PART I. BROMINATION STUDIES IN STEROIDAL SAPOGENINS

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PART II. CHEMICAL INVESTIGATIONS OF DIPHLORYNCHUS MOSSAMBICENSIS

by

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ABSTRACT

Desoxytigogenin was prepared by oxidation followed by Wolff-Kishner reduction of tigogenin. A number of methods were employed to open the side chain of desoxytigogenin to the corresponding dihydrodesoxytigogenin. Oxidation of dihydrodesoxytigogenin yielded the corresponding C_{26} aldehyde, which was isolated in pure form and characterised unambiguously. Bromination studies under varying conditions have been made on this aldehyde but the results have not be completed as yet.

LIST OF CONTENTS

ACKNOWLEDGEMENTS

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PART I.

BROMINATION STUDIES IN STEROIDAL SAPOGENINS

INTRODUCTION(1)

Among the naturally occurring derivatives of the steroids are a large number of glycosides found widely distributed in the plant kingdom and known as saponins.

The saponins are extremely effective as detergents, medicinals, foaming agents in fire extinguishers and as fish poison. Hydrolysis of the saponins with acids or enzymes produces sapogenins, which are C_{27} steroids, and various sugars. The sugars have been regarded as united in a straight chain with the terminal unit attached to the hydroxyl group of the sapogenin and this view has been confirmed. (2).

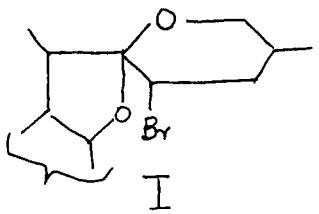
The general skeleton and the numbering system of steroidal sapogenins is indicated in Figure 1, whereas the nomenclature is illustrated in Table 1. Although the stereochemistry of the nucleus in various steroidal sapogenins has been well established, the stereochemistry of the E and F rings is relatively less well known. The asymmetric centers at C-20, C-22 and C-25 permit the existence of eight side chain diastereoisomers. Therefore, various workers have shown considerable interest in the stereochemistry of the side chain.

Although the stereochemistry of C-20 (3,4) and the configuration of C-25 (5,6) seem well established now, the question of the configuration of C-22 and the related matter of the conformation of the methyl group attached to C-25 are still not very well settled.

A. Bromination

Bromination of the steroidal sapogenin side chain has received much attention in the past twenty years. During the course of their investigations to obtain hormone intermediates from the sapogenins, Marker and Rohrmann (7) found that the two oxygen atoms in the sapogenin side chain are inert only in neutral or alkaline media but in acid media they are unusually reactive. They postulated the presence of a potential carbonyl group existing as a spiroketal. These workers found that sarsasapogenin acetate, sarsasapogenin and sarsasapogenone all react with one molecule of bromine in acetic acid to give crystalline mono-bromo derivatives with evolution of hydrogen bromide. Sarsasapogenin acetate did not react with more than one molecule of bromine. Bromo sarsasapogenin upon Clemmensen reduction yielded tetrahydrosarsasapogenin, indicating that the bromo compound still contained the spiroketal grouping.

In order to determine the position of the bromine atom, Marker and Turner (8) oxidised the acetate of bromosarsasapogenin and obtained a C-22 keto acid (3-hydroxy-16 keto-bis-norcholanic acid) from the acidic fraction, while from the neutral fraction unchanged bromosarsasapogenin was recovered. The isolation of the C-22 keto acid indicated that the bromine is at C-23 rather than at C-20 and the structure of the side chain in the bromo sarsasapogenin may be represented by I.



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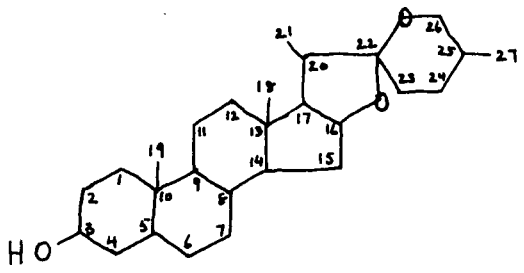
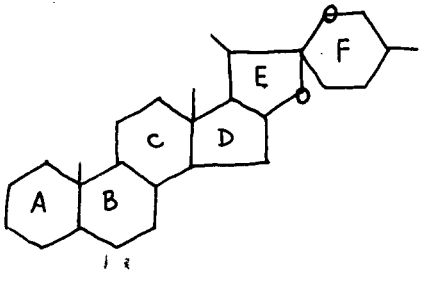


FIGURE 1. THE SKELETON AND THE NUMBERING SYSTEM OF STEROIDAL SAPOGENINS

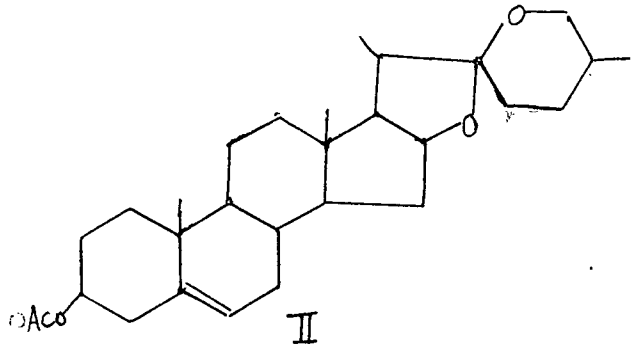
TABLE 1

THE NOMENCLATURE OF SOME STEROIDAL SAPOGENINS



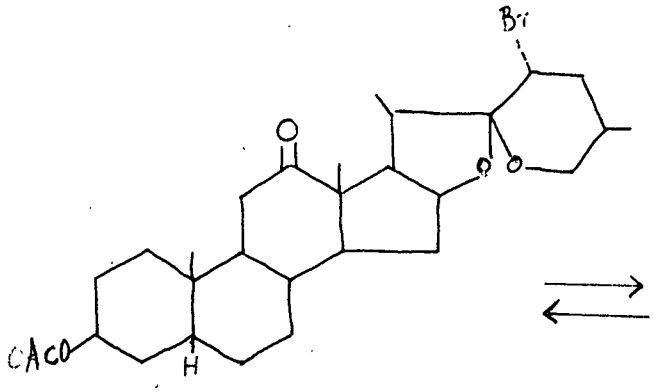
SUBSTITUENTS	C_{25}	C_5	NAME
3β-OH	neo	β	SARSASAPOGENIN
3β-OH	iso	β	SMILAGENIN
3β-OH	iso	α	TIGOGENIN
3β-OH	neo	α	NEOTIGOGENIN
3β-OH	neo	Δ^5	YAMOGENIN
3β-OH	iso	Δ^5	DIOSGENIN
3β-OH, 12 CO	neo	α	NEOHECOGENIN
3β-OH, 12 CO	iso	α	HECOGENIN

Marker and Turner found it possible to brominate diosgenin acetate (II) obtaining 5,6,23-tribromo diosgenin acetate.



II

Mueller and Norton (9) isolated two isomeric monobromo hecogenin acetates and derived the 11α-(V) and 11β-23-dibromo hecogenin acetates (VI) from one of them (III). The second pair of dibromides from (IV) yielded a 9,11-dehydro-hecogenin acetate (VIII) thus linking the isomerism to a bromine atom in the side chain. They substantiated this postulate by isolating two bromoketols, each yielding 3β, 12β, dihydroxy-5α, 22 a-spirostan-11-one (10) on debromination, pointing towards this type of isomerism. These workers, however, were unable to isolate a C_{22} keto acid. They thus designated the isomeric bromo-isosapogenin in which the carbon-bromine linkage has a different configuration from that in the original 23-bromo-isosapogenins as 23b-bromo-22a-spirostan (IV), the original being 23a-bromo-22a-spirostan (III). Dickson (11) supported this finding in that the infra-red spectra of the two groups differ considerably in the 1050-850 $cm.^{-1}$ region and they identified the absorption bands as characteristic of 23a (727 $cm.^{-1}$) and 23b (654 $cm.^{-1}$) bromo isomers.



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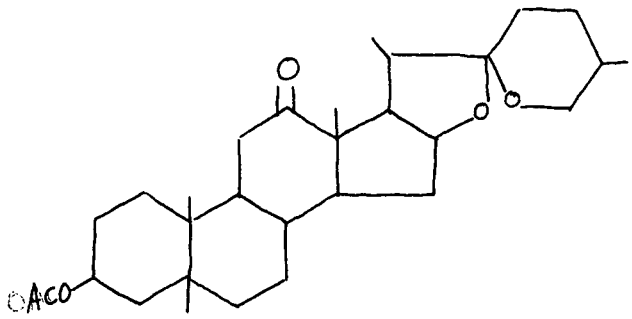
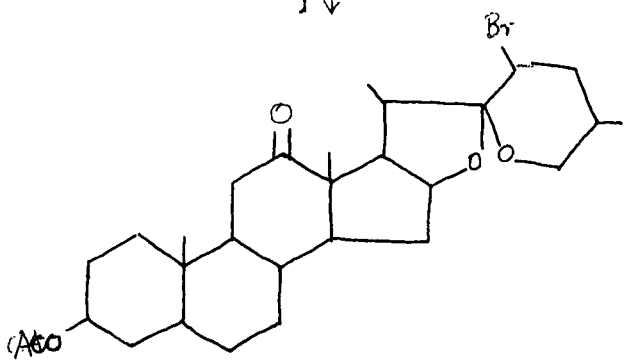


HECOGENIN ACETATE



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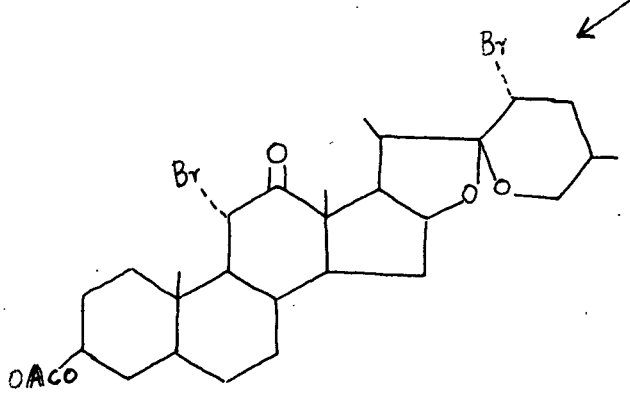
III



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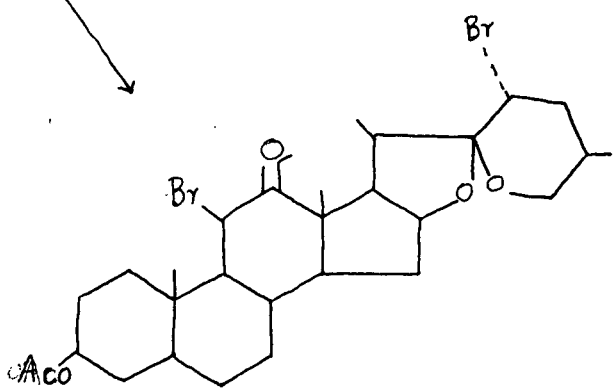

