T H E S I S

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DEGREE OF DOCTOR OF PHILOSOPHY

by

HENRY S. WATSON.

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

A C K N O W L E D G E M E N T.

The author wishes to thank Professor F. S. Spring, F.R.S., for his continued interest and encouragement during the course of this work.

STUDIES IN STEROIDS

and

TETRACYCLIC TRITERPENOIDS

C O N T E N T S.

PART I.

PART II.

PART I.

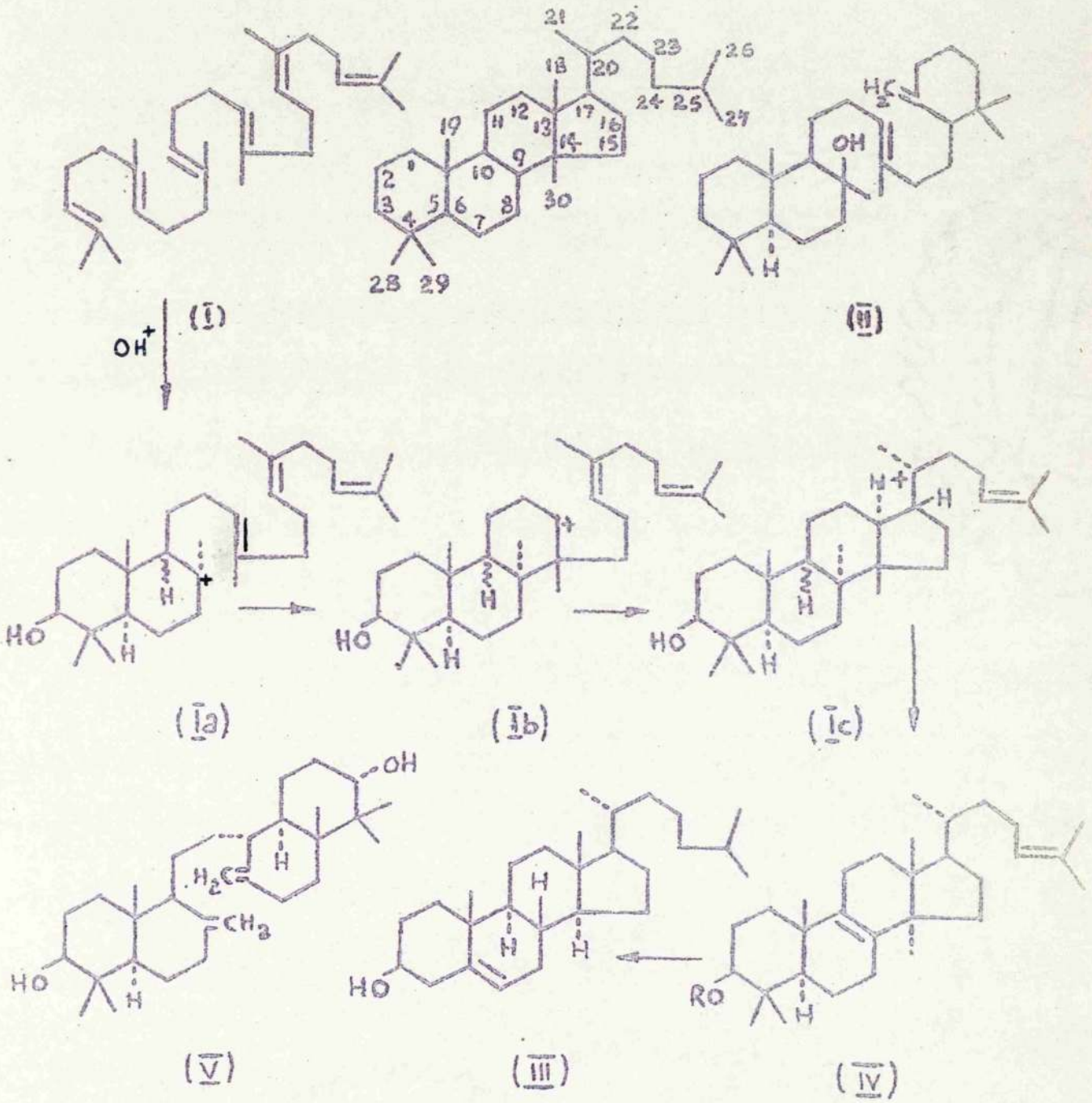
INTRODUCTION.

The triterpenoid group of naturally occurring compounds may be classified according to the number of rings contained in their carbocyclic structures. There are no known mono- or dicyclic triterpenoids, but acyclic and tricyclic triterpenoids are represented by squalene (I) and ambrein (II) respectively. The pentacyclic triterpenoids include the α-amyrin, β-amyrin, lupeol, taraxasterol and taraxerol series, and form the largest group. Individual members of this group possess structures which are divisible into six isopentane units and thus they conform to the "isoprene rule". In contrast, members of the smaller tetracyclic group of triterpenoids do not formally conform to the empirical isoprene rule and moreover, some members of this group have recently been shown to contain thirty one carbon atoms. Consequently the tetracyclic triterpenoids can be regarded as tri- or tetramethyl steroids.

The widely occurring sterol, cholesterol (III), is synthesised *in vivo* from acetic acid through the intermediation of squalene (I)[1]. The biosynthesis of the tetracyclic triterpenoids[2] may be considered to involve the cyclisation of squalene[3], motivated by attack of the cation OH^+ at position $C_{(3)}$ and proceeding synchronously by the formation of the intermediates (Ia), (Ib) and (Ic). Lanosterol (IV, R = H) is obtained from (Ic) by movement of the $C_{(13)}$-hydrogen atom to

$C_{(20)}$, accompanied by the simultaneous migrations of the β-methyl group at $C_{(14)}$ to position $C_{(13)}$, and of the α-methyl group at $C_{(8)}$ to position $C_{(14)}$. Cholesterol is formed biogenetically from lanosterol by loss of three carbon atoms. Other ionic mechanisms have been proposed[2] for the biosynthesis of the pentacyclic group of triterpenoids from squalene.

The work described in Part I of this thesis is concerned solely with the tetracyclic group of triterpenoids.



CLASSIFICATION OF THE TETRACYCLIC TRITERPENOIDS.

With one exception, those tetracyclic triterpenoids which have been fully formulated possess the perhydro-1:2-cyclopentenophenanthrene ring system of the steroids, and they may be subdivided into two series represented by lanosterol and

euphol respectively[4]. Members of each series undergo reactions which are characteristic of their sub-group. Thus with lanosterol and related compounds, the formation of a conjugated 7:9(11)-diene from the 8(9)-ene is accompanied by a positive change in molecular rotation, while in the euphol series, the same conversion results in a large negative change. The conjugated 7:9(11)-dienes of the lanosterol and euphol series also exhibit slightly different ultra-violet light absorption spectra. The influence of acid on the nuclear Δ^8 double bond is equally characteristic of both series. In the lanosterol group, acid isomerisation of the 8:9-double bond produces an equilibrium mixture of the Δ^7 and Δ^8 isomers. Acid isomerisation of the 8:9-double bond in the euphol group, however, effects a molecular rearrangement in which the 8:9-double bond moves to the 13:17-position with simultaneous methyl group migrations.

Recently a new type of tetracyclic structure has been discovered in the diol onocerin (V)[5] which may be included in the squalene (I) group of triterpenoids.

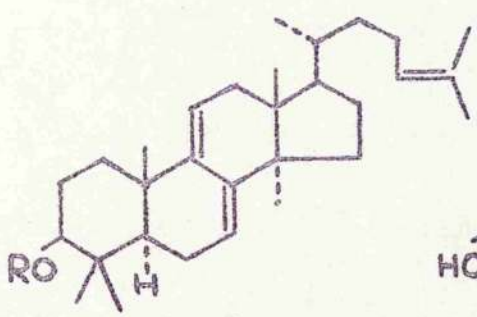
(i) The lanosterol group includes lanosterol, dihydrolanostero agnosterol, dihydroagnosterol, cycloartenol, cyclolaudenol, eburicoic acid, the polyporenic acids A, B and C, and pinicolic acid A.

Lanosterol (lanosta-8:24-dien-3β-ol) (IV, R = H) and agnosterol (lanosta-7:9(11):24-trien-3β-ol) (VI, R = H) were first

isolated by Windaus[6] from the "isocholesterol" mixture obtained from sheep wool wax. Dihydrolanosterol and dihydroagnosterol were both obtained from the same source by Ruzicka[7], and may be prepared by hydrogenation of the side chain double bond of (IV, R = H) and (VI, R = H) respectively.

cycloArtenol (handianol), isolated from the fruit of Artocarpus integrefolia[8], Strychnos nux-vomica L.[9], and various Euphorbiaceae[10,11], was the first triterpenoid found to contain a cyclopropane ring, and it was identified as 9:19-cyclolanost--24-en-3β-ol (VII) by Barton[8,12] and Spring[9,13].

The closely related C-31 triterpenoid, cyclolaudenol, occurring in opium marc, was shown to be 24b-methyl-9:19-cyclolanost-25-en-3β-ol (VIII)[14].



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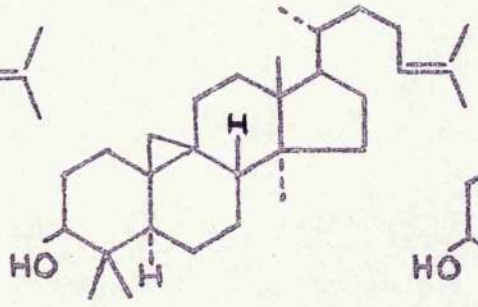

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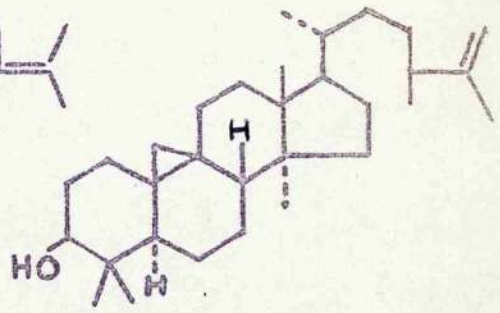
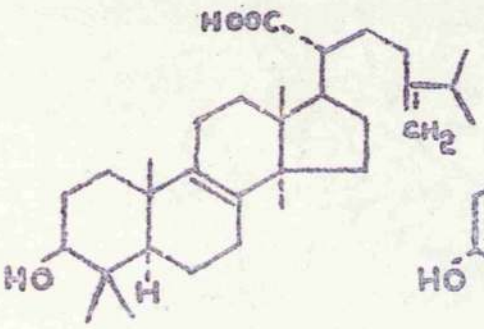


(VIII)

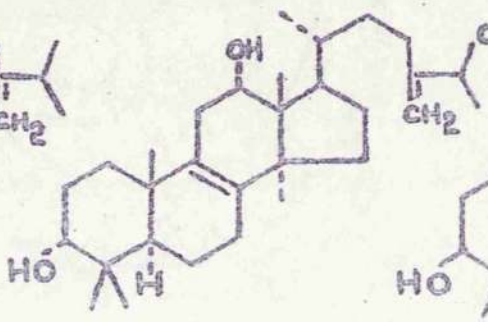
The remaining members of the lanosterol group are produced by the metabolism of various wood rotting fungi, in particular those of the Basidiomycetes and Polyporous classes. The structures of these closely related fungal acids which have been fully elucidated are shown in table A. Both eburicoic acid

and tumulosic acid occur together with their corresponding dehydro-compounds which are related to them in the same way as agnosterol is to lanosterol.

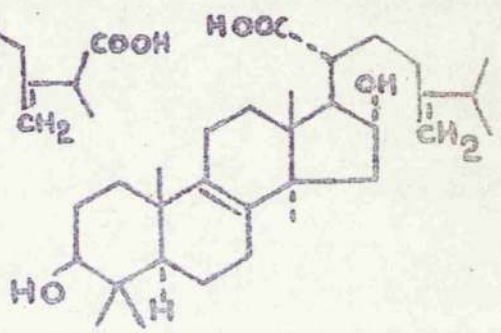
TABLE A.



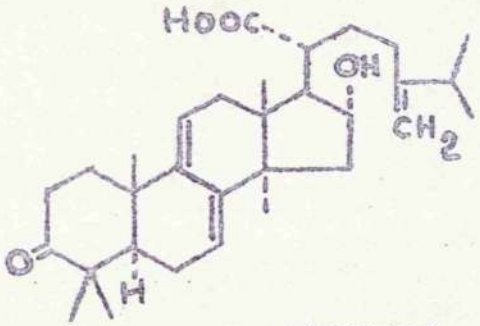
Eburicoic Acid[15,16]



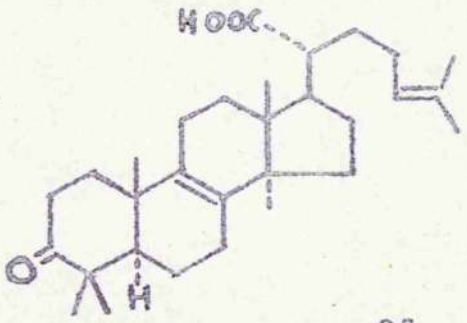
Polyporenic Acid[17,20]



Tumulosic Acid[17,21,22]



Polyporenic Acid C[17,23,24]



Pinicolic Acid A[25]

(ii) The euphol group of tetracyclic triterpenoids includes euphol, tirucallol, euphorbol, elemadienolic acid and elemadienoic acid.

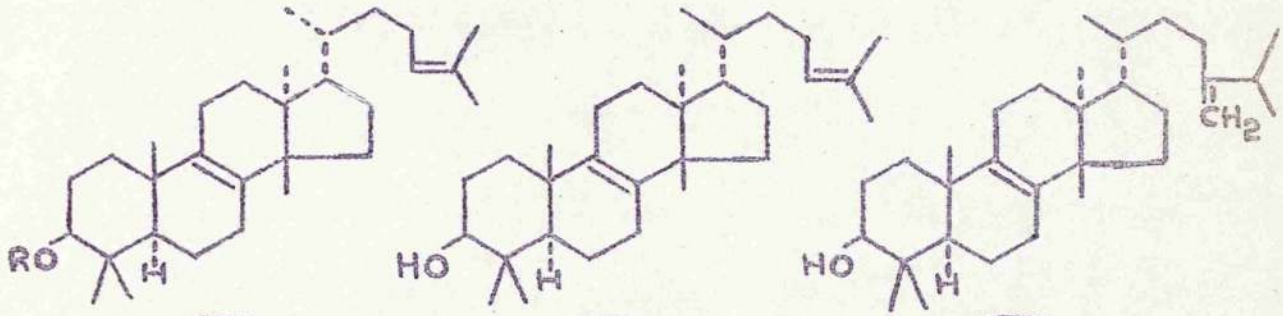
Euphol (eupha-8:24-dien-3β-ol), $C_{30}H_{50}O$, occurs in the latex of several Euphorbiaceae, and was first isolated by Newbold and Spring[26]. Its structure has finally been

established as shown in (IX, R = H)[27], and the important aspects of its chemistry are described in a later section.

Tirucallol was originally isolated from Euphorbia tirucalli L.[28] and shown to be 20-isoeuphol (X)[29,30]. The configurations at $C_{(17)}$ and $C_{(20)}$ were confirmed by side chain degradation studies[31,32].

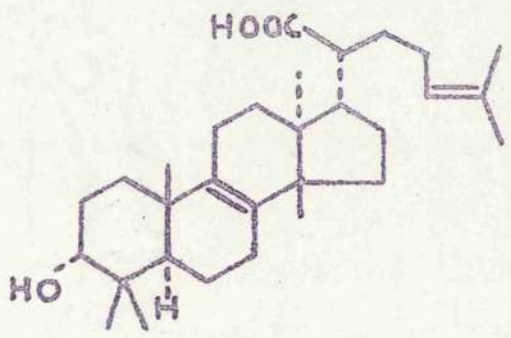
Euphorbol (XI), a C-31 triterpenoid occurring together with euphol in various Euphorbiaceae[26], was related to tirucallol by elimination of the side chain methylene group[30,33].

The elemadienolic ($C_{30}H_{48}O_3$) and elemadienoic acids, obtained from the resin of Manila elemi, were isolated and inter-related by Ruzicka[34]. The conversion of both acids into

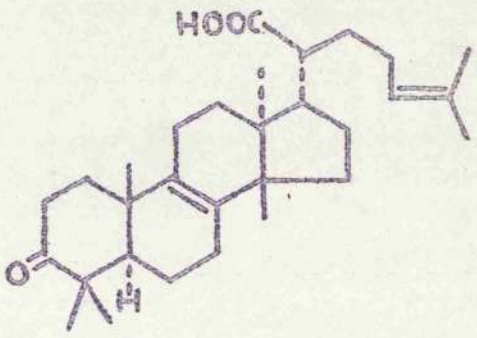


(IX) (X) (XI)

epielemenol[38] and its identity with dihydrotirucallol[33] led to the formulation of elemadienolic acid and elemadienoic acid as (XII) and (XIII) respectively[30].



(XII)



(XIII)

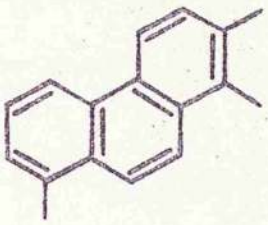
THE CONSTITUTION OF LANOSTEROL.

Early studies[7,28,36] showed lanosterol, $C_{30}H_{50}O$, to be a diethenoid, secondary, 3β-alcohol and consequently tetracyclic. One of the double bonds is present as an isopropylidine group[37] which can be catalytically reduced to give dihydrolanosterol (lanostenol). Dihydrolanosterol contains the less reactive nuclear double bond.

The lanosterol nucleus. The skeletal structure of lanosterol was partly revealed by dehydrogenation experiments. Pyrolysis of lanostadienol with selenium gives 1:2:8-trimethylphenanthrene (XIV)[7,38]. Later, Barton[39] showed that dehydrogenation of the "lanostene" (lanost-8-ene and lanosta-7:9(11)-diene) and "lanostenol" (lanost-8-en-3β-ol and lanosta-7:9(11)-dien-3β-ol) mixtures also gave (XIV), but in higher yield from the former, so that the two adjacent methyl groups in (XIV) are not derived from the gem-dimethyl groups at $C_{(4)}$ by a

retropinacoline rearrangement[40], and must correspond to those at $C_{(13)}$ and $C_{(14)}$ in the original molecule.

The tetrasubstituted nature of the less reactive double bond of lanosterol was shown from infra-red absorption measurements[41], and confirmed by an ultra-violet examination of lanost-8-ene[39]; its environment was largely deduced from a study of the products arising from a variety of oxidation reactions. Mild chromic acid oxidation of lanost-8-enyl acetate (XV, R = Ac) gives both the αβ-unsaturated ketone (XVI, R = Ac) and the characteristic yellow, transoid 1:4-dion-2-ene (XVII)[7,40].



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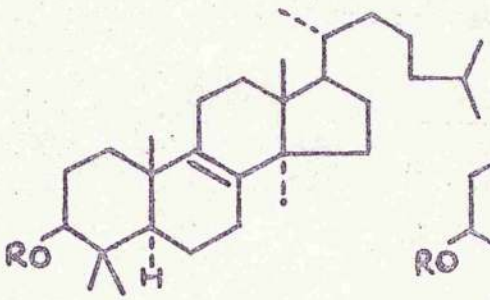

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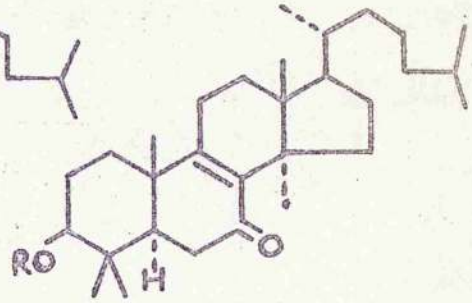
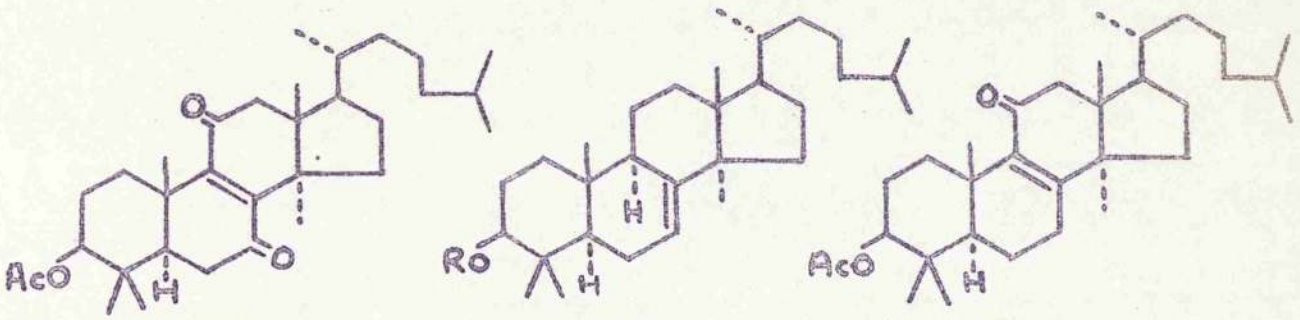


(XVI)

Under more drastic conditions, 7:11-dioxolanost-8-enyl acetate (XVII) is the major product from this reaction, and it is also obtained from 7-oxolanost-8-enyl acetate (XVI, R = Ac) by further oxidation[42-44]. In the presence of mineral acid, lanost-8-enyl acetate (XV, R = Ac) forms an equilibrium mixture with lanost-7-enyl acetate (XVIII, R = Ac)[39,45,46], and it is preferentially oxidised when the mixture is treated with chromic

acid under mild conditions[45]. Stronger oxidant mixtures, however, convert lanost-7-enyl acetate into the dionenyl acetate (XVII)[46].

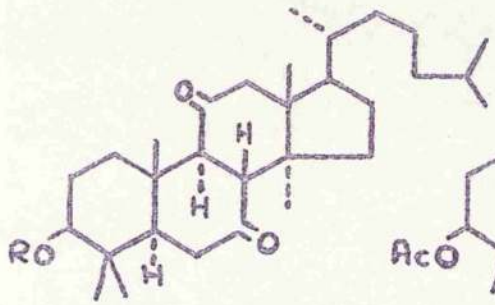
Of the two carbonyl groups in 7:11-dioxolanost-8-enyl acetate (XVII), that at position $C_{(7)}$ is much more reactive, and is selectively reduced by the Wolff-Kishner method to give the Δ^{8} -$C_{(11)}$-ketone (XIX).



(XVII) (XVIII) (XIX)

Treatment of the dionenyl acetate (XVII) with zinc dust in acetic acid saturates the ethylenic bond to give 7:11-dioxo-lanostanyl acetate (XX, R = Ac)[42]. The formation of 7:11--dioxolanost-8-enyl acetate (XVII) from lanost-8-enyl acetate (XV) suggested that the nuclear double bond of lanostenyl acetate was flanked by two methylene groups[7]. From an infra-red examination of the anediones (XX, R = Ac), it could be inferred that the carbonyl groups in this acetate were both present in six membered rings[40]. These deductions were confirmed by the formation of further oxidation products. Treatment of

7:11-dioxolanost-8-enyl acetate (XVII) with selenium dioxide gives 7:11-dioxolanosta-5:8-dienyl acetate (XXI)[40,44]. 7:11:12--Trioxolanosta-5:8-dienyl acetate (XXII, R = Ac) is obtained by the selenium dioxide oxidation of (XXI)[40], or by treatment of 7-oxolanosta-5:8:11-trienyl acetate (XXIII) with chromium trioxide[44].



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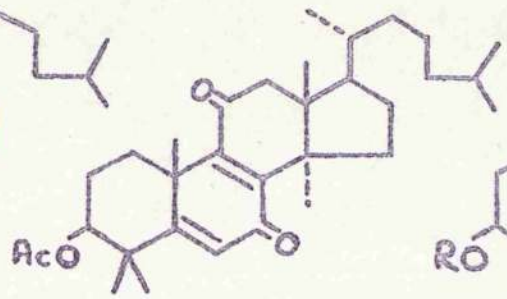
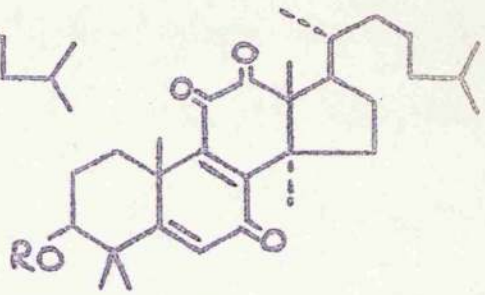
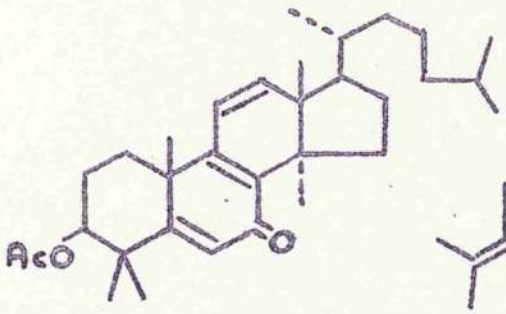


(XXI)



(XXII)

The position of the nuclear double bond of lanosterol was related to that of the hydroxyl group by the conversion of the dienetrione (XXII, R = H) into 7:11:12-trioxo<u>iso</u>lanosta-3:5:8--triene (XXIV) in which the conjugated system has been extended to ring A[39]. This relationship was also confirmed by the conversion of (XXIV) into the dicarboxyketone(XXV).



