# CHEMISTRY OF *cyclo*ARTENOL, *cyclo*LAUDENOL and BUTYROSPERMOL

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THESIS

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C O N T E N T S.

Introduction.

The triterpenoids are a group of naturally occurring compounds containing thirty carbon atoms, the molecular structures of which are divisible into six isoprene units. The majority of these compounds have a five-ring carbocyclic skeleton but the aliphatic squalene and the tricyclic ambrein are also known. There are no known mono- or dicyclic triterpenoids.

The structures of the tetracyclic group of triterpenoids, however, do not conform to the isoprene rule, and in addition some members of this group have recently been shown to contain, not thirty, but thirty-one carbon atoms. This failure to conform to the isoprene rule suggests that it may be more correct to regard the tetracyclic compounds as trimethyl and tetramethyl steroids rather than as triterpenoids.

As the work described in this thesis is concerned solely with the chemistry of tetracyclic triterpenoids, it is not proposed to include in this historical review a summary of the chemistry of the large group of pentacyclic triterpenoids, comprising the α-amyrin, the β-amyrin and the lupeol series.

The tetracyclic triterpenoids which have hitherto been examined in detail have been shown to have the perhydrocyclopentenophenanthrene ring system of the steroids. According to Dawson, Halsall and Swayne (1), members of the tetracyclic group may be subdivided into two series, typified by lanosterol and euphol respectively. The change in molecular rotation associated with the conversion of an 8:9-ene into the corresponding 7:9(11)--diene indicates the series to which it belongs. Thus with lanosterol and related compounds, the formation of the diene causes a positive change in rotation, while in the euphol series, a large negative change results. The ultra-violet absorption spectra of the dienes of the two series are distinct. Again, the behaviour of the nuclear (8:9)-double bond when treated with acid, is characteristic for each group.

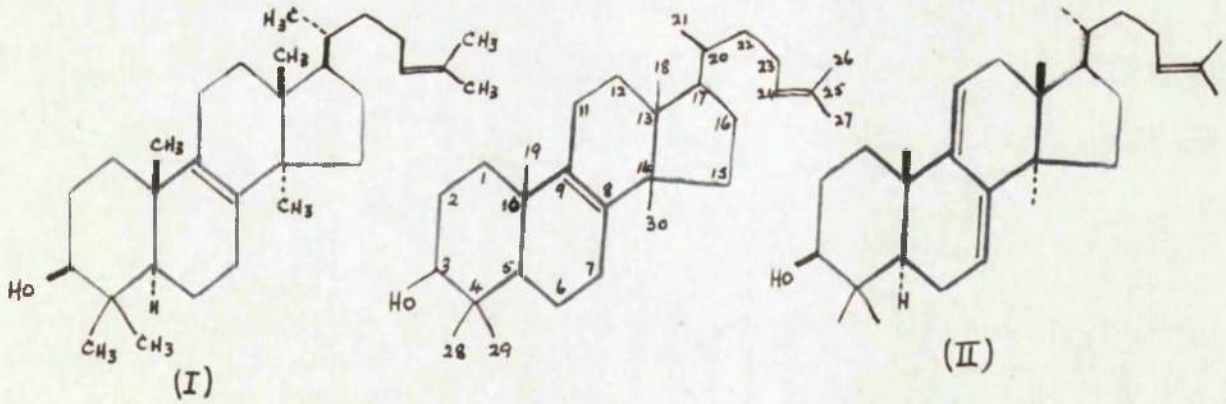
Classification of the Tetracyclic Triterpenoids.

(i) The lanosterol group consists of lanosterol, dihydrolanosterol, agnosterol, dihydroagnosterol, eburicoic acid, the polyporenic acids A, B and C and pinicolic acid A. Eburicoic acid and the polyporenic acids each contain thirty-one carbon atoms; the remaining members of the

series each have thirty carbon atoms.

Lanosterol (lanosta-8:24-dien-3β-ol) was first isolated by Windaus (2) from "isocholesterol", obtained from the neutral fraction of sheep wool wax. The constitution (I) of lanosterol has been established. The chemistry of lanosterol is discussed in a later part of this section.

Agnosterol was also isolated from "isocholesterol" by Windaus (2). Formula (II) represents the structure of agnosterol.



(I) (II)

Dihydrolanosterol and dihydroagnosterol were both obtained from wool wax by Ruzicka (3) and may be prepared by hydrogenation of the side-chain double bond of lanosterol and agnosterol respectively. Ruzicka (3) also showed that lanosterol and dihydrolanosterol constitute 50% of

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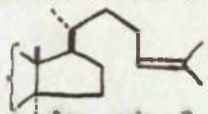
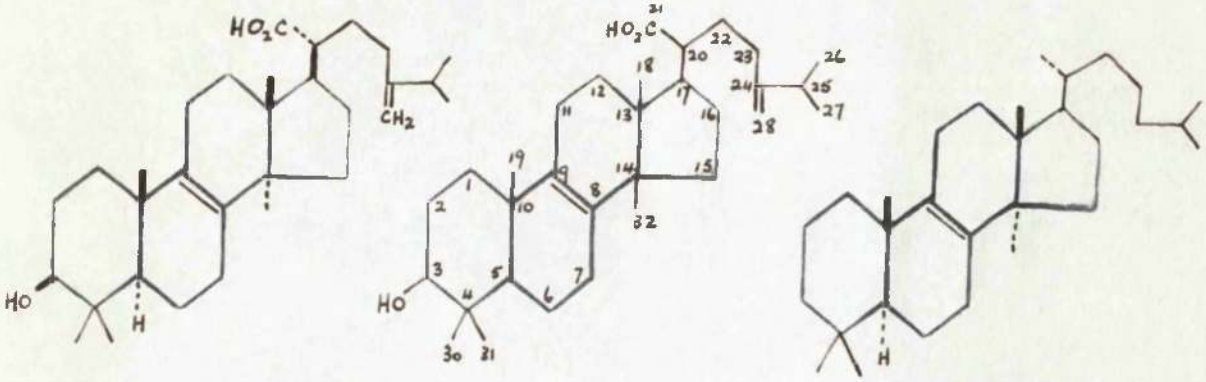
A dotted bond between $C_{(20)}$ and $C_{(21)}$,  , denotes the same configuration at $C_{(20)}$ as in cholesterol.

"isocholesterol", dihydroagnosterol 20%, while agnosterol is only present in small amounts.

Eburicoic acid was first isolated by Japanese workers (4) from the mycelium of the fungus Fomes officinalis Fr. Robertson and his co-workers (5) subsequently showed that this acid could be isolated from five species of fungi of the Basidiomycetes class and that in two of these species, Lentinus dactyloides Clel. and Fomes officinalis Fr., eburicoic acid occurred together with dehydroeburicoic acid, which is related to the former as agnosterol is to lanosterol. The structure of eburicoic acid, a $C_{(31)}$- triterpenoid, has been established by the elegant work of Robertson (5) and is as represented in (III). In numbering the carbon atoms of this acid and its derivatives, the $C_{(28)}$ has been reserved for use in the, at present unknown, compounds having the stigmasterol type of side chain. Final proof

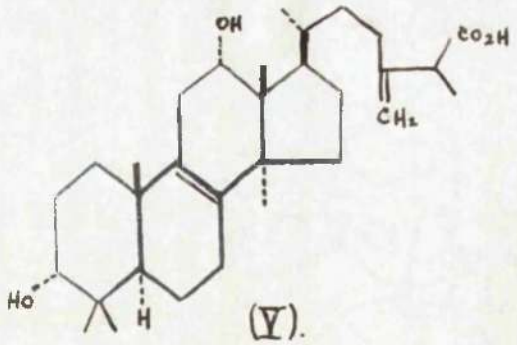



(III). (IV).

of the correctness of formula (III) was provided by the conversion (5) of eburicoic acid into lanost-8-ene (IV), using methods unlikely to cause stereochemical rearrangement in the molecule. The stereochemistry of eburicane is thus considered to be identical with that of lanostane.

The polyporenic acids A, B and C were first isolated from the widely occurring birch fungus, Polyporus betulinis Fr. by Cross, Elliott, Heilbron and Jones (6).

Polyporenic acid A, $C_{31}H_{50}O_4$, has been characterised as a diethenoid, dihydroxy-monocarboxylic acid, and was assigned the structure (V) by Jones and his collaborators as a result of extensive degradative studies (8-11). Jeger (12)

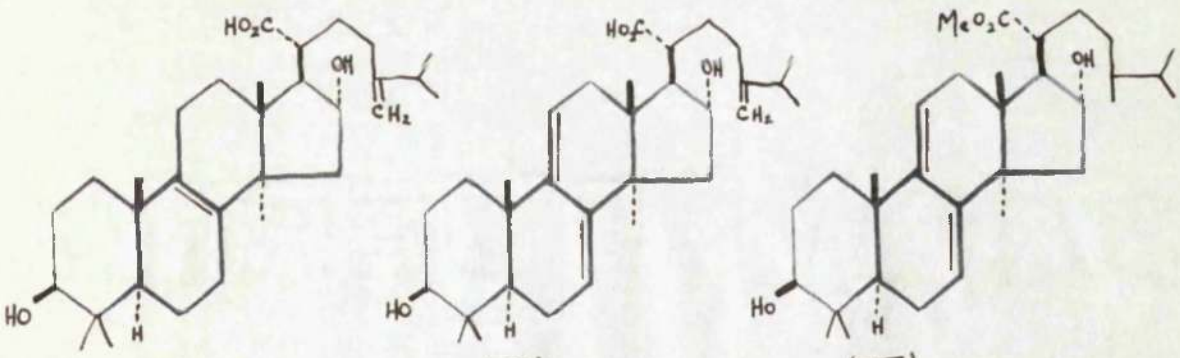



(V).

has since confirmed this structure by degradation of polyporenic acid A and lanosterol to a common derivative, and more recently Halsall and Hodges (13) have converted polyporenic acid A and lanosterol to a different common intermediate.

Polyporenic acid B, has recently been shown by Jones (14) to consist of a difficultly separable mixture of 3β:16α-

-dihydroxyeburic-3:24(28)-dien-21-oic acid (VI) and smaller amounts of the corresponding dehydro-derivative (VII). Jones did not resolve this mixture, but converted the dihydro methyl ester into the dehydro-material which was found to be identical with methyl 3β:16α-dihydroxyeburic-7:9(11)-dien-21-oate (VIII) previously obtained from polyporenic acid C (IX) (15).

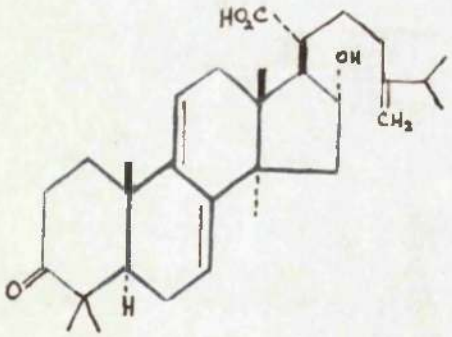



(VI) (VII) (VIII).

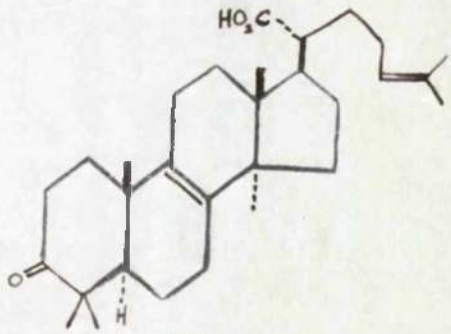
Robertson and his co-workers (16) have recently described the isolation of (VI) which they term tumulosic acid, from the fungus Polyporus tumulosus Cooke. As the acid in this case was eventually separated from its dehydro-derivative, Robertson proposes the retention of the name tumulosic acid for (VI).

Polyporenic acid C, $C_{31}H_{46}O_4$, has been characterised as a triethenoid hydroxy-keto-carboxylic acid (IX). Apart from the configuration of the hydroxyl group at $C_{(16)}$,

which was incorrectly assigned the β-configuration, Jones (15) established this structure by the conversion of polyporenic acid C into a derivative of eburicoic acid. In a more recent communication, Bowers, Halsall and Sayer (17) have shown, from a study of molecular rotation data, that the $C_{(16)}$ hydroxyl group has the α-configuration. They have also submitted convincing evidence that the parent hydrocarbon of polyporenic acid C is eburicane, which, as indicated above, has the same stereochemistry as lanostane.




(IX)

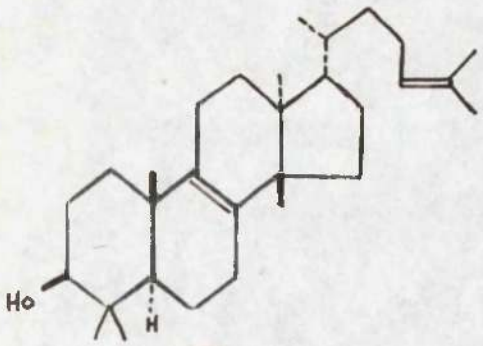



(X)

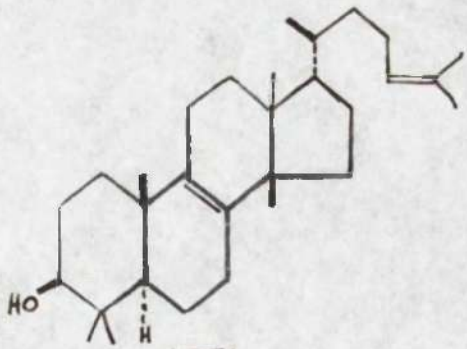
Pinicolic acid A, $C_{30}H_{46}O_3$, a keto-carboxylic acid has recently been isolated from the pine-rotting fungus Polyporus pinicola Fr. by Jones and his co-workers (18), and the structure established as (X) by conversion into a known derivative of eburicoic acid. Pinicolic acid A is the first example of a fungal acid having the lanostane carbon skeleton.

(ii) The euphol group of naturally occurring compounds consists of euphol, euphorbol, elemadienolic acid, elemadienonic acid, tirucallol and butyrospermol.

Euphol (euphadienol), $C_{30}H_{50}O$, is found in the Euphorbiaceae and was first isolated by Newbold and Spring (19). The structure has now been established as (XI), although the configuration at $C_{(17)}$ has recently been questioned by Barton. The chemistry of euphol is discussed in a later part of this section.



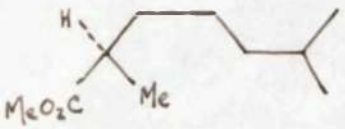

(XI).



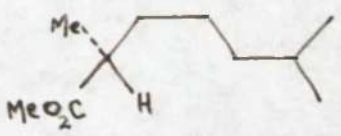

(XII).

Tirucallol, $C_{30}H_{50}O$, was first obtained from Euphorbia tirucalli L. by Haines and Warren (20) and characterised as a diethenoid secondary alcohol (21). In a recent communication (22) Arigoni, Jeger and Ruzicka have established the structure as (XII), 20-isoeuphol, following a series of degradative reactions which produced two acid fragments, isolated as the methyl esters. The first, L-(+)-2 :6-dimethyl-oenanthic acid

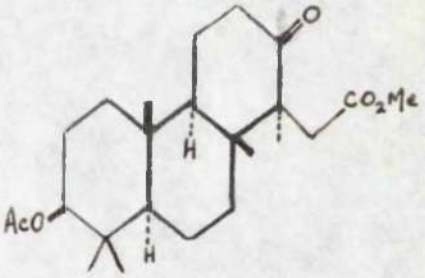
methyl ester (XIII), $[\alpha]_D$ + 18°, is the optically active enantiomorph of D-(-)-2 : 6-dimethyl-oenanthic acid methyl ester (XIV), $[\alpha]_D$ - 17°, previously isolated from euphadienol in a similar series of reactions (23). The second product proved to be identical with the tricylic acetoxy-methyl ester (XV), also obtained from euphadienol (23).




(XIII)




(XIV).

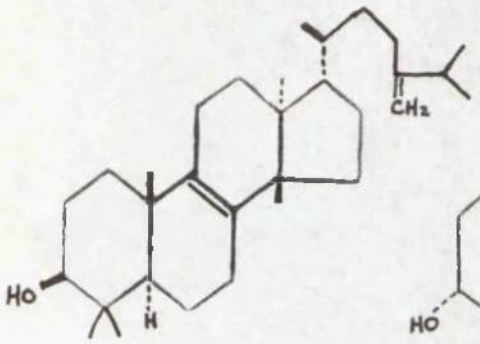



(XV).

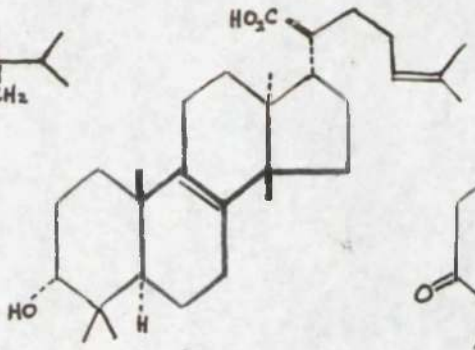
Euphorbol, $C_{31}H_{52}O$, occurs together with euphol in the Euphorbiaceae (19) and was recently related to tirucallol by elimination of the extra methylene group located in the side chain (24). Following the elucidation of the structure of tirucallol, Arigoni, Jeger and Ruzicka (22) formulated euphorbol as (XVI).

Elemadienolic and elemadienonic acids were isolated from Manila elemi resin by Ruzicka (25), who characterised the former as a doubly unsaturated, monohydroxy acid, $C_{30}H_{48}O_3$, and the latter as the corresponding ketone. Ruzicka and Hausermann (26) converted both to epielemenol,

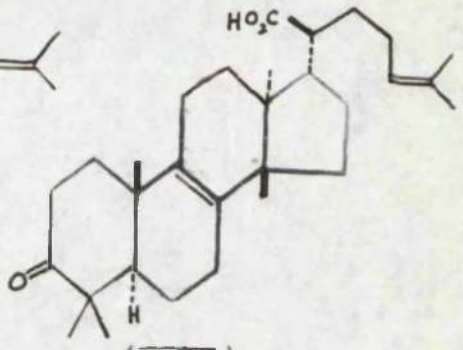
which was later identified as being identical with tirucallenol (dihydrotirucallol) (24). Elemadienolic acid is now therefore formulated as (XVII) and elemadienonic acid as (XVIII) (22).




(XVI)




(XVII)



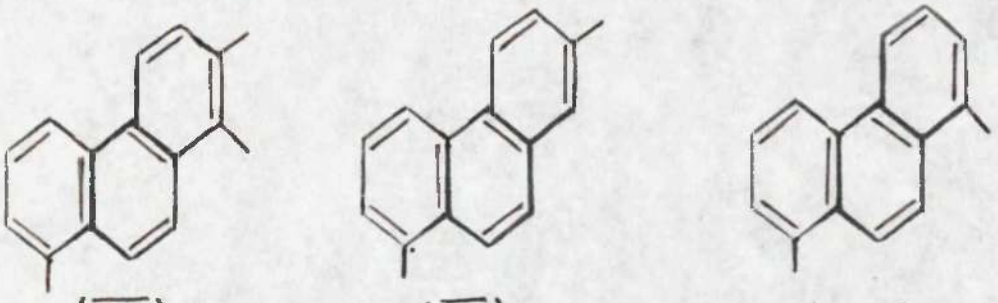

(XVIII)

Butyrospermol, a tetracyclic triterpenoid from shea nut fat is established as a member of the euphol series by experiments described in this thesis; results of earlier investigations are discussed in the Theoretical Section.

Dehydrogenation Reactions.

The tetracyclic triterpenoids undergo dehydrogenation on pyrolysis with selenium. The main product from the dehydrogenation of lanostadienol was first identified by Schulze in 1936 (27) as 1:2:3-trimethylphenanthrene (XIX). Three rings in the lanosterol molecule were thus accounted for. Ruzicka (28) also obtained (XIX) from "isocholesterol", together with other unidentified products.

Barton (30) has dehydrogenated "lanostene" (containing lanost-8-ene and lanost-9(11)-diene) and "lanosterol" (containing lanost-8-enol and lanost-7:9(11)-dienol), both of which were obtained from "isocholesterol". 1:2:8-Trimethylphenanthrene was obtained from both sources but in considerably better yield from "lanostene". The two adjacent methyl groups in (XIX) cannot therefore be derived from the gem-dimethyl groups at $C_{(4)}$ by a retropinacoline rearrangement as was postulated by Ruzicka (29); they must correspond to the methyl groups at $C_{(13)}$ and $C_{(14)}$ in the original molecule.

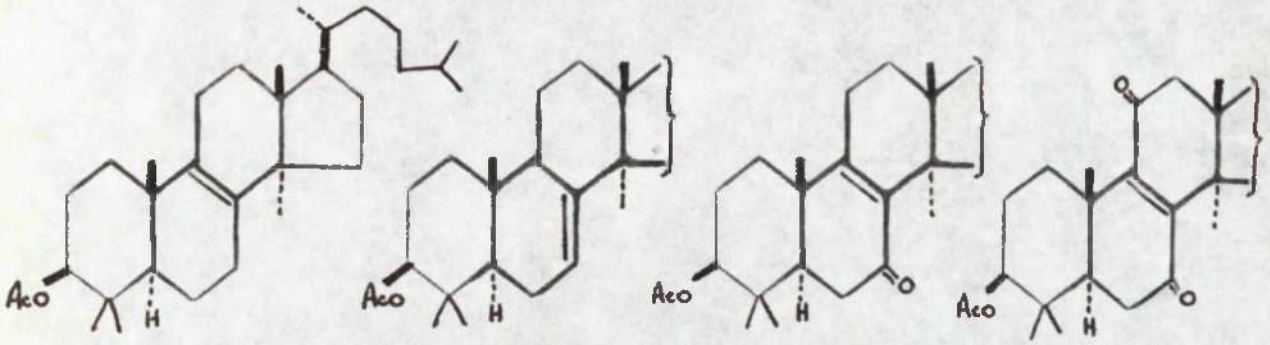


(XIX) (XX) (XXI)

In the euphol series, Warren (31) dehydrogenated eupha-8:24-dienol and identified the main product as 1:2:8--trimethylphenanthrene (XIX). Jeger (32) later dehydrogenated eupha-8:24-diene and isolated 1:2:8-trimethyl-, 1:7-diemthyl- (XX) and 1:8-dimethylphenanthrene (XXI).

Reactions in the Lanosterol Nucleus.

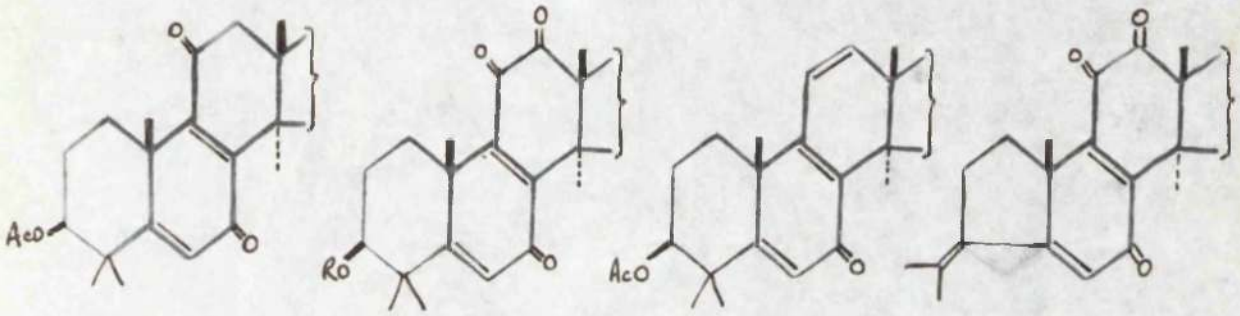
The majority of the tetracyclic triterpenoids have a fully substituted nuclear double bond and on oxidation with chromic acid give characteristic transoid 1:4-dione--enes, typified by (XXV). The first oxidations in the lanosterol nucleus were carried out by Ruzicka (23), who found that oxidation of lanost-8-enyl acetate (XXII) with chromic acid gave two products, 7:11-dioxolanost-8-enyl acetate (XXV) and 7-oxolanost-8-enyl acetate (XXIV). It was subsequently shown (33-36) that under more drastic conditions, (XXV) is the major product produced and that (XXIV) is readily converted to (XXV) on further oxidation (33).




(XXII) (XXIII) (XXIV) (XXV)

Lanost-8-enyl acetate when treated with mineral acid, forms an equilibrium mixture containing equal proportions of lanost-8-enyl acetate (XXII) and lanost-7-enyl acetate (XXIII) (30, 37, 38). Marker, Wittle and Mixon (37) showed that when this mixture is treated with chromic acid

under mild conditions, the $\Delta^8$ isomer is preferentially oxidised. Lanost-7-enyl acetate, however, may be oxidised using stronger conditions when (XXV) is formed (38).

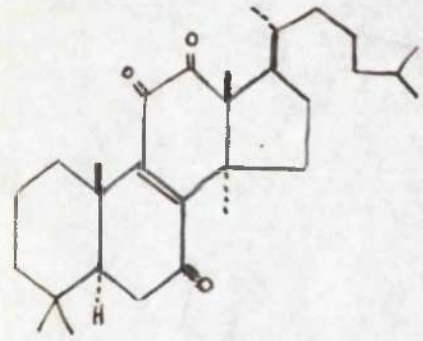



(XXVI). (XXVII). (XXVIII). (XXIX).

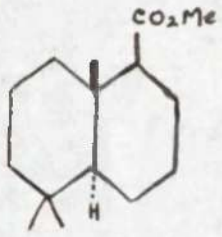
Oxidation of (XXV) with selenium dioxide gives 7:11--dioxolanosta-5:8-dienyl acetate (XXVI) (29, 33), which on further, more drastic, selenium dioxide oxidation gives 7:11:12-trioxolanost-5:8-dienyl acetate (XXVII, R = Ac) (29). The latter compound may also be prepared by oxidation of 7-oxolanost-5:8:11-trienyl acetate (XXVIII) with chromic acid (33). Barton (30) found that (XXVII, R = H), on dehydration with phosphorus pentachloride, underwent a retropinacoline rearrangement to form 7:11:12--trioxo*iso*lanost-3:5:8-triene (XXIX), in which the conjugated system has been extended to ring A. The position of the hydroxyl group in lanosterol was thus related to that of the nuclear double bond.

Jeger (39) recently degraded the ene-trione (XXX) to

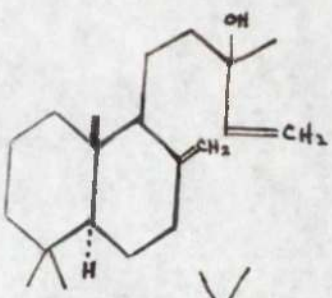
the ester (XXXI) which was also obtained from the diterpene manool (XXXII). Since manool had previously been related to abietic acid (XXXIII) (40), ambrein (XXXIV) (41) and oleanolic acid (XXXV) (42), it was concluded that lanosterol has the same constitution and configuration in rings A and B as a great many other naturally occurring terpenoids.



(XXX)



(XXXI)



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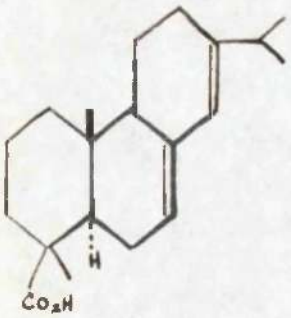
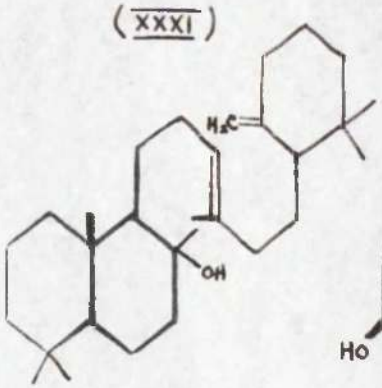
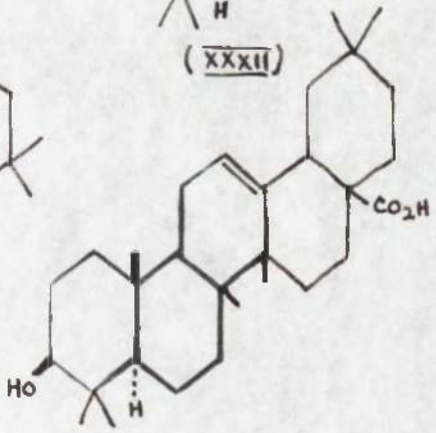


(XXXIII)



(XXXIV)



(XXXV)

The Side Chain of Lanosterol.

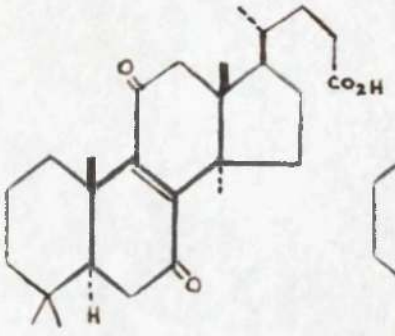
As early as 1933 (43) and 1937 (37), it was noticed that when lanost-8-enyl acetate was subjected to vigorous oxidation with chromic acid, small amounts of a sweet smelling, steam volatile substance were produced. More

recently, Barton, McGhie and co-workers (44, 45) have isolated this material in higher yield and by careful chromatography of the derived 2:4-dinitrophenylhydrazones, showed that it is a mixture of acetone and 6-methylheptan--2-one (XXXVI). As the reactive double bond of lanostadienol had previously been shown to be present in an isopropylidene group (66), it was concluded that the side chain of the lanosterol molecule is isooctenyl in nature, as represented in (XXXVII).

