrangendo gestores da America Central e o Caribe, e em Barquisimeto, Venezuela.

Actividades programadas para el segundo semestre de 1998

•**Jornada Anual de Gestão Tecnológica na Ibero-América.** Tem o propósito de criar um espaço para disseminar informações e fomentar o intercâmbio de experiências em torno do tema, o Sub Programa XVI prepara sua jornada de atividades, que acontecerá em 18 de Novembro de 1998, em São Paulo/SP/ Brasil, como parte da programação do XX Simposio de Gestão da Inovação Tecnológica. Paineis e reuniões paralelas com especialistas ibero-americanos em gestão tecnológica compõem a programação. As discussões, em reunião aberta, deverão ser concentradas no tema âncora da jornada, ou seja: «O papel dos Sistemas de Inovação na Governabilidade Democratica». A programação contemplara ainda reuniões de estudiosos, pesquisadores e especialistas em Gestão da Cooperação Empresa-Universidade e Indicadores de Ciência e Tecnologia. Temas que constituem as Redes de atuação do Sub Programa, sob a coordenação dos Profs. Mario Albornóz e Guilherme Ary Plonski. Informações complementares sobre o evento poderão ser obtidas com a Coordenação Internacional - Av. Prof. Luciano Gualberto, 908 Sala I-137 - Cep 05508-900- São Paulo/SP/Brasil. Tels: 55.011.211.4633/210.4640/818.5849/5837. Fax: 55.011.816.8044/814.0439 ou E-mail: jmarcovitch@usp.br

NOTICIAS IBEROEKA

LOS Proyectos IBEROEKA se han constituido en un importante y efectivo instrumento de cooperación empresarial, industrial y tecnológica, a través de proyectos de innovación conjuntos orientados al desarrollo de productos, procesos y servicios con un mercado potencial y basado preferentemente, en nuevas tecnologías. En ellos participan empresas de, al menos dos países, de Iberoamérica, asociados, en lo posible, a centros de investigación y tienen como objetivos el incremento de la productividad y la competitividad de la industria y la economía en la región iberoamericana. Tienen una gran flexibilidad en cuanto al diseño y a la organización interna de las distintas actividades.

Actualmente, de los 201 Proyectos presentados; tal como se aprecia en la Figura 1, el **número total de Proyectos IBEROEKA Certificados** en ejecución suman 102, en los cuales participan activamente 329 organizaciones iberoamericanas, con una inversión total de 170, 5 millones de dólares americanos. La inversión promedio por proyecto es de 1,7 millón de dólares.

De las 329 organizaciones participantes, 240 corresponden a empresas, 46 a Centros de Investigación y 43 a Universidades, las mismas que representan el 73%, 14% y 13%, respectivamente. Ver Figura 2.

En cuanto a las áreas temáticas en las cuales están enmarcados, el 52% se ubican en Tecnologías de la Información y Comunicaciones, 33% en Tecnología de la Salud y Alimentación, 10% en Tecnologías de Materiales, 3% en Recursos Energéticos y 2% en Medio Ambiente. Ver Figura 3.

La evolución que ha experimentado IBEROEKA desde su puesta en marcha hasta la fecha se representa en la Figura 4, cuya línea de crecimiento se corresponde con el número de Proyectos IBEROEKA aprobados en las respectivas Reuniones del Consejo Técnico Directivo y Asambleas Generales del Programa CYTED realizadas en las ciudades mencionadas.

Los 99 Proyectos restantes se encuentran en diversas fases de su gestión, en procura de su próxima concreción.

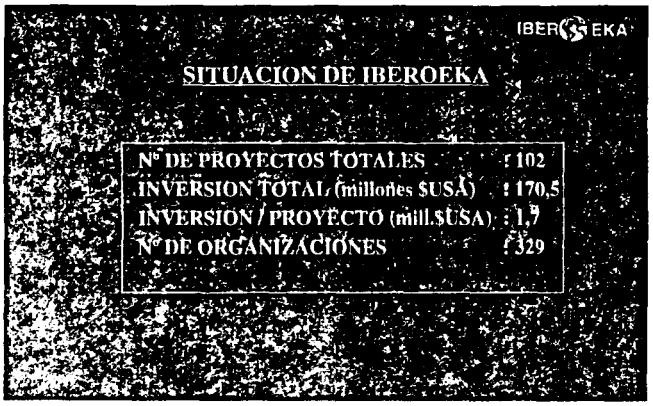


Figura 1.

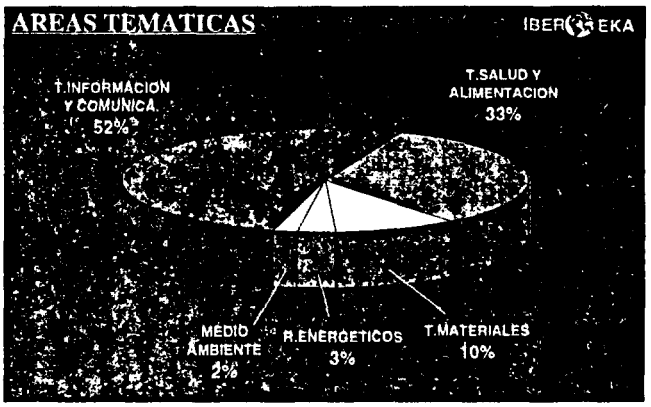


Figura 3.

Figura 2.

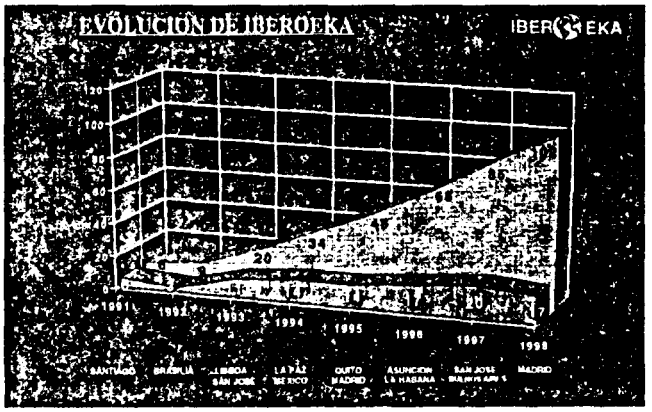


Figura 4.

NUEVOS PROYECTOS IBEROEKA CERTIFICADOS

LA XXIX Reunión del Consejo Técnico Directivo, celebrada en Madrid, España, el 1 de Junio de 1998, certificó los siguientes Proyectos IBEROEKA

IB058	Producción de fibra de coco para sustrato agrícola (IBERCOCO)	Brasil, España, México
IB148	Materiales termoplásticos cargados reforzados con fibras vegetales (MATFIVE)	Brasil, España
IB151	Desarrollo de formas de liberación de medicamentos (LICEL)	Argentina, España
IB153	Desarrollo de estación bioclimática de control de condiciones ambientales, con tecnología Internet (ESTACIÓN BIOLÓGICA)	Brasil, España
IB155	Investigación y desarrollo de una nueva gama de bombas eléctricas destinada a ejercer múltiples y específicas prestaciones (INCHESPA)	Chile, España
IB156	Desarrollo de transformadores de medida para el mercado americano (TRAFOMED)	España, México
IB158	Aplicación de las tecnologías de atmósfera modificada y pasteurización al vacío de la pesca y acuicultura en Colombia (AMPAFISH)	Colombia, España
IB165	Insecticida y fungicida integrado en producto único, de amplio espectro y ecotoxicológicamente neutro, para la protección de la madera verde (FKR MANA)	Colombia, España, Venezuela
IB167	Proyecto IBEROEKA de Sistema integrado de fabricación y control de PYMES (CONPYM)	Brasil, España
IB172	Sistema Integrado de Ayuda Asistencial - 2 (Gestión financiera y administrativa) (SIAS-2)	Cuba, España
IB173	Investigación y desarrollo de detectores de gases a alta temperatura basados en cerámicas electrónicas para aplicación en automóviles (DEGASAUTO)	Brasil, España
IB174	Desarrollo de bioactivadores para nutrición vegetal en base a residuos orgánicos renovables (PHYTACTIV)	Colombia, España
IB175	Desarrollo de un sistema de gestión integral de puentes (SIGP)	Colombia, España
IB179	Desarrollo de tecnologías de reciclaje de cromo en la industria galvanoplástica (RECICROM)	Cuba, España
IB180	Microencapsulación de un hibridoma productor de anticuerpo monoclonal anticaderina VE para la inhibición del desarrollo de metástasis tumorales (ACM CADERINA VE/MC)	Cuba, España
IB185	Recuperación, tratamiento y transformación de vinazas en lignosulfonatos (LIGNOVINAZA)	Colombia, España
IB192	Sistema integral de gestión y control de tráfico en vías rápidas (ELVIRA)	Brasil, España

EXPERIENCIAS EN IBEROEKA

Proyecto IBEROEKA IB-139 BIOADHESIVOS OFTÁLMICOS «Adhesivos como alternativa a las suturas en la cirugía oftálmica»

EN toda herida e intervención quirúrgica en las que hay una solución de continuidad de planos y estructuras, el cierre de las mismas se debe realizar generalmente con suturas en piel y mucosas en partes blandas, o tornillos, grapas o similares en estructuras rígidas.

El uso de substancias adhesivas no está muy extendido, aunque ha aumentado su uso paulatinamente desde la Segunda Guerra Mundial. Las razones que lo han favorecido son la homogeneidad en la distribución de la tensión en la unión y el hecho de no producir deformaciones de la herida. A pesar de ello, existen muchas reticencias en los sectores industriales y médicos en el uso de adhesivos como alternativa a otras tecnologías de unión tradicional, las cuales derivan de la escasa fortaleza de las uniones adhesivas.

Instalaciones del Instituto Oftalmológico de Alicante.

En oftalmología ocular los ahesivos que existen comercialmente se han venido utilizando en la clínica oftalmológica únicamente en casos de urgencia de perforación ocular. Los trabajos en clínica humana son apenas inexistentes. En otros campos de la oftamología ocular, vías lagrimales, estrabismo, cirugía de la catarata, etc., la experiencia se reduce a cirugía experimental en animales.

El grupo del Instituto Oftalmólogico de Alicante utilizó los adhesivos comerciales ya existentes anteriormente a base de fibrinógeno y cianoacrilato en incisiones esclerales de la cirugía de la catarata, comprobando que, aunque rápidos, no ofrecían las características ideales esperadas. Los adhesivos de fibrina bien tolerados no poseen una gran fuerza tensil y por tanto no son capaces de soportar determinadas tensiones de los tejidos implicados en la unión; así como, su potencial transmisiín de enfermedades por tratarse de un hemoderivado. Los adhesivos inorgánicos de base cianoacrílica con mayor fuerza tensil y no transmisores de infecciones provocan por su gran rigidez una peor tolerancia por parte de los pacientes.

Con todo ello surgió la idea de obtener una serie de adhesivos, distintos según el tejido ocular a sellar, donde variaría su rigidez en pro de una mayor o menor fuerza tensil a realizar y una mejor tolerancia ocular.

El adhesivo ideal por tanto debe ser una substancia totalmente de síntesis, biodegradable en el tiempo que se estipule como suficiente, tortalmente reemplazable por tejido cicatricial normal (alrededor de un mes o más según la capa ocular donde se aplique); biocompatible y muy bien tolerada por el paciente, con mínimos fenómenos irritativos y sin sensación de disconfort ocular.

El adhesivo debe ser de fácil manejo y aplicación, facilitando el acceso a zonas donde dar un punto de sutura es difícil, o donde hay una desestructuración de la capa en la que prácticamente es imposible el acceso a las agujas de sutura. Por todo ello, los adhesivos aportan grandes ventajas disminuyendo costes y tiempo quirúrgico a las soluciones actuales a base de suturas.

Este adhesivo o adhesivos ideales son los que pueden conseguir el equipo que trabaja en este ambicioso proyecto, que finalizará por completo en 1999, en el que participan desde Alicante, el Profesor J.L. Alió, Director Médico del Instituto Oftalmológico de Alicante, el Profesor M. Martín, del Departamento de Química Inorgánica de la Universidad de Alicante, en colaboración con el equipo cubano, dirigido por el Profesor R. Álvarez y su Departamento de Biomateriales (BIOMAT) de la Universidad de La Habana, con experiencia previa en el uso de adhesivos en otras ramas de la Medicina, como son la Estomatología o la Ginecología. Forman un gran equipo con intercambio de experiencias enriquecedoras que se ha traducido ya en la aparición de toda una serie de adhesivos que suponen un gran avance en el campo de la Oftalmología.

IX CONFERENCIA IBEROAMERICANA DE COORDINACIÓN DEL PROGRAMA CYTED

SE celebró en el Centro Iberoamericano de Formación de Santa Cruz de la Sierra, Bolivia, los días 22 y 23 de Mayo de 1998 y contó con la participación de los Coordinadores Internacionales de los Subprogramas del Programa CYTED, Doctores Manuel Murillo (II), Roberto E. Cunningham (IV), Paulino Andreu (V), José Antonio Cordero (VII), Carlos Mammana (IX), Mahabir P. Gupta (X), Efrén Parada (XI), Gonzalo Halffter (XII), Roberto Cerrini Villas Boas (XIII), Silvio Ríos (XIV) y Leonardo Uller (XV); D. Horacio Díaz del Barco, Secretario General y D. Francisco Ferrándiz, Secretario Técnico del Programa CYTED; y, los Coordinadores de los Grupos de Trabajo, integrados por D. Gonzalo León, Subdirector General de Planeamiento y Seguimiento de la Oficina de Ciencia y Tecnología de España, como Coordinador General; Dª Clara Morán, del Consejo Nacional de Ciencia y Tecnología de México y Coordinadora Nacional CYTED y D. Ignacio Ávalos, Presidente del Consejo Nacional de Investigaciones Científicas y Tecnológicas de Venezuela, como Coordinadores de los Grupos I y II, respectivamente.

Uno de los temas tratados ampliamente fue la Presentacion y discusión del Documento *«Propuesta de Reestructuración del Programa CYTED»*, que estuvo a cargo del Coordinador General de los Grupos de Trabajo. Se recogieron las sugerencias efectuadas por los Coordinadores Internacionales a dicha propuesta, las mismas que se incorporaran en un documento de *reflexión y recomendaciones para los órganos de dirección del Programa.*

Otros temas tratados fueron: *Problemática de los Presupuestos 1997 y 1998. Seguimiento de las aportaciones de los Organismos Signatarios; Coordinación de las Actividades de Redes Temáticas y Proyectos de Investigación Precompetitiva; Análisis de las posibilidades de transferencia de tecnología y su relación con los Proyectos de Innovación IBEROEKA.*

De pie, izquierda a derecha: Roberto E. Cuningham, Efrén Parada, Mahabir P. Gupta, Silvio Ríos, Paulino Andreu, Francisco Ferrándiz, José Antonio Cordero, Roberto Cerrini Villas Boas, Gonzalo Halffter, Leonardo Uller, Carlos Mammana, João Melo Borges y Manuel M. Murillo. Sentados, izquierda a derecha: Ignacio Ávalos, Gonzalo León, Clara Morán y Horacio Díaz del Barco.

RESULTADOS DE CYTED

PROYECTO X. 1 BÚSQUEDA DE PRINCIPIOS BIOACTIVOS EN PLANTAS DE LA REGIÓN

Doctor Roberto Pinzón S. (*)

DESDE su inicio en 1989, bajo la coordinación del Doctor Ceterino Sánchez y posteriormente del Doctor Mahabir P. Gupta, el Subprograma X Química Fina Farmacéutica, se ha planteado como uno de sus objetivos la búsqueda de moléculas bioactivas que pudieran transformarse en nuevos medicamentos y, con este fin, se diseñó el Proyecto X - 1 Búsqueda de Principios Bioactivos en Plantas de la Región.

Diversas consideraciones motivaron el planteamiento de este proyecto, entre ellas las siguientes: la región dispone de la mayor y más rica biodiversidad por lo cual existe un lugar privilegiado para la investigación, existe una importante tradición etnofarmacológica y un uso frecuente de las plantas por grandes núcleos de la población, el potencial terapéutico de estos recursos no ha sido estudiado, existen mejores y más apropiados bioensayos y un notorio avance en las tecnicas para los estudios fitoquímicos, se observa un especial interés de los organismos internacionales vinculados al área de la salud y de algunos sectores de la industria farmacéutica por el estudio de las plantas medicinales y, por último, en la región hay grupos de investigadores con una infraestructura adecuada para realizar las tareas de investigacion.

Es ampliamente conocido el hecho de que la mayor parte de las especies vegetales existentes en los países iberoamericanos no ha sido estudiada, por lo cual se ignora un importante potencial de compuestos, no solamente con actividad biológica, sino también útiles en sectores industriales y alimenticios. Adicionalmente, los países latinoamericanos importan cantidades considerables de fármacos, muchos de los cuales se encuentran como recursos naturales en los mismos países.

Por otra parte, el estudio de las plantas medicinales se ha constituido en una preocupación permanente de los organismos que tijan políticas sobre la salud de la población, y últimamente se ha hecho reconocimiento de la importancia que ellas tienen en los sistemas de salud de los países en desarrollo, principalmente.

El Proyecto tuvo un enfoque integral e interdisciplinario y su éxito dependio en buena parte de la voluntad de realizar trabajos conjuntos entre los grupos de investigadores y de intercambiar información, muestras y resultados entre ellos. Su objetivo final fue la obtención de pistas o cabezas de serie con actividad biológica las cuales, ellas o sus análogos, puedan tener uso terapéutico. Se perseguía obtener el mayor conocimiento posible de las especies vegetales desde el punto de vista biológico y químico, promover una mejor y más racional utilización de estas plantas y confirmar o descubrir nuevos efectos biológicos.

El Proyecto se inició en Noviembre de 1991 y terminó en Noviembre de 1996. Inicialmente se vincularon al mismo, 11 grupos de investigadores de ocho países; posteriormente se integraron 9 grupos más para completar 20 grupos con 246 investigadores de instituciones de 11 países: Argentina, Bolivia, Chile, Colombia, Costa Rica, Ecuador, España, Guatemala, Panamá, Perú y Portugal. Se trataba de grupos iberoamericanos que estaban desarrollando trabajos en el área de los productos naturales de uso medicinal, que disponían de una infraestructura adecuada y de recursos económicos para llevar a cabo las investigaciones.

Se adoptó una metodología común para todos los grupos, diseñada en cuatro fases con el fin de facilitar la vinculación de un mayor número de grupos. La metodología estaba orientada hacia la realización de estudios bioguiados, y comprendía desde la selección de las especies, la ejecución de las pruebas de actividad biológica hasta el aislamiento y la caracterización química de los compuestos presentes en las especies seleccionadas. La selección de las especies se hizo teniendo en cuenta prioritariamente la información sobre su uso popular como medicinal y los aspectos quimiotaxonómicos. La revisión bibliográfica fue prolija y el estudio de las plantas solamente se inició cuando fue efectuada esta revisión en forma sistemática.

A través de la realización del Proyecto se estudiaron 238 especies vegetales, pertenecientes a 56 familias, provenientes de 14 países iberoamericanos. A estas especies se les hicieron ensayos de actividad biológica y un cribado químico. Con base en los resultados obtenidos en estos ensayos se adelantaron investigaciones más profundas con algunas de las especies estudiadas. Se tienen resultados de 568 pruebas de actividad biológica, entre ellas actividad antiinflamatoria, antiprotozoaria, analgésica, antimalárica, antimicótica, moluscicida, antibacteriana, antipirética, cardiovascular, antiherpética, antidiarreica, hipoglucemiante, sobre el sistema nervioso central, diurética, hipnótica, antisida, citotóxica, espasmolítica, virucida, atrapadora de radicales libres y hepatoprotectora. También se realizaron estudios de toxicidad aguda y subcrónica. Se han aislado y caracterizado químicamente 65 compuestos conocidos y 15 compuestos nuevos, a los cuales se les realizan pruebas para determinar posibles actividades farmacológicas. Entre estos últimos compuestos se mencionan los siguientes: 1,3,6-trihidroxi-2,5-dimetoxixantona, 2-metilpropenoato de 11,13-dihidromelitensina, 4-trihidroxi 6-metoxichalcona, 6-desacetoxi -2-beta-hidroxi-korberina B.

Los resultados obtenidos son muy positivos y con la continuación de algunos de los estudios en marcha se prevé su posible utilización en terapeutica, puesto que se ha avanzado considerablemente y se ha llegado a un importante punto de cooperación entre los grupos de investigadores que permite presagiar un aumento en la obtención de resultados importantes. Estos resultados constituyen, además, un valioso aporte para la realización de los Proyectos X-2: *Síntesis de Moléculas Bioactivas Análogas de Productos Naturales de Origen Iberoamericano* y X-3: *Evaluación de la Biodiversidad de Países Iberoamericanos como Fuente de Agentes Inmunomoduladores y Quimioterapéuticos*.

La divulgación de los resultados de los trabajos realizados se ha hecho a través de 26 publicaciones científicas, 56 ponencias en congresos científicos, 20 tesis de Postgrado y 73 tesinas. Entre las publicaciones citamos, a manera de ejemplo, las siguientes: Cytotoxic Activities of Colombian Plants Extracts on Chinese Hamster Lung Fibroblasts, Analgesic and Antiinflamatory Effects of *Drymonia serrulata* (Jacq.) Mart., Screening of Panamanian Medicinal Plants for Brine Shrimp Toxicity, Crown Gall Tumor Inhibition, Cytotoxicity and DNA Intercalation, Pharmacological and Phytochemical Studies of *Cephaelis axillaris*, Antiinflamatory and Analgesic Activity of *Baccharis trinervis*: Identification of its Active Constituents, Actividad Antimicrobiana de Diez Plantas Nativas Usadas en Guatemala, Inhibitory Effects of Various Extracts of Argentine Species on Free Radical Mediated Reaction and Human Neutrophil Functions.

Doctor Roberto Pinzon.

Uno de los logros más importantes del Proyecto tiene relación con acciones de cooperación entre grupos de investigadores. Una actividad resultante de la cooperación entre los grupos es su participación en algunos programas patrocinados por la Comunidad Europea y la vinculación a una red en el marco del Programa Alfa. Asimismo, ha sido de gran importancia la participación de algunos grupos de investigadores en Proyectos IBEROEKA.

Especial relevancia se le dio al aprendizaje de nuevas técnicas por parte de los investigadores vinculados al Proyecto. En cumplimiento de esta estrategia se ha desarrollado un exitoso programa de movilidad de científicos y se ha estimulado la participación de éstos en talleres programados por el Subprograma X Química Fina Farmacéutica y en algunos otros que se consideraron de interés para su formación y/o perfeccionamiento. 30 científicos de 13 países han efectuado trabajos en centros de investigación de grupos pertenecientes al Proyecto, con una duración aproximada de 70 meses. En lo relacionado con los talleres, más de 150 investigadores tomaron parte en los 20 organizados por el Subprograma, entre los cuales podemos mencionar los siguientes: Bioensayos Quimioterapéuticos de Productos Naturales con Énfasis en Actividad Antiviral, Técnicas de Estudio de Fármacos Activos a nivel de Inflamación y otros Procesos Mediados por Radicales Libres, Bioensayos Enzimáticos de Productos Naturales con Énfasis en Actividad Anticáncer, Utilización de Productos Naturales en la Industria Farmacéutica, Resonancia Magnética Nuclear Aplicada a Productos Naturales, Estrategias en el Aislamiento de Productos Naturales, Nuevos Avances en la Metodología de Screening Anticáncer e Informática en Productos Naturales, Quimioterapia Antiparasitaria: Malaria, Leishmania y Enfermedad de Chagas.

El Proyecto cumplió con los objetivos propuestos y deja como una contribución importante, además de los resultados mencionados, la conformación de una masa crítica de investigadores en el área de los productos naturales de uso medicinal que, seguramente lograran exitosas contribuciones en aspectos relacionados con la terapéutica moderna.

(*) *Jefe del Proyecto X.1, Investigador del Departamento de Farmacia, Universidad Nacional de Colombia.*

RED IBEROAMERICANA DE INDICADORES DE CIENCIA Y TECNOLOGÍA (RICYT)

TALLER DE OBTENCION DE INDICADORES BIBLIOMÉTRICOS

Madrid, 23-25 de Febrero de 1998

Doctora Isabel Gómez (*)
Doctora Rosa Sancho (**)

Doctora Isabel Gómez Caridad.

Doctora Rosa Sancho.

LA RICYT fue creada en 1995 como una red del Subprograma XVI de CYTED, «*Gestión de la Investigación y el Desarrollo Tecnológico*», con el apoyo de la Organización de los Estados Americanos (OEA). Su objetivo es reunir indicadores de inversiones a los sistemas de Ciencia y Tecnología de la región (financiación, gasto y personal dedicado a CyT). Se definieron los parámetros comunes para la medición de las actividades científicas y tecnológicas, y se intenta armonizar los conceptos utilizados y normalizar las técnicas estadísticas empleadas.

Hasta la fecha se han publicado dos volúmenes con el título «*Indicadores de Ciencia y Tecnología Iberoamericanos/Interamericanos*», que cubren un total de 30 indicadores de inversiones, de los periodos 1990-95 y 1990-96, con datos de 25 países (América Latina y Caribe, España, Portugal, EEUU y Canadá).

En Octubre de 1996, se decidió introducir indicadores cuantitativos de resultados científicos y técnicos adecuados a estos países, para lo que previamente se organizó un Taller especializado en indicadores bibliométricos de producción científica, en el Centro Nacional de Información y Documentación Científica (CINDOC), del Consejo Superior de Investigaciones Científicas (CSIC), de Madrid, con la participación de 14 expertos iberoamericanos y 12 del propio CINDOC.

Dicho Taller se estructuró en tres sesiones. La primera se centró en los indicadores bibliométricos obtenidos a partir de bases de datos internacionales, tanto de carácter multidisciplinar (Science Citation Index, SCI), como especializadas en diversas disciplinas (Química, «Chemical Abstracts»; Física «Physics Abstracts»; Medicina, «Medline»; Ingeniería, «Engineering Index»; Biología, «Biological Abstracts», etc). Se puso de manifiesto que las bases de datos habitualmente empleadas en los países desarrollados para la obtención de indicadores de producción científica, específicamenet el SCI, presentan serias limitaciones al ser aplicados a los países iberoamericanos, debido, principalmente, a la escasa cobertura de revistas locales y a que no recogen habitualmente otro tipo de literatura científica comunmente empleadas en estos países, como son los informes técnicos. Por tanto, los indicadores obtenidos de estas bases de datos deben ser complementados con información procedente de otras fuentes.

La segunda sesión se ocupó de indicadores bibliométricos a partir de bases de datos multicéntricas, regionales o nacionales, en las cuales la cobertura de literatura iberoamericana es más exhaustiva y la selección de las fuentes se realiza en los propios países. Se presentaron las bases de datos multicéntrica *AGRIS* de Agricultura, la regional *LILACS* de Medicina y las nacionales *PERIÓDICA* y *CLASE* de México, relativas a Ciencias Naturales y Ciencias Sociales y Humanidades, respectivamente, *CUBACIENCIA* de Cuba, multidisciplinar, y las españolas *ICYT* e *ISOC*, también de Ciencias Naturales y Ciencias Sociales y Humanidades, respectivamente.

Se pusieron de manifiesto los problemas de control de calidad de las bases de datos nacionales, la falta de coordinación entre los centros cooperantes cuando se trata de las bases de datos multicéntricas o regionales y la falta de metodología en la recopilación e introducción de datos. Se resaltaron también los perjuicios derivados de la demora temporal entre la edición de la revista y su inclusión en la base de datos. Asimismo, se destacó la conveniencia de evitar duplicaciones de esfuerzos con una mejor coordinación de las actividades de creación de base de datos.

La tercera sesión se centró en las revistas locales como fuentes de las bases de datos. Se presentó el Informe del II Taller de Publicaciones Científicas en América Latina celebrado en Guadalajara, México, en Noviembre de 1997 y el Proyecto LATINDEX para la elaboración de un catálogo de publicaciones periódicas de la región. Se expuso la experiencia española en la evaluación de la calidad de revistas científicas y se discutieron los criterios para evaluar la calidad científica de las publicaciones seriadas iberoamericanas. Se destacó el importante papel de los editores para conseguir una difusión internacional de las revistas nacionales.

Como conclusiones generales del Taller se destacaron las siguientes:

1) No existe una única base de datos que recoja toda la producción científica de calidad de los países iberoamericanos. El SCI empleado universalmente para medir la producción presenta una reducidísima cobertura de revistas de estos países, y su empleo da lugar a falsas interpretaciones.
2) Los indicadores bibliométricos obtenidos de las bases de datos multidisciplinarias y especializadas de ámbito internacional; así como de las nacionales, serán paraciales y complementarias entre sí.
3) La falta de normalización de las bases de datos, que deriva del hecho de que han sido diseñadas con fines bibliográficos y no bibliométricos, llevó al acuerdo de sugerir a sus creadores que las adapten para la obtención de indicadores bibliométricos, es decir, recojan los lugares de trabajo de todos los autores, identifiquen cada autor con su institución y hagan figurar el país de la institución en un campo de consulta independiente.
4) Se propone sugerir a los editores de revistas científicas que se adapten a las normas internacionales para la edición de este tipo de publicaciones, incluir los nombres y direcciones de todos los firmantes de los trabajos, y distribuir las revistas que publican en el mayor número de bibliotecas y bases de datos, tanto locales como de otros países; así como en las de carácter internacional, a fin de conseguir una mayor difusión de los trabajos en ellas publicados.
5) Se propone que el esfuerzo que se está llevando a cabo para hacer un repertorio de revistas científicas y técnicas iberoamericanas y evaluar la calidad científica de éstas, se ampare en un marco institucional supranacional, como podría ser el Programa CYTED.
6) Se planteó la necesidad de un estudio teórico para diseñar un sistema de indicadores más específicos para Iberoamérica que responda a los objetivos de la ciencia en esos países y complemente a los indicadores bibliométricos.
7) Se destacó el hecho de que los indicadores bibliométricos los origina un colectivo de sociólogos y documentalistas, mientras que es otro colectivo diferente, que desconoce las limitaciones y márgenes de error de dichos indicadores, quien los emplea para fines de política científica. Por tanto, es importante resaltar que se trata de indicadores parciales, cuya fiabilidad es mayor en ciencia básica que en aplicada, su validez difiere de unos temas a otros por el empleo de diferentes vehículos de difusión de resultados, y su validez es mayor cuando se aplican a países o grandes colectivos que cuando se consideran pequeños grupos o individuos.

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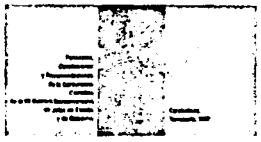
PUBLICACIONES CYTED

LOS DESAFÍOS ÉTICOS DE LA INVESTIGACIÓN CIENTÍFICA Y TECNOLÓGICA EN IBEROAMÉRICA

Secretaría General del Programa CYTED. Madrid, España.
Mayo 1997.
223 pp.



LOS DESAFÍOS ÉTICOS DE LA INVESTIGACIÓN CIENTÍFICA Y TECNOLÓGICA EN IBEROAMÉRICA

La publicación comprende las Ponencias, Conclusiones y Recomendaciones de la Conferencia Científica de la VII Cumbre Iberoamericana de Jefes de Estado, celebrada en Caraballeda, Venezuela, del 8 al 10 de Octubre de 1997 y cuyo tema central se encuentra reflejado en el título de esta publicación.

La publicación contiene:

Presentación
Discursos del Acto Inaugural de la Conferencia Científica
Ponencias y Relatoría de la Sesión I «LA BIOÉTICA EN LA INVESTIGACIÓN CIENTÍFICA Y TECNOLÓGICA»
Ponencias y Relatoría de la Sesión II «PROBLEMAS ÉTICOS DE LA CONSERVACIÓN Y USO DE LA BIODIVERSIDAD»
Ponencias y Relatoría de la Sesión III «INTEGRIDAD ÉTICA DE LA INVESTIGACIÓN CIENTÍFICA»
Ponencias y Relatoría General de la Sesión IV CONCLUSIONES Y RECOMENDACIONES.

Además contiene dos Anexos:

Anexo I Contribuciones escritas a la Conferencia Científica
Anexo II Relación de Participantes

La presentación es una interesante reflexión sobre las implicancias del progreso moral frente al progreso tecnológico de la sociedad y como inciden estos aspectos en la investigación científica.

Interesados en disponer de esta publicación, pueden dirigirse a la Secretaría General del Programa CYTED. Avenida de Reyes Católicos, 4. 28040 Madrid. España. Teléfono: (341) 583 82 68 - 336 04 29. Fax (34.1) 583 84 10/11/13 E-mail: cyted@aecl.es - cyted@cicyt.es

REPAIR AND REHABILITATION OF REINFORCED CONCRETE STRUCTURES: THE STATE OF THE ART

Walter F. Silva Araya, Oladis Trocónis de Rincón, Luis Pumarada O'Neill. (Editores)

NUEVAS TENDENCIAS EN LA REPARACIÓN Y REHABILITACIÓN DE ESTRUCTURAS DE CONCRETO REFORZADO.
Seminario Internacional, Taller y EXPOCENTRO'97
ISBN 0-7844-0299-X
ASCE 1997
241 pp

Esta publicación contiene los trabajos que fueron expuestos en el Seminario Taller Internacional referido, celebrado en Maracaibo, Venezuela, del 27 de Abril al 1 de Mayo de 1997, al que asistieron 150 participantes de 12 países: *ingenieros, contratistas, funcionarios gubernamentales, profesores, estudiantes e investigadores involucrados en el desarrollo y aplicación de tecnologías en los temas de discusión.*

El evento fue auspiciado por el Programa CYTED, la *National Science Foundation*, el *Centro de Estudios para la Corrosión* de la Universidad de Zulia y el Centro para la Cooperación Hemisférica en Investigación y Educación en Ingeniería y Ciencias Aplicadas (Co-Hemisi) de la Universidad de Puerto Rico. El libro fue revisado en su contenido, *aprobado y publicado por la American Society of Civil Engineers* (ASCE).

También contiene el sumario de los trabajos expuestos, las necesidades de investigación, procedimientos para la rehabilitación y reforzamiento de estructuras de concreto, desde diversos puntos de vista y en los diferentes países. El evento también sirvió para crear un marco de intercambio de experiencias, posibilidades de desarrollar proyectos conjuntos de investigación, exhibición de productos y servicios por compañías locales e internacionales, discusiones de los *grupos para identificar temas de interés para la colaboración* internacional, así como, proveer de un mecanismo de transferencia tecnológica y colaboración internacional.

Esta publicación resulta beneficiosa para investigadores e ingenieros, ya que es un compendio del estado del arte y retos futuros en este campo. Los sumarios de los trabajos están publicados en inglés y español.

La publicación contiene lo siguiente:

Foreword
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Workshop
Workshop Program
Participant Information
Breakout Group Topics and Leaders
Breakout Group Participants
Workshop Summary (English Version)
Workshop Summary (Spanish Version)
Papers
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Advanced Computational Model for Sacrificial Cathodic Protection of Tertel Submerged Reinforced Concrete Marine Footers
Alberto Sagues, S.C. Kranc, Francisco Pressuel-Moreno.
Protection Corrosion Mechanisms Involved in Repair Systems with Surface Coatings on Reinforced Cement
E. Pazini and C. Andrade
Practical Application of Cathodic Protection Systems for Reinforcing Steel Substructures in Marine Environment
Ivan R. Lasa, Rodney G. Powers, Richard J. Kessler
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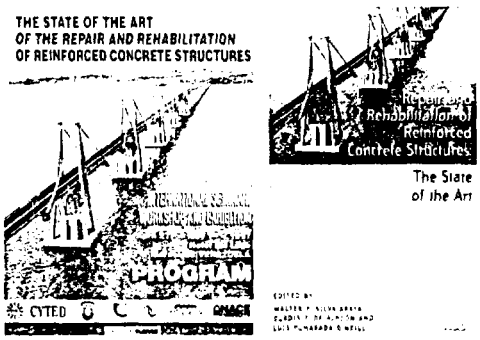


Para mayor información, dirigirse a: Doctora Oladis Trocónis de Rincón, Coordinadora Internacional de la Red Iberoamericana de Corrosión en Estructuras de Concreto Armado (DURAR), Directora del Centro de Estudios de Corrosión, Facultad de Ingeniería, Universidad de Zulia, Avenida Goajira, Apartado Postal 10.482, Estado de Zulia, Venezuela. Teléfono: (58.61) 51 21 97, Fax: (58.61) 52 57 32/59 85 27.

MANUAL DE INSPECCIÓN, EVALUACIÓN Y DIAGNÓSTICO DE CORROSIÓN EN ESTRUCTURAS DE HORMIGÓN ARMADO

DURAR Red Temática XV.B Durabilidad de la Armadura - CYTED
Subprograma XV Corrosión Impacto ambiental sobre Materiales
Comité Editorial. Oladis Trocónis de Rincón. Aleida Romero de Carruyo. Carmen Andrade. Paulo Helene. Isabel Díaz

ISBN 980-296-541-3
Abril 1997
208 pp

La obra refleja el esfuerzo realizado por especialistas de 10 países iberoamericanos en la elaboración del presente manual que aborda aspectos normativos para el mantenimiento de estructuras de hormigón armado.

La obra comprende, además del prólogo e introducción, seis capítulos que tratan sobre los aspectos relacionados con esta temática. Estos son:

Capítulo I. Fundamentos Generales de Corrosión
1. Generalidades
2. Corrosión de la Armadura en el Hormigon
3. Tipos de Corrosión
4. Factores que afectan y desencadenan la corrosión de las armaduras.
5. Métodos de Prevención y Protección contra la Corrosión.
6. Vida útil y Vida Residual

Capítulo II Procedimientos de Inspección
1. Generalidades
2. Inspección Preliminar
3. Inspección detallada

Capítulo III Descripción de Métodos de Ensayos
1. Análisis físico-químicos del Hormigon
2. Evaluación del Estado de la Armadura

Capítulo IV Diagnóstico General desde el punto de vista de Corrosión
1. Generalidades
2. Bases del Diagnóstico
3. *Procedimiento General del Diagnostico*

Capítulo V. Pronóstico y Evaluación de la Vida Residual de la Estructura

VI. Orientación para una correcta reparación y rehabilitación
1. Generalidades.
2. Procedimiento General de Reparación.
3. Alternativas de Reparación.
4. Procedimiento detallado de la Reparación.

BIBLIOGRAFÍA
GLOSARIO
NOMENCLATURA
ANEXO

En cada capítulo y epígrafe hay subapartados que tratan sobre aspectos más específicos relacionados con cada uno de los temas.

Para mayor información, dirigirse a: Red Iberoamericana de Corrosion en Estructuras de Concreto Armado (DURAR). Doctora Oladis Trocónis de Rincón. Coordinadora Internacional de la Red. Directora del Centro de Estudios de Corrosión. Facultad de Ingeniería. Universidad de Zulia. Avenida Goajira. Apartado Postal 10.482. Estado de Zulia. Venezuela. Teléfono: (58.61) 51 21 97. Fax: (58.61) 52 57 32 / 59 85 27.

TEMAS EN TECNOLOGÍA DE ALIMENTOS. VOLUMEN I

Red Iberoamericana de Propiedades Físicas de Alimentos Relevantes para el Diseño Industrial (RIPFADI)
CYTED

José Miguel Aguilera (Editor)
Instituto Politécnico Nacional
México, 1997
ISBN 970-18-0934-3
337 pp

La tecnología de alimentos constituye un área del conocimiento muy importante en Latinoamérica. Entre las razones para la preeminencia del area destacan: la trascendencia de la agricultura y del procesamiento primario de productos agropecuarios en la economía de países de la región; la urgencia por contar con alimentos en cantidad y calidad suficiente para hacer frente a los requerimientos nutricionales crecientes de la población, en particular para los grupos más vulnerables, la necesidad de preservar alimentos en condiciones climáticas adversas, que por ajenas al mundo desarrollado no se investigan; la gran variedad de materias primas autóctonas, comidas étnicas y procesamientos milenarios que aguardan una respuesta tecnológica para hacer frente al modernismo; la premura para dar mayor agregado a las exportaciones agropecuarias y marítimas; la relevancia en los mercados mundiales de productos agrícolas de la región y por último, la creciente tecnificación de la alimentación en los grandes centros urbanos.

El libro es fruto de la experiencia académica y profesional de un distinguido grupo de científicos y profesores de Argentina, Chile, España, México y Uruguay, asociados al esfuerzo emprendido por el Programa CYTED en materia de tecnología de alimentos e interesado en transmitir a los jóvenes estudiantes, técnicos y profesionales relacionados con los temas de la actividad del agua, las nuevas o renovadas técnicas de conservación, la textura y las propiedades físicas de los alimentos, que en conjunto nos acercan a nuevos enfoques de la tecnología y la ingeniería de los alimentos. En esta publicación cuya coordinación de temas, revisión y edición han estado a cargo del Doctor José Miguel Aguilera, aparecen los trabajos de especialistas iberoamericanos muy reconocidos internacionalmente en los diversos campos de la tecnología de alimentos.

El contenido de la publicación es el siguiente:

Prólogo
Efrén Parada Arias (México)

Actividad del Agua. Concepto y aplicación en alimentos con alto contenido de humedad
Jorge Welti Ch. y Fidel Vergara B. (México)

Preservación I

Alimentos conservados por factores combinados
Stella Maris Alzamora (Argentina)

Preservación II

Atmósferas controladas y modificadas
José Manuel del Valle y María Teresa Palma (Chile)

Presevación III

Congelación de alimentos
Noemí E. Zaritzky (Argentina)

Fritura de Alimentos
José Miguel Aguilera (Chile)

Propiedades Físicas I

Reología de sólidos y textura
Elvira Costell, Susana M. Fiszman y Luis Durán (España)

Propiedades Físicas II

Ópticas y color
Carlos Calvo y Luis Durán (España)

Propiedades Físicas III

Caracterización de alimentos en polvo
Gustavo V. Barbosa Cánovas, Humberto Vega Mercado y Enrique Ortega Rivas (México)

Para mayor información, dirigirse al Doctor José Miguel Aguilera, Coordinador Internacional de la Red RIPFADI. Jefe del Departamento de Ingeniería Química. Pontificia Universidad Católica de Chile. Vicuña Mackenna 4860 - Casilla 306. Santiago 22. Chile. Teléfono: (562) 552 23 75 Anexo 4256. Fax: (562) 686 58 03-E-mail: 240395@puevaa.cl

PRINCIPALES INDICADORES DE CIENCIA Y TECNOLOGÍA

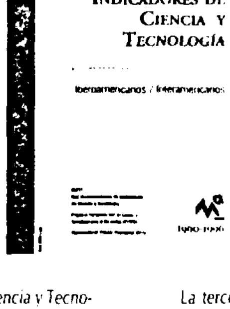
IBEROAMERICANOS / INTERAMERICANOS. 1990 - 1996

Red Iberoamericana de Indicadores de Ciencia y Tecnología

Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo (CYTED)

Organización de los Estados Americanos (OEA)

Buenos Aires, Argentina
Febrero 1998
ISSN 0329-4838
239 pp



Este informe fue realizado en base a la respuesta brindada por los paises al requerimiento de 1997. En algunos casos, debido a la falta de información más recientes, se han tomado en consideración los datos surgidos del requerimiento de 1996. *Los datos han sido presentados por los Organismos Nacionales de Ciencia y Tecnología.* El diseño de los instrumentos y la estructura del presente trabajo, son frutos de las recomendaciones surgidas de los talleres metodológicos de la RICYT. Se han tomado en cuenta, particularmente, las pautas del Manual de Frascati de la OCDE y las conclusiones de una reunión preparatoria realizada en Sao Paulo, Brasil, a fines de 1996, auspiciada por el Ministerio de Ciencia y Tecnología de Brasil.

El hecho de que los indicadores cuantitativos no contaron con un nivel de respuesta uniforme se debe a que se solicitó a los paises que los valores referidos al gasto *fueran desglosados en función de los conceptos de actividades científicas y tecnológicas* (ACT) e *investigación y desarrollo exprimental* (I+D). En el relevamiento realizado en 1996 por la RICYT, no estaba presente la introducción de parametros vinculados al conceptio de ACT.

El informe consta de tres partes. En la primera se presenta una descrpción de la imagen de la ciencia y la tecnología en América Latina tal como surge de la información obtenida y se analizan conceptualmente los indicadores seleccionados.

La segunda parte tiene como objeto permitir el análisis de la Información de cada pais por separado. Se reseñan para cada pais los rasgos principales del sistema institucional de ciencia y tecniologogía, en base a cinco puntos: 1) Estructura institucional; 2) Marco legal; 3) Instituciones que ejecutan I+D; Instituciones que realizan servicios científicos y tecnológicos; 5) Datos del organismo nacional de ciencia y tecnología. A ésto se suma la presentación de 30 indicadores cuantitativos.

La tercera parte presenta estos indicadores en forma comparativa para el conjunto de paises.

Para mayor información, dirigirse al Doctor Mario Albornoz, Coordinador Internacional de la Red RICYT, Instituto de Estudios Sociales de la Ciencia y la tecnología, Universidad de Quilmes, Grupo REDES A.C. - Rivadavia 2358, 6° Piso, 1034 Buenos Aires, Argentina. Telefax: (541) 951 82 21 / 2431. E-mail: ricyt@ricyt.edu.ar

I SEMINARIO INTERNACIONAL SOBRE MEJORAMIENTO Y REORDENAMIENTO DE ASENTAMIENTOS URBANOS PRECARIOS MEJORHAB

Proyecto XIV. 4 CYTED.
Caracas, 1997
367 pp

La publicación ha sido realizada bajo la coordinación del Comité Organizador del Seminario y contiene los textos de las conferencias y los trabajos aprobados por dicho Comité y presentados durante el Seminario.

El contenido de la publicación es el siguiente:

CONFERENCIAS

- CONSORCIO CATUCHE

José Virtuoso. César Martín, Mary Gloria Olivo (Venezuela)

- **Hacia la generación de políticas populares autogeneratorias de rehabilitación edilicia en la ciudad de Buenos Aires. 2 Casos: Cooperativas San Telmo y La Unión, y 1 Organización: El Movimiento de Ocupantes e Inquilinos.**

Néstor Jeifetz (Argentina)

- **Mejoras Urbanas, Alternatvas y Ciudadanía**

Paulo Eduardo Fonseca de Campos (Brasil)

- **Auto-Gestión y Tecnología**

Alexander Svoei Yamaguti (Brasil)

- **Convenios de Programación para el Mejoramiento Integral del Hábitat**

Ignacio Canales Molina (Chile)

- **Acciones Gubernamentales en el Mejoramiento y Reordenamiento de Asentamientos Urbanos Precarios. La experiencia de dos programas chilenos.**

Rubén Patricio Sepúlveda Ocampo (Chile)

- **Los Actores de la Rehabilitación de espacios urbanos deteriorados.**

Ana Sugraves (Chile)

- **Rescate de zonas precarias para la ciudad**

Ricardo Muttoni (Uruguay)

- **Mejoramiento integral de los barrios a través de la autogestión**

Josefina Baldó Ayala, Federico Villanueva Brandt (Venezuela)

- **Tecnologías no convencionales aplicadas a la infraestructura física de los asentamientos urbanos en zonas precarias.**

Carmen Yanes M. (Venezuela)

TRABAJOS

- **Paneles aislantes de concreto armado, como elementos portantes de cierre para casas de interés social.**

Alexandra Araujo Bertini, Elov Ferraz Machado Junior (Brasil)

- **Del Urbanismo de Centro al Urbanismo de Borde**

Helen Barroso, Francisco Mustieles y Araxi Latchinián (Brasil)

- **Política Habitacional en Municipio Carente: Franco da Rocha**

M. Albertina, J. Carvalho, Lucas Fehr (Brasil)

- **Concreto con Agregado Grueso Reciclado de residuos de concreto, un nuevo material de construcción para componentes habitacionales de interés social.**

Machado Junior, Elov Ferraz (Brasil)

- **Aspectos tecnológicos para la producción de concretos de alto rendimiento. Un medio para la calificación de un equipo de alto rendimiento.**

Jefferson B.L. Liborio, Isac José da Silva, Aluiso Braz de Melo (Brasil)

- **El encofrado incorporable a los elementos estructurales de concreto armado, como una alternativa a los sistemas de encofrado tradicionales.**

Jefferson B.L. Liborio, Isac Jose da Silva, Aluiso Braz de Melo, Mestre Rui Ferraz de Almeida Prado Massoni (Brasil)

- **La producción de prefabricados livianos, mediante secado térmico (vapor) bajo presión atmosférica, destinados a elementos estructurales.**

Jefferson B.L. Liborio, Isac José da SZilva, Aluiso Braz de Melo (Brasil)

- **Evaluación del rendimiento de sistemas constructivos innovadores: Estudio de caso.**

Fabiana Lopes de Oliveira, Antônio Abreu Filho (Brasil)

- **Uso de revestimientos resistentes de concreto armado en la rehabilitación de construcciones de mampostería.**

Fabiana Lopes de Oliveira, João Bento de Hanai (Brasil)

- **Residuos de construcción y demolición, una fuente auxiliar de agregados gruesos para concretos estructurales de baja resistencia.**

Machado Junior Elov Ferraz, Luciano M. Latterza (Brasil)

- **Aplicación de técnicas de texturización y coloración de mezclas y concretos en la rehabilitación de habitaciones.**

Osny Pellegrino Ferreira, João Bento de Hanai (Brasil)

- **Aplicaciones de resina poliuretana de origen vegetal para cobertura de losas.**

Osny Pellegrino Ferreira, Francisco Artthur da Silva Vecchia (Brasil)

- **Dimensiones del conjunto habitacional y la segregación espacial**

Eduardo Piza Pereira Gomes, Leandro de Oliveira (Brasil)

- **Génesis y consolidación: Hacia una tipificación del espacio público abierto. Caso de Estudio: Barrios «23 de Marzo y Virgen del Carmen», Parroquia Idelfonso Vásquez, Maracaibo.**

Tomás Perez Valecillos, César Castellano (Venezuela)

- **Una experiencia de mejoras y reorganización del área periurbana.**

Marcos J. A. Santana, Luiz R. Moraes (Brasil)

- **Empleo de agregados obtenidos de escombros en la construcción de una vivienda económica.**

Andrés Francisco Urrutia Rodriguez (Venezuela)

- **Satisfacción Residencial en viviendas unifamiliares de área reducida.**

Fabiola Vivas, Oscar Moros (Venezuela)

- **Condiciones para un Proyecto de Autoconstrucción comunitaria de viviendas derivaciones de una experiencia con un grupo de familias damnificadas.**

Esther Wiesenfeld (Venezuela)

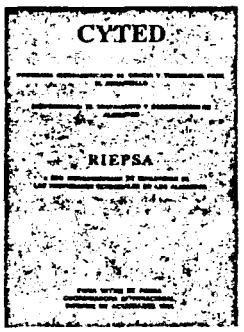
Para mayor información, dirigirse al Doctor Paulo Eduardo Fonseca de Campos, Jefe del Proyecto XIV.4- ABCP, Associação Brasileira de Cimento Portland, Avenida Torres de Oliveira, 76- Jaquaré, 05347-902 Sao Paulo, Brasil. Telefono: (55.11) 268 51 11 Extensión 125 y 184. Fax: (55.11) 376 053 20- E-mail: petcyted@fractal.com.br

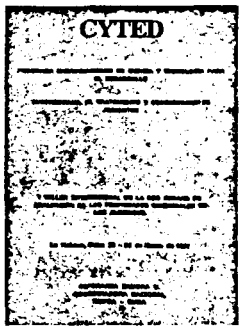
RIEPSA. RED IBEROAMERICANA DE EVALUACIÓN DE LAS PROPIEDADES SENSORIALES DE LOS ALIMENTOS. INFORME DE ACTIVIDADES 1996

Emma Witting de Penna, Coordinadora Internacional
Santiago, Chile, Marzo 1997
134 pp

I TALLER INTERNACIONAL DE LA RED CUBANA DE EVALUACIÓN DE LAS PROPIEDADES SENSORIALES DE LOS ALIMENTOS
Esperanza Zamora U. Coordinadora Nacional RIEPSA Cuba
La Habana, Cuba, Marzo 1997

39 pp
Subprograma XI Tratamiento y Conservación de Alimentos
CYTED





Ambas publicaciones corresponden a una de las actividades del Subprograma XI. Tratamiento y Conservación de Alimentos, la Red RIEPSA, que cuenta con 316 centros asociados distribuidos en todos los países de la Región Iberoamericana.

En la primera de las publicaciones se presenta el Informe Anual 1996 de la Red que contiene la información referida a las Reuniones de coordinación RIEPSA, cursos dictados, viajes de coordinación, estadías de investigación, reuniones de coordinación, directorio de entidades copatrocinadoras, actividades de divulgación. También figura la información sobre la Reunión Internacional de Coordinadores, programa y acta; así como, las actividades realizadas por los países que integran la Red: Argentina, Bolivia, Brasil, Colombia, Costa Rica, Cuba, Chile, Ecuador., El Salvador., España, Guatemala, México, Nicaragua, Panamá, Paraguay, Perú, Portugal, República Dominicana, Uruguay y Venezuela . También se incluye un Directorio de Coordinadores Nacionales, Programa de Actividades 1997, Relatorio Técnico de «SENSIBER'96- I Simposio Iberoamericano de Análisis Sensorial « realizado en Campinas, Sao Paulo, Brasil, del 30 de Junio al 3 de Julio de 1997 y como anexos la información sobre cursos, talleres, boletines, etc. que aparecen en esta publicación.

En la segunda publicación se presenta una monografía correspondiente a los anales del I Taller Internacional de Evaluación Sensorial» celebrado en La Habana, Cuba, el 20 de Marzo de 1996 dentro del marco de CICTA V.

La recopilación de las conferencias y de los trabajos presentados en forma de poster, corresponde al trabajo de la Coordinadora Nacional de la Red en Cuba, Profesora Esperanza Zamora, del Instituto de Investigaciones de la Industria Alimenticia.

El contenido de la publicación es el siguiente:

CONFERENCIAS

- **RIEPSA. Logros y perspectivas futuras.**
  E. Witting de Penna
- **Desarrollo de la Evaluación Sensorial en Cuba**
  E. Zamora
- **La estadística en el desarrollo de la Evaluación Sensorial.**
  I. Rodríguez

ACTIVIDAD CENTRAL DEL DIES

- **Reseña histórica, presente y futuro de la Evaluación Sensorial**
  L. Castillo
- **Actualización de metodologías de Evaluación Sensorial**
  L. Castillo

TRABAJOS EN POSTERS EN CICTA V

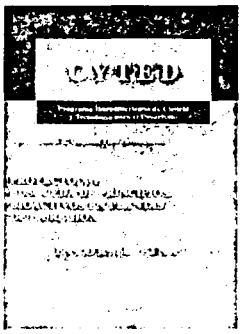
- **Aceptabilidad del yogurth de soya**
- **Adiestramiento de evaluadores para cambios de color**
- **Cómo determinar expectativas de aceptación y preferencias**
- **Comparación de evaluación de dos rones cubanos**
- **Análisis descriptivo cuantitativo en conservación en diferentes envases**
- **Caracterización sensorial de plátano Burro cv.crema**
- **Estudio organoléptico del sabor carne en pasta**
- **Evaluación de atributos y defectos del sabor de vinos mexicanos**
- **Formación de catadores para evaluación de textura de alimentos**
- **Aceptación de lomitos en conserva**
- **Sabor y textura en algunas variedades de queso**
- **Características sensoriales y composición química de rones**
- **Verificación de jueces**
- **Guía general para adiestramiento de evaluadores de rones cubanos**
- **Control de calidad sensorial de figuras bañadas en chocolate**
- **Vocabulario para tipificación de vinos mexicanos**
- **Optimización de alimentos para la tercera edad.**

Para mayor información, dirigirse a la Doctora Emma Witting de Penna, Coordinadora Internacional de la Red RIEPSA. Facultad de Ciencias Químicas y Farmacéuticas. Universidad de Chile. Vicuña Mackenna 20 . Casilla 233 Santiago. Chile. Teléfono: (56.2) 222 82 27 Fax: (56.2) 222 79 00

PROYECTO X.1
BÚSQUEDA DE PRINCIPIOS BIOACTIVOS EN PLANTAS DE LA REGIÓN. INFORME FINAL

Subprograma X Química Fina Farmacéutica
CYTED

Junio 1997
232 pp



La publicación incluye un compendio de lo que fue la trayectoria del Proyecto a través de su realización, cuya jefatura internacional estuvo a cargo del Doctor Roberto Pinzón de la Universidad Nacional de Colombia. En ella se anotan los objetivos, la metodología seguida, los grupos e investigadores participantes, las actividades realizadas y los logros obtenidos en el Proyecto. Se agregan además, algunas de las publicaciones realizadas, en las cuales pueden observarse los resultados de las investigaciones efectuadas.

En el Proyecto han participado 20 grupos de investigación con 246 investigadores de instituciones de 11 países: Argentina, Bolivia, Chile, Colombia, Costa Rica, Ecuador, España, Guatemala, Panamá, Perú y Portugal. Se han estudiado 238 especies, a las que se les realizaron ensayos de actividad biológica y un cribado químico. Con los resultados de los ensayos preliminares se adelantaron investigaciones más profundas con algunas de las especies estudiadas. Se tienen los resultados de 568 pruebas de actividad biológica. De las plantas estudiadas se han aislado y caracterizado 65 compuestos conocidos y 15 compuestos nuevos a los cuales se les realizan en la actualidad pruebas para determinar posibles actividades farmacológicas.

Actualmente, se encuentra en trámite una patente sobre un extracto vegetal con posible actividad en algunas afecciones relacionadas con el SIDA. Los trabajos fueron realizados conjuntamente por grupos de investigadores de la Universidad de Buenos Aires (Argentina) y de la Universidad de Navarra (España).

Se han efectuado 26 publicaciones científicas, 56 Ponencias en congresos científicos, 20 tesis de postgrado y 73 tesinas.

Entre los más importantes logros del Proyecto se pueden señalar la cooperación lograda entre grupos de investigadores, entidades nacionales e internacionales; la participación de los grupos en programas de la Unión Europea, dentro del Programa ALFA a través de la Red RELAPLAMED en la que participan seis grupos del Proyecto y dos de la UE (Italia y Portugal), cuya actividad inicial es la realización de programas de doctorado y posteriormente llevarán a cabo proyectos de investigación conjuntos.

En cuanto a la acciones de formación y perfeccionamiento, se ha dado especial importancia al aprendizaje de nuevas técnicas por parte de los investigadores vinculados al Proyecto, a través de un exitoso programa de movilidad de científicos y a su participación en talleres programados por el Subprograma y en otros considerados de interés para su formación y/o perfeccionamiento. En este sentido, 30 científicos de 13 países han efectuado trabajos en centros de investigación de los grupos participantes en el Proyecto, con una duración media de 70 meses. En los talleres, más de 150 investigadores participaron en los 20 organizados por el Subprograma.

Los resultados se encuentran reseñados en los anexos respectivos que contiene la publicación.

Además de los resultados referidos, otros logros se refieren a la puesta en marcha del Proyecto X.2 «Síntesis de Moléculas Bioactivas Análogas de Productos Naturales de Origen Iberoamericano» al cual el Proyecto X.1 ha aportado los resultados de sus investigaciones.

Para mayor información, dirigirse al Doctor Roberto Pinzón. Departamento de Farmacia Universidad Nacional de Colombia. Apartado 14.490. Bogotá, Colombia. Teléfono: (57.1) 368 75 30 51 21 97. Fax: (57.1) 222 51 81 / 250 78 80.

NOMBRAMIENTOS Y DISTINCIONES CYTED

JOSÉ ANTONIO CORDERO, NUEVO SECRETARIO GENERAL DEL PROGRAMA CYTED

EN la XV Asamblea General Extraordinaria del Programa CYTED, celebrada en Madrid, España, los días 3 y 4 de Junio de 1998, ha sido propuesto el Doctor José Antonio Cordero Martín, como Secretario General, en sustitución de D. Horacio Díaz del Barco. El Doctor Cordero ha desarrollado una amplia y reconocida trayectoria en el campo de la investigación y desarrollo, tanto como Director del Instituto de Automática Industrial del Consejo Superior de Investigaciones Científicas de España, como Coordinador Internacional del Subprograma VII Electrónica e Informática Aplicadas de CYTED, en el que su labor ha sido muy fructífera en los resultados obtenidos, en la generación de diversas actividades de I+D y en la conformación de un amplio equipo de investigadores iberoamericanos que participan en dichas actividades.

El nuevo Secretario General asume el cargo en un momento muy especial en CYTED, con una nueva configuración en su estructura y orientación pragmática acorde con los cambios que han experimentado los diversos Sistemas Nacionales de Ciencia y Tecnología de Iberoamérica. En ese sentido, su amplia experiencia y vocación de trabajo desea volcarlos en esta tarea de potenciar un mejor y mayor aprovechamiento de los innumerables beneficios que aporta el CYTED a la comunidad científica y tecnológica de la región iberoamericana.

D. José Antonio Cordero.

CONDECORACIÓN OTORGADA A PAULINO ANDREU, COORDINADOR INTERNACIONAL CYTED

EL Doctor Paulino Andreu, Coordinador Internacional del Subprograma V «Catálisis y Adsorbentes» del Programa CYTED ha recibido del Señor Presidente de la República de Venezuela, Doctor Rafael Caldera, la imposición de la Orden Francisco de Miranda en su Tercera Clase, en reconocimiento a su meritoria labor en pro de la construcción y puesta en marcha de la Planta Productora de Derivados Sanguíneos. El acto de imposición tuvo lugar el 19 de Mayo de 1998 durante la presentación e inauguración de la Planta.

Dicha imposición representa un reconocimiento a su gran labor como científico y por sus invalorables aportes a la investigación y desarrollo en Venezuela. Nuestras felicitaciones y reconocimiento por la fructífera gestión que realiza al frente del Subprograma.

D. Paulino Andreu.

DESIGNACIÓN DE JOÂO CARLOS LOPES DE MELO BORGES, COMO SECRETARIO ADJUNTO 1998 DEL PROGRAMA CYTED

D. João Carlos Lopes de Melo de Borges, destacado miembro del Instituto de Investigação Científica Tropical, IICT, de Portugal, ha sido designado como Secretario Adjunto 1998 del Programa CYTED. Licenciado en Economía, con una Maestria en Desarrollo y Cooperación Internacional y Especialización en Gestión de Ciencia y Tecnología, realizadas en Portugal, posee una amplia experiencia en la gestión de proyectos, servicos, información, informática, políticas de cooperación económica con África y la Comunidad Europea, formulación y gestión de proyectos, etc. Su carrera profesional la ha desarrollado en el Instituto de Apoio ao Retorno de Nacionais (IARN), Instituto de Investigação Científica Tropical (IICT) y el Gabinete para as Comunidades Europeias do Ministério do Equipamento, Planeamento e Administração do Território (MEPAT), desempeñando en cada uno de ellos importantes cargos.

Su concurso y aportes como Secretario Adjunto serán muy valiosos en la preparación de la próxima Conferencia Científica de la VIII Cumbre Iberoamericana de Jefes de Estado y de Gobierno, a llevarse a cabo en Porto, Portugal, los días 21 y 22 de Septiembre de 1998, cuyo tema central es ***«Ciencia Global e Intereses Locais/Ciencia Global e Intereses Locales»***. La organización de la Conferencia está a cargo de la Secretaría General del Programa CYTED y del Instituto de Cooperação Científica e Tecnológica Internacional , ICCTI, de Portugal.

D. João Melo Borges.

¿QUÉ ES EL PROGRAMA CYTED?

El Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo (CYTED) es un programa internacional y multilateral, creado en 1984, mediante un Acuerdo Marco interinstitucional entre los 21 países de habla española y portuguesa de uno y otro lado del Atlántico. En CYTED participan además como Organismos Observadores, el Banco Interamericano de Desarrollo, la Comisión Económica para América Latina de Naciones Unidas, la Organización de Naciones Unidas para la Educación, la Ciencia y la Cultura y la Organización de Estados Americanos. CYTED pretende ser un instrumento para facilitar el desarrollo tecnológico y la innovación, mediante la coordinación de los recursos existentes y la cooperación entre Universidades, Centros de Investigación y Desarrollo y Empresas innovadoras de la Región Iberoamericana. Constituye un medio para promover la modernización productiva y la mejora de la calidad de vida de los países iberoamericanos y actúa, adicionalmente, como puente para la Cooperación entre América Latina y Europa.

Sus objetivos son el fomento de la cooperación en el campo de la investigación aplicada y el desarrollo tecnológico para la obtención de resultados científicos y tecnológicos transferibles a los sistemas productivos y las políticas sociales de los países iberoamericanos. Participan grupos de investigación, científicos y tecnólogos de Universidades, Centros de I+D y de Empresas innovadoras de los países signatarios de CYTED. La organización y gestión del Programa corresponde a un modelo organizativo descentralizado, con una clara distinción entre el marco político o institucional, representado por los Organismos Signatarios de CYTED, y el marco técnico o funcional, constituido por los Coordinadores Internacionales de Subprogramas. Existe una Secretaría General que coordina y gestiona globalmente el Programa. La dirección del Programa es asumida por la Asamblea General y el Consejo Técnico Directivo.

Su financiamiento está basado en: a) los recursos que asignan los países participantes, a través de sus instrumentos nacionales o de la cooperación internacional, para financiar a los grupos nacionales de I+D que actúan en el ámbito de las actividades multilaterales de CYTED; b) el Programa tiene una financiación adicional por parte de España, que garantiza una aportación no inferior al 50% del total del presupuesto. Esta aportación procede, a partes iguales, de la Comisión Interministerial de Ciencia y Tecnología y de la Agencia Española de Cooperación Internacional; c) contribuciones voluntarias de los diversos países para financiar las actividades de gestión y cooperación que se llevan a cabo en ellos (reuniones de coordinación, seminarios, experiencias conjuntas, etc.). Este modelo de financiación del Programa consigue un efecto de sinergia que potencia los recursos existentes en la Región Iberoamericana. A este respecto, una evaluación del Programa realizada en 1992 por un Comité Internacional, señaló en su informe final que CYTED es uno de los programas de cooperación internacional que presenta un mejor balance coste/beneficio.

