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INVESTIGATION OF PRODUCTION OF CONTRACEPTIVES

IS/CUB/75/008

CUBA.

TECHNICAL REPORT:
Steroid Chemistry

Prepared for the Government of Cuba by the
United Nations Industrial Development Organization,
executing agency for the
United Nations Development Programme

United Nations Industrial Development Organization

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INVESTIGATION OF PRODUCTION OF CONTRACEPTIVES
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CUBA

Technioal report: Steroid chemistry

Prepared for the Government of Cuba
by the United Nations Industrial Development Organization,
executing agency for the United Nations Development Programme

Based on the work of Gerald Blunden, expert in steroid ohemistry

United Nations Industrial Development Organization
Vienna, 1976

Explanatory notes

References to dollars ($) are to United States dollars, unless otherwise stated.

A full stop (.) is used to indicate decimals.

A comma (,) is used to distinguish thousands and millions.

References to "tons" are to metric tons, unless otherwise specified.

The following technical abbreviations are used in this report:

ha	hectare
µm	micrometre
v/v	volume per volume

ABSTRACT

This is the report of a mission to Cuba undertaken as part of the project "Investigation on the Production of Contraceptives" (IS/CUB/75/008) of which the United Nations Industrial Development Organization (UNIDO) was the executing agency.

The leaves of *Agave fourcroydes* and *Solanum laciniatum* and the tubers of *Polyanthus tuberosa* have been studied in Cuba as potential economic sources of steroidal drug precursors. *A. fourcroydes* and *P. tuberosa* yield hecogenin, which is a valuable precursor for the production of corticosteroids and *S. laciniatum* yields solasodine, which is an ideal precursor for a wide range of steroids, including the oral contraceptives. A large area of *A. fourcroydes* is cultivated in Cuba and from the juice expressed from the leaves, hecogenin is obtained in sufficient quantity and quality to make the plant a valuable industrial source of hecogenin. For industrial use, the crude sapogenin mixture obtained from *P. tuberosa* contains an unacceptably high proportion of sapogenins other than hecogenin, in particular $\Delta^{9(11)}$-hecogenin. Moreover, increased cultivation of the species would be necessary if sufficient plant material were to be available for industrial use and hence it is suggested that *P. tuberosa* is unlikely to be an economically useful source of hecogenin. *S. laciniatum* is a highly promising source of solasodine, but more agricultural information is necessary before the economic value of the plant for Cuba can be assessed. However, the annual yield of leaves and splasodine obtained to date make the prospects for the plant very good.

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INTRODUCTION

In 1975 UNIDO conducted a global survey on contraceptives, which included the supply of raw materials and local production. This survey involved visits to developed and developing countries to collect accurate data for the preparation of the report. During the visit to Cuba the development of useful sources of steroid drug precursors that can be obtained from Cuban plants was discussed and a draft document on the establishment of a pilot plant for the production of these precursors was finalized.

The project "Investigation on the Production of Contraceptives" (IS/CUB/75/008) was requested by the Government of Cuba in April 1975 and approved by the United Nations Development Programme (UNDP) in July 1975. The Ministry of Public Health was designated as the government co-operating agency and the United Nations Industrial Development Organization (UNIDO) as the executing agency. The purpose of the project was to investigate the possibilities of expanding the production of contraceptives from locally available raw materials and of establishing the production of intrauterine devices.

The expert was engaged in order:

- To analyse the locally available plants for steroidal sapogenin contents
- To evaluate the locally available plants for their suitability as sources of steroid-drug precursors
- To advise the experimental laboratory on methods of extracting steroid-drug precursors from plants
- To prepare a training programme for technicians

Cuba has a well-developed pharmaceutical industry which produces approximately 800 different products. Although the Government does not have a family planning programme, the country's policy is to provide contraceptives for those who wish to plan their family; therefore the pharmaceutical industry has been producing oral contraceptives from imported bulk materials since 1968. As the demand for contraceptives has increased, the Government wishes to expand the existing production with the aim of satisfying the country's needs and, if possible, for export purposes. The Government wishes to develop the production of both oral contraceptives and of other steroid drugs based on locally-available raw materials. This requires the evaluation of both indigenous and introduced plants, the large-scale extraction of suitable compounds and their transformation into steroids of medicinal use. The pharmaceutical industry in Cuba currently has the facilities for the formulation, tabletting and packaging of oral contraceptives.