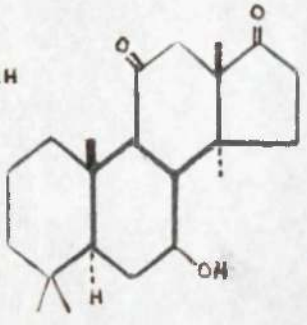
(XXXVI)

(XXXVII)

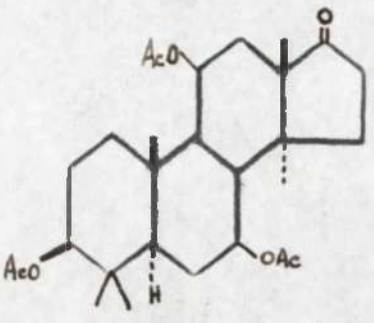
Ruzicka and his collaborators (46) independently reached the same conclusion by step-wise degradation of the side chain. Oxidation of lanosta-8:24-diene with chromic acid gave trisnor-7:11-dioxolanost-8-enoic acid (XXXVIII) which was converted in seven stages to the diketo-alcohol (XXXIX), the infra-red spectrum of which indicated the presence of a carbonyl group in a five-membered ring. From the infra-red



(XXXVIII)



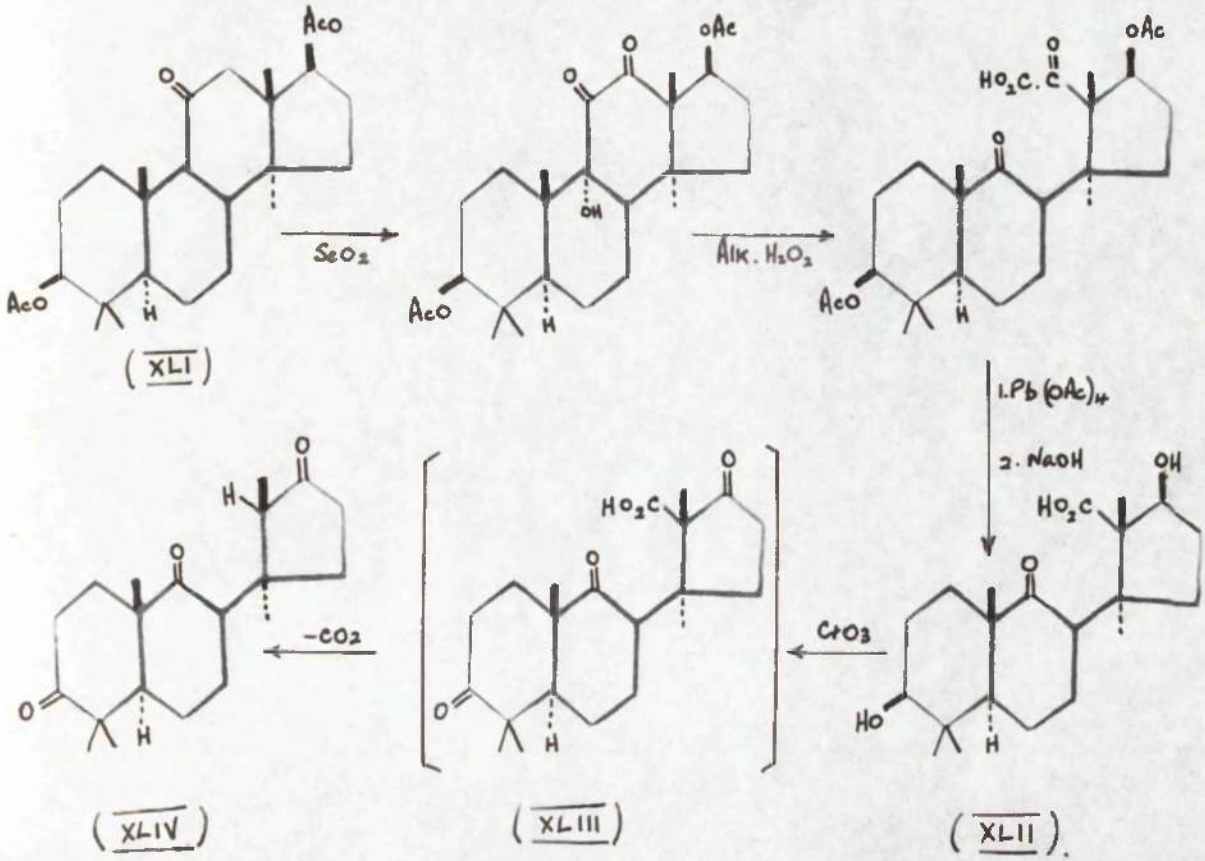
(XXXIX)



(XL)

spectrum of a similar degradation product (XL), Barton (48) was able to show that only one methylene group is adjacent to the carbonyl function. The point of attachment of the side chain must therefore be either $C_{(15)}$ or $C_{(17)}$.

That the side chain is in fact attached at $C_{(17)}$ was proved by Ruzicka (49) from the series of reactions outlined below. The degradation product (XLI) was converted in several stages to the dihydroxy-keto-acid (XLII). On mild oxidation with chromic acid, this underwent spontaneous decarboxylation, giving the triketone (XLIV); (XLII) must therefore be a β-hydroxy-acid, which on oxidation forms the intermediate β-keto-acid (XLIII). This evidence



requires that the hydroxyl group in (XLII), and hence the side chain of lanosterol, be located at $C_{(17)}$.

The Stereochemistry of Lanosterol.

The configuration at each asymmetric centre in the lanosterol molecule was established in 1952 by Curtis, Fridrichsons and Mathieson (50), from an X-ray analysis of lanosterol iodoacetate. In 1953, Barton (51) confirmed these findings by chemical means, using generalised conformational and molecular rotation arguments. These arguments are outlined in this section.

The hydroxyl group at $C_{(3)}$ is considered to have the β-configuration because of its stability towards attempted epimerisation (52) and its regeneration on reduction of the derived ketone with sodium and alcohol (37). On dehydration with phosphorus pentachloride, ring A undergoes a retropinacoline rearrangement, a reaction again known to be characteristic of a 3β-alcohol (53,54).

Klyne (55) first showed that rings A and B were trans-fused from a study of molecular rotation data. This was confirmed by Jeger (39) who, as explained above, isolated the fragment corresponding to rings A and B from both lanosterol and manool. Lanosterol was thus

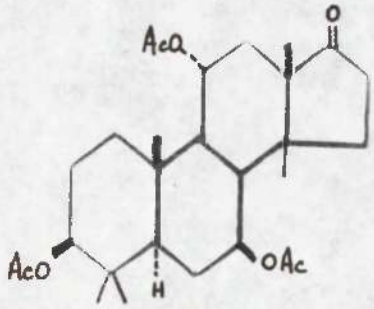
related to oleanolic acid, in which rings A and B had previously been shown to be trans-fused (56)

In an earlier communication (30), Barton postulated that the methyl group at $C_{(13)}$ was on the same side of the molecule as that at $C_{(10)}$, to explain the difference in steric hindrance of the keto-groups in 7:11-dioxo-lanostanol. The $C_{(13)}$-methyl group on this basis must have the β-configuration.

The two possible configurations for the $C_{(14)}$-methyl group are as shown in the part formulae (XLV) and (XLVI) for 3β:7β:11α-triacetoxylanostan-17-one (XLVII) (57). On Wolff-Kishner reduction of the 17-keto grouping, it is


(XLV)


(XLVI)




(XLVII)

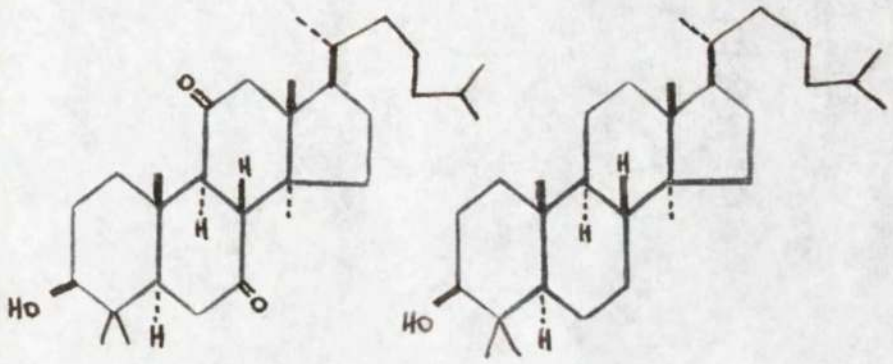
calculated (55) that the change in molecular rotation for structure (XLV) would be approximately + 500°, and for (XLVI), + 250°. The actual rotation difference is + 110° and therefore the $C_{(14)}$-methyl group is considered to be fused α, as in (XLVI).

The nuclear double bond between rings B and C may be

saturated through intermediates having the 7:11-diketo--grouping. The stability of 7:11-dioxolanostan-3-ol (XLVIII) to vigorous treatment with alkali, indicates that the hydrogens attached to carbon atoms $C_{(8)}$ and $C_{(9)}$ have the thermodynamically more stable configurations. On the basis of conformational arguments previously advanced by Barton (53), the configurations at $C_{(8)}$ and $C_{(9)}$ are shown to be <u>trans</u> relative to each other, but <u>anti</u> relative to $C_{(14)}$ and $C_{(10)}$.

The stereochemistry at $C_{(17)}$, the point of attachment of the side chain, is determined from a study of molecular rotation data. In the lanosterol series, the molecular rotation differences between compounds with the <u>iso</u>octyl type of side chain and those in which the side chain is replaced by -COMe, are in good agreement with those for corresponding steroid compounds. Therefore in lanosterol as in cholesterol, the side chain is considered to have the 17β-configuration.

The configuration of the saturated lanosterol molecule, lanostanol is therefore as in (XLIX).

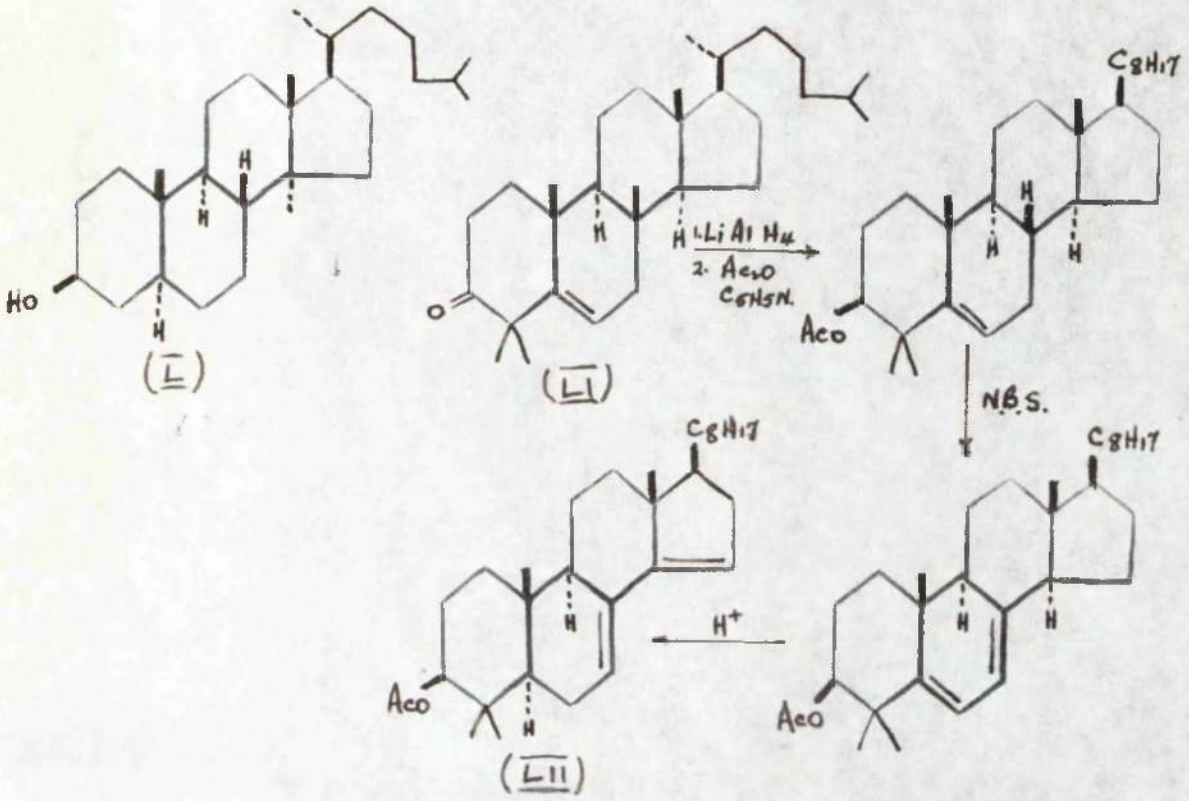


XLVIII XLIX

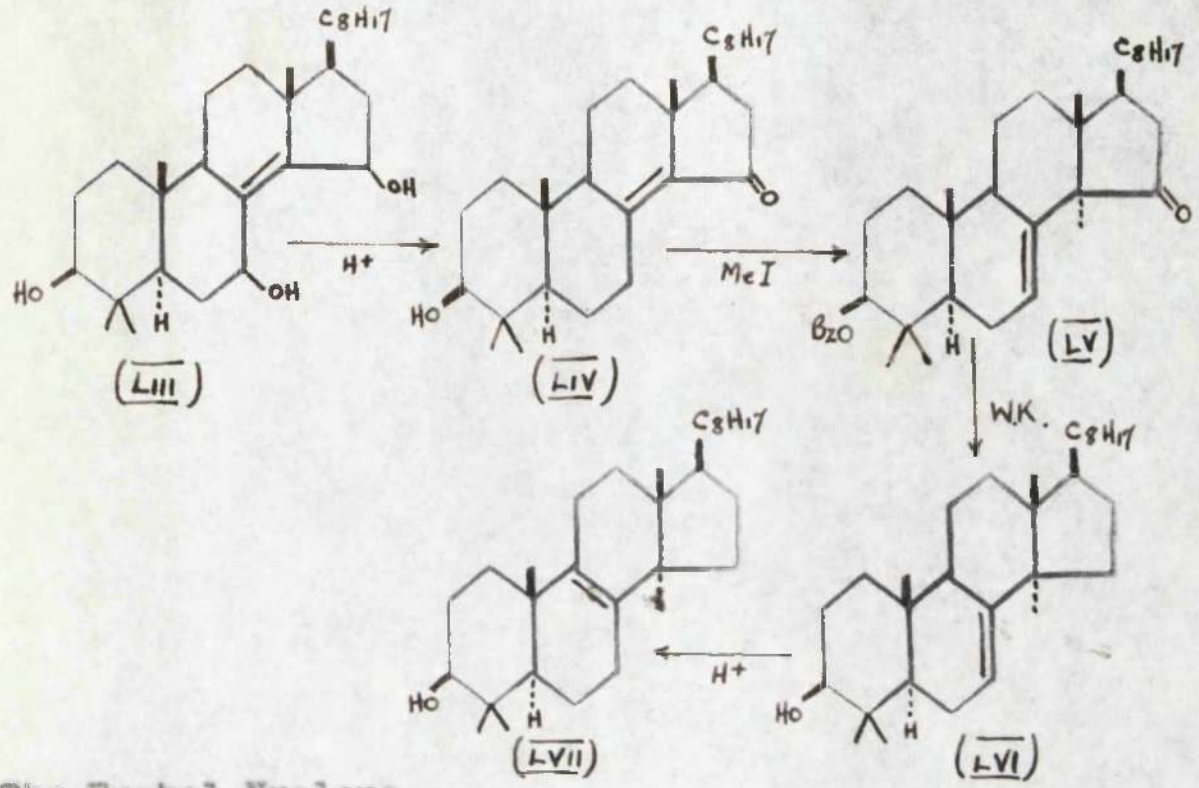
Synthesis of Lanostenol.

Rigorous confirmation of the structure and stereochemistry of lanosterol, as established from the degradative and deductive studies outlined above, has now been provided in the synthesis of lanostenol (dihydrolanosterol) from cholesterol, recently reported by Barton, Woodward and co-workers (58). The first direct inter-relationship between lanosterol and cholesterol was established by Barton (59), who converted lanosterol into 14-methylcholestanol (L). More recently, Barton and Woodward (60) have also obtained (L) from cholesterol. The synthesis of lanostenol is outlined below.

Methylation of cholest-5-en-3-one with methyl iodide and potassium tert.-butoxide gave 4:4-dimethylcholest-5-en--3-one (LI) which was converted by the steps shown to the diene (LII). On oxidation of (LII) with peracid,

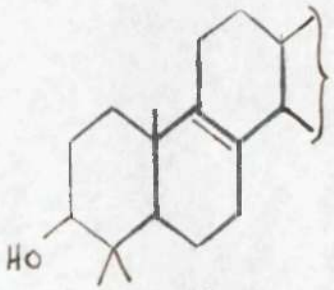



followed by hydrolysis, a triol probably (LIII) was obtained. This triol on treatment with mineral acid gave an αβ-unsaturated ketone (LIV), the benzoate of which was methylated, as above, to give (LV). By reduction under drastic Wolff-Kishner conditions, (LV) was converted into (LVI), which proved to be identical with lanost-7-enol. Treatment of the benzoate with hydrogen chloride and careful chromatography of the resulting mixture, gave lanost-8-enyl benzoate, hydrolysis of which yielded lanostenol (LVII). The above work constitutes the first total synthesis of a tetracyclic triterpenoid.




The Euphol Nucleus.

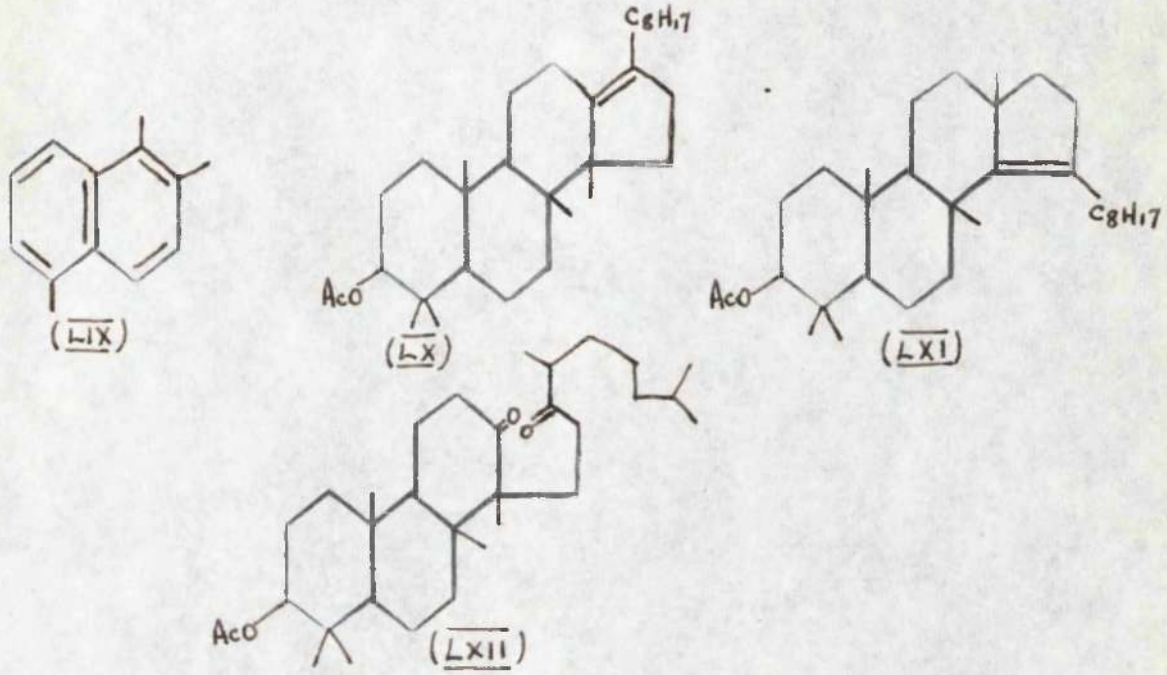
The close structural relationship between rings A, B and C of euphol and rings A, B and C of lanosterol, is established by the exactly analogous behaviour of derivatives of both compounds in reactions involving these rings. Euphol and its derivatives readily undergo the same dehydration and oxidation reactions (32) as those already described for the lanosterol nucleus, thus allowing rings A, B and C to be formulated as in (LVIII).




(LVIII).

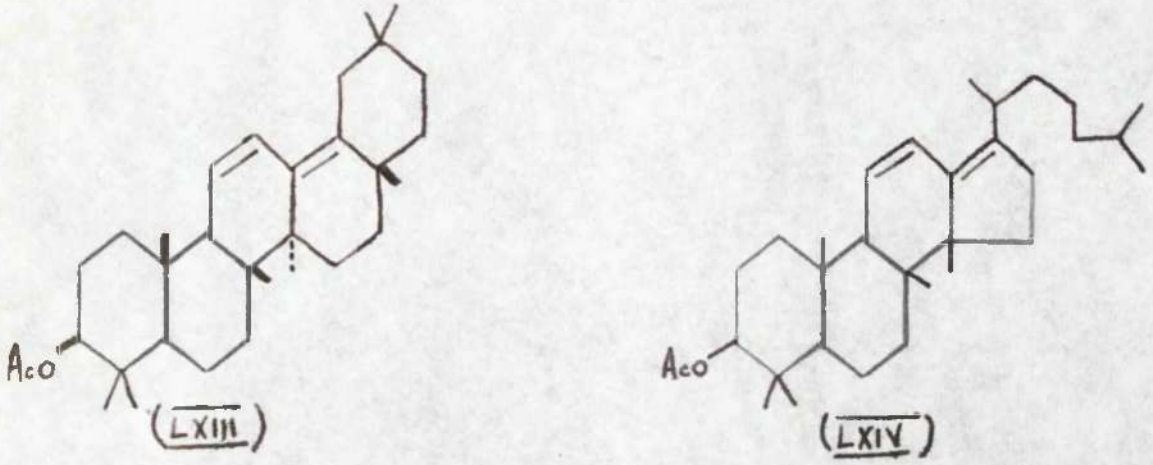
From a study of infra-red absorption data, Barton (61) has recently established that ring D of the euphol molecule is five-membered, as was first suggested by Ruzicka (62). The presence of a methyl group at $C_{(13)}$ and at $C_{(14)}$ follows from the dehydrogenation experiments already described.

The nature of the isomerisation of euphenol to isoeuphenol has now been fully explained by Barton (61) and the point of attachment of the side chain shown to be $C_{(17)}$. This rearrangement was first described by Vilkas (63) who found that, the $\Delta^8$ double bond in euphenyl acetate moves to another fully substituted position when euphenyl acetate is treated with mineral acid. From selenium dehydrogenation of isoeuphadiene, Barton established that this isomerisation involves methyl group migrations. The isolation of 1:2:5-trimethylnaphthalene (LIX) as the sole reaction product was attributed to migration of the $C_{(14)}$-methyl group to $C_{(8)}$, and allowed isoeuphenyl acetate to be formulated as (LX) or (LXI).




(LIX) (LX) (LXI) (LXII)

Structure (LX) is favoured from the following considerations. First, the diketone formed on ozonolysis of isoeuphenyl acetate (32) must have the structure (LXII) and be derived from (LX) as the infra-red absorption spectrum shows the presence of two $-CH_2CO$ groups. There is also chemical evidence that (LX) is correct. Secondly, the dehydro-derivative, isoeuphadienyl acetate (65) is very similar to oleana-11:13(18)-dienyl acetate (LXIII) in chemical reactivity and ultra-violet and infra-red absorption spectra, and must be formulated as (LXIV).



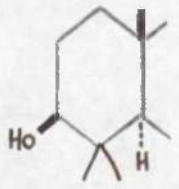

(LXIII) (LXIV)

Jeger, Ruzicka and co-workers (23) independently determined the size of ring D and the nature of the isomerisation of euphenol to isoeuphenol from a series of degradative reactions.

The Stereochemistry of Euphol.

The stereochemistry at each centre of asymmetry in the euphol molecule has now been established, with the possible exception of that at $C_{(17)}$.

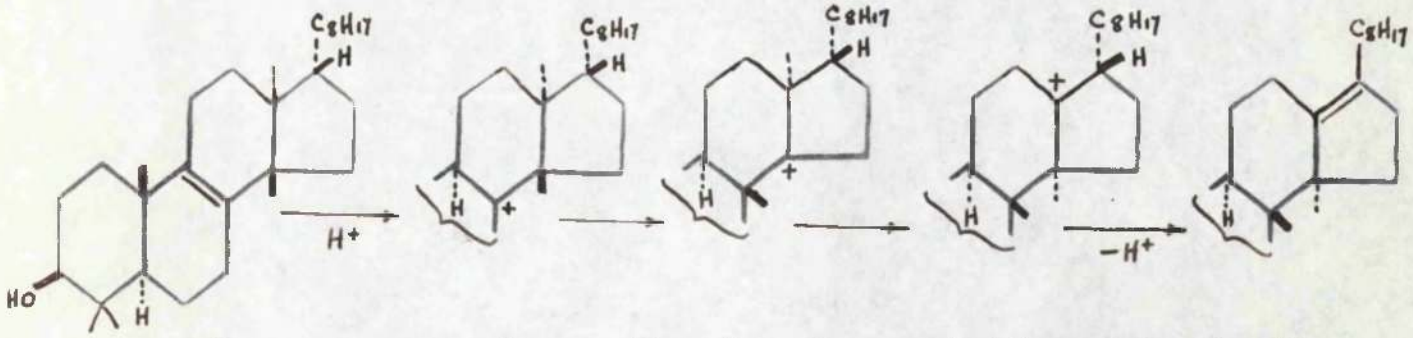
The configurations at $C_{(3)}$, $C_{(5)}$ and $C_{(10)}$ are stated by Barton (64) to be as in (LXV) from the facts that the hydroxyl group at $C_{(3)}$ is equatorial and has the molecular rotation properties of a normal 3β-alcohol.




LXV

On the basis of conformational arguments outlined below, Barton (61,64) and Ruzicka (23) consider the methyl group at $C_{(13)}$ to have the α-configuration and that at $C_{(14)}$, the β-configuration. With this stereochemistry, rings B and C are forced to assume the unfavourable conformation of two 'half-boats'. This steric strain provides a 'conformational driving force'

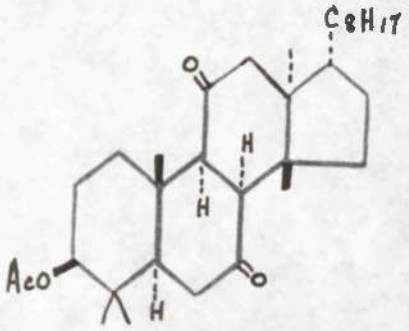
for migration of the methyl groups at $C_{(13)}$ and $C_{(14)}$, whereby the molecule can adopt the isoeuphenol type of structure in which rings A, B and C are in the more favourable 'all-chair' conformation. In the lanosterol series, the methyl groups do not migrate when a carbonium ion is formed at $C_{(8)}$ as all three six-membered rings are already in the 'all-chair' conformation and the molecule is not subjected to a conformational driving force. On the basis of a totally concerted mechanism for the isoeuphenol rearrangement, the side chain at $C_{(17)}$ and the methyl group at $C_{(13)}$ must both have the α-configuration and the methyl group at $C_{(14)}$ the β-configuration. The mechanism may then be represented by the following synchronous stages.




From a comparison of molecular rotation data in the lanosterol and euphol series, Barton (64) confirmed that the side chain at $C_{(17)}$ has the α-configuration. On the same basis, the configuration at $C_{(20)}$ was claimed to be opposite to that at $C_{(20)}$ in lanosterol. Jeger and

Ruzicka (23), however, have shown from degradative studies that the configuration at $C_{(20)}$ is the same as that in lanosterol. In a more recent communication (61), Barton now asserts that if the method of molecular rotation differences cannot be applied, then no definite configuration can be assigned to $C_{(17)}$. The Swiss group (23), however, regard the side chain as being fused α on the basis of a completely concerted mechanism for the isoeuphenol rearrangement, and it is with this configuration at $C_{(17)}$ that the formulae given in this thesis are written.

Compounds having rings B and C saturated may be prepared via intermediates having the 7:11-diketo-grouping. 7:11-Dioxoeuphanyl acetate, however, on treatment with selenium dioxide (32) or with alkali (32) regenerates 7:11-dioxoeuph-8-enyl acetate; the two hydrogen atoms at $C_{(8)}$ and $C_{(9)}$ are therefore considered to be cis relative to each other (67), and are probably in the α-configuration as in (LXVI).




(LXVI)

# THEORETICAL.

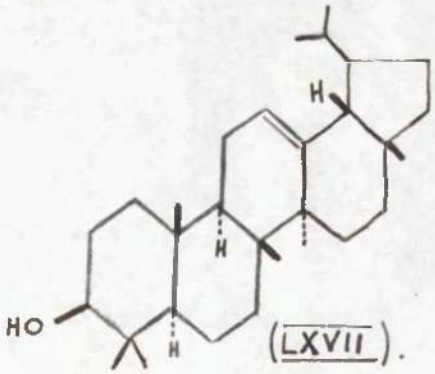
## Section I.

