STEREOCHEMICAL CONSIDERATIONS IN TRITERPENOID CHEMISTRY

T H E S I S

submitted to

THE UNIVERSITY OF GLASGOW

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requirements of the

DEGREE OF DOCTOR OF PHILOSOPHY

by

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

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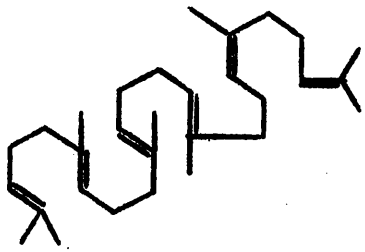
INTRODUCTION

The triterpenes have been the subject of much interest over the past forty years. Fundamentally, they can be regarded as low molecular weight polymers, comprising six isoprene units joined in a regular or an irregular fashion, and forming a skeleton of thirty carbon atoms. The recognition of a certain number of compounds containing thirty-one carbon atoms, as belonging to the triterpene class, has resulted in the adoption of the more comprehensive term triterpenoid.

Depending upon the degree of cyclisation of their parent hydrocarbon squalene, the triterpenoids can be divided into three main groups.

(i) The squalenoid group includes the aliphatic hydrocarbon, squalene (I) and the tricyclic alcohol, ambrein (II), both of which are of animal origin. The symmetrical diol, onocerin (III) was isolated from a plant source and also falls within this group. Presumably, it is the product of a simultaneous cyclisation of both ends of the squalene chain.

(ii) The tetracyclic group, which bears a close structural relationship to the steroids, can be divided into three sub-groups, typified by lanosterol (IV), euphol (V) and tirucallol (VI).



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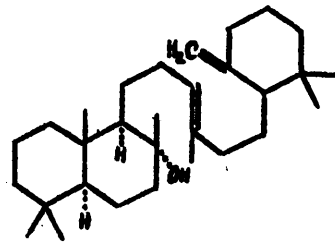

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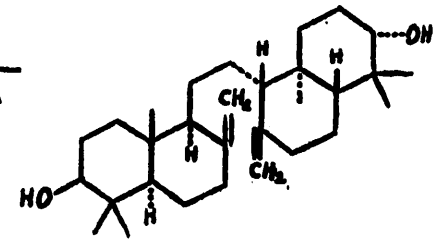

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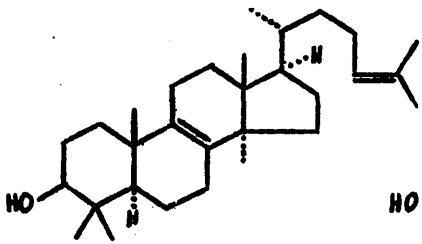

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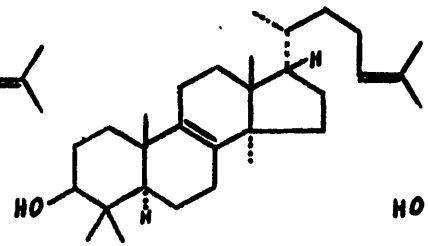

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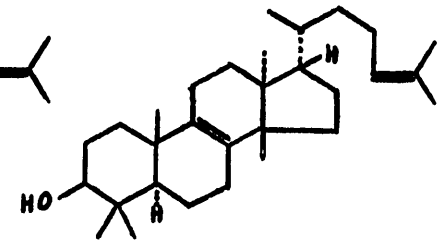
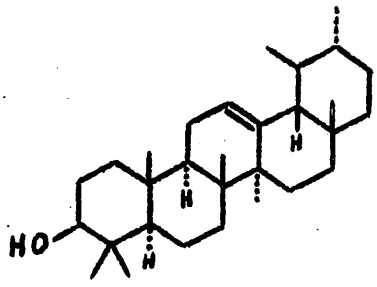


VI

Into this group also falls the pentacyclic compounds cycloartenol and cyclolaudenol, in which a cyclopropane bridge replaces a nuclear double bond of the true tetracyclic triterpenoid.

(iii) The third, and largest group is the pentacyclic triterpenoids of which well over fifty compounds of known constitution have been reported. All of these compounds can be derived from one of the five basic carbon skeletons represented by α-amyrin (VII), β-amyrin (VIII), lupeol (IX), taraxasterol (X) and hydroxyhopanone (XI), either through direct substitution, or slight structural variations of the carbon skeleton.



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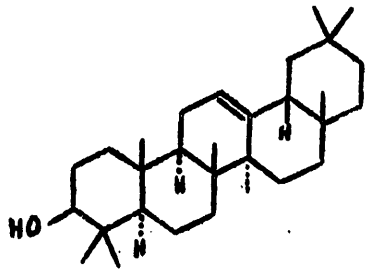
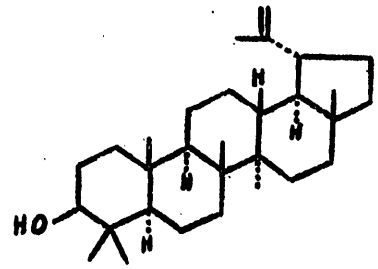


VIII



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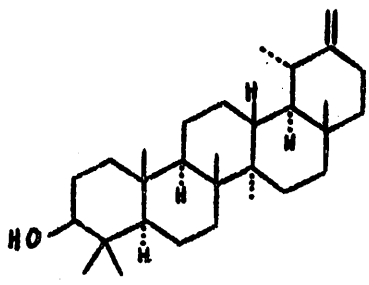

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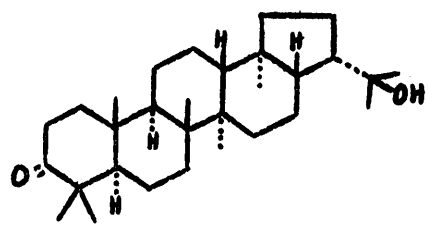
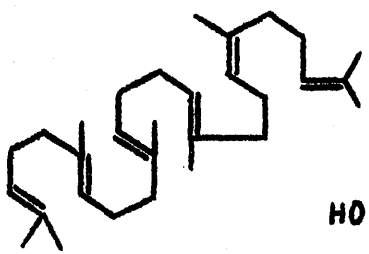


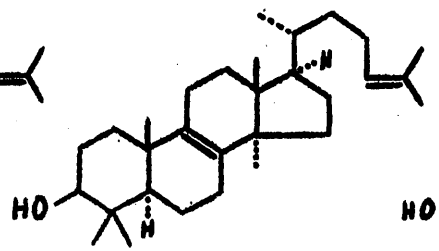
XI

Biogenesis of the Triterpenoids.

As a result of the work of a number of eminent chemists,[1-12] it has how been firmly established that squalene (I) is the immediate precursor of the triterpenoids and steroids. Using labelled acetic acid, squalene has been synthesised and subsequently converted into lanosterol (IV).[2,3] Degradative studies[8] of the cholesterol (XII) derived from this labelled lanosterol have shown that the mode of cyclisation of squalene follows the route proposed by Woodward and Bloch.[9]



I



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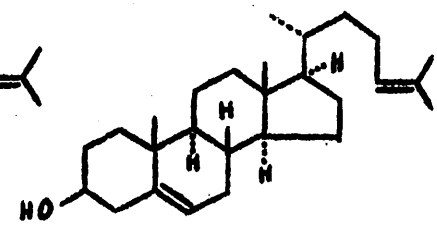
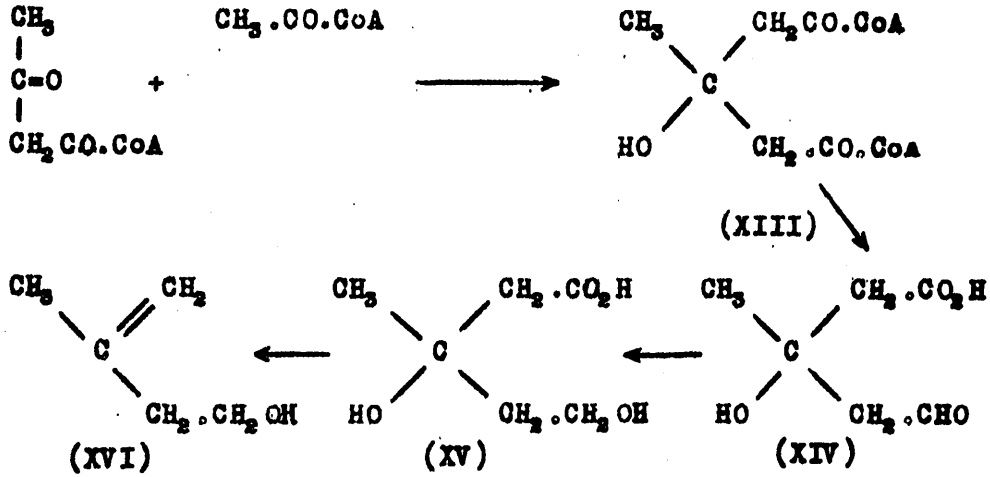


XII

The cyclisation involves a fully concerted process[8,9,10] without formation of any stabilised intermediates, and is supported by the transoid nature[11] of the squalene chain. Since ^{18}O appeared in ring A of the lanosterol isolated when an $^{18}O_2$ atmosphere was used, Tchen and Bloch[5] showed that aerobic conditions were necessary for the cyclisation and that the reaction was probably initiated by the approach of an OH^+ cation, derived from molecular oxygen, to the double bond at one end of the squalene chain.

Squalene itself is considered to be built up from acetate in the following manner.[12-16] Condensation of acetyl co-enzyme A with aceto-acetyl co-enzyme A yields β-hydroxy-β-methyl-glutaryl co-enzyme A (XIII) which affords mevalonic

acid (XV) by way of mevaldic acid (XIV). Decarboxylation and elimination of water then gives 3-methyl-but-3-enol (XVI), the pyrophosphate ester of which is regarded[14] as the isoprene building block upon which the steroids and triterpenoids are based. A fully concerted mechanism is proposed[16,17] for this

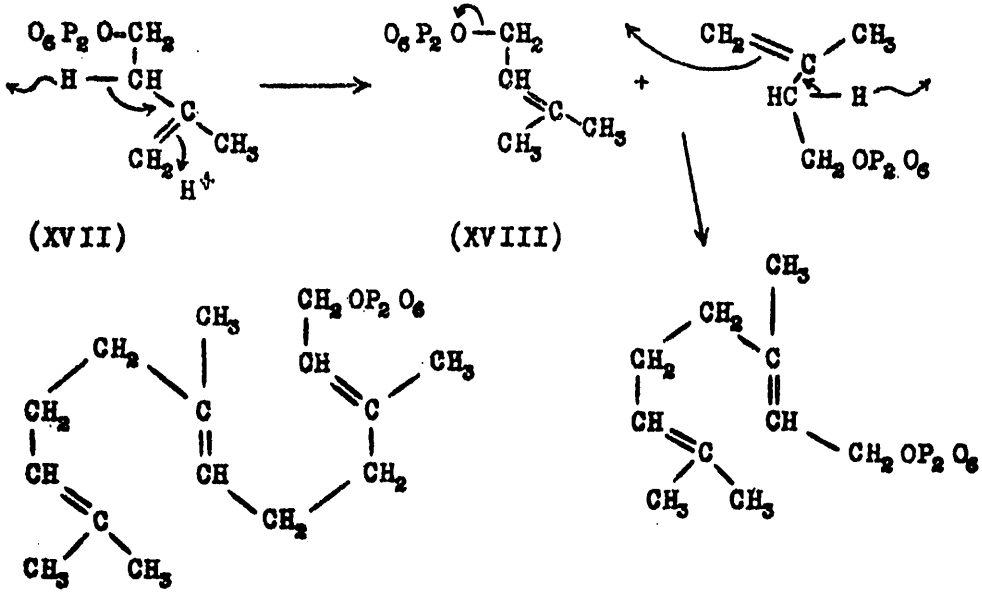


simultaneous decarboxylation and elimination, since no deuterium uptake was found to take place when the reaction was carried out in deuterium oxide.

Confirmation of the general validity of the above route was obtained by the isolation of several phosphorylated intermediates[18-20] in the biosynthesis of squalene, one of which was identified[20] as 3-methyl-but-3-enyl pyrophosphate. The structure of this compound was rigidly established by synthetic methods.[21,22]

The use of labelled mevalonic acid has shown[23,24,25] that head to tail condensation of two units occurs, $C_{(2)}$ of one

molecule linking to $C_{(5)}$ of the other, without loss or exchange of the hydrogen atoms attached to those carbon atoms. Rilling and Bloch[16] proposed a mechanism for the condensation in which the initial step is isomerisation of the isopentenyl derivative (XVII) to the dimethyl-allyl pyrophosphate ester (XVIII). The latter then condenses with an isopentenyl unit to yield geranyl pyrophosphate ester (XIX). Confirmation was later obtained by



(XVII) (XVIII)

(XX) (XIX)

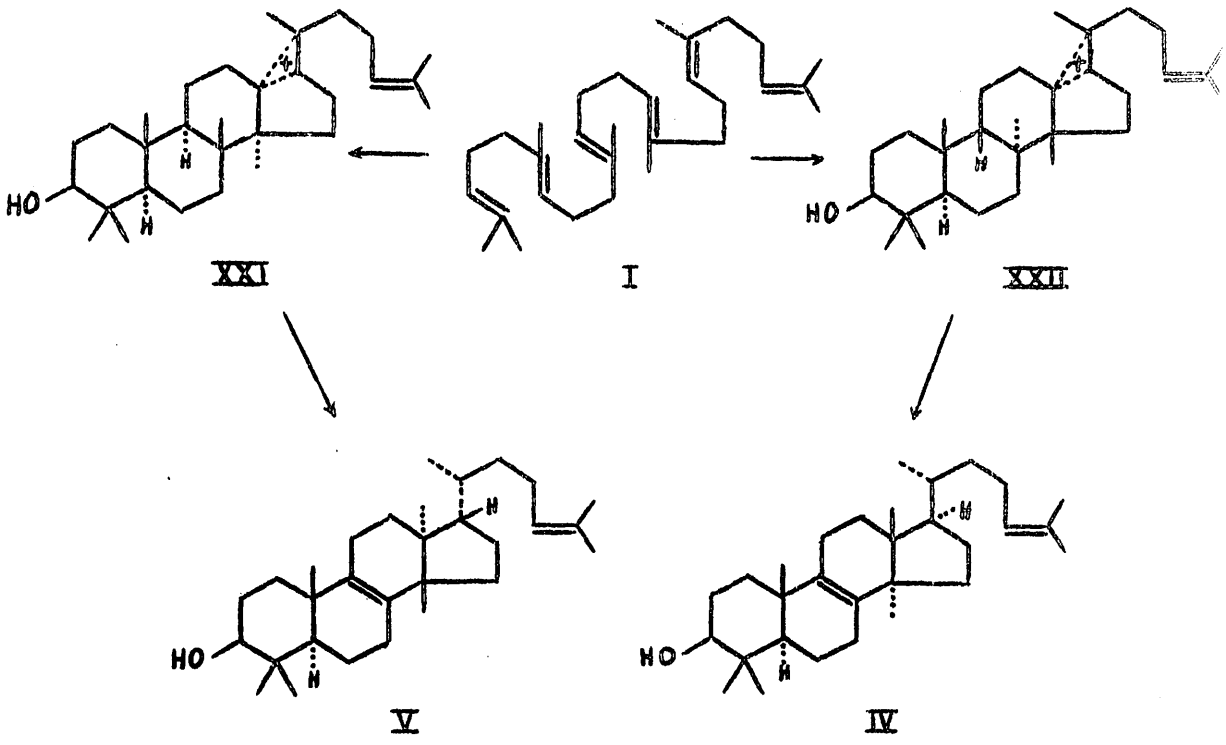
the enzymatic isomerisation[21,26] of 3-methyl-but-3-enyl pyrophosphate to dimethyl-allyl pyrophosphate. Further support in favour of this mechanism came from the condensation of 3-methyl-but-3-enyl and dimethyl-allyl pyrophosphates to yield, as a first product, geranyl pyrophosphate (XIX) which then reacted with another isopentenyl unit to form the farnesyl derivative (XX).[14]

Squalene is considered to arise from the tail to tail condensation of two farnesyl molecules.

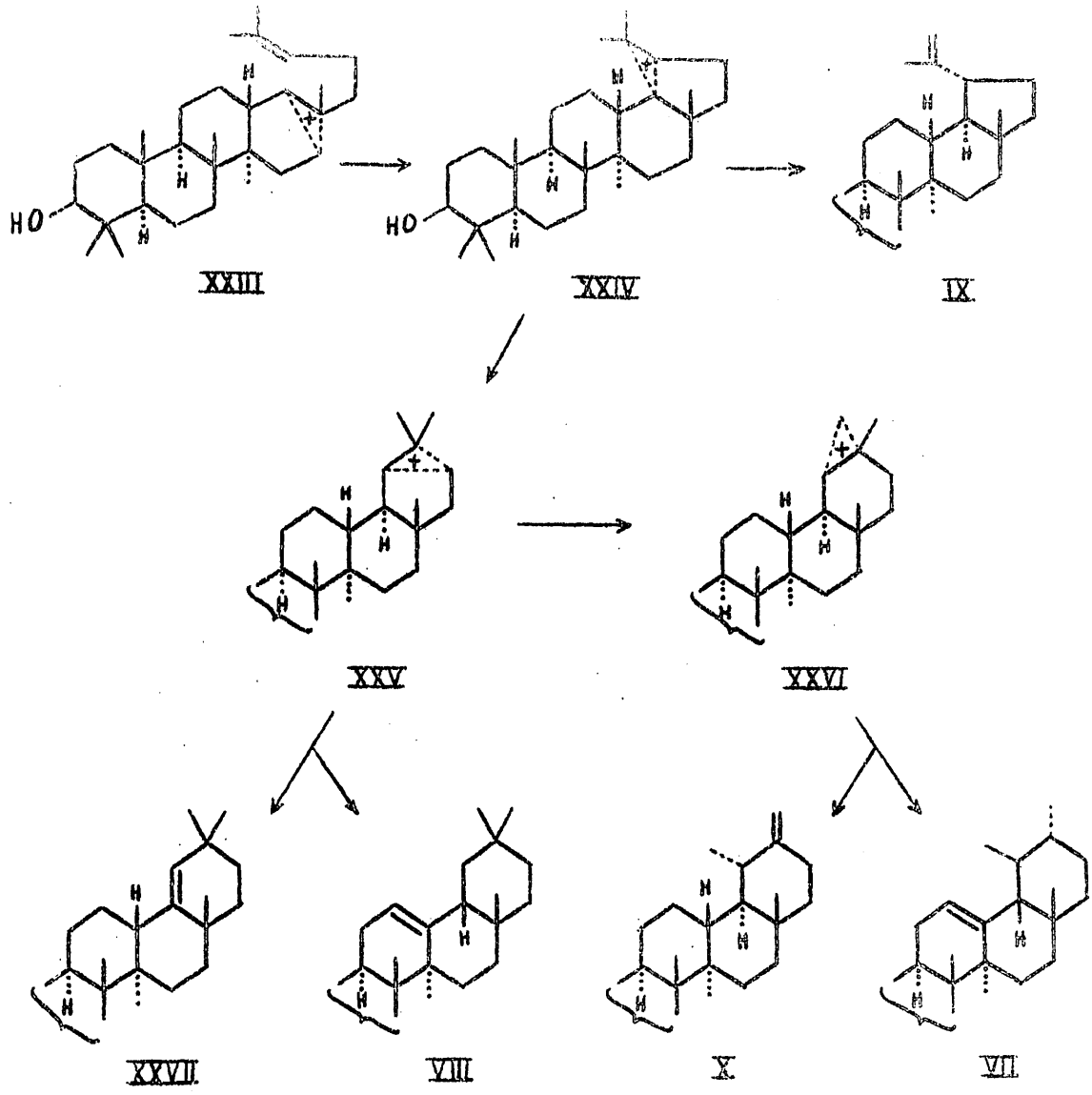
The scheme proposed initially[1] for the cyclisation of squalene did not embrace any stereochemical considerations but due to the work of Eschenmoser et al.[27], a biogenetic pathway leading to the correct stereochemistry in the steroids and the triterpenoids was eventually advanced. The enzymatic folding of the all-trans squalene chain into specific boat and chair conformations is the prime factor in determining the nature of the final product.

A chair-boat-chair-boat conformational sequence gives rise on cyclisation, to the carbonium ion (XXII) which has the requisite stereochemistry to yield lanosterol (IV) on migration of the methyl groups attached to $C_{(8)}$ and $C_{(14)}$ and of the hydrogen atom at $C_{(13)}$, followed by loss of a proton from $C_{(9)}$. Recently,[26,29] it was shown that displacement of the methyl groups takes place by two 1:2-shifts and not by one 1:3-shift.

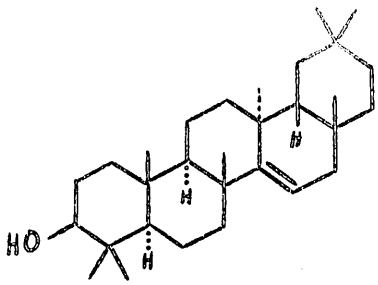
Folding of the squalene chain in a chair-chair-chair-boat conformational sequence leads to the carbonium ion (XXI) which is the precursor of the euphol-tirucallol group. Again, by 1:2-shifts of the appropriate methyl groups and hydrogen atom, and loss of a proton from $C_{(9)}$, there obtains the stereochemistry apparent in the euphol series (V).



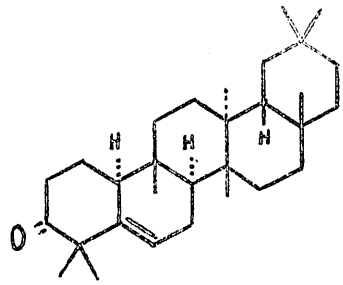
Rearrangement of (XXI) by a Wagner-Meerwein shift leads to the carbonium ion (XXIII) from which all the pentacyclic triterpenoids are derived by further cyclisation of the side chain folded in a boat conformation. The cation (XXIV) so formed can immediately eliminate a proton to afford lupeol (IX) or rearrange through the carbonium ion (XXV) to (XXVI) which subsequently yields the α-amyrin (VII) and taraxasterol (X) derivatives by migration of one of the methyl groups at $C_{(22)}$ followed by proton loss.



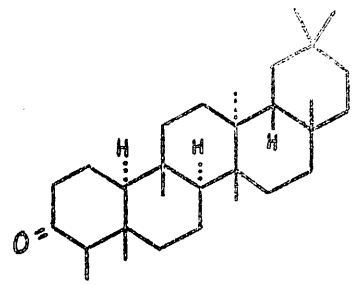
The cation (XXV) leads directly to germanicol (XXVII), and the other β-amyrin, (VIII), derivatives can be formed by hydrogen shifts and proton elimination. Further 1:2-shifts of the axial methyl groups and hydrogen atoms are necessary for the formation of the taraxerol (XXVIII), glutinone (XXIX) and friedelin (XXX) derivatives.



XXVIII

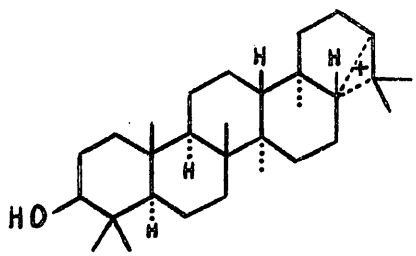


XXIX

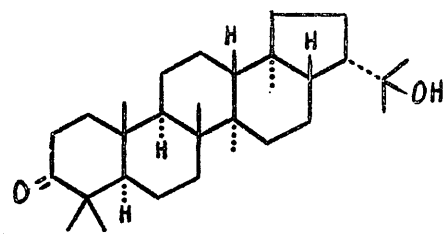


XXX

To explain the structure (XI)[30-34] for hydroxyhopanone, Ruzicka[12] suggested that the squalene chain is folded in a chair-chair-chair-chair-boat sequence which on cyclisation, would yield the intermediate carbonium ion (XXXI). The occurrence of the squalenoid triterpenoids, ambrein (II) and onocerin (III) are explained by a cyclisation mechanism which involves cationic attack simultaneously at both ends of the squalene chain.



XXXI



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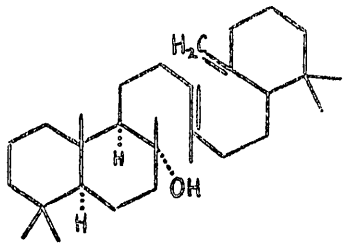

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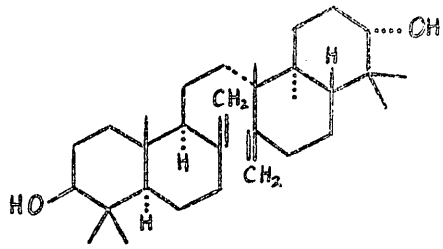


III

THEORETICAL

SECTION I

The Chemistry of Glutinone.

The $C_{(3)}$-epimers of glutin-5- and -5(10)-en-3-ols have been prepared and their stereochemistry discussed. 'Glutinol-II', the alcohol derived from an isomer of glutinone, is identified as glutin-5(10)-en-3β-ol while the isolation of epiglutinol (glutin-5-en-3β-ol) from Nature supports the biogenetic theory, which predicts the natural occurrence of this epimer.[42] The structure of dihydroglutinyl acetate has been established as 5β-glutinan-3α-yl acetate. Experiments on 'glutinone-III' are described and tentative conclusions concerning the structures of this isomer and its derivatives, have been drawn.

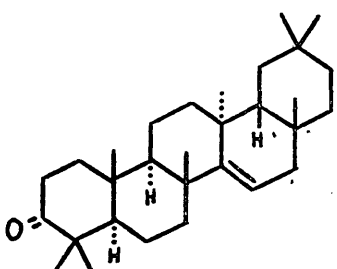
Historical

The isolation of a new triterpenoid ketone from alder bark (*Alnus glutinosa* L.) was first reported in 1953 by Chapon and David[35] who established the molecular formula, $C_{30}H_{48}O$, and later named the compound glutinone.[36] Beaton, Spring and Stevenson,[37] working independently from the French authors, also isolated this ketone from the same source although they termed it alnusenone. Reduction of glutinone with lithium aluminium hydride[36,37] or sodium in alcohol[37] gave the same alcohol, glutinol, in which the hydroxyl group must have an equatorial orientation since the alcohol was recovered unchanged after prolonged refluxing with sodium amyloxide in air.[38] That the carbonyl group in glutinone was relatively unhindered was demonstrated by the ease of formation of an oxime[37] and by Wolff-Kishner reduction[37,39] to the unsaturated hydrocarbon, glutinene. The infrared spectrum[37] of glutinone includes a band at 1702 $cm.^{-1}$, characteristic of a carbonyl group in a six-membered ring.

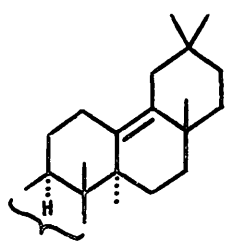
The presence of a double bond in the ketone was inferred from its ultraviolet absorption spectrum[37] and from the yellow colour formed with tetranitromethane. Monoperphthalic acid oxidation to yield an epoxide[38] confirmed this view. Glutinyl acetate absorbed only one mole of hydrogen when reduced catalytically[37,40] and the product,

dihydroglutinyl acetate, was transparent to ultraviolet light and gave no colour with tetranitromethane. Dihydroglutinol, obtained by hydrolysis of the acetate, yielded the corresponding ketone on oxidation with chromic acid. Wolff-Kishner reduction[37] of the latter material afforded the saturated parent hydrocarbon, glutinane, also obtained on catalytic reduction of glutinene.

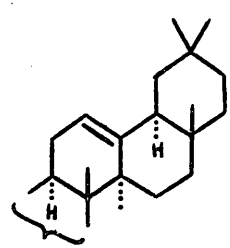
Since glutinone occurs in nature closely associated with the isomeric taraxerone (XXXII), Beaton et al.,[38] assumed the triterpenoid nature of the ketone. Their analytical figures confirmed the molecular formula $C_{30}H_{48}O$ and assuming the presence of a carbonyl group and a single ethylenic linkage, it became apparent that glutinone must be classified as a pentacyclic triterpenoid. This was readily established[38] when glutinene rearranged to the equilibrium mixture of olean-13(18)-ene (XXXIII) and 18α-olean-12-ene (XXXIV) on treatment of the hydrocarbon with mineral acid. This same hydrocarbon mixture is also the product of the mineral acid rearrangement of olean-12-ene, olean-13(18)-ene, olean-18-ene (germanicene) and friedel-3-ene.[41]



XXXII



XXXIII

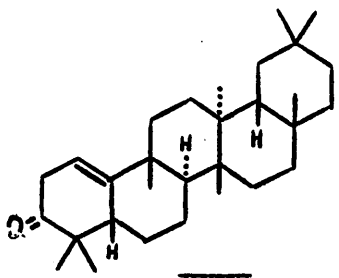


XXXIV

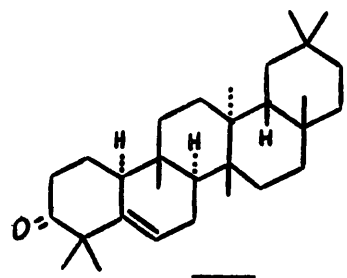
Beaton et al.[38] now turned their attention to the location of the double bond in glutinone. Although the ultraviolet absorption spectrum[37] showed that the double bond was not in conjugation with the carbonyl group, the above authors deduced from a consideration of the ethylenic absorption curves of glutinone and glutinyl acetate that the two functional groups must be in juxtaposition. The tri-substituted nature of the double bond was suggested by the intensity of the absorption maximum of the acetate[37] and by a band at 788 cm.$^{-1}$ in its infrared spectrum.[38] This was confirmed when a triol diacetate was formed on treatment of glutinyl acetate with osmium tetroxide and subsequent acetylation.[38] The stability of the triol diacetate to the chromic-acetic acid reagent at room temperature indicated the tertiary nature of the non-acylable hydroxyl group.