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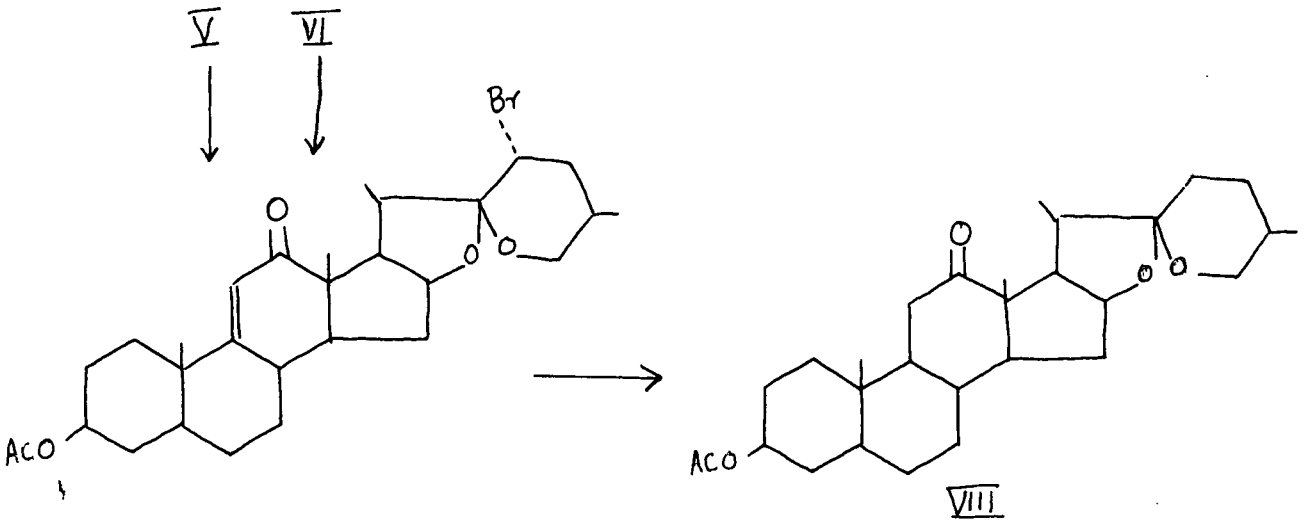

VIII

IV

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MIXTURE OF DIBROMIDES

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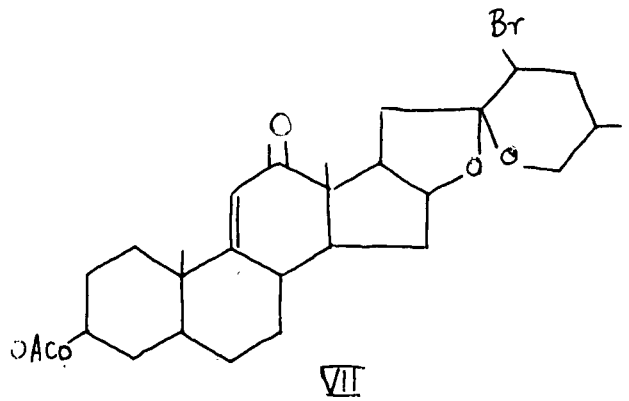


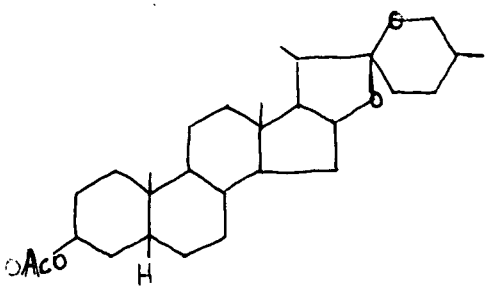
VII

Djerassi et al. (12) in their investigations of the synthesis of cortisone from steroidal sapogenins found that if spirostan-3β-yl-acetate (IX) was treated with two molecules of bromine in acetic acid, 23,23-dibromo-spirostan-3β-yl acetate could be isolated in essentially quantitative yield but 22-iso-allospirostan-3β-ol (Xa) and 22-isoallospirostan (Xa) on similar bromination yielded only a 23-monobromo derivative. They thus concluded from these observations that the steroidal sapogenin side chain can be dibrominated in the normal (neo) configuration but only monobrominated when it possesses the 22-iso configuration.

Wall and Serota (13) showed that sarsasapogenin and smilagenin (XIIa) are isomeric at C_{22} and designated the actual configuration of sarsasapogenin and smilagenin at C_{22}. They reasoned that on account of the hindrance of the methyl group attached to C_{20} it is impossible to construct a 23-dibromide of XIa and therefore it is smilagenin. By analogy they assigned configuration XIb and XIIb to 20-isosmilagenin and 20-iso-sarsasapogenin respectively.

Although as seen from the account given above there has been a considerable amount of work done on the bromination reaction, there is no real evidence to confirm the position of bromine substitution in the side chain.

The stabilising influence of a bromine substituent in the spiroketal side chain has been effectively illustrated during the investigations for the conversion of hecogenin to cortisone. Hecogenin was found to be a readily accessible raw material for cortisone



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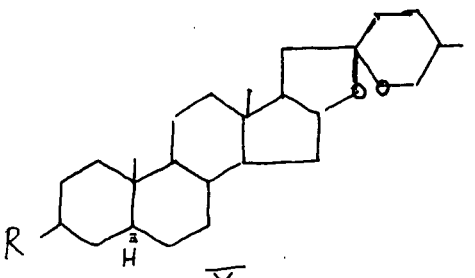
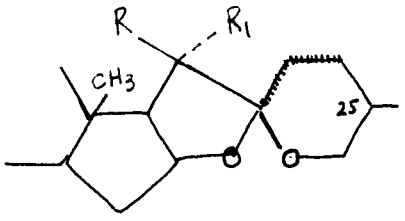


X

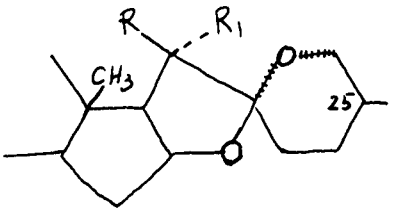
X a R = H

X b R = OH



XI a R = H, $R_1 = CH_3$

XI b R = CH_3, R_1 = H



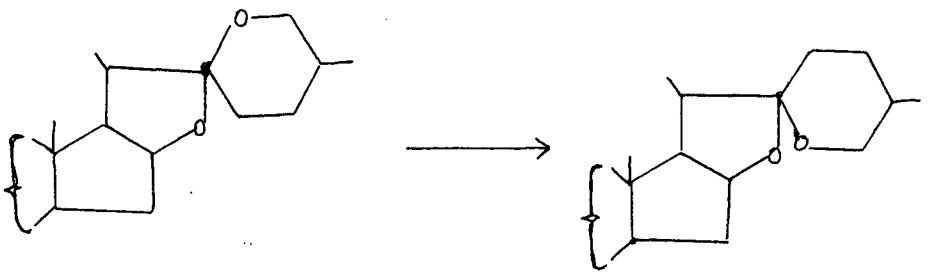
XII a R = H, $R_1 = CH_3$

XII b R = CH_3, R_1 = H

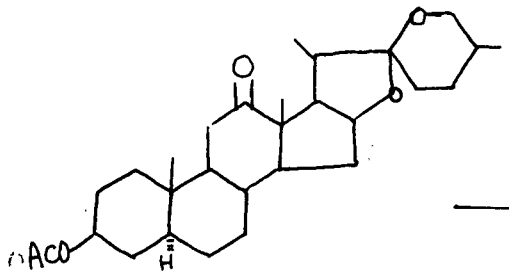
production but methods initially reported for conversion of hecogenin to cortisone left something to be desired in the yield and number of steps required. Djerassi, Ringold and Rosenkranz (14) applied the method used by Gallagher for transposition of a 12-keto group to the 11-position via a Marker-Lawson type ketol. Hecogenin acetate (XIII) was converted to the 11,23-dibromide (XIV), which was then treated with alkali to effect hydrolysis at C_{11} and isomerisation to the most stable ketol. The bromine substituent in the side chain was finally removed by reduction with zinc dust to yield (XV). Oxidation of the ketol (XV) with bismuth oxide in refluxing acetic acid gave the 11,12-diketone (XVI) as a mixture of the keto and enol forms. Reaction of this mixture with ethane dithiol in the presence of hydrogen chloride gas gave the 12-ethylenethioketal and this on desulfurisation afforded the 11-keto-sapogenin (XVII), a known cortisone intermediate.

B. The Iso Reaction

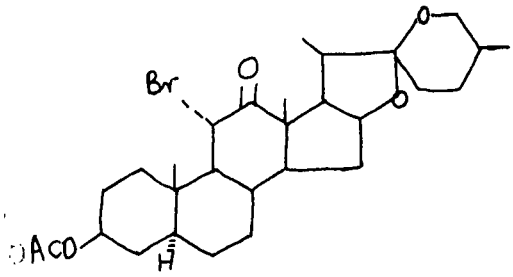
Marker and Rohrmann (7) discovered the conversion of the "normal" sapogenin, sarsasapogenin into the "iso" sapogenin, smilagenin by prolonged treatment with 2N ethanolic hydrochloric acid. They suggested that the reaction involves the opening and reclosure of the terminated oxide ring.



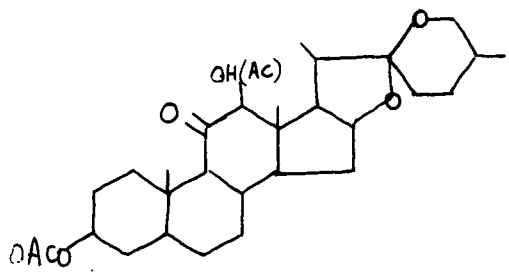
"Normal" sapogenin "Iso" sapogenin



XIII



XIV



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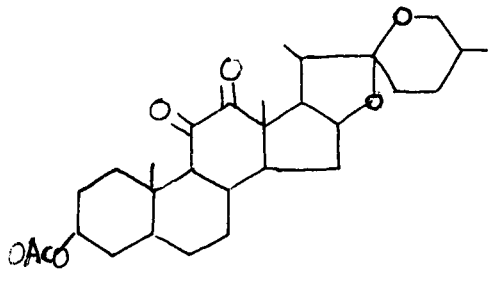
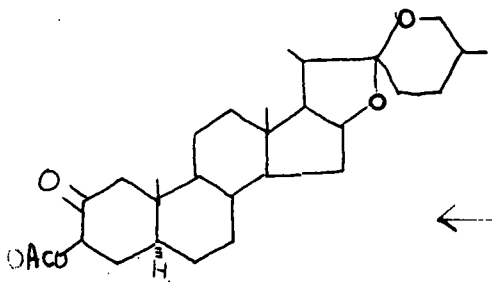


XVI



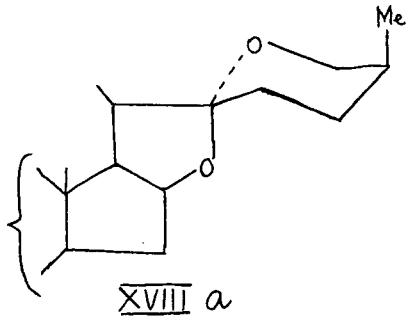
XVII

Wall and Serota (17) did some further investigations on the isomerisation of sarsasapogenin and showed that the reaction yields a mixture containing sarsasapogenin, smilagenin and the corresponding Δ^2- or Δ^3-3-desoxysapogenins. They also observed that the isomerisation is an equilibration in which the formation of the more stable "iso" sapogenin is favored. Similar hydrochloric acid treatment of the rare diosgenin isomer, yamogenin (5,22{-25L-spirostene-3β-ol) resulted in formation of $\Delta^{3,5}$ desoxytigogenin (3,5,22{-25D-spirostadiene).