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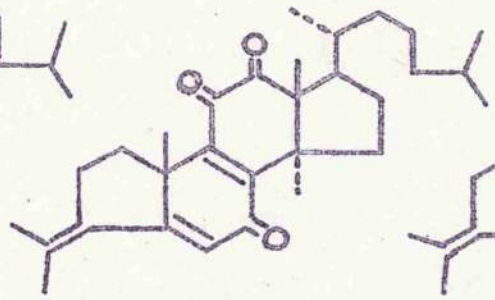

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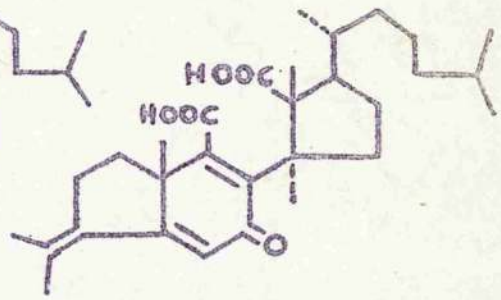
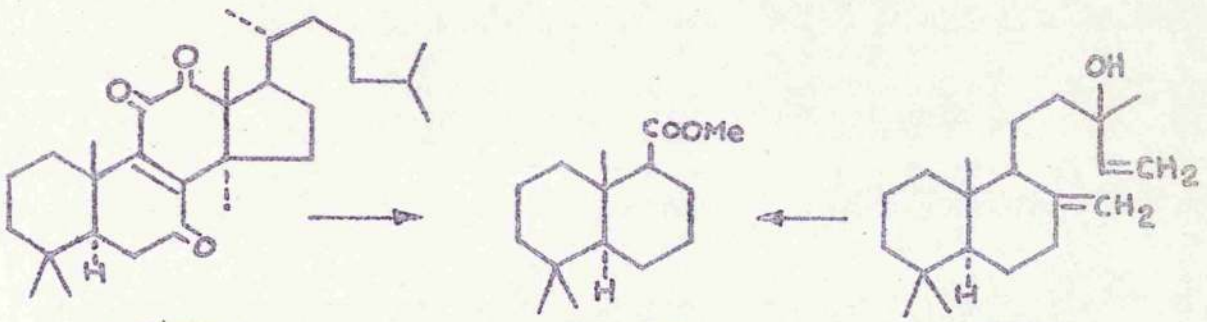


(XXV)

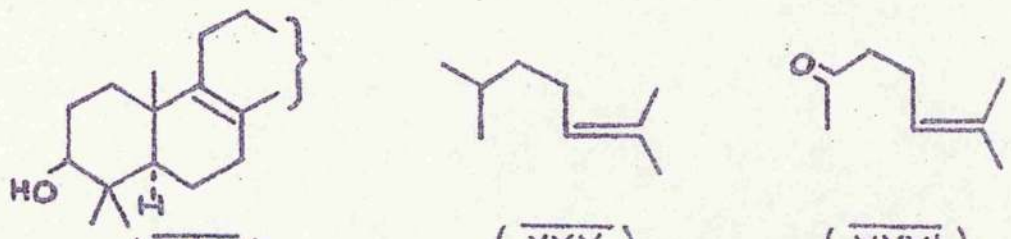
The constitution and configuration of rings A and B of lanosterol were revealed by the degradation of the enetrione (XXVI) to the bicyclic ester (XXVII)[48] which was also obtained from the diterpene manool (XXVIII), previously related to triterpenoids of established structure[49].

The reactions described above allowed the partial formulation of the lanosterol nucleus as shown in (XXIX).



(XXVI) (XXVII) (XXVIII)

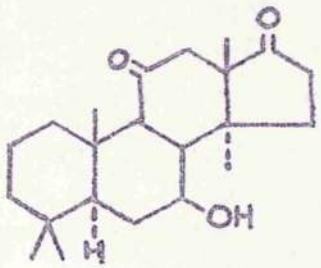
The side chain of lanosterol. The presence of an isooctenyl side chain (XXX) in lanosterol was shown by the formation of acetone and 6-methylheptan-2-one (XXXI) from the vigorous oxidation of lanost-8-enyl acetate (XV, R = Ac)[48,50,51]. Moreover, lanostadienol was shown to contain an isopropylidine group[37].



(XXIX) (XXX) (XXXI)

Lanosta-8:24-diene was converted in eight stages to the diketo-

-alcohol (XXXII), the infra-red absorption spectrum of which indicated the presence of a carbonyl group located in a five membered ring[52]. From a similar degradation product (XXXIII), Barton[53] showed that only one methylene group is adjacent to the carbonyl function so that the point of attachment of the side chain of lanosterol must be at $C_{(17)}$. The same conclusion was also reached by Ruzicka[54].



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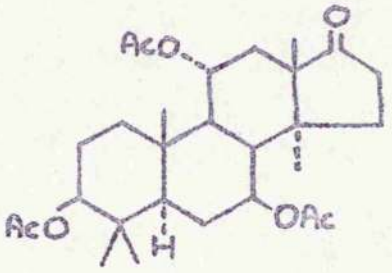

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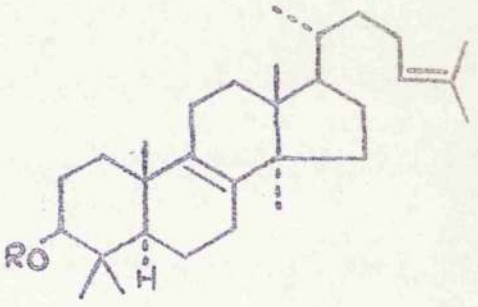


(IV)

The stereochemistry of lanosterol. The stereochemistry of the lanosterol molecule was established to be as is shown in (IV, R = H) from an X-ray analysis of its iodoacetate[55] and was confirmed by chemical means[56]. The β-(equatorial) configuration of the $C_{(3)}$-hydroxyl group was deduced from its stability towards epimerisation[57], its regeneration from the 3-ketone by reduction with sodium and alcohol[45], and by dehydration when ring A underwent a retropinacoline rearrangement; a reaction which requires the hydroxyl group to have the β-(equatorial) configuration[58]. The α-configuration of the

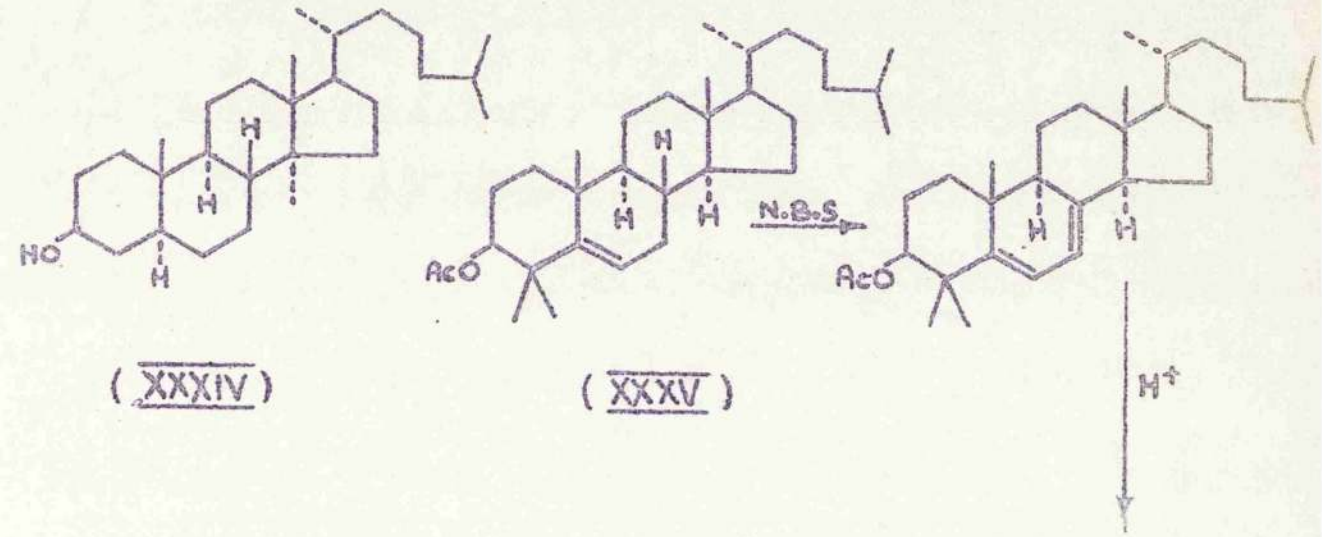
hydrogen atom at $C_{(5)}$ was established from a study of molecular rotation data[59], and by the degradation of the enetrione (XXVI) to a product common to mancol.

To account for the hindered nature of the $C_{(11)}$-carbonyl group in 7:11-dioxolanostanol (XX, R = H), the $C_{(13)}$-methyl group was allocated the β-configuration[39], and molecular rotation considerations favoured the α-configuration for the $C_{(14)}$-methyl group[59]. The stereochemistry at position $C_{(17)}$ was also determined from a study of molecular rotation data. It was found that molecular rotation differences between compounds in the lanosterol series having the isooctenyl type side chain and those in which it is replaced by a β-COMe group, were in agreement with similar differences for the corresponding steroid compounds. The side chain of lanosterol is therefore considered to have the 17β-configuration as in cholesterol (III).

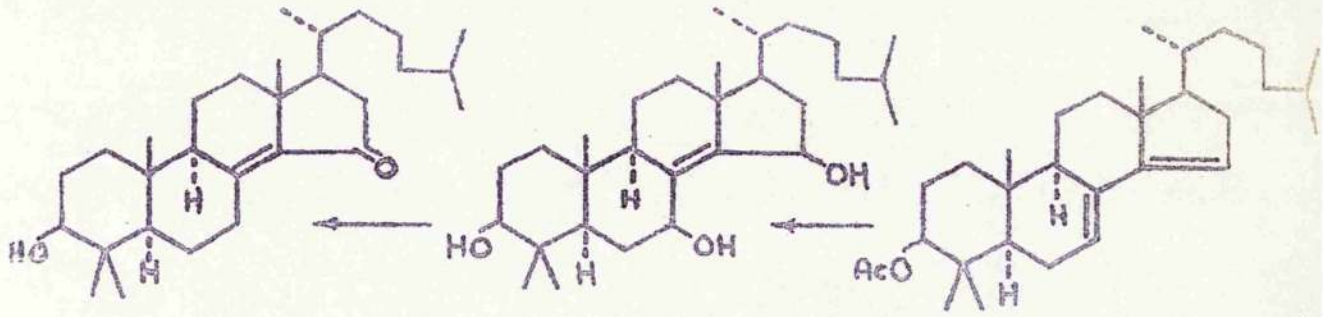
The stability of 7:11-dioxolanostanol (XX, R = H) to vigorous treatment with alkali shows that the hydrogen atoms at $C_{(8)}$ and $C_{(9)}$ have the more thermodynamically stable configurations which are mutually trans-relative but anti-relative to $C_{(14)}$ and $C_{(10)}$ respectively[60].

The synthesis of lanostenol. The constitution and stereochemistry of lanosterol have now been rigorously confirmed by the synthesis of lanostenol from cholesterol by Barton,

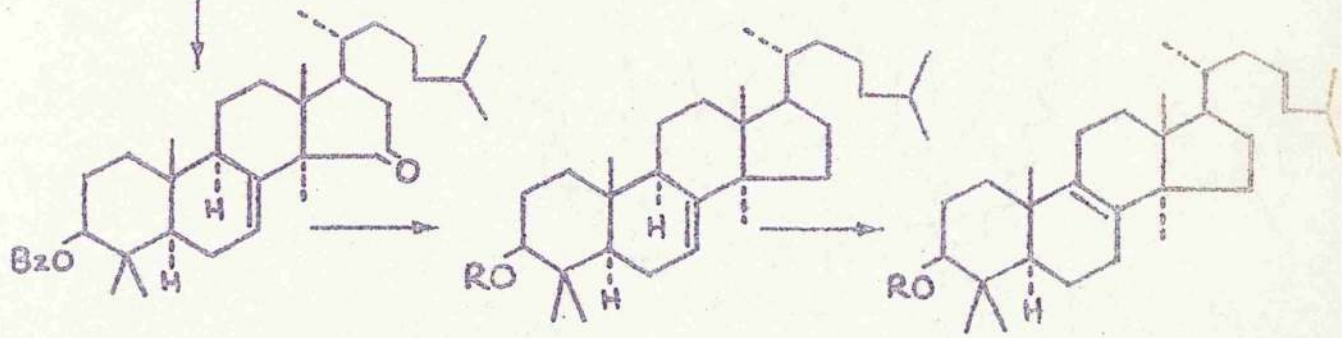
Woodward, and co-workers. This achievement constitutes the first total synthesis of a tetracyclic triterpenoid. The synthesis, which is outlined below, followed from the conversion of lanosterol into 14α-methylcholestanol (XXXIV)[62]. 14α-Methylcholestanol has also been prepared from cholesterol[63]. Methylation of cholest-5-en-3-one with methyl iodide and potassium tert-butoxide, followed by hydride reduction and acetylation of the product, gave 4:4'-dimethylcholest-5-enyl acetate (XXXV) which was converted by the steps shown to the dienyl acetate (XXXVI). On oxidation of (XXXVI) with per-acid and subsequent hydrolysis of the product, a triol, probably (XXXVII) was obtained. With mineral acid, the triol dehydrated to give the αβ-unsaturated ketone (XXXVIII), the benzoate of which was methylated as before to give (XXXIX). By reduction under drastic Wolff-Kishner conditions, (XXXIX) was converted into (XVIII, R = H) which proved to be identical with lanost-7-en-3β-ol. Treatment of the benzoate (XVIII, R = Bz) with hydrogen chloride and careful chromatography of the resulting mixture gave lanost-8-enyl benzoate (XV, R = Bz), hydrolysis of which gave lanost-8-en-3β-ol (XV, R = H).



(XXXIV) (XXXV)



(XXXVIII) (XXXVII) (XXXVI)

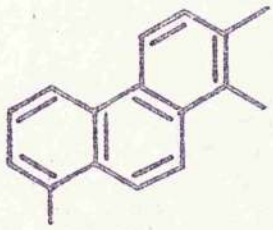


(XXXIX) (XVIII) (XV)

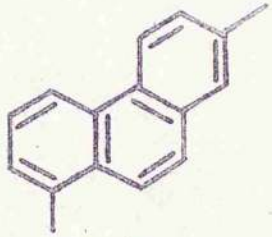
THE CONSTITUTION OF EUPHOL.

Newbold and Spring[26] first characterised euphol as a tetracyclic triterpenoid alcohol, $C_{30}H_{50}O$, containing two double bonds, one of which is readily reduced by platinum and hydrogen. This ethylenic linkage was later found to be present as an isopropylidine group[64,66]. The structural elucidation of euphol followed on closely similar lines to those described for lanosterol.

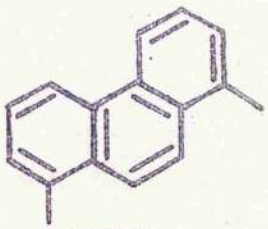
The nucleus and side chain of euphol. Dehydrogenation of euphadienol gives 1:2:8-trimethylphenanthrene (XIV)[66], while euphadiene yields both 1:7-dimethyl-(XL) and 1:8-dimethyl-phenanthrene (XLI) as well[66], so that euphol must contain a methyl group at $C_{(13)}$ and $C_{(14)}$.



(XIV)



(XL)

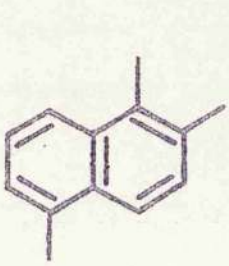


(XLI)

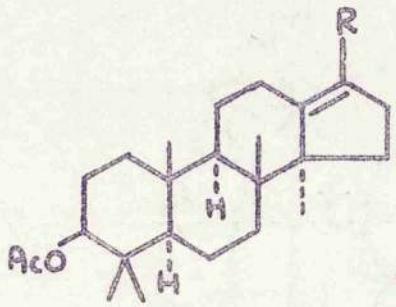
The structures of rings A, B and C, and the location of the less reactive double bond of euphol were shown to be the same (XXIX) as that in lanosterol by Jeger and his co-workers[66,67]. They found that euphol and its derivatives underwent the same dehydration and oxidation reactions to yield analogous compounds

to those obtained in the lanosterol series. Ring D of euphol was shown to be five membered[68] by Barton[27] from a study of the infra-red spectra of the hydrocarbons euph-8-ene and lanost-8-ene, from which it follows that both have the same number of methyl groups. The side chain structure was also elucidated by a series of degradation reactions similar to those reported for lanosterol[67,69-71].

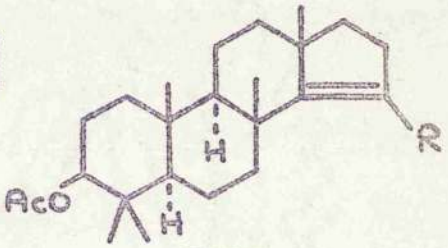
The major difference in structure between euphol and lanosterol therefore became confined to the region of ring D, and its nature, as well as the position of attachment of the side chain[67,72], followed from a study of the acid isomerisation of euphol which was first described by Vilkas[73]. When treated with mineral acid, the 8:9-double bond of euphol was found to move to a second fully substituted position (isoeuphenol). From the selenium dehydrogenation of isoeuphadiene, Barton[27] attributed the isolation of 1:2:5-trimethylnaphthalene (XLIII) to the migration of the $C_{(14)}$-methyl group to position $C_{(8)}$, which permitted isoeuphenyl acetate to be formulated as either (XLIV, R = C_8H_{17}) or (XLV, R = C_8H_{17}). Formula (XLV, R = C_8H_{17}) for isoeuphenyl acetate was excluded when the diketone formed by the ozonolysis of this acetate was shown to be (XLVI, R = C_8H_{17}) from its infra-red absorption spectrum, from the unhindered nature of the carbonyl groups, and by its absorption of five mols. of bromine[66].



(XLIII)

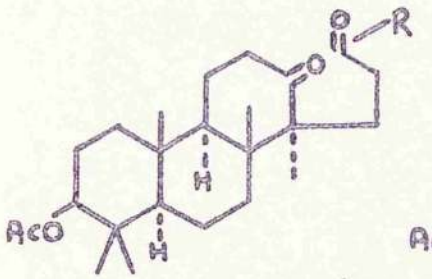


(XLIV)

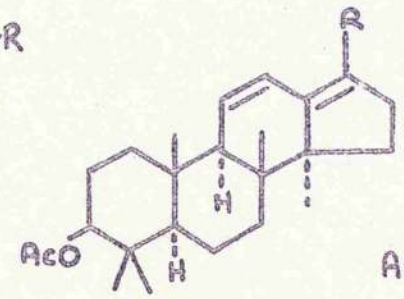


(XLV)

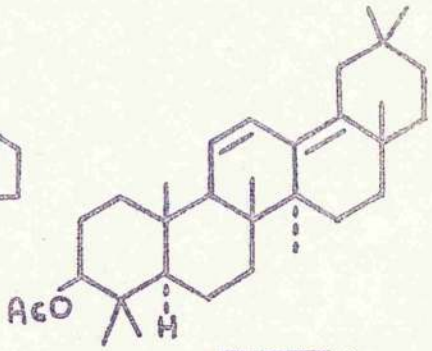
Evidence supporting the formulation of isoeuphenyl acetate as (XLIV, R = C_8H_{17}) was obtained from the close similarity in ultra-violet light absorption spectra between the conjugated diene, isoeuphadienyl acetate (XLVII, R = C_8H_{17}), and the well known pentacyclic diene, oleana-11:13(18)-dienyl acetate (XLVIII). The same conclusion as to the nature of ring D and



(XLVI)



(XLVII)

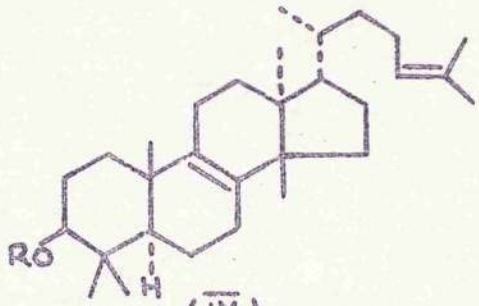


(XLVIII)

the isomerisation of euphenol to isoeuphenol was reached by Jeger and Ruzicka[78] from a series of extensive degradation reactions.

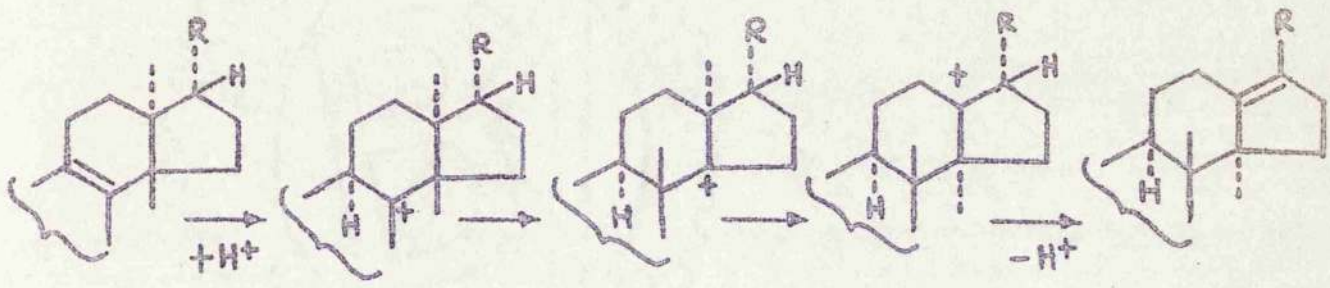
The stereochemistry of euphol. The stereochemistry at each asymmetric centre in the euphol molecule has now been established as is shown in (IX, R = H). Barton[27] has allocated

the configurations at $C_{(3)}$, $C_{(5)}$ and $C_{(10)}$ since the hydroxyl group is equatorial and has the molecular rotation properties of a normal 3β-alcohol. The α- and β- configurations of the methyl groups at positions $C_{(13)}$ and $C_{(14)}$ respectively were assigned on the basis of a conformational interpretation of the euphenol to isoeuphenol isomerisation[27,75]. According to Barton[27], the euphol molecule (IX, R = H) has rings B and C in an unfavourable conformation of two 'half-boats', and the resulting steric strain provides a conformational driving force



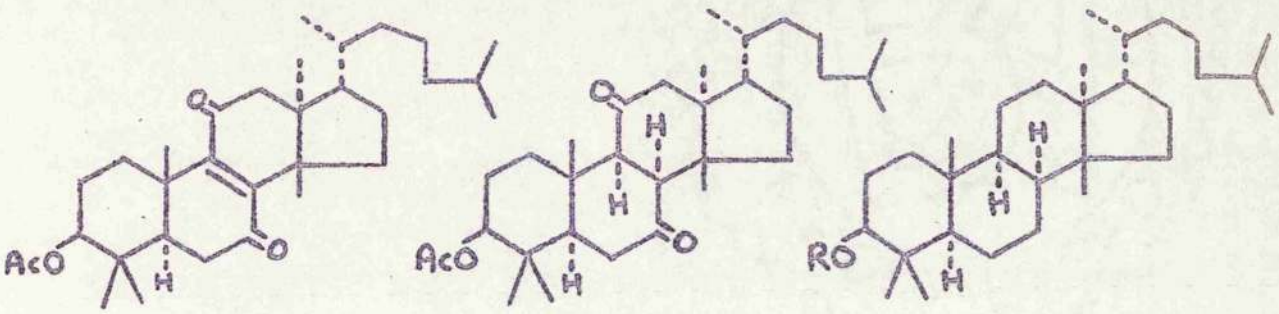
(IX)

for the subsequent migration of the $C_{(13)}$- and $C_{(14)}$-methyl groups to form the isoeuphenol system in which rings A, B and C are in the more stable 'all-chair' conformation. A critical examination of molecular models, however, shows that (IX) may exist in a 'half-chair' conformation. The isoeuphenol rearrangement, when based on a totally concerted mechanism as represented by the synchronous stages shown below, ($R = C_8H_{17}$), establishes the α-configuration of the side chain at position $C_{(17)}$ and of the $C_{(13)}$-methyl group, while the methyl group at $C_{(14)}$ must have the β-configuration.



After controversial evidence based on comparisons of molecular rotation differences in the lanosterol and euphol series[27], Ruzicka[78] showed from degradative studies that the configuration at $C_{(20)}$ in euphol is the same (α) as that in lanosterol. Confirmation of the configurations at $C_{(17)}$ and $C_{(20)}$ in the euphol molecule was obtained by the establishment of the α-configuration of the side chain of tirucallol (20-isoeuphol) (X)[31]. Warren[32] also showed that euphol is 20-isotirucallol by degradation of the side chain of tirucallol and euphol to corresponding derivatives in which the symmetry at $C_{(20)}$ is destroyed. These degradative products from euphol and tirucallol proved to be identical.

Euphol derivatives in which rings B and C are saturated may be prepared by treatment of the dionene (XLIX) with zinc dust and acetic acid. The hydrogen atoms at $C_{(8)}$ and $C_{(9)}$ in 7:11-dioxoeuphanyl acetate (L) are considered to be mutually cis-relative[72] since treatment of (L) with selenium dioxide or with strong alkali[66] regenerates 7:11-dioxoeuph-8-enyl acetate (XLIX). The saturated alcohol, euphanol (LI, R = H), is at present unknown.



(XLIX) (L) (LI)

Recent extensive reviews of the tetracyclic group of triterpenoids are given by Gascoigne and Simes[76], and Halgall and Jones[77].

THEORETICAL.

SECTION I. The Constitution of Butyrospermol.

Experiments designed to elucidate the constitution and stereochemistry of butyrospermol are described. These and related reactions show that butyrospermol is 9α-eupha-7:24-dien-3β-ol.

INTRODUCTION.

In 1934, Heilbron, Moffet and Spring[78] isolated the acetate of an alcohol of approximate molecular formula $C_{30}H_{50}O$, and which they named basseol, from the non-saponifiable fraction of shea nut fat (Butyrospermum parkii). Basseol was characterised as a tetracyclic diethenoid secondary alcohol containing a vinylidine group, and its acetate was converted into β-amyrin acetate by a variety of acidic reagents[79].

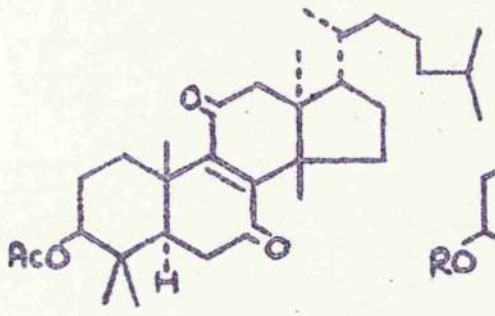
A subsequent re-examination of shea nut fat by Heilbron, Jones and Robins[80] led to the isolation of an alcohol, butyrospermol, the chemical properties of which differed markedly from those reported for basseol. In particular, butyrospermyl acetate could not be converted into β-amyrin acetate, and it contained an isopropylidine group and not a vinylidine group. Other investigations[81,82] failed to reveal the presence of basseol in shea nut fat, and it is now accepted that basseol acetate was butyrospermyl acetate contaminated with ca. 16% of β-amyrin acetate[82-84]. The physical constants of butyrospermol, however, are in excellent agreement with those reported for basseol, which in contrast to basseol acetate, is considered to have been a pure compound[84]. Butyrospermol has also been found in the fruit of Artocarpus integrefolia[8].