On reviewing the evidence gathered by them, it became apparent to Beaton et al.[38] that only the structures (XXXV) and (XXIX) were compatible with the facts so far established and the former structure was excluded on the basis of the following results. Neither acid nor base treatment of glutinone afforded an αβ-unsaturated ketone as would have been expected in the case of (XXXV). The formation of the non-conjugated enol-acetate (XXXVI) supports the view that the structure (XXIX)

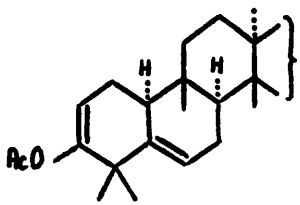
represents glutinone and this was further confirmed when a non-conjugated diene (XXXVII) was obtained on pyrolysis of glutinyl benzoate. Since the hydroxyl group in glutinol is



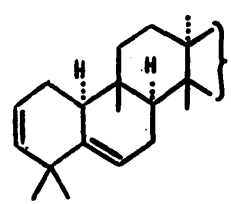
XXXV



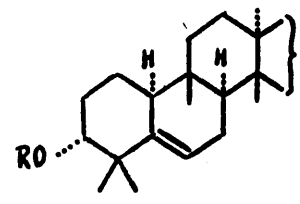
XXIX



XXXVI



XXXVII

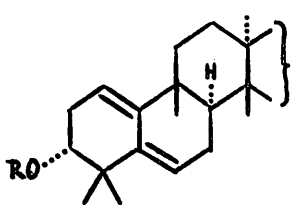


XXXVIII

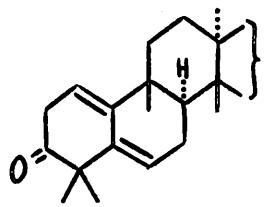
equatorial, it must have the α-configuration in this type of structure and leads to the formulation of the alcohol as glutin-5-en-3α-ol (XXXVIII, R = H).

Confirmation of the structure of glutinone came from a study of the reactions of glutina-1(10):5-dien-3α-yl acetate (XXXIX, R = Ac), the dienyl acetate derived from glutinyl acetate

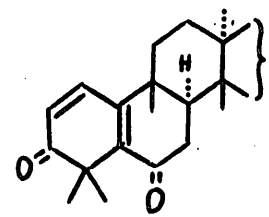
by selenium dioxide oxidation.[38,39] Hydrolysis of the dienyl acetate with lithium aluminium hydride gave glutina-1(10):5-dien-3α-ol (XXXIX, R = H) which was oxidised with the chromium trioxide-pyridine complex in the expectation of forming the corresponding dienone (XL). The product, however, was a fully conjugated dioxo-diene which exhibited absorption bands in the ultraviolet at 2180 and 3190 Å. and was formulated as glutina-1:5(10)-dien-3:6-dione (XLI).



XXXIX



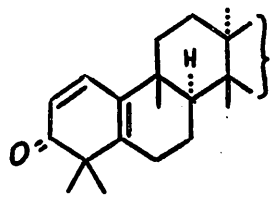
XL



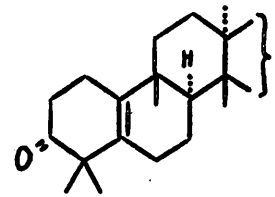
XLI

In an attempt to prepare a $C_{(3)}$-oxygenated oleanane derivative, glutinone was treated with mineral acid[38] under the same conditions which successfully isomerised the hydrocarbon glutin-5-ene to the equilibrium mixture of olean-13(18)-ene and 18α-olean-12-ene, but no homogeneous material could be isolated. When milder reaction conditions were employed an isomeric ketone, $C_{30}H_{48}O$, m.p. 251-253°, was formed. Chapon[39] reported the formation of this isomer, called 'ketone-II', on treatment of glutinone with a mixture of sulphuric and acetic

acids. 'Ketone-II' was also the product of an attempted Clemmensen reduction of glutinone.[39] An examination of the infrared spectrum[39] of the derived hydrocarbon, 'glutinene-II', revealed that the double bond was fully substituted and similar studies by Beaton et al.[38] on the 'acetate-II' confirmed this view. The structure of this isomer was established by the latter authors who obtained a fully conjugated dienone on successive treatment of 'ketone-II' with bromine and potassium acetate. Since the conjugated dienone could be represented only by the structure (XLII), it followed that 'ketone-II' must be glutin-5(10)-en-3-one (XLIII).



XLII

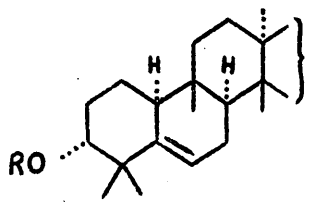


XLIII

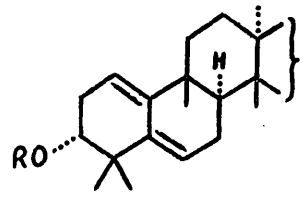
The Epimeric Glutin-5(10)-en-3-ols.

Although previous workers[38,39] were able to prepare the corresponding 'alcohol-II' and 'acetate-II', no attempt was made to assign a configuration to the oxygen function at $C_{(3)}$ in these compounds. On reviewing this work, it became evident to the author that an equatorial attachment of the

hydroxyl group to ring A could safely be assumed as the same glutin-5(10)-en-3-ol was obtained on reduction of glutin-5(10)-en-3-one (XLIII) with either sodium and alcohol[38] or lithium aluminium hydride.[38,39] Reference to the known equatorial alcohol, glutin-5-en-3α-ol (XXXVIII, R = H), would imply a similar 3α-configuration for the hydroxyl group in 'alcohol-II' but this implication was contradicted by the behaviour of 'glutinyl-II acetate' on oxidation with selenium dioxide. The product of this reaction, 'glutinadienyl-II acetate', had absorption maxima in the ultraviolet region at 2320, 2380 and 2470 Å., characteristic of the heteroannular 1(10):5-diene system, but the melting point and specific rotation of this compound were quite different from those of the known glutina-1(10):5-dien-3α-yl acetate (XXXIX, R = Ac). That both dienyl



XXXVIII

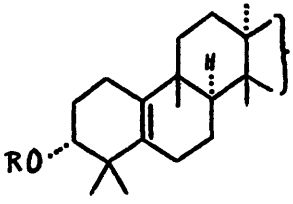


XXXIX

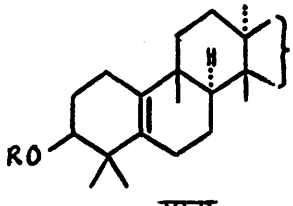
acetates contain the same unsaturated system was fully evidenced by the striking similarity of their ultraviolet absorption spectra, and this led inevitably to the conclusion that the difference

in properties observed between them can be attributed only to their $C_{(3)}$-acetate groups having distinct and separate configurations. From this, it is obvious that a configurational difference must also exist in the $C_{(3)}$-acetate groups of their parent compounds, 'glutinyl-II acetate ' and glutin-5-en-3α-yl acetate, albeit both had been shown to possess equatorial acetate groups. Accordingly, 'glutinyl-II acetate' must be formulated as glutin-5(10)-en-3β-yl acetate (XLV, R = Ac). The following explanation was devised to satisfy this apparently anomalous situation and was subsequently proved correct.[42]

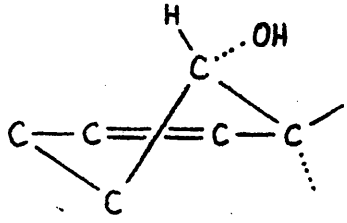
In the epimeric glutin-5(10)-en-3α- (XLIV, R = H) and -3β-ol (XLV, R = H), the double bond forces carbon atoms $C_{(1)}$, $C_{(4)}$, $C_{(5)}$ and $C_{(10)}$ to be coplanar, or nearly so, and depending upon the position of $C_{(3)}$ relative to this plane, ring A will adopt one or the other of the two possible half-chair conformations represented by the structures (XLIVa) and (XLVa).



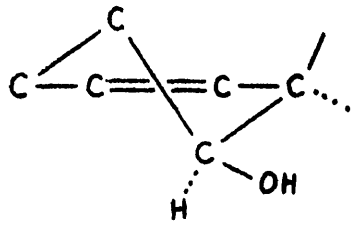
XLIV



XLV

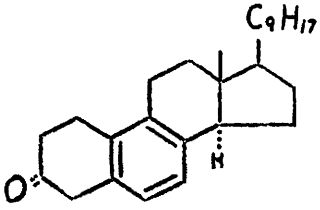


XLIVa

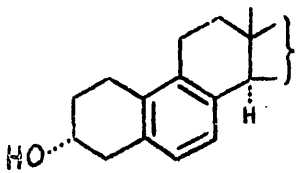


XLVa

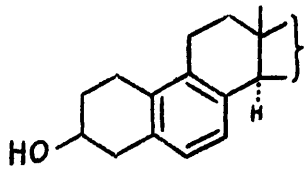
As shown schematically, the hydroxyl group is α-orientated in the former and β-orientated in the latter conformation while retaining an equatorial attachment in either structure. The steric situation is clearly analogous to that of the epimeric neoergosterols[43] where coplanarity of the carbon atoms $C_{(1)}$, $C_{(4)}$, $C_{(5)}$ and $C_{(10)}$ is induced by the aromatic nature of ring B. Reduction of neoergosterone (XLVI) led to a mixture of the 3α- and 3β- alcohols, (XLVII) and (XLVIII), both of which were shown to possess equatorial hydroxyl groups.



XLVI



XLVII

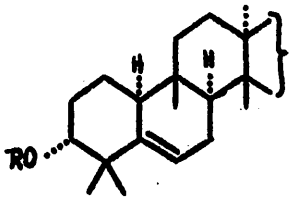


XLVIII

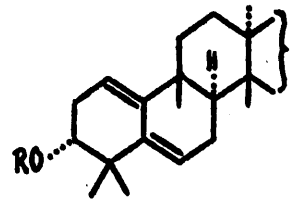
A study of molecular models of the two half-chair conformations indicated that each had the same number of 1:3-diaxial interactions and consequently, each should possess similar conformational stabilities. In view of this, the

reduction of glutin-5(10)-en-3-one with lithium aluminium hydride was repeated and, after acetylation of the product, there was again obtained the glutin-5(10)-enyl acetate, m.p. 297-299°, $[\alpha]_D$ - 23°, described by Beaton *et al.*[38] Close examination of the mother liquors revealed the presence of an isomeric acetate, m.p. 209-210°, $[\alpha]_D$ - 49°, which was isolated as a minor product of the reaction. Since hydrolysis of these acetates followed by oxidation, regenerated glutin-5(10)-en-3-one, they are the epimeric glutin-5(10)-en-3-yl acetates.

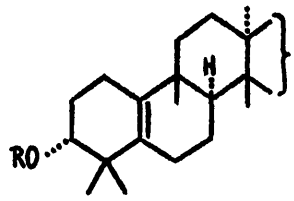
The identity of the acetate, m.p. 209-210°, as glutin-5(10)-en-3α-yl acetate (XLIV, R = Ac) followed from its relationship with glutin-5-en-3α-yl acetate (XXXVIII, R = Ac). Oxidation of the latter compound with selenium dioxide in acetic acid gives the conjugated dienyl acetate (XXXIX, R = Ac) which likewise was formed on similar treatment of the acetate, m.p. 209-210°. As this reaction does not affect the configuration of the acetate group, it could readily be concluded that this material is glutin-5(10)-en-3α-yl acetate (XLIV, R = Ac). Consequently, the other isomer, m.p. 297-299°, 'glutinyl-II acetate', must be glutin-5(10)-en-3β-yl acetate (XLV, R = Ac). The synthesis of each of these epimers by alternative, unambiguous routes, as described below, provided further confirmation of their structures.



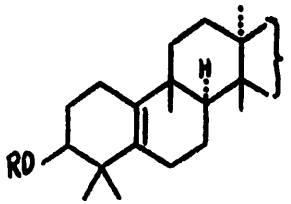
XXXVIII



XXXIX



XLIV



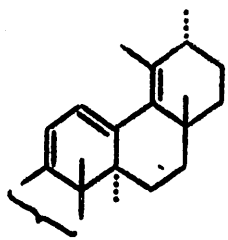
XLV

Glutin-5(10)-en-3α-yl Acetate.

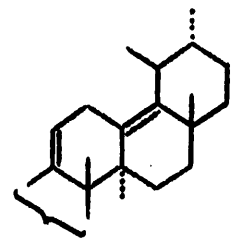
Catalytic hydrogenation of glutina-1(10):5-dien-3α-yl acetate (XXXIX, R = Ac) in glacial acetic acid yielded the fully saturated dihyroglutinyl acetate, but when a mixed solvent medium was used, only partial reduction occurred,[38,40] the product being glutin-5-en-3α-yl acetate (XXXVIII, R = Ac). In order to obtain the required glutin-5(10)-en-3α-yl acetate (XLIV, R = Ac), a method of reduction was necessary in which a 1:4-addition across the diene system would take place.

Reduction of the conjugated trienyl system in ursa-9(11):12:18-trien-3β-yl acetate (XLIX) with lithium in liquid

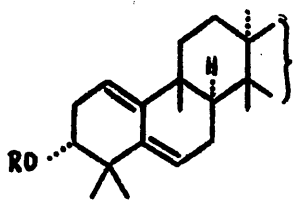
ammonia[44] resulted in such an addition across the latter part of the triene to give ursa-9(11):13(18)-dien-3β-yl acetate (L). This method of reduction was applied to glutina-1(10):5-dien-3α-yl acetate (XXXIX, R = Ac) and, after acetylation and chromatography of the product, glutin-5(10)-en-3α-yl acetate (XLIV, R = Ac) was obtained in approximately 10% yield. Under the basic conditions of the reduction, the dienyl acetate is



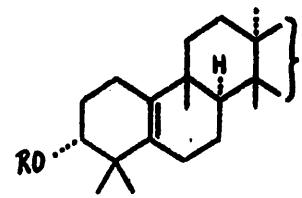
XLIX



L



XXXIX



XLIV

hydrolysed to the corresponding alcohol and it was believed that the poor yield of product was due to rearrangements caused by the strong base. To overcome this difficulty, the tetrahydro-

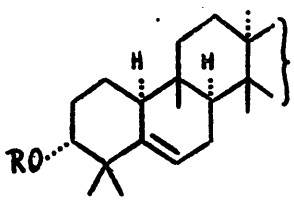
pyranyl ether of glutina-1(10):5-dien-3α-ol (XXXIX, R = H) was prepared according to the method of Sondheimer, Velasco and Rosenkranz.[45] This ether linkage is known to be stable under alkaline conditions and has been used successfully in the steroid series for the protection of hydroxyl groups.[46] Although a new centre of asymmetry was introduced at the point of attachment to the pyran ring, no attempt was made to separate the mixture of stereo-isomers thereby formed as subsequent cleavage of the ether bond in the reduced product would have made this unnecessary.

3α-2'-Tetrahydropyranyloxy-glutina-1(10):5-diene showed maximal absorption at 2340, 2400 and 2480 Å. and when reduced with lithium in liquid ammonia followed by acid hydrolysis and subsequent chromatography of the product, glutin-5(10)-en-3α-ol (XLIV, R = H) was obtained in approximately 40% yield. This alcohol was also the only isolable product of an attempted equilibration of glutina-1(10):5-dien-3α-ol, using potassium tert-butoxide in tert-butanol.

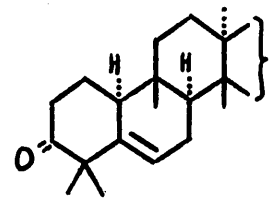
Glutin-5(10)-en-3β-yl Acetate.

The configuration of glutin-5(10)-en-3β-yl acetate ('glutinyl-II acetate') was established by a different route. Reduction of the $C_{(3)}$-ketone groups of triterpenoids with aluminium isopropoxide in isopropanol is known to give a mixture of epimers,[47] separable by chromatography on alumina. Glutin-5-en-3-one (XXIX) was reduced in this fashion and the

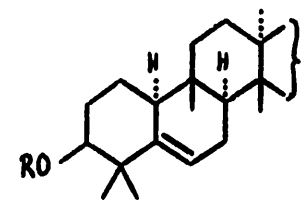
product in light petroleum was chromatographed on alumina. Elution with light petroleum - benzene yielded two homogeneous alcohols, the more strongly adsorbed alcohol being recognised as glutin-5-en-3α-ol (XXXVIII, R = H). The less strongly adsorbed component is an isomeric alcohol, $C_{30}H_{50}O$, m.p. 210.5-211.5°, $[\alpha]_D$ + 64°, which was characterised as its acetate, m.p. 192-194°, $[\alpha]_D$ + 79°.



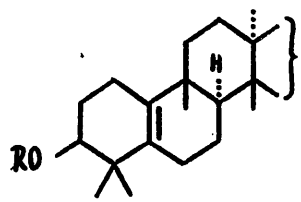
XXXVIII



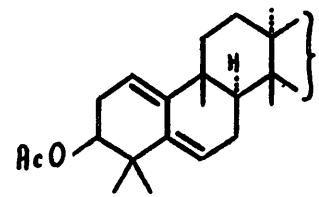
XXIX



LI

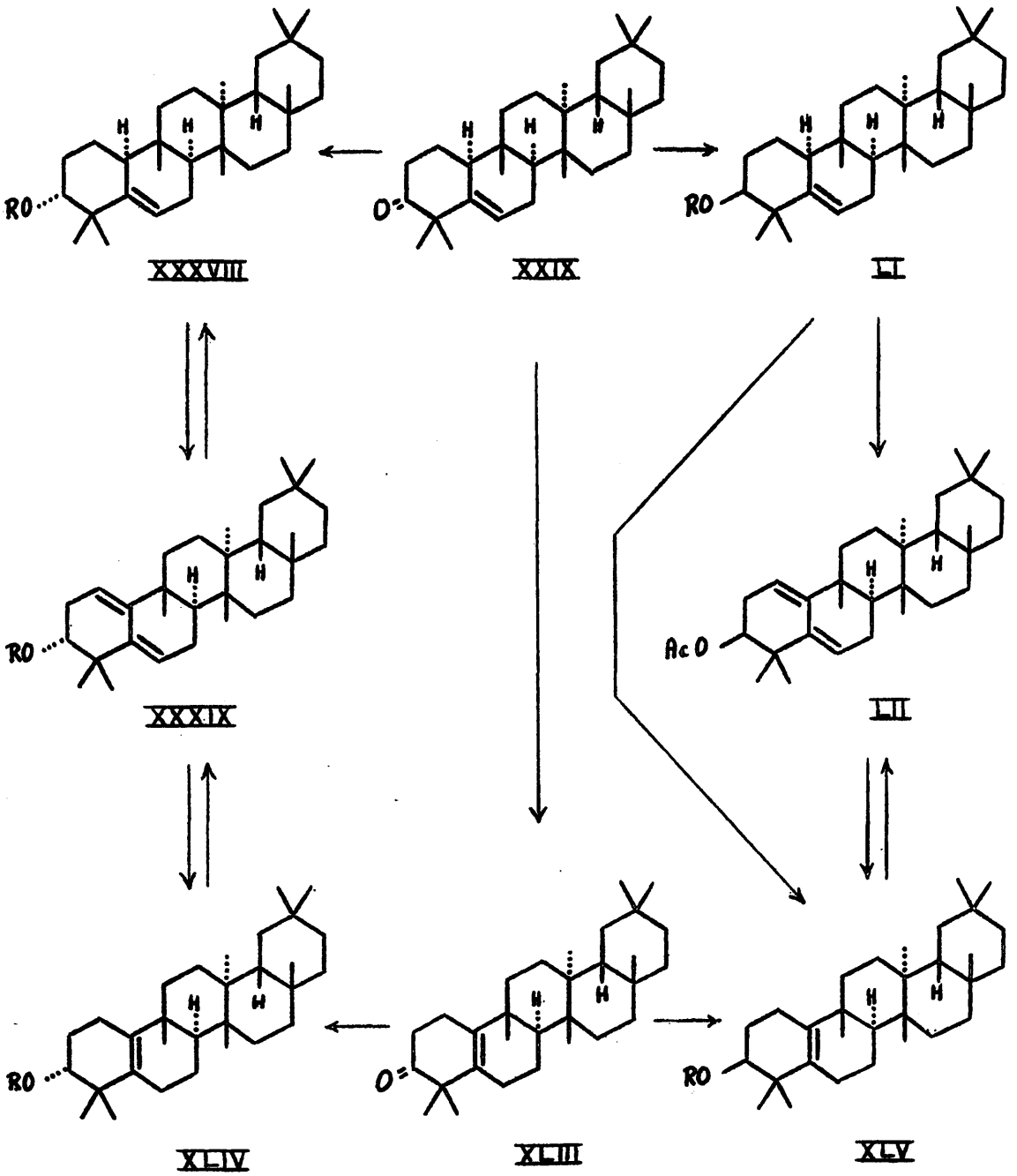


XLV



LII

Since this alcohol regenerates glutin-5-en-3-one on oxidation with the chromium trioxide - pyridine complex, it is identified as glutin-5-en-3β-ol (epiglutinol) (LI, R = H). It was related to 'glutinyl-II acetate' by oxidation with



XXXVIII XXIX LI

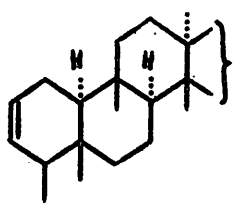
XXXIX LII

XLIV XLIII XLV

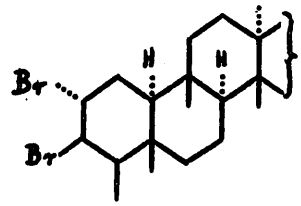
selenium dioxide. The product, glutina-1(10):5-dien-3β-yl acetate (LII), was identical with the dienyl acetate derived from 'glutinyl-II acetate' on similar oxidation. Further evidence adduced from the fact that when dry hydrogen chloride was passed through a solution of glutin-5-en-3β-yl acetate (LI, R = Ac) in chloroform at 0°, it was smoothly isomerised to glutin-5(10)-en-3β-yl acetate (XLV, R = Ac), the melting point of which was not depressed on admixture with 'glutinyl-II acetate'. The final structures and relationships established between the two series are summarised in the accompanying illustration.

Glutina-1(10):5-diene.

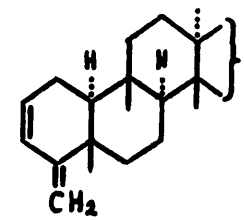
During the course of the above work, the preparation of a stable dibromide (LIV) from friedel-2-ene (LIII) was reported[48] and on dehydrobromination with alkali, this yielded a conjugated diene, λ max. 2410 Å (ε, 19,950), which was formulated as friedela-2:4-diene (LV). Using the usual empirical rules,[49] the position calculated for the absorption maximum of this diene system occurs at 2290 Å. The difference of 120 Å between the observed and calculated values seemed too great to be reconcilable with even this approximate method of calculation. This fact, and also the observation[38,48] that dehydrobromination of 4-bromo-friedelin (LVI) had led to a



LIII

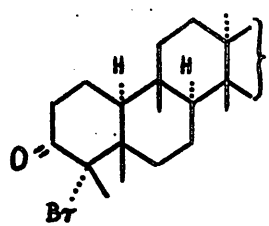


LIV

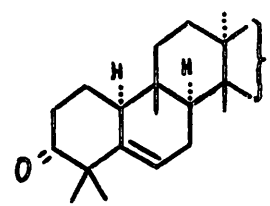


LV

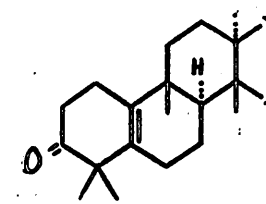
mixture of glutin-5- and -5(10)-en-3-ones (XXIX and XLIII), a reaction involving shift of the $C_{(5)}$-methyl group of the friedelin derivative to $C_{(4)}$ of the glutinone derivative, suggested the possibility of a similar rearrangement having taken place during the dehydrobromination of the dibromide (LIV). The product, in this case, would be glutina-1(10):5-diene (LVIII) and the value (λ max. 2440 Å.) calculated for the ultraviolet absorption maximum of this structure compares



LVI



XXIX

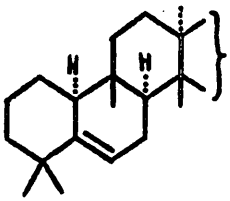


XLIII

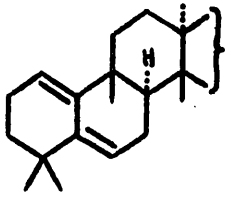
more favourably with the value (λmax. 2410 Å.) observed by Corey and Ursprung.[48]

In view of this, glutin-5-ene (LVII), prepared by Wolff-Kishner reduction of glutinone, was treated with selenium

dioxide in acetic acid to yield glutina-1(10):5-diene (LVIII). The ultraviolet absorption of this compound showed a principal maximum at 2400 Å. but the melting point and specific rotation differed considerably from those reported for friedela-2:4-diene (LV).



LVII

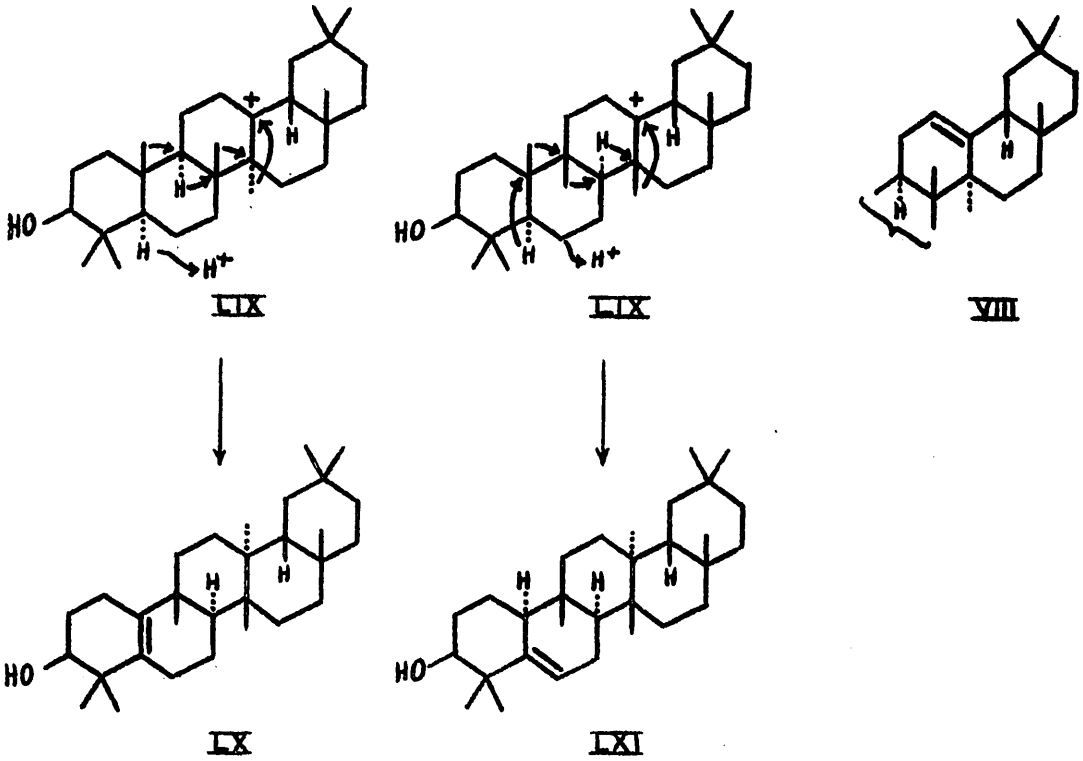


LVIII

The Natural Occurrence of Glutin-5-en-3β-ol.

It is a generally accepted view[27] that in the biogenesis of the triterpenoids, the cyclisation of squalene is initiated by the approach of an OH^+ cation to $C_{(3)}$ of the folded chair form of the molecule. One of the intermediates of the cyclisation and rearrangement is the carbonium ion (LIX) which is the immediate precursor of β-amyrin (VIII). By a series of 1:2-shifts of the axial methyl groups and hydrogen atoms attached to $C_{(14)}$, $C_{(8)}$, $C_{(9)}$, and $C_{(10)}$ followed by loss of a proton from $C_{(5)}$, or by a further shift of the $C_{(5)}$-hydrogen atom and elimination of a proton from $C_{(6)}$, there obtains the compounds (LX) and (LXI) which represent glutin-5(10)-en-3β-ol

and glutin-5-en-3β-ol (epiglutinol), respectively.



Thus, it is apparent that any naturally occurring alcohol of the glutinone series would have a β-orientation of the hydroxyl group attached to $C_{(3)}$ if this biogenetic pathway were followed. Confirmation came from an examination of the alcoholic fractions extracted from the bark of the black alder (Alnus glutinosa L.). Chromatography yielded a product which was in no way distinguishable from synthetically prepared glutin-5-en-3β-ol (LXI) (see p.26) and with which it was

identified through mixed melting point and infrared spectroscopic studies.