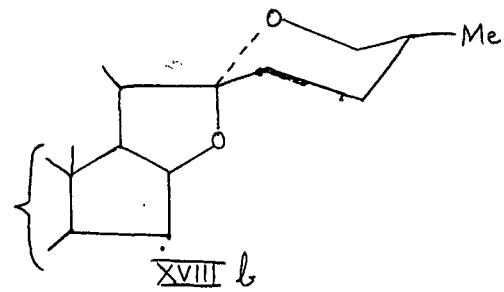
Callow and James (18) investigated alternative conditions of isomerisation and found that the reaction takes place in hot dioxane containing hydrochloric acid and also occurs in the presence of acetic anhydride under similar anhydrous reactions.

James (19) related the configuration at C_{25} to glyceraldehyde and it has been proved that neotigogenin (25L) and tigogenin (25D) have the same configuration at all asymmetric centers except C_{25}.

Callow and Massy-Beresford (20) showed by a series of reactions that the configuration at C_{22} is the same in both series and structures (XVIIIa) and(XVIIIb) have been proposed for the 25L-and 25D-sapogenins.



XVIII a

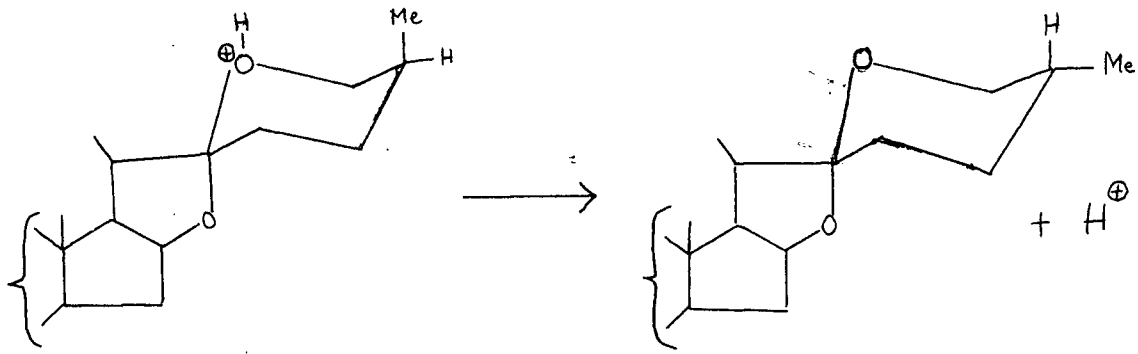


XVIII b

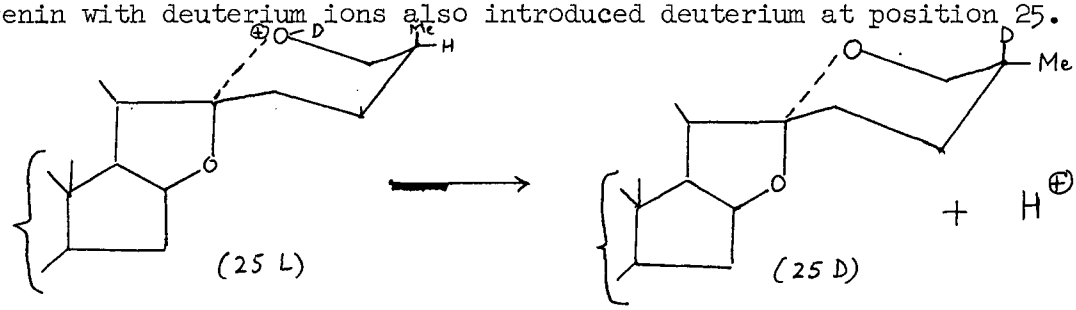
They concluded that the isomerisation XVIIIa ⟶ XVIIIb consisted of an inversion of configuration at C_{25} in which the methyl group attached to

C_{25} changes from the axial to the equatorial conformation.

Cornforth (21) suggested that this inversion takes place by the addition of a proton to the oxygen atom of ring F of the 25L-sapogenin, the proton thus being well placed to initiate a displacement reaction with inversion of configuration at C_{25}.



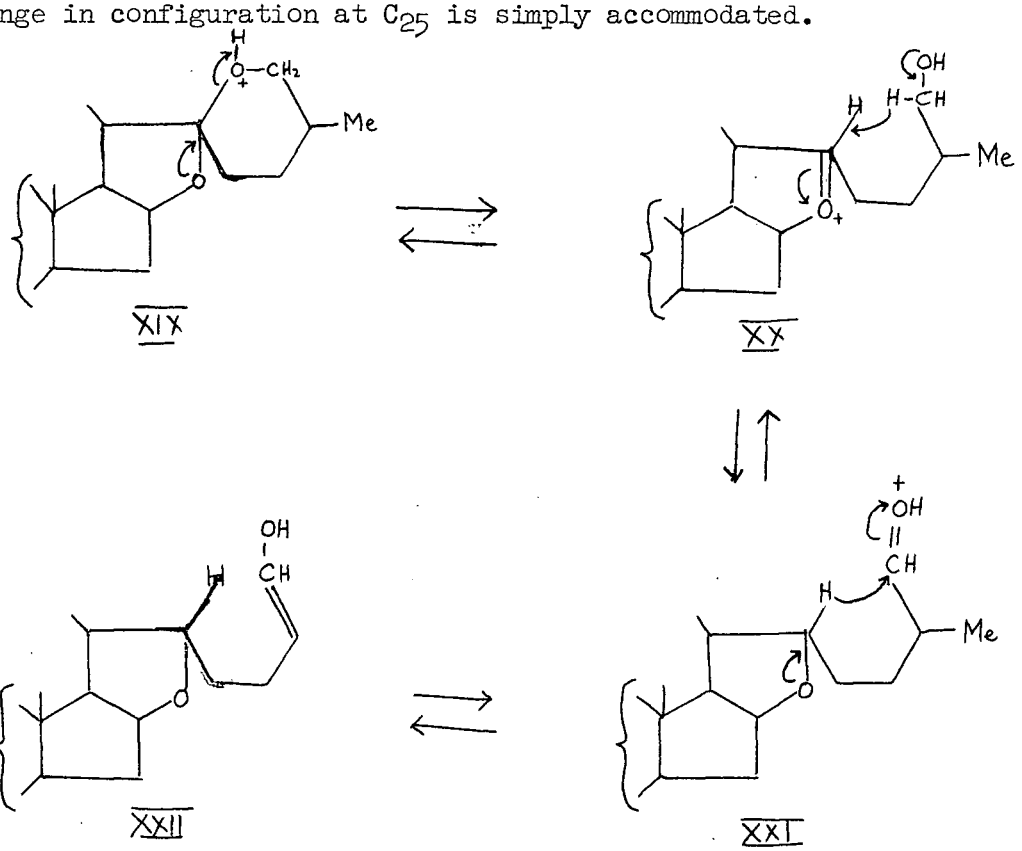
Callow and Massy-Beresford (22) supported Cornforth's hypothesis. They showed that the treatment of 25L-sapogenin with deuterium ions gave a 25D-sapogenin containing, at position 25, one deuterium atom stable towards brief treatment with acids. Similar treatment of a 25-D-sapogenin with deuterium ions also introduced deuterium at position 25.



Woodward, Sondheimer and Mazur (23) consider that the isomerisation proceeds by an oxidation-reduction mechanism. The key step in the change is a reversible hydride transfer involving the

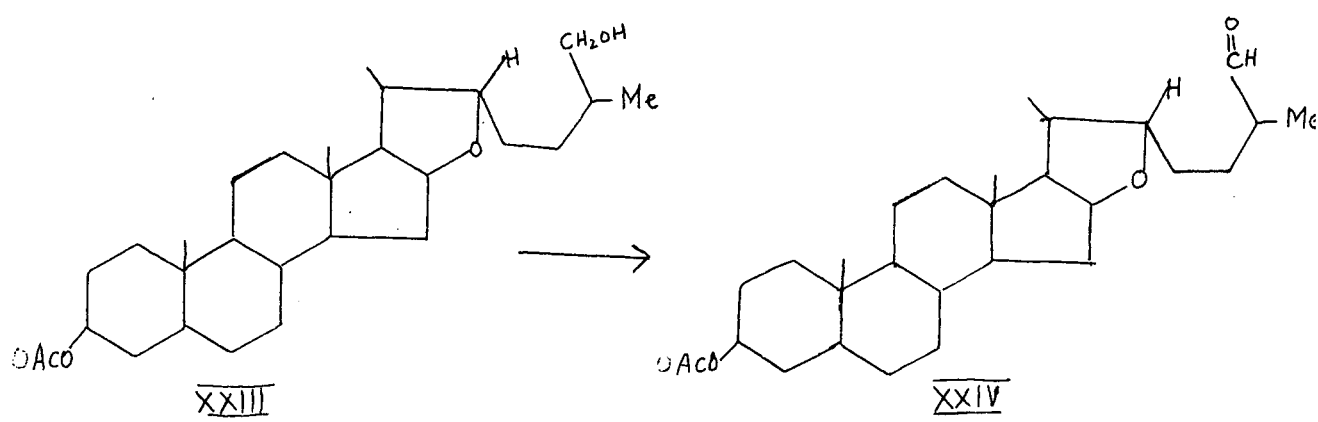
oxonium compound (XX) and (XXI).

Since the conjugate acid (XXI) may be expected to be in readily established equilibrium with the corresponding enol (XXII), change in configuration at C_{25} is simply accommodated.



XIX XX XXII XXI

They further found powerful support experimentally for this mechanism by carrying out the following conversions.



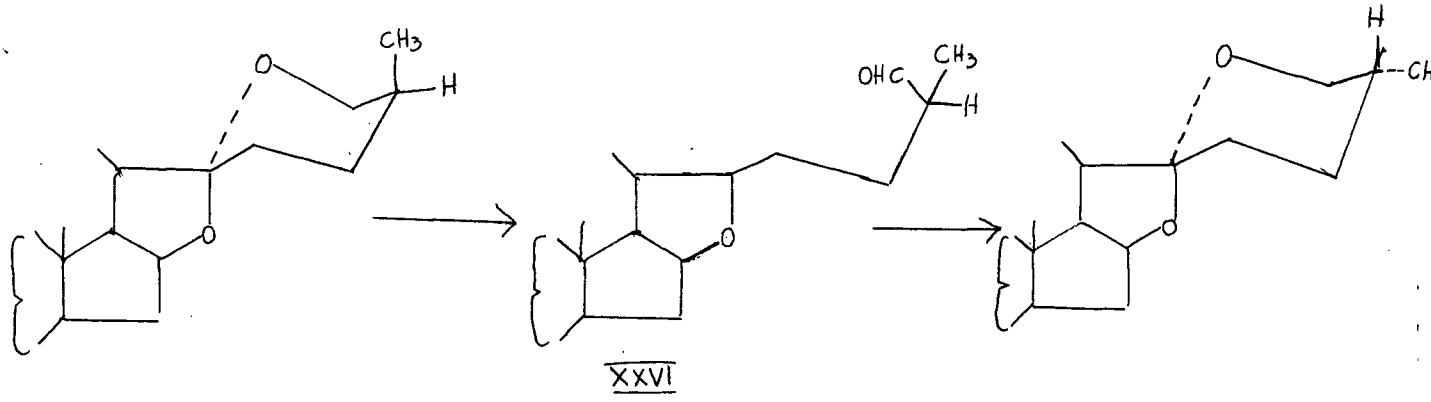
XXIII XXIV

Dihydrotigogenin-3-monoacetate (XXIII) was oxidized with sodium dichromate to give an oily mixture of aldehydes (XXIV) isomeric at C-25. When the mixture was subjected to conditions ordinarily used to bring about interconversion of XVIIIa ⟶ XVIIIb (concentrated hydrochloric acid in boiling ethanol), tigogenin mixed with some neotigogenin was produced in over 75 per cent yield.

