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ESTABLISHMENT OF ADVANCED STEROIDS PRODUCTION

DP/CUB/81/013

CUBA

Technical report: UNIDO contract No. 91/070*

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

Based on the work of Dr. W.N. Walker & Co.
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* This document has not been edited.

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CONTENTS

SUMMARY

This summary covers the three Interim Reports of 31 May, 1991, 16 September, 1991, 6 December, 1991 and this Final Report of 9 December, 1991.

1. A well-installed pilot production unit for the processing of steroid products has been established within the confines of the Establishment Empresa Labatorio Farmaceutica, "Mario Muñoz" situate Hacendados Nun 1, Habana Vieja, C. Habana, Cuba.

2. This unit may be considered as multi-purpose and is very versatile, a considerable range of unit operations and chemical processes having already been demonstrated.

   Further extention of the versatility of the unit could be readily incorporated in the event of need to introduce further novel processes.

3. The pilot production unit has been satisfactorily commissioned on a mechanical level with only limited modifications being necessary or shortfalls encountered. Inadequacy of services, particularly refrigeration and to some degree vacuum, have caused most problems.

4. A wide range of unit operations and processes have been commissioned and operated in the unit satisfactorily. While several processing problems were encountered, in several cases due to raw material supplies, good experience has been gained in the general satisfactory resolution of these problems.

5. During commissioning tests productions of four finished products were acheived, analysing according to the pharmacopoeia standards necessary for further use in formulation.
   These products were :- Testosterone propionate
   Testosterone enanthate
   Triamcinolone acetonide
   Ethinyl estradiol

6. The production of the intermediate "diene acetate", to be used as an intermediate in the longer term for corticosteroid

production, was not completed within the project period due to serious chemical and fuel supply situations. Commissioning was satisfactory so far as completed and had previously been acheived on pilot scale-up level in production equipment.

7. For the fully satisfactory exploitation of economic production of steroids in Cuba, both for domestic consumption and as an export potential, several recommendations are put forward.

RECOMMENDATIONS.

1. The most important recommendation relates to the future of satisfactory and economical use of the valuable and proved pilot steroid production unit resulting from this project. While originally production of all steroids, other than advanced corticosteroids, was besed on diosgenin this is no longer an economic starting material in Cuba since agricultural production of suitable sources has not been established. An alternative raw material which Cuba can provide on most advantageous terms has to be sought. Cuba is potentially in a very favourable position to do so, largely from currently waste material.

   It is recommended that an urgent Techno-economic Study be performed in Cuba to assess the utilization of sugar cane wastes for the production of phytosterols and the subsequent conversion by fermentation to products most useful for steroid syntheses using modern technologies. At the same time the study should also consider the fermentative conversion of a second, and supportative, source for the same steroid starting materials - cholesterol. Cuba has the experience and potential of producing this from animal products - cattle spinal cord.

Further recommendations refer to improvements and necessary addition to the production unit to enable it to work satisfactorily and economically on regular production of the bulk pharmaceutical products at its appropriate capacity and to the highest pharmacopoeial standards necessary.

2. An absolute necessity to instal a small centrifuge (500mm diameter stainless steel) for the production of the product testosterone enanthate for which the highest domestic demand exists.
3. Installation of a larger (800 - 1000mm diameter stainless steel) centrifuge for general processing work.
4. Installation of a dedicated crystallisation unit to ensure regular production of top grade pharmaceutical products.
5. Installation of a supplementary dedicated vacuum service (water ring pump) for the steroid plant.
6. Improvement of heat exchange facility in conjunction with circulating pump, particularly for cooling of the glass reaction unit GR 100.
7. For regular production, the containment of solvent recovery operations within the production facility is needed. For this purpose a small pot still with fractionating column, manually operated, is required.

INTRODUCTION.

This Final Report should be considered not as a closing document of the work performed during the project life. It should rather be considered as an 'aide mémoire' available as a general guide and working document for the processing of steroids in the plant established at Empresa Labatorio Farmaceutica, "Mario Muñoz" situate Hacendados Nun 1, Habana Vieja, C. Habana, Cuba.

The detailed results of commissioning have all been reported in the three Interim Technical Reports published on 31 May, 1991, 16 September, 1991 and 6 December 1991 and are not repeated as such in this final report. The Third Interim Technical Report in particular summarises all processes commissioned.

The three Interim Technical Reports and the Final Report should be considered together as a composite report on the project and not in isolation.

The Final Report will present the ultimate situation regarding the commissioning and at the same time provide a general summary of commissioned processes including essential criteria, possible problems and solutions as well as suggestions for obtaining more usable materials from minor crops as they accumulate.

Sections are included covering some specific aspects of production and a general summary of Good Manufacturing Practice and Good Laboratory Practice.

Consideration is given to Project Consolidation and Development and the report closes with Conclusions and Recommendations.

1. PROCESS SUMMARY.

This section is presented as a summary of all stages commissioned and documents any prescription variations, criteria for successful processing, possible problems and solutions as well as minor crop processing. It is also intended as a reference guide.

TRIAMCINOLONE SYNTHESIS:

Stage 1: Production of prednisolone acetate from prednisolone.

a) Method reference: MPT 1/4

b) Prescription validation: Validated as written.

c) Essential criteria: Dry pyridine. Use moisture protection.

d) Possible problems: Only under-acetylation due to incorrect quantities or omission. Re-acetylate to correct.

e) Minor crops: Not applicable.

Stage 2: Production of pregna-1,4,9(11)-triene-17α,21-diol-3,20-dione, 21-acetate (1,4,9-triene) from prednisolone acetate.

a) Method reference: MPT 5A/8A

b) Prescription validation: Prescription validated as written at laboratory level. Further commissioning batch needed, but not possible due to lack of material.

c) Essential criteria: Temperature control most important and, by implication, rate control of sulphur dioxide injection. Pharmaceutical grade N-bromo-succinimide (Peboc) should be employed, if possible, for optimum result.

d) Possible problems: Lack control or incorrect quantities and quality of N-bromo-succinimide can lead to low quality product. If low quality obtained it is usually best to proceed directly to the next stage of epoxide formation and then purify. A laboratory check should be made before proceeding.

An alternative processing method has also been supplied for comparison.

e) Minor crops: Not applicable.