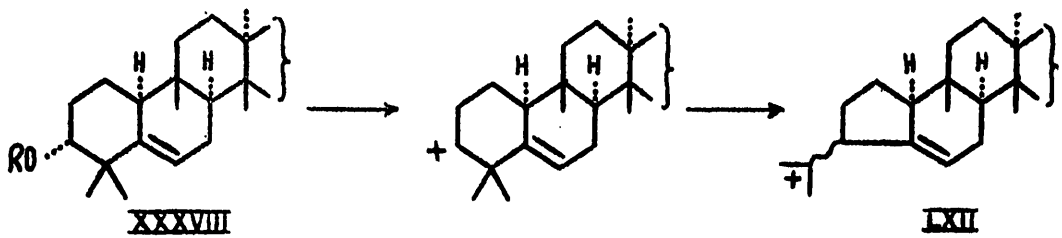
Acid-induced Isomerisations of the Glutinyl Acetates.

All attempts[38] to isomerise glutinone derivatives having a $C_{(3)}$-oxygen function, to the corresponding oxygenated oleanane derivatives have so far proved unsuccessful. Nevertheless, it was with this objective that the following experiments were carried out.

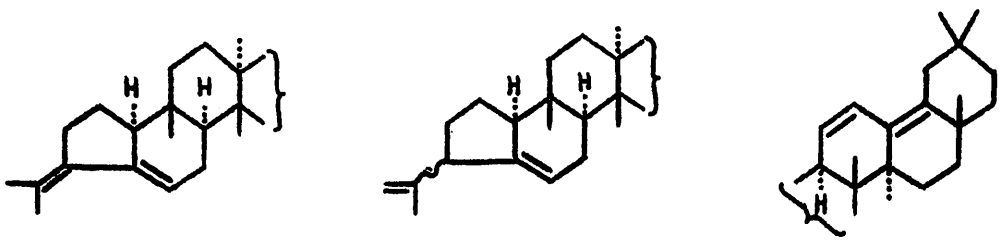
Mild acid treatment of glutin-5-en-3α-yl acetate (XXXVIII, R = Ac) under the conditions that readily isomerised glutin-5-en-3β-yl acetate (LI, R = Ac) to glutin-5(10)-en-3β-yl acetate (XLV, R = Ac), resulted only in the recovery of unchanged starting material. When somewhat stronger acid conditions were used, there was obtained an intractable gum, the ultraviolet absorption spectrum of which indicated the presence of a conjugated diene system, with well-defined maxima at 2410, 2500 and 2580 Å. The hydrocarbon nature of this material was suggested by its behaviour on chromatography, the major portion being eluted in the first fraction with light petroleum.

Formation of the hydrocarbon can be postulated as occurring through ionic elimination of the $C_{(3)}$-acetate group, followed by ring contraction to give the carbonium ion (LXII). Rearrangement and proton elimination could lead to several conjugated and non-conjugated dienes, of which two are shown:

(LXIII) and (LXIV). However, the ultraviolet absorption spectrum closely resembles that of oleana-11:13(18)-dienyl acetate (LXV), λ max. 2420, 2500 and 2600 Å, and suggests that an isomerisation, similar to the rearrangement of glutin-5-ene to the equilibrium mixture of olean-13(18)-ene and 18α-olean-12-ene,[88] has taken place, leading to a ring A contracted structure which includes an 11:13(18)-diene chromophore.



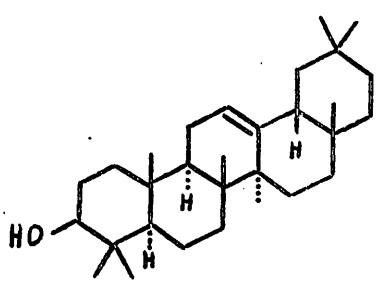
XXXVIII LXII



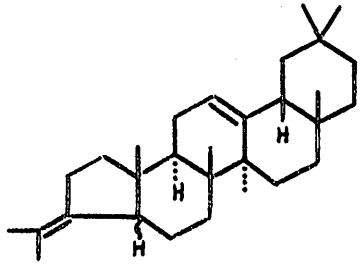
LXIII LXIV LXV

The movement of the ring B double bond in (LXIII) and (LXIV) to ring C can easily be envisaged by 1:2-shifts of the angular methyl groups and hydrogen atoms along the backbone of the molecule, but difficulty arises when the exo- and acyclic double bonds in these compounds are considered.

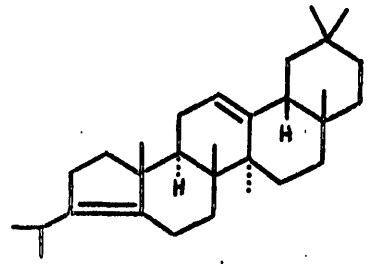
Dehydration of β-amyrin (VIII) with phosphorus pentachloride[50-54] yields a non-conjugated diene, 'β-amyrilene-I' (LXVI), which can be converted into its isomer (LXVII) on acid treatment.[55] The non-rearrangement[54,55] of the latter compound to an



VIII



LXVI

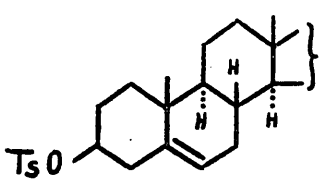


LXVII

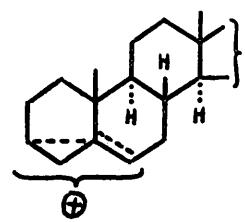
11:13(18)-diene precludes the possibility of (LXIII) or (LXIV) being formed initially during the acid-catalysed rearrangement of glutin-5-en-3α-yl acetate, unless a synchronous movement of both double bonds along the molecular back-bone is postulated. Such a movement, however, would result in a double displacement of the angular methyl groups and hydrogen atoms and the likelihood of this occurring is, in the author's opinion, doubtful.

The necessity for postulating initial formation of the structures (LXIII) and (LXIV), or their carbonium ion derivatives, can be obviated if an analogy is drawn between this rearrangement and one that has been extensively studied in the sterol series.[56-70]

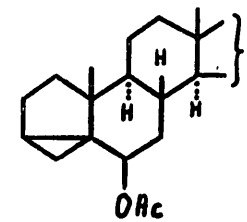
Under mildly acidic conditions, 3β-cholesterol derivatives are known to undergo a solvolytic rearrangement whereby the $C_{(3)}$-functional group is transferred to $C_{(6)}$ with complete retention of configuration, concurrent with the formation of a cyclopropane bridge between $C_{(3)}$ and $C_{(6)}$. Thus, cholesteryl tosylate (LXVIII) when treated with potassium acetate and acetic anhydride[58,59] yields i-cholesteryl acetate (LXX). Current views[67-69] favour the carbonium ion (LXIX), produced through ionic elimination of the $C_{(3)}$-functional group, as participating in this rearrangement. Usually, an alkaline



LXVIII



LXIX



LXX

metal acetate is employed in this reaction to prevent the development of high acidity as the i-cholesterol product will revert to the original cholesterol derivative under more strongly acidic conditions.[64] In the acid-catalysed rearrangement of glutinyl acetate, the acidity can develop to its full potential and the author suggests that, instead of reverting to glutinyl acetate, the structure (LXXI) undergoes the following

rearrangement in an effort to relieve the steric strain induced by the cis-locking of rings D and E. Protonation of the cyclopropane bridge with ring opening yields the carbonium ion (LXXII) and the ensuing movement of the positive centre along the molecular back-bone by the usual mechanism of 1:2-shifts of the axial groups and loss of a proton from $C_{(11)}$ leads to (LXXIII). Elimination of the $C_{(6)}$-acetate group, followed by a 1:3-shift, or two 1:2-shifts of the neighbouring hydrogen atoms, and 1:2-shifts of the axial methyl groups at $C_{(14)}$ and $C_{(13)}$ yields the carbonium ion (LXXIV). If, as Courtney, Gascoigne and Szumer[71] suggest, loss of a proton occurs synchronously and in an anti-parallel direction to the movement of the $C_{(13)}$-methyl group, then only the 12α-hydrogen atom satisfies this geometric requirement and accordingly, proton elimination will yield 8:10:14-trimethyl-5α-novoleana-9(11):12-diene (LXXV)[¤]. Under the influence of strong acid, this would rearrange to the corresponding 11:13(18)-dienyl derivative (LXXVI), analogous to the conversion[80] of oleana-9(11):12-dienyl acetate (LXXVII) to oleana-11:13(18)-dienyl acetate (LXV).

The biogenetic pathway outlined previously (p.30), predicts the natural occurrence of 3β-hydroxylated glutinone

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¤ The nomenclature of this hydrocarbon is based on that proposed by Allan et al (see ref. 54)

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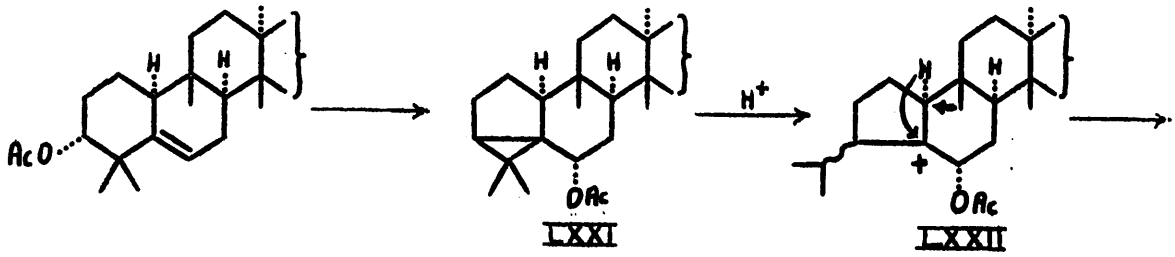
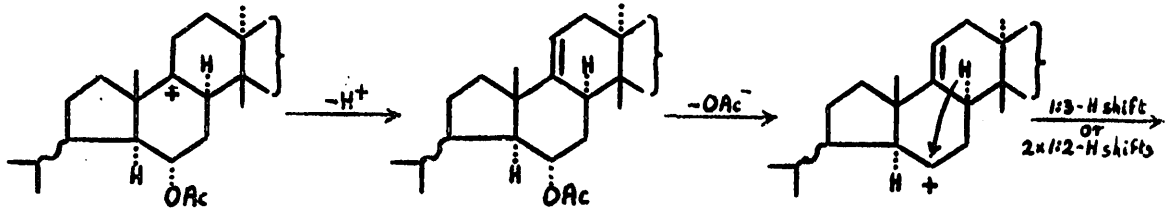
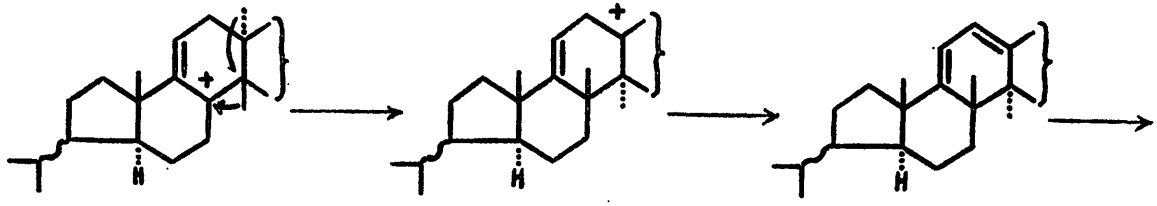


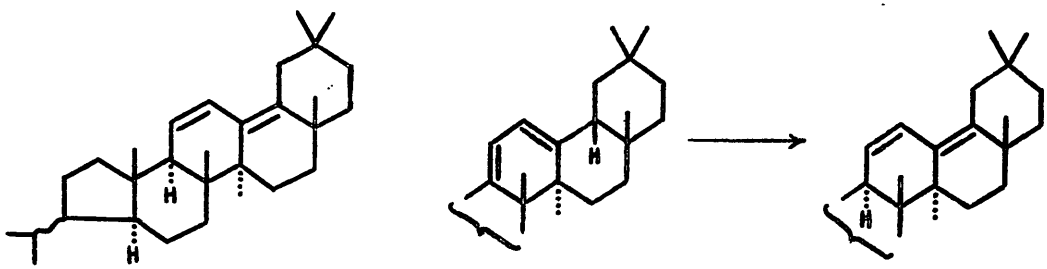
LXXI LXXII



LXXIII



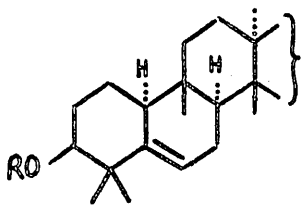
LXXIV LXXV



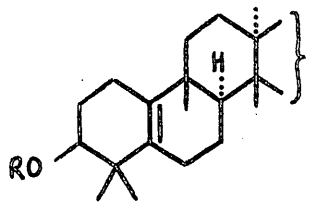
LXXVI LXXVII LXV

derivatives and is supported by the isolation of epiglutinol from Alnus glutinosa. It is apparent, therefore, that if this natural process is to be reversed, then the more logical starting point would be with the glutin-5- or -5(10)-en-3β-ol derivatives. This conclusion is favoured by the ease with which glutin-5-en-3β-yl acetate (LI, R = Ac) is isomerised to glutin-5(10)-en-3β-yl acetate (XLV, R = Ac) under mild acid conditions whereas only unchanged starting material is recovered from similar treatment of glutin-5-en-3α-yl acetate (XXXVIII, R = Ac).

Glutin-5(10)-en-3β-yl acetate (XLV, R = Ac), however, proved to be stable towards strong mineral acid, being recovered unchanged after prolonged treatment at 100°.



LI



XLV

That the $C_{(3)}$-acetate group exerts some influence over the ethylenic double bond is shown clearly by the ready isomerisation of glutin-5- and -5(10)-ene to oleanane derivatives[38] whilst under the same conditions, the corresponding glutinyl acetates either remain unchanged or only rearrange following ionic elimination of the acetate group. The ultraviolet absorption

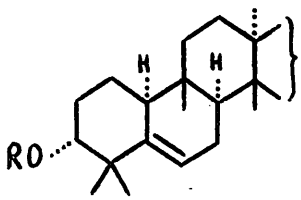
spectra of glutinone and glutinyl acetate had previously suggested that some such interaction exists, as Beaton et al.[38] deduced from this data the proximity of the double bond and the $C_{(3)}$-oxygen function. This influence is discussed further in a later section (p. 57).

The Stereochemistry of Glutinane Derivatives.

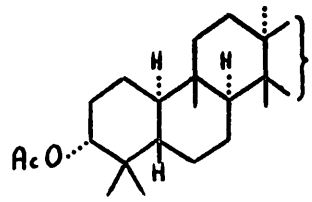
The double bond of glutin-5-en-3α-yl acetate (XXXVIII, R = Ac) is readily reduced catalytically[37,40] to give dihydroglutinyl acetate, from which the corresponding saturated alcohol and ketone have been prepared. Chapon[40] reported that catalytic reduction of glutina-1(10):5-dien-3α-yl acetate yielded a saturated acetate different from that derived from glutinyl acetate but, on repetition of this work, Beaton et al.[38] showed that the two saturated acetates were identical although they could make no assignment of configuration to the $C_{(5)}$-hydrogen atom in the reduced product.

Addition of hydrogen to the double bond in glutin-5-en-3α-yl acetate can be accomplished in two ways. If attack is from the front of the molecule, the product will be 5β-glutinan-3α-yl acetate (LXXVIII). On the other hand, if the double bond is attacked from the rear, 5α-glutinan-3α-yl acetate (LXXIX) will be formed. Both structures possess all-chair conformations but the former, having a trans- anti- trans locking of rings A, B and C, allows the 3α-acetoxy group to retain its equatorial

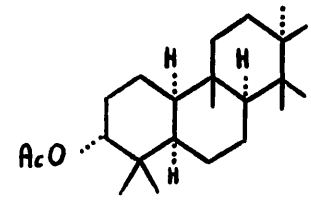
configuration whereas the cis-locking of rings A and B in the latter structure forces the acetate group into an axial



XXXVIII

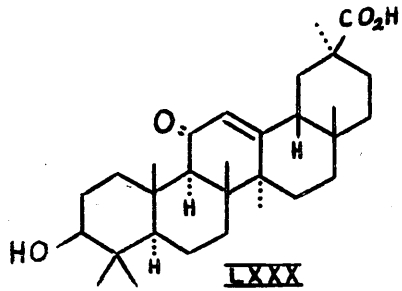


LXXVIII

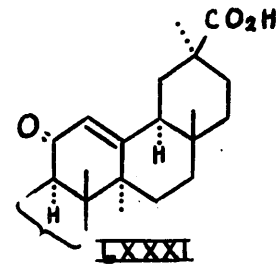


LXXIX

configuration. This situation is analogous to that of the carboxyl group in glycyrrhetic acid (LXXX) and 18α-glycyrrhetic acid (LXXXI).[72] The possibility of the acetate group of 5α-glutinan-3α-yl acetate (LXXIX) attaining an equatorial



LXXX



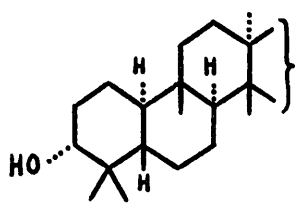
LXXXI

configuration by constraining ring B into a boat shape was considered but dismissed on the grounds of the thermodynamic instability of the boat conformation. This assumption and also the knowledge that on chromatography of a mixture of $C_{(3)}$-epimeric alcohols, the more hindered axial

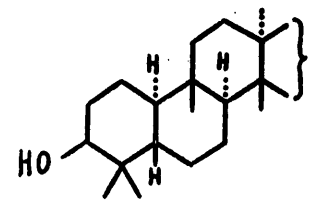
alcohol will be eluted before the more strongly adsorbed equatorial one, enabled the author to show that the addition of hydrogen to the double bond of glutin-5-en-3α-yl acetate (XXXVIII, R = Ac) was unilateral and also to assign a β-configuration to the $C_{(5)}$-hydrogen atom of the product.

Catalytic hydrogenation of glutinyl acetate again yielded dihydroglutinyl acetate which after hydrogenolysis with lithium aluminium hydride, was subjected to careful chromatography. No difference in melting point and specific rotation was observed between the first and the last fractions nor was there a depression of melting point on admixture of the two, and accordingly, the addition of hydrogen to the double bond must have taken place unilaterally. Chromic acid oxidation of dihydroglutinol gave dihydroglutinone, the homogeneity of which was demonstrated by chromatography. The saturated ketone was reduced with aluminium isopropoxide in isopropanol to a mixture of its $C_{(3)}$-epimers which were separated by chromatography on alumina. The more strongly adsorbed component was recognised as dihydroglutinol and, since it is the equatorial epimer, it must therefore be 5β-glutinan-3α-ol (LXXXII). The less strongly adsorbed component is an isomeric alcohol, $C_{30}H_{52}O$, m.p. 266-268°, $[\alpha]_D$ + 33°, which was characterised as its acetate, m.p. 230-232°, $[\alpha]_D$ + 52°. This alcohol regenerates dihydroglutinone

on oxidation with the chromium trioxide-pyridine complex and is accordingly identified as dihydroepiglutinol (5β-glutinan-3β-ol) (LXXXIII).



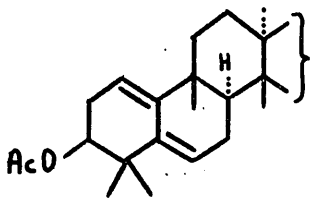
LXXXII



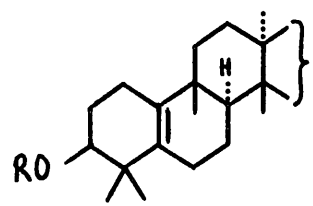
LXXXIII

Several attempts were made to prepare 5α-glutinane derivatives by catalytic reduction of those glutinone derivatives possessing a 3β-acetate group. It was reasoned that the steric hindrance caused by the β-orientated methyl groups at $C_{(4)}$, $C_{(9)}$, and $C_{(14)}$ and also the acetate group at $C_{(3)}$, would prevent formation of the normal 5β-glutinane derivatives by forcing the hydrogen to attack the molecule from the rear to give the corresponding 5α-glutinane derivatives. When a solution of glutina-1(10):5-dien-3β-yl acetate (LII) in acetic acid was shaken with hydrogen over platinum catalyst, a crystalline precipitate appeared within a few minutes and the mixture had to be warmed before complete solution was again obtained. After nineteen hours, the product was isolated and separated by means of crystallisation into a material, m.p. 210-220°, which showed no selective absorption in the ultraviolet above 2000 Å,

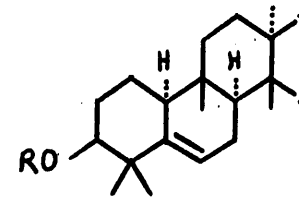
and an unsaturated compound which was identified as glutin-5(10)-en-3β-yl acetate (XLV, R = Ac). The latter substance which Chapon[40] stated to be inert to catalytic reduction, is much less soluble than the dienyl acetate and is probably formed within the first few minutes of the reaction, as evidenced by the separation of the crystalline precipitate. Glutin-5(10)-en-3β-yl acetate may well be the product of a 1:4-addition of hydrogen across the diene system but it is more



LII



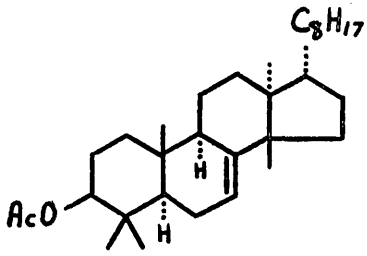
XLV



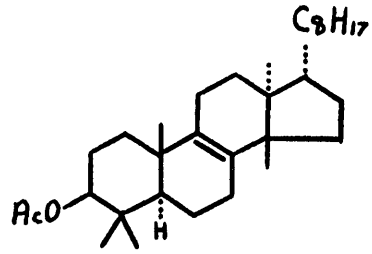
LI

probable that the 1(10)-double bond of the dienyl acetate is reduced first and the resulting glutin-5-en-3β-yl acetate (LI, R = Ac) then isomerises under the acid conditions to glutin-5(10)-en-3β-yl acetate. This is borne out by the observation[38] that partial catalytic reduction of glutina-1(10):5-dien-3α-yl acetate yields glutin-5-en-3α-yl acetate while complete reduction gives 5β-glutinan-3α-yl acetate (LXXVIII). Further support for this belief was supplied by treatment of glutin-5-en-3β-yl acetate with hydrogen and

platinum catalyst which gave a mixture of glutin-5(10)-en-3β-yl acetate and the same saturated material as was obtained previously. An analogy can be drawn between this reaction and the isomerisation of 9α-euph-7-enyl acetate (LXXXIV) to euph-8-enyl acetate (LXXXV) under similar conditions.[73,74] Attempted hydrogenation of glutin-5(10)-en-3β-yl acetate in acetic acid yielded a mixture



LXXXIV



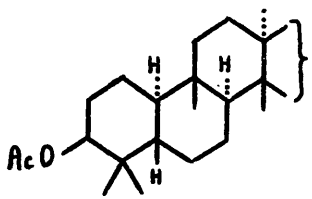
LXXXV

of unchanged starting material and the saturated substance which was now examined in more detail.

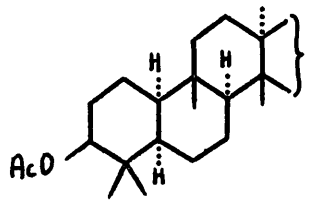
This material, which apparently is obtained only by hydrogenation of those glutinone derivatives possessing 3β-acetate groups, was fully saturated, showing no absorption in the ultraviolet above 2000 Å. and giving no colour with tetranitromethane. The melting point range of the substance suggested that it was a mixture but neither repeated recrystallisation nor chromatography could accomplish a separation. However, hydrolysis of the material followed by careful chroma-

tography of the product, yielded a small amount of 5β-glutinan-3β-ol (LXXXIII). The remainder was an inseparable mixture.

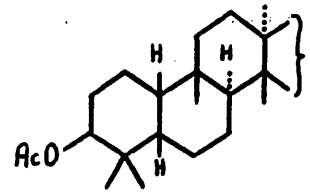
Since glutin-5(10)-en-3β-yl acetate can be isolated from the hydrogenation of both glutin-5-en-3β-yl acetate and glutina-1(10):5-dien-3β-yl acetate, it is reasonable to assume that this compound is an isolable intermediate in these reductions and that it is the addition of hydrogen to the 5(10)-double bond which results in the formation of the saturated material. It is probable then, that the mixture consists of the three possible stereo-isomers (LXXXVI to LXXXVIII) which can arise by varying modes of cis-addition of hydrogen to the 5(6)- and 5(10)- double bonds.



LXXXVI



LXXXVII



LXXXVIII

To prevent rearrangement of glutin-5-en-3β-yl acetate to its 5(10)-isomer, and hence possible formation of the 10β-glutinane derivative on reduction, the hydrogenation was attempted in neutral media, but only unchanged starting material

was recovered even when increased pressure was employed.

Although no 5α-glutinane derivatives have been isolated, the steric hindrance caused by the β-orientated groups at $C_{(3)}$, $C_{(4)}$, $C_{(9)}$ and $C_{(14)}$ has been amply demonstrated in the above experiments.

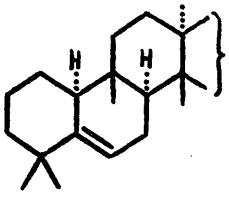
The Structure of 'Glutinone-III'.

The isomerisation of glutinone to glutin-5(10)-en-3-one on treatment with either sulphuric and acetic acids,[39] or hydrochloric and acetic acids,[38] has already been mentioned. Chapon[39] reported that a second isomer, m.p. 183°, $[\alpha]_D$ -18°, and designated 'ketone-III', was isolated in approximately 10% yield when glutinone was treated for a longer period of time with a stronger mixture of acids. An attempt to prepare the corresponding hydrocarbon was unsuccessful.

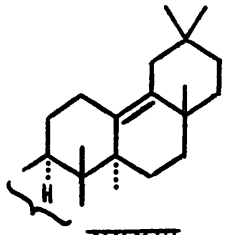
The author has repeated this isomerisation and, after chromatography and several recrystallisations of the product, has obtained a compound, m.p. 182-184°, in 10% yield but having a specific rotation ($[\alpha]_D$ = 34°) which differed considerably from that reported by Chapon.[39] A small quantity of olean-13(18)-en-3-one, identified by mixed melting point and spectroscopic methods, was isolated as a minor product of the isomerisation. Analysis of 'glutinone-III' confirmed the molecular formula, $C_{30}H_{48}O$, and its infrared spectrum showed a band at

1706 cm.$^{-1}$ characteristic of a carbonyl group in a six-membered ring. The poor yield of this isomer induced the author to seek improved methods of preparation.

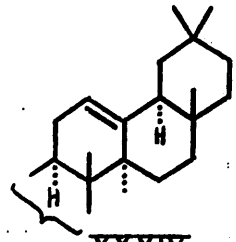
Treatment of glutinone with a refluxing mixture of hydrochloric and acetic acids, conditions under which glutin-5-ene (LVII) was readily isomerised[38] to the equilibrium mixture[41] of olean-13(18)-ene (XXXIII) and 18α-olean-12-ene (XXXIV), gave only a mixture from which no homogeneous material could be isolated. However, during a study of the friedelene-oleanene rearrangment, Courtney, Gascoigne and Szumer[71] observed that treatment of friedel-3-ene (LXXXIX) with dry hydrogen chloride in refluxing acetic acid solution for one hour yielded glutin-5(10)-ene (XC). Similar treatment for several hours resulted in a mixture of hydrocarbons, two of which were identified as



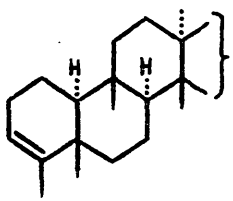
LVII



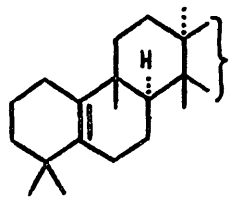
XXXIII



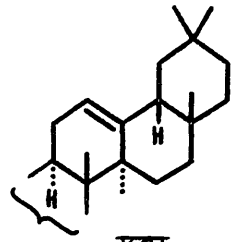
XXXIV



LXXXIX



XC



XCI

glutin-5(10)-ene and olean-12-ene (XCI). A comparison of the infrared spectrum of the mixture with those of 18α-olean-12-ene and olean-13(18)-ene suggested that these hydrocarbons also were present.

Under the somewhat milder conditions of this reaction, it was hoped that glutinone would be successfully isomerised to its isomer 'ketone-III' in improved yield. Crystallisation of the product, however, yielded a material, m.p. 251-253°, $[\alpha]_D$ - 81°, which did not depress the melting point of glutin-5(10)-en-3-one. The specific rotation is considerably lower than that of pure glutin-5(10)-en-3-one ($[\alpha]_D$ - 96°) and repeated recrystallisation did not appreciably raise this value. While it is possible that the product may contain a small amount of 'ketone-III', it is more likely, in the author's opinion, that the low specific rotation is due to the presence of unchanged glutinone. It is of interest to note that Chapon[39] quotes a specific rotation of -84° for the 'ketone-II' prepared by their method.