I. FINDINGS

A. Analysis of locally-available plants for steroidal sapogenin contents

In Cuba, several plant species have received preliminary chemical investigations to determine their content of the steroidal sapogenins that are known to be readily converted into medicinally-useful steroid drugs. The plants studied are *Polyanthus tuberosa*, *Agave fourcroydes*, *Solanum laciniatum* and, to a lesser extent, *Dioscorea* species.

Polyanthus tuberosa

This plant is cultivated in Cuba for its ornamental flowers. From the tubers, the sapogenins hecogenin, $\Delta^{9(11)}$-hecogenin and tigogenin have been isolated. The total sapogenin yield from 2 year-old tubers was about 2%, calculated from the dry weight. About 70% of the total crude sapogenin mixture was reported to be hecogenin, about 10%-15% $\Delta^{9(11)}$-hecogenin and 3%-5% tigogenin. Hecogenin is a valuable precursor for the production of corticosteroids, but it is of little value for the production of oral contraceptives.

During the assignment, three different samples of *P. tuberosa* tubers were processed and assayed for hecogenin content using a thin-layer chromatographic method. The results obtained are shown below:

Sample	Hecogenin content (%)
6 month-old tubers	1.46
15 month-old tuber (1 plant)	0.83
18 month-old tubers (mixed sample)	1.58

In all three samples, the quantity of tigogenin was small (less than 4% of the total sapogenin), but the proportion of $\Delta^{9(11)}$-hecogenin was 15%-20% of that of hecogenin.

Agave fourcroydes (Henequen)

This species is already cultivated in Cuba as a source of the fibre henequen. Preliminary studies in Cuba have shown that the leaves of *A. fourcroydes* contain steroidal sapogenins, in particular hecogenin and tigogenin. Hecogenin was found to be the predominant steroid and tigogenin formed from 10%-20% of the total sapogenin. For industrial use hecogenin must be as free from tigogenin as possible. The percentage of tigogenin should not exceed about 10% of the total sapogenin and preferably should be less than about 7%.

Previous experience with Agave sisalana has shown that the hecogenin content is highest in the basal part of the leaves, where the tigogenin content is very low. In the upper parts of the leaf, the quantity of tigogenin is high. To determine whether this applied also to A. fourcroydes, two leaf samples, one from a 5-year-old plant and one from a 9-year-old plant, were taken and divided into basal, middle and upper regions. The basal regions of the 5-and 9-year-old leaves contained respectively 0.24% and 0.47% hecogenin, calculated from the dry weight of leaf, whereas their respective tigogenin contents were 0.01% and 0.03%. In both cases, therefore, the proportion of tigogenin in the total sapogenin was less than 7%. The tigogenin contents of the total sapogenin in the middle and upper regions of the 9-year-old leaf, however, were 33% and 62% respectively. The sapogenin yields from the middle and upper regions of the 5-year-old leaf were too small for accurate assessment, but the same trend towards high tigogenin content was apparent.

Having established the low tigogenin content of the basal part of the leaves, several different leaf samples were selected for determination of the sapogenin contents in their basal parts. The results are shown in table 1.

Table 1. Sapogenin content of the basal part of A. fourcroydes leaves

Age of the plant (years)	Sapogenin content (% of dry weight)		Tigogenin content as % of total sapogenins
	Hecogenin	Tigogenin	
6	0.60	0.06	9
8	0.12	0.01	8
9	0.38	0.02	5
11	0.46	0.04	8

If A. fourcroydes were to be used as an industrial source of hecogenin, the sapogenin would be extracted from juice expressed prior to decortication of the leaves for the production of fibre. The basal parts of the leaves are very thick, whereas the middle and upper parts are considerably thinner. As a result, the expressed juice would be expected to have come mainly from the basal region. Juice samples prepared from leaves of different ages were assayed for hecogenin and tigogenin content. The results are shown in table 2.