Stage 3: Production of 9β,11β-epoxy-pregna-1,4-diene-17α,21-diol-3,20-dione from pregn-1,4,9(11)-triene-17α,21-diol-3,20-dione, 21-acetate.

a) Method reference: MPT 9A/12A

b) Prescription validation: Prescription validated as written, but inclusion of analysis check on strength of alkali used.

c) Essential criteria: Purified tetrahydrofuran to be used as scheduled. Temperature control also important. Pharmaceutical grade N-bromo-succinimide (Peboc) recommended for best results. Strength of alkali solution used should be checked by analysis.

d) Possible problems: Quality due to failure of observation of essential criteria or use incorrect quantities or low quality input of steroid.
If low quality product obtained at any time purification by crystallisation (ethanol) should be considered before proceeding with fluorination.

e) Minor crops: Not normally applicable.
If crystallisation performed minor crops may be obtained from mother liquors. Purification by column chromatography might need to be investigated and applied.

Stage 4: Production of 9α-fluoro-prednisolone from 9β,11β-epoxy-pregn-1,4-diene-17α,21-diol-3,20-dione.

a) Method reference: MPT 13/16

b) Prescription validation: Prescription validated as written. Also new prescription will be written for increased batch size as commissioned. An amendment to the corresponding analysis method MPTA 13/16 referring to sampling technique, preparation of sample and plate interpretation has already been issued. The mobile phase was also changed (from toluene:ethyl acetate 1:1 to 1:2)

c) Essential criteria: Most important is adequate strength of aqueous hydrogen fluoride prepared and this must be checked by analysis before steroid addition.

Wearing protective clothing is particularly important and appropriate first aid treatment must be available. No unauthorized entry to the work area must be permitted and notices clearly posted on both doors to fluorination room.

d) Possible problems: No problems envisuaged when correct conditions, including temperature control, are applied.

e) Minor crops: Crude product normally processed so not normally applicable.
If product should be required for sale (or in the event of a particularly low quality product) crystallisation from methanol or ethanol may be applied.

Column chromatography would probably be the best method of obtaining useful material from minor crops or liquors.

Stage 5: Production of 9α-fluoro-prednisolone -11β,17α,21-tri-acetate from 9α-fluoro-prenisolone.

a) Method reference: MPT 17A/20A

b) Prescription validation: Process method validated though yield low, but comparable with control. Laboratory has acheived scheduled yield but also some low observed. Centrifuge should be used at this stage. Liquors thoroughly checked before discharge.

c) Essential criteria: Calcium chloride protection should be applied. Temperature control.
Checking of product filtrates for presence of dissolved steroid before discharge.

d) Possible problems: Lack of temperature control might lower yields - no cure. Only other likely problem might be incomplete acetylation and could be corrected in situ by the addition of more reagent and/or catalyst.

N.B. Suggested this reaction should be investigated using methane sulphonic acid (MSA) in place of p-tolume sulphonic acid (PTSA). MSA claimed as stronger acid and possibly more economic reagent plus possibly less side products.

e) Minor crops: Not applicable.

Stage 6: Production of 9α-fluoro-pregna-1,4,16-triene-3,20-dione, 11β,21-diacetate, crude and purified from 9α-fluoro-prednisolone-11β,17α,21-triacetate.

a) Method reference: MPT 21/24 & MPT 25/27

b) Prescription validation: The prescription for preparation was validated as written but using the alternative proceedure with solvent carrier as standard.
The purification prescription was also validated producing good quality product, but due to input quality did not meet scheduled yield.

c) Essential criteria: Control of temperature is most important, but using alternative proceedure this is automatically controlled provided solvent is not lost during reflux.
Anhydrous potassium acetate is also important. Note: only the potassium salt promotes satisfactory reaction.

d) Possible problems: Determination of the optimal reaction time is the main problem. A HPLC analysis method for determination of completion is of high priority.

e) Minor crops: No minor crops involved at crude stage. Mother liquors from purification contain many impurities determined by efficiency of earlier stages. No method has currently been developed to obtain useful material.

Stage 7: Production of triamcinolone-11β,21-diacetate from 9α-fluoro-pregna-1,4,16-triene-11β,21-diol-3,20-dione, 11β,21-diacetate.

a) Method reference: MPT 28/33

b) Prescription validity: Prescription validated as written although operating conditions could not be fully acheived. Prescribed temperature conditions need to be realized in subsequent runs when adequate refrigeration capacity and process materials are available.

c) Essential criteria: Quality of acetone and potassium permanganate are important. Visual control of reaction is most efficient. Temperature control of reaction of paramount importance - hence the efficiency and capacity of refrigeration.

Some control variation possible by varying number of plates of heat exchanger used in pre-cooling steroid solution, i.e. plates for pre-cooling may be increased with decrease of after-cooling (may even be eliminated with minor piping alteration).

d) Possible problems: Over-oxidation products are lost in water during work-up lowering yield. At most only a trace of starting material should be observed in reaction mixture. If starting material is excessive due to under-oxidation the product should be crystallised before proceeding. Mother liquors, in principle, can be re-cycled through oxidation stage.

e) Minor crops: Not normally applicable. If crystallisation needed crops from mother liquors could be re-cycled provided no other significant impurities are present.

Stage 8: Production of triamcinolone-16α,17α-acetonide, 11β,21-diacetate from triamcinolone-11β,21-diacetate.

a) Method reference: MPT 34/37

b) Prescription validation: Prescription validated, but with minor work-up modification excluding distillation.

c) Essential criteria: Use of nitrogen and pure acetone important.

d) Possible problems: Only problem might be in-complete formation of acetonide. Re-reaction should be applied.

e) Minor crops: Not applicable.

Stage 9: Production of triamcinolone-16α,17α-acetonide, crude from triamcinolone-16α,17α-acetonide, 11β,21-diacetate.

a) Method reference: MPT 38/41

b) Prescription validation: Prescription validated as written.

c) Essential criteria: Passage of nitrogen through liquid and exclusion of moisture important. Check of alkali concentration before use is advised. Temperature control.

d) Possible problems: Under-saponification could be resolved by proceeding with crystallisation, if product has been isolated, and further saponification of mother liquors. If under-saponification of isolated crop is serious it may be best to re-saponify all product. If the under-saponification is detected before work-up addition of more alkali and further reaction time should resolve.

e) Minor crops: No minor crops normally, but filtrates should be checked for presence of steroid content and might provide a small useful minor crop on evaporation. These would probably need re-saponifying.

Stage 10: Production of release quality triamcinolone-16α,17α-acetonide from crude.

a) Method reference: MPT 42/46

b) Prescription validation: Prescription validated with issued amendment to work-up.

c) Essential criteria: Checking clarity of filtrates and protection from atmospheric pollution. Use correct nylon filter cloth. Check mass balance.

d) Possible problems: Crystallisation in filter or lines. Overcome by correct equipment and preferably performed in dedicated crystallisation plant.

e) Minor crops: Include vessel washings in this category. Working is covered in prescription - further crystallisation or re-saponification as appropriate.