CYTED divide el ámbito potencial de actuación dentro de la Ciencia y la Tecnología en una serie de áreas o *Subprogramas*, que van modificándose en el tiempo de acuerdo a los intereses de los países participantes. Actualmente, la relación de Subprogramas operativos es la siguiente:

SUBPROGRAMAS TEMÁTICOS

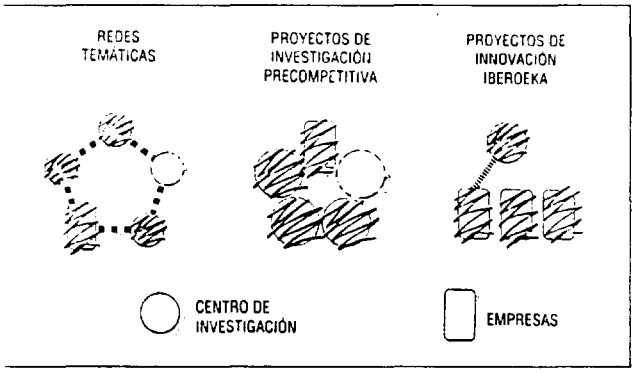
- **Acuicultura**
- **Biomasa como Fuente de Productos Químicos y Energía.**
- **Nuevas Fuentes y Conservación de la Energía.**
- **Tecnología de Materiales.**
- **Química Fina Farmacéutica.**
- **Diversidad Biológica.**
- **Tecnología para Viviendas de Interés Social.**
- **Biotecnología.**
- **Catálisis y Adsorbentes.**
- **Electrónica e Informática Aplicadas.**
- **Microelectrónica.**
- **Tratamiento y Conservación de Alimentos.**
- **Tecnología Mineral.**
- **Corrosión/Impacto Ambiental sobre Materiales.**

SUBPROGRAMAS HORIZONTALES

- **Metodología en Ciencia y Tecnología.**
- **Gestión de la Investigación y el Desarrollo Tecnológico.**

Las modalidades de actuación dentro de CYTED

- **Redes Temáticas**. Facilitan la interacción, la cooperación y la transferencia de conocimientos y tecnologías entre grupos que trabajan en temas similares. Uno de sus objetivos principales es la generación de Proyectos de Investigación Precompetitiva.



- **Proyectos de Investigación Precompetitiva**. Se trata de proyectos de investigación aplicada realizados en colaboración entre grupos de investigación y de empresas de diversos países que constituyen un equipo internacional. Uno de sus propósitos es permitir la transferencia de sus resultados a los sistemas productivos de los países participantes; así como, poner las bases para que dicha transferencia se produzca.
- **Proyectos de Innovación IBEROEKA**. Persiguen la cooperación entre empresas de diversos países a través de proyectos de innovación conjuntos. Su objetivo es el incremento de la productividad y la competitividad de la industria y la economía.

El Programa tiene una gran flexibilidad en cuanto al diseño y a la organización interna de las distintas actividades. En estos momentos existen más de 100 Redes Temáticas y Proyectos de Investigación Precompetitiva en funcionamiento y hay presentados más de 200 Proyectos de Innovación, la mitad de ellos están en ejecución y el resto pendiente de la obtención de la certificación IBEROEKA.

ORGANISMOS SIGNATARIOS DEL PROGRAMA CYTED

ARGENTINA	Secretaría de Ciencia y Tecnología, SECyT
BOLIVIA	Consejo Nacional de Ciencia y Tecnología, CONACYT
BRASIL	Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq
COLOMBIA	Instituto Colombiano para el Desarrollo de la Ciencia y la Tecnología, COLCIENCIAS
COSTA RICA	Ministerio de Economía, Comercio, Ciencia, Tecnología e Industrias
CUBA	Ministerio de Ciencia, Tecnología y Medio Ambiente
CHILE	Comisión Nacional de Investigaciones Científicas y Tecnológicas, CONICYT
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ESPAÑA	Oficina de Ciencia y Tecnología Agencia Española de Cooperación Internacional, AECI
GUATEMALA	Consejo Nacional de Ciencia y Tecnología, CONCYT
HONDURAS	Consejo Hondureño de Ciencia y Tecnología, COHCYT
MÉXICO	Consejo Nacional de Ciencia y Tecnología, CONACYT
NICARAGUA	*Ministerio de Economía y Desarrollo*
PANAMÁ	Secretaría Nacional de Ciencia, Tecnología e Innovación, SENACYT
PARAGUAY	Instituto Nacional de Tecnología y Normalización, INTN
PERÚ	Consejo Nacional de Ciencia y Tecnología, CONCYTEC
PORTUGAL	Ministério de Ciência e Tecnologia. Instituto de Cooperação Científica y Tecnológica Internacional, ICCTI
REPÚBLICA DOMINICANA	Oficina Nacional de Planificación, ONAPLAN
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«Catálisis y Adsorbentes»	Paulino Andreu	Petróleos de Venezuela, S. A.	Venezuela
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GENERAL GUIDANCE FOR THE PRECLINICAL DEVELOPMENT OF MEDICINAL PRODUCTS

Those involved in the development of medicinal products should bear in mind that preclinical safety studies carried out to support the use of a new medicinal product should correspond to a real necessity and not be done according to a check-list. In order to facilitate such an approach, the following question-based guidance could be applied:

- What information is the animal study expected to provide?
- Is this information essential in the reassurance on safety for this particular medicinal product?
- If this information is essential, could it be obtained by experiments not involving intact animals?
- If information is essential and can only be obtained from live animals, is it possible to decrease numbers of animals used without compromising the usefulness of the data?
- Is there overall justification for the final protocol?

TABLE 1A: Procedures Required by the Japanese Guidelines (Category A)

A. Studies which should normally be conducted for all test substances to assess the overall profile of the general pharmacological effects:

1) Effects on general activity and behavior
   — The general activity of animals should grossly be observed. The grossly observable changes should be evaluated in detail in order to thoroughly clarify the effects of the test substance.
2) Effects on the central nervous system
   — Effects of the test substance on spontaneous locomotor activity should be examined.
   — General anesthetic effects should be assessed.
   In addition to the effects of the test substance on conscious intact animals, it is necessary to assess the test substance for effects synergistic with or antagonistic to general anesthetics.
   — Effects of the test substance on convulsions should be assessed.
   In addition to an assessment of proconvulsive activity of the test substance alone in intact animals, it is necessary to determine whether the test substance has effects synergistic with or antagonistic to the means used to provoke convulsions.
   — Analgesic action should be assessed.
   — Effects of the test substance on the body temperature should be assessed.
3) Effects on the autonomic nervous system and smooth muscle
   — Effects of the test substance on the isolated ileum should be assessed. Those effects of the test substance itself and interaction with agonists should be assessed.
4) Effects on the respiratory and cardiovascular systems
   — Effects of the test substance on respiration, blood pressure, blood flow, heart rate and electrocardiogram should be assessed.
   Normally, anesthetized animals are used. Conscious animals are also used, when necessary.
5) Effects on the digestive system
   — Effects of the test substance on gastrointestinal transit should be assessed.
   Intestinal transit time should be investigated; gastric emptying time should also be examined, if appropriate.
6) Effects on water and electrolyte metabolism
   — The urinary volume and urinary concentrations of sodium, potassium, chloride, etc., should be determined.
7) Other important pharmacological effects
   — Studies which are not included in the principal pharmacological studies and in category A, 1) to 6) above should also be undertaken to assess for significant pharmacological properties which are expected either from the known properties of other drugs which are chemically or pharmacologically related to the test substance or from toxicological or clinical findings.

B. Studies to be conducted, if necessary, in the light of the results of the studies in the category A.:

1) Effects on the central nervous system
   - — Effects of the test substance on the spontaneous electroencephalogram should be assessed. Computed-aided analysis of the data may be used.
   - — Effects of the test substance on the spinal reflex should be assessed.
   - — Effects of the test substance on the conditioned avoidance response should be assessed.
   - — Effects of the test substance on coordinated locomotor activity should be assessed.
2) Effects on the somatic nervous system
   - — Effects of the test substance on the neuromuscular junction should be assessed.
   - — Muscular relaxation potential should be assessed.
   - — Local anesthetic effects should be assessed.
3) Effects on the autonomic nervous system and smooth muscle
   - — Effects of the test substance on the pupillary diameter and on nictitating membrane contraction should be assessed.
   - — The examinations should also be conducted with isolated organs and tissues such as blood vessel, trachea, vas deferens, uterus, etc.
4) Effects on the respiratory and cardiovascular systems
   - — Effects of the test substance on changes in blood pressure and heart rate induced by autonomic drugs, vagal stimulation, common carotid artery occlusion, etc., should be assessed.
   - — Examinations should also be made with the heart in situ.
   - — Effects of the test substance on isolated organs and tissues such as heart, atrium, papillary muscle, vascular bed, etc., should be assessed.
5) Effects on the digestive system
   - — Effects of the test substance on the secretion of gastric juice, saliva, bile and pancreatic juice should be assessed.
   - — Effects of the test substance on the motility, in vitro, of stomach and intestine should be assessed.
   - — Effects of the test substance on the motility of the gastrointestinal tract in situ should also be assessed.
   - — Effects of the test substance on the gastroduodenal mucous membrane should be assessed.
6) Other effects
   - — Effects of the test substance on the blood coagulation system should be assessed.
   - — Effects of the test substance on platelet aggregation should be assessed.
   - — Hemolytic potential should be assessed.
   - — Effects of the test substance on renal function should be assessed.

PROPOSAL FOR NEW GUIDANCE

"Fixed combinations of herbal medicinal products with long-term marketing experience"
Guidance to facilitate mutual recognition and use of biobliographic data

Note for Guidance concerning the application of section C.6 Part 4 of the Annex to Directive 91/507/EEC as amended, with a view to the submission of an application for a marketing authorisation for a well-established herbal medicinal product. This guideline should be read in conjunction with current EU guidelines.

1. JUSTIFICATION

1.1 Applicants will be required to justify the particular combination of active substances proposed. Fixed combination products will only be considered acceptable if the proposed combination is based on valid therapeutic principles.

1.2 For any individual fixed combination it is necessary to assess the potential advantages in the clinical situation against possible disadvantages, in order to determine whether the product meets the requirements of the standards and protocols with respect to efficacy and safety.

1.3

POTENTIAL ADVANTAGES OF FIXED COMBINATIONS INCLUDE ONE OF THE FOLLOWING:

a) an improvement of the benefit/risk assessment due to:

i. the addition or potentiation of therapeutic activities of their substances, which results in:

- a level of efficacy similar to the one achievable by each active substance used alone at higher doses than in combination, but associated with a better safety profile

or

- a level of efficacy above the one achievable by a single substance with an acceptable safety profile.

ii. the counteracting by one substance of an adverse reaction produced by another one.

b) a simplification of therapy

A fixed combination of active substances is acceptable if it achieves a similar level of efficacy to the one achievable by each active substance used alone at higher doses than in combination but improves patient compliance.

DISADVANTAGES OF FIXED COMBINATIONS INCLUDE:

i. the fact that even a combination which meets the needs of the average patient is unlikely to be ideally adjusted for the needs of each individual patient;

ii. the addition of the different adverse reactions specific to each substance.

1.3 General rules

Combinations, in principle, may not be considered rational if the duration of action of the substances differ significantly. This may not necessarily apply where it can be shown that the combination is clinically valid despite differences in this respect, e.g. if one substance is intended to enhance absorption of the other or where the substances are intended to exert their effects successively.

Each substance of the fixed combination must have documented contribution within the combination.

The inclusion of a substance to counteract an adverse reaction of another substance may be considered justified, but only if the adverse reaction is a serious or a commonly occurring one.

The inclusion of a substance intended to produce unpleasant adverse effects as a means of preventing abuse in undesirable.

Substances having a critical dosage range or a narrow therapeutic index are unlikely to be suitable for inclusion in fixed combinations.

2. INDICATIONS

The indications claimed for a fixed-combination medicinal product should be such that the presence of each active substance makes a contribution to the claimed effect. The product should be formulated so that the dose and proportion of each substance present is appropriate for the intended use.

An indication must be a well-recognised disease state, modification of a physiological state, dysfunctional state, syndrome or pathological entity. The individual substances of a fixed combination may be intended to relieve simultaneously different symptoms of such a disease state. In this case, it should be a prerequisite that these symptoms regularly occur simultaneously in a clinically relevant intensity and for a relevant period of time. It will not be proper to regard each individual symptom as an indication for the fixed combination, since it may also occur in other diseases and for treating this symptom alone the other substances may be irrelevant.

3. PHARMACODYNAMIC AND PHARMACOKINETIC DATA

The possibility of interactions between the active substances should always be considered and discussed in the expert report. When data on interactions are available, they have to be submitted and discussed by the expert.

3.1 Pharmacodynamic data

The expert report should address the question of the addition or the potentiation of the pharmacodynamic effects of the various active substances.

3.2 Pharmacokinetic data

The expert report should address the question of the various pharmacokinetic parameters of each active subtance.

4. SAFETY AND EFFICACY

It is permissible to distinguish between the extent of the studies required in the case of those fixed combinations which correspond closely to combinations which are already in widespread use provided these are thoroughly and reliably documented, and those studies required in the case of those combinations which are essentially new :

a) When the fixed combination corresponds closely to combinations that are already in widespread use, a well founded bibliographical data analysis should be submitted. Provided that the respective data are thoroughly and reliably documented, this analysis may be helpful in reducing the amount of clinical trials to be performed and could facilitate the selection of doses for each substance and the proposed dose range of the fixed combination.

b) When the fixed combination is essentially new (active substances not usually combined, unusual quantitative composition of usually combined substances or one substance entirely new), the data needed are similar to a new chemical entity in the situation where the fixed combination is to be proposed (first line or second line therapy). Existing experience with the substances should be taken into account.

4.1 Safety aspects

Where there are grounds to expect that a fixed-combination product may be substantially more harmful or give rise to much more frequent adverse effects than any individual active substances given alone, the applicant should provide evidence that this does not occur in therapeutic use, or that the advantages of the combination e.g. increased efficacy, outweigh such disadvantages.

This should be read in conjunction with the *draft* guidance "Non-clinical testing of herbal drug preparations with long-term marketing experience - Guidance to facilitate mutual recognition and use of bibliographic data".

4.2 Therapeutic data

The efficacy and the safety of use of the fixed combination must be evident from clinical trials or bibliographic data submitted by the applicant.

4.3 Composition and dosage regimen

The proposed dosage regimen must be justified.

The dosage of each active substance within the fixed combination must be such as the combination is safe and effective for a significant population subgroup and the benefit/risk assessment of the fixed combination is equal or exceeds the one of each of its active substances taken alone.

Where active substances are intended to relieve simultaneously different symptoms or to prevent different diseases, selected doses of each active substance are often those commonly used for the treatment of each symptom or the prevention of each disease.

5. COMBINATION PACKS

The principles applicable to fixed-combination products will also be applied in the assessment of preparations consisting of different medicinal products in combination packs where the products are intended for simultaneous or sequential administration.

PHARMACOLOGY OF RECEPTORS

- Introduction : Neurotransmission and transmission of hormonal information
  - ⇒The 'actors'
  - ⇒Biochemical approach
  - ⇒Genetic approach
  - ⇒Computer modelling and mutagenesis
  - ⇒Biophysical approach
- Direct identification by radioligand binding
  - ⇒Saturation binding
  - ⇒Competition binding
  - ⇒Kinetic experiments
- Functional experiments
- Examples
- Angiotensin II receptors
- Serotonin receptors

Neurotransmission and transmission of hormonal information: introduction

Fysiological role:

adaptation to the environment

(stress response : adrenalin)

adequate response to fysiological changes

(high blood glucose : insulin)

(low blood pressure : Angiotensin II)

sensory functions:

(pain reflex, vision, smell ....)

effector functions:

(heart contraction, gland secretion ...)

brain functions:

(memory, thinking ...)

Pathofysiological role

diabetes (deficient insulin)

high blood pressure (high angiotensin II level)

PHARMACOLOGY OF RECEPTORS

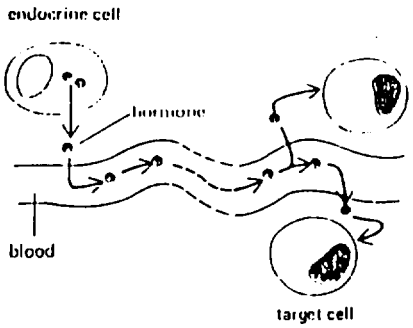
Examples of radioligand binding assays:

Receptor subtypes	radioligand of choice	potential therapeutic target: agonist	antagonist
Angiotensin II	[^{3}H]-angiotensin		antihypertensive
AT_1	[^{3}H]-losartan		
AT_2	[^{125}I]-CGP42112		antiproliferative
GABA			
Benzodiazepine	[^{3}H]-flunitrazepam	anxiolytic	
$GABA_A$	[^{3}H]-GABA		anxiolytic
$GABA_B$	[^{3}H]-Baclofen		anxiolytic
glutamate			
non-selective	[^{3}H]-glutamate		anti-ischemia
NMDA	[^{3}H]-CPP		anti-ischemia
NMDA/PCP	[^{3}H]-MK801		antipsychotic
quisqualate	[^{3}H]-AMPA		anti-ischemia
kainate	[^{3}H]-kainate		anti-ischemia
Neuropeptide Y	[^{3}H]-NPY		
Y_1	[^{3}H]-BIBP3226		antihypertensive
Y_2, Y_4, Y_5	[^{3}H]-NPY		appetite suppressant
Opiates			
μ (OP_3)	[^{3}H]-DAMGO	analgesic	
δ (OP_1)	[^{3}H]-DADLE		immunosupr.
κ (OP_2)	[^{3}H]-U-50,488	analgesic	appetite supressant

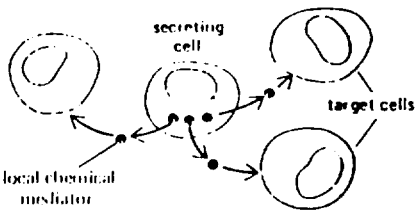
Examples of radioligand binding assays:

Receptor subtypes	radioligand of choice	potential therapeutic target agonist	antagonist
Serotonin	[^{3}H]-5-HT		
5-HT_{1a}	[^{3}H]-8-OHDPAT		antidepressant
5-$HT_{1b,1d}$	[^{125}I]-cyanopindolol		
5-HT_{2a}	[^{3}H]-ketanserin		antidepressant
5-$HT_{2b,c}$	[^{3}H]-mesulergin		antidepressant
5-HT_3	[^{3}H]-GR65630		antiemetic
5-HT_4	[^{3}H]-GR113808		
5-$HT_{5,5a,6}$	[^{3}H]-LSD, [^{3}H]-5-CT		
Acetylcholine	[^{3}H]-acetylcholine		
Musc M_1	[^{3}H]-pirenzepine	memory	antiulcer
Musc M_2	[^{3}H]-AFDX116		memory
Musc $M_{3,4,5}$	[^{3}H]-QNB		
	[^{3}H]-N-MethylScopolamine		
Nicotine	[^{125}I]-bungarotoxin		
dopamine	[^{3}H]-dopamine		
D_1	[^{3}H]-SCH23390		antiemetic
D_2	[^{3}H]-spiperone		antipsychotic
D_3			
adrenalin			
$\alpha_{1a,1b,1d}$	[^{3}H]-prazosin		antihypertensive
$\alpha_{2a,2b,2c}$	[^{3}H]-idazoxan	antihypertensive	
	[^{3}H]-rauwolscine		antidepressant
	[^{3}H]-clonidine		
β_1	[^{3}H]-alprenolol		
	[^{125}I]-cyanopindolol		antihypertensive
β_2			
β_3		lipolysis	

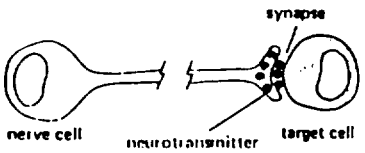
ENDOCRINE = HORMONE

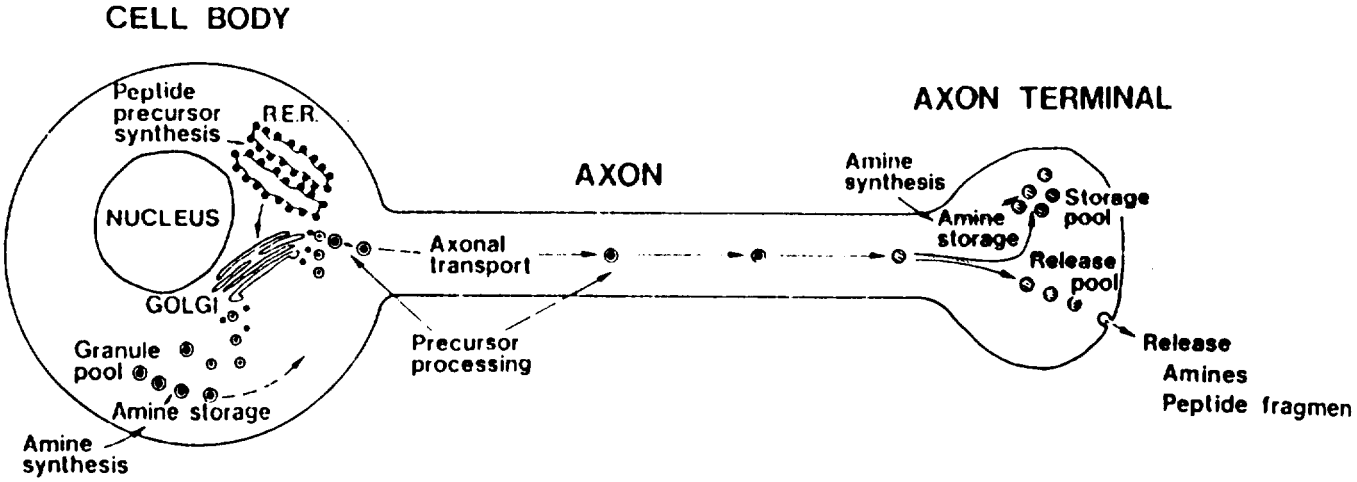


PARACRINE = LOCAL HORMONE



SYNAPTIC = NEURO-TRANSMITTER





. Diagram illustrating the biosynthesis, packaging and release of peptides and amines in neurones. The peptides are generated from large precursor molecules produced in the rough endoplasmic reticulum (RER) and packaged in secretory granules or vesicles in the Golgi stacks. The granules are transported out from the cell body to the terminals (axonal transport) where they release their contents by exocytosis upon stimulation. In contrast, amines are produced in the cytosol of the cell body, axon and terminal and packaged by uptake in preformed granules or vesicles. As a result amines and peptides may coexist in granules although the proportions in molar terms may vary depending on the circumstances.

NEUROTRANSMISSION:

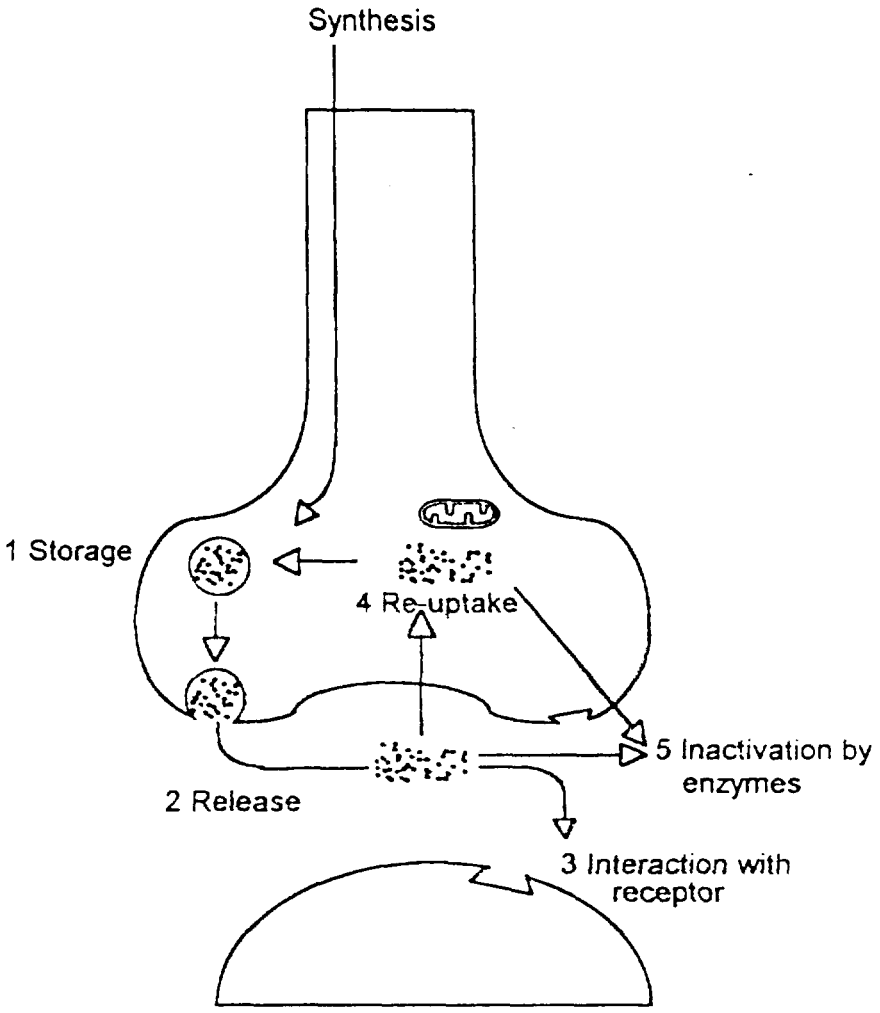
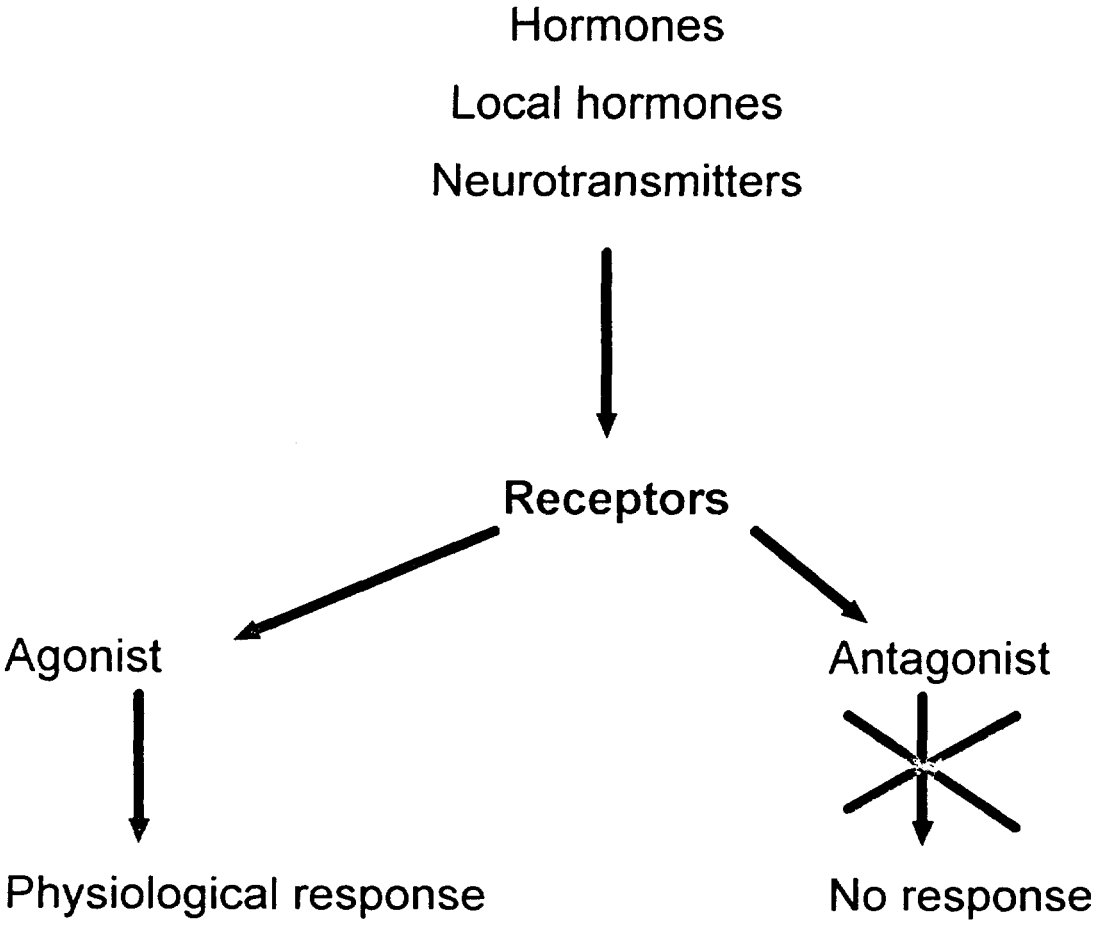
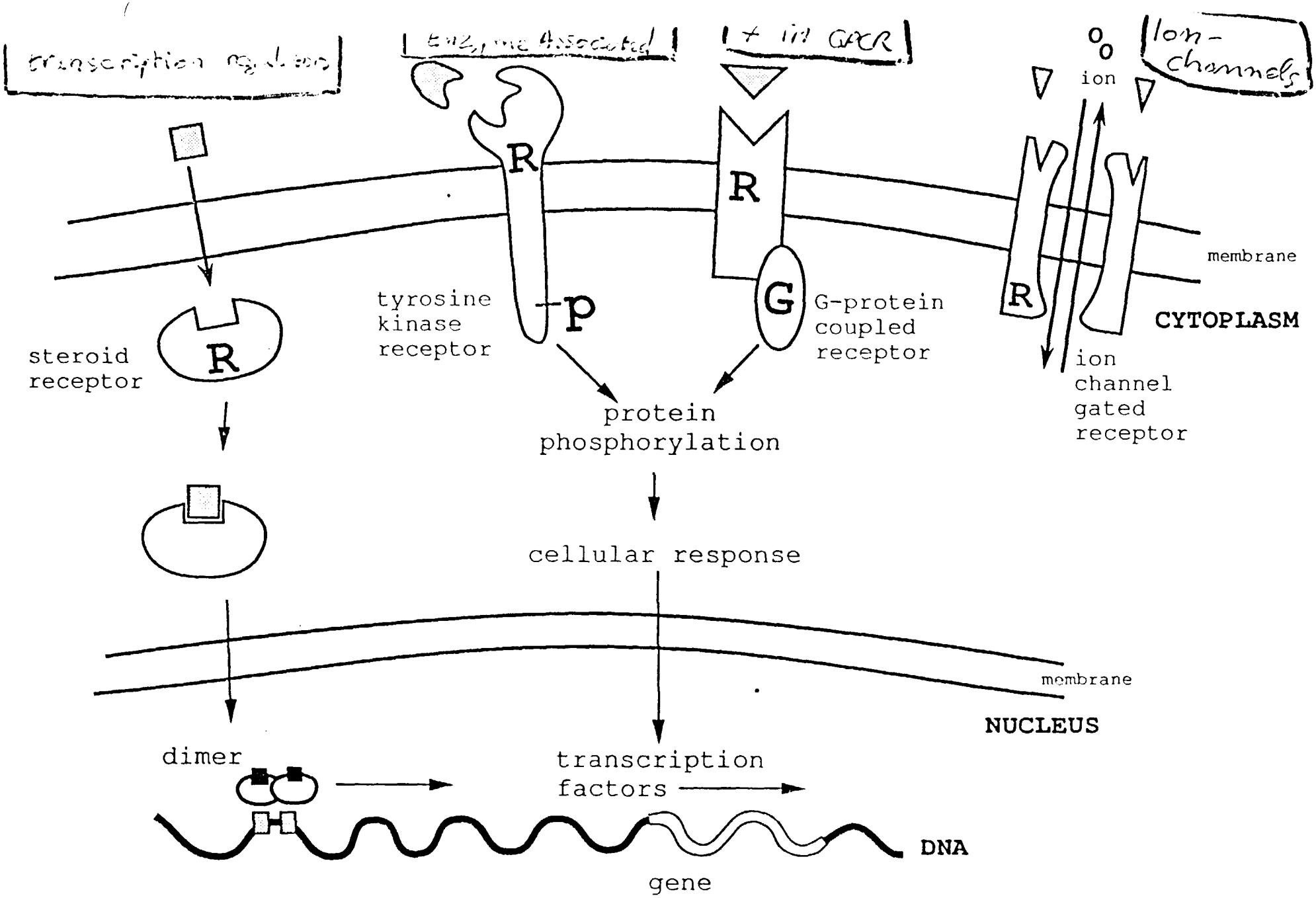


Fig.3. Storage, release and re-uptake of serotonin.

Neurotransmission and transmission of hormonal information: the 'actors'




transcription regulators
Enzyme Associated
+ ive GPCR
ion-channels
ion
membrane
R
R
R
R
G
steroid receptor
tyrosine kinase receptor
P
G-protein coupled receptor
CYTOPLASM
ion channel gated receptor
protein phosphorylation
cellular response
membrane
NUCLEUS
dimer
transcription factors
DNA
gene

PHARMACOLOGY OF RECEPTORS:

biochemical approach

- Natural and synthetic compounds : a 'ligand'

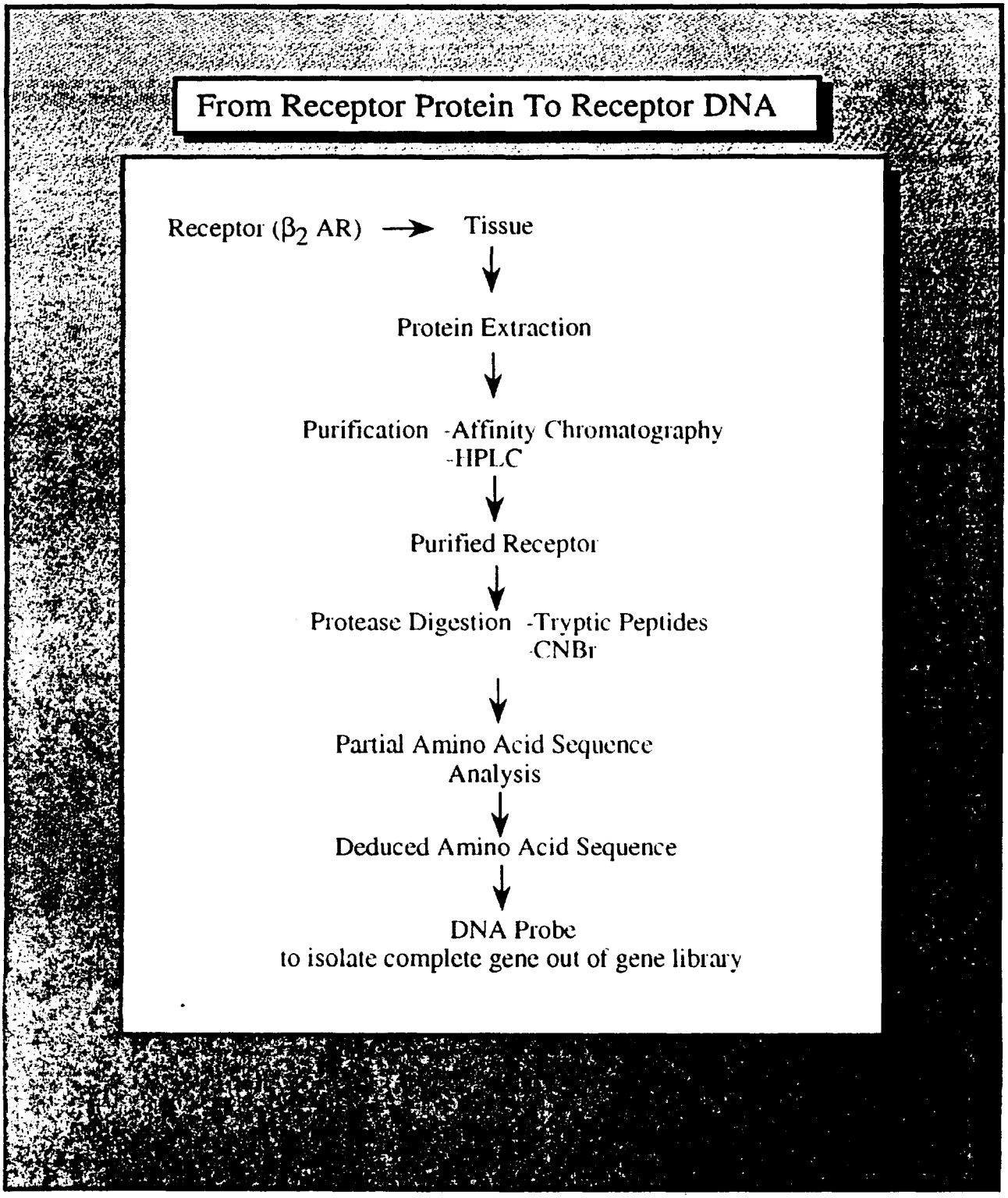
- Direct binding using a radiolabelled ligand : 'radioligand'

- Binding of different ligands is a typical property of a certain receptor : 'order of potency' : a fingerprint

- Functional experiments reveal the 'intrinsic activity' of a ligand, whether it is a 'agonist'or a 'antagonist'

- Endogeneous ligands can bind to different related receptors : 'receptor subtypes' ;new receptor subtypes can be discovered and can be correlated with the benificial or side effects of certain drugs

FROM BIOCHEMISTRY TO GENETICS



PHARMACOLOGY OF RECEPTORS:

genetic approach I

- Purification of a receptor protein and partial sequencing, followed by the synthesis of a 'cDNA probe'

- Isolation of the 'receptor gene'

- Stable expression of the receptor gene in a cell line and selection of a 'single cell clone'

- Measurement of the biochemical properties of the receptor in these cell lines

- Study the expression of a receptor gene by detection of its mRNA by 'in situ hybridization'

PHARMACOLOGY OF RECEPTORS:

genetic approach II

- Sequence alignement of the receptor genes to determine relationships between 'receptor subtypes' and the definition of 'receptor families'

- Based on the structural resemblance with bacterio-rhodopsin computer generated 3D models of GPCR can be generated

- 'computer modelling' in combination with 'site directed mutagenesis' can give information on the morphology of the binding site and contribute to 'rational drug design'

- Confirmation of these models awaits 'receptor cristallography'

GURE 24
mparison of the amino acid sequences of drenergic receptors (β-AR) from turkey throcytes and hamster lung and bovine odopsin (Rhod). Hydrophobic domains are noted M1 to M7.

FIGURE 25
Spatial structure and hydropathy index (i.e. localisation of hydrophobic segments in the polypeptide chain) of bacteriorhodopsin from ref 1 (p 293 and 444)

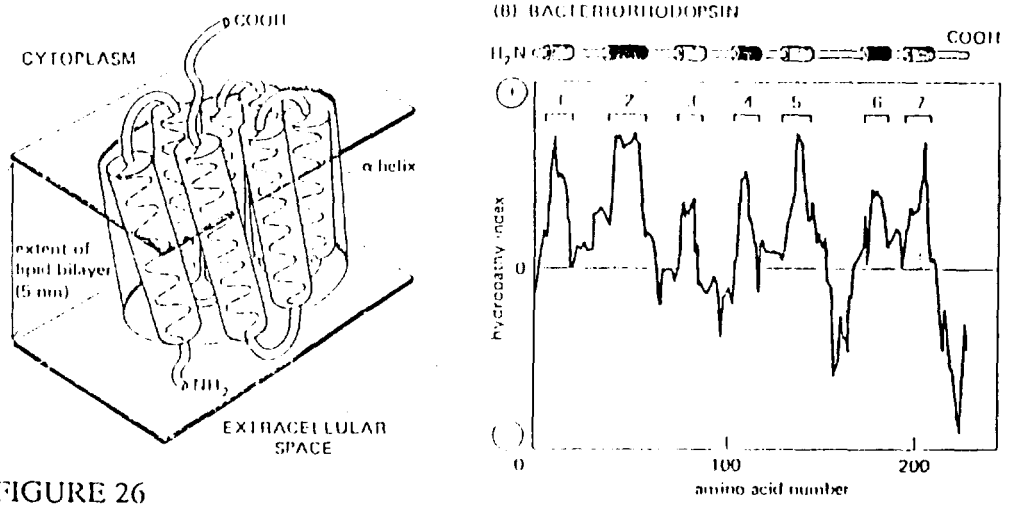
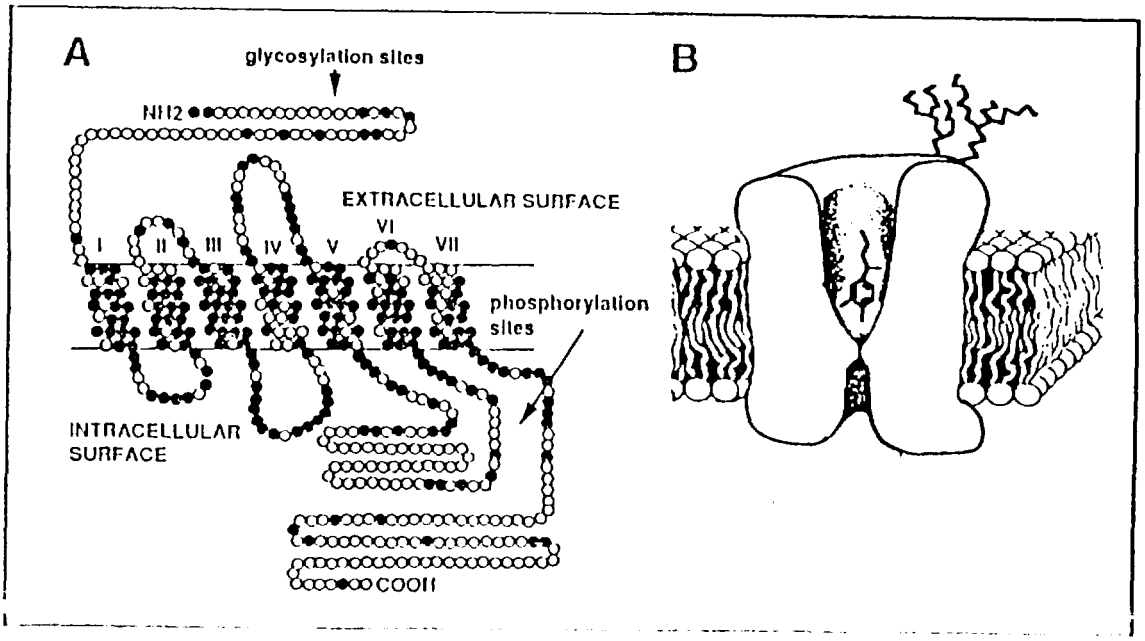


FIGURE 26
Proposed secondary structure (A) and spatial structure (B) of β-adrenergic receptors.



PHARMACOLOGY OF RECEPTORS:

- Direct identification by radioligand binding

  ⇒Saturation binding

  ⇒Competition binding

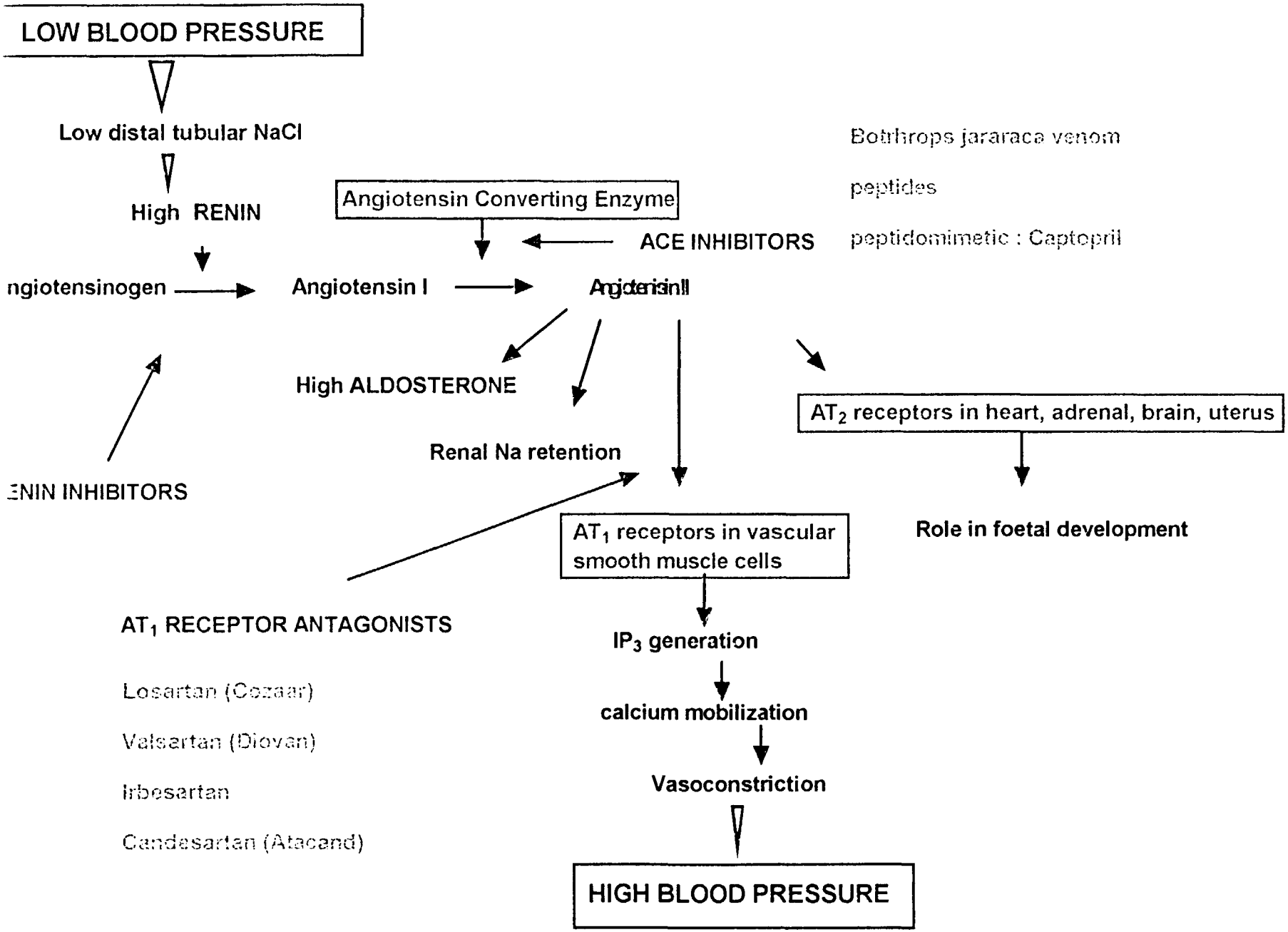
  ⇒Kinetic experiments

- Functional experiments

- Examples of radioligands

- Angiotensin II receptors

- NPY receptors


LOW BLOOD PRESSURE
Low distal tubular NaCl
High RENIN
Angiotensin Converting Enzyme
ACE INHIBITORS
Bothrops jararaca venom
peptides
peptidomimetic : Captopril
ngiotensinogen
Angiotensin I
Angiotensin II
High ALDOSTERONE
Renal Na retention
ENIN INHIBITORS
AT2 receptors in heart, adrenal, brain, uterus
Role in foetal development
AT1 receptors in vascular smooth muscle cells
AT1 RECEPTOR ANTAGONISTS
Losartan (Cozaar)
Valsartan (Diovan)
Irbesartan
Candesartan (Atacand)
IP3 generation
calcium mobilization
Vasoconstriction
HIGH BLOOD PRESSURE

IUPHAR PROPOSED RECEPTOR CODE:

Humphrey P. et al. Pharmacol.Rev.,50,217-277,1998

- 1.1 - 1.8. Ion channels
  - 1.1 Cys-looped receptors (GABA, AcCH,5-HT)
  - 1.2.Glutamate gated (NMDA, AMPA, kainate)
  - 1.3.cAMP and IP_3 gated channels
  - 1.4.P2X receptors and H+gated channels
- 2.1 - 2.3. 7 TM GPCR
  - 2.1 'Rhodopsin' like
  - 2.2. 'secretin' like
  - 2.3. metabotropic and $GABA_B$
- 3.1.- 3.4. Enzyme associated
  - 3.1.Tyrosine kinase
  - 3.2.Tyrosine kinase associated
  - 3.3.Ser/Thr kinase
  - 3.4.guanylate cyclase
- 4.1.- 4.2. Transcription regulators
  - 4.1.non-steroid (vitD,thyroid)
  - 4.2.steroid receptors

STRUCTURAL CLASS

2.1. 5HT.01A.RNO.00.00 **rat $5HT_{1A}$**

1.1. 5HT.03 .HSA .00.00S **human $5HT_3$**

RECEPTOR TYPE CODE

RECEPTOR FAMILY

5HT	serotonin
ANG	angiotensin
NPY	neuropeptide Y
ADR	adrenalin
etc....	

01A	5-HT_{1A}
01B	5-HT_{1B}
02C	5-HT_{2C}
01	D_1
02	D_2
etc....	

SPLICE VARIANT CODE

00
01
02

EXTRA CODE

spare code

SPECIES CODE

HSA	human
RSO	rat
etc...	

FINAL LETTER CODE

P	provisional
S	receptor subunit
M	multimeric receptor (known)

Receptor type	5-HT$_2$
Receptor subtype	5-HT$_{2C}$
Receptor code	2.1.5HT.02C.000.00.00
Previous name(s)	5-HT$_{1C}$
Structural information	h 377aa, P28221, chr. Xq24 r 377aa, P49145 m 374aa, P28565
Functional assays	IP$_1$ accumulation in homogenates of choroid plexus (porcine)
Selective agonists	none non-selective: (±)-α-Me-5-HT (±)-DOB
Agonist potency ratios	5-HT = α-Me-5-HT > DOI > RU24969 = quipazine > 5-CT > sumatriptan
Selective antagonists	RS102221 non-selective: mesulergine
Antagonist potencies	RS102221 (p*K* 8.6) non-selective: mesulergine (8.8)
Radioligands	[^{3}H]-mesulergine [^{125}I]-LSD
Radioligand assays	recombinant receptor; choroid plexus; cortex homogenate
Ligand affinities	5-HT (p*K* 8.7), mesulergine (9.1), DOI (7.4), ketanserin (7.6)
Transduction mechanism(s)	G_q/G_{11} preferentially increases PI hydrolysis and elevates [Ca^{2+}]$_i$
Receptor distribution	choroid plexus, raphe nuclei, pons, striatum, hippocampus (CA1, CA3), hypothalamus, spinal cord; no peripheral distribution identified
Tissue function(s)	possibly involved in modulation of transferrin production and regulation of CSF volume

COMMENTS

Receptor type	5-HT$_3$
Receptor subtype	—
Receptor code	1.1.5HT.01.000.00.00.5
Previous name(s)	'M' receptor
Structural information	4TM h 5-HT$_{3A}$, 478aa, P46098, chr. 11 r 5-HT$_{3A}$, 483aa, P35563 m 5-HT$_{3A}$, 487aa, P23979
Functional assays	contraction of ileum via myenteric acetylcholine release (guinea-pig); depolarisation of isolated vagus nerve (guinea-pig, rat, rabbit); cardioexcitation via sympathetic noradrenaline release (rabbit); von Bezold-Jarisch reflex (various species)
Selective agonists	2-Me-5-HT 1-(m-chlorophenyl) biguanide (m-CPB)
Agonist potency ratios	m-CPB > 5-HT > 2-Me-5-HT >> 5-MeOT
Selective antagonists	tropisetron ondansetron granisetron
Antagonist potencies	tropisetron (p*K* 10–11) ondansetron (8–10) granisetron (10)
Radioligands	[^{3}H]-(S)-zacopride [^{3}H]-BRL43694 [^{3}H]-GR65630 [^{3}H] LY278584
Radioligand assays	recombinant α subunit
Ligand affinities	granisetron (p*K* 8.8), ondansetron (8.3), M-CPB (7.2), 5-HT (6.9), 2-Me-5-HT (6.7)
Transduction mechanism(s)	intrinsic transmitter-gated ion channel promotes increased [Ca^{2+}]$_i$
Receptor distribution	striatum, hippocampus (CA1), substantia nigra, globus pallidus; post-ganglionic sympathetic neurones, sensory neurones
Tissue function(s)	sympathetic and parasympathetic neuroexcitation (various species); vagal neuroexcitation: provocation of emesis (ferret)
COMMENTS	Pentamer of four subunits. Human, rat and mouse orthologues of a single subunit (5-HT$_{3A}$) have been cloned. Species-differences in pharmacology and kinetics of activation and inactivation exist.

The IUPHAR Receptor Code

P. P. A. Humphrey and E. A. Barnard

A generally accepted system for defining, characterizing and classifying transmitter and hormone receptors is desirable for several reasons. First, pharmacologists need a common language so that ambiguity is not created by varied and inconsistent terminology. A significant fraction of the mammalian genome codes for receptor proteins and subunits; this leads to the prediction of several thousands of individual signal-transducing receptors, even without consideration of the very large group of olfactory and gustatory receptors that exists[1]. Thus, an internationally acceptable classification system for receptors is both desirable and necessary. Second, the development of a systematic scheme of classification is in itself an important generic research approach in biological science. It can bring focus, highlight relationships, and stimulate the recognition and investigation of features common to various classes and groups. It can also indicate deficiencies in our information and add an evolutionary perspective, which may bring its own insights. It remains to be determined why there is, in many cases, such a large variety of receptors for a given chemical messenger transmitter. Importantly, we need to determine whether all these receptors are functional. It is also interesting to consider how they evolved and how many more endogenous ligands, known and unknown, are to be anticipated. Third, drug discovery benefits greatly from the systematic analysis of the growing body of information on receptors and their subclassification, with the knowledge that each receptor type provides a potential new drug target. Thus, it is now recognised that the identification of well-defined receptors often leads to highly specific drugs with new clinical indications and/or fewer undesirable side-effects. It seems reasonable to assume that future computer-based analyses or predictions of drug selectivities will benefit greatly from an established universal framework of a systematic receptor classification.

The term 'receptor' is in use in various broad senses, but for pharmacological purposes the term is used specifically[2,3] and refers to proteins that are 'signal transducing receptors', as defined and discussed by Kenakin *et al.*[4]. That is, each such protein specifically recognises and is activated by a native agonist, which is one of numerous different messenger molecules that specifically relay a signal into a cell or between compartments within a cell. Thus, receptors in the pharmacological sense may be in one of three locations: (a) in the plasma membrane (the receptors for neurotransmitters, trophins, growth factors and cytokines, other immuno-mediators, morphogens, sensory stimulants and chemoattractants, and circulating hormones); (b) in an organelle membrane (e.g. those receptors where the transduction process involves the release of Ca^{2+} from an intracellular store); and (c) in the cytosol. Case (c) can involve migration of the ligand-bound receptor to the cell nucleus, as is the case for the receptors that belong to the superfamily of ligand-regulated transcription factors, whose ligands are steroid hormones and certain other fat-soluble hormones. For all of the natural signalling agonists involved, the general term 'transmitter' can be employed.

As a consequence of the considerations listed above, much effort has been dedicated in the recent past to the classification of the pharmacologically better-known neurotransmitter receptors. However, molecular biology has created a dramatic increase in information on the existence and structure of receptors, often preceding any data on their function, thereby reversing the traditional order of pharmacological data acquisition. The IUPHAR Committee for Receptor Nomenclature and Drug Classification (NC-IUPHAR), with its various subcommittees, has been constituted to review current approaches to receptor characterization and to define a generic scheme of nomenclature[5–7]. Although progress has been made in standardizing trivial names in a number of receptor areas (and this initiative will continue), it is suggested that an all-embracing, rational system of coding receptors should be introduced (an alphanumeric receptor code, RC), analogous to the EC (Enzyme Commission) enzyme codes established by IUBMB (International Union of Biochemistry and Molecular Biology). It will differ from the EC system (which was introduced before the era of

molecular biology) in that the proposed codes are intended to convey structural information (relevant to the mode of receptor transduction) together with additional elements relating to operational characteristics, set out in a hierarchical order. This should provide a unique, unambiguous and authoritative descriptor for each receptor protein. Each RC, assigned by NC-IUPHAR, is intended to be used in publications for definitive identification, in conjunction with a suitable (ideally, approved) trivial name. RCs will also be used in *The IUPHAR Receptor Database*, currently being developed, which will uniquely link nucleotide and protein databases to comprehensive data on receptor function and drug-related characteristics.

The details presented here constitute a proposal, sanctioned by NC-IUPHAR (see acknowledgement), which provides a basis for a broad and full debate within the pharmacological community at large and with scientists from other disciplines and their representative bodies.

CRITERIA FOR RECEPTOR CHARACTERIZATION

It is generally acknowledged that studies towards the pharmacological characterization and classification of receptors for hormones, neurotransmitters and autacoids should involve work on both function and structure[3,5,6,8,9]. Receptor structure, in terms of its amino acid sequence, is unambiguous and will allow the allocation of a database code to the unequivocally identified protein. Function is equally important and not necessarily sufficiently predictable from structure, although it may be. Thus, one amino acid difference may make important differences in the drug recognition characteristics of a receptor (cf. the rat and human neurokinin NK_1 receptors) or by contrast, significant differences in receptor sequence homology may make little such difference (e.g. in the case of human 5-HT_{1B} and 5-HT_{1D} receptors or somatostatin receptors sst_1 and sst_2)[9-11]. It is therefore essential to establish which drugs are specific and selective for a particular receptor, either as agonists or antagonists, and to provide appropriate quantitative measurements of key parameters[4,12]. The affinities (as dissociation equilibrium constants) of ligands are often measured from radioligand binding studies and such data (at least for antagonists) should be equivalent to the corresponding data from functional studies. It has been argued that parameters from both types of study clearly cannot be considered on semantic grounds under the umbrella term 'functional' and hence the term 'operational' has been introduced[3,6]. Doubts about the use of the term operational in this context have been expressed because of its prior (and currently accepted) use in reference to agonism and agonist-specific parameters, which involve an efficacy component with more than just a binding (and recognition) parameter[4,13]. However, this strengthens the argument for using the term operation which, importantly, implies an added involvement of receptor activation and hence transduction. The alternative term 'recognition' more specifically refers to the binding characteristics or binding affinities of drugs (both agonist and antagonist) for a given receptor but does not necessarily encompass all aspects of receptor function, as the term operational undoubtedly does.

Transduction in the context of receptor characterization is intended to refer to the steps which allow the binding of an agonist at the receptor to be linked to the transmission of a signal into a cell or between compartments of a cell. This will necessarily involve specific changes in the receptor proteins (if it is in the ion-channel class) or, in other cases, relayed to associated proteins which execute the primary signalling step. Transductional data should not involve the categorisation of more downstream second message cascades themselves, although such information might be used judiciously to infer events upstream. Even if more tightly defined, the use of transductional data to characterize receptors is controversial but it has been invaluable nevertheless in the classification of 5-HT receptors[8,9]. Thus 5-HT_{1C} (now called 5-HT_{2C}) and 5-HT_2 (now called 5-HT_{2A}) receptors were predicted to belong to the same receptor group on the basis of shared transduction mechanisms. This was later fully confirmed when both receptor genes were cloned and the respective proteins were shown to share a high degree of homology[9]. By contrast, although both 5-HT_3 and 5-HT_4 receptor types can be blocked by tropisetron (albeit at different concentrations), it was obvious on the basis of transduction that the two receptors were quite distinct even before both genes had been cloned[8,9]. Thus, at the very least, consideration of the transduction mechanism involved will distinguish between a transmitter-gated ion-channel receptor and a G protein-coupled receptor (i.e. the 5-HT_3 and 5-HT_4 receptor, respectively). It should be noted that the structural classes proposed here (Table 2) reflect known fundamentally different transduction mechanisms for each. It follows that a knowledge of receptor transduction mechanisms from functional studies is impor-

tant but how much value should be attached to such operational data specifically for receptor characterization purposes in isolation remains to be determined. However, there is a growing view that the unique intracellular face of each membrane-bound receptor protein will dictate preferred stoichiometric interactions with adjacent proteins, which will be characteristic of the receptor type involved. On the basis of these arguments, essential operational data for receptor characterization would include all drug recognition and drug interaction data, from both functional as well as radioligand binding studies, together with data on receptor transduction mechanisms directly related to receptor activation.

In summary, we propose that the pharmacological criteria for receptor characterization will depend on the integration of data from studies on both receptor structure and receptor operation. When such an integrated pharmacological profile is sufficiently detailed it will be possible to register an IUPHAR Receptor Code (RC) with confidence.

PROPOSAL FOR A SYSTEMATIC RECEPTOR CODE

A systematic numbering system for all known pharmacological receptors allows definitive labelling of a particular receptor by an international pharmacological authority (NC-IUPHAR), while providing valuable classified information about its characteristics. Each RC would provide a unique identifier for each receptor within the NC-IUPHAR receptor database, containing extensive pharmacological information on receptor characterization and classification. If necessary such codes could be changed when new information and understanding dictated that change was essential. A full alphanumeric code could provide a universally accessible record of numbering and subclassification of receptors which would reflect the state of current knowledge for the structure and characteristics of each receptor. The coding system would not replace trivial names, which would normally be used, but it would circumvent some of the problems associated with attempts to standardize such trivial names.

The proposed RC would consist of a set of divisions, separated from each other by full points; each division conveys a different category of information about the receptor, as summarised in Table 1. Thus, according to the system proposed, the rat 5-HT_{1A} receptor (as a G protein-coupled recombinant receptor for 5-HT, classified pharmacologically as the 1A subtype) would have a receptor code (RC) of 2.1.5HT.01A.RNO.00.00 and the human 5-HT$_3$ (as the first transmitter-gated cation channel receptor subunit for 5-HT) an RC of 1.1.5HT.01.HSA.00.00.S. Other examples to illustrate the coding system are the rat muscarinic acetylcholine receptor, which would have an RC of 2.1.ACH.01.RNO.00.00, and the human $P2X_1$ receptor subunit, which would have an RC of 1.4.NUCT.01.HSA.00.00.S. These RCs are based on the assignment of the coded information explained in the following sections.

An RC will be reserved for the polypeptide product(s) of a single orthologous gene. Species variants will share a common RC but will be differentiated by a three-letter species code (Table 8).

A provisional RC can be assigned without cloning of the relevant gene providing it has been robustly characterized in whole tissues (e.g. histamine H_4 receptor). This may prove difficult in the brain, or in other tissues if multiple sub-types co-occur. Where this is so, the minimum requirement for a recombinant receptor to be accepted as a functional entity will be that robust operational (which must include transductional) data are provided for the heterologously expressed receptor and that its mRNA, or better the protein itself, is shown to occur *in vivo*. Recombinant receptors which have not been shown to be functional in whole tissues (e.g. 5-ht_{4b}) would only be assigned a provisional RC designated by a terminal upper case 'P' (e.g. 2.1.5HT.05B.RNO.00.00.P).

For structural classes where hetero-oligomeric receptors occur, RCs for individual subunits will often be represented instead of the entire multimeric receptor, since the composition of the latter is usually indeterminate at present. Subunit RCs will be indicated by a terminal upper case 'S' (e.g. 1.4.NUCT.01.RNO.00.00.S in the case of the rodent $P2X_1$ receptor subunit for ATP).

Where the subunit composition of an endogenous heteromeric receptor becomes known, that receptor would be assigned its own RC (indicated by a terminal upper case 'M' to represent a 'multimeric' receptor); its subunit composition would be indicated in the database by listing the component RCs (all indicated by the terminal S), and their stoichiometry when known. For many transmitter-gated ion-channel receptors in structural class 1.0, where a subunit combinatorial system occurs, the exact stoichiometry is not currently known. However, the situation is less complex for hetero-oligomers occurring in structural class 3.0 where there is normally a fixed composition of subunits for each receptor type.

Table 1. Categories in the IUPHAR Receptor Code.

Structural class	1.1. – 4.4.
indicated by first two numbers	(see Tables 2–6, for description of associated codes)
Receptor family alphanumeric code (up to six upper case characters, but commonly three)	an abbreviation of the endogenous agonist, family of agonists, or collective term (e.g. ACH for acetylcholine) (see Table 7 for recommended codes)
Receptor type alphanumeric code (up to five upper case characters)	consistent with the approved trivial name or number and its associated recognition and transduction characteristics (see Receptor type codes)
Species abbreviations three upper case letters	species abbreviations used by the Human Genome Database (e.g. HSA for human) (see Table 8 for abbreviations)
Splice or other sequence variant pair of numbers after species code	.00. – .99. (see Splice variant codes)
Extra category pair of numbers after splice variant code	.00. – .99. reserved for further subclassification purposes if considered desirable in the future; where this and the preceding sub-division are not assigned, the zeros can be omitted for most purposes
Final letter code single upper case letter	.P – provisional RC .S – receptor subunit .M – multimeric receptor of known composition

Table 2. Main structural class codes.

Code	Structural class
1.0.	Ion-channel receptors
2.0.	Seven-transmembrane domain, G protein-coupled receptors
3.0.	Enzyme-associated receptors (with subunits having one membrane-inserted domain)
4.0.	Transcriptional regulator receptors

Each structural class is subdivided as outlined in Tables 3–6. If the subclassification of a receptor is uncertain, it will be provisionally assigned the code for the class, e.g.1.0., 2.0., etc.
It should be recognised that these structural classes are provisional and that additional classes (and subclasses) may eventually be necessary as more information on receptors becomes available.
Literature references on receptors in many of the subclasses listed below can be found in this compendium.

Table 3. Subclasses within structural class 1.0: Ion-channel receptors

Code	Subclass	Examples
1.1.	**superfamily of Cys-loop receptors**[14,15] includes ion channels gated by γ-aminobutyric acid (GABA), glycine, 5-HT, acetylcholine (nicotinic) and glutamate (anion channels)[16–18]	1.1.GABA. 1.1.GLY. 1.1.5HT. 1.1.ACH. 1.1.GLU.
1.2.	**glutamate-gated cation channels** includes NMDA and non-NMDA receptors	1.2.GLU.
1.3.	**related to voltage-gated cation channels**[a] includes receptors for cyclic nucleotides and for IP_3 as well as the 'ryanodine receptor'	1.3.IP3.
1.4.	**related to epithelial Na^+ channels; non-peptide-gated** includes P2X receptors for ATP (ref. 19) and proton-gated cation channels[20]	1.4.NUCT.
1.5.	**related to epithelial Na^+ channels; peptide-gated** e.g. Phe-Met-Arg-Phe amide (FMRF)-gated Na^+ channel[21]	1.5.FMRF.
1.6.	**related to inward rectifier K^+ channels**[b,c] e.g. ATP-activated K^+ channel[22,23] and the ATP-antagonised K^+ channel (K_{ATP})[24–26]	1.6.NUCT.
1.7.	**related to ATPase-linked transporters**[b,d] e.g. cystic fibrosis transmembrane regulator (CFTR) ATP-activated anion channel[27]	1.7.NUCT.
1.8.	**related to neurotransmitter transporters**[b,d] e.g. glutamate-activated Cl^- channel/excitatory amino acid transporter [28,29]	1.8.GLU

[a] It should be noted that subclass 1.3. will contain at least two protein superfamilies, which do not share any sequence homology. One comprises the cyclic nucleotide receptors and the second comprises the IP_3 and the ryanodine receptors.