Table 2. Hecogenin and tigogenin content of juice samples of A. fourcroydes leaves

Age of the plant (years)	Sapogenin yield from juice (mg/100 ml)		Tigogenin as % of total sapogenin
	Hecogenin	Tigogenin	
6	97	8	8
7	75	3	4
9	68	7	9
12	30	3	9

In all four juice samples analysed, the tigogenin formed less than 10% of the total sapogenin. Each juice sample was taken from only one leaf and no significance should be placed on the fact that the hecogenin content was highest in the juice of the youngest plant and lowest in that of the oldest plant.

The insoluble residue obtained after acid hydrolysis of the juice is known as "coffee grounds" and this material from Agave sisalana is a commercial product. One way to measure the quality of "coffee grounds" is to analyse the material for hecogenin and tigogenin content. A sample of "coffee grounds" was prepared from equal volumes of the four juice samples used in table 2. The sample was found to contain hecogenin 7.0%, tigogenin 0.7%; tigogenin as percentage of total sapogenin 9%. This result was consistent with yields normally obtained from A. sisalana. Juice from A. sisalana has an average hecogenin content of 130-180 mg/100 ml and the "coffee grounds" prepared from such material by the method used in this experiment have an average hecogenin content of 12%-15%. This shows that the hecogenin content of both the juice and "coffee grounds" samples of A. fourcroydes are on average one half the values obtained for A. sisalana.

Solanum laciniatum

There are a number of Solanum species that grow in Cuba, but these have as yet received only cursory examination. Trial work has been undertaken, however, on S. laciniatum, a rich source of solasodine. Solasodine is a valuable precursor of steroidal drugs, including the oral contraceptives. From the leaves of S. laciniatum grown in experimental plots, a maximum of 2% solasodine was obtained after 11 months of growth. In a single harvest 15 tons, wet weight (approximately 1.5 tons dry weight), of leaves were harvested from 1 ha after 11 months. It is hoped, however, to collect leaves at least twice during the year when total yields of 20-25 tons per hectare are expected. Leaves collected after 5 months growth, when it would be expected to make the first harvest, contained approximately 1% solasodine.

During the expert's assignment, one mixed sample of S. laciniatum leaves from plants of various ages was assayed for solasodine content, which was found to be 0.57% of the dry weight. This yield appeared to be somewhat lower than expected, but the analytical method used was not completely satisfactory for the extract used.

Dioscorea species

There are many Dioscorea species growing in Cuba. The tubers of some Dioscorea species are rich sources of diosgenin, which is the most desirable steriodal sapogenin for use as a precursor of medicinally-useful steriod drugs, particularly of the oral contraceptives. Some screening work is in progress, as well as studies to determine the variations in diosgenin yield depending on the age of the plant and on the time of the harvest. This programme is in its early stages and no comment can be passed on its chances of economic success.

B. Evaluation of the suitability of Polyanthus tuberosa, Agave fourcroydes and Solanum laciniatum as sources of steroid-drug precursors

Polyanthus tuberosa

From the tubers of P. tuberosa a good yield of sapogenin can be obtained, the major compound being hecogenin, which is a valuable starting material for the production of steroid drugs. However, as well as hecogenin, the extracted sapogenin contains $\Delta^{9(11)}$-hecogenin, tigogenin and probably some di-hydroxy sapogenin. The proportion of these other steroids, of which $\Delta^{9(11)}$-hecogenin is the prinicpal one, is of the order of 15%-20%. For industrial use it would be necessary to separate hecogenin from these other compounds, which is difficult and leads to a loss in the quantity of hecogenin isolated.

P. tuberosa is grown in Cuba for its flowers and after 2 years' growth the tubers are dugup, the old ones rejected and only the new tubers, which grow around the old ones, are selected for replanting. The old tubers would be available for extraction of sapogenins, but the quantity of plant material currently available would be insufficient for industrial use and further areas would need to be planted.

Because of the high proportion of sapogenins other than heoogenin, in particular $\Delta^{9(11)}$-hecogenin, the need to increase the area under cultivation, and the availability of Agave fourcroydes (see below), which is potentially a considerably better economic source of hecogenin, it is not considered that P. tuberosa is a viable economic source of hecogenin for Cuba.