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ETHINYL ESTRADIOL SYNTHESIS.

Stage 1: Production of androsta-1,4-diene-3,17-dione, 17-ethylene ketal (ADD ketal) from androsta-1,4-diene-3,17-dione (ADD).

a) Method reference: EM 19/22

b) Prescription validation: Prescription validated, but modified to suit longer reaction time needed in production equipment. Reduction of crystallisation volume from 5 volumes to 2 volumes incorporated.

c) Essential criteria: Efficient water removal. Control of optimum reaction time by thin layer chromatography using quantitative reference standards (5% & 10%). Volume of crystallisation of product.

d) Possible problems: Incompleteness of ketalization corrected by use of more catalyst, additional benzene for azeotroping or re-ketalization of whole batch. At present lack of a small crystallisation equipment is a problem.

e) Minor crops: Minor crops are best re-kecalized unless excessive impurities build up when the ketal should be split (acid treatment) and recovered ADD purified.

Stage 2: Production of estrone from androsta-1,4-diene-3,17-dione, 17-ethylene ketal (ADD ketal).

a) Method reference: EM 23/27

b) Prescription validation: Process validated including changes made with respect to replacement of benzene. Also concentration of steroid decreased. Prescription and flow sheets have been up-dated with inclusion of estrone purification from acetone. Prescription yield not validated on production scale due to lack of materials.

c) Essential criteria: Reaction very sensitive to oxygen and ouality of solvents (peroxides and moisture to be absent). Tetrahydrofuran solvent must be freshly purified for this reaction. Moisture exclusion to be applied and use dry oxygen free nitrogen (use pyrogallol scrubbing & gas drying recommended)

d) Possible problems: No operational problems expected. Experience showed check on adequate supply lithium metal be made prior to starting reaction. Possibility of shortfall should also be taken into account when scheduling raw material supplies.

Any problems experienced likely to be due only from deviation from essential criteria and would manifest in low yield.

e) Minor crops: Essentially dealt with in the prescription. Quality of any second crop estrone needs careful checking as often most suitable for conversion to estradiol (laboratory method has been supplied), rather than purification for ethinyl estradiol.

Stage 3: Production of ethinyl estradiol, crude from estrone.

a) Method reference: EM 28/31

b) Prescription validation: Prescription fully validated in the laboratory. Revised procedure for plant as alternative to be written after further commissioning when materials available. Will include improved potassium handling technique.

c) Essential criteria: Handling of potassium only in presence, or by, technically qualified person.
Quality and dryness of solvents important. Acetylene quality of prime importance - policy should be to perform laboratory test conversion for each cylinder before use (or until such times as facilities are available for on-site analysis of gas).
The full prescribed gas purification system to be used as minimum -i.e. any other purification proposed may be added but not used as substitute.
During this reaction and particularly potassium alkoxide formation a stand-by nitrogen cylinder should be coupled in parallel to that in use, so that immediate switch-over can be made in event of cylinder emptying (This should be general policy whenever use of gas is an important factor in reaction - here safety is the prime consideration).

Use of appropriate level of estrogenic protection clothing after reaction commencement, during work-up and thereafter. Special care with dry product.

d) Possible problems: Due to nature of reaction consistency in prescribed method some stirring problems might interfere with efficiency of gas distribution in reactor used. An alternative method supplied has been proved in the laboratory which may be preferable. Reaction time is also shorter. This will be proved on plant when material is available.

e) Minor crops: Not applicable.

Stage 4: Production of release quality ethinyl estradiol from ethinyl estradiol, crude.

a) Method reference: EM 32/34

b) Prescription validation: Prescription validated (in course of work-up of sub-standard material but at non-standard yield due to abnormal quality input.

c) Essential criteria: Estrogen handling techniques must be applied rigorously. Special care during crude solid charging, dry solid un-loading and packaging.

d) Possible problems: With dimethylformamide, particularly hot, ensure contact only with stainless steel. Check well beforehand all valves and flanges are leak-proof. Ensure hot filter & lines to prevent crystallisation (jacketted filter useful).
Not acheiving release quality material directly - most likely, but not often encountered, with respect to colour. Resolve by further crystallisation.
Three different methods of purification have been supplied and the most appropriate may be selected.

e) Minor crops: Minor crops should be obtained by concentration of appropriate mother liquors. Crops containing significant amounts of estrone which cannot be removed by crystallisation may be subjected to re-ethinylation.

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TESTOSTERONE AND ESTERS SYNTHESIS.

Stage 1: Production of pregn-5-en-3β,17β-diol, 3β-acetate (mono-acetate) from dehydroepiandrosterone acetate (DHA).

a) Method reference: TM 1/4

b) Prescription validation: Validated as written.

c) Essential criteria: Sodium borohydride quality must be assured. Important solvent is free of aldehydes and ketones.

d) Possible problems: No problem foreseen if criteria met. In event of under-reduction add more borohydride for completion. If isolated product repeat reduction proceedure.

e) Minor crops: Not applicable.

Stage 2: Production of pregn-5-en-3β,17β-diol, 3β-acetate, 17β-benzoate (acetate-benzoate) crude & washed from pregn-5-en-3β,17β-diol, 3β-acetate (mono-acetate).

a) Method reference: TM 5/8 & TM 9/11

b) Prescription validation: Prescription TM 5/8 validated as written.
Prescription TM 9/11 for washed product validated but with the replacement of methanol by 96% ethanol (1t/1t).

c) Essential criteria: Quality pyridine (dry) important. Protect reaction with calcium chloride drying tube.

d) Possible problems: None likely and only might be under-esterification. Add more reagent if detected in reaction mixture or if afer isolation re-solve at washing stage to produce good quality but more minor crop.

e) Minor crops: Not applicable to TM 5/8. Method for further working of mother liquors and minor crops to androst-enediols provided.

Stage 3: Production of pregn-5-en-3β,17β-diol, 17β-benzoate (mono-benzoate) from pregn-5-en-3β,17β-diol, 3β-acetate, 17β-benzoate (washed acetate-benzoate).

a) Method reference: TM 12/15

b) Prescription validation: Prescription validated but including replacement of methanol by 96% ethanol (1t/1t).

c) Essential criteria: Temperature control and pre-adjustment of pH to phenolphthalein.

d) Possible problems: In event of sub-standard material due to under- or over- saponification purify by methanol or ethanol crystallisation.

e) Minor crops: Minor crops may be obtained from ethanol washes. Also if crystallisation of sub-standard material is performed the mother liquors need treatment. In both cases saponification to a mixture of 17β- and 17α-androstenediols should be performed and products may be combined with materials from stage 3.