[b] It is recognised that the definition of subclasses 1.6., 1.7. and 1.8. may require modification as more knowledge of the component receptors becomes available, but they serve to illustrate the full potential of the proposed coding system.

[c] Several K_{ATP} channel subtypes are known in different tissues, differing greatly in their response to inhibitory sulphonylureas (SUR) and to channel-opening drugs such as diazoxide. Their structures as known so far by DNA cloning contain two unrelated subunits: an inward rectifier K^+ channel (Kir) protein (from the Kir.6 series), and a sulphonylurea and nucleotide-binding protein (SUR). A family of SURs produce variations in the K_{ATP} pharmacology[35]. Therefore, K_{ATP} channel subunits will be numbered in two series; for the KIR (6.1), etc., subunits as 1.6.KIR.61.S [or 62.S, etc.] and for the SUR1, etc., subunits 1.6.SUR.01.S [or 02.S etc.], and the entire K_{ATP} channel as 1.6.NUCT.01.M [or 02.M, etc.] when definitive evidence for the subunit composition is available.

[d] Although genes for members of subclasses 1.7. and 1.8. have each been cloned, expressed and shown to correspond to channels seen in native tissues[27,28], the precise relationship of the ion channel to the transporter is at present unclear. Transporters are not necessarily transmitter-gated ion channels: however, in addition to the case of glutamate transporters, transporters of 5-HT and of dopamine incorporate, respectively, a 5-HT-gated channel[36] or a dopamine-gated channel[37], and this principle may hold also for noradrenaline and GABA transporters[38].

Table 4. Subclasses within structural class 2.0: G protein-coupled receptors.

Code	Subclass	Examples
2.1.	**'rhodopsin'** the vast majority of seven-transmembrane domain, G protein-coupled receptors[a]	2.1.ADR.
2.2.	**secretin-related receptors** the second largest subclass; comprises receptors for calcitonin, CGRP, corticotropin-releasing factor, gastric inhibitory peptide, glucagon, glucagon-like peptide, growth hormone-releasing factor, PACAP, parathyroid hormone, secretin and vasoactive intestinal peptide	2.2.SEC. 2.2.CGRP.
2.3.	**metabotropic glutamate and $GABA_B$ receptors**	2.3.GLU.

[a] From the strict structural viewpoint, class 2.1. is not homogeneous. All of its members comprise a 7TM amino acid chain, but in a very few, the active receptor is formed from this by a proteolytic cleavage. The first described was the thrombin receptor (2.1.THR.), in which the agonist thrombin specifically cleaves the receptor chain to liberate a new N-terminal segment and activate the receptor[39]. Others are the thyrotropin receptor (2.1.TSH.), where a peptidase produces two extracellular chains[40] and other protease-activated receptors where the natural agonist is an unidentified trypsin-like protease[41,42].
The subclasses shown have been classified according to their protein sequences such that within a subclass all receptor types share significant similarity (i.e. ≥20% sequence identity) throughout the predicted hydrophobic transmembrane domains[30] (see also *The G protein-Coupled Receptor Database* at www.gcrdb.ulthscsa.edu/).

Table 5. Subclasses within structural class 3.0: enzyme-associated single-transmembrane domain receptors.

Code	Subclass	Examples
3.1.	**receptors with intrinsic tyrosine kinase (TK) activity** e.g. single-subunit TK receptors with extracellular Ig domains; single-subunit TK receptors without extracellular Ig domains; multiple-subunit TK receptors formed by post-translational cleavage; Trk receptors for neurotrophins	3.1.PDGF. 3.1.EGF. 3.1.INS. 3.1.NT1.
3.2.	**non-enzyme-containing receptors associating with extrinsic tyrosine kinase** comprises a wide range of multi-subunit receptors with a ligand-specific α-subunit and a subunit type for signal transduction[31,32]; includes: (a) receptors that utilize Janus Kinase (JAK)-type kinases, (b) receptors that associate with other tyrosine kinases and (c) tyrosine-kinase-associated receptors with the ligand-binding subunit membrane anchored by a glycolipid, such as that for ciliary neurotrophic-factor	3.2.IL1. 3.2.GH. 3.2.NTN. 3.2.GDNF. 3.2.CNTF.
3.3.	**receptors with serine/threonine kinase activity** e.g. receptors for transforming growth factor β	3.3.TGF.
3.4.	**intrinsic cyclase receptors** e.g. receptors with guanylate cyclase activity	3.4.ANP.

Table 6. Subclasses within structural class 4.0: transcriptional regulator receptors[a].

Code	Subclass	Examples
4.1.	**non-steroid receptors** comprises the heterodimeric receptors for non-steroid ligands including retinoic acid, thyroid hormone and vitamin D	4.1.TH. 4.1.VITD3.
4.2.	**steroid receptors** comprises the homodimeric receptors for steroids including cortisone, aldosterone, progesterone and testosterone	4.2.PROG.

[a] Nuclear receptors constitute a distinct class that is of great therapeutic relevance. With the discovery of multiple orphan nuclear receptors and hence of other potential structural subclasses[33,34], efforts are being applied by a group of experts in the field to further subclassification on the basis of the integrated pharmacological approach proposed by NC-IUPHAR.

Table 7. Receptor family codes.

Acetylcholine	**ACH**	Insulin	**INS**
Adenosine	**ADO**	Inositol 1,4,5-trisphosphate	**IP3**
Adenosine and uridine triphosphates	**NUCT**	Melatonin	**MLT**
Angiotensin	**ANG**	Nerve growth factor	**NGF**
Atrial natriuretic peptide	**ANP**	Neurokinins	**NK**
Bradykinin	**BK**	Neuropeptide Y	**NPY**
Calcitonin	**CALC**	Neurotrophins	**NT1** (etc.)
Cannabinoids	**CBD**	Neurotensin	**NTSN**
Cholecystokinin	**CCK**	Neurturin	**NTN**
Calcitonin gene-related peptide	**CGRP**	Noradrenaline/adrenaline	**ADR**
Cysteinyl leukotrienes	**CLT**	Olfactory	**OLF**
Ciliary neurotrophic factor	**CNTF**	Opioids	**OP**
Corticotropin-releasing factor	**CRF**	Oxytocin	**OXY**
Dopamine	**DA**	Pituitary adenylate cyclase-activating polypeptide	**PACAP**
Epithelial-derived growth factor	**EGF**	Platelet-derived growth factor	**PDGF**
Endothelins	**ET**	Progesterone	**PROG**
Gamma-aminobutyric acid	**GABA**	Prostaglandins	**PG**
Glial-derived nerve factor	**GDNF**	Secretin	**SEC**
Glutamate	**GLU**	Somatostatin	**SRIF**
Glycine	**GLY**	Transforming growth factor β	**TGF**
Growth hormone	**GH**	Thrombin	**THR**
Gustatory	**GUS**	Thyroid hormone	**TH**
Histamine	**HIST**	Thyrotropin	**TSH**
Hydroxy leukotrienes	**BLT**	Vasopressin	**VASO**
5-hydroxytryptamine (serotonin)	**5HT**	Vasoactive intestinal peptide	**VIP**
Interleukins	**IL, IL1** (etc.)	Vitamin D3	**VITD3**

This list of abbreviations for the endogenous agonist(s), or a collective term to describe receptor families (e.g. gustatory, cannabinoid) is not exhaustive but represents receptor families for which classifications are well developed and a few illustrations of newer receptor families not well classified.

RECEPTOR TYPE CODES

The use of an alphanumeric abbreviation for the receptor type category would allow either recognisable reference to trivial names or for more recently identified receptors direct reference to the simple numbering system used by molecular biologists. Parenthetically, it is suggested that in some cases, for example the muscarinic acetylcholine and dopamine receptor families, consideration should be given as to whether the current schemes are appropriate[44]. Regardless, the two options for designating an RC are outlined below and each subcommittee could choose one for their receptor family.

(1) For well established classifications, the receptor type category will be represented by an upper case alphanumeric code that is recognisable as analogous to the trivial nomenclature, e.g. for the G protein-coupled 5-HT receptors[9,45]:

2.1.5HT.01A. for the 5-HT_{1A} receptor
2.1.5HT.01B. for the 5-HT_{1B} receptor
2.1.5HT.01D. for the 5-HT_{1D} receptor
2.1.5HT.02A. for the 5-HT_{2A} receptor
2.1.5HT.07. for the 5-HT$_7$ receptor,
etc.
2.1.ADR.A1A. for the α_{1A}-adrenoceptor
2.1.ADR.B1. for the β_1-adrenoceptor,
etc.

(2) Where subclassification has not been attempted, a simple chronological numeric code may be assigned although this approach seems scientifically parsimonious and circumvents an opportunity to provide additional classifying information (e.g. as for dopamine receptors[44]):

2.1.DA.01.
2.1.DA.02., etc.

It is recognised that there is a strongly held view by some that an arbitrary numeric code should be used for all receptor types, which would circumvent past problems associated with incorrectly assigning trivial names. However, after much discussion at NC-IUPHAR meetings, it was agreed that many pharmacologists would not readily accept a system which, for example, groups together the adrenoceptor family without distinguishing between α-adrenoceptors and β-adrenoceptors, with their very different pharmacological characteristics. Nevertheless, if consistent yet arbitrary numeric codes were considered desirable in the future, it would be a simple exercise to number each receptor type according to its established position in the database.

SPLICE VARIANT CODES

For splice variants, it was agreed that the variants will be chronologically numbered according to identification within a species, e.g. EP_3 receptors for prostaglandins[45,46] can be coded as:

2.1.PG.EP3.HSA.01.
2.1.PG.EP3.HSA.02.
2.1.PG.EP3.HSA.03.
2.1.PG.EP3.HSA.04.
2.1.PG.EP3.OCU.01., etc.

Splice variants for a given receptor may have been identified in one species but not in others, e.g. two mouse splice variants have been unequivocally demonstrated for the somatostatin sst_5 receptor but not the human homologue[47,48]. They can be coded as:

2.1.SRIF.01A.HSA.00.
2.1.SRIF.01A.MMU.01.
2.1.SRIF.01A.MMU.02.

No code for trinucleotide repeats is recommended; these can be described in the text associated with the relevant RC.

Table 8. Species codes.

BBO	*Bos bovinus* (cow)
CAE	*Cercopithecus aethiops* (African green monkey)
CFA	*Canis familiaris* (dog)
CGR	*Cricetulus griseus* (hamster)
CJA	*Callithrix jacchus* (marmoset)
CPO	*Cavia porcellus* (guinea-pig)
FCA	*Felis catus* (cat)
HSA	*Homo sapiens* (man)
MML	*Macaca mulatta* (Rhesus monkey)
MMU	*Mus musculus* (mouse)
MPU	*Mustela putorius furo* (ferret)
MRU	*Macropus rufus* (red kangaroo)
OCU	*Oryctolagus cuniculus* (rabbit)
OOV	*Ovies ovies* (sheep)
PPA	*Papio papio* (baboon)
PTR	*Pan troglodytes* (chimpanzee)
RNO	*Rattus norvegicus* (rat)
SSC	*Sus scrofa* (pig)

These abbreviations are proposed in order to establish and maintain consistency with existing internationally authoritative databases (e.g. GDB, MEDLINE). Alternative abbreviations to denote the species when using common receptor names have been published by NC-IUPHAR and these are still appropriate for use in textual discussion referring to common names[7].

FUTURE DIRECTIONS

We have provided justification for a systematic method of coding receptors of all structural types. The RC system proposed is designed not just to be informative but also to provide a distinct alphanumeric descriptor for each receptor protein. It is intended that each individual RC will not only define each receptor type unambiguously by way of a simple reference for publication purposes but that it will also

associate automatically with a large body of information in an authoritative pharmacological database. This database would supply an urgent need of pharmacologists and other scientists interested in the correlation and integration of data on receptor operation with that on receptor structure. *The IUPHAR Receptor Database* will link not only with existing databases already established for gene nucleotide sequences and amino acid sequences of receptor proteins, but will also uniquely provide detailed pharmacological data on the characteristics of receptor recognition and transduction. The latter data will be approved by international panels of experts in each of the many existing and future NC-IUPHAR subcommittees established for the different receptor families.

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INTRODUCTION TO TABLES

In the following contributions, a complete classification – integrating all available data for a receptor family – constitutes a chapter. The status of both the field and the subcommittee determine the content of each chapter. Where a full classification is not available, a synopsis of the current position (a summary statement) has been provided. Newly constituted subcommittees, or those currently reassessing a classification, are acknowledged by listing the membership. It is intended that complete classifications for all major receptor families of interest to pharmacologists will be provided in the next edition of the compendium.

A standard tabular format is used for each classification of a receptor family.

The NC-IUPHAR-recommended trivial nomenclature is provided, together with the IUPHAR Receptor Code (RC). Although the tables include an entry for receptor subtype, it is not recommended that this term is used, except for highly homologous receptor proteins within a family (see earlier chapters, Recommendations for Receptor Nomenclature; The IUPHAR Receptor Code)

Precedence is given to data from studies on human receptors, but many data (clearly differentiated) are also provided for receptors from common laboratory species, notably rat and mouse Note that the preliminary receptor code allocated to each receptor is intentionally incomplete (i.e. it does not define the species) to take account of this feature of the tables. Data from non-mammalian species are not included.

Particular emphasis has been placed on providing referenced quantitative data for operational characteristics obtained from well-controlled, rigorous experimentation. Quantitative agonist data are preferably given as equi-effective molar ratios (see the earlier chapter, Terms and Symbols in Quantitative Pharmacology) where the standard agonist = 1. Unless stated, antagonist potencies are given as pK_B values for competitive antagonists or occasionally as IC_{50} values when competitive antagonists are unavailable. Binding affinities are given as pIC_{50} values or K_d values, where possible.

Receptor type	nomenclature for a structurally and operationally distinct receptor in a given family
Receptor subtype	nomenclature for a receptor with strong structural homology to other types, but with distinct operational characteristics
Receptor code	a preliminary receptor code (RC), without the species abbreviation
Previous name(s)	outdated names that exist in the literature, but which are no longer the recommended nomenclature
Structural information	the number of transmembrane domains (TM) and amino acids (aa), and accession numbers for the human (h), rat (h), and mouse (m) receptors where available; the chromosomal location (chr.) for the human receptor
Functional assays	pharmacological test systems (whole tissues or isolated cells) in which a response can be firmly attributed to the function of a defined receptor type or subtype
Selective agonists	agonists that are selective for the receptor type; specific agonists, which are not selective between receptor types in the family, are included if useful
Agonist potency ratios	quantitative agonist data are preferably given as equi-effective molar ratios (EMR) where standard agonist = 1, defined in terms of a functional response in a particular tissue or cell type; potency ratios (the reciprocal of EMR) are alternatively provided
Selective antagonists	antagonists that are selective for the receptor type; specific antagonists, which are not selective between receptor types in the family, are included if useful
Antagonist potencies	expressed as pK_B or pA_2 values from functional assays, or as IC_{50} values (if no competitive antagonists are available)
Radioligands	available radioligands (agonist or antagonist, selective or non-selective)
Radioligand assays	cell lines expressing cloned receptors, or any tissues or cell lines expressing endogenous receptors
Ligand affinities	binding affinities for key ligands are given as pIC_{50} (or IC_{50}) values or expressed as pK_d (or K_d) values unless otherwise stated
Transduction mechanism(s)	the preferred receptor signalling pathway or mechanism, when established; any identified alternative mechanisms
Receptor distribution	central and peripheral distribution of the receptor (and the identifying techniques)
Tissue function(s)	the physiological response mediated by the receptor if established in whole tissue, preferably *in vivo*
COMMENTS	additional information, particularly structural information on splice variants, and anomalies in operational characteristics

Receptor type	5-HT_2
Receptor subtype	5-HT_{2C}
Receptor code	2.1.5HT.02C.000.00.00
Previous name(s)	5-HT_{1C}
Structural information	h 377aa, P28221, chr. Xq24 r 377aa, P49145 m 374aa, P28565
Functional assays	IP_3 accumulation in homogenates of choroid plexus (porcine)
Selective agonists	none non-selective: (±)-α-Me-5-HT (±)-DOB
Agonist potency ratios	5-HT = α-Me-5-HT > DOI > RU24969 = quipazine > 5-CT > sumatriptan
Selective antagonists	RS102221 non-selective: mesulergine
Antagonist potencies	RS102221 (p*K* 8.6) non-selective: mesulergine (8.8)
Radioligands	[^{3}H]-mesulergine [^{125}I]-LSD
Radioligand assays	recombinant receptor; choroid plexus; cortex homogenate
Ligand affinities	5-HT (p*K* 8.7), mesulergine (9.1), DOI (7.4), ketanserin (7.6)
Transduction mechanism(s)	G_q/G_{11} preferentially increases PI hydrolysis and elevates $[Ca^{2+}]_i$
Receptor distribution	choroid plexus, medulla, pons, striatum, hippocampus (CA1, CA3), hypothalamus, spinal cord; no peripheral distribution identified
Tissue function(s)	possibly involved in modulation of transferrin production and regulation of CSF volume

COMMENTS

Receptor type	5-HT_3
Receptor subtype	—
Receptor code	1.1.5HT.01.000.00.00.S
Previous name(s)	'M' receptor
Structural information	4TM h 5-HT_{3A}, 478aa, P46098, chr. 11 r 5-HT_{3A}, 483aa, P35563 m 5-HT_{3A}, 487aa, P23979
Functional assays	contraction of ileum via myenteric acetylcholine release (guinea-pig); depolarisation of isolated vagus nerve (guinea-pig, rat, rabbit); cardioexcitation via sympathetic noradrenaline release (rabbit); von Bezold-Jarisch reflex (various species)
Selective agonists	2-Me-5-HT 1-(m-chlorophenyl)-biguanide (m-CPB)
Agonist potency ratios	m-CPB > 5-HT > 2-Me-5-HT >> 5-MeOT
Selective antagonists	tropisetron ondansetron granisetron
Antagonist potencies	tropisetron (p*K* 10–11) ondansetron (8–10) granisetron (10)
Radioligands	[^{3}H]-(*S*)-zacopride [^{3}H]-BRL43694 [^{3}H]-GR65630 [^{3}H]-LY278584
Radioligand assays	recombinant α subunit
Ligand affinities	granisetron (p*K* 8.8), ondansetron (8.3), M-CPB (7.2), 5-HT (6.9), 2-Me-5-HT (6.7)
Transduction mechanism(s)	intrinsic transmitter-gated ion channel promotes increased [Ca^{2+}]$_i$
Receptor distribution	*striatum, hippocampus* (CA1), *substantia nigra, globus pallidus;* post-ganglionic sympathetic neurones, sensory neurones
Tissue function(s)	sympathetic and parasympathetic neuroexcitation (various species); vagal neuroexcitation: provocation of emesis (ferret)
COMMENTS	Pentamer of four subunits. Human, rat and mouse orthologues of a single subunit (5-HT_{3A}) have been cloned. Species-differences in pharmacology and kinetics of activation and inactivation exist.

DETERMINATIO OF ESSENTIAL OILS IN VEGETABLE DRUGS *(2.8.12.)*

- Aniseed : ≥ 20 ml/kg
- Caraway fruit : ≥ 30 ml/kg
- Chamomile flower, roman : > 7 ml/kg
- Cinnamon: > 12 ml/kg
- Clove: > 150 ml/kg
- Fennel, bitter : > 40 ml/kg
- Fennel, sweet : > 20 ml/kg
- Matricaria flower: > 4 ml/kg
- Peppermint leaf : > 12 ml/kg
- Star anise : > 70 ml/kg
- Thyme : > 12 ml/kg
- Valerian Root : > 5 ml/kg

MICROBIAL CONTAMINATION

- Acacia : total viable aerobic count *(2.6.12.)* < 10^4 /gram bacteria; TEST *E.coli (2.6.13.)*
- Agar : total viable aerobic count *(2.6.12.)* < 10^3 /gram bacteria; TEST *E.coli* and *Salmonella (2.6.13.)*
- Gelatin : total viable aerobic count *(2.6.12.)* < 10^3 /gram bacteria; TEST *E.coli* and *Salmonella (2.6.13.)*
- Maize starch : total viable aerobic count *(2.6.12.)* < 10^3 /gram bacteria and < 10^2/gram fungi; TEST *E.coli (2.6.13.)*
- Potato starch : total viable aerobic count *(2.6.12.)* < 10^3 /gram bacteria and < 10^2/gram fungi; TEST *E.coli (2.6.13.)*
- Wheat starch : total viable aerobic count *(2.6.12.)* < 10^3 /gram bacteria and < 10^2/gram fungi; TEST *E.coli (2.6.13.)*
- Rice starch : total viable aerobic count *(2.6.12.)* < 10^3 /gram bacteria and < 10^2/gram fungi; TEST *E.coli (2.6.13.)*
- Tragacanth : total viable aerobic count *(2.6.12.)* < 10^4 /gram bacteria; TEST *E.coli* and *Salmonella (2.6.13.)*
- Guar gallactomannan : total viable aerobic count *(2.6.12.)* < 10^3 /gram bacteria; TEST *E.coli* and *Salmonella (2.6.13.)*

Arachis oil : < 5.0
Olive oil : < 5.0 (also for parenteral dosage forms)
Sesame oil : < 5.0
Wool fat : < 20
Wool fat, hydrous : < 15

- Saponification value *(2.5.6.)*

Carnauba wax (own method used) : 78 to 95
Castor oil : 176 to 187
Peru Balsam : 230 to 255
Wool fat : 90 to 105
Wool fat, hydrous : 67 to 79

- Unsaponifiable matter *(2.5.7.)*

Almond oil : < 0.7 %
Almond oil (refined) : < 0.7 %
Arachis oil : < 1.0 %
Castor oil : < 0.8 %
Olive oil (own method) : < 1.5 %
Sesame oil : < 2.0 % (Ph.Eur. Suppl. 1998)
Soya-bean oil : < 1.5 %

DETERMINATION OF WATER *(2.5.12.)*

If intended for use in the manufacture of parentral dosage forms

Almond oil, refined : 0.3 %
Arachis oil : 0.3 %
Castor oil : 0.3 %
Olive oil : 0.1 %
Sesame oil : 0.05 %
Soya-bean oil : 0.3 %

RESIDUE ON EVAPORATION OF ESSENTIAL OILS *(2.8.9.)*

- Lemon oil

SOLUBILITY OF ALCOHOL OF ESSENTIAL OILS *(2.8.10.)*

- Eucalyptus oil
- Clove oil

ASSAY OF 1,8- CINEOLE IN ESSENTIAL OILS *(2.8.11.)*

- Eucalyptus oil

4. shake aqueous layer with 2 x 50 ml light petroleum; combine light petroleum layers
5. wash with 3 x 50 ml alcohol (50 %)
6. evaporate light petroleum and dry residue at 100-105 °C (15 min) : a gram
7. dissolve residue in 20 ml alcohol (neutralised to bromophenol blue solution)
8. if necessary, titrate with 0.1 M HCl (b ml)
9. % of unsaponifiable matter: 100 (a - 0.032 b)/m

Carnauba wax : Acid value : 2-7 ; Saponification value : 78-95

1. 2.000 g (m gram) + 40 ml xylene : reflux until completely dissolved
2. + 20 ml alcohol + 1 ml phenolphthalein solution
3. titrate hot solution with 0.5 M alcoholic KOH (n1 ml); carry out a blank test (n2 ml)
4. calculate acid value : 28.05 (n1 - n2)/m

1. add to titrated solution (acid value) 20.0 ml 0.5 M alcoholic KOH; reflux for 3 h
2. add 1 ml phenolphthalein solution; titrate hot solution with 0.5 M HCl (n3 ml)
3. carry out blank (n4 ml)
4. calculate saponification value : 28.05 (n4 - n3)/m + acid value

- Acid value *(2.5.1.)*

Almond oil : < 2.0
Almond oil, refined : < 0.5
Anise oil : < 1.0
Arachis oil : < 0.6
Carnauba wax : 2 to 7 (own method)
Olive oil : < 2.0 ; if intended for use in manufacture of parenteral dosage forms : < 0.5
Peppermint oil : < 1.4
Sesame oil : < 0.6 ; if intended for use in manufacture of parenteral dosage forms : < 0.3
Wool fat : < 1.0
Wool fat, hydrous : < 0.8

- Ester value *(2.5.2.)*

Beeswax, white : 17 to 24 (own method)
Beeswax, yellow : 17 to 22 (own method)

- Hydroxyl value *(2.5.3.)*

Castor oil *(Ricini oleum)* : method A : > 150

- Iodine value *(2.5.4.)*

Castor oil : 82 to 90

- Peroxide value *(2.5.5.)*

Almond oil : < 10.0
Almond oil, refined : < 5.0

7. + NH_3 (alkaline) + chloroform extraction + evaporate chloroform extract to dryness
8. dissolve residue in 20.0 ml H_2SO_4 0.01 M
9. titrate excess acid with NaOH 0.02 M on methyl red mixed solution (n ml)
10. total alkaloids, as hyosyamine :

$$57.88 \times (20-n)/(100-d) \times m$$

Lemon oil : ≥ 2.2 % and ≤ 4.5 % carbonyl compounds, calculated as citral

1. 9.000 g + 20 ml ethanol
2. add 10.0 ml hydroxylamine.HCl + bromophenol blue solution
3. titrate with 0.5 M alcoholic KOH (from yellow to olive-green)
4. allow to stand for 5 min.; titrate again if necessary
5. 1 ml 0.5 M alcoholic KOH = 76.1 mg carbonyl compounds, as citral

Ichthammol : ≥ 4.5 % and ≤ 7.0 % total ammonia

1. dissolve 2.50 g in warm water
2. rinse solution into a 250 ml volumetric flask + add 200 ml NaCl-solution + dilute to 250 ml
3. filter (discard first 20 ml)
4. 100.0 ml clear filtrate + 25 ml formaldehyde solution (neutralised to phenolphthalein)
5. titrate with 0.1 M NaOH until faint pink colour is obtained
6. 1 ml 0.1 M NaOH = 1.703 mg NH_3

- TESTS

Gelatin : Sulphur dioxide: ≤ 200 ppm

1. cfr. Apparatus for determination of SO_2
2. boil gelatin with dilute HCl for 1 h.
3. collect SO_2 in test tube with 10 ml (neutralised) dilute hydrogen peroxide solution
4. heat contents of test tube for 15 min.
5. titrate with 0.1 M NaOH on bromophenol blue R

Eucalyptus oil : aldehydes

1. 10 ml oil + 5 ml toluene and 4 ml alcoholic $NH_2OH.HCl$ solution (contains methylorange)
2. shake
3. titrate with 0.5 M KOH in alcohol (60 %) until red colour changes to yellow
4. continue titration with shaking
5. end-point reached : permanent pure yellow colour of indicator in lower layer
6. repeat titration on further 10 ml with first determination liquid as reference
7. maximum 2.0 ml 0.5 M KOH in alcohol (60 %)

Olive oil : unsaponifiable matter : ≤ 1.5 %

1. 5.0 g oil + 50 ml 2 M alcoholic KOH : reflux for 1 h (+ shaking)
2. + 50 ml water; shake; cool ; transfer to separating funnel
3. + 50 ml light petroleum ; shake; transfer aqueous layer to second separating funnel

TITRATIONS

- ASSAYS

Ipecacuanha root : ≥ 2.0 % total alkaloids (emetine + cephaëline), calculated as emetine

1. 7.5 g powder + NH_3 and ether extraction
2. residue : dissolve in neutralised alcohol (90 %)
3. + 15.0 ml HCl 0.1 M
4. titrate excess acid with NaOH 0.1 M on methyl red mixed solution
5. 1 ml HCl 0.1 M = 24.03 mg total alkaloids, as emetine

Belladonna leaf : ≥ 0.30 % total alkaloids (hyoscyamine + hyoscine = scopolamine), calculated as hyoscyamine

1. determine loss on drying at 100-105°C (d)
2. 10.0 g powder (m) + NH_3 and ether-alcohol extraction
3. percolation with chloroform-ether (1/3 vol.)
4. concentrate percolate to about 50 ml
5. transfer to a separating funnel; add 2.1 volumes of ether (density < water density)
6. extraction with 3 x 20 ml H_2SO_4 0.25 M; separate H_2SO_4-layer
7. + NH_3 (alkaline) + chloroform extraction + evaporate chloroform extract to dryness
8. dissolve residue in 20.0 ml H_2SO_4 0.01 M
9. titrate excess acid with NaOH 0.02 M on methyl red mixed solution (n ml)
10. total alkaloids, as hyosyamine :

$$57.88 \times (20-n)/(100-d) \times m$$

Hyoscyamus leaf : ≥ 0.05 % total alkaloids (hyoscyamine + hyoscine = scopolamine), calculated as hyoscyamine

1. determine loss on drying at 100-105°C (d)
2. 40.0 g powder (m) + NH_3 and ether-alcohol extraction
3. percolation with chloroform-ether (1/3 vol.)
4. concentrate percolate to about 50 ml
5. transfer to a separating funnel; add 2.1 volumes of ether (density < water density)
6. extraction with 3 x 20 ml H_2SO_4 0.25 M; separate H_2SO_4-layer
7. + NH_3 (alkaline) + chloroform extraction + evaporate chloroform extract to dryness
8. dissolve residue in 20.0 ml H_2SO_4 0.01 M
9. titrate excess acid with NaOH 0.02 M on methyl red mixed solution (n ml)
10. total alkaloids, as hyosyamine :

$$57.88 \times (20-n)/(100-d) \times m$$

Stramonium leaf : ≥ 0.25 % total alkaloids (hyoscyamine + hyoscine = scopolamine), calculated as hyoscyamine

1. determine loss on drying at 100-105°C (d)
2. 10.0 g powder (m) + NH_3 and ether-alcohol extraction
3. percolation with chloroform-ether (1/3 vol.)
4. concentrate percolate to about 50 ml
5. transfer to a separating funnel; add 2.1 volumes of ether (density < water density)
6. extraction with 3 x 20 ml H_2SO_4 0.25 M; separate H_2SO_4-layer

SULPHATED ASH *(2.4.14.)*

- Ichtammol : < 0.3 %
- Linseed : < 6.0 %
- Liquorice root : < 10.0 %
- Maize starch : < 0.6 %
- Potato starch : < 0.6 %
- Rice starch : < 1.0 %
- Valerian root : < 15.0 %
- Wheat starch : < 0.6 %
- Wool fat : < 0.15 %
- Wool fat, hydrous : < 0.1 %

LOSS ON DRYING *(2.2.32.)*

- Acacia - 100-105 °C: max. 15.0 %
- Acacia, spray dried - 100-105 °C : max. 10.0 %
- Agar - 100-105 °C : max. 20.0 %
- Aloes, barbados - 100-105 °C : max. 12.0 %
- Aloes, cape - 100-105 °C : max. 10.0 %
- Aloes, dry extract, standardised - 100-105 °C / 3 h : max. 4.0 %
- Bearberry leaf - 100-105 °C / 2 h : max. 10.0 %
- Belladona leaf (% not given; determined in the ASSAY) + prepared - 100-105 °C : max. 5.0 %
- Cascara - 100-105 °C / 2 h : max. 10.0 %
- Devil's claw root - 100-105 °C : max. 12.0 %
- Digitalis leaf - 100-105 °C : max. 6.0 %
- Extracts : **Dry residue** : 100-105 °C / 3 h
- Frangula bark - 100-105 °C/ 2 h : max. 10.0 %
- Gelatine - 100-105 °C : max. 15 %
- Guar galactomannan - 100-105 °C / 5 h : max. 15.0 %
- Hamamelis leaf - 100-105°C / 4 h : max. 10.0 %
- Hyoscyamus leaf (% not given; determined in the ASSAY) + prepared - 100-105 °C : max. 5.0 %
- Ipecacuanha, prepared - 100-105 °C : max. 5.0 %
- Ipecacuanha, root - 100-105 °C : max. 10.0 %
- Lime flower - 100-105 °C / 2 h : max. 12.0 %
- Maize starch - 100-105 °C : max. 15.0 %
- Marshmallow root - 100-105°C / 2 h : max.12.0 %
- Opium, raw -100-105 °C / 4 h : max. 15.0 %
- Potato starch - 100-105 °C : max. 20.0 %
- Psyllium seed - 100-105 °C / 2 h : max. 14.0 %
- Rhubarb - 100-105 °C : max. 12.0 %
- Rice starch - 100-105 °C : max. 15.0 %
- Senna leaf - 100-105 °C / 2 h : max. 12.0 %
- Senna pods, Alexandrian - 100-105 °C / 2 h : max. 12.0 %
- Senna pods, Tinnevelly - 100-105 °C / 2 h : max. 12.0 %
- Shellac - 40-45 °C / 24 h : max. 2.0 %
- Stramonium leaf (% not given; determined in the ASSAY) + prepared - 100-105 °C : max. 5.0 %
- Wheat starch - 100-105 °C : max. 15.0 %
- Wool fat - 100-105 °C / 1 h : max. 0.5 %

GAS CHROMATOGRAPHY *(2.2.28.)*

- Almond oil : Foreign fatty oils *(2.4.22.)* (fatty-acid fraction in %); Sterols *(2.4.23.):Determination of the sterols : after preparative TLC on silicagel*
- Almond oil, refined : Foreign fatty oils *(2.4.22.)* (fatty-acid fraction in %); Sterols *(2.4.23.):Determination of the sterols : after preparative TLC on silicagel*
- Anise oil : Chromatographic profile
- Arachis oil : Foreign fatty oils *(2.4.22.)*
- Clove oil : Chromatographic profile
- Fennel, bitter : Estragole (TESTS) and Anethole and Fenchone (ASSAY)
- Fennel, sweet : Estragole and Fenchone (TESTS) and Anethole (ASSAY)
- Olive oil : Foreign fatty oils *(2.4.22.)*; Sterols *(2.4.23.):Determination of the sterols : after preparative TLC on silicagel*
- Peppermint oil : Chromatographic profile
- Pesticide residues *(2.8.13.)*: Organo phosphorus insecticides, Organochlorine and Pyrethroid insecticides : no monographs
- Sesame oil : Foreign fatty oils *(2.4.22.)*
- TEST for methanol and 2-propanol *(2.9.11.)* : extracts and tinctures
- Wool fat : Butylhydroxytoluene (< 200 ppm)
- Wool fat, hydrous : Butylhydroxytoluene (< 150 ppm)

LIQUID CHROMATOGRAPHY *(2.2.29.)*

- Devil's claw root : ASSAY : Harpagoside (1.2 %) : detection at 278 nm ; C18-column
- Opium, raw : ASSAY : Morphine (> 10.0 %), Codeine (> 2.0 %), Thebaine (< 3.0 %) : detection at 280 nm ; C8-column
- Sesame oil : TESTS : Composition of triglycerides : refractometer detection; 2 C18-columns

(Ph.Eur. - Supplem. 1988)

SIZE-EXCLUSION CHROMATOGRAPHY *(2.2.30.)*

- Purification of Organochlorine, Organophosphorus and Pyrethroid insecticides in **PESTICIDE RESIDUES** *(2.8.13.)*

DETECTION IN DAYLIGHT AFTER SPRAYING

- Acacia : 2 runs ; ***spray : aminohippuric acid reagent + heat*** : galactose + arabinose + rhamnose (no glucose)
- Bearberry leaf : ***spray : 1 % solution dichloroquinonechlorimide in methanol + 2 % solution of anhydrous sodium carbonate*** : arbutin + gallic acid + hydroquinone
- Belladonna leaf : ***spray : potassium iodobismuthate solution*** : hyoscyamine + hyoscine; ***spray : sodium nitrite solution*** : hyoscyamine; TEST : no atropine
- Carnauba wax : ***spray : phosphomolybdic acid solution in alcohol*** : triacontanol + blue zones
- Fatty oils : identification *(2.3.2.)* : on octadecylsilyl silica gel : ***spray : 10 % phosphomolybdic acid solution in alcohol + heat***
- Guar galactomannan : 1 run ; ***spray : aminohippuric acid reagent + heat*** : galactose + mannose
- Hamamelis leaf : ***spray : ferric chloride solution*** : tannic acid + gallic acid + phenolic compounds
- Hyoscyamus leaf : ***spray : potassium iodobismuthate solution*** : hyoscyamine + hyoscine; ***spray : sodium nitrite solution*** : hyoscyamine; TEST : no atropine
- Opium, raw : ***spray : potassium iodobismuthate solution + 0.4 % sulfuric acid*** : morphine + codeine + papaverine + noscapine + thebaine
- Rhubarb : TEST : Rheum rhaponticum : ***spray : phosphomolybdic acid solution*** : no rhaponticin
- Senega root : ***spray : anisaldehyde solution + heat*** : saponosides (red zones); ***spray : 20 % phosphomolybdic acid in ethanol + heat*** : saponosides (blue zones)
- Senna leaf ***: spray : 20 % nitric acid solution + heat + spray : 5 % KOH in alcohol (50%)*** : sennosides B, A, D and C + rhein-8-glucoside.
- Senna pods, Alexandrian***: spray : 20 % nitric acid solution + heat + spray : 5 % KOH in alcohol (50%)*** : sennosides B, A, D and C + rhein-8-glucoside.
- Senna pods, Tinnevelly: ***spray : 20 % nitric acid solution + heat + spray : 5 % KOH in alcohol (50%)*** : sennosides B, A, D and C + rhein-8-glucoside.
- Star anise : ***spray : 20 % phosphomolybdic acid in alcohol + heat*** : anethole + triglycerides; TEST : no myristicine (Illicium anisatum)
- Sterols in Olive oil : separation of sterol fraction : ***spray : potassium permanganate solution***
- Stramonium leaf : ***spray : potassium iodobismuthate solution*** : hyoscyamine + hyoscine; ***spray : sodium nitrite solution*** : hyoscyamine; TEST : no atropine
- Tragacanth : ***spray : aminohippuric acid reagent + heat*** : galactose + arabinose + xylose + fucose
- Valerian root : TEST : ***spray : anisaldehyde solution + heat*** : valereinc acid + valtrate + isovaltrate + acetoxyvalerenic acid

TESTS

- Almond oil : 0.100 g in 10.0 ml cyclohexane : **absorbance between 264 nm and 276 nm ≤ 0.20**
- Lemon oil : **absorbance between 260 nm and 400 nm : 0.20 to 0.96**
- Olive oil : 1.00 g in 100.0 ml cyclohexane : **absorbance at 270 nm ≤ 0.20 ; ratio absorbanc at 232 nm/270 nm > 8**

PHENOLS AND POLYPHENOLS

ASSAY

- **Hamamelis leaf** : (protected from light)

1. 0.750 g powder (m in gram) + 150 ml water. Heat to boiling; cool; transfer and dilute to 250.0 ml
2. filter; discard first 50 ml of the filtrate

total polyphenols:

1. 5.0 ml filtrate + 25.0 ml water; mix 5.0 ml with 2.0 ml phosphotungstic acid solution; dilute to 50.0 ml with sodium carbonate solution
2. measure (after 3 min) absorbance (A1) at 715 nm

polyphenols not absorbed by hide powder:

1. 20.0 ml filtrate + 0.20 g hide powder CRS; shake for 60 min; filter
2. dilute 5.0 ml filtrate to 25.0 ml with water
3. mix 5.0 ml with 2.0 ml phosphotungstic acid solution; dilute to 50.0 ml with sodium carbonate solution
4. measure (after 3 min) absorbance (A2) at 715 nm

standard:

1. dissolve 50.0 mg pyrogallol R in water; dilute to 100.0 ml
2. dilute 5.0 ml solution to 100.0 ml with water
3. mix 5.0 ml with 2.0 ml phosphotungstic acid solution; dilute to 50.0 ml with sodium carbonate solution
4. measure (after 3 min) absorbance (A3) at 715 nm

% tannins: **13.12 x (A1 - A2)/ A3 x m**

- **Rhatany root :**

cfr. Hamamelis leaf

- **Thyme :**

1. dilute essential oil from assay to 50.0 ml alcohol (90.0 %)
2. 5.0 ml solution + 40 ml alcohol (90 %) + dilute with water to 100.0 ml
3. 5.0 ml in separating funnel + 45 ml water + 0.5 ml dilute ammonia + 1 ml 2 % aminopyrazolone; mix ; + 4 ml 2 % potassium ferrcyanide; mix;
4. + 25 ml methylene chloride; shake; separate methylene chloride layer
5. shake aqueous layer with 2 x 25 ml and 10 ml methylene chloride and filter
6. dilute to 100.0 ml with methylene chloride; measure absorbance at 450 nm
7. calculate % phenols, expressed as thymol; **(specific absorbance = 805)**

TESTS : Gelatin : phenolic preservatives + pentachlorophenol by TLC

7. cfr. 2-5
8. apply to TLC-plates as bands 20 mm x 3 mm 2 x 60 µl of test solution and 2 x 60 µl of reference solution; develop plate 2 x over 15 cm; dry; examine under 254 nm
9. mark zones, corresponding to ***β-glycyrrhetic acid*** in the chromatograms.
10. remove coatings; transfer to 25 ml flasks; add 5.0 ml ethanol; shake for 15 min.
11. filter each solution into 10 ml volumetric flask; rinse filter; dilute to 10.0 ml with EtOH.
12. prepare blank from plate
13. measure absorbance; calculate % glycyrrhizinic acid : **A1 x m2 x C/A2 x m1**

- **Rhatany root** : *Total polyphenols, Polyphenols not adsorbed by hide powder* as tannins with pyrogallol as Standard : **absorbance at 715 nm**

- **Rhubarb** : ≥ 2.2 % *Hydroxyanthracene derivatives* as rhein : **absorbance at 515 nm**

1. 0.100 g powder (m gram) + 30.0 ml water; mix and weigh; reflux for 15 min.
2. cool; add 50 mg $NaHCO_3$; weigh; adjust to original mass with water
3. centrifuge and transfer 10.0 ml liquid to reflux flask; add 20 ml ferric chloride solution; mix
4. reflux for 20 min., add 1 ml HCl, heat for further 20 min. shaking frequently; cool; transfer to separating funnel
5. shake with 3 x 25 ml ether, used to rinse the flask; combine ether extracts; wash with 2 x 15 ml water
6. filter ether extracts and dilute to 100.0 ml with ether
7. evaporate 10.0 ml to dryness; dissolve residue in 10.0 ml 0.5 % Mg-acetate in MeOH
8. measure absorption; calculate % rhein: **A x 0.64/ m (specific absorbance = 468)**

- **Senna leaf** : ≥ 2.5 % *Hydroxyanthracene glycosides* as sennoside B : **absorbance at 515 nm**

1. 0.150 g powder (m gram) + 30.0 ml water; mix and weigh; reflux for 15 min.
2. cool; weigh; adjust to original mass with water
3. centrifuge and transfer 20.0 ml supernatant liquid to separating funnel; add 0.1 ml dilute HCl; shake with 3 x 15 ml chloroform; discard chloroform layer;
4. add 0.10 g $NaHCO_3$; shake 3 min.;centrifuge; transfer 10.0 ml supernatant for reflux
5. add 20 ml ferric chloride solution; mix; reflux for 20 min.; add 1 ml HCl; heat for further 20 min.; shake to dissolve the precipitate
6. cool, transfer to separating funnel; shake with 3 x 25 ml ether, used to rinse the flask
7. combine ether layers; wash with 2 x 15 ml water; transfer ether layers and dilute to 100.0 ml with ether.
8. evaporate 10.0 ml ether to dryness; dissolve residue in 10.0 ml 0.5 % Mg-acetate in MeOH
9. measure absorbance; calculate % sennoside B : **A x 1.25/ m (specific absorbance = 240)**

- **Senna pods, Alexandrian** : *Hydroxyanthracene glycosides* as sennoside B : **absorbance at 515 nm**

1. cfr. Senna leaf

- **Senna pods, Tinnevelly** : *Hydroxyanthracene glycosides* as sennoside B : **absorbance at 515 nm**

1. cfr. Senna leaf

- **Thyme** : *Phenols* as Thymol : **absorbance at 450 nm** (cfr. Phenols and polyphenols)

THIN-LAYER CHROMATOGRAPHY *(2.2.27.)*

DETECTION IN ULTRAVIOLET LIGHT AT 254 nm :

- Anise oil : UV : anisaldehyde , anethol ; ***spray : vanillin reagent + heat*** : linalol, anethol, monoterpene hydrocarbons
- Aniseed : UV : anethol ; ***spray : phosphomolybdic acid + heat*** : anethol + triglycerides
- Cinnamon : 254 nm : cinnamaldehyde + eugenol ; 365 nm : o-methoxy-cinnamaldehyde ; ***spray : phloroglucinol solution*** : cinnamaldehyde +o-methoxy-cinnamaldehyde
- Clove : UV : eugenol + acetyleugenol; ***spray : anisaldehyde solution + heat*** : eugenol + acetyleugenol + caryophyllene
- Clove oil : UV : eugenol + acetyleugenol; ***spray : anisaldehyde solution + heat*** : eugenol + acetyleugenol + caryophyllene
- Devil's claw root : UV : harpagoside; ***spray : phloroglucinol solution + hydrochloric acid + heat*** : harpagoside
- Fennel bitter : UV : anethole; ***spray : sulphuric acid + heat*** : fenchone + anethole + terpenes
- Fennel sweet : UV : anethole; ***spray : sulphuric acid + heat*** : anethole + terpenes
- Gelatine : TEST : amino acid derivatives : derivatisation on the plate with ***dimethylaminonaphthalene-sulphonyl chloride + disodium tetraborate + dry at 60°C***; develop : 365 nm ; phenolic preservatives (ethyl-, methyl-, propyl parahydroxybenzoate, pentachlorophenol) : 254 nm
- Gentian root : UV : amarogentine; ***spray : fast red B salt; 10 min.*** : amarogentine; + ammonia vapour : amarogentine; TEST : other Gentiana species
- Lemon oil ***(no spray)***: UV 254 nm: citral + citropten + bergamotin + 5-genaryloxy-7-methoxycoumarin + psoralen derivative + biakangelicin; UV 365 nm : psoralen derivative, citropten, 5-genaryloxy-7-methoxycoumarin, bergamotin. TEST : adulterants : UV 254 nm : methyl anthranilate + methyl salicylate + chalcones ; UV 365 nm ; ***HCl-vapour + daylight***: chalcones + other adulterants
- Liquorice root : TESTS : UV : β-glycyrrhetic acid ; ***spray : anisaldehyde solution***; daylight: zones with Rf of 0.6 + β-glycyrrhetic acid; ASSAY: isolation by TLC of β-glycyrrhetic acid + absorbance at 250 nm
- Matricaria flower : UV : en-yne-dicycloether + matricin; ***spray : anisaldehyde solution + heat*** : bornyl acetate + matricin + bisabolol + en-yne-dicycloether + terpenes
- Peppermint leaf : UV : carvone + pulegone; ***spray : anisaldehyde solution + heat***; ***daylight*** : menthol + cineole + carvone + pulegone + isomenthone + menthyl acetate + menthone + hydrocarbons
- Peppermint oil : UV : carvone + pulegone; ***spray : anisaldehyde solution + heat***; ***daylight*** : menthol + cineole + carvone + pulegone + isomenthone + menthyl acetate + menthone + hydrocarbons + menthofuran
- Peru balsam : UV : benzyl benzoate + benzyl cinnamate; ***spray : phosphomolybdic acid + heat***; nerolidol ; no colophony
- Sterols in fatty oils : Separation of the sterol fraction from unsaponifiable matter *(2.4.23.)*: ***spray : dichlorofluorescein solution in ethanol + UV*** (before further GC analysis of isolated strerols)
- Shellac : TEST : colophony : UV ; ***spray : anisaldehyde solution + heat***
- Thyme : UV : thymol + quenching zones ; ***spray : anisaldehyde solution + heat*** : thymol + carvacrol + cineole + linalol + borneol

DETECTION IN ULTRAVIOLET LIGHT AT 365 nm :

- Aloes, barbados : ***spray : 10 % KOH in methanol + UV*** : barbaloin + aloesine ; ***heat*** : violet fluorescence zone
- Aloes, cape : ***spray : 10 % KOH in methanol, heat, UV*** : barbaloin + aloinosides A and B + aloesine; TEST : barbados aloes : no zone of violet fluorescence
- Aloes, dry extra extract standardised : ***spray : 10 % KOH in methanol,*** UV : barbaloin + aloesine + aloinosides A and B + violet fluorescence zone
- Cascara : ***spray : 5 % KOH in alcohol 50% + heat + UV*** : cascarosides (several zones with same fluorescence); TEST : other species of Rhamnus; anthrones: ***spray : 5 % KOH in alcohol 50% + heat + UV***: no zones of blue or orange-brown fluorescence; ***spray : 0.5 % nitrotetrazolium blue solution in methanol*** : no violet or greyish-blue zones
- Chamomille flower, roman : ***spray : solution of diphenylboric acid aminoethyl ester + macrogol 400 solution + stand for 30 min. + UV*** : apigenin + apigenin-7-glucoside + luteolin + apiin
- Cinchona bark : ***spray : anhydrous formic acid R + UV*** : quinine + quinidine ; ***spray : iodoplatinate reagent*** : quinine + quinidine + cinchonine + cinchonidine
- Cinnamon : 254 nm : cinnamaldehyde + eugenol ; 365 nm : o-methoxy-cinnamaldehyde ; ***spray : phloroglucinol solution*** : cinnamaldehyde +o-methoxy-cinnamaldehyde
- Digitalis leaf : ***spray : mixture of solution of chloramine + solution of trichloroacetic acid in alcohol + heat + UV*** : purpureaglycoside B + A + gitoxin + digitoxin
- Eucalyptus oil : ***spray : anisaldehyde solution + heat + UV*** : 1,8-cineole ; no citronellal
- Frangula bark : ***spray : 5 % KOH in alcohol 50% + heat + daylight*** : glucofrangulins + frangulins (several brownish-red zones); TEST : other species of Rhamnus; anthrones: ***spray : 5 % KOH in alcohol 50% + heat + UV*** : no yellow or blue fluorescence zones; ***spray : nitrotetrazolium blue solution in methanol*** : no violet or greyish-blue zones
- Gelatine : TEST : <u>amino acid derivatives</u> : derivatisation on the plate with dimethylaminonaphthalene-sulphonyl chloride + disodium tetraborate + dry at 60°C; develop ; 365 nm ; <u>phenolic preservatives</u> (ethyl-, methyl-, propyl parahydroxybenzoate, pentachlorophenol) : 254 nm
- Ipecacuanha root : ***spray : solution of iodine in alcohol + heat + daylight*** : emetine + cephaëline ; UV : intense yellow fluorescence : emetine +cephaëline
- Lemon oil *(no spray)* : UV 254 nm: citral + citropten + bergamotin + 5-genaryloxy-7-methoxycoumarin + psoralen derivative + biakangelicin; UV 365 nm : psoralen derivative, citropten, 5-genaryloxy-7-methoxycoumarin, bergamotin. TEST : adulterants : UV 254 nm : methyl anthranilate + methyl salicylate + chalcones ; UV 365 nm ; HCl-vapour : daylight: chalcones + other adulterants
- Lime flower : ***spray : solution of diphenylboric acid aminoethyl ester + macrogol 400 solution + stand for 30 min. + UV*** : rutine + hyperoside + different zones of fluorescence (pattern description)
- Rhubarb : UV : emodin + physcione + chrysophanol + rhein + aloe-emodin ; ***spray : 10 % KOH in methanol*** : zones become red to violet

- **Digitalis leaf** : ≥ 0.3 % *Cardenolic glycosides* as digitoxin : **absorbance at 540 nm**

1. shake 0.250 g powder with 50.0 ml water for 1 h.; add 5.0 ml 150 g/l Pb-acetate solution; shake; add after a few minutes 7.5 ml 4 % Na_2HPO_4 solution
2. filter; reflux 50.0 ml filtrate with 5 ml HCl (15 %) for 1 h.
3. transfer to separating funnel, rinse, shake with 3 x 25 ml chloroform
4. dry combined chloroform layers with anh. Na_2SO_4; dilute to 100.0 ml with chloroform
5. evaporate 40.0 ml to dryness; dissolve residue in 7 ml alcohol (50 %); add 2 ml dinitrobenzoic acid solution + 1 ml NaOH 1 M
6. prepare reference solution : 50.0 mg digitoxin CRS in 50.0 ml alcohol; dilute 5.0 ml to 50.0 ml; 5.0 ml dilution + 25 ml water + 3 ml HCl (15 %); reflux for 1 h.
7. transfer to separating funnel, rinse, shake with 3 x 25 ml chloroform
8. dry combined chloroform layers with anh. Na_2SO_4; dilute to 100.0 ml with chloroform
9. evaporate 40.0 ml to dryness; dissolve residue in 7 ml alcohol (50 %); add 2 ml dinitrobenzoic acid solution + 1 ml NaOH 1 M
10. measure absorbance of the 2 solutions during 12 min. until maximum
11. calculate content of cardenolic glycosides

- **Frangula bark** : ≥ 7.0 % *Glucofrangulins* as glucofrangulin A : **absorbance at 515 nm**;

1. weigh 0.250 g powder (m gram) + 25.0 ml 70 % methanol; mix; weigh again
2. reflux for 15 min; cool; weigh and adjust to first mass with 70 % methanol
3. filter; transfer 5.0 ml filtrate to separating funnel; + 50 ml water + 0.1 ml HCl;
4. shake with 5 x 20 ml light petroleum; transfer aqueous layer to volumetric flask
5. wash organic layers with 2 x 15 ml water; add water to aqueous layer in 100 ml volumetric flask
6. add 5 ml 5 % sodium carbonate; dilute to 100.0 ml with water; discard light petroleum;
7. 40.0 ml aqueous solution + 20 ml 20 % ferric chloride solution; reflux for 20 min.; add 2 ml HCl; reflux further for 20 min.; shake to dissolve precipitate; cool
8. transfer to separating funnel; shake with 3 x 25 ml ether; combine ether extracts; wash with 2 x 15 ml water
9. dilute ether layer to 100.0 ml; evaporate 20.0 ml to dryness;
10. dissolve residue in 10.0 ml 0.5 % Mg-acetate solution in MeOH
11. measure absorbance : **A x 3.06/ m (specific absorbance = 204)**

- **Hamamelis leaf** : *Total polyphenols, Polyphenols not adsorbed by hide powder* as tannins with pyrogallol as Standard : **absorbance at 715 nm** (cfr. Phenols and polyphenols)

- **Liquorice root** : ≥ 4.0 % glycyrrhizinic acid : **absorbance at 250 nm after preparative TLC**

1. test solution: 1.00 g powder (m1) + 25 ml 1 M HCl + 2.5 ml dioxan in 100 ml flask; reflux for 2 h.; cool; filter; discard filtrate
2. rinse flask and filter with 5 x 20 ml water; discard rinsings
3. dry flask and filter at 105 °C for 20 min
4. transfer filter to the flask; add 50 ml chloroform; reflux for 5 min.; filter warm chloroform solution; repeat extraction with 3 x 25 ml chloroform; filter warm chloroform solutions;
5. evaporate combined chloroformic extracts to dryness; transfer residue quantitavely with chloroform-methanol (1/1 vol.) to 10.0 ml flask; rinse beaker with chloroform, evaporate to 2 ml; add to 10.0 ml flask; dilute to 10.0 ml with chloroform-methanol (1/1 vol.)
6. reference solution: 50.0 mg glycyrrhizinic acid CRS (m2) of C % declared content + 25 ml HCl + 2.5 ml dioxan in 100 ml flask; reflux for 2 h.; cool; filter; discard filtrate

- **Cascara** : ≥ 8.0 % *Hydroxyanthracene glycosides of which ≤ 40 % other than cascarosides* as cascaroside A : **absorbance at 515 nm ; absorbance ratio at 515 nm to 440 nm > 2.4**

1. 1.00 g powder in 100 ml boiling water + stirring for 5 min.
2. cool, dilute to 100.0 ml with water, shake, filter, discard first 20 ml
3. 10.0 ml filtrate to separating funnel + 0.1 ml 1 M HCl; shake with 2 x 20 ml ether-hexane (1/3 vol)
4. wash organic layer with 5 ml water; discard organic layer; add rinsings to aqueous layer
5. shake aqueous layers with 4 x 30 ml ethylacetate; combine ethylacetate extracts
6. aqueous layer ⇒ assay of cascarosides
7. evaporate organic layer to dryness
8. dissolve residue in 0.3-0.5 ml MeOH; transfer to volumetric flask; rinse with warm water
9. cool; dilute to 50.0 ml with water; transfer 20.0 ml to round-bottomed flask with 2 g ferric chloride and 12 ml HCl.
10. reflux for 4 h; cool, transfer to separating funnel and rinse with 3-4 ml NaOH 1 M and 3-4 ml water; add rinsings to separating funnel
11. shake with 3 x 30 ml ether-hexane (1/3 vol); wash organic layers with 2 x 10 ml water; discard water
12. dilute organic phase to 100.0 ml ether-hexane (1/3 vol); evaporate 20.0 ml
13. dissolve residue in 10.0 ml 0.5 % Mg-acetate solution in MeOH
14. measure absorbance; calculate : **A x 6.95/ m (specific absorbance = 180)**

- **Cascara** : ≥ 8.0 % *Hydroxyanthracene glycosides of which ≥ 60.0 % Cascarosides,* as cascaroside A **: absorbance at 515 nm ; absorbance ratio at 515 nm to 440 nm > 2.7**

1. aqueous layer ⇒ assay of cascarosides : dilute to 50.0 ml with water
2. transfer 20.0 ml to round-bottomed flask with 2 g ferric chloride and 12 ml HCl.
3. reflux for 4 h; cool, transfer to separating funnel and rinse with 3-4 ml NaOH 1 M and 3-4 ml water; add rinsings to separating funnel
4. shake with 3 x 30 ml ether-hexane (1/3 vol); wash organic layers with 2 x 10 ml water; discard water
5. dilute organic phase to 100.0 ml ether-hexane (1/3 vol); evaporate 20.0 ml
6. dissolve residue in 10.0 ml 0.5 % Mg-acetate solution in MeOH
7. measure absorbance; calculate : **A x 6.95/ m (specific absorbance = 180)**

- **Cinchona bark** : ≥ 6.5 % *total alkaloids of which ≥ 30 % and ≤ 60 % as quinine-type alkaloids; relative content of Quinine-type alkaloids* : **absorbance at 316 and 348 nm**

1. 1.000 g powder (m gram) + 10 ml water + 7 ml dilute HCl; heat for 30 min.; cool; add 25 ml chloroform, 50 ml ether, 5 ml 20 % NaOH
2. shake for 30 min.; add 3 g tragacanth powder; shake until solution becomes clear
3. filter, rinse flask with 5 x 20 ml chloroform-ether (1/2 vol); combine filtrate and washings
4. evaporate to dryness; dissolve residue in 10.0 ml ethanol; evaporate 5.0 ml to dryness
5. dissolve residue in 0.1 M HCl and dilute to 1000.0 ml
6. prepare 2 reference solutions (30.0 mg quinine and 30.0 mg cinchonine) in 0.1 M HCl and dilute to 1000.0 ml
7. measure absorbances of the 3 solutions at 316 nm and 348 nm; cfr formulas

ABSORPTION SPECTROPHOTOMETRY, ULTRAVIOLET AND VISIBLE *(2.2.25.)*

ASSAY *(with reference to dried drug)*

- **Aloes dry extract, dry extract standardised** : ≥ 19.0 % and ≤ 21.0 % *Hydroxyanthracene derivatives*, as barbaloin : **absorbance at 512 nm**

1. 0.400 g powder (m) + 2 ml MeOH + 5 ml water(warm), mix,+ 75 ml water(60°C); shake 30 min
2. Cool, filter, rinse, filter, add rinsings and dilute to 1000.0 ml with water
3. 10.0 ml solution + 1 ml 60% ferric chloride solution + 6 ml HCl.; reflux for 4 h; cool
4. Cool, transfer to separating funnel (quantitav) + 4 ml NaOH 1 M; shake with 3 x 20 ml ethe:
5. wash ether layers with 2 x 10 ml water; discard washings; dilute to 100.0 ml with ether
6. evaporate 20.0 ml ether phase; dissolve residue in 10.0 ml 0.5 % Mg-acetate in MeOH
7. measure absorbance; calculate : **A x 19.6/ m (specific absorbance = 255)**

- **Aloes, barbados** : ≥ 28.0 % *Hydroxyanthracene derivatives*, as barbaloin : **absorbance at 512 nm**

1. 0.300 g powder (m) + 2 ml MeOH + 5 ml water(warm), mix,+ 75 ml water(60°C); shake 30 min
2. Cool, filter, rinse, filter, add rinsings and dilute to 1000.0 ml with water
3. 10.0 ml solution + 1 ml 60% ferric chloride solution + 6 ml HCl.; reflux for 4 h; cool
4. Cool, transfer to separating funnel (quantitav) + 4 ml NaOH 1 M; shake with 3 x 20 ml ether
5. wash ether layers with 2 x 10 ml water; discard washings; dilute to 100.0 ml with ether
6. evaporate 20.0 ml ether phase; dissolve residue in 10.0 ml 0.5 % Mg-acetate in MeOH
7. measure absorbance; calculate : **A x 19.6/ m (specific absorbance = 255)**

- **Aloes, cape** : ≥ 18.0 % *Hydroxyanthracene derivatives*, as barbaloin : **absorbance at 512 nm**

1. 0.400 g powder (m) + 2 ml MeOH + 5 ml water(warm), mix,+ 75 ml water(60°C); shake 30 min
2. Cool, filter, rinse, filter, add rinsings and dilute to 1000.0 ml with water
3. 10.0 ml solution + 1 ml 60% ferric chloride solution + 6 ml HCl.; reflux for 4 h; cool
4. Cool, transfer to separating funnel (quantitav) + 4 ml NaOH 1 M; shake with 3 x 20 ml ether
5. wash ether layers with 2 x 10 ml water; discard washings; dilute to 100.0 ml with ether
6. evaporate 20.0 ml ether phase; dissolve residue in 10.0 ml 0.5 % Mg-acetate in MeOH
7. measure absorbance; calculate : **A x 19.6/ m (specific absorbance = 255)**

- **Bearberry leaf** : ≥ 8.0 % *Hydroquinones* as anhydrous arbutin : **absorbance at 455 nm**

1. 0.400 g powder (m gram) + 50 ml water; reflux for 30 min.; cool; dilute to 250.0 ml
2. 5.0 ml solution in separating funnel + 45 ml water + 1.0 ml 2 % aminopyrazolone + 0.5 ml dilute NH_3 + 1.0 ml 8 % potassium ferricyanide solution; stand for 5 min.
3. shake with 25 ml methylene chloride; filter; repeat extraction with 3 x 25 methylene chloride
4. dilute to 100.0 ml with methylene chloride
5. measure absorbance; calculate : **A x 7.716/ m (specific absorbance = 648)**

GENERAL METHODS, APPLIED IN PHARMACOPOEIAL HERBAL MONOGRAPHS
(Ph. Eur. 1997 : 69 monographs)

CHEMICAL REFERENCE STANDARDS (CRS)

- Digitalis leaf : ***digitoxin CRS*** : ASSAY : cardenolic glycosides, expressed as digitoxin
- Hamamelis leaf : ***hide powder CRS*** : ASSAY : tannins
- Ipeca root : ***emetine.2HCl CRS + cephaëline.2HCl CRS*** : IDENTIFICATION : TLC
- Liquorice root : ***glycyrrhizinic acid CRS*** : ASSAY : TLC + absorbance at 250 nm
- Rhatany root : ***hide powder CRS*** : ASSAY : tannins
- Senna leaf + pots : ***Senna extract CRS*** : IDENTIFICATION : TLC

REFRACTIVE INDEX *(2.2.6.)*

- Anise oil : 1.552 to 1.561
- Clove oil : 1.528 to 1.537
- Eucalyptus oil : 1.458 to 1.470
- Lemon oil : 1.474 to 1.476 + TEST for foreign essential oils (destillate max. 0.003 less)
- Peppermint oil : 1.457 to 1.467
- Sesame oil : 1.472 to 1.476

OPTICAL ROTATION *(2.2.7.)*

Angle of optical rotation

- Clove oil : 0 to -2°
- Eucalyptus oil : 0 to +10°
- Lemon oil : +57° to 70° + TEST for foreign essential oils (destillate max. 6° less)
- Peppermint oil : -10° to -30°

VISCOSITY *(2.2.8. , 2.2.9. and 2.2.10.)*

- Guar gallactomannan : ***with rotating viscosimeter*** : > 75 % and < 140 % of labeled value

DROP POINT *(2.2.17.)*

- Beeswax, white : 61 to 65 °C
- Beeswaw, yellow : 61 to 65 °C
- Wool fat : 38 to 44 °C
- Wool fat, hydrous : 38 to 44° C

FREEZING POINT *(2.2.18.)*

- Anise oil : 15 to 19 °C

PROPOSAL FOR NEW GUIDANCE

"Fixed combinations of herbal medicinal products with long-term marketing experience"
Guidance to facilitate mutual recognition and use of biobliographic data

Note for Guidance concerning the application of section C.6 Part 4 of the Annex to Directive 91/507/EEC as amended, with a view to the submission of an application for a marketing authorisation for a well-established herbal medicinal product. This guideline should be read in conjunction with current EU guidelines.

1. JUSTIFICATION

1.1 Applicants will be required to justify the particular combination of active substances proposed. Fixed combination products will only be considered acceptable if the proposed combination is based on valid therapeutic principles.

1.2 For any individual fixed combination it is necessary to assess the potential advantages in the clinical situation against possible disadvantages, in order to determine whether the product meets the requirements of the standards and protocols with respect to efficacy and safety.