By varying the reaction conditions employed in the sulphuric-acetic acid method, an improved yield of 'ketone-III' was obtained when the isomerisation was carried out at 40°, at which temperature the reaction time was considerably shortened. As had been anticipated, glutin-5(10)-en-3-one was found to serve equally well as starting material in this isomerisation and gave similar yields of 'ketone-III'.

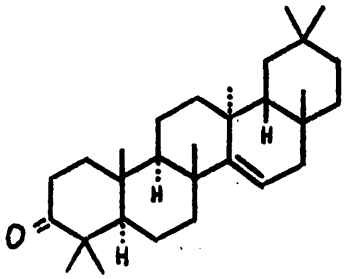
This isomer, as isolated by the author, had m.p. 182-184°, $[\alpha]_D$ - 34°. Wolff-Kishner reduction gave the corresponding 'hydrocarbon-III', obtained pure only after many recrystallisations, and lithium aluminium hydride reduction afforded the 'alcohol-III' which crystallised poorly. Acetylation followed by chromatography and repeated recrystallisation gave 'glutinyl-III acetate', m.p. 208-210°, $[\alpha]_D$ + 38°.

The ultraviolet absorption spectrum of the acetate indicated that its double bond was triply substituted and this was borne out by a band at 820 $cm.^{-1}$ in its infrared spectrum. The 'hydrocarbon-III', on the other hand, did not display this band in the infrared and its ultraviolet absorption maximum was much more intense, being located at 2040 Å. (ε 12,000). An intensity of this magnitude is characteristic of a fully substituted double bond. The ultraviolet absorption spectrum of 'ketone-III' itself was intermediate between those of the acetate and the hydrocarbon. This evidence and the difficulty experienced in purifying all three compounds, suggested that 'ketone-III' was not a homogeneous substance but was, in fact, an equilibrium mixture of two or more ketones. On this basis, 'glutinyl-III acetate' is probably derived from one component of the mixture while 'glutinene-III' arises from another component.

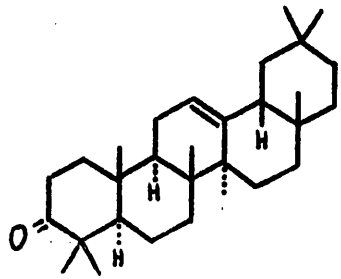
Compound	ε values at				
	2050	2100	2150	2200	2300 Å.
Glutinone-III	6400	4040	2270	1200	0
Glutinyl-III acetate	2900	1680	440	0	0
Glutinene-III	11,900	8800	5660	3670	413

The observation that the hydrocarbons glutin-5-ene and -5(10)-ene are both isomerised[38] to the equilibrium mixture of oleanane derivatives, led the author to believe at first that 'ketone-III' was composed of a mixture of the corresponding ketones, a view that was supported to some extent by the isolation of olean-13(18)-en-3-one from the mother liquors of 'ketone-III'. However, this was easily disproved by comparing their specific rotations. The specific rotation of 'ketone-III' is -34° whilst 18α-olean-12-en-3-one has a positive value and olean-13(18)-en-3-one has only a small negative rotation of -10°. For the same reason, 'ketone-III' could not consist of a mixture of the other $C_{(3)}$-ketones which have a double bond in the region of $C_{(13)}$ [taraxer-14-enone (XXXII), olean-12-enone (XCII) and olean-18-enone (XCIII)], since they all have positive rotations.

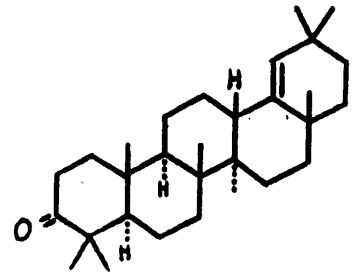
Not too much significance can be attached to the isolation of olean-13(18)-en-3-one in this isomerisation as glutinone occurs in nature closely associated with two isomeric ketones, taraxerone and lupenone, from which it is separated by



XXXII



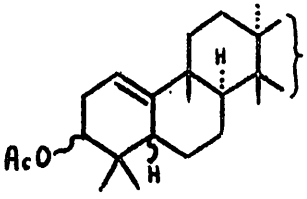
XCII



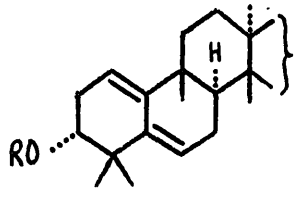
XCIII

chromatographic and chemical techniques. Under the same conditions employed in the isomerisation of glutinone, lupenone yields an isomer, 'lupenone-II', which has been identified[79] as olean-13(18)-en-3-one. The ultraviolet absorption spectrum of this isomer indicated that the material was contaminated by a diene impurity, postulated[79] as the 11:13(18)-diene analogue of olean-13(18)-en-3-one, and this same diene impurity was observed in the ultraviolet absorption spectrum of the olean-13(18)-en-3-one isolated in the glutinone isomerisation. As the yield of this compound amounted to less than 1.5% of the total, it could quite conceivably have arisen from the presence of a minor quantity of lupenone in the starting material. Close examination of the products of similar isomerisations, using different samples of glutinone, failed to reveal the presence of any olean-13(18)-en-3-one and hence, this isomer is regarded as being a product of lupenone, rather than glutinone, isomerisation.

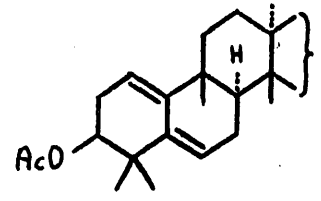
The positions 1(10)-, 7-, 8- and 9(11)- were now considered as possible sites for the location of the double bond in 'glutinyl-III acetate' but the first of these (XCIV) was eliminated on the grounds that selenium dioxide oxidation of the acetate did not give rise to either of the two known glutina-1(10):5-dienyl acetates (XXXIX, R = Ac) and (LII). The



XCIV



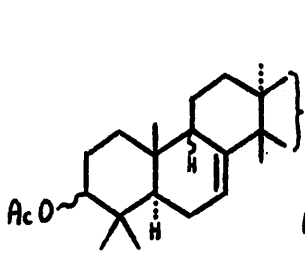
XXXIX



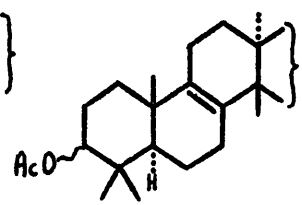
LII

other three possible structures (XCV), (XCVI) and (XCVII) have the same configurations at $C_{(5)}$, $C_{(10)}$, $C_{(13)}$, $C_{(14)}$ and $C_{(18)}$ as do the corresponding compounds (XCVIII) in the euphane series and hence it can be expected that they would behave in a similar fashion.

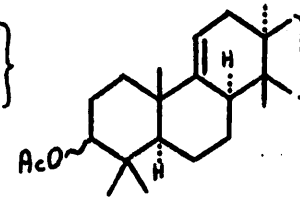
Euph-7-, euph-8- and euph-9(11)-enyl acetates when treated with selenium dioxide in acetic acid, all give eupha-7:9(11)-dienyl acetate[75-78] (XCIX, R = C_8H_{17}) which has a characteristic ultraviolet absorption spectrum with maxima at 2320, 2390 and 2460 Å.



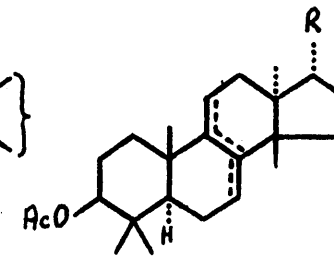
XCV



XCVI

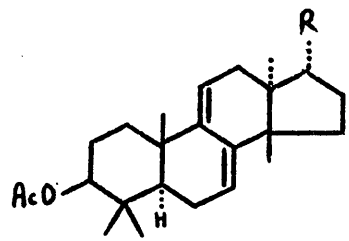


XCVII

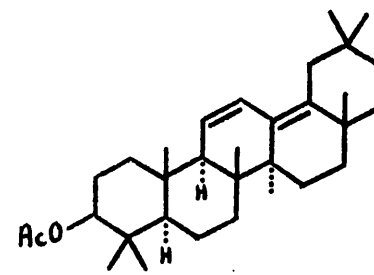


XCVIII

'Glutinadienyl-III acetate' exhibits maxima at 2430, 2500 and 2600 Å and it is considered unlikely that this large bathochromic shift of 100 Å. could be attributed to the transition from a five to a six-membered ring D. This spectrum, on the other hand, closely resembles that of oleana-11:13(18)-dienyl acetate (LXV), λ max. at 2420, 2500 and 2600 Å, although the melting points and specific rotations were quite different.



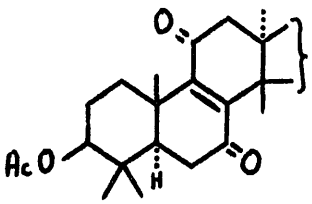
XCIX



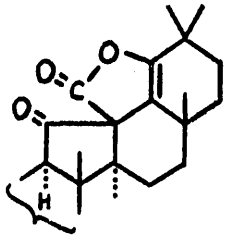
LXV

In order to establish which diene system was present, viz., the 7:9(11)- or the 11:13(18)-diene, 'glutinadienyl-III acetate' was oxidised with chromium trioxide in acetic acid at 80°. Under these conditions, eupha-7:9(11)-dienyl acetate is converted[80,81] into the ene-dione (C) which has an absorption

maximum at 2710 Å in the ultraviolet, while oleana-11:13(18)-dienyl acetate gives the 'O_8-acetate'[82,85], which has the structure of a β,δ-unsaturated lactone (CI) and shows selective absorption at 2300 Å with a low intensity inflection at 3000 Å.



C



CI

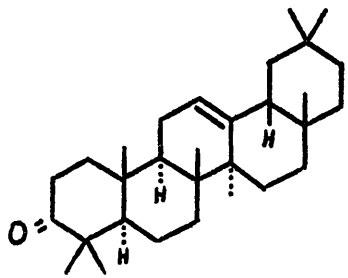
The product, isolated in small yield, absorbed in the ultraviolet at 2250 Å, with a low intensity inflection at 2700 Å. A comparison of its infrared spectrum with that of the 'O_8-acetate' showed that they had identical carbonyl systems, both displaying sharp bands at 1786, 1748 and 1695 $cm.^{-1}$. Consequently, 'glutinadienyl-III acetate' must include an 11:13(18)-diene system and from this, it follows that the double bond in 'glutinyl-III acetate' must be located in the region of $C_{(13)}$, as was originally postulated.

It was mentioned previously that the 'ketone-III' could not consist of a mixture of olean-12-en-3-one, olean--13(18)-en-3-one, 18α-olean-12-en-3-one, taraxerone or germanicone because of its negative specific rotation but this

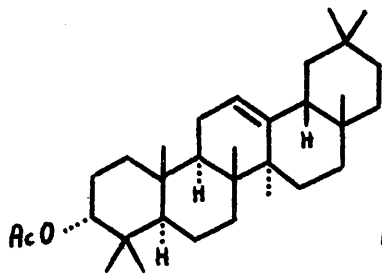
does not preclude the possibility of one or more of these ketones being present together with some other ketone, as yet unknown, and having a strong negative rotation. The lithium aluminium hydride reduction of the 'ketone-III' may well have formed a mixture of $C_{(3)}$-epimers and in the subsequent work-up of the acetylated product, the axial 3α-acetoxy derivative of one of these ketones may have been isolated as 'glutinyl-III acetate'. This would then explain the presence in 'glutinadienyl-III acetate' of an 11:13(18)-diene system while its physical constants differed from those of oleana-11:13(18)-dienyl acetate.

Reduction of olean-12-en-3-one (β-amyrenone) (XCII) with aluminium isopropoxide and isopropanol gave a mixture of the 3β- and 3α-alcohols which was separated in the usual way by chromatography on alumina. Oxidation of olean-12-en-3α-yl acetate (CII) with selenium dioxide in acetic acid gave the corresponding dienyl acetate (CIII) which, although having the same light absorption and specific rotation as 'glutinadienyl-III acetate', had a higher melting point. A comparison of their infrared spectra and a mixed melting point determination proved their non-identity.

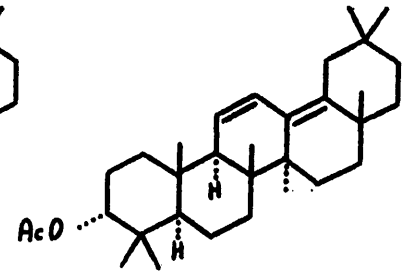
The ultraviolet and infrared spectra of 'glutinyl-III acetate' indicated that the double bond was tri-substituted and confirmation of this was obtained by comparing the difference in



XCII



CII



CIII

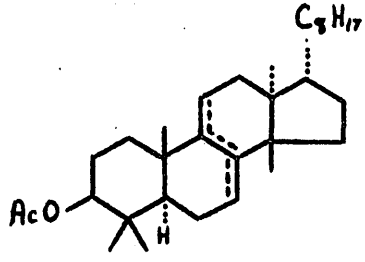
molecular rotation between 'glutinyl-III acetate' and 'glutinadienyl-III acetate' with the corresponding differences between the 3β-acetates of the ketones, olean-12-en-3-one, olean-13(18)-en-3-one, 18α-olean-12-en-3-one, taraxerone and germanicone, and their derived dienyl acetate, oleana-11:13(18)-dien-3β-yl acetate (LXV).

Series	M_D		Δ
	Acetate	Dienyl Acetate	
'Ketone-III'	+178°	-549°	-727°
Olean-12-en-3-one			
(3β-series)	+427	-308	-735
(3α-series)	+182	-554	-736
Olean-13(18)-en-3-one	-164	-308	-144
18α-Olean-12-en-3-one	+248	-308	-556
Taraxerone	+56	-308	-364
Germanicone	+89	-308	-397

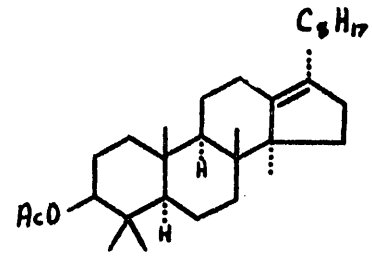
As can be seen from the table, the rotational change in the 'ketone-III' series is in close agreement with the changes between olean-12-en-3β- and -3α-yl acetates and their dienyl acetates. From this, it can be deduced that the double bond in 'glutinyl-III acetate' lies between $C_{(12)}$ and $C_{(13)}$ and furthermore, when the molecular rotational change in the 18α-oleanane series is taken into consideration, it is apparent that the $C_{(18)}$-hydrogen atom must still have the β-configuration.

The ready isomerisation[38] of the glutinene hydrocarbons by mineral acid, to the mixture of olean-13(18)-ene and 18α-olean-12-ene while similar treatment of the corresponding ketones gives a product which does not belong to the oleanene series, makes it clear that the $C_{(3)}$-carbonyl group must exert some influence over the course of the isomerisation. This view receives some support from the previously described acid isomerisation experiments on the glutinyl acetates where the presence of an acetate group at $C_{(3)}$ successfully prevented migration of the double bond away from its proximity until elimination of the oxygen function had taken place. Since the three euphenyl acetates (XCVIII) are all converted on mineral acid treatment[77,78,81,84] into isoeuph-13(17)-enyl acetate (CIV) through 1:2 shifts of the axial methyl groups at $C_{(13)}$ and $C_{(14)}$,

then the $C_{(3)}$-oxygen function can no longer have any effect on the isomerisation once the double bond has reached the 7-, 8- or 9(11)-positions. Accordingly, 'glutinyl-III acetate' can differ from β-amyrin acetate only in the regions of rings A and B.

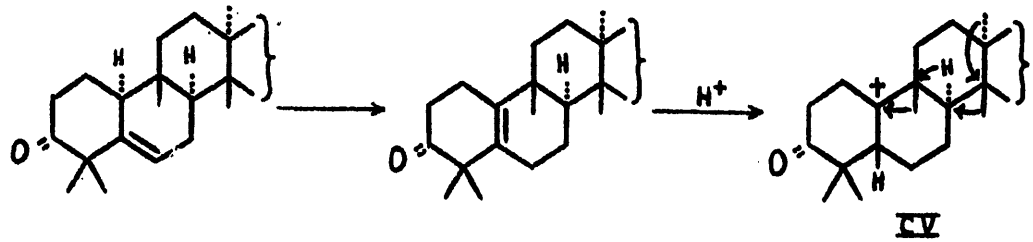


XCVIII

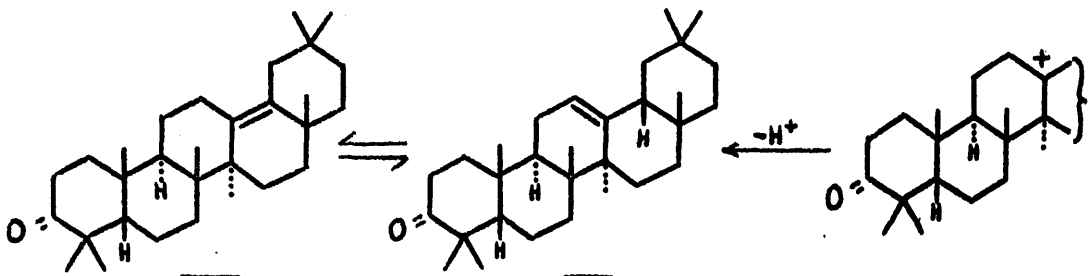


CIV

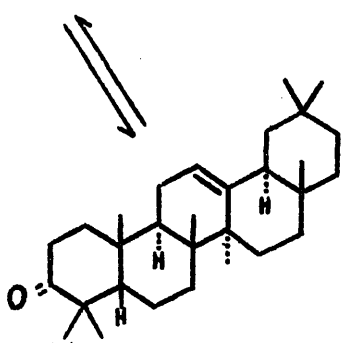
A reasonable conclusion to be drawn is that rings A and B are cis-β-fused in 'glutinyl-III acetate' and hence in the parent compound, 'glutinone-III'. Such a structure can arise through normal migration of the 5(6)-double bond in glutinone to the 5(10)-position, followed by insertion of a 5β-hydrogen atom by means of protonation from above, to yield the carbonium ion (CV). Rearrangement in the usual way and loss of a proton from $C_{(12)}$ (the 12α-hydrogen atom, according to Courtney et al.[71]) would result in 5β-olean-12-en-3-one (CVI) which, under the influence of strong acid, would probably form a mixture of the 12- and 13(18)-double bond isomers. The possibility of 5β:18α-olean-12-en-3-one (CVIII) also being present, cannot be discounted.



CV



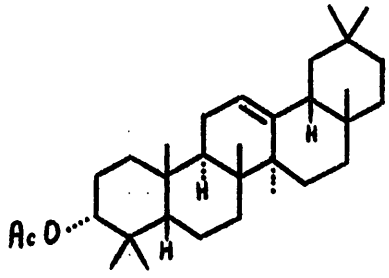
CVII CVI



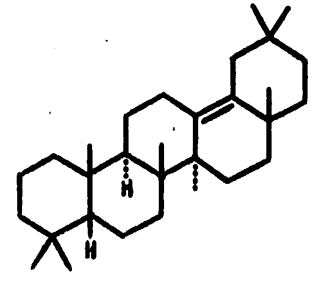
CVIII

It is the author's belief that 'glutinyl-III acetate' is derived from the 5β-olean-12-en-3-one component and, since metal hydride reduction of a relatively non-hindered ketonic group yields predominantly the equatorial epimer, it can

tentatively be identified as 5β-olean-12-en-3α-yl acetate (CIX). Correspondingly, the hydrocarbon 'glutinene-III' is considered to arise from the 5β-olean-13(18)-en-3-one (CVII) component, on the basis of spectroscopic evidence, and can be formulated as 5β-olean-13(18)-ene (CX).



CIX



CX

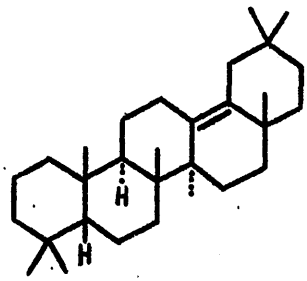
Lack of material prevented positive identification of these compounds by chemical means, but the molecular rotation data outlined below lends support to the above conclusions.

Comparisons of the changes in rotational values between 'glutinyl-III acetate' and the olean-12-en-3α- and -3β-yl acetates, and between the coprostanol and cholestanol acetates appeared, at first sight, to be a simple means of determining not only whether the rings A and B are cis-β-fused but also the configuration of the $C_{(3)}$-substituent. Unfortunately, the interposition of a gem-dimethyl group between the bridge-head atom and the $C_{(3)}$-acetate group tends to make such comparisons

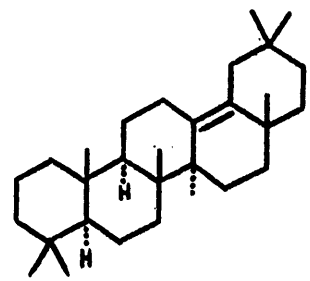
unreliable. The 19-hydroxy-oleanane and 19-hydroxy-18α-oleanane derivatives, which seemingly could also be used for comparison purposes, suffer from having their hydroxyl groups flanked by ternary and quaternary carbon atoms and hence the environment of these groups differs radically from that of corresponding groups located at $C_{(3)}$.

Similarly, attempts to correlate the molecular rotation changes accompanying inversion of the 5α-hydrogen atom in the olean-13(18)-ene hydrocarbon series with the corresponding changes observed between the fully saturated oleanan-3β-yl acetate and its 18α-oleanan-3β-yl acetate analogue are negated by the dissimilar natures of their ring fusions. Thus, rings A, B, C and D are fused cis-anti-trans-anti in 5β-olean-13(18)-ene (CX) and, in olean-13(18)-ene (XXXIII), the fusion is trans-anti-trans-anti whereas in oleanan-3β-yl acetate (CXI) and 18α-oleanan-3β-yl acetate (CXII), rings E, D, C and B are fused cis-syn-trans-anti and trans-anti-trans-anti, respectively.

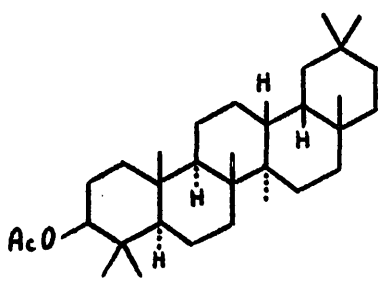
Notwithstanding these difficulties, a clearer insight into the stereochemical nature of 'glutinyl-III acetate' was gained through a consideration of the molecular rotation contributions of 3α- and 3β-acetoxy groups. Klyne and Stokes[85] have shown that in the triterpenoid series, a 3α-acetate group contributes a large negative value to the molecular rotation of the unsubstituted



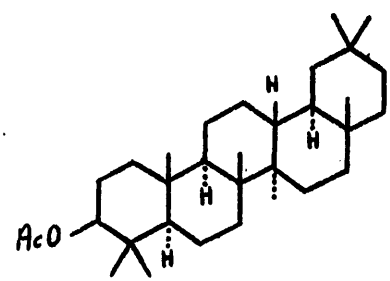
CX



XXXIII



CXI

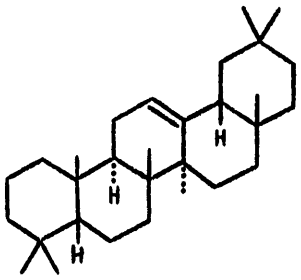


CXII

hydrocarbon whilst the contribution of a 3β-acetate group is smaller but of opposite sign, cf. olean-12-ene and the olean-12-en-3α- and -3β-yl acetates: for the 3α-acetate, Δ = -203° and for the 3β-acetate, Δ = +42°.

If an assumption is made that 'glutinene-III' is represented by 5β-olean-13(18)-ene (CX), then a comparison of its molecular rotation [M_D = -156°] with that of olean-13(18)-ene [M_D = -197°] reveals that inversion of the $C_{(5)}$-hydrogen atom contributes a small positive value of +41°. Extrapolating this

value from olean-12-ene [M_D = +385°] to the hypothetical 5β-olean-12-ene (CXIII), the molecular rotation of the latter compound is

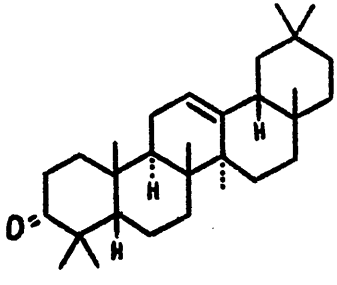


CXIII

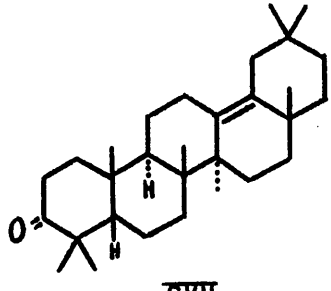
calculated to be +426°. Comparison of the rotational values of 'glutinyl-III acetate' and 5β-olean-12-ene indicates a large negative contribution of -248° from the acetate group. The data of Klyne and Stokes[88] would thus dictate a 3α-configuration for the substituent group in 'glutinyl-III acetate' and this is further borne out by the molecular rotation changes in the series illustrated below.

	Compound	M_D	Δ_1	Δ_2
	Glutinene-III	-156°		
1.	Glutinyl-III acetate	+178°	+334°	
2.	Glutinadienyl-III acetate	-549°		-393°
	Olean-13(18)-ene	-197°		
1.	Olean-12-en-3α-yl acetate	+182°	+379°	
2.	Oleana-11:13(18)-dien-3α-yl acetate	-554°		-357°
	Olean-13(18)-ene	-197°		
1.	Olean-12-en-3β-yl acetate	+427°	+624°	
2.	Oleana-11:13(18)-dien-3β-yl acetate	-308°		-111°

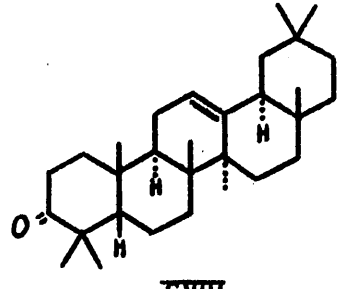
In the oleanane series, a ketonic group located at $C_{(3)}$ is not subject to steric hindrance unless rings A and B are cis-α-fused, whereupon some interaction between the carbonyl group and the axial methyl groups at $C_{(10)}$ and, to a lesser extent, at $C_{(8)}$ might be expected. As this type of ring fusion is considered highly improbable in 'glutinone-III', it is apparent that the ketonic group is relatively non-hindered and reduction with lithium aluminium hydride should give the equatorial epimer. Accordingly, 'glutinyl-III acetate' must also be considered equatorial. This supports our initial assumption of a cis-β-fused ring system in 'glutinone-III' as the geometry of 5β-olean-12-en-3α-yl acetate (CIX) is such that the 3α-acetate group must be in the more stable equatorial configuration.



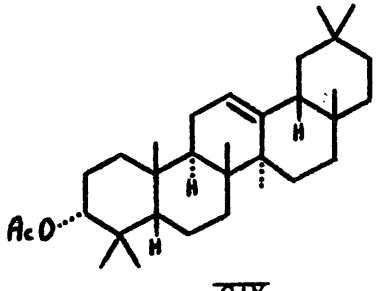
CVI



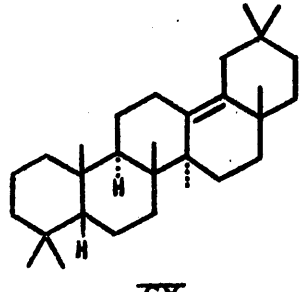
CVII



CVIII



CIX



CX

To summarise the foregoing evidence, 'glutinone-III' can best be represented, in the author's opinion, as an equilibrium mixture of 5β-olean-12-en-3-one (CVI) and 5β-olean-13(18)-en-3-one (CVII). Although not detected, the possible presence of some 5β:18α-olean-12-en-3-one (CVIII) in this mixture should not be ignored. The derivatives 'glutinyl-III acetate' and 'glutinene-III', are considered to arise from the two components (CVI) and (CVII) respectively, and are tentatively identified as 5β-olean-12-en-3α-yl acetate (CIX) and 5β-olean-13(18)-ene (CX).

S E C T I O N II

The Constituents of Elder Bark (Sambucus nigra).

From the non-saponifiable fractions of light petroleum and ether extracts of elder bark, have been isolated α-amyrenone, α-amyrin, betulin, β-sitosterol and oleanolic acid. This is the first recorded instance of α-amyrenone occurring in nature.

The only comprehensive examination of elder bark (*Sambucus nigra*) reported in the literature is that of Zellner,[86] in 1926, carried out during a survey of the barks of many species. He was able, however, to identify only a few of the components, the remainder being unknown to him. Zellner saponified the light petroleum extract of the bark and recrystallised the non-saponifiable material several times from ethyl acetate, by this means achieving a partial separation into an ethyl acetate soluble and an ethyl acetate insoluble fraction. The latter, m.p. 72°, proved to be a mixture of aliphatic alcohols which Zellner found impossible to separate although he managed to establish that the main component was ceryl alcohol.

After removal of the residual aliphatic alcohols from the ethyl acetate soluble fraction, it was fractionally crystallised from alcohol to yield two further materials, one of which, m.p. 134° (acetate, m.p. 118°), was identified as a phytosterol. The second material was recrystallised from ethyl acetate to give an apparently homogeneous product, m.p. 216°, the analysis of which corresponded to the molecular formula $C_{23}H_{42}O_2$. From the mother liquors was isolated yet another material, m.p. 179°, (acetate, m.p. 216°), of molecular formula $C_{27}H_{48}O$.