Stage 4: Production of testosterone benzoate, purified from pregn-5-en-3β,17β-diol, 17β-benzoate (mono-benzoate).

a) Method reference: TM 16/18 & TM 19/21

b) Prescription validation: Prescription TM 16/18 validated as written.
Prescription TM 19/21 validated but including replacement of methanol by 96% ethanol (1t/1t).

c) Essential criteria: Distilling reaction mixture dry before reaction and good quality aluminium isopropoxide are important. Technique of steam distillation and close attention during this operation. Careful checking of distillate for product.

d) Possible problems: No chemical problems foreseen except for under-oxidation (some starting material always remains). If serious add more aluminium isopropoxide.

e) Minor crops: Processing of these is covered in prescription TM 19/21.

Stage 5: Production of testosterone, crude from testosterone benzoate, purified.

a) Method reference: TM 22/25

b) Prescription validation: Prescription validated as written.

c) Essential criteria: Passage of nitrogen during reaction is most important and also during reflux to prevent by-product and resin formation as well as producing good colour.

d) Possible problems: If in-adequate quality obtained it is normally resolved by crystallisation. Usually increased washing of lower quality product is found necessary initially and good quality first isolated material results at depressed yield. The re-crystallisation is then more relevant to the low quality second crop produced. Such problems are only likely to occur in event of failure of supply of nitrogen or inadequate purging. A standby nitrogen cylinder in parallel to the supply in operation is most important.

e) Minor crops: Second crop material is usually also suitable for testosterone propionate production. Otherwise it may be upgraded by crystallisation. If testosterone benzoate seen to be present - re-saponify. In case of significant impurities column chromatography can be considered.

Stage 6: Production of testosterone, pure from testosterone, crude.

a) Method reference: TM 25A/25B

b) Prescription validation: Prescription validated as written.

c) Essential criteria: Pre-heating of filter and lines to prevent crystallisation. Mass balance established while retaining cakes and liquors.
Availability of dedicated small crystallisation unit would be very benificial.

d) Possible problems: In-line crystallisation. Passing of charcoal through filter.

e) Minor crops: May be dealt with similarly to stage 5.

Stage 7(A): Production of testosterone propionate, crude from testosterone crude.

a) Method reference: TM 26/28

b) Prescription validation: Prescription validated as written.

c) Essential criteria: Use of good quality dry pyridine and anhydride. Calcium chloride moisture protection.

d) Possible problems: Only foreseen problem might be under-esterification due to incorrect measuring or weighing and could be corrected in-situ by addition more anhydride.

e) Minor crops: Not applicable.

Stage 7(B): Production of testosterone propionate, release quality from testosterone propionate, crude.

a) Method reference: TM 29/32

b) Prescription validation: Prescription validated as written. Product released to USP XX11 & BP 88.

c) Essential criteria: Checking clarity of filtered solution before crystallisation. Filtration of wash solvent. Protection from atmospheric or other pollution.

d) Possible problems: Mechanical problems of in-line crystallisation or passage of charcoal through filter. Unlikely following laid down procedures.
Failure to reach release quality directly dealt with by re-crystallisation.

e) Minor crops: If re-crystallisation of minor crops to release quality is not possible they should be saponified together with final mother liquors to recover testosterone for further purification or re-use. For saponification the proceedure testosterone benzoate to testosterone TM 22/25 should be used.

Stage 8: Production of testosterone enanthate, release quality from tesosterone, pure.

a) Method reference: TM 33A/36A

b) Prescription validation: Prescription TM 33/36 is not validated. A revised prescription TM 33A/36A has been issued. Product-of crude product has been fully validated on plant scale, but final isolation of release quality product has only been validated on pilot laboratory scale due to equipment shortfall. Product quality meets BP 88 and, only with exception of moisture content, USP XX11.

c) Essential criteria: Quality of dry pyridine and enanthic (Heptanoic) acid anhydride important. Quantity of enanthic anhydride was optimalized.
Facility of a centrifuge for isolation of final crystallised product appears to be a necessary requirement for satisfactory isolation on production scale.

d) Possible problems: No esterification problems envisuaged following correct procedure.
Main problem revolves around establishing product isolation in good crystal form. This depends on efficient removal solvent from easily filterable crystal form. Technique of crystal form needs close attention governed by suitable agitation during the period of crystal formation.
In initial laboratory separations by vacuum a special drying technique was necessary initially drying in a stream of cold air before final vacuum drying. Using the centrifuge the product can be largely dried in situ in the basket and transferred directly to the vacuum drier for final attention. The provision of a centrifuge for regular production is essential.

e) Minor crops: Second crops can be obtained from mother liquors by static crystallisation and decantation of supernatant liquor. These crystals may be of satisfactory quality or can be up-graded by further crystallisation. If they cannot be up-graded they should be combined with mother liquors for low temperature vacuum distillation and saponification to recover testosterone (use testosterone benzoate to testosterone procedure).

Alternatively work should be performed to check the possibility of use of the first crop filtrates as solvent for the subsequent batch to optimalize yield. It is very likely that filtrates could be re-cycled several times. When any impurities start to appear in the crop the filtrates should be treated by evaporation and saponification.

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DIENE SYNTHESIS.

Stage 1: Production of 11β-bromo-hecogenin.

a) Method reference: HD 1/4

b) Prescription validation: Prescription validated as written, except limits of water in ethanol waived from 0.05% to 0.3% and hydrogen chloride concentration in reaction mixture increased from 0.4N to 0.5N.

c) Essential criteria: Paramount importance is quality of ethanol employed. Water content as low as possible (original specification 0.05%) and absolute maximum should be 0.3% (by Karl Fischer titration).
Moisture check on solvent when it is in reactor is also advised.
Technique and conditions for precipitation to be strictly observed.
Bromine usage to be recorded by weight and volume. Check in separating funnel should be made on bromine to ensure no water enters head tank for addition.
Temperature controls important.

d) Possible problems: No problems likely if conditions followed. Incomplete solution at end of 1 hour after completion of bromine addition indicates sub-standard ethanol. Complete loss of solution colour indicates shortage of bromine. This latter can be corrected by addition of small portions of bromine. The former might also be corrected by adding excess bromine and additional ethanol but a more impure product is likely.
It is not advised to proceed to the

next step with seriously sub-standard 11β-bromo-hecogenin.
Not following the correct watering out procedure may lead to sticky product and although probably this will ultimately solidify it will result in very slow filtration and very wet product.

e) Minor crops: Not applicable.