The non-saponifiable fraction from the seed fat of *Strychnos nux-vomica* L. has been shown to contain the triterpenoids α-amyrin and *cycloartenol*. *cyclo*Artenol has been identified as 9:19-*cyclo*lanost--24-en-3β-ol.

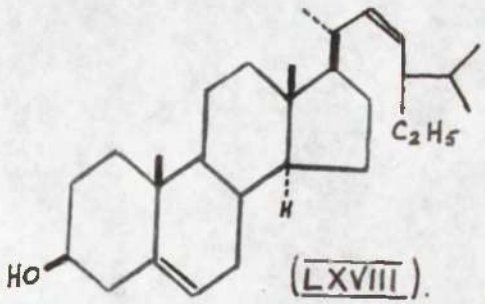
The seeds of Strychnos nux-vomica L. are processed commercially for the alkaloid poisons strychnine and brucine, which were first isolated by Pelletier and Caventou over a hundred years ago. S. nux-vomica also contains one to two percent of a fat, which in 1815 was shown by Meyer (68) to consist largely of the glycerides of capric, caprylic, caproic, butyric and palmitic acids. In 1912 and 1915, Heiduschka and Wallenreuter (69, 70) made a detailed examination of the non-saponifiable fraction, which constitutes approximately twenty per cent of the fat. The fraction was acetylated, using acetic anhydride, and by fractional crystallisation from the same solvent, three crystalline acetates were isolated and characterised as the alcohols. The first was described as a phytosterol, m.p. 158°, analysis of which corresponded to the formula $C_{27}H_{46}O,H_2O$. The second alcohol, m.p. 115° (99° solvated; acetate m.p. 123-124°) was formulated as $C_{32}H_{54}O$ and the third, m.p. 186° (acetate m.p. 221°) as $C_{35}H_{58-60}O$. The alcohol, m.p. 186°, was characterised by the preparation of several esters and it was reported that the acetate, m.p. 221°, on oxidation with chromic acid, gave a product resembling "oxy-amyrin acetate".

Through the courtesy of the Directors of Messrs. T. and H. Smith Ltd., Edinburgh, supplies of S. nux-vomica seed fat were made available for further investigation. In the commercial extraction of strychnine and brucine, the crushed seeds are extracted with boiling benzene and the alkaloid bases isolated by extraction of the benzene solution with hydrochloric acid. The fat, obtained as a dark brown gum on evaporation of the benzene, was refluxed for 4 hours in 5 per cent methanolic sodium hydroxide and the non-saponifiable fraction isolated by dilution with water and extraction with ether. The resulting orange, waxy solid was acetylated and the product dissolved in light petroleum and chromatographed, to give four well-defined fractions. The first, a sweet-smelling, colourless oil, has not been examined further. The second was crystallised several times from chloroform-methanol to give plates, m.p. 223-225°, and on hydrolysis these gave an alcohol which separated from aqueous methanol as needles, m.p. 185°. The alcohol and acetate, which gave a strong yellow colour with tetranitromethane and a pink colour in the Liebermann-Burchardt test, were shown to be identical with α-amyrin (LXVII) and α-amyrin acetate respectively. They

must correspond to the alcohol m.p. 136° and the acetate m.p. 221°, originally described by Heiduschka and Wallenreuter. The final fraction was purified by M.B.E. Fayez (71) who obtained an acetate, m.p. 142.5-143°, which on hydrolysis gave an alcohol, m.p. 165.5-167.5° shown, by direct comparison, to be identical with stigmasterol (LXVIII). The phytosterol, m.p. 153°, described by Heiduschka and Wallenreuter was, in all probability, an impure sample of stigmasterol.




(LXVII).




(LXVIII).

The third fraction was examined in detail by the author. After many crystallisations from chloroform--methanol plates, m.p. 122-124° were obtained, analysis of which indicated the formula $C_{32}H_{52}O_2$. The yield of this acetate amounted to 12 per cent of the total acetylated non-saponifiable fraction. With the Liebermann-Burchardt reagent, the acetate gave a blood red solution having a strong green fluorescence. The presence of an unsaturated centre was indicated by the strong yellow

colour given with tetranitromethane and by the ultra-violet light absorption which showed a maximum at 2090 Å. ($\varepsilon$, 1500). The parent alcohol, $C_{30}H_{50}O$ (m.p. 115°; 99° when solvated), is almost certainly identical with one of the components originally described by Heiduschka and Wallenreuter.

The acetate $C_{32}H_{52}O_2$, m.p. 122-124°, on hydrogenation at room temperature in the presence of platinum catalyst, absorbed one molecular proportion of hydrogen to give a dihydro-acetate, $C_{32}H_{54}O_2$, m.p. 130-132°, hydrolysis of which gave a dihydro-alcohol, $C_{30}H_{52}O$. The presence of a secondary alcohol group in the dihydro-alcohol (and consequently in the parent alcohol) was established by oxidation to a ketone $C_{30}H_{50}O$. All the dihydro-derivatives gave a faint but distinct yellow colour with tetranitromethane in chloroform but showed no selective light absorption in the ethylenic region.

The double bond was shown to be present in an isopropylidene grouping by ozonolysis of the acetate $C_{32}H_{52}O_2$, m.p. 122-124°, to give acetone, isolated as the 2:4-dinitrophenylhydrazone. The non-volatile fragment was obtained as a trisnor-aldehyde acetate $C_{29}H_{46}O_3$, m.p. 155-157°, which on mild oxidation with chromic acid, gave a

trisnor-acid acetate $C_{29}H_{46}O_4$, m.p. 221.5-223°. This acid was also obtained by chromic acid oxidation of the acetate m.p. 122-124°, and on hydrolysis gave the parent hydroxy-trisnor-acid $C_{27}H_{44}O_3$, m.p. 218-220°.

From the results described above, some general features of the structure of the parent alcohol were established. Analysis of derivatives indicated the formula $C_{30}H_{50}O$ for the alcohol. A pentacyclic structure of the amyrin or lupeol type was considered unlikely from the low melting points of the alcohol and its derivatives and also from the ozonolysis and oxidation reactions which supported a tetracyclic structure having a side chain terminating in an <u>iso</u>propylidene group, as in lanosterol and euphol. The Liebermann-Burchardt test was also markedly different from that given by the pentacyclic triterpenoids. The formula $C_{30}H_{50}O$ for the parent alcohol, however, requires that the molecule be doubly unsaturated, if it is tetracyclic. The only evidence of further unsaturation in the dihydro- series was the faint but distinct yellow colour given with tetranitromethane; no light absorption in the range 1950 to 2200 Å. was apparent.

Treatment of the dihydro-acetate in chloroform with

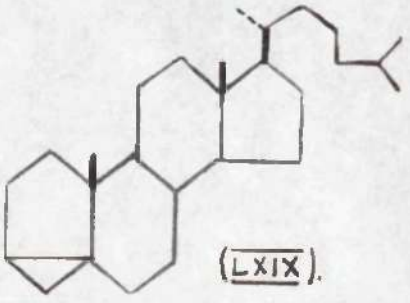
hydrogen chloride at low temperature gave a product, m.p. 137-157°, which unlike the dihydro-acetate gave a strong yellow colour with tetranitromethane and showed absorption maximum at 2080 Å. ($\epsilon$, 3600). At this point in the investigation, the presence of a cyclopropane ring was suspected and suggested a comparison of the alcohol from S. nux-vomica with cycloartenol, which Barton (73) had recently isolated from the fruit of Artocarpus integrifolia and which contains a cyclopropane ring. A comparison of the physical constants of the S. nux-vomica alcohol and its derivatives with those of the corresponding derivatives of cycloartenol was made, and this strongly suggested their identity. The identities of the alcohol from S. nux-vomica and cycloartenol were established by mixed melting point determinations, carried out by Professor D. H. R. Barton (Table A.).

TABLE A.

| | From S. nux-vomica | | From Artocarpus integrifolia | | |
|---|---|---|---|---|---|
| | M.p. | $[\alpha]_D$ | M.p. | $[\alpha]_D$ | Mixed M.p. |
| cycloArtenol | 115° (99°solvated) | +54° | 85-92° | +43° | 112-113.5° (sinters 110°) |
| Acetate | 122-124° | +60° | 122.5-123.5° | +58° | 121.5-122.5° |

cycloArtenol has since been isolated from Euphorbia balsamifera by Chapon and David (74) and has recently been shown to be identical with handianol, isolated from Euphorbia handiensis, by Gonzalez and co-workers (75).

Barton (73) found that Artocarpus integrifolia contained mainly the ketone, cycloartenone, together with smaller amounts of cycloartenol and butyrospermol. The conclusions reached concerning the structure of cycloartenol were very similar to those outlined above. Barton also observed that derivatives of the dihydro--alcohol, cycloartanol, gave a pale yellow colour with tetranitromethane; carefully purified i-cholestane (LXIX), was also found to give a similar colour with tetranitromethane and to be transparent to ultra-violet light in the range 1950 to 2100 Å. from which it is



(LXIX).

concluded that the pale yellow colour with tetranitromethane is a characteristic of the cyclopropane ring.

The resistance of cycloartanyl acetate to oxidation was demonstrated by Barton; the acetate was recovered

unchanged on treatment with selenium dioxide and with hot peracetic acid under conditions known to attack the very unreactive double bond of α-amyrin (76). The presence of a cyclopropane ring was further evidenced by the infra-red absorption spectra of cycloartenone and cycloartenyl acetate, both of which showed absorption near 1000 $cm.^{-1}$, although somewhat masked by neighbouring bands of high intensity. i-Cholestane also showed absorption at 1010 $cm.^{-1}$, and Derfer, Pickett and Boord (77) had previously demonstrated that alkyl-substituted cyclopropanoid compounds are characterised by intense absorption in the region 1020-1000 $cm^{-1}$.

Barton treated cycloartanyl benzoate with hydrogen chloride and obtained a constant melting isomer, artenyl benzoate, m.p. 197-198°. The derived alcohol, artenol, also melted sharply m.p. 152-154°, but the corresponding acetate required many crystallisations before artenyl acetate, m.p. 165-167° was obtained. All these products showed characteristics of ethylenic unsaturation and although they were presumably claimed to be homogeneous, Barton added that artenol and its benzoate might possibly be mixtures of double-bond isomers, very difficult to separate on crystallisation.

As mentioned above treatment of cycloartanyl

acetate with hydrogen chloride gave a product, m.p. 137-157° having the characteristics of ethylenic unsaturation and which proved to be extremely difficult to purify. Similar treatment of cycloartenyl acetate also gave a product which melted over a range, m.p. 144-154°. This material gave a strong yellow colour with tetranitromethane in chloroform and was shown by the Beilstein test to contain halogen, no doubt due to the addition of hydrogen chloride to the isopropylidene group. Continued recrystallisation resulted in a gradual increase in melting point with considerable loss of material. It was concluded from these results that fission of the cyclopropane ring of cycloartenol and its derivatives with acid gives a difficultly separable mixture of double-bond isomers.

A solution of the acetate mixture m.p. 137-157° in acetic acid was shaken with hydrogen and platinum catalyst at 30° for 2 hours, by which time absorption of hydrogen had ceased. The product, m.p. 137-154° still gave a yellow colour with tetranitromethane and showed selective absorption in the ultra-violet region of reduced intensity: maximum at 2060 Å. ($\varepsilon$, 2000). Complete hydrogenation of the acetate mixture, m.p. 137-157°, was attempted using more forcing hydrogenation conditions, but in each case the partially reduced product was obtained. It was concluded

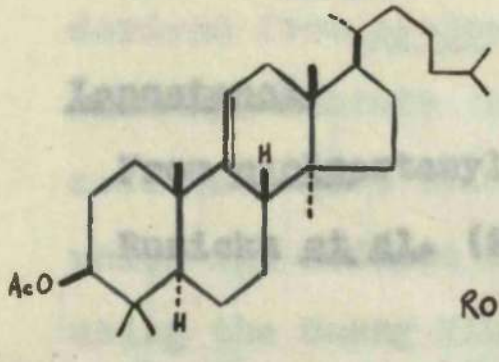
that approximately 50 per cent of the acid isomerisation product of cycloartanyl acetate is inert to hydrogenation. Careful chromatography of the partly reduced acetate mixture and of the derived alcohol and benzoate mixtures, failed to give a homogeneous product. The partly saturated product was oxidised with chromic acid in acetic acid using vigorous conditions, and the product chromatographed. The first fraction on crystallisation gave, in 60 per cent yield, a saturated acetate, $C_{32}H_{56}O_2$, m.p. 155-156° which gave no colour with tetranitromethane and showed no selective light absorption. Hydrolysis gave a saturated alcohol, $C_{30}H_{54}O$, m.p. 180-181°. On further elution, a second fraction was obtained which gave in 10 per cent yield, a pale yellow compound $C_{32}H_{50}O_4$, m.p. 155-156° which showed no tetranitromethane colour and showed absorption maximum at 2700 Å. (ε, 8700). This light absorption is characteristic of a fully transoid 1:4-dione-ene grouping, as in 7:11-dioxolanost-8-enyl acetate (LXXII). In a subsequent experiment in which hydrogenation was carried out at room temperature for 8 hours, the two products described above were accompanied by smaller amounts of a third compound $C_{32}H_{52}O_3$, m.p. 134-135°. This material, which gave no colour with tetranitromethane and had an

absorption maximum at 2420 Å. (ε, 9000), was found to be identical with a β,β-disubstituted αβ-unsaturated ketone derived from one of the constituents of the original acetate mixture as outlined below.

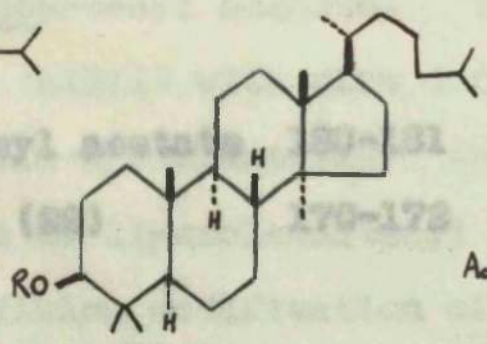
J. A. Henry (71) isolated a homogeneous product from the acetate mixture by a procedure analogous to that used by Marker, Wittle and Mixon (37) in the isolation of lanost-7-enyl acetate from the mixture obtained on acid rearrangement of lanost-8-enyl acetate. The mixture m.p. 137-157° was oxidised with chromic acid in acetic acid under very mild conditions and the product chromatographed. Early fractions contained the component which largely escaped oxidation, and on crystallisation yielded a well-defined product $C_{32}H_{54}O_2$, m.p. 170-172°, in 50 per cent yield. The compound gave a strong yellow colour with tetranitromethane and showed selective absorption in the ultra-violet region with a maximum at 2060 Å.(ε, 4300). Further elution gave a small amount of material having the ultra-violet absorption characteristics of a 1:4-dione-ene, but which could not be obtained pure. A third product m.p. 184-185° was also obtained in low yield; it showed ultra-violet absorption at 2420 Å. (ε, 9200), characteristic of an αβ-unsaturated ketone, and was prepared

in high yield by more vigorous oxidation of the acetate m.p. 170-172° with chromic acid.

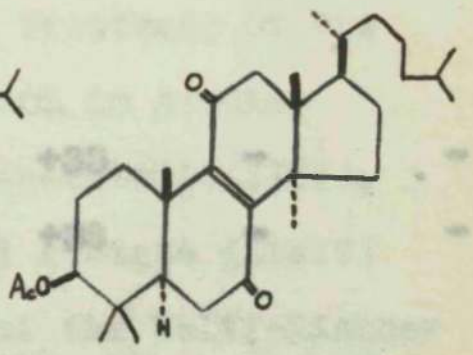
The physical constants and properties of the homogeneous compounds obtained from the mixture resulting on acid fission of the cyclopropane ring in cycloartanyl acetate were compared with those of possibly corresponding derivatives in the lanosterol and euphol series. This comparison strongly suggested a relationship with the lanosterol derivatives; the unsaturated acetate m.p. 170-172° appeared to be identical with lanost-9(11)--enyl acetate (LXX), the saturated acetate m.p. 155-156° with lanostanyl acetate (LXXI, R = Ac), the saturated alcohol m.p. 180-181° with lanostanol (LXXI, R = H) and the dione-ene with 7:11-dioxolanost-8-enyl acetate (LXXII).




(LXX)




(LXXI)




(LXXII)

Comparison of the physical constants is set out in Table B.

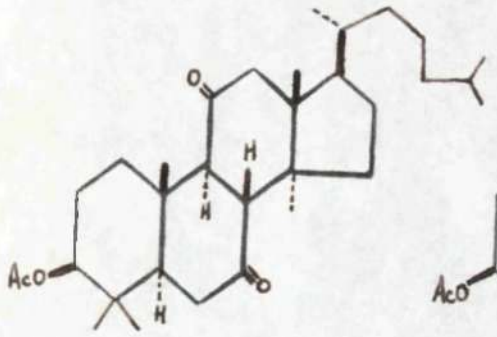
TABLE B.

| | M.p. | $[\alpha]_D$ | $\lambda$ max. | $\varepsilon$. |
|---|---|---|---|---|
| Lanost-9(11)-enyl Acetate | | | | |
| From cycloartanyl acetate | 170-172° | +85° | 2060 Å. | 4300 |
| Ruzicka et al. (29) | 170-171 | +81 | - | - |
| McGhie et al. (1) | 177-178 | +83 | - | - |
| 7:11-Dioxolanost-3-enyl Acetate. | | | | |
| From cycloartanyl acetate | 155-156 | +91 | 2700 | 3700 |
| Ruzicka et al. (3) | 156-158 | +90.5 | - | - |
| McGhie et al. (34) | 158-159 | +91.6 | 2750 | 3710 |
| Lanostanyl Acetate | | | | |
| From cycloartanyl acetate | 155-156 | +41 | - | - |
| Ruzicka et al. (29) | 150-151 | +41 | - | - |
| McGhie et al. (78) | 156-157 | +46 | - | - |
| Lanostanol | | | | |
| From cycloartanyl acetate | 130-131 | +33 | - | - |
| Ruzicka et al. (29) | 170-172 | +36 | - | - |

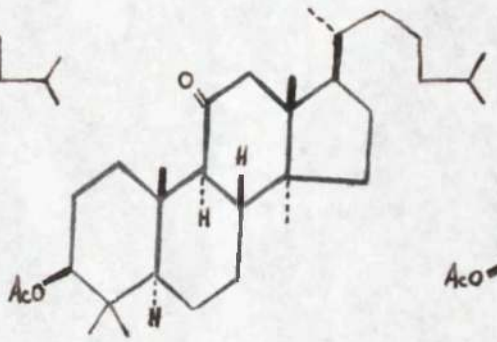
In order to confirm identity by direct comparison, the lanosterol derivatives described above were prepared from a sample of "isocholesteryl acetate" kindly supplied by

Dr. C. L. Hewitt of Organon Laboratories Ltd. "isoCholesterol" has been shown (3) to be a mixture composed of lanosterol, dihydrolanosterol, agnosterol and dihydroagnosterol. The acetate mixture was hydrogenated in acetic acid using platinum catalyst to give a mixture of dihydrolanosteryl and dihydroagnosteryl acetates. It is known that oxidation of both dihydrolanosteryl and dihydroagnosteryl acetates with chromic acid gives 7:11-dioxolanost--8-enyl acetate (33). Accordingly, the mixture of dihydro-acetates was treated with chromic acid in acetic acid following the procedure used by Cavalla and McGhie (34) in the oxidation of lanost-8-enyl acetate. A homogeneous product, 7:11-dioxolanost--8-enyl acetate (LXXII) was obtained in good yield and shown to be identical with the dionenyl acetate derived from cycloartanyl acetate. Treatment of the dionenyl acetate (LXXII) with zinc dust in acetic acid (36) gave 7:11-dioxolanostanyl acetate (LXXIII), which was reduced to 11-oxolanostanyl acetate (LXXIV) using the Huang Minlon modification of the Wolff-Kishner reaction as described by McGhie, Pradhan and Cavalla (78). Reduction of (LXXIV) with lithium aluminium hydride and monoacetylation of the product according to

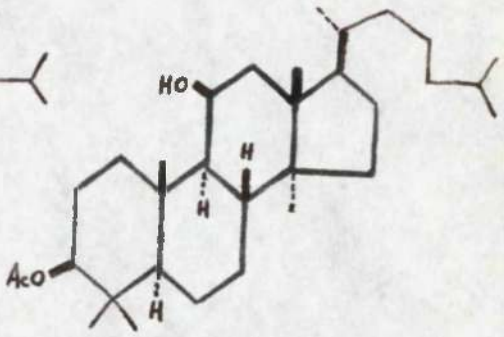
Ruzicka and co-workers (29), gave 11-hydroxylanostanyl acetate (LXXV). The conversion of (LXXV) into lanost--9(11)-enyl acetate (LXX) was effected by treatment with phosphorus oxychloride in pyridine solution, under conditions more vigorous than those described by Ruzicka (29). The unsaturated acetate m.p. 170-172° derived from cycloartanyl acetate proved to be identical with lanost-9(11)-enyl acetate. Hydrogenation of the 9(11)-enyl acetate at 80° for 24 hours (29) gave lanostanyl acetate (LXXI, R = Ac), hydrolysis of which furnished lanostanol (LXXI, R = H). The saturated acetate m.p. 155-156° obtained from cycloartanol as described above and the derived alcohol, m.p. 180-181°, were found to be identical with lanostanyl acetate and lanostanol respectively.




(LXXIII)




(LXXIV)



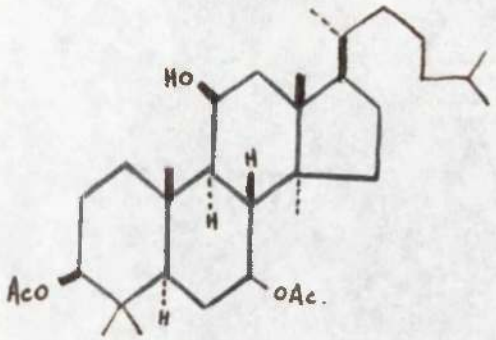

(LXXV)

It is probable that the artenyl acetate and the dihydro-derivative, artanyl acetate, described by Barton, were largely composed of lanost-9(11)-enyl acetate and lanostanyl acetate respectively. The comparison of physical constants quoted by Barton (73) with those obtained in this work is given in Table C.

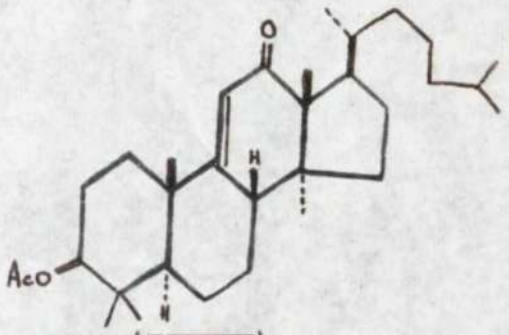
TABLE C

| | M.p. | $[\alpha]_D$ | $\lambda$ max. | $\varepsilon$ |
|---|---|---|---|---|
| Artenyl Acetate (73) | 165-167° | +73° | 1995 Å. | 3600 |
| Lanost-9(11)-enyl Acetate (this work) | 170-172 | +85 | 2060 | 4300 |
| Artanyl Acetate (73) | 143-149 | - | - | - |
| Lanostanyl Acetate (this work) | 155-156 | +41 | - | - |

Reduction of 7:11-dioxolanostanyl acetate (LXXIII) using the Huang Minlon modification of the Wolff-Kishner reaction, gave 11-oxolanostanyl acetate (LXXIV), as described above; when the normal Wolff-Kishner reaction was used, however, the reaction proceeded in a different direction. The product proved to be identical with 3:7-diacetoxylanostan-11-ol (LXXVI), and was undepressed in melting point when mixed with an authentic sample prepared by reduction of 7:11-dioxolanostanyl acetate with lithium aluminium hydride (29)




(LXXVI)




(LXXVII)

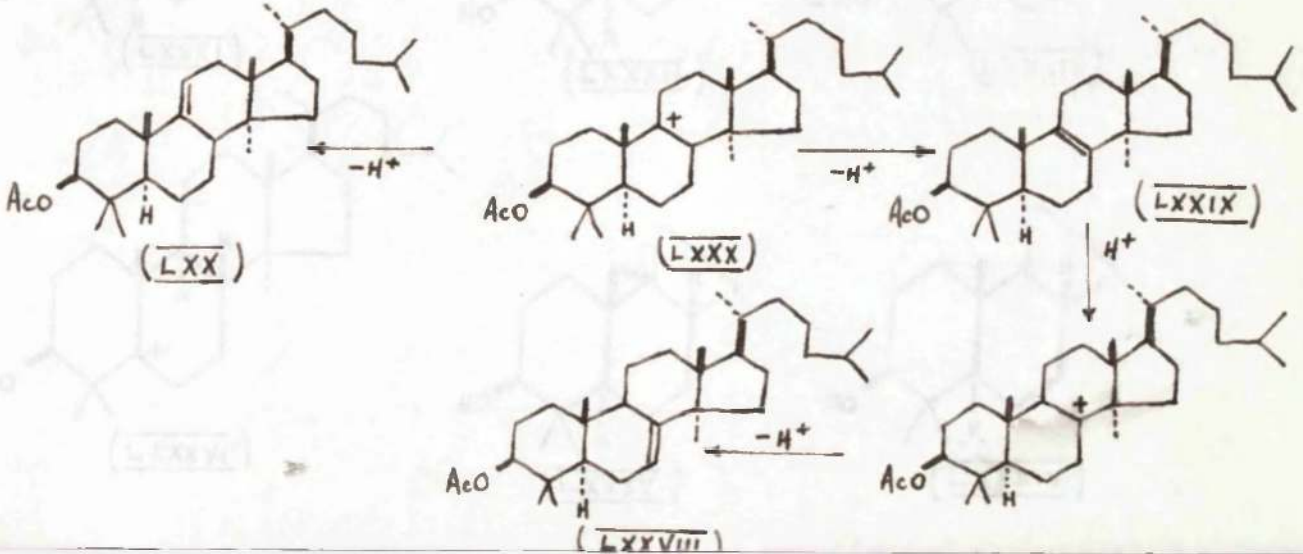
Identification of the unsaturated acetate, m.p. 170-172° as lanost-9(11)-enyl acetate requires that the β,β-disubstituted α,β-unsaturated ketone m.p. 184-185° formed from it on oxidation with chromic acid, must be 12-oxolanost-9(11)-enyl acetate (LXXVII). J. A. Henry (72) found that vigorous hydrogenation of (LXXVII) gave lanostanyl acetate; when the reduction was stopped after the absorption of two molecular proportions of hydrogen, lanost-9(11)-enyl acetate was obtained.

The nature of the acid rearrangement product from cycloartanyl acetate was established from the following considerations. The isolation of lanost-9(11)-enyl acetate in 50% yield plus small amounts of 12-oxolanost--9(11)-enyl acetate by partial oxidation of the acetate mixture, together with the isolation of lanostanyl acetate in 60% yield and 7:11-dioxolanost-8-enyl acetate in approximately 10% yield by oxidation of the hydrogenated mixture, indicates that lanost-9(11)-enyl acetate is the major product of the rearrangement.

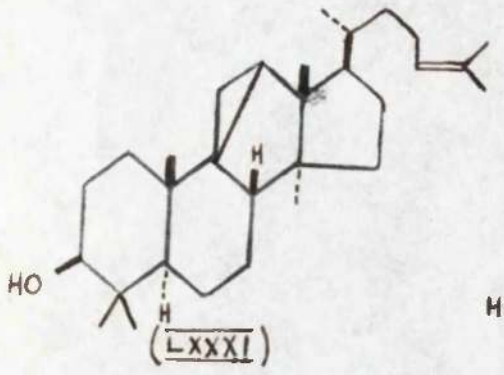
As already mentioned, oxidation of lanost-9(11)-enyl acetate with chromic acid gives 12-oxolanost-9(11)-enyl acetate. Careful chromatography of the product failed to reveal any trace of 7:11-dioxolanost-8-enyl acetate which is, however, produced in good yield by oxidation of both lanost-7-enyl acetate (LXXVIII) and lanost-8--enyl acetate (LXXIX). Again lanost-9(11)-enyl acetate can be readily hydrogenated giving lanostanyl acetate, whereas lanost-7- and -8-enyl acetates cannot be hydrogenated. In the reaction described above, in which lanostanyl acetate and 7:11-dioxolanost-8-enyl acetate were isolated, it would appear that the former was formed by hydrogenation of lanost-9(11)-enyl acetate and the latter by oxidation of lanost-7- or -8-enyl acetate. Since, however, treatment of either the $\Delta^7$ or the $\Delta^8$ isomer with hydrogen chloride under conditions exactly analogous to those used in the rearrangement of cycloartanyl acetate, gives an equilibrium mixture of both isomers, the rearrangement product of cycloartanyl acetate must be a mixture of the $\Delta^{9(11)}$, $\Delta^8$ and $\Delta^7$ isomers. Although only approximately 70% of the acetate mixture has been accounted for as lanost-9(11)-enyl acetate (60%) and lanost-7- and -8-enyl acetates (approximately 10%), the rearrangement product is considered to contain only these three components.

A synthetic mixture of lanost-9(11)-enyl acetate (60%) and the equilibrium mixture of lanost-7- and -8-enyl acetates (40%) was prepared to confirm this view; the product closely resembled the original acetate mixture, m.p. 137-157°.