Early studies[80,82] showed that butyrospermol is a

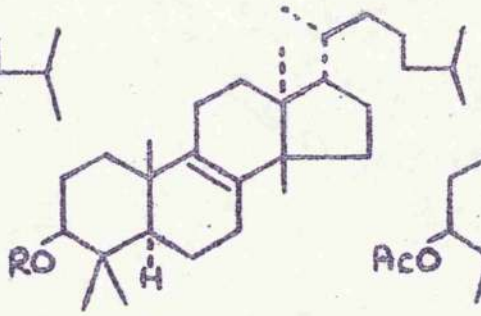
diethenoid, secondary alcohol, $C_{30}H_{50}O$ (or a near homologue), and it is consequently tetracyclic. One of the double bonds is present as an isopropylidine group and is readily reduced to give dihydrobutyrospermol. The second (less reactive) double bond is not reduced by platinum and hydrogen, and from spectroscopic evidence it was concluded to be tetrasubstituted[81,83].

Dawson et al.[82] showed that treatment of dihydrobutyrospermyl acetate with dry hydrogen chloride at 0° gives dihydroisobutyrospermyl acetate. A consideration of the reactions of this isomer led them to suggest that its double bond was trisubstituted.

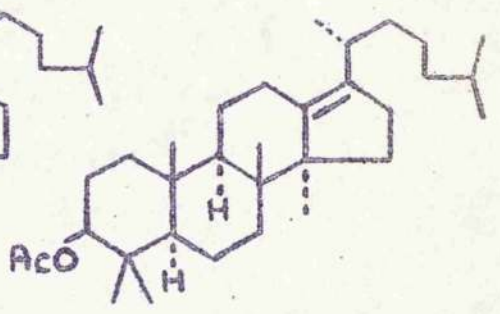
The principal features of the constitution of butyrospermol were elucidated by Spring and his co-workers[84,86] who found that dihydroisobutyrospermyl acetate is identical with euph-8-enyl acetate (LII, R = Ac). The identity was confirmed by the conversion of dihydroisobutyrospermyl acetate into 7:11-dioxoeuph-8-enyl acetate (XLIX) by chromic acid oxidation; into isoeuph-13(17)-enyl acetate (LIII) by isomerisation with hydrochloric-acetic acid, and into 8ξ:9ξ-epoxyeuphanyl acetate (LIV) by treatment with perbenzoic acid.



(XLIX)

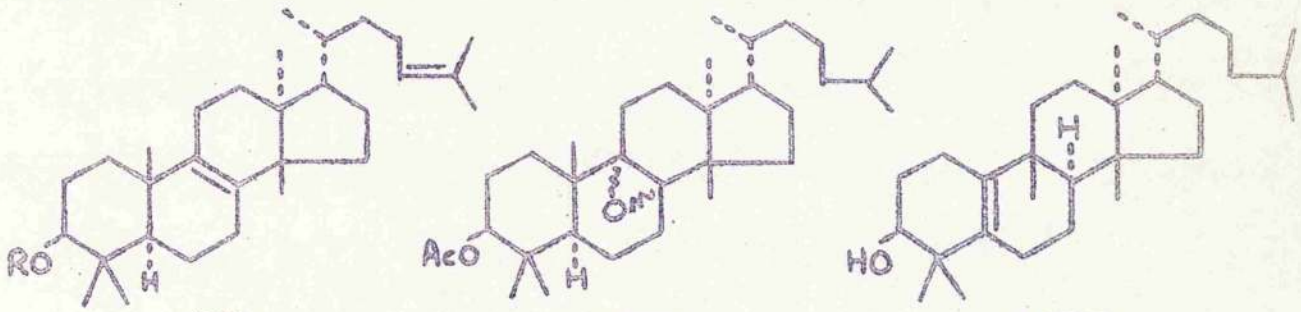


(LII)



(LIII)

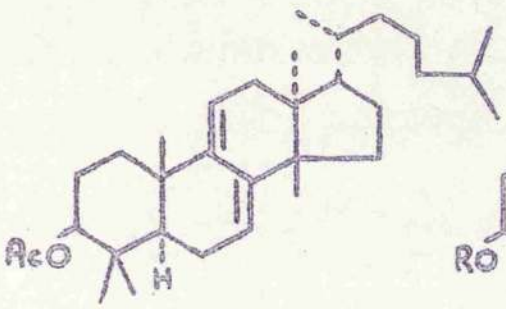
The relation between butyrospermol and euphol was confirmed by the conversion of the former into the latter. Addition of one mol. of bromine to butyrospermyl acetate in chloroform and treatment of the solution with hydrogen chloride, followed by debromination of the product with zinc, gave euphyl acetate (IX, R = Ac). The isomerisation of dihydrobutyrospermyl acetate into euph-8-enyl acetate (LII, R = Ac) is also effected by shaking it with platinum and hydrogen in acetic acid, a reaction which suggested that the less reactive double bond of butyrospermol is not tetrasubstituted[81,82,85] as represented by (LV).



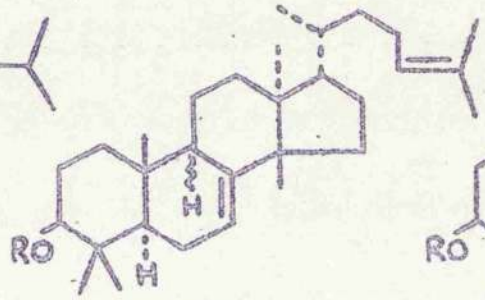
(IX) (LIV) (LV)

Treatment of dihydrobutyrospermyl acetate with osmic acid, followed by acetylation, gives a saturated triol diacetate, from which it can be concluded that the less reactive double bond of butyrospermol is tri- and not tetrasubstituted. The triol diacetate is readily converted into cupha-7:9(11)-dienyl acetate (LVI) under a variety of conditions. Dihydrobutyrospermyl acetate is therefore a double bond isomer of euph-8-enyl acetate (LII, R = Ac) in which the unsaturated centre is either between

$C_{(7)}$ and $C_{(8)}$ or between $C_{(9)}$ and $C_{(11)}$. Accordingly, butyrospermol was identified by these workers as either 9ξ-eupha-7:24-dien-3β-ol (LVII, R = H) or 8ξ-eupha-9(11):24-dien-3β-ol (LVIII, R = H).



(LVI)



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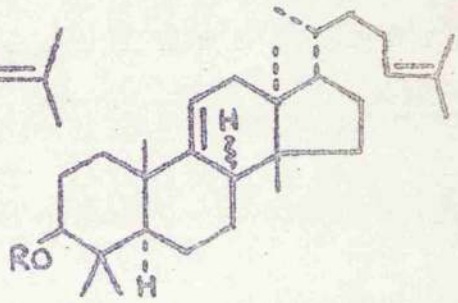
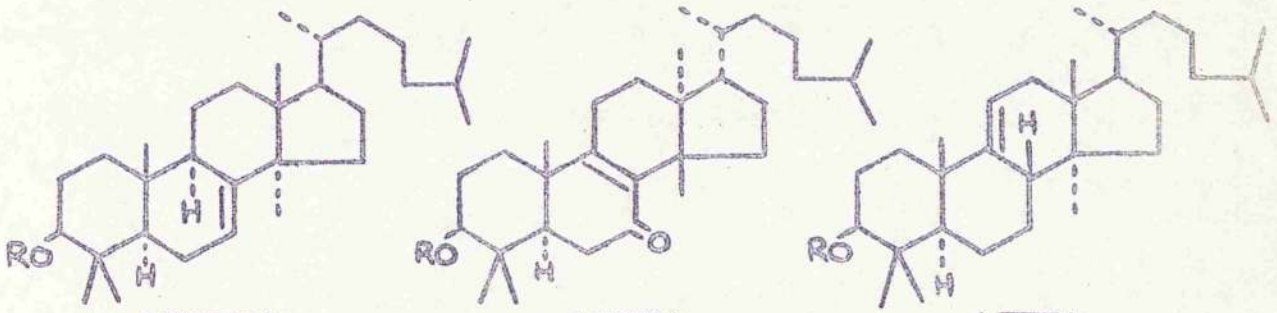


(LVIII)

A similar conclusion was reached by Jones and his collaborators[77,83] who converted dihydrobutyrospermyl acetate into 7-oxoeuph-8-enyl acetate (LIX, R = Ac) by treating it with an excess of perbenzoic acid. A detailed examination of the infra-red spectra of dihydrobutyrospermyl acetate and related compounds also led these authors to the view that the less reactive double bond of butyrospermol is tri- and not tetra-substituted. No conclusive evidence was available which indicated whether this ethylenic linkage is located at the 7:8- or the 9:11-position. A comparison of the behaviour of dihydrobutyrospermyl acetate with lanost-7-ene and lanost-9(11)--ene derivatives in corresponding reactions did not allow a satisfactory choice to be made between structures (LVII, R = H) (LVIII, R = H) for butyrospermol. In the case of lanost-7-enyl acetate (XVIII, R = Ac), the double bond is inert towards

hydrogenation and it is partly isomerised to the 8:9-position on treatment with mineral acid[37,45,46]. Lanost-9(11)-enyl acetate (LX, R = Ac) is stable towards treatment with mineral acid and can be catalytically reduced to lanostanyl acetate[37]. These considerations favoured structure (LVII, R = H) for butyrospermol, for which there are two possible isomeric structures depending on whether the hydrogen atom at $C_{(9)}$ has the α- or β-configuration. Barton[27] has recently described the preparation of the dihydro--acetate of (LVII, R = H) which is not identical with dihydro-butyrospermyl acetate and which Halsall and Jones[77] assume to have the α-configuration at $C_{(9)}$. To explain the unusual, but similar,



(XVIII) (LIX) (LX)

molecular rotation differences observed when butyrospermol and cycloartenol (VII), which has a 9β-methylene group, are oxidised to the corresponding ketones, these workers expressed a tentative preference for the 9β-form of (LVII, R = H) for butyrospermol. The following, and related experiments, show that butyrospermol is 9α-eupha-7:24-dien-3β-ol and that the euph-7-enyl acetate described by Barton[27] is 9β-euph-7-enyl acetate.

Dihydrobutyrospermyl Acetate.

As already mentioned, basseol acetate, m.p. 141°, $[\alpha]_D$ + 22.4°[78], is now considered to be butyrospermol acetate contaminated with ca. 16% of β-amyrin acetate[84]. The isolation of pure butyrospermyl acetate from this mixture by crystallisation procedures is attended by large losses. These observations have been confirmed by the author and an improved method for the separation of the basseol acetate mixture is reported.

Hydrolysis of shea nut fat using alcoholic potassium hydroxide solution gave the non-saponifiable fraction as a yellow, low melting solid in 3% yield. This material was refluxed with acetic anhydride and the solution left overnight at room temperature. The semi-crystalline mass which separated during this interval was removed, and the filtrate kept at 0° for several days when a second crop of solid separated. The latter product was crystallised repeatedly from an ethanol-ethyl acetate mixture to give stout needles which had physical constants similar to those reported for basseol acetate, viz. m.p. 135-136° $[\alpha]_D$ + 25°. Although the bulk of these crystals melted sharply at 135-136°, solid persisted in the melt until above 180°, as had already been observed by Jones[80]. Pure butyrospermyl acetate, m.p. 144°, $[\alpha]_D$ + 12°, was obtained from the mixture only after many crystallisations from relatively dilute solutions of the same solvent mixture.

Dihydrobutyrospermyl acetate was obtained by the reduction

of butyrospermyl acetate using platinum and hydrogen in neutral solvent. The observation that euphenyl acetate is easily separated from β-amyrin acetate by chromatography of the mixture[88] suggested that the separation of a mixture containing the acetates of dihydrobutyrospermol and β-amyrin in this way may be equally effective. "Basseol acetate" was shaken for nine hours with platinum and hydrogen, in ethyl acetate solution, and the resulting product chromatographed. The initial fractions eluted from the column were found to consist of pure dihydrobutyrospermyl acetate, m.p. 135-136°, $[\alpha]_D$ + 10.8°. The yield of dihydrobutyrospermyl acetate obtained from basseol acetate by this method is almost quantitative, and is three times greater than the yield of pure butyrospermol obtained by the repeated crystallisation of basseol acetate .

It has already been shown that dihydrobutyrospermyl acetate is isomerised to euph-8-enyl acetate (LXI, R = Ac) by its brief treatment with dry hydrogen chloride at 0°[84,86]. Under stronger acid conditions, viz. hydrochloric-acetic acid mixtures at 100°, euph-8-enyl acetate is converted into isoeuph-13(17)-enyl acetate (LXII)[27]. However no direct conversion of dihydrobutyrospermyl acetate into isoeuph-13(17)-enyl acetate by the latter reaction has been reported. The result of this reaction was required for comparison with the behaviour of 9ξ-euph-7-enyl acetate (LXI, R = Ac) under the same conditions. It was found that isoeuph-13(17)-enyl acetate is isolated as the sole reaction product when dihydrobutyrospermyl acetate is treated with an

hydrochloric-acetic acid mixture at 100°. The theoretical implications of this conversion are discussed in the following section.

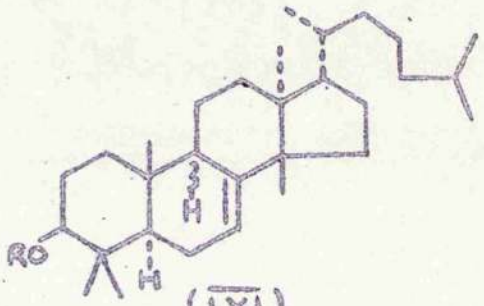
9β-Euph-7-enyl Acetate.

The possible stereochemical relationship which could exist between 9ξ-euph-7-enyl acetate (LXI, R = Ac)[27] and dihydrobutyrospermyl acetate, and the considerations which led Halsall and Jones[77] to express a tentative preference for the 9β-form of structure (LVII, R = H) for butyrospermol, have already been discussed. It was hoped that a critical examination of the euph-7-enyl acetate would reveal its relationship to dihydrobutyrospermyl acetate, and so establish, or otherwise, the constitution of butyrospermol as 9ξ-eupha-7:24-dien-3β-ol (LVII, R = H).

Barton et al.[27] have described the formation of euph-7-enyl acetate from 7-oxoeuph-8-enyl acetate (LIX, R = Ac) by Wolff-Kishner reduction, followed by acetylation of the product. Treatment of euph-7-enyl acetate with selenium dioxide gives eupha-7:9(11)-dienyl acetate (LVI), a conversion which showed that the former is a simple double bond isomer of euph-8-enyl acetate (LII, R = Ac). The configuration of the hydrogen atom at $C_{(9)}$ in euph-7-enyl acetate (LXI, R = Ac) was not established by these workers. 9ξ-Euph-7-enyl acetate (LXI, R = Ac) was prepared from euphol (IX, R = H) in five steps; results differing from those reported by Barton et al.[27]

are described.

Extraction of Euphorbia resin with boiling acetone gave the well known "euphorbone" mixture (8.3%) as a yellow wax which was extracted with boiling light petroleum. The purified material obtained by this method was chromatographed on alumina



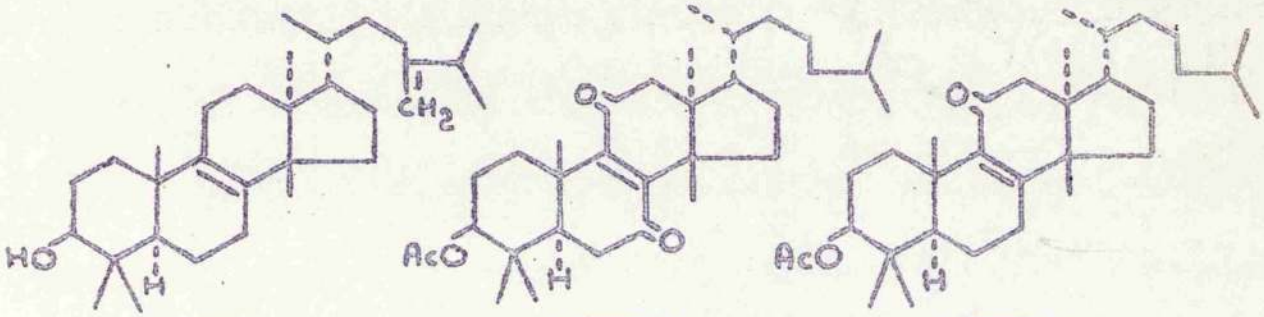
(LXI)

which had previously been deactivated by prolonged exposure to the air. These modifications of the method described by Newbold and Spring[26] led to improved yields (10%) of pure euphol (LX, R = H), and its ready separation from euphorbol (XI). After acetylation, the side chain double bond of euphol was reduced using platinum and hydrogen in ethyl acetate solution to give euph-8-enyl acetate (LXI, R = Ac).

The Ozonolysis of Euph-8-enyl Acetate.

Barton and his co-workers[27] have reported that the ozonolysis of euph-8-enyl acetate gives 7-oxoeuph-8-enyl acetate (LIX, R = Ac) as the sole isolated product in 25% yield. This reaction was repeated under various conditions and was found to be of a complex nature. Treatment of euph-8-enyl acetate with ozone at between -5° and -10°[27] afforded a mixture, the constituents of which were separated by careful chromatography.

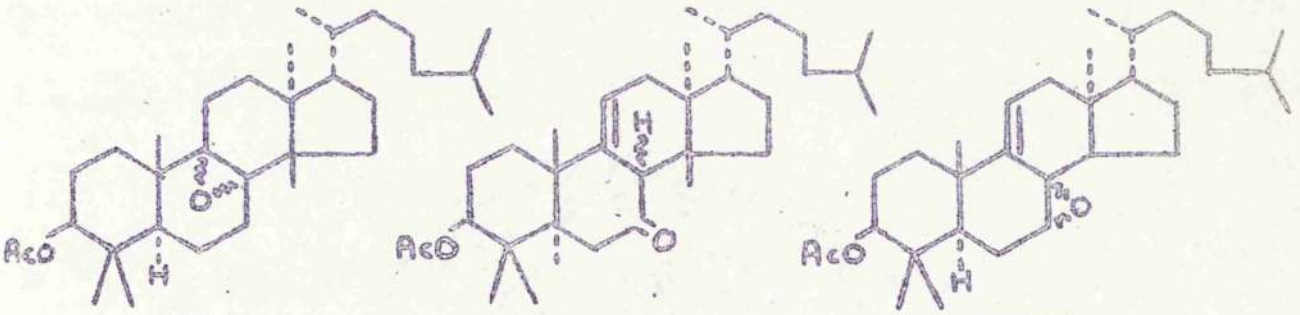
7:11-Dioxoeuph-8-enyl acetate (XLIX), 11-oxoeuph-8-enyl acetate (LXII) and 7-oxoeuph-8-enyl acetate (LIX, R = Ac) were eluted from the column in that order and in yields of 5, 5, and 10% respectively.



(XI) (XLIX) (LXII)

At lower temperatures (ca. - 40°), the ozonolysis of euph-8-enyl acetate gave starting material (30%) together with equal quantities of 7-oxoeuph-8-enyl acetate (LIX, R = Ac) and a crystalline product m.p. 96-98°. The latter material was examined in some detail. Its ultra-violet absorption spectrum showed the presence of both the αβ-unsaturated carbonyl system (ϵ_{256} = 4,300) and an isolated ethylenic bond (ϵ_{205} = 4,700). Moreover, the absorption wavelength (256 mu) of the αβ-unsaturated ketone corresponds to 11-oxoeuph-8-enyl acetate (LXII) rather than 7-oxoeuph-8-enyl acetate (LIX, R = Ac) which was found to absorb at 252 mu. Treatment of the acetate mixture, m.p. 96-98°, with a sulphuric-acetic acid mixture increased the proportion of αβ-unsaturated ketone by 50%, and subsequent chromatography of the product gave both (LIX, R = Ac) and (LXII). Consequently the crystals, m.p. 96-98°, consist of a mixture of 11-oxoeuph-8-enyl

acetate (LXII) and an acetate which is either a non-conjugated ketone (LXIII) or an unsaturated oxide (LXIV). Barton[27] has obtained such an intermediate (LXIII or LXIV) by the treatment of eupha-7:9(11)-dienyl acetate (LVI) with monoperphthalic acid, and has shown that it is readily converted into 7-oxoeuph-8-enyl acetate (LIX, R = Ac) by its brief treatment with mineral acid. These observations suggest that the oxidation of euph-8-enyl acetate (LII, R = Ac) by ozonolysis proceeds through the mediation of eupha-7:9(11)-dienyl acetate (LVI) which is formed from the initial epoxide, 8ξ:9ξ-epoxyeuphanyl acetate (LIV) by the presence of small amounts of acid in the solvent.



(LIV) (LXIII) (LXIV)

9ξ-Euph-7-enyl Acetate.

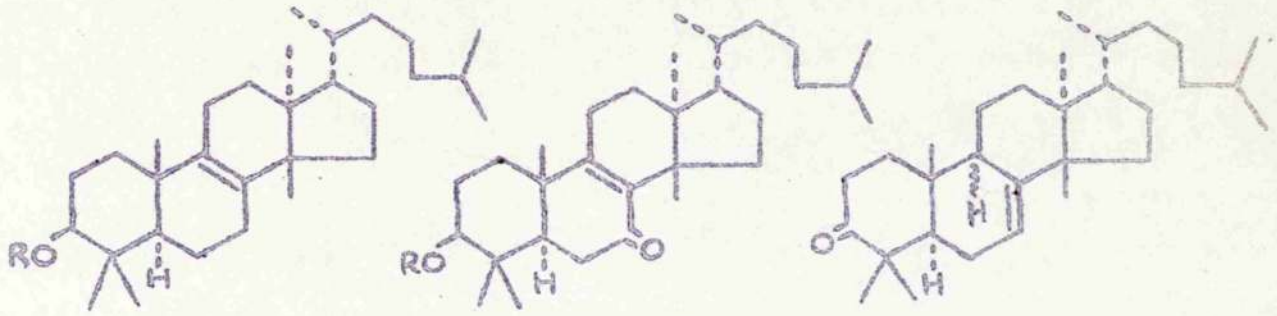
The position of the nuclear double bond of 9ξ-euph-7-enyl acetate (LXI, R = Ac)[27] followed from its mode of formation, by which the elimination of the $C_{(7)}$-carbonyl group of 7-oxoeuph-8--enyl acetate (LIX, R = Ac) is accompanied by movement of the double bond from the 8:9- to the 7:8-position (cf. 9α-lanost-7--enyl acetate)[39]. This accepted mechanism was confirmed when the

double bond of the αβ-unsaturated ketone (LIX, R = Ac) was found to be stable under strong alkaline conditions. 7-Oxoeuph-8-enyl acetate was recovered unchanged after prolonged treatment with 10% alkali.

7-Oxoeuph-8-enyl acetate (LIX, R = Ac) was reduced according to the method described by Barton et al.[27], who obtained 9ξ-euph-7-enyl acetate (LXI, R = Ac), m.p. 92-94°, $[\alpha]_D$ - 60°. The acetylated reaction product, after a preliminary filtration through alumina, crystallised as needles, m.p. 92-95°, $[\alpha]_D$ - 28°, and was shown to be a mixture when a homogeneous compound, 9ξ-euph-7-enyl acetate, separated from the filtrate as blades, m.p. 78-79°, $[\alpha]_D$ - 98°. That the initial reaction product is a mixture was confirmed by its careful chromatography. After the elution of 9ξ-euph-7-enyl acetate, m.p. 78-79°, $[\alpha]_D$ - 98°, fractions were obtained which ranged in melting point from 90° to 105°, and which failed to yield a pure acetate on subsequent crystallisation. The lower specific rotation of the mixture, when compared with that of 9ξ-euph-7-enyl acetate (LXI, R = Ac), is best explained by the presence of euph-8-enyl acetate (LII, R = Ac), $[\alpha]_D$ + 35°, as the second constituent.

The homogeneity of the 9ξ-euph-7-enyl acetate (LXI, R = Ac) obtained as described above was established by its hydrolysis to the alcohol (LXI, R = H), m.p. 62-64°, $[\alpha]_D$ - 110°, using lithium aluminium hydride; reacetylation of this alcohol gave

the same acetate. 9ξ-Euph-7-en-3-one (LXV), m.p. 121-122°, $[\alpha]_D$ - 168°, and 9ξ-euph-7-enyl benzoate (LXI, R = PhCO), m.p. 118-119°, $[\alpha]_D$ - 61°, were prepared from 9ξ-euph-7-en-3β-ol (LXI, R = H) by oxidation with the pyridine-chromium trioxide complex, and by treatment with benzoyl chloride in pyridine, respectively.

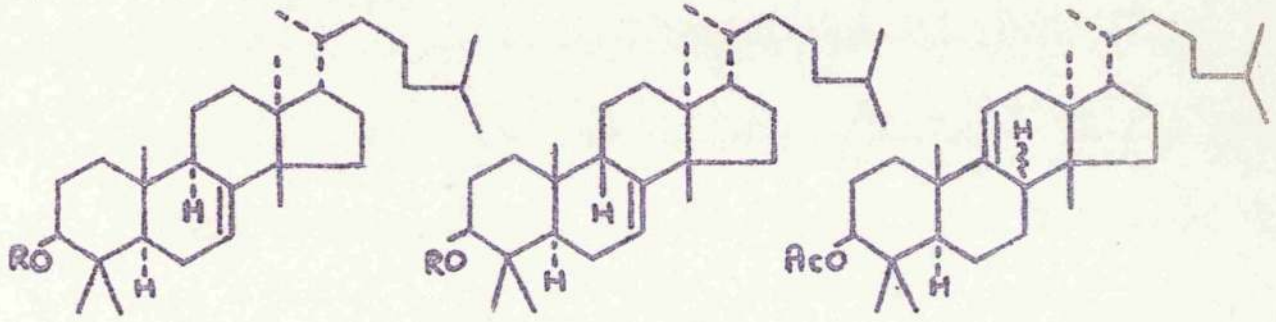


(LII) (LIX) (LXV)

The Configuration at $C_{(9)}$ in 9ξ-euph-7-enyl acetate and dihydrobutyrospermyl acetate.