An excellent confirmation of the above mechanism was provided by Djerassi et al. (26) who isolated the thioketal of the intermediate aldehyde.

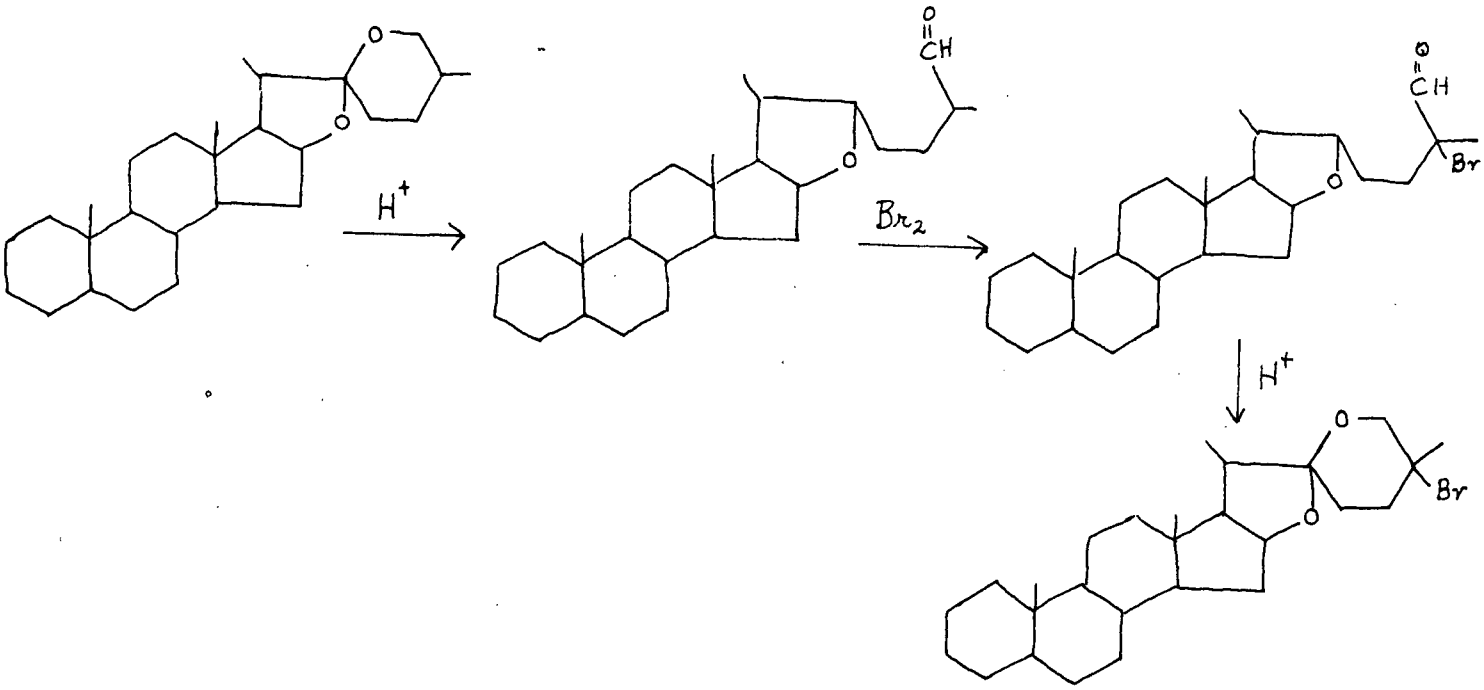
DISCUSSION

Woodward and Sondheimer's proposed mechanism for the acid catalysed isomerization of steroidal sapogenins at C_{25} included as an intermediate the aldehyde XXVI, which subsequently cyclised again to yield the isosapogenin isomeric only at C_{25}.

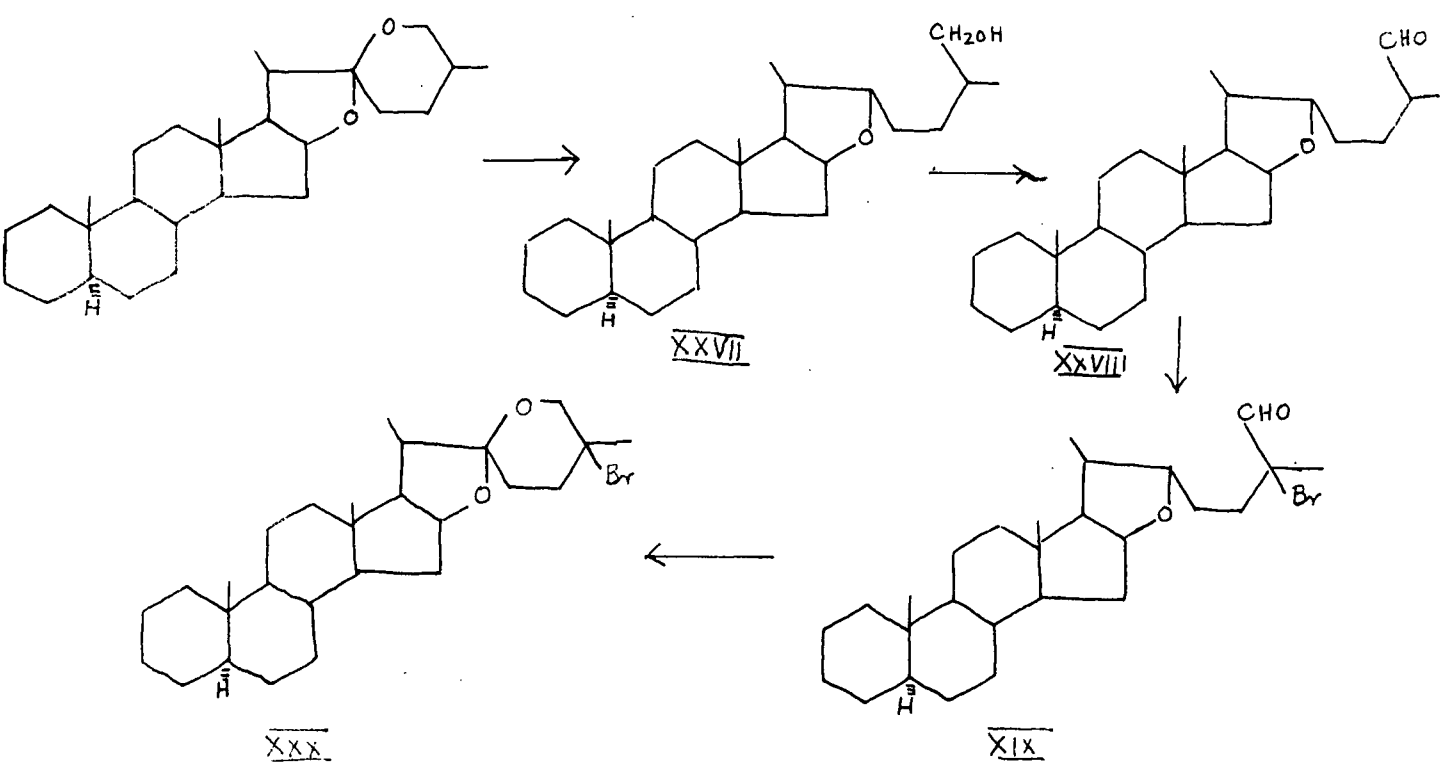


XXVI

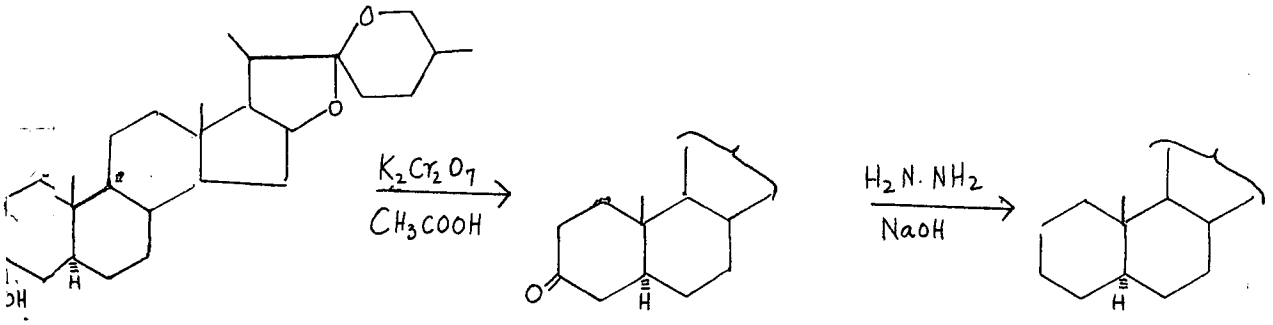
It thus became of interest to the present authors to find out whether the ring opening of the side chain took place during the acidic conditions normally employed in bromination reactions. If this was in fact the case, it was noted that the bromine atom would substitute at C_{25} and not at C_{23}, as previously supposed by other workers. The series of reactions which would take place are outlined below.



In order to test this hypothesis and provide conclusive evidence as to the actual structure of the bromosapogenins, we decided to synthesize unambiguously a C_{25} bromosapogenin and then compare the synthetic product with the product resulting from direct bromination of the steroidal sapogenin. Using desoxytigogenin as a starting material, the following sequence was chosen.



Desoxytigogenin (3-desoxy-5α-spirostan) was prepared from tigogenin by oxidation with potassium dichromate in acetic acid and then subjected to a Wolff-Kishner reduction with hydrazine hydrate and sodium hydroxide in diethylene glycol to yield 65 per cent crude desoxytigogenin. This material was chromatographed



on activated Merck alumina to yield 25 per cent of pure crystalline desoxytigogenin, identical in melting point, infra-red spectrum and optical rotation with an authentic sample of desoxytigogenin, kindly supplied by the Syntex laboratories. This starting material was subjected to a number of reactions designed to open the spiroketal side chain.

It has been reported (24) that lithium aluminium hydride in the presence of anhydrous hydrogen chloride reduces the side chain to a dihydrosapogenin. We made several attempts under varying conditions to employ this reagent in our sequence, but were not very successful. In each case the product was mainly the starting material.

A successful reduction was however possible under catalytic hydrogenation conditions. In the presence of Adams catalyst and acetic acid under 60 pounds of hydrogen pressure for a few hours (4), desoxytigogenin was converted to dihydrodesoxytigogenin (XXVII). This

material, after purification by chromatography on alumina, melted at 103-104°.

Another reaction subsequently employed for the preparation of dihydrodesoxytigogenin (XXVII) was an aluminium chloride catalysed reduction with lithium aluminium hydride (25). This method gave a good yield of dihydrodesoxytigogenin identical with the sample prepared by catalytic reduction and is advantageous because larger amounts can be conveniently handled.

The next step in the sequence was to oxidize the dihydrodesoxytigogenin (XXVII) to the corresponding aldehyde (XXVIII) by means of potassium dichromate in concentrated sulfuric acid and acetic acid. This reaction does not proceed to completion, and not unexpectedly, some unreacted alcohol as well as some acid are isolated along with the aldehyde. The aldehyde can be conveniently isolated after chromatography on deactivated alumina. Since all attempts to crystallise the aldehyde (obtained as colorless viscous oil) failed, characterization was obtained via its crystalline derivatives. Treatment of the aldehyde with ethane dithiol and boron trifluoride did not yield a clean reaction. The corresponding thioketal could however be isolated in very poor yield, after a number of careful chromatographic separations on deactivated alumina. This thioketal was found to be identical with the corresponding thioketal (XXV) formed when desoxytigogenin was treated with ethane dithiol and boron trifluoride (26). This served to establish the structure of our synthetic aldehyde. A crystalline ketal, melting point

102-103° was obtained when the aldehyde was treated with ethyleneglycol and p-toluenesulfonic acid as the catalyst.