An ether extract of the bark yielded two substances,

one of which could not be obtained crystalline and had a decomposition point of 298°. It appeared to be acidic in character and analysed for a molecular formula of $C_{22}H_{38}O_3$. The second substance, m.p. 245°, obtained crystalline from a hydrochloric acid - alcohol mixture, gave a violet colouration in the Liebermann- Burchard test. For its analysis, Zellner quoted figures which correspond to the molecular formula, $C_{24}H_{48}O_3$.

In our hands, extraction of the dried bark with light petroleum yielded a dark brown resin (2-3% by weight) which was refluxed with methanolic potassium hydroxide to afford the non-saponifiable fraction as an orange gum. Unlike Zellner's experience, the author found this material to be extremely soluble in ethyl acetate. Chromatography of a solution of this on alumina in light petroleum, gave five main fractions The first of these was crystallised from chloroform-methanol and then aqueous acetone several times to afford a pure substance, m.p. 124-126°, $[\alpha]_D$ + 107°, which exhibited a strong band in the infrared at 1701 $cm.^{-1}$ characteristic of a ketone group in a six-membered ring. It was identified by mixed melting point methods and infrared spectroscopy as α-amyrenone (CXIV). Confirmation of its identity was obtained by the preparation of the corresponding alcohol and acetate.

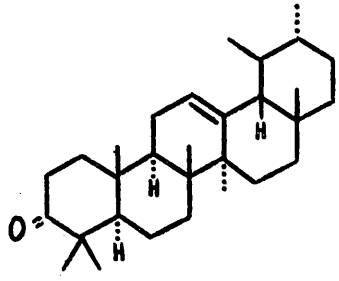
This is the first recorded instance of α-amyrenone occurring in nature.

The second fraction, eluted with benzene - ether mixtures, failed to crystallise from methanol but, over a period of several weeks, an orange gum and clusters of white needles were deposited. The needles were collected, acetylated and chromatographed in light petroleum on alumina. Fractions eluted with the same solvent crystallised from chloroform-methanol in lustrous plates, m.p. 224-225°, $[\alpha]_D$ + 80°, the infrared spectrum of which was identical with that of α-amyrin acetate (CXV). Hydrolysis of the acetate yielded α-amyrin, m.p. 188-190°, and oxidation of the alcohol with the pyridine-chromium trioxide complex gave α-amyrenone. This alcohol probably corresponds with the substance, m.p. 179°, isolated by Zellner.[86]

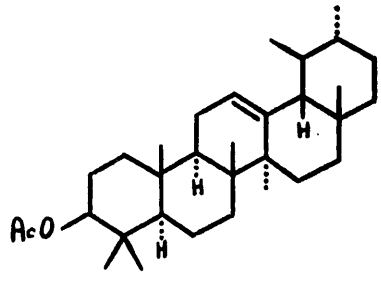
The third fraction failed to give a positive reaction in the Liebermann-Burchard test. Crystallisation from aqueous acetone gave small plates, m.p. 67-71°, $[\alpha]_D \pm 0°$, and it undoubtedly corresponds to the mixture of aliphatic alcohols obtained by Zellner.

The fourth fraction was recrystallised from chloroform-methanol to give needles, m.p. 256-259°, $[\alpha]_D$ + 16°, whose

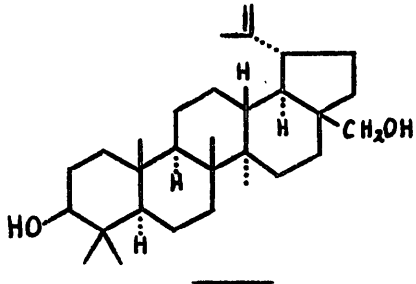
infrared absorption spectrum was identical with that of betulin (CXVI). Treatment of the alcohol with pyridine and acetic anhydride gave the corresponding betulin diacetate.



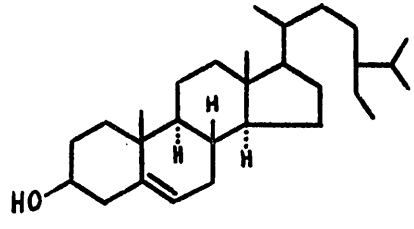
CXIV



CXV



CXVI

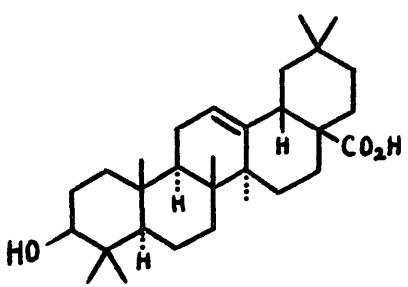


CXVII

The final fraction, also eluted with benzene-ether, gave a blue-green colour in the Liebermann-Burchard test and was obtained from chloroform-methanol as plates, m.p. 138-140°, (acetate, m.p. 129-130°). The infrared absorption spectrum was identical with that of β-sitosterol (CXVII). The mother liquors of all the fractions were combined, acetylated and rechromatographed on alumina to yield further quantities of α-amyrin acetate, betulin diacetate and β-sitosteryl acetate.

No trace could be found of the material, m.p. 216°, isolated by Zellner, and in the author's opinion, it probably consisted of an impure sample of betulin.

An ether extract of the bark yielded a dark brown tar (1% by weight of dried bark) which was saponified in the usual manner and extracted with ether. The brown solid which separated at the interface of the ether and water phases, was collected, dissolved in hot methanol and treated with concentrated hydrochloric acid. Recrystallisation of the product from methanol gave needles, m.p. 310-312°, $[\alpha]_D$ + 73°, whose infrared spectrum was identical with that of oleanolic acid (CXVIII). Esterification with diazomethane gave the corresponding methyl ester and treatment of the latter with pyridine and acetic anhydride yielded the methyl ester acetate, both of which did not depress the melting points of authentic specimens.



CXVIII

The ether solution containing the non-saponifiable portion of the extract, was concentrated and allowed to stand overnight whereby a further small quantity of oleanolic acid was deposited. A solution of the residue in light petroleum-benzene was chromatographed on alumina but yielded only small amounts of α-amyrin, betulin and β-sitosterol.

Extraction of the bark with 80% alcohol yielded on acid hydrolysis, a moderate amount of a dark brown solid which was tentatively identified as belonging to the lignin family.

S E C T I O N III

The Constitution of Soyasapogenol B.

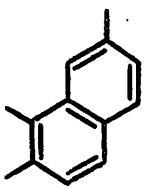
The facile conversion of the dioxo-dienyl derivative of epimeric sapogenol B triacetate into sapogenol B dioxo-dienyl triacetate has been represented as a reversed aldol : direct aldol intramolecular sequence and led previous workers[103] to the conclusion that sapogenol B is 3β:21α:24-trihydroxy-olean-12-ene. However, the recognition[105] of sapogenol D as the methyl ether of isomeric sapogenol B, and the relative ease with which it undergoes solvolytic reactions, strongly suggests that the ring E hydroxyl group in sapogenol B is located at $C_{(22)}$. The experiments described in this section furnish evidence that the latter hypothesis is correct and accordingly, sapogenol B is identified as 3β:22β:24-trihydroxy-olean-12-ene.

The soya bean has proved to be a fruitful source of many compounds[87-92] of which one, hispidic acid, isolated in 1923 by Murumatsu,[87] was later[93-95] shown to be a saponin which on acid hydrolysis yielded a sapogenin mixture. This mixture was not fully resolved until 1937 when Ochiai et al.[96], employing column chromatography as a means of separation, were able to isolate four homogeneous compounds which were designated soyasapogenols A, B, C and D. More recently, the presence of sapogenol B in alfalfa,[97] of sapogenols A, B and C in ladino clover (Trifolium repens)[98] and of sapogenol C in whin (Ulex europaeus)[99] has been reported.

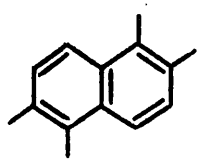
Elemental analyses indicated the molecular formula $C_{30}H_{46}(OH)_4$ for sapogenol A and $C_{30}H_{47}(OH)_3$ for sapogenol B while the other two sapogenols, C and D, had analyses corresponding to $C_{30}H_{46}(OH)_2$ and $C_{30}H_{48}O(OH)_2$ respectively. These figures suggested that the sapogenols were triterpenoid in nature and confirmation was forthcoming when three typical triterpenoid degradation products were obtained on selenium dehydrogenation[96] of sapogenol B, viz. 1,2,7-trimethyl-naphthalene (CXIX), 1,2,5,6-tetramethyl-naphthalene (CXX) and 1,8-dimethyl-picene (CXXI). The presence of an unreactive ethylenic linkage in each of the sapogenols was demonstrated[93-95]

with tetranitromethane and sapogenol C was shown to possess an additional double bond which was readily reduced on catalytic hydrogenation. The nature of the third oxygen atom in sapogenol D was not rigidly established but Ochiai *et al.*[96] suggested that it was present as an oxide bridge.

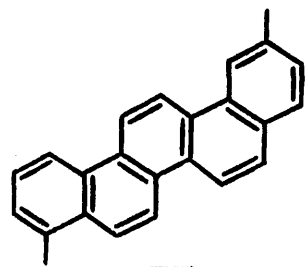
Triterpenoid chemistry was in its initial stages during this period and little could be done towards establishing the



CXIX

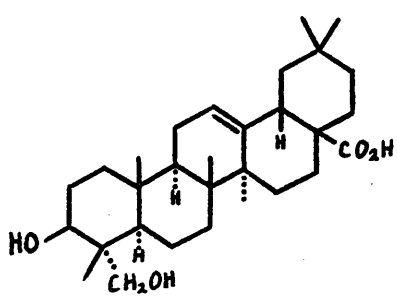


CXX



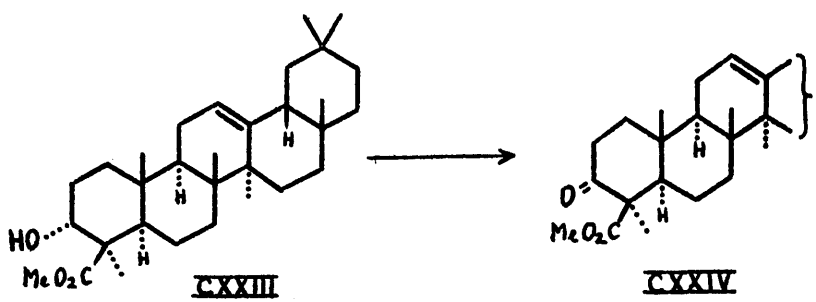
CXXI

structures of the sapogenols. However, oxidative studies and high temperature dehydrogenations[100] led to the observation that the sapogenols behaved in a manner analogous to that of hederagenin (CXXII) and probably contained a similar type of 1,3-glycol system.

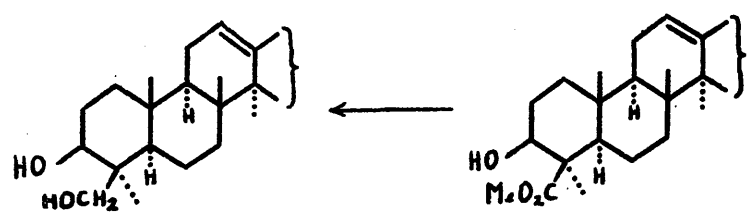


CXXII

Little more work was done until 1950 when the first major advance in sapogenol chemistry was gained[101] through a correlation of the sapogenols with the oleanane group of triterpenoids. Oxidation[102] of the methyl ester of α-boswellic acid (CXXIII) either with chromic acid or by the Oppenauer method, yielded the corresponding keto-ester (CXXIV), catalytic hydrogenation of which lead to the 3β-hydroxy ester (CXXV), the methyl ester of epi-α-boswellic acid. Lithium aluminium hydride reduction of this hydroxy ester gave 3β,24-dihydroxy-olean-12-ene (CXXVI) which was found to be identical with dihydrosapogenol C. This relationship firmly established



CXXIII CXXIV



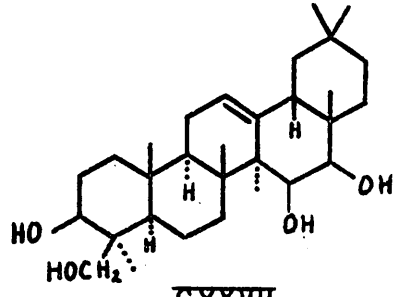
CXXVI CXXV

the structure and stereochemistry of sapogenol C except for the location of the double bond susceptible to catalytic hydrogenation.

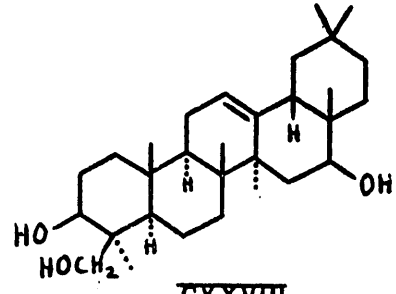
Meyer et al.[101] showed that the structure of sapogenol A follows from that of sapogenol C since osmium tetroxide oxidation of the latter followed by acetylation of the product, yielded a mixture of sapogenol A tetra-acetate, a stereo-isomeric tetra-acetate and the 11-oxo derivative of the latter compound. From this, it was concluded that the cis-glycol system in sapogenol A must occupy the site of the reducible double bond in sapogenol C and furthermore, since sapogenol A consumes only one mole of the lead tetra-acetate reagent on oxidation, the cis-glycol grouping cannot be located in ring A.

The formation of a 12:19-dioxo-9(11);13(18)-diene derivative from sapogenol A tetra-benzoate on selenium dioxide oxidation precluded the positions $C_{(9)}$, $C_{(11)}$, $C_{(12)}$, $C_{(13)}$, $C_{(18)}$ and $C_{(19)}$ for the cis-glycol group, and of the remaining three positions, that is, $C_{(6)}$ and $C_{(7)}$, $C_{(15)}$ and $C_{(16)}$, and $C_{(21)}$ and $C_{(22)}$, the first was eliminated on the grounds of steric hindrance.[101] Although the above authors could obtain no experimental evidence to support their views, they favoured the positions $C_{(15)}$ and $C_{(16)}$ and accordingly formulated the structure (CXXVII) for sapogenol A. The structure of

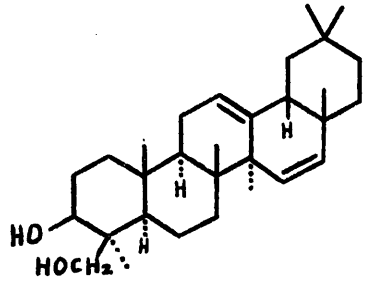
sapogenol C (CXXIX) followed from that of sapogenol A, with a double bond replacing the α-glycol group.



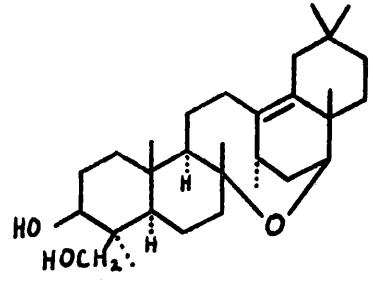
CXXVII



CXXVIII



CXXIX



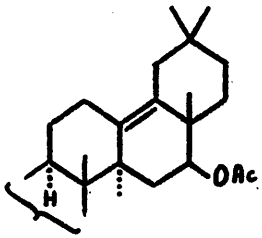
CXXX

From the experimental evidence outlined below, Meyer et al.[101] postulated the structures (CXXVIII) and (CXXX) for sapogenols B and D. In the latter sapogenol, the presence of an oxide linkage was established through infrared studies and treatment of this sapogenol with a variety of ether-splitting reagents. Both ultraviolet and infrared spectra suggested that the double bond in sapogenol D, in contrast to the other sapogenols, was fully substituted. The molecular

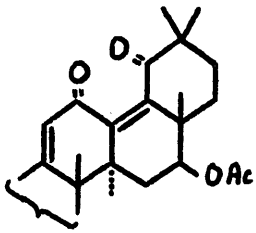
formula, $C_{30}H_{50}O_3$, does not allow a pentacyclic structure to be drawn for this compound but its structure must closely resemble that of the other sapogenols since they can be converted into common derivatives.

Treatment of sapogenol D diacetate with boron trifluoride and acetic anhydride afforded a triacetate, $C_{36}H_{56}O_6$. This was formulated as the 13(18)-double bond isomer (CXXXI) of sapogenol B triacetate (CXXXIII) since both gave the same dioxodienyl derivative (CXXXII) on vigorous selenium dioxide oxidation. It was concluded from this, that one end of the oxide linkage in sapogenol D must be located at the position in sapogenol B of its third hydroxyl group. This, in turn, was related indirectly to the reactive double bond in sapogenol C through the preparation of a derivative common to both sapogenols C and D. Sapogenol D diacetate on treatment with hydrochloric and acetic acids yielded a pentacyclic chloro compound formulated as (CXXXIV), and on further treatment of this material with sodium iodide and acetone, the non-conjugated diene (CXXXV) was obtained. Mild selenium dioxide oxidation gave the triene (CXXXVI) which proved to be identical with that prepared through a similar oxidation of sapogenol C diacetate.

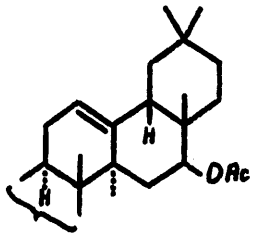
The preparation of a dioxo-diene derivative of sapogenol D diacetate in which the oxide linkage remained intact was further evidence in favour of a structure analogous to that



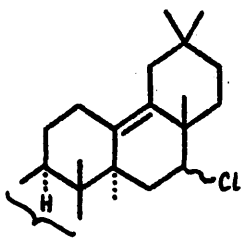
CXXXI



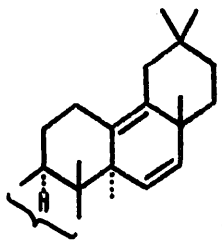
CXXXII



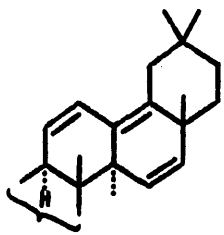
CXXXIII



CXXXIV

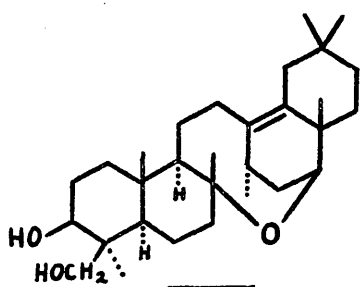


CXXXV



CXXXVI

of the other sapogenols and it was on the basis of this and related experimental evidence that Meyer *et al.*[101] postulated the structure (CXXX) for sapogenol D.

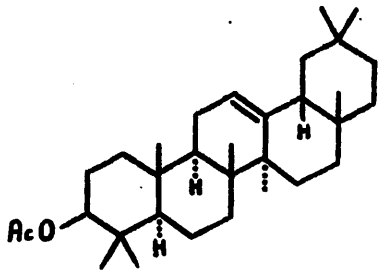


CXXX

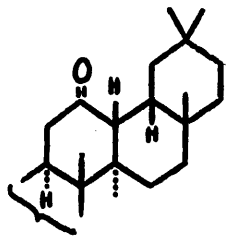
If this structure is accepted, then the conversion of sapogenol D into the derivatives of sapogenols B and C must involve cleavage of an ether linkage and concurrent ring closure

to give a pentacarbocyclic structure. This type of rearrangement, which had hitherto been unknown in triterpenoid chemistry, aroused considerable interest.

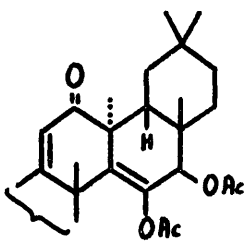
In particular, the group of Smith, Smith and Spring[103] attempted to determine the true nature of the oxide ring in sapogenol D. As a preliminary step, they applied to sapogenol A a series of reactions designed to test the hypothesis that the 1:2-glycol group was located in ring D. Performic acid oxidation of β-amyrin acetate (CXXXVII) gives the saturated 12-ketone (CXXXVIII) which on treatment with bromine is converted into the αβ-unsaturated ketone (CXXXIX). Oxidation of this material with selenium dioxide forms 12-oxo-taraxera-9(11):14-diene (CXL).[104] When this reaction scheme was applied to sapogenol A tetra-acetate, there was obtained as final product, a tetra-acetoxy-12-oxo-taraxera-9(11):14-diene whose spectral properties and changes in molecular rotation were in agreement with those of (CXL). If Meyer's structure for sapogenol A is correct, then this tetra-acetoxy dienone must be represented by (CXLI) which contains an enol-acetate group. However, the tetra-acetoxy dienone is recovered unchanged after alkaline hydrolysis and reacetylation, and consequently the cis-glycol system in sapogenol A cannot be located at $C_{(15)}$ and $C_{(16)}$.



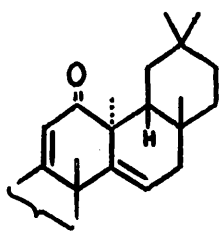
CXXXVII



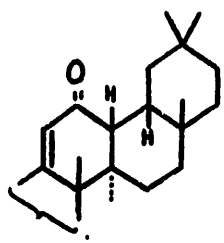
CXXXVIII



CXLI

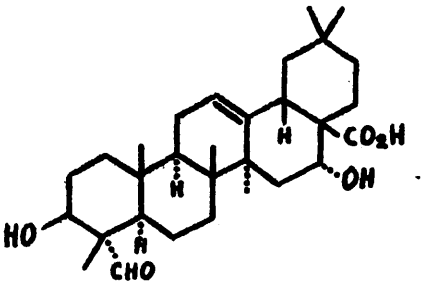


CXL

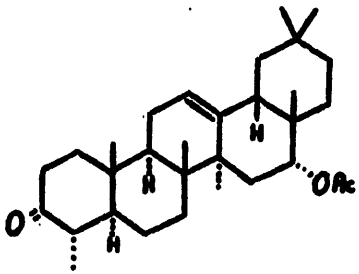


CXXXIX

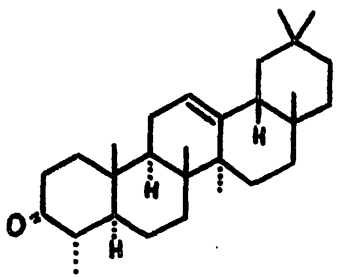
This conclusion was later confirmed[105] by the preparation of a 24-noroleana-12:15-dien-3-one (CXLIV) by pyrolysis of 16α-acetoxy-24-norolean-12-en-3-one (CXLIII), the latter compound having previously[106,107] been prepared from quillaic acid (CXLII). This nor-dienone (CXLIV) proved to be different from the nor-dienone obtained earlier[100] by copper-bronze dehydrogenation of sapogenol C. Both nor-dienones gave the same 24-norolean-12-en-3-one (CXLV) on catalytic hydrogenation and consequently their structures could differ only in the location of the reducible double bond.



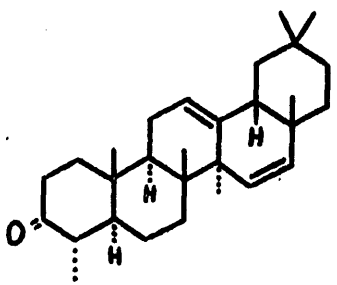
CXLII



CXLIII



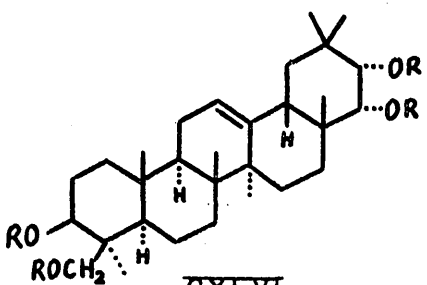
CXLV



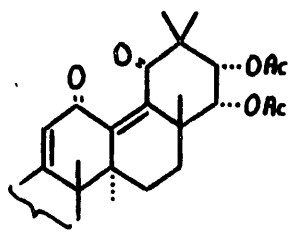
CXLIV

A study of the behaviour of the 12:19-dioxo-9(11):13(18)-dienyl derivatives of sapogenol A tetra-acetate enabled Smith et al.[103] to deduce that the cis-glycol group was situated at $C_{(21)}$ and $C_{(22)}$ and that sapogenol A was in fact 3β:21α:22α:24-tetrahydroxy-olean-12-ene (CXLVI, R = H). Vigorous selenium dioxide oxidation of sapogenol A tetra-acetate (CXLVI, R = Ac) gave the corresponding dioxo-dienyl derivative (CXLVII), m.p. 265-266°, which was not identical with the dioxo-dienyl

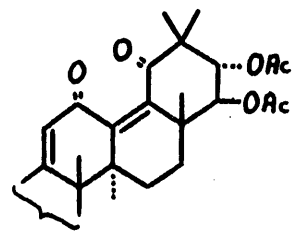
tetra-acetate (CXLVIII), m.p. 323-324°, prepared previously by Meyer et al.[101] from sapogenol A tetra-benzoate by similar oxidation followed by alkaline hydrolysis and acetylation. When the tetra-acetoxy dioxo-diene (CXLVII), m.p. 265-266°, was hydrolysed with alkali and reacetylated, the higher melting isomer (CXLVIII) could be isolated in good yield.



CXLVI



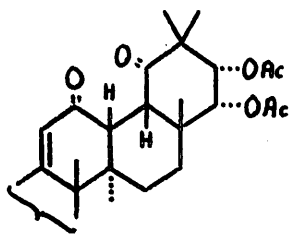
CXLVII



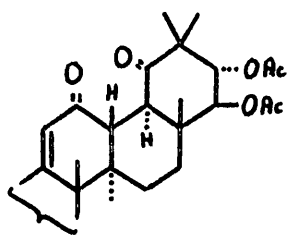
CXLVIII

This conversion suggested to Smith et al.[103] that a base induced epimerisation of the cis-glycol system had occurred and they attributed this to the influence of the 12:19-dioxo-9(11):13(18)-diene chromophore since sapogenol A tetra-acetate was itself recovered unchanged on similar treatment. The 12-keto-9(11)-ene part of the chromophore was known to be inert as the tetra-acetoxy 12-oxo-olean-9(11)-ene (CLIII) was unaffected by alkaline hydrolysis and the above authors showed the 19-carbonyl group to be activating species in this epimerisation through the following experiments.

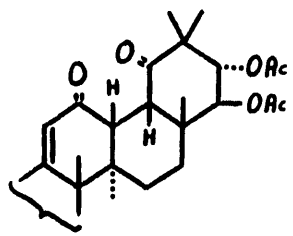
Zinc and ethanol reduction of the lower melting tetra-acetoxy dioxo-diene (CXLVII) gave the 13β:18β-dihydro-derivative (CXLIX) whilst similar treatment of the higher melting isomer (CXLVIII) yielded its corresponding dihydro-derivative (CLI). Alkaline hydrolysis of each and subsequent reacetylation afforded the same 3β:21α:22β:24-tetra-acetoxy-12:19-dioxo-18α-olean-9(11)-ene (CL) in which inversion of the $C_{(18)}$-hydrogen atom had occurred, and also, in one case, of the 22α-acetoxy group.



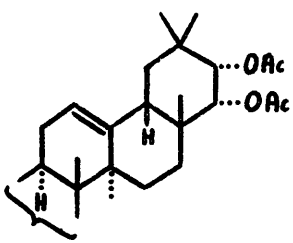
CXLIX



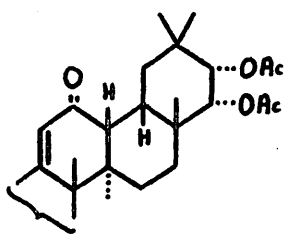
CL



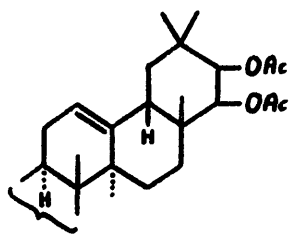
CLI



CLII



CLIII



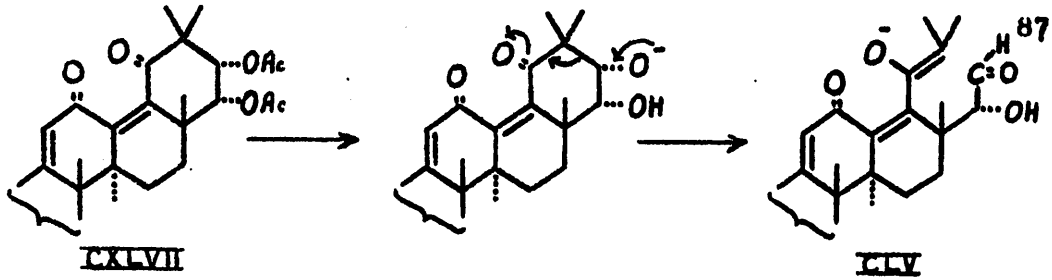
CLIV

On the basis of this evidence, Smith et al.[103] asserted that not only was the 19-carbonyl group playing an active part in this epimerisation but that the cis-glycol system must be

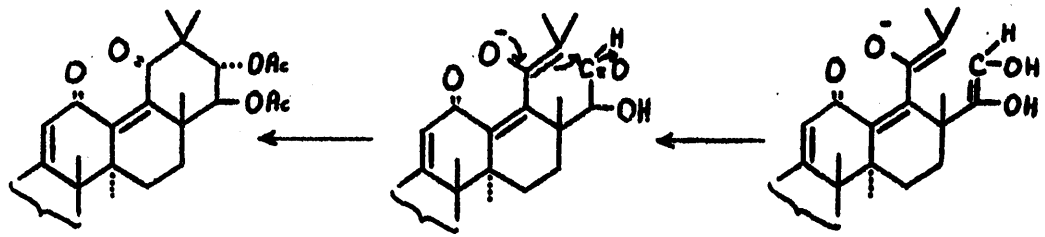
located at $C_{(21)}$ and $C_{(22)}$ since the ketone could not influence any other site. The α-configuration assigned to this cis-glycol group was based on the isolation of sapogenol A tetra-acetate as the minor product of the oxidation of sapogenol C with osmium tetroxide.[101,103] This is represented as attack at the less hindered β-face of the molecule to form predominantly the stereoisomeric sapogenol A tetra-acetate (CLIV) while attack at the more hindered α-face leads to (CLII).