Stage 2: Production of delta-9(11)-hecogenin from 11β-bromo-hecogenin.

a) Method reference: HD 5/8

b) Prescription validation: Prescription has been validated as written except using reactor VS 102 in place of VS 103. To meet scheduling requirements the use of a centrifuge is considered essential.

c) Essential criteria: Experience from other dehydrobromination reactions suggests that calcium carbonate should always be charged to dmf solvent before wet steroid. Poor vacuum during distillation resulted in varying temperature restriction during this stage during commissioning (from 95°C to 110°C). Further increase should be resisted and better vacuum applied if possible.
Avoid caking of product on walls.

d) Possible problems: Slow filtration main problem and centrifuge should be used.
Alternative isolation methods involving removal of calcium carbonate prior to watering out should be further considered. Filtration of hot dmf and addition of water to warm dmf is most promising but good stirring essential.
Only use stainless steel for hot dmf and check security of valves and flanges.
Poor quality material (determined by low melting point and U/V analysis may need to be crystallised accord- to prescription HD 9/11.
Alternatively it may be preferable to consider simple washing of the product with either methanol or 96% ethanol.
Materials having a melting point 188°/210°C and/or molecular extinction in the U/V of E(1%;1cm) >240 may be processed directly according to prescription HD 12/15.

e) Minor crops: Only applicable if low quality delta-9(11)-hecogenin is encountered. Further useful material can probably only be obtained by column chromatography.

Stage 3: Production of crystallised delta-9(11)-hecogenin from crude delta-9(11)-hecogenin.

a) Method reference: HD 9/11

b) Prescription validation: Not validated as produced products of adequate quality for direct processing.

c) Essential criteria: Hot filter and lines if crystallisation ever employed.

d) Possible problems: Only crystallisation in lines and filter and associated mechanical problems.

e) Minor crops: Second crops might be possible but further useful material probably only recoverable by column chromatography.

Stage 4: Production of delta-9(11)-tigogenin from delta-9(11)-hecogenin.

a) Method reference: HD 12/15

b) Prescription validation: Not validated on prescription scale, but process validated on small pilot scale in alternative production equipment.

c) Essential criteria: Removal of water during initial distillation.

d) Possible problems: Avoid cooling batch below $80^{o}C$ before transferring to watering out as batch may become rather thick.

e) Minor crops: Not applicable.

Stage 5: Production of crystallised delta-9(11)-tigogenin from crude delta-9(11)-tigogenin.

a) Method reference: HD 16/19

b) Prescription validation: Not validated on prescription scale, but validated on pilot scale in production equipment.

c) Essential criteria: Distillation must be performed at atmospheric pressure and distillation at 64°C maintained for at least 15 minutes. If necessary add more methanol to maintain prescribed volume.

d) Possible problems: None envisuaged.

e) Minor crops: Many impurities usually present in mother liquors make it difficult to isolate useful material. Preparation of acetate and column chromatography of acetate might be useful if significant desired product can be detected in thin layer chromatography.

Stage 6: Production of diene acetate from crystallised delta-9(11)-tigogenin.

(A) Pseudomerisation
(B) Oxidation
(C) Saponification

a) Method reference: HD 20/29

b) Prescription validation: Not validated on prescription scale, but validated on smaller scale in alternative production eouipment.

c) Essential criteria: (A) - Thorough drying of methylamine hydrochloride important. Dry minimum 24 hours, preferably 48 hours at 105°C. Keep in closed dry container until used and use as soon as possible.
(B) - temperature control important.
(C) - use anhydrous sodium acetate.

d) Possible problems: (A) if slow completion of pseudomerisation seen add more methylamine hydrochloride and react longer.

(B) avoid formation of emulsions during extractions.

(C) handling of solid residue in equipment to ensure satisfactory breaking-up and complete removal of dichloromethane.

e) Minor crops: Mother liquors may provide second crops which should up-grade by crystallisation. For further material investigation necessary.

For other delta-16 products it has been possible to form sulphonate derivatives with sodium metabisuphite as a means of obtaining further useful material and this might be possible in this series.

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2. SOME GENERAL NOTES ON PRODUCTION.

A) Anhydrous reactions.

Where anhydrous reactions are operated it is a good practice to protect the reaction contents as much as possible from atmospheric moisture. This is particularly applicable in the frequent high humidity conditions existing in Cuba.

This is best accomplished by fitting calcium chloride drying tubes, chambers or bottles to the reactor vent outlets. In some cases the reactor may be simply sealed off but care must be taken that no pressure or vacuum condition is developed.

Charging holes should be kept closed as far as possible during reaction.

Appropriate reactions for which these precautions are appropriate are esterifications, anhydrous saponifications and reactions containing chemicals specially sensitive to moisture e.g. potassium alkoxides, borohydrides etc.

Protection is appropriate in the following cases :-

Triamcinolone synthesis	Testosterone & esters synthesis	Ethinyl estradiol synthesis	Diene synthesis
MPT 1/4	TM 5/8	EM 28/31	HD 1/4
MPT 5A/8A	TM 12/15		HD 20/22
MPT 17A/20A	TM 22/25		
MPT 21/23	TM 26/28		
MPT 38/40	TM 33/37		

B) Solvent purifications and 'pure' solvents.

In certain reactions the quality of solvent used is particularly important. These are referred to in the prescriptions as either 'purified','pure' or 'dry'.

Where 'purified' solvent is indicated this should be purified 'in-house' according to the appropriate method even if the manufacturers analysis appears good. The 'purified' solvents should be used immediately after preparation or within any time limit indicated.

This applies particularly to the following where purification is considered absolutely essential :-

Tetrahydrofuran	in	MPT 9A/12A
		EM 23/27
		EM 28/31

In some prescriptions solvents are specified as 'pure' or 'dry'. For 'pure' solvents the manufacturers and receipt analyses can normally be accepted as indicating suitability for use provided all general and any special requirements are met. For example, for MPT 28 the permanganate test for presence of oxidisable impurities is most important and stringent and must be complied with. However, in some cases an analysis should also be performed by, or on behalf of, the production department just prior to use. It is suggested this should be done for any 'dry' solvent specification and essential for ethanol used in HD 1/4. Here where quality is so essential a Karl Fischer moisture determination of solvent actually in the reactor is recommended.

C) SOLVENT RECOVERIES.

In many cases recovered solvents may be re-used directly. In some cases they may be suitable for use in any process, in others it may be specified that they are for re-use only in a specific stage or stages.

All recovered solvents must be analysed (for this the availability of GLC is to be highly recommended) whether essentially pure or as re-usable mixtures, clearly marked and stored. While boiling point and density are useful criteria for pure solvents (moisture content should always be reported also) for mixtures, apart from the best guide of GLC, density and refractive index are useful parameters.

Whenever recovered solvents are re-used in processes it should be clearly recorded in the operating log sheets.

D) PROCESSING WITH GASES.