1.3

POTENTIAL ADVANTAGES OF FIXED COMBINATIONS INCLUDE ONE OF THE FOLLOWING:

a) an improvement of the benefit/risk assessment due to:

i. the addition or potentiation of therapeutic activities of their substances, which results in:

- a level of efficacy similar to the one achievable by each active substance used alone at higher doses than in combination, but associated with a better safety profile

or

- a level of efficacy above the one achievable by a single substance with an acceptable safety profile.

ii. the counteracting by one substance of an adverse reaction produced by another one.

b) a simplification of therapy

A fixed combination of active substances is acceptable if it achieves a similar level of efficacy to the one achievable by each active substance used alone at higher doses than in combination but improves patient compliance.

DISADVANTAGES OF FIXED COMBINATIONS INCLUDE:

i. the fact that even a combination which meets the needs of the average patient is unlikely to be ideally adjusted for the needs of each individual patient;

ii. the addition of the different adverse reactions specific to each substance.

1.3 General rules

Combinations, in principle, may not be considered rational if the duration of action of the substances differ significantly. This may not necessarily apply where it can be shown that the combination is clinically valid despite differences in this respect, e.g. if one substance is intended to enhance absorption of the other or where the substances are intended to exert their effects successively.

Each substance of the fixed combination must have documented contribution within the combination.

The inclusion of a substance to counteract an adverse reaction of another substance may be considered justified, but only if the adverse reaction is a serious or a commonly occurring one.

The inclusion of a substance intended to produce unpleasant adverse effects as a means of preventing abuse in undesirable.

4.2 Therapeutic data

The efficacy and the safety of use of the fixed combination must be evident from clinical trials or bibliographic data submitted by the applicant.

4.3 Composition and dosage regimen

The proposed dosage regimen must be justified.

The dosage of each active substance within the fixed combination must be such as the combination is safe and effective for a significant population subgroup and the benefit/risk assessment of the fixed combination is equal or exceeds the one of each of its active substances taken alone.

Where active substances are intended to relieve simultaneously different symptoms or to prevent different diseases, selected doses of each active substance are often those commonly used for the treatment of each symptom or the prevention of each disease.

5. COMBINATION PACKS

The principles applicable to fixed-combination products will also be applied in the assessment of preparations consisting of different medicinal products in combination packs where the products are intended for simultaneous or sequential administration.

- **NTA 2B, Part I C 1: Expert Report on Chemical, Pharmaceutical and Biological Documentation**

The requirements are found adequate for herbal medicinal products. Tabular formats specific to Herbal Medicinal Products are proposed (pages 5 to 27).

- **NTA 2B, Part II: Concerning Chemical, Pharmaceutical and Biological Documentation for Vegetable Medicinal Products.**

This part of the NTA is found not well adapted to herbal medicinal products. Many parts are unclear and need redrafting. Modifications are proposed and appear in bold italics (pages 28 to 32).

Pharmaceutical Expert Report

Format 2B102A/HERBAL

Name of Company : **Name of Finished Herbal Medicinal Product :** **Name of Active substance(s) :**	**Tabular format Referring to Part of the Dossier**	**(For National Aut Use only)**
PART II A: DOSAGE FORM - DEVELOPMENT PHARMACEUTICS		
Product Development Studies Summary[1] : Volume Page(s)		**(For National Au Use Only) COMM**
Explanation of choice of the composition : Volume Page(s)		
Explanation of optimisation of concentrations of the additives in the composition : Volume Page(s)		
Summary of studies on compatibility data with other products (if necessary) : Volume Page(s)		

[1] The phytochemical overview is included in Format 2B110A and 2B111A

Pharmaceutical Expert Report

Format 2B103A/HERBAL

Name of Company : Name of Finished Herbal Medicinal Product : Name of Active substance(s) :	Tabular format Referring to Part of the Dossier	(For National Authority Use only)
PART II A : DOSAGE FORM - DEVELOPMENT PHARMACEUTICS		
Summary of studies on compatibility with the container/ closure : **Volume Page(s)**		**(For National Authority Use only) COMMENTS**
Summary of in vivo bioavailability/bioequivalence studies : **Volume Page(s)**		
In vitro dissolution data on products used in the in vivo bioavailability studies : Volume Page(s)		

Pharmaceutical Expert Report

Format 2B104A/HERBAL

Name of Company : Name of Finished Herbal Medicinal Product : Name of Active substance(s) :	Tabular format Referring to Part of the Dossier	(For National Authority Use only)

PART II B : METHOD OF PREPARATION		
Manufacturing formula : Volume Page(s)		(For National Authority Use only) COMMENTS
Batch size : **Formula :**		
Manufacturing process (including in process control and assembly) : Volume Page(s)		

Pharmaceutical Expert Report

Format 2B105A/HERBAL

Name of Company : Name of Finished Herbal Medicinal Product : Name of Active substance(s) :	Tabular format Referring to Part of the Dossier	(For National Authority Use only)
PART II B : METHOD OF PREPARATION - PROCESS VALIDATION		
Summary of experimental studies : Volume Page(s)		**(For National Authority Use only) COMMENTS**

Name of Company : **Name of Finished Herbal Medicinal Product :** **Name of Active substance(s) :**	**Tabular format Referring to Part of the Dossier**	**(For National Authority Use only)**

PART II C : CONTROL OF STARTING MATERIALS - ACTIVE SUBSTANCE(S) : HERBAL DRUG PREPARATION[2]

Specifications and routine tests : **Volume** **Page(s)**	**(For National Authority Use only) COMMENTS**
Definition of a production batch : **Volume** **Page(s)**	
(a) Herbal drug preparation(s) described in a pharmacopoeia[1] : (b) Herbal drug preparation(s) not described in a pharmacopoeia :	
Summary of specifications and Routine Tests - Characteristics : **Volume** **Page(s)**	
Identification tests : **Volume** **Page(s)**	
Purity tests : **Volume** **Page(s)**	
Potential contamination: Loss on drying/Dry residue: Residual solvents: Relative density:	
Other tests : **Volume** **Page(s)**	
Assay/Other evaluation of potency : **Volume** **Page(s)**	
Constituents with known therapeutic activity: or Markers: or other justified determination: Compliance with Pharmacopoeia[1]:..... ☐	

[1] Reference to the Pharmacopoeia used should be given; see CD 75/318 Annex Part 2 C.1.1

[2] A monograph or comprehensive specification of the herbal drug has to be presented (see Format 2B106Aii)

Pharmaceutical Expert Report

Format 2B106Aii/HERBAL

Name of Company : **Name of Finished Herbal Medicinal Product :** **Name of Active substance(s) :**	**Tabular format Referring to Part of the Dossier**	**(For National Authority Use only)**

PART II C : CONTROL OF STARTING MATERIALS - ACTIVE SUBSTANCE(S) : HERBAL DRUG	
Specifications and routine tests : Volume Page(s)	**(For National Authority Use only) COMMENTS**
Definition of a production batch : Volume Page(s)	
(a) Herbal drug(s) described in a pharmacopoeia[1] : (b) Herbal drug(s) not described in a pharmacopoeia :	
Summary of specifications and Routine Tests - Characteristics : Volume Page(s)	
Identification tests : Volume Page(s)	
Purity tests : Volume Page(s)	
Foreign matter: Pharmacopoeia[1].....☐ Loss on drying/Water: Pharmacopoeia[1].....☐ Total ash/Ash insoluble in hydrochloric acid: Pharmacopoeia[1].....☐ Microbial contamination: Pharmacopoeia[1].....☐ Other potential contamination:	
Other tests : Volume Page(s)	
Assay/Other evaluation of potency : Volume Page(s)	
Constituents with known therapeutic activity: or Markers: or other justified quantitative determination: Compliance with Pharmacopoeia[1]:..... ☐	

[1] Reference to the Pharmacopoeia used should be given; see CD 75/318 Annex Part 2 C.1.1

Pharmaceutical Expert Report

Format 2B107A/HERBAL

Name of Company : **Name of Finished Herbal Medicinal Product :** **Name of Active substance(s) :**	**Tabular format Referring to Part of the Dossier**	**(For National Authority Use only)**

PART II C : CONTROL OF STARTING MATERIALS - ACTIVE SUBSTANCE(S): HERBAL DRUG/HERBAL DRUG PREPARATION - SCIENTIFIC DATA

1. Nomenclature : **Volume** **Page(s)**	**(For National Authority Use only) COMMENTS**
Scientific name of the plant, with the name of the authority, variety and chemotype : Parts of the plant : Definition of herbal drug/herbal drug preparation : Quantity or ratio of the herbal drug to the herbal drug preparation : Extraction solvent(s) : Other name : Laboratory code :	

2. Description : **Volume** **Page(s)**	
Physical form :	
Constituents with known therapeutic activity:	Justification of the choice of markers:
Other substance(s):	

Pharmaceutical Expert Report

Format 2B108A/HERBAL

Name of Company : **Name of Finished Herbal Medicinal Product :** **Name of Active substance(s) :**	**Tabular format Referring to Part of the Dossier**	**(For National Authority Use Only)**

PART II C : CONTROL OF STARTING MATERIALS - ACTIVE SUBSTANCE(S): HERBAL DRUG/HERBAL DRUG PREPARATION - SCIENTIFIC DATA		
3. Manufacture :	**Volume** **Page(s)**	**(For National Authority Use Only) COMMENTS**
Plant Production :	**Volume** **Page(s)**	
Geographical source : Name(s) and address(es) of the grower(s) and/or the supplier(s) of the drug : Cultivation/Collection from wild sources : Pre-harvest chemical treatment : Harvest : Drying : Cutting and milling : Other processes :		
Name(s) and address(es) of the manufacturer of the preparation :	**Volume** **Page(s)**	
Manufacturing route :	**Volume** **Page(s)**	

Name of Company : Name of Finished Herbal Medicinal Product : Name of Active substance(s) :	Tabular format Referring to Part of the Dossier	(For National Authority Use only)

PART II C : CONTROL OF STARTING MATERIALS - ACTIVE SUBSTANCE(S) : HERBAL DRUG/HERBAL DRUG PREPARATION - SCIENTIFIC DATA

Description of process : Volume Page(s)	(For National Authority Use Only) COMMENTS
Extraction solvents, reagents : Volume Page(s)	
Purification stages : Volume Page(s)	
Standardisation (adjusted to a defined content of constituents with known therapeutic activity): Volume Page(s)	
Range of the inert material or herbal drug preparation added:.....-.....%	
4. Quality control during manufacture : Volume Page(s)	
Starting materials : Volume Page(s)	
Control tests on intermediate products : Volume Page(s)	

Pharmaceutical Expert Report

Format 2B110A/HERBAL

Name of Company : **Name of Finished Herbal Medicinal Product :** **Name of Active substance(s) :**	**Tabular format Referring to Part of the Dossier**	**(For National Authority Use only)**

PART II C : CONTROL OF STARTING MATERIALS - ACTIVE SUBSTANCE(S) : (DEVELOPMENT) HERBAL DRUG - SCIENTIFIC DATA	
Description of the herbal drug: **Volume** **Page(s)**	**(For National Authority Use Only) COMMENTS**
Macroscopic description: Pharmacopoeia[1] ..... ☐ Microscopic description: Pharmacopoeia[1] ..... ☐ Additional tests: (e.g. thin-layer chromatography): Pharmacopoeia[1] ..... ☐	
Composition and analytical research for constituents and physical characteristics: **Volume** **Page(s)**	
Tests for adulterants and for known toxic constituents: **Volume** **Page(s)**	
Summary of Analytical Development and Validation Studies: **Volume** **Page(s)**	

[1] Reference to the Pharmacopoeia used should be given; see CD 75/318 Annex Part 2 C.1.1

Name of Company : Name of Finished Herbal Medicinal Product : Name of Active substance(s) :	Tabular format Referring to Part of the Dossier	(For National Authority Use only)

PART II C : CONTROL OF STARTING MATERIALS - ACTIVE SUBSTANCE(S) : (DEVELOPMENT) HERBAL DRUG PREPARATION - SCIENTIFIC DATA

Herbal drug preparations such as extracts	(For National Authority Use Only) COMMENTS
Analytical chemical profile: Volume Page(s)	
Detection of known toxic constituents/adulterants: Volume Page(s)	
Summary of Analytical Development and Validation Studies: Volume Page(s)	

Pharmaceutical Expert Report

Format 2B112A/HERBAL

Name of Company : **Name of Finished Medicinal Product :** **Name of Active substance(s) :**	**Tabular format Referring to Part of the Dossier**	**(For National Authority Use only)**

PART II C : CONTROL OF STARTING MATERIALS - ACTIVE SUBSTANCE(S) : (IMPURITIES) HERBAL DRUG/HERBAL DRUG PREPARATION - SCIENTIFIC DATA	
Potential impurities originating from cultivation harvesting/collection, storage: Page(s) :	**Test procedures and their limits of detection or limits of quantitation :**
Microbial contamination:	Pharmacopoeia[1] ..... ☐
Pesticide residues:	Pharmacopoeia[1] ..... ☐
Heavy metals/other toxic elements:	Pharmacopoeia[1] ..... ☐
Mycotoxins:	Pharmacopoeia[1] ..... ☐
Fumigation agents:	
Radioactivity:	
Potential impurities arising during the production and purification : Page(s) :	**Test procedures and their limits of detection or limits of quantitation :**
Residual solvents:	Pharmacopoeia[1] ..... ☐
Other substance(s):	
Potential impurities/contaminants in herbal drug preparations :	**Test procedures and their limits of detection or limits of quantitation :**
Microbiological quality :	Pharmacopoeia[1] ..... ☐
Other Substance(s) :	
Potential substitution and adulterants:	**Test procedures and their limits of detection or limits of quantitation :**

COMMENTS : (For National Authorities Use Only)

[1] Reference to the Pharmacopoeia used should be given; see CD 75/318 Annex Part 2 C.1.1

Pharmaceutical Expert Report

Format 2B113Ai/HERBAL

Name of Company : **Name of Finished Herbal Medicinal Product :** **Name of Active substance(s) :**	**Tabular format Referring to Part of the Dossier**	**(For National Authority Use only)**

PART II C : CONTROL OF STARTING MATERIALS - ACTIVE SUBSTANCE(S) : (BATCH ANALYSIS) - HERBAL DRUG PREPARATION[1]

Batches tested :	**Volume**	**Page(s)**	**(For National Authority Use Only) COMMENTS**
Date(s) of manufacture : Place(s) of manufacture : Batch size : Batch (Lot) number : Use of batches (incl.: preclinical & clinical testing) :			
Results of tests :	**Volume**	**Page(s)**	
Batch Nos :			
Characteristics : Identification tests : Purity tests : Potential contaminants : Other tests : Assay(s) / potency :			
Reference standard (analytical results) :	**Volume**	**Page(s)**	
Origin/supplier Characteristics : Identification tests : Purity tests : Potential contaminants : Other tests : Assay(s) / potency :			

[1] The batch analysis of the herbal drug has to be presented (see Format 2B113Aii)

Pharmaceutical Expert Report

Format 2B113Aii/HERBAL

Name of Company : **Name of Finished Herbal Medicinal Product :** **Name of Active substance(s) :**	**Tabular format Referring to Part of the Dossier**	**(For National Authority Use only)**

PART II C : CONTROL OF STARTING MATERIALS - ACTIVE SUBSTANCE(S): (BATCH ANALYSIS) - HERBAL DRUG

Batches tested :	**Volume**	**Page(s)**	**(For National Authority Use Only) COMMENTS**
Date(s) of manufacture : Place(s) of manufacture : Batch size : Batch (Lot) number : Use of batches (incl. : preclinical & clinical testing)			
Results of tests :	**Volume**	**Page(s)**	
Batch Nos :			
Characteristics : Identification tests : Purity tests : Potential contaminants : Other tests : Assay(s) / potency :			
Reference standard (analytical results) :	**Volume**	**Page(s)**	
Origin/supplier : Characteristics : Identification tests : Purity tests : Potential contaminants : Other tests : Assay(s) / potency :			

Name of Company : **Name of Finished Herbal Medicinal Product :** **Name of Active substance(s) :**	**Tabular format Referring to Part of the Dossier**	**(For National Authority Use only)**

PART II C : CONTROL OF STARTING MATERIALS - EXCIPIENT(S) : SPECIFICATIONS AND ROUTINE TESTS

1. Excipient(s) described in a pharmacopoeia[1]: **Volume Page(s)**	**(For National Authority Use Only) COMMENTS**

2. Excipient(s) not described in a pharmacopoeia : **Volume Page(s)**	
Characteristics : Identification tests : Purity tests : - Physical - Chemical - Biological / Immunological Other tests : Assay(s) and/or evaluations:	

[1] Reference to the Pharmacopoeia used should be given; see CD 75/318 Annex Part 2 C.1.1

Pharmaceutical Expert Report

Format 2B115A/HERBAL

Name of Company : **Name of Finished Herbal Medicinal Product :** **Name of Active substance(s) :**	**Tabular format Referring to Part of the Dossier**	**(For National Authority Use only)**
PART II C : CONTROL OF STARTING MATERIALS - EXCIPIENT(S) : SCIENTIFIC DATA		
Summary of studies : Volume Page(s)		**(For National Authority Use Only) COMMENTS**

Name of Company : **Name of Finished Herbal Medicinal Product :** **Name of Active substance(s) :**	**Tabular format Referring to Part of the Dossier**	**(For National Authority Use only)**

PART II C : CONTROL OF STARTING MATERIALS - PACKAGING MATERIAL (IMMEDIATE PACKAGING)

Specifications and routine tests : **Volume** **Page(s)**	**(For National Authority Use Only) COMMENTS**
Type of material : Construction : Quality specifications (routine tests and summary of control methods) :	
Scientific data : **Volume** **Page(s)**	
Summary of development studies on packaging materials : Batch analysis (analytical results) :	

Pharmaceutical Expert Report

Format 2B117A/HERBAL

Name of Company : **Name of Finished Herbal Medicinal Product :** **Name of Active substance(s) :**	**Tabular format Referring to Part of the Dossier**	**(For National Authority Use only)**
PART II D : CONTROL TESTS ON INTERMEDIATE PRODUCTS **Volume Page(s)**		**(For National Authority Use Only) COMMENTS**
PART II E : CONTROL TESTS ON THE FINISHED PRODUCT : **Volume Page(s)**		
Product specifications and tests for release at time of manufacture :		
General product characteristics : Identification tests : Quantitative determination of constituents with known therapeutic activity or of markers : Purity tests : Pharmaceutical tests :		
Identification and determination of excipient(s) : Volume Page(s)		
Approved colouring materials : Determination of antimicrobial or chemical preservatives: Other additives :		

Name of Company : **Name of Finished Herbal Medicinal Product :** **Name of Active substance(s) :**	**Tabular format Referring to Part of the Dossier**	**(For National Authority Use only)**

PART II E : CONTROL TESTS ON THE FINISHED PRODUCT - SCIENTIFIC DATA

Summary of analytical development and validation studies : **Volume** **Page(s)**	**(For National Authority Use Only) COMMENTS**
Batch Analysis: **Volume** **Page(s)**	
Batches tested : Batch (Lot) number : Date(s) of manufacture : Place(s) of manufacture : Batch size : Use of batches :	
Results of Batch Analysis : **Volume** **Page(s)**	
Batch Nos :	
Tests :	
Reference standard : **Volume** **Page(s)**	

Pharmaceutical Expert Report

Format 2B119A/HERBAL

Name of Company : **Name of Finished Herbal Medicinal Product :** **Name of Active substance(s) :**	**Tabular format Referring to Part of the Dossier**	**(For National Authority Use only)**

PART II F : STABILITY - STABILITY TESTS ON ACTIVE SUBSTANCE(S)
Batches tested : **Volume** **Page(s)**
Batch Nos :
General test methodology : **Volume** **Page(s)**
Accelerated test conditions (temperature ° C, % RH, light) : Normal test conditions (temperature ° C, % RH, light) :
Analytical test procedures : **Volume** **Page(s)**
Assay techniques and validation : Determination of degradation products/chromatographic profile :
Validation of all test procedures including limits of detection (including initial results): **Volume** **Page(s)**
Results of tests : **Volume** **Page(s)**
Proposed storage conditions and Duration of Storage to be permitted before re-testing : **Volume** **Page(s)**
COMMENTS (For National Authorities Use Only)

Name of Company : **Name of Finished Herbal Medicinal Product :** **Name of Active substance(s) :**	**Tabular format Referring to Part of the Dossier**	**(For National Authority Use only)**

PART II F : STABILITY - STABILITY TESTS ON THE FINISHED PRODUCT

Batches tested and packaging used :	**Volume**	**Page(s)**
Stability study methods :	**Volume**	**Page(s)**
Real time studies (temperature °C, % RH, light) : Studies under other conditions :		
Characteristics studied :	**Volume**	**Page(s)**
Physical characteristics : Microbiological characteristics : Chemical characteristics : Chromatographic characteristics : Packaging characteristics :		
Evaluation methods and validation :	**Volume**	**Page(s)**
Results of tests :	**Volume**	**Page(s)**
Interpretation of results :	**Volume**	**Page(s)**
Proposed shelf-life and storage conditions (incl. after reconstitution or after first opening if appropriate) :	**Volume**	**Page(s)**
Ongoing stability studies :	**Volume**	**Page(s)**
COMMENTS : (For National Authorities Use Only)		

Pharmaceutical Expert Report

Format 2B121A/HERBAL

Name of Company : **Name of Finished Herbal Medicinal Product :** **Name of Active substance(s) :**	**Tabular format Referring to Part of the Dossier**	**(For National Authority Use only)**

PART II G : BIOAVAILABILITY/BIOEQUIVALENCE	**Volume**	**Page(s)**
Give reference to relevant sections in Part IV.		

PART II Q : OTHER INFORMATION

Summary of analytical test procedures used in metabolism and bioavailability studies, and validation studies :	**Volume**	**Page(s)**

Summary of synthesis of radiolabelled active substance(s) used in metabolic and/or pharmacokinetic studies :	**Volume**	**Page(s)**

COMMENTS (For National Authority Use Only)

Part II: Concerning Chemical, Pharmaceutical and Biological Documentation for *Herbal* Medicinal Products

The principle of GMP and the detailed guidelines are applicable to all operations, which require the authorisation referred to in **Article 16 of Directive 75/319/EEC** as modified. They are also relevant for all other large scale pharmaceutical manufacturing processes, such as that undertaken in hospitals, for the preparations of products for use in clinical trials, and for wholesaling, where applicable.

All analytical test procedures described in the various sections of the Part II chemical, pharmaceutical and biological documentation must be described in sufficient detail to enable the procedures to be repeated if necessary (e.g. by an official laboratory). All procedures need to be validated and the results of the validation studies must be provided.

PART II A: COMPOSITION

1. COMPOSITION OF THE MEDICINAL PRODUCT

NAMES OF INGREDIENTS	UNIT AND/OR PERCENTAGE FORMULA	FUNCTION	REFERENCE TO STANDARDS
Active substance(s)			
Excipient(s)			

2. CONTAINER (BRIEF DESCRIPTION)

Nature of container materials; qualitative composition; method of closure; method of opening.

3. CLINICAL TRIAL FORMULA(E)

4. *DOSAGE FORM* - DEVELOPMENT PHARMACEUTICS

Explanation with regard to the choice of formulation, composition, ingredients and container, supported, if necessary, by data on development pharmaceutics. The overage, with justification thereof, should be stated. Tests carried out during pharmaceutical development must be described in detail, e. g. in vitro dissolution studies for solid pharmaceutical forms.

PART II B: METHOD OF PREPARATION

1. **MANUFACTURING FORMULA** (including details of batch size)

2. **MANUFACTURING PROCESS** (including in-process control and the pharmaceutical assembly process)

If ***herbal drug*** preparations are the starting material, the description of their manufacturing process and their control belong to section C.
A flow-chart of the manufacturing process should be given.

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E_Mail: mail@emea.eudra.org http://www.eudra.org/emea.html

3. VALIDATION OF THE PROCESS

Validation of the process should be carried out when a non-standard method of manufacture is used or for steps of the manufacturing process which are critical for the product described in the finished product specifications (experimental data showing that the manufacturing process, using materials of the stated quality and the types of manufacturing equipment specified, is a suitable one and will consistently yield a product of the desired quality).

PART II C: CONTROL OF STARTING MATERIALS

1. ACTIVE SUBSTANCE(S)

1.1. Specifications and routine tests

*1.1.0 **Definition of a production batch***

1.1.1 Active substance(s) described in a pharmacopoeia
For herbal drug preparations additional specifications as set out in 1.1.2 may be required.

1.1.2 Active substance(s) not described in a pharmacopoeia

- Characteristics
- Identification tests
- ***Purity tests***
  - * Potential contamination by micro-organisms, products of micro-organisms, pesticides, toxic metals, radioactivity, fumigants, etc.
  - * Physical
  - * Chemical
- Other tests
- Assay(s) of ***constituents*** with known therapeutic activity ***or of markers***.

In the case of a herbal drug preparation, a monograph on the herbal drug has to be presented.

1.2. Scientific Data

1.2.1 Nomenclature

- Scientific name of plant, with the name of the authority, variety and chemotype
- ***Parts of the plant***
- ***Definition of the herbal drug/herbal drug preparation***
- ***Quantity or ratio of the herbal drug to the herbal drug preparation***
- ***Extraction solvent(s)***
- Other name
- Laboratory code

1.2.2 Description

- Physical form
- ***Constituents with known therapeutic activity or markers***
  - • Structural formula (including conformational data for macromolecules)
  - • Molecular formula
  - • Relative molecular mass
  - • Chirality
- ***Other substance(s)***

1.2.3 Manufacture

- ***Plant production***
- Geographical source of active substance ***of herbal origin***
- Name(s) and address(es) of the ***manufacturer of the preparation***

- ***Manufacturing route***
- Description of process
- Solvents, reagents
- Purification stages
- ***Standardisation***

1.2.4 Quality control during manufacture

- Starting materials
- Control tests on intermediate products (where appropriate)

1.2.5 Development (for active substance(s) of ***herbal*** origin)

1.2.5.1 ***Herbal drugs, herbal drug preparations such as powders***

- Description of the ***herbal drug or of the herbal drug preparation***
  - macroscopic
  - microscopic
  - ***Additional tests***
- Composition and analytical research for ***constituents*** and physical characteristics
- ***Tests for adulterants and for known toxic constituents***
- ***Summary of*** analytical development and validation ***studies***, commentary on the choice of routine tests and specifications

1.2.5.2 ***Herbal drug preparations such as extracts***

- Analytical chemical profile (qualitative and quantitative)
- Detection of ***known*** toxic ***constituents (e.g. pyrrolizidine alkaloids, ginkgolic acids, estragole, thujone, ...)*** /adulterants
- ***Summary of*** analytical development and validation ***studies***, commentary on the choice of routine tests and specifications.

1.2.6 Impurities

- Potential impurities originating from the ***cultivation, harvesting/collection, storage***
- Potential impurities arising during the production and purification, ***residual solvents***
- Methods detecting potential contamination of the active substance(s) ***of herbal origin*** by micro-organisms and products of micro-organisms, pesticides, fumigation agents, toxic metals, radioactivity etc.
- Potential ***substitution and adulterants***

1.2.7 Batch analysis

- Batches tested (date of manufacture, place of manufacture, batch size, and use of batches including batches used in preclinical and clinical testing)
- Results of tests
- Reference ***standard*** (analytical results), primary and others

2. EXCIPIENT(S)

2.1. Specifications and routine tests

2.1.1 Excipient(s) described in a pharmacopoeia

2.1.2 Excipient(s) not described in a pharmacopoeia

- Characteristics
- Identification tests
- Purity tests (including limits for named, total, other single, unidentified single and unidentified total impurities)
  - physical
  - chemical
  - ***biological/immunological***
- Other tests

- Assay(s) and/or evaluations (where necessary)

2.2. Scientific data

Data, where necessary, for example on excipient(s) used for the first time in medicinal products (see II C.1.2).

3. PACKAGING MATERIAL (IMMEDIATE PACKAGING)

3.1 Specifications and routine tests

- Type of material
- Construction
- Quality specifications (routine tests and ***summary of control methods***)

3.2 Scientific data

- Development studies on packaging materials
- Batch analysis, analytical results

PART II D: CONTROL TESTS ON INTERMEDIATE PRODUCTS (IF NECESSARY)

A distinction should be made between in-process controls (Part II B) and control tests on intermediate products.

PART II E: CONTROL TESTS ON THE FINISHED PRODUCT

1. SPECIFICATIONS AND ROUTINE TESTS

1.1 Product specifications and tests for release at time of manufacture (general characteristics, specific standards)

1.2 Control Methods

1.2.1 Test procedures for identification and quantitative determination ***of constituents with known therapeutic activity or of markers.***

It must be described in detail (including biological and micro-biological methods where relevant), together with other tests which include those in the appropriate general monograph for the type of dosage form in the European Pharmacopoeia:

- Identification tests
- Quantitative determination of active substance(s); and additionally for ***herbal drugs*** and ***herbal drug preparations***, quantitative determination of ***constituents*** with known therapeutic activity ***or of markers***
- Purity tests
- Pharmaceutical tests (e.g. dissolution)

1.2.2 Identification and determination of excipient(s)

- Identification tests for approved colouring materials
- Determination of antimicrobial or chemical preservatives (with limits)
- ***Other additives***

2. SCIENTIFIC DATA

2.1 Analytical validation of methods and comments on the choice of routine tests and standards (e.g. working standards)

2.2 Batch analysis

- Batches tested (date of manufacture, place of manufacture, batch size and use of batches)
- Results of ***batch analysis***
- Reference ***standard*** (analytical results), primary and others

PART II F: STABILITY

1. STABILITY TESTS ON ACTIVE SUBSTANCE(S)

- Batches tested
- General test methodology
  - accelerated test conditions
  - normal test conditions
- Analytical test procedures
  - assay ***techniques and validation***
  - determination of degradation products/***chromatographic profile***
- Validation of all test procedures including limits of detection (including initial results)
- Results of tests
- Conclusions

Data on stability tests on active substances may not be required if justified by the applicant, provided that the finished product is manufactured immediately after production of the active substance(s).

2. STABILITY TESTS ON THE FINISHED PRODUCT

- Quality specification for the proposed shelf-life
- Batches tested and packaging
- ***Stability*** study methods
  - real time studies
  - studies under other conditions
- Characteristics studied
  - physical characteristics
  - microbiological characteristics
  - chemical characteristics
  - chromatographic characteristics
  - characteristics of the packaging (container/closure interaction with the product)
- Evaluation test procedures
  - description of test procedures
  - validation of test procedures
- Results of tests (including initials and reference to degradation products)
- Conclusions
  - shelf-life and storage conditions
  - shelf-life after reconstitution or first opening of the product
- Ongoing stability studies

PART II G: BIOAVAILABILITY/ BIOEQUIVALENCE

Give reference to relevant sections in Part IV.

PART II Q: OTHER INFORMATION

This part is intended for information not covered by any of the previous parts, e.g. the analytical tests used for the pharmaceutical development of the product, studies concerning metabolism and bioavailability, etc.

QUALITY AND VALIDATION

QUALITY

*DEFINITIONS

*AIM IMPLEMENTATION QUALITY SYSTEM

*GUIDELINES :
- GLP
- EN45001
- ISO

VALIDATION

*WHICH VALIDATION PARAMETERS HAVE TO BE VALIDATED ?

*LINEARITY

*PRECISION

*ACCURACY

*OTHER PARAMETERS

QUALITY

1 DEFINITIONS

Quality : How well products, processes or services fit to the requirements for their intended use

Quality System : Laboratory activities aimed at producing accurate work and a high-quality work product

Quality Control : Planned activities designed to provide a quality product

Quality Assurance : Planned activities designed to ensure that the quality control activities are being properly implemented

Good Laboratory Practices : Official rules and operating procedures that are considered to be minimum requirements for the promotion of quality and integrity of the work product

Laboratory accreditation : Formal recognition of a laboratory by an independent science-based organization that the laboratory is competent to perform specific tests

2 AIM of the IMPLEMENTATION of a QUALITY SYSTEM

**improvement of the laboratory activities* : rationalisation, standardisation and uniformisation of the activities of the laboratory by means of STANDARD OPERATING PROCEDURES (SOP's)

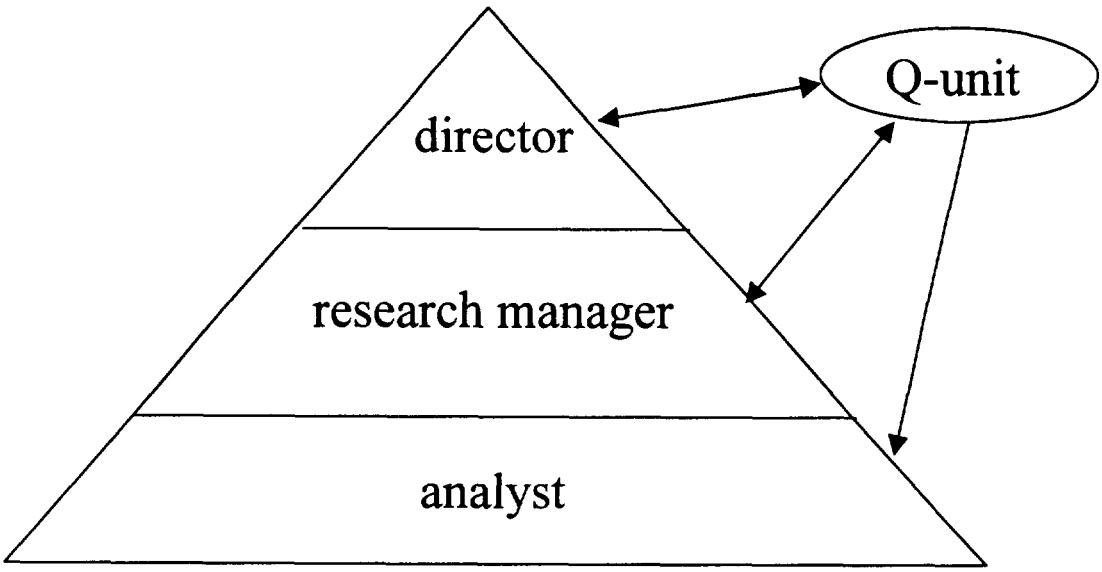
**higher confidentiality of the results (trust)*

3 GUIDELINES

3.1 GLP : principles of good laboratory practice

* Personnel
  - levels : director – research manager – analyst
  - function / job discription
  - training (initial – permanent)

* Quality assurance programm
  - QA-manager :
  - independent !
  - control of :
    - work done according to SOP's
    - reports
  - internal audits



* Equipment / material / laboratory space
    enough
    high quality
    fit for their intended use
    maintenance
    storage
    ...

* Procedures
    SOP's

* Research-analysis –plan
    discription of research – analysis
    personnel involved
    methods
    reports

* Reports
    raw data ⟶ final report

* Archives
    samples
    plan
    raw data
    final report
    ...

3.2 EN45001 – BELTEST – ACCREDITATION

« ROUTINE WORK »

QUALITY SYSTEM

QUALITY MANUAL content

* declaration of the director

* structure of the laboratory

* function discription

* general procedures :
  - treatement of the request of analysis
  - price-time table
  - order
  - samples
  - equipment
    - acchievement, maintenance
    - documentation file
    - calibration
  - calibration – verification
  - methods of analysis
    - Pharmacopeia
    - validation
  - reports
  - deviations from SOP's

* evaluation of the lab
    interlaboratory testing (e.g. Proficiency Test Scheme of the Council of Europe)

* non-complience – corrective action

* complaints
    personnel
    clients

3.3 International Organisation for Standardisation (ISO)

* GENERAL guidelines

* need to be ADAPTED to the different fields of work

* DEMANDS of custommer ➡ quality supplier ↑

ISO 9000 (1994) : Quality management and quality assurance standards

ISO 9001 (1994) : Quality Systems – Model for quality assurance in design, development, production, installation and servicing

ISO 9002 (1994) : Quality Systems – Model for quality assurance in production, installation and servicing

ISO 9003 (1994) : Quality Systems – Model for quality assurance in final inspection and test

ISO 9004 (1994) : Quality management and quality system elements

Validation of analytical methods

1. Which validation characteristics have to be validated?

According to ICH (International Conference of Harmonisation) guideline:

Type of analytical procedure / **Characteristics**	**IDENTIFI -CATION**	**TESTING FOR IMPURITIES**		**ASSAY** dissolution (measurement only) content/potency
		quantitative	limit	
Accuracy	-	+	-	+
Precision				
Repeatability	-	+	-	+
Interm.	-	+ (1)	-	+ (1)
precision	+	+	+	+
Specificity (2)	-	- (3)	+	*
Detection limit	-	+	-	*
Quantitation	-	+	-	+
limit	-	+	-	+
Linearity				
Range				

- : signifies that this characteristic is normally not evaluated
+ : signifies that this characteristic is normally evaluated
(1) in cases where reproducibility has been performed, intermediate precision is not needed
(2) lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s)
(3) may be needed in some cases

2. LINEARITY

2.1 DEFINITION

The **linearity** of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of the analyte in the sample.

The **range** is the interval between the lower and the upper analyte concentration for which it has been demonstrated that the analytical procedure has a suitable level of accuracy, precision and linearity.

2.2 EXPERIMENTAL DESIGN / CALCULATIONS

At least 5 standards are injected in duplo. (50 to 200% of the expected amount)

⇨ Correlation coefficient

$$r = \frac{\sum_i [(x_i - \bar{x}).(y_i - \bar{y})]}{\sqrt{\left[\sum_i (y_i - \bar{y})^2\right]\left[\sum_i (x_i - \bar{x})^2\right]}}$$

⇨ Least squares line: linear regression

$$b = \frac{\sum_i [(x_i - \bar{x}).(y_i - \bar{y})]}{\sqrt{\sum_i (x_i - \bar{x})^2}}$$

$$a = \bar{y} - b.\bar{x}$$

$$s_{y/x} = \sqrt{\left[\frac{\sum_i (yi - \hat{yi})^2}{n-2}\right]}$$

$$s_b = \frac{s_{y/x}}{\sqrt{\sum_i (x_i - \bar{x})^2}}$$

$$s_a = s_{y/x} \cdot \sqrt{\frac{\sum_i x_i^2}{n.\sum_i (x_i - \bar{x})^2}}$$

t-test to compare the intercept with (0,0)

In this test we check whether or not (0,0) is included in the 95% confidence interval on the intercept.{50}

$H_o : \alpha = 0$

$t = |a| / s_a < t_{(\alpha;N-2)}$ with $\alpha = 0.05 \Rightarrow H_0$ is accepted;

there is no significant difference with (0,0)

Testing the significance of the regression coefficient

$H_o : \beta = 0$

$t_s = (b-0)/s_b > t_{(\alpha;N-2)}$ with $\alpha = 0.05 \Rightarrow H_1$ is accepted

ANOVA - lack of fit

Source of variation	SS	df	MS	F
Due to regression	SS_{REG}	1	MS_{REG}	
Residuals				
- lack of fit	SS_{LOF}	k-2	MS_{LOF}	MS_{LOF}/MS_{PE}
- pure error	SS_{PE}	$\sum_i n_i$-k	MS_{PE}	
Total	SS_T	$\sum_i n_i$-1		

The linearity test is performed by calculating: $F=MS_{LOF}/MS_{PE}$, which is compared with $F^{(0.05;k-2;\Sigma\ ini-k)}$.

$H_o: F=MS_{LOF}/MS_{PE} < F^{(0.05;k-2;\Sigma ini-k)}$

The F-test is easily significant when the method is very precise. (e.g. when auto-injector is used and the method has very limited sample-pretreatment) In these cases the QC is also calculated.

Quality coefficient

$$QC(mean) = \sqrt{\frac{\sum_{i}^{n}(\frac{y_i - \hat{y}_i}{\bar{y}})}{n-1}}.100$$

The calculated QC should be smaller than 2.5%

3. PRECISION

3.1 DEFINITION

The **precision** of an analytical method expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under prescribed conditions. The precision differs depending on the experimental conditions.
System precision is restricted to the error due to the measurement itself and can be determined by repeatedly analysing a sample ready for the measurement within a short period of time.

The **repeatability** expresses the precision under the same operating conditions over a short interval of time.

The **intermediate precision** expresses within-laboratories variations: different days, different analysts, different equipment, etc. One specifies M factor different intermediate precision (M = 1, 2, 3), according to which factor (time, operator, equipment) is (are) changed.

The **reproducibility** expresses the precision between laboratories (e.g. collaborative studies, usually applied to standardisation of methodology)

3.2.EXPERIMENTAL DESIGN / CALCULATIONS

Analyse 6 samples on 3 days

The **repeatability** is expressed as :
- repeatability standard deviation (s_r)
- repeatability variance or mean square (s_r^2) = MS_{within}
- repeatability coefficient of variation (VC_r)

The repeatability variance is estimated by the within-day mean square (MS).

The **time intermediate precision** is expressed as:
- time intermediate precision standard deviation (s_R)
- time intermediate precision variance or mean square (s_R^2) = $MS_{between}$
- time intermediate precision coefficient of variation (VC_R)

The mean squares necessary to perform the calculations are obtained when an ANOVA single factor is performed in EXCEL (analysis tools, ANOVA single factor)

When no outliers are rejected and there are 6 values of every day s^2_R can be simplified:
$s^2_R = (MS_{between} - MS_{within}) / n_j$ n_j = number of measurements per day

After analysis of variance (one way, single factor, α= 0.05) one can investigate whether or not there is a significant difference between the results of the different days by an F-test.

4. ACCURACY

4.1. DEFINITION

The **accuracy** of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

4.2.EXPERIMENTAL DESIGN / CALCULATIONS

The accuracy is tested on **at least three concentration levels in triplo.**
To 50% of the sample ± 50%, ± 100% and ± 125% standard is added. These points have to fall within the validated linear range of the method.

The recovery, when using the single-point standard addition, is given by :

$$\text{Recovery}\% = \frac{X_{after} - X_{before}}{X_{added}}$$

Guidelines for Good Agricultural Practice (G.A.P.) of Medicinal and Aromatic Plants

5 August 1998
Final Europam Version

General Part

Introduction

0.1. Scope. The guidelines for the Good Agricultural Practice of Medicinal and Aromatic (Culinary) herbs is intended to apply to the growing and primary processing of all such plants traded and used in the European Union. Hence it applies to the production of all plant materials used in the food, feed, medicinal, flavouring and perfume industries. It also applies to all methods of production including organic production in accordance with the European regulations.

0.2. The Environment. Growers involved in the production of herbs must ensure that they avoid damage to existing wildlife habitats, and that they make efforts to maintain and to enhance the biodiversity of their farms. Wild crafting might be regulated by a specific guideline.

0.3. The present Good Agricultural Practice Guidelines provide additional standards for the production and processing of raw materials insofar as they mainly focus on identifying those critical production steps (measures) that are needed to comply with good quality. In this respect, it will be attempt to minimize insufficient quality by prevention.

0.4. A main aim is to ensure that the plant raw material meets the demands of the consumer and as such the standards of the highest quality. Especially important aspects are that they:

- are produced hygienically, in order to reduce microbiological load to a minimum,

- are produced with care, so that the negative impacts affecting plants during cultivation, processing and storage can be limited,

As in the course of the production process medicinal and aromatic plants and their products are exposed to a large number of both microbiological and other contaminants, the main aim of present guidelines is to provide guidance for producers to reduce plant (raw material) contamination to the greatest extent.

0.5. All participants of the production process (from primary producers to traders) are required to comply with these guidelines voluntarily and to elaborate practical measures in order to realize them.

Producers, traders and processors of medicinal and aromatic plants, especially of tea-like products and herbal medicinal products, should comply with the GAP Guidelines, document this, by a Way Bill (batch documentation) and demand that their partners also meet these requirements.

Principles and Guidelines for Good Agricultural Practice (G.A.P.)

1. Seeds and propagation material

1.1. Seeding materials are to be identified botanically, indicating plant variety, cultivar, chemotype and origin[1]. The material used should be 100 % traceable. The same applies to vegetatively propagated starting material. Starting material used in organic production has to be certified organic.

1.2. Starting material should meet the requirements/standards concerning purity and germination (wherever available: certified seed/propagation material should be used). The starting material should be as free as possible of pests and diseases in order to guarantee healthy plant growth. When resistant or tolerant species or origins are available, they should be preferred.

1.3. The occurrence of not species/variety-identical plants and parts of plants has to be controlled in the course of the entire production process (cultivation, harvest, drying, packaging). Such impurites have to be eliminated promptly. Plants material or seeds derived from or comprising Genetically Modified Organisms have to be in accordance with national and European regulations.

2. Cultivation

2.1 Depending on the mode of cultivation e.g. conventional or organic, growers should be allowed to follow different Standard Operating Procedures for cultivation (to be elaborated). In general, care should be taken to avoid environmental disturbances. The principles of good crop husbandry must be followed including an appropriate rotation of crops.

2.2. Soil and Fertilization

2.2.1. Medicinal and aromatic plants cannot be grown in soils that are contaminated by sludge. Soils should furthermore not be contaminated by heavy metals, residues of plant protection products and other not naturally occuring chemicals, etc. For this reason, minimum effective chemical input should be achieved.

2.2.2. The manure applied should be void of human feces and prior to application it should be thoroughly composted.

2.2.3. All other fertilizing agents should be applied sparingly and in accordance with the demands of the plant and the particular species (including application between harvests). The use of fertilizers should be in accordance with efforts to minimise leaching.

---

[1] These specifications can vary, as follows:

a) Species: Chamomilla recutita Rauschert; cultivar: Bodegold; chemotype: bisabololoxide A/Chamazulene. Origin: Seed Company, 1996. Charge No 4711

b) Species: Chamomilla recutita Rauschert; self-collected seed from the arc in front of the ranch: Delightful Herbs, No. 5, Harvester Str., 91222 Chamomille Town, California, on 26.6.1991.; since that time propagated

2.3. Irrigation

2.3.1. Irrigation should be minimized as much as possible and applied according to the needs of the plant.

2.3.2. Irrigation-water should be in accordance with national and potential European quality standards and should be as free as possible of contaminants, such as faeces, heavy metals, pesticides, herbicides and toxicologically hazardous substances.

2.4. Crop maintenance and plant protection

2.4.1. Tillage should be adapted to plant growth and requirements.

2.4.2. Pesticide and herbicide application should be avoided as far as possible. When necessary they should be carried out using the minimum effective rates of approved plant protection products. Products for chemical plant protection have to conform with the European Union's maximum residue limits (European Pharmacopoeia, European Directives, Codex Alimentarius). Application and storage of plant protection products has to be in accordance with the recommendations of manufacturers and the authorities.

The application should be carried out only by qualified staff using approved equipment. Application should precede the harvest by a period either defined by the buyer or indicated by the producer of the plant protection product.

The use of pesticides and herbicides has to be documented.

2.4.3. All measures regarding nutrient supply and chemical plant protection, should secure the marketability of the product. It is obligatory that the buyer of the batch be informed of the brand, quantity and date of pesticide use in a written form.

3. Harvest

3.1. The harvest should take place when the plants are of the best possible quality according to the different utilizations.

3.2. Harvest should preferably take place under the best possible conditions (wet soils, dew, rain or exceptionally high air humidity can be unfavourable). If harvest is performed under wet conditions, extra care should be taken in order to avoid the unfavourable influence of moisture.

3.3. Equipment should be in a both a clean and technically perfect working order. Those machine parts including their housings that have a direct contact with the harvested crop, should be regularly cleaned and kept free of oil and other contamination (including plant left-overs).

3.4. Cutting devices of harvesters must be adjusted so that the collection of soil particles can be reduced to a minimum.

3.5. In the course of harvest, care should be taken to ensure that no toxic weeds can mix with the harvested crop.

3.6 Damaged and perished plant parts must be promptly eliminated

3.7. All containers used in the harvest must be clean and must be kept free of the remnants of previous crops; containers out of use, must also be preserved in a dry condition, free of pests and inaccessible for mice/rodents as well as livestock and domestic animals.

3.8. The harvested crop should not be exposed to direct contact with the soil. It must be promptly collected and under dry, clean conditions (e.g. sacks, baskets, trailers and containers, etc.) submitted to transport.

3.9. Mechanical damage and compacting of the crop that would result in undesirable quality changes must be avoided. In this respect, attention must be paid to
- avoiding the overfilling of the sacks,
- the stacking up of sacks should not result in thickening of the crop,
- the harvested crop should be transported and kept in containers or bags in such way that the occurence of heating is prevented.

3.10. Delivery of freshly harvested plant material must occur as quickly as possible to the processing facility in order to prevent heating.

3.11. The harvested crop must be protected from pests, mice/rodents and domestic animals. Pest control measures should be documented.

4. Primary processing

Primary processing includes steps of processing such as washing, freezing, distilling, drying, etc.. All these processes whether for food or medicinal use must conform to European and national regulations.

4.1. Arriving at the processing facility the harvested crop has to be promptly unloaded and unpacked. Prior to processing the material should not be exposed directly to the sun (except in case there is a specific need e.g. for distillation) and it must be protected from rainfall.

4.2. Buildings used in the processing of harvested crops must be clean, as well as thoroughly aerated and must never be used for housing livestock.

4.3. Buildings must be constructed so as to provide protection for the harvested crop against birds, insects, rodents as well as domestic animals. In all storage (including packaging stores) and processing areas suitable pest control measures such as baits and electric insect killing machines must be operated and maintained by professionally qualified staff or contractors.

4.4. Processing equipment must be maintained clean and must be regularly serviced.

4.5. In the case of natural open air drying, the crop must be spread out in a thin layer. In order to secure unlimited air circulation, the drying frames must be located at a sufficient distance from the ground. Attempts must be made to achieve uniform drying of the crop and as a consequence to avoid mould formation.

When drying with oil, the exhaust fumes should not be reused for drying. Direct drying should not be allowed except with butane, propane, or natural gas.

4.6. Except in the case of natural open air drying, the conditions (e.g. temperature, duration, etc.) must be selected taking into consideration the type (e.g. root, leaf or flower) and active substance content (e.g. essential oils and others) of the crude drug to be produced.

Drying conditions should be documented.

4.7. Drying directly on the ground or under direct exposure to the sun-light should be avoided unless it is required for a particular plant.

4.8. All material must be inspected or sieved in order to eliminate sub-standard products and foreign bodies. Sieves must be maintained in a clean state and should be serviced regularly.

4.9. Clearly marked waste-bins should be kept ready, emptied daily and cleaned.

4.10. In order to protect it and to reduce the risk of pest attacks, the product should be promptly packaged.

5. Packaging

5.1. After the repeated control and eventual elimination of low-quality materials and foreign bodies, the product should be packaged in clean and dry, preferably new sacks, bags or cases. The label must be clear, permanently fixed and made from non-toxic material. Information must conform with the European and national labelling regulations.

5.2. Packaging materials should be stored in a clean and dry place that has to be free of pests and inaccessible for livestock and domestic animals. It must be guaranteed that no contamination of the product takes place by the use of packaging material, particularly in the case of fibre bags.

5.3. Reusable packaging materials should be well cleaned and perfectly dried prior to their usage. It must be guaranteed that no contamination takes place by reusing bags.

6. Storage and Transport

6.1. Packaged dried materials and essential oils should be stored in a dry, well aerated building, in which the daily temperature fluctuations are limited and good aeration is given. Fresh products (except Basil) should be stored between 1°C and 5°C while frozen products should be stored below –18°C (or below –20°C for longer term storage). Essential oil storage must conform to the appropriate chemical storage standards.

6.2. As a protection against pests, birds, rodents and domestic animals, the window and door openings are to be protected, e.g. by wire netting.

6.3. It is recommended that the packaged dry crop will be stored:
- in buildings with concrete or similar easy to clean floors,
- on pallets,
- with a sufficient distance to the wall,
- thoroughly separated from other crops to avoid cross-contamination.
Organic products must be stored separately.

6.4. In the case of bulk transport, it is important to secure dry conditions and furthermore, in order to reduce the risk of mould formation or fermentation, it is extremely advisable to use aerated containers. As a substitute, the use of sufficiently aerated transport vehicles and other aerated facilities is recommended. Essential oil transport must conform to appropriate regulations. National and European regulations on transport have to be respected.

6.5. Fumigation against pest attack should be carried out only in the case of necessity and it must be carried out exclusively by licensed personnel. Only registered chemicals must be used. Any fumigation against pest attack should be reported in the documentation.

6.6. For fumigation of warehouses, only permitted substances should be used, according to European or national regulations.

6.7. When frozen storage or saturated steam is used for pest control, humidity of the material must be controlled after treatment.

7. Equipment

7.1. Equipment used in plant cultivation and processing should be easy to clean, in order to eliminate the risk of contamination.

7.2. All machinery should be mounted in an easily accessible way. They must be well serviced and regularly cleaned. Fertilizer and pesticide application machinery must be regularly calibrated.

7.3. Preferably non-wooden equipment should be used unless tradition demands wooden material. When wooden equipment (such as e.g. pallets, hoppers, etc.) is used, it should not come into direct contact with chemicals and contaminated/infected materials, so that infection of the plant material can be prevented.

8. Personnel and Facilities

8.1. Personnel should receive adequate botanical education before performing tasks that require this knowledge.

8.2. All processing procedures should fully conform with both EU-Guidelines on Food Hygiene and the General Principles for food hygiene of Codex Alimentarius as well as the European Directive on Good Manufacturing Practice.

8.3. Personnel entrusted with the plant material should be required to have a high degree of personal hygiene (including personnel working in the fields) and have received adequate training regarding their hygiene responsibilities.

The buildings where the plant processing is carried out, have to be provided with changing facilities as well as toilets including hand washing facilities, according to the respective regulations..

8.4. Persons suffering from known infectious diseases transmittable via food, including diarrhoea, or being transmitters of such diseases, must be suspended from areas where they are in contact with the plant material, according to the respective regulations.

8.5. Persons with open wounds, inflammations and skin-infections should be suspended from the areas where plant processing takes place, or have to wear appropriate protecting clothing or gloves, until their complete recuperation.

8.6. Personnel should be protected from contact with toxic or potentially allergenic plant materials by means of adequate protective clothing.

8.7. The welfare of all staff involved in the growing and processing shall be ensured.

9. Documentation

9.1. All starting materials and processing steps have to be documented including the location of cultivation. Field records showing previous cropping and inputs should be maintained by all growers.

9.2. All batches from coherent areas should be unambiguously and unmistakably labelled (e.g. by the application of a batch number). This should take place as early as possible.

9.3. Batches from differing areas shall be mixed only if it is guaranteed that the mixture in itself will be homogenous. Such mixing procedures should also be documented.

9.4. It is essential to document the type, quantity and the date of harvest of the crop, as well as the chemicals and other substances (e.g. fertilizers, pesticides and herbicides, growth regulators, etc.) used during production.

9.5. The application of the fumigation agents such as phosphin must be entered into batch documentation.

9.6. All processes and procedures that could bear an impact on the quality of the product must be entered into the batch documentation.

9.7. All agreements (production guidelines, contracts, etc.) between producer and buyer should be fixed in a written form.

It should be documented in a Way Bill (batch documentation) that cultivation, harvesting and production have been performed in accordance with the GAP Guideline. Minimum information included in the Way Bill should cover geographical definition of growth place, country of origin and responsible producer.

9.8. The results of audits should be documented in an Audit Report (copies of all documents, Schlagkartei, Audit Reports, Analyse Reports) to be stored for a minimum of 10 years.

9.9 Special circumstances during the growth period which may influence the chemical composition like extreme weather conditions, pests - particularly in the harvest period - must be documented.

10. Education

It is extremely advisable to educate all personnel dealing with the crop or those engaged in the direction of the production regarding production techniques and the appropriate use of herbicides and pesticides.

11. Quality Assurance

Agreements between producers and buyers of medicinal and aromatic plants, with regard to quality questions, e.g. active principles and other characteristic ingredients, optical and sensoric properties, limit values of germ numbers, plant protection chemical residues and heavy metals, must be based on internationally recognized or national specifications and should be laid down in a written form.

OVERVIEW OF THE HIGHLIGHTS OF THE EUROPEAN PHARMACOPOEIA (1)

1964 : CREATION OF THE EUROPEAN PHARMACOPOEIA COMMISSION BY 8 MEMBER COUNTRIES OF THE COUNCIL OF EUROPE (EUROPEAN TREATY SERIES N°. 50)

1969 : PUBLICATION OF THE FIRST VOLUME OF THE FIRST EDITION

1972 : MONOGRAPHS OF THE FIRST EDITION BECOME OFFICIAL IN ALL MEMBER STATES (JURICIDAL ACT)

1975 : DIRECTIVE 75/318/EEC AMENDED BY DIRECTIVE 83/570/EEC : FOR MARKETING AUTHORIZATION OF PRODUCTS FOR HUMAN USE, IT IS MANDATORY FOR APPLICANTS TO USE THE MONOGRAPHS OF THE EUROPEAN PHARMACOPOEIA

1983 : PUBLICATION OF THE SECOND EDITION

1987 : DIRECTIVE 87/20/EEC : IDEM FOR PRODUCTS FOR VETERINARY USE

1988 : FIRST PUBLICATION OF THE JOURNAL, PHARMEUROPA, FORUM OF THE EUROPEAN PHARMACOPOEIA

1989 : 25th ANNIVERSARY OF THE EUROPEAN PHARMACOPOEIA, INTERNATIONAL CONFERENCE AT STRASBOURG

1992 : FIRST JOINT PHARMACOPOEIAL OPEN CONFERENCE ON INTERNATIONAL HARMONIZATION OF EXCIPIENT STANDARDS, ORLANDO, USA

1994 : PROCEDURE FOR THE CERTIFICATION OF SUITABILITY OF PH. EUR. MONOGRAPHS BECOMES OPERATIONAL

OVERVIEW OF THE HIGHLIGHTS OF THE EUROPEAN PHARMACOPOEIA (2)

1995 : OPENING OF THE EUROPEAN MEDICINES EVALUATION AGENCY (EMEA) IN LONDON

TODAY (1996) :

* 23 MEMBER STATES AND 10 OBSERVER STATES

* THE COMPLETE EUROPEAN PHARMACOPOEIA :
  2ND EDITION : 1 PART AND 19 FASCICULES
  (MAY, 1995) :

  - ABOUT 1025 MONOGRAPHS

  - ABOUT 668 CHEMICAL REFERENCE SUBSTANCES (1994)
    YEARLY DISTRIBUTION : ABOUT 21.000 (1994)

* PHARMACOPOEIAL INSTITUTE (5000 m^2 ; 41 STAFF) INCLUDING PERMANENT SECRETARIAT AND LABORATORY

* SOLID BASE FOR THE PROTECTION OF THE HEALTH OF ABOUT 500 MILLION CITIZENS IN 23 MEMBER STATES

* CD-ROM VERSION OF THE EUROPEAN PHARMACOPOEIA 2ND EDITION AVAILABLE

* 3ND EDITION OF THE EUROPEAN PHARMACOPOEIA : SCHEDULED FOR JUNE 1996

PHARMACOPOEIAL MONOGRAPHS ON VEGETABLE DRUGS AND PREPARATIONS THEREOF (1)

* AIMS

CONTROL OF PRODUCT QUALITY IN TERMS OF

* PURITY
* SAFETY
* EFFICIENCY
* SUITABILITY FOR USE (EXCIPIENTS)

* GUIDE FOR THE TECHNICAL CONTENT OF MONOGRAPHS ON VEGETABLE DRUGS AND PREPARATIONS OF VEGETABLE ORIGIN (1993)

1. VEGETABLE DRUGS
2. PLANT RAW MATERIALS OBTAINED AFTER TREATMENT
   - 2.1. VOLATILE OILS
   - 2.2. BALSAMS, RESINS AND GUMS
   - 2.3. STARCHES
3. TINCTURES AND EXTRACTS

PRESENT STATUS AND PROSPECTS OF VEGETABLE DRUGS OF THE EUROPEAN PHARMACOPOEIA (1)

* PRESENT STATUS

MONO-GRAPHS	EUR.PH. 1997	EUR.PH. 1998	DAB 10	PH.F. 10	IT.PH. 9	JAP.PH. 12
TOTAL NUMBER	1099	112	965	-	855	-
VEGETABLE DRUGS	69	16	127	141	103	47 (E.PH) ↑ 113 ↓ 66 (NON-E.PH)
PERCENTAGE VEGETABLE DRUGS	6.3%	14.2%	13	-	12	-

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EUROPEAN PHARMACOPOEIA 1997 + 1998	MONOGRAPHS ALREADY PUBLISHED	MONOGRAPHS UNDER STUDY	TOTAL
VEGETABLE DRUGS	48	50	98
PLANT RAW MATERIALS OBTAINED AFTER TREATMENT	22	15	37
TINCTURES AND EXTRACTS	5	17	22
VEGETABLE OILS AND WAXES	10	10	20
TOTAL	85(7.0%)	92	177

* VOLATILE OILS, BALSAMS, RESINS, GUMS AND STARCHES

PRESENT STATUS AND PROSPECTS OF VEGETABLE DRUGS OF THE EUROPEAN PHARMACOPOEIA (2)

1. VEGETABLE DRUGS

1.1. MONOGRAPHS ALREADY PUBLISHED : **48**

1	*Allii sativi bulbus pulvis*	25	*Ipecacuanhae pulvis normatus*
2	*Althaeae radix*	26	*Levistici radix*
3	*Anisi fructus*	27	*Lini semen*
4	*Anisi stellati fructus*	28	*Liquiritae radix*
5	*Belladonnae folium*	29	*Lupuli flos*
6	*Belladonnae pulvis normatus*	30	*Matricariae flos*
7	*Betulae folium*	31	*Menthae piperitae folium*
8	*Carvi fructus*	32	*Opium crudum*
9	*Caryophylli flos*	33	*Orthosiphonis folium*
10	*Chamomillae romanae flos*	34	*Polygalae radix*
11	*Cinchonae cortex*	35	*Psyllii semen*
12	*Cinnamomi cortex*	36	*Ratanhiae radix*
13	*Crataegi fructus*	37	*Rhamni purshianae cortex*
14	*Cyamopsidis seminis pulvis*	38	*Rhei radix*
15	*Digitalis purpureae folium*	39	*Sambuci flos*
16	*Foeniculi amari fructus*	40	*Sennae folium*
17	*Foeniculi dulcis fructus*	41	*Sennae fructus acutifoliae*
18	*Frangulae cortex*	42	*Sennae fructus angustifoliae*
19	*Gentiaenae radix*	43	*Stramonii folium*
20	*Hamamelidis folium*	44	*Stramonii pulvis normatus*
21	*Harpagophyti radix*	45	*Tiliae flos*
22	*Hyoscyami folium*	46	*Thymi herba*
23	*Hyoscyami pulvis normatus*	47	*Uvae ursi folium*
24	*Ipecacuanhae radix*	48	*Valerianae radix*

PRESENT STATUS AND PROSPECTS OF VEGETABLE DRUGS OF THE EUROPEAN PHARMACOPOEIA (3)

1. VEGETABLE DRUGS (Continued)

1.1. MONOGRAPHS UNDER STUDY : 50

1	*Absinthii herba*	26	*Juniperi fructus*
2	*Alchemillae herba*	27	*Lavandulae flos*
3	*Arnicae flos*	28	*Lichen islandicus*
4	*Aurantii amari flos*	29	*Malvae flos*
5	*Boldo folium*	30	*Marrubii herba*
6	*Calendulae flos*	31	*Melissae folium*
7	*Capsici fructus*	32	*Menthae arvensis folium*
8	*Cardui mariae fructus*	33	*Millefolii herba*
9	*Centaurii herba*	34	*Origani herba*
10	*Coriandri fructus*	35	*Passiflorae herba*
11	*Crataegi folium cum flore*	36	*Plantaginis ovatae folliculis seminis*
12	*Curcumae xanthorrhizae rhizoma*	37	*Plantaginis ovatae semen*
13	*Droserae rotundifoliae herba*	38	*Primulae radix*
14	*Echinaceae purpureae radix*	39	*Pruni africanae cortex*
15	*Eleutherococci senticosi radix*	40	*Rosmarini folium*
16	*Equiseti herba*	41	*Salicis cortex*
17	*Eucalypti folium*	42	*Salviae officinalis folium*
18	*Filipendulae ulmariae flos*	43	*Tanaceti parthenii herba*
19	*Foenigraeci semen*	44	*Taraxaci officinalis herba*
20	*Fucus vesiculosus*	45	*Taraxaci officinalis herba cum cortex*
21	*Ginkgo bilobae folium*	46	*Tormentillae rhizoma*
22	*Ginseng radix*	47	*Verbasci flos*
23	*Graminis rhizoma*	48	*Verbenae herba*
24	*Hippocastani semen*	49	*Violae tricoloris herba*
25	*Hyperici herba*	50	*Zingiberis rhizoma*

PRESENT STATUS AND PROSPECTS OF VEGETABLE DRUGS OF THE EUROPEAN PHARMACOPOEIA (4)

2. PLANT RAW MATERIALS OBTAINED AFTER TREATMENT

2.1. MONOGRAPHS ALREADY PUBLISHED : **22**

1	*Acaciae gummi*	12	*Aurantii amari floris aetheroleum*
2	*Acaciae gummi dispersione dessicatum*	13	*Balsamum peruvianum*
3	*Agar*	14	*Caryophylii floris aetheroleum*
4	*Aloe barbadensis*	15	*Eucalypti aetheroleum*
5	*Aloe capensis*	16	*Guar galactomannanum*
6	*Amylum maydis*	17	*Ichtammolum*
7	*Amylum oryzae*	18	*Lacca*
8	*Amylum pregelificatum*	19	*Limonis aetheroleum*
9	*Amylum solani*	20	*Menthae piperitae aetheroleum*
10	*Amylum triticii*	21	*Tragacantha*
11	*Anisi aetheroleum*	22	*Xanthani gummi*

2.2. MONOGRAPHS UNDER STUDY : **15**

1	*Amylum solubile*	9	*Foeniculi dulcis aetheroleum*
2	*Aurantii corticis amari aetheroleum*	10	*Lavandulae aetheroleum*
3	*Aurantii corticis dulcis aetheroleum*	11	*Menthae arvensis aetheroleum*
4	*Balsamum benzoe*	12	*Myrrha*
5	*Carrageenanum*	13	*Pinus silvestris aetheroleum*
6	*Citri sinensis aetheroleum*	14	*Podophyllotoxinum*
7	*Cyamopsis gummi*	15	*Thymi aetheroleum*
8	*Foeniculi amari aetheroleum*		

PRESENT STATUS AND PROSPECTS OF VEGETABLE DRUGS OF THE EUROPEAN PHARMACOPOEIA (5)

3. EXTRACTS AND TINCTURES

3.1. MONOGRAPHS ALREADY PUBLISHED : **5**

1	*Aloes extractum siccum normatum*
2	*Extracta*
3	*Frangulae corticis extractum siccum normatum*
4	*Sennae folii extractum siccum normatum*
5	*Tincturae*

3.2. MONOGRAPHS UNDER STUDY : **17**

1	*Aesculi extractum siccum*	10	*Hyperici herbae extractum siccum normatum*
2	*Belladonnae extractum siccum*	11	*Ipecacuanhae extractum fluidum*
3	*Belladonnae tinctura*	12	*Liquiritiae extractum siccum solubile*
4	*Boldo extractum siccum normatum*	13	*Opii extractum 20 per centum*
5	*Cascarae extractum siccum*	14	*Opii tinctura*
6	*Crataegi extractum siccum*	15	*Passiflorae extractum siccum normatum*
7	*Fucus vesiculosus extractum normatum*	16	*Rhamni purshianae extractum*
8	*Harpagophyti extractum siccum normatum*	17	*Valerianae extractum siccum*
9	*Hippocastani extractum siccum normatum*		

PRESENT STATUS AND PROSPECTS OF VEGETABLE DRUGS OF THE EUROPEAN PHARMACOPOEIA (6)

4. VEGETABLE OILS AND WAXES

4.1. MONOGRAPHS ALREADY PUBLISHED : **10**

1	*Amygdalae oleum*
2	*Amygdalae oleum raffinatum*
3	*Arachidis oleum*
4	*Arachidis oleum hydrogenatum*
5	*Cera carnauba*
6	*Olivae oleum*
7	*Ricini oleum*
8	*Sesami oleum*
9	*Sojae oleum*
10	*Sojae oleum hydrogenatum*

4.2. MONOGRAPHS UNDER STUDY : **10**

1	*Arachidis oleum hydrogenatum*
2	*Gossypii oleum*
3	*Gossypii oleum hydrogenatum*
4	*Helianthi annui oleum*
5	*Maidis oleum*
6	*Olivi oleum raffinatum*
7	*Rapae oleum*
8	*Ricini oleum*
9	*Ricini oleum hydrogenatum*
10	*Tritici aestivi oleum*

Increasingly used Herbal Medicines in the East and West World

The total sales volume of herbal medicines

In China	US$	1.8	billion	in 1995
In the US	US$	0.86	billion	in 1990
	US$	2.1	billion	in 1995
In EU	US$	6	billion	in 1995

WHO TRADITIONAL MEDICINE PROGRAMME

Major objectives are to :

- facilitate the integration of traditional medicine into the national healthcare system

- promote the rational use of traditional medicine through development of technical guidelines and international standards in the field of herbal medicines and acupuncture

- act as a clearing house for the dissemination of information on various forms of traditional medicine

WHA 1987 RESOLUTION

Member states were urged:

- to initiate comprehensive programmes for the identification, evaluation, preparation, cultivation and conservation of medicinal plants used in traditional medicine;

- to ensure quality control of drugs derived from traditional plant remedies by using modern techniques and applying suitable standards and good manufacturing practices.