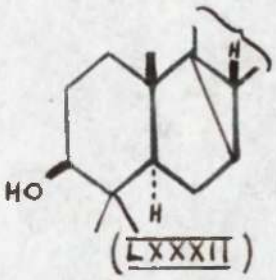
It has been shown (71) that lanost-9(11)-enyl acetate is unchanged after treatment with hydrogen chloride in chloroform; this isomer was not obtained on similar treatment of lanost-7- or -8-enyl acetate. It would therefore appear that lanost-9(11)-enyl acetate and lanost-8-enyl acetate are formed simultaneously by the loss of a proton from the carbonium ion formed on breaking the cyclopropane ring, and that the -8-enyl acetate subsequently equilibrates to a mixture with the -7-enyl acetate. The simplest explanation of these reactions is that the initial product formed is the carbonium intermediate (LXXX), the subsequent fate of which is depicted below:



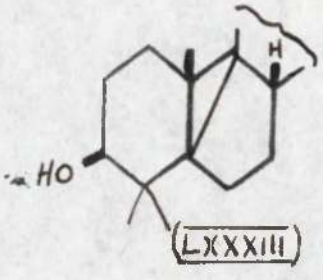
The considerations outlined above eliminate structures from which the carbonium ion (LXXX) is derived by rearrangement of intermediates in which $C_{(8)}$ or $C_{(11)}$ is the electron deficient centre and, unless modifications of the lanostane carbon skeleton are to be considered, establish that one end of the cyclopropane bridge is located at $C_{(9)}$. The evidence available at this stage, however, did not allow a satisfactory choice to be made from the five structures (LXXXI)-(LXXXV), all of which were considered possible for cycloartenol. Structure (LXXXIII) appeared improbable since the acetate mixture, m.p. 137-157° did not contain any appreciable amount of rearrangement products derived from the carbonium ion (LXXXVI).



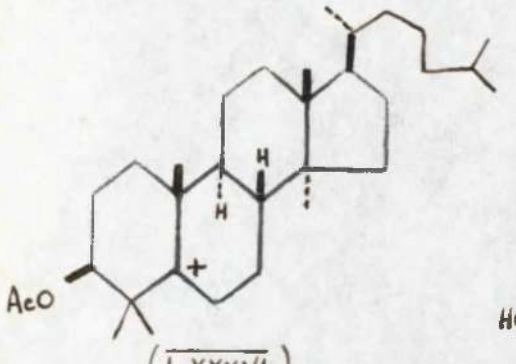

(LXXXI)



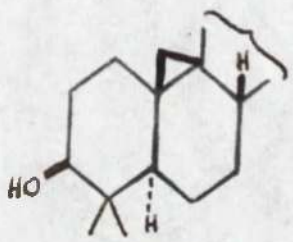

(LXXXII)



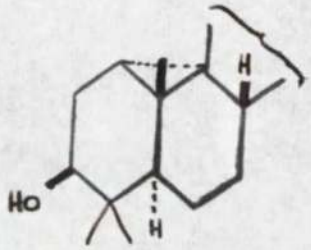

(LXXXIII)




(LXXXVI)




(LXXXV)



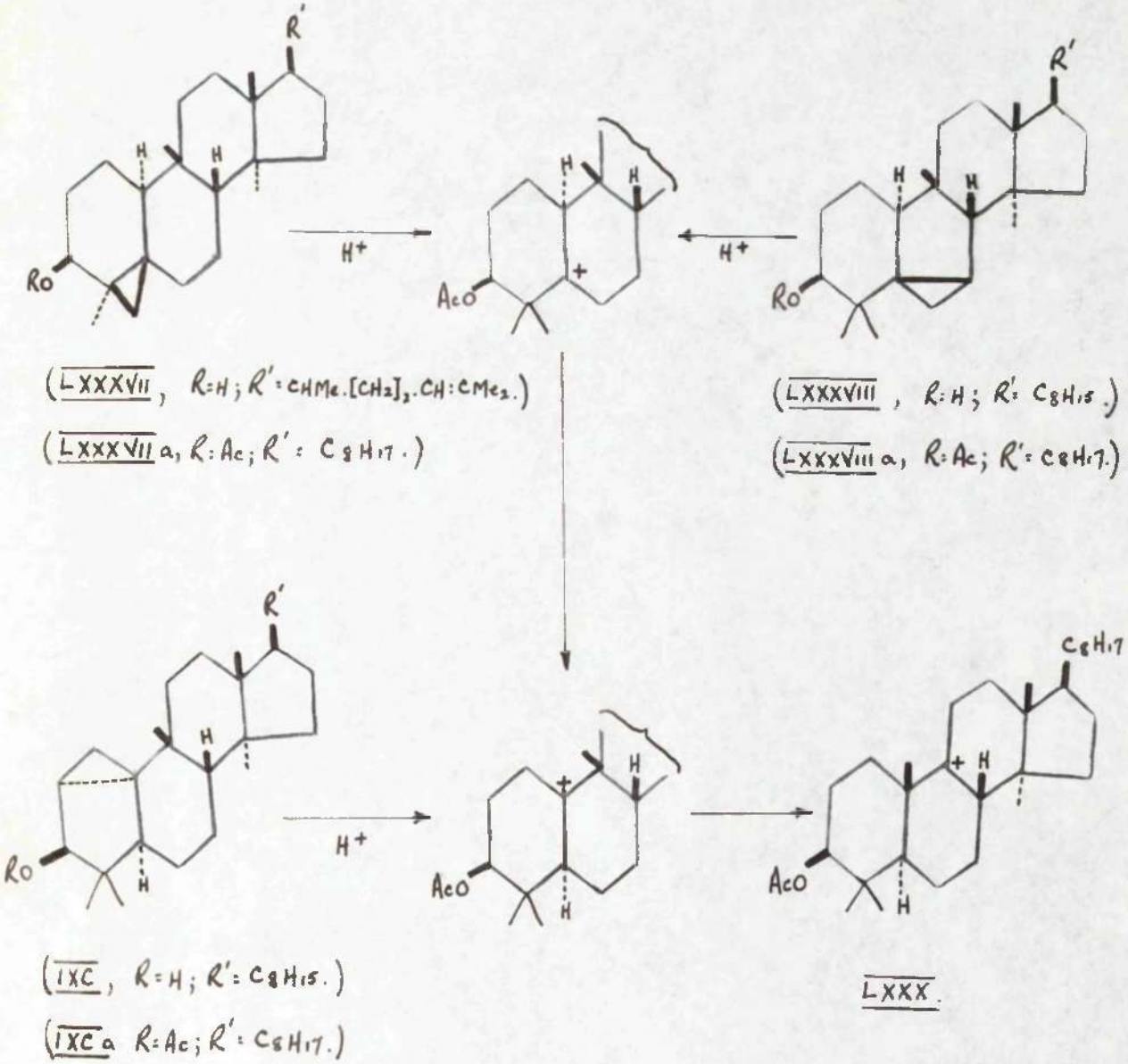

(LXXXIV)

Having established that the cyclopropane ring includes $C_{(9)}$, it was hoped that by treatment of cycloartanyl acetate with a cation other than $H^+$, the other end of the bridge would be located. Attempted chlorination and bromination of cycloartanyl acetate was, however, unsuccessful; using a moderate excess of halogen, the starting material only was recovered, while more vigorous treatment yielded intractable gums. J. A. Henry (72) attempted to rearrange cycloartanyl acetate using the pyridine stabilised iodine salt of stearic acid and t-butyl hypochlorite, but recovered starting material in both cases.

A further limitation to the number of possible structures for cycloartenol was imposed in September, 1953, when, in a valuable contribution to the chemistry of cyclopropane compounds, Cole (79) showed that an infra-red absorption band at 3042 $cm.^{-1}$ is characteristic of an unsubstituted methylene group included in a cyclopropane ring; such a band was shown to be present in the spectrum of cycloartenol and in that of cycloartanol, thus reducing the five possible structures to two, i.e. (LXXXI) and (LXXXV).

If, however, simple modifications of the lanostane carbon skeleton are not to be excluded, three additional

formulae (LXXXVII)-(IXC) qualify for consideration as representing cycloartenol. The path to the carbonium ion (LXXX) by proton induced rearrangement of cycloartanyl acetate is shown below for each of these additional structures [(LXXXVIIa), (LXXXVIIIa) and (IXCa)]:




(LXXXVII, R=H; R'=CHMe.[CH2]3.CH:CMe2.)

(LXXXVIIa, R=Ac; R'=C8H17.)

(LXXXVIII, R=H; R'=C8H15.)

(LXXXVIIIa, R=Ac; R'=C8H17.)

(IXC, R=H; R'=C8H15.)

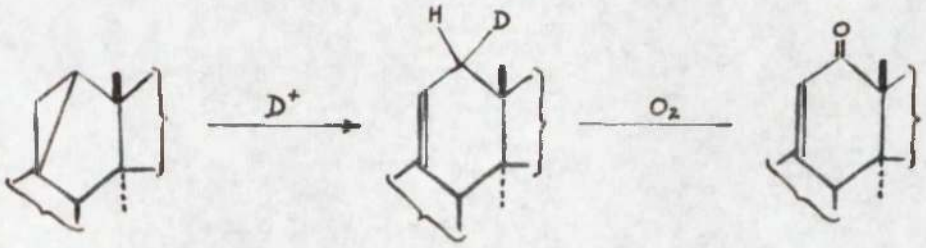
(IXCa R=Ac; R'=C8H17.)

LXXX.

Formulae (LXXXVII) and (IXC) were eliminated for two reasons: First, the ultra-violet absorption spectrum of cycloartanone is characteristic of a compound containing an isolated carbonyl function and shows no exaltation attributable to conjugation between such a grouping and a cyclopropane ring (81). Secondly, J. A. Henry (80) showed that cycloartanone readily forms an enol acetate, the ultra-violet absorption of which is that of a normal enol acetate; no exaltation attributable to conjugation between a cyclopropane ring and an ethylenic linkage (81) was observed.

J. A. Henry (80) tested the validity of structure (LXXXI) using a method based on that recently described by Barton and de Mayo (83) in the treatment of a similar structural problem, namely the position of the cyclopropane ring in phyllanthol, a triterpenoid which has a cyclopropane ring and which is related to α-amyrin as cycloartanol is related to lanost-9(11)-enol. Treatment of cycloartanyl acetate with deuterium chloride and a trace of deuterium oxide gave a mixture of deuterated double-bond isomers, from which an x-deuterolanost-9(11)--enyl acetate was isolated by application of the partial oxidation technique previously used in the preparation of the undeuterated isomer. Analysis of the product

indicated the presence of 0.8 gram-atom deuterium per mole. If cycloartenol is correctly represented by (LXXXI), the deuterated compound must be 12-deutero-lanost-9(11)-enyl acetate, oxidation of which should eliminate deuterium to give 12-oxolanost-9(11)-enyl acetate:



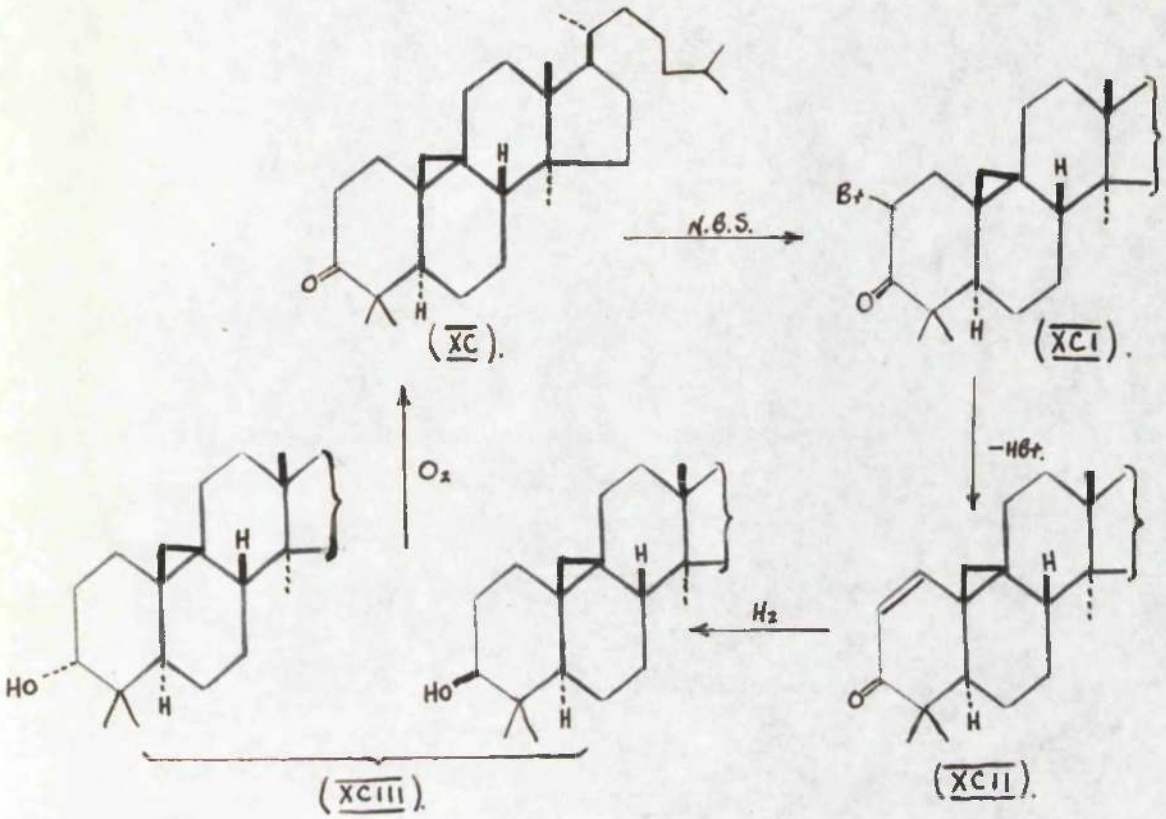

Oxidation of deuterated lanost-9(11)-enyl acetate, however, gave a product, the physical constants of which were identical with those of 12-oxolanost-9(11)--enyl acetate, but which still contained 0.8 gram-atom deuterium per mole.

cycloArtenol cannot therefore have the structure (LXXXI) and can only be adequately represented by (LXXXV) or (LXXXVIII).

At this stage, Barton, Warnhoff and Page (84) reported an infra-red examination of the mixture of deuterated lanostenes obtained on treatment of cyclo-artane with deuterium chloride. Comparison of the intensity of absorption at 1372 $cm.^{-1}$ with that given by undeuterated lanost-9(11)-ene, indicated that the

deuterated carbon atom obtained on fission of the cyclopropane ring is present as a deuteromethyl group, other than one of the gem-methyl groups. The only structure compatible with this observation is (LXXXV), which Barton and his colleagues concluded was the true representation of cycloartenol.

A proof that cycloartenol is (LXXXV) was obtained by the author in the following manner. Treatment of cycloartanone (XC) in carbon tetrachloride with N-bromosuccinimide in the presence of a fine suspension of calcium carbonate, gave 2-bromocycloartanone (XCI). On refluxing with collidine, (XCI) was dehydrobrominated to give cycloart-1-en-3-one (XCII). The ultra-violet absorption spectrum of (XCII) shows a well-defined maximum at 2690 Å. ($\varepsilon$, 8700), which is located at a considerably higher wavelength than the absorption maximum of a simple $\alpha\beta$-unsaturated ketone. This exaltation is considered to be adequate proof that the $\alpha\beta$-unsaturated carbonyl group in cycloart-1-en-3-one is directly conjugated with the cyclopropane ring, as in (XCII). That these reactions had left the cyclopropane ring intact was established by hydrogenation of the unsaturated ketone (XCII); this treatment not only saturated the ethylenic linkage but also reduced the




carbonyl group to a mixture of the 3α- and 3β-alcohols (XCIII). Mild oxidation of the mixture with chromic acid in acetic acid regenerated cycloartanone.

These experiments eliminate formula (LXXXVIII)and prove conclusively that cycloartenol is correctly represented as 9:19-cyclolanost-24-en-3β-ol (LXXXV).

## Section II.

A new triterpenoid alcohol, cyclolaudenol, and (+)-n-nonacosan-10-ol have been isolated from the non-saponifiable fraction of a chloroform extract of opium marc. cycloLaudenol has been shown to be 24b-methyl-9:19-cyclolanost-25-en-3β-ol.

Opium is the product obtained by cutting the unripe capsules of certain varieties of poppies and allowing the sap which exudes to dry spontaneously. Since early in the eighteenth century, opium has been the subject of detailed chemical examination and from it, about thirty different alkaloids have been isolated and their structures elucidated. A high level of efficiency has now been achieved in the commercial extraction of these alkaloids, although the method at present used is essentially that described by Gregory in 1833 (35, cf. 36, 37). In this, the opium is stirred with water, treated with a hot, concentrated solution of calcium chloride and filtered. The press--cake or opium marc is washed with water, the washings and filtrate combined and processed for alkaloids. The marc which constitutes approximately 20 per cent of the original opium, has been shown to contain the calcium salts of lactic, meconic and sulphuric acids, but apart from this observation, no other examination of the non-alkaloidal constituents of opium has been reported.

Through the courtesy of the Directors of Messrs. T. and H. Smith Ltd., Edinburgh, supplies of opium marc were made available for further examination.

Chloroform extracts of the marc were freed of residual alkaloids, which consisted mainly of narcotine, and evaporated to give a brown gum, corresponding in quantity to 18 per cent of the dried marc. Hydrolysis of this fat with potassium hydroxide gave the non--saponifiable fraction in 24 per cent yield, which, when chromatographed over activated alumina, gave only inseparable mixtures. The non-saponifiable fraction, however, separated from concentrated acetone solution as a partly crystalline solid, a solution of which in light petroleum was chromatographed into two well--defined fractions, m.p. 81-82° and m.p. 123-125° respectively.

The first fraction on crystallisation gave an optically inactive alcohol $C_{28}H_{60}O$, m.p. 81-82° which gave no colour with tetranitromethane and showed no selective light absorption in the ultra-violet region. The Liebermann-Burchardt test was also negative. This alcohol was characterised by the preparation of an acetate $C_{31}H_{62}O_2$, m.p. 44.5-45.5; and was shown to contain a secondary hydroxyl group (72) by oxidation to a ketone $C_{29}H_{58}O$, m.p. 74.5-75.5°, reduction of which under Wolff-Kishner conditions gave a hydrocarbon $C_{29}H_{60}$, m.p. 63-64°. The physical constants of the alcohol and

its derivatives were very similar to those of corresponding derivatives of (+)-n-nonacosan-10-ol, an aliphatic alcohol first isolated from apple cuticle wax by Chibnall and co-workers (88). Although (+)-n-nonacosan-10-ol itself does not show any appreciable optical rotation, it has been reported that the hydrogen phthalate in concentrated solution (20% in chloroform) has a very slight dextrorotation: $[\alpha]_{5461}$, + 0.62°. (+)-n-Nonacosan--10-ol has also been isolated from the fruit of Ginkgo biloba (89, 90). A comparison of the melting points of the alcohol and its derivatives obtained from the three sources, is given in Table D.

TABLE D.

| | Opium | Apple Cuticle | Ginkgo biloba. |
|---|---|---|---|
| | M.p. | M.p. | M.p. |
| (+)-n-Nonacosan--10-ol | 81-82° | 81.9-82.2° | 82.5° |
| (+)-n-Nonacosan--10-yl acetate | 44.5-45.5 | 44.5-45 | 43-43.5 |
| n-Nonacosan-10-one | 74.5-75.5 | 74.7-74.9 | 74 |
| n-Nonacosane. | 63-64 | 62.7-63 | - |

The melting points of the alcohol from opium and its acetate were undepressed when mixed with samples of the corresponding compounds derived from apple cuticle wax,

supplied by Professor A. C. Chibnall F.R.S. Identity of the two alcohols was further confirmed by their infra-red absorption spectra, which were identical.

The second fraction from the chromatogram was crystallised from methanol to give long needles, m.p. 123-124°, in quantity corresponding to 35 per cent of the solid chromatographed. This compound was named cyclolaudenol for reasons which will become apparent in the sequel. In the Liebermann-Burchardt test, cyclolaudenol gave a blood red solution with a strong green fluorescence, exactly as for cycloartenol. Unsaturation in the alcohol was evidenced by a yellow tetranitromethane colour and by the ultra-violet absorption spectrum which showed an apparent maximum at 2060 Å. ( $\varepsilon$ 1,500). Analyses of cyclolaudenol and its derivatives did not allow a satisfactory choice to be made from the formulae $C_{30}H_{50}O$, $C_{31}H_{52}O$ and $C_{32}H_{54}O$ for the parent alcohol. It was later established from degradative experiments, however, that cyclolaudenol has the empirical formula $C_{31}H_{52}O$, and therefore in this discussion, formulae based on this will be quoted, although much of the work was carried out without prior knowledge of the exact molecular weight.

cycloLaudenol was characterised by the preparation

of an acetate, cyclolaudenyl acetate $C_{33}H_{54}O_2$, m.p. 120-121° and was shown to be a secondary alcohol (91) by oxidation to a ketone, cyclolaudenone, $C_{31}H_{50}O$. J. A. Henry (91) established that cyclolaudenol contains one reactive double bond from the following observations. cycloLaudenyl acetate when shaken with hydrogen over a platinum catalyst, readily forms a dihydro-derivative, cyclolaudanyl acetate. The first-named acetate also absorbs one mole of bromine to give a dibromide, and on treatment with perbenzoic acid forms a monoxide, cyclolaudenyl acetate oxide. cycloLaudanyl acetate and cyclolaudenyl acetate oxide did not show any appreciable light absorption in the ethylenic region but, in contrast to their apparently saturated nature, both gave a faint but distinct yellow colour with tetranitromethane.

At this stage, a close structural relationship with cycloartenol was suggested by a number of considerations, which indicated that cyclolaudenol might also have a tetracyclic triterpenoid nucleus containing a cyclopropane ring and a reactive double bond in the side chain. First, the dihydro-derivatives of both cyclolaudenol and cycloartenol give a distinct pale yellow colour with tetranitromethane, but do not show

selective absorption in the ultra-violet region. Secondly, molecular rotation differences between corresponding derivatives of both series are very similar, as shown in Table E.

TABLE E.

$[M]_D$

| | Alcohol | Acetate | Benzoate | Ketone | $\Delta_1$ | $\Delta_2$ | $\Delta_3$ |
|---|---|---|---|---|---|---|---|
| cycloLaudenol | +206° | +265° | +343° | +83° | +59° | +137° | -123° |
| cycloArtenol | +230 | +280 | +400 | +93 | +50 | - | -137 |
| cycloLaudanol | +190 | +242 | - | - | +52 | - | - |
| cycloArtanol | +214 | +277 | - | +102 | +63 | - | -112 |

The change in molecular rotation accompanying oxidation of cyclolaudenol to cyclolaudenone is strongly negative, as is found in the formation of cycloartenone from cycloartenol. Barton (73) first pointed out that cycloartenol is, in this respect, markedly different from the majority of the naturally occurring triterpenoid alcohols, oxidation of which normally gives rise to a positive change in molecular rotation. The only other compounds which share this anomaly are butyrospermol and agnosterol. Barton and Jones (92, 93) have shown that members of the tetracyclic group of triterpenoids which contain a reactive double bond, show little change in

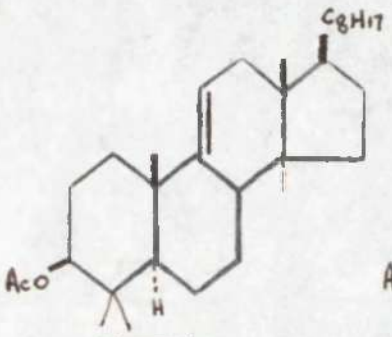
molecular rotation on hydrogenation. On this evidence, cyclolaudenol, like cycloartenol, can be considered as a member of this group. Again, it has been observed (94) that acetylation of a 3β-hydroxy-triterpenoid is accompanied by a positive change in molecular rotation; the corresponding change for a 3α-hydroxy-triterpenoid is, on the other hand, strongly negative. As can be seen from Table E, acetylation of the hydroxyl group in cyclolaudenol and in cyclolaudanol is accompanied by a positive change in rotation, from which it is concluded that if cyclolaudenol resembles cycloartenol in being a 3-hydroxy-triterpenoid, the hydroxyl group has the β-configuration.

J. A. Henry (91) showed that cyclolaudenol does contain a cyclopropane ring by treatment of cyclolaudanyl acetate with hydrogen chloride. The product melted over a range, m.p. 145-155° and, in contrast to cyclolaudanyl acetate, gave a strong yellow tetranitromethane colour and showed ethylenic absorption in the ultra-violet region with a maximum at 2080 Å. (ε 3,100). The presence of a cyclopropane ring in cyclolaudenol was confirmed by an examination of the infra-red absorption spectra of this alcohol and its derivatives, carried out by Dr. A. R. H. Cole of the University of Western Australia.

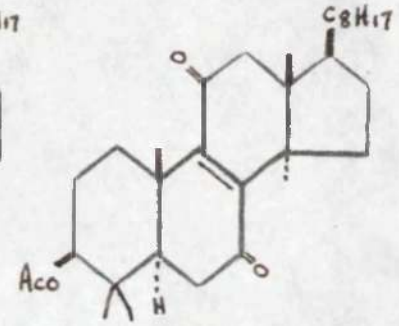
In carbon tetrachloride solution, cyclolaudenol, its acetate and cyclolaudanyl acetate showed absorption at 3040 cm.$^{-1}$ as had previously been observed for cycloartenol, cycloartanol and i-cholestane (Theoretical, Part I) and which is known to be characteristic of an unsubstituted methylene group included in a cyclopropane ring (79).

J. A. Henry (91) found that the following reactions involving the nucleus of cyclolaudenol are exactly similar to those of cycloartenol. Mild oxidation of the acetate mixture m.p. 145-155°, obtained on treatment of cyclolaudanyl acetate with mineral acid, gave three distinct products. The first, laudenyl acetate, $C_{33}H_{56}O_2$, showed characteristics of ethylenic unsaturation and is the analogue of lanost-9(11)-enyl acetate (XCVI). The second product, dioxolaudenyl acetate, $C_{33}H_{52}O_4$, was yellow in colour and showed absorption in the ultra-violet region with a maximum at 2700 Å. ($\varepsilon$ 7,400), characteristic of a fully transoid 1:4-dione-ene. It is an analogue of 7:11-dioxolanost-8-enyl acetate (XCVII) and like it, is reduced to a colourless diketone on treatment with zinc in acetic acid. The third product, oxolaudenyl acetate $C_{33}H_{54}O_3$, was isolated only in low yield and had the ultra-violet absorption characteristics of a $\beta,\beta$-disubstituted $\alpha\beta$-unsaturated ketone. It is an analogue of

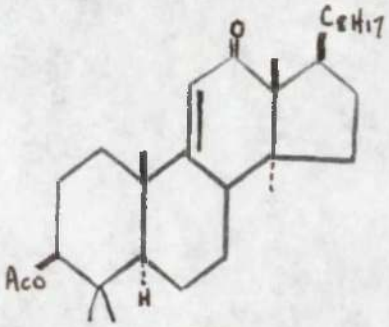
12-oxolanost-9(11)-enyl acetate (XCVIII) and is formed in high yield by oxidation of laudenyl acetate with chromic acid. Again, catalytic hydrogenation of the acetate mixture m.p. 145-155°, and oxidation of the product with chromic acid, gave a saturated acetate, laudanyl acetate $C_{33}H_{53}O_2$, together with smaller amounts




(XCVI)




(XCVII)




(XCVIII)

of dioxolaudenyl acetate. From laudanyl acetate, laudanol, laudanone and laudane were prepared. This striking similarity between cyclolaudenol and cycloartenol extends to molecular rotation relations between corresponding compounds in both series.

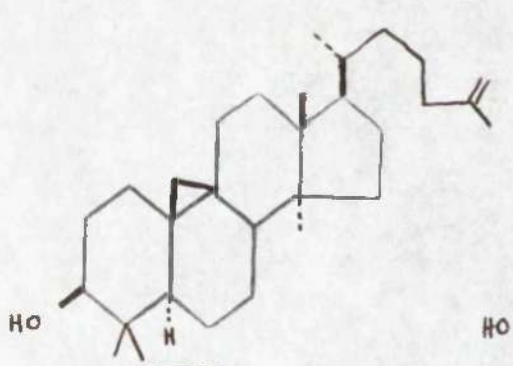
The saturated alcohol laudanol, however, was not identical with lanostanol; nor was dioxolaudenyl acetate identical with either of the corresponding derivatives in the lanosterol or euphol series. From these results, it was concluded that cyclolaudenol and cycloartenol have the same pentacyclic nuclear structure and that the difference between the two alcohols lies solely in the

nature of the side chain.