Having characterized the aldehyde, bromination studies were carried out under a variety of conditions. The oily nature of the products and the small quantities available did not allow complete characterization at this stage.

CONCLUSION

A series of reactions have been employed for opening ring F of the steroidal sapogenin side chain. These reactions provide an unambiguous synthesis of an aldehyde (XXVII), which is an important intermediate in studying reactions of the steroidal sapogenin side chain. The synthetic aldehyde has been completely characterized and its structure established by direct comparison with known substances.

Bromination studies on this aldehyde have been carried out, although characterization has not been completed. Due to small quantities of the starting material available, it was not possible to continue this investigation at the present time. However, it is hoped that these investigations will be resumed and completed in the very near future.

EXPERIMENTAL

Melting points were determined on a Fisher-Johns apparatus and were uncorrected. Rotations were measured in chloroform solution. Infra-red spectra were run on a Perkin Elmer model 21 spectrophotometer.

Preparation of Desoxytigogenin (27)

Tigogenin (10 g.) recrystallised from ethanol was dissolved in chloroform (50 ml.) and the solution cooled to 15°C. A solution of chromium trioxide (5 g.) in 90% acetic acid (30 ml.) was also cooled to 15°C. and added dropwise to the tigogenin solution with vigorous stirring. The temperature rose to 25°C. and the oxidation mixture allowed to stand for one hour. After dilution with water (100 ml.) the crude tigogenone was extracted five times with 30 ml. portions of chloroform. The combined chloroform extract was washed with water, 10% aqueous NaOH (15 ml.) and again with water. The extract was dried over sodium sulphate (anhydrous) and evaporated to yield tigogenone (9 g.).

The crude tigogenone, without further purification, was taken up in a mixture of diethylene glycol (80 ml.) and ethanol (500 ml.) to which were added 40 ml. hydrazine hydrate and 8.0 g. of sodium hydroxide. The mixture was refluxed half an hour and volatiles under 150° removed. After addition of diethylene glycol (80 ml.) the mixture was heated two hours at 190°C. Water (500 ml.) was added to the reaction mixture, and this was then extracted with eight 50 ml. portions of benzene. The benzene extract was finally washed with water. After drying over

anhydrous sodium sulphate and evaporation of solvent in vacuo, a greenish yellow product (6.5 g.) was obtained.

The crude desoxytigogenin was then chromatographed on a column packed with activated Merck alumina (200 g.) in petroleum ether. The crude product was dissolved in a 1:1 mixture of petroleum ether (30°-60°) and benzene (120 ml.).

Elution with petroleum ether benzene (4:1) (5,000 ml.) yielded white crystals of desoxytigogenin (1.6 g.) m.p. 168-71°; infra-red ($CHCl_3$) 1450, 1375, 1050, 990, 930 and 900 Cm^{-1}. $[\alpha]_D$ - 63°.

Elution with ether (100%) (2500 ml.) yielded a yellow crystalline material (4.7 g.) m.p. 210°C. $[\alpha]_D$ - 45.4°.

A mixed melting point of the lower melting material with a sample from Syntex laboratories showed no depression and infra-red comparison of the two samples were found to be identical.

Lithium Aluminium Hydride-Hydrogen Chloride Reaction on Desoxytigogenin (24)

Desoxytigogenin (6 g., recrystallised after purification by chromatography) was dissolved in anhydrous ether (625 ml.) and the stirred solution was saturated at room temperature with anhydrous hydrogen chloride for half an hour. To the vigorously stirred solution lithium aluminium hydride (5.0 g.) was added piece by piece allowing sufficient time for each piece to react.

Anhydrous hydrogen chloride was passed through the solution during the hydride addition. The solution was then refluxed for two hours with anhydrous hydrogen chloride being passed throughout the refluxing period. After the refluxing was complete, the reaction mixture was cooled

and water was added dropwise to hydrolyse any excess lithium aluminium hydride. The suspension which formed immediately upon addition of water cleared up by standing overnight. The aqueous layer was separated and washed several times with ether. The combined ether extracts were washed with water until the water washings were neutral to litmus. The extract was then dried over anhydrous sodium sulphate and evaporated to yield a white crystalline material (0.92 g.). The infra-red spectrum showed characteristic bands of the sapogenin side chain.

The crystalline material was dissolved in benzene (15 ml.) and chromatographed on alumina (50 g., deactivated by adding 1.5 ml. 10% aqueous acetic acid). Elution with petroleum ether-benzene (9:1) (1000 ml.) yielded white crystals, which were found to be desoxytigogenin.

Catalytic Hydrogenation of Desoxytigogenin (4)

Desoxytigogenin (1 g.) was dissolved in glacial acetic acid (300 ml.) and warmed on a steam bath. After cooling, platinum oxide (1 g.) was added to the solution. The suspension was hydrogenated in a Paar Hydrogenator with shaking for 21 hours at 50 lbs. pressure.

After the hydrogenation was complete, the catalyst was filtered off and water (350 ml.) was added to the reaction mixture. The mixture was then extracted four times with 100 ml. portions of ether. The combined ether extracts were washed with water, 5% sodium hydroxide and finally again with water. The ethereal solution was dried over anhydrous magnesium sulphate and evaporated on a steam bath.

The oily residue from above was dissolved in methanol (40 ml.) and 10% methanolic potassium hydroxide (10 ml.) was added to it and refluxed for one and a half hours. The methanol was evaporated under vacuo on a steam bath and a pale yellow oily residue, which crystallised on standing, was obtained. This residue was taken up in water (25 ml.) and extracted four times with 25 ml. portions of ether. The combined ether extracts were washed with water, dried over anhydrous sodium sulphate and evaporated to yield crude dihydro desoxytigogenin (0.92 g.) m.p. 86-96°; infra-red in chloroform: ($CHCl_3$) 1450, 1360, 1150, 1040, 920 cm^{-1}.

The dihydrodesoxytigogenin (0.92 g.) was dissolved in a 1:1 petroleum ether-benzene mixture (10 ml.) and chromatographed on Shawinigan alumina (30 g., directly from the bottle).

Elution with petroleum ether (100%) (350 ml.) yielded a small amount of oil which crystallised on standing and was found to be desoxytigogenin.

Elution with ether-benzene (1:1) (600 ml.) yielded an oily substance (0.80 g.) which showed disappearance of the sapogenin side chain absorption bands in the infra-red spectrum. This oil solidifies on standing and melts at 100-102°, with some softening at 80°. To obtain the pure crystals of dihydrodesoxytigogenin, the solid was crystallized with ether m.p. 103-104°, $\alpha_D^{-43°}$

Lithium Aluminium Hydride-Aluminium Chloride Reduction of Desoxytigogenin (25)

Anhydrous aluminium chloride (38.4 g.) in 300 ml. anhydrous ether was added slowly, in portions, to an ice-cold mixture of lithium aluminium hydride (2.7 g.) in anhydrous ether (300 ml.)

A solution of desoxytigogenin (3.0 g., recrystallised after purification from chromatography) in dry ether (150 ml.) was added to the ice-cold (5-10°C.) stirred mixture dropwise over a period of 30 minutes. After addition was complete, stirring was continued for one hour at 5-10°C. and then refluxed for four hours. After cooling, the mixture was treated cautiously with water and dilute hydrochloric acid. The aqueous phase was extracted several times with ether. The combined ether extracts were dried over anhydrous sodium sulphate, evaporated to yield a clear viscous oil (3.0 g.) which solidified upon standing for about one hour without solvent.

This material was taken up in acetone and recrystallised to yield rectangular crystals m.p. 105-7°. A second crystallisation of the substance yielded a second crop m.p. 107.5-109°.

The material was then dissolved in 25 ml. of petroleum ether-benzene (1:1) and chromatographed on alumina (65 g., taken directly from bottle) in petroleum.

Elution with petroleum-ether (100%) (350 ml.) yielded small amounts of oil. Elution with benzene-ether (1:1) (600 ml.) yielded clear viscous oil (2.55 g.,) which crystallised on standing: m.p. 107.5-108.5°; infra-red in chloroform; 1450, 1360, 1150, 1040, 920 cm^{-1}; α_D - 4.3°.

Calcd. for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52; O, 7.95. Found: C, 80.52; H, 11.80; O, 7.90.

Oxidation of Dihydrodesoxytigogenin

Dihydrodesoxytigogenin (2.4 g.) (XXVI) was dissolved in benzene (100 ml.) and cooled at 5°C.

Potassium dichromate (12 g.) was dissolved in 60% acetic acid (300 ml.) and sulphuric acid (4 ml.) and this solution, after being cooled at 5°C., was added dropwise to the cooled mixture of dihydrodesoxytigogenin. After the addition was complete, the reaction mixture was stirred for about two hours at 5°C.

To decompose any excess oxidant, an aqueous solution of ferrous sulphate was added and the resulting dark mixture extracted several times with benzene. The combined benzene extract was washed successively with water, sodium carbonate and again with water. The extract was then dried over anhydrous sodium sulphate and evaporation of the solvent on steam bath under vacuo yielded crude aldehyde (1.80 g.). The infra-red spectrum in chloroform 3400, 1700 $cm.^{-1}$.

The crude aldehyde was dissolved in a 1:1 petroleum ether-benzene mixture (20 ml.) and chromatographed on Shawinigan alumina (60 g; deactivated by addition of 3 cc. of 10% aqueous acetic acid - activity II-III) in petroleum ether.

Elution with petroleum ether (100%)(1250 ml.) yielded small traces of an oil. Elution with petroleum ether-benzene (1:1) (2500 ml.) yielded 0.480 g. of a viscous oil which was the 5α - furostan - 26 -

aldehyde. Infra-red in chloroform; 2900, 2800 (shoulder), 1695, cm^{-1}.

Elution with ether-benzene (1:1) (1500 ml.) yielded another oily substance (1.15 g.) found to be a mixture of unreacted alcohol with some aldehyde. Infra-red in chloroform; 3400, 1700 $cm.^{-1}$

Preparation of 5α-Furostan-26-Ethylene Thioketal

The chromatographed aldehyde (1.3 g.) was treated with ethanedithiol (3 ml.) and a drop of perchloric acid and allowed to stand for five and a half hours. The reaction mixture was then treated with 10% sodium hydroxide after being diluted with ether (50 ml.) and finally washed with water. The combined ether extract was dried over anhydrous sodium sulphate and the solvent evaporated on a steam bath to yield a crude pale yellow oil (1.15 g.).

The crude material was dissolved in benzene and chromatographed on activated alumina (25 g.) in petroleum ether.

Elution with petroleum ether (100%) yielded a small amount of viscous oil. Elution with petroleum ether-benzene (8:2) mixture (500 ml.) yielded a yellow viscous oil (710 mg.) which still showed a carbonyl absorption in the infra-red spectrum.

The oil from the first chromatography was dissolved in petroleum ether (20 ml.) containing benzene (1 ml.). A very small amount of undissolved flocculent solid was filtered off and the clear solution was put on activated Merck alumina (15 g.) in petroleum ether for a more careful chromatography.