The evidence which shows that the 9ξ-euph-7-enyl acetate, prepared by the above method, is the 9β-epimer of (LXI, R = Ac) is dependent upon the reactions which show that dihydrobutyrospermyl acetate is 9α-euph-7-enyl acetate (LXVI, R = Ac) and vice-versa. Such evidence was obtained from molecular rotation considerations and by a comparison of the acid-induced isomerisations of 9β-euph-7-enyl acetate (LXVII, R = Ac) with those of dihydrobutyrospermyl acetate under the same conditions. 8ξ-Euph-9(11)-enyl acetate (LXVIII) has also been prepared and is found

to differ from both 9β-euph-7-enyl acetate (LXVII, R = Ac) and dihydrobutyrospermyl acetate. Rigorous proof of the structure of dihydrobutyrospermyl acetate was obtained simultaneously by W. Lawrie[88], the principal features of which are also described.

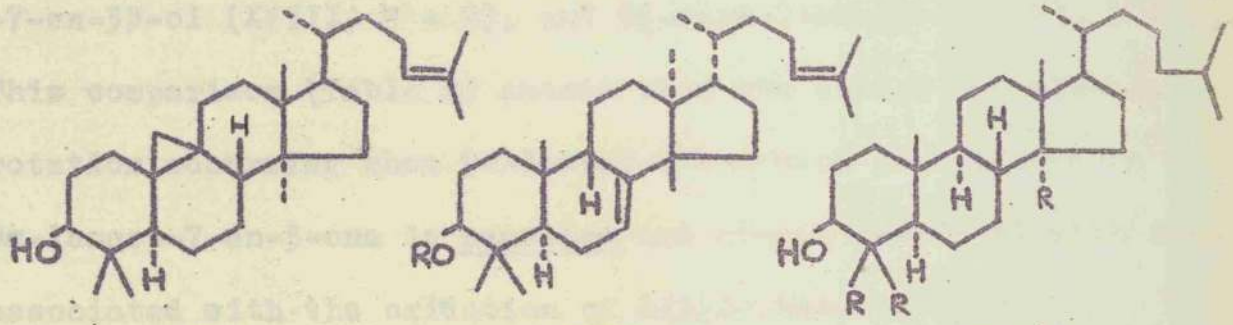


(LXVI) (LXVII) (LXVIII)

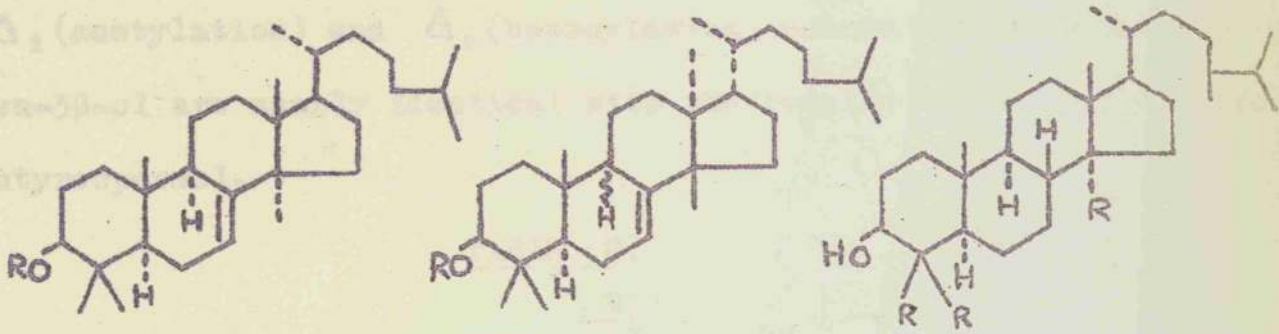
(i) Molecular rotation considerations. Barton[6] has observed that the change in molecular rotation (Δ_3) on oxidation of butyrospermol and cycloartenol to the corresponding ketones is in each case negative, an unusual feature in triterpenoid compounds, and for this reason he suggested that the two alcohols may be related. The establishment of the constitution and stereochemistry of cycloartenol as (VII)[9,12,13] led Jones and his collaborators[77,83] to suggest that the similarity in the Δ_3 values is based on a common configuration at $C_{(9)}$ and they tentatively formulated butyrospermol as 9β-eupha-7:24-dien-3β-ol (LXIX, R = H). This argument is invalidated by a closer study of the general and widely accepted guiding principles used in the

application of molecular rotation relationships to structural analysis. Terminal rings of the same type make contributions to the molecular-rotation which are very approximately independent of the rest of the molecule, provided that the adjacent ring is a saturated unsubstituted cyclohexane ring[59,69]. It is also recognised that non-angular methyl groups have little effect on the contribution of a terminal ring to the molecular-rotation. These principles have been illustrated by Klyne[59]. Thus, in general, the change in molecular-rotation (Δ_{CO}) produced by the introduction of a 3-carbonyl oxygen atom into many 5α-steroid and 5α-triterpenoid hydrocarbons is positive, and the molecular-rotation change (Δ_3) accompanying the oxidation of a 3β-hydroxy-5α-steroid or corresponding triterpenoid to the corresponding ketone is also positive. However, two exceptions to this rule are found in lanostanol (LXX, R = Me) and laudanol (LXXI, R = Me) since the change in rotation when these alcohols are oxidised to the corresponding ketones is in each case negative, as is the change accompanying the conversion of lanostane and laudane into the corresponding 3-ketones. In table B, molecular rotation changes for lanostanol (LXX, R = Me) and laudanol (LXXI, R = Me) are compared with corresponding changes for cholestanol (LXX, R = H) and ergostanol (LXXI, R = H). It is seen that the introduction of methyl groups at $C_{(4)}$ and $C_{(14)}$ has a substantial effect upon the molecular-rotation

contribution of the terminal rings in both cholestanol and ergostanol.



(VII) (LXIX) (LXX)



(XVIII) (LXI) (LXXI)

TABLE B.

	M_D^o				
	3β-alcohol	Hydrocarbon	3-ketone	Δ_3	Δ_{oc}
Lanostanol (LXX, R = Me)[40]	+150	+149	+116	-34	-33
Laudanol (LXXI, R = Me)[14]	+93	+107	+62	-31	-45
Cholestanol (LXX, R = H)[90]	+93	+91	+159	+66	+68
Ergostanol (LXXI, R = H)[91]	+64	+66	+140	+76	+74

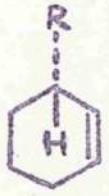
The recognition of this effect led to a comparison of the molecular-rotation changes associated with the reactions of dihydrobutyrospermol with corresponding changes for 9α-lanost-7-en-3β-ol (XVIII, R = H), and 9ξ-euph-7-en-3β-ol (LXI, R = H). This comparison (Table C) showed that the change in molecular rotation occurring when 9α-lanost-7-en-3β-ol is oxidised to 9α-lanost-7-en-3-one is <u>negative</u> and almost identical with that associated with the oxidation of dihydrobutyrospermol and butyrospermol to the corresponding 3-ketones. Moreover, the Δ_1 (acetylation) and Δ_2 (benzoylation) values for 9α-lanost-7-en-3β-ol are nearly identical with the related values for dihydrobutyrospermol.

TABLE C.

	M_D°						
	Alcohol.	Acetate.	Benzoate.	Ketone.	Δ_1	Δ_2	Δ_3
9α-Lanost-7-en-3β-ol (XVIII, R = H)[60]	+45	+156	+266	-85	+111	+221	-130
Dihydrobutyrospermol[60]	-60	+56	+164	-182	+116	+224	-122
9ξ-Euph-7-en-3β-ol (LXI, R = H)[60]	-471	-460	-325	-716	+11	+146	-245

In contrast, the corresponding values for 9ξ-euph-7-en-3β-ol are widely divergent from both those of 9α-lanost-7-en-3β-ol and of dihydrobutyrospermol. These results constitute the first substantial evidence which indicates that dihydrobutyrospermol

is 9α-euph-7-en-3β-ol (LXVI, R = H) and 9ξ-euph-7-en-3β-ol is its 9β-epimer (LXVII, R = H). The large negative change in molecular rotation accompanying the conversion of 9β-euph-7-en--3β-ol (LXVII, R = H) into the corresponding 3-ketone was at first a surprising result. However, Jones and his co-workers[92] have pointed out that in the case of substituted 3-alkyl cyclohex-1-enes, the one having the $C_{(3)}$-hydrogen atom in the β-configuration (LXXII) has a much more negative rotation than its 3α-epimer (LXXIII)[93], and for this reason they suggested that the specific rotation relationship between 9β-euph-7-enyl acetate (LXVII, R = Ac) and dihydrobutyrospermyl acetate is explained if these acetates can be regarded as a pair of substituted cyclohex--1-enes.



(LXXII)

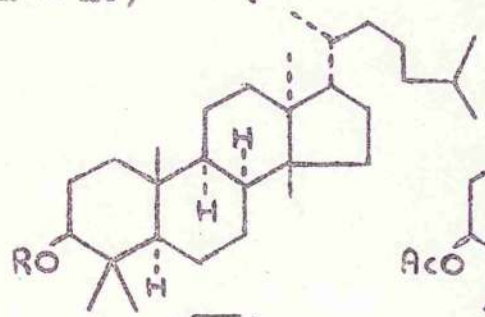
(LXXIII)

(ii) The reactions of 9ξ-euph-7-enyl acetate. The method used in the preparation of 9ξ-euph-7-enyl acetate (LXI, R = Ac) strongly suggested that this acetate has the more stable configuration at $C_{(9)}$. From an inspection of molecular models, the more stable configuration at this asymmetric centre is β-, since the stereochemistry of 9α-euph-7-enyl acetate (LXVI, R = Ac)

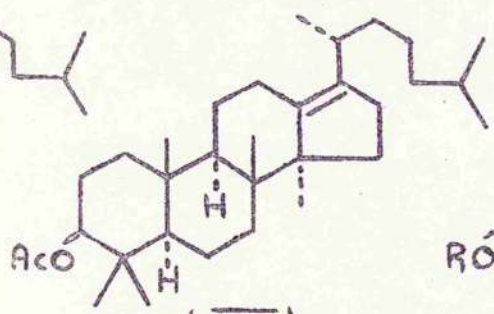
constrains the molecule to adopt a conformation which includes a boat (or half-boat); whereas 9β-euph-7-enyl acetate (LXVII, R = Ac) can assume a more stable 'all-chair' (or half-chair) conformation. Accordingly, dihydrobutyrospermyl acetate should be 9α-euph-7-enyl acetate and 9ξ-euph-7-enyl acetate should be its β-epimer; a conclusion which was established experimentally by comparing the chemical stability of these two acetates.

The behaviour of dihydrobutyrospermyl acetate towards varying acidic conditions has been described in a previous section. 9ξ-Euph-7-enyl acetate (LXI, R = Ac) was recovered unchanged after treatment with dry hydrogen chloride at 0° for 2 hours, conditions which convert dihydrobutyrospermyl acetate into euph-8-enyl acetate (LII, R = Ac). As already described, dihydrobutyrospermyl acetate was quantitatively isomerised to isoeuph-13(17)-enyl acetate (LIII) in the presence of an hydrochloric-acetic acid mixture at 100° for 3 hours. Application of this reaction to 9ξ-euph-7-enyl acetate effected a partial change; starting material, a mixture, and isoeuph-13(17)-enyl acetate were isolated from the reaction product. This result shows in fact that 9ξ-euph-7-enyl acetate is only partly isomerised to euph-8-enyl acetate (LII, R = Ac) by stronger acidic conditions than those required for the total conversion of dihydrobutyrospermyl acetate into the same acetate (LII, R = Ac). The formation of isoeuph-13(17)-enyl acetate (LIII) from 9ξ-euph-

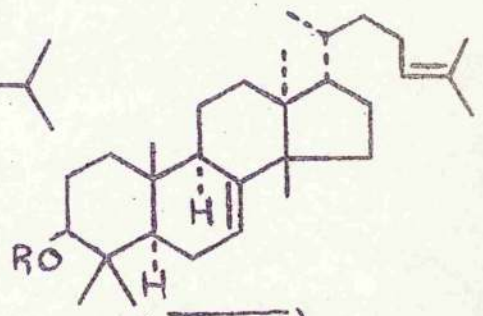
-7-enyl acetate is interpreted as a carbonium ion induced, partial movement of the 7:8-double bond to the 8:9-position to give an equilibrium mixture of the corresponding isomers, of which euph-8-enyl acetate is converted into isoeuph-13(17)--enyl acetate (LIII) by the '<u>iso</u>euphenol' rearrangement[27]. The behaviour of 9ξ-euph-7-enyl acetate in this reaction closely resembles that of 9α-lanost-7-enyl acetate (XVIII, R = Ac) which equilibrates with its Δ^8-isomer when it is treated with chloroformic hydrogen chloride[37,45,46]. In an attempt to prepare the unknown saturated euphanyl acetate (LI, R = Ac), 9ξ-euph-7-enyl acetate was shaken with hydrogen and platinum in acetic acid over a prolonged period. The acetate, however, was recovered unchanged. Under similar conditions, dihydrobutyrospermyl acetate is isomerised to euph-8-enyl acetate (LII, R = Ac)[83,84].



(<u>LI</u>)



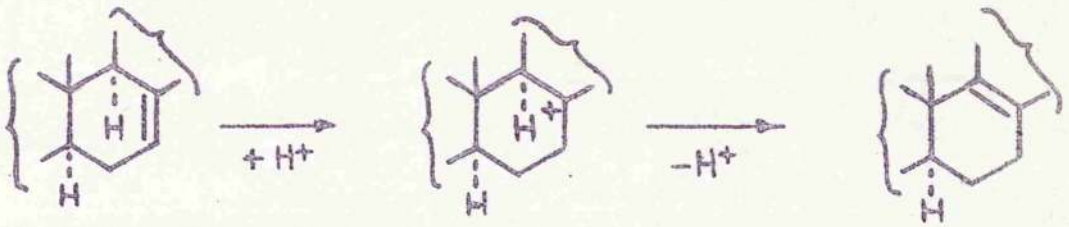
(<u>LIII</u>)



(<u>LXXIV</u>)

The experiments described above illustrate the relatively greater ease with which dihydrobutyrospermyl acetate is converted into euph-8-enyl acetate when compared with 9ξ-euph-7-enyl

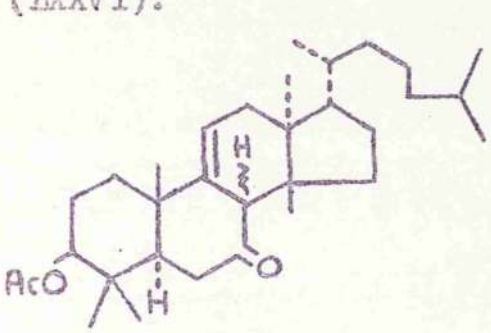
acetate (LXI, R = Ac). Consequently the strained conformation of the dihydrobutyrospermyl acetate molecule constitutes the driving force motivating its irreversible conversion into euph-8-enyl acetate. Dihydrobutyrospermyl acetate and 9ξ-euph-7-enyl acetate are therefore 9α-euph-7-enyl acetate (LXVI, R = Ac) and 9β-euph-7-enyl acetate (LXVII, R = Ac) respectively, and it follows that butyrospermol is 9α-eupha-7:24-dien-3β-ol (LXXIV, R = H).



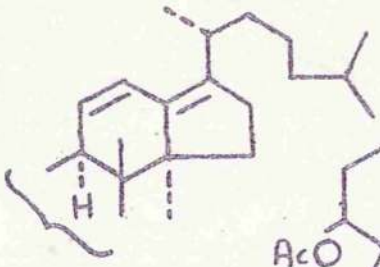
(LXVI) (LII)

(iii) Oxoapoeuphenyl acetate. The above conclusions are in agreement with the direct evidence obtained from a study of the oxidation product of dihydrobutyrospermyl acetate. W. Lawrie[88] showed that oxidation of this acetate with chromium trioxide gave, as the major product, a non-conjugated, unsaturated oxo-acetate which he named oxoapoeuphenyl acetate. Treatment of oxoapoeuphenyl acetate with hydrochloric-acetic acid yields an oxoisoeuph-13(17)-enyl acetate, which was identified by its Wolff-Kishner reduction, followed by acetylation, to isoeuph-13(17)-enyl acetate (LIII). The carbonyl group of oxoisoeuph-13(17)-enyl acetate,

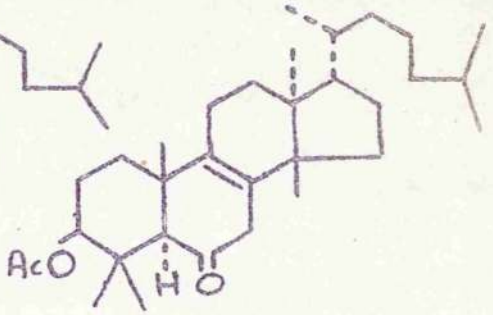
and of oxoapoeuphenyl acetate, was shown to be at positions $C_{(6)}$ or $C_{(7)}$ since oxoisoeuph-13(17)-enyl acetate is not an αβ-unsaturated ketone, and it is converted into an oxoisoeupha--11:13(17)-dienyl acetate (LXXV) by oxidation with selenium dioxide. The formation of oxoapoeuphenyl acetate from dihydrobutyrospermyl acetate involves a molecular rearrangement, otherwise oxoapoeuphenyl acetate is represented by (LXIII) or (LXXVI).



(LXIII)



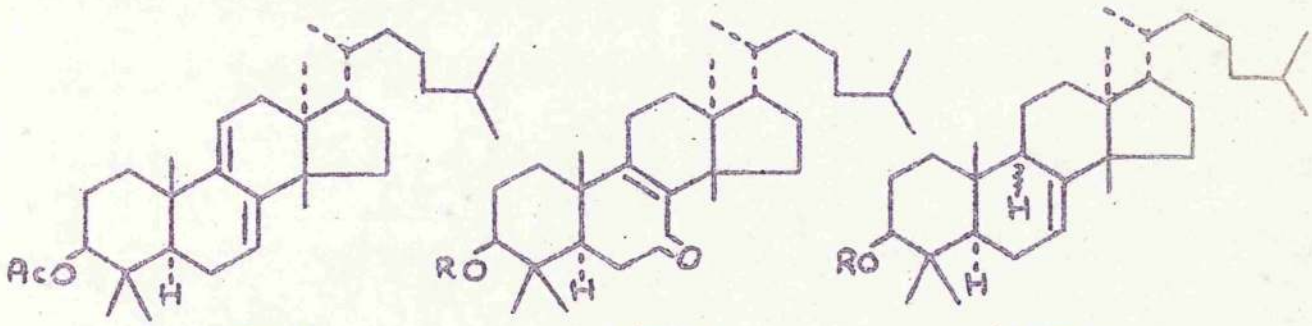
(LXXV)



(LXXVI)

8ξ-7-Oxoeuph-9(11)-enyl acetate (LXIII) can only be obtained from a euph-7-enyl acetate or a euph-9(11)-enyl acetate via eupha-7:9(11)-dienyl acetate (LVI), since a double bond will not move out of conjugation with a carbonyl group under the conditions in which oxoapoeuphenyl acetate is formed[27,65,74], and 7-oxoeuph-8-enyl acetate (LIX, R = Ac) is stable to mineral acid[27]. Structure (LXIII) for oxoapoeuphenyl acetate was therefore excluded when oxidation of eupha-7:9(11)-dienyl acetate (LVI) under the conditions used for the oxidation of dihydrobutyrospermyl acetate into oxoapoeuphenyl acetate failed to

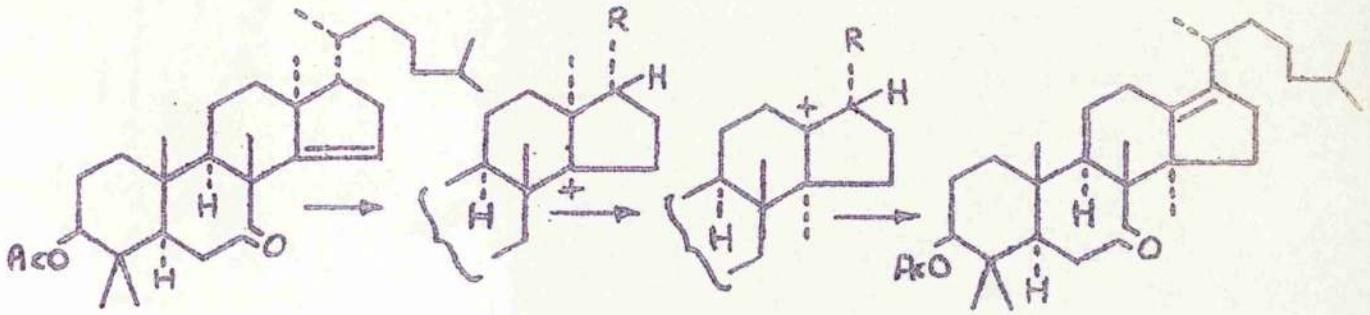
produce a non-conjugated, unsaturated ketone. Formula (LXXVI) could not represent oxoapoeuphenyl acetate since an infra-red examination of this acetate indicated the presence of a non-fully substituted double bond, and reduction of oxoapoeuphenyl acetate by the Wolff-Kishner method, followed by acetylation, gave a new isomer, apoeuphenyl acetate. The decision that oxoapoeuphenyl acetate is neither (LXIII)or (LXXVI) is supported by the observation that oxoapoeuphenyl acetate is not isomerised to an αβ-unsaturated ketone by treatment with either alkali or mineral acid. Consequently the carbonyl group of oxoapoeuphenyl acetate is located at $C_{(7)}$ and dihydrobutyrospermyl acetate is 9β-euph-7-enyl acetate (LXI, R = Ac)



(LVI) (LIX) (LXI)

Support for the view that a molecular rearrangement is included in the oxidation of dihydrobutyrospermyl acetate to 7-oxoapoeuphenyl acetate was obtained when treatment of apoeuphenyl acetate with hydrogen chloride at 0° for two hours gave iseuph-13(17)-enyl acetate (LIII). After the same treatment euph-8-enyl acetate (LXI, R = Ac) is unchanged and dihydrobutyrospermyl acetate is simply converted into euph-8-enyl

acetate[84,86]. The formation of 7-oxoapoeuphenyl acetate from dihydrobutyrospermyl acetate is represented as a synchroncus reaction in which oxidation at the 7:8-double bond is accompanied by movement of the $C_{(14)}$-methyl group to $C_{(8)}$. The initiated reaction may proceed via two paths. Firstly, the movement of the $C_{(14)}$-methyl group to $C_{(8)}$ is accompanied by, and the path terminates in, the loss of a proton from $C_{(15)}$. Following the alternative route, the migration of the 14β-methyl group to $C_{(8)}$ is accompanied by the movement of the 13α-methyl group to $C_{(14)}$ and loss of the 12β-hydrogen atom as a proton Whichever path is followed, the carbonyl group in oxoapoeuphenyl acetate is at $C_{(7)}$, and according to the first mechanism, 7-oxo-apoeuphenyl acetate is (LXXVII), and its conversion into 7-oxoiso-euph-13(17)-enyl acetate (LXXVIII), by treatment with mineral acid, involves the protonation of the 14:15-double bond, simultaneous movement of the 13α-methyl group to $C_{(14)}$ and loss of the 17β-hydrogen atom as a proton:-

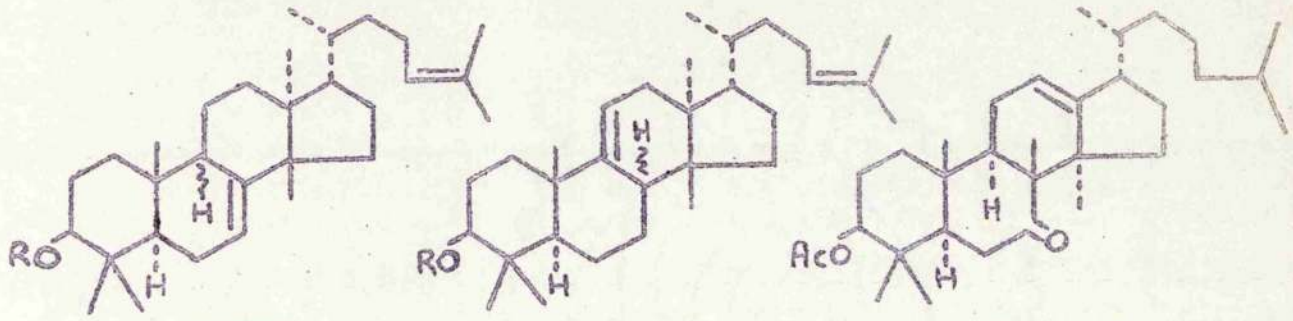


(LXXVII) (LXXVIII)

If the second path is followed, 7-oxoapoeuphenyl acetate is (LXXIX) and its subsequent isomerisation to 7-oxoisoeuph-13(17)-enyl acetate (LXXVIII) is a simple carbonium ion induced movement of the double bond from the $C_{(12)}$ to the 13:17-position. W. Lawrie[88] also obtained evidence which favoured structure (LXXVI) for 7-oxoapoeuphenyl acetate although a decision between this structure and (LXXIX) is not pertinent to the argument, which is that these mechanisms require that the $C_{(9)}$-hydrogen atom in dihydrobutyrospermyl acetate is not involved in the oxidation of this acetate to 7-oxoapoeuphenyl acetate. The configuration of the hydrogen atom at $C_{(9)}$ of dihydrobutyrospermyl acetate is therefore the same (α) as that in 7-oxoisoeuph-13(17)-enyl acetate (LXXVIII). Accordingly dihydrobutyrospermyl acetate is 9α-euph-7-enyl acetate (LXVI, R = Ac) and butyrospermol is 9α-eupha-7:24-dien-3β-ol (LXXIV, R = H).