When gases are specified for use in reactions they may be employed as a reactive chemical e.g. acetylene, hydrogen, carbon dioxide, methyl bromide etc, or as a protecting agent to limit unwanted side reactions e.g. nitrogen or for safety protection e.g. nitrogen, carbon dioxide.

In all cases, before starting reactions, it should be checked that at least one full standby cylinder is available. This should be coupled in parallel to the main supply line for immediate switch over in event of a cylinder emptying (ideally a flow alarm should be fitted as warning). This is particularly important in the case of nitrogen in sensitive saponification reactions, for ethinylations and particularly when being supplied as a safety cover, e.g. preparing alkoxide from potassium metal and Grignard reactions.

Purification and drying procedures must be applied as specified in prescriptions.

E) FINAL PRODUCT FILTRATION, ISOLATION AND DRYING.

The general procedure employed is described in various technologies and should be strictly followed to minimise the possibility of re-work.
The procedure involves preparation of an 'in-line' filter with cloth, filter aid and paper. This should be first tested for security by filtering a small volume of appropriate solvent (i.e. similar to that to be used in crystallisation) containing charcoal through it under pressure and checking for leaks and clarity of solution. Filtrate is re-cycled as necessary until clarity is observed. Clarity test is best performed in the laboratory filtering a sample over a paper of some 1 - 1½cm diameter in a small Hirsch funnel. Extraneous particles of charcoal can be readily seen in a pattern on the paper.

When the main batch is filtered the filtrate must again be tested for clarity before it is allowed to pass to the crystallising vessel or evaporator. For this purpose a valved 'T'-piece on the outlet of the filter is very beneficial so that flow can be continuous avoiding any chance of pad disturbance

which could cause clarity to be lost.
Filtration of final products should be performed on nylon cloth and all washing solvents used pre-filtered before use.

It is recommended that a specific crystallisation unit be installed, incorporating the piping system to permit continuos flow during filtration. The unit could be used for all products and reserved for the unit operations of dissolution, charcaol treatment under reflux, clarification, concentration and crystallisation.

F) PLANT MARKING.

It is recommended that all vessels in use carry a card indicating 'batch in progress' with number of batch and any other information considered important, e.g. batch number, operation, operators in charge, hazards etc.

Similarly all drying equipment, static, vacuum etc., as well as trays carrying indentification should also indicate outside the materials being dried, entry time with date and drying restrictions.

G) HAZARD MARKINGS.

The current practice of indicating work in progress and no unauthorized entry should continue including smoking prohibition not only in the process area but also the vicinity.

ESTROGENS: when estrogens are being produced or handled in vessels, filters, tanks, driers, packing room, laboratory etc clear signs indicating presence of such materials should be posted.
Clothing used, especially when un-loading driers or packaging of dry estrogens should be washed down before un-dressing and placed in bags for laundering. Full instructions for working with estrogens have been provided.

FLUORINATION: particular attention should be given to indication of work being performed in the fluorination room. Both entry doors should be posted with notices.

H) PLANT CLEARANCE FOR MAINTENANCE.

When any work is to be performed in the plant involving welding or other potential source of ignition, the plant and surrounding area must be cleared of all inflammable solvents. Vessel interiors and other ancilliaries must be cleared in an appropriate manner to the contents finishing with water washing and complete filling of vessels with water and discharging to ensure all pockets of solvent are removed. Interiors of vessels and exterior areas should be checked with the available explosion meter (MSA Explosimeter 2E). Use of Draeger tubes to determine levels of specific vapours is also a useful precaution for indicating safety against not only explosion but also personal exposure.

A signed clearance certificate should be issued before work is commenced in the production area whether this is carried out by factory employed or contracted maintenance staff.

Fuses must always be removed to prevent inadvertant operation of agitators or other moving parts before entering any vessel.

I) PRODUCTION AIDS.

All reactors have been calibrated with volume measuring sticks. Such aids should be carefully stored and be readily available for use when plant is being operated. In absence of flow meters for most operations these measuring aids provide adequate assistance in determining batch transfer times. Such times of often of importance for optimal processing.

Temperature recording is often very important and it is essential that good recorde are kept on log sheets or wherever possible use made of temperature recording charts. Such records should be marked with batch number, dated and retained with log sheets.

In particular when cleaning units the cleanliness and satisfactory operation of valves (especially bottom valves of reactors) should be ascertained.

3. A BRIEF GUIDE TO GOOD MANUFACTURING PRACTICE (GMP) & GOOD LABORATORY PRACTICE (GLP).

This section is not included to imply that the above practices are not appreciated by the senior staff who will have to administer them. It is hoped, however, this will be a useful guide having particular reference to production of bulk pharmaceutical active ingredients and related laboratory activities.

GMP - the following headings are considered :-

a) Quality
b) Personnel & training
c) Documentation.

a) Quality: the quality of a product must meet a standard appropriate to its intended use and a comprehensive system must exist, so designed, documented and implemented to produce such quality. Appropriate personnel and equipment, and facilities must be furnished to acheive this position.

Attainment requires involvement and commitment of all concerned.

Some terms encountered relative to 'Quality' may need defining, such as :- Quality assurance, Quality control and in total Good Manufacturing Practice.

Quality assurance: is the total of all organized arrangements made to ensure appropriate quality is acheived. It is the product of good manufacture and other factors such as original product design, development and validation.

In the overall production of pharmaceutical products (and of bulk active ingredient) it encompasses the following in ensuring the quality assurance of the product :-

1) correct ingredients contained in correct proportions
2) is of purity required
3) has been correctly processed
4) is contained in the proper container
5) such containers will bear the correct labels or be appropritely marked
6) is stored and distributed so quality is maintained

Good Manufacturing Practice: is that part of quality assurance aimed at ensuring products are consistantly manufactured in a quality appropriate to the intended use. It is concerned with both manufacturing and quality control procedures.

Basic requirements are that :-

1. the manufacturing process is defined before commencement of any activity
2. necessary facilities are provided including
   (a) approproately trained personnel
   (b) adequate premises and space
   (c) suitable equipment
   (d) correct materials
   (e) approved procedures
   (f) suitable storage (& transport)
3. procedures are written in instructional form, in clear and unambiguous language and are applicable to the facilities provided
4. operators are trained to carry out the procedures correctly
5. records are kept during manufacture to demonstrate all required steps in the defined procedure were in fact taken and that quantity and quality produced were those expected
6. records of manufacture (and distribution) which will enable the complete history of any batch to be traced are retained in legible and accessible form
7. a system is available to re-call any batch of product should this be necessary (this is of particular importance & relevance to final formulated products)

Quality control: is that part of Good Manufacturing Practice concerned with sampling, specification and testing and with the organization, documentation and release procedures which ensure that the necessary and relevant tests are, in fact, carried out. Also that materials are not released for use, nor products released for sale or supply until their quality has been judged to be satisfactory. 'Quality control' is also used in the sense of the organizational entity which has the responsibility for these functions.