Elution with petroleum ether-benzene (9:1) (480 ml.) yielded a colorless viscous oil, which was practically odorless in earlier fractions of the chromatography. However, as the chromatography proceeded, a sulfur odor appeared in the later fractions of the viscous oil. The earlier fractions showed a strong carbonyl absorption in the infra-red while the later fractions had no carbonyl absorption. The fractions showing no carbonyl absorption were combined and taken up in acetone. After standing in the refrigerator for two days, white crystals were obtained, m.p. 90-96°.

Thioketal from Desoxytigogenin (26)

Desoxytigogenin (3.15 g.) was treated with ethanedithiol (6.4 g.) and borontrifluoride etherate (5.7 ml.). After standing for two hours, the reaction mixture was diluted with benzene, washed with 5% sodium hydroxide and water successively. The extract was cloudy in appearance and was allowed to stand overnight over anhydrous sodium sulphate after which time a clear solution was obtained. On evaporation of the solvent under vacuo, a crude oily viscous material (3.0 g.) was obtained.

This crude product was dissolved in 20 ml. petroleum ether-benzene (7:3) mixture and chromatographed on activated Merck alumina (90 g.) in petroleum ether.

Elution with petroleum ether-benzene (8:2) (300 ml.) yielded a white crystalline substance (1.0 g.) (m.p. 167-69°) which was unreacted

desoxytigogenin.

Elution with petroleum ether-benzene (6:4) (250 ml.) yielded white crystals (1.82 g.) (m.p. 86-95°). These crystals were combined and recrystallised to yield white crystals of 5α-furostan-26-ethylene thioketal, m.p. 96-98°.

Ethylene Ketal from 5α-furostan-26-aldehyde

The aldehyde, 5α-furostan-26-aldehyde (XXVII) (0.5 g.) was dissolved in freshly distilled benzene (500 ml.) and to this were added ethylene glycol (12 ml.) and p-toluene sulfonic acid monohydrate (100 mg.). The reaction mixture was refluxed for 72 hours using a water separator to remove the water as an azeotrope. The reaction mixture was then concentrated on a steam bath in vacuo and finally evaporated completely.

The crude material from the above reaction (0.45 g.) was taken up in petroleum ether (10 ml.) and chromatographed on Shawinigan alumina (50 g. directly from bottle) in petroleum ether. Elution with 1:1 petroleum ether-benzene (750 ml.) yielded a colorless oil (180 mg.), which solidified upon standing without solvent. The solid was taken up in methanol and recrystallised to give sharp melting crystals of 5α-furostan-26-ethylene ketal, m.p. 102-103°.

Calcd. for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88; O, 10.79

Found: C, 78.22; H, 10.76; O, 10.59

Bromination of 5α-furostan-26-aldehyde

A stock solution of bromine (2.64g; 10.9 ml.) in glacial acetic acid (100 ml.) was prepared.

The aldehyde (0.55 g.) was dissolved in glacial acetic acid (110 ml.) and cooled in an ice bath, until the acetic acid began to solidify. The ice bath was then removed and to this stirred solution, an aliquot (10 ml.) from the stock bromine solution was added dropwise.

After the addition was complete, water (300 ml.) was added to the reaction mixture and the mixture was extracted four times with 50 ml. portions of ether. The combined ether extracts were washed with water, dilute sodium bicarbonate solution and again with water. The extract was then dried over anhydrous sodium sulfate and evaporated in vacuo to yield a viscous oil (0.53 g.)

The viscous oil was dissolved in a petroleum ether (20 ml.) and benzene (5 ml.) mixture and put on a column packed with silica gel (15 g.) in petroleum ether.

Elution with petroleum ether (100%) (450 ml.) yielded small traces of an oily substance (71 mg.). Elution with 9:1 petroleum ether-benzene (700 ml.) mixture yielded an oily substance (101 mg.). Elution with 1:1 petroleum ether-benzene (1000 ml.) yielded an oily substance (155 mg.) and finally elution with chloroform (100%) yielded a yellowish viscous oil (176 mg.).

The oily substance (155 mg.) eluted with 1:1 petroleum ether-benzene mixture in the above chromatography was rechromatographed carefully on a column packed with silica gel (4.5 g.). Elution with 3:2 petroleum ether-benzene (175 ml.) gave a small amount of oily substance,

which on analysis, was found to contain 5.89% bromine. Elution with 3:1 benzene-petroleum ether (250 ml.) gave small traces of oily substance and finally elution with benzene (100%) (100 ml.) gave a viscous oily substance, which was found to contain 2.44% bromine on analysis.

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PART II.

CHEMICAL INVESTIGATIONS OF DIPHLORYNCHUS MOSSAMBICENSIS

ABSTRACT

The ground material from the bark of Diphlorynchus Mossambicensis was extracted with methanol, and methanol soluble concentrate was obtained. In addition a green gummy material, sparingly soluble in methanol, was obtained. The methanol concentrate was separated into acid, basic and neutral fractions and preliminary chemical investigations were made on these fractions.

Two crystalline substances of empirical formulas $C_{30-35}H_{44-54}O_2$ and $C_{37-38}H_{52-56}O_2$ have been isolated, separated and purified from the green gummy material. Spectral and analytical data have been collected and a few chemical reactions have been made on these two compounds.

INTRODUCTION

The Apocynaceae family has in recent years received much attention from virtually every area of medicinal and chemical interest. The reason for this is due to the fact that this family provides access to a large number of interesting substances many of which are important in the medicinal field. Perhaps the most striking example of this can be taken from the large amount of work recently carried out on the genus Rauwolfia, a rather large member of the Apocynaceae family. In particular, the species Rauwolfia serpentina has generated a tremendous amount of interest during the last ten years and this is due mainly to the isolation of reserpine, an alkaloid with pronounced hypotensive and sedative activity. This substance is presently finding important application in the treatment of mental diseases, lowering of blood pressure, etc.,

In connection with our investigation of natural products from the Apocynaceae family, we became interested in initiating chemical studies on an African plant of the genus Diplorhynchus in order to see whether some interesting substances could be isolated. The particular species which we obtained was Diplorhynchus mossambicensis (1,2) kindly supplied to us by Dr. J. L. C. Marais, Department of Chemistry, Witwatersrand University, Johannesburg.

DISCUSSION

To the best of our knowledge, there has been no chemical work on this genus and thus it became necessary to develop an isolation procedure.

The ground plant material was extracted with methanol and a concentrate was obtained. In view of our interest in isolating any alkaloids which may be present, this concentrate was separated into acid, basic, and neutral fractions and extracted with a number of solvents. However, there was essentially no basic material isolated and we focussed our attention on the acidic and neutral fractions, which appeared to be quite interesting on the basis of spectra data. After numerous unsuccessful attempts were made to isolate solid substances from these fractions, we attempted some chemical reactions on these oily materials. However, the oily nature of the reaction products prevented proper characterization and we discontinued this area of work for the present time.

In addition to a large portion of the concentrate which was highly soluble in methanol, there was obtained a green gummy material which deposited on the walls of the flask during the evaporation of methanol. This material was only sparingly soluble in methanol but more soluble in acetone and ether. This gummy substance was washed with alkali and chromatographed on deactivated alumina to yield a colourless oily substance. When this oil was taken up in petroleum ether and the solution allowed to stand in the refrigerator, a white solid was deposited. This solid material was separated, by means of fractional crystallisation, into two substances, a higher melting compound termed compound A and a lower

melting compound termed compound B.

The compound A, m.p. 225°, $[\alpha]_D$ + 8.2°, is less soluble in petroleum ether and ethanol. The analytical figures at the present time allow us only to propose an empirical formula, $C_{30-35}H_{44-54}O_2$, although a C_{35} formulation seems to be quite definitely favored on the basis of our later experiments. The infra-red spectrum of this material shows a carbonyl absorption at 1705 cm.$^{-1}$ and a double bond peak at 1650 cm.$^{-1}$. The intensity of the latter appears to indicate that the double bond is either conjugated with the carbonyl function or that a conjugated double bond system is present. The ultra-violet spectrum shows a strong absorption band at 275 mμ as well as an interesting triplet (222, 216, 204 mμ) at lower wave lengths. The occurrence of the latter three bands appears reminiscent of certain diene chromophores particularly where steric overcrowding is prevalent (3).

Reduction studies on compound A have provided some interesting results. Catalytic hydrogenation of A yields a new crystalline substance, m.p. 202-204° and the analytical results on this product are in agreement with an empirical formula, $C_{34-35}H_{56-58}O_2$. The infrared spectrum indicated a disappearance of the double bond absorption but the carbonyl band was still present. The ultraviolet spectrum was considerably changed and a broad absorption in the 260-280 mμ region was observed. Lithium aluminium hydride reduction of A also yielded a crystalline substance, m.p. 186-88°. The product had no absorption in the ultraviolet region of the spectrum and its infrared spectrum indicated a disappearance of the carbonyl absorption. A new band in the hydroxyl region of the spectrum was apparent. Consequently it may be suggested that the carbonyl functional group must be a part of the chromophore which absorbs in the ultraviolet.

The lower melting compound B, m.p. 138°, $[\alpha]_D$ + 10.1° is more readily soluble in petroleum ether and ethanol. The analytical results on this material agree with the empirical formula $C_{37-38}H_{52-56}O_2$. Its infrared and ultraviolet spectra are very similar to those of A and it may be proposed, at present, that a very close structural similarity exists between A and B. However, the reduction products resulting from B are quite different from those obtained in the higher melting series. Catalytic hydrogenation yielded a new crystalline compound, m.p. 101-102°, which possessed similar spectral properties to the analogous substances in the A series. The infrared spectrum indicated a disappearance of the double bond absorption and the ultraviolet spectrum possessed a broad absorption in the 260-280 mμ region. The reaction product from the lithium aluminium hydride reduction was an oily substance, which failed to crystallize. This product showed a new band in the hydroxyl region and a disappearance of the carbonyl absorption in the infrared spectrum.

An interesting reaction appears to have occurred during our attempts to prepare 2,4-dinitrophenylhydrazone derivatives of compounds A and B. The products from these reactions were yellow crystalline compound, m.p. 158-160° and 101-102° respectively. Although it was first assumed that these represented the expected derivatives, the analytical results excluded this possibility. The analytical values showed a very low percentage of nitrogen quite unlike the value expected for the normal 2,4-dinitrophenylhydrazone derivatives. Further confirmation of this fact was obtained when an ultraviolet spectrum was taken. It is well known (4) that these derivatives normally possess a very intense absorption in the 360-380 mμ. The spectra of our products showed only a very weak absorption

in this region but did still possess strong absorption at 275 mμ. The infrared spectra of these substances still possessed the typical carbonyl absorption and consequently it was obvious that the carbonyl function in our compounds cannot be ketonic in the nature. Although it can be concluded that the products are not phenylhydrazones, there appears little question that these substances are not the starting materials. It therefore appears that some change, perhaps an acid catalysed isomerisation, has taken place.

EXPERIMENTAL

Melting points were determined on a Fisher-Johns apparatus and were uncorrected. Rotations were run in chloroform solution. Infra-red spectra were taken on the Perkin Elmer Model 21 spectrophotometer. Ultra-violet spectra were done on the Cary Model 14 spectrophotometer.

Extraction

The ground plant material (950 g.) was treated with methanol (1.5 litres) and the material refluxed for 10 to 12 hours. The brown methanol layer was decanted, fresh solvent was added to the flask containing the plant material, refluxed again, decanted, etc. The entire process was repeated seven times. The combined methanol extracts were treated with a small amount of glacial acetic acid and evaporated on a steam bath under vacuo until a concentrate (about 400 ml.) was obtained.