Agave fourcroydes

A. fourcroydes is fairly extensively cultivated in Cuba as a source of the fibre henequen. The juice, obtained by pressing the leaves prior to their decortication for the production of fibre, is available as a source of steroids. It has been estimated that about 30 million litres of juice would be available every year for the extraction of sapogenins. From the limited number of juice samples analysed, the average hecogenin content was found to be 675 mg/l, which would yield theoretically 20,250 kg hecogenin. If only 10,000 kg of hecogenin could be recovered, the price of which is $70-$100 per kilogram, the project would seem to be economically sound.

The total sapogenin extracted from A. fourcroydes juice is characterized by having hecogenin as its major component, with the other sapogenins present in small amounts. In particular, the tigogenin proportion of the total averaged only 7%, which is economically satisfactory.

Although it is to be expected that Cuba will eventually produce final steroid drugs from hecogenin, in the early stages it should be possible to sell the insoluble residue obtained after acid hydrolysis of the juice ("coffee grounds"), as well as hecogenin.

In summary, A. fourcroydes appears to be a highly promising source of steroid-drug precursors because of the ready availability of plant material, the adequate hecogenin content and the low tigogenin content of the juice, and because the technology for the treatment of Agave juice to produce either "coffee grounds" or hecogenin is well established and is comparatively simple. Hecogenin, however, is only suitable for the production of oorticosteroids and is of little use for the production of oral contraceptives.

Solanum laciniatum

S. laciniatum is a well-known source of the azosteroid solasodine, which is an ideal precursor for the synthesis of pharmacologically-active steroid drugs, including the oral contraceptives. The results obtained in the Cuban research programme indicate that the plant produces economic quantities of solasodine and an 85% isolation rate has been achieved from the dried plant material. The synthesis group at the Adalberto Pesant Laboratories (MINSAP) has converted solasodine into 16-dehydro-pregnenolone in high yield.

The results obtained to date indicate that S. laciniatum has great promise as a source of solasodine. However, before deciding whether the plant is a viable economic source of steroids for Cuba, much agricultural research work needs to be done. It is neoessary to determine over several years the yields of both plant material and solasodine per hectare. The different times of harvest proposed need to be examined carefully to determine their effects on the yields of leaves and their solasodine content. So far the trial work has been done on an experimental basis in trial plots, but experience from other countries has shown that big decreases in the yield of leaves can be expected under normal agricultural conditions. Moreover, S. laciniatum is an introduced species and it has to be established whether plant pests such as nematodes, fungi and viruses will adversely effect the plants when grown for several years. Until this information is available it is impossible to assess the economic future of S. laciniatum in Cuba.

C. Methods of extraction of steroidal sapogenins from plant materials

Various methods of processing and extracting plant materials for the isolation of steroidal sapogenins were discussed and relevant papers and other information were given to the Cuban research workers. The discussions covered the following major points of importance relating to the extraction of sapogenins on the laboratory scale.

Initial processing of plant materials

When fresh plant material containing saponins is disintegrated in water, enzymes present increase the yield of sapogenin that can be isolated from the plant. It was recommended that this activity should be utilized to obtain maximum yields of sapogenin from the plants. The enzyme or enzymes are stable and will still function after the plant material has been dried at 65°C, powdered and incubated in water.

Methods of hydrolysis of saponins

Any increase in the yield of sapogenin that may occur, allowing the disintegrated fresh or powdered dry plant material to stand overnight in water at room temperature produces considerable enzymic hydrolysis of the saponins present. Complete hydrolysis of the saponin is then achieved by refluxing in 2N aqueous hydrochloric acid for two hours. The use of alternative acids, such as sulphuric, and different times of hydrolysis were discussed, but the use of hydrochloric acid as described was recommended.

Treatment of acid-insoluble residue

After acid-hydrolysis, the mixture is filtered and the acid-insoluble residue is washed with water, alkali and water. The use of an alkali, such as ammonia, was advocated particularly if it extracted substantial quantities of unwanted materials and if Δ^5-sapogenins were being isolated.