To acheive effective control of quality the following are necessary :-

a) adequate facilities and staff should be available for sampling, inspecting and testing starting materials, packaging materials, intermediate, bulk and finished products (as defined in producing pharmaceuticals) and, where appropriate, control of the environment.

b) samples of starting materials (including recovered materials), packaging materials, intermediate, bulk and finished products should be taken by personnel and by approved sampling methods under the direction of the Quality Controller.

c) results of the inspection and testing of materials, and of intermediates, bulk or finished products should be formally assessed against specification by the Quality Controller (or persons designated by such) before materials are released for use or products released for further processing, sale or supply. Product assessment should include a review and evaluation of relevant manufacturing (including packaging) documentation.

d) sufficient reference samples of products should be retained (when appropriate in final pack) to permit future examination if necessary.

b) PERSONNEL & TRAINING.

Principle: Sufficient personnel should exist at all levels with the ability, training, experience, and when appropriate the professional/technical qualifications relevant to the tasks allocated to them. Duties and responsibilities should be clearly defined and explained as well as being recorded in job descriptions or by other suitable means.
Training should cover not only specific tasks but also Good Manufacturing Practice in general including especially importance of personal hygeine.

General: Key personnel are the Production Manager and person responsible for Quality Control. Different persons should occupy these positions, neither being responsible to the other. Both have a responsibilfty for acheiving the requisite quality. Duties of the person responsible for Quality Control are wider than those necessarily suggested by titles "Chief Analyst" or "Laboratory Head". Part time workers and Consultants should only be appointed to the above positions in unavoidable or exceptional circumstances.

All organizations should clearly define allocation of responsibilities they lay down, though such may vary from establishment to establishment. However, the person responsible for Quality Control should have the authority to establish, verify and implement all quality control procedures. He should have the authority, independant of production although in consultation with Production, to approve materials and products. Also to

reject, as he sees fit, starting materials, packaging materials etc. as well as intermediate, bulk and finished goods which do not comply with the relevant specification or which were not manufactured in accordance with the approved methods.

The Production Manager, in addition to his responsibilities for production areas, equipment, operations and records of management of production personnel and manufacture of products in accordance with Master formula or methods, will have other responsibilities bearing on quality which he should share - or exercise jointly -with che person responsible for Quality Control. Such might include monitoring and control of manufacturing environment, plant hygeine, approval of suppliers of materials, protection of products and materials against spoiling and deterioration and retention of records.

Hygeine and protection of personnel should include provision of convenient washing facilities close to plant and use of them encouraged.

All persons entering production areas (i.e. including visitors, maintenance workers and senior management) should wear protective clothing including especially headwear appropriate to the area. Garments should be regularly laundered. Changing rooms should be provided.

Direct contact between operators hands and all starting materials, intermediate products and final products (other than when in packages) should be avoided.

For operators pre-employment medical checks and regular follow-up medical checks should be applied. Staff should be encouraged to report open lesions and supervisory staff should look for the presence of such.

Eating, drinking, chewing and smoking are not to be permitted in a manufacturing area.

c) DOCUMENTATION: This is a prime necessity in any system of Quality Assurance. It is used to define the system of control, reduce risk of error and permit investigation and tracing of

defective products. The system should be such that the history of any batch of product can be traced. This includes utilization and disposal of starting material, packaging material, intermediate, bulk and finished products.

Commonly used documents include :

Raw material specifications
Packaging materials specifications
Master formula and methods
Batch manufacturing records
Records of receipt, examination and issue of starting materials & packaging materials
Records of testing and release of intermediates, bulk and finished goods
Sampling procedures
Analytical methods and test results
Standard procedures for equipment operation, maintenance and cleaning

Documents should contain all necessary, but no superfluous data.

The marking of materials is of great importance. Apart from clear indication of the material by name (or code number if deemed necessary for security or other control purposes), materials should be clearly marked using a coloured label system to indicate un-tested materials, materials under test, materials rejected and materials passed for use. Various colour codes are acceptable although it is more or less universally applied to use green labels for passed materials, red labels for failed materials. Other colours such as yellow or white may also be employed. In some instances differentiation in state or degree may be made by either using full colour labels or simply white edged in colour.

Quarantine areas should be provided for intermediate states and products under test.

With regards raw materials it is most important to record the date of receipt and analysis and validity date by which time material must be used or subjected to re-analysis.

GLP : Good Laboratory Practice.

While these comments are most pertinent to the Quality Control laboratory they should also apply where relevant to the Production control and other laboratories.

Suitable facilities and properly trained and motivated staff are essential to provide reliable results from any analytical or other laboratory.

Buildings: should be designed, equipped and maintained at a level to suit operations to be performed in them. Special attention should be given to avoid cross contamination within laboratories as well as production areas; to safe disposal of water and effluent wastes (particularly after contact with highly pharmacologically active drugs e.g. estrogens) and fume cupboard discharges; provision of adequate storage space for equipment, reagents, retention samples and records. Microbiological laboratories and biological laboratories must be separate from chemical laboratories.

Staffing & training: Staff must demonstrate capability of performing the required work. Training programmes for all levels of staff should be in operation and cover non-qualified personnel who may perform in-process testing.

Equipment: Should be well serviced and, when appropriate, calibrated regularly and records kept of such. Clear operating instructions should be available for each item of specialised equipment. Suitable conditions should be provided to protect sensitive equipment against e.g. humidity, temperature, dust, vibration etc.

Reference standards: Reagents and solutions should only be prepared by competant persons following laid-down procedures. Such materials, unless for immediate use, should be marked with date of preparation together with the signature of the person who prepared them.

Records of test results: These should be kept in such a way that consequetive results may be compared easily.

Sampling procedures: Should be clearly described in written instructions and particularly ensure that representative samples are taken. Samples of final products should be taken only by the Quality Control staff in appropriate quantity and in sample containers clearly labelled indicating contents and batch or reference number with date. Samples should be analysed in accordance with with the methods detailed or referred to, in relevant specifications or pharmacopoeias. Validity of results must always be checked (including checking of calculations) before any material is either released or rejected.

Certificates of analysis should be provided together with appropriate labels for all packages of the batch.

4. PROJECT CONSOLIDATION AND DEVELOPMENT.

While a flexible, essentially multi-purpose, pilot steroid production plant is now available in Cuba, and in which products of pharmacopoeial quality can be produced, it is necessary to consider its future economical use.