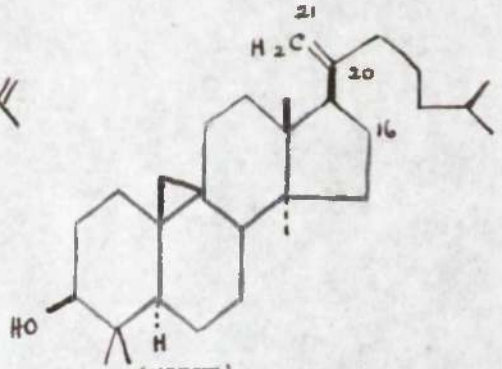
The side chain of cyclolaudenol has been examined in detail by the author and its constitution and configuration established. Ozonolysis of cyclolaudenyl acetate in chloroform solution at low temperature gave formaldehyde, which was isolated as the dimedone derivative, and a ketone, oxonorcyclolaudanyl acetate $C_{32}H_{52}O_3$, m.p. 140-141°, which resisted further oxidation with chromic acid. Thus in contrast to cycloartenol, which contains an isopropylidene group $>C = C(Me)_2$, cyclolaudenol contains a vinylidene group $>C = CH_2$. Dr. Cole's infra-red measurements confirmed the presence of a vinylidene group in cyclolaudenol; the infra-red spectra of cyclolaudenol and its acetate showed absorption bands at 3071 cm.$^{-1}$ in carbon tetrachloride solution and at 887 cm.$^{-1}$ in carbon disulphide, which bands are characteristic of the group $>C = CH_2$. These bands were absent from the spectrum of cyclolaudanol.

At this stage, the possibility that cyclolaudenol differs from cycloartenol solely in the position of the double bond in the side chain, was considered. The two possible structures for cyclolaudenol then become (XCIV) and (XCV). If however, (XCIV) represents cyclolaudenol, then cyclolaudanyl acetate should be identical with

cycloartanyl acetate, whereas the two are distinct. If cyclolaudenol is (XCV), the non-identity of cyclolaudanyl acetate and cycloartanyl acetate requires that hydrogenation of the double bond in (XCV) has proceeded quantitatively to give the unnatural configuration at $C_{(20)}$. This was considered to be extremely unlikely, and was finally disproved by experiments described in the sequel. Derivatives of polyporenic acid C, which is




(XCIV.)

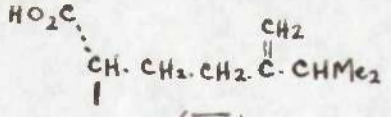



(XCV)

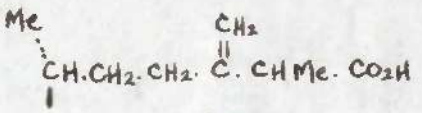
related to lanosterol, have recently been described (17) in which the unnatural configuration at $C_{(20)}$ can be stabilised by hydrogen bonding between e.g. an α-hydroxyl group at $C_{(16)}$ and a methoxy-carbonyl group at $C_{(21)}$; these iso-compounds cannot, however, be formed when no hydrogen bonding is possible between $C_{(16)}$ and $C_{(21)}$.

It was considered possible that cyclolaudenol might be related to a higher homologue of cycloartenol in that it is a $C_{31}$- or $C_{32}$-triterpenoid. Eburicoic acid (5) and the polyporenic acids A (8-11), B (14) and C (15, 17)

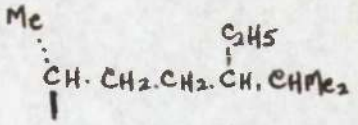
have recently been shown to be $C_{31}$-triterpenoids of the lanostane series, the fundamental structural differences between which and lanosterol lie, not in the nucleus, but in the side chain. The $C_9$- side chains of eburicoic acid and the polyporenic acids B and C are as represented in (IC) and that of the related polyporenic acid A in (C). Triterpenoids having the side chain found in stigmasterol (CI) had not previously been described, but nevertheless, this type of structure was also considered for cyclolaudenol.



(IC)



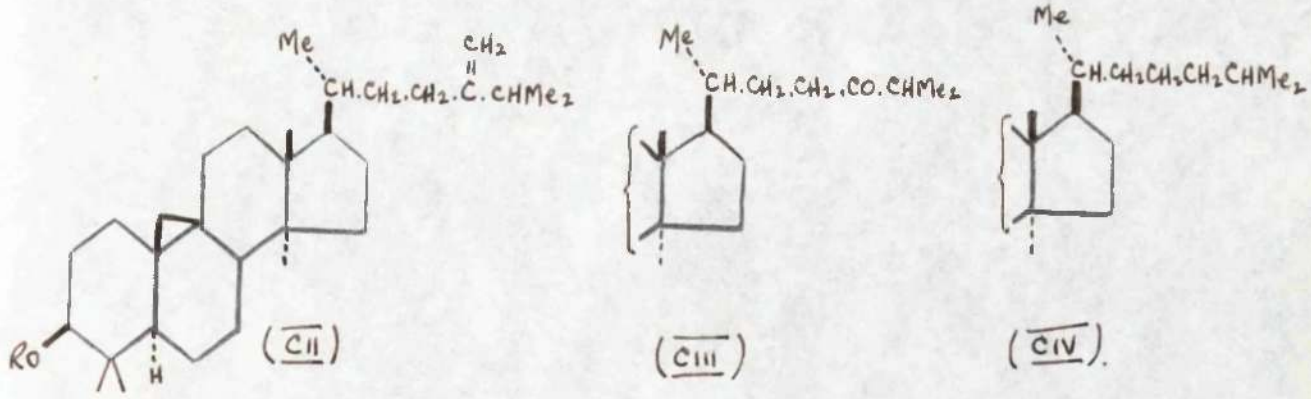
(C)



(CI)

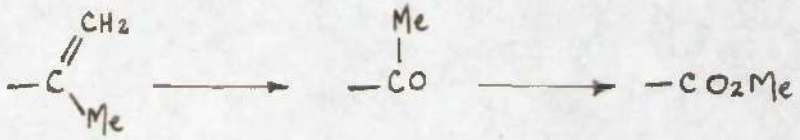
An attractive hypothesis to explain the non-identity of cyclolaudanyl acetate and cycloartanyl acetate presented itself in the possibility that cyclolaudenol is a $C_{31}$-triterpenoid which, in common with the other $C_{31}$-triterpenoids mentioned above, has a side-chain double-bond located between $C_{(24)}$ and $C_{(25)}$, as in (CII, R = H) The following experiments, however, showed that the latter part of this hypothesis is incorrect. Oxonor-cyclolaudanyl acetate, the keto-acetate obtained on ozonolysis of cyclolaudenyl acetate, was reduced by the Wolff-Kishner method to norcyclolaudanyl acetate $C_{32}H_{54}O_2$,

which was converted by standard methods into the derived alcohol, norcyclolaudanol, and the ketone, norcyclolaudanone. If cyclolaudenol is correctly represented by (CII, R = H), then oxonorcyclolaudanyl acetate will be (CIII, R = Ac) and norcyclolaudanyl acetate should be identical with cycloartanyl acetate (CIV, R = Ac), whereas the two are distinct, as are the corresponding alcohols. The possibility that norcyclolaudanol differs from cycloartanol simply in configuration at $C_{(3)}$ was considered unlikely from molecular rotation considerations, and was shown to be untenable by the non-identity of norcyclolaudanone and cycloartanone.




Oxonorcyclolaudanyl acetate was next examined in greater detail. Oxidation of this acetate with potassium hypobromite gave an acid, 3β-acetoxybisnorcyclolaudanoic acid, which could not be obtained crystalline but which was isolated as the methyl ester.

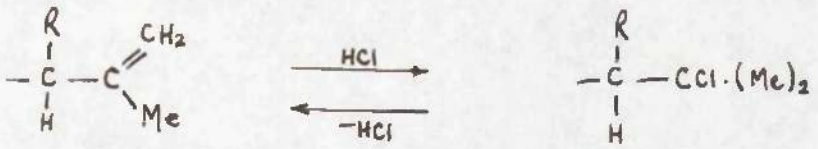
Oxonorcyclolaudanyl acetate must, therefore, be a methyl ketone from which it follows that the side chain of cyclolaudenol terminates in an isopropenyl group. The transformation of cyclolaudenyl acetate into oxonorcyclolaudanyl acetate and thence to methyl 3β-acetoxybisnorcyclolaudanoate can then be represented by the following partial formulae:




It had been planned to degrade the side chain of cyclolaudenol further, by subjecting the methyl ester of this bisnor acid to a Barbier-Wieland type degradation. The hypobromite oxidation of the methyl ketone was, however, very slow, the acid product could not be obtained crystalline and the ester was isolated in low yield only after a tedious purification process.

This proposed route was therefore abandoned and, as an alternative, an attempt was made to isomerise the side-chain double bond of cyclolaudenol by the addition and elimination of hydrogen chloride in the hope that it would be converted into the isopropylidene isomer. It was realised that this isomerisation would also result in fission of the cyclopropane ring with the

formation of a mixture of double-bond isomers; however, it was hoped that ozonolysis of the product would result in preferential attack of the side-chain double bond. Treatment of cyclolaudenyl acetate with hydrogen chloride in chloroform gave "laudenyl acetate hydrochloride" $C_{33}H_{55}O_2Cl$ which melted sharply, m.p. 166-170° and which was dehydrochlorinated by refluxing with acetic anhydride. Ozonolysis of the product, which also melted sharply, m.p. 152-156°, gave formaldehyde as the sole volatile product; no trace of acetone could be detected. As far as the side chain is concerned, the reactions described above have followed the course depicted below:




A homogeneous product could not be isolated from the non-volatile fragment of the reaction product, no doubt due to simultaneous attack by the oxidising agent on the side-chain double bond and the mixture of nuclear double-bond isomers.

Further examination of the methyl ketone, oxonorcyclolaudanyl acetate established that $C_{(24)}$ in cyclolaudenol (numbering as for lanosterol) carries an alkyl

group substituent. Using two different methods, attempts were made to form an enol-acetate of oxonor-cyclolaudanyl acetate, which could then be used as starting material for further degradation of the side chain. Neither method, however, gave the expected product; in each case a keto-acetate (m.p. 123-125°, $[\alpha]_D$ + 52°) was obtained which was isomeric with, but different from oxonorcyclolaudanyl acetate (m.p. 140-141°, $[\alpha]_D$ + 61°). Hydrolysis of oxonorcyclo-laudanyl acetate and acetylation of the product, again gave this isomer m.p. 123-125°, which was also formed on treatment of oxonorcyclolaudanyl acetate with chromic acid at room temperature. These reactions can only be explained if there is a centre of asymmetry adjacent to the carbonyl function in oxonorcyclolaudanyl acetate; $C_{(24)}$ in this keto-acetate, and consequently in cyclolaudenol, must therefore carry a methyl or ethyl group substituent. Assuming the designation b* for the configuration of the substituent at $C_{(24)}$ in cyclo-laudenol, the isomer m.p. 123-125° was named oxonorcyclo--24ab-laudanyl acetate, since experiments described in the sequel indicate that it is a difficultly separable

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* This configuration is established from considerations discussed in a later part of this section.

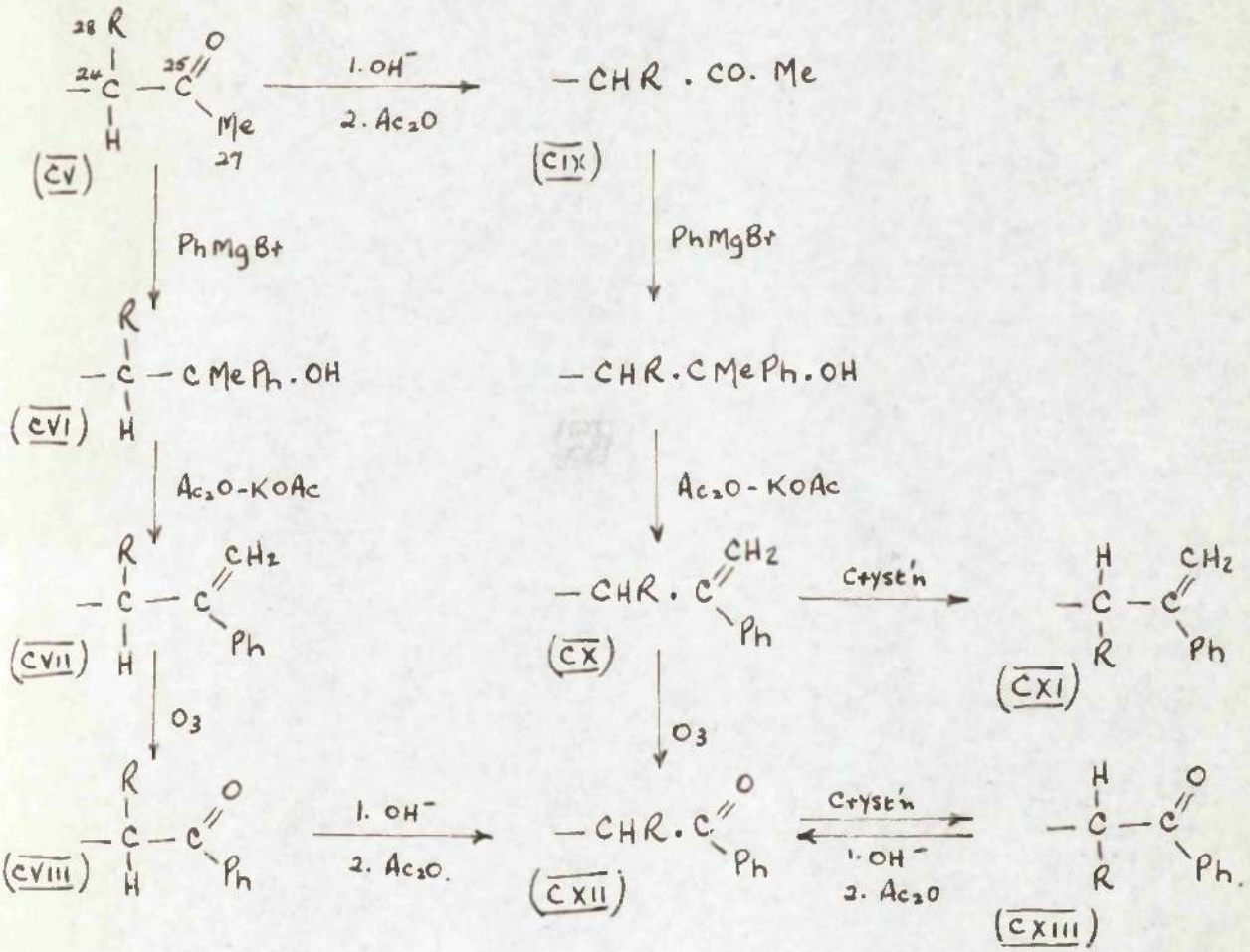
mixture of the 24a- and 24b- isomers.

Oxonorcyclolaudanyl acetate (CV) on treatment with six molecular proportions of phenylmagnesium bromide and acetylation of the product, gave a phenyl methyl carbinol, 25-hydroxy-25-phenylnorcyclolaudanyl acetate (CVI), which on dehydration with acetic anhydride and potassium acetate, gave the styryl compound 25-phenylnorcyclolaud-25(27)-enyl acetate (CVII). The absorption spectrum showed maxima at 2080 and 2360 Å. ($\epsilon$ 12,200 and 8,100). This is believed to be the sterically pure 24b-isomer, since the reactions involved in its formation are considered unlikely to have brought about any change in configuration at $C_{(24)}$. That dehydration had proceeded in the direction indicated was established by ozonolysis of the styryl compound to give formaldehyde, isolated in high yield as the dimedone derivative, and the 24b-phenyl ketone, 25-oxo-25-phenyl-bisnorcyclolaudanyl acetate (CVIII), which gave but a pale yellow tetranitromethane colour and showed ultra-violet absorption maxima at 2050 and 2420 Å. ($\epsilon$ 13,000 and 11,750). This last compound is also considered to be sterically homogeneous. It was expected that dehydration would result in elimination of the hydrogen atom at $C_{(24)}$; failure to do so may be attributed to the

proximity of the alkyl substituent at $C_{(24)}$ and the phenyl group at $C_{(25)}$.

Oxonorcyclo-24ab-laudanyl acetate (CIX) was treated in a similar manner with phenylmagnesium bromide and the resulting phenyl methyl carbinol dehydrated without further purification. The product proved to be a difficultly separable mixture of the 24a-and 24b- styryl compounds and was designated 25-phenylnorcyclo-24ab--laud-25(27)-enyl acetate (CX). The ultra-violet absorption spectrum was identical with that of the 24b-isomer. Extensive recrystallisation of this material yielded the pure 24a-isomer, 25-phenylnorcyclo--24a-laud-25(27)-enyl acetate (CXI) which also showed absorption maxima at 2080 and 2360 Å. ($\varepsilon$ 13,000 and 8,700). Ozonolysis of the 24ab-styryl compound again gave a high yield of formaldehyde and a ketone (CXII), which showed ultra-violet absorption characteristics of a phenyl ketone with maxima at 2050 and 2420 Å. ($\varepsilon$ 13,000 and 11,250). This must be a mixture of the 24a- and 24b- phenyl ketones, designated 25-oxo-25-phenylbisnor-cyclo-24ab-laudanyl acetate (CXII). After many crystallisations, this gave the pure 24a-isomer, 25-oxo--25-phenylbisnorcyclo-24a-laudanyl acetate (CXIII) which had the same absorption spectrum as the 24b-isomer.

Treatment of either the 24a-phenyl ketone or its 24b-isomer with alkali, and reacetylation of the product, gave in each case the 24ab-phenyl ketone (CXII) identical with that originally obtained from the 24ab-methyl ketone.




The physical constants of the various members of the 24a-, 24b- and 24ab- series are given in Table F.

TABLE F.

| | | 24b Series. | | | 24ab-Series. | | | 24a-Series. | |
|---|---|---|---|---|---|---|---|---|---|
| | | M.p. | $[\alpha]_D$ | | M.p. | $[\alpha]_D$ | | M.p. | $[\alpha]_D$ |
| Norketone. | (CV) | 140-141° | +61° | (CIX) | 123-125° | +52° | | - | |
| Phenyl methyl carbinol. | (CVI) | 152-154 | +37 | | - | | | - | |
| Styryl compound. | (CVII) | 101-102 | +56 | (CX) | 99-114 | +50 | (CXI) | 138-139° | +44° |
| Phenyl ketone. | (CVIII) | 112-114 | +58 | (CXII) | 99-102 | +49 | (CXIII) | 110-111 | +44 |

Attempts to isolate the 24a-isomer from oxonorcyclo--24ab-laudanyl acetate (CIX) by careful chromatography and by crystallisation were unsuccessful. From a study of the molecular rotation data given in Table F, it follows that members of the 24ab-series are composed of approximately equal proportions of the 24a- and 24b-isomers. Oxonorcyclo-24ab-laudanyl acetate was retreated with 3% alkali for 3 hours and the product acetylated in an attempt to convert the 24b-component into its 24a-isomer. The product, however, had the same physical constants as the original 24ab-ketone (CIX). On treatment under more forcing conditions, namely heating at 170° with 20% potassium hydroxide and at 200° with sodium ethoxide, intractable gums were obtained.

Having established that $C_{(24)}$ in cyclolaudenol carries an alkyl group substituent, identification of this attachment was attempted by further degradation of the 24ab-phenyl ketone (CXII). Reaction of the last named compound with phenylmagnesium bromide gave a diphenyl carbinol (CXIV) which was treated, without purification, with potassium acetate and acetic anhydride to give a homogeneous product, the diphenylethylene (CXV), in which the asymmetric centre at $C_{(24)}$ has been destroyed. The diphenylethylene gave a strong yellow tetranitromethane colour and showed absorption maxima at 2050 and 2430 Å. ($\varepsilon$ 29,300 and 13,400). Ozonolysis of the diphenylethylene at low temperature gave a steam-volatile fraction, which was identified as benzophenone and characterised as its 2,4-dinitrophenylhydrazone; the non-volatile fraction of the reaction product crystallised from methanol to give a ketone (CXVI), which was characterised as its oxime. The ketone gave a pale yellow colour with tetranitromethane and was transparent to ultra-violet light. It follows from its

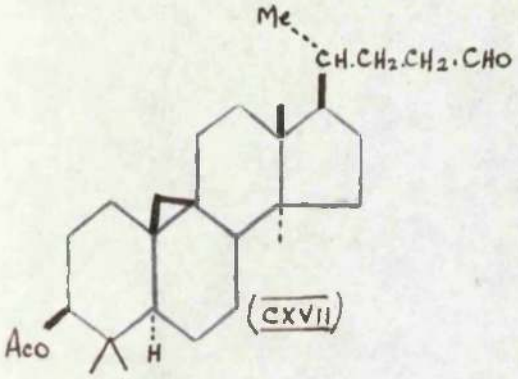
$$-CHR.CO.Ph \longrightarrow -CHR.CPh_2.OH \longrightarrow -CR=CPh_2 \longrightarrow -CO.R$$

(CXII) (CXIV) (CXV) (CXVI)

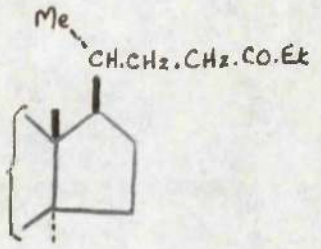
method of preparation that this ketone is 24-oxotrisnorcyclolaudanyl acetate and its relationship to cyclolaudenyl acetate is represented by the partial formulae shown below, in which R = $CH_3$ or $C_2H_5$.


Two methods were available at this stage in the investigation for the identification of the group attached to $C_{(24)}$. The first is a further Barbier--Weiland degradation and the second is an attempted conversion of cycloartenol into the degradation product, 24-oxotrisnorcyclolaudanyl acetate (CXVI). It was evident that the success of the second method would depend on the correctness of the hypothesis that cyclolaudenol and cycloartenol differ only in the nature of the side chain, but since supplies of cyclolaudenol were very limited, it was decided to examine this method. In the degradation of cyclolaudenol to 24-oxotrisnorcyclolaudanyl acetate, the reactions employed were chosen to ensure that the labile cyclopropane ring remained intact; a similar requirement was necessary for reactions employed in the degradation of cycloartenol.

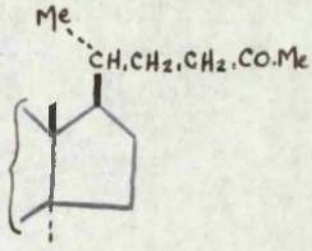
Since it was believed that the $C_{(24)}$-attachment was either a methyl or ethyl group, it was necessary




(CXVII)



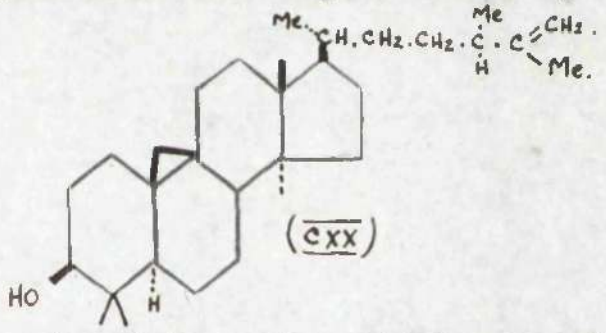

(CXVIII)




(CXIX)

to convert cycloartenol into the methyl ketone (CXIX) and the ethyl ketone (CXVIII), in order to compare these with 24-oxotrisnorcyclolaudanyl acetate. Ozonolysis of cycloartenyl acetate gives the tris-nor aldehyde; 3β-acetoxytrisnorcycloartan-24-ol (CXVII) (see Theoretical, Part I). On treatment of the aldehyde with diazoethane, the ethyl ketone (CVIII), 24-oxonorcycloartanyl acetate was formed (72), but which was different from 24-oxotrisnorcyclolaudanyl acetate. Similar reaction of the tris-nor aldehyde (CXVII) with diazomethane gave the methyl ketone (CXIX), 24-oxobisnorcycloartanyl acetate, which was characterised by its oxime and by its stability to chromic acid at room temperature. The physical constants of the methyl ketone (CXIX) were identical with those of 24-oxonorcyclolaudanyl acetate and its oxime; identity

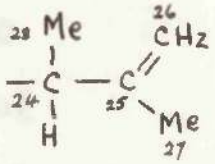
was established by mixed melting point determinations. Thanks to the co-operation of Professor E. R. H. Jones, F.R.S., and Dr. G. D. Meakins of Manchester University, the infra-red absorption spectra of 24-oxotrisnor*cyclo*laudanyl acetate and 24-oxobisnor*cyclo*artanyl acetate were compared and shown to be identical. *cyclo*Laudenol must therefore have a methyl group substituent at $C_{(24)}$ and have the constitution represented by (CXX).



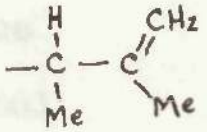

(CXX)

The stereochemistry of *cyclo*laudenol can also be deduced. The conversion of *cyclo*laudenol and *cyclo*artenol into the same degradation product (CXIX), establishes the structural and steric identity of the pentacyclic nucleus and the side chain up to and including $C_{(23)}$ of the two alcohols. The only feature of the stereochemistry of *cyclo*laudenol which remains to be defined, namely the configuration at $C_{(24)}$, was established from molecular rotation considerations. The two possible configurations at $C_{(24)}$ are as shown in (CXXI) and (CXXII) which represent the terminal carbon atoms in the side chain of *cyclo*laudenol; using the accepted nomenclature, these are designated 24b (CXXI)

and 24a (CXXII) respectively.




(CXXI)




(CXXII)

Bergmann and Low (95) have shown that the introduction of a 24a-methyl group into cholestanol, cholesterol and their esters, results in a positive change in molecular rotation ($\Delta \simeq +22°$), whereas a negative change ($\Delta \simeq -29°$) is produced by the introduction of a 24b-methyl group. The nomenclature at present in use requires that the configurational indices used by Bergmann and Low should be interchanged. Table G shows a comparison of the molecular rotations of derivatives of the lanostane series (96) and <u>cyclo</u>artenol series with those of derivatives of the laudane series (91). In each case, the molecular rotation difference ($[M]_D$ laudane$-[M]_D$ lanostane derivative) is negative, from which it is concluded that the $C_{(24)}$--methyl group in <u>cyclo</u>laudenol has the b-configuration. <u>cyclo</u>Laudenol is therefore correctly described as 24b--methyl-9:19-<u>cyclo</u>lanost-25-en-3β-ol (CXXI; cf. CXX).

TABLE G.

| | $[M]_D$ | | $[M]_D$ | Δ |
|---|---|---|---|---|
| Lanostane | +145° | Laudane | +107° | -38° |
| Lanostanol | +150 | Laudanol | +93 | -57 |
| Lanostanyl acetate | +193 | Laudanyl acetate | +155 | -38 |
| Lanostanone | +116 | Laudanone | +62 | -54 |
| cycloArtanol | +214 | cycloLaudanol | +191 | -23 |
| cycloArtanyl acetate | +277 | cycloLaudanyl acetate | +242 | -35 |

The configuration of the $C_{(24)}$-methyl group in eburicane, the saturated parent of the eburicoic acid series (5), which as yet has not been prepared from this source, can also be deduced by similar reasoning. Table H shows a comparison of the molecular rotations of lanost-8-enol (96) and eburic-8-enol derivatives (97, 98).

TABLE H.

| | $[M]_D$ | | $[M]_D$ | Δ |
|---|---|---|---|---|
| Lanost-8-enol | +261° | Eburic-8-enol | +238° | -23° |
| Lanost-8-enyl acetate | +275 | Eburic-8-enyl acetate | +271 | -4 |
| Lanost-8-ene | +272 | Eburic-8-ene I | +234 | -38 |
| | | Eburic-8-ene II | +218 | -54 |

Once again the differences in molecular rotation are negative, and, although the data is more limited in this

case than in the laudane series, it is concluded that eburic-8-enol is 24b-methyl lanost-8-enol. Eburicane must, therefore, have the same constitution and configuration as laudane.

## SECTION III.

The principal features of the constitution and configuration of butyrospermol, a tetracyclic triterpenoid from shea nut fat, have been determined, and a close structural relationship to euphol established.

In 1934, Heilbron, Moffet and Spring (99) reported the isolation of a new tetracyclic diethenoid secondary alcohol, $C_{30}H_{50}O$, from the non-saponifiable fraction of shea nut fat. This alcohol, which was subsequently named basseol (100), occurred together with the hydrocarbon illipene (101) and the pentacyclic triterpenoids β-amyrin and lupeol, and was initially isolated as the acetate. In a later communication, Heilbron, Beynon and Spring (102) showed that basseol contained one reactive double bond, which was present as a vinylidene group. The conversion of basseol into β-amyrin on treatment with a variety of acidic reagents was also reported. In 1949, Heilbron, Jones and Robins (103) re-examined shea nut fat and in addition to illipene, β-amyrin and lupeol, isolated a new tetracyclic, diethenoid secondary alcohol, named butyrospermol, which was isomeric with, but different from, basseol; no trace of the latter alcohol was found. Butyrospermol, which was shown to contain an isopropylidene group and an unreactive double bond, differed markedly from basseol in that it was not isomerised to β-amyrin on treatment with mineral acid. In the same year, Seitz and Jeger (104) also isolated butyrospermol from shea nut fat and deduced that the unreactive nuclear bond was fully

substituted from a study of the infra-red absorption spectrum of the hydrocarbon, dihydrobutyrospermadiene. Butyrospermol has also been found in the fruit of Artocarpus integrifolia (73).

More recently, Dawson, Halsall, Jones and Robins (105) reported that rigorous examination of samples of fat from different varieties of shea nuts had failed to reveal any trace of basseol. Jones further pointed out that impure samples of butyrospermyl acetate (m.p. 140-143°, ca. 3° low, $[\alpha]_D$ + ca. 20°), containing 15-20 per cent of β-amyrin acetate, only give pure butyrospermyl acetate (m.p. 146-147°, $[\alpha]_D$ + 11°) after continued recrystallisation, and suggested that the basseol acetate (m.p. 141°, $[\alpha]_D$ + 22.4°) originally described by Heilbron, Moffet and Spring (99), was in fact, an impure sample of butyrospermyl acetate containing approximately 16 per cent β-amyrin acetate.