Extraction of sapogenins

From the dried, acid-insoluble residue, sapogenins are extracted with either light petroleum or chloroform. The light petroleum extract has far fewer contaminants than that of chloroform, but extraction takes 16-24 hours with light petroleum and 3-4 hours with chloroform. For the chemical isolation and separation of sapogenins, the former solvent is recommended, but for analytical purposes chloroform may be better because of the time factor. However, the chloroform extracts of some plants contain too many coloured impurities for the extracts to be analysed accurately by the thin-layer chromatographic method used during this mission. In the cases of *Agave fourcroydes* and *Polyanthus tuberosa*, the chloroform extracts were ideal.

Because of the potential economic value of *A. fourcroydes*, emphasis was placed on large scale methods of processing the juice for the production of "coffee grounds". In particular, the well-established "fermentation" method currently employed in one area of the United Republic of Tanzania was examined and the possibilities of both pressure hydrolysis of saponins and acid hydrolysis were reviewed. In addition to this method, a new large-scale procedure for the preparation of "coffee grounds" was described. This involves the passage of air through the *Agave* juice in a tall column and the resulting foam produced by the saponins is carried from the top of the column into tanks of hot acid, which breaks the foam by hydrolysing the saponins. The "coffee grounds" produced by this "foaming" procedure are of better quality and with a considerably higher hecogenin content than those produced by the "fermentation" method, but the recovery of hecogenin from the juice is lower. The "foaming" method is protected by patent.

D. Preparation of a training programme for technicians

The training programme devised and discussed with the Cuban research team would apply to the practical training of graduates in the techniques required for the processing, extraction, isolation and analysis of steroidal sapogenins in plant materials. Papers giving details of many of these procedures were supplied and others were to be sent from the United Kingdom. The methods of processing, extraction and analysis of steroidal sapogenins were demonstrated practically to the Cuban research group working on plant steroids, who also used these procedures when assisting the expert in the work conducted during the programme.

The laboratory work was undertaken in the research laboratory at the Laboratorio Dr. Mario Muñoz. The classical methods of processing and extraction of plant materials for steroidal sapogenins were being used by the Cuban research group and only minor modifications were suggested. As written earlier in the report, the effects of enzymes in the initial processing of plant materials both for increasing the yield of sapogenin extraction and for partial hydrolysis of the saponins was suggested as a routine procedure. The optimum methods of acid hydrolysis and extraction of sapogenins from the acid-insoluble residue were already used by the Cubans. However, the use of chloroform for the more rapid extraction of the sapogenins from *A. fourcroydes* and *P. tuberosa* was found to be highly suitable when the extracts were analysed.

For routine analyses of steroidal sapogenin contents of extracts of plant materials, including *Agave* species, *P. tuberosa* and *Dioscorea* species, a thin-layer chromatographic method was suggested and used during the assignment.

This method uses air-dried layers of silica gel G, 250 μm wet thickness. A constant volume of plant extract prepared from a known weight of plant material is applied several times to the layer using a micro-pipette. Between these spots, gradually increasing volumes are applied of a solution of the required reference sapogenin of known concentration. The development solvent varies with the sapogenin being estimated, but chloroform-ethanol 95:5 v/v is a useful solvent system for many plant extracts and double development in n-hexane-ethyl acetate 4:1 v/v is ideal for the separation of tigogenin from substantial quantities of hecogenin. After development, the compounds are located by spraying with either 50% aqueous sulphuric acid or 300% antimony trichloride solution in concentrated hydrochloric acid and heating at 130°-150°C until the spots have developed a deep colour. The intensities of the various spots are compared by eye. Although only semi-quantitative, the degree of replication obtained is good, the method is applicable to a wide range of plant materials and high results owing to contaminants are not obtained as in some analytical methods using estimations based on techniques such as infra-red and ultra-violet spectroscopy and colorimetry.

An easy method for the routine separation of individual sapogenins from a crude mixture was described. This method uses preparative thin-layer chromatography and the solvent systems required for the separation of many different sapogenins were given. Although large quantities cannot be isolated in this way sufficient pure material can be obtained for the characterization of the compounds.

II. RECOMMENDATIONS

1. Because of the quantity and quality of hecogenin obtainable from the juice of Agave fourcroydes in Cuba, it is considered that this plant is a valuable source of an ideal precursor for the synthesis of corticosteroids. The juice could be made available in large quantity and pilot-scale production should be commenoed as soon as possible.