For regular production of top quality bulk steroids. and at levels to satisfy even initial requirements it is desirable to augment facilities with the addition of certain plant items. One of these, a centrifuge for production of testosterone enanthate is essential. The most desirable additions recommended to improve throughput and provide best conditions for production regularly of highest quality products are detailed later in the list of recommendations.

It should be noted that the plant is already very flexible and versatile, but it is possible to envisuage the need for an even wider range of reactions being considered. One such could be low temperature reaction employing liquid ammonia which gives possible entry into a range of products, 19-norsteroids, particularly employed in various contraceptive formulations. Only very minor additions would be involved in providing facilities for such reactions.

The main factor, however, to ensure the most successful utilization of the plant facility is to provide it with starting materials particularly advantageous for economic production. Such should be domestically derived starting materials if possible.

The original concept for the Cuban steroid plant was to supply it with diosgenin derived from plants grown in Cuba for production of non-corticoid steroids and with hecogenin produced from the waste liquor of Henequen to produce an intermediate, diene acetate, which could later be used to produce the corticosteroids.

The production of 'coffee ground' from which hecogenin is isolated was implemented under UNIDO project DP/CUB/78/003. The use of the isolated hecogenin for the production of diene acetate may still be considered valid.

The production of diene acetate from hecogenin commissioning is near completion. Further research development work involved in the conversion of diene acetate to a later and more valuable intermediate,common with one produced during the project from prednisolone, has been completed showing promising results. This now awaits scale-up and optimalization work.

On the other hand the use of diosgenin as a starting material for non-corticosteroids as originally intended is no longer considered to be an economic strategy. Firstly progress has not been made into the growing of any suitable species of plant for the domestic production of diosgenin. Diosgenin, unless available at specially favourable cost price, is probably now only appropriate as a starting material for basic corticosteroids by utilization of some fermentation stages and not very competitive for classical steroids. For these latter and also the diuretic spironolactone, which is of significant importance to Cuba, recent developments have shown androstadienedione (AD) to be a preferred and more economical starting material while androstadienedione (ADD) is preferred for the synthesis of estrogens.

It would be of profound benefit for Cuba to be able to produce these starting materials themselves at acceptably beneficial cost and at the same time provide a great degree of self-dependancy.

Cuba is, in fact, in a potentially favourable situation of having an essentially waste by-product from the sugar industry which contains phytosterols which can be converted by fermentation into both androstenedione (AD) and androstadienedione (ADD). A secondary product, cholesterol, is also potentially available in Cuba being obtained from cattle spinal cord and for which technology and capacity are available. Cholesterol is also a suitable starting material for conversion by fermentation into AD & ADD.

It is considered, for the successful utilization of the steroid plant installed in Cuba and further development of steroid products that it is most urgent that a Techno-economic study be carried out to determine the potential of supplies of phytosterols and cholesterol and their conversion by fermentation into AD & ADD in Cuba.

This is the prime recommendation of this report.

5. CONCLUSIONS AND RECOMMENDATIONS.

A) CONCLUSIONS.

1. The project DP/CUB/81/013 has been satisfactorily concluded.
2. A good basic general purpose unit for the production of bulk steroid products for pharmaceutical use and intermediates on which to build and develop in the future is now available in Cuba.
3. The ability and capability to produce four bulk active steroid products to the highest pharmacopoeia standards has been demonstrated.
4. The variety of operations performed and processes covered has demonstrated the flexibility of the plant. However it should be noted that minor modifications and additions could be incorporated readily in the future to further extend the versatility. An example is the possibility of entering into the production of 19-norsteroids which could be an interesting extension to the current range of products.
5. The ultimate real success of economic production and expansion of the Advanced Steroids Production Unit in Cuba would be enhanced by considering the recommendations now put forward.

B) RECOMMENDATIONS.

1. That an urgent Techno-economic Study be performed in Cuba to assess the utilization of sugar cane wastes for the production of phytosterols and the subsequent conversion by fermentation to produce products most useful for steroid syntheses using modern technologies. At the same time the study should also consider the fermentative conversion of a second, and supportative, source for the same steroid starting materials - cholesterol
2. An absolute necessity to instal a small centrifuge (500mm diameter stainless steel) for the production of the product testosterone enanthate for which the highest domestic demand exists.

3. Installation of a larger (800 - 1000mm diameter stainless steel) centrifuge for general processing work.
4. Installation of a dedicated crystallisation unit to ensure regular production of top grade pharmaceutical products.
5. Installation of a supplementary dedicated vacuum service (water ring pump) for the steroid plant.
6. Improvement of heat exchange facility in conjunction with circulating pump, particularly for cooling, of glass reaction unit GR 100.
7. For regular production, the containment of solvent recovery operations within the production facility is needed. For this purpose a small pot still with fractionating column, manually operated, is required.

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UNIDO's comments on Dr. N. Walker's final report
DP/CUB/81/013

The expert presented a summary report after completion of his third visit to the field for the performance of running tests and demonstration of the technological processes in the recenly concluded pilot plant for the processing of steroid products.

During the commissioning period, satisfactorily production as per USP XXII standards for the following products were achieved:

- Testosterone propionate
- Testosterone enanthate
- Triamcinolone acetonide
- Ethenyl estradiol

In order to improve the economical benefits of the production, it is advisable to look for the substitution of diosgenin for locally available raw materials for the production of non-corticosteroid active principles. The utilization of waste by-products from sugar industry could be satisfactorily utilized.

The performance of a techno-economic study for the utilization of sugar cane wastes for the production of phytosterols and their subsequent microbiological and synthetic transformation. The study should also consider the utilization of cholest rol from cattle spinal cord with similar purposes.

In order to improve the production possibilities of the plant, it is necessary to complete in the first stage the installation with a small centrifuge (500 ml diameter) which will allow the proper production of testosterone enanthate.

Other investments will be necessary, such as installation of an industrial centrifuge, a crystallization unit, improvement of vacuum service, refrigeration facilities and definitive solution for the recovery of the utilized solvents.

The production processes for the following active substances were validated.

- The whole triamcinolone synthesis including 10 synthetic stages
- The whole ethenyl estradiol synthesis including 5 synthetic stages.
- Synthesis of Testosterone and its esters including 9 synthetic stages.
- Diene synthesis starting from Hecogenin including 6 synthetic stages.

Detailed recommendations were given in connection with safety procedures to be followed while working with estrogens, fluorination gases, solvents, etc. Also the expert emphasized the application of the G.M.P. and G.L.P. regulations and on the proper understanding of the technical and working personnel on the subject. Specific guidelines for quality control procedures for raw materials, processes and final products.

The work performed by the expert was satisfactorily evaluated by UNIDO substantive area.