Jones and co-workers (105) also examined the action of mineral acid on butyrospermol. Treatment of butyrospermyl acetate with hydrogen chloride in chloroform at 0°, gave a "hydrochloride", dehydrochlorination of which gave isobutyrospermyl acetate, in which the original isopropylidene group has been replaced by an isopropenyl group. The dihydro-derivative, however, dihydroisobutyrospermyl

acetate was not identical with dihydrobutyrospermyl acetate, indicating that the nuclear double bond of butyrospermol also rearranges on treatment with mineral acid. Dihydroisobutyrospermyl acetate was also formed in high yield by treatment of dihydrobutyrospermyl acetate with hydrogen chloride. Jones deduced that the nuclear double bond in these iso-compounds is trisubstituted from a study of their ultra-violet absorption spectra and from the results of perbenzoic acid titrations, and concluded that it occupies a similar cyclic position to that of "iso-lanosteryl acetate" (lanost-?-enyl acetate).

Butyrospermol has again been isolated from shea nut fat by the author and has been shown to be a tetracyclic triterpenoid, closely related to euphol.

Hydrolysis of shea nut fat with alcoholic potassium hydroxide gave the non-saponifiable fraction as a dark yellow gum in 3 per cent yield. A solution of this gum in acetic anhydride was refluxed for 3 hours and left overnight at room temperature. The semi-crystalline mass which separated was removed and the filtrate kept at 0° for 2 days, when a second crop of solid separated. This material was crystallised twice from ethanol-ethyl

acetate to give stout needles which had the same physical constants as those quoted for basseol acetate (m.p. 138-139°, $[\alpha]_D$ + 22°). Although the bulk of the material melted at 138-139°, some solid persisted in the melt until above 180°, as had already been observed by Jones (103). Only after many crystallisations was butyrospermyl acetate (m.p. 143.5-145°, $[\alpha]_D$ + 11°) obtained.

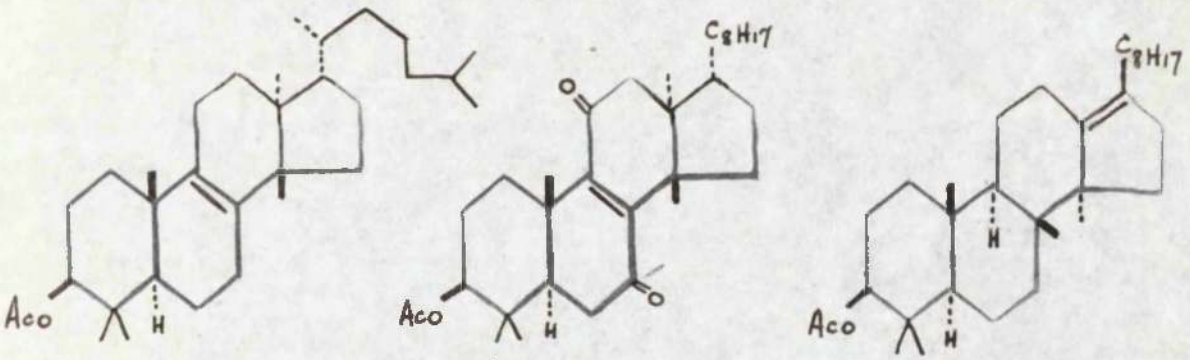
The constants quoted by Heilbron, Moffet and Spring (99) for the alcohol basseol (m.p. 109.5°, $[\alpha]_D$ - 11.9°) were observed to be very similar to those given by Jones (103) for butyrospermol (m.p. 111-113°, $[\alpha]_D$ - 12°); this similarity suggested that impure samples of butyrospermyl acetate might be more readily purified by crystallisation of the derived alcohol. On hydrolysis of material of m.p. 130-135°, $[\alpha]_D$ + 30° and + 25°, the higher melting component of the mixture readily separated as the least soluble fraction on crystallisation from aqueous acetone; pure butyrospermol (m.p. 109-110°, $[\alpha]_D$ -12.5°) was then obtained on concentration of the mother liquors. Furthermore, hydrolysis of impure butyrospermyl acetate (m.p. 138-139°, $[\alpha]_D$ + 22°) i.e. "basseol acetate", and crystallisation of the product from aqueous acetone, readily gave butyrospermol as the least soluble fraction,

the high melting component remaining in the mother liquor. The derived acetate was identical with butyrospermyl acetate (m.p. 143-145°, $[\alpha]_D$ + 11°) originally obtained by the tedious recrystallisation process. From these results it is concluded that basseol was in fact a homogeneous compound, being identical with butyrospermol.

As was mentioned in the Historical Section, the vast majority of the tetracyclic triterpenoids contain a nuclear double bond, oxidation of which results in the formation of characteristic 1:4-dione-enes. The behaviour of the nuclear double bond in dihydrobutyrospermyl and dihydro*iso*butyrospermyl acetates on oxidation was examined in the hope that a 1:4-dione--ene would be formed which would enable a relationship to the other tetracyclic triterpenoids to be established.

Dihydrobutyrospermyl acetate when oxidised with chromic acid gave an uncrystallisable gum, having the ultra-violet absorption characteristics of an αβ--unsaturated ketone. Comparable oxidation of dihydro--*iso*butyrospermyl acetate, however, gave a pale yellow product, m.p. 110-111°, which showed absorption in the ultra-violet region with a maximum at 2710 Å. (ε 8,100), characteristic of a fully *transoid* 1:4-dione-ene. This

compound proved to be identical with 7:11-dioxoeuph-8--enyl acetate (CXXIV), and was undepressed in melting point when mixed with an authentic sample prepared by oxidation of euph-8-enyl acetate. Dihydroisobutyrospermyl acetate was quickly shown to be identical with euph-8-enyl acetate (CXXIII), a mixture of both preparations showing no depression in melting point. The



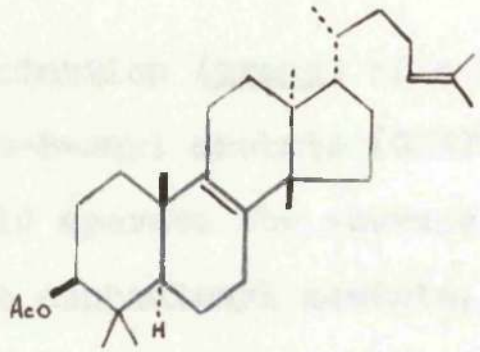

(CXXIII) (CXXIV) (CXXV)

formation of euph-8-enyl acetate from dihydrobutyrospermyl acetate by treatment with hydrogen chloride was rather surprising in view of the fact that euph-8-enyl acetate is itself isomerised to isoeuphenyl acetate (CXXV) in the presence of mineral acid. A model experiment showed, however, that euph-8-enyl acetate is recovered unchanged on treatment with hydrogen chloride under the conditions quoted for the isomerisation of dihydrobutyrospermyl acetate to dihydroisobutyrospermyl acetate (105). The identity of

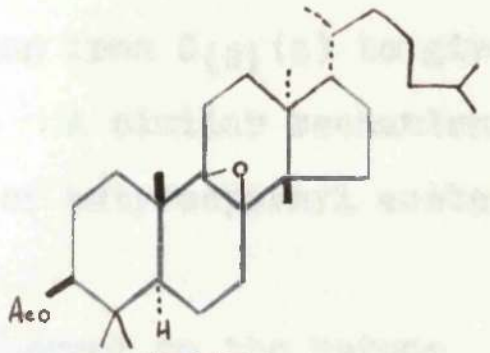
dihydroisobutyrospermyl acetate and euph-8-enyl acetate was further confirmed by the conversion of both to isoeuphenyl acetate (CXXV), under more drastic acid conditions.

One of the factors influencing Jones and co-workers (105) in their decision that dihydroisobutyrospermyl acetate contains a trisubstituted double bond, was the alleged unreactivity of this acetate to perbenzoic acid titration. Dihydroisobutyrospermyl acetate has been shown, however, to consume one molecular proportion of perbenzoic acid, giving 8:9-epoxyeuphanyl acetate (CXXVII), identical with a sample prepared from euph-8-enyl acetate in a similar manner.

Rigorous confirmation of the identity of dihydroisobutyrospermyl acetate and euph-8-enyl acetate, was obtained in the conversion of butyrospermyl acetate into the acetate of euphol (euphadienyl acetate) (CXXVI). Reaction of butyrospermyl acetate with 1 mol. of bromine in acetic acid gave butyrospermyl acetate dibromide which was treated, without purification, with hydrogen chloride in chloroform under mild conditions. The product was debrominated with zinc in acetic acid to give euphadienyl acetate in high yield.

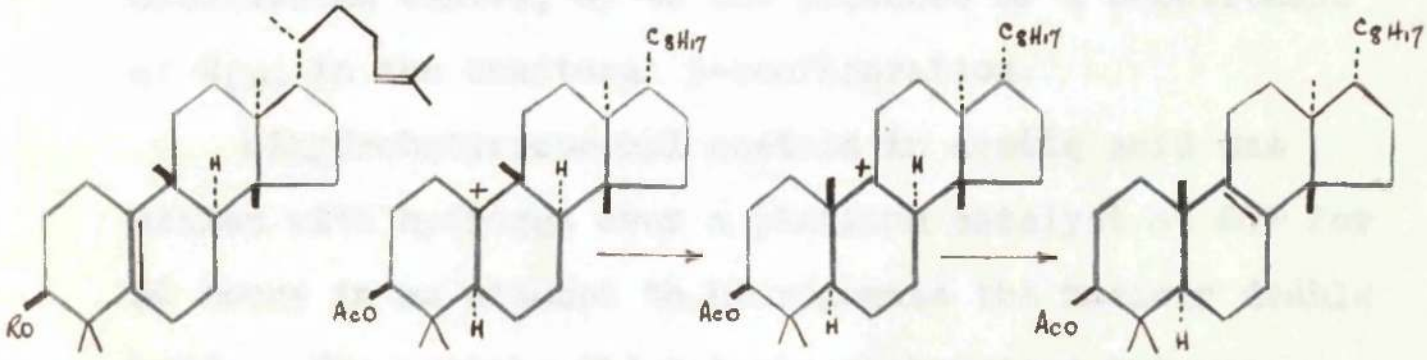



(CXXVI)




(CXXVII)

According to Seitz and Jeger (104) the nuclear double bond of butyrospermol is tetrasubstituted. If this is correct, the only possible structure for butyrospermol, compatible with the ready isomerisation of dihydrobutyrospermyl acetate to euph-8-enyl acetate is (CXXVIII). The conversion of (CXXVIII) into euph--8-enyl acetate (CXXIII) would then be represented as




(CXXVIII) (CXXIII)

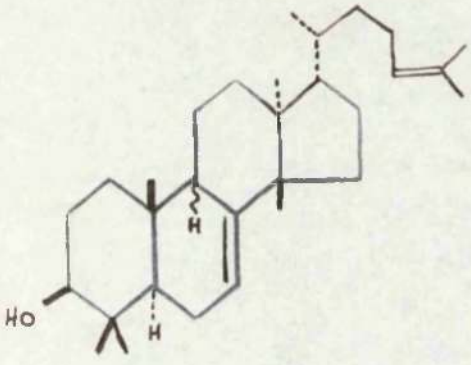
saturation of the side-chain double bond in (CXXVIII) with hydrogen to give dihydrobutyrospermyl acetate, followed by approach of a proton to the rear (α) side of the nuclear double bond with synchronous movement of the methyl group attached to $C_{(9)}$ (β) to $C_{(10)}$ and

elimination (trans) of a proton from $C_{(8)}$(α) to give euph-8-enyl acetate (CXXIII). A similar mechanism would operate the conversion of butyrospermyl acetate into euphadienyl acetate.

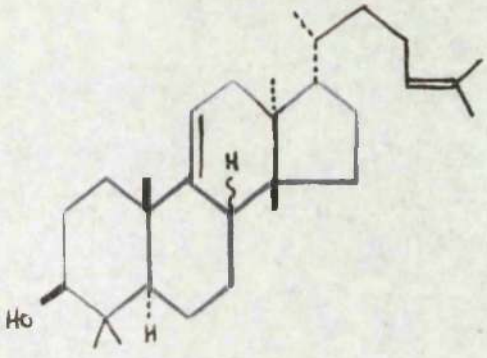
The oxidation of butyrospermol to the ketone butyrospermone is accompanied by a large negative change in molecular rotation (103, 73). cycloArtenol and cyclolaudenol (Theoretical, Parts I and II respectively) behave similarly on oxidation, in contrast to all other naturally occurring triterpenoids (with the exception of agnosterol) oxidation of which gives rise to a positive change in rotation. This may be due to the proximity of the oxygen function at $C_{(3)}$ to an unsaturated centre, or to the presence of a substituent at $C_{(9)}$ in the unnatural β-configuration.

Dihydrobutyrospermyl acetate in acetic acid was shaken with hydrogen over a platinum catalyst at 80° for 30 hours in an attempt to hydrogenate the nuclear double bond. The acetate did not absorb hydrogen but was converted into euph-8-enyl acetate. This ease of isomerisation did not in itself invalidate structure (CXXVIII) for butyrospermol, but it did suggest strongly that two other structures (CXXIX) and (CXXX) qualified for consideration as representing butyrospermol in that the acetyl dihydro-derivatives of both could readily

form euph-8-enyl acetate on isomerisation under mild conditions. A compound corresponding to the acetyl




(CXXIX)

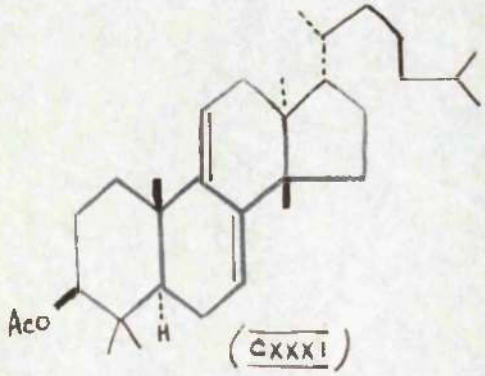



(CXXX)

dihydro-derivative of (CXXIX), euph-7-enyl acetate, in which the hydrogen at $C_{(9)}$ is probably in the α-configuration, has recently been described by Barton (61), but it is not identical with dihydrobutyrospermyl acetate. It is possible, however, that butyrospermol is (CXXIX) in which the hydrogen at $C_{(9)}$ has the β-configuration.

Formula (CXXVIII) for butyrospermol has been shown to be untenable from the considerations outlined below, which indicate that the nuclear double bond is not tetra- but trisubstituted, and must be in the $\Delta^{7}$-(CXXIX) or $\Delta^{9(11)}$ -position (CXXX). Treatment of dihydrobutyrospermyl acetate with osmium tetroxide, followed by acetylation at room temperature, gave a product m.p. 181-182°, analysis of which indicated it to be a triol--diacetate. The compound gave no tetranitromethane

colour and was transparent to ultra-violet light. On refluxing with acetic anhydride and potassium acetate, this triol-diacetate was converted into a product, m.p. 111-112°, which gave a red-brown colour with tetranitromethane and showed ultra-violet absorption maxima at 2320, 2400 and 2470 Å. (ε 15,000, 17,000 and 10,500). This product was shown to be identical with the heteroannular diene, eupha-7:9(11)-dienyl acetate (CXXXI) and was undepressed in melting point on mixing with an authentic sample, prepared from 8:9-epoxyeuphanyl acetate (CXXVII) by treatment with sulphuric acid in acetic acid (65). Eupha-7:9(11)-dienyl acetate was also obtained from the triol-diacetate, m.p. 181-182° by heating at 100° for 2 days, by sublimation and by treatment with zinc dust in acetic acid.




(CXXXI)

The conversion of dihydrobutyrospermyl acetate into eupha-7:9(11)-dienyl acetate cannot be explained by formula (CXXVIII) for butyrospermol; in addition the formation of a triol-diacetate indicates that the nuclear

double bond is trisubstituted, not tetrasubstituted. The possible structures for butyrospermol then became (CXXIX) and (CXXX). It is not considered possible to differentiate between (CXXIX) and (CXXX) by comparison of the chemical reactivity of dihydrobutyrospermyl acetate with that of two possibly corresponding derivatives in the lanosterol series, lanost-9(11)-enyl acetate and lanost-7-enyl acetate on account of the differences in configuration at $C_{(13)}$ and $C_{(14)}$. Thus the nuclear double bond of dihydrobutyrospermyl acetate is inert to hydrogenation, gives an αβ-unsaturated ketone on oxidation with chromic acid and is isomerised to the 8:9-position on treatment with mineral acid. The nuclear double bond of lanost-9(11)-enyl acetate, although giving an αβ-unsaturated ketone on oxidation, can be readily hydrogenated and is unchanged after treatment with mineral acid, while that of lanost-7--enyl acetate cannot be hydrogenated but gives a transoid 1:4-dione-ene on oxidation and, with mineral acid, forms an equilibrium mixture of the $\Delta^{7}$- and $\Delta^{8}$-isomers.

The evidence at present available, therefore, does not allow a satisfactory choice to be made between the formulae (CXXIX) and (CXXX) for butyrospermol.

# EXPERIMENTAL.

Melting points are uncorrected except in the section concerning butyrospermol. Specific rotations were measured in chloroform solution in a 1 dm. tube at room temperature and ultra-violet absorption spectra were measured in absolute ethanol solution using a Unicam SP.500 spectrophotometer. Grade II alumina and a light petroleum fraction of b.p. 60-80° were used for chromatography unless otherwise specified. The analysts were Dr. A. C. Syme and Mr. Wm. McCorkindale of The Royal Technical College, Glasgow.

Hydrolysis of S. Nux-vomica Seed Fat. - A solution of sodium hydroxide (160 g.) in water (200 ml.) was added to a boiling solution of the dark brown fat (1 kg.) in ethanol (3 l.) and the mixture refluxed for 4 hours, concentrated to half bulk and mixed with warm water (12 l.). The resulting suspension was extracted with ether (3 x 2.5 l.), the extracts combined, washed with water and evaporated. Removal of water from the product by co-distillation using benzene, gave the dry, non-saponifiable fraction as an orange, gummy solid (125 g.)

Chromatography of Acetylated Non-saponifiable Fraction. - The non-saponifiable matter (90 g.) in dry pyridine (250 ml.) and acetic anhydride (100 ml.) was

kept at room temperature for 24 hours. The mixture was diluted with water, the product extracted with ether and the extract washed with N hydrochloric acid, water and dried ($Na_2SO_4$). The acetylated product was obtained as an orange-coloured solid (96 g.), a solution of which in light petroleum (1 l.) was percolated through a column (5.5 x 132 cm.) of alumina (3 kg.). Fractions of 1 litre were collected, and the chromatogram developed as shown.

| Fraction | | Weight | Description | M.p. |
|---|---|---|---|---|
| 1 | Light petroleum | - | - | - |
| 2 | " | Trace | Colourless oil | |
| 3 | " | 0.74 g. | " | |
| 4 | " | 1.41 | " | |
| 5 | " | 3.21 | " | |
| 6 | Light petroleum: benzene (1:1) | 1.15 | " | |
| 7 | " | Trace | Colourless gum | |
| 8 | " | 18.05 | White solid | 217-219° |
| 9 | " | 12.16 | " | 216-217° |
| 10 | " | 7.09 | " | 212-214° |
| 11 | " | 4.17 | White solid and colourless gum | |
| 12 | " | 2.49 | Colourless gum | |

| Fraction | Eluant | Weight | Description | M.p. |
|---|---|---|---|---|
| 13 | Benzene | 2.00 | Colourless gum | |
| 14 | " | 1.50 | " | |
| 15 | Benzene: (Ether (9:1) | 3.00 | Yellow gum | 110-112° |
| 16 | " | 2.00 | " | 110-111° |
| 17 | " | 2.52 | Yellow gum and crystals | 115-118° |
| 18 | " | 16.15 | Pale yellow solid | 118-119° |
| 19 | " | 2.32 | Yellow gum and crystals | 97-109° |
| 20 | " | 0.35 | Yellow gum | |
| 21 | " | Trace | " | |

Final elution of the column with acetone (6 l.) gave a brown, viscous oil (16.34 g.), which was rechromatographed by M. B. E. Fayez (71) and stigmasteryl acetate, m.p. 142.5-143°, isolated.

Fractions (2-6) were combined to give a colourless oil having a faint lemon odour but which has not been examined further.

α-Amyrin Acetate. - Fractions (8-10) from the foregoing chromatogram were combined, several crystallisations from chloroform-methanol giving α-amyrin acetate

(30 g.) as blades, m.p. 223-225°, alone or mixed with an authentic specimen, $[\alpha]_D$ + 80° (c,1.2), which gave a pink Liebermann-Burchardt reaction and a strong yellow colour with tetranitromethane.

Found: C,81.3; H,11.2

Calc. for $C_{32}H_{52}O_2$: C,82.0; H,11.2%.

Hydrolysis of this acetate (0.5 g.) by refluxing in ethanolic potassium hydroxide solution (100 ml., 3%) and isolation of the product by means of ether, gave α-amyrin which separated from aqueous methanol as needles (0.4 g.), m.p. 185°, alone or mixed with an authentic specimen, $[\alpha]_D$ + 82° (c,2.0).

Found: C,84.5; H,11.9

Calc. for $C_{30}H_{50}O$: C,84.4; H,11.8%.

cyclo<u>Artenyl Acetate</u>. - Fractions (15-18) (above) were combined and crystallised several times from chloroform-methanol to give <u>cyclo</u>artenyl acetate (12 g.) as irregular plates, m.p.122-124°, $[\alpha]_D$ + 60° (c,1.6), showing a strong yellow colour with tetranitromethane. Light absorption: Max. at 2090 Å. ($\epsilon$, 1,500).

Found: C,81.3; H,11.2

Calc. for $C_{32}H_{52}O_2$: C,82.0; H,11.2%

With the Liebermann-Burchardt reagent, the compound initially

gave a pale yellow solution, which slowly developed to a blood red colour, having a strong green fluorescence. Professor D. H. R. Barton found that a mixture with cycloartenyl acetate, m.p. 121.5-122.5°, from Artocarpus integrifolia had m.p. 121.5-122.5°

cycloArtanyl Acetate. - cycloArtenyl acetate (5 g.) in glacial acetic acid (350 ml.) was shaken, at atmospheric pressure and room temperature, with hydrogen in the presence of platinum catalyst (from 1 g. platinum oxide) for 2 hours, by which time absorption had ceased. The solution was filtered, evaporated under reduced pressure and the residue crystallised from chloroform-methanol to yield long needles, m.p. 127-130°. Further crystallisation from chloroform-methanol gave cycloartanyl acetate as long needles, m.p. 131-132°, $[\alpha]_D$ + 59.5° (c,1.8). The compound gave a pale yellow colour with tetranitromethane in chloroform and showed no selective ultra-violet light absorption.

Found: C,81.7; H,11.9

Calc. for $C_{32}H_{54}O_2$: C,81.6; H,11.7%.

cycloArtanol. - A solution of cycloartanyl acetate (1 g.) in 3% methanolic potassium hydroxide (200 ml.) was heated under reflux for 2 hours, cooled and

diluted with water. The ether extract was washed with water, dried ($Na_2SO_4$) and evaporated. Crystallisation of the residue from methanol gave cycloartanol as plates, m.p. 98° (solvated), $[\alpha]_D$ + 50° (c,1.1).

Found: C,83.7; H,12.5

Calc.for $C_{30}H_{52}O$: C,84.0; H,12.2%.

cycloArtanone. - A solution of cycloartanol (1.3 g.) in stabilised acetic acid (150 ml.) was stirred at room temperature and chromic acid (250 mg.) in acetic acid (20 ml.) added during 1 hour. The solution was kept overnight at room temperature, methanol (5 ml.) added and the mixture diluted with water. The product was collected in ether, washed with 10% aqueous potassium carbonate, water and dried ($Na_2SO_4$). The ether was evaporated and the residue crystallised from methanol to give cycloartanone as plates, m.p. 110°, $[\alpha]_D$ + 23° (c,0.9).

Found: C,84.3; H,12.1

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

Ozonolysis of cycloArtenyl Acetate. - Ozonised oxygen (2 mols. ozone) was passed through a solution of cycloartenyl acetate (1.6 g.) in dry chloroform (150 ml.)

at - 45°. The solution was allowed to attain room temperature (1 hour), acetic acid (25 ml.) was added and then zinc dust (2 g.) during 30 minutes with stirring. The filtered solution was washed with water and the washings retained. The residue obtained on evaporation of the chloroform solution, crystallised from acetone--water to give 3β-acetoxy-25:26:27-trisnorcycloartan-24-ol as dense prisms (1 g.) m.p. 155-157° (decomp.), $[\alpha]_D$ + 59.5° (c,2.0).

Found: C,78.7; H,10.5

$C_{29}H_{46}O_3$ requires: C,78.7; H,10.5%

The aqueous washings were distilled, and to the distillate (300 ml.) an aqueous solution of 2:4-dinitrophenylhydrazine hydrochloride was added. Acetone 2:4-dinitrophenylhydrazone (0.21g., 26%) was isolated, which separated from ethanol as golden needles, m.p. 127-128°, alone or mixed with an authentic specimen.

Found: C,45.3; H,4.2; N,23.4

Calc. for $C_9H_{10}O_4N_4$: C,45.3; H,4.2; N,23.5%.

3β-Acetoxy-25:26:27-trisnorcycloartan-24-oic Acid. - (1) 3β-Acetoxy-25:26:27-trisnorcycloartan-24-ol (356 mg.) in acetic acid (100 ml.) was treated with chromic acid in acetic acid (10 ml., 6.4 mg./ml., 1.2 mols.) during 30 minutes and the solution left overnight at room temperature.

Methanol was added, the solution mixed with water and extracted with ether. The extract was washed with water, dried ($Na_2SO_4$) and the product crystallised from acetone-water as prismatic needles (230 mg.), m.p. 216-218°. Further crystallisation gave 3β-acetoxy-25:26:27-trisnorcycloartan-24-oic acid as short prismatic needles, m.p. 221.5-223°, $[\alpha]_D$ + 62 (c,0.9).

Found: C,76.2; H,10.3

$C_{29}H_{46}O$ requires: C,76.0; H,10.1%

(ii) Chromic acid (2.8 g.) in water (3 ml.) and acetic acid (35 ml.) was added dropwise during 90 minutes to a boiling solution of cycloartenyl acetate (2 g.) in acetic acid (100 ml.). The mixture was evaporated and the dry residue shaken with sulphuric acid (2%; 250 ml.) and ether. The ethereal solution was washed with water (100 ml.) and with sodium hydroxide solution (3%; 3 x 100 ml.); a sodium salt separated at the solvent interface. The salt was collected, suspended in water (100 ml.), the mixture acidified with hydrochloric acid, and the solid collected by means of ether. 3β-Acetoxy-25:26:27-trisnorcycloartan-24-oic acid separated from aqueous acetone as prismatic needles, m.p. 221-223°, alone or mixed with the sample described above, $[\alpha]_D$ + 62° (c,1.1).

Methyl 3β-Acetoxy-25:26:27-trisnorcycloartan--24-oate. - 3β-Acetoxy-25:26:27-trisnorcycloartan-24-oic acid (70 mg.) in dry ether (20 ml.) was treated with ethereal diazomethane until effervescence had ceased. The solution was evaporated and the residue crystallised from methanol to give methyl 3β-acetoxy-25:26:27-trisnorcycloartan-24-oate as needles (44 mg.), m.p. 122-124°, $[\alpha]_D$ + 55.6°.

Found: C,76.0; H,10.2

$C_{33}H_{48}O_4$ requires: C,76.2; H,10.2%.

3β-Hydroxy-25:26:27-trisnorcycloartan-24-oic acid. - 3β-Acetoxy-25:26:27-trisnorcycloartan-24-oic acid (100 mg.) was heated under reflux with 3% methanolic potassium hydroxide (100 ml.) for 2 hours. The solution was diluted with 3N hydrochloric acid and the product isolated by means of ether. 3β-Hydroxy-25:26:27-trisnorcycloartan-24-oic acid separated from aqueous acetone as prisms (80 mg.), m.p. 213-220°, $[\alpha]_D$ + 54° (c,0.6 in pyridine).

Found: C,77.5; H,10.7

$C_{27}H_{44}O_3$ requires: C,77.8; H,10.7%

Treatment of cycloArtenyl Acetate with Hydrogen Chloride. - cycloArtenyl acetate (500 mg.) in acetic acid (100 ml.) and concentrated hydrochloric acid (3ml.) was

heated at 95° for 3 hours. The solvent was removed under reduced pressure and the residue treated with acetic anhydride (10 ml.) and pyridine (5 ml.) on the steam bath for 3 hours. The product crystallised from chloroform-methanol as blades, m.p. 144-154°. After five crystallisations, blades m.p. 154-163°, $[\alpha]_D$ + 72° (c,1.1) were obtained.

Treatment of cycloArtanyl Acetate with Hydrogen Chloride. - A solution of cycloartanyl acetate (1 g.) in dry chloroform (30 ml.) at 0° was treated with a vigorous stream of dry hydrogen chloride for 3 hours. The solvent was evaporated under reduced pressure at 30° and the residue crystallised from chloroform-methanol as blades (910 mg.) m.p. 137-157°, $[\alpha]_D$ + 68° (c,1.6). The material gave a strong yellow colour with tetranitromethane in chloroform.