2. In the early stages, while production facilities are developed for the transformation of hecogenin into final steroid drugs, either the "coffee grounds" or hecogenin produced should be readily salable on the international market. The time required for the production of "coffee grounds" on a large scale should be comparatively short, whereas considerably longer time will be required before final steroid compounds are produced. It is suggested that in the interim period, the "coffee grounds" or hecogenin produced should be sold.

3. Consideration should be given to starting pilot-scale production of either "coffee grounds" or hecogenin, as well as to the technical requirements of converting hecogenin into pharmaceutically-useful steroid drugs.

4. It is necessary to determine the relationship between the hecogenin content of A. fourcroydes leaf and the time of year. In East Africa, production of "coffee grounds" often ceases at one period of the year because the tigogenin percentage of the total sapogenin is unacceptably high. It is recommended that A. fourcroydes be studied to determine whether a similar phenomenon occurs in Cuba, in which case production may have to be discontinued for that period.

5. It is necessary to determine the hecogenin and tigogenin content of the leaves of A. fourcroydes of different ages. The tigogenin content of young leaves of A. sisalana (1-2 years after planting) is unacceptably high. A. fourcroydes is not cut until 5 years after planting-out, so this problem should not apply, but it is recommended that the study should be made.

6. A method of possibly increasing the yield of hecogenin from the leaves of A. fourcroydes was discussed and it is suggested that this possibility be tried. The leaves should first be lightly pressed and the juice collected, but after 10-20 minutes the leaves should be tightly pressed, when the bulk of the juice should be obtained. The preliminary damage to the cells of the leaves will, it is hoped, increase the yield of available hecogenin.

7. Solanum laciniatum shows considerable promise as a source of solasodine. However, it is too early to decide whether the project will be economically viable. More research is necessary before a decision can be made, particularly on agricultural aspects. It is necessary to obtain more data on annual yields per hectare of S. laciniatum leaves and their solasodine content. This study should be continued over several years and the trials must be on a sufficiently large scale that the results can be used to estimate the likely yields, if the plant were to be grown under normal farming conditions. Accurate costing will be necessary to determine the combined expenses of planting, fertilizer treatment, the possible use of anti-pest sprays, harvesting and drying.