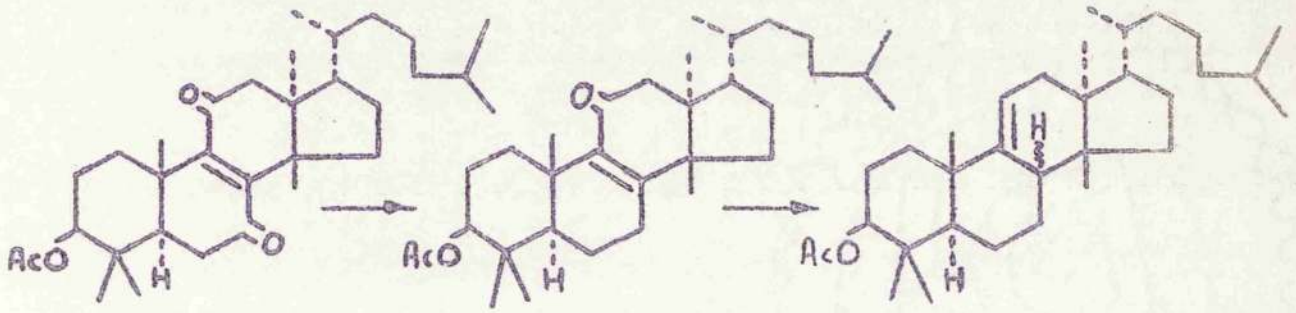
8ξ-Euph-9(11)-enyl acetate.

As previously described, earlier studies[83,84,86] identified butyrospermol as either 9ξ-eupha-7:24-dien-3β-ol (LVII, R = H) or 8ξ-eupha-9(11):24-dien-3β-ol (LVIII, R = H). The synthesis of 8ξ-euph-9(11)-enyl acetate (LXXX) was therefore undertaken in the hope of proving its identity, or otherwise, with dihydrobutyrospermyl acetate.



(LVII) (LVIII) (LXXIX)

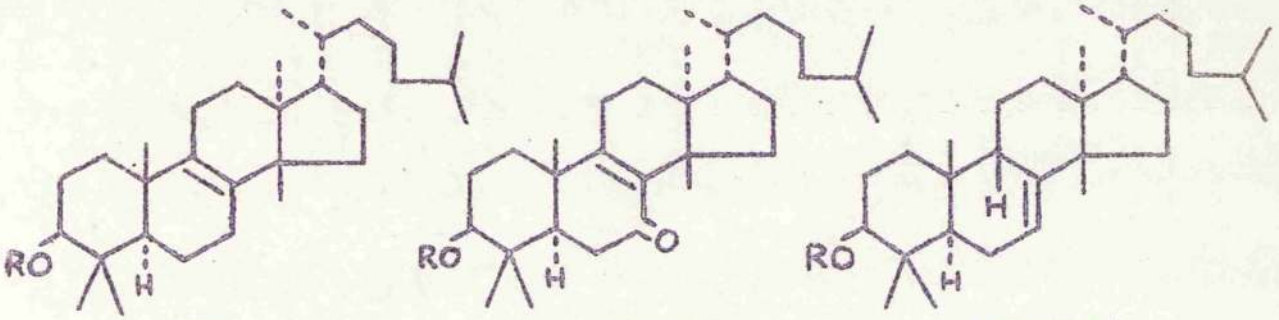
7:11-Dioxoeuph-8-enyl acetate (XLIX) was obtained by the oxidation of euph-8-enyl acetate (LII, R = Ac) with chromium trioxide according to the method reported by Christen et al.[66]. Using the Huang-Minlon modification of the Wolff-Kishner reaction[8] followed by acetylation, the more reactive carbonyl group at $C_{(?)}$ in 7:11-dioxoeuph-8-enyl acetate was reduced to give 11-oxoeuph--8-enyl acetate (LXII)[27]. The $C_{(11)}$-carbonyl group of these acetates is sterically hindered by the close proximity of the β-methyl group at $C_{(13)}$. However, it was found that application of the forcing variant of the Wolff-Kishner reaction[95] to 11-oxo-euph-8-enyl acetate (LXII), and acetylation of the product, effected a reduction of the carbonyl group accompanied by the simultaneous movement of the double bond from the 8:9-position to the 9:11-position to give 8ξ-euph-9(11)-enyl acetate (LXXX), m.p. 100-101°, $[\alpha]_D$ - 58.6°. The acetate (LXXX) was only obtained after extensive purification of the crude product which had a specific rotation of - 27°. It is probable, therefore, that



(XLIX) (LXII) (LXXX)

some euph-8-enyl acetate (LII, R = Ac), $[\alpha]_D$ +35°, is also formed during the reduction of 11-oxoeuph-8-enyl acetate (LXII); an observation already recorded in the formation of 9β-euph-7--enyl acetate (LXVII, R = Ac) from 7-oxoeuph-8-enyl acetate (LIX, R = Ac) by the same method. 8ξ-Euph-9(11)-enyl acetate (LXXX) shows the characteristic ultra-violet light absorption maximum of an isolated double bond (ξ_{204} = 3,830) and gives a yellow colour with tetranitromethane. The melting point of the acetate is largely depressed by 9β-euph-7-enyl acetate and the physical constants of 8ξ-euph-9(11)-enyl acetate differ widely from those found for dihydrobutyrospermyl acetate. 8ξ-Euph-9(11)--enyl acetate was shown to be a simple double bond isomer of euph-8-enyl acetate by its oxidation with selenium dioxide. An ultra-violet examination of the resulting product revealed the presence of eupha-7:9(11)-dienyl acetate (LVI). The configuration of the hydrogen atom at $C_{(8)}$ of 8ξ-euph-9(11)-enyl acetate has

not been established. However it has been shown that the Wolff-Kishner reduction of 7-oxoeuph-8-enyl acetate (LIX, R = Ac)



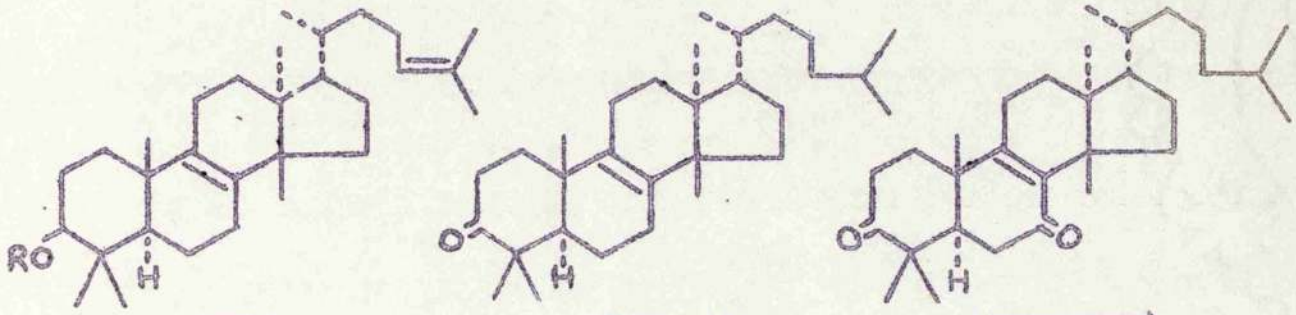
(LII) (LIX) (LXVII)

leads to the formation of the more stable epimer which is 9β-euph-7-enyl acetate (LXVII, R = Ac). The $C_{(8)}$-hydrogen atom of 8ξ-euph-9(11)-enyl acetate is therefore tentatively assigned the α-configuration on the basis that with such a stereochemistry the molecule can adopt a stable 'all-chair' conformation. The synthesis of this acetate, and its non-identity with dihydrobutyrospermyl acetate or with 9β-euph-7-enyl acetate, gave support to the view that the nuclear double bond of dihydrobutyrospermyl acetate is located at the 7:8-position rather than the 9:11-position.

7-Oxoeuph-8-en-3-one.

7-Oxoeuph-8-enyl acetate (LIX, R = Ac) has been prepared by the low temperature ozonolysis of euph-8-enyl acetate (LII, R = Ac)[27]. An alternative method for the introduction of the $\Delta^{8:9}$-unsaturated, 7-oxo-system was encountered during experiments

designed to prepare euph-8-en-3-one (LXXXI). Euph-8-en-3β-ol (LII, R = H), obtained by the catalytic reduction of euphol (IX, R = H) in neutral solvent, was treated with the chromium trioxide-pyridine complex described by Poos et al.[96], and which is generally considered to be a specific reagent for the conversion of an hydroxyl group into a carbonyl group. The resulting mixture was readily separated by chromatography when light petroleum eluted the desired ketone, euph-8-en-3-one (LXXXI). Elution of the column with benzene gave yellow crystals (14%) which after purification afforded a second colourless compound, $C_{30}H_{48}O_2$. It gives no colour with tetranitromethane and shows the characteristic ultra-violet light absorption maximum (ε_{252} = 10,200) of an αβ-unsaturated ketone. It has been observed during the course of this work that in the euphol series, the absorption maxima and intensities of the two $\Delta^{8:9}$, αβ-unsaturated ketones are distinctly different. 7-Oxoeuph-8-enyl acetate (LIX, R = Ac) shows maximum absorption between 252 mu and 254 mu ($\varepsilon \doteq$ 10,000), while 11-oxoeuph-8-enyl acetate (LXII) absorbs at 256-257 mu ($\varepsilon \doteq$ 8,000). Accordingly, and since position $C_{(7)}$ is more reactive than the sterically hindered $C_{(11)}$-position, the oxidation product, $C_{30}H_{48}O_2$, is formulated as 7-oxoeuph-8-en-3-one (LXXXII).



(IX) (LXXXI) (LXXXII)

This conclusion was confirmed by the preparation of 7-oxoeuph--8-en-3-one from 7-oxoeuph-8-enyl acetate (LIX, R = Ac). Alkaline hydrolysis of (LIX, R = Ac) and subsequent oxidation of the product gave 7-oxoeuph-8-en-3-one (LXXXII), which was found to be identical with the diketone, $C_{30}H_{48}O_2$, obtained from euph-8-en-3β-ol (LII, R = H).

SECTION II.

A Partial Synthesis of Parkeol : Lanosta-9(11):24-dien-3β-ol.

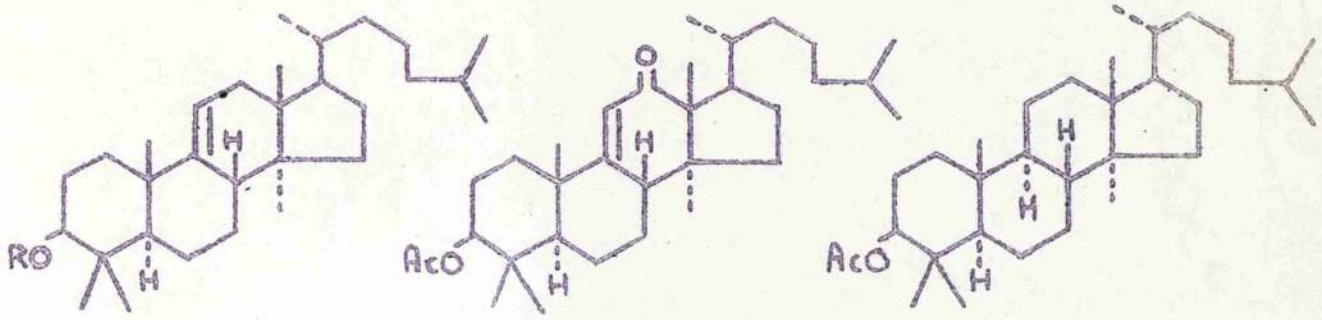
Parkeol, a tetracyclic triterpenoid isolated from shea nut fat, has been identified as lanosta-9(11):24-dien-3β-ol by its synthesis from 'isocholesterol'.

INTRODUCTION.

In 1934, Bauer and Moll[97] reported the isolation from shea nut fat, of a new alcohol, $C_{30}H_{50}O$ (or a near homologue), which they named parkeol. The alcohol, m.p. 164°, was characterised by the preparation of its acetate, m.p. 154°, and benzoate, m.p. 197°. Later, Dawson et al.[62] obtained an acetate, m.p. 154-157°, $[\alpha]_D$ + 95°, together with butyrospermyl acetate, from the acetylated non-saponifiable fraction of shea nut fat. They concluded that this compound is identical with parkeyl acetate and they prepared the parent alcohol, m.p. 162-165°, $[\alpha]_D$ + 65°. No subsequent investigation of parkeol has been reported.

A further examination of parkeol was undertaken in these laboratories by W. Lawrie[98], who obtained parkeyl acetate, m.p. 159-160°, $[\alpha]_D$ + 86°, by chromatography of the acetylated non-saponifiable fraction of shea nut fat. Parkeol, m.p. 159-160°, $[\alpha]_D$ + 76.8°, and parkeyl benzoate, m.p. 200-201°, $[\alpha]_D$ + 95.4°, were also prepared. Parkeyl acetate gives a strong yellow colour with tetranitromethane and shows ethylenic absorption in the ultra-violet region (ε_{206} = 5,100). On catalytic hydrogenation under neutral conditions, parkeyl acetate was found to absorb one mol. of hydrogen to give dihydroparkeyl acetate which is unsaturated (ε_{205} = 4,900). Parkeol is therefore a diethenoid alcohol, containing a readily reducible double bond, and consequently is tetracyclic. Dihydroparkeyl acetate was

found to be stable to mineral acid and to treatment with selenium dioxide. Oxidation of dihydroparkeyl acetate with chromium trioxide gave oxodihydroparkeyl acetate which was identified as an αβ-unsaturated ketone from its ultra-violet light absorption spectrum. Dihydroparkeyl acetate was reduced to a saturated acetate, tetrahydroparkeyl acetate, by catalytic hydrogenation at 80°. These results are analagous to the behaviour of lanost-9(11)-enyl acetate (LX, R = Ac), and strongly suggested that the less reactive double bond of parkeol is located at the 9:11-position. A direct comparison between dihydroparkeyl acetate and lanost-9(11)-enyl acetate showed no depression in melting point. Moreover, oxodihydroparkeyl acetate and tetrahydroparkeyl acetate did not depress the melting points of 12-oxolanost-9(11)-enyl acetate (LXXXIII) and lanostanyl acetate (LXXXIV) respectively. A comparison between the physical constants of corresponding derivatives in the dihydroparkeol and lanost-9(11)-enol series is given in table D.



(LX) (LXXXIII) (LXXXIV)

TABLE D.

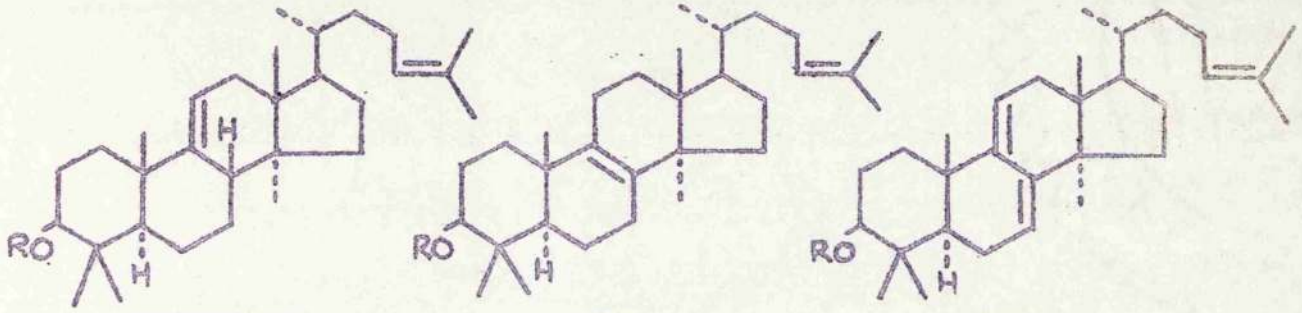
Acetate	m.p.°	$[\alpha]_D^\circ$
Dihydroparkeyl acetate	171-172	+87
Lanost-9(11)-enyl acetate (LX, R = Ac)	171-172	+89
Oxodihydroparkeyl acetate	181-183	+90
12-Oxolanost-9(11)-enyl acetate (LXXXIII)	180-182	+94
Tetrahydroparkeyl acetate	161.5-162.5	+40.5
Lanostanyl acetate (LXXXIV)[47]	156-157	+45

It followed from these results that dihydroparkeyl acetate is lanost-9(11)-enyl acetate (LX, R = Ac). The synthesis of lanosta-9(11):24-dien-3β-ol (LXXXV, R = H) was undertaken to establish the location of the more reactive double bond of parkeol.

Lanosta-9(11):24-dien-3β-ol.

The starting material used for the synthesis of lanosta-9(11):24-dien-3β-ol (LXXXV, R = H) was the 'isocholesterol' mixture obtained from the neutral fraction of sheep wool wax[6]. 'isoCholesterol' has been shown to contain lanosterol (IV, R = H) (20%), dihydrolanosterol (XV, R = H) (20%), agnosterol (VI, R = H) (20%), and a small proportion of dihydroagnosterol (LXXXVI, R = H)[99,100]. Recently the isolation of pure lanosterol from this mixture has been reported[101,102]. Voser, Jeger and Ruzicka[103] have described the preparation of 7:11-dioxolanost-24-enyl acetate (LXXXVII) from 'isocholesterol' and this conversion

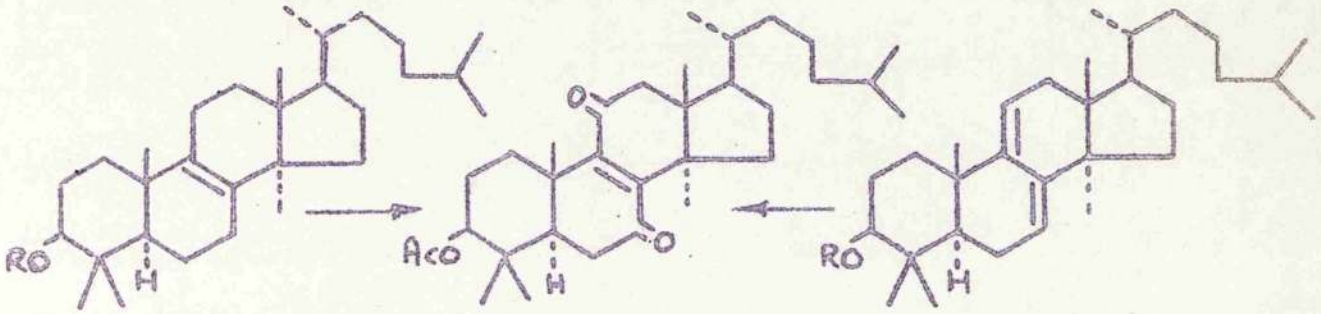
constitutes the preliminary steps in the synthesis of lanosta-9(11):24-dien-3β-ol (LXXXV, R = H).



(LXXXV) (IV) (VI)

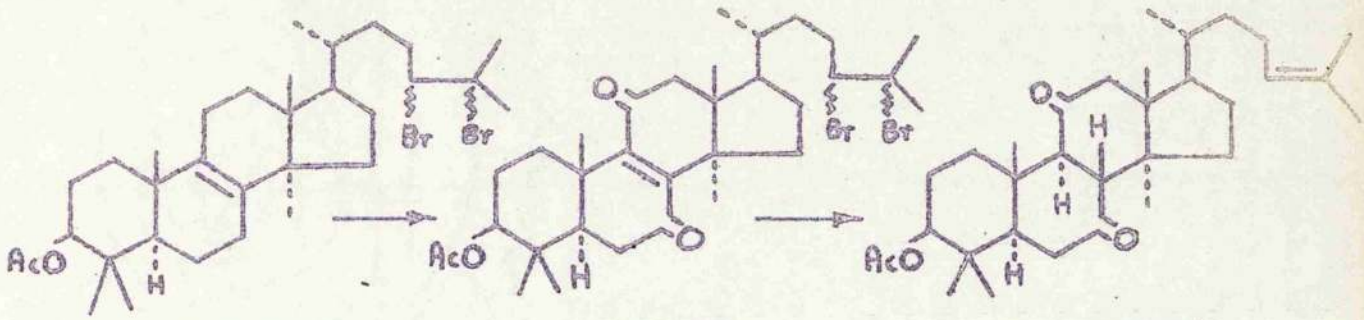
A sample of 'isocholesterol', kindly given to us by Dr. C. L. Hewitt of Organon Laboratories Ltd., was purified by acetylation, and from the ultra-violet light absorption spectrum of the product, was found to contain less than 1% of the heteroannular dienes (VI, R = Ac) and (LXXXVI, R = Ac). Treatment of the 'isocholesteryl acetate' mixture with bromine in acetic acid at room temperature gave the corresponding mixture of 24ξ:25ξ-dibromo-acetates, which had m.p. 129-134°. A pure dibromide, $C_{32}H_{52}O_2Br_2$, separated from the filtrate after collection of the dibromo-acetate mixture. It gave a positive Beilstein test, a strong yellow colour with tetranitromethane, and showed only ethylenic absorption in the ultra-violet region. Consequently it is formulated as a 24ξ:25ξ-dibromolanost-8-enyl acetate (LXXXVIII) and it is probably identical with the stereoisomeric dibromide ('B') recently isolated by Lewis and McGhie from a similar bromination of 'isocholesterol'[102].

Lanost-8-enyl acetate (XV, R = Ac) and lanosta-7:9(11)-dienyl acetate (LXXXVI, R = Ac) are both converted into 7:11-dioxolanost--8-enyl acetate (XVII) by treatment with chromium trioxide[44]. The mixture of dibromo-acetates, m.p. 129-134°, was therefore



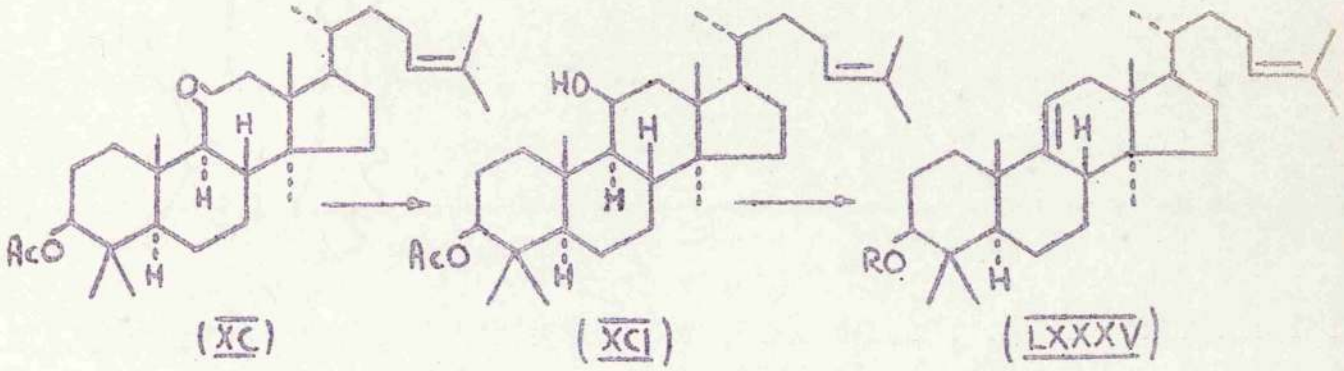
(XV) (XVII) (LXXXVI)

oxidised in this way without further purification, and both 24ξ:25ξ-dibromo-7:11-dioxolanost-8-enyl acetate (LXXXIX) and 7:11-dioxolanost-8-enyl acetate (XVII) were isolated from the neutral fraction of the reaction product. The formation of (XVII) by oxidation of the dibromide mixture shows that this product also contains lanost-8-enyl acetate (XV, R = Ac). Reduction of the dibromodionenyl acetate (LXXXIX) with zinc dust in acetic acid gave 7:11-dioxolanost-24-enyl acetate (LXXXVII) by simultaneous debromination and saturation of the nuclear double bond[103].



(LXXXVIII) (LXXXIX) (LXXXVII)

7:11-Dioxolanost-24-enyl acetate (LXXVII) was converted into 11-oxolanost-24-enyl acetate (XC) using the Huang-Minlon modification of the Wolff-Kishner reaction[94], followed by acetylation of the resulting product. The infra-red absorption spectrum of (XC) showed the presence of a six-ring carbonyl group. Reduction of 11-oxolanost-24-enyl acetate (XC) with lithium aluminium hydride in ether, followed by acetylation of the crude diol at room temperature, gave 11β-hydroxylanost-24-enyl acetate (XCI). The conversion of (XCI) into lanosta-9(11):24--dienyl acetate (LXXXV, R = Ac) was effected by its vigorous treatment with phosphorous oxychloride in pyridine. Lanosta--9(11):24-dien-3β-ol (LXXXV, R = H) was prepared by hydrolysis of the acetate (LXXXV, R = Ac) using lithium aluminium hydride; the benzoate (LXXXV, R = PhCo) was obtained from the alcohol by treatment with benzoyl chloride in pyridine.



(XC) (XCI) (LXXXV)

The physical constants of parkeol and lanosta-9(11):24--dien-3β-ol, and their corresponding derivatives are shown in Table E. It was found that parkeol and lanosta-9(11):24-dien--3β-ol exhibit the same specific rotation and a mixture of these alcohols shows no depression in melting point. The same relationship exists between parkeyl acetate and lanosta-9(11):24--dienyl acetate (LXXXV, R = Ac), and between parkeyl benzoate and lanosta-9(11):24-dienyl benzoate (LXXXV, R = PhCo). Consequently parkeol is identified as lanosta-9(11):24-dien-3β-ol (LXXXV, R = H).

TABLE E.

Compound.	m.p.°	$[\alpha]_D^\circ$
Parkeol	159-160	+76.8
Lanosta-9(11):24-dien-3β-ol (LXXXV, R = H)	157-158	+76
Parkeyl acetate	159-160	+86
Lanosta-9(11):24-dienyl acetate (LXXXV, R=Ac)	161-162	+86
Parkeyl benzoate	200-201	+95.4
Lanosta-9(11):24-dienyl benzoate (LXXXV, R=PhCO)	199-200	+94

The same conclusion was also reached by W. Lawrie[90] who found that treatment of parkeyl acetate with osmium tetroxide followed by oxidation of the resulting product with lead tetra-acetate gave acetone, which was isolated as its 2:4-dinitrophenylhydrazone. In this way he showed that parkeol contains an isopropylidine group and not a vinylidine group. The formulation of parkeol as lanosta-9(11):24-dien-3β-ol has also been confirmed by infra-red studies. The infra-red spectra of parkeol and lanosta-9(11):24-dien-3β-ol, and their corresponding derivatives, measured in Nujol or carbon tetrachloride, were found to be identical.

E X P E R I M E N T A L.