WHA 1991 RESOLUTION

Requests the Director-General :

- to continue to recognise the high importance of this programme and to mobilise increased financial and technical support as required;

- to ensure that the contribution of scientifically proven traditional medicines is fully exploited within all of the WHO programmes where plant-derived and other natural products may lead to the discovery of new therapeutic substances;

- to seek appropriate partnerships with governmental bodies and non-governmental organisations as well as with industry in implementing this resolution.

WHO's Documents regarding Herbal Medicines

The guidelines for assessment of herbal medicines

The objective

- to define basic criteria for the evaluation of the quality, safety and efficacy of herbal medicines and
- to assist national regulatory authorities, scientific institutions and manufacturers to undertake an assessment of the documentation/submission/dossier in respect of such products

RECENT WHO DOCUMENTS ON MEDICINAL PLANTS

- Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines - Regional Office for the Western Pacific (1993)

- Guidelines on the Conservation of Medicinal Plants (1993)

- Quality Control Methods for Medicinal Plant Materials (1994)

- Selection of Essential Medicinal Plants - Regional Office for the Eastern Mediterranean (1995)

- Monographs on selected Medicinal Plants (1996/1997)

The Objective of Monographs

- to provide scientific information on the safety, efficacy and quality control of widely used medicinal plants for facilitating the proper use of herbal medicines in the Member States;
- to provide models for assisting Member States to develop their own monographs on these and additional herbal medicines;
- to facilitate information exchange among the Member States.

The Information Contained in the WHO Monographs on Selected Medicinal Plants

Part I

- botanical features
- quality control
- major active chemical constituents

Part II

- medical applications
- pharmacology
- posology
- possible contra-indications and precautions.

WHO Monographs on Selected Medicinal Plants

(Volume I, 1996)

List of Monographs

1. Bulbus alii cepae
2. Bulbus alii sativi
3. Aloe
4. Aloe vera gel
5. Radix astragali
6. Fructus bruceae
7. Radix bupleuri
8. Herba centellae
9. Flos chamomillae
10. Cortex cinnamomi
11. Rhizoma coptidis
12. Rhizoma curcumae longae
13. Radix echinaceae
14. Herba echinaceae purpureae

WHO Monographs on Selected Medicinal Plants

(Volume I, 1996)

List of Monographs

15. Herba ephedrae
16. Folium gingko
17. Radix ginseng
18. Radix glycyrrhizae
19. Radix paeoniae
20. Semen plantaginis
21. Radix platycodi
22. Radix rauwolfiae
23. Rhizoma rhei
24. Folium sennae
25. Fructus sennae
26. Herba thymi
27. Radix valerianae
28. Rhizoma zingiberis

WHO Monographs on Selected Medicinal Plants

(Draft Volume II, 1997)

List of Medicinal Plants

1. Aesculus hippocastanum
2. Althaea officinalis
3. Angelica sinensis
4. Arctostaphylos uva ursi
5. Calendula officinalis
6. Capsicum annuum
7. Chrysanthemum parthenium
8. Cimicifuga racemosa
9. Crataegus monogyna C. laevigata
10. Eleutherococcus senticosus
11. Eucalyptus globulus
12. Hamanelis virginiana
13. Harpagophtum procumbens
14. Andrographidis paniculata ***
15. Hypericum perforatum
16. Melaleuca alternifolia

WHO Monographs on Selected Medicinal Plants

(Draft Volume II, 1997)

List of Medicinal Plants

17. Melissa officinalis
18. Mentha piperita
19. Ocimum sanctumy
20. Oenothera biennis
21. Piper methysticum
22. Polygala senega
23. Prunus (Pygeum) africana
24. Angelica sinensis ***
25. Rhamnus purshiana
26. Rhamnus frangula
27. Salvia miltiorrhiza
28. Sambucus nigra
29. Serenoa repens
30. Silybum marianum
31. Syzygium aromaticum
32. Urtica dioica, U. urens

INTERNATIONAL CONFERENCE OF DRUG REGULATORY AUTHORITIES (ICDRA)

1991: Endorsement of WHO Guideline on the Assessment of Herbal Medicines

1994: 10 points for future priorities

1996: Endorsement of the WHO monographs on selected medicinal plants

ESTABLISHMENT AND GOALS OF ESCOP (1)

ESTABLISHMENT

EUROPEAN SCIENTIFIC COOPERATIVE ON PHYTOTHERAPY (ESCOP)

FOUNDED ON 18 JUNE 1989 : OFFICIAL DOCUMENT SIGNED AT A MEETING IN COLOGNE, GERMANY BY **SIX FOUNDER MEMBERS**

* SOCIETE BELGE DE PHYTOTHERAPIE/BELGISCHE VERENIGING VOOR PHYTOTHERAPIE (SBP), **BELGIUM**
* ASSOCIATION FRANCAISE POUR LE MEDICAMENT DE PHYTOTHERAPIE (AFMP) **FRANCE**
* GESELLSCHAFT FUR PHYTOTHERAPIE, e.V. (GfP), **GERMANY**
* NEDERLANDSE VERENIGING VOOR FYTOTHERAPIE (NFV), **THE NETHERLANDS**
* SCHWEIZERISCHE MEDIZINISCHE GESELLSCHAFT FUR PHYTOTHERAPIE (SMGP), **SWITZERLAND**
* BRITISH HERBAL MEDICINE ASSOCIATION (BHMA), **UNITED KINGDOM**

FULL MEMBERS

* SOCIETY OF PHYTO- AND AROMATHERAPY, **GREECE** (SINCE 1992)
* IRISH ASSOCIATION ON PHYTOTHERAPY, **IRELAND** (SINCE 1992)
* SOCIETA ITALIANA DI FITOCHIMICA (SIF), **ITALY** (SINCE 1991)
* SWEDISH PHYTOCHEMICAL SOCIETY, **SWEDEN**
* TURK FITOTERAPI DERMEGI, **TURKEY**

ASSOCIATE MEMBERS

AUSTRIA, DENMARK, NORWAY, PORTUGAL

AFFILIATE MEMBERS

AUSTRALIA, INDIA, SOUTH-AFRICA, USA (2X)

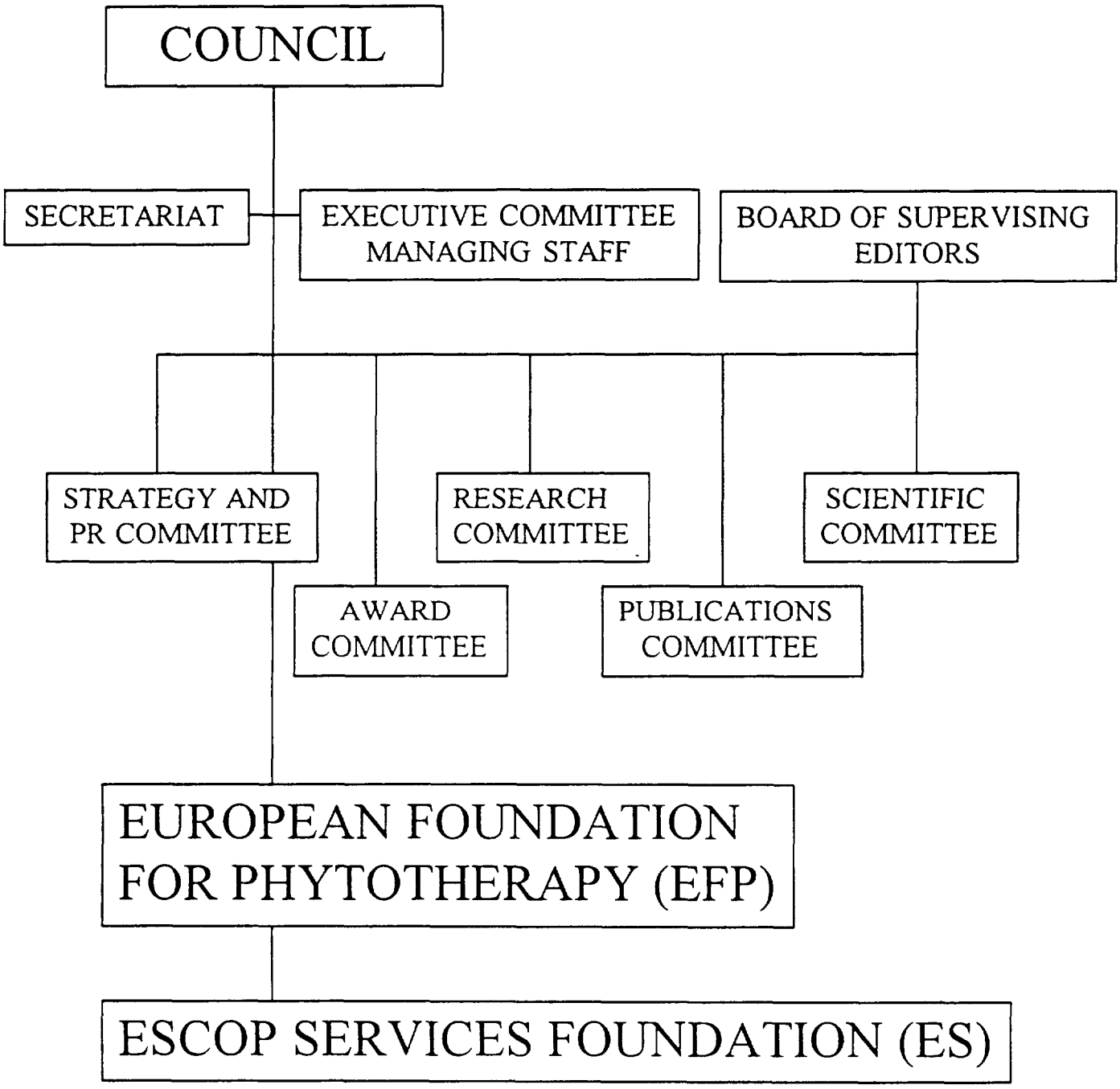
ESTABLISHMENT AND GOALS OF ESCOP (2)

GOALS OF ESCOP

* **GENERAL AIMS**
  * ADVANCE **THE SCIENTIFIC STATUS** OF PHYTOMEDICINES
  * ASSIST WITH **THE HARMONIZATION** OF **THEIR REGULATORY STATUS** AT THE **EUROPEAN LEVEL**

* **MAJOR AIMS AND OBJECTIVES**
  * DEVELOP A COORDINATED SCIENTIFIC FRAMEWORK TO ASSESS PHYTOMEDICINES
  * PROMOTE THE ACCEPTANCE OF PHYTOMEDICINES, ESPECIALLY WITHIN THE THERAPY OF GENERAL MEDICINAL PRACTITIONERS
  * SUPPORT AND INITIATE CLINICAL AND EXPERIMENTAL RESEARCH IN PHYTOTHERAPY
  * IMPROVE AND EXTEND THE INTERNATIONAL ACCUMULATION OF SCIENTIFIC AND PRACTICAL KNOWLEDGE IN THE FIELD OF PHYTOTHERAPY
  * FURTHER COOPERATION AMONG ASSOCIATONS OF PHYTOTHERAPY TO ADVANCE THESE AIMS INCLUDING THE SCIENTIFIC AND REGULATORY STATUS OF PHYTOMEDICINES WORLDWIDE

Scheme of ESCOP Organisation 1993



EUROPEAN FOUNDATION FOR PHYTOTHERAPY (EFP)

* ESTABLISHED IN AMSTERDAM ON 19 JANUARY 1993 BY THE BRITISH HERBAL MEDICINE ASSOCIATION, THE GESELLSCHAFT FUR PHYTOTHERAPIE AND THE NEDERLANDSE VERENIGING VOOR FYTOTHERAPIE

* CONSTITUTED AS AN EUROPEAN ECONOMIC INTEREST GROUPING (EEIG) TO SUPPORT ESCOP

* **CONCERTED ACTION** DEVELOPED BY THE EFP IN COLLABORATION WITH THE CENTRE FOR COMPLEMENTARY HEALTH STUDIES OF THE UNIVERSITY OF EXETER, UNITED KINGDOM

DETERMINING EUROPEAN STANDARDS FOR THE SAFE AND EFFECTIVE USE OF PHYTOMEDICINES

* ACCEPTED BY THE EUROPEAN COMMUNITY IN THE FRAMEWORK OF THE

BIOMEDICAL AND HEALTH RESEARCH PROGRAMME

IN JULY 1993 (BMH1-CT93-1238)

RESEARCH PROGRAMME OF THE EUROPEAN FOUNDATION FOR PHYTOTHERAPY

BIOMEDICAL AND HEALTH RESEARCH PROGRAMME OF THE EUROPEAN COMMUNITY (1993-1996)

AIM

TO DETERMINE EUROPEAN STANDARDS FOR THE SAFE AND EFFECTIVE USE OF PHYTOMEDICINES

OBJECTIVES

* PRODUCE A TOTAL OF **50 HARMONIZED MONOGRAPH PROPOSALS** MEETING THE REQUIREMENTS OF THE COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS (CPMP) AND OTHER REGULATORY AGENCIES

* PILOT AND COMMENCE **A PHARMACOVIGILANCE SYSTEM** FOR ADVERSE REPORTS ON PHYTOMEDICINES, A SYSTEM WITH ACCOMPANYING VALIDATION MEASURES, AND LINKED TO ESTABLISHED PRACTICAL PHARMACOVIGILANCE CENTRES FOR EXTERNAL REFERENCE

* CONVERSE **AN EXPERT PANEL** OF CLINICIANS AND OTHER SPECIALISTS TO PROVIDE BOTH **OPERATIONAL GUIDELINES FOR THOSE INITIATING CLINICAL TRIALS** AND **ONGOING SUPPORT** FOR SUCH WORK

DEVELOPMENT OF SCIENTIFIC FRAMEWORK FOR ASSESSMENT

BY ESCOP (1)

* THE SCIENTIFIC COMMITTEE OF ESCOP PUBLISHED 15 MONOGRAPHS (5 IN 1990, 10 IN 1992), SUMMARIZING THE MEDICINAL USES OF PLANT DRUGS (INCLUDING THEIR SAFETY) AND SUBMITTED THEM TO THE COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS (CPMP) FOR ASSESSMENT

* ON THE ADVICE OF THE CPMP, THE FORMAT OF **THE SUMMARY OF PRODUCT CHARACTERISTICS** (SPC) WAS ADOPTED FOR SUBSEQUENT DOCUMENTATION (SINCE NOVEMBER 1992)

THE SPC IS AN INTEGRAL PART OF **AN APPLICATION FOR AUTHORIZATION** TO MARKET A MEDICINAL PRODUCT FOR HUMAN USE IN ANY OF THE 15 MEMBER STATES OF THE EUROPEAN UNION, AS LAID DOWN IN DIRECTIVE 65/65/EEC.

ACCORDING TO **THE NOTICE TO APPLICANTS**, PUBLISHED IN 1993, THE SPC IS A DEFINITIVE STATEMENT BETWEEN THE COMPETENT AUTHORITY AND THE MARKETING AUTHORIZATION HOLDER AND IT IS THE COMMON BASIS OF COMMUNICATION BETWEEN THE AUTHORITIES OF ALL THE MEMBER STATES.

DATA SHEETS FOR MEDICINAL PRODUCTS ARE BASED ON THE SPC, WHICH IS THUS ALSO THE BASIS ON INFORMATION FOR THE PRESCRIBER OR SUPPLIER OF THE PRODUCT.

DEVELOPMENT OF SCIENTIFIC FRAMEWORK FOR ASSESSMENT

BY ESCOP (2)

* **ESCOP PROPOSAL FOR THE SUMMARY OF PRODUCT CHARACTERISTICS (SPC)**

1. NAME OF THE MEDICINAL PRODUCT
2. QUALITATIVE AND QUANTITATIVE COMPOSITION
   - 2.1. ACTIVE INGREDIENT
     - 2.1.1. DEFINITION
     - 2.1.2. CONSTITUENTS
3. PHARMACEUTICAL FORM
4. CLINICAL PARTICULARS
   - 4.1. THERAPEUTIC INDICATIONS
   - 4.2. POSOLOGY AND METHOD OF ADMINISTRATION
     - 4.2.1. DOSAGE
     - 4.2.2. METHOD OF ADMINISTRATION
     - 4.2.3. DURATION OF ADMINISTRATION
   - 4.3. CONTRA-INDICATIONS
   - 4.4. SPECIAL WARNINGS AND SPECIAL PRECAUTIONS FOR USE
   - 4.5. INTERACTION WITH OTHER MEDICAMENTS AND OTHER FORMS OF INTERACTION
   - 4.6. PREGNANCY AND LACTATION
   - 4.7. EFFECTS ON ABILITY TO DRIVE AND USE MACHINES
   - 4.8. UNDESIRABLE EFFECTS
   - 4.9. OVERDOSE

DEVELOPMENT OF SCIENTIFIC FRAMEWORK FOR ASSESSMENT

BY ESCOP (3)

* **ESCOP PROPOSAL FOR THE SUMMARY OF PRODUCT CHARACTERISTICS (SPC)**

5. PHARMACOLOGICAL PROPERTIES

5.1. PHARMACODYNAMIC PROPERTIES

- *IN VITRO* STUDIES
- *IN VIVO* STUDIES
- CLINICAL STUDIES

5.2. PHARMACOKINETIC PROPERTIES

5.3. PRECLINICAL SAFETY DATA

- ACUTE TOXICITY
- REPEATED DOSE TOXICITY
- SUBCHRONIC TOXICITY
- MUTAGENICITY

6. PHARMACEUTICAL PARTICULARS

6.1. LIST OF EXICIPIENTS

6.2. INCOMPATIBILITIES

6.3. SHELF LIFE

6.4. SPECIAL PRECAUTIONS FOR STORAGE

6.5. NATURE AND CONTENTS OF CONTAINER

6.6. INSTRUCTIONS FOR USE/HANDLING

6.7. NAME OR STYLE AND PERMANENT ADDRESS OR REGISTERED PLACE OF BUSINESS OF THE HOLDER OF THE MARKETING AUTHORIZATION

7. MARKETING AUTHORIZATION NUMBER

8. DATE OF APPROVAL/REVISION OF SPC

9. REFERENCES

* ESCOP MONOGRAPHS ARE AVAILABLE FOR DISTRIBUTION AND CONTAIN ALL SCIENTIFIC DATA OF SPC EXCEPT DETAILS RELEVANT ONLY TO A PRODUCT I.E. NO. 6, 7 AND 8

DEVELOPMENT OF SCIENTIFIC FRAMEWORK FOR ASSESSMENT BY ESCOP (4)

PREPARATION OF SPC DRAFTS BY ESCOP

* PREPARATION OF A FIRST DRAFT BY A SINGLE AUTHOR WITH PARTICULAR EXPERTISE ON THE SUBJECT

* CIRCULATION OF THIS DRAFT TO MEMBERS OF ONE OF THE TWO SUBCOMMITTEES OF THE ESCOP SCIENTIFIC COMMITTEE FOR THEIR OWN CONSIDERATION

* DISCUSSION OF THESE DRAFTS, DURING THE MEETINGS OF THE SCIENTIFIC COMMITTEE (6 TIMES A YEAR) AND PRODUCTION OF A SECOND DRAFT. AN ACKNOWLEDGED ACADEMIC EXPERT ON EACH DRUG IS INVITED TO JOIN THE MEETINGS

* CIRCULATION OF THE SECOND DRAFT TO THE SUPERVISING EDITORS OF ESCOP

* COMPILATION OF COMMENTS OF SUPERVISING EDITORS AND REDISCUSSION AT THE SUBCOMMISSION MEETINGS

* FINAL PROPOSAL IS PUBLISHED AND SUBMITTED TO THE CPMP FOR FEEDBACK

ESCOP MONOGRAPHS ON THE MEDICINAL USES OF PLANT DRUGS (1)

1. PHARMACOLOGICAL PROPERTIES

* ***MATRICARIAE* FLOS** (MATRICARIA FLOWER)

5.1. PHARMACODYNAMIC PROPERTIES

- NOT A SINGLE ACTIVE PRINCIPLE
- BOTH VOLATILE SESQUITERPENES AND WATER SOLUBLE FLAVONOIDS HAVE BENEFICIAL EFFECTS

5.2. PHARMACOKINETIC PROPERTIES

(-)-α-BISABOLOL IS READILY ABSORBED BY THE SKIN. AFTER PERCUTANEOUS ADMINISTRATION OF ^{14}C(-)-α-BISABOLOL, 82% OF THE RADIOACTIVITY WAS FOUND IN THE URINE OF MICE [40, 40a]. AFTER ORAL ADMINISTRATION OF APIGENIN-7-GLUCOSIDE, FREE APIGENIN WAS DETECTED IN URINE [41]. IN GERM FREE RATS NO HYDROLYSIS OF FLAVONE GLYCOSIDES COULD BE OBSERVED. OBVIOUSLY INTESTINAL MICROFLORA CAN EFFECT THE CLEAVAGE OF THE GLYCOSIDIC BONDS [41, 42]. FURTHERMORE, ORALLY ADMINISTERED APIGENIN WAS DETECTED IN THE SERUM OF ANIMALS [43].

ESCOP MONOGRAPHS ON THE MEDICINAL USES OF PLANT DRUGS (2)

2. CLINICAL USEFULNESS

* ***ALLII SATIVI* BULBUS (GARLIC BULB)**

2.1.2. CONSTITUENTS

CAREFULLY DRIED, POWDERED MATERIAL CONTAINS ABOUT 1 PERCENT ALLIIN [(+)-S-ALLYL-L-CYSTEINE-SULPHOXIDE] [2] OR THE MAIN SULPHUR CONTAINING AMINO ACID. OTHER CHARACTERISTIC, GENUINE CONSTITUENTS ARE (+)-S-METHYL-L-CYSTEINE SULPHOXIDE, GAMMA-GLUTAMYL PEPTIDES, UBIQUITOUS AMINO ACIDS, STEROIDS AND ADENOSINE [3]. IN THE PRESENCE OF THE ENZYME ALLIINASE, ALLIIN WILL BE CONVERTED TO ALLICIN (A MG OF ALLICIN IS CONSIDERED TO BE EQUIVALENT TO 0.45 MG OF ALLICIN) [4].

IN TURN, ALLICIN IS THE PRECURSOR OF VARIOUS TRANSFORMATION PRODUCTS, INCLUDING AJOENES, VINYLDITHIINES, OLIGOSULPHIDES AND POLYSULPHIDES, DEPENDING ON THE CONDITIONS APPLIED [4].

MATERIAL DERIVED FROM GARLIC BY STEAM DISTILLATION OR EXTRACTION IN AN OILY MEDIUM CONTAINS VARIOUS ALLICIN TRANSFORMATION PRODUCTS [4-6].

4.1. THERAPEUTIC INDICATIONS

PROPHYLAXIS OF ATHEROSCLEROSIS [7-11]. TREATMENT OF ELEVATED BLOOD LIPID LEVELS INSUFFICIENTLY INFLUENCED BY DIET [12-32]).

IMPROVEMENT OF THE CIRCULATION IN PERIPHERAL VASCULAR DISEASE [33].

RESPIRATORY INFECTIONS AND CATARRHAL CONDITIONS [4, 34-36].

ESCOP MONOGRAPHS ON THE MEDICINAL USES OF PLANT DRUGS (3)

2. CLINICAL USEFULNESS

* ***CRATAEGUS* (HAWTHORN)**

5.1. PHARMACODYNAMIC PROPERTIES

* CLINICAL STUDIES

CLINICAL RESULTS DEMONSTRATE AN INCREASE IN CARDIAC PERFORMANCE AND OUTPUT [28-30], A DECREASE IN PERIPHERAL VASCULAR RESISTANCE [28-31], A DECREASE IN PULMONARY ARTERIAL AND CAPILLARY PRESSURE (31), A REDUCTION IN THE PRESSURE RATE PRODUCT AT REST [32-35] AND DURING EXERCISE [33-36], A RISE IN ERGOMETRIC TOLERANCE [28, 33, 35, 37] AND AN IMPROVEMENT IN METABOLIC PARAMETERS [34, 38].

* THERAPEUTIC INDICATIONS

DECLINING CARDIAC PERFORMANCE EQUIVALENT TO STAGES I AND II OF THE NYHA (NEW YORK HEART ASSOCIATION) CLASSIFICATION. CASES OF SENILE HEART WHILE DIGITALIS IS NOT YET REQUIRED [28, 29, 32-34, 39-44].

ESCOP MONOGRAPHS ON THE MEDICINAL USES OF PLANT DRUGS (4)

3. TOXICOLOGICAL ASPECTS

* ***VALERIANAE* RADIX (VALERIAN ROOT)**

2.1.2. CONSTITUENTS

VALEPOTRIATES MAY BE PRESENT IN THE ROOT [7-10] BUT ARE UNSTABLE AND UNLIKELY TO BE PRESENT IN FINISHED PRODUCTS [3, 4, 11].

SINCE VALEPOTRIATES ARE GENERALLY ABSENT FROM PREPARATIONS, THEIR PHARMACOLOGICAL AND TOXICOLOGICAL PROPERTIES HAVE BEEN EXCLUDED FROM SECTION 5.

* ***HYPERICI* HERBA (ST. JOHN'S WORT)**

4. CLINICAL PARTICULARS

4.8. UNDESIRABLE EFFECTS

NONE CONFIRMED AT DOSE LEVELS UP TO 1 MG OF TOTAL HYPERICIN [48-50]. PHOTOSENSITIZATION MIGHT OCCUR AT HIGHER DOSAGE [51].

4.9. OVERDOSE

PHOTOSENSITIZATION AT HIGH DOSAGE HAS BEEN REPORTED DURING EXPERIMENTAL ANTIVIRAL TREATMENT WITH SYNTHETIC HYPERICIN (35 MG IV) IN HIV-POSITIVE PATIENTS [51]. TYPICAL PHOTOTOXIC SYMPTOMS INCLUDE RASH, PRURITIS AND ERYTHEMA 24 HOURS AFTER EXPOSURE TO UV LIGHT. TREATMENT CONSISTS OF AVOIDING EXPOSURE TO LIGHT.

RESOLUTION

THE PARTICIPANTS OF THE THIRD INTERNATIONAL SCIENTIFIC ESCOP SYMPOSIUM, "RESEARCH AND THERAPY WITH PHYTOMEDICINES", HELD AT THE HAGUE-SCHEVENINGEN, THE NETHERLANDS, MARCH 18th 1994, AS WELL AS THE REPRESENTATIVES OF SCIENTIFIC ASSOCIATIONS FOR PHYTOTHERAPY FROM NINETEEN COUNTRIES SUBMIT THE FOLLOWING RESOLUTION :

1. ESCOP STIMULATES SCIENTIFIC RESEARCH INTO SAFETY AND EFFICACY OF PHYTOMEDICINES BY MEANS OF THE BIOMEDICAL AND HEALTH RESEARCH PROGRAMME OF THE EUROPEAN UNION. THIS PROGRAMME CONTAINS THE DEVELOPMENT OF EUREOPAN STANDARDS FOR SAFETY AND EFFICACY OF PHYTOMEDICINES. WITHIN THIS FRAMEWORK OF RESEARCH, THE COMPLEX NATURE OF PHYTOMEDICINES IS TAKEN INTO ACCOUNT.

2. ESCOP OFFERS WELL-CONSIDERED AND SCIENTIFICALLY BASED EXPERTISE AND INFORMATION CONCERNING PHYTOMEDICINES. IN THE INTEREST OF PUBLIC SAFETY. ESCOP HAS A COMPREHENSIVE PHARMACOVIGILANCE PROGRAMME TO MONITOR THE USE OF PHYTOMEDICINES AND PROVIDE ACCURATE INFORMATION TO REGULATORY AUTHORITIES, THE MEDIA AND GENERAL PUBLIC.

3. ESCOP SUPPORTS CLINICAL RESEARCH WITH PHYTOMEDICINES. WITHIN THIS RESEARCH THE RESULTS WITH THE USED AND CHARACTERIZED PHYTOMEDICINES SHOULD BE APPLICABLE TO COMPARABLE PREPARATIONS.

4. ESCOP IS OF THE OPINION THAT THE TREATMENT BY MEANS OF PHYTOMEDICINES IS BOTH EFFECTIVE AND ECONOMIC. THEREFORE PHYTOMEDICINES SHOULD CONTINUE TO BE A PART OF NATIONAL HEALTH CARE SYSTEMS IN EUROPE.

5. ESCOP HAS BEEN WORKING ON EUROPEAN CRITERIA FOR THE ASSESSMENT OF PHYTOMEDICINES IN ORDER TO ASSIST THE PROMOTION OF THE HARMONIZATION PROCESS SINCE 1989. TO THIS END ESCOP MONOGRAPHS HAVE BEEN PUBLISHED AND SUBMITTED TO THE CPMP.

THE HAGUE-SCHEVINGEN, THE NETHERLANDS
MARCH 18th, 1994

THIS RESOLUTION HAS BEEN ACCLAIMED BY THE COUNCIL OF ESCOP IN ITS MEETING AT THE HAGUE-SCHEVINGEN, MARCH 20th, 1994.

ACHIEVEMENTS OF ESCOP (1989-1996)

* DEVELOPMENT OF A DEFINITION FOR PHYTOMEDICINES, WHICH WAS LATER ON ADOPTED BY OTHER ORGANISATIONS.

* PRODUCTION OF PROPOSALS FOR EUROPEAN MONOGRAPHS UNDER THE FORMAT OF SUMMARY OF PRODUCT CHARACTERISTICS (SPC).

* PUBLICATION OF AN OFFICIAL NEWSLETTER CALLED EUROPEAN PHYTOTELEGRAM.

* CONTRIBUTION TO THE BIRTH OF THE WHO GUIDELINES FOR THE ASSESSMENT OF HERBAL MEDICINAL PRODUCTS.

* PARTICIPATION IN THE BIOMEDICAL AND HEALTH RESEARCH PROGRAMME (BIOMED) OF THE EUROPEAN UNION TO "DETERMINE EUROPEAN STANDARDS FOR THE SAFE AND EFFECTIVE USE OF PHYTOMEDICINES".

* ORGANISATION OF FOUR INTERNATIONAL SCIENTIFIC SYMPOSIA ON THE COURSE OF "EUROPEAN HARMONY IN PHYTOTHERAPY", OCTOBER 1990, BRUSSELS, MARCH 1992, MILAN ; MARCH 1994, THE HAGUE AND MARCH 1996, COLOGNE.

* ACCLAMATION OF A RESOLUTION ON THE OCCASION OF THE THIRD INTERNATIONAL SYMPOSIUM OF ESCOP (1994) AND DISTRIBUTION AFTERWARDS AMONGST OTHERS TO MEMBERS OF THE EUROPEAN COMMISSION, EUROPEAN PARLEMENT AND CPMP.

HYPERICI HERBA

St. John's Wort

DEFINITION

St. John's Wort consists of the dried flowering top or dried aerial part of *Hypericum perforatum* L. collected shortly before or during the flowering period. It contains not less than 0.04 per cent of naphthodianthrones of the hypericin group (so called total hypericin), calculated as hypericin ($C_{30}H_{16}O_8$; M_r 504.5).

The material complies with the Pharmacopée Française or with the Deutscher Arzneimittel-Codex [1,2].

CONSTITUENTS

Characteristic constituents are naphthodianthrones (usually 0.1-0.15 %), mainly hypericin and pseudohypericin. Lower levels than 0.1 % may result from harvesting of lower parts of the herb [3]. Further characteristic substances are the biosynthetic precursors of hypericin and pseudohypericin, i.e. protohypericin and protopseudohypericin, which are transformed into the cyclic compounds by exposure to light [4-7]. A minor component is cyclopseudohypericin [8].

Another group of constituents consists of flavones and flavonols (2-4 %), mainly quercetin glycosides including hyperoside (0.7 %), quercitrin, isoquercitrin and rutin (0.3 % each); also the aglycones quercetin, kaempferol, luteolin and myricetin [9-12]. Biflavonoids, such as 3,8´´-biapigenin and 3´,8´´-biapigenin (0.01-0.5 %), are mainly present in the flowers [13,14]. Other constituents include: essential oil (0.1-1 %) containing mainly higher n-alkanes [9,15]; characteristic xanthones (up to 10 ppm), mainly 1,3,6,7-tetra-hydroxyxanthone (norathyriol) [16]; tannins of the catechin-type (6.5-15 %) [15,17]; procyanidins [18]; and phloroglucinol derivatives, principally hyperforin (2-4 %) which is unstable [17,19].

The level of naphthodianthrones correlates with the level of hyperforin, flavones, flavonols [20] and procyanidins [21] and the level of hypericin correlates with the level of pseudohypericin [21].

CLINICAL PARTICULARS

Indications

Mild to moderate depressive states (ICD-10* category F32.0, F32.1), somatoformic

* International Classification of Diseases, Tenth Revision, Chapter V (F). World Health Organization, 1991.

disturbances including symptoms such as restlessness, anxiety and irritability [22-45].

Posology and method of administration

Dosage
Adults: standardized tinctures or fluid extracts [22,23,26-30,40,41,44], or standardized, dried hydroethanolic [24,42,43] or hydromethanolic [25,31-39] extracts, equivalent to 0.2-1 mg of total hypericin (determined by specific methods) daily; 2-4 g of the drug daily for tea infusions [45].
Elderly: dose as for adults.
Children from 6 to 12 years under medical supervision only: half the adult dose.

Method of administration
For oral administration; for local application.

Duration of administration
Usually no restriction.

Contra-indications

None known.

Special warnings and special precautions for use

If a significant treatment response in depressive disorders is not apparent after 4 to 6 weeks, the medication should be discontinued. On the other hand, as with other antidepressants, an antidepressive effect is not expected before 10 to 14 days of treatment.

Interaction with other medicaments and other forms of interaction

None reported.

Pregnancy and lactation

No data available. In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines

Clinical studies indicate no negative influence on general performance or the ability to drive [46,47].

Undesirable effects

None confirmed at dose levels up to 1 mg of total hypericin [48-50]. Photosensitization might occur at much higher dosages [51].

Overdose

Photosensitization at high dosage has been reported during experimental antiviral treatment with synthetic hypericin (35 mg intravenously) in HIV-positive patients [51]. Typical phototoxic symptoms include rash, pruritus and erythema 24 hours after exposure to ultraviolet light. Treatment consists of avoiding exposure to light.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamics

In vitro experiments

Hydroethanolic preparations of St. John's Wort have shown selective type A monoamine oxidase (MAO) inhibitory effects [52,53]. The highest activity is attributed to several flavonoid aglycones and to quercitrin. Xanthones, which are present only in minor quantities (up to 10 ppm), also appear to have a high MAO-inhibiting activity, whereas hypericin and St. John's Wort preparations have a rather low activity [54,55]. Inhibition of the enzyme catechol-O-methyl-transferase (COMT) is reported in St. John's Wort fractions containing mainly flavonoids [54].

Receptor binding studies with a hydroethanolic extract containing approx. 0.15 % of total hypericin have revealed moderate interactions with the $GABA_A$/benzodiazepine receptor/chloride-ionophore complex (displacement of ^{3}H-muscimol, ^{3}H-flunitrazepam and ^{35}S-TBPS binding). Pure hypericin resulted in potentiation of binding at the $GABA_A$ and benzodiazepine receptors and at a serotonin ($5\text{-}HT_1$) receptor [56]. The biflavonoid amentoflavone has binding activity at the benzodiazepine receptor [57].

Another hydroethanolic extract as well as pure hypericin inhibited in vitro the enzyme dopamine-β-hydroxylase [58].

A hydromethanolic extract showed a reduced expression of serotonin receptors in a neuroblastoma cell line model [59]. The same extract resulted in a massive suppression of interleukin-6 release in human blood samples after phytohaemagglutinin stimulation [60].

In vivo experiments

Animal studies in mice with hydroethanolic preparations of St. John's Wort containing known amounts of hypericin (corresponding to 2-12 mg/kg orally) have revealed CNS activities which can be interpreted as an antidepressant effect. Aggressive behaviour was significantly reduced after 3 weeks of daily treatment equivalent to 6 mg/kg and 12 mg/kg p.o. hypericin. Physical activity was enhanced, and no undesired anticholinergic effects were found. A significant increase in physical activity was also observed after administration of pure hypericin 20 mg/kg i.p. [61]. Typical sedative effects of standardized St. John's Wort preparations on potentiating the ethanol-induced sleeping time in mice have also been demonstrated (equivalent to 2, 4 and 6 mg/kg hypericin p.o.). Oral administration of an extract equivalent to 1, 2 or 3 mg/kg, but not 6 mg/kg, of hypericin in mice resulted in a reserpine antagonism, which is also indicative of antidepressant effects [61].

Externally applied St. John's Wort preparations have been reported to have antiinflammatory and antibacterial effects [75,76,78]. The antibiotic effect has been attributed to the presence of hyperforin [79]. A hydroethanolic extract equivalent to 2.8 g herb was tested in rodents for its antiinflammatory properties in the croton oil test and reduced oedema significantly by 50 % compared to control [80]. In the same study it was concluded from fractionation experiments that the antiinflammatory principle is

concentrated in the lipophilic fractions.

In different in vitro and in vivo models strong antiviral effects of synthetic hypericin (0.5 mg/kg in vivo) have been found [62-70].

Pharmacological studies in humans

A significant increase in urinary neurotransmitter metabolites was observed 2 hours after oral administration of a standardized hydroethanolic preparation of St. John´s Wort to 6 women with depressive symptoms [71]. The same preparation, equivalent to 0.5 mg total hypericin or 1.4 g herb, was studied for effects on the electroencephalogram (EEG) of 40 depressive patients after 4 weeks of treatment [41]. The results have been interpreted as predominantly relaxing effects (increase in theta-activity, decrease in alpha-activity and no change in beta-activity). Compared to the decrease of alertness after bromazepam this particular effect on alpha waves was much smaller after administration of St. John´s Wort [41].

In another EEG study in 12 healthy volunteers, after 6 weeks medication with 900 mg of a hydromethanolic extract a reduction in the alpha and an increase in the theta and beta frequencies, as well as reduction of audio-visual latencies in evoked potentials, was shown. The study was conducted in a double-blind, placebo controlled cross-over design with two weeks of medication-free time between the two phases [72]. 4 weeks treatment with 300 mg t.i.d. with another hydromethanolic preparation improved sleep quality with an increase in deep sleep phases [73].

In a study with 24 healthy volunteers, the effects of the same hydromethanolic preparation (300 mg t.i.d. over 4 weeks) on the resting electroencephalogram as well as on visually and acoustically evoked potentials were compared with maprotiline (10 mg t.i.d.). In resting EEG these medications revealed oppositely directed changes in the theta frequencies (increase with St. John´s Wort and decrease with maprotiline) and mainly similarly directed changes in alpha and beta frequencies. The overall results of the study have been interpreted as a tendency to improvement of cognitive functions due to the St. John´s Wort preparation [74].

In another study with 13 healthy volunteers, a significant increase in the nocturnal melatonin plasma concentration was observed after 3 weeks administration of a hydroethanolic St. John´s Wort preparation at a daily dosage equivalent to 0.53 mg of total hypericin. The increased production of melatonin (particularly the increase in nocturnal amplitude) is in accordance with the subchronic effects on melatonin profiles in depressive patients treated with desipramine and amitriptyline [77].

Clinical studies

Besides numerous case reports and drug monitoring studies with more than 5000 patients on the efficacy and safety of standardized hydroalcoholic St. John´s Wort preparations [22-25], 19 controlled double blind and open studies have been conducted, involving more than 2000 patients and 7 different hydroalcoholic St. John´s Wort preparations [26-44]. The major indication in the studies was mild to moderate depressive disorders. In most of the studies a signifi-

cant improvement of main symptoms (mood, loss of interest and activity) and other symptoms (sleep, concentration, somatic complaints) of the depressive syndrome have become evident. The activity was proven against placebo and against different antidepressants and tranquillizers (amitriptyline, imipramine, maprotiline, diazepam, bromazepam).

In a preliminary study of a St. John´s Wort preparation combined with light therapy in patients with seasonal affective disorders the antidepressant effect of St. John´s Wort was enhanced by light therapy [39].

Pharmacokinetics

The absorption and distribution of orally administered radioactively labelled ^{14}C-hypericin and ^{14}C-pseudohypericin were studied in mice [81]. It could be demonstrated that 6 hours after application 80 % of hypericin and 60 % of pseudohypericin were absorbed. The distribution was not indicative of selective accumulation in certain organs and the main radioactivity was found in blood. Radioactivity was also identified in the brain.

The bioavailability of hypericin and pseudohypericin has been studied in two investigations, with 14 and 12 volunteers respectively. The doses were equivalent to 0.1 % and 0.3 % respectively of total hypericin in a hydromethanolic extract (300-1800 mg). The following results were obtained for hypericin with the 0.1 % extract: t_{max} 2.5 hours, c_{max} 4.3 ng/ml, plasma half-life approximately 6 hours [82]. With the 0.3 % extract the parameters for hypericin and pseudohypericin were as follows: hypericin t_{max} 4-6 hours, c_{max} 1.5-14.2 ng/ml, plasma half-life 24.8-26.5 hours; pseudohypericin t_{max} 2-4 hours, c_{max} 2.7-30.6 ng/ml, plasma half-life 16.3-36 hours [83]. Data on excretion of hypericin are not reported.

Preclinical Safety Data

There are no systematic studies on single dose toxicity, on reproductive toxicity or on carcinogenicity.

At therapeutically relevant dosages of total hypericin, i.e. up to 1 mg daily over 8 days, it was shown in an experimental, double-blind, placebo-controlled study with 40 volunteers that photo-sensitivity was not induced [50]. In a study with the i.v. application of synthetic hypericin in HIV-infected patients (reversible) symptoms of phototoxicity were observed at the highest dosage regime which was 35 times higher than the highest oral dosage of total hypericin used in the therapy of depressive disorders [51].

Photosensitization caused by St. John´s Wort is mainly known from veterinary studies. Phototoxic symptoms occurred in a dose-dependent manner in light-coloured cattle after substantial feeding on fresh St. John´s Wort [84-87]. From this finding, it is estimated that 30 times the therapeutic dose would be necessary to produce the first phototoxic symptoms in humans.

The genotoxicity of hydroethanolic St. John´s Wort extracts containing 0.2 to 0.3 % of total hypericin and usually less than 0.1 % quercetin has been studied in different in vitro and in vivo test systems with

mammalian cells [88-92]. The in vitro investigations were performed with the Ames test, the HGPRT (hypoxanthine guanidine phosphoribosyl transferase)-test (up to 4 µl/ml *Hypericum* extract), UDS (unscheduled DNA synthesis)-test (up to 1.37 µl/ml *Hypericum* extract) and with the cell transformation test using Syrian hamster embryo cells (up to 10 µl/ml *Hypericum* extract). As in vivo tests, the spot test of the mouse (up to 10 ml/kg of extract), the chromosome aberration test with bone marrow cells of the Chinese hamster (oral dose of extract 10 ml/kg) and the micronucleus test with bone marrow of mice (oral dose of extract up to 2000 mg/kg) were employed. All in vivo and most in vitro test results were negative, indicating no mutagenic potential of defined St. John´s Wort preparations prepared from hydroethanolic extraction. The observed positive findings in the Ames mutagenicity test were attributed solely to the presence of quercetin [90].

The potential genotoxicity of quercetin, an ubiquitous substance in many fruits and vegetables (estimated daily intake from food: at least 25 mg), has been studied extensively [93,94]. Most of the in vivo studies yielded negative results. A recent 2-year carcinogenicity study at the U.S. National Institutes of Health resulted in an increased rate of renal tubular cell adenomas, but only in the highest dosage group (4 %) and only in male rats [95]. The results have been interpreted as not relevant to humans [96,97].

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URTICAE RADIX

Nettle Root

DEFINITION

Nettle root consists of the whole, cut or powdered, dried roots and rhizomes of *Urtica dioica* L., *Urtica urens* L., their hybrids or mixtures of these.

The material complies with the Deutsches Arzneibuch [1].

CONSTITUENTS

Urtica dioica agglutinin (UDA) approx. 0.1 % [2-6], a lectin which can be separated into 6 isolectins [7]; a polysaccharide mixture (2 glucans, 2 glucogalacturonans and 1 arabinogalactan) [6,8,9]; scopoletin approx. 0.002-0.01 % [10-12]; 3-β-sitosterol approx. 0.2-1 % [10-12], sitosterol-3-β-D-glucoside approx. 0.05-0.2 % [11,12], similar sterols and sterol glucosides [12,13]; phenyl propanes (homovanillyl alcohol and its glucoside) [12,14]; lignans such as neo-olivil and its derivatives [12,14] and lignan glucosides [12,14-16]; ceramides [17]; fatty acids including (10E,12Z)-9-hydroxy-10,-12-octadecadienoic acid [18]; and also monoterpene diols and glucosides [19].

CLINICAL PARTICULARS

Therapeutic indications

Symptomatic treatment of micturition disorders (nocturia, pollakisuria, dysuria, urine retention) in benign prostatic hyperplasia (BPH) of stages I and II as defined by Alken [20-40].

Posology and method of administration

Dosage

Daily dose: 4-6 g of the drug as an infusion [35,40]; 600-1200 mg of a dried extract preparation (5:1, 20 % methanol) [20,21, 23,24,26-28,30-32,39]; 1.5-7.5 ml fluid extract (1:1, 45 % ethanol) [37], or 5 ml ethanolic extract (1:5, 40 % ethanol) [38].

Method of administration

For oral administration.

Duration of administration

No restriction.

Contra-indications

None known.

Special warnings and special precautions for use

Difficulties in micturition require at all times clarification by a physician and regular medical controls in order to exclude the need for other treatment, e.g. surgical intervention. Consultation with a physician is necessary especially in cases of blood in the urine and acute urine retention.

Interaction with other medicaments and other forms of interaction

None reported.

Pregnancy and lactation

Not applicable.

Effects on ability to drive and use machines

None known.

Undesirable effects

Gastric complaints [21,24].
Rare cases of allergic skin reactions [21].

Overdose

No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

There are three main hypotheses about the pathogenesis of BPH:

(1) An increased production of dihydrotestosterone (DHT) by the prostate leads to an alteration of the androgen/oestrogen-ratio with an increase of oestrogens. A possible therapy might be the inhibition of 5-α-reductase which reduces testosterone to dihydrotestosterone and the inhibition of aromatase which converts testosterone into 17-β-oestradiol [40-44].

(2) An increased concentration of prostaglandins and leucotrienes is another possible cause of BPH. Therapy could consist of an inhibition of the eicosanoid metabolism by inhibiting phospholipase, prostaglandin synthetase and/or lipoxygenase [40-42].

(3) An increased binding capacity of the sex hormone binding globulin (SHBG) to testosterone and dihydrotestosterone results in hyperplasia as a compensation for a decrease in hormones and in an increase of 5-α-reductase activity [45,46]. The therapy could be a reduction of the SHBG binding capacity for androgens. Although this hypothesis seems to be the most probable explanation, the mechanism is still unknown, and many biochemical pathways seem to play a role in BPH [22,40,42].

<u>In vitro experiments</u>
A significant suppression (average 67 %) of the SHBG binding capacity in the presence of an Urtica root extract preparation (5:1, 20 % methanol) could be shown in vitro. It appears that the 5-α-DHT binding to proteins can be influenced by the extract [46]. An aqueous extract of nettle roots inhibited binding of SHBG to solubilized receptors from human prostatic tissue [47].

The lignan secoisolariciresinol as well as a mixture of isomeric (11E)-9,10,13-trihydroxy-11-octadecenoic and (10E)-9,12-, 13-trihydroxy-10-octadecenoic acids isolated from *Urtica dioica* root extract reduced binding activity of human SHBG. So did the mixture of the latter two after methylation [48].

(10E,12Z)-9-hydroxy-10,12-octadecadienoic acid isolated from an aqueous-methanolic root extract inhibited aromatase activity [18,49]. On the other hand, aromatase inhibition by 5 other compounds isolated from nettle root extract was only weak (less than 1 % compared to 4-hydroxy-androst-4-ene-3,17-dione) [50].

A polysaccharide fraction obtained from an aqueous extract was shown to be active in the lymphocyte transformation test. Isolated polysaccharides produced a dose-dependent reduction of haemolysis in the classical and alternative complement test. From these results an antiinflammatory and immunomodulating effect was deduced [6,8,51]. Isolated *Urtica dioica* lectins were found to stimulate the proliferation of human lymphocytes in the lymphocyte transformation test [6,8].

Organic solvent extracts of *Urtica dioica* root gave 28-82 % inhibition of Na/K-ATP ase activity of human BPH-tissue cells. Steroidal compounds of the root, such as stigmast-4-en-3-one, stigmasterol and campesterol, inhibited the enzyme activity by 23.0-67.0 % at concentrations ranging from 10^{-3} to 10^{-6} M. These results suggest that some hydrophobic constituents such as steroids inhibit the membrane Na/K-ATPase activity of the prostate which may subsequently suppress prostate-cell metabolism and growth [52].

The lectin fraction gave a 53 % inhibition of the binding of epidermal growth factor (EGF) to EGF-receptors in cultivated cells from human prostatic tissue [6]. Fractions from the 20 % methanolic extract of Urtica root gave a statistically significant growth inhibition of cultured human BPH-tissue cells [53]. UDA from an *Urtica dioica* root extract showed a dose-dependent inhibition of EGF-binding to human A 431 epidermal cancer cell membranes [54].

In vivo experiments

An average decrease of 30 % of prostate volume after a 100-day treatment with 90 mg of a 20 % methanolic extract (5:1) of Urtica root per kg body weight was shown in 10 dogs suffering from BPH [55]. The same extract did not inhibit testosterone and dihydrotestosterone stimulated growth of the prostate in castrated rats [56].

A polysaccharide fraction obtained from an aqueous extract of *Urtica dioica* root was found to be active in the carrageenan rat paw oedema model [6,8,51]. A crude extract from nettle root containing 4 different polysaccharides was shown to possess anti-inflammatory activity comparable to indomethacin in the rat paw oedema test 5 hours after oral administration [57].

Pharmacological studies in humans

31 men between 58 and 82 years with BPH stages I to II were treated with 1200 mg of a dried standardized Urtica root extract preparation (5:1; 20 % methanol) daily for

20 weeks. By fine needle aspiration biopsies of the prostate at four-weekly intervals a morphologically relevant effect on the prostate adenoma cells was found that may be due to a competitive inhibition of the SHBG binding capacity of the extract [58].

Prostatic cells taken from 33 BPH patients by needle biopsy were treated with Urtica root extract. A decrease of homogenous granules was detected by fluorescence microscopy, thus showing that the biological activity in these hyperplasic cells had decreased [59]. The presence of Urtica root extract constituents or their metabolites in BPH tissue was demonstrated by fluorescence microscopy after in vivo and in vitro application of the extract [60].

Morphological examination of BPH tissue before and after therapy with Urtica root extract confirmed ultrastructural changes in the smooth muscle cells of the prostate [61]. The extract also caused a decrease of cell proliferation in tissue cultures taken from BPH patients [62].

It has been shown that UDA binds to the cell membrane of prostatic adenoma cells [63] and inhibits their proliferation [64].

Clinical studies

The following clinical studies have been performed with dried standardized extract (5:1, 20% methanol) preparations of Urtica root:

50 BPH I-II patients were enrolled in a double-blind, controlled study. 25 patients were treated with 600 mg extract daily during 9 weeks, 25 received placebo. A significant ($p<0.05$) improvement of micturition volume (44 % increase) was observed as well as a highly significant decrease in serum levels of SHBG ($p=0.0005$). The latter is probably due to the SHBG binding capacity of the extract [24].

In a double-blind, controlled study with 40 patients (n=20: 1200 mg extract/day, n=20: placebo) a statistically significant ($p\leq0.05$) decrease of micturition frequency and of SHBG levels was demonstrated in the verum group after 6 months [39].

A 14 % improvement of urinary flow and 40-53 % improvement of residual urine were observed in 32 BPH patients who received 600 mg extract per day during 4-6 weeks in a controlled (placebo: n=35), double-blind study [28].

In a field study with 5492 patients receiving 600-1200 mg extract per day during 3-4 months significant improvements in nycturia and micturition frequency were observed [21]. A 50 % decrease in nycturia was shown in a field study with 4051 BPH patients who received 1200 mg extract per day during 10 weeks [23]. 4480 BPH patients received 600-1200 mg extract per day during 20 weeks in another field study. There was a significant ($p<0.01\%$) improvement of symptoms such as impaired urinary flow and residual urine [32].

111 BPH patients with nycturia received 1200 mg extract per day for 10 weeks. Nocturnal micturition frequency decreased in 55 % of the cases [26]. 37 out of 39 BPH I-III patients experienced an improvement of urinary flow and a reduction of residual

urine, nycturia and pollakisuria after a 6-month treatment with 600-1200 mg extract per day [27]. 67 out of 89 BPH patients receiving 600 mg extract per day during 3-24 months in an open study had decreased residual urine [20].

A decrease of prostate volume and of residual urine as well as a significant ($p<0.05$) decrease of SHBG, oestradiol and oestrone were observed in an open study with 253 BPH patients who received 1200 mg extract per day during 12 weeks [30]. A decrease of prostate volume in 54 % of the cases and a decrease of residual urine in 75 % of the cases were observed in an open study with 26 BPH patients who received 1200 mg extract per day during 4-24 weeks [31].
The following studies have been performed with other preparations:

4087 BPH and prostatitis patients received 600-1200 mg of a mixed extract of Urtica root and herb. A 36 % improvement of urinary flow was observed in 49 % of the cases as well as a decrease of residual urine in 62 % of the cases [29].

30 days treatment with 30-150 drops of a fluid extract (Ph.F., 45% ethanol) led to a 66 % decrease in residual urine in a study with 10 BPH patients [37].

In a study with 67 BPH patients a reduction of nocturnal micturition frequency was observed after 6 months treatment with a daily dose of 5 ml extract (1:5, 40 % ethanol) [38].

Pharmacokinetic properties

No data available.

Preclinical safety data

No data available.

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LEGAL STATUS OF PHYTOMEDICINES IN NORTHERN EUROPE

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PHYTOMEDICINES IN THE EEC

HISTORICAL PERSPECTIVE

*	MARCH, 1978	COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS **(CPMP)** WORKING GROUP
*	NOVEMBER, 1988	NOTE FOR GUIDANCE **"QUALITY OF HERBAL REMEDIES"**
*	OCTOBER, 1990	INAUGURAL **ESCOP** SYMPOSIUM IN BRUSSELS FIRST SERIES OF DRAFT MONOGRAPHS ON HERBAL REMEDIES
*	JANUARY, 1991	**PHARMACEUTICS COMMITTEE** OF THE EEC DISCUSSION ON EVALUATION OF HERBAL DRUGS
*	MARCH, 1991	START IN CPMP
*	NOVEMBER, 1992	COMMENTS TO ESCOP
*	MAY, 1993	NEW SUMMARY OF PRODUCT CHARACTERISTICS **(SPC)** BY ESCOP
*	FEBRUARY, 1994	COMMENTS BY MEMBER STATES
*	MARCH, 1994	PROPOSED **FINAL SPCs** FOR ASSESSMENT
*	JUNE, 1994 -	ADOPTION BY THE CPMP OF **FOUR SPCs** ON HERBAL LAXATIVES (*FRANGULA* AND *SENNA*(3))
	-	ADOPTION OF *MATRICARIAE* FLOS AND *VALERIANAE* RADIX DELAYED
*	DECEMBER, 1996	COMPLETION OF **50 HARMONIZED SPC PROPOSALS**

DEFINITION OF HERBAL REMEDIES

WHO SIXTH INTERNATIONAL CONFERENCE

OF DRUG REGULATORY AUTHORITIES

OTTAWA, OCTOBER 1991

"FINISHED, LABELLED MEDICINAL PRODUCTS THAT CONTAIN AS ACTIVE INGREDIENTS AERIAL OR UNDERGROUND PARTS OF PLANTS, OR OTHER PLANT MATERIAL, OR COMBINATIONS THEREOF, WHETHER IN THE CRUDE STATE OR AS PLANT PREPARATIONS.

PLANT MATERIAL INCLUDES JUICES, GUMS, FATTY OILS, ESSENTIAL OILS AND ANY OTHER SUBSTANCES OF THIS NATURE.

HERBAL MEDICINES MAY CONTAIN EXCIPIENTS IN ADDITION TO THE ACTIVE INGREDIENTS.

MEDICINES CONTAINING PLANT MATERIAL COMBINED WITH CHEMICALLY DEFINED ACTIVE SUBSTANCES, INCLUDING CHEMICALLY DEFINED, ISOLATED CONSTITUENTS OF PLANTS, ARE NOT CONSIDERED TO BE HERBAL MEDICINES".

ARE PHYTOMEDICINES CONSIDERED AS MEDICINES WITHIN THE EUROPEAN FRAMEWORK OF LEGISLATION? (1)

EUROPEAN DIRECTIVE 65/65 EEC (26.01.1965)

ARTICLE 1

MEDICINAL PRODUCT

ANY SUBSTANCE OR COMBINATION OF SUBSTANCES PRESENTED FOR TREATING OR PREVENTING DISEASE IN HUMAN BEINGS OR ANIMALS.

ANY SUBSTANCE OR COMBINATION OF SUBSTANCES WHICH MAY BE ADMINISTERED TO HUMAN BEINGS OR ANIMALS WITH A VIEW TO MAKING A MEDICINAL DIAGNOSIS OR TO RESTORING, CORRECTING OR MODIFYING PHYSIOLOGICAL FUNCTIONS IN HUMAN BEINGS OR IN ANIMALS IS LIKEWISE CONSIDERED AS MEDICINAL PRODUCT

ARE PHYTOMEDICINES CONSIDERED AS MEDICINES WITHIN THE EUROPEAN FRAMEWORK OF LEGISLATION? (2)

EUROPEAN COURT OF JUSTICE

JUDGMENT OF 28 OCTOBER 1992

1. "... A PRODUCT RECOMMENDED OR DESCRIBED AS HAVING **PROPHYLACTIC** OR **THERAPEUTIC** PROPERTIES IS A MEDICINAL PRODUCT WITHIN THE MEANING OF THE FIRST SUBPARAGRAPH OF

ARTICLE 1(2) OF CD 65/65 EEC

... EVEN IF IT IS GENERALLY REGARDED AS **A FOODSTUFF** AND IN THE CURRENT STATE OF SCIENTIFIC KNOWLEDGE HAS **NO KNOWN THERAPEUTIC EFFECT**"

2. "... A PRODUCT WHOSE THERAPEUTIC PROPERTIES ARE DESCRIBED ONLY IN DOCUMENTATION SUCH AS A BROCHURE SENT ON DEMAND ... AFTER SALE, OR BY THE MANUFACTURER, OR THE SUPPLIER OF THE PRODUCT, OR BY A THIRD PARTY MAY, WHERE IN THE LATTER CASE THE THIRD PARTY IS NOT ACTING TOTALLY INDEPENDENTLY OF THE MANUFACTURER OR SUPPLIER, BE DESCRIBED AS A MEDICINAL PRODUCT ..."

HERBAL REMEDIES AS MEDICINAL PRODUCTS (1)

HERBAL REMEDIES ARE MEDICINAL PRODUCTS

ARTICLE 1 OF CD 65/65 EEC

AS FINISHED MEDICINAL PRODUCTS

THEY ARE LIABLE TO APPROVAL

FOLLOWING ARTICLE 3 OF CD 65/65 EEC

COUNCIL DIRECTIVE 65/65 (26.01.1965)

ARTICLE 3

NO PROPRIETARY MEDICINAL PRODUCT MAY BE PLACED ON THE

MARKET IN A MEMBER STATE UNLESS AN AUTHORISATION HAS BEEN

ISSUED BY THE COMPETENT AUTHORITY OF THE MEMBER STATE

HERBAL REMEDIES AS MEDICINAL PRODUCTS (2)

A MARKETING AUTHORISATION ACCORDING **TO ARTICLE 4 OF CD 65/65 EEC** IS OBLIGATORY

THE APPLICANT HAS TO SUBSTANTIATE **QUALITY, SAFETY** AND **EFFICACY** OF HIS PRODUCT FOLLOWING

CD 75/318 EEC

AT A EUROPEAN LEVEL, THE DEFINITIVE STATEMENT ON THE RESULTS OF THE ASSESSMENT PROCESS OF A MEDICINAL PRODUCT IS **THE SUMMARY OF PRODUCT CHARACTERISTICS, SPC**

CONTENT OF SPC

* **DEFINITION**
  * **CONSTITUENTS**
  * **CLINICAL PARTICULARS**
    - THERAPEUTIC INDICATIONS
    - POSOLOGY AND METHOD OF ADMINISTRATION
    - CONTRA-INDICATIONS
    - INTERACTIONS
    - UNDESIRABLE EFFECTS
  * **PHARMACOLOGICAL PROPERTIES**
    - PHARMACODYNAMICS
    - PHARMACOKINETICS
    - PRECLINICAL SAFETY DATA

HERBAL REMEDIES AS MEDICINAL PRODUCTS (3)

1.QUALITY

* EEC-NOTE FOR GUIDANCE **"QUALITY OF HERBAL REMEDIES"**

  A CLEAR BOTANICAL IDENTIFICATION AND A DETAILED DEFINITION AND CONTROL OF THE STEPS OF THE MANUFACTURING PROCESS IS NECESSARY TO ASSURE A **CONSISTENT QUALITY** OF HERBAL MEDICINAL PRODUCTS.

* MONOGRAPHS OF **THE EUROPEAN PHARMACOPOEIA** SHOULD NOT ONLY **COVER VEGETABLE DRUGS**, BUT ALSO PLANT **RAW MATERIALS** OBTAINED AFTER TREATMENT AND **TINCTURES** AND **EXTRACTS**.