Light absorption: Max. at 2060 Å. ($\epsilon$ 3,600).

Lanostanyl Acetate. - (i) A solution of the acetate mixture, m.p. 137-157° (340 mg.) in acetic acid (150 ml.) was shaken with hydrogen and platinum catalyst (500 mg.) for 24 hours at 80°. The solution was filtered and evaporated to give a crystalline solid, m.p. 137-154°, which gave a yellow colour with tetranitromethane and

showed light absorption at 2060 Å. (ε 2,000). Chromic acid (320 mg.) in acetic acid (25 ml.) was added during 30 minutes with stirring to a solution of the hydrogenation product (840 mg.) in acetic acid (100 ml.) heated on the steam bath, and heating was continued for 90 minutes. The cooled solution was mixed with methanol (25 ml.) and evaporated to dryness, and the residue diluted with water (200 ml.). The neutral product (820 mg.), isolated by means of ether, in light petroleum (50 ml.) was filtered through a column (2 x 12 cm.) of alumina (25 g.). Elution with light petroleum-benzene (4:1, 375 ml.) gave a solid (480 mg.), which on crystallisation from chloroform--methanol gave lanostanyl acetate as needles, m.p. 155-156°, $[\alpha]_D$ + 141° (c,1.8). The melting point was undepressed on mixing with an authentic specimen, prepared from "iso-cholesterol" as outlined below. The compound gave no tetranitromethane colour and was transparent to ultra--violet light.

Found: C,81.3; H,12.0

Calc. for $C_{32}H_{56}O_2$: C,81.3; H,11.9%.

Hydrolysis with 3% ethanolic potassium hydroxide under reflux for 3 hours and isolation of the product by means of ether, gave lanostanol, needles from chloroform-methanol, m.p. and mixed m.p. 180-181°, $[\alpha]_D$ + 33° (c,1.1).

Found: C,83.8; H,12.7

Calc. for $C_{32}H_{54}O$: C,83.7; H,12.6%.

Elution with light petroleum-benzene (1:4, 100 ml.) and with benzene (150 ml.) gave a partly crystalline product (83 mg.) which on crystallisation from methanol, gave 7:11-dioxolanost-8-enyl acetate as pale yellow plates, m.p. and mixed m.p. 155-156°, $[\alpha]_D$ + 91° (c,0.6). Light absorption: Maxima at 2060 and 2700 Å. ( $\varepsilon$ 3,800 and 7,600).

Found: C,77.2; H,10.4

Calc. for $C_{32}H_{50}O_4$: C,77.1; H,10.1%.

(ii) The acetate mixture, m.p. 137-157° (1.4 g.) was shaken with hydrogen over a platinum catalyst (1.5 g.) for 8 hours at room temperature. The product in acetic acid (250 ml.) was treated with chromic acid (960 mg.) as described above, and a solution of the product in light petroleum (50 ml.) chromatographed on alumina (50 g.). Elution with light petroleum (750 ml.) gave lanostanyl acetate (needles from chloroform-methanol), m.p. and mixed m.p. 155-156°, $[\alpha]_D$ + 41.5 (c,1.2). The fraction (212 mg., m.p. 160-165°) eluted with light petroleum--benzene (2:3, 1200 ml.) could not be purified but showed the ultra-violet absorption characteristics of 7:11-dioxolanost-8-enyl acetate with a maximum at 2700 Å. ( $\varepsilon$ 5,200).

Elution with benzene-ether (9:1, 525 ml.) gave a fraction (220 mg.) which crystallised from methanol as needles, m.p. 178-181°. Further crystallisation from the same solvent gave 12-oxolanost-9(11)-enyl acetate as needles, m.p. and mixed m.p. 184-185°, $[\alpha]_D$ + 91° (c,0.8). Light absorption: Max. at 2420 Å. (ε 9,900).

Hydrogenation of "isoCholesteryl" Acetate. - A solution of "isocholesteryl acetate (m.p. 125°, 30 g.) in glacial acetic acid (380 ml.) was shaken with hydrogen for 4 hours at 70° in the presence of platinum catalyst (previously reduced from 2 g. platinum oxide). The solution was filtered, evaporated under reduced pressure and the product crystallised from chloroform-methanol as long blades (26 g.), m.p. 118-120°.

7:11-Dioxolanost-8-enyl Acetate. - (cf. 34). - The hydrogenated product (20 g.) from the preceding experiment was dissolved in stabilised acetic acid (1 l.), stirred on the steam bath and a solution of chromic acid (13 g.) in water (30 ml.) added during 30 minutes. Heating was continued for 1.5 hours, methanol (5 ml.) added and the cooled solution diluted with ice water (4 l.). The resulting suspension was coagulated by addition of sodium chloride, filtered and washed with water. A solution of this solid in ether (500 ml.) was washed with

5% aqueous sodium hydroxide, water and dried ($Na_2SO_4$). The product was dissolved in benzene (250 ml.) and percolated through a short column of alumina (200 g.) which was further eluted with benzene. Evaporation yielded a yellow solid which readily crystallised from methanol as yellow plates (12.5 g.), m.p. 151-154°. Further crystallisation from methanol yielded 7:11--dioxolanost-8-enyl acetate as yellow plates, m.p. 155-156°, $[\alpha]_D$ + 92° (c,1.2). Light absorption: Max. at 2700 Å. ($\varepsilon$ 7,600).

Found: C,77.0; H,10.4.

Calc. for $C_{32}H_{50}O_4$: C,77.1; H,10.1%.

7:11-Dioxolanostanyl Acetate. - (38) Zinc dust (60 g.) was added portionwise during 20 minutes to a refluxing solution of 7:11-dioxolanost-8-enyl acetate (3.5 g. in acetic acid (450 ml.) and heating continued for 1 hour. The acetic acid was decanted and the zinc further extracted with boiling acetic acid (300 ml.). The combined extracts were concentrated (400 ml.), poured into water and the solid product collected, washed with water and dissolved in ether. The ether solution was washed with 10% aqueous sodium carbonate, water and dried ($Na_2SO_4$). The product crystallised from chloroform-methanol as plates (6.5 g.), m.p. 218-219°. Further crystallisation from the same

solvent gave 7:11-dioxolanostanyl acetate as plates, m.p. 220-222°, $[\alpha]_D$ + 57° (c,1.3). The compound gave no colour with tetranitromethane in chloroform, and showed no selective light absorption in the ultra-violet region.

Found: C,77.1; H,10.4

Calc. for $C_{32}H_{52}O_4$: C,76.8; H,10.4%.

11-Oxolanostanyl Acetate. - (15). A solution of 7:11-dioxolanostanyl acetate (7.5 g.) in redistilled diethylene glycol (250 ml.) was heated with hydrazine hydrate (100%; 3.8 ml.) at 200° for 1 hour. To the cooled mixture was added a solution of sodium (7.5 g.) in diethylene glycol (70 ml.) and the whole maintained at 220-230° for 6 hours. The mixture was cooled, poured into water, acidified with hydrochloric acid and extracted with ether. The ether solution was washed with water, dried ($Na_2SO_4$) and evaporated to give a yellow solid (7.0 g.), a solution of which in pyridine (50 ml.) and acetic anhydride (50 ml.) was heated on the steam bath for 3 hours. The product (6.5 g.), isolated by means of ether, was dissolved in light petroleum-benzene (9:1, 100 ml.) and percolated through a column (1.5 x 20 cm.) of alumina (30 g.). Continued elution with the same solvent (150 ml.) gave a fraction (5.5 g.), m.p. 138-140°, which

on recrystallisation from chloroform-methanol gave 11-oxolanostanyl acetate as needles, m.p. 143-144°, $[\alpha]_D$ + 64° (c,1.1).

Found: C,79.2; H,11.4

Calc. for $C_{32}H_{54}O_3$: C,79.0; H,11.2%.

11-Hydroxylanostanyl Acetate. - (29). 11-Oxolanostanyl acetate (2.4 g.) in dry ether (100 ml.) was added to a suspension of lithium aluminium hydride (2.5 g.) in dry ether (150 ml.) and the mixture refluxed for 2.5 hours. The solution was cooled, diluted with ether and excess hydride destroyed by the cautious addition of ice water. The suspension was treated with 5N sulphuric acid and the ether solution washed with water and dried ($Na_2SO_4$). The product crystallised from chloroform-methanol as needles (1.5 g.), m.p. 190-191°. A solution of this material in pyridine (20 ml.) and acetic anhydride (20 ml.) was kept overnight at room temperature and the product, isolated by means of ether, crystallised from chloroform--methanol, to give 11-hydroxylanostanyl acetate as needles, m.p. 216°, $[\alpha]_D$ + 22° (c,2.0).

Found: C,78.8; H,11.5

Calc. for $C_{32}H_{56}O_3$: C,78.6; H,11.2%.

Lanost-9(11)-enyl Acetate. - A solution of 11-hydroxylanostanyl acetate (650 mg.) in dry pyridine (40 ml.) was heated with phosphorus oxychloride (5 ml.) at 100° for 3 hours. The mixture was mixed with water, extracted with ether and the ether solution washed with 3N sulphuric acid, 10% aqueous sodium carbonate, water and dried ($Na_2SO_4$). A solution of the product (640 mg.) in light petroleum (100 ml.) was filtered through a column (1.5 x 16 cm.) of alumina (30 g.) and the fraction (500 mg.) eluted with light petroleum benzene (4:1, 450 ml.) crystallised from chloroform-methanol to give lanost-9(11)--enyl acetate which separated as regular, hexagonal plates, m.p. 173-174°, $[\alpha]_D$ + 34° (c,1.3). The compound gave a strong yellow colour with tetranitromethane in chloroform. Light absorption: Max. at 2060 Å. ($\varepsilon$ 4,300).

Found: C,81.4; H,11.3

Calc. for $C_{32}H_{54}O_2$: C,81.6; H,11.7%.

Lanostanyl Acetate. - (78). Lanost-9(11)-enyl acetate (200 mg.) in acetic acid (150 ml.) was shaken with hydrogen and platinum catalyst (previously reduced from 300 mg. platinum oxide) at 30° for 24 hours. The solution was filtered, evaporated under reduced pressure and the residue crystallised from chloroform-methanol as fine needles (160 mg.), m.p. 154-155°. Further crystallisation

from the same solvent yielded lanostanyl acetate as needles, m.p. 155-156°, $[\alpha]_D$ + 41° (c,1.2). The compound gave no colour with tetranitromethane in chloroform and showed no selective light absorption in the ultra-violet region.

Found: C,81.4; H,12.1

Calc. for $C_{32}H_{56}O_2$: C,81.3; H,11.9%.

Lanostanol. - A solution of lanostanyl acetate (100 mg.) in 3% methanolic potassium hydroxide (50 ml.) was refluxed for 3 hours, cooled and poured into water. The ether extract was washed with water, dried ($Na_2SO_4$) and evaporated. The residue crystallised from chloroform--methanol to give lanostanol as fine needles, m.p. 130-131°, $[\alpha]_D$ + 33° (c,1.1).

Found: C,83.7; H,12.8

Calc. for $C_{30}H_{54}O$: C,83.7; H,12.6%.

Wolff-Kishner Reduction of 7:11-Dioxolanostanyl Acetate. - A mixture of 7:11-dioxolanostanyl acetate (5 g.) and 100% hydrazine hydrate (11 ml.) in dry ethanol (130 ml.) was boiled under reflux for 2 hours, a solution of sodium (12 g.) in ethanol (150 ml.) was added, and the mixture kept at 200° for 16 hours. The cooled mixture was diluted with water, and the product (4.9 g.) collected by means of ether and acetylated on the steam bath for

45 minutes with acetic anhydride (20 ml.) and pyridine (30 ml.). A solution of the acetylated product in light petroleum-benzene (4:1, 200 ml.) was filtered through a column (3.8 x 24 cm.) of alumina (210 g.). The combined, partly crystalline fractions (4.1 g.) eluted by light petroleum-benzene (1:4, 1600 ml.), benzene (1300 ml.) and benzene-ether (9:1, 1000 ml.), when crystallised from methanol, yielded 3:7-diacetoxy-lanostan-11-ol as fine needles, m.p. 236-237°, $[\alpha]_D$ + 58° (c,1.0). The melting point was undepressed on mixing with an authentic sample, prepared by the method of Voser (29), which had m.p. 236-237°, $[\alpha]_D$ + 57° (c,0.95).

Found: C,74.8; H,10.8

Calc. for $C_{34}H_{58}O_5$: C,74.7; H,10.7%.

Treatment of cycloArtanyl Acetate with Bromine. - (i) cycloArtanyl acetate (100 mg.) in carbon tetrachloride (100 ml.) was treated at 0° with bromine (100 mg., 3 mols.) in carbon tetrachloride (3 ml.) and the solution left overnight at 0°. The mixture was diluted with carbon tetrachloride, washed with 10% sodium thiosulphate solution, water and dried ($Na_2SO_4$). The residue obtained on removal of the solvent was crystallised from acetone to give needles, m.p. 130-131°, alone or mixed with starting

material, $[\alpha]_D$ + 59° (c,1.1).

(ii) cycloArtanyl acetate (150 mg.) in carbon tetrachloride (35 ml.) was treated in a similar manner with excess bromine (1 g.) in carbon tetrachloride (10 ml.). No crystalline product was obtained.

2-Bromocycloartanone. - A solution of cycloartanone (625 mg.) in carbon tetrachloride (20 ml.) was treated with N-bromosuccinimide (314 mg., 1.2 mols.) in the presence of suspended calcium carbonate; the mixture was illuminated with a 250-w lamp. The mixture was diluted with carbon tetrachloride, filtered, washed with water, dried over $Na_2SO_4$ and evaporated. The residue separated from methanol as a light brown solid, m.p. 100-110°, and after several crystallisations from the same solvent, gave 2-bromocycloartanone as needles, m.p. 116°, $[\alpha]_D$ + 43° (c,1.3). The compound gave a pale yellow tetranitromethane colour.

Found: C,71.3; H,9.7

$C_{30}H_{49}OBr$ requires: C,71.3; H,9.8%.

Dehydrobromination of 2-Bromocycloartanone. - 2-Bromocycloartanone (200 mg.) in collidine (3 ml.) was heated under reflux for 2 hours, left overnight at room temperature and filtered. The solution was diluted with water and a solution of the product, collected by means

of ether, in light petroleum (15 ml.) percolated through a column (1.25 x 7.5 cm.) of alumina (6 g.). The fractions eluted by light petroleum (40 ml.) and light petroleum-benzene (4:1; 100 ml.) were combined and crystallised from methanol to give cyclo*art*-1-*en*-3-*one* as prisms, m.p. 100°, $[\alpha]_D$ - 40° (c,1.3). Light absorption: maxima at 2060 and 2690 Å. ($\varepsilon$ 5,000 and 3,700).

Found: C,35.0; H,11.7

$C_{30}H_{48}O$ requires: C,34.8; H,11.4%

*Reduction of* cyclo*Art*-1-*en*-3-*one*. - cyclo*Art*--1-en-3-one (200 mg.) in acetic acid (125 ml.) was shaken with hydrogen and platinum (from 200 mg. platinum oxide) for 3 hours, by which time absorption of hydrogen had ceased. The solution was filtered and evaporated and the residual gum crystallised from methanol as blades, m.p. 76-87°. A solution of the crude mixture of 3α- and 3β- alcohols (205 mg.) in acetic acid (125 ml.) was treated with chromic acid in acetic acid (6.35 ml., 6.4 mg./ml., 1.2 mols.) and left overnight at room temperature. The neutral product was isolated in the normal manner and on crystallisation from methanol gave *cyclo*artanone as blades (142 mg.), m.p. 110°, alone or mixed with an

authentic specimen, $[\alpha]_D$ + 23° (c,0.3).

Found: C,84.3; H,12.1

Calc. for $C_{30}H_{50}O$: C,84.4; H,11.8%.

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Non-saponifiable Fraction of Opium Marc. - Opium marc (1 kg.) was extracted with boiling chloroform (2 x 4 l.), the extracts combined and evaporated. The residue was redissolved in ether (4 l.) and washed with 3N hydrochloric acid ( 2 x 3l.) and water (3 l.). The brown gum (180 g.), obtained on removal of the solvent, was extracted with a mixture of boiling benzene (300 ml.) and ethanol (2 l.), and the extract decanted from a quantity (10 g.) of undissolved, rubber-like material. A solution of potassium hydroxide (120 g.) in water (200 ml.) was added to the boiling extract and the mixture refluxed for 3 hours. The hot solution was added to water (10 l.), the resulting suspension extracted with ether (5 l., 2 x 2.5 l.) and the combined extracts washed with water. The residue obtained on evaporation was freed from water by co-distillation using benzene, to give an orange gum (43 g.). This non-saponifiable fraction was dissolved in warm acetone (100 ml.), the solution kept at room temperature for 4 hours and the crystalline solid separating (18.5 g.) collected. A second crop of solid (2.5 g.) was obtained on leaving the mother liquor overnight at room temperature.

Chromatography of Acetone-crystallised Fraction. - A solution of the combined acetone-crystallised fractions

(21 g.) in benzene (500 ml.) was percolated through a column (4.5 x 32 cm.) of alumina (520 g.) and the chromatogram developed as shown.

| Fractions | Eluant | Volume | Weight | Description |
|---|---|---|---|---|
| 1 - 8 | Benzene | 4.0 l. | 1.17g. | Gum |
| 9 - 20 | Benzene:ether (19:1) | 6.0 | 3.21 | White Solid |
| 21 - 25 | " | 2.5 | 0.92 | Clear Gum |
| 26 - 43 | " (9:1) | 9.0 | 7.11 | " |
| 44 - 52 | " (4:1) | 4.5 | 2.85 | " |
| 53 - 58 | " (1:1) | 3.0 | 1.51 | " |
| 59 - 67 | " | 4.5 | 1.16 | " |
| 68 - 70 | Benzene:Methanol (9:1) | 1.5 | 2.72 | Brown Gum |

Fractions (1 - 8) and (59 - 70) could not be obtained crystalline and were not examined further.

(+)-n-Nonacosan-10-ol. - Fractions (9 - 20) were combined and crystallised from chloroform as soft, lustrous plates (1.4 g.), m.p. 80-82°. Further crystallisation from ethyl acetate gave (+)-n-nonacosan--10-ol as prisms, m.p. 81-82°, $[\alpha]_D \pm 0°$ (c,2.0), showing no colour with tetranitromethane in chloroform and no selective ultra-violet light absorption. No colour was obtained in the Liebermann-Burchardt test.

Found: C,81.8; H,14.2

Calc. for $C_{29}H_{60}O$: C,82.0; H,14.2%

(+)-n-Nonacosan-10-yl acetate. - (+)-n--Nonacosan-10-ol (200 mg.) in pyridine (10 ml.) and acetic anhydride (5 ml.) was heated on the steam bath for 2 hours. The product was isolated by means of ether and on crystallisation from ethyl acetate, gave (+)-n-nonacosan-10-yl acetate as plates (130 mg.), m.p. 44.5-45.5°.

Found: C,79.6; H,13.3

Calc. for $C_{31}H_{62}O_2$: C,79.8; H,13.4%

cycloLaudenol. - Fractions (21 - 58) of the above chromatogram were combined and crystallised from methanol as needles (7 g.), m.p. 121-123°, $[\alpha]_D$ + 48° (c,1.8). Further crystallisation from methanol gave cyclolaudenol as stout needles, m.p. 123-125°, $[\alpha]_D$ + 46° (c,1.5). The compound gave a pale yellow colour with tetranitromethane. Light absorption: Max. at 2050 Å. ( ε 1,500)

Found: C,84.6; H,12.0

$C_{31}H_{52}O$ requires: C,84.5; H,11.9%

cycloLaudenyl Acetate. - cycloLaudenol (6 g.) in pyridine (25 ml.) and acetic anhydride (12 ml.) was

heated on the steam bath for 2 hours. The solution was cooled, mixed with water and extracted with ether. The extract was washed with 3N hydrochloric acid, saturated sodium bicarbonate solution, water and dried ($Na_2SO_4$). Evaporation of the ether gave a solid which crystallised from chloroform-methanol as blades (5.5 g.), m.p. 117-119°, $[\alpha]_D$ + 52° (c,1.7). Further crystallisation from methanol gave cyclolaudenyl acetate, which separated as elongated plates, m.p. 120-121°, $[\alpha]_D$ + 55° (c,0.8) and gave a pale yellow colour with tetranitromethane. Light absorption: Max. at 2060 Å. ($\epsilon$ 1,500).

Found: C,82.0; H,11.4

$C_{33}H_{54}O_2$ requires: C,82.1; H,11.3%.

Ozonolysis of cycloLaudenyl Acetate. - A solution of cyclolaudenyl acetate (3 g.) in dry chloroform (150 ml.) was treated at - 45° with ozonised oxygen (9.3 ml. $O_3$/min., 2 mols.) for 30 minutes. After attaining room temperature (1 hour), acetic acid (25 ml.) was added, and then zinc dust (6 g.) portionwise during 30 minutes with stirring. After stirring for a further hour, the filtered solution was washed with water (5 x 200 ml.). The aqueous washings were combined, adjusted to pH 7.0, and treated with a saturated aqueous solution of dimedone (200 ml.). After 24 hours at 0°, the

separated formaldehyde dimethone (0.84 g., 35%) was collected (m.p. 188-189°) and crystallised from ethanol, from which it separated as needles, m.p. 189-190°, alone or mixed with an authentic sample.

Found: C,68.5; H,8.4

Calc. for $C_{17}H_{24}O$: C,69.8; H,8.3%.

Evaporation of the dried chloroform solution (above) gave a partly crystalline solid (2.96 g.) which was dissolved in light petroleum (200 ml.) and percolated through a column (2.75 x 18 cm.) of alumina (90 g.). Elution with light petroleum (1,000ml.) and light petroleum--benzene (3:1, 1,400 ml.) gave a fraction (2 g.) which crystallised from methanol as needles, 140-141°. Further crystallisation from methanol gave oxonorcyclolaudanyl acetate as needles, m.p. 140-141°, $[\alpha]_D$ + 61° (c,1.3). The compound gave a pale yellow colour with tetranitromethane and showed no selective absorption in the ultra--violet region.

Found: C,79.2; H,10.8

$C_{32}H_{52}O_3$ requires: C,79.3; H,10.8%.

To a refluxing solution of oxonorcyclolaudanyl acetate (100 mg.) in methanol (25 ml.) was added hydroxylamine hydrochloride (200 mg.) and sodium acetate (400 mg.) in water (1 ml.) and methanol (10 ml.). Refluxing was

continued for 2 hours and the mixture diluted with water and extracted with ether. The ether solution was washed with water, dried ($Na_2SO_4$) and the product crystallised from methanol to give the oxime as needles, m.p. 160-161°, $[\alpha]_D$ + 54° (c,0.3).

Found: C,77.0; H,10.6

$C_{32}H_{53}O_3N$ requires: C,76.9; H,10.7%.

Wolff-Kishner Reduction of 25-Oxo-26-norcyclolaudanyl Acetate. - A mixture of 25-oxo-26-norcyclolaudanyl acetate (250 mg.), 100% hydrazine hydrate (2.5 ml.) and sodium ethoxide (from 312 mg. of sodium) in ethanol (15 ml.) was kept at 200° for 12 hours. The reaction mixture was cooled, diluted with water and extracted with ether. The ether extract was washed with 3N hydrochloric acid, water and dried ($Na_2SO_4$). The product was treated on the steam bath for 3 hours with acetic anhydride (5 ml.) and pyridine (5 ml.). A solution of the dried acetylated product (236 mg.), isolated in the normal manner, in light petroleum (25 ml.) was filtered through a column (1 x 8 cm.) of alumina (6 g.). The fraction eluted with light petroleum (125 ml.) was crystallised from methanol to give needles (134 mg.), m.p. 116-118°. Further crystallisation from the same solvent gave 26-norcyclolaudanyl acetate as needles, m.p. 118-120°, $[\alpha]_D$ + 58° (c,1.1). A mixture with

cycloartanyl acetate had m.p. 117-128°.

Found: C,81.3; H,11.6

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

26-Norcyclolaudanol. - A solution of 26-norcyclolaudanyl acetate (200 mg.) in 3% methanolic potassium hydroxide (50 ml.) was refluxed for 2 hours, cooled, diluted with water and extracted with ether. The ether extract was washed with water, dried ($Na_2SO_4$) and evaporated. The product was crystallised from methanol to give 26-norcyclolaudanol as needles (176 mg.), m.p. 114-115°, $[\alpha]_D$ + 49° (c,1.6). A mixture with cycloartanol had m.p. 98-110°.

Found: C,83.9; H,12.2

$C_{30}H_{52}O$ requires: C,84.0; H,12.2%.

26-Norcyclolaudan-3-one. - A solution of 26-norcyclolaudanol (236 mg.) in stabilised acetic acid (70 ml.) and benzene (15 ml.) was stirred at room temperature and chromic acid in acetic acid (10 ml., 4.3 mg./ml., 1.2 mols.) added during 30 minutes, and the solution left overnight. Methanol was added, the mixture poured into water and extracted with ether. The product crystallised from methanol as blades (172 mg.), m.p. 99-101°, and on further crystallisation gave 26-norcyclolaudan-3-one as blades, m.p. 100-101°, depressed to 92-97° when mixed with

cycloartanone, $[\alpha]_D$ + 22° (c,2.2).

Found: C,84.0; H,11.9

$C_{30}H_{48}O$ requires: C,84.4; H,11.8%

26-Norcyclolaudanone (95 mg.) in methanol (15 ml.) was refluxed with a solution of hydroxylamine hydrochloride (200 mg.) and sodium acetate (400 mg.) in water (1 ml.) and methanol (10 ml.) for 2 hours. The solution was diluted with water and the product collected with ether. Crystallisation from chloroform-methanol gave the oxime as needles, m.p. 203°, $[\alpha]_D$ - 9.8° (c,0.6).

Found: C,81.7; H,11.3

$C_{30}H_{51}ON$ requires: C,81.6; H,11.6%.

Methyl 3β-acetoxy-26:27-bisnorcyclolaudan-25-oate. - 25-Oxo-26-norcyclolaudanyl acetate (375 mg.) in dioxan (15 ml.) was shaken with bromine (1 ml.), potassium hydroxide (15 g.), and water (20 ml.) at room temperature for 14 days. The solution and solid which separated during the reaction, were mixed with water and shaken with ether; a potassium salt separated at the solvent interface. The salt was collected, suspended in water (100 ml.), the mixture acidified (methyl-red) with hydrochloric acid, and the solid collected by means of ether. The resulting gum (284 mg.) was treated on the steam bath for 2½ hours with acetic anhydride (5 ml.) and pyridine (5 ml.). The

solution was cooled, diluted with water and the product, an uncrystallisable gum, isolated by means of ether. A solution of the acetylated acid (313 mg.) in dry ether (20 ml.) was treated with a dry ether solution (15 ml.) of diazomethane (from 1.5 g. N-nitrosomethyl urea). The reaction mixture was kept overnight at room temperature and then evaporated under reduced pressure to give a gum which was dissolved in light petroleum (25 ml.) and percolated through a column (1.75 x 5 cm.) of alumina (9 g.). Elution with light petroleum-benzene (2:3, 125 ml.; 1:4, 75 ml.) gave a fraction (62 mg.) which, on crystallisation from methanol, yielded methyl 3β-acetoxy-26:27-bisnorcyclolaudan-25-oate as needles, m.p. 115-116°, $[\alpha]_D$ + 57° (c,1.1).

Found: C,76.8 ; H,10.5

$C_{32}H_{52}O_4$ requires: C,76.8; H,10.5%.

Treatment of cycloLaudenyl Acetate with Hydrogen Chloride. - cycloLaudenyl acetate (2 g.) in dry chloroform (40 ml.) was treated with a stream of dry hydrogen chloride at 0° for 3 hours. The solid obtained on removal of the solvent, crystallised from methanol, giving a "hydrochloride" (1.7 g.) as needles, m.p. 166-170° (decomp.), $[\alpha]_D$ + 64° (c,1.3). The compound gave a strong yellow colour with tetranitromethane.

Found: C,76.0; H,10.8

$C_{33}H_{55}O_2Cl$ requires: C,76.3; H,10.7%.

A solution of the "hydrochloride" in acetic anhydride (15 ml.) was refluxed for 15 hours, then diluted with water. The product, isolated in the usual manner, separated from chloroform-methanol as needles (1.1 g.), m.p. 152-156°, $[\alpha]_D$ + 61° (c,1.1).

Found: C,81.8; H,11.0

$C_{33}H_{54}O_2$ requires: C,82.1; H,11.3%.

Ozonised oxygen was then passed through a solution of this product (1.3 g.) in acetic acid (170 ml.) at room temperature for 1 hour. The solution was diluted with water (1 l.), treated with 10% ferrous sulphate solution (20 ml.), and steam distilled. From the distillate (500 ml.), formaldehyde (22%) was isolated as its dimethone, needles (from ethanol), m.p. and mixed m.p. 190-191°.

Found: C,69.7; H,8.2

Calc.for $C_{17}H_{24}O_4$: C,69.8; H,8.3%.

The filtrate from the dimethone derivative was again steam distilled, and the distillate (250 ml.) treated with aqueous 2:4-dinitrophenylhydrazine hydrochloride; no hydrazone separated. The non-volatile product was treated with chromic acid by the method used for the preparation of lanost-9(11)-enyl acetate from the mixture

obtained by treatment of cycloartanyl acetate with hydrogen chloride (see Theoretical, Part I), but no crystalline product was isolated.