All melting points are uncorrected. Specific rotations were measured in chloroform solution in a 1 dm. tube at room temperature and ultra-violet light absorption spectra were measured in an absolute ethanol solution using a Unicam S.P. 500 spectrophotometer. Grade II alumina and a light petroleum fraction of b.p. 60-80° were used for chromatography, unless otherwise specified. Colour reactions with tetranitromethane were observed in chloroform solutions. Infra-red absorption spectra were measured by Dr. G. T. Newbold and the analyst was Mr. W. McCorkindale of the Chemistry Department of the Royal College of Science and Technology, Glasgow. C.1.

I. EUPHOL DERIVATIVES.

Saponification of Shea Nut Fat. - A solution of shea nut fat (5.5 kg.) in ethanolic potassium hydroxide solution (10%, 15 l.) was heated under reflux for 5 hours, poured into water, and the mixture extracted with ether. The ethereal extract was washed with aqueous ethanol (30%), and water, and dried (Na_2SO_4). Evaporation in vacuo gave the non-saponifiable fraction as a yellow, low melting solid (166 g., 3%).

Butyrospermyl Acetate. - The non-saponifiable fraction (166 g.) was refluxed with acetic anhydride (830 c.c.) for 4 hours and the solution kept at room temperature for 24 hours.

The semi-crystalline mass was collected and the filtrate kept at 0° for 3 days, when a second crop (A, 11.1 g.) separated. Acetic anhydride (520 c.c.) was added to the first crop; the mixture heated under reflux for 4 hours, kept at 20° for 12 hours, and filtered. The filtrate was kept at room temperature for 3 days, and the separated solid (B, 8.2 g.) collected. Three crystallisations of the solid A from ethanol-ethyl acetate (10:3) gave 'basseol acetate' as needles (3.8 g.), m.p. 135-136°, $[\alpha]_D$ + 26° (c, 1.4). Similarly three crystallisations of fraction B from the same solvent mixture gave 'basseol acetate' as needles (3.5 g.), m.p. 135-136°, $[\alpha]_D$ + 24.5° (c, 2.5). These crystals were combined and crystallised six times from the same solvent mixture to give butyrospermyl acetate (1.1 g.) as stout needles m.p. 143-144°, $[\alpha]_D$ + 12.5° (c, 3.5); the melting point and specific rotation did not change after repeated crystallisation.

Dihydrobutyrospermyl Acetate. - (1) Butyrospermyl acetate (1.01 g.) in ethyl acetate (75 c.c.) was shaken with hydrogen and a suspension of pre-reduced platinum catalyst (300 mg.) in ethyl acetate (15 c.c.) at room temperature for 4 hours. Evaporation of the filtered solution under reduced pressure, and crystallisation of the residue from chloroform--methanol gave dihydrobutyrospermyl acetate (933 mg.) as prismatic needles, m.p. 133-134°, $[\alpha]_D$ + 11.5° (c, 1.1).

(2) 'Basseol acetate (m.p. 135-136°, $[\alpha]_D$ + 23.5°, 4.9 g.) in ethyl acetate (200 c.c.) was shaken with hydrogen and platinum catalyst (300 mg.) at 20° for 9 hours. Removal of the catalyst and evaporation of the filtrate *in vacuo* gave a gum (5.1 g.) which was purified by chromatography of its solution in light petroleum on alumina (220 g.). The fraction (3.74 g.) eluted with the same solvent (3.75 l.) crystallised from chloroform-methanol to give dihydrobutyrospermyl acetate (2.74 g.) as prismatic needles, m.p. and mixed m.p. 134-135°, $[\alpha]_D$ + 10° (*c*, 4.5).

Treatment of Dihydrobutyrospermyl Acetate with Hydrochloric-Acetic Acid. - Dihydrobutyrospermyl acetate (65 mg. was dissolved in a solution of hydrochloric acid in acetic acid (1:20, 3 c.c.) and the mixture kept at 100° for 3 hours. The product, isolated by means of ether, was crystallised twice from chloroform-methanol when isoeuph-13(17)-enyl acetate (33 mg.) separated as leaflets, m.p. 110-111°, $[\alpha]_D$ - 9.7° (*c*, 1.0). Its melting point was undepressed by an authentic specimen prepared by similar acid treatment of euph-8-enyl acetate.

Euphol (eupha-8:24-dien-3β-ol). - Finely ground euphorbium resin (3.0 kg.) was extracted with boiling acetone (6 l.) for 3 hours, the hot mixture filtered, and the filtrate kept at room temperature for 2 days. The wax (250 g.) which separated during this period was collected, and after drying,

heated under reflux with light petroleum (2.5 l.) for 1 hour. After cooling, the mixture was filtered and the filtrate evaporated under reduced pressure. 'Euphorbone' was obtained as a yellow, low melting solid (234 g., 8%). A solution of this material (95 g.) in light petroleum (4 l.) was percolated through a column (71 x 7.5 cm.) of alumina (Grade III, 3 kg.). Elution with this solvent (11 l.) and benzene-light petroleum (1:9, 6 l.) yielded a colourless wax (3.6 g.) which was not examined. The fractions eluted by benzene-light petroleum (1:4, 19 l.) crystallised from acetone to give euphol (23.0 g.) as long needles, m.p. 114-116°, $[\alpha]_D$ + 33° (c, 1.7).

Newbold and Spring[26] give m.p. 116°, $[\alpha]_D$ + 32° for euphol. Continued elution of the column with the same solvent mixture (10 l.) and crystallisation of the eluate from acetone gave euphorbol (15.0 g.) as rosettes, m.p. and mixed m.p. 118-122°

Euphyl Acetate (eupha-8:24-dienyl acetate). - Euphol (22.0 g.) was heated on the steam bath with acetic anhydride (20 c.c.) and pyridine (20 c.c.) for 2 hours, the solution poured into water, and the mixture extracted with ether (2 l.). After washing successively with water, dilute acid (3N HCl) and water, the extract was dried (Na_2SO_4) and evaporated to dryness. The residue crystallised from chloroform-methanol to give euphyl acetate (20.0 g.) as long needles, m.p. 107-109°, $[\alpha]_D$ + 39° (c, 2.1). Newbold and Spring[26] give m.p. 109°, $[\alpha]_D$ + 41°.

Euph-8-enyl Acetate. - A solution of euphyl acetate (19.0 g.) in ethyl acetate (250 c.c.) was shaken with hydrogen and a suspension of pre-reduced platinum catalyst (1.0 g.) at room temperature for 3 hours. Evaporation of the filtered solution in vacuo and crystallisation of the residue from chloroform-methanol gave euph-8-enyl acetate (15.0 g.) as long needles, m.p. 124-125°, $[\alpha]_D$ + 34.9° (c,3.1). Newbold and Spring[26] give m.p. 124°, $[\alpha]_D$ + 34.5°.

Ozonolysis of Euph-8-enyl Acetate. - (1) At -5° to -10° (a) A solution of euph-8-enyl acetate (10.1 g.) in ethyl acetate (300 c.c.) was treated with a slow stream of ozonised oxygen for 2 hours, during which the temperature of the solution was kept between -5° and - 10°. After washing successively with aqueous ferrous sulphate, saturated sodium bicarbonate solutions, and water; evaporation of the dried (Na_2SO_4) solution gave the product as a yellow gum (10.6 g.) which was adsorbed from light petroleum on alumina (330 g.). Elution of the column with benzene-light petroleum (1:1, 3.2 l.) and crystallisation of the fraction from methanol yielded yellow needles (490 mg.), m.p. 93-103°, repeated crystallisation of which gave 11-oxoeuph-8-enyl acetate (120 mg.) as blades, m.p. 127-128°, $[\alpha]_D$ + 25° (c,0.8). Light absorption: max. at 257 mu (ε = 8,200). It does not give a colour with tetranitromethane.

Barton *et al.*[27] give m.p. 130-131°, $[\alpha]_D$ + 28° for this acetate.

Continued elution of the alumina with benzene-light petroleum (3:1, 4 l.) and benzene (4.8 l.), followed by three crystallisations of the combined eluates from methanol, afforded 7-oxoeuph-8-enyl acetate (716 mg.) as small needles, m.p. 163-165°, $[\alpha]_D$ + 35.3° (*c*, 1.4). Light absorption: max. at 254 mu (ε = 10,010). It gives no colour with tetranitromethane.

Barton *et al.*[27] give m.p. 162-164°, 168-170°, $[\alpha]_D$ + 35°.

(b) Euph-8-enyl acetate (1.25 g.) was treated with ozone under the conditions described above, and the product chromatographed on alumina (30 g.). The gum (214 mg.) eluted with benzene-light petroleum (1:4, 200 c.c.) crystallised from methanol to give 7:11-dioxoeuph-8-enyl acetate (100 mg.) as yellow needles, m.p. and mixed m.p. 107°. Light absorption: max. at 272 mu (ε = 8,600). Continued elution with the same solvent mixture (500 c.c.) and benzene-light petroleum (1:1, 400 c.c.) gave 7-oxoeuph-8-enyl acetate (80 mg.) as needles from methanol, m.p. and mixed m.p. 163-166°.

(2) *At* -35°. Euph-8-enyl acetate (6.6 g.) in ethyl acetate (300 c.c.) was ozonised at -35° for 2 hours. The product, isolated as described above, was obtained as a yellow gum (7.1 g.) which was chromatographed on alumina (220 g.). Crystallisation of the combined fractions eluted with light petroleum (4.5 l.)

and benzene-light petroleum (1:4, 2.25 l.) from chloroform--methanol gave euph-8-enyl acetate (2.25 g.) as stout needles, m.p. 125-126° (no depression), $[\alpha]_D$ + 34.6° (c,3.0). Elution of the column with benzene-light petroleum (1:1, 1.25 l.) yielded a fraction (520 mg.) which, after three crystallisations from methanol, was obtained as pale yellow needles, m.p. 96-98°. Light absorption: max. at 205 mu (ε = 4,680) and 256 mu (ε = 4,340). This product was treated with sulphuric acid as described below. Benzene-light petroleum (3:1, 2.25 l.) and benzene (2 l.) eluted fractions which gave 7-oxoeuph-8-enyl acetate (400 mg.) from methanol as small needles, m.p. 166-168°, $[\alpha]_D$ +35.5° (c,0.8). Light absorption: max. at 254 mu (ε = 10,000).

Treatment of the Fraction, m.p. 96-98°, with Sulphuric--Acetic acid. - A solution of the above fraction (500 mg.) in acetic acid (17 c.c.) containing concentrated sulphuric acid (2 drops) was heated under reflux for 10 minutes. The product, isolated by means of ether as a brown gum, light absorption: max. at 202 mu (ε = 3,440) and 256 mu (ε = 8,800), was dissolved in light petroleum and the solution filtered through a column of alumina (15 g.). Elution with the same solvent (600 c.c.) and crystallisation of the fraction from methanol gave 11-oxoeuph--8-enyl acetate (143 mg.) as blades, m.p. 127-128° (no depression), $[\alpha]_D$ + 26.2° (c,2.2). Light absorption: max. at 257 mu (ε = 8,400). Benzene-light petroleum (1:4, 200 c.c.) eluted a yellow

gum (43 mg.) which did not crystallise. Continued elution with the same solvent mixture (250 c.c.) and benzene-light petroleum (1:1, 300 c.c.) yielded a fraction which was crystallised twice from methanol. 7-Oxoeuph-8-enyl acetate (52 mg.) separated as small needles, m.p. 162-164°, $[\alpha]_D$ +35.3° (c, 1.4). Light absorption: max. at 254 mu (ε = 10,000).

Treatment of 7-Oxoeuph-8-enyl Acetate with Alkali. - The acetate (60 mg.) was heated under reflux with methanolic potassium hydroxide solution (10%, 15 c.c.) for 24 hours and the alcohol, isolated by ether extraction, was treated with acetic anhydride (5 c.c.) and pyridine (5 c.c.) at 100° for 1 hour. The product was obtained as a yellow gum which crystallised from methanol to give 7-oxoeuph-8-enyl acetate (41 mg.) as rosettes, m.p. 162° (no depression).

9β-Euph-7-enyl Acetate. - A solution of 7-oxoeuph-8-enyl acetate (690 mg.) in redistilled diethylene glycol (30 c.c.) containing hydrazine hydrate (100%, 1.0 c.c.) was kept at 185° for 1 hour, then cooled to 70° and treated with a solution prepared from sodium (700 mg.) and diethylene glycol (12 c.c.). The reaction solution was distilled until it refluxed gently at 210-220° and maintained at this temperature for 5 hours. The product (640 mg.) was isolated by means of ether and heated on the steam bath with acetic anhydride (5 c.c.) and pyridine (5 c.c.) for 2 hours.

(1) A solution of the dry acetylated material, obtained as a brown gum (712 mg.), in light petroleum was percolated through a short column of alumina (22 g.). Crystallisation of the combined fractions eluted by the same solvent (650 c.c.) from acetone-methanol gave 9β-euph-7-enyl acetate (210 mg.) as thin blades, m.p. 78-79°, $[\alpha]_D$ - 98° (c,0.4). Light absorption: max. at 206 mu (ξ = 3,060). The acetate gives a yellow colour with tetranitromethane.

(Found: C,81.3; H,11.5. Calc. for $C_{32}H_{54}O_2$: C,81.6; H,11.6%).

Barton *et al.*[27] give m.p. 92-94°, $[\alpha]_D$ - 60° for this acetate.

Continued elution of the column with light petroleum gave fractions which crystallised from acetone-methanol as stout needles (75 mg.), m.p. 91-93°, $[\alpha]_D$ - 22° (c,1.1). Light absorption: max. at 205 mu (ξ = 5,810). A homogeneous acetate could not be obtained by repeated crystallisation of the crystals.

(2) Alternatively, a solution of the dry acetylated gum in light petroleum was filtered through a column of alumina. Evaporation of the solution and crystallisation of the residue from acetone-methanol gave an acetate mixture as needles, m.p. 92-95°, $[\alpha]_D$ - 28° (c,1.1). 9β-Euph-7-enyl acetate separated from the filtrate as blades, m.p. 78-79°, $[\alpha]_D$ - 97° (c,0.9).

9β-Euph-7-en-3β-ol. - 9β-Euph-7-enyl acetate (202 mg.) in dry ether (50 c.c.) was heated under reflux with lithium

aluminium hydride (500 mg.) for 30 minutes. Excess hydride was destroyed by the addition of ice and the solution washed successively with dilute acid (5N H_2SO_4) and water, and dried (Na_2SO_4). Evaporation *in vacuo* gave the product as a clear gum which crystallised from methanol to give 9β-*euph*-7-*en*-3β-*ol* (140 mg.) as felted needles, m.p. 62-64°, $[\alpha]_D$ - 110° (*c*, 0.7). Light absorption: max. at 206 mu (ε = 3,200). It gives a yellow colour with tetranitromethane.

(Found: C, 83.9; H, 12.0. $C_{30}H_{52}O$ requires C, 84.0; H, 12.2%).

Acetylation of the alcohol (174 mg.) in the usual manner, followed by crystallisation from acetone-methanol gave 9β-euph-7-enyl acetate (120 mg.) as long blades, m.p. 76-78° (no depression), $[\alpha]_D$ - 93° (*c*, 0.8).

9β-*Euph*-7-*enyl* *Benzoate*. - A solution of 9β-euph-7-en-3β-ol (59 mg.) in pyridine (5 c.c.) containing benzoyl chloride (2 c.c.) was kept at 100° for 3 hours. The product, isolated by ether extraction as a yellow gum (91 mg.), was adsorbed from light petroleum on a column of alumina (2.7 g.). Crystallisation of the combined fractions eluted with this solvent (125 c.c.) from acetone-methanol gave 9β-*euph*-7-*enyl* *benzoate* (50 mg.) as leaflets, m.p. 118-119°, $[\alpha]_D$ - 61° (*c*, 1.0).

(Found: C, 83.4; H, 10.5. $C_{37}H_{58}O_2$ requires C, 83.4; H, 10.6%).

9β-*Euph*-7-*en*-3-*one*. - The suspension prepared by treating chromium trioxide (120 mg.) with pyridine (2 c.c.) was

added to a solution of 9β-euph-7-en-3β-ol (117 mg.) in pyridine (5 c.c.), and the mixture allowed to stand at room temperature for 12 hours with occasional shaking. Ether extraction in the normal manner gave the product as a gum which was chromatographed on alumina (4 g.). Light petroleum (250 c.c.) eluted fractions (75 mg.) which crystallised from methanol to give 9β-euph-7-en-3-one (31 mg.) as blades, m.p. 121-122°, $[\alpha]_D$ - 168° (c, 0.6). Light absorption: max. at 205 mu (ε = 3,810). The ketone gives a yellow colour with tetranitromethane.

(Found: C, 84.4; H, 11.8. $C_{30}H_{50}O$ requires C, 84.4; H, 11.8%).

Treatment of 9β-Euph-7-enyl Acetate with Hydrogen Chloride. - A solution of 9β-euph-7-enyl acetate (113 mg.) in chloroform (15 c.c.), and cooled to 0°, was treated with a stream of dry hydrogen chloride for 2 hours. After washing with dilute alkali and water, the solution was dried (Na_2SO_4), and the solvent removed in vacuo. Two crystallisations of the residue from acetone-methanol gave 9β-euph-7-enyl acetate (68 mg.) as blades, m.p. 76-78° (no depression), $[\alpha]_D$ - 95° (c, 0.9).

Treatment of 9β-Euph-7-enyl Acetate with Hydrochloric-Acetic Acid. - The acetate (57 mg.) was dissolved in a mixture of hydrochloric and acetic acids (1:20, 3 c.c.) and the solution heated on the steam bath for 3 hours. The solution was poured into water and the product, isolated by means of ether, was chromatographed on a short column of alumina (2.0 g.). Elution

with light petroleum (25 c.c.) gave a fraction (11.4 mg.) which crystallised from acetone-methanol to yield 9β-euph-7-enyl acetate as blades, m.p. 76-78° (no depression). Continued elution (25 c.c.) and crystallisation of the eluate (27 mg.) from acetone-methanol gave a mixture as plates, m.p. 80-95°. Crystallisation of the fraction (8 mg.) eluted with a further 50 c.c. of light petroleum from methanol afforded isoeuph-13(17)-enyl acetate as leaflets, m.p. and mixed m.p. 110-111°.

Attempted Hydrogenation of 9β-Euph-7-enyl Acetate. - A solution of 9β-euph-7-enyl acetate (58 mg.) in stabilised acetic acid (50 c.c.) was shaken with hydrogen and pre-reduced platinum catalyst (100 mg.) for 18 hours at room temperature. Evaporation of the filtered solution under reduced pressure and crystallisation of the residue from acetone-methanol gave 9β-euph-7-enyl acetate (38 mg.) as blades, m.p. 76-78° (no depression), $[\alpha]_D$ - 97° (c, 0.9)

7:11-Dioxoeuph-8-enyl Acetate. - A solution of chromium trioxide (15.0 g.) in acetic acid (95%, 220 c.c.) was added dropwise, over 2½ hours, to a stirred solution of euph-8-enyl acetate (15.1 g.) in a mixture of acetic acid (300 c.c.) and methylene chloride (75 c.c.). The reaction solution was kept at 50° for 4 hours when excess oxidant was destroyed by the addition of methanol. Ether extraction of the neutral product in the usual manner yielded a brown gum (15.0 g.), a solution of

which in light petroleum (100 c.c.) was percolated through a column of alumina (400 g.). The combined fractions (8.4 g.) eluted with benzene-light petroleum mixtures (1:4, 3 l., 2:3, 0.9 l., 1:1, 1.2 l., 3:1, 0.9 l.) and benzene (1.2 l.) crystallised from methanol to give 7:11-dioxoeuph-8-enyl acetate (6.0 g.) as yellow needles, m.p. 112-113°, $[\alpha]_D$ + 23.2° (c, 1.7). Light absorption: max. at 272 mu (ε = 8,650).

Christen et al.[66] give m.p. 113-114°, $[\alpha]_D$ + 20°.

11-Oxoeuph-8-enyl Acetate. - 7:11-Dioxoeuph-8-enyl acetate (4.8 g.) in redistilled diethylene glycol (245 c.c.) containing hydrazine hydrate (100%, 4.9 c.c.) was heated at 185-190° for 1 hour, then treated with a solution prepared by reacting sodium (4.8 g.) with diethylene glycol (81 c.c.), and the mixture refluxed at 220° for 6 hours. The product, isolated by means of ether as a yellow gum (4.2 g.), was heated on the steam bath with acetic anhydride (10 c.c.) and pyridine (10 c.c.) for 1.5 hours. A solution of the dry acetylated material (4.6 g.) in light petroleum was chromatographed on alumina (140 g.). The fractions eluted with this solvent (1.8 l.), benzene-light petroleum mixtures (1:3, 1.5 l., 1:1, 1.2 l., 3:1, 0.6 l.) and benzene (1.5 l.) were combined and crystallised twice from methanol. 11-Oxoeuph-8-enyl acetate (2.6 g.) separated as large blades, m.p. 128-129°, $[\alpha]_D$ + 27.9° (c, 1.7). Light absorption: max. at 257 mu (ε = 8,400). It gives no colour with tetranitromethane.

Barton *et al.*[27] give m.p. 130-131°, $[\alpha]_D$ + 28° for this acetate.

8ξ-*Euph*-9(11)-*enyl Acetate*. - 11-Oxoeuph-8-enyl acetate (2.36 g.) in redistilled diethylene glycol (150 c.c.) was treated with a solution obtained by reacting sodium (6.80 g.) with diethylene glycol (390 c.c.), and the mixture heated to 200°. Anhydrous hydrazine was distilled (under nitrogen) into the mixture until it refluxed at 180°. After 18 hours at this temperature, excess hydrazine was removed by distillation until the mixture refluxed at 212°. The mixture was maintained at this temperature for a further 36 hours. The product was isolated by means of ether as a brown gum (2.16 g.) which was treated with acetic anhydride (20 c.c.) and pyridine (20 c.c.) at 100° for 2 hours. A solution of the dry acetylated material (2.20 g.) in light petroleum (75 c.c.) was filtered through a column (18 x 3 cm.) of alumina (75 g.). Elution with light petroleum (600 c.c.) gave fractions (200 mg.) which crystallised from acetone-methanol to give 8ξ-*euph*-9(11)-*enyl acetate* as needles, m.p. 100-101°, $[\alpha]_D$ - 58.6° (c,1.2). Light absorption: max. at 204 mu (ε = 3,830). It gives a yellow colour with tetranitromethane. A mixture with 9β-euph-7-enyl acetate (m.p. 78-79°) had m.p. 76-80°.

(Found: C,81.5; H,11.6. $C_{32}H_{54}O_2$ requires C,81.6; H,11.6%).

Continued elution with the same solvent (1.8 l.) and benzene-light

petroleum (1:9, 300 c.c.) yielded fractions (413 mg.), crystallisation of which from acetone-methanol gave an acetate mixture as blades, m.p. 91-95°, $[\alpha]_D$ - 26° (c, 1.4). Recrystallisation of this mixture gradually changed its melting point and specific rotation; a homogeneous product could not be obtained thereby.

Treatment of 8ξ-Euph-9(11)-enyl Acetate with Selenium Dioxide. - A solution of 8ξ-euph-9(11)-enyl acetate (93 mg.) in acetic acid (15 c.c.), containing selenium dioxide (60 mg.), was heated under reflux for 9 hours, poured into water and the mixture extracted with ether in the usual way. The product was obtained as a yellow gum (83 mg.). Light absorption: max at 232 mu (ξ = 8,700), 240 mu (ξ = 9,110) and 247 mu (ξ = 6,150).

Euph-8-en-3β-ol. - Eupha-8:24-dien-3β-ol (10.0 g.) in ethyl acetate (125 c.c.) was added to a suspension of pre-reduced platinum catalyst (1.0 g.) in ethyl acetate (25 c.c.), and the mixture shaken with hydrogen at 20° for 2 hours. Evaporation of the filtered solution under reduced pressure and crystallisation of the residue from chloroform-methanol gave euph-8-en-3β-ol (8.7 g.) as long needles, m.p. 120-121°, $[\alpha]_D$ + 31.5° (c, 1.1).

Newbold and Spring[26] give m.p. 120°, $[\alpha]_D$ + 34° for this alcohol.

Oxidation of Euph-8-en-3β-ol with Chromium Trioxide and Pyridine. - A solution of the alcohol (26.8 g.) in pyridine (270 c.c.) was added to the suspension obtained by treating chromium trioxide (26.8 g.) with pyridine (270 c.c.), the mixture thoroughly shaken over 3 hours, and allowed to stand at room

temperature for 48 hours when it was poured into water and extracted with ether. After washing with dilute acid (3N HCl) and water, the extract was dried (Na_2SO_4) and evaporated under reduced pressure to give the reaction product as a brown oil (25.7 g.). A solution of the product in light petroleum (500 c.c.) was percolated through a column of alumina (300 g.). The fractions (20.0 g.) eluted with this solvent (7.5 l.) crystallised from acetone (at 0°) to give euph-8-en-3-one (10.2 g. as small needles, m.p. 65-66°, $[\alpha]_D$ + 64° (c, 1.2). Light absorption: max. at 207 mu (ε = 5,800). It gives a yellow colour with tetranitromethane.

Roth and Jeger[41] give m.p. 68°, $[\alpha]_D$ + 66° for this ketone.

The fraction (4.3 g.) eluted with benzene (2.5 l.) crystallised from methanol as yellow blades (2.0 g.), m.p. 104-107°. Four crystallisations from the same solvent gave 7-oxoeuph-8-en-3-one (1.0 g.) as colourless blades, m.p. 137-138°, $[\alpha]_D$ + 7° (c, 1.2). Light absorption: max. at 252 mu (ε = 10,200). The compound gives no colour with tetranitromethane. (Found: C, 81.4; H, 11.2. $C_{30}H_{48}O_2$ requires C, 81.8; H, 11.0%).