HERBAL REMEDIES AS MEDICINAL PRODUCTS (4)

2. SAFETY

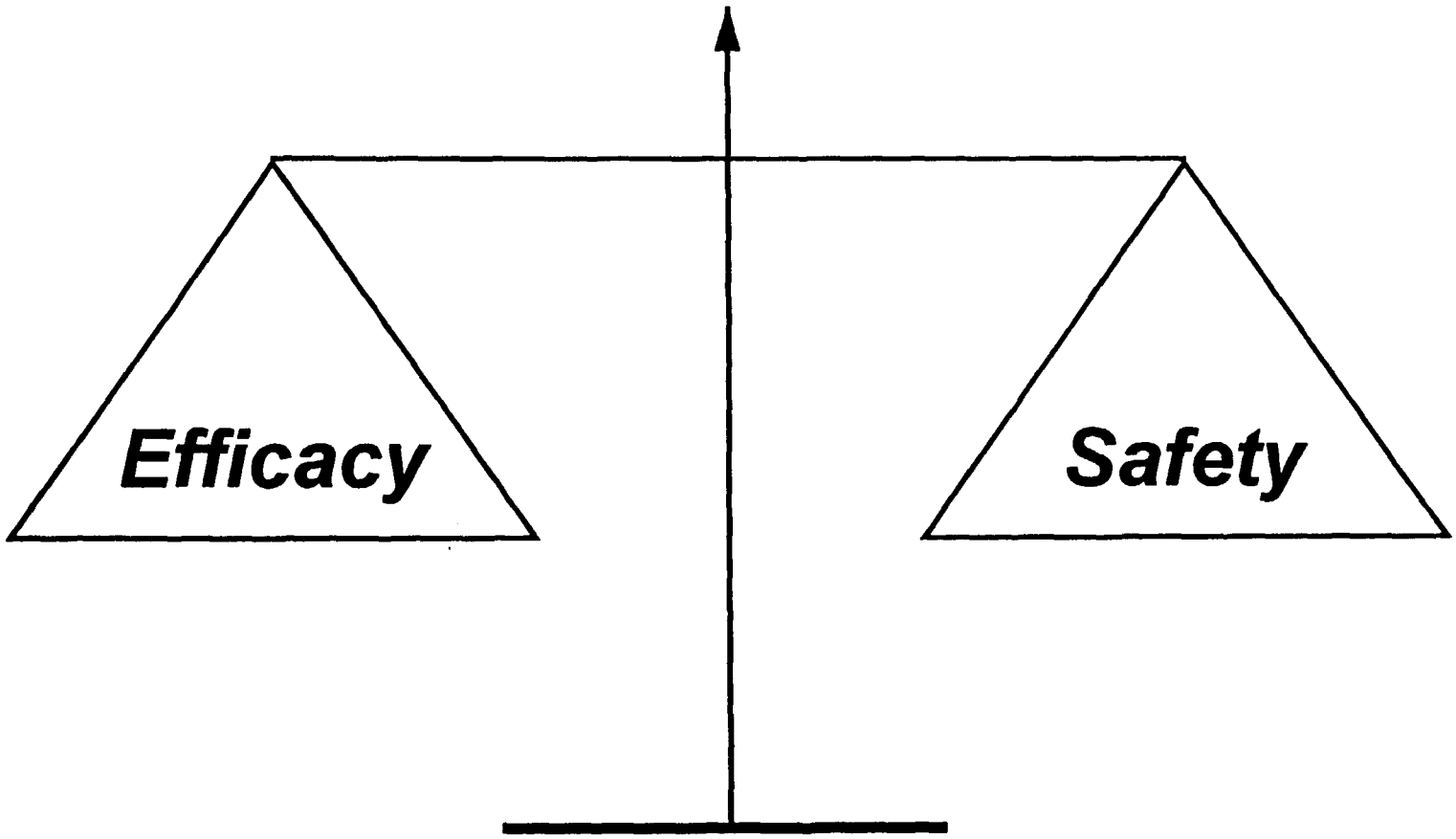
* A **CLEAR DEFINITION** OF A HERBAL DRUG AND **QUALITY CONTROL** ARE FUNDAMENTAL STEPS TOWARDS A **SAFE USE** OF HERBAL REMEDIES

* IN SOME CASES MORE DATA RELATED TO UNWANTED INGREDIENTS OR **"NEGATIVE MARKERS"** ARE NECESSARY TO ASSESS THE **SAFETY** OF A HERBAL MEDICINAL PRODUCT.
A HARMONISATION OF OPINIONS ON SAFETY ASPECTS MUST BE BASED ON A **HARMONIZED DEFINITION** OF HERBAL DRUGS

* A LIST HAS BEEN MADE OF HERBAL DRUGS WITH **MORE SERIOUS RISKS AND NO PROVEN BENEFIT**, WHICH ARE NOT ACCEPTABLE FOR A MARKETING AUTHORISATION AT THE PRESENT STATE OF KNOWLEDGE

* HERBAL DRUGS WITH A PARTICULARLY HIGH RISK REQUIRE A MORE DETAILED DISCUSSION CONCERNING **THE BENEFIT TO RISK RATIO**. THEREFORE, **A SYSTEMATIC RISK EVALUATION** SHOULD INCLUDE THE PLAUSIBLE PROOF **OF EFFICACY**

The Balance



HERBAL DRUGS WITH SERIOUS RISKS WITHOUT ANY ACCEPTED BENEFIT (GERMANY) (1)

PLANT NAME	PLANT PART	TOXIC INGREDIENT(S)
ACONITUM SP.	ALL PARTS	ACONITINE
ANGELICA ARCHANGELICA L.	FRUIT, HERB	FURANOCOUMARINES
ARITSTOLOCHIA SP.	ALL PARTS	ARTISTOLOCHIC ACIDS
ARTEMISIA CINA (BERG) WILLK.	FLOWER-BUDS	SANTONIN
BERBERIS VULGARIS L.	BARK, ROOT	BERBERINE
BORAGO OFFICINALIS L.	HERB, FLOWERS	PYRROLIZIDINES
BRYONIA SP.	ROOT	CURCURBITACINES
CHENOPODIUM AMBROSIOIDES L.	ESSENTIAL OIL	ASCARIDOLE
CHRYSANTHENUM VULGARE (L.) BERN.	FLOWER, HERB	THUJONE
CITRULLUS COLOCYNTHIS (L.) SCHRAD.	FRUIT	CUCURBITACINES
CONVOLVULUS SCAMMONIA L.	RESIN	GLYCORESINS
CROTON TIGLIUM L.	SEED	PHORBOL ESTERS
CYNOGLOSSUM OFFICINALE L.	HERB	PYRROLIZIDINES
DRYOPTERIS FILIX MAS (L.) SCHOTT	RHIZOME	PHLOROGLUCINOLS
EXEGONIUM PURGA (WEND.) BENTH	ROOT, RESIN	RESIN
JUGLANS REGIA L.	FRUIT SHELL	JUGLONE
JUNIPERIS SABINA L.	HERB	ESSENTIAL OIL
LEDUM PALUSTRE L.	HERB	ESSENTIAL OIL

HERBAL DRUGS WITH SERIOUS RISKS WITHOUT ANY ACCEPTED BENEFIT (GERMANY) (2)

PLANT NAME	PLANT PART	TOXIC INGREDIENT(S)
MALLOTUS PHILLIPENSIS (LAM.) MÜLL ARG	GLAND, TRICHOMES	PHLOROGLUCINOLS
OCIMUM BASILICUM L.	ESSENTIAL OIL	ESTRAGOL
PETASITES HYBRIDUS (L.) GART. *PETROSELINUM CRISPUM* (HILL) NYM. EX. A.W.HILL *PULSATILLA VULGARIS* MILL.	LEAF FRUIT HERB	PYRROLIZIDINES APIOL ALKALOIDS
RUTA GRAVEOLENS L. *RUBIA TINCTORUM* L.	HERB, LEAF HERB, LEAF	FURANOCOUMARINES LUCIDIN
SASSAFRAS ALBIDUM (NUTT) NEES *SENECIO* SP. *STRYCHNOS NUX VOMICA* L. *SYMPHYTUM* SP.	WOOD, ROOTS HERB, ROOTS SEED HERB, LEAVES, ROOTS	SAFROLE PYRROLIZIDINES STRYCHNINE PYRROLIZIDINES
TEUCRIUM CHAMAEDRIS L. *TUSSILAGO FARFARA* L.	HERB FLOWERS, ROOTS	HEPATOTOXIC PYRROLIZIDINES
VINCA MINOR L.	HERB, LEAVES	HEMATOLOGICAL DISORDERS

HERBAL REMEDIES AS MEDICINAL PRODUCTS (5)

3. EFFICACY(1)

* A MEDICINAL PRODUCT IS ESSENTIALLY CHARACTERIZED BY ITS **THERAPEUTIC INDICATIONS**, WHICH ARE BASED ON KNOWN LEVEL OF **EFFICACY**.
  YET PROOF OF EFFICACY IS NOT ONLY PROVIDED BY RECENT **DOUBLE BLIND RANDOMISED CLINICAL TRIALS**

COUNCIL DIRECTIVE 65/65
OF 26 JANUARY 1965

ARTICLE 4

(No. 8a)
THE APPLICANT SHALL NOT BE REQUIRED TO PROVIDE THE RESULTS OF PHARMACOLOGICAL AND TOXICOLOGICAL OR THE RESULTS OF CLINICAL TRIALS IF HE CAN DEMONSTRATE :

(ii) ... BY DETAILED REFERENCE TO **PUBLISHED SCIENTIFIC LITERATURE** PRESENTED IN ACCORDANCE WITH THE SECOND ARTICLE OF **DIRECTIVE 75/318 EEC** THAT THE CONSTITUENT OR CONSTITUENTS OF THE PROPRIETARY MEDICINAL PRODUCT HAVE **A WELL ESTABLISHED MEDICINAL USE**, WITH **RECOGNIZED EFFICACY** AND AN **ACCEPTABLE LEVEL OF SAFETY**

HERBAL REMEDIES AS MEDICINAL PRODUCTS (6)

3. EFFICACY(2)

* A **CLEAR DEFINITION** OF THE HERBAL DRUG PREPARATION IS THE BASIS FOR ANY DISCUSSION OF **EFFICACY**.

  ONE HERBAL PREPARATION IS CONSIDERED AS **ONE ACTIVE CONSTITUENT** EVEN IF IT IS COMPOSED OF MANY DIFFERENT CHEMICALLY DEFINED SUBSTANCES

* IN SOME CASES, WHEN ACTIVE INGREDIENTS OF HERBAL PREPARATIONS ARE WELL KNOWN,

  A **REPRODUCIBLE THERAPEUTIC ACTIVITY** CAN BE ASSURED BY A **STANDARDISATION** OF THESE ACTIVE INGREDIENTS

  EVEN IN THOSE CASES, THE THERAPEUTIC ACTIVITY MAY BE INFLUENCED BY OTHER CONSTITUENTS OF THE HERBAL PREPARATION, WHICH MAY ENHANCE, PREVENT OR PROLONG **ABSORPTION**

* AS A CONSEQUENCE, EVERY ASSESSMENT OF EFFICACY MUST BE BASED **ON THE INDIVIDUAL HERBAL DRUG PREPARATION**

  THIS MAY INCLUDE ONE ASSESSMENT FOR A NUMBER OF SIMILAR PREPARATIONS COVERED BY A MONOGRAPH OF THE EUROPEAN PHARMACOPOEIA

* A **CLEAR CORRELATION** BETWEEN THE **PREPARATION**, THE **DOSAGE** AND THE **THERAPEUTIC EFFECT** MUST BE ESTABLISHED IN CASES WHERE **BIBLIOGRAPHIC DATA** ARE PRESENTED TO SUBSTANTIATE **EFFICACY** AND **SAFETY**

HERBAL REMEDIES AS MEDICINAL PRODUCTS (7)

3. EFFICACY(3)

* SEVERAL MEMBER STATES (**BELGIUM, FRANCE**) HAVE INTRODUCED DIFFERENT LEVELS IN THE WORDING OF **THERAPEUTIC INDICATIONS** ON THE BASIS OF KNOWN LEVEL OF EFFICACY

  - THERAPEUTIC INDICATION PRECEDED BY THE TERM **"TRADITIONALLY USED IN ..."**
  - THERAPEUTIC INDICATION PRECEDED BY THE TERM **"USED IN ..."**
  - THERAPEUTIC INDICATION **STATED DIRECTLY** WITH NO PARTICULAR TERM

* THE **FIRST TWO CATEGORIES** ARE COVERED BY A NOTICE TO APPLICANTS FOR MARKETING AUTHORIZATION.

  - BULLETIN OFFICIEL (B.O.) 90/22 BIS, 1990 (FRANCE)
  - CIRCULAIRE ADMINISTRATIVE NO. 387, 22.09.1989
  - CIRCULAIRE MINISTERIELLE, 30.11.1994
    (MONITEUR BELGE, 10.02.1995) (BELGIUM)

* WITH THE EXCEPTION OF **LAXATIVE MEDICINES** BASED ON **VEGETABLE DRUGS** THE THIRD CATEGORY IS NOT COVERED BY THIS NOTICE

HERBAL REMEDIES (1)

NOTICE TO APPLICANTS FOR MARKETING AUTHORIZATION

(B.O. 90/22, 1990, FRANCE)

1. THERAPEUTIC INDICATIONS PRECEDED BY TERM **"TRADITIONALLY USED IN....** (1)

1.1. LIST OF THERAPEUTIC INDICATIONS

- THE RECOMMENDED THERAPEUTIC INDICATIONS ARE PRESENTED IN THE FORM OF A **LIST**

- THESE INDICATIONS ARE NUMBERED AND CONTAIN WORDING INTENDED FOR **THE MEDICAL PROFESSION** ON THE ONE HAND AND **FOR THE PUBLIC** ON THE OTHER

1.2. VEGETABLE DRUGS AND VEGETABLE DRUG PREPARATIONS

- THE RECOMMENDED DRUGS ARE SHOWN IN A **LIST** WHICH EXCLUDES ALL **TOXIC PLANTS**

- THIS LIST IS NOT DEFINITIVE, NEW PROPOSALS MAY BE FORMULATED BY THE APPLICANT PROVIDED THAT THE APPLICATION IS SUPPORTED BY A SCIENTIFIC DOSSIER, PROOF OF SAFETY IS FURNISHED AND A SUFFICIENT PERIOD OF USAGE IS SHOWN

HERBAL REMEDIES (2)

NOTICE TO APPLICANTS FOR MARKETING AUTHORIZATION

(B.O. 90/22, 1990, FRANCE)

1. THERAPEUTIC INDICATIONS PRECEDED BY TERM **"TRADITIONALLY USED IN....** (2)

1.3. REQUIREMENTS FOR THE DOSSIER FOR A MARKETING AUTHORIZATION APPLICATION

- **CHEMICAL** AND **PHARMACEUTICAL** DOCUMENTATION

- **TOXICOLOGICAL** DOCUMENTATION

* A TOXICOLOGICAL DOSSIER IS **NOT REQUIRED** FOR PREPARATIONS CORRESPONDING TO HERBAL **TEAS** OR PREPARED WITH **ALCOHOL** OF A STRENGTH OF LOWER THAN **30 PERCENT** V/V, OR TO TRADITIONALLY USED **TINCTURES** AND **EXTRACTS**

* ALL OTHER PREPARATIONS INVOLVING SPECIAL CONSTITUENTS, AS WELL AS PLANT **POWDERS** AND **TINCTURES** WITH **NON-TRADITIONAL USAGE**, SHALL BE SUBJECT TO A LEVEL 2 TOXICOLOGICAL STUDY, EXCEPT WHEN JUSTIFIED

- **PHARMACOLOGICAL** AND **CLINICAL** DOCUMENTATION

NO EVALUATION IS REQUIRED **AT THE MOMENT** IF THE THERAPEUTIC INDICATIONS ARE THOSE IN THE LIST

HERBAL REMEDIES (3)

NOTICE TO APPLICANTS FOR MARKETING AUTHORIZATION

(B.O. 90/22, 1990, FRANCE)

2. THERAPEUTIC INDICATIONS PRECEDED BY TERM **"USED IN** ....

* WHEN A WIDE USAGE EXISTS ALTHOUGH NO TRADITIONAL USE CAN BE ACCEPTED BECAUSE OF INSUFFICIENT PERIOD OF TIME, **ADDITIONAL TOXICOLOGICAL, PHARMACOLOGICAL** AND **CLINICAL** DATA MUST BE SUPPLIED, ON A **CASE-BY-CASE BASIS** IN ORDER TO CONFIRM THE VALUE OF THE MEDICINAL PRODUCT

3. PREPARATIONS DERIVED FROM **FRESH PLANTS**

* THE ANALYTICAL PART OF THE PHARMACEUTICAL DOCUMENTATION SHALL INCLUDE ESPECIALLY **TLC AND HPLC** AND/OR **GC PROFILES** (QUALITATIVE AND QUANTITATIVE ASPECTS) AND COMPARATIVE CHROMATOGRAMS BETWEEN THE FRESH DRUG, THE PREPARATION OBTAINED FROM IT AND THE CORRESPONDING DRIED DRUG

* WHEN PREPARATIONS ARE DERIVED FROM PLANTS **TRADITIONALLY USED AS FOODS**, THE **PHARMACEUTICAL** DOSSIER MUST BE **COMPLETE**, BUT THE **TOXICOLOGICAL** DOSSIER IS **NOT REQUIRED**

* IN ALL OTHER CASES, **A TOXICOLOGICAL DOSSIER** MUST BE SUPPLIED AND AT LEAST BE IN LINE WITH WHAT IS REQUIRED FOR THE **POWDERED FORM**, EXCEPT WHEN JUSTIFIED

HERBAL REMEDIES (4)

* FRANCE

* NUMBER OF THERAPEUTIC INDICATIONS : **36**
* NUMBER OF PLANTS : **174**
* FIXED COMBINATIONS

1. MIXTURES ARE POSSIBLE BETWEEN DRUGS OR THEIR PREPARATIONS WITH **SIMILAR** AND **COMPLEMENTARY** USAGES
2. MIXTURES OF DRUGS FOR **HERBAL TEAS** : MAXIMUM OF **TEN** DRUGS
   * FIVE BASIC DRUGS CONSIDERED TO BE SUPPORTING THE **ACTIVITY**
   * THREE DRUGS TO IMPROVE **TASTE**, WITH A MAXIMUM OF 15 PERCENT W/W
   * TWO DRUGS TO IMPROVE **APPEARANCE**, WITH A MAXIMUM OF 10 PERCENT W/W
3. MIXTURES OF **OTHER PREPARATIONS** : MAXIMUM OF **SIX** DRUGS
   * FOUR BASIC DRUGS
   * TWO PREPARATIONS TO IMPROVE TASTE AND APPEARANCE

* BELGIUM

* NUMBER OF THERAPEUTIC INDICATIONS : **21** IN **19** LISTS
* NUMBER OF PLANTS : **127**
* FIXED COMBINATIONS

1. MIXTURES ARE ONLY POSSIBLE BETWEEN DRUGS OR PREPARATIONS WITH **SIMILAR** USAGES, WITH A MAXIMUM OF **THREE** DRUGS. OTHER MIXTURES MUST BE SCIENTIFICALLY PROVEN.
2. MIXTURES OF DRUGS FOR **HERBAL TEAS** : MAXIMUM OF **SIX** DRUGS
   - THREE BASIC DRUGS CONSIDERED TO BE SUPPORTING **THE SAME ACTIVITY**
   - THREE PREPARATIONS TO IMPROVE TASTE AND APPEARANCE

HERBAL REMEDIES (5)

EXAMPLES OF ACCEPTED THERAPEUTIC INDICATIONS AND CORRESPONDING MEDICINAL PLANTS (1)

FRANCE			
INFORMATION FOR THE MEDICAL PROFESSION	INFORMATION FOR THE PUBLIC	NUMERICAL CODE	
		ORALLY	EXTERNAL USE
• TRADITIONALLY USED IN THE SYMPTOMATIC TREATMENT OF FUNCTIONAL DISORDERS OF CUTANEOUS FRAGILITY, SUCH AS ECCHYMOSIS, PETECHIASIS, ETC...	TRADITIONALLY USED IN MANIFESTATIONS OF FRAGILITY OF THE SMALL VESSELS OF THE SKIN	015	016
015 *AESCULUS HIPPOCASTANUM* L. (COMMUN HORSE CHESTNUT) (SEED, STEM BARK) ; *CITRUS SINENSIS* (L.) PERS. (SWEET ORANGE) (PEEL OF FRUIT) ; *KRAMERIA TRIANDRA* RUIZ LOPEZ ET PAVON (RATANHIA) (ROOT) ; *MELILOTUS OFFICINALIS* (LAM.) DESR. (FIELD MELILOT) (FLOWERING TOP) ; *RIBES NIGRUM* L. (BLACK CURRANT) (FRESH FRUIT) ; *VACCINIUM MYRTILLUS* L. (BLUEBERRY) (FRESH FRUIT) ; *VIBURNUM PRUNIFOLIUM* L. (BLACK HAW) (STEM BARK) ; *VITIS VINIFERA* L. (VINE) (LEAF) **016** = 015 WITHOUT *CITRUS SINENSIS* (L.) PERS. ; PLUS *ARNICA MONTANA* L. (MOUNTAIN ARNICA), *A. CHAMISSONIS* L. (LEAF ARNICA) (FLOWERHEAD) ; *CENTELLA ASIATICA* (L.) . URBAN (CENTELLA) (WHOLE PLANT)			
• TRADITIONALLY USED IN THE SYMPTOMATIC TREATMENT OF MINOR PAINFUL ARTICULAR MANIFESTATIONS	TRADITIONALLY USED IN PAINFUL ARTICULAR MANIFESTATIONS, TENDITINIS, SPRAINS	131	132
131 *CONYZA CANADENSIS* (L.) CRONQ. (AERIAL PARTS) ; *FILIPENDULA ULMARIA* (L.) MAXIM (DROPWORT) (FLOWER, FLOWERY TOP) ; *FRAXINUS EXCELSIOR* L. (COMMON ASH) (LEAF) ; *HARPAGOPHYTUM PROCUMBENS* DC. (DEVIL'S CLAW) (SECONDARY TUBEROUS ROOT) ; *RIBES NIGRUM* L. (BLACK CURRANT) (LEAF) ; *SALIX ALBA* L. (WILLOW), *S.PURPUREA* L., *S.VIMINALIS* L. (STEM BARK) ; *SCROPHULARIA NODOSA* L. (COMMON FIGWORT) (ROOT, FLOWERING TOP) ; *STACHYS OFFICINALIS* (L.) TREV. ST. LEON (BETONY) (LEAF) ; *URTICA DIOICA* L. (STINGING NETTLE) (AERIAL PARTS) **132** = 131 WITHOUT *FRAXINUS EXCELSIOR* L., AND *CONYZA CANADENSIS* (L.) CRONQ.			

HERBAL REMEDIES (6)

EXAMPLES OF ACCEPTED THERAPEUTIC INDICATIONS AND CORRESPONDING MEDICINAL PLANTS (2)

BELGIUM

LIST 1

* THIS HERBAL REMEDY CAN BE USED TO REDUCE EXCITABILITY IN ADUTS AND CHILDREN, PARTICULARLY IN CASES OF DISORDERS OF SLEEP, AFTER ELIMINATION OF ALL SERIOUS PATHOLOGIES AND ALTHOUGH ITS EFFICACY HAS NOT BEEN PROVEN YET ACCORDING TO THE ACTUALLY EXISTING EVALUATION CRITERIA

  CITRUS AURANTIUM L. SSP. *AURANTIUM* (BITTER ORANGE) (LEAF, FLOWER) ; *HUMULUS LUPULUS* L. (HOPS) (CONE, FLOWERING TOP, LUPULINE), *LAVANDULA VERA* DC (LAVENDEL) (FLOWER), *LIPPIA CITRIODORA* H.B.K. (VERVAIN) (LEAF) ; *MELISSA OFFICINALIS* L. (BALM) (LEAF), *PAPAVER RHOEAS* L. (RED POPPY) (FLOWER); ; *PASSIFLORA INCARNATA* L. (PASSION FLOWER) (WHOLE PLANT) ; *TILIA* SP. (LIME TREE) (INFLORESCENCE), *VALERIANA OFFICINALIS* L. (VALERIAN) (ROOT)

LIST 14

* THIS HERBAL REMEDY CAN BE USED AS **ADJUVANT** IN THE TREATMENT OF BENIGN PROSTATE HYPERPLASIA (BPH), AFTER ELIMINATION OF ALL SERIOUS PATHOLOGIES AND ALTHOUGH ITS EFFICACY HAS NOT BEEN PROVEN YET ACCORDING TO THE ACTUALLY EXISTING EVALUATION CRITERIA

  PYGEUM AFRICANUM HOOK (BARK) ; *SERENOA REPENS* (BATR.) SMALL (SABAL) (FRUIT), *URTICA DIOICA* L. (STINGING NETTLE) (ROOT)

LIST OF ACCEPTED THERAPEUTIC INDICATIONS (BELGIUM, UPDATED TILL 1997) (1)

CAN BE USED .....
AFTER ELIMINATION OF ALL SERIOUS PATHOLOGIES

I	TO REDUCE EXCITABILITY IN ADULTS AND CHILDREN OLDER THAN 12 YEARS, PARTICULARLY IN CASES OF DISORDERS OF SLEEP
II	**A.** AS STIMULANT LAXATIVE **B.** AS BULK LAXATIVE
III	TO PROMOTE THE RENAL ELIMINATION OF WATER * ADJUVANT IN THE TREATMENT OF BENIGN URINARY TRACT INFECTIONS
IV	IN PAINFUL ARTICULAR MANIFESTATIONS
V	FOR OCCASIONAL BENIGN COUGHS. IF THE COUGH PERSISTS FOR MORE THAN THREE DAYS, CONSULT A DOCTOR
VI	TO STIMULATE APPETITE
VII	IN THE SYMPTOMATIC TREATMENT OF DIGESTIVE DISORDERS
VIII	AS A CHOLAGOGUE
IX	LOCALLY (MOUTH AND THROAT WASHES, PASTILLES) FOR PAIN RELIEF AND ORAL HYGIENE IN AFFECTIONS OF THE ORAL CAVITY AND/OR THE OROPHARYNX
X	TOPICALLY AS A SOOTHING, ANTIPRURIGINOUS, WOUND HEALING AND ANTIINFLAMMATORY APPLICATION FOR DERMATOLOGICAL AILMENTS AND FUNCTIONAL DISORDERS OF CAPILLARY FRAGILITY

LIST OF ACCEPTED THERAPEUTIC INDICATIONS
(BELGIUM, UPDATED TILL 1997) (2)

CAN BE USED .....
AFTER ELIMINATION OF ALL SERIOUS PATHOLOGIES

XI IN DISORDERS OF CARDIAC ERETHISM IN THE ADULT (HEALTHY HEART)

XII IN THE SYMPTOMATIC TREATMENT OF FATIGUE

- ° **INFORMATION FOR THE MEDICAL PROFESSION:** THIS USE DOES NOT EXCLUDE THE NECESSITY FOR A FURTHER ETHIOLOGICAL SEARCH, ESPECIALLY AFTER TWO WEEKS OF TREATMENT
- ° **INFORMATION FOR THE PUBLIC:** CONSULT THE DOCTOR IN ORDER TO FIND THE REASON OF YOUR FATIGUE
- * ADJUVANT IN THE SYMPTOMATIC TREATMENT OF POST-INFECTIOUS FATIGUE

XIII TO PREVENT CARDIOVASCULAR DISEASES DUE TO AGEING

XIV AS ADJUVANT IN MICTURITION DISORDERS DUE TO BENIGN PROSTATE HYPERPLASIA

TO BE USED ONLY AFTER MEDICAL DIAGNOSIS

XV IN THE SYMPTOMATIC TREATMENT OF MILD DIARRHOEA

- ° IF THE DIARRHOEA PERSISTS FOR MORE THAN TWO DAYS, CONSULT A DOCTOR
- ° ANY SEVERE DIARRHOEA MAY LEAD TO A SERIOUS RISK OF DEHYDRATATION ESPECIALLY IN CHILDREN UNDER SIX YEARS OF AGE AND REQUIRES THE ADVICE OF A DOCTOR

LIST OF ACCEPTED THERAPEUTIC INDICATIONS (BELGIUM, UPDATED TILL 1997) (3)

CAN BE USED .....
AFTER ELIMINATION OF ALL SERIOUS PATHOLOGIES

XVI	IN THE SYMPTOMATIC TREATMENT OF CEREBRAL DISORDERS OF THE AGED
XVII	IN CASES OF EYE IRRITATION OR DISCOMFORT DUE TO VARIOUS CAUSES (SMOKY ATMOSPHERE, SUSTAINED VISUAL EFFORT, SEA OR SWIMMING POOL BATHES ETC.) PRECAUTION : USE ONLY FOR MILD AFFECTIONS. IF THE SYMPTOMS INCREASE OR PERSIST FOR MORE THAN TWO DAYS, CONSULT A DOCTOR. DO NOT USE : WHEN THE IRRITATION IS ACCOMPANIED BY PUS ; IN CASES OF SHARP PAIN : IN CASES OF DIRECT IMPACT OR OF INJURY
XVIII	AS ADJUVANT IN HEPATIC DISORDERS TO BE USED ONLY AFTER MEDICAL DIAGNOSIS
XIX	**A.** : IN SUBJECTIVE MANIFESTATIONS OF VENOUS INSUFFICIENCY SUCH AS HEAVY LEGS **B.** : IN THE SIGNS AND SYMPTOMS OF HAEMORRHOIDS
XX	AS ADJUVANT IN FUNCTIONAL PREMENSTRUEL DISORDERS AND PREMENOPAUSAL DISORDERS
XXI	IN THE SYMPTOMATIC TREATMENT OF PSYCHOSOMATIC DISORDERS
XXII	TO ASSIST LOSS OF WEIGH IN ADDITION TO DIETING
XXIII	LOCALLY FOR TREATMENT OF WARTS

CURRENT STATUS OF PHYTOMEDICINES IN EUROPE

(FRANCE AND BELGIUM)

I. PHYTOMEDICINES PLACED ON THE MARKET **WITHOUT THERAPEUTIC CLAIMS**

- FOOD SUPPLEMENTS, NEUTRACEUTICALS, PHARMAFOODS, HEALTH FOODS, FUNCTIONAL FOODS, ETC ...
- FOOD REGULATION IS APPLICABLE
  I.E. **NOTIFICATION** INSTEAD OF **REGISTRATION**
- SOLD IN "HEALTH FOOD STORES", "DIET SHOPS" AND "SUPER-MARKETS"

II. PHYTOMEDICINES PLACED ON THE MARKET **WITH THERAPEUTIC CLAIMS**

CLASS 1 : STATUS OF DRUGS

- **SAFETY** AND **EFFICACY** HAVE BEEN PROVEN ACCORDING TO GCP-GUIDELINES

CLASS 2 : SPECIAL CLASS OF PLANT-BASED DRUGS

- **SAFETY** AND **EFFICACY** HAVE **NOT** BEEN PROVEN ACCORDING TO GCP-GUIDELINES, BUT ARE SUPPORTED BY **PUBLISHED SCIENTIFIC LITERATURE**
- APPROPIATE LABELLING IS REQUIRED
- STANDARDS OF QUALITY AND CONSTANCY OF QUALITY ARE GUARANTEED BY APPROVAL OF AN APPROPIATED REGISTRATION DOSSIER
- ONLY SOLD IN PHARMACIES

HERBAL REMEDIES (7)

NOTICE TO APPLICANTS FOR MARKETING AUTHORIZATION

(B.O. 90/92, 1990, FRANCE)

CONTENT OF THE DOSSIER (1)

PART I.

I.A. ADMINISTRATIVE DATA

I.B. SUMMARY OF PRODUCT CHARACTERISTICS

I.C. EXPERT REPORTS ON THE **CHEMICAL** AND **PHARMACEUTICAL** DOCUMENTATION AND IF APPROPIATE ON THE **TOXICOLOGICAL, PHARMACOLOGICAL** AND **CLINICAL** DOCUMENTATION

PART II. CHEMICAL AND **PHARMACEUTICAL** DOCUMENTATION

II.A. COMPOSITION

1. COMPOSITION OF THE PROPRIETARY MEDICINAL PRODUCT

2. CONTAINER

3. IF RELEVANT, THE FORMULA USED FOR TOXICOLOGICAL TRIALS MUST BE SPECIFIED

4. PHARMACEUTICAL DEVELOPMENT

II.B. METHOD OF PREPARATION

1. MANUFACTURING FORMULA

2. MANUFACTURING PROCESS

HERBAL REMEDIES (8)

NOTICE TO APPLICANTS FOR MARKETING AUTHORIZATION

(B.O. 90/92, 1990, FRANCE)

CONTENT OF THE DOSSIER (2)

PART II. CHEMICAL AND **PHARMACEUTICAL** DOCUMENTATION

II.C. CONTROL OF STARTING MATERIALS

1. ACTIVE INGREDIENTS

1.1. SPECIFICATIONS AND ROUTINE TESTS

1.1.1. ACTIVE INGREDIENTS DESCRIBED IN A PHARMACOPOEIA
1.1.2. ACTIVE INGREDIENTS NOT DESCRIBED IN A PHARMACOPOEIA

1.2. SCIENTIFIC DATA

1.2.1. NOMENCLATURE
1.2.2. DESCRIPTION
1.2.3. PRODUCTION
1.2.4. QUALITY CONTROL DURING MANUFACTURE
1.2.5. DEVELOPMENT OF HERBAL REMEDIES

a. VEGETABLE DRUGS
b. VEGETABLE DRUG PREPARATIONS

1.2.6. IMPURITIES
1.2.7. BATCH ANALYSIS

2. OTHER INGREDIENTS

2.1. SPECIFICATIONS AND ROUTINE TESTS
2.2. SCIENTIFIC DATA
2.3. BATCH ANALYSIS

3. PACKAGING MATERIAL (PRIMARY PACKAGING)

3.1. SPECIFICATIONS AND ROUTINE TESTS
3.2. SCIENTIFIC DATA
3.3. BATCH ANALYSIS, ANALYTICAL RESULTS

HERBAL REMEDIES (9)

NOTICE TO APPLICANTS FOR MARKETING AUTHORIZATION

(B.O. 90/92, 1990, FRANCE)

CONTENT OF THE DOSSIER (3)

PART II. CHEMICAL AND **PHARMACEUTICAL** DOCUMENTATION

II.D. CONTROL TESTS ON INTERMEDIATE PRODUCTS (IF NECESSARY)

II.E. CONTROL TESTS ON THE FINISHED PRODUCT

1. SPECIFICATIONS AND ROUTINE TESTING
   - 1.1. PRODUCT SPECIFICATIONS
   - 1.2. CONTROL METHODS
     - 1.2.1. IDENTIFICATION, ASSAY AND OTHER TESTS
     - 1.2.2. IDENTIFICATION AND DETERMINATION OF EXCIPIENTS

2. SCIENTIFIC DATA
   - 2.1. ANALYTICAL VALIDATION
   - 2.2. BATCH ANALYSIS

II.F. STABILITY

1. STABILITY TESTS ON THE ACTIVE INGREDIENT(S)
2. STABILITY TESTS ON THE FINISHED PRODUCT AND/OR, IF APPROPIATE, ON THE VEGETABLE DRUG(S) PREPARATIONS

II.G. OTHER INFORMATION

HERBAL REMEDIES (10)

NOTICE TO APPLICANTS FOR MARKETING AUTHORIZATION

(B.O. 90/92, 1990, FRANCE)

CONTENT OF THE DOSSIER (4)

PART III. TOXICOLOGICAL DOCUMENTATION

CATEGORY 1

* **NO TOXICOLOGICAL** STUDY IS REQUIRED, WHETHER THE MEDICINAL PRODUCTS ARE COMPOSED OF ONE OR SEVERAL ACTIVE INGREDIENTS

* THESE ARE :
  - DRUGS FOR HERBAL TEAS
  - AQUEOUS EXTRACTS
  - AQUEOUS ALCOHOLIC EXTRACTS PREPARED WITH ETHYL-ALCOHOL OF A LOW STRENGTH (LESS THAN OR EQUAL TO 30 PERCENT V/V)
  - AQUEOUS ALCOHOLIC EXTRACTS PREPARED WITH ETHYL-ALCOHOL OF A STRENGTH OF MORE THAN 30 PERCENT V/V AND TINCTURES, WHEN THEY ARE TRADITIONALLY USED IN THE FRENCH AND/OR EUROPEAN PHARMACOPOEIAS
  - LAXATIVE PLANT DRUGS LISTED

HERBAL REMEDIES (11)

NOTICE TO APPLICANTS FOR MARKETING AUTHORIZATION

(B.O. 90/92, 1990, FRANCE)

CONTENT OF THE DOSSIER (5)

PART III. TOXICOLOGICAL DOCUMENTATION

CATEGORY 2

* IN PRINCIPLE, **AN ABRIDGED TOXICOLOGICAL STUDY** IS REQUIRED, EXCEPT WHEN JUSTIFIED BY THE APPLICANT

* THIS APPLIES TO OTHER PLANT BASED MEDICINAL PRODUCTS **"TRADITIONALLY USED IN ..."** INCLUDING
  - MOST POWDERS OF WHOLE DRUGS
  - TINCTURES OF A NON-TRADITIONAL USAGE
  - MOST AQUEOUS ALCOHOLIC EXTRACTS PREPARED WITH ETHYLALCOHOL OF HIGH STRENGTH (MORE THAN 30 PERCENT V/V)

* THIS EVALUATION MUST INCLUDE
  - A BRIEF REVIEW BY THE EXPERT ON THE **MODE OF EXTRACTION** OF THE DRUG (SOLVENTS USED)
  - AN **ACUTE ORAL TOXICITY** TRIAL IN RATS
  - A **4 WEEKS ORAL TOXICITY TRIAL** IN RATS, CONSISTING OF A STUDY OF BEHAVIOUR AND GROWTH, AND INVESTIGATION OF HAEMATOLOGICAL, BIOCHEMICAL AND HISTOLOGICAL PARAMETERS (AT LEAST 15 ORGANS SUBJECTED TO INVESTIGATION)

CATEGORY 3

* THIS RELATES TO PLANT-BASED MEDICINAL PRODUCTS **"USED IN ..."** AND CONCERNS PLANT-BASED MEDICINAL PRODUCTS WITH NON-TRADITIONAL USE

HERBAL REMEDIES (12)

NOTICE TO APPLICANTS FOR MARKETING AUTHORIZATION

(B.O. 90/92, 1990, FRANCE)

CONTENT OF THE DOSSIER (6)

PART IV. CLINICAL DOCUMENTATION

* **NO CLINICAL** EVALUATION OF ACTIVITY IS REQUIRED IF THE THERAPEUTIC INDICATIONS CLAIMED ARE THESE MENTIONED IN THE LIST AND IF THE PLANT CONCERNED IS INCLUDED IN THE LIST

* IN OTHER CASES AN EVALUATION IS NECESSARY AND IS TO BE JUSTIFIED ON A CASE-BY-CASE BASIS

* FOR CERTAIN DRUGS OR PREPARATIONS DETAILS CONCERNING DOSAGE MUST BE SCRUPULOUSLY RESPECTED

* DISTINCT RULES HAVE BEEN ESTABLISHED FOR HERBAL TEAS AND OTHER PREPARATIONS TAKING INTO ACCOUNT TRADITIONAL USE :
  - **FOR HERBAL TEAS** :
    THE RECOMMENDED ORAL DOSAGE VARIES ACCORDING TO THE CASE. IT IS GENERALLY ABOUT ONE CUP, TWO TO FIVE TIMES A DAY (BETWEEN 250 ML AND 1 L OF TEA A DAY)

  - **FOR PREPARATIONS** OTHER THAN HERBAL TEAS
    - IF THE PHARMACEUTICAL FORM IS TO BE DISSOLVED IN WATER BEFORE ADMINISTRATION, THE DAILY DOSAGE IS THAT OF THE CORRESPONDING TEA

    - FOR OTHER PREPARATIONS, THE APPLICANT WILL JUSTIFY THE CLAIMED DOSAGE. IT IS RECOMMENDED THAT TRADITIONALLY USED DOSAGE BE TAKEN INTO ACCOUNT

PART V. SPECIAL PARTICULARS

PART V.A. DOSAGE FORM

PART V.B. SAMPLES

PART V.C. MANUFACTURER AUTHORIZATION(S)

PART V.D. MARKETING AUTHORIZATION(S)

NUTRACEUTICALS (USA) (1)

* DIETARY SUPPLEMENTS
  * TRADITIONALLY COMPOSED OF ESSENTIAL NUTRIENTS SUCH AS VITAMINS, MINERALS AND PROTEINS
  * NLEA (104 STAT. 2353, NOVEMBER 1990) (NUTRITION LABELING AND EDUCATION ACT) ADDED HERBS AND SIMILAR SUBSTANCES
  * DSHEA (108 STAT. 4325, OCTOBER 1944) (DIETARY SUPPLEMENT HEALTH AND EDUCATION ACT) ESTABLISHED A FINAL DEFINITION FOR DIETARY SUPPLEMENTS
    * "A PRODUCT (OTHER THAN TOBACCO) THAT IS INTENDED TO SUPPLEMENT THE DIET THAT BEARS OR CONTAINS ONE OR MORE OF THE FOLLOWING DIETARY INGREDIENTS : A VITAMIN, A MINERAL, AN HERB OR OTHER BOTANICAL, AN AMINO ACID, A DIETARY SUBSTANCE FOR USE BY MAN TO SUPPLEMENT THE DIET BY INCREASING THE TOTAL DAILY INTAKE OR A CONCENTRATE, METABOLITE, CONSTITUENT, EXTRACT OR COMBINATIONS OF THOSE INGREDIENTS
    * IS INTENDED FOR INGESTION IN PILL, CAPSULE, TABLET OR LIQUID FORM
    * IS NOT REPRESENTED FOR USE AS A CONVENTIONAL FOOD OR AS THE SOLE ITEM OF A MEAL OR DIET
    * IS LABELED AS A DIETARY SUPPLEMENT
    * INCLUDES PRODUCTS SUCH AS AN APPROVED NEW DRUG, CERTIFIED ANTIBIOTIC, OR LICENSED BIOLOGIC THAT WAS MARKED AS A DIETARY SUPPLEMENT OR FOOD BEFORE APPROVAL, CERTIFICATION OR LICENSE" (PROVISION STILL UNDER REVIEW BY U.S. SECRETARY OF HEALTH AND HUMAN SERVICES)"

NUTRACEUTICALS (USA) (2)

* NUTRACEUTICALS

"NUTRACEUTICALS ARE FOOD INGREDIENTS, ADDITIVES, FORMULATED PRODUCTS, OR STAND-ALONE SUPPLEMENTS WHICH COMBINE A PRODUCT'S NUTRITIONAL VALUE WITH A THERAPEUTIC BENEFIT, BEYOND THAT OBTAINED THROUGH A TRADITIONAL DIET".
NUTRACEUTICALS CAN RANGE FROM ISOLATED NUTRIENTS TO DIETARY SUPPLEMENTS, SPECIAL DIETS WITH GENETICALLY INGENEERED DESIGNER FOODS, HERBAL PRODUCTS, PROCESSED FOODS

SOME ADDITIONAL TERMS FOR NUTRACEUTICALS ARE : DESIGNER FOODS, FITNESS FOODS, FOODACEUTICALS, FUNCTIONAL FOODS, LONGEVITY FOODS, MEDICAL FOODS, NUTRITIONAL FOODS, PHARMACEUTICAL FOODS, PHARMACOFOODS, PHARMAFOODS, PHARMAFOODICALS, PRESCRIPTION FOODS, SUPER FOODS AND THERAPEUTIC FOODS

NUTRIENT	CONDITION
BETA CAROTENE	LUNG CANCER
NIACIN	RECURRENT HEART ATTACKS
PYRIDOXINE	DEPRESSION
VITAMIN A	MEASLES
MAGNESIUM	HYPERTENSION
GARLIC	ARTERIOSCLEROSIS
FISH OIL	HYPERTENSION
CALCIUM	HYPERTENSION, OSTEOPOROSIS
ANTIOXIDANTS	REDUCES DAMAGE FROM HEART ATTACKS

NUTRACEUTICALS (USA) (3)

* FUNCTIONAL FOODS

  * IT IS INTERESTING TO NOTE THE ASPECT OF FUNCTIONAL FOODS AND COMPARE FUNCTIONAL FOODS TO NUTRACEUTICALS

  * NUTRACEUTICALS FOCUS ON SPECIFIC INGREDIENT COMPONENTS AND FORMULATED PRODUCTS

  * FUNCTIONAL FOODS DO NOT HAVE A REGULATORY DEFINITION BUT ARE CONSIDERED FOODS CONTAINING SIGNIFICANT LEVELS OF BIOLOGICALLY ACTIVE COMPONENTS THAT IMPART HEALTH BENEFITS BEYOND BASIC NUTRITION WHEN CONSUMED IN TYPICAL OR OPTIMAL SERVING SIZES

  * FUNCTIONAL ATTRIBUTES OF MANY TRADITIONAL FOODS ARE BEING DISCOVERED WHILE OTHER FOODS ARE BEING ENGINEERED TO INCORPORATE BENEFICIAL COMPONENTS

  * BY THE USE OF DIFFERENT TERMINOLOGY, THE POTENTIAL FOR CONFUSION IS CLEAR AS WELL AS THE POSSIBLE ABUSE OR MISUSE OF THESE TYPES OF PRODUCTS POSED TO CONSUMERS WITH A MIXED KNOWLEDGE OF NUTRITION EDUCATION

NUTRACEUTICALS (USA) (4)

EXAMPLES OF FUNCTIONAL FOODS

FOOD COMPONENT	POTENTIAL BENEFIT
ALLYL (GARLIC)	REGULAR INTAKE MAY REDUCE THE RISK OF CANCER AND LOWER BLOOD PRESSURE AND CHOLESTEROL LEVELS
BETA GLUCAN (OATS)	MAY HELP REDUCE THE RISK OF CARDIOVASCULAR DISEASE
BIFIDOBACTERIA	MAY ENHANCE (IN YOGURT AND OTHER DIARY PRODUCTS) GASTROINTESTIONAL FUNCTION
CATECHIN (TEA)	MAY REDUCE THE RISK OF CANCER
INSOLUBLE FIBER (WHEAT BRAN)	MAY HELP REDUCE THE RISK OF BREAST AND COLON CANCER
ISOFLAVONES (SOY)	REGULAR CONSUMPTION MAY LOWER CHOLESTEROL IN INDIVIDUALS WITH HIGH CHOLESTEROL LEVELS
LYCOPENE (WHICH PRODUCES THE RED COLOR IN TOMATOES, TOMATO SAUCE, RED GRAPEFRUIT, RED PEPPERS)	HIGH INTAKE MAY REDUCE THE RISK OF PROSTATE AND CERVICAL CANCER
OLIGOSACCHARIDES (BULK SUGAR SUBSTITUTES TO REPLACE THE TASTE AND TEXTURE OF SUGAR IN CONFECTIONS)	MAY IMPROVE THE QUALITY OF INTESTINAL MICROFLORA AND DECREASE RISK OF TOOTH DECAY

NUTRACEUTICALS (USA) (5)

* REGULATORY ACTIVITY

  * HISTORICAL PERSPECTIVE

    RESPONSABILITIES OF USFDA

    * MONITORING BOTH FOOD AND DRUG SAFETY INCLUDING ESTABLISHING STANDARDS, FORMING INSPECTION SYSTEMS AND SETTING GUIDELINES FOR ADDITIVES

    * 1938 : EXPANDED WITH THE FOOD, DRUG AND COSMETIC ACT

    * 1958 : REQUIREMENTS OF SAFETY FOR NEW FOOD ADDITIVES BY THE MANUFACTURERS I.E. PROHIBITION OF APPROVAL OF ANY FOOD ADDITIVE SHOWN TO INDUCE CANCER IN ANIMALS OR HUMANS → ISSUANCE OF A WARNING LETTER POSTED ON PUBLIC DISPLAY AT USFDA HEADQUARTERS → MUCH MEDIA ATTENTION FOR THE COMPANIES INFERRING THAT THEY ARE IN SERIOUS VIOLATION OF THE LAW AND IN DANGER OF AN ENFORCEMENT ACTION

NUTRACEUTICALS (USA) (6)

* CURRENT REGULATORY ACTIVITY

  * 1940 : NUTRITION, LABELING AND EDUCATION ACT (NLEA) : REQUIRES ALL PACKAGED FOOD TO BEAR NUTRITION LABELING AND ALL HEALTH CLAIMS FOR FOODS TO BE CONSISTENT WITH THE TERMS DEFINED BY THE SECRETARY OF HEALTH AND HUMAN SERVICES

    CRITICISMS :

    - RESTRICTIONS ON HEALTH CLAIMS
    - ABILITY TO DISQUALIFY ON HEALTH CLAIMS BASED ON SPECIFIC TRANSGRESSIONS

  * 1994 : DIETARY SUPPLEMENT HEALTH AND EDUCATION ACT (DSHEA) :

    - PROVIDES A MORE COMPREHENSIVE DEFINITION OF DIETARY SUPPLEMENTS
    - ESTABLISHES SPECIFIC LABELING REQUIREMENTS FOR DIETARY SUPPLEMENTS
    - PROVIDES A REGULATORY FRAMEWORK
    - AUTHORITIES USFDA TO PROMOTE GOOD MANUFACTURING PRACTICE REGULATIONS FOR DIETARY SUPPLEMENTS AND INGREDIENTS
    - CLASSIFIES DIETARY SUPPLEMENTS AS FOOD
    - ESTABLISHES A COMMISSION TO REGULATE LABEL CLAIMS

* ISSUES OF INTEREST

- SUGGESTED CHANGE TO REPLACE US RECOMMENDED DAILY ALLOWANCES (RDAs) WITH REFERENCE DAILY INTAKES (RDIs) EFFECTIVE JANUARY 1997

- CLAIMS CAN BE COMMUNICATED BUT WITH A DISCLAIMER STATING : "THIS STATEMENT HAS NOT BEEN EVALUATED BY THE FOOD AND DRUG ADMINISTRATION. THIS PRODUCT IS NOT INTENDED TO DIAGNOSE, TREAT, CURE OR PREVENT ANY DISEASE"

- USFDA PROPOSALS TO AMEND REGULATIONS ON CERTAIN NUTRIENT CONTENT CLAIMS TOOK EFFECT JANUARY 1996

- EXACT LABELING OF DIETARY SUPPLEMENTS IS UNDER REVIEW BY THE COMMISSION ON DIETARY SUPPLEMENT LABELS. RULES RELATING TO FOOD LABELING AND REGULATIONS FOR DIETARY SUPPLEMENTS BECOME EFFECTIVE MARCH 23, 1999. THE COMPLIANCE DEADLINE FOR THE FOOD LABELING REGULATIONS IS JANUARY 1, 2000

- THE OFFICE OF DIETARY SUPPLEMENT RESEARCH UNDER THE NATIONAL INSTITUTES OF HEALTH WAS ESTABLISHED TO EXPLORE THE ROLE OF SUPPLEMENTS TO IMPROVE HEALTH AND PROMOTE THE SCIENTIFIC STUDY OF THEIR BENEFIT IN MAINTAINING HEALTH AND PROMOTING DISEASE. THE OFFICE IS FUNDED US$ 5M ANNUALLY

- THE FOOD CODE OF 1995 HAS BEEN REVISED AND UPDATED AS THE FOOD CODE 1997 AND IS AVAILABLE FROM FDA

- THE FDA MODERNIZATION AND ACCOUNTABILITY ACT OF 1997 HAS GIVEN THE FDA A DIRECTIVE TO DEVELOP REGULATIONS FOR THEIR OWN GUIDANCE AND FUTURE MISSION

- THE U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES HAS PREPARED PROPOSALS TO EXPAND FDA's CAPABILITIES REGARDING FOOD SAFETY, THEIR AUTHORITY TO RECALL FOOD, THEIR AUTHORITY TO IMPOSE CIVIL MONETARY PENALTIES FOR VIOLATIONS UNDER THE FEDERAL FOOD, DRUG AND COSMETIC ACT

NUTRACEUTICALS (USA) (8)

USFDA DECISIONS ON HEALTH CLAIMS TO DATE

ACCEPTED CLAIMS	DISALLOWED CLAIMS
CALCIUM AND OSTEOPOROSIS	DIETARY FIBER AND CANCER
FOLIC ACID AND NEURAL TUBE BIRTH DEFECTS	DIETARY FIBER AND CARDIO-VASCULAR DISEASE
	ANTIOXIDANT VITAMINS AND CANCER
	ZINC AND IMMUNE FUNCTION IN THE ELDERLY
	OMEGA-3 FATTY ACIDS AND CORONARY HEART DISEASE

SOURCE : DRUG TOPICS, JANUARY 24, 1994

SOME FUNCTIONAL FOODS IN JAPAN

FOOD	HEALTH CLAIM
GREEN TEA	REDUCES THE EFFECTS OF OLD AGE, SUPPRESSES CARCINOGENS, LOWERS CHOLESTEROL, PREVENTS CAVITIES, HELPS ELIMINATE STRONTIUM 90
OLIGO CC (A CARBONATED BEVERAGE)	INCREASES LEVELS OF LYSIS BACTERIA TO GIVE DIGESTION BALANCE
LIFO VITAN	HIGH ENERGY VITAMIN DRINK
VEGETAKI	NUTRIENTS FOR ENERGY
TAKOSU INNER YOU	CALCIUM AND IRON SUPPLEMENT
ROYAL JELLY	ENERGY AND SEXUAL ENHANCEMENT
RELAX GUM	CALMATIVE WITH VALERIAN
KARAOKE NO-DOSE	IMPROVES VOICE OF SINGERS
DRINK JELLY	
PISNIK GUM	FOR RELAXATION AND ENERGY, TO BE 'ELEGANT'
POCHARSWEPSE DIA	ENHANCES ATHLETIC PERFORMANCE

NUTRACEUTICALS (USA) (9)

* **RESULTS OF THE FIRST INTERNATIONAL CONFERENCE ON EAST-WEST PERSPECTIVES ON FUNCTIONAL FOODS (SEPTEMBER 1995)**

  * FUNCTIONAL FOODS SHOULD BE DESCRIBED AS FOODS THAT IMPROVE OR AFFECT BODY FUNCTIONS OVER AND ABOVE THEIR NORMAL NUTRITIONAL VALUES

  * MEDICAL CLAIMS SHOULD NOT BE MADE FOR THESE FOODS

  * THE FUNCTIONAL EFFECTS MUST BE SCIENTIFICALLY PROVEN AND SUBSTANTIATED

  * THE DEVELOPMENT OF RELEVANT AND GOOD BIOLOGIC MARKERS FOR BODY FUNCTIONS SHOULD BE ENCOURAGED

  * MORE RESEARCH SHOULD BE CONDUCTED ON EPIDEMIOLOGICAL STUDIES FOR FUNCTIONAL FOODS

  * A FUNCTIONAL CLAIM SHOULD BE ALLOWED FOR WELL DOCUMENTED AND STUDIED FUNCTIONAL FOOD

* *CAROL McBRIDE : DRAGOCO REPORT, 4, 191 (1998)*

  * IT IS A VERY SERIOUS RESPONSIBILITY TO GUARD THE PUBLIC SAFETY BY OVERSEEING THE FOOD SUPPLY

  * TO CONCLUDE THIS ARTICLE, I WOULD LIKE TO OFFER THE FOLLOWING INSIGHTS INTO SOME OF THE ISSUES THAT ALL COUNTRIES MUST CONSIDER IN REGARD TO NUTRACEUTICALS

    * THE ROUTINE USE OF SUPPLEMENTS IS NOT RECOMMENDED BY ANY BODY OF EXPERTS

    * NUTRITION IS NOT THE ONLY FACTOR THAT INFLUENCES HEALTH, WELL BEING AND RESISTANCE TO DISEASE

    * FOOD IS MORE THAN THE SUM OF ITS NUTRIENTS

    * OPTIMAL NUTRIENT USE LEVELS ARE VERY DIFFICULT TO DETERMINE

    * TAKING SUPPLEMENTS OF SINGLE NUTRIENTS MAY HAVE DETRIMENTAL HEALTH EFFECTS

    * DIETARY SUPPLEMENTS VARY IN QUALITY AND DIGESTIVE BIOAVAILABILITY

    * SUPPLEMENTS ARE PROMOTED BY COMMERCIAL SOURCES CURRENTLY ON THE BASIS OF INCOMPLETE SCIENCE

    * FOCUSING ON SUPPLEMENTS CAN BE A TOO EASY ANSWER THAT TAKES ATTENTION AWAY FROM SERIOUSLY IMPROVING ONE'S LIFESTYLE

HERBAL REMEDIES

STANDARD LICENSES IN GERMANY (1)

* **ARZNEIMITTELGESETZ (MEDICINES ACT) : AMG 78**

  - FROM 01.01.1978 ON ALL PREPARED MEDICINES REQUIRE A SPECIAL LICENSE SHOWING NOT ONLY THEIR **QUALITY**, BUT ALSO THEIR **ACTIVITY** AND **HARMLESSNESS** (§ 22 AMG)

  - THIS LICENSE IS DELIVERED BY THE **BUNDESGESUNDHEIT-SAMT (BGA)** (FEDERAL MINISTRY OF HEALTH)

  - REMEDIES ON THE MARKET AT THAT TIME COULD IN CONFORMITY WITH THE TRANSITIONAL REGULATIONS (ART. III § 7 AMG) BE NOTIFIED AND GIVEN A **"FICTITUOUS"** LICENCE I.E. A LICENSE **"AS OF RIGHT"**, WHICH WOULD EXPIRE IN 31.12.1989

* **SCREENING OF THE SCIENTIFIC EVIDENCE FOR HERBAL DRUGS IS DONE BY THE GERMAN COMMISSION E FOR HUMAN MEDICINE, SECTION OF PHYTOTHERAPY**

  - SPECIFIC MONOGRAPHS ARE PREPARED AND PUBLISHED IN THE **BUNDESANZEIGER (BAnz)** (FEDERAL GAZETTE)

  - EXAMPLE : *VALERIANAE RADIX* (VALERIAN ROOT) = BAnz No. 90, DATED 15.03.1985

    - NAME
    - CONSTITUENTS
    - USES
    - CONTRA-INDICATIONS
    - SIDE EFFECTS
    - INTERACTIONS
    - DOSAGE
    - MANNER OF USE
    - EFFECTS

  - THE MONOGRAPHS FORM THE FOUNDATION FOR THE LICENSING AND THE SO CALLED RE-LICENSING

HERBAL REMEDIES

STANDARD LICENSES IN GERMANY (2)

EXAMPLE OF A COMMISSION E PREPARED MONOGRAPH

***VALERIANAE RADIX* (VALERIAN ROOT)**

NAME OF THE DRUG :
VALERIANAE RADIX, VALERIAN ROOT

CONSTITUENTS OF THE DRUG :
VALERIAN ROOT, CONSISTING OF THE UNDERGROUND PARTS OF THE COLLECTIVE SPECIES *VALERIANA OFFICINALIS* LINNE, FRESH OR CAREFULLY DRIED AT OR BELOW 40°C, AND ITS PREPARATIONS IN ACTIVE DOSES.
THE ROOTS CONTAIN ESSENTIAL OIL WITH MONO- AND SESQUITERPENES (VALERENIC ACIDS).
THE THERMO- AND CHEMOLABILE GENUINE VALEPOTRIATES ARE NOT PRESENT IN THE USUAL THERAPEUTICALLY USED FORMULATIONS (INFUSION, EXTRACT, FLUID EXTRACT, TINCTURE)

USES :
RESTLESSNESS, NERVOUS DISTURBANCES OF SLEEP

CONTRA-INDICATIONS :
NONE KNOWN

SIDE EFFECTS :
NONE KNOWN

INTERACTIONS :
NONE KNOWN

DOSAGE :
IF NOT OTHERWISE PRESCRIBED :
INFUSION : 2-3 G DRUG TO A CUP, ONE OR MORE TIMES A DAY
TINCTURE : ½-1 TEASPOONFUL (1-3 ML), ONE OR MORE TIMES A DAY
EXTRACTS : CORRESPONDING TO 2-3 G DRUG, ONE OR MORE TIMES A DAY

MANNER OF USE :
INTERNALLY : AS EXPRESSED JUICE, TINCTURE, EXTRACTS, AND OTHER GALENICAL PREPARATIONS
EXTERNALLY : AS A BATH ADDITIVE

EFFECTS :
CALMING, PROMOTING READINESS TO SLEEP

HERBAL REMEDIES

STANDARD LICENSES IN GERMANY (3)

* **SCREENING OF SCIENTIFIC EVIDENCE BY THE GERMAN COMMISSION E FOR HUMAN MEDICINE, SECTION OF PHYTOTHERAPY RESULTS IN POSITIVE AND NEGATIVE MONOGRAPHS DEPENDING ON THEIR BENEFIT-RISK RATIO**

  - **NEGATIVE MONOGRAPHS** : COMMISSION E CAME TO THE CONCLUSION NOT TO ADVOCATE THERAPEUTIC USE

    "THIS, IN NO WAY, PROHIBITS THEIR USE, BUT THE PHARMACIST IS HIS DISCUSSIONS WITH THE PATIENTS WILL BE HESITANT IN RECOMMENDING OR WILL INFORM THEM OF THE FACT"

  - THE COMMISSION E FOR HUMAN MEDICINE, SECTION OF PHYTOTHERAPY, DID NOT WORK FROM JULY 1994 TILL OCTOBER 1995

* **RESULTS OF THE SCREENING WORK BY THE COMMISSION E**

  - PERIOD : 1978-1992

    - **285** PUBLISHED MONOGRAPHS
    - **188** (66%) SHOWED TO EXHIBIT SOME RISKS
    - **58** (22%) SHOWED NO PROOF OF EFFICACY AND WERE CONSIDERED TO HAVE A NEGATIVE BENEFIT-RISK RATIO
    - **58** (22%) SHOWED TO EXHIBIT ALLERGIC SIDE EFFECTS EG. ASTERACEAE
    - **35** (12%) SHOWED TO EXHIBIT GASTRO-INTESTINAL DISTURBANCE DUE TO THE PRESENCE OF SAPONINS

  - SOME CONSTITUENTS WERE **MUTAGENIC** OR **CARCINOGENIC** E.G. PYRROLIZIDINES, ARISTOLOCHIA ACIDS

MOLECULAR PHARMACOLOGY OF HORMONE- AND NEUROTRANSMITTER RECEPTORS.

BY

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PHARMACOLOGY OF RECEPTORS.

A) INTRODUCTORY COMMENTS.

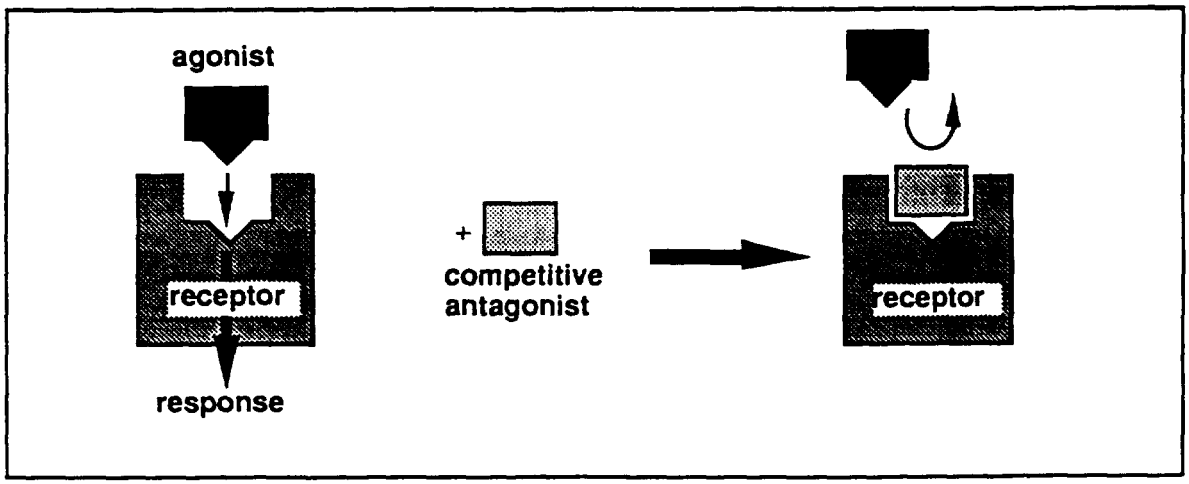
Neurotransmission and hormonal information is extremely important for the well being of higher organisms. It is therefore not surprising that certain diseases may result from (or be associated with) anomalies in hormone- or neurotransmitter concentrations, or from the inability of their target cells to respond adequately. Administration of the messengers themselves (e.g. insulin) and of synthetic analogs is therefore often carried out to counteract these pathophysiological conditions, and in some instances also to alter normal physiological conditions (e.g. contra-ception). An important branch of the activities of the pharmaceutical industry is therefore implicated in the development of drugs which mimic or block the action of natural messengers; i.e.

- **The agonists**. These compounds bind to the receptors and elicit the physiological responses. They include the endogenous messengers and synthetic molecules (Fig. 51).
- **The antagonists** or "**blockers**". These compounds are all synthetic or derived from other organisms (e.g. present in plants and animal toxins). They bind to the receptor but this interaction does not elicit the physiological response.
  • The most common antagonists compete in a reversible fashion with the endogenous chemical messengers for binding to their receptors, thereby preventing the target cells to respond to the presence of these messengers. Such reversible competitive antagonists bind to the same site of the receptor as the agonist (Fig. 51). Alternative types of antagonism are discussed at the end of this chapter.

Agonists and antagonists are of medical interest if they show pronounced affinity and selectivity towards one or more specific receptors. The discovery of such drugs usually requires the synthesis of a considerable number of structurally related molecules and the screening of their toxicity and biological activity. The derived structure- toxicity and structure- activity relationships can then be used for the design of even more efficient compounds. In the past, most of the structure- activity relationships were carried out by measuring the drug- induced physiological responses *in vivo* or in intact tissues or organs. The last decades have, however, been characterised by the development of new biochemical techniques such as radioligand binding , by which it has become possible to investigate drug-receptor interactions on isolated cells or even on isolated membrane preparations.

This allows the fast screening of the affinity of newly synthesised drugs for the receptor, or receptors of interest. Possible species- related differences in drug action were initially be avoided by using human blood cells or on post-mortem obtained tissues. The latest trend is to circumvent the use of human tissues by transfecting tumour cell lines (e.g. Chinese hamster ovary cells) with human DNA coding for the desired receptor, and to use the expressed receptors for screening tests. Finally, much effort is nowadays devoted towards the determination of the exact molecular structure of the receptors (and especially of the binding site of the chemical messenger). The dream of the up to date pharmacologist is the development of the ideally fitting drug on the basis of such knowledge without needing long and expensive structure-activity relationship studies.

FIGURE 51
Agonists and competitive antagonists



The pharmaceutical industry has synthesised a considerable number of structurally related molecules, and this has greatly contributed to our knowledge about receptors. Indeed, the "**binding site**" of each receptor possesses a unique spatial arrangement of amino acid residues with which certain parts of the "**ligand**" (i.e. messenger or drug) can interact. The effectivity of such interactions differs from one drug to another, so that the affinity of a receptor is different for every drug. The order of affinities (often called "**order of potencies**") of a series of drugs or for a specific receptor (i.e. its "pharmacological profile") therefore serves as a useful "fingerprint" for that receptor. Such fingerprints allow:

- the positive identification of a receptor,
- the discrimination of one receptor from another
- the discovery of new receptors

Messengers are often capable to recognise a whole series of different receptors. These receptors are often specific for that messenger (e.g. the receptors for acetylcholine bind no other messenger), and they are usually referred to as "**receptor subtypes**". Occasionally, such a receptor family may be shared by a limited number of messengers (e.g. the adrenergic receptors can be stimulated by both adrenaline and noradrenaline, but by no other messenger). A newly discovered receptor often recognises a known messenger, so that it can be referred to as a "receptor subtype".