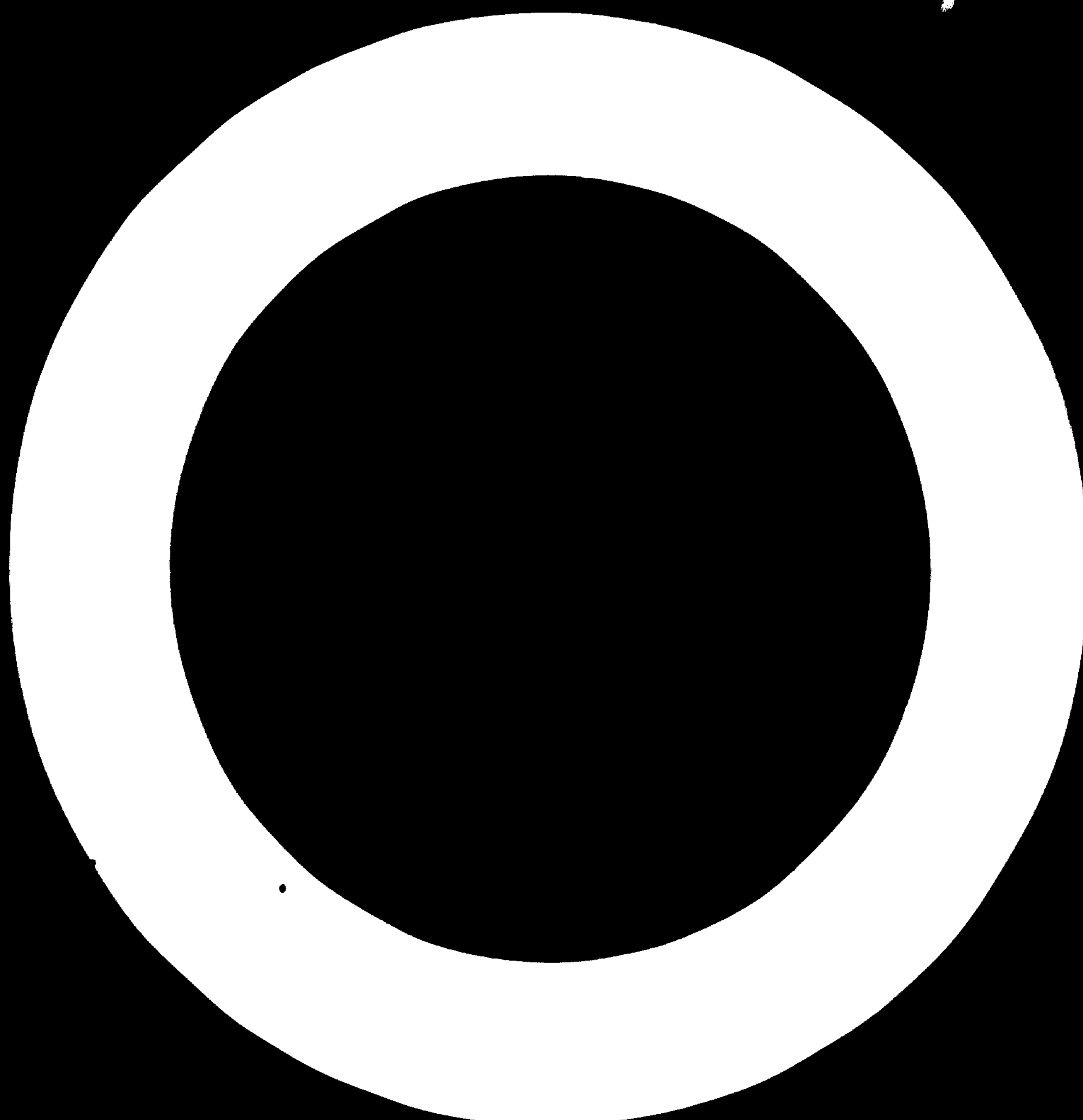
8. During the field trials, careful note should be taken of any damage to the crop by pests such as nematodes, insects and fungi and to determine whether the incidence of attack increases year by year.

9. It has been proposed that two harvests of leaves should be made every year. It is necessary to study the amounts of leaves obtained in this way and the effects this has on the annual solasodine yield per hectare.

10. The S. laciniatum project should be actively continued and development work on the conversion of solasodine into pharmaceutically-useful steroidal compounds pursued further.

11. Because of the high proportion of other steroidal compounds, in particular $\Delta^{9(11)}$-hecogenin, in the crude sapogenin mixture obtained from the tubers of Polyanthus tuberosa, it is considered that this plant is not a suitable source of hecogenin for industrial use.

12. It is recommended that the reference compounds and equipment necessary for the analysis of plant materials be purchased and sent to Cuba, where they will be required quiokly for their future work.



Annex I

COUNTERPART PERSONNEL

Dr. Luis R. Capo, Director Desarrollo Técnico, Vice Ministerio Industria Farmacéutica, who conducted the overall co-ordination of the project in Cuba

Dra. Angela Sánchez Morales, who assisted in the practical work

Lie. Humbertor A. Lastra, who works on the Polyanthus tuberosa project

Lie. Caridad Robaira, who works on the Agave fourcroydes project

Lie. Carlos E. Timor, who works on the Solanum laciniatum project

Annex II

DRAFT PROJECT FOR A PILOT PLANT FOR HECOGENIN PRODUCTION

Project title:	Establishment of a Pilot Plant for Hecogenin Production in Cuba
Categories:	Research and development in pharmaceutical industry
Project duration:	9 months - UNIDO with assistance from a voluntary contribution from a Government
Project budget:	$US 130,000
	$US 100,000 - Equipment
	$US 24,000 - Expert
	$US 6,000 - Training

A. Background and supporting information

Justification for the project

Cuba is rich in medicinal plants and plants which contain compounds suitable for industrial use as precursors of medicinally-useful steroids. The Government of Cuba wishes to develop and utilize these resources, and research is in progress to evaluate many indigenous and introduced species as economic sources of steroid drug precursors. In particular, two plant species are being extensively studied, (a) *Solanum laciniatum* for its content of solasodine, which is an ideal precursor of a wide range of useful steroid drugs, including oral contraceptives and (b) *Agave fourcroydes*, which yields hecogenin, a valuable precursor for the production of corticosteroids.