7-Oxoeuph-8-en-3-one from 7-Oxoeuph-8-enyl Acetate. - 7-Oxoeuph-8-enyl acetate (211 mg.) was heated under reflux with methanolic potassium hydroxide solution (3%, 15 c.c.) for 3 hours. The alcohol, isolated by means of ether as a yellow gum, separated from light petroleum as an amorphous powder (116 mg.), m.p. 92-94°

a solution of which in pyridine (10 c.c.) was treated with the mixture prepared from chromium trioxide (100 mg.) and pyridine (2 c.c.). After occasional shaking, the mixture was kept at room temperature for 12 hours. Ether extraction in the usual manner gave the product as a gum which crystallised from methanol to give 7-oxoeuph-8-en-3-one (52 mg.) as blades, m.p. 136-137°, $[\alpha]_D$ + 6° (c, 0.9). Light absorption: max. at 252 mu (ε = 10,000) It gives no colour with tetranitromethane and a mixture with the specimen obtained from euph-8-en-3β-ol as described above, had m.p. 136-138° (no depression).

II. LANOSTEROL DERIVATIVES.

24ξ:25ξ-Dibromolanost-8-enyl Acetate. - 'isoCholesterol' (50.0 g.) was acetylated by heating on the steam bath with acetic anhydride (50 c.c.) and pyridine (60 c.c.) for 3 hours. A solution of the product, obtained by ether extraction, in benzene (3 l.) was filtered through a column of alumina (400 g.). Evaporation of the filtrate in vacuo and crystallisation of the residue from chloroform-methanol gave the acetate mixture (40.0 g. as large needles, m.p. 121-125°, $[\alpha]_D$ + 60.5° (c, 2.0). Light absorption: max. at 207 mu (ε = 6,750), 235 mu (ε = 1,000), 243 mu (ε = 1,110), and 252 mu (ε = 740). A solution of bromine (5.7 g.) in acetic acid (156 c.c.) was added dropwise to a stirred solution of this acetate mixture (28.0 g.) in ether (120 c.c.). Ether was distilled off under reduced pressure at 20° until crystallisation commenced, when the reaction mixture was allowed

to stand at room temperature overnight. The mixture of dibromo-acetates (19.1 g.), after washing with acetic acid, was collected as colourless needles, m.p. 129-134°, and was used for the next reaction without further purification.

Voser, Jeger, and Ruzicka[103] give m.p. 120-123° for this mixture. A second crop (2.3 g.) separated from the filtrate as needles, m.p. 154°, several crystallisations of which from chloroform-methanol gave 24ξ:25ξ-dibromolanost-8-enyl acetate as large plates, m.p. 171-172°, $[\alpha]_D$ + 6.3° (c, 1.8). Light absorption: max. at 205 mu (ε = 6,200). It gives a strong yellow colour with tetranitromethane.

(Found: C, 61.1; H, 8.8. Calc. for $C_{32}H_{52}O_2Br_2$: C, 61.1; H, 8.4%).

Lewis and McGhie[102] give m.p. 176-177°, $[\alpha]_D$ + 7° for a 24:25-dibromolanost-8-enyl acetate ('B').

24ξ:25ξ-Dibromo-7:11-dioxolanost-8-enyl Acetate. - Stabilised acetic acid (2.3 l.) was added with stirring to a solution of the dibromo-acetate mixture (m.p. 129-134°, 102 g.) in methylene chloride (120 c.c.) and the suspension treated dropwise with a solution of chromium trioxide (100 g.) in acetic acid (90%, 500 c.c.). The warm solution was allowed to remain at 20° for 18 hours, when excess oxidant was destroyed by the addition of methanol, and after evaporation to small bulk, the mixture was poured into water (3 l.). Isolation of the neutral product by means of ether yielded a yellow solid which crystallised from chloroform-methanol as needles (38.0 g.), m.p. 168-175°.

(1) Two crystallisations of this material from chloroform-methanol gave 24ξ:25ξ-dibromo-7:11-dioxolanost-8-enyl acetate (14.0 g.) as deep yellow needles, m.p. 188-190° (dec.). Light absorption: max. at 270 mu (ε = 8,620).

(2) A solution of the crystals, m.p. 168-175°, (8.1 g.), in benzene-light petroleum (1:4) was chromatographed on alumina (240 g.). Elution of the column with the same mixture (2.1 l.) and benzene-light petroleum (1:1, 0.6 l.) yielded fractions (1.13 g. which crystallised from methanol to give 7:11-dioxolanost-8-enyl acetate (0.9 g.) as yellow leaflets, m.p. 158-159°, alone or when mixed with an authentic specimen, $[\alpha]_D$ + 94° (c, 1.7). Light absorption: max. at 270 mu (ε = 8,150). Continued elution of the alumina with the latter solvent mixture (1.5 l.) and benzene-light petroleum (4:1, 1.2 l.) eluted fractions (1.6 g.) which gave 24ξ:25ξ-dibromo-7:11-dioxolanost-8-enyl acetate (1.1 g.) as deep yellow needles from chloroform-methanol, m.p. 183-185°, $[\alpha]_D$ + 91° (c, 1.0). Light absorption: max. at 270 mu (ε = 9,100).

Voser, Jeger, and Ruzicka[103] give m.p. 188-189°, $[\alpha]_D$ + 100° for this compound.

7:11-Dioxolanost-24-enyl Acetate. - Zinc dust (1.0 g.) was added to a boiling solution of 24ξ:25ξ-dibromo-7:11-dioxolanost-8-enyl acetate (1.0 g.) in acetic acid (150 c.c.) and the mixture heated under reflux for 1 hour. The filtered solution was poured into water and a solution of the precipitate in ether washed with saturated sodium bicarbonate solution and water. Evaporation of

the dried (Na_2SO_4) ether solution gave the residue as a white solid which crystallised from chloroform-methanol. 7:11-Dioxolanost-24-enyl acetate (660 mg.) separated as leaflets, m.p. 199-202°, $[\alpha]_D$ + 57.5° (c,1.0). Light absorption: max. at 203 mu (ε = 5,060). It gives a yellow colour with tetranitromethane.

Voser, Jeger, and Ruzicka[103] give m.p. 203-204°, $[\alpha]_D$ + 58°.

11-Oxolanost-24-enyl Acetate. - 7:11-Dioxolanost-24-enyl acetate (3.75 g.) and hydrazine hydrate (100%, 1.9 c.c.) in diethylene glycol (125 c.c.) was kept at 200° for 1 hour, then treated with a solution prepared by reacting sodium (3.8 g.) with diethylene glycol (35 c.c.). The reaction mixture was maintained at 230° for 5 hours. The product (3.56 g.), isolated by means of ether in the usual way, was heated on the steam bath with acetic anhydride (20 c.c.) and pyridine (20 c.c.) for 3 hours. A solution of the dry, crude acetate (4.0 g.) in light petroleum was percolated through a column of alumina (120 g.). Benzene-light petroleum mixtures (1:9, 1 l., 1:4, 1.2 l., 1:1, 1 l.) eluted fractions (1.3 g.) which crystallised from methanol to give 11-oxolanost-24-enyl acetate (1.0 g.) as needles, m.p. 156-157°, $[\alpha]_D$ + 62° (c,1.7). Light absorption: max. at 203 mu (ε = 4,000); infra-red (nujol): bands at 1739 $cm.^{-1}$ (3β-acetate) and 1695 $cm.^{-1}$ (11-carbonyl). It gives a yellow colour with tetranitromethane. (Found: C,79.0; H,10.9. $C_{32}H_{52}O_3$ requires C,79.3; H,10.8%).

11β-Hydroxylanost-24-enyl Acetate. - A solution of 11-oxolanost-24-enyl acetate (840 mg.) in dry ether (150 c.c.) was heated under reflux with lithium aluminium hydride (1.0 g.) for 2.5 hours. After dilution, the ethereal solution was washed successively with water, dilute acid (SN H_2SO_4) and water, and dried (Na_2SO_4). Evaporation under reduced pressure gave the crude diol as a white solid, a solution of which in pyridine (10 c.c.) was treated with acetic anhydride (10 c.c.) at 20° overnight. The solid product was isolated by ether extraction and crystallised from chloroform-methanol when 11β-hydroxylanost-24-enyl acetate (670 mg.) separated as needles, m.p. 189-190°, $[\alpha]_D$ + 63° (c, 1.7). Light absorption: max. at 203 mu (ε = 3,900); infra-red (nujol): bands at 1709 $cm.^{-1}$ (3β-acetate) and 3440 $cm.^{-1}$ (11β-hydroxyl). It gives a yellow colour with tetranitromethane. (Found: C, 79.2; H, 11.4. $C_{32}H_{54}O_3$ requires C, 79.0; H, 11.2%).

Lanosta-9(11):24-dienyl Acetate (parkeyl acetate). - Phosphorous oxychloride (3 c.c.) was added to a solution of 11β-hydroxylanost-24-enyl acetate (450 mg.) in dry pyridine (30 c.c.) and the mixture heated on the steam bath for 3 hours, then cooled and poured into ice-water. The product, isolated by extraction with ether, crystallised from chloroform-methanol to give lanosta-9(11):24-dienyl acetate (370 mg.) as plates, m.p. 161-162°, $[\alpha]_D$ + 86° (c, 1.5). Light absorption: max. at 204 mu (ε = 7,200). It gives a strong yellow colour with tetranitromethane. A mixture with parkeyl acetate, m.p. 159-160°,

$[\alpha]_D$ + 86°, had m.p. 160-162° (no depression).

(Found: C,81.6; H,11.3. $C_{32}H_{52}O_2$ requires C,82.0; H,11.2%).

Lanosta-9(11):24-dien-3β-ol (parkeol). - Lanosta-9(11):24--dienyl acetate (250 mg.) was hydrolysed by heating under reflux for 30 minutes with a suspension of lithium aluminium hydride (500 mg.) in dry ether (50 c.c.). The product, obtained in the usual manner, crystallised from methanol. Lanosta-9(11):24-dien--3β-ol (194 mg.) separated as felted needles, m.p. 157-158°, $[\alpha]_D$ + 76° (c,1.0). Light absorption: max. at 204 mu (ε = 9,450). It gives a strong yellow colour with tetranitromethane. A mixture with parkeol, m.p. 159-160°, $[\alpha]_D$ + 76.8°, had m.p. 158-160° (no depression).

(Found: C,84.6; H,12.0. $C_{30}H_{50}O$ requires C,84.4; H,11.8%).

Lanosta-9(11):24-dienyl Benzoate (parkeyl benzoate). - A solution of lanosta-9(11):24-dien-3β-ol (84 mg.) in dry pyridine (7 c.c.) containing benzoyl chloride (3 c.c.) was heated at 100° for 3 hours, then poured into water and the mixture allowed to stand overnight. Ether extraction of the product gave a brown solid (144 mg.), a solution of which in benzene-light petroleum (1:4) was chromatographed on alumina (4.0 g.). This solvent mixture (160 c.c.) eluted fractions (88 mg.) which crystallised from chloroform-methanol to give lanosta-9(11):24-dienyl benzoate (65 mg.) as needles, m.p. 199-200°, $[\alpha]_D$ + 94° (c,1.2). A mixture with parkeyl benzoate, m.p. 200-201°, $[\alpha]_D$ + 95.4°, had m.p. 199-201° (no depression).

(Found: C,83.6; H,10.5. $C_{37}H_{54}O_2$ requires C,83.7; H,10.3%).

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PART II

THE STEROID SAPOGENINS.

INTRODUCTION.

The saponins, first detected by Schmiedeberg in 1875[1], are a group of naturally occurring plant glycosides, which on hydrolysis give a sapogenin and a sugar, or mixture of sugars. Steroid sapogenins are characterised by dehydrogenation to Diels' hydrocarbon, and have the perhydro-1:2-cyclopentenophenanthrene ring system of the steroids. In contrast, the triterpenoid group of sapogenins give mainly naphthalene and picene derivatives under the same conditions.

This section describes the isolation of certain steroid sapogenins and no review of the triterpenoid group of sapogenins is included. The term sapogenin, therefore, infers steroid sapogenin.

The sapogenin nucleus. A relationship between the sapogenins and the sterols was first suggested by Ruzicka[2] and Jacobs[3], who showed that when sarsasapogenin is submitted to dehydrogenation with selenium, a fragrant volatile ketone, similar to that obtained from cholesterol, is produced. This observation also indicated the attachment of a C-8 side chain to the sapogenin nucleus, and later[4], the non-volatile residue from this reaction was shown to contain Diel's hydrocarbon(I) In 1935, Tschesche[5,6] related tigogenin, gitogenin and digitogenin by interconversion, and degraded tigogenin to etioallobilianic acid (II). By a similar procedure, sarsasapogenin was converted

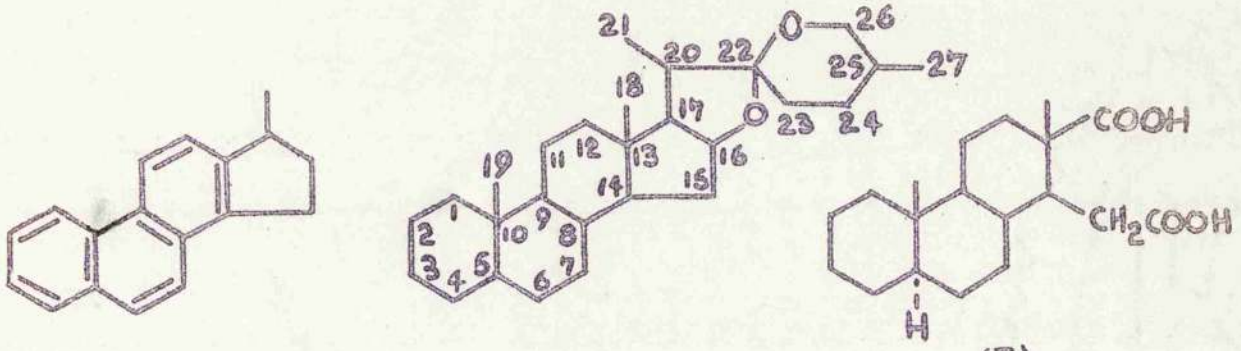
into etiobilianic acid of the coprostane (5β-)steroid series. The nuclear hydroxyl group common to these sapogenins was shown to be at position-$C_{(3)}$ by the dehydrogenation of the methyl carbinol of sarsasapogenone to 7-methyl-1:2-cyclopenteno-phenanthrene[7,8]. During the period 1939-1947, Marker and his co-workers[9,10] isolated and elucidated the parent nuclear structures of most of the sapogenins. The structure of the earliest known sapogenin, digitogenin, has only recently been established as is shown in (III)[11,12].

Variations in the nuclear structure of the sapogenins arise from:

(i) the number, position, and configuration of hydroxyl groups. In addition to the usual 3β-hydroxyl group, positions-$C_{(2)}$, $C_{(6)}$, $C_{(12)}$, and $C_{(15)}$ may be hydroxylated. Djerassi[12] has shown the existence of both a cis-(2β:3β) and trans-(2α:3β) glycol series. A number of closely related alcohols have been isolated[9,13] which differ from the natural sapogenins in that they possess an open ketal side chain. A $C_{(27)}$-hydroxysapogenin has recently been described[14].,

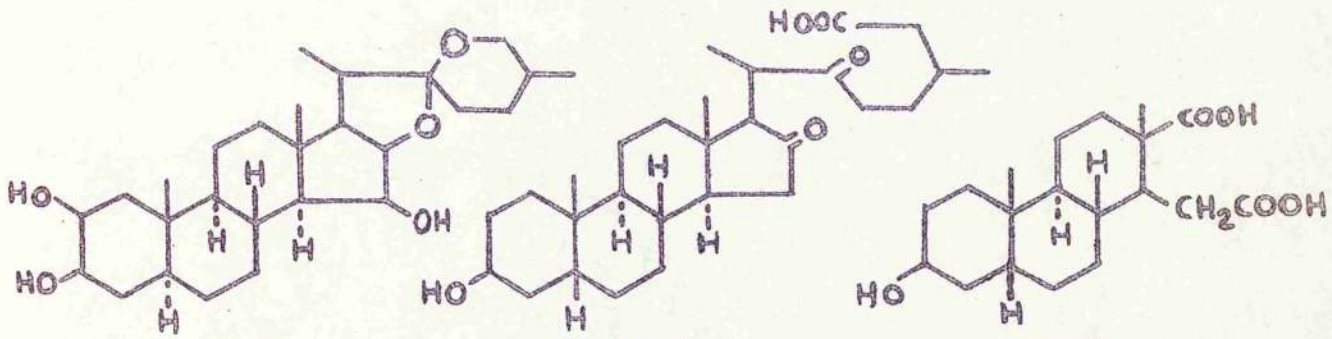
(ii) the presence of a carbonyl group at the $C_{(12)}$-position, and

(iii) the mode of union, cis- or trans-, of rings A and B. All natural unsaturated sapogenins have a double bond in the 5:6-position.



(I) (II)

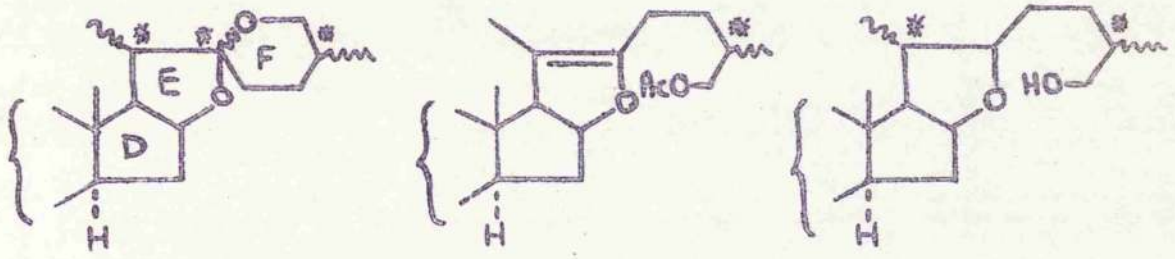
The sapogenin side chain. The structure of the sapogenin spiroketal side chain (IV) was established by the stepwise degradation of sarsasapogenoic acid (V) to 3β-hydroxy-etiobilianic acid (VI)[18]. The complex problem of the configuration at the asymmetric carbon atoms $C_{(20)}$, $C_{(22)}$, and $C_{(25)}$ has not been attacked until recently, and its partial elucidation has followed mainly from a study of the sensitivity of the side chain towards acid.



(III) (V) (VI)

Certain naturally occurring pairs of sapogenins exist, one member, the normal sapogenin, of each pair being isomerised to the other, isosapogenin, by vigorous treatment with mineral acid[16-21]. Ring opening of the side chain in both the normal

and iso-series is effected by treatment of the sapogenin with acetic anhydride at 200°, when the χ-sapogenin diacetate (VII)[22] is formed with loss of symmetry at $C_{(20)}$ and $C_{(22)}$. The χ-sapogenin reverts to the original sapogenin by treatment with strong mineral acid. Catalytic hydrogenation of a natural sapogenin gives the corresponding dihydrosapogenin (VIII). Marker[17] attributed the acid isomerisation of the normal



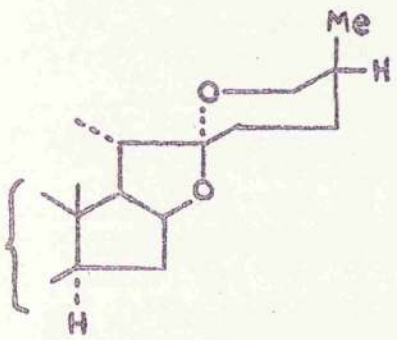
(IV) (VII) (VIII)

sapogenin into the isosapogenin to inversion at $C_{(22)}$ in the normal sapogenin, on the basis that the χ- and dihydro-derivatives obtained from both the normal and isosapogenins are identical. Wall and his co-workers[19] and Scheer et al.[23], however, found that oxidation of the χ-sapogenins of the normal and iso-series gave (+) and (-)-α-methyl glutaric acid respectively. The conversion of a normal sapogenin into an isosapogenin, by treatment with mineral acid, therefore involves inversion at $C_{(25)}$, and James[24] deduced that the normal and isosapogenins have the L- and D-configurations (relative to D-glyceraldehyde)

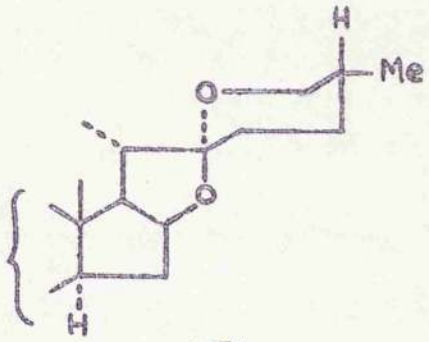
respectively at this centre.

Although the configuration of the sapogenins at the $C_{(25)}$-asymmetric carbon atom has thus been established, the assignment of configurations at the remaining centres $C_{(20)}$ and $C_{(22)}$ rests on less secure evidence. A remarkable resurgence of interest in this problem within the last few years[19-21, 25-29] has led to the description of a new isomer, prepared by mild acid treatment of the χ-sapogenin, from both the normal and iso-series and resulting in recyclisation. This new class of isomers has been variously called "neosapogenin"[20,25], "20-isosapogenin"[19], "anasapogenin"[26], and "cyclo-χ-sapogenin"[28], and differs from the original sapogenin only in configuration at $C_{(20)}$. From a study of the mechanism of formation of this isomer, its reactions, and an examination of molecular models, it appears likely that both series of the natural sapogenins have a $C_{(20)}$-methyl group in the α-configuration as in cholesterol[19,25]. With regard to the configuration at $C_{(22)}$, there is the evidence[23] that the dihydrosapogenins of the normal and iso-series have the same configuration at $C_{(22)}$, and that inversion at $C_{(25)}$ in the natural sapogenins takes place without opening of ring F[21]. Consequently both series of natural sapogenins have the same configuration at $C_{(22)}$, a conclusion recently established by Callow and Massy-Beresford[30]. They have prepared, from two sapogenins diastereoisomeric at $C_{(25)}$, compounds in which the

centre of symmetry at $C_{(25)}$ has been destroyed by the introduction of a double bond between $C_{(24)}$ and $C_{(25)}$. The complete identity of these compounds indicates that the normal and isosapogenins have identical configurations at all centres of symmetry except $C_{(25)}$. The absolute configuration at $C_{(22)}$ has not yet been established. From a study of molecular models and conformational analysis, these workers favour the assignment of structures (IX) and (X) to the normal (25L) and iso-(25D)-sapogenins respectively.



(IX)

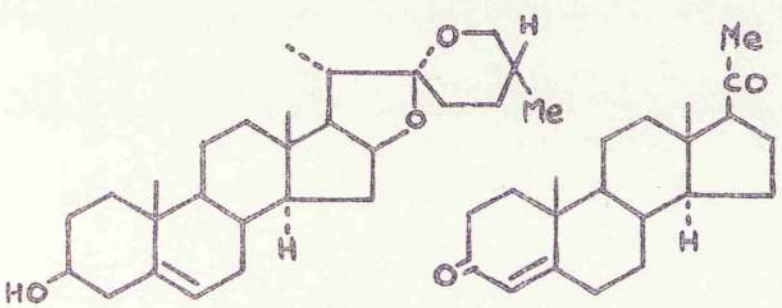


(X)

This formulation has been adopted in the following text. The prefix neo- does not refer to the 20-isosapogenins[20,25], but to the $C_{(25)}$-isomer of a natural sapogenin.

The conversion of sapogenins into sex-hormones and 11-oxygenated steroids. - Since 1946[31], a number of degradations have been reported describing the conversion of sapogenins into members of the pregnane and androstane series of sex-hormones, using methods based on the side chain cleavage described by Marker[32] in the conversion of diosgenin (XI) into progesterone (XII).

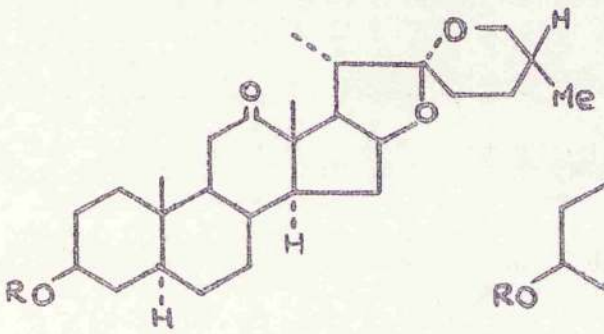
As well as being important materials for the production of sex-hormones, the sapogenins offered a potential solution to the problem of introducing a carbonyl group at position -$C_{(11)}$ in the steroid nucleus, and led to a more economic partial synthesis of the clinically important compound, cortisone (17α-hydroxy-11--dehydrocorticosterone, XIII). Hecogenin (XIV, R = H), a



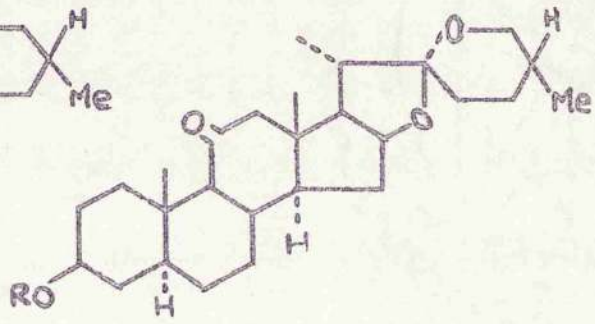
(XI) (XII) (XIII)

readily accessible material[33,34], was converted into 11-oxotigogenin (XV, R = H) by a variety of methods[35,36,37]. The principal method[36] is based on the application of bromination procedures which have been established for the replacement of a $C_{(12)}$--carbonyl group by one at $C_{(11)}$ in the steroid nucleus, and has formed the basis of a partial 28 stage synthesis used in the manufacture of cortisone from hecogenin. At the time of the author's investigation, hecogenin (XIV, R = H) was being commercially extracted from the sisal plant Agave sisalana Perrine[33,34] and little was known of the other sapogenin constituents. An examination of the hecogenin residues from this plant was instigated, consequently, with the particular hope of isolating a $C_{(11)}$ -oxygenated sapogenin which would be invaluable as an

intermediate in the synthesis of adrenocortical and sex-hormones.



(XIV)



(XV)

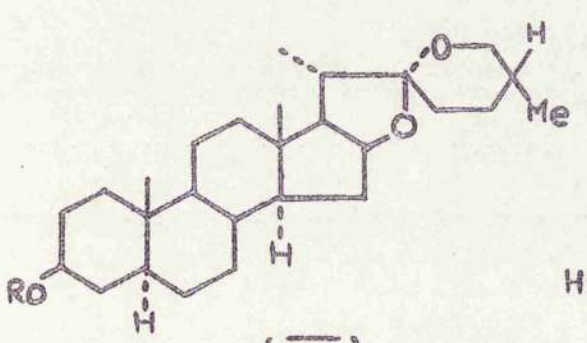
THEORETICAL.