Smith et al.[103] postulated the conversion of 3β:21α:22α:24-tetra-acetoxy-12:19-dioxo-oleana-9(11):13(18)-diene (CXLVII) into the corresponding 3β:21α:22β:24-tetra-acetoxy derivative (CXLVIII) as a retro-aldol condensation followed by a direct intramolecular aldol condensation in which the axial 22α-substituent is inverted to the more stable equatorial 22β-configuration through enolisation of the intermediate aldehyde (CLV).

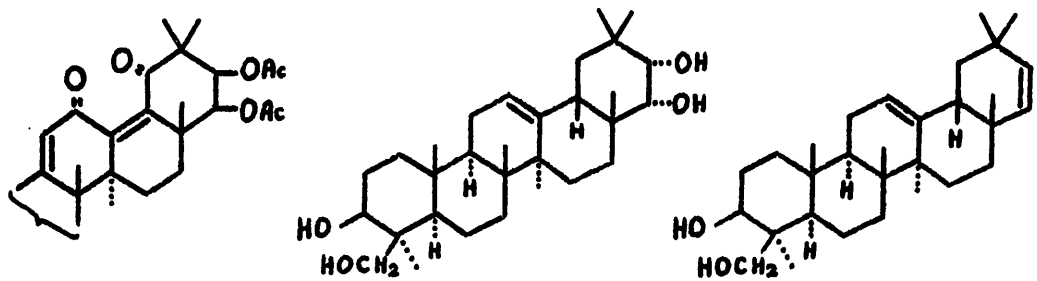
The preparation of the dioxo-dienyl derivative (CLVI) of the stereoisomeric sapogenol A tetra-acetate and its conversion into the same tetra-acetoxy dioxo-diene (CXLVIII) on base treatment lent support to the reaction mechanism outlined above and resulted in the formulation of sapogenol A as (CLVII) and sapogenol C as (CLVIII).



CXLVII CLV



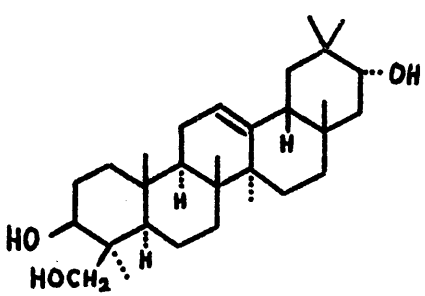
CXLVIII



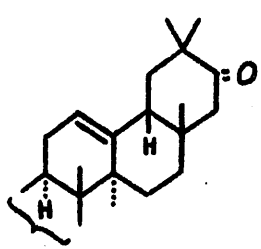
CLVI CLVII CLVIII

A similar study of the dioxo-dienyl derivatives of sapogenol B led Smith et al.[103] to the conclusion that this sapogenol was 3β:21α:24-trihydroxy-olean-12-ene (CLIX). A direct relationship was established between sapogenols B and C when dehydration of the isopropylidene derivative of sapogenol B with phosphorus oxychloride in pyridine and

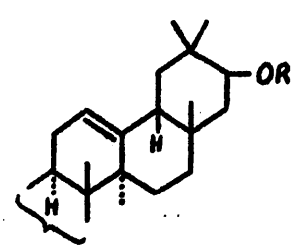
subsequent acid hydrolysis, afforded sapogenol C in good yield. Oxidation of the 3β:24-isopropylidene derivative of sapogenol B gave the 21-ketone (CLX), reduction of which with lithium aluminium hydride afforded a mixture of the 21α- and 21β-alcohols. Chromatography on alumina enabled the above authors[105] to separate this epimeric mixture into its components. Acid hydrolysis and acetylation of the 21β-epimer (CLXI, R = H) thus obtained, yielded the stereoisomeric sapogenol B triacetate (CLXI, R = Ac) which was converted in to its dioxo-diene derivative (CLXII) in the usual way. Base hydrolysis and reacetylation of the latter compound inverted the 21β-substituent to yield 3β:21α:24-triacetoxy-12:19-dioxo-oleana-9(11):13(18)-diene (CLXIII). This compound was also derived from sapogenol B triacetate on vigorous selenium dioxide oxidation.



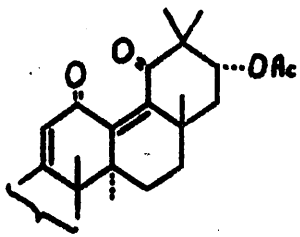
CLIX



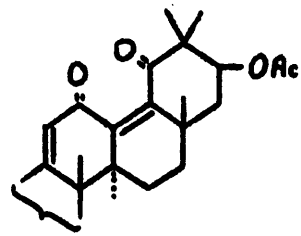
CLX



CLXI



CLXIII

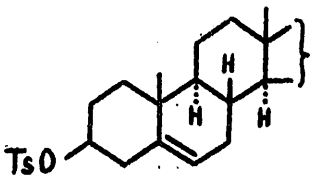


CLXII

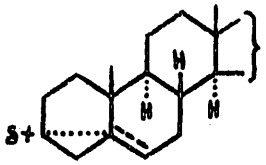
This base induced epimerisation was again represented as a retro-aldol: direct aldol type of condensation whereby an axial substituent is converted into its more stable equatorial form. The mechanism can only hold true if the dioxo-diene structure includes a β-hydroxy system and accordingly, Smith et al.[103] assigned the ring E hydroxyl group in sapogenol B to position 21. The stereochemistry of this group was already known to be axial from dehydration experiments and so led to the structure (CLIX).

Meanwhile, the Swiss group of Jeger et al.[105] had been working further on the problem of sapogenol D. More accurate elemental analyses revised the molecular formula to $C_{31}H_{52}O_3$ and an O-methyl determination indicated the presence of one methoxyl group. In the light of this new evidence, the earlier work on sapogenol D was reviewed and it became apparent that this sapogenol was in fact the methyl ether of the 13(18)-double bond isomer of sapogenol B. However, the

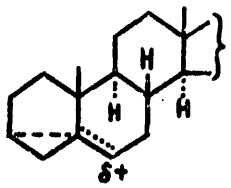
ease with which this ether linkage could be broken suggested that this occurred through a solvolytic process and introduced doubts that the methoxyl group was attached to $C_{(21)}$. Jeger *et al.*[105] proposed that a more likely location would be at the $C_{(22)}$-position where the 13(18)-double bond can participate in the solvolytic process in a manner analogous to cholesteryl tosylate (LXVIII) and similar systems.[56-70]



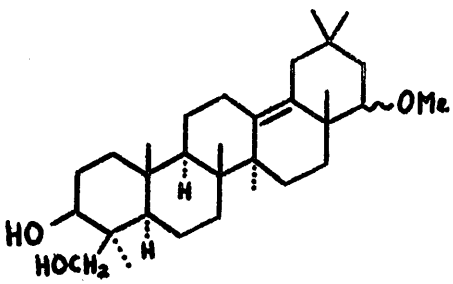
LXVIII



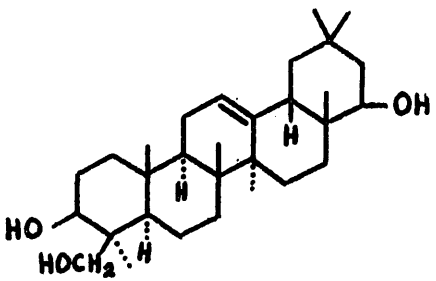
CLXIV



CLXV



CLXVI

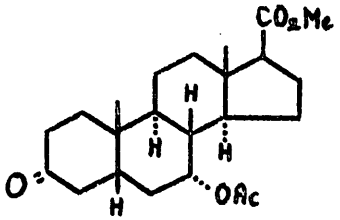


CLXVII

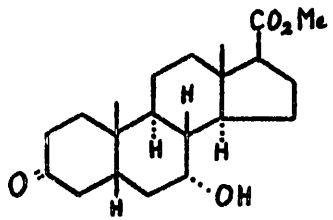
Here the double bond enhances the rate of solvolysis through the formation of a resonating cation (CLXIV) and (CLXV).

From the proposal[105] of structure (CLXVI) for sapogenol D, it subsequently followed that sapogenol B must be 3β:22β:24-trihydroxy-olean-12-ene (CLXVII). To account for the

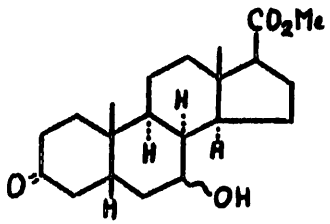
epimerisation of the dioxo-dienyl derivative of the stereo-isomeric sapogenol B triacetate on alkaline hydrolysis, Jeger et al.[105] drew an analogy between this and the formation of the derivatives (CLXIX) and (CLXX) on treatment of methyl 7α-acetoxy-3-oxo-5β-etianate (CLXVIII) with methanolic potassium hydroxide.[108] The corresponding 12-oxo derivative of (CLXVIII) also gave similar products under these conditions.



CLXVIII

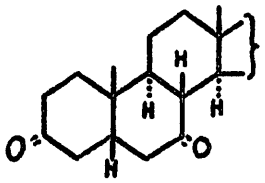


CLXIX

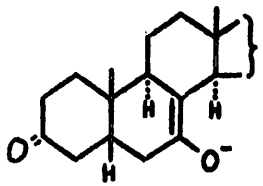


CLXX

The inversion of the $C_{(8)}$-hydrogen atom is, in this case, however, due to a reduction-oxidation type[109,110] of reaction in which the enolate anion (CLXXII) plays its part. The initial step in this epimerisation is apparently oxidation of the 7α-hydroxyl group by the alcohol in presence of its alkoxide in a manner similar to the Oppenauer oxidation.[111] Enolisation of the intermediate keto-compound (CLXXI) results in inversion of the 8β-hydrogen atom and subsequent reduction yields the 5β:8α-derivative (CLXX).



CLXXI

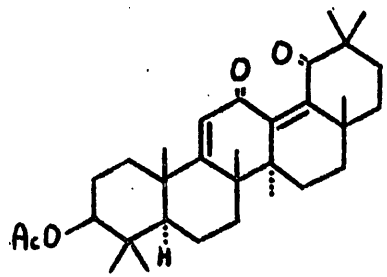


CLXXII

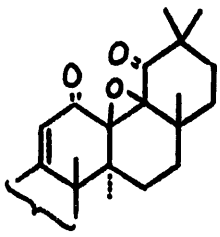
If this reduction-oxidation reaction is the influencing factor in the epimerisation of the stereoisomeric sapogenol B dioxo-dienyl derivative, then inversion of the 22β-acetoxy group should occur whether the 12:19-dioxo-9(11):13(18)-diene chromophore is present or not. Sapogenol B is, however, stable to the basic reaction conditions employed in these epimerisations and consequently the validity of the mechanism proposed by Jeger *et al.*[105] is to be questioned.

In order to differentiate between the position 21 and 22 for the ring E hydroxyl group in sapogenol B, the author attempted to apply to this sapogenol a sequence of reactions which had been fully investigated[112-115] in the β-amyrin, oleanolic acid and glycyrrhetic acid series. Mild chromic acid oxidation of 12:19-dioxo-oleana-9(11):13(18)-dien-3β-yl acetate (CLXXIII) gives a neutral product, the structure of which was established as the 13(18)-oxide derivative (CLXXIV). Vigorous alkaline treatment of the oxide results in cleavage of the 18(19)-carbon to carbon bond, to give the diketo-carboxylic

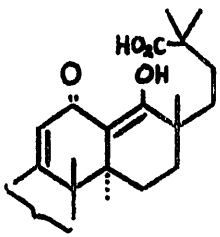
acid (CLXXV). This is a tautomeric mixture existing mainly in the enolic form (CLXXVI) and was characterised as the methyl ester and the methyl ester mono-acetate.



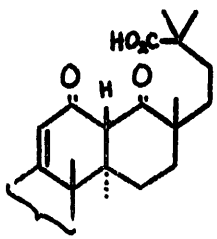
CLXXIII



CLXXIV

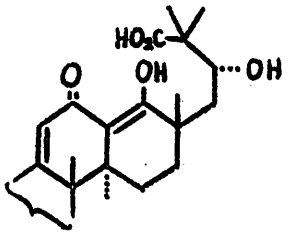


CLXXVI

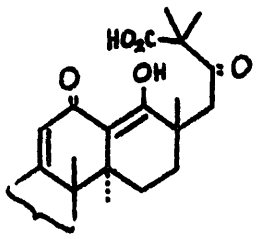


CLXXV

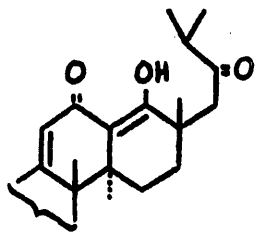
If the third hydroxyl group in sapogenol B were located at $C_{(21)}$, then the final product of the above reaction scheme would be the β-hydroxy acid (CLXXVII). Further oxidation of this material should then yield the β-keto acid (CLXXVIII) which would readily decarboxylate to give the neutral product (CLXXIX). On the other hand, a $C_{(22)}$-hydroxyl group in sapogenol B would result in a stable 22-keto derivative (CLXXXI) on oxidation of the corresponding hydroxy-diketo acid (CLXXX).



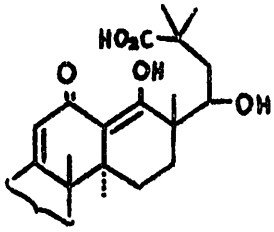
CLXXVII



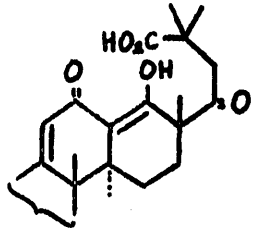
CLXXVIII



CLXXIX



CLXXX



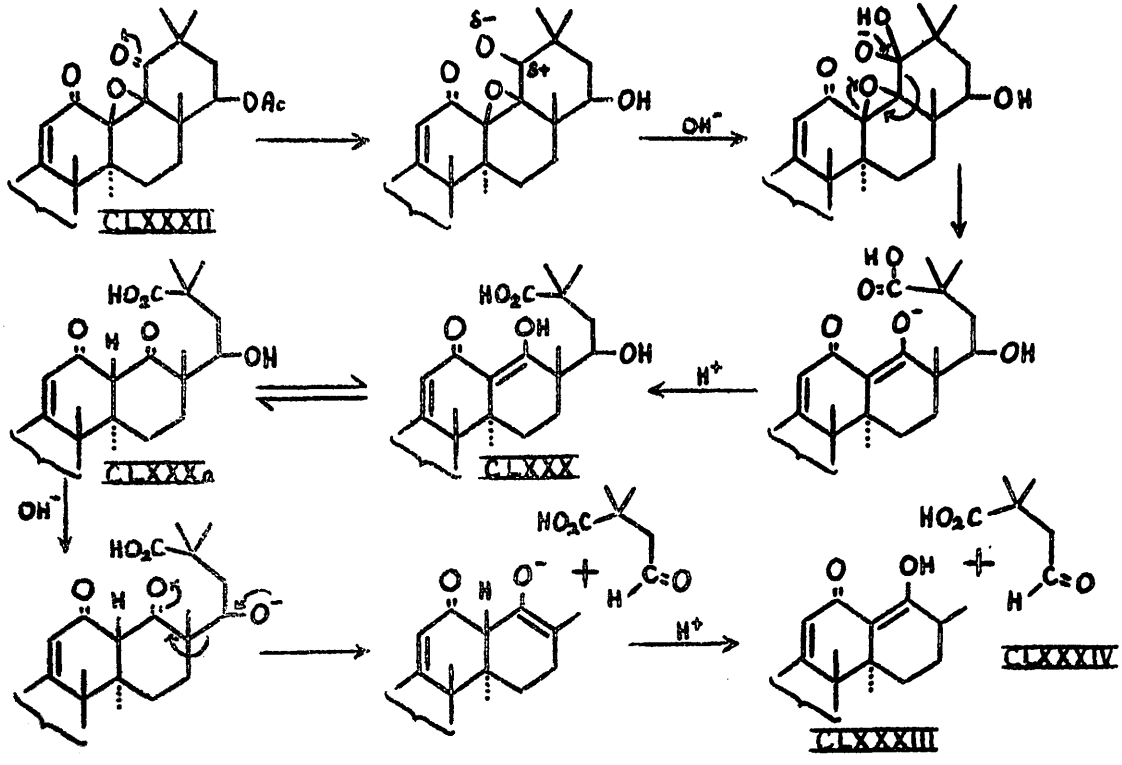
CLXXXI

Oxidation of sapogenol B dioxo-dienyl triacetate with chromium trioxide in acetic acid at 90° gave, in good yield, the 13(18)-oxido derivative (CLXXXII) whose spectral properties, λ max. 2560 Å, agreed well with those of the β-amyradioxo-dienyl oxide (CLXXIV). Treatment of the oxido compound with methanolic potassium hydroxide at 150° for 7 hr. gave a product separated into neutral and acidic fractions, neither of which could be obtained crystalline even after acetylation and chromatography.

As a modification[116] of the base treatment, the 13(18)-oxido compound was refluxed with sodium amyloxide in amyl

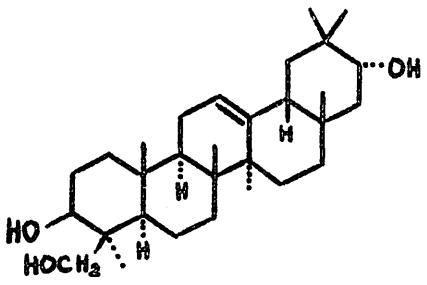
alcohol for 90 min. Again the product was separated into a neutral and an acidic fraction, both of which proved intractable.

In retrospect, it appears likely that cleavage of the 18(19)-bond did occur and that the resultant 22β-hydroxy acid (CLXXX) must have been further broken down into the two fragments (CLXXXIII) and (CLXXXIV) by a retro-aldol mechanism since the β-hydroxy ketone system in (CLXXXa) would not be expected to be stable under the strongly alkaline reaction conditions. The following mechanism can be postulated for the formation of the acid and its degradation products.

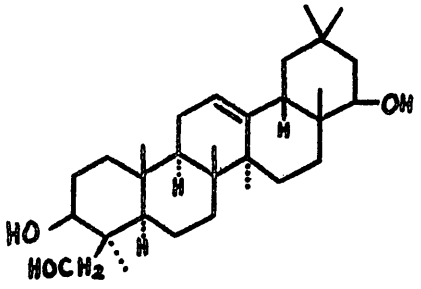


In favour[116] of this argument, it was noted that the infrared spectrum of the neutral fragment exhibited bands at 1670 and 1610 $cm.^{-1}$ and its ultraviolet absorption maximum was located at 2380 Å.

The failure of this method refocussed our attention on the dioxo-dienyl derivatives of sapogenol B triacetate. The structure (CLIX) for sapogenol B was based on the facile interconversion of the epimeric dioxo-dienyl derivatives and on the representation of this epimerisation as a retro-aldol: direct aldol condensation sequence. Jeger et al.[105], on the other hand, favoured the 22β-hydroxy structure (CLXVII) and asserted that epimerisation occurred through a reduction-oxidation type of mechanism. However, it had been shown previously[103] that the 12:19-dioxo-9(11):13(18)-diene chomophore must support this epimerisation since under analogous conditions,



CLIX



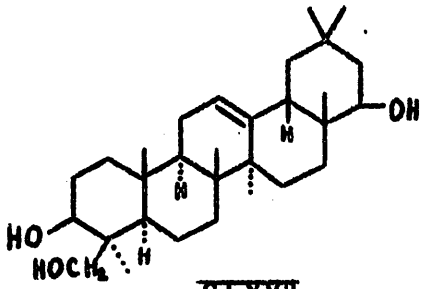
CLXVII

both sapogenol B triacetate and sapogenol A tetra-acetate are recovered unchanged.

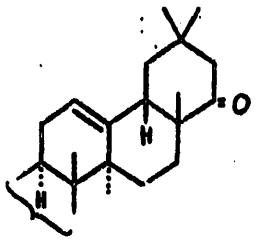
If the former author's conjecture is correct, then reduction of the 13(18)-double bond would not prevent epimerisation, by their mechanism, of a 21β-acetoxy group whereas, if a 22α-acetoxy group were present, no inversion can take place. The results of the following experiments demonstrated that the latter possibility does occur and that accordingly, sapogenol B must be formulated as 3β:22β:24-trihydroxy-olean-12-ene (CLXVII)

Treatment of sapogenol B with acetone and sulphuric acid gave the isopropylidene derivative which was oxidised to the 22-ketone (CLXXXV) with the chromium trioxide-pyridine complex. Reduction of the latter compound with lithium aluminium hydride gave the mixture of stereo-isomers separable by chromatography on alumina into the isopropylidene derivatives of sapogenol B and its 22α-epimer (CLXXXVI). Brief acid treatment of the former, followed by acetylation, yielded sapogenol B triacetate, which was oxidised with selenium dioxide to 3β:22β:24-triacetoxy-12:19-dioxo-oleana-9(11):13(18)-diene (CLXXXVII). Zinc and ethanol reduction of this dioxo-diene gave the 13β:18β-dihydro derivative (CLXXXVIII), m.p. 265-267°, $[\alpha]_D$ + 126°, which on alkaline hydrolysis and reacetylation afforded 3β:22β:24-triacetoxy-12:19-dioxo-18α-olean-9(11)-ene (CLXXXIX),

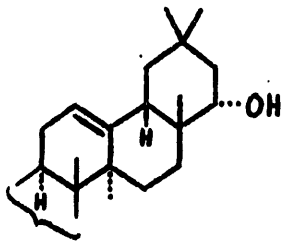
m.p. 305-307°, $[\alpha]_D$ + 53°. The change in the configuration of the $C_{(18)}$ -hydrogen atom was reflected in a displacement of the ultraviolet absorption maximum from 2460 Å to 2420 Å.



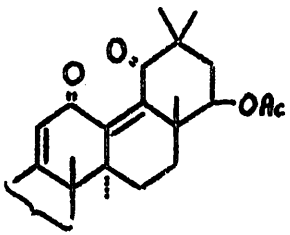
CLXXVII



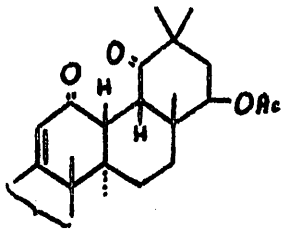
CLXXXV



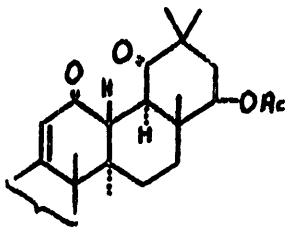
CLXXXVI



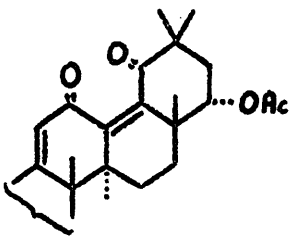
CLXXXVII



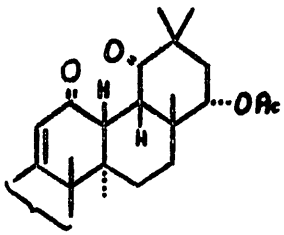
CLXXXVIII



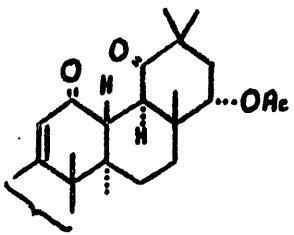
CLXXXIX



CXC



CXCI



CXCII

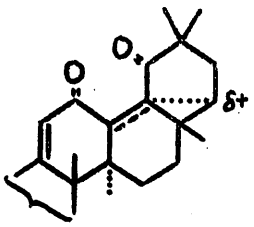
The 22α-hydroxy epimer (CLXXXVI) afforded 3β:22α:24-triacetoxy-12:19-dioxo-oleana-9(11):13(18)-diene (CXC) on brief acid treatment followed by acetylation and selenium dioxide oxidation. The corresponding 13β:18β-dihydro derivative (CXCI), m.p. 234-236°, $[\alpha]_D$ + 90°, was obtained on zinc-ethanol reduction, and alkaline hydrolysis followed by reacetylation of this material gave 3β:22α:24-triacetoxy-12:19-dioxo-18α-olean-9(11)-ene (CXCII), m.p. 215-217°, $[\alpha]_D$ + 75°. Again, inversion of the $C_{(18)}$-hydrogen atom resulted in a hypsochromic shift of the ultraviolet absorption maximum from 2450 Å. to 2420 Å.

Since the final products (CLXXXIX) and (CXCII) were not identical either with each other or with (CLXXXVIII) and (CXCI), it followed that not only had inversion of the $C_{(18)}$-hydrogen atom occurred but that the 22-acetoxy group had retained its configuration throughout in each case.

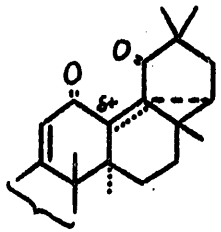
With the formulation of sapogenol B as 3β:22β:24-trihydroxy-olean-12-ene (CLXVII), the mechanism by which the dioxo-dienyl derivative (CXC) of epimeric sapogenol B triacetate is converted into its isomer (CLXXXVII) on base treatment, becomes somewhat obscure. The influence of the 13(18)-double bond on the epimerisation is clearly seen from the experiments outlined above and one possibility that suggests itself, is that the double bond activated the 22-acetoxy group in a manner

similar to that in the i-sterol series[56-70] and in the solvolysis reactions[101,105] of sapogenol D.

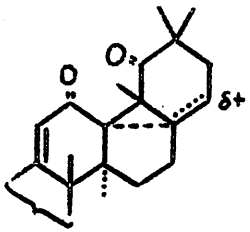
In this, a carbonium ion having a cyclopropane bridge is set up and stabilisation occurs through the resonance forms (CXCIII) and (CXCIV) in which the cyclopropane bridge is in conjugation with the ketone group. The structure (CXCV) may also help to stabilise the carbonium ion. There are several objections to this mechanism, not the least of which is, that the electrons of the 13(18)-double bond are closely held in the heavily conjugated dioxo-diene chromophore and are unlikely to participate in such a process. Furthermore, the epimerisation is carried out in a basic medium where anion (CXCVI) rather than carbonium ion, formation is to be expected.



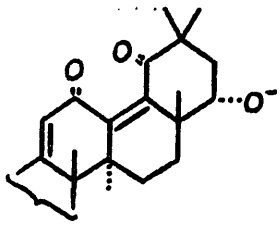
CXCIII



CXCIV



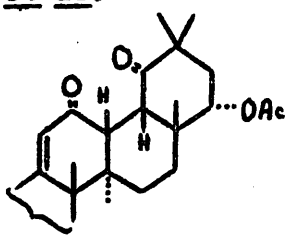
CXCV



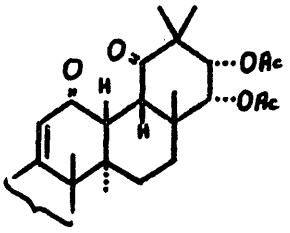
CXCVI

An alternative and a much more likely explanation is that the epimerisation is the result of a modified reduction-oxidation type of reaction. The planarity induced in the molecule by the 12:19-dioxo-9(11):13(18)-diene grouping imposes a considerable amount of strain, which can be partially relieved by inversion of the 22-acetoxy group. It is suggested that due to this strained state of the molecule, the kinetic energy requirements necessary to initiate this epimerisation are lowered to such an extent thatthe reaction conditions employed can supply them. In the 13β:18β-dihydro-derivative (CXCI), on the other hand, saturation of the 13(18)-double bond leads to a relatively strain-free structure which would require the application of more energy than the system can provide, to accomplish the epimerisation. The failure of sapogenol B to epimerise can be explained on similar terms.