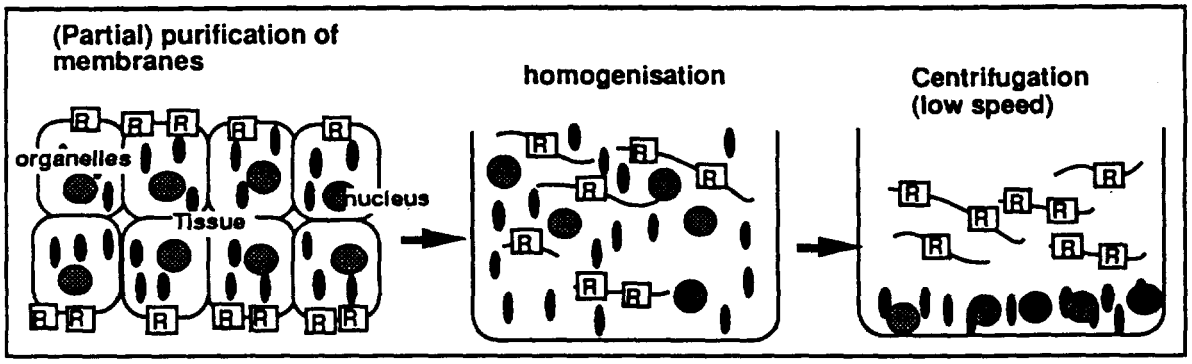
The investigation of receptors has first relied on physiological approaches, then on biochemical approaches and nowadays on genetic approaches. The introduction of each new approach has always provided an impulse for the discovery of new receptors or receptor subtypes. These discoveries are very beneficial for the medical treatment of diseases, since it authorises the use of more- and more selective drugs and, hence, with less possible side effects.

D) DIRECT IDENTIFICATION OF RECEPTORS BY RADIOLIGAND BINDING

TECHNICAL ASPECTS.

For a long time, hormone and neurotransmitter receptors remained abstract concepts whose existence was proposed only to explain pharmacological effects on target tissues. Since the mid-seventies, it has become possible to investigate of the receptor molecules themselves by the means of radioligand binding. This technique also allows the direct evaluation of the binding properties of any compound for a given membrane-bound receptor.

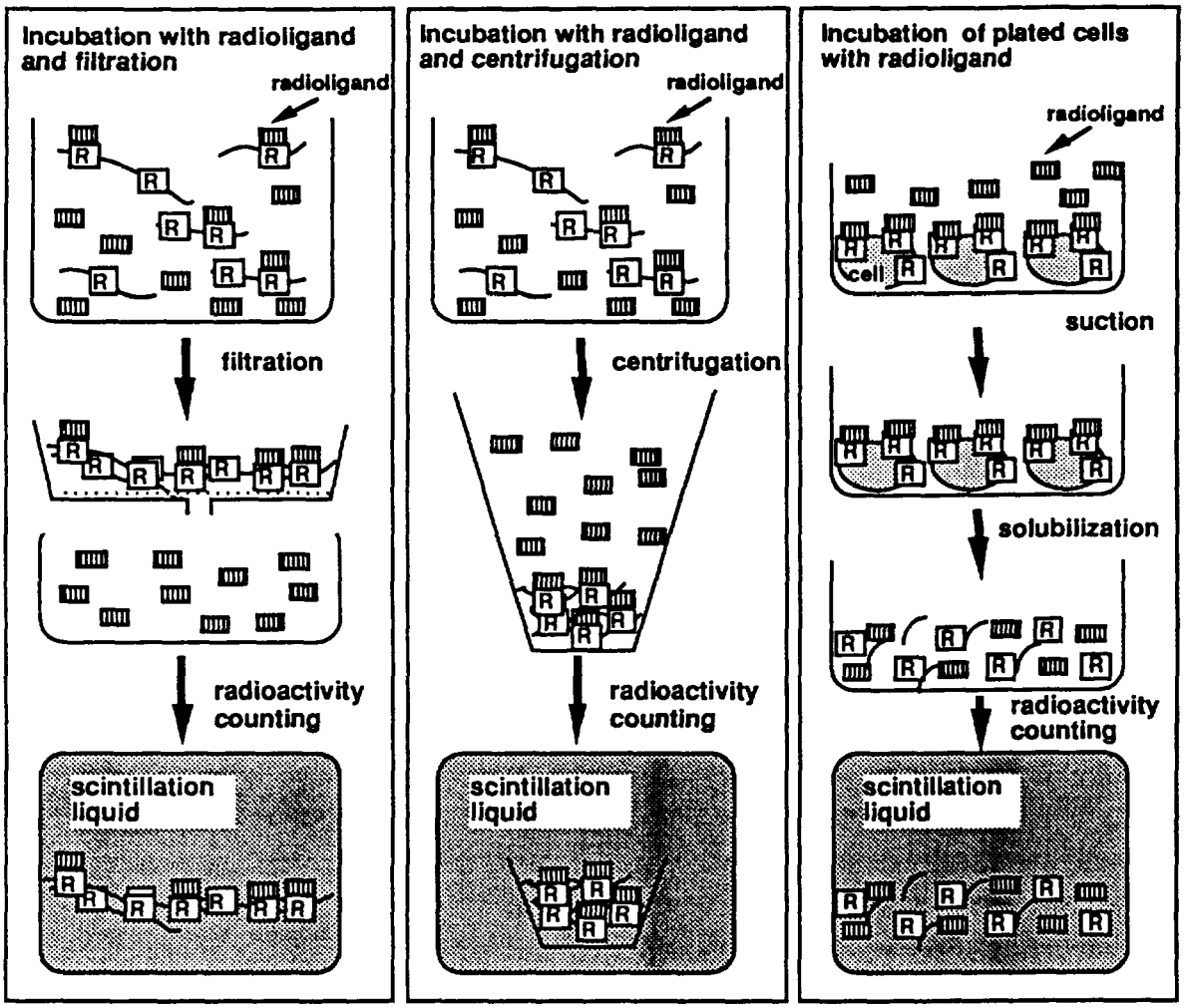
FIGURE 60A
Preparation of cell membranes



Radioligand binding comprises two major steps.

- Incubation of cells, cell homogenates or purified plasma membrane preparations with an adequate radioactively labelled drug ; the "**radioligand**". Adequate radioligands can be selected out of the wide variety of commercially available agonists and antagonists. Obviously, these radioligands should display high affininty and selectivity towards the receptor of interest. If no such radioligands are available, ligands can be custom- labelled by the investigator (for radioiodination) or by specialised institutions.
  •Tritium [^{3}H] and iodine [^{125}I] are the most frequently used isotopes. Because of the long half-life of tritium (12.3 years), the tritiated ligand does not have to be resynthesized or repurchased frequently. However, because of the relatively low specific radioactivity of tritium (29 Ci/mmol), tritiated ligands are only suitable when the biological material contains sufficient amounts of the desired receptor.
  •If not, radioiodinated ligands are more suitable because of the relatively high specific radioactivity of ^{125}I (2125 Ci/mmol). However, the short half-life (60 days), the exposure of the investigator to gamma rays and the fact that the pharmacological and physicochemical properties of the iodinated ligand may deviate considerable from those of the original ligand constitute major drawbacks of this isotope.

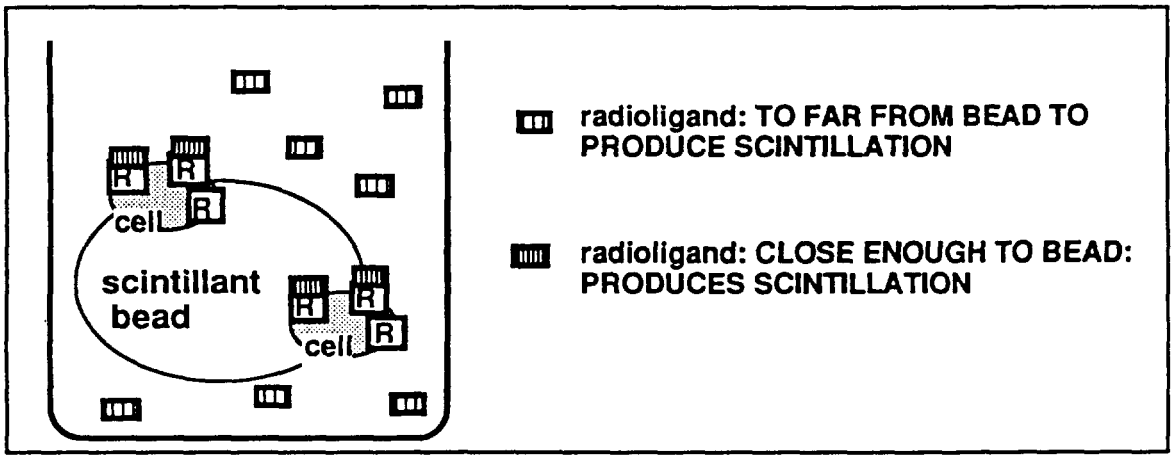
FIGURE 60B
Measurement of radioligand binding by different methods involving the removal of free radioligand.



- Separation of free and receptor-bound drug. This is commonly done by one of the three following techniques:
  •Filtration (Fig. 60B): the free radioligand passes through the filter whereas the receptor-bound radioligand remains on the filter. Quantitation of the amount of receptor-bound radioligand is done by counting the radioactivity on the filter. This technique is usually employed when using membrane preparations and when performing radioligand binding to intact cells in suspension. The popularity of this technique results from the ability to handle a large number of samples with relative ease as well as the commercial availability of a variety of filtration devices. Moreover, the filters can be washed thouroughly and rapidly with fresh buffer (preferentially ice-cold-to prevent dissociation of the radioligand-receptor complex). This allows the removal of remaining traces of free radioligand. The filters are usually of glass fibre, but sometimes it is also necessary to coat them with polyethyleneimine or to siliconize them to prevent radioligand absorption to the filter. For 'high throughput screening', the radioligand binding may be performed in 96 well microtiter plates. After the incubation, the contents of the wells are filtered simultaneously with a cell harvester. The receptor-bound radioligand is then present at 96 different locations on a large rectangular filter (10 by 20 cm) and, after application of the scintillant, the radioactivity of these locations is counted.

•Centrifugation (Fig. 60B): membranes or cells precipitate whereas the free radioligand remains in solution, and can be discarded. Quantitation of the amount of receptor-bound radioligand is done by counting the radioactivity of the pellet. Since no thorough washing is involved, this technique is especially useful when the radioligand-receptor complex dissociates rapidly. However, this technique results in high background radioactivity due to the trapping of radioligand in the pellet. Manual manipulations and the resulting risk of contamination constitute additional disadvantages of the technique.
•Suction (Fig. 60B). Binding to intact cells may be achieved by using cells which are plated on the bottom of each well in (e.g. 24 well) multiwell plates. After the incubation, the free radioligand is removed by suction, the cells may then be washed with fresh buffer (preferentially ice-cold-to prevent dissociation of the radioligand-receptor complex), and the remaining receptor-bound radioligand in each well is counted. For this purpose, plated cells are often treated with a detergent solution to solubilise the membranes. The radioactivity gets into solution and can then be counted easily. Here again, many manual manipulations are required.

FIGURE 60C
Measurement of radioligand binding by the SPA technique.



The recently available scintillation proximity assay (SPA) technique demands even less manipulations since the separation between free- and bound radioligand is avoided. For this technique (Fig 60C), small scintillant-containing beads are already present in the incubation tube/well. When these beads are also coated with wheat germ agglutinin (WGA), they will attach intact cells or membranes. The principle of the technique is based on the assumption that the overwhelming majority of the free radioligand molecules are to far from the beads for the scintillant to be activated whereas the receptor-bound radioligand is in close proximity of bead and, hence, capable of stimulating the scintillant. Therefore, the measured scintillation will mainly arise from receptor-bound radioligand molecules.

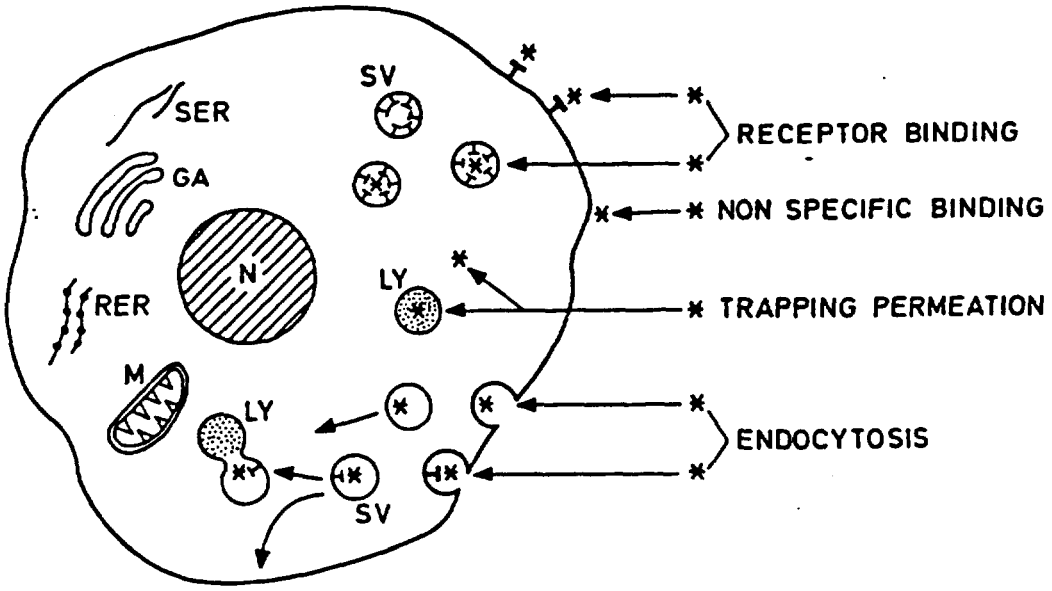
SPECIFIC AND NON-SPECIFIC BINDING

Binding of a radioligand to a physiologically relevant receptor (i.e. '**specific binding**') should at least obay the following criteria:

- The binding should be saturable, since a finite number of receptors are expected in a biological preparation.
- The specificity of unlabelled ligands in competing with the radioligand for binding to the receptor should parallel their potency to provoke (for agonists) or block (for antagonists) receptor- mediated physiological responses.

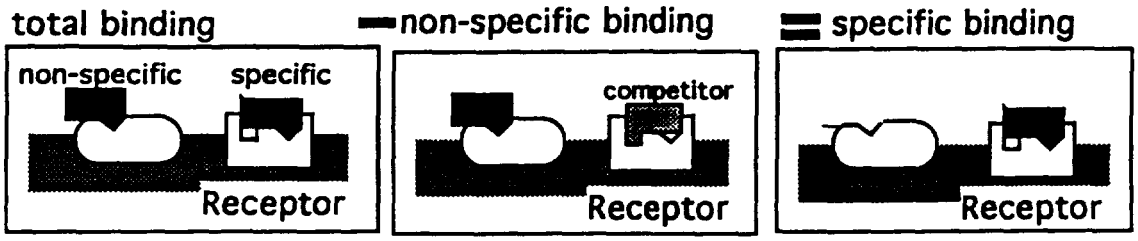
Radioligands will bind to their receptor, but also to some extent to other receptors and to non-receptor sites such as carrier proteins, enzymes, cell components recognising certain chemical moieties of the radioligand (e.g. the catechol moiety for radiolabelled catecholamines) (Fig 61 A) or even separation materials such as filters or test tubes. This binding is called '**non-specific binding**'. One of the major problems in developing a suitable binding assay is the selection of a radioligand that shows enough specificity towards the receptor. In general, a hydrophilic (to avoid partitioning in the lipid bilayer of the membrane) radioligand with high affinity for the desired receptor may be a good candidate. However, more or less of the measured binding will always be non-specific. To deal with this problem, radioligand binding experiments always comprise two determinations: total binding and non-specific binding and the **non-specific binding** must be subtracted from the **total binding** to obtain the **specific binding**; i.e. binding to the receptor of interest (Fig. 61B).

FIGURE 61A
Possible interaction between radioligands and different cellular sites



The obtention of a correct non-specific binding constitutes the most delicate aspect of a radioligand binding technique. In theory, non-specific binding can simply be obtained by adding an excess of competitor to the incubation mixture, so that binding of the radioligand to the receptors is completely displaced. In practice, care must be taken to choose a competitor which displaces the radioligand from the receptor only, and not from the other, non-specific sites. It is recommended to choose a potent competitor whose chemical structure is quite distinct from that of the radioligand.

FIGURE 61B
Determination of total and non-specific binding and the calculation of specific binding.



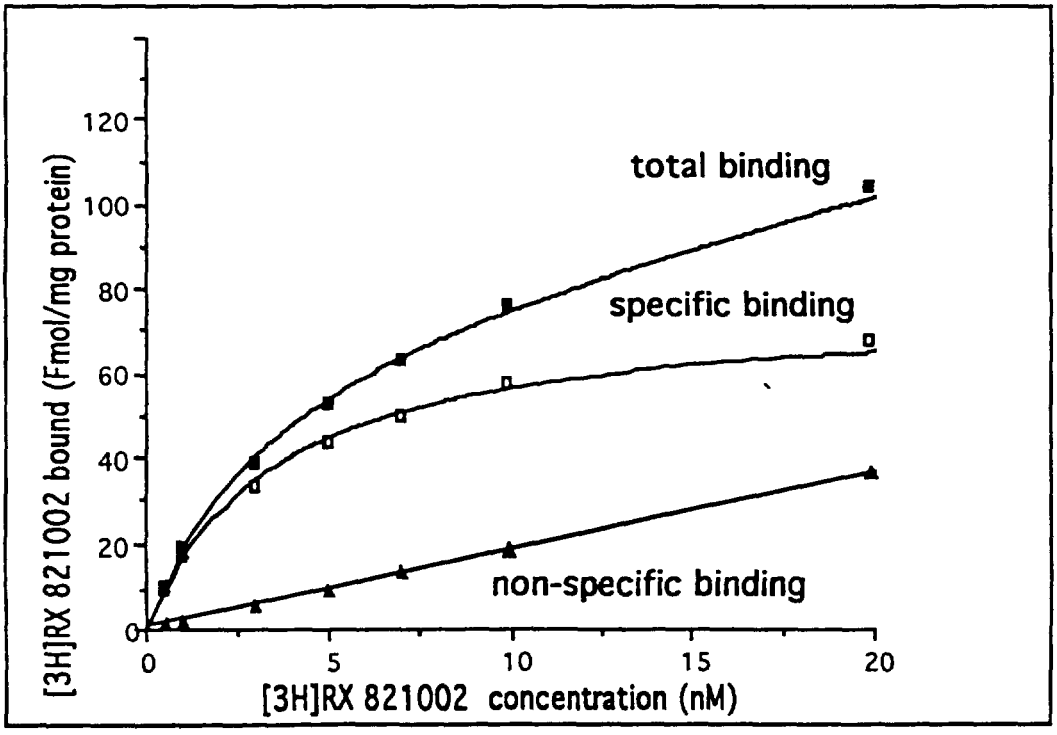
TYPES OF RADIOLIGAND BINDING EXPERIMENTS.

Radioligand binding studies provide three main categories of information: saturation binding data, competition binding data and kinetic data.

a) Saturation binding

These experiments provide information about the concentration of a receptor. They are solicited to compare the concentrations of different receptors in a given tissue and to monitor variations in receptor concentration as a result of normal physiological regulation, medication and pathophysiological conditions.

FIGURE 62A
Saturation binding of the α_2-adrenergic antagonist [^{3}H]RX 821002 to α_2-adrenergic receptors in membranes from the human frontal cortex.



For saturation binding experiments, constant amounts of membrane suspension are incubated with increasing concentrations of radioligand. Obviously, both total and non-specific binding should be measured at each concentration of radioligand. In the example shown in Fig. 62A, Binding is expressed as a function of the free concentration of radioligand by a **saturation binding plot.** Obviously, only the specific binding is of interest.

To analyse these saturation binding data, it is necessary to advance a relevant molecular model for the radioligand-receptor interaction. In the simple (and fortunately the most common) situation, the interaction of the radioligand (L) with the receptor (R) can be expressed as a reversible bimolecular reaction that obeys the law of mass action : i.e.

$$L + R \underset{k-1}{\overset{k1}{\rightleftharpoons}} L\text{-}R \qquad (1)$$

Where k1 and k-1 are the association and dissociation rate constants, respectively. The equilibrium dissociation constant (K_D) is given by :

$$K_D = k\text{-}1 / k1 = [L] \cdot [R] / [L\text{-}R] \qquad (2)$$

The relationship between the amount of occupied receptors and the free radioligand concentration (i.e. the saturation binding plot) is as follows:

$$[L\text{-}R] = [R_{tot}] / (1 + K_D / [L]) \qquad (3)$$

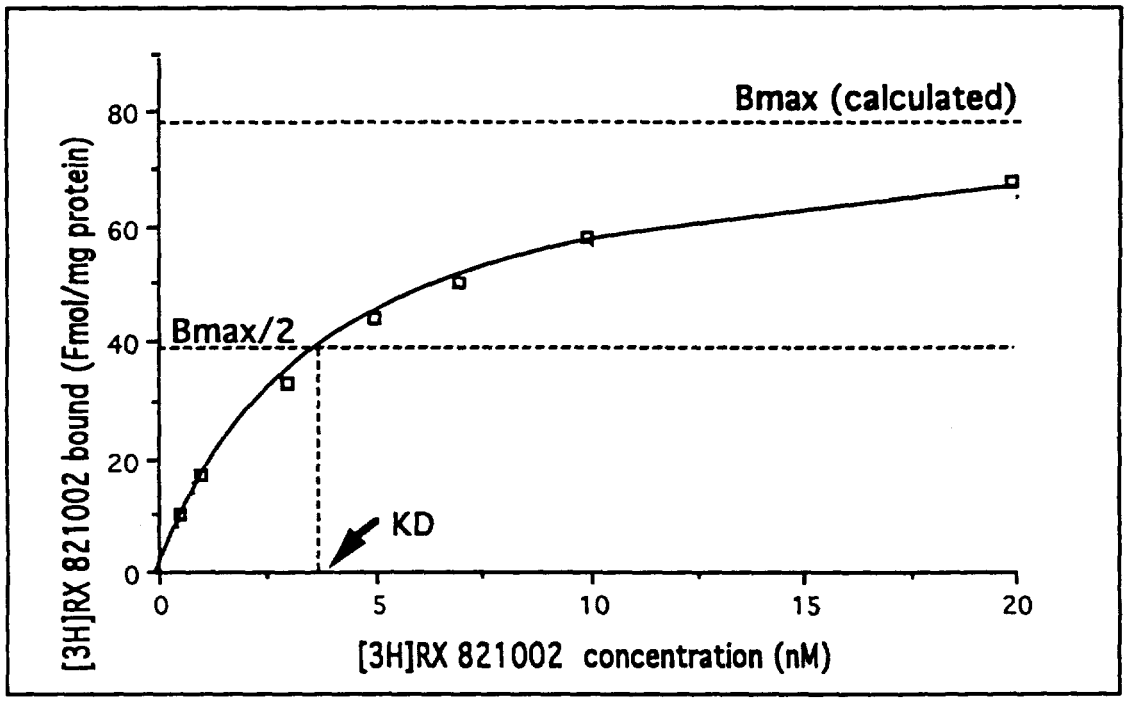
Where $[R_{tot}]$ is the total number of receptor sites. [L-R] refers to the amount of bound ligand, and $[R_{tot}]$ to the maximum binding. These notations are usually replaced by "B" and "B_{max}" (which are often expressed as fmol/mg protein). Equation 3 becomes then:

$$B = B_{max} / (1 + K_D / [L]) \qquad (3)$$

This equation is analogous to the Michaelis-Menten equation of enzyme kinetics and describes a rectangular hyperbola (Fig 62B). Initially, B increases almost linearly with L. Then B tends to level off when L is further increased. The limit value is B_{max}. It is important to notice that this that B_{max} will be attained only at infinite concentrations of L. Thus, one will never observe B_{max} experimentally; Bmax may be approached but never attained.

Half-maximal binding is obtained when $L = K_D$ (since equation 3 becomes: $B = B_{max}/2$). In other words,the K_D of a radioligand corresponds to its concentration for which half of the receptors are occupied. The K_D- value is thus an "inverse" measure for the radioligand's affinity for the receptor: a low K_D corresponds to high affinity and a high K_D to low affinity.

FIGURE 62B
Graphical analysis of the saturation binding plot of the α_2-adrenergic antagonist [^{3}H]RX 821002 to α_2-adrenergic receptors in membranes from the human frontal cortex (i.e specific binding from Fig. 62A).



B_{max} and K_D cannot be easily determined by graphical analysis of the saturation binding plot since equation 3 is a non-linear relationship and since B_{max} is, in fact, only reached when $L = \infty$. This equation can, however, be transformed mathematically to yield a linear **'Scatchard plot'** corresponding to the following equation:

$$B/[L] = -B/K_D + B_{max}/K_D \qquad (4)$$

The Scatchard plot of the saturation binding data from Fig. 62 is represented in Fig. 63A. The relationship between B/[L] (the ordinate) and B (the abscissa) is linear. K_D corresponds to the negative reciprocal of the line. The intercept of the line with the abscissa (i.e. when B/[L] =0) is B_{max}. Thus, it is relatively easy to calculate K_D and B_{max} values by linear regression analysis of the Scatchard plot.

The relationship described by equation 4 is for the simplest case; i.e. a single class of non-interacting receptor sites. However, it is possible that the radioligand binds to two different receptors with different affinity or even that one receptor is present in two or more (non-interconverting) affinity states for the radioligand. This situation will result in a non-linear Scatchard plot, show a upward concavity such as in Fig. 63B.

Moreover, certain receptors (e.g. ion channel- gating receptors which make part of a larger structure or receptors which possess multiple binding sites such as the insulin receptor) are also able to influence each-other's binding characteristics.
This may result in either negative or positive cooperative interactions among the

binding sites. In other words, binding of the radioligand to one site decreases (for negative cooperativity) or increases (for positive cooperativity) the affinity of the radioligand for other sites. This will also result in non- linear Scatchard plots with respectively upward concavity (for negative cooperativity) or downward concavity (for positive cooperativity) (Fig. 63B).

FIGURE 63A
Scatchard plot of the saturation binding data shown in Fig. 62. (B in fmol/mg protein, F in nM)

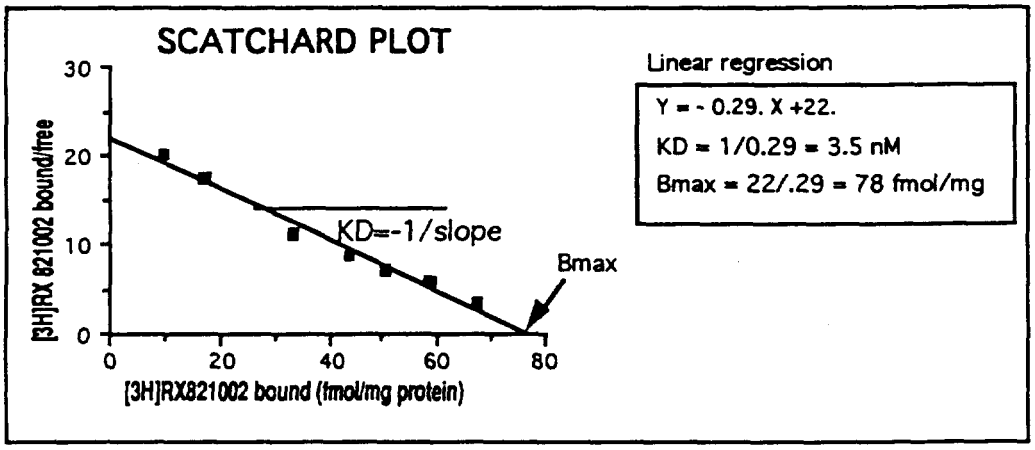
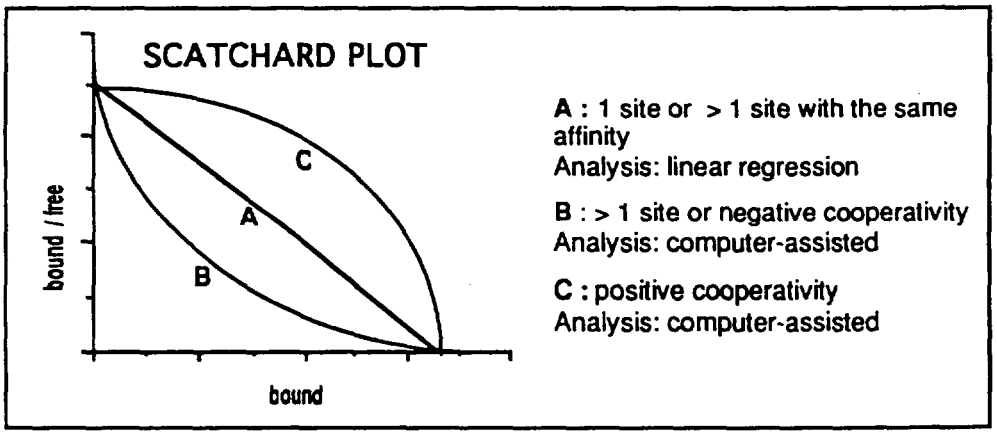


FIGURE 63B
Scatchard plots: different possibilities



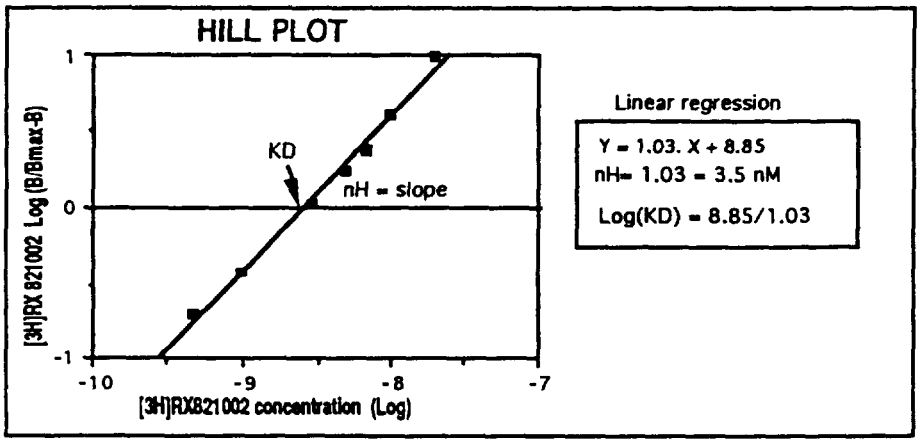
A more sensitive method to ascertain whether radioligand binding obeys the law of mass action is to analyse the 'Hill plot' (Fig. 64) of the saturation binding data. The Hill equation is, in fact, a logarithmic transformation of equation 3.

$$\text{Log}(B/(B_{max} - B)) = nH.\text{Log}([L]) - \text{Log}(K_D) \qquad (5)$$

$\text{Log}(B/(B_{max} - B))$ is the ordinate and $\text{Log}([L])$ is the abscissa of the Hill plot. The slope corresponds to the Hill coefficient: "nH". The law of mass action is obeyed if nH = 1 (in practice, values between 0.8 and 1.2 will do). This means that the radioligand binds with the same affinity to all the sites. nH < 1 is indicative for either negative

cooperativity or for the existence of binding sites with different affinity. nH > 1 is indicative for positive cooperativity, i.e. where radioligand binding to one site increases the affinity of the radioligand for other sites.

FIGURE 64
Hill plot of the saturation binding data shown in Fig. 62. (B in fmol/mg protein, F in nM)



A practical disadvantage of a Scatchard plot is that the extrapolation of data obtained over an insufficient concentration range of L may give an artificial impression of the lack of complexity of the radioligand-receptor interactions. This may result in inaccurate Hill plots since they rely on a correct estimation of the B_{max} value. Although Scatchard and Hill plots are still often shown for demonstrative purposes, K_D, B_{max} and nH values are now almost always calculated by computer programmes which are based on non-linear regression analysis of the saturation binding data. In the case of two non-interconverting binding sites, they even allow the calculation of the concentration of each site and its respective affinity of the radioligand. Among those, Graph-Pad (available both for Windows and Macintosh) is one of the most performant.

Finally, certain important considerations need to be taken into account before correctly analyzing saturation binding data; they include:

- The data must represent an equilibrium situation. In practice, this means that the incubation must have occurred long enough for an equilibrium to be reached. This can be checked by investigating binding of a given concentration of radioligand as a function of the incubation time. This binding will increase time-wise until a plateau value (corresponding to the equilibrium situation) is reached. Equilibrium binding is often obtained within minutes at usual incubation temperatures (20-37°C) but that it may become considerably longer when temperature is lowered to (0-4°C).
- binding is expressed as a function of the free concentration of radioligand. This concentration may be set equal to the concentration of radioligand added (i.e. [L] = [L_{init}]) if only a small fraction of the added radioligand is bound (in other words, if most of the added radioligand still remains free). If a more substantial amount of radioligand is bound (e.g. > 5 %), then [L] is smaller than [L_{init}], and its correct value should be calculated by the equation: [L] = [L_{init}] - [L-R].
- The ligand must not aggregate at higher centrations to a dimer or multimer.

b) Competition binding

Radioligands are fairly expensive and only very few of them are specific enough for the purpose of receptor identification. Fortunately, radiolabelling of a drug is not strictly required for determining its affinity for a given receptor. This parameter can indeed be determined on basis of the drug's ability to compete with a (specific) radioligand for binding to that receptor. These competition binding experiments are now widely used by pharmacologists as a screening tool to evaluate the affinity of newly synthetised compounds (or of natural substances) for one or more receptors of interest. This approach has several advantages over the measurement of physiological responses. First, the same experimental setup can be used to investigate the affinity of a drug affinities for different receptors, whereas physiological responses may be very diverse and, hence, need to be monitored by different techniques. Second, the affinity of a drug for a specific receptor can be determined without ambiguity, whereas physiological responses are remote events which may be triggered by different receptors or even be modulated at steps which are intermediate between receptor-stimulation and the final response.

It is important to note that the terms **'competition binding'** and **'competitor'** (for the non-radioactive substance) are commonly utilized irrespective of whether the 'competitor' is truly competitive or not. This semantic problem merits proper attention.

For competition binding experiments, constant amounts of membrane suspension are incubated with a fixed concentration of radioligand and increasing concentrations of the non-radioactive substance to be tested (the competitor), after which binding of the radioligand is measured. Binding of the radioligand is expressed as a function of the free concentration of competitor by a **competition binding plot**, such as the one shown in Fig 65. Competitor concentrations might span several orders in magnitude, so that they are often expressed on a logarithmic scale. In the simple situation (in which the competitor is truly 'competitive') the radioligand and the competitor bind in a reversible fashion to the same site of the receptor. The radioligand (L)- receptor (R) and the competitor (I)- receptor (R) interactions can be expressed as reversible bimolecular reactions: i.e.

$$\begin{matrix} L \\ + R \\ I \end{matrix} \quad \rightleftharpoons \quad \begin{matrix} L\text{-}R \\ \\ I\text{-}R \end{matrix} \qquad (6)$$

The equilibrium dissociation constants for these interactions are denoted as K_D for the radioligand and K_i (with i instead of D, to avoid confusion) for the competitor. The relationship between the amount of radioligand binding (B) and the competitor concentration (i.e. the competition binding plot) obeys the following equation:

$$B = B_{control} - B_{control}/(1 + K_i \cdot (1 + [L]/K_D)/[I]) \qquad (7)$$

Control binding ($B_{control}$) represents binding of the radioligand in the absence of competitor. An interesting situation occurs when the competitor has decreased control binding by 50% (i.e. when $B = B_{control}/2$). This situation occurs when the concentration of competitor (usually denoted as IC_{50}) is equal to $K_i.(1 + [L]/K_D)$. The

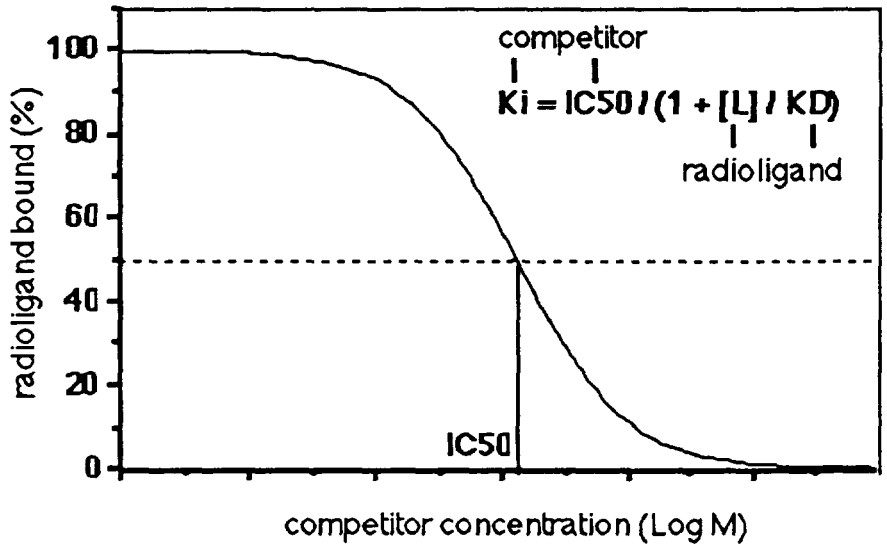
competitor's K_i can thus be calculated from the experimental IC_{50}-value by the following equation (equation of **Cheng and Prusoff**) :

$$K_i = IC_{50}/(1 + [L]/ K_D) \qquad (8)$$

[L] is known and K_D is obtained from saturation binding experiments. Please note that K_i is a constant, but that the IC_{50}-value is dependent on the concentration and the K_D of the radioligand used (Fig. 66). Accordingly:

- K_i-values of different competitors may always be compared with each other (e.g. to give a rank order of affinities) since they represent affinity constants.
- IC_{50}-values may only be compared with each other when the competition binding experiments are carried out under identical conditions; i.e. when the same radioligand is used at the same concentration for all the experiments.

FIGURE 65
Competition binding curve (100% binding is binding in the absence of competitor) and determination of the competitor's K_i from the IC_{50}



A practical example of the utility of competition binding curves for finding out whether a radioligand truly binds to the desired receptor is shown in Fig. 67 and 68. In these experiments, various unlabelled drugs compete with the tritiated β-adrenergic antagonist [^{3}H]-dihydroalprenolol for binding to turkey erythrocyte membranes. The experiments were performed under identical conditions (i.e the radioligand concentration was 10 nM) so that the IC_{50}-values of the curves can be compared with each other. The affinity of the agonists decreases in the order: (-)-isoproterenol > (-)-noradrenaline > (-)-adrenaline (Fig. 67). The nonselective α-adrenergic antagonist phentolamine has only very low affinity and no competition can be demonstrated for the non-bioactive compounds catechol and 3,4-dihydroxy phenylglycol (Fig. 67). These characteristics fit with those obtained for β_1-adrenergic receptors by functional studies. Moreover, in agreement with the known stereoselectivity of β-adrenergic receptors for antagonists such as propranolol and agonists such as adrenaline, the dextrorotary isomers display lower affinity than the levorotary isomers (Fig. 68).

FIGURE 66
Effect of the [L]/K_D-ratio of the radioligand on the competition curve of a drug with K_i = 0.1 µM

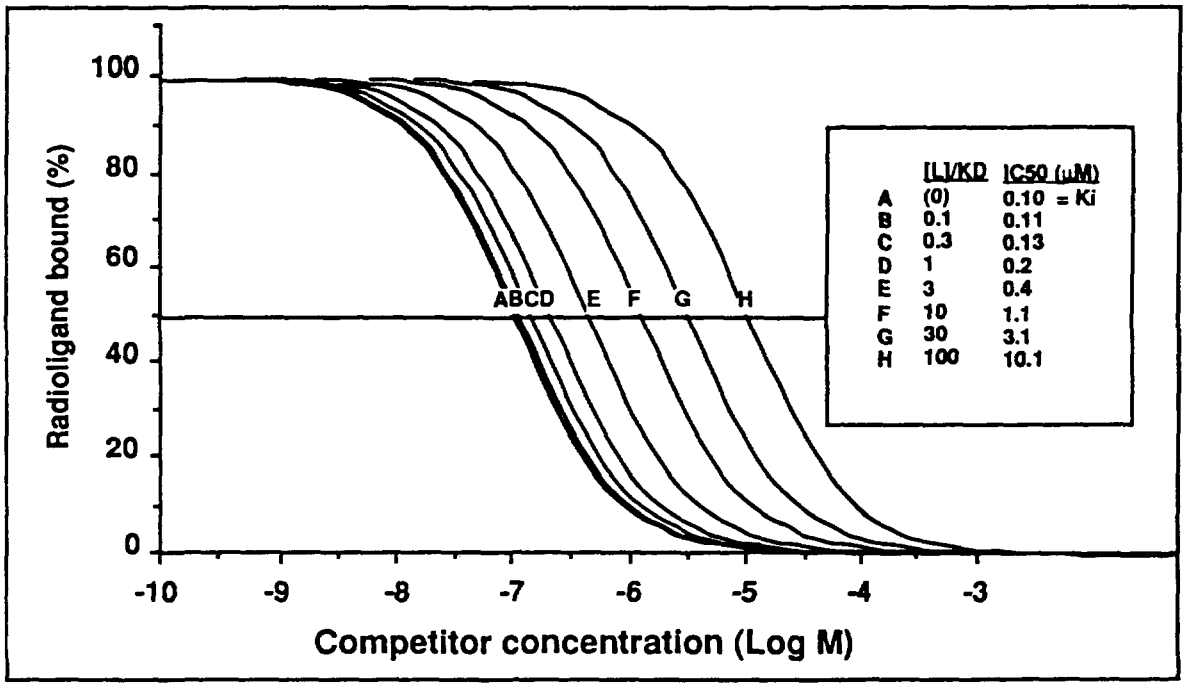


FIGURE 67
Competition binding curves for β_1-adrenergic receptors in turkey erythrocyte membranes

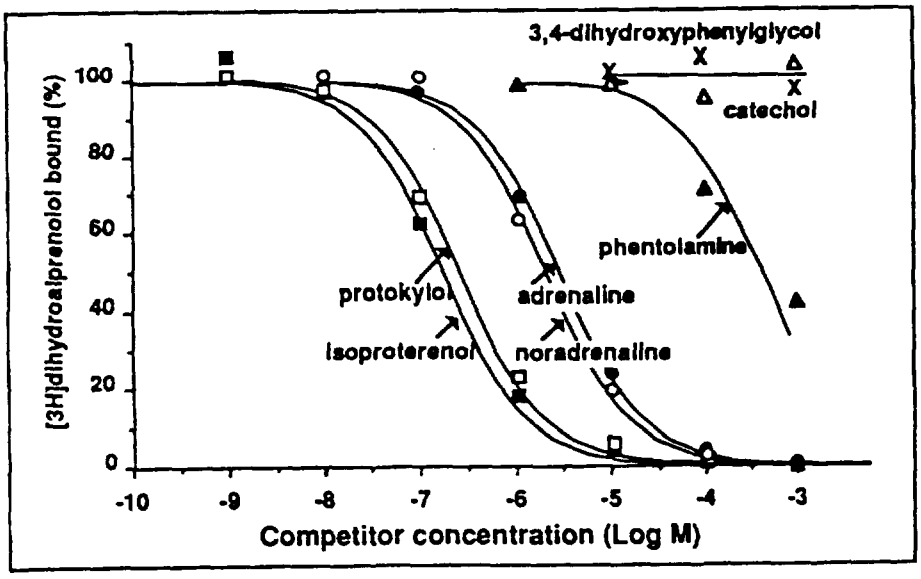
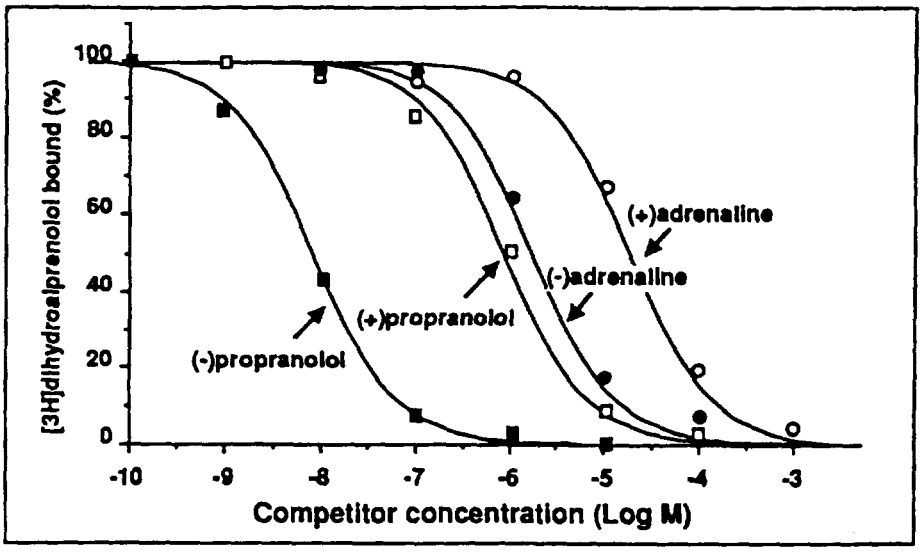


FIGURE 68: Stereoselective competition binding curves for β_1-adrenergic receptors



When the radioligand and the competitor bind in a reversible fashion to a single population of non-interacting receptors (i.e. in the simple situation), the competition binding curve should have a steep sigmoidal shape. (with 11, 50 and 89 % decrease in radioligand binding when the competitor concentration is 1/10th, equal or 10 times its IC_{50} -value). Hill coefficients that are calculated from such plots (in where the binding of the competitor ($B_i = B_o - B$) is taken in consideration: i.e. $Log(B_i/(100 - B_i)) =$

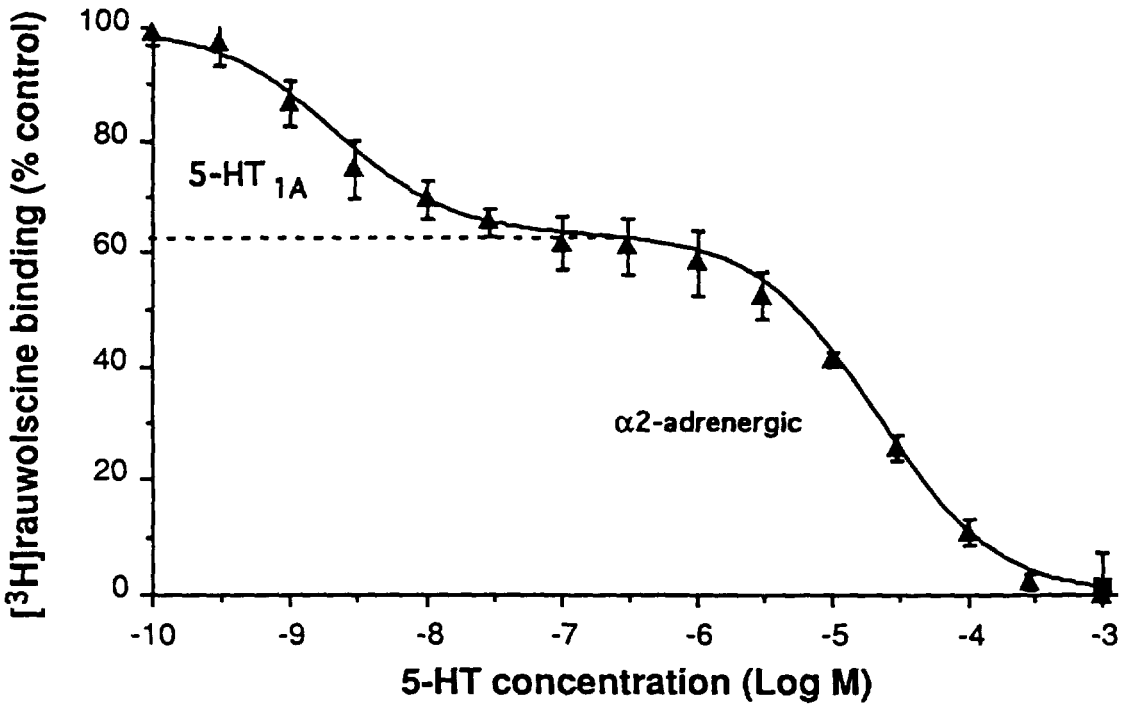
$nH.Log([I]) - Log(IC_{50})$ are equal to 1.

Radioligands may possess the same affinity for two (or more) receptors, receptor subtypes or even receptor subpopulations. When such different receptors coexist in the same cells or membrane preparation, they will not be discriminated from each other by the radioligand. Indeed, the saturation binding curves appear as if the radioligand binds to be a single class of non-cooperative sites. However, these different receptors (subtypes, subpopulations) may possess different affinity for certain unlabelled drugs, so that they can be detected and discriminated from each other by competition binding experiments with these drugs. In such cases, the nH-values of such curves will be less than one. There are two situations:

- First, the competitor displays a large (> 1000-fold) difference in affinity for the different receptors,, subtypes or subpopulations. In this situation, the competition binding curve will be biphasic (i.e. with a plateau) and the parameters of each component (% of binding, IC_{50}) are easy to measure. This is the case for [^{3}H]-rauwolscine which binds with the same affinity to α_2-adrenergic receptors and (5-HT_{1A}) serotonergic receptors. Serotonin possesses much higher affinity for its own receptor as for the α_2-adrenergic receptors and can be used to distinguish both receptors from each other in e.g. human frontal cortex membranes (Fig. 70). At low concentrations it will first occupy the 5-HT_{1A} receptors and, only when its concentration gets high enough, it will start to occupy the α_2-adrenergic receptors. 5-HT_{1A} receptors represent 40 % of the binding and α_2-adrenergic receptors 60 %). The K_i values of serotonin for these receptors can be calculated from the IC_{50}-values according to the equation of Cheng and Prusoff.

FIGURE 70

Competition binding curve of 5-HT for α_2-adrenergic and 5-HT_{1A} serotonergic receptors in membranes from human frontal cortex



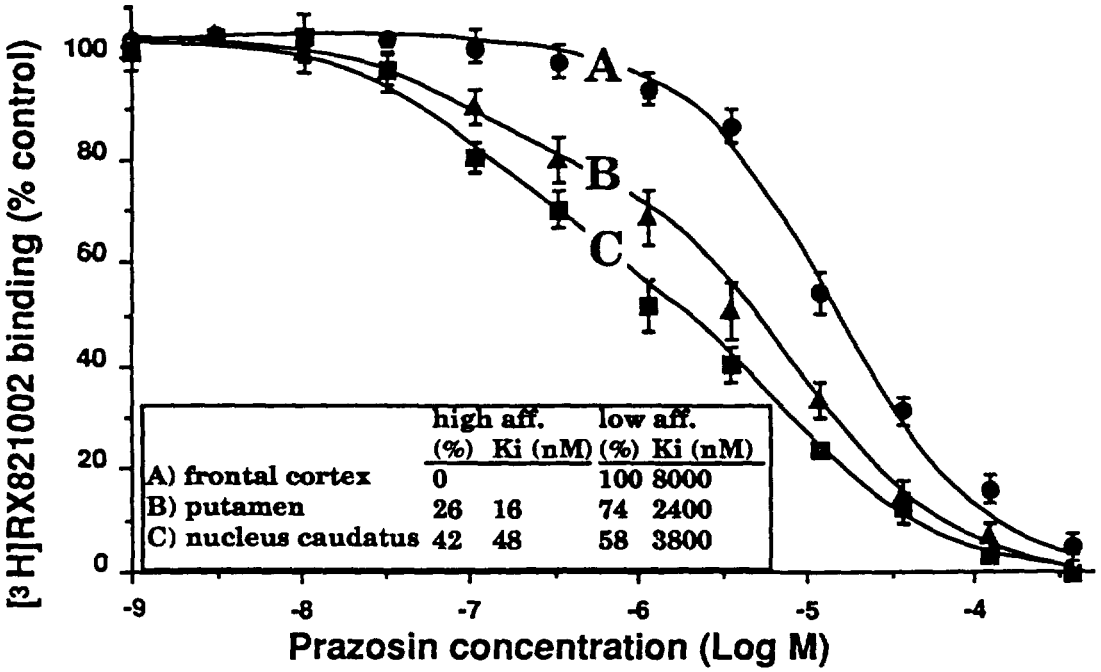
- Second, the competitor only possess a limited (<100 times) difference in affinity for the different receptors, subtypes or subpopulations. Such competition curves are shallow (nH < 1) but, since both components are not separated by a distinct plateau, it is necessary to calculate the competition binding parameters of each component (% of binding, IC_{50}) by computer-assisted analysis. This is illustrated in Fig. 71 for α_{2A}- and α_{2B}-adrenergic receptors. Radioligands such as the antagonist [3H]-RX821002 are unable to discriminate between them but certain antagonists such as prazosin possesses a relatively weak selectivity for the α_{2B} receptors. Prazosin competition binding curves for different human brain areas are shown in Fig 71.

  • For the nucleus caudatus, the competition curve is quite shallow (nH = 0.48). This indicates that α_{2A}- and α_{2B} receptors are both present. The simplest way to describe such curve is to give its nH and IC_{50}. However, since K_i's refer to individual competitor- receptor interactions, it is not possible to calculate any K_i from this IC_{50}. Computer-assisted analysis is necessary to calculate the proportion of α_{2A}- and α_{2B} receptors and their IC_{50} (and K_i) for prazosin.

  • The curve is also shallow for the putamen but it is steep (nH = 1.01) for the cortex (Fig. 71). For the cortex, the curve can be analysed according to a single-site model and the high K_i of prazosin indicates that only α_{2A} receptors are present in this brain region.

FIGURE 71
Competition binding curve of prazosin (α_{2B}- subtype- selective antagonist) for α_2-adrenergic

receptors in membranes from different human brain regions.



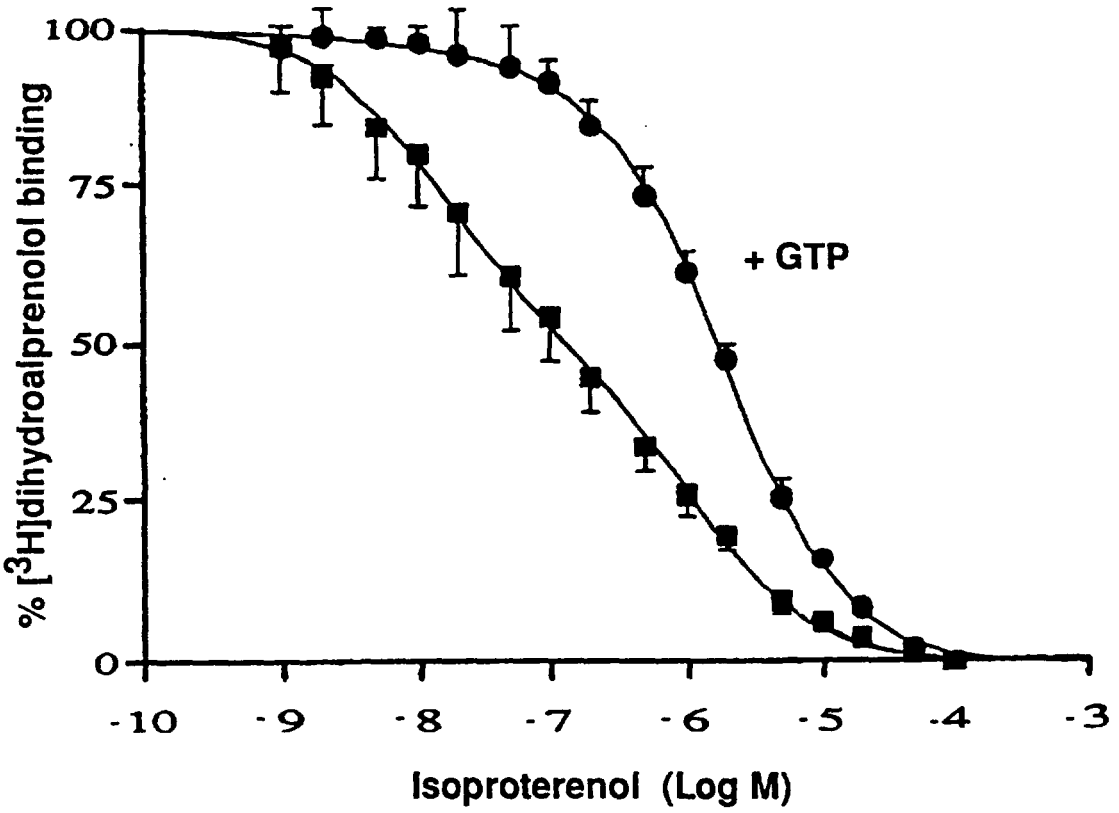
An interesting situation is observed for G-protein- linked receptors in broken cell preparations. These are often split into two subpopulations with different affinity for agonists, but with the same affinity for antagonists. This heterogeneity towards agonists is not related to differences in primary amino acid sequence, but rather to their capability to undergo functional coupling to the G-proteins. The receptor subpopulation which is able to undergo functional coupling to G-proteins (coupling-prone receptors) possess high agonist affinity. The receptor subpopulation which is unable to couple (non-coupled receptors) possess low agonist affinity.

- If the radioligand is an antagonist, it will regard the receptors as a single class of non- cooperative sites. Antagonist competition binding curves will be steep but agonist competition binding curves will be shallow.
- If the radioligand is an agonist, it will preferentially label the coupling-prone receptors (especially at low concentrations).

Shallow competition binding curves for agonists may thus reflect two distinct phenomena: the presence of different receptors, or functional receptor heterogeneity. Fortunately, it is possible to distinguish between these two possibilities by using guanine nucleotides such as GTP. These compounds break up agonist-receptor- G protein complexes, so that the receptors return to the the uncoupled, low agonist affinity form. In practice, GTP is thus capable to produce a rightward shift and steepening of the agonist competition binding curve, at least if the high affinity is related to functional coupling of the receptor to the G-proteins.

This is the case for the isoproterenol (agonist)/ [3H]-dihydroalprenolol (antagonist) competition binding curve in rat lung membranes (Fig. 72) The initially shallow curve is steepened and shifted to the right by GTP.

FIGURE 72
Competition binding curve of isoproterenol (β- adrenergic antagonist) for β-adrenergic receptors in rat lung membranes: effect of GTP.



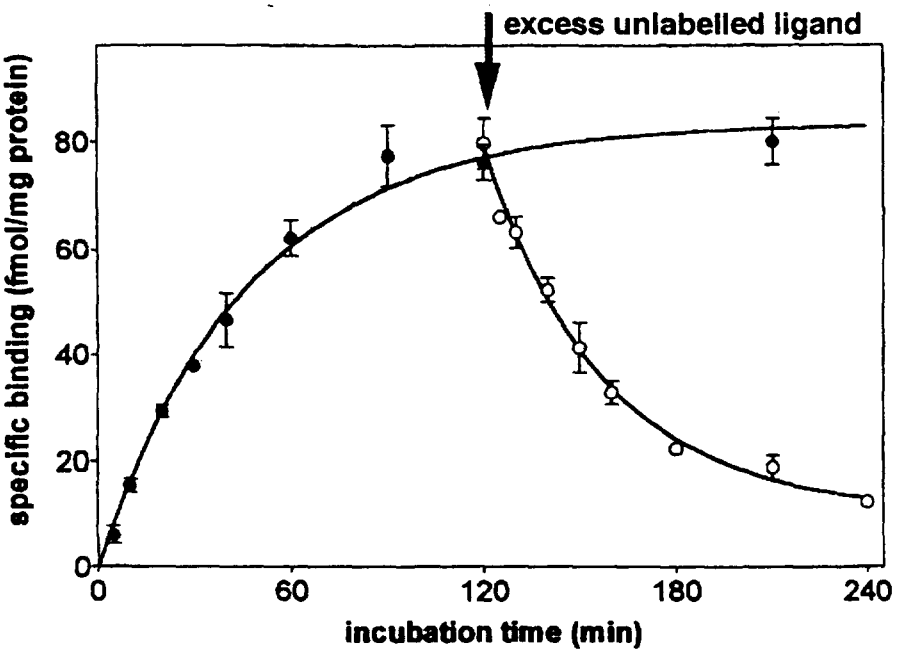
c) Kinetic experiments.

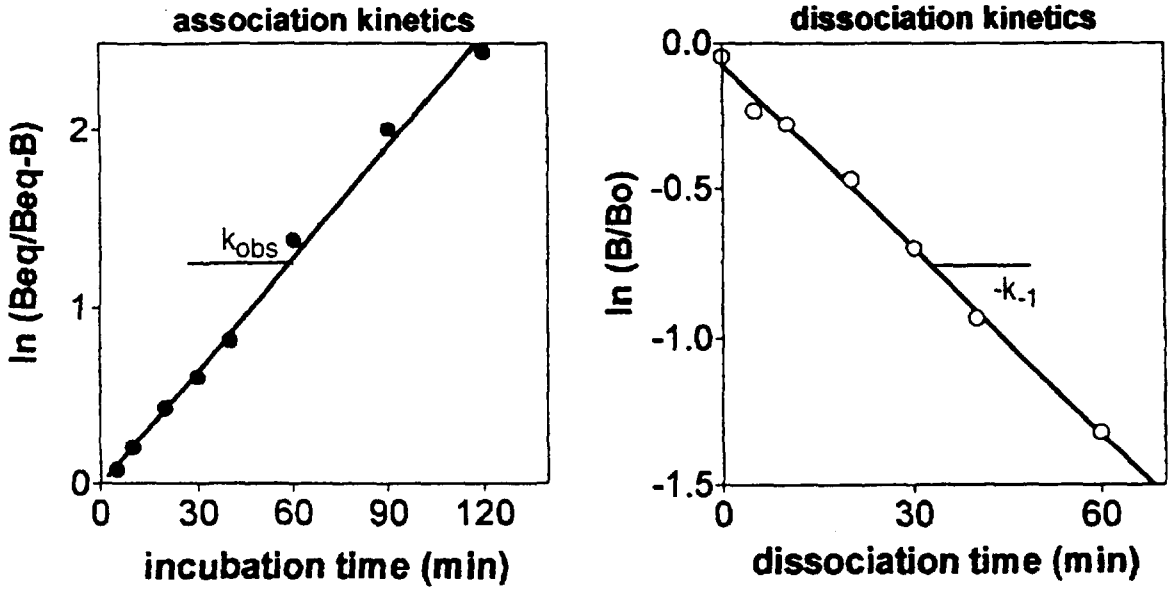
Unlike the saturation and competition binding experiments, kinetic studies provide information about the time-course of the binding. These studies usually comprise two types of experiments (Fig. 73).

- Determination of dissociation rate constant: in these experiments the radioligand is incubated with the receptor and the dissociation is initiated either by adding an excess of unlabelled ligand (so that free receptors are immediately occupied and no longer accessible to the radioligand) or by dilution (usually, after washing away the free radioligand, so that its free concentration is to low to undergo noticeable re-association). Then, the amount of binding is measured after different periods of time. First-order reactions occur when the radioligand-receptor complex is a single bimolecular species (L-R). Binding decreases exponentially and the dissociation rate constant (k-1) can be shown to be related to the time it takes for half of the L-R complexes to dissociate ($t_{1/2}$) by the equation $k\text{-}1 = 0.693/t_{1/2}$.
  Dissociation data can easily be calculated by plotting $\ln(B/B_{t=0})$ versus the dissociation time (Fig. 73). In the case of a first-order reaction, the plot will be linear and the slope corresponds to the negative value of k-1 (usualy expressed in

min^{-1}).

FIGURE 73
Association and dissociation binding of [^{3}H]NPY to the Y_1- receptors in the human SK-N-MC cells





- Determination of association rate constant: in these experiments the radioligand is incubated with the receptor and the amount of binding is measured after different periods of time. Binding will increase until equilibrium (equilibrium binding B_{eq})

- Determination of association rate constant: in these experiments the radioligand is incubated with the receptor and the amount of binding is measured after different periods of time. Binding will increase until equilibrium (equilibrium binding B_{eq}) is reached. Under circumstances where [L] is added at concentrations in considerable excess of [R] (as is most often the case), [L] can be assumed not to change throughout the incubation. In contrast, as only [R] decreases the rate of association may be regarded as being a 'pseudo first-order' reaction. When plotting $\ln(B_{eq}/(B_{eq} - B))$ versus the association time, the pseudo first-order rate constant (k_{obs}) is given by the slope of the plot (Fig. 73). Since the radioligand also undergoes dissociation from the receptor in this type of experiments, it ensues that k_{obs} reflects both the association and the dissociation of the radioligand. The true, bimolecular association rate constant k1 (usualy expressed in $M^{-1}.min^{-1}$) can be obtained by the following equation:

$$k1 = (k_{obs} - k\text{-}1)/[L]. \qquad (9)$$

Kinetic data allow the discrimination between fast reversible, slowly reversible and irreversible ligands (dissociation kinetics). Both the association and dissociation constants provide an estimation of the equilibrium dissociation constant (K_D) independently of saturation binding experiments: i.e.

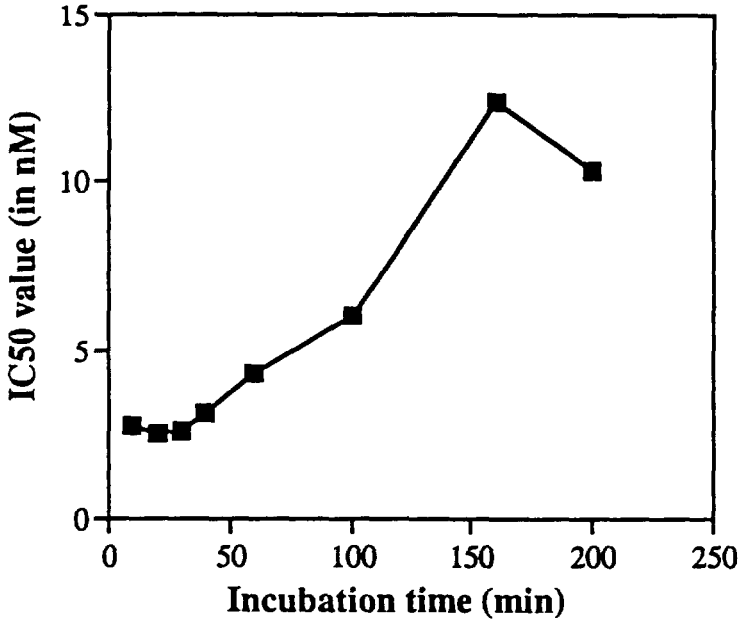
$$K_D = [L].[R]/[L\text{-}R] = k\text{-}1/k1 = [L].k\text{-}1/(k_{obs} - k\text{-}1) \qquad (10)$$

When the k-1/k1 ratio is quite distinct from the K_D obtained from saturation binding experiments, it may be that the ligand induces a time-dependent change in receptor conformation leading to an increase, or decrease, in receptor affinity.