On standing, some solid material began to form on the walls of the flask. This solid was filtered off and the filtrate was diluted with water (1 l.), acidified with 10% hydrochloric acid (100 ml.) and extracted several times with 500 ml. portions of petroleum ether (3.5 l.), benzene (3 l.), ether (3 l.) and chloroform (2.5 l.) in that order. These acid extracts were washed with water, then concentrated by evaporation to about 500 ml., dried over anhydrous sodium sulfate and evaporated completely to give a petroleum ether extract (PE 1), a benzene acid extract (BZ 1), an ether acid extract (Et 1) and a chloroform acid extract ($CHCl_3$ 1) respectively.

To the combined aqueous layers and washings from the above acid

extracts, sodium hydroxide pellets were added until the solution turned alkaline. This alkaline solution was then extracted several times with 500 ml. portions of benzene (2.5 l.), ether (2.5 l.) and chloroform (2 l.). These alkaline extracts were washed with water, concentrated to about 500 ml., dried over anhydrous sodium sulfate and finally evaporated down to obtain a benzene basic extract, an ether extract and a chloroform basic extract.

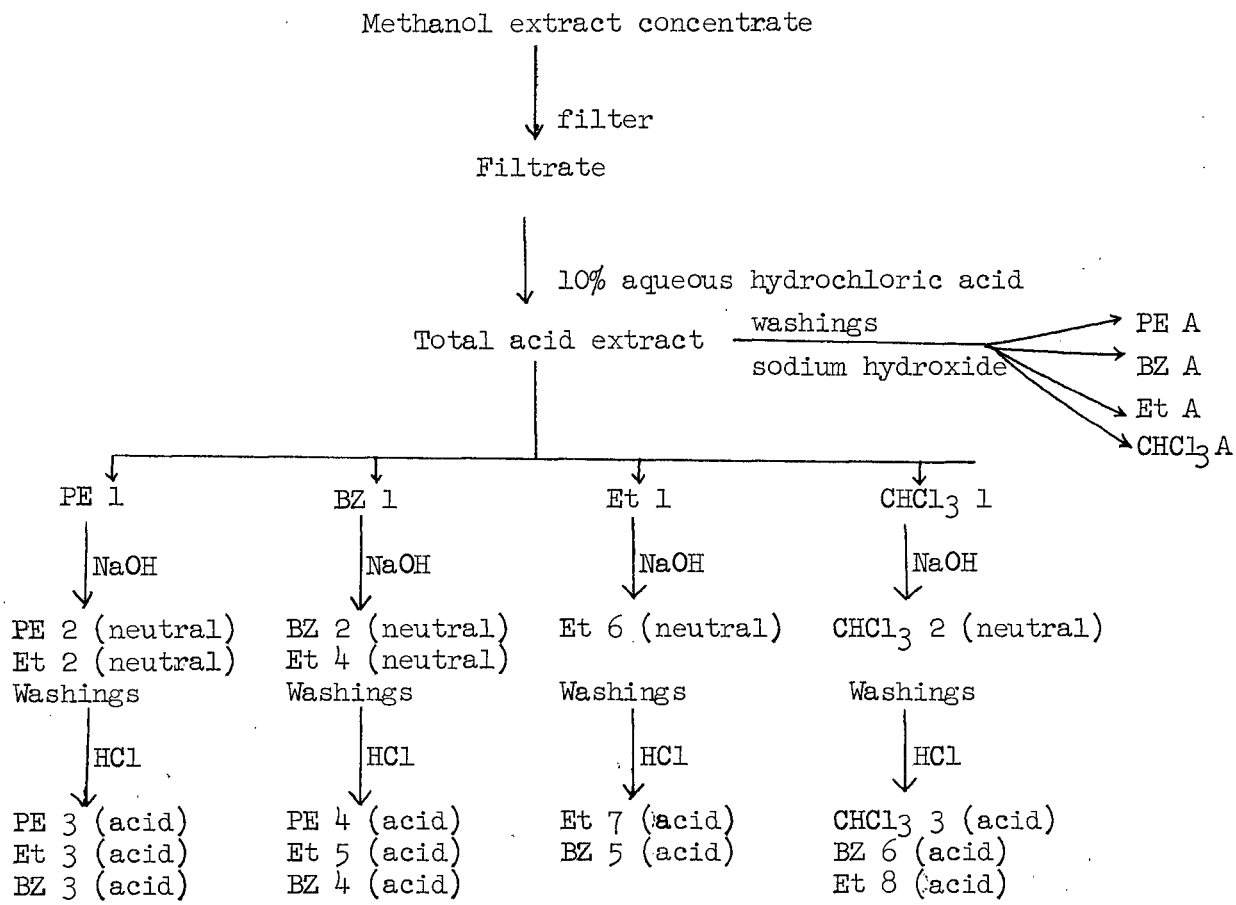
On addition of a small amount of ether to the petroleum ether, benzene, ether and chloroform acid extracts, some crude solid material was obtained.

The petroleum ether and benzene acid extracts (PE 1 and BZ 1) were diluted with more petroleum ether and benzene and washed with aqueous sodium hydroxide solution until alkaline. These extracts were washed with water and dried over anhydrous sodium sulfate. The alkaline aqueous washings from benzene acid extract and petroleum ether acid extract were also extracted with ether and the ether layer dried over sodium sulfate. On evaporation of the solvent some residue remained. These extracts were termed petroleum ether neutral (PE 2), benzene neutral (BZ 2), Et 2 and Et 4.

The alkaline washings of the petroleum ether acid extract from the above operation were acidified with hydrochloric acid and extracted with petroleum ether, ether and benzene, dried and evaporated to give acidified extracts - PE 3, Et 3 and BZ 3. Similarly, the alkaline washings from the benzene acid extract were also acidified with hydrochloric acid and extracted several times with benzene, petroleum ether and ether, washed with water, dried over anhydrous sodium sulfate and the solvent evaporated to give acidified extracts - Et 5 and BZ 4.

The chloroform acid fraction ($CHCl_3$ 1) solidified on standing with ether. The ether was then evaporated and to the solid residue, more chloroform was added till all the residue dissolved. The solution was then treated with aqueous sodium hydroxide until alkaline, extracted with chloroform, washed with water, dried over anhydrous sodium sulfate and evaporated to give the chloroform neutral fraction ($CHCl_3$ 2). The aqueous and alkaline washings from the above operation were acidified with hydrochloric acid and extracted with chloroform, ether and benzene, dried and evaporated to give acidified extracts - $CHCl_3$ 2, BZ 6 and Et 8.

Similarly, the ether acid extract which on addition of some more ether had deposited some solid material, was taken up in ether, basified with aqueous sodium hydroxide solution and then extracted several times with ether. This extract was washed with water, dried over anhydrous sodium sulfate and the solvent evaporated to give the ether neutral fraction (Et 6). The alkaline and aqueous washings from above were acidified with hydrochloric acid and extracted with ether and benzene. These solutions were dried over anhydrous sodium sulfate and evaporated to give acidified extracts - Et 7 and BZ 5.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filter
Filtrate
10% aqueous hydrochloric acid
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PE A
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CHCl3 A
PE 1
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Et 1
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Washings
BZ 2 (neutral)
Et 4 (neutral)
Washings
Et 6 (neutral)
Washings
CHCl3 2 (neutral)
Washings
HCl
HCl
HCl
HCl
PE 3 (acid)
Et 3 (acid)
BZ 3 (acid)
PE 4 (acid)
Et 5 (acid)
BZ 4 (acid)
Et 7 (acid)
BZ 5 (acid)
CHCl3 3 (acid)
BZ 6 (acid)
Et 8 (acid)

The weights of the various fractions from the extraction were as follows:

$CHCl_3$ 2 (neutral)	0.867 g.
PE 2 (neutral)	1.420 g.
BZ 2 (neutral)	0.648 g.
Et 6 (neutral)	0.292 g.
Et 7 (acid)	0.970 g.
BZ 4 (acid)	0.568 g.
$CHCl_3$ 3 (acid)	0.625 g.

Spectral data on the various fractions from the extraction:

$CHCl_3$ 2 - infra-red in chloroform: 3300, 1650 $cm.^{-1}$

PE 2 - infra-red in chloroform: 3500, 1730 (broad), 1650 $cm.^{-1}$; λmax. 272 mμ

BZ 2 - infra-red in chloroform: 3400, 1725 (broad) $cm.^{-1}$; λmax. 280 mμ (shoulder)

Et 6 - infra-red in chloroform: 3400, 1700 $cm.^{-1}$

Et 7 - infra-red in chloroform: 1700 $cm.^{-1}$; λmax. 285 mμ

BZ 4 - infra-red in chloroform: 1700 $cm.^{-1}$; λmax. 275 mμ

$CHCl_3$ 3 - infra-red in chloroform: 1700 $cm.^{-1}$; λmax. 275 mμ

Chromatography of $CHCl_3$ 2 extract

The $CHCl_3$ 2 extract (0.767 g.) was dissolved in benzene (10 ml.) with slight warming and chromatographed on Shawinigan alumina (60 g.; deactivated by 1.8 ml. of 10% aqueous acetic acid.).

Elution with benzene-ether (4:1) (500 ml.) yielded a yellowish oily substance (325 mg.); infra-red in chloroform 3400, 1650 $cm.^{-1}$; λ max. 332 and 227 mμ . This is termed C-2A.

Elution with chloroform-methanol (95:5) (500 ml.) yielded an oily substance (125 mg.); infra-red in chloroform 1650 $cm.^{-1}$; λ max. 310, 295 and 227 mμ . This fraction is termed C-2B.

Hydrogenation of fraction C-2A

The chromatographed material (56 mg.) from benzene-ether (4:1) elution was dissolved in glacial acetic acid (10 ml.) and platinum oxide catalyst (50 mg.) added to it. The solution was hydrogenated at atmospheric pressure. A total of 16 cc. of hydrogen was taken up during a three hour period.

After the hydrogenation was complete (no further uptake of hydrogen), the catalyst was filtered off and the acetic acid was evaporated in vacuo on a steam bath. To the residue in the flask, water (25 ml.) was added and several extractions with fresh portions of ether were carried out. The ethereal extract was washed, dried over anhydrous sodium sulfate and evaporated to yield an oily substance (40 mg.); infra-red in chloroform showed disappearance of bands at 1650 $cm.^{-1}$; λ max. 320, 290 and 275 mμ .

Chromatography of fraction PE 2

The petroleum ether neutral fraction (PE 2) (1.2 g.) was dissolved in petroleum ether (25 ml.) and benzene (10 ml.) and chromatographed on Shawinigan alumina (45 g.; deactivated with 1.3 cc. of 10% aqueous acetic acid.)

Elution with petroleum ether (500 ml.) yielded an oily material (260 mg.); infra-red in chloroform shows no carbonyl absorption; λ max. 252 mμ. This fraction is termed P-2A.

Elution with petroleum ether-benzene (1:1) (350 ml.) yielded an oily substance (200 mg.); infra-red in chloroform 1700, 1625 cm.$^{-1}$; λ max. 272 mμ. This fraction is termed P-2B.

Elution with benzene (500 ml.) yielded a viscous oil (180 mg.); infra-red in chloroform 1700, 1625 cm.$^{-1}$; λ max. 272 mμ. This fraction is termed P-2C.