Cuba has a well-developed pharmaceutical industry producing, among other products, oral contraceptives from imported bulk chemicals. The Government wishes to develop production of both oral contraceptives and other steroid drugs based on locally-available raw materials to supply both the home market and for export.

The first phase of a research and development programme was carried out with aid from SIS and executed by UNIDO. The main recommendation from this study was that, from the *A. fourcroydes* leaves cultivated in Cuba, pilot-scale production of both the crude sapogenin concentrate known as "coffee grounds" and of hecogenin should be commenced. This programme will be carried out by UNIDO with the assistance of a voluntary donation from another Government.

Institutional framework

The research laboratory in Havana will provide counterparts to the UNIDO experts carrying out the project. In addition, the research laboratory will be used for the analysis of *A. fourcroydes* plant material and of the "coffee grounds" and hecogenin produced.

Provision of Government follow-up

The Government will undertake marketing of either "coffee grounds" or hecogenin until these materials can be utilized in Cuba for the production of corticosteroids.

Other related activities

The Government of Cuba, parallel with the development of production of "coffee grounds" and hecogenin, will develop the transfer of hecogenin by chemical transformations into pharmaceutically-useful steroid drugs.

Future UNIDO assistance

Such assistance will be envisaged after completion of phase 2 of the project.

Other sources of funding

The Government has not requested external assistance for this project from any agency or programme other than SIS for phase 1 and UNIDO, with a voluntary contribution from another Government, for phase 2.

B. Objectives of the project

Long-range objective

The long-range objective is to develop a pharmaceutical steroid industry based on available raw materials in Cuba for both domestic and export purposes. Hecogenin is to be developed for the production of corticosteroids and, if field trials are satisfactory, solasodine from *Solanum laciniatum* for oral contraceptives.

Immediate objectives

Based on the results of Phase 1, the immediate objectives for Phase 2 are as follows:

(a) To establish a pilot plant for the production of "coffee grounds" and hecogenin from the juice obtained from the leaves of Agave fourcroydes;

(b) To work out a production programme on the pilot-plant scale;

(c) To establish a quality control laboratory for the analysis of "coffee grounds" and hecogenin produced;

(d) To train the Cuban technicians;

(e) To study the possibilities of transfer of hecogenin produced into corticosteroid drugs.

C. Work plant

Project activities for phase 2	Location	Duration
1. Purchase of equipment and machinery and delivery to Cuba	Vienna	9 months
2. Expert in the field for establishment of pilot plant and the elaboration of the production programme	Cuba	1 month
Establishment of the quality control laboratory		
Elaboration of control methods		
3. Erection of equipment and machinery	Cuba	3 months
4. Expert in the field for pilot-scale production and development	Cuba	5 months
Training of personnel for production, quality control and maintenance of equipment		

Other UNIDO/government input

Assignment of international staff to phase 2

An international expert for the establishment of the pilot plant and production.

A G.P.O. to be provided by the assiting Government.

A mechanical engineer for the erection and maintenance of equipment to be provided by the Cuban Government.

Training provision

A 4-month training period will be provided to study:

Methods of extraction, purification and separation of plant steroids

Quality control

Maintenance of equipment

Equipment

The equipment required for the project is as below. The prices are approximate and are subject to increases due to inflation.

Item	Cost ($)
Stainless steel press (e.g. Protessor)	13,000
2 tanks made of fibre glass or of steel plate, capacity 1,000 litre (can be locally built)	1,000
Slurry pump	1,000
Hydrolysis tank, glass line, with slow-speed stirrer and motor, and heat exchanger	50,000
Continuous action centrifuge	9,000
Neutralizing tank (can be locally built)	400
Drying oven	2,600
Heat source (steam boiler) - if not already available	-
Pumps, piping and fittings	13,000
Fibre glass foaming column and receivers (could be made locally)	10,000
Total	100,000

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