Treatment of 25-Oxo-26-norcyclolaudanyl Acetate with Alkali. - 25-Oxo-26-norcyclolaudanyl acetate (m.p. 140-141°, $[\alpha]_D$ + 61°, 200 mg.) was refluxed with 5% methanolic potassium hydroxide (150 ml.) for 5 hours. The solution was cooled, mixed with water and extracted with ether. The product crystallised from aqueous methanol to yield 25-oxo-26-norcyclo-24ab-laudanol (140 mg.) as short, thick needles, m.p. 130-141°, $[\alpha]_D$ + 43° (c,1.4)

Found: C,81.0; H,11.4

$C_{31}H_{50}O_2$ requires: C,81.4; H,11.4%.

This alcohol (110 mg.) was treated for 12 hours at room temperature with acetic anhydride (5 ml.) and pyridine (5 ml.), and the product isolated in the normal manner. Two crystallisations from methanol gave 25-oxo-26-norcyclo--24ab-laudanyl acetate as needles (60 mg.), m.p. 123-125°, unchanged on further crystallisation, $[\alpha]_D$ + 52° (c,1.5). A mixture with 25-oxo-26-norcyclolaudanyl acetate had m.p. 123-135°.

Found: C,79.3; H,10.7

$C_{32}H_{52}O_3$ requires: C,79.3; H,10.8%

25-Oxo-26-norcyclo-24ab-laudanyl acetate (150 mg.) in methanol (23 ml.) was refluxed with a solution of hydroxylamine hydrochloride (300 mg.) and sodium acetate (600 mg.) in water (2 ml.) and methanol (15 ml.) for 2½ hours. The product was isolated in the normal manner and on crystallisation from methanol gave the oxime as short needles, m.p. 153-154°, $[\alpha]_D$ + 50° (c,1.5).

Found: C,76.6; H,10.5

$C_{32}H_{53}O_3N$ requires: C,76.9; H,10.7%.

Treatment of 25-Oxo-26-norcyclolaudanyl Acetate with Chromic Acid. - 25-Oxo-26-norcyclolaudanyl acetate (100 mg.) in acetic acid (25 ml.) was stirred at room temperature and treated with chromic acid in acetic acid (9 ml., 2 mg./ml., 1.3 mols.) during 30 minutes. The reaction was left overnight at room temperature and the neutral product isolated with ether. Crystallisation from methanol gave 25-oxo-26-norcyclo-24ab-laudanyl acetate as needles (72 mg.), m.p. 123-125° alone or mixed with the sample described above, $[\alpha]_D$ + 53° (c,1.2).

Attempted Enol-acetylation of 25-Oxo-26-norcyclo-laudanyl Acetate. - (i) A mixture of 25-oxo-26-norcyclo-laudanyl acetate (100 mg.), acetic anhydride (2 ml.) and freshly fused potassium acetate (100 mg.) was kept at

132° for 10 hours. The product, isolated by means of ether, gave 25-oxo-26-norcyclo-24ab-laudanyl acetate, which separated from methanol as needles (87 mg.), m.p. 123-125°, $[\alpha]_D$ + 51° (c,1.2).

(ii) A solution of the same acetate (100 mg.) in isopropenyl acetate (20 ml.) and one drop of concentrated sulphuric acid, was kept at 100° for 3 hours. Potassium carbonate was added, the solvent evaporated under reduced pressure, the residue mixed with water and the product collected with ether. After two crystallisations from methanol, 25-oxo-26-norcyclo-24ab-laudanyl acetate was obtained as needles (82 mg.), m.p. 123-125°, $[\alpha]_D$ + 51° (c,1.2).

Treatment of 25-Oxo-26-norcyclolaudanyl Acetate with Phenylmagnesium Bromide. - The acetate (2 g., 1 mol.) in ether (80 ml.) was added during 45 minutes to a solution of phenylmagnesium bromide (4.5 g., 6 mols.) in ether (100 ml.), and the mixture refluxed for 30 minutes. The cooled solution was poured into 3N hydrochloric acid and the product isolated by means of ether. The product was steam-distilled for 1 hour and a solution of the non-volatile fraction (2.4 g.) in acetic anhydride (15 ml.) refluxed with freshly fused potassium acetate (0.5 g.) for 1 hour. A solution of the product in light petroleum (100 ml.) was percolated through a column (2.75 x 15 cm.) of

alumina (75 g.) and the fraction (0.6 g.) eluted by light petroleum (900 ml.) crystallised from methanol to give 25-phenyl-26-norcyclolaud-25(27)-enyl acetate as plates, m.p. 101-102°, $[\alpha]_D$ + 56° (c,1.2). The styryl compound gave a strong yellow colour with tetranitromethane. Light absorption: maxima at 2080 and 2360 Å. ( ε,12,200 and 8,100)

Found: C,83.9; H,10.4

$C_{38}H_{56}O_2$ requires: C,83.8; H,10.4%.

The fractions eluted by light petroleum-benzene (1:4, 1050 ml.; 1:1, 1350 ml.) were combined (1.5 g.) and twice crystallised from acetone-water to give the phenyl methyl carbinol, 25-hydroxy-25-phenyl-26-norcyclolaudanyl acetate as small needles (0.7 g.), m.p. 152-154°, $[\alpha]_D$ + 37° (c,1.1). It gave a pale yellow colour with tetranitromethane. Light absorption: Max. at 2080 Å. ( ε,7250).

Found: C,81.0; H,10.4

$C_{38}H_{58}O_3$ requires: C,81.1; H,10.4%.

A solution of the alcohol and freshly fused potassium acetate (0.5 g.) in acetic anhydride (15 ml.) was heated under reflux for 4 hours and left overnight. The product in light petroleum (80 ml.) was chromatographed on alumina (40 g.), and the fraction eluted by

light petroleum (330 ml.) crystallised from methanol to give the styryl compound described above as needles (0.7 g.), m.p. and mixed m.p. 101-102°, $[\alpha]_D$ + 55.6° (c,1.1).

Ozonolysis of 25-Phenyl-26-norcyclolaud--25(27)-enyl Acetate. - A solution of the styryl compound (1 g.) in dry chloroform (150 ml.) was treated with ozonised oxygen at - 45° (2 mols. ozone). The solution was allowed to attain room temperature (1 hour), acetic acid (15 ml.) added, and then zinc dust (1.5 g.) added during 30 minutes with stirring. After filtration, the solution was washed with water and the washings retained. The product obtained on removal of the solvent, was steam-distilled, and a solution of the residue (0.9 g.) in light petroleum (70 ml.) chromatographed on a column (2.25 x 8 cm.) of alumina (30 g.). The fraction (0.5 g.) eluted by light petroleum-benzene (4:1, 640 ml.) gave 25-oxo-25-phenyl-23:27-bisnorcyclolaudanyl acetate, which separated from methanol as needles, m.p. 112-114°, $[\alpha]_D$ + 57.5° (c,1.2). The phenyl ketone gave a pale yellow colour with tetranitromethane. Light absorption: maxima at 2050 and 2420 Å. ($\varepsilon$ 13,000 and 11,750)

Found: C,81.1; H,10.0

$C_{37}H_{54}O_3$ requires: C,81.3; H,10.0%.

To the aqueous washings, saturated aqueous dimedone (150 ml.) was added and formaldehyde isolated as its dimethone (240 mg., 44%) needles (from ethanol) m.p. and mixed m.p. 190-191°.

Found: C,69.7; H,8.4

Calc. for $C_{17}H_{24}O_4$: C,69.8; H,8.3%

25-Oxo-25-phenyl-26:27-bisnorcyclolaudanyl acetate (100 mg.) in methanol (15 ml.) was refluxed for 2 hours with a solution of hydroxylamine hydrochloride (200 mg.) and sodium acetate (400 mg.) in water (1 ml.) and methanol (10 ml.). The product was crystallised three times from methanol to give the oxime as short, thick needles, m.p. 177-179°, $[\alpha]_D$ + 55° (c,1.3).

Found: C,73.8; H,9.7

$C_{37}H_{55}O_3N$ requires: C,79.1; H,9.9%.

Treatment of 25-Oxo-26-norcyclo-24ab-laudanyl Acetate with Phenylmagnesium Bromide. - 25-Oxo-26--norcyclo-24ab-laudanyl acetate (3.7 g.) was treated with phenylmagnesium bromide (3.2 g.; 6 mols.) as described above. The crude alcohol was readily dehydrated by refluxing with acetic anhydride (30 ml.)and freshly fused potassium acetate (1 g.) for 4 hours, and the product isolated in the normal manner. A solution of the product (4.4 g.) in light petroleum (150 ml.) was filtered

through a column (4 x 12.5 cm.) of alumina (130 g.). The fractions (2.3 g.) eluted by light petroleum (600 ml.) and light petroleum-benzene (4:1, 3 l.) were combined and crystallised from ethanol, from which 25-phenyl-26-norcyclo-24ab-laud-25(27)-enyl acetate separated as fine needles (2 g.), m.p. 99-114°, $[\alpha]_D$ + 49.8° (c,1.2), which gave a strong yellow colour with tetranitromethane. Light absorption: maxima at 2080 and 2360 Å. (ε 13,000 and 8,700). Nine crystallisations from ethanol gave 25-phenyl-26-norcyclo-24a-laud-25(27)-enyl acetate as fine needles, m.p. 138-139°, $[\alpha]_D$ + 44° (c,1.1). Light absorption: maxima at 2080 and 2360 Å. (ε 13,700 and 8,750).

Found: C,83.7; H,10.2

$C_{38}H_{56}O_2$ requires: C,83.8; H,10.4%.

Ozonolysis of 25-Phenyl-26-norcyclo-24ab-laud--25(27)-enyl Acetate. - Ozonised oxygen (2 mols. ozone) was passed through a solution of the 24ab-styryl compound (2 g.; m.p. 99-114°) in dry chloroform (150 ml.) at - 45°, and the ozonide decomposed with zinc dust and acetic acid as described above. The product (1.9 g.) was isolated by means of chloroform and the aqueous washings retained. After removal of chloroform, the residue was steam-distilled for 1 hour. The non-volatile

fraction was dissolved in light petroleum (150 ml.) and filtered through a column (2.75 x 11 cm.) of alumina (60 g.). The fractions (1.1 g.) eluted by light petroleum-benzene (1:1, 1800 ml.; 3:1, 500 ml.) and benzene (400 ml.) were combined and crystallised from methanol, to yield 25-oxo-25-phenyl-26:27-bisnorcyclo--24ab-laudanyl acetate as needles (0.7 g.), m.p. 99-102°, $[\alpha]_D$ + 49° (c,1.4). Light absorption: maxima at 2050 and 2420 Å. ($\varepsilon$ 13,000 and 11,250). Seven crystallisations from methanol gave 25-oxo-25-phenyl-26:27-bisnorcyclo-24a-laudanyl acetate, m.p. 110-111°, $[\alpha]_D$ + 44° (c,1.7). A mixture with 25-oxo-25-phenyl--26:27-bisnorcyclolaudanyl acetate (m.p. 112-114°) had m.p. 98-103°, i.e. approximately the same as the 24ab--mixture described above.

Found: C,81.1; H,10.0

$C_{37}H_{54}O_3$ requires: C,81.3; H,10.0%.

From the aqueous washings, formaldehyde (67%) was isolated as its dimethone, needles (from ethanol), m.p. and mixed m.p. 190-191°

Found: C,69.6; H,8.6

Calc. for $C_{17}H_{24}O_4$: C,69.8; H,8.3%.

The filtrate from the dimethone was again distilled and the distillate treated with 2:4-dinitrophenylhydrazine; no precipitate was obtained.

Treatment of the 24a- and 24b- Phenyl Ketones with Alkali. - (i) Hydrolysis of the 24a- phenyl ketone (116 mg.) with 3% methanolic potassium hydroxide, and treatment of the product with acetic anhydride ( 5 ml.) and pyridine (5 ml.) at 95° for 1½ hours, yielded the 24ab-phenyl ketone which separated from methanol as needles (84 mg.), $[\alpha]_D$ + 47° (c,1.2), m.p. 96-101°, alone or mixed with the specimen of m.p. 99-102°.

(ii) The 24b-phenyl ketone (110 mg.) was treated with 5% methanolic potassium hydroxide under reflux for 6 hours and the product reacetylated in the normal manner. Crystallisation from methanol gave the 24ab--phenyl ketone (71 mg.), $[\alpha]_D$ + 46° (c,1.0), m.p. 96-100°, alone or mixed with a specimen of m.p. 99-102°.

25:25-Diphenyl-26:27-bisnorcyclolaud-24-enyl Acetate. - The 24ab-phenyl ketone (1.4 g.; m.p. 98-102°) was treated with phenylmagnesium bromide (2.7 g.) as described above. The alcohol was dehydrated (without purification) by refluxing for 4 hours with acetic anhydride (15 ml.) and potassium acetate (0.5 g.). A solution of the product (1.4 g.) in light petroleum

(100 ml.) was chromatographed on a column (3 x 8.5 cm.) of alumina (45 g.). The fraction eluted by light petroleum-benzene (4:1; 560 ml.) crystallised from chloroform-methanol, from which the diphenylethylene separated as plates (0.4 g.), m.p. 170°, $[\alpha]_D$ + 53° (c,1.4). It gave a strong yellow colour with tetranitromethane. Light absorption: maxima at 2050 and 2430 Å. ($\varepsilon$ 29,300 and 13,400).

Found: C,85.0; H,9.5

$C_{43}H_{58}O_2$ requires: C,85.1; H,9.6%.

24-Oxo-25:26:27-trisnorcyclolaudanyl Acetate. - A solution of the diphenylethylene (350 mg.) in dry chloroform (100 ml.) was treated with ozonised oxygen at -45° ( 2 mols. ozone) and the ozonide treated with zinc and acetic acid. The product (350 mg.) was isolated by means of chloroform in the usual manner and the water-washings were retained. The residue obtained on removal of the solvent was steam-distilled, and a solution of the non-volatile fraction in light petroleum-benzene (1:1, 80 ml.) percolated through a column (1.75 x 6.75cm.) of alumina (12 g.). The fraction (77mg.) eluted by light petroleum-benzene (1:3, 250 ml.) crystallised from methanol to give 24-oxo-25:26:27-trisnorcyclolaudanyl acetate as plates, m.p. 170°, $[\alpha]_D$

+ 53° (c,1.2). The compound gave a pale yellow tetranitromethane colour and showed no selective light absorption.

Found: C,78.7; H,10.8

$C_{30}H_{48}O_3$ requires: C,78.9; H,10.6%

The acetate (45 mg.) in methanol (3 ml.) was refluxed with a solution of hydroxylamine hydrochloride (100 mg.) and sodium acetate (200 mg.) in water (0.5 ml.) and methanol (5 ml.) for 2 hours. The product crystallised from chloroform-methanol, yielding the oxime as plates, m.p. 219-220°, $[\alpha]_D$ + 50.2° (c,0.8).

Found: C,76.2; H,10.2

$C_{30}H_{49}O_3N$ requires: C,76.4; H,10.5% .

Ether extraction of the aqueous washings from the ozonolysis gave a clear gum (62 mg.) with a fragrant odour. A solution in methanol (2 ml.) on treatment with Brady's reagent, yielded benzophenone 2:4-dinitrophenylhydrazone, which separated from acetic acid as orange plates (89 mg., 43%), m.p. and mixed m.p. 235-236°. Light absorption: maxima at 2060, 2240, 2500 and 3800 Å. (ε 37,300, 26,400, 18,500 and 30,000).

Found: C,63.1; H,3.9; N,15.6

Calc. for $C_{19}H_{14}O_4N_4$: C,63.0; H,3.9; N,15.5%.

24-Oxo-26:27-bisnorcycloartanyl Acetate. - 3β-Acetoxy-25:26:27-trisnorcycloartan-24-ol (1 g.) in dry ether (20 ml.) and pure dioxan (10 ml.) was treated with ethereal diazomethane (50 ml.; from 5 g. of nitrosomethylurea) at room temperature for 3 days. The solvent was evaporated under reduced pressure and a solution of the product in light petroleum-benzene (2:1, 90 ml.) percolated through a column (2.5 x 9 cm.) of alumina (30 g.). The fraction (350 mg.) eluted by light petroleum-benzene (2:1, 800 ml.; 1:1, 300ml.) crystallised from methanol as plates, m.p. 166-167°. Further crystallisation gave 24-oxo-26:27-bisnorcycloartanyl acetate as plates, m.p. 170° alone or mixed with 24-oxo--25:26:27-trisnor-cyclolaudanyl acetate, $[\alpha]_D$ + 58° (c,1.2).

Found: C,78.9; H,10.6

$C_{33}H_{48}O_3$ requires: C,78.9; H,10.6%.

24-Oxo-26:27-bisnorcycloartanyl acetate (100 mg.) in methanol (15 ml.) was refluxed for 2½ hours with a solution of hydroxylamine hydrochloride (200 mg.) and sodium acetate (400 mg.) in water (1 ml.) and methanol (10 ml.). Two crystallisations from chloroform-methanol gave the oxime as needles, m.p. 219-220°, alone or mixed with the oxime of 24-oxo-25:26:27-trisnorcyclolaudanyl

acetate, $[\alpha]_D$ + 50° (c,0.9).

Found: C,76.2; H,10.0

$C_{23}H_{49}O_3N$ requires: C,76.4; H,10.5%.

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Saponification of Shea Nut Fat. - A solution of shea nut fat (5.5 kg.) in 10% ethanolic potassium hydroxide (15 l.) was heated under reflux for 5 hours, the solution mixed with water and extracted with ether. The ether extract was washed with water, dried ($Na_2SO_4$) and evaporated to give the non-saponifiable fraction as a yellow-brown gum (163 g.; 3%).

Butyrospermyl Acetate. - The non-saponifiable fraction (160 g.) was refluxed for 4 hours with acetic anhydride (300 ml.) and the solution allowed to stand overnight at room temperature. The semi-crystalline mass (200 g.) which separated was collected and the filtrate kept at 0° for a further 2 days. A second crop of solid (14 g.) which separated was collected and crystallised from ethanol-ethyl acetate (10:3) as stout needles (6.2 g.), m.p. 129-131°. One further crystallisation gave needles, m.p. 138-139°, $[\alpha]_D$ + 22° (c,2.1). After a total of nine recrystallisations from ethanol-ethyl acetate, butyrospermyl acetate (1.4 g.), m.p. 145°, $[\alpha]_D$ + 11° (c,2.0) was obtained.

Found: C,81.6; H,11.4

Calc. for $C_{32}H_{52}O_2$: C,81.9; H,11.2%.

Fractional crystallisation of the first crop of solid obtained from the acetylation, gave a further small quantity of butyrospermyl acetate.

Butyrospermol. - (i) Impure butyrospermyl acetate (m.p. 138-139°, $[\alpha]_D$ + 22°, 1 g.) was hydrolysed by refluxing with 3% methanolic potassium hydroxide for 2 hours. The product was crystallised three times from aqueous acetone to give butyrospermol (400 mg.), m.p. 109-110°, $[\alpha]_D$ - 12.5 (c,1.1). Acetylation with acetic anhydride and pyridine refurnished butyrospermyl acetate, m.p. 143-145°, $[\alpha]_D$ + 11° (c,1.6).

(ii) Hydrolysis of impure butyrospermyl acetate (m.p. 130-135°, $[\alpha]_D$ + 25°) and crystallisation of the product from aqueous acetone gave needles, m.p. 185-210°. A second crop, m.p. 107-109°, $[\alpha]_D$ - 7.5° (c,1.4) was obtained from the mother liquor and on one further crystallisation from aqueous acetone gave butyrospermol, m.p. 109-110°, $[\alpha]_D$ - 12° (c,1.2). Butyrospermol was also obtained by similar treatment of acetate, m.p. 130-135°, $[\alpha]_D$ + 30°.

Dihydrobutyrospermyl Acetate. - Butyrospermyl acetate (1.4 g.) in ethyl acetate (200 ml.) was shaken with hydrogen over an acid-free platinum catalyst (500 mg.) for

12 hours at room temperature. The filtered solution was evaporated and the residue crystallised from chloroform-methanol as prismatic needles, m.p. 128.5--131.5°. Further crystallisation from the same solvent gave dihydrobutyrospermyl acetate as prismatic needles, m.p. 135-136.5°, $[\alpha]_D$ + 10.7 (c,2.0).

Dihydroisobutyrospermyl Acetate. - A solution of dihydrobutyrospermyl acetate (200 mg.) in dry chloroform (25 ml.) was externally ice-cooled and treated with a vigorous stream of dry hydrogen chloride for 2 hours. The solution was diluted with chloroform, washed with 10% potassium carbonate, water and dried ($Na_2SO_4$). The product on crystallisation from chloroform-methanol, gave dihydroisobutyrospermyl acetate (euph-8-enyl acetate) as needles (162 mg.), m.p. 124.5--125.5°, $[\alpha]_D$ + 33.6° (c,2.3). The melting point was undepressed on mixing with an authentic sample of euph--8-enyl acetate.

Treatment of Euph-8-enyl Acetate with Hydrogen Chloride at 0°. - Euph-8-enyl acetate (200 mg.) was treated with hydrogen chloride at 0° exactly as for dihydrobutyrospermyl acetate. Starting material was recovered in high yield (140 mg.) m.p. and mixed m.p.

124-125°, $[\alpha]_D$ + 33° (c,1.2).

Treatment of Dihydroisobutyrospermyl Acetate with Hydrochloric Acid in Acetic Acid. - Dihydroisobutyrospermyl acetate (82 mg.) was heated on the steam bath for 3 hours with a solution of hydrochloric acid in acetic acid (1:20, 3 ml.). The product was isolated by means of ether and on crystallisation from chloroform-methanol, gave isoeuphenyl acetate as plates (53 mg.), m.p. 111° alone or mixed with an authentic sample prepared from euph-3-enyl acetate in a similar manner, $[\alpha]_D$ -10° (c,1.6).

Oxidation of Dihydrobutyrospermyl Acetate with Chromic Acid. - Dihydrobutyrospermyl acetate (100 mg.) in benzene (5 ml.) and acetic acid (30 ml.) was treated with chromic acid in acetic acid (10.1 ml., 7.1 mg./ml., 4 mols.) during 30 minutes and the solution left overnight at room temperature. The neutral product was obtained as a pale yellow gum which failed to crystallise, and which showed ultra-violet absorption with a maximum at 2480 Å. This gum was again treated with chromic acid (5 mols.) and the product chromatographed but no crystalline product was obtained.

Oxidation of Dihyroisobutyrospermyl Acetate with Chromic Acid. - Dihydroisobutyrospermyl acetate (137 mg.) in acetic acid (80 ml.) was stirred on the steam bath and treated with a solution of chromic acid in acetic acid (10 ml., 8 mg./ml., 4.2 mols.) during 30 minutes; heating was continued for 1½ hours. The neutral product (140 mg.) was dissolved in light petroleum (15 ml.) and filtered through a column (1.25 x 6 cm.) of alumina (5 g.). The fraction (40 mg.) eluted by light petroleum-benzene (4:1, 300 ml.) was crystallised three times from methanol to give 7:11--dioxoeuph-8-enyl acetate as yellow needles, m.p. 110-111°, alone or mixed with an authentic sample (32), $[\alpha]_D$ + 19.7° (c,1.0). Light absorption: Max. at 2710 Å. ($\varepsilon$ 8,100).

Treatment of Dihydroisobutyrospermyl Acetate with Perbenzoic Acid. - Dihydroisobutyrospermyl acetate (100 mg.) in a chloroform solution of perbenzoic acid (118 mg./ml., 4 ml.) was kept at 0° for 2 days and at room temperature for 2 days. The solution was diluted with chloroform, washed with 10% potassium carbonate solution, water and dried ($Na_2SO_4$). Three crystallisations of the product from methanol gave 8:9-epoxyeuphanyl acetate as needles (64 mg.), m.p. and mixed m.p. 175-177°, $[\alpha]_D$ + 62° (c,0.9). The product gave no colour with

tetranitromethane and was transparent to ultra-violet light.

Conversion of Butyrospermyl Acetate into Euphadienyl Acetate. - Butyrospermyl acetate (200 mg.) in chloroform (25 ml.) was treated at 0° with a 1% solution of bromine in chloroform (6.82 ml., 1 mol.). The colourless solution was treated with a vigorous stream of dry hydrogen chloride at 0° for 1½ hours and the product isolated in the normal manner. The resulting clear gum failed to crystallise and was heated under reflux with acetic acid (27 ml.) and zinc dust (2 g.) for 2 hours. The filtered solution was diluted with water and the product isolated by means of ether. After four crystallisations from chloroform-methanol, euphadienyl acetate was obtained as needles, m.p. and mixed m.p. 106.5-107.5°, $[\alpha]_D$ + 40° (c,0.8).

Hydrogenation of Butyrospermyl Acetate in Acetic Acid. - Butyrospermyl acetate (20 mg.) in acetic acid (100 ml.) was shaken with hydrogen over a platinum catalyst (200 mg.) for 30 hours at 60°. The filtered solution was evaporated and the residue crystallised from chloroform-methanol as needles, m.p. 115-116°. Two further crystallisations gave euph-8-enyl

acetate as needles, m.p. and mixed m.p. 120-122°, $[\alpha]_D$ + 25° (c,0.6).

Treatment of Dihydrobutyrospermyl Acetate with Osmium Tetroxide. - Osmium tetroxide (1.62 g., 1.5 mols.) in dry ether (20 ml.) was added to a solution of dihydrobutyrospermyl acetate (2 g.) in pure pyridine (20 ml.), and the mixture kept in the dark for 12 days at room temperature. The resulting brown-green, needle-like complex and solution were diluted with ether (100 ml.) and treated under reflux with lithium aluminium hydride (4.5 g.) added during 30 minutes. Heating was continued for 1 hour, excess lithium aluminium hydride destroyed by the addition of ethyl acetate and water and the mixture finally mixed with water. The dark brown suspension which resulted was extracted with ether and the extract washed with water and dried ($Na_2SO_4$). The product was treated with acetic anhydride (25 ml.) and pyridine (25 ml.), and a solution of the dry acetylated product in light petroleum (150 ml.) percolated through a column (2.7 x 10 cm.) of alumina (60 g.). The fraction (310 mg.) eluted by light petroleum (900 ml.) gave dihydrobutyrospermyl acetate as prismatic needles, m.p. and mixed m.p. 134-135°, $[\alpha]_D$ + 10° (c,1.4).

Elution with light petroleum-benzene (4:1, 900ml.; 1:1, 750 ml.) gave a fraction (1.12 g.) which crystallised from chloroform-methanol as prismatic needles, m.p. 177-178°. Further crystallisation gave a triol-diacetate as prismatic needles, m.p. 181-182°, $[\alpha]_D$ - 82° (c,1.2). The product gave no tetranitromethane colour and was transparent to ultra-violet light.

Found: C,75.2; H,10.9

$C_{34}H_{52}O_5$ requires: C,74.7; H,10.7%.

Eupha-7:9(11)-dienyl Acetate. - (i) The triol-diacetate, m.p. 181-182° (200 mg.) was refluxed for 4 hours with acetic anhydride and freshly fused potassium acetate (300 mg.) and the solution left overnight at room temperature. The product, isolated by means of ether, was dissolved in light petroleum (50 ml.) and filtered through a column (1.25 x 9 cm.) of alumina (6 g.). Elution with light petroleum (75 ml.) gave eupha-7:9(11)-dienyl acetate, which separated from methanol as needles (60 mg.), m.p. 111-112°, alone or mixed with an authentic specimen (65), $[\alpha]_D$ - 78° (c,1.0). The product gave a red-brown colour with tetranitromethane. Light absorption: Maxima at 2320, 2400 and 2470 Å. ($\varepsilon$ 15,000, 17,000 and 10,500).

(ii) The triol-diacetate, m.p. 181-182° (125 mg.) was heated under vacuum for 2 days at 100°. The material then had m.p. 120-155° and light absorption: maxima at 2320, 2400 and 2470 Å. (ε 5,600, 6,500 and 4,200). A solution of the product in light petroleum (25 ml.) was chromatographed on alumina (4 g.) and the fraction (40 mg.) eluted with light petroleum (75 ml.) crystallised from methanol to give eupha-7:9(11)--dienyl acetate as needles, m.p. and mixed m.p. 111-112°. Elution with light petroleum-benzene (4:1, 50 ml.; 1:1, 100 ml.) and benzene (25 ml.) gave unchanged starting material (50 mg.) m.p. and mixed m.p. 177-178°.

(iii) The triol-diacetate, m.p. 181-182° (100 mg.) was sublimed in high vacuum at 160-180° and the product (m.p. 70-90°) chromatographed on alumina (3 g.). The fraction (47 mg.) eluted by light petroleum gave eupha--7:9(11)-dienyl acetate, m.p. and mixed m.p. 111-112°. Further elution with benzene (150 ml.) gave starting material, m.p. and mixed m.p. 177-178°.

(iv) Zinc dust (600 mg.) was added to a solution of triol-diacetate (100 mg.) in acetic acid (10 ml.) and the mixture heated on the steam bath for 2 hours. The filtered solution was diluted with water and the product

isolated by means of ether. Crystallisation from methanol gave eupha-7-9(11)-dienyl acetate as needles, m.p. and mixed m.p. 109-111°.

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