Finally, kinetic data also provide information about the time required for binding of a radioligand to reach equilibrium (association kinetics). This information is crucial for the set-up of saturation and competition binding experiments. Indeed, the K_D and K_i -values which are derived from such experiments are only meaningful when, at any concentration of radioligand and competitor, binding is at or at least close to equilibrium. When the incubation time is to short, it could:

- produce a false estimation of B_{max} and K_D- values for saturation binding.
- produce a false estimation of IC_{50} and K_i- values for competition binding experiments. The example in Fig 74 shows an increase in experimental IC_{50} of a rapidly associating/dissociating competitor with the incubation time (i.e. the competition binding curve shifts to the right) when a slowly associating/ dissociating radioligand is used. The opposite (i.e. the competition binding curve shifts to the left) may be encountered for a slowly associating/ dissociating competitor and a rapidly associating/dissociating radioligand.

FIGURE 74
Effect of the incubation time on the IC_{50} of Y_1-receptors-selective antagonist BIBP3227 for competing with [^{3}H]NPY for binding to Y_1-receptors in rat forebrain membranes.

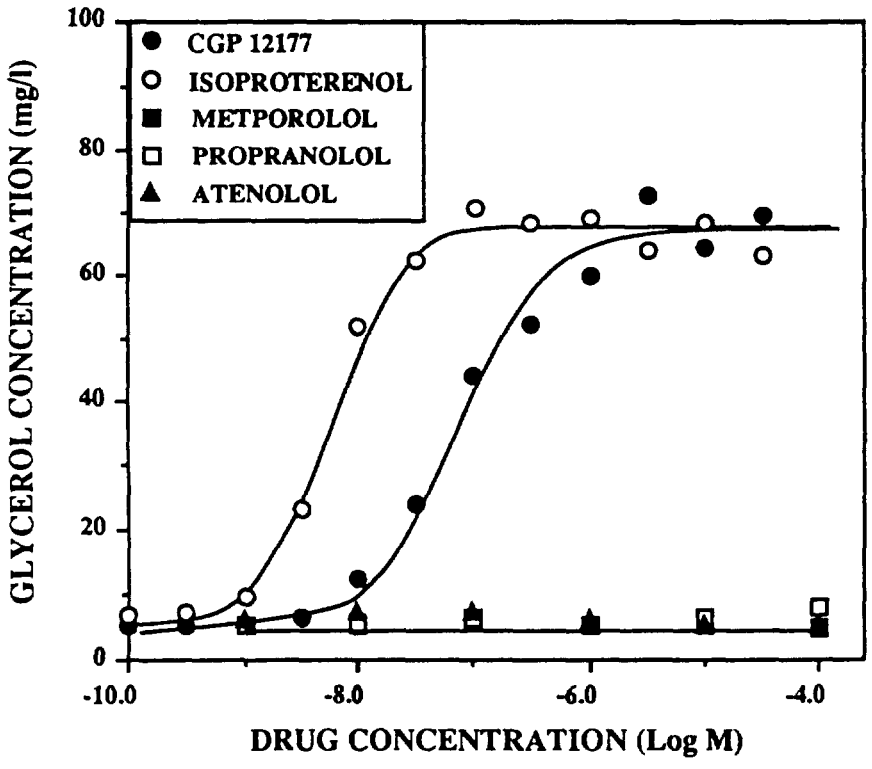


E) MEASUREMENT OF AGONIST-EVOKED RESPONSES.

Stimulation of a membrane-bound receptor by an agonist will provoke the onset of a whole chain of intracellular events. These events will ultimately lead to a "physiological" response. This response, as well as intermediate intracellular events can be measured to obtain (indirect) information about the receptor. For example, to investigate the effect of β-adrenergic drugs on the heart, the most proximate "biochemical" response is adenylate cyclase stimulation. The activity of this enzyme can be measured either in broken cell preparations or purified membranes (i.e. measurement of the conversion of [^{32}P]-ATP into [^{32}P]-cAMP) as well as in intact cell or whole organ preparations (measurement of the cAMP concentration). The more distant "physiological" events comprise the positive inotropic (i.e. increased force of contraction) and positive chronotropic (i.e. increased rate of contraction) responses. The relationship between the drug-evoked response and receptor occupancy is often complex, especially when both phenomena are separated from each other by a long chain of intermediary events. In an attempt to define such relationships, pharmacologists have introduced concepts such as "intrinsic activity", "efficacy" and "receptor reserve".

FIGURE 77

Dose-dependent effect of isoproterenol (β-adrenergic agonist) and propranolol (antagonist) on the lipolysis (measured by the amount of released glycerol) in rat fat cells



Ligands may be roughly divided into agonists and antagonists. Fig. 77 compares the ability of the β-adrenergic agonist isoproterenol and of the antagonist propranolol to stimulate lipolysis in (i.e. glycerol release from) rat adipose cells. As expected, the antagonist propranolol produces no response, even at very high concentrations. In

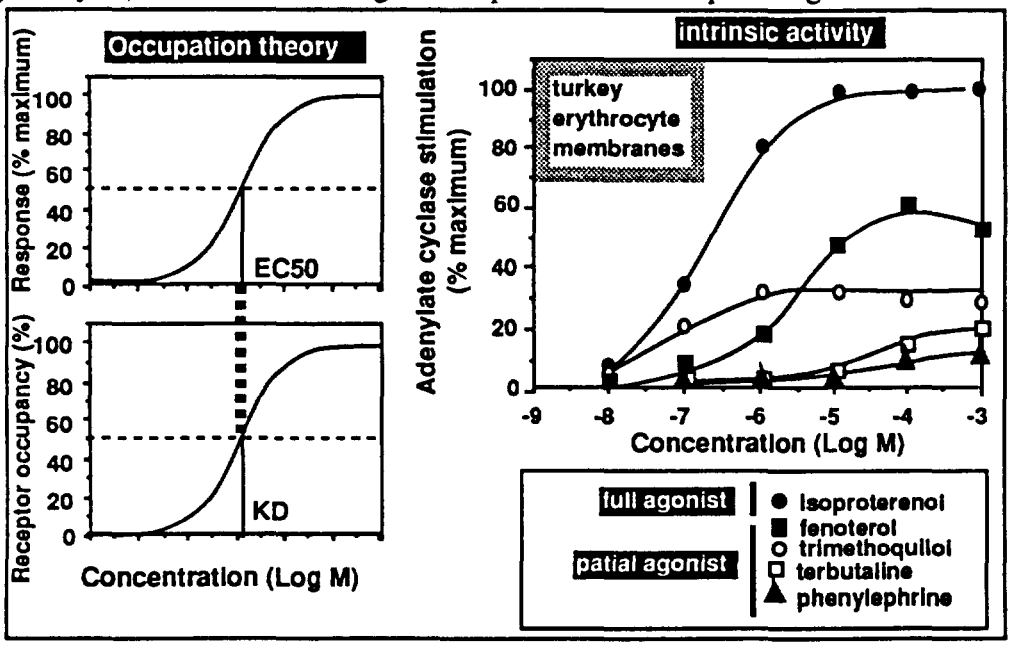
contrast, the response increases with the isoproterenol concentration until a plateau value is reached. Such representation, wherein the response (ordinate) is expressed in function of the ligand concentration (abscissa), is called a "**dose-response curve**" (the concentration is usually expressed in a logarithmic scale since it spans several orders in magnitude). In 1926, Clark developed the "**occupation theory**", wherein he proposed that the agonist-mediated response should be proportional to the number of occupied receptors. The concentration which causes half-maximal stimulation (denoted as "EC_{50}") was therefore equal to the K_D of the agonist for the receptor.

a) Problems with dose-response curves.

A more subtle distinction between agonists became necessary after the realisation that agonists do not necessarily produce the same maximal response. As a typical example, Fig. 78 (right pannel) compares dose-response curves of different β-adrenergic agonists to produce adenylate cyclase stimulation in turkey erythrocyte membranes. The maximal degree of adenylate cyclase stimulation is clearly different from one agonist to another.

In addition, the maximal response is, in many tissues, already attained when only part of the receptors are occupied (i.e. $EC_{50} < K_D$). In such situations, the occupation theory is no longer valid.

FIGURE 78
(left) Representation of Occupation theory (right) Dose-response (adenylate cyclase stimulation in turkey erythrocytes) curves of the full agonist isoproterenol and of partial agonists



A close examination of the molecular events which link receptor-occupation by the ligand and the final ligand-evoked response (sections b to e) provides a better insight concerning the actual physical meaning of dose-response curves and concepts such as "intrinsic activity", "efficacy" and "receptor reserve".

b) Models for receptor activation.

The distinction between agonists and antagonists has been explained by the ability of agonists, but not of antagonists, to initiate (or favour) a conformational modification of the receptor molecule (or molecular complex) and that this modification is the first step in the initiation of the cellular response. This first step represents the **stimulus**. Major current models assume that receptors can only adopt one active conformation and that the stimulus of a ligand reflects the fraction of occupied receptors residing in this active conformation.

The simplest model to deal with such situation is the "**two-step model**": In this model, the bound agonist induces a conformational change of the receptor by reducing the difference in free energy between both receptor conformations. The ligand (L) binds first to the non-active receptor (Rn) with the "microscopic" equilibrium dissociation constant (K), and this non-active ligand-receptor complex (L-Rn) is in equilibrium with the active complex (L-Ra). This latter equilibrium represents a first-order reaction with the "microscopic" equilibrium dissociation constant (K' = [L-Rn]/[L-Ra]).

$$\underset{}{} \quad \overset{K}{} \quad \overset{K'}{}$$
$$L + Rn \quad \Leftrightarrow \quad L\text{-}Rn \quad \Leftrightarrow \quad L\text{-}Ra \qquad (12)$$

This model corresponds to the classical representation of H-R-G complex formation; i.e.

$$\underset{}{} \quad \overset{K}{} \quad \overset{K'}{}$$
$$H + R + G \quad \Leftrightarrow \quad H\text{-}R + G \quad \Leftrightarrow \quad H\text{-}R\text{-}G \qquad (13)$$

The second equilibrium forms the key element for discriminating between agonists and antagonists:

- For antagonists: the second equilibrium is completely shifted to the left (i.e. K' >> 1): all of the occupied receptors remain in the non-active conformation.
- For agonists: The second equilibrium is shifted more to the right for strong agonists as for weak agonists, so that more of the occupied receptors reside in the active conformation; i.e. K' (strong agonist) < K' (weak agonist).

The fraction of occupied receptors residing in the active conformation is related to K' by the following equation:

$$[L\text{-}Ra]/([L\text{-}Ra] + [L\text{-}Rn]) = 1/(K' + 1) \qquad (14)$$

Several authors have proposed an alternative "**allosteric model**" which is derived from the Monod-Wyman-Changeux "Plausible Model". In this model, both receptors conformations are in equilibrium, even in the absence of ligand. Here, the agonist "favorses" a conformational change of the receptor because of its higher affinity for the active conformation. The equilibrium constant for the transition between the two forms of the receptor (K' = [Rn]/[Ra]) is very high since the great majority of receptors are inactive in the absence of drug. Nevertheless, this model allows unoccupied receptors to produce a small stimulus. Ligands are able to bind both to Rn and Ra with the "microscopic" equilibrium dissociation constants Kn and Ka, respectively.:

$$
\begin{array}{ccc}
L + Rn & \Leftrightarrow & L + Ra \\
\Uparrow & K' & \Uparrow \\
Kn & & Ka \\
\Downarrow & & \Downarrow \\
L\text{-}Rn & & L\text{-}Ra
\end{array}
\qquad (15)
$$

Certain receptors have been shown to "pre-couple" to G proteins, so that their mode of activation may be more adequately described by the allosteric model. Here, both R-G and H-R-G represent the active receptor; i.e.

$$
\begin{array}{ccc}
H + R + G & \Leftrightarrow & H + R\text{-}G \\
\Uparrow & K' & \Uparrow \\
Kn & & Ka \\
\Downarrow & & \Downarrow \\
H\text{-}R + G & & H\text{-}R\text{-}G
\end{array}
\qquad (16)
$$

In this model, agonists can be discriminated from antagonists on basis of differences between their in binding affinities for the active and non-active receptors. This model provides also an explanation for the existence of so-called "**inverse agonists**".

- Antagonists are supposed to bind with equal affinity to both receptor conformations (i.e. Kn = Ka); the [Ra]/[Rn] ratio remains the same as in the basal situation.
- Agonists bind with higher affinity to Ra as compared to Rn (i.e. Kn > Ka) so that the whole equilibrium will be pulled to the right, resulting in an increase in the [Ra]/[Rn] ratio. The Kn/Ka- ratio is higher for strong agonists as for weak agonists.
- Inverse agonists bind with higher affinity to Rn as compared to Ra (i.e. Ka > Kn) so that the whole equilibrium will be pulled to the left, resulting in a decrease of the [Ra]/[Rn] ratio. Some of the compounds which interact with benzodiazepine receptors (see Chapter 1) are inverse agonists: they decrease the affinity of GABA for the GABA-A receptor.

The fraction of occupied receptors residing in the active conformation is related to Ka, Kn and K' by the following equation:

$$[L\text{-}Ra]/([L\text{-}Ra] + [L\text{-}Rn]) = 1/(1 + K' \cdot Ka/Kn) \qquad (17)$$

Transfection studies during the past few years have led to the introduction of even more complex models to explain the activation of G-protein coupled receptors. A model such as the one below allows:

- the receptor to be in the active (R*) conformation even when it is not coupled to a G protein
- the receptor to couple to a G protein even in the absence of agonist

$$
\begin{array}{ccc}
 & K & \\
H + R + G & \Leftrightarrow & HR + G \\
\Uparrow & & \Uparrow \\
J & & \beta J \\
\Downarrow & \beta K & \Downarrow \\
H + R^* + G & \Leftrightarrow & HR^* + G \\
\Uparrow & & \Uparrow \\
M & & \alpha M \\
\Downarrow & \alpha\beta K & \Downarrow \\
H + R^*G & \Leftrightarrow & HR^*G
\end{array}
\qquad (18)
$$

c) From receptor-occupation "[L-R]" to stimulus "S".

The capability of the bound ligand to stimulate the receptor has been termed the "**intrinsic efficacy**" (ε) of the ligand by Furchgott (1966). ε Is proportional to the fraction of occupied receptors residing in the active conformation in the above "two-step" and "allosteric" models; i.e. $\varepsilon \approx [L\text{-}Ra]/([L\text{-}Ra] + [L\text{-}Rn])$.

The **stimulus** (S) is dependent on the amount of occupied receptors ([L-R]) and on the intrinsic efficacy (ε) of the ligand (Fig. 79): i.e.

$$S = \varepsilon \cdot [L\text{-}R] \qquad (19)$$

Substitution of [L-R] by $[R_{tot}]/(1 + K_D/[L])$ yields:

$$S = \varepsilon \cdot [R_{tot}]/(1 + K_D/[L]) \qquad (20)$$

S is dependent on properties of the ligand- receptor interaction: ε and K_D. S is also dependent on $[R_{tot}]$, a tissue- dependent property.

Equation 20 was first presented in a more rudimentary form by Stephenson (1956): $\varepsilon \cdot [R_{tot}]$ was expressed as a single term, the "efficacy" (e), which is dependent on the tissue (because of $[R_{tot}]$) as well as on the ligand- receptor interaction (because of ε).

d) From stimulus "S" to response "E/E_{max}": case of a linear relationship

To deal with the many steps which might succeed this initial stimulus, the "**response**" (E) should considered to be an undefined function (F) of S: i.e.

$$E = F(S) = F(\varepsilon . [L\text{-}R]) = F(\varepsilon . [R_{tot}]/(1 + K_D/[L])) \quad (21)$$

A special case of equation 21 occurs when E is proportional to the stimulus. This equation can then be written as:

$$E \sim \varepsilon . [L\text{-}R] = \varepsilon . [R_{tot}]/(1 + K_D/[L]) \quad (22)$$

The maximal response of the most active agonist known (i.e. with ε_{max}) is:

$$E_{max} \sim \varepsilon_{max} . [R_{tot}] \quad (23)$$

When the response of an agonist is expressed relative to this maximum, we have:

$$E/E_{max} = (\varepsilon/\varepsilon_{max})/(1 + K_D/[L]) \quad (24)$$

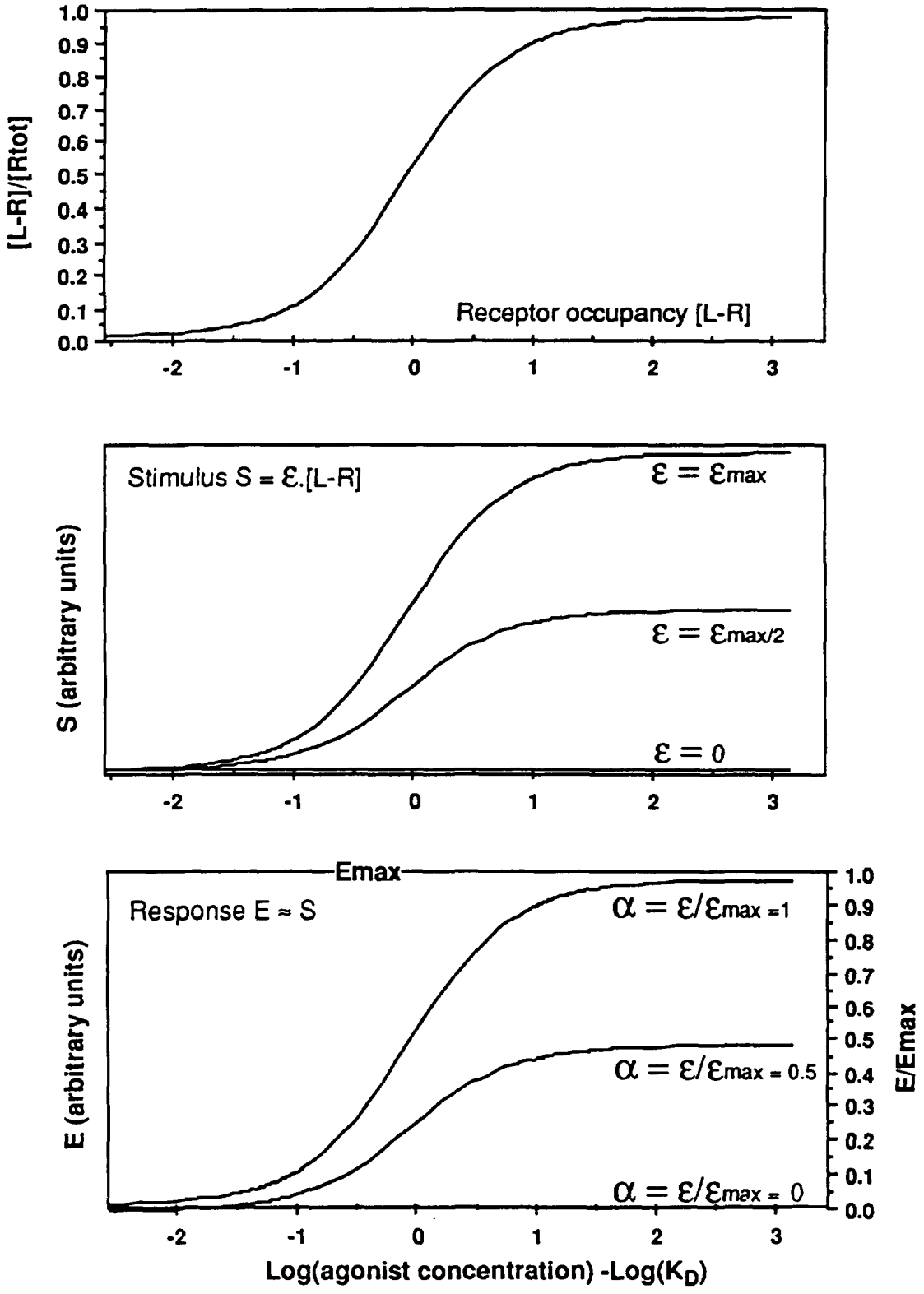
This equation is similar to the one originally proposed by Ariens (1954) to deal with the fact that different agonists do not necessarily produce the same maximal response.

$$E/E_{max} = \alpha/(1 + K_D/[L]) \quad (25)$$

He introduced the term "**intrinsic activity**" (α) as a parameter to indicate the maximal response of an agonist compared to the most potent agonist known. α Is an experimental parameter but, under the particular condition of a linear stimulus-response relationship, it is proportional to its intrinsic efficacy. It corresponds to the ratio betwee the intrinsic efficacy of the agonist of interest and the intrinsic activity of the most active agonist known to date ($\alpha = \varepsilon/\varepsilon_{max}$) Depending of the value of α, ligands can be divided into three categories:

- $\alpha = 1$. This category of ligands produces the maximal degree of response (E_{max}). They are called "full agonists". Adrenaline, noradrenaline and isoproterenol are all full agonists for β-adrenergic receptors (Fig. 78).
- $0 < \alpha < 1$. These ligands are denoted as "partial agonists". The rank order of the a values for the partial agonists presented in Fig. 78 is : phenylephrine (0.09) < terbutaline (0.20) < trimethoquilol (0.33) < fenoterol (0.64). Fig. 78 also clearly shows that there is no correlation between a drug's EC_{50} and its intrinsic activity.
- $\alpha = 0$. This situation occurs for antagonists. These ligands bind to the receptor without eliciting a response.

FIGURE 79
Relationship between receptor occupancy, stimulus and response
case of a linear stimulus-response relationship



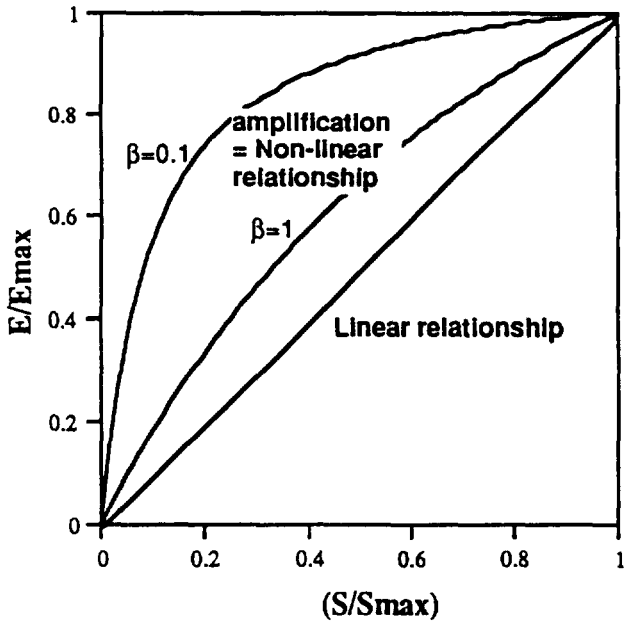
e) From stimulus "S" to response "E/E_{max}": non-linear relationship

The equation of Ariens represents only a special case of the general equation 22: i.e. when E/E_{max} is proportional to the stimulus. Quite often, however, the number of activated receptors will exceed the maximal response capacity of the system. In other words, the maximal response is already attained when only part of the receptors are occupied. In such situations, α is no longer proportional to $\varepsilon/\varepsilon_{max}$.

In equation 22, which was developed by Stephenson (1956) and Furchgott (1966), the response is an undefined function of the stimulus. F is, in principle, undefined for two potential reasons:
- the undefined nature of the cascade of cellular events which follow the initial stimulus
- the undefined relationship between consecutive events.

Although many of these cellular events are already known in great detail, the relationship between consecutive events appears, very often, not to be a linear one. A common reason for such non-linear relationship is that cellular events are capable to amplify the signal (stimulus) to an extend which exceeds the response capacity of the subsequent event. In other words, the response capacity of the second event becomes saturated even before the magnitude of the first event has reached its maximum (Fig. 80).

FIGURE 80
Linear- and non-linear relationship between E/E_{max} and S



The stimulus-response relationship may thus be composed of any number of saturable and linear functions arranged in sequence. An overall saturable output will still be expected. The classical (and simplest) way to describe F, is to represent it as a rectangular hyperbolic function: i.e. $E/E_{max} = S/(S + 1)$. However, F should also reflect

the efficiency of the cellular events converting receptor stimulus into tissue response, as well as the number of events (i.e. the greater the number of saturable steps, the greater the global amplification). A fitting parameter (β) which deals with the number and efficiency of the intermediate cellular events is therefore introduced in the stimulus-response relationship: i.e. $E/E_{max} = S/(S + \beta)$ Fig. 80 shows the relationship for different values of β. Please note that the amplification becomes more pronounced as β decreases.

Equation 22 can now be represented as:

$$E/E_{max} = \varepsilon.[L\text{-}R]/(\varepsilon.[L\text{-}R] + \beta) \quad (26)$$

$$= \varepsilon.[R_{tot}]/(1 + K_D/[L])/(\varepsilon.[R_{tot}]/(1 + K_D/[L]) + \beta)$$

$$= \varepsilon.[R_{tot}]/(\varepsilon.[R_{tot}] + \beta + \beta.K_D/[L]) \quad (27)$$

Computer-simulated graphic representation of equation 27 shows the consequences of varying ε, $[R_{tot}]$ and β.

Variation of ε and $[R_{tot}]$ (Fig. 81,82).

Variation of ε occurs, for example, when one compares the dose-response curves of different ligands by using the same experimental system (i.e. when $[R_{tot}]$ and β are constant). In Fig. 81, all the ligands bind with the same K_D to facilitate comparison. This standardised binding curve is presented by the dots. Ligands will behave as:

- Antagonists: when ε is very small. Their $EC_{50} \sim K_D$ for binding.
- Partial agonists: when ε increases (around $0.1 < \varepsilon.[R_{tot}] < 10$). Their $EC_{50} < K_D$ for binding.
- Full agonists: when ε is very high. Their $EC_{50} << K_D$ for binding.

In other words, increasing the intrinsic efficacy of a ligand will increase its "intrinsic activity" (here only considered to be an experimental parameter) until a limit value (which corresponds to full agonism) is reached. At the same time, the drug's EC_{50} will decrease (Fig. 82).

$[R_{tot}]$ refers to the total concentration of the receptors which are functionally active (i.e. coupled to a response mechanism). Receptor densities can vary dramatically from one tissue to another, and even within a given tissue. Receptor desensitisation (Chapter 3) constitutes a typical example wherein the cells defend themselves against prolonged stimulation by agonists by decreasing $[R_{tot}]$, both by decreasing the total receptor number and the fraction of functionally active receptors. Fig. 81 compares the dose-response curves of ligands for different $[R_{tot}]$ (when ε and β are kept constant). Decreasing $[R_{tot}]$ may have profound effects on different types of ligands:

- Full agonists (i.e. with $\alpha = 1$ to start with) may undergo a large increase in EC_{50} or even become partial agonists, with a small increase in EC_{50}.
- Partial agonists (i.e. with $\alpha < 1$ to start with) may become antagonists, with little variation of EC_{50}.

FIGURE 81

Theoretical dose-response curves of an agonist for different values of ε.Rtot. Dots correspond to

receptor occupancy and $\beta = 1$.

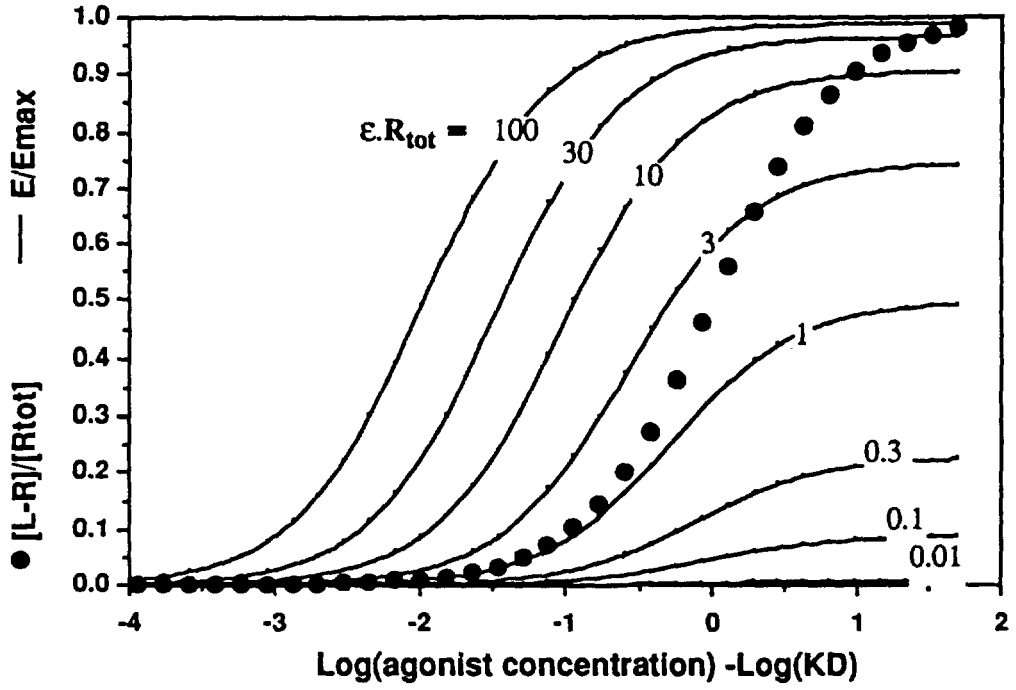
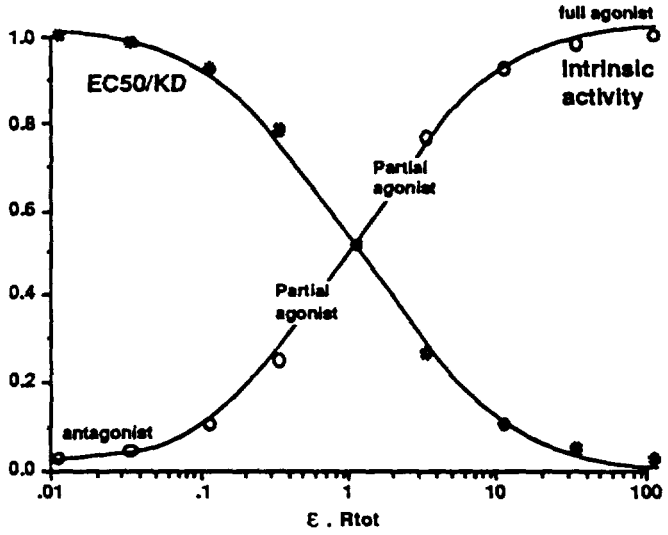


FIGURE 82
Effect of $\varepsilon.R_{tot}$ (with $\beta = 1$) on the EC_{50}/K_D ratio and on the intrinsic activity



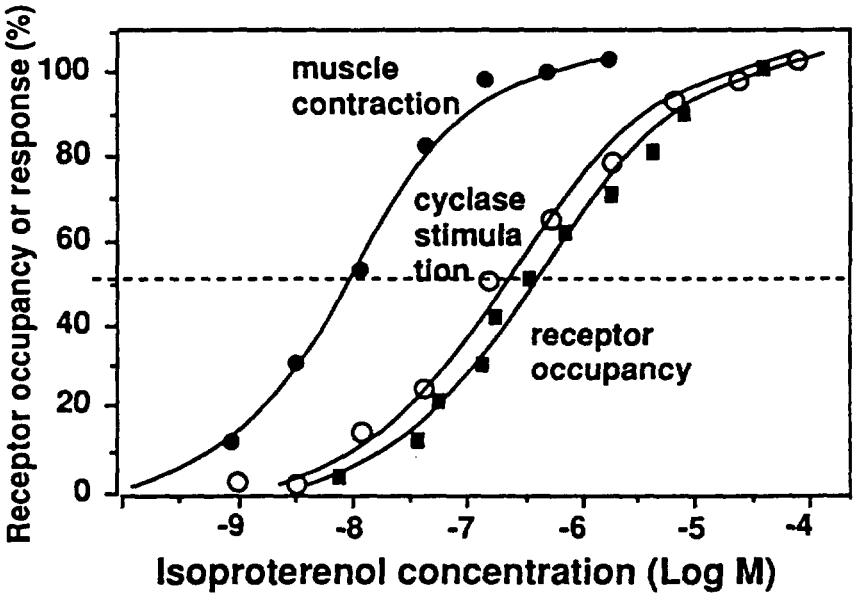
Chronic agonist treatment may lead to receptor desensitisation (chapter 3). This has important therapeutic implications. If the administrated agonist has high efficacy, then the effect of desensitization (mainly an increase in EC_{50}) can be overcome by increasing the dosage of the agonist. However, if the administrated agonist has low ε, increasing the dosage will not necessarily overcome the loss of functional receptorsand even prevent the action of any other potential stimulus.

Variation of β: (Fig. 83)

β is a "fitting parameter" which is inversely related to the global cellular amplification. It is dependent on the efficiency of intermediary cellular evens to amplify the stimulus (if the same response is measured in different cells or even under different experimental conditions). It also reflects the "distance" between the stimulus and the measured response, since an increased number of intermediary cellular events may cause further amplification of the stimulus.

Increasing the cellular amplification (i.e. decreasing β) has the same consequences on the agonist dose-response curve as increasing $[R_{tot}]$. In the heart, for example, the dose-response curve for the isoproterenol- mediated inotropic effect is shifted to the left by about one order in magnitude when compared to the less distant adenylate cyclase response (Fig. 83). The value of β for the inotropic effect is thus 10 times lower as for the adenylate cyclase response. This corresponds to a (virtual!) 10-fold increase in $[R_{tot}]$.

FIGURE 83
Comparison between β-adrenergic receptor occupancy (from binding studies) and isoproterenol-mediated adenylate cyclase stimulation and heart muscle contraction. From ref. 13



f) "Receptor reserve"

As depicted in Fig. 81,83 agonist dose-response curves may be shifted well to the left of the actual binding curves (i.e. $EC_{50} << K_D$) when $\varepsilon \cdot [R_{tot}]$ is large. This means (at first glance) that the response may be maximal when only part of the receptors are occupied by the agonist. In other words, cellular amplification systems allow agonists (the natural messengers as well as synthetic drugs) to produce a maximal response at receptor subsaturating levels. The terms "**receptor reserve**" or "**spare receptor**" were introduced as an attempt to describe this phenomenon: the receptor reserve is the fraction of receptors greater than that required to produce the maximal tissue response by an agonist. The receptor reserve is greater for certain agonists (i.e. those with higher intrinsic efficacy) than others (Fig. 81), it decreases with desensitization (Fig. 81) and it becomes more pronounced when the measured response is more distant from the initial agonist-receptor interaction (Fig. 83).

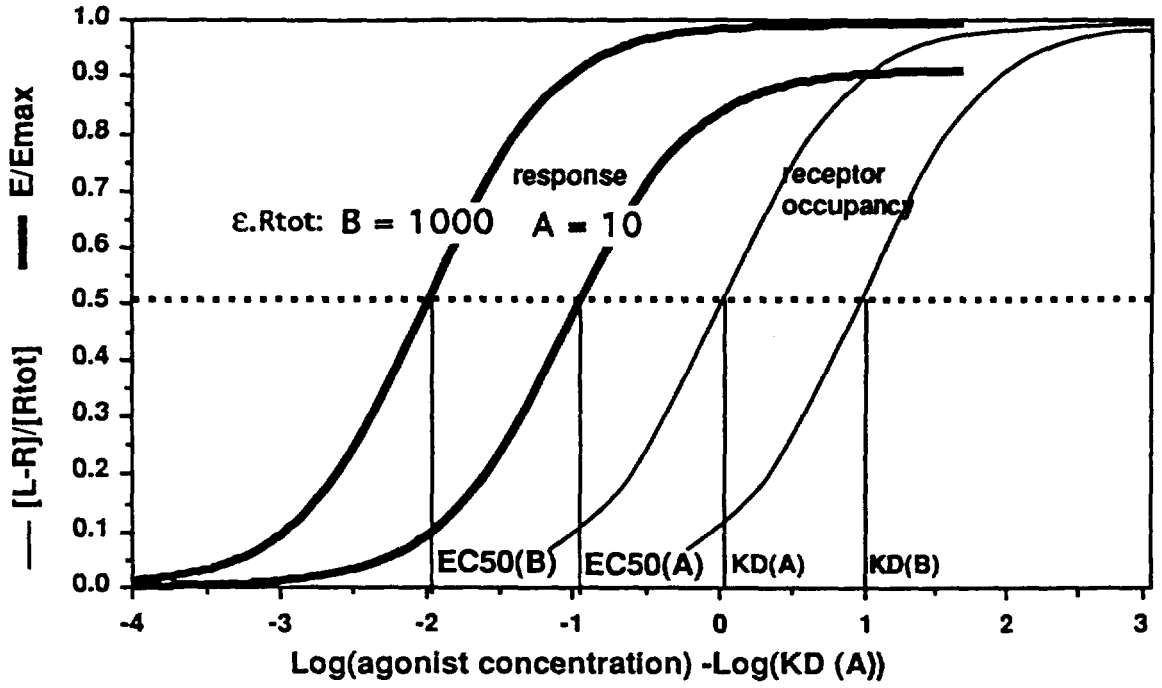
However, one must be aware that this definition is ambiguous since, strictly speaking, all of the receptors should be required to produce a maximal response. Obviously, this can never be attained experimentally since it should require [L] to be infinitely high.

g) Receptor classification.

Adrenergic receptors have been initially classified into the α- and β-subtypes on basis of differences in the order of potencies of agonists (Fig. 54). However, because the EC_{50}-values of agonist dose-response curves do not necessarily reflect their K_D, such classification is now recognised to be hazardous. This is well illustrated in the following example. Consider that agonist A has 10 times the affinity of B, but that the intrinsic efficacy (ε) of B is 100 times that of A. Fig. 84 shows that the competition binding curves for the two agonists will truly reflect their difference in K_D. However, the dose- response curves for these agonists will show that B has 10-fold higher potency than A. This reversal of potency pattern might eventually lead to the conclusion that the receptors which are labelled by the radioligand are different from those which are implicated in the agonist-mediated response.

EC_{50}-values, as well as intrinsic activities of agonists constitute weak arguments for classifying receptors because these parameters also reflect tissue- dependent factors besides the actual agonist-receptor interaction. In contrast, the agonist- related parameters intrinsic efficacy (ε) and affinity (K_D) are fixed and specific for each agonist-receptor interaction. A valid comparison of receptors in different physiological systems can thus be performed on basis of these parameters. When there is a cellular amplification system, these parameters can only be obtained with great difficulty (beyond the scope of this course) form dose-response curves but it is easy to obtain the K_D (or K_i) of different ligands by radioligand binding experiments.

FIGURE 84
Different potency ratios for agonists may be obtained by binding studies and physiologic experiments (agonist A has 10 times the affinity of B, but the intrinsic efficacy (ε) of B is 100 times that of A)



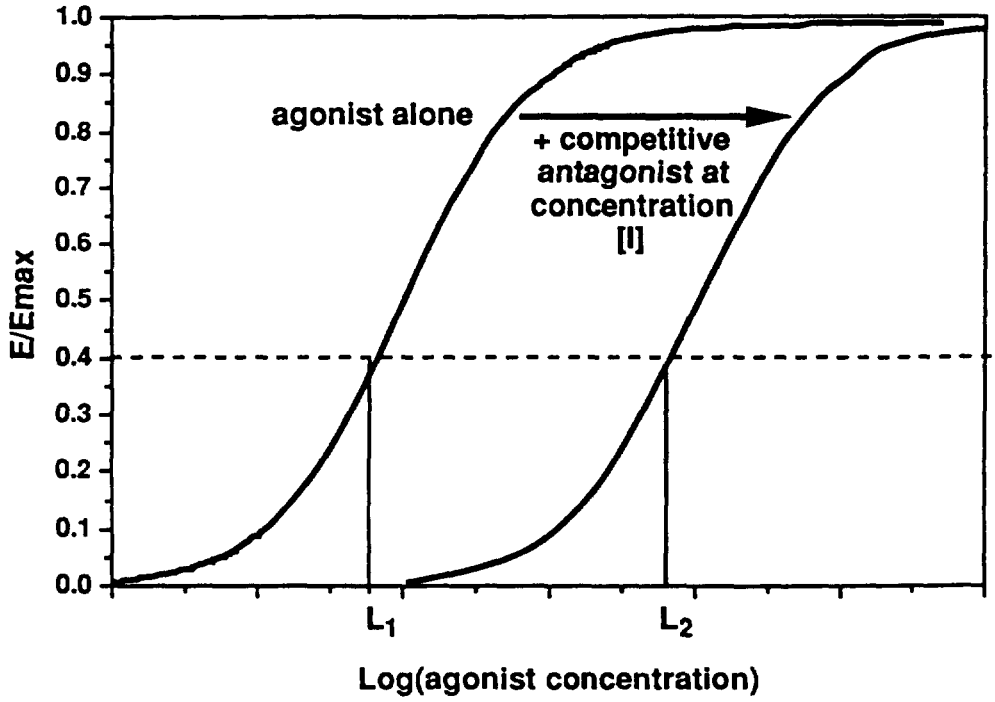
h) Antagonist affinity.

Since agonist dose-response experiments do not allow the direct estimation of their K_D, it is not possible to calculate antagonist K_i-values from their IC_{50} from competition experiments either. This problem can, however, be circumvented by the method developped by **Schild** and coworkers. This method is based on the fact that the dose-response curve of an agonist will undergo a parallel rightward shift when the experiment is done in the presence of a constant concentration of a competitive antagonist (Fig. 85).

The rationale for this method is that an agonist may produce a certain response at concentration $[L_1]$, and the same response at a higher concentration $[L_2]$ in the presence of a competitive antagonist at concentration [I] (Fig. 85). The ratio of these equiactive agonist concentrations ($[L_2]/[L_1]$, often referred to as "dose ratio") is related to the K_i of the antagonist by the equation:

$$[L_2]/[L_1] = 1 + [I]/K_i \qquad (28)$$

FIGURE 85
Agonist dose-response curve: effect of a fixed concentration of a competitive antagonist



In practice, agonist dose-response curves are constructed for different antagonist concentrations. Fig. 86 shows the dose-response curve of CGP12177 to stimulate lipolysis in rat adipocytes, and the ability of increasing concentrations of the β-adrenergic antagonist metoprolol to produce rightward shifts of this curve. This figure clearly shows that the dose-ratio will be more pronounced when the antagonist concentration increases. The $[L_1]$ value for the control curve (i.e. without agonist) corresponds to the agonist concentration producing an arbitrarily chosen response (e.g. 40 % of the maximum for Fig 85). $[L_2]$- values of the agonist (i.e. concentations at which the responses are the same) are then measured for the curves that are obtained in the presence of the different concentrations of the antagonist. The antagonist - $Log(K_i)$ (also referred to as "pA_2") can then be determined with high accuracy by linear regression analysis of the plot which corresponds to equation 29 (which is a logarithmic transformation of equation 28).

$$Log([L_2]/[L_1] - 1) = Log([I]) - Log(K_i) \qquad (29)$$

Fig. 87 shows the Schild plot of the experiment shown in Fig. 86. Regressions according to equation 29 are called **Schild regressions**. They represent the most useful physiological tool for pharmacologic receptor classification. It is, for example, on basis of such studies that the β-adrenergic receptors in rat adipocytes were discovered to constitute a new subclass, possessing unusually low affinity for antagonists. Indeed, the K_i-value of metoprolol (1.6 nM) is well above values which are typical for β_1- and β_2-adrenergic receptors.

FIGURE 86
CGP12177 (β3-selective agonist) dose response (lypolysis in rat fat cells) curve: effect of 0.04, 0.2 and1 mM metoprolol (β1-selective antagonist)

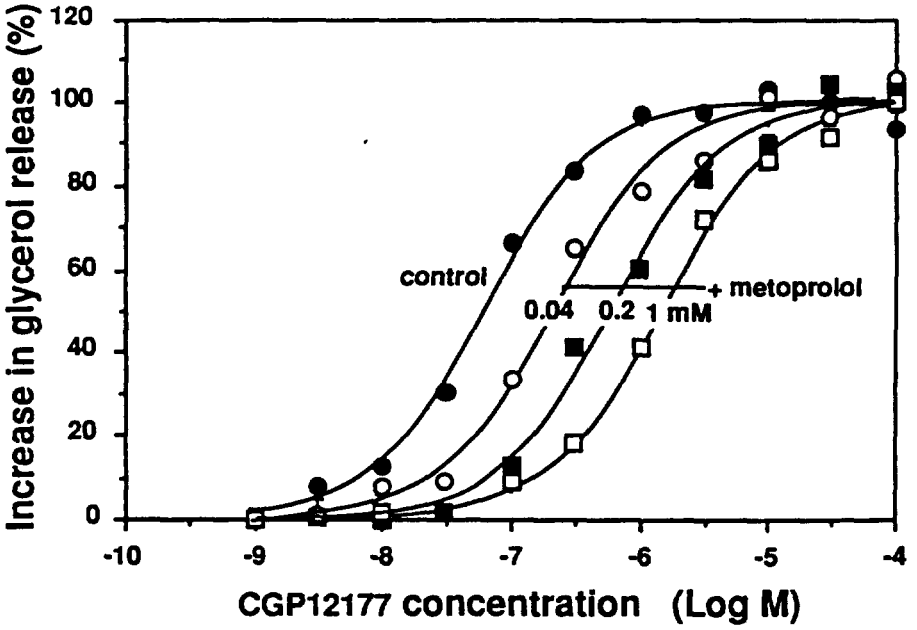
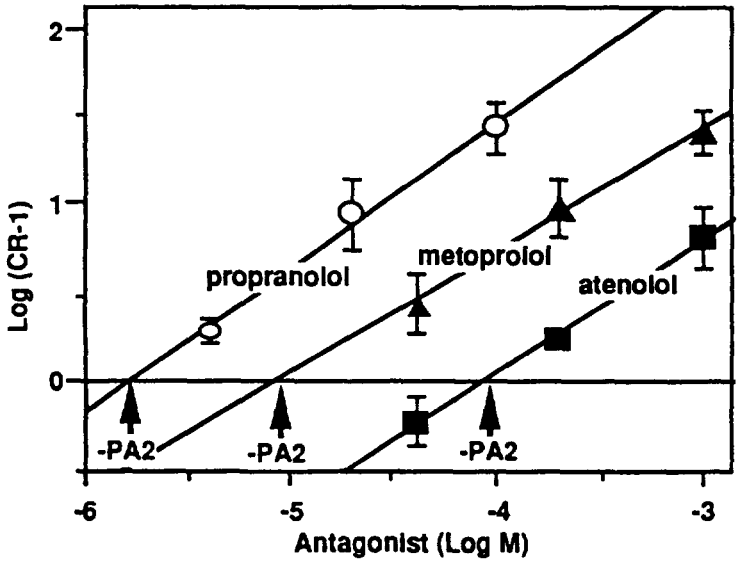


FIGURE 87:
Schild plot of the shifted dose- response curves of Fig.86 (for metoprolol) and for similar experiments with the antagonists propranolol and atenolol



i) Non-competitive versus insurmountable antagonists.

Competitive antagonists produce a leftward shift of agonist dose-response curves (Fig 88). Competitivity is obvious when the agonist and the antagonist bind to the same or to partially overlapping binding sites at the receptor so that the binding of the one excludes the binding of the other (Fig. 89). Accordingly, agonists may displace antagonists from the receptor sites and this displacement becomes more effective as the agonist concentration increases.

Yet, some antagonists may decrease the maximal response anyhow. Such antagonists are commonly referred to as **non-competitive antagonists.** It is important to notice that this denomination is only valid when agonists and antagonists are added together to the experimental system (i.e. cells, tissues); i.e. when agonists and antagonists are given a chance to compete with each other for binding to the receptor (Fig 88).

At least two mechanisms may provide a molecular basis for **non-competitive antagonism:**

- Antagonists may bind to a site of the receptor that is different from the binding site of the agonist (Fig 89). This antagonist- receptor interaction may result in a conformational change of the receptor, resulting in its inability (or at least reduced ability) to bind the agonist. Such **allosteric** mechanisms occur especially in the case of mediator-operated channels which have multiple binding sites (such as the NMDA receptor).
- Antagonists may also block an intracellular event that is triggered by the agonist-receptor interaction and thereby impair the chain of events linking the stimulus to the measured response (Fig 89). These antagonists are denoted as **functional antagonists.** They do not bind to the receptor (and thus do not block agonist binding and receptor activation). Since a particular response may be triggered by a variety of different receptors in the same tissue (e.g. α-adrenergic, angiotensin II, Neuropeptide Y, serotonin, prostaglandin and endothelin receptors trigger vascular smooth muscle contraction), functional antagonists are likely to block the responses of all these receptors. Therefore, if an antagonist is found to be non-competitive for a given receptor, its ability to affect the action of related receptors (i.e. giving the same response) is usually checked to find out whether it is a functional antagonist or not.

Non-competitive antagonists are best detected and studied in a system possessing a linear stimulus- response coupling. Indeed, as shown in Fig. 81, non-competitive antagonists may be falsely identified as competitive ones if the system possesses a large receptor reserve.

Radioligand binding studies can also distinguish between competitive, allosteric and functional antagonists. For this purpose, saturation binding curves of a radiolabelled agonist should be obtained in the absence (as control) or presence of a given concentration of antagonist. Here again, it is imperative for the agonist and the antagonist to be added together to the experimental system. The Scatchard curve should be unaffected for functional antagonists, undergo an

Figure 88:
antagonists are denoted as (non)-competitive or (in)surmountable depending on the incubation protocol.

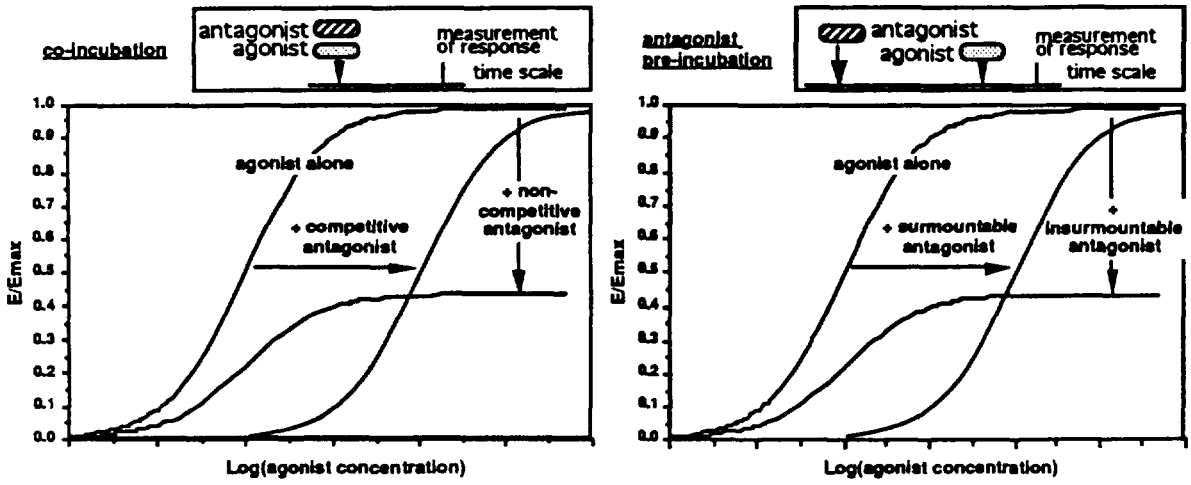
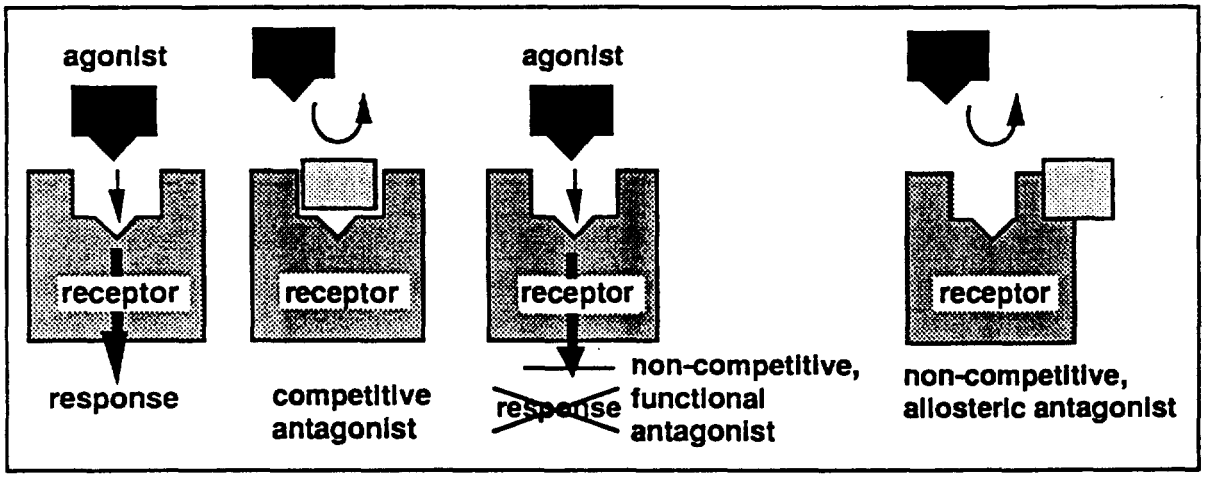


Figure 89:
Molecular mechanisms resulting in competitive versus non-competitive antagonism.



apparent increase in K_D without change in B_{max} for competitive antagonists and undergo a decrease in B_{max} without change in K_D (i.e. undego a parallell shift) for allosteric antagonists (Fig. 90). Allosteric antagonists can also be detected by kinetic experiments in which the dissociation of the radiolabelled agonist is initiated by an excess of the unlabelled agonist without (as control) or with the antagonist of interest. The antagonist is likely to be allosteric if it produces a further increase in the dissociation rate (Fig. 91).

Figure 90:
Effect of competitive and non-competitive antagonists on the Scatachard plot of radiolabelled agonist saturation binding.

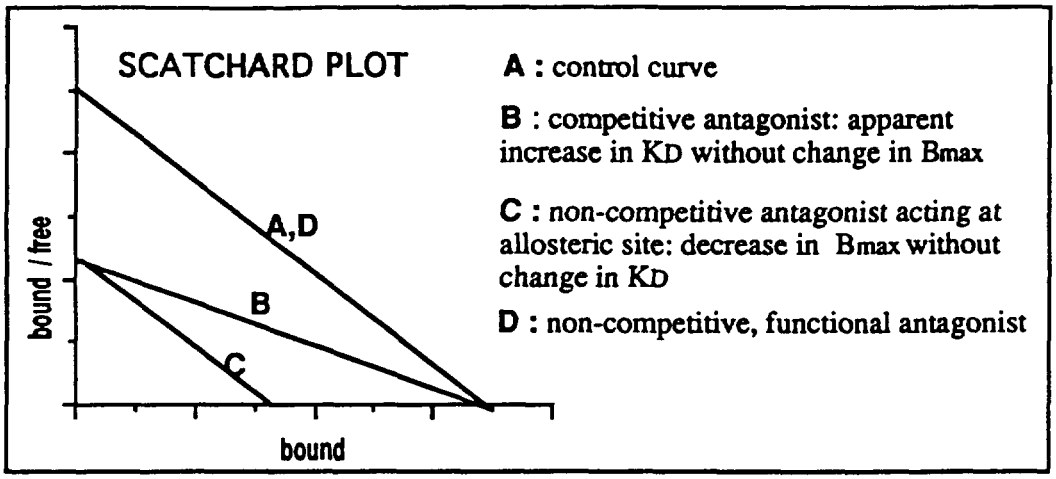
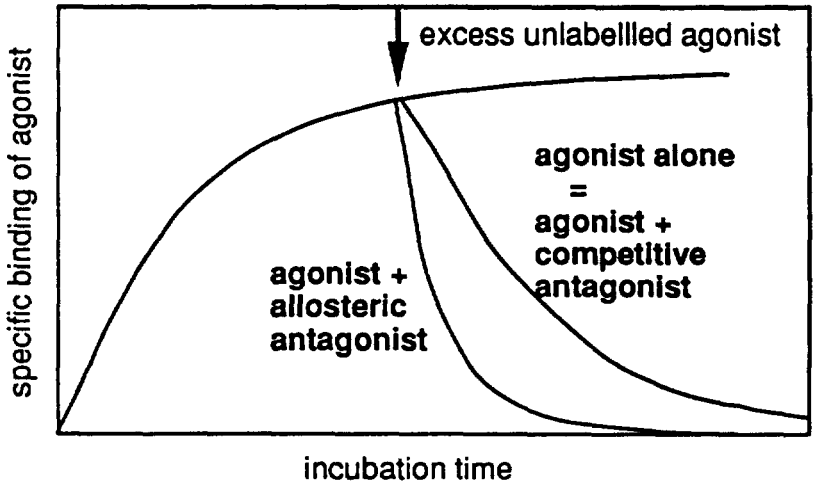


Figure 91:
Effect of competitive and allosteric antagonists on the dissociation of a radiolabelled agonist.



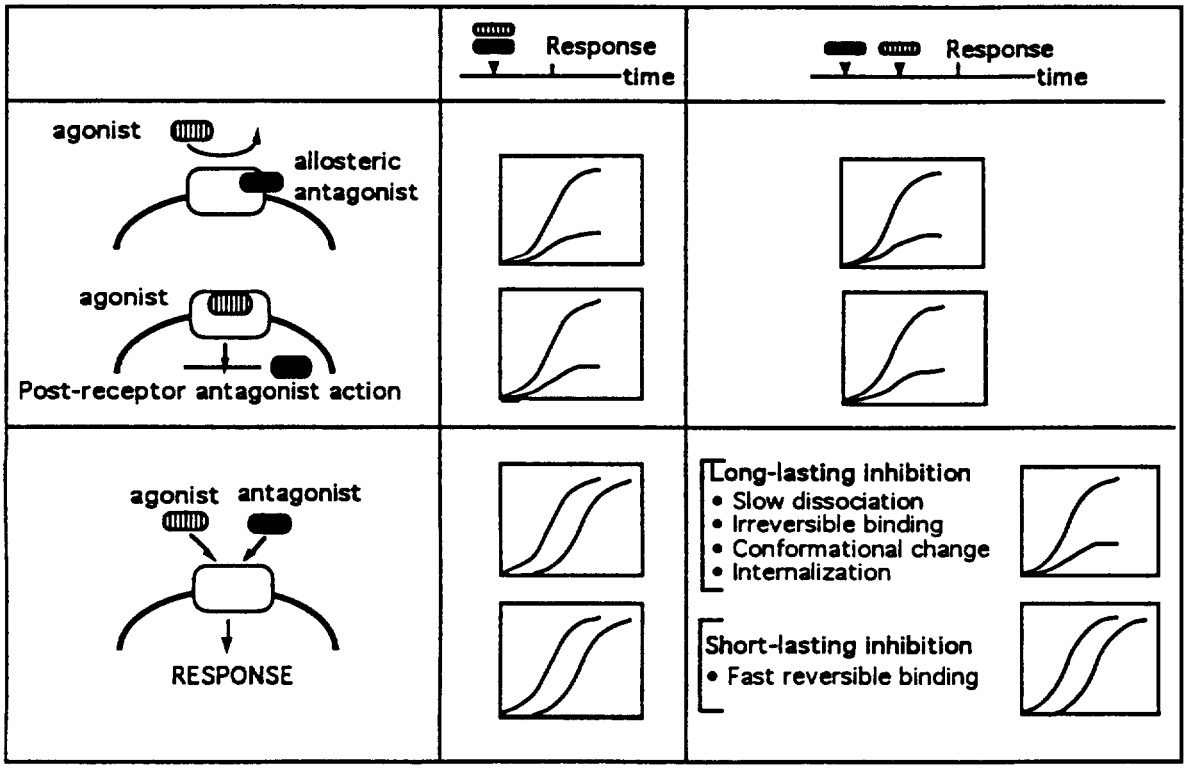
However, functional studies very often include a preincubation step, in where the receptors are pre- equilibrated with the antagonist. Subsequently, the agonist will be added and the response measured. This implies that the antagonist had the opportunity to interact with the receptor for some time without any interference of the agonist. Therefore, one prefers to speak in terms of **surmountable** (parallell shifts of the dose-response curves) and **insurmountable** antagonism (depression of the maximal response) rather than in terms of competitive/non- competitive antagonism (Fig. 88). The relationship between the surmountability and competitivity of antagonists is as follows (Fig. 92):

Surmountable antagonists are **competitive** antagonists which dissociate sufficiently fast from the receptor. This allows the subsequently added agonist to occupy all receptor sites, at least when its concentration is high enough.

Insurmountable antagonists refer to :

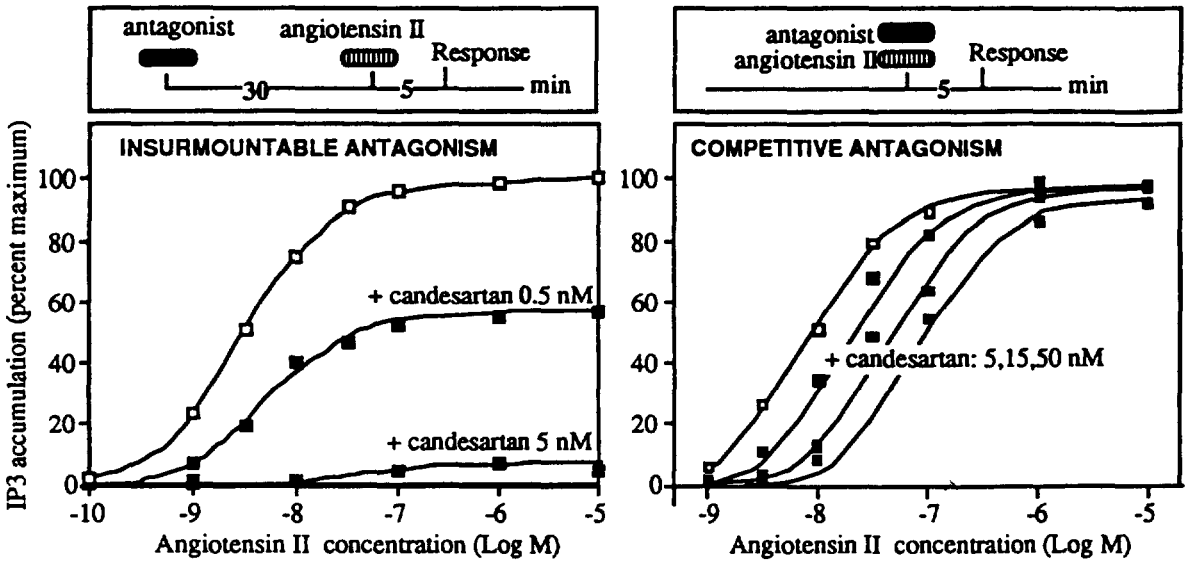
- **non-competitive** antagonists (both allosteric and functional) as well as to
- **competitive** antagonists but with such long-lasting action that the subsequently added agonist does not get the opportunity to occupy (stimulate) all the receptor sites at the time the response is measured. In other words: the agonist is not long enough in contact with the receptors to surmount the antagonist's action. This type of antagonists are thus likely to display irreversible (i.e. covalent) or slowly reversible binding characteristics in radioligand binding studies. However, according to at least two theories, the antagonist does not need to remain bound to the receptor to produce a long- lasting effect. According to those theories, certain antagonists induce a conformational change of the receptor into an inactive state and, subsequent to the dissociation of the antagonist, the receptor only gets slowly back to its original conformation.

Figure 92:
relationship between (non)-competitive and (in)surmountable antagonists.



When one observes a reduction of the maximal response in functional studies which include a preincubation step, it is therefore not possible to discern whether the antagonist is truly non- competitive or not. However, this distinction can easily be made on basis of experiments in where the receptors are co- incubated with agonist and antagonist. The angiotensin II AT_1 receptor blocker candesartan represents a typical example of an insurmountable, yet competitive antagonist (Fig. 93).

Figure 93:
The angiotensin II AT_1 receptor blocker candesartan represents a typical example of an insurmountable, yet competitive antagonist (response is production of inositol phosphates in Chinese Hamster Ovary cells expressing the cloned human angiotensin II receptor of the AT1 subtype (CHO-AT1 cells))



j) Concluding comments:

Physiological experiments constituted for a long time the sole approach to test ligand-receptor interactions. Because of the indirect nature of the results (i.e. a "distant" response is measured), information about the ligand-receptor interaction cloud be biassed by nature of the experimental system. The positive side of this is that physiological experiments in intact organs or even *in vivo* are rather close to the clinical reality. The negative side of this is that physiological experiments do not provide clear-cut information about ligand-receptor interactions, so that they only constitute marginal tools for the purpose of receptor classification and identification. Indeed, EC_{50}-values and intrinsic activities of agonists are easy-to measure, but tissue-dependent. K_D-values and intrinsic efficacies (ε) describe the agonist-receptor interactions more accurately, but they are difficult to obtain (at least in the absence of radioligand binding studies). Schild regressions of shifted dose-response curves provide an accurate determination of antagonist K_i-values, and they may represent the most useful physiological tool for pharmacologic receptor classification.

The more recent radioligand binding studies provide direct information about agonist and antagonist- affinities for receptors. They also allow to detect the coexistence of receptor subclasses in a given tissue and, in certain instances, even to discriminate between agonists and antagonists. Because of its simplicity and accuracy, the radioligand binding approach is a very useful tool for the identification, classification and the discovery of receptors, as well as for investigating the affinity and specificity of new potential drugs for receptors of interest. However, radioligand binding experiments provide only crude information about the physiological actions and the therapeutic benefit of the investigated drugs.