One objection is, that the dihydro-dioxo-dienyl derivative (CXLIX) of sapogenol A tetra-acetate is known[103] to epimerise on alkaline hydrolysis. Here however, there is no reason to suppose that this inversion cannot follow the course proposed by Smith *et al.*[103]

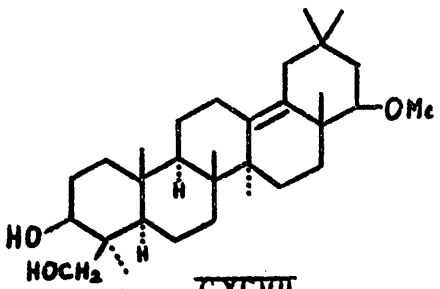


CXCI

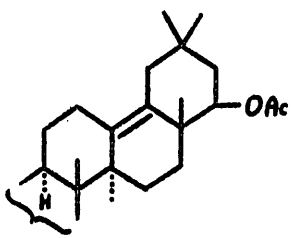


CXLIX

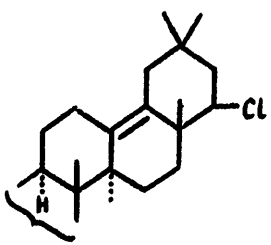
From the formulation of sapogenol B as (CLXVII), it naturally followed that sapogenol D had to have the structure of a 3β:24-dihydroxy-22β-methoxy-olean-13(18)-ene (CXCVII). The attachment of the methoxyl group in the β-configuration was reasoned from the solvolytic reactions of sapogenol D. Thus, 3β:22β:24-triacetoxy-olean-13(18)-ene (CXCVIII) can be prepared from this sapogenol either through the chloro derivative (CXCIX) or directly from the sapogenol itself.[101] This connotes an equatorial (β) configuration for the methoxyl group since it had been shown in the cholesterol series that elimination[63,65] to give the diene (CC) occurred when the substituent was attached axially.



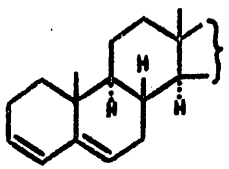
CXCVII



CXCVIII



CXCIX



CC

EXPERIMENTAL

Grade II alumina and light petroleum, b.p. 60-80°, were used for chromatography. The melting points were determined in the Kofler apparatus, except for those marked (C) which were determined in open capillaries. Specific rotations were measured in chloroform solution in a 1 dm. tube. Ultraviolet absorption spectra were measured in ethanol solution, using a Unicam S.P. 500 and a Hilger H.700.307 spectrophotometer. Infrared absorption spectra were measured by Dr. G. T. Newbold, Miss N. Caramando and Miss J. Goldie. Thanks are due to Dr. A. C. Syme and Mr. Wm. McCorkindale of the Royal College of Science and Technology, Glasgow, C.1., for the micro-analyses.

I. Glutinone.

Extraction of Alder Bark. - Finely chopped bark (2.72 kg.) was extracted (5 x 15 hr.) with boiling light petroleum (5 x 5 l.) and evaporation of the solvent from the combined extracts gave a dark brown resinous solid (93 g.). A solution of the resin (164.5 g.) from two batches of the bark (total weight, 5.45 kg.) in light petroleum (2 l.) was chromatographed on alumina (3.5 kg.). Light petroleum (22.5 l.) and light petroleum-benzene (9:1, 3 l.) eluted intractable gums (3.2 g.) whereafter light petroleum-benzene mixtures yielded fractions (50.6 g.) which were

crystallised from chloroform-methanol to give blades, m.p. 210-235°. Several recrystallisations of this material yielded the glutinone - taraxerone mixture (9 g.) as blades, m.p. 236-240°, $[\alpha]_D$ + 25° (c,1.6). From the mother liquors was isolated a further quantity of glutinone - taraxerone (2.5 g.) and lupenone (9 g.) as prismatic needles, m.p. and mixed m.p. 171-172°, $[\alpha]_D$ + 57° (c,2.4). A yellow gum which contaminated the crystalline material was saponified and chromatographed to yield a small amount of lupenone (0.5 g.).

Benzene (6 l.) and benzene-ether mixtures (22.5 l.) eluted intractable coloured gums (10 g.) then benzene-ether (1:1, 3.75 l.) eluted fractions (2.31 g.) which were recrystallised several times from chloroform-methanol to give epiglutinol (1.5 g.) as plates, m.p. and mixed m.p. 210-211°, $[\alpha]_D$ + 63° (c,1.2). (see p.109). Acetylation of the alcohol with pyridine and acetic anhydride gave the corresponding acetate (55 mg.), m.p. and mixed m.p. 192-194°, $[\alpha]_D$ + 78° (c,2.4). Oxidation with pyridine - chromium trioxide at room temperature and crystallisation of the product from chloroform-methanol gave glutinone as blades, m.p. and mixed m.p. 244-246°, $[\alpha]_D$ + 31° (c,1.1).

Continued elution of the column with ether and ether-methanol (9:1, 3 l.) yielded fractions (48.3 g.) whose melting points enabled them to be grouped into three larger fractions -

fraction A (m.p. 160-210°), fraction B (m.p. 185-245°) and fraction C (m.p. 130-190°).

Isolation of Lupeol. - The crystalline material from fraction A was collected, and its solution in light petroleum (250 c.c.) chromatographed on alumina (300 g.). Light petroleum - benzene (2:1, 3 l.) eluted a fraction (0.54 g.) which was recrystallised from chloroform-methanol to yield epiglutinol as plates, m.p. and mixed m.p. 210-211°, $[\alpha]_D$ + 62° (c,2.2).

Elution with light petroleum-benzene (1:1, 6.5 l.) afforded a mixture of alcohols (1.77 g.) which could not be separated by crystallisation. The same solvent mixture (1:1, 7.5 l. and 1:2, 5 l.) yielded fractions (2.48 g.) which after several recrystallisations from chloroform-methanol gave lupeol (0.79 g.) as needles, m.p. and mixed m.p. 215-217°, $[\alpha]_D$ + 28° (c,1.64). Treatment of the alcohol (72 mg.) with pyridine-acetic anhydride (1:1) on the steam-bath for 30 min. yielded lupenyl acetate (53 mg.), m.p. and mixed m.p. 219-220°, $[\alpha]_D$ + 46° (c,1.0). Oxidation of the alcohol (330 mg.) with chromium trioxide and pyridine at room temperature overnight gave lupenone (from chloroform-methanol) as needles, m.p. and mixed m.p. 172-173°, $[\alpha]_D$ + 58° (c,1.95).

Light petroleum-benzene (1:2, 4 l.) eluted an inseparable

mixture of alcohols before the same solvent mixture (7.5 l.) eluted fractions (1.14 g.) which were recrystallised from chloroform-methanol to yield taraxerol as plates, m.p. and mixed m.p. 283-285°, $[\alpha]_D \pm 0°$ (c,0.6).

Isolation of Taraxerol. - The top crops from fraction B were recrystallised from aqueous acetone and then several times from chloroform-methanol to give taraxerol (2.3 g.) as plates, m.p. and mixed m.p. 283-285°, $[\alpha]_D \pm 0°$ (c,0.5).

The alcohol (100 mg.) was treated with pyridine-acetic anhydride (1:1) at 100° for 1 hr. and the product crystallised from chloroform-methanol to give taraxeryl acetate (82 mg.) as plates, m.p. and mixed m.p. 304-306°, $[\alpha]_D + 10°$ (c,0.8).

Oxidation of the alcohol (95 mg.) with the pyridine-chromium trioxide complex and crystallisation of the product from chloroform-methanol yielded taraxerone (47 mg.) as plates, m.p. and mixed m.p. 242-244°, $[\alpha]_D + 8°$ (c,0.7).

Isolation of β-Sitosterol. - Several recrystallisations of the solid material from fraction C from aqueous acetone and then from chloroform-methanol yielded β-sitosterol as blades, m.p. and mixed m.p. 138-140°, $[\alpha]_D - 28°$ (c,1.0).

Treatment of the alcohol (105 mg.) with pyridine and acetic anhydride for 1 hr. on the steam-bath and crystallisation of the product from chloroform-methanol gave β-sitosteryl

acetate as lustrous plates, m.p. and mixed m.p. 128-130°, $[\alpha]_D$ - 37° (c,1.3).

Isolation of Betulin. - The mother liquors of fractions A, B and C were combined, evaporated to dryness and a solution of the residue (15.9 g.) in light petroleum-benzene (4:1, 750 c.c was adsorbed on a column or alumina (300 g.). Elution with light petroleum-benzene mixtures (18 l.) yielded a mixture of alcohols (4.9 g.) from which, after repeated recrystallisation, was isolated a small amount (130 mg.) of taraxerol, m.p. and mixed m.p. 284-286°, $[\alpha]_D \pm 0°$ (c,0.6).

Light petroleum-benzene (1:1, 8.5 l.) eluted an intractable gum (1.6 g.). The same solvent mixture (1:2, 4.5 l.) and benzene (7.5 l.) then eluted fractions (2.4 g.) which were crystallised from chloroform-methanol to yield betulin (485 mg.) as needles, m.p. and mixed m.p. 253-256°, $[\alpha]_D$ + 20° (c,1.8). Acetylation in the usual manner gave betulin diacetate as needles, m.p. and mixed m.p. 223-224°, $[\alpha]_D$ + 21.5° (c,0.8).

No further crystalline material was eluted from the column.

Glutin-5-en-3α-yl Acetate. - A suspension of the glutinone-taraxerone mixture (11.5 g.) in dry ether (900 c.c.) was refluxed with lithium aluminium hydride (2.1 g.) for 45 min. The mixed alcohols, isolated in the usual way, were acetylated

with pyridine and acetic anhydride at 100° for 2 hr., and the product fractionally crystallised from chloroform-methanol to yield plates. These were recrystallised several times from chloroform-methanol to give taraxeryl acetate (2.1 g.) as plates, m.p. 303-305°, $[\alpha]_D$ + 11° (c, 1.2).

Concentration of the mother liquors afforded needles which were repeatedly recrystallised from chloroform-methanol to yield pure glutinyl acetate (glutin-5-en-3α-yl acetate) (5.7 g.), m.p. and mixed m.p. 235-236°, $[\alpha]_D$ + 46° (c, 1.8).

Glutin-5-en-3α-ol. - A suspension of glutin-5-en-3α-yl acetate (6 g.) in dry ether (500 c.c.) was refluxed gently with lithium aluminium hydride (2.5 g.) for 30 min. and the product crystallised from methanol to yield glutin-5-en-3α-ol (4.3 g.) as needles, m.p. and mixed m.p. 201-203°, $[\alpha]_D$ + 61° (c, 1.4).

Glutin-5-en-3-one. - A solution of glutin-5-en-3α-ol (3.1 g.) in dry pyridine (30 c.c.) was treated with the chromium trioxide-pyridine complex (4 g. in 40 c.c.) at room temperature for 16 hr. The product, isolated in the usual manner, was crystallised from chloroform-methanol to yield glutin-5-en-3-one (2.6 g.) as plates, m.p. and mixed m.p. 244-246°, $[\alpha]_D$ + 31° (c, 1.8).

Glutin-5-en-3β-ol.- A mixture of glutinone (950 mg.) and aluminium isopropoxide (1.25 g.) in absolute isopropanol (12.5 c.c.) was distilled slowly, with the addition of isopropanol

to maintain constant volume. After 4½ hr., the distillate no longer contained acetone and the solution was evaporated to dryness. The product, isolated in the usual way by means of ether, was dissolved in light petroleum (30 c.c.) and chromatographed on alumina (30 g.). Elution with light petroleum-benzene (2:1, 1150 c.c.) yielded fractions (246 mg.) which crystallised from chloroform-methanol to give glutin-5-en-3β-ol (epiglutinol)(see p.104) as needles, m.p. 210.5-211.5°, $[\alpha]_D$ + 64° (c,0.9) (Found: C,84.2; H,12.0. $C_{30}H_{50}O$ requires C,84.4; H,11.8%). A mixture with glutin-5-en-3α-ol had m.p. 194-196°.

Continued elution with the same solvent mixture (1500 c.c.) yielded mixtures whereafter light petroleum-benzene (3500 c.c.) gave fractions (460 mg.) which crystallised from chloroform-methanol to yield glutin-5-en-3α-ol as needles, m.p. and mixed m.p. 201-203°, $[\alpha]_D$ + 62° (c,1.9).

Glutin-5-en-3β-yl Acetate. - Glutin-5-en-3β-ol (109 mg.) was treated with pyridine-acetic anhydride (1:1) at 100° for 90 min. The product, isolated in the usual manner, crystallised from chloroform-methanol to give glutin-5-en-3β-yl acetate (77 mg.) as plates, m.p. 192-194°, $[\alpha]_D$ + 79° (c,1.1) $\lambda_{max.}$ 2050 Å. (ε 4050) (Found: C,82.25; H,11.4. $C_{32}H_{52}O_2$ requires C,82.0; H,11.2%).

Hydrolysis of the acetate (34 mg.) with lithium aluminium hydride (50 mg.) in dry ether (20 c.c.) gave glutin-5-en-3β-ol which crystallised from chloroform-methanol as needles, m.p. and mixed m.p. 210-211°, $[\alpha]_D$ + 63° (c, 0.9)

Oxidation of Glutin-5-en-3β-ol.- The yellow complex from chromium trioxide (100 mg.) and pyridine (1 c.c.) was added to a solution of glutin-5-en-3β-ol (32 mg.) in pyridine (0.5 c.c.) and the mixture kept at room temperature for 17 hr. A solution of the product, isolated in the usual way, in light petroleum (10 c.c.) was filtered through a column of alumina (5 g.) and the column eluted with the same solvent, (300 c.c.). The eluate crystallised from chloroform-methanol to give glutin-5-en-3-one as plates, m.p. and mixed m.p. 244-245°, $[\alpha]_D$ + 31° (c, 1.2).

Glutin-5(10)-en-3-one. - A solution of glutin-5-en-3-one (1.5 g.) in glacial acetic acid (750 c.c.) was treated with concentrated hydrochloric acid (18 c.c.) and the mixture heated on the steam-bath for 20 hr. The solvent was removed under reduced pressure and the product, isolated by means of ether, crystallised several times from chloroform-methanol to yield glutin-5(10)-en-3-one (790 mg.) as plates, m.p. 251-253°, $[\alpha]_D$ -91.5° (c, 1.5).

Glutin-5(10)-en-3β-yl Acetate. - Lithium aluminium hydride (450 mg.) was added to a suspension of glutin-5(10)-en-3-one (750 mg.) in dry ether (200 c.c.) and kept at room temperature for 1 hr. The product, isolated in the usual way, was treated with pyridine-acetic anhydride (1:1, 10 c.c.) at 100° for 30 min. and the crystals which separated on cooling, were collected (mother liquor A) and recrystallised from chloroform-methanol to give glutin-5(10)-en-3β-yl acetate (450 mg.) as plates, m.p. 297-299°, $[\alpha]_D$ - 23° (c,1.7). Beaton et al.[38] give m.p. 290-293°, $[\alpha]_D$ - 27° for 'alnus-5(10)-en-3ξ-yl acetate'.

A suspension of glutin-5(10)-en-3β-yl acetate (55 mg.) in dry ether (25 c.c.) was treated with lithium aluminium hydride (50 mg.) and refluxed for 5 min. The product was isolated in the usual manner and crystallised from light petroleum, to give glutin-5(10)-en-3β-ol as needles, m.p. 244-245°, $[\alpha]_D$ -42.5° (c,0.8). Beaton et al.[38] give m.p. 241-242°, $[\alpha]_D$ - 42° for 'alnus-5(10)-en-3ξ-ol'. (Found: C,84.35; H,11.5. Calc. for $C_{30}H_{50}O$. C,84.4; H,11.8).

Glutin-5(10)-en-3α-yl Acetate. - The pyridine-acetic anhydride mother liquor A (above) was diluted with water and extracted with ether. The extracted solid (225 mg.) crystallised from chloroform-methanol to give glutin-5(10)-en-3α-yl acetate (50 mg.) as needles, m.p. 209-210°, $[\alpha]_D$ - 49°

(c,1.4) $\lambda_{max.}$ 2050 Å. (ε 5100); (Found: C,82.0; H,11.5. $C_{32}H_{52}O_2$ requires C,82.0; H,11.2%).

Treatment of glutin-5(10)-en-3α-yl acetate (45 mg.) with lithium aluminium hydride (50 mg.) and crystallisation of the product from chloroform-methanol gave glutin-5(10)-en-3α-ol as plates, m.p. 258-259°, $[\alpha]_D$ - 42° (c,1.6) $\lambda_{max.}$ 2060 Å. (ε 4300) (Found: C,83.4; H,11.9. $C_{30}H_{50}O$, 1/3MeOH requires C,83.4; H,11.8%). A mixture with glutin-5(10)-en-3β-ol had m.p. 238-242°.

Glutin-5(10)-en-3-one. - (a) A solution of glutin-5(10)-en-3α-ol (38 mg.) in pyridine (1 c.c.) was added to the complex from chromium trioxide (40 mg.) and pyridine (0.5 c.c.) and the mixture kept at room temperature for 15 hr. A solution of the product, isolated in the usual way, in light petroleum (50 c.c.) was chromatographed on alumina (6 g.). Elution with the same solvent (300 c.c.) yielded a fraction (30 mg.) which crystallised from chloroform-methanol to give glutin-5(10)-en-3-one as plates, m.p. and mixed m.p. 251-253°, $[\alpha]_D$ - 97° (c,1.3).

(b) A solution of glutin-5(10)-en-3β-ol (55mg.) in pyridine (0.5 c.c.) was treated with the chromium trioxide-pyridine reagent (50 mg. in 0.5 c.c.) for 4 days at room temperature. The product, isolated by means of ether, was dissolved in light

petroleum (25 c.c.) and filtered through a column of alumina (5 g.). Light petroleum (500 c.c.) eluted a fraction (30 mg.) which crystallised from chloroform-methanol to give glutin-5(10)-en-3-one as plates, m.p. and mixed m.p. 251-253°, $[\alpha]_D$ - 96° (c,1.6°).

Glutina-1(10):5-dien-3α-yl Acetate.-(a) Selenium dioxide (1.0 g.) in water (1 c.c.) and acetic acid (25 c.c.) was added to a solution of glutin-5-en-3α-yl acetate (1.0 g.) in glacial acetic acid (250 c.c.) and the mixture warmed at 60-70° for 1 hr. A solution of the product, isolated in the usual way, in light petroleum (100 c.c.) was adsorbed on a column of alumina (30 g.). Elution with the same solvent (500 c.c.) gave a fraction (868 mg.) which crystallised from chloroform-methanol to yield glutina-1(10):5-dien-3α-yl acetate as needles, m.p. 164-165°, $[\alpha]_D$ + 33° (c,1.5) $\lambda_{max.}$ 2330 and 2380 Å. (ε16,500 and 17,000); inflection at 2480 A. (ε 10,500).

(b) A solution of glutin-5(10)-en-3α-yl acetate (35 mg.) in glacial acetic acid (25 c.c.) was refluxed with selenium dioxide (35 mg.) in water (0.5 c.c.) for 5 hr. The product, isolated in the usual way, was chromatographed in light petroleum (5 c.c.) on a column of alumina (5 g.). The same solvent (150 c.c.) eluted fractions which crystallised from chloroform-methanol to give glutina-1(10):5-dien-3α-yl acetate as needles, m.p. and mixed m.p. 164-166°, $[\alpha]_D$ + 35° (c,0.5) $\lambda_{max.}$ 2310 and 2380 Å. (ε15,000 and 17,000); point of inflection at 2480 Å. (ε 10,000).

Glutina-1(10):5-dien-3α-ol. - A solution of the dienyl acetate (392 mg.) in dry ether (100 c.c.) was refluxed with lithium aluminium hydride (400 mg.) for 25 min. The product was worked up in the usual manner and crystallised from chloroform methanol to give glutina-1(10):5-dien-3α-ol (295 mg.) as prismatic needles, m.p. and mixed m.p. 198-200°, $[\alpha]_D$ + 84° (c,1.6) $\lambda_{max.}$ 2330 and 2400 Å. (ε16,000 and 18,000); point of inflection at 2480 Å. (ε 11,000). Beaton et al.[38] give m.p. 195-197°, $[\alpha]_D$ + 83°.

Glutina-1(10):5-dien-3β-yl Acetate. - (a) A solution of glutin-5-en-3β-yl acetate (82 mg.) in glacial acetic acid (20 c.c.) was treated with selenium dioxide (80 mg.) in water (0.1 c.c.) and acetic acid (2 c.c.) and kept at 60-70° for 1hr. The filtered solution was evaporated to dryness and a solution of the residue in light petroleum (5 c.c.) was adsorbed on alumina (10 g.). Elution with the same solvent (750 c.c.) yielded fractions which crystallised from chloroform-methanol to give glutina-1(10):5-dien-3β-yl acetate (68 mg.) as blades, m.p. 209-210°, $[\alpha]_D$ + 112.5° (c,1.1) $\lambda_{max.}$ 2320 and 2380 Å. (ε 15,000 and 16,500); point of inflection at 2470 Å. (ε 10,000); (Found: C,82.3; H,10.9. $C_{32}H_{50}O_2$ requires C,82.3; H,10.8%). It gives a deep orange colour with tetranitromethane.

(b) A solution of selenium dioxide (100 mg.) in water (0.1 c.c.) was mixed with a solution of glutin-5(10)-en-3β-yl acetate

(100 mg.) in acetic acid (50 c.c.). After 3 hr. refluxing, the solvent was removed and a solution of the product in light petroleum (10 c.c.) was chromatographed on alumina (8 g.). Crystallisation of the fractions eluted by light petroleum (175 c.c.) from chloroform-methanol gave glutina-1(10):5-dien-3β-yl acetate (75 mg.) as plates, m.p. and mixed m.p. 209-210°, $[\alpha]_D$ + 112° (c,1.0) $\lambda_{max.}$ 2310 and 2380 Å. (ε 16,500 and 18,000); point of inflection at 2460 Å. (ε 11,000).

Glutina-1(10):5-dien-3β-ol. - A solution of the dienyl acetate (43 mg.) in dry ether (15 c.c.) was refluxed with lithium aluminium hydride (100 mg.) for 30 min. The product was dissolved in light petroleum-benzene (2:1, 15 c.c.) and chromatographed on alumina (5 g.). Benzene-ether (20:1, 100 c.c.) eluted a fraction (36 mg.) which crystallised from chloroform-methanol to give glutina-1(10):5-dien-3β-ol as needles, m.p. 195-197°, $[\alpha]_D$ + 110° (c,0.6) $\lambda_{max.}$ 2320 and 2390 Å. (ε 15,000 and 16,000); point of inflection at 2470 Å (ε 10,000); (Found: C,84.9; H,11.7. $C_{30}H_{48}O$ requires C,84.8; H,11.4%).

3α-2'-Tetrahydropyranyloxyglutina-1(10):5-diene. - p-Toluene sulphonic acid (0.5 mg.) was added to a solution of glutina-1(10):5-dien-3α-ol (345 mg.) in benzene (15 c.c.) and dihydropyran (1.5 c.c.) and the mixture kept at room temperature for 16 hr. A solution of the product, isolated in the usual way,

in light petroleum (40 c.c.) was chromatographed on alumina (30 g.). The fractions eluted by light petroleum (300 c.c.) crystallised from chloroform-methanol to give 3α-2'-tetrahydropyranyloxyglutina-1(10):5-diene as needles (after gelation), m.p. 171-173°, $[\alpha]_D$ + 70° (c,1.6) $\lambda_{max.}$ 2340 (inflex.), 2400 and 2480 (inflex.) Å. (ε 13,500, 14,500 and 9,600); (Found: C,82.9; H,11.3. $C_{35}H_{56}O_2$ requires C,82.6; H,11.1%).

Glutin-5(10)-en-3α-ol. - (a) Lithium (300 mg.) was added to a solution of 3α-2'-tetrahydropyranyloxyglutina-1(10):5-diene (285 mg.) in ether (150 c.c.) and liquid ammonia (300 c.c.) and the mixture stirred for 1 hr. Ethanol (10 c.c.) was added and the product, isolated in the usual way, was refluxed in benzene (10 c.c.), ethanol (20 c.c.) and concentrated hydrochloric acid (0.25 c.c.) for 90 min.; the hydrolysed product was then chromatographed in light petroleum (20 c.c.) on alumina (10 g.). Elution with light petroleum-benzene (1:6, 425 c.c.) yielded fractions which crystallised from chloroform-methanol, to give glutin-5(10)-en-3α-ol as plates, (95 mg.), m.p. and mixed m.p. 258-259°, $[\alpha]_D$ - 42° (c,1.6) $\lambda_{max.}$ 2040 Å. (ε4300). This alcohol with pyridine and acetic anhydride at 100° gave its acetate as needles, m.p. and mixed m.p. 209-210°, $[\alpha]_D$ - 49° (c,1.4).

(b) Lithium (150 mg.) was added to a solution of glutina-1(10):5-dien-3α-yl acetate (100 mg.) in ether (50 c.c.) and liquid

ammonia (100 c.c.) and the mixture stirred for 1 hr. After addition of ethanol (10 c.c.), the ammonia was allowed to evaporate. The product, isolated in the usual way, was acetylated with pyridine and acetic anhydride and chromatographed on alumina (10 g.). Elution with light petroleum (175 c.c.) yielded a fraction (15 mg.) which after three recrystallisations from chloroform-methanol gave glutin-5(10)-en-3α-yl acetate as needles, m.p. and mixed m.p. 207-208°, $[\alpha]_D$ - 48° (c,0.3).

(c) Potassium (6.5 g.) was added to a solution of glutina-1(10):5-dien-3α-ol (100 mg.) in tert.-butanol (100 c.c.), the mixture was heated at 100° for 18 hr., then diluted with water, and the product isolated in the usual way and chromatographed in light petroleum-benzene (9:1, 15 c.c.) on alumina (10 g.), Elution with this solvent mixture (125 c.c.) yielded fractions which crystallised from chloroform-methanol, to give glutin-5(10)-en-3α-ol as plates (12 mg.), m.p. 247-251°, $[\alpha]_D$ - 36° (c,0.7), raised to m.p. and mixed m.p. 254-256° on recrystallisation. A mixture of the alcohol and glutin-5(10)-en-3β-ol had m.p. 235°.

Conversion of Glutin-5-en-3β-yl Acetate into Glutin-5(10)-en-3β-yl Acetate. - Dry hydrogen chloride was passed through a solution of glutin-5-en-3β-yl acetate (86 mg.) in chloroform (10 c.c.) for 5 hr. The product crystallised from chloroform-methanol to give glutin-5(10)-en-3β-yl acetate (60 mg.) as plates, m.p. and mixed m.p. 298-300°, $[\alpha]_D$ - 24° (c,1.0).

Glutin-5-ene. - Glutin-5-en-3-one (1.06 g.), sodium methoxide solution (from 700 mg. sodium and 40 c.c. dry methanol) and hydrazine hydrate (100%, 5 c.c.) were heated in an autoclave at 200° for 12 hr. The product, purified by filtration through alumina, crystallised from chloroform-methanol to give glutin-5-ene (778 mg.) as blades, m.p. and mixed m.p. 180-182°, $[\alpha]_D$ + 57° (c, 1.5). Beaton et al.[37] give m.p. 181-181.5°, $[\alpha]_D$ + 56°, for 'alnusene'.

Glutina-1(10):5-diene. - Selenium dioxide (100 mg.) in water (0.1 c.c.) and acetic acid (3 c.c.) was added to a solution of glutin-5-ene (104 mg.) in acetic acid (25 c.c.) and the mixture kept at 100° for 1 hr. The filtered solution was evaporated to dryness and a solution of the product in light petroleum (10 c.c.) was chromatographed on alumina (8 g.). Light petroleum (50 c.c.) eluted fractions which crystallised from chloroform-methanol to give glutina-1(10):5-diene (64 mg.) as plates, m.p. 182-183°, $[\alpha]_D$ + 127° (c, 1.6) $\lambda_{max.}$ 2340 and 2400 Å. (ε 14,500 and 15,500); point of inflection at 2470 Å. (ε10,000). (Found: C, 88.5; H, 11.9. $C_{30}H_{48}$ requires C, 88.2; H, 11.8%).

Acid Treatment of Glutin-5-en-3α-yl Acetate. - (a) Dry hydrogen chloride was passed through a solution of glutin-5-en-3α-yl acetate (100 mg.) in chloroform (10 c.c.) for 2 hr. The product was isolated in the usual manner and crystallised from chloroform-methanol to yield unchanged starting material, m.p.

and mixed m.p. 234-236°, $[\alpha]_D$ + 45° (c,1.7).

(b) Concentrated hydrochloric acid (6 c.c.) was added to a solution of glutin-5-en-3α-yl acetate (500 mg.) in acetic acid (250 c.c.) and the mixture heated on the steam-bath for 18 hr. The solvent was removed under reduced pressure and the product, isolated in the usual way, was dissolved in light petroleum (20 c.c.) and adsorbed on a column of alumina (12 g.). Elution with light petroleum (100 c.c.) yielded an intractable gum (420 mg.) which exhibited absorption in the ultraviolet at 2080, 2410 and 2500 Å; point of inflection at 2570 Å.

Acid Treatment of Glutin-5(10)-en-3β-yl Acetate. - A solution of glutin-5(10)-en-3β-yl acetate (96 mg.) in benzene (5 c.c.) and acetic acid (50 c.c.) was treated with concentrated hydrochloric acid (1.2 c.c.) and the mixture heated at 100° for 20 hr. Crystallisation of the product from chloroform-methanol yielded unchanged glutin-5(10)-en-3β-yl acetate (75 mg.) as plates, m.p. and mixed m.p. 298-300°, $[\alpha]_D$ - 24° (c,1.1).

5β-Glutinan-3α-yl Acetate. - A solution of glutin-5-en-3α-yl acetate (750 mg.) in glacial acetic acid (300 c.c.) was shaken with platinum (from 300 mg. PtO_2) and hydrogen for 20 hr. at room temperature. The product crystallised from chloroform-methanol to give 5β-glutinan-3α-yl acetate (670 mg.) as plates, m.p. 262-263°, $[\alpha]_D$ + 11.5° (c,1.0). Beaton et al.[37]

give m.p. 264-265°, $[\alpha]_D$ + 11.5° for 'alnusanyl acetate'. It shows no selective absorption above 2000 Å. in the ultraviolet.