The Steroid Constituents of Agave sisalana Perrine.

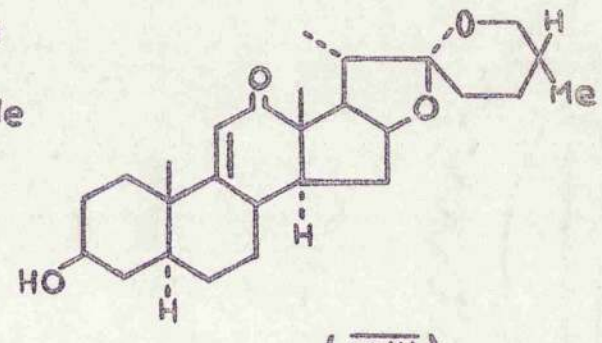
The sisal plant, Agave sisalana Perrine, has been found to contain the steroid sapogenins, hecogenin, neohecogenin, neotigogenin, and a new sapogenin, isomeric with hecogenin.

In an extensive examination of the steroid glycosidic content of Mexican plant species, Marker and his co-workers[9] have isolated and identified many naturally occurring sapogenins. Hecogenin, the 3β-hydroxy-12-oxo-sapogenin formulated as (XIV, R = H), and later shown to belong to the iso-series (25D)[24], was obtained from American species of the plant type Agave, the genus which includes the sisal plant Agave sisalana Perrine which is widely cultivated in East Africa. Marker[9] has also shown that the sapogenin content of the plant may vary widely with its species and age.

The commercial extraction of hecogenin from this plant species was described in 1951-52[33,34]. The presence of a non-ketonic sapogenin, $C_{27}H_{44}O_3$, and an unsaturated sapogenin was also reported, and it was suggested that these were tigogenin (XVI, R = H) and 9-dehydrohecogenin (XVII) respectively. In the process of isolation, the fibre is removed from the sisal plant and the residue is compressed to give a juice which contains the sapogenin glycosides. After acid hydrolysis of the juice, the sapogenins are extracted from the mixture with isopropyl ether, and separate on concentration of the extract. The collected material is heated under reflux with acetic anhydride and the addition of methanol to the resulting solution precipitates crude hecogenin acetate. Supplies of the filtrate from the crude



(XVI)



(XVII)

hecogenin acetate were made available for further examination through the courtesy of the directors of T. and H. Smith Ltd., Edinburgh.

The mother liquors were concentrated and a green amorphous solid, fraction A, separated on cooling. This material was collected and total evaporation of the filtrate gave a brown gum, fraction B. Both of these fractions were examined separately.

FRACTION A.

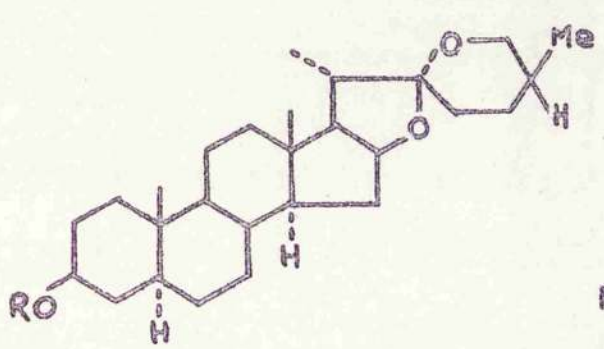
A preliminary attempt to separate the ketonic and non-ketonic acetates by treatment of fraction A with 'Girard P' reagent (pyridinium acetohydrazide chloride) was unsuccessful. A sample of fraction A was hydrolysed, but no effective separation of constituent alcohols could be obtained by crystallisation procedures. Fractional crystallisation of fraction A, firstly from acetic anhydride, then from ethanol, yielded several semi-crystalline products, from the final mother liquors of which separated a mixture of a white amorphous powder and green

octahedral crystals. After mechanical separation of the crystals and purification by chromatography, a colourless acetate, $C_{29}H_{46}O_4$, m.p. 175-178°, $[\alpha]_D$ - 86°, was obtained. The acetate gave no colour with tetranitromethane, a red colour in the Liebermann-Burchardt test, and was transparent to ultra-violet light. Alkaline hydrolysis of the acetate gave the corresponding alcohol, $C_{27}H_{44}O_3$, m.p. 198-202°, $[\alpha]_D$ - 73°. The physical constants of the alcohol and acetate are in good agreement with those reported for neotigogenin (XVIII, R = H) and its acetate (XVIII, R = Ac) respectively. neoTigogenin was first isolated by Goodson and Noller[38] from chlorogalum pomeridianum together with its $C_{(25)}$-epimer, tigogenin (XVI, R = H). neoTigogenin has been shown to belong to the normal (25L) series of sapogenins[24] and use was made of this fact in confirming the identity of the isolated alcohol, m.p. 198-202°, as such. Prolonged refluxing of the isolated acetate, m.p. 175-178°, with strong mineral acid, followed by acetylation, gave tigogenin acetate (XVI, R = Ac) in good yield[39], by inversion of the $C_{(25)}$-methyl group from the axial to the more stable equatorial conformation. Tigogenin (XVI, R = H), m.p. 203-205°, $[\alpha]_D$ - 74°, was obtained by alkaline hydrolysis of the corresponding acetate. Tigogenin acetate (XVI, R = Ac) has not been isolated from the acetylated sisal residues.

The remaining semi-crystalline fractions obtained by the crystallisation of fraction A were combined according to similarity in their melting point ranges. Chromatography of these new fractions gave further quantities of the sapogenin acetates also isolated from fraction B.

FRACTION B.

The residual gum (fraction B) was chromatographed after acetylation and the first fraction gave pure neotigogenin acetate (XVIII, R = Ac). Subsequent fractions, though crystalline, were mixtures which separated by extensive fractional crystallisation. The more insoluble component of the mixtures was identified as hecogenin acetate (XIV, R = Ac), the major sapogenin constituent of Agave sisalana Perrine[33], by direct comparison with an authentic specimen supplied by Messrs. T. and H. Smith Ltd. A second acetate, $C_{29}H_{44}O_5$, m.p. 220-222°, $[\alpha]_D$ - 14°, isomeric with hecogenin acetate, and which gave an alcohol, $C_{27}H_{42}O_4$ m.p. 238-240°, $[\alpha]_D$ - 4°, separated from the mother liquors as the more soluble component of the acetate mixtures. From a comparison of physical constants, the alcohol, m.p. 238-240°, is identified as neohecogenin (XIX, R = H)[40], the $C_{(25)}$-epimer of hecogenin.



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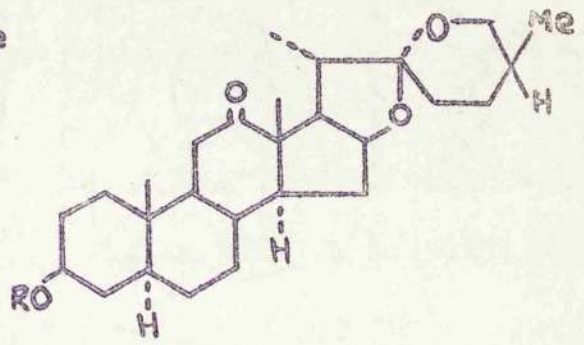


(XIX)

Callow and James[21] have also made an examination of sisal juice, and have isolated a number of sapogenins from this source. They have adopted the name sisalagenin for neohecogenin to avoid confusion with the 20-isosapogenins. The physical constants found for the isolated sapogenins and their acetates are compared with those reported by Callow and James in the following table:-

Compound	This work		Callow and James[21]	
	m.p.°	$[\alpha]_D$°	m.p.°	$[\alpha]_D$°
neoTigogenin	198-202	-73	197-203	-75
neoTigogenin acetate	175-178	-86	175-181	-79
Hecogenin acetate	244-245	-2.5	237-243	+ 4
neoHecogenin	238-240	-3.8	244-246	- 4.5
neoHecogenin acetate	220-222	-14	228-232	-12

= The footnote in this publication refers to the work described in this section.

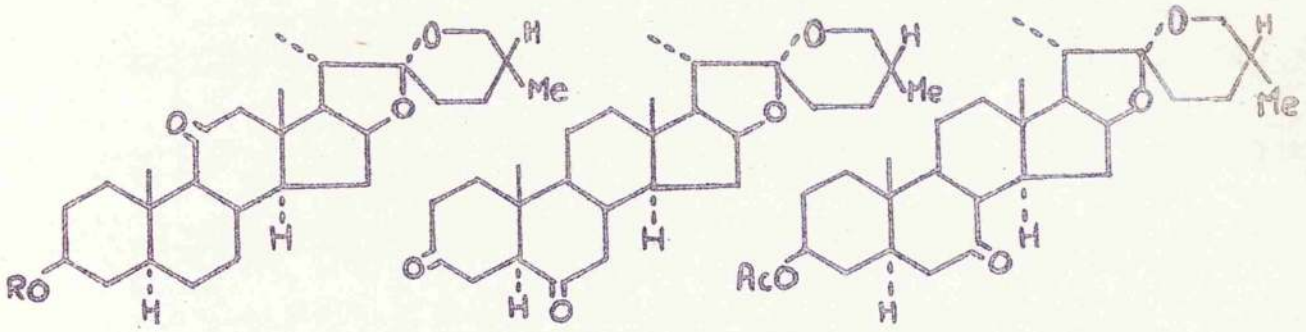
The strongly adsorbed residue from fraction B, obtained by elution of the above column with methanol, was reacetylated. On working up in the usual manner with ether, a separation into ether-soluble and ether-insoluble fractions was effected, both of which were chromatographed separately. The ether-soluble fraction gave hecogenin acetate (XIV, R = Ac) as the only pure compound. The ultra-violet light absorption spectra of subsequent fractions showed maximum absorption at 238 mu (ε = 3,000-13,000) and consequently they contain an αβ-unsaturated ketone. This observation has already been made by Cornforth *et al.*[33] who suggest the presence of 9-dehydrohecogenin (XVII, R = H), which does not depress the melting point of hecogenin[41]. Attempts to purify the αβ-unsaturated ketone were unsuccessful.

The ether-insoluble fraction, on chromatography, gave a similar acetate mixture containing 10% of an αβ-unsaturated ketone. A pure acetate, $C_{29}H_{44}O_5$, m.p. 238-241°, $[\alpha]_D$ + 1°, was obtained from the mixture by repeated crystallisation. Alkaline hydrolysis of the acetate gave the alcohol, $C_{27}H_{42}O_4$, m.p. 240-242°, $[\alpha]_D$ - 6°, which was converted into the corresponding diketone, $C_{27}H_{40}O_4$, m.p. 236°, $[\alpha]_D$ + 22°, by mild oxidation with chromium trioxide. The acetate readily formed a crystalline 2:4-dinitrophenylhydrazone, $C_{35}H_{48}N_4O_8$, m.p. 150°. The physical constants of the alcohol and these derivatives do not agree with those reported for any of the known sapogenins and their corresponding derivatives. The acetate gives no colour with

tetranitromethane and shows no selective absorption in the ultra-violet region. The presence of a carbonyl group is deduced from the formation of a 2:4-dinitrophenylhydrazone acetate. These observations strongly suggested that the $C_{27}H_{42}O_4$ alcohol is isomeric with hecogenin (XIV, R = H). Wolff-Kishner reduction of the $C_{29}H_{44}O_5$ acetate, followed by acetylation, gave tigogenin acetate (XVI, R = Ac), identical with a specimen prepared from neotigogenin acetate (XVIII, R = Ac) Consequently the $C_{29}H_{44}O_5$ acetate is an oxotigogenin acetate.

The position of the carbonyl group in this new sapogenin has not been established, but provided it has the same stereochemistry as hecogenin, several locations can be excluded from the following considerations. Oxidation of the sapogenin gives the corresponding 3-ketone, $C_{27}H_{44}O_4$, which must contain two carbonyl groups, and since it does not give a colour with ferric chloride in ethanol, the dicarbonyl system is not situated in ring A. The ketone group in the original sapogenin acetate cannot therefore be located at carbon atoms $C_{(1)}$, $C_{(2)}$ or $C_{(4)}$. Position $C_{(6)}$ is also excluded since the diketone is not identical with 6-oxotigogenone (chlorogenone, XX). Similarly, the sapogenin acetate is not identical with 7-oxotigogenin acetate (XXI) or 11-oxotigogenin acetate (XV, R = Ac). The ease of formation of a 2:4-dinitrophenylhydrazone from the $C_{29}H_{44}O_5$ acetate also excludes position $C_{(11)}$ for the carbonyl group. 6-Oxotigogenone and 7-oxotigogenin acetate were prepared for

comparisons by Mr. J. I. Shaw of this Department, while 11-oxotigogenin acetate was supplied by Messrs. Glaxo Ltd. Neogenin acetate (12-oxotigogenin acetate, XIV, R = Ac) was found to depress the melting point of the sapogenin acetate, and position $C_{(15)}$ is excluded since the infra-red absorption spectrum of the sapogenin shows the carbonyl group to be present in a six-membered ring. Consequently it appears that the carbonyl group of the sapogenin is located at position $C_{(23)}$ or $C_{(24)}$.



(XV) (XX) (XXI)

This conclusion may be criticised on the grounds that isomerisation may have occurred during the Wolff-Kishner reduction of the sapogenin acetate to tigogenin acetate. An infra-red examination of the corresponding $\underline{\gamma}$-sapogenin diacetate, prepared from the sapogenin by treatment with acetic anhydride at 200°, would distinguish between a carbonyl group in the $C_{(23)}$-position and one at $C_{(24)}$.

EXPERIMENTAL.

Notes given in Part I describing experimental procedures also apply to this section.

Treatment of the Residues from Agave sisalana Perrine. - The residual liquors (75 l.) were evaporated under reduced pressure until solid separated, when the mixture was allowed to cool and the green amorphous mass (fraction A, 2.0 kg.) collected. Complete evaporation of the filtrate gave a viscous gum (fraction B, 1.35 kg.) which solidified on standing.

neoTigogenin Acetate. - Fraction A (35.0 g.) was crystallised from acetic anhydride (1 l.) and gave three crops of amorphous solid. The first crop (20.0 g.) was crystallised twice from ethanol (1.2 l., and 0.4 l.) to give a further three crops which were collected. The combined ethanolic filtrates, on reduction to low bulk (400 c.c.), crystallised as a mixture of an amorphous powder (1.5 g.), m.p. 200-223°, and dense green octahedra (1.0 g.), m.p. 167-171°. After washing with hot ethanol (20 c.c.), a solution of the crystals in light petroleum was chromatographed on a short column of alumina (20 g.). Elution with the same solvent (800 c.c.) and benzene-light petroleum (1:1, 700 c.c.), and two crystallisations of the combined eluates from acetone gave neotigogenin acetate (400 mg.) as small needles, m.p. 175-178°, $[\alpha]_D$ - 85.7° (c, 2.0). It does not give a colour with tetranitromethane and shows no selective light absorption in the ultra-violet region.

(Found: C,75.7; H,10.3. Calc. For $C_{29}H_{46}O_4$: C,75.9; H,10.1%). Goodson and Noller[38] give m.p. 174-176°, $[\alpha]_D$ - 73° for this compound.

neo<u>Tigogenin</u>. - The acetate (137 mg.) was hydrolysed by heating under reflux with ethanolic potassium hydroxide solution (3%, 7 c.c.) for 2 hours. The product, isolated by means of ether, was crystallised three times from methanol to give <u>neo</u>tigogenin (35 mg.) as needles, m.p. 198-202°, $[\alpha]_D$ - 73° (<u>c</u>,1.1). (Found: C,77.8; H,10.8. Calc. for $C_{27}H_{44}O_3$: C,77.8; H,10.7%). Goodson and Noller[38] give m.p. 202-203°, $[\alpha]_D$ - 65° for the alcohol.

<u>Tigogenin Acetate</u>. - A solution of <u>neo</u>tigogenin acetate (1.0 g.) in ethanol (100 c.c.) containing hydrochloric acid (10N, 20 c.c.) was heated under reflux for 20 hours, then poured into water (800 c.c.). The solid was collected, and, after drying, acetylated by boiling with acetic anhydride (5 c.c.) for 30 minutes. The product, which separated on cooling, crystallised from acetone to give tigogenin acetate (600 mg.) as long needles, m.p. 203-205°, $[\alpha]_D$ - 68° (<u>c</u>,1.2). It gives no colour with tetranitromethane and is transparent to ultra-violet light.

(Found: C,75.8; H,10.3. Calc. for $C_{29}H_{46}O_4$: C,75.9; H,10.1%). Jacobs and Fleck[42] give m.p. 200-202°, $[\alpha]_D$ - 57° (in pyridine).

<u>Tigogenin</u>. - Tigogenin acetate (100 mg.) was heated under reflux with ethanolic alkali solution (3% KOH, 7 c.c.) for 2 hours and the alcohol isolated by means of ether. Tigogenin separated from methanol as plates, m.p. 203-205°, $[\alpha]_D$ - 75° (<u>c</u>,1.2). (Found: C,74.9; H,10.6. Calc. for $C_{27}H_{44}O_3 . CH_3OH$: C,74.9; H,10.8%).

Jacobs and Fleck[42] give m.p. 203-204°, $[\alpha]_D$ = 49° (in pyridine).

Chromatography of Fraction A. - The fractions obtained by the crystallisation of fraction A, firstly from acetic anhydride and then from ethanol, as described above, were combined according to similarity in melting point ranges to give three fractions I, II, and III, each of which was chromatographed on alumina in the usual way. Light petroleum and benzene-light petroleum (1:1) eluted neotigogenin acetate. Benzene eluted a crystalline mixture of hecogenin acetate and neohecogenin acetate which was separated by fractional crystallisation from chloroform--methanol. Pure hecogenin acetate was eluted with benzene-ether (3:1). The yields of sapogenin acetates obtained from fractions I, II, and III are shown below:

Fraction No.	Fraction Wt.	m.p.	Sapogenin Acetate	Wt.	m.p. and mixed m.p.
I	5.5 g.	200-240°	neoHecogenin acetate	0.3 g.	220-222°
			Hecogenin acetate	1.2 g.	244-245°
II	3.5 g.	165-185°	neoTigogenin acetate	1.6 g.	175-178°
			Hecogenin acetate	0.5 g.	243-245°
III	11.0 g.	150-170°	neoHecogenin acetate	1.7 g.	175-178°
			Hecogenin acetate	2.3 g.	243-245°

Hecogenin Acetate and neoHecogenin Acetate. - A solution of fraction B (100 g.) in pyridine (100 c.c.) was treated with acetic anhydride (100 c.c.) overnight. Extraction by means of ether gave the product as a brown solid, a solution of which in benzene-light petroleum (1:3, 1.5 l.) was percolated through a

column (5.5 x 121 cm.) of alumina (2.5 kg.). Elution with benzene (1 l.) yielded a fraction (2.5 g.) which was crystallised from chloroform-methanol, then acetone. neoTigogenin acetate (600 mg.) separated as plates, m.p. 175-178° (no depression). Continued washing with the same solvent mixture (16 l.) gave a fraction (25.8 g.), m.p. 190-210°, repeated crystallisation of which from chloroform-methanol yielded hecogenin acetate (5.2 g.) as needles, m.p. and mixed m.p. 244-245°, $[\alpha]_D$ - 2.5° (c,3.9).

(Found: C,73.5; H,9.4. Calc. for $C_{29}H_{44}O_5$: C,73.7; H,9.4%).

Several crystallisations from the mother liquors gave neohecogenin acetate (2.0 g.) as plates, m.p. 220-222°, $[\alpha]_D$ - 14° (c,1.1). It gives no colour with tetranitromethane and is transparent to ultra-violet light.

(Found: C,73.4, 73.6; H,9.6, 9.4. Calc for $C_{29}H_{44}O_5$: C,73.7; H,9.4%)

Callow and James[21] give m.p. 228-232°, $[\alpha]_D$ - 12° for this acetate.

Elution of the column with benzene-ether (19:1) furnished a fraction (13.7 g.), m.p. 190-208°, which was separated as described above to give hecogenin acetate (1.6 g.), m.p. 243-245° (no depression), $[\alpha]_D$ - 2.5° (c,1.1), and neohecogenin acetate (420 mg.) m.p. 218-222° (no depression), $[\alpha]_D$ - 13° (c,1.1). Benzene-ether (1:1, 6 l.) and ether (3 l.) eluted intractable gums (3.6 g.) which were not further examined.

neoHecogenin. - A solution of neohecogenin acetate (145 mg.) in dilute ethanolic alkali (3% KOH, 7 c.c.) was heated under reflux for 2 hours. The product, isolated by means of ether, crystallised from aqueous acetone when neotigogenin (70 mg.) separated as fine needles, m.p. 238-240°, $[\alpha]_D$ - 3.8° (c, 1.1). (Found: C, 75.6; H, 10.0. Calc. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8%). Callow and James[21] give m.p. 244-246°, $[\alpha]_D$ - 4.5° for neohecogenin.

Chromatography of the Adsorbed Residue. - (i) Elution of the above column with ether-methanol (9:1, 3 l.) gave a brown gum (46.0 g.) which was heated on the steam bath with pyridine (50 c.c.) and acetic anhydride (25 c.c.) for 3 hours. The product was isolated by means of ether (2 l.) in the normal manner, and on standing at 0°, a semi-crystalline mass (20.0 g.) separated from the extract. The filtrate from the collection of this material was evaporated in vacuo to give a gum (25.5 g.), a solution of which in light petroleum (100 c.c.) was filtered through a column (6 x 26 cm.) of alumina (1 kg.). Elution with benzene-light petroleum (1:2, 15 l.) and six crystallisations of the fraction from chloroform-methanol gave hecogenin acetate (200 mg.) as long needles, m.p. 243-245° (no depression), $[\alpha]_D$ -2.6 (c, 1.5). An inseparable mixture (1.9 g.), m.p. 186-190°, light absorption: max. at 238 mu (ε = 3,000), was obtained by continued washing with the same solvent mixture. Ether (2 l.) eluted a gum (400 mg.) which crystallised from chloroform-methanol as plates (160 mg.), m.p. 216-219°. Light absorption:

max. at 238 mu (ε = 13,100). A homogeneous acetate could not be obtained by further crystallisation of the crystals.

(ii) The $C_{29}H_{44}O_5$ Acetate. (The following experiments are with Mr. J. I. Shaw). - A solution of the ether-insoluble fraction (6.5 g.), obtained from the adsorbed residue as described above, in benzene-light petroleum (1:1, 200 c.c.) was chromatographed on alumina (190 g.). Elution of the column with benzene-light petroleum (4:1), benzene, and benzene-ether (1:1) mixtures gave fractions (5.0 g.) which crystallised from chloroform-methanol as needles, m.p. 230-240°. Light absorption: max. at 238 mu (ε = 1,200). Repeated crystallisation of the crystals from the same solvent mixture yielded an acetate (600 mg.) as plates, m.p. 238-241°, $[\alpha]_D$ + 1° (c,1.7). Light absorption: it shows no selective light absorption in the ultra-violet region; infra-red (nujol), band at 1706 $cm.^{-1}$ (six-ring ketone). It does not give a colour with tetranitromethane.

(Found: C,73.6; H,9.7. $C_{29}H_{44}O_5$ requires C,73.7; H,9.4%).

A mixture of the acetate with hecogenin acetate, m.p. 244-245°, $[\alpha]_D$ - 2.5°, had m.p. 205-211°.

The $C_{27}H_{42}O_4$ Alcohol. - A solution of the acetate (30 mg.) m.p. 238-241°, in ethanolic potassium hydroxide solution (3%, 5 c.c.) was heated under reflux for 2 hours. The alcohol, isolated by means of ether, crystallised from methanol as prisms (17 mg.), m.p. 240-242°, $[\alpha]_D$ - 6° (c,1.0). It does not give

a colour with tetranitromethane and is transparent to ultra-violet light.

(Found: C,75.2; H,9.4. $C_{27}H_{42}O_4$ requires C,75.3; H,9.8%).

The $C_{27}H_{40}O_4$ Diketone. - A solution of chromium trioxide (45 mg.) in acetic acid (95%, 100 c.c.) was added dropwise to a stirred solution of the alcohol, m.p. 240-242°, (200 mg.) in acetic acid (50 c.c.), and the mixture kept at 20° for 16 hours. The product, obtained by ether extraction, crystallised from acetone to give the diketone (75 mg.), m.p. 236°, $[\alpha]_D$ + 22° (c,1.0). It does not give a colour with ferric chloride in ethanol and shows no selective ultra-violet light absorption.

(Found: C,75.1; H,9.5. $C_{27}H_{40}O_4$ requires C,75.6; H,9.4%).

A mixture of the diketone with 6-oxotigogenone, m.p. 233-235°, $[\alpha]_D$ - 75°, had m.p. 229°.

The 2:4-Dinitrophenylhydrazone Acetate. - The above acetate, m.p. 238-241°, (100 mg.) in ethanol (40 c.c.) was treated with an excess of Brady's reagent, and after heating, the solution allowed to crystallise. A solution of the dried product in benzene was filtered through a short column of alumina and the filtrate evaporated under reduced pressure. The residue crystallised from chloroform-methanol to give the 2:4-dinitrophenylhydrazone acetate as orange plates, m.p. 250° (dec.).

Light absorption: max. at 370 mu (ξ = 26,000).

(Found: C,63.3; H,7.4. $C_{35}H_{48}N_4O_8$ requires C,64.4; H,7.4%).

Tigogenin Acetate from the $C_{29}H_{44}O_6$ Acetate. - The above acetate, m.p. 238-241°, (500 mg.) was treated with a solution prepared by reacting sodium (1.0 g.) with methanol (10 c.c.) and containing hydrazine hydrate (100%, 5 c.c.) at 200° for 17 hours. The mixture was poured into water and the product isolated by means of ether in the usual way. A solution of the product in pyridine (2 c.c.) was heated on the steam bath for 2 hours with acetic anhydride (2 c.c.). The acetylated material (480 mg.) crystallised from methanol to give tigogenin acetate as needles, m.p., alone or with a specimen prepared from neotigogenin acetate, 203-205°, $[\alpha]_D$ - 68.5° (c, 1.2).

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