Finally elution with benzene-ether (1:1) (500 ml.) yielded a viscous oil (375 mg.); infra-red in chloroform 1700, 1625 cm.$^{-1}$; λ max. 272 mμ. This fraction is termed P-2D.

Hydrogenation of fraction P-2D

The chromatographed P-2D fraction (52 mg.) was dissolved in glacial acetic acid and added to pre-reduced platinum oxide (50 mg.) suspended in glacial acetic acid. The reaction mixture was hydrogenated at room temperature and an uptake of hydrogen (8 cc.) was recorded.

After hydrogenation was complete, the catalyst was filtered off and acetic acid evaporated under vacuo. The residue was taken up in

ether and washed with 10% sodium bicarbonate solution and then with water. The ether extract was dried over anhydrous sodium sulfate and evaporated to yield an oily substance (39 mg.); infra-red in chloroform 1705, 1650 cm.$^{-1}$; λmax. 243 mμ .

Lithium aluminium hydride reduction of fraction P-2D

Lithium aluminium hydride (150 mg.) was introduced into a 250 ml. flask which was equipped with a condenser, a dropping funnel, and a mechanical stirrer, and contained anhydrous ether (75 ml.). The solution was stirred for 15 minutes and refluxed for another 15 minutes.

A solution of fraction P-2D (100 mg.) was added at a rate of 2 to 3 drops per second and then the reaction mixture was refluxed with vigorous stirring for an hour, and allowed to stand at room temperature for four hours.

Moist ether was added to decompose the excess lithium aluminium hydride, followed by addition of water. The ethereal layer was separated and the aqueous layer extracted several times with ether. The combined ether extracts were washed with water, dried over anhydrous magnesium sulfate and evaporated to yield an oily material (127 mg.); infra-red in chloroform 3500, 1625 cm.$^{-1}$; λmax. 232 mμ .

Preparation of 2,4-dinitrophenylhydrazone of P-2D

A stock solution of 2,4-dinitrophenylhydrazine in alcohol was prepared.

The chromatographed P-2D fraction (50 mg.) was evacuated on the high vacuum pump to remove any traces of solvents. Thissample was then dissolved in ethanol (2 ml.) and 2,4-dinitrophenylhydrazine reagent (1.5 ml.) from the above stock solution added to it, until the mixture turned turbid. The reaction mixture was allowed to stand for about an hour during which time orange crystals were formed. These crystals were filtered off and dried under vacuum. On several recrystallisations from ethanol, the pure orange crystals were obtained, m.p. 115-116°C.; λ max. 355 and 258 mμ .

Found: C, 45.86; H, 4.40; N, 23.40; O, 26.50; mol. wt. (Rast) 393.

Hydrogenation of fraction E-7

The ether acid fraction (E-7) (53 mg.) was dissolved in glacial acetic acid (12 ml.) and added to pre-reduced platinum oxide (50 mg.) which was also taken in glacial acetic acid. The reaction mixture was then hydrogenated at atmospheric pressure.

After a few hours, the catalyst was filtered off and acetic acid evaporated under vacuo. The residue was washed with water, dried over anhydrous sodium sulfate and evaporated to yield an oily substance 40 mg. ; infra-red in chloroform 3500, 1705, 1650 cm.$^{-1}$; λ max. 297 mμ .

Chromatography of fraction E-7

The ether acid fraction (E-7) (800 mg.) was dissolved in a mixture of ether-petroleum ether (4:1) and chromatographed on a column packed with silica gel (30 g.)

Elution with petroleum ether-ether (1:4) (50 ml.) gave a yellow oil (400 mg.); infra-red 1705, 1600 cm.$^{-1}$

Further elution with petroleum ether-ether (1:4) (450 ml.) gave a yellowish oil (180 mg.); infra-red 1705, 1600 cm.$^{-1}$.

Lithium aluminium hydride reduction of fraction E-7

An attempt to reduce the ether acid fraction (E-7) (200 mg; chromatographed fraction from above) with lithium aluminium hydride in refluxing ether failed and hence more drastic conditions were used.

The unreacted E-7 fraction recovered from above reduction was dissolved in freshly redistilled tetrahydrofuran (20 ml.). Lithium aluminium hydride (200 mg.) was dropped into a three-necked flask containing dry tetrahydrofuran (100 ml.) and solution was refluxed with stirring for one hour. The solution containing E-7 was then added dropwise and allowed to reflux for ten hours with stirring.

At the completion of the refluxing, most of tetrahydrofuran was distilled off and the excess lithium aluminium hydride decomposed by moist ether and water. The reaction mixture was then extracted several times with ether. To dissolve the solid aluminium hydroxide, an aqueous solution of sulfuric acid was added to the mixture and further extracted with ether. The combined ether extract was washed with water, dried over anhydrous sodium sulfate and evaporated to give pale yellowish oily material (95 mg.); infra-red in chloroform 3500, 1600 cm.$^{-1}$.

Isolation, purification and separation of two crystalline substances

The ground plant material was treated with methanol and the methanol extract was concentrated as outlined on page . except that no

acetic acid was added to the extract before concentration.

During the evaporation of the methanol extract under vacuo, a thick green gummy material was deposited on the walls of the flask. This material was only very slightly soluble in methanol and after removing the methanol soluble portion, it was dissolved in acetone and was removed from the flask. The acetone was evaporated and a thick gummy residue (18 g.) containing some pale yellow powder was obtained.

This material was taken up in ether and washed with 10% sodium hydroxide and water. The pale yellow powder seems to be soluble in the alkaline solution. The ether extract was dried over anhydrous sodium sulfate and evaporated to give a viscous greenish oil (15 g.).

The greenish oil (15 g.) dissolved in a 3:2 mixture of petroleum ether-benzene (250 ml.) and was chromatographed on Shawinigan alumina (450 g.; deactivated by 14.5 ml. of 10% aqueous acetic acid).

Elution with petroleum ether-benzene (4:1) yielded colorless oil (6.5g.); infrared in chloroform 1700, 1650 $cm.^{-1}$; λmax. 275 mμ .

Elution with ether (2.5 l.) gave a green oil (5.8 g.) which was set aside without further investigation.

The colorless oil from the first fraction of the chromatography was taken up in petroleum ether and kept in a refrigerator overnight. The next day, white crystals (m.p. 140-180°) were deposited, but on filtration were found to be rather sticky. However, concentration of the mother liquors from above yielded white crystals (525 mg.) (m.p. 125-30°), which on further recrystallisation from petroleum ether and then ethanol gave good sharp melting crystals, m.p. 138°. These sticky crystals from the first crop were further crystallised from petroleum ether to yield a crystalline compound (800 mg.) (m.p. 220-225°), which on subsequent recrystallisation

from petroleum ether and ethanol gave a good crystalline substance, m.p. 226-27°. This compound is termed compound A. Again concentration of the mother liquor from the above operation gave no more of the high melting substance but low melting substance (m.p. 138°), termed as compound B, which was further purified by recrystallisation from ethanol.

In all 700 mg. of compound A and 1.3 g. of compound B were obtained.

Analytical and spectral data of the two compounds were as follows:

Compound A; infrared in potassium bromide 1705, 1635 cm.$^{-1}$; λmax. 279 mμ λshoulder 222, 216, 204 m ; $[\alpha]_D^{CHCl_3}$ + 8.2°

Found: C, 82.89; H, 10.12; O, 7.17. Empirical formula $C_{15.3}H_{22.4}O$ (Bernhardt)
Found (UBC): C, 82.19; H, 10.63. Empirical formula $C_{17.2}H_{26.6}O$

Compound B; infrared spectrum in potassium bromide 1705, 1635 cm.$^{-1}$; λmax. 279 m ; λshoulder 222, 216, 204 m ; $[\alpha]_D^{CHCl_3}$ + 10.1°

Found: C, 83.72; H, 10.12; O, 5.95. Empirical formula $C_{18.6}H_{27.3}O$
molecular weight (Rast), 512.

Lithium aluminium hydride reduction of compound A

Lithium aluminium hydride (200 mg.) was placed in a flask containing anhydrous ether (100 ml.) and refluxed with stirring for half an hour. A solution of compound A (100 mg.) was dissolved in ether and added to the refluxing solution at a rate of 2-3 drops per second. After completion of the addition the reaction mixture was refluxed with stirring for two hours.

The excess lithium aluminium hydride was decomposed with water. Some dilute sulfuric acid was added to break up the solid aluminium hydroxide, and the whole mixture was extracted several times with ether.

The ether extract was washed with water, dried over anhydrous magnesium sulfate and evaporated to yield a semi-solid product (55 mg.). This material was crystallised twice from ethanol, m.p. 186-88°; infrared in potassium bromide 3500, 1635 $cm.^{-1}$; no ultraviolet absorption.

Hydrogenation of compound A

A solution of compound A (50 mg.) in glacial acetic acid was hydrogenated over platinum oxide (50 mg.) at room termperature and atmospheric pressure for three hours. During this time there was an absorption of 14 cc. of hydrogen.

After no more hydrogen was taken up, the catalyst was filtered off and the acetic acid was removed by evaporation in vacuo. The residue was washed with water, extracted several times with ether and the combined ethereal layer washed with water, dried over anhydrous sodium sulfate and evaporated. White crystals were obtained in the flask, and these on further purification and recrystallisation from ethanol yielded a white crystalline substance (32 mg.), m.p. 202-204°; infrared in potassium bromide 1725 $cm.^{-1}$; λmax. 260-280 mμ (broad).

Found: C, 82.06; H, 11.42; O, 6.31. Empirical formula $C_{17.4}H_{29.2}O$

Isolation of a new compound during attempt to prepare 2,4-dinitrophenylhydrazone of compound A

The compound A (50 mg.) was dissolved in ethanol (a small amount remained insoluble) after heating on a steam bath. To this solution 2,4-dinitrophenylhydrazine reagent (2 ml.) was added and allowed to stand. After ten minutes, pale yellowish crystals were formed. These crystals were filtered off and recrystallised twice from ethanol to give yellow crystals, m.p. 152-54°; infrared in potassium bromide 1705, 1635 $cm.^{-1}$; λmax. 275 mμ ; λshoulder 222, 216, 204 mμ.

Found: C, 78.21; H, 9.38; O, 11.30; N, 1.11

Lithium aluminium hydride reduction of compound B

The compound B (100 mg.) was reduced according to the procedure outlined on page for compound A, to give an oily substance (82 mg.) which failed to crystallize; infrared in chloroform 3500, 1635 $cm.^{-1}$

Hydrogenation of compound B

The compound B (50 mg.) was hydrogenated at atmospheric pressure with platinum oxide as a catalyst under the conditions outlined forhydrogenation of compound A. After the usual work up, an oily substance (48 mg.) was obtained, which crystallized from ethanol. Recrystallization from ethanol yielded a white crystalline substance, m.p. 100-101°; infrared in potassium bromide 1705 $cm.^{-1}$; λmax. 260-280 mμ.

Isolation of a new compound during attempted preparation of a 2,4-dinitrophenylhydrazone of compound B

The compound B (50 mg.) was dissolved in ethanol after heating on a steam bath. To this solution, 2,4-dinitrophenylhydrazine reagent (2 ml.) was added and allowed to stand. After half an hour, orange yellowish crystals were formed and filtered off. Recrystallisation from ethanol yielded orange crystals, m.p. 101-102°; infrared in potassium bromide 1705, 1635 $cm.^{-1}$; λmax. 275 mμ , λshoulder 222, 216, 204 mμ.
Found: C, 73.91; H, 9.77; O, 13.91; N, 2.41

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