5β-Glutinan-3α-ol. - A suspension of the acetate (1.4 g.) in dry ether (400 c.c.) was refluxed gently with lithium aluminium hydride (500 mg.) for 30 min. A solution of the product in light petroleum-benzene (4:1, 150 c.c.) was carefully chromatographed on alumina (50 g.) but no difference was observed between the melting points of the first and final fractions. Crystallisation of the fractions from chloroform-methanol yielded 5β-glutinan-3α-ol (985 mg.) as fine needles, m.p. 252-253°, $[\alpha]_D$ + 27.5° (c, 1.8)

5β-Glutinan-3-one.[a] A stirred solution of 5β-glutinan-3α-ol (985 mg.) in benzene (250 c.c.) and stabilised acetic acid (500 c.c.) at room temperature was treated dropwise over a period of 30 min. with a solution of chromium trioxide (143 mg.) in stabilised acetic acid (28.6 c.c.). Stirring was continued for a further 30 min., methanol (10 c.c.) was added, the solvent removed under reduced pressure and a solution of the neutral product in light petroleum (75 c.c.) was chromatographed on alumina (20 g.). Light petroleum (1500 c.c.) and light petroleum-benzene (1:1, 250 c.c.) eluted a fraction (819 mg.) which was crystallised from chloroform-methanol to yield 5β-glutinan-3-one (672 mg.) as needles, m.p. 228-229°, $[\alpha]_D$

+ 51° (c,1.0). Beaton et al.[37] give m.p. 228-230°, $[\alpha]_D$ + 52° for 'alnusanone'.

Elution of the column with ether (250 c.c.) yielded a fraction (194 mg.) which crystallised from chloroform-methanol to give 5β-glutinan-3α-ol as needles, m.p. and mixed m.p. 252-253°, $[\alpha]_D$ + 28° (c,1.2).

(b) 5β-Glutinan-3α-ol (336 mg.) in dry pyridine (16 c.c.) was treated with the chromium trioxide-pyridine complex (446 mg. in 4 c.c.) for 44 hr. at room temperature. The product, isolated in the usual manner, was dissolved in light petroleum (25 c.c.) and adsorbed on a column of alumina (10 g.). Light petroleum (800 c.c.) eluted a fraction (281 mg.) which was recrystallised from chloroform-methanol to yield 5β-glutinan-3-one (210 mg.) as needles, m.p. and mixed m.p. 228-229°, $[\alpha]_D$ + 50.5° (c,0.8).

5β-Glutinan-3β-ol. - A mixture of the saturated ketone (715 mg.) and aluminium isopropoxide (957 mg.) in absolute isopropanol (15 c.c.) was distilled slowly, with the addition of isopropanol to maintain constant volume. After 4 hr., the distillate no longer contained acetone and the reaction mixture was evaporated to dryness. A solution of the product, isolated by means of ether, in light petroleum (100 c.c.) was chromatographed on alumina (30 g.). Light petroleum-benzene (2:1, 2900 c.c.) eluted a fraction (429 mg.) which crystallised from chloroform-methanol to yield 5β-glutinan-3β-ol (295 mg.)

as plates, m.p. 266-268°, $[\alpha]_D$ + 33° (c,1.7); (Found: C,84.3; H,12.3. $C_{30}H_{52}O$ requires C,84.0; H,12.2%).

Elution of the column with light petroleum-benzene (1:1, 1000 c.c.) yielded mixtures. The same solvent mixture (2500 c.c.) then gave fractions (174 mg.) which crystallised from chloroform-methanol to yield 5β-glutinan-3α-ol (145 mg.) as fine needles, m.p. 250-251°, $[\alpha]_D$ + 27° (c,1.1).

5β-Glutinan-3β-yl Acetate. - 5β-Glutinan-3β-ol (104 mg.) was treated with pyridine and acetic anhydride at 100° for 1 hr. The product, isolated in the usual manner, crystallised from chloroform-methanol to give 5β-glutinan-3β-yl acetate (72 mg.) as plates, m.p. 230-232°, $[\alpha]_D$ + 52° (c,1.7) (Found: C,81.9; H,11.7. $C_{32}H_{54}O_2$ requires C,81.6; H,11.6%).

A solution of the acetate (40 mg.) in dry ether (50 c.c.) was refluxed with lithium aluminium hydride (50 mg.) for 30 min. and the product crystallised from chloroform-methanol to yield 5β-glutinan-3β-ol as plates, m.p. and mixed m.p. 266-268°, $[\alpha]_D$ + 32° (c,0.8).

Oxidation of 5β-Glutinan-3β-ol. - A solution of the alcohol (52 mg.) in dry pyridine (0.8 c.c.) was treated with the chromium trioxide-pyridine complex (75 mg. in 3 c.c.) and the mixture kept at room temperature for 13 hr. The product, isolated by means of ether, was dissolved in light petroleum

(25 c.c.) and filtered through a column of alumina (5 g.). Elution with the same solvent (450 c.c.) gave a fraction (29 mg.) which crystallised from chloroform-methanol to yield 5β-glutinan-3-one as needles, m.p. and mixed m.p. 227-229°, $[\alpha]_D$ + 52° (c,1.8).

Hydrogenation of Glutina-1(10):5-dien-3β-yl Acetate. - (a) A solution of glutina-1(10):5-dien-3β-yl acetate (102 mg.) in glacial acetic acid (200 c.c.) was shaken with hydrogen over platinum black catalyst (from 75 mg. PtO_2) for 19 hr. at 60°. The product crystallised from chloroform-methanol to yield glutin-5(10)-en-3β-yl acetate (38 mg.) as plates, m.p. and mixed m.p. 297-299°, $[\alpha]_D$ - 24° (c,1.7). From the mother liquors a material (36 mg.), m.p. 210-222° was isolated which showed no selective absorption above 2000 Å.

(b) A solution of the dienyl acetate (49 mg.) in glacial acetic acid (100 c.c.) was shaken with hydrogen and platinum (from 214 mg. PtO_2) for 18 hr., at 60°. The product was crystallised from chloroform-methanol to yield a material, m.p. 204-214°, which was transparent to ultraviolet light.

The saturated material (78 mg.) in dry ether (50 c.c.) was hydrolysed by treatment with lithium aluminium hydride (100 mg.) and a solution of the product in light petroleum (25 c.c.) was chromatographed on alumina (8 g.). Light petroleum-benzene (2:1, 525 c.c.) eluted a fraction (26 mg.)

which crystallised from chloroform-methanol to yield 5β-glutinan-3β-ol as plates, m.p. and mixed m.p. 266-268°, $[\alpha]_D$ + 33° (c,1.1). Treatment with pyridine and acetic anhydride at 100° for 1 hr. gave the acetate m.p. and mixed m.p. 230-231°, $[\alpha]_D$ + 51° (c,0.7).

Continued elution of the column yielded no other homogeneous material.

Hydrogenation of Glutin-5(10)-en-3β-yl Acetate. - A solution of glutin-5(10)-en-3β-yl acetate (99 mg.) in glacial acetic acid (200 c.c.) was shaken with hydrogen and platinum (from 193 mg. PtO_2) for 18 hr. at 60°. The product was crystallised several times from chloform-methanol to yield unchanged starting material (18 mg.), m.p. and mixed m.p. 298-299°, $[\alpha]_D$ - 23° (c,1.1).

From the mother liquors a saturated material (45 mg.), m.p. 215-216° was isolated which was hydrolysed and a solution of the product in light petroleum (25 c.c.) chromatographed on alumina (5 g.). Light petroleum-benzene (2:1, 350 c.c.) eluted a fraction (15 mg.) which crystallised from chloroform-methanol to yield 5β-glutinan-3β-ol as plates, m.p. and mixed m.p. 266-267°, $[\alpha]_D$ + 30° (c,0.5)

No other homogeneous material was obtained from the column.

Hydrogenation of Glutin-5-en-3β-yl Acetate. - (a) A solution of glutin-5-en-3β-yl acetate (400 mg.)in glacial acetic acid (250 c.c.) was shaken with hydrogen and platinum (from 205 mg. PtO_2) for 18 hr. at room temperature. The product was recrystallised several times from chloroform-methanol to yield glutin-5(10)-en-3β-yl acetate (27 mg.) as plates, m.p. and mixed m.p. 298-300°, $[\alpha]_D$ - 17° (c,1.8).

A saturated material (232 mg.), m.p. 208-222°, $[\alpha]_D$ + 49°, which was isolated from the mother liquors, was hydrolysed with lithium aluminium hydride and a solution of the product in light petroleum (50 c.c.) was chromatographed on alumina (30 g.). A fraction (64 mg.), eluted with light petroleum-benzene (1:1, 1500 c.c.), crystallised from chloroform-methanol to yield 5β-glutinan-3β-ol as plates, m.p. and mixed m.p. 265-267°, $[\alpha]_D$ + 31° (c,1.2).

(b) A solution of glutin-5-en-3β-yl acetate (127 mg.) in ethyl acetate (50 c.c.) was shaken with hydrogen and platinum (from 108 mg. PtO_2) for 22 hr. at room temperature. Crystallisation of the product from chloroform-methanol afforded unchanged starting material.

(c) A solution of the acetate (150 mg.) in ethyl acetate (100 c.c.) was shaken with hydrogen and platinum (from 143 mg. PtO_2) for 6 hr. at room temperature and 4 atmos. pressure. The product crystallised from chloroform-methanol to yield

glutin-5-en-3β-yl acetate (115 mg.) as plates, m.p. and mixed m.p. 192-193°, $[\alpha]_D$ + 79°, (c,1.4).

Isomerisation of Glutin-5-en-3-one. - Dry hydrogen chloride was passed through a refluxing solution of glutin-5-en-3-one (250 mg.) in acetic acid (100 c.c.) for 3 hr. The solvent was removed under reduced pressure and the product recrystallised from chloroform-methanol to yield glutin-5(10)-en-3-one (218 mg.) as plates, m.p. 251-253°, $[\alpha]_D$ - 81° (c,2.5). The melting point was undepressed on admixture with a pure specimen of glutin-5(10)-en-3-one. Chapon[39] gives m.p. 248°, $[\alpha]_D$ - 84°, for 'ketone-II'.

Glutinone-III. - (a) Concentrated sulphuric acid (30 c.c.) in acetic acid (170 c.c.) was added to a suspension of glutin-5-en-3-one (1.0 g.) in benzene (30 c.c.) and the mixture was heated at 40° for 17 hr. Undissolved solid (70 mg.) was collected and recrystallised from chloroform-methanol to yield unchanged glutin-5-en-3-one, m.p. and mixed m.p. 244-246°, $[\alpha]_D$ -30° (c,1.2).

The dark red-brown filtrate was diluted with water, extracted with ether and a solution of the product in light petroleum (10 c.c.) was chromatographed on alumina (30 g.). Light petroleum (1700 c.c.) eluted fractions which were recrystallised several times from chloroform-methanol to give

'glutinone-III' (195 mg.) as striated plates, m.p. 182-184°, $[\alpha]_D$ - 34° (c,0.9) $\lambda_{max.}$ 2030 Å. (ε 6440). Chapon[88] reports m.p. 184°, $[\alpha]_D$ - 18°, for 'ketone-III'. From the mother liquors were isolated large needles which crystallised from chloroform-methanol to yield olean-13(18)-en-3-one (12 mg.), m.p. and mixed m.p. 199-201°, $[\alpha]_D$ - 8° (c,0.2). The infrared spectrum was identical with that of an authentic specimen of olean-13(18)-en-3-one.

(b) A solution of glutin-5(10)-en-3-one (104 mg.) in benzene (3 c.c.) was treated with concentrated sulphuric acid (1.5 c.c.), in acetic acid (8.5 c.c.) and the mixture was allowed to stand at room temperature for 12 days. Crystals (48 mg.) which separated from the dark brown solution, were collected and recrystallised from chloroform-methanol to yield unchanged glutin-5(10)-en-3-one, m.p. and mixed m.p. 251-253°, $[\alpha]_D$ - 83° (c,1.4).

Dilution of the filtrate with water and extraction with ether gave a brown gum (52 mg.) which was dissolved in light petroleum (20 c.c.) and adsorbed on a column of alumina (5 g.). A fraction, eluted with light petroleum (125 c.c.) crystallised from chloroform-methanol to give glutin-5(10)-en-3-one (15 mg.) m.p. and mixed m.p. 252-253°, $[\alpha]_D$ - 84° (c,0.6). The same solvent (625 c.c.) eluted fractions (30 mg.) which crystallised

from chloroform-methanol to yield 'glutinone-III' as plates, m.p. 181-183°, $[\alpha]_D$ - 36° (c,0.2).

Glutinene-III. - A solution of 'glutinone-III' (100 mg.) in sodium methoxide solution (from 300 mg. sodium and 25 c.c. dry methanol) and hydrazine hydrate (100%; 2 c.c.) were heated in an autoclave at 180° for 18 hr. The product, purified by filtration through alumina, was recrystallised several times from chloroform-methanol to give 'glutinene-III' (32 mg.) as prismatic needles, m.p. 163-164°, $[\alpha]_D$ - 38° (c,1.2) $\lambda_{max.}$ 2040 Å. (ε 12,000) (Found: C,87.6; H,12.6. $C_{30}H_{50}$ requires C,87.7; H,12.3%).

Glutinyl-III Acetate. - A suspension of the ketone (195 mg.) in dry ether (50 c.c.) was refluxed with lithium aluminium hydride (150 mg.) for 25 min. The product, isolated in the usual way, was treated with pyridine and acetic anhydride at 100° for 1 hr. Several recrystallisations of the acetylated product from chloroform-methanol yielded 'glutinyl-III acetate' (55 mg.) as plates, m.p. 208-210°, $[\alpha]_D$ + 38° (c,2.2) $\lambda_{max.}$ 2050 Å. (ε 2900). (Found: C,81.8; H,11.4. $C_{32}H_{52}O_2$ requires C,82.0; H,11.2%).

Glutinadienyl-III Acetate. - 'Glutinyl-III acetate' (66 mg.) in acetic acid (50 c.c.) was treated with a solution of selenium dioxide (75 mg.) in water (0.1 c.c.) and acetic acid

(2 c.c.) and the mixture was heated at 100° for 1 hr. The filtered solution was evaporated to dryness and a solution of the residue in light petroleum (10 c.c.) was chromatographed on alumina (8 g.). Light petroleum (650 c.c.) eluted a fraction (62 mg.) which crystallised from chloroform-methanol to yield 'glutinadienyl-III acetate' as plates, m.p. 144-145°, $[\alpha]_D$ - 118° (c,2.4), or needles, m.p. 163-164°, $[\alpha]_D$ - 117° (c,1.2). $\lambda_{max.}$ 2430, 2500 and 2600 Å. (ε 23,600, 28,400 and 18,000) (Found: C,82.4; H,10.85. $C_{32}H_{50}O_2$ requires C,82.3; H,10.8%). These two crystalline forms were interconvertible and the lower melting material was observed to resolidify on continued heating to a needle-like form, which melted sharply at 163-164°.

Oleana-11:13(18)-dien-3β-yl Acetate. - A solution of β-amyrin acetate (204 mg.) in acetic acid (100 c.c.) was treated with a solution of selenium dioxide (200 mg.) in water (0.2 c.c.) and acetic acid (4 c.c.) and the mixture was refluxed for 3 hr. A solution of the product, isolated in the usual manner, in light petroleum (25 c.c.) was purified by chromatography on alumina (8 g.). Crystallisation of the eluate from chloroform-methanol yielded oleana-11:13(18)-dien-3β-yl acetate (145 mg.) as plates, m.p. 228-229°, $[\alpha]_D$ - 66.5° (c,1.6). $\lambda_{max.}$ 2420, 2500 and 2600 Å. (ε 27,600, 31,500 and 20,300).

Olean-12-en-3α-ol. - A mixture of β-amyrenone (750 mg.)

and aluminium isopropoxide (1.0 g.) in absolute isopropanol (10 c.c.) was distilled slowly with the addition of isopropanol to maintain constant volume. When the distillate no longer contained acetone (3 hr.), the solution was evaporated to dryness and the product was isolated by means of ether. A solution of the product in light petroleum (100 c.c.) was chromatographed on a column of alumina (30 g.). Light petroleum-benzene (2:1, 900 c.c.) eluted a fraction (175 mg.) which crystallised from methanol to yield olean-12-en-3α-ol (130 mg.) as prismatic rods, m.p. 226-228°, $[\alpha]_D$ + 74° (c,1.4).

Continued elution with the same solvent mixture (1200 c.c.) yielded mixtures whereafter light petroleum-benzene (2:1, 1500 c.c.) gave a fraction which crystallised from methanol to yield olean-12-en-3β-ol (200 mg.) as needles, m.p. and mixed m.p. 198-200°, $[\alpha]_D$ + 93° (c,1.2).

Olean-12-en-3α-yl Acetate. - Olean-12-en-3α-ol (170 mg.) was treated with pyridine-acetic anhydride (1:1) at 100° for 90 min. The product, isolated in the usual manner, crystallised from chloroform-methanol to give olean-12-en-3α-yl acetate (124 mg.) as needles, m.p. 135-136°, $[\alpha]_D$ + 38° (c,1.4).

Oleana-11:13(18)-dien-3α-yl Acetate. - Selenium dioxide (100 mg.) in water (0.1 c.c.) and acetic acid (2 c.c.) was added to a solution of olean-12-en-3α-yl acetate (108 mg.) in acetic acid (50 c.c.) and the mixture was refluxed for 3 hr. The

product, isolated in the usual way, was purified by chromatography on alumina (8 g.) and crystallised from chloroform-methanol to give oleana-11:13(18)-dien-3α-yl acetate (68 mg.) as needles, m.p.185-187°, $[\alpha]_D$ - 119° (c,2.1) $\lambda_{max.}$ 2420, 2510 and 2600 Å. (ε 27,500, 31,700 and 20,500) (Found: C,82.6; H,10.9. $C_{32}H_{50}O_2$ requires C,82.3; H,10.8%. A mixture with 'glutinadienyl-III acetate' had m.p. 151-168°.

'O_s-Acetate'. - A solution of chromium trioxide (20 mg.) in stabilised acetic acid (0.94 c.c.) was added over a period of 10 min. to a refluxing solution of oleana-11:13(18)-dien-3β-yl acetate (17 mg.) in stabilised acetic acid (5 c.c.). The mixture was refluxed for a further 60 min. and a solution of the neutral product, isolated in the usual manner, in light petroleum (5 c.c.) was chromatographed on alumina (5 g.). Benzene (250 c.c.) eluted a fraction (12 mg.) which crystallised from chloroform-methanol to yield the 'O_s-acetate' as needles m.p. and mixed m.p. 261-261.5°, $[\alpha]_D$ + 33° (c,0.8). $\lambda_{max.}$ 2260 Å. (ε 4000) and a point of inflection at 3000 Å. (ε 440). Infrared absorption: strong bands at 1786, 1747 and 1695 $cm.^{-1}$.

Oxidation of Glutinadienyl-III Acetate. - 'Glutinadienyl-III acetate' (74 mg.) in stabilised acetic acid (20 c.c.) was treated at 80° with a solution of chromium trioxide (45 mg.) in stabilised acetic acid (2.13 c.c.), added dropwise over 10 min. and stirring was continued for 1 hr. The solvent was removed

under reduced pressure and the neutral product, isolated in the usual manner, chromatographed in light petroleum (5 c.c.) on alumina (8 g.). Elution with light petroleum-benzene (1:2, 200 c.c.) afforded a fraction (22 mg.) which crystallised from chloroform-methanol to give the oxidation product as thick plates, m.p. 194-195° (resolidifying to flat needles, m.p 203-204°), $[\alpha]_D$ + 51° (c,0.8) $\lambda_{max.}$ 2250 Å. (ε 3700); with inflections at 2600 and 3000 Å. (ε 1900 and 440). Infrared absorption: strong bands at 1786, 1748 and 1695 $cm.^{-1}$.

II. The Constituents of Elder Bark.

Extraction. - The chopped dried bark (1.7 kg.) was extracted in the soxhlet apparatus with light petroleum (14 l.) for 17 hr. and the dark coloured solution evaporated to dryness under reduced pressure to yield a greenish-brown resinous solid (44.5 g.). A solution of the extract in benzene (500 c.c.) and methanol (1750 c.c.) was refluxed for 5 hr. with potassium hydroxide (75 g.) in water (250 c.c.). The volume of the solution was reduced by evaporation and, after the addition of water, the mixture was extracted with ether (6 x 1.5 l.). The ether extract was washed with dilute hydrochloric acid (15%), sodium carbonate solution, water, dried (Na_2SO_4) and evaporated to dryness, giving an orange-red gum (19.0 g.) which failed to crystallise from ethyl acetate.

The aqueous alkaline solution from the saponification was acidified with concentrated hydrochloric acid and extracted with ether, yielding a dark brown gum (1.4 g.) which was not examined.

Chromatography of the Non-saponifiable Fraction. - A solution of the orange-red gum (19.0 g.) in light petroleum-benzene (4:1, 1500 c.c.) was chromatographed on alumina (600 g.). Elution with light petroleum-benzene (4:1, 9 l.) yielded non-crystalline gums (2.8 g.), the infrared spectrum of which

showed no triterpenoid characteristics. Continued elution with the same solvent mixture (8 l.) gave fractions (1 1 g.) which crystallised from chloroform-methanol to afford amorphous material (fraction A), the melting points of which ranged between 81° and 122° and whose infrared spectra showed absorption in the carbonyl region (1701 $cm.^{-1}$).

Elution with light petroleum-benzene mixtures (28 l.) and benzene (5 l.) yielded orange coloured gums which failed to crystallise. Benzene-ether (4:1, 9 l.) eluted fractions (3.6 g.) which after long standing (6 weeks) deposited clusters of needles from methanol solution (fraction B).

The same solvent mixture (4 :1, 5 l.) eluted a low melting solid (2.5 g.) fraction C.

Continued elution (4:1, 6 l.) yielded fractions (1.7 g.) which had melting points ranging between 225° and 257° (fraction D). Further elution (4:1, 5 l.) yielded yet another material (1.9 g.) which gave a typical sterol reaction in the Liebermann-Burchard colour test - fraction E.

Ether and ether-methanol mixtures eluted no further crystalline material.

Examination of the Fractions.

Fraction A. - The solid material from these fractions was collected and recrystallised several times from chloroform-methanol and then from aqueous acetone to give small blades,

(302 mg.), m.p. 124-126°, $[\alpha]_D$ + 106.5° (c,1.6) λ max.2040 Å. (ε 5600). A mixed melting point with authentic α-amyrenone showed no depression and their infrared spectra were identical.

Fraction B. - This material, after standing for 6 weeks in methanol, slowly deposited a brown gum and clusters of needles, the melting points of which ranged from 161° to 175°. These were collected, dried under vacuum and treated with pyridine and acetic anhydride (1:1) for 1 hr. on the steam-bath. The acetylated product (1.3 g.), isolated in the usual way, was dissolved in light petroleum (50 c.c.) and adsorbed on a column of alumina (30 g.). Elution with light petroleum (1400 c.c.) afforded fractions which crystallised from chloroform-methanol as lustrous plates, (645 mg.), m.p. 224-225°, $[\alpha]_D$ + 80° (c,1.6) λ max.2050 Å. (ε 3800). On admixture with a specimen of α-amyrenyl acetate, the melting point was undepressed and their infrared spectra were identical.

A solution of the acetate (192 mg.) in dry ether (125 c.c.) was refluxed with lithium aluminium hydride (200 mg.) for 35 min. and the product, isolated by means of ether, was crystallised from aqueous acetone to give α-amyrin as needles, m.p. 188-190° $[\alpha]_D$ + 90° (c,1.2), identified by mixed melting point and infrared spectroscopy.

The alcohol (50 mg.) in dry pyridine (1.2 c.c.) was

treated with the chromium trioxide-pyridine complex (100 mg. in 1 c.c.) at room temperature for 17 hr. and the product crystallised from aqueous acetone to yield small plates, m.p. and mixed m.p. 125-127°, $[\alpha]_D$ + 115° (c,1.0). The infrared spectrum was identical with that of α-amyrenone.

Fraction C. - The crystalline material from these fractions was recrystallised several times from aqueous acetone to give small plates, m.p. 67-71°, $[\alpha]_D \pm 0°$, which gave a negative colour reaction in the Liebermann-Burchard test.

Fraction D. - These fractions were combined and recrystallised from chloroform-methanol to give needles, m.p. 256-259°, $[\alpha]_D$ + 16° (c,1.2). The melting point was undepressed on admixture with a specimen of betulin and their infrared spectra were identical.

The alcohol (196 mg.) was treated with pyridine-acetic anhydride for 90 min. at 100° and a solution of the product in light petroleum (50 c.c.) was filtered through a column of alumina (20 g.). Light petroleum (4200 c.c.) eluted fractions which crystallised from chloroform-methanol to give betulin diacetate as prismatic needles, m.p. and mixed m.p. 223-224°, $[\alpha]_D$ + 22° (c,1.7), $\lambda_{max.}$ 2070 Å. (ε 2150). It was identified by comparison of its infrared spectrum with that of authentic betulin diacetate.

Fraction E. - In the Liebermann-Burchard test, this fraction gave the blue-green colour characteristic of a steroid alcohol and, on being recrystallised (charcoal) several times from chloroform-methanol, yielded plates, m.p. 138-140°, $[\alpha]_D$ - 31° (c,1.4), $\lambda_{max.}$ 2040 Å. (ε 2300). The melting point was undepressed by β-sitosterol with which it was identified by infrared spectroscopy.

A sample (100 mg.) of the alcohol was treated with pyridine and acetic anhydride (1:1) at 100° for 30 min. and the product, isolated by means of ether, was recrystallised from chloroform-methanol to give β-sitosteryl acetate as blades, m.p. and mixed m.p. 128-130°, $[\alpha]_D$ - 38° (c,1.2).

Isolation of Oleanolic Acid. - The bark, previously extracted with light petroleum, was exhaustively extracted with ether in the soxhlet apparatus for 83 hr. The ether solution was evaporated to dryness, yielding a dark brown tar (18 g.), a solution of which in benzene (200 c.c.) and methanol (1150 c.c.) was refluxed for 5 hr. with potassium hydroxide (75 g.) in water (150 c.c.). The mixture was diluted with water, concentrated (to ca. 2 l.) and extracted with ether. Material which separated at the interface was collected, washed with dilute hydrochloric acid, water and dried, yielding an amorphous substance (770 mg.). This material was dissolved in boiling methanol, filtered free from suspended bark dust and the

filtrate acidified with concentrated hydrochloric acid. After three days, the crystalline product was collected, washed well with water and recrystallised several times (charcoal) from chloroform-methanol to give needles, m.p. 310-312°, $[\alpha]_D$ + 73° (c,0.9) $\lambda_{max.}$ 2060 Å. (ε 3600), identical with oleanolic acid (mixed m.p. and infrared).

A suspension of the acid (200 mg.) in dry ether (100 c.c.) was treated for 16 hr. with excess diazomethane in ether. The product crystallised from methanol to give methyl oleanolate (150 mg.) as needles, m.p. and mixed m.p. 198-200°, $[\alpha]_D$ + 72.5° (c,1.6). The infrared spectrum was identical with that of an authentic specimen of methyl oleanolate.

The methyl ester (100 mg.) was acetylated with pyridine and acetic anhydride for 1 hr. on the steam-bath and the product crystallised from chloroform-methanol to give methyl oleanolate acetate as plates, m.p. 221-223°, $[\alpha]_D$ + 71° (c,0.5).

The Non-saponifiable Fraction. - The ether solution, containing the non-saponifiable fraction, was worked up in the usual way, concentrated (to ca. 300 c.c.) and allowed to stand overnight at room temperature. The precipitate which settled out, was collected washed with ether and dried, yielding a grey solid (202 mg.), m.p. 299-307°. Concentration of the filtrate afforded a second crop (35 mg.), m.p. 296-305°. These were combined, dissolved in hot methanol and acidified with

concentrated hydrochloric acid. The amorphous material which settled out on cooling, was recrystallised several times from methanol to yield oleanolic acid (85 mg.) as needles, m.p. and mixed m.p. 310-312°, $[\alpha]_D$ + 75° (c,1.2).

The ether filtrate was evaporated to dryness, yielding a brown gum (3.2 g.) which was dissolved in light petroleum-benzene (4:1, 500 c.c.) and chromatographed on alumina (120 g.). Elution with the same solvent (3 l.) yielded fractions (798 mg.) consisting of an oily material which failed to crystallise. Fractions (1.3 g.) eluted with light petroleum-benzene mixtures (7 l.) crystallised from chloroform-methanol to give a mixture of plates (m.p. 40-65°) and needles (m.p. 150-159°) - fraction A.

Continued elution with light petroleum-benzene mixtures (4 l.) yielded a brown gum (91 mg.) which crystallised from chloroform-methanol to give blades, m.p. 138-140° - fraction B.

Benzene-ether mixtures (3 l.) eluted a brown gum (231 mg.) which crystallised from chloroform-methanol as an amorphous solid, m.p. 242-253° - fraction C.

Ether-methanol mixtures and methanol eluted no further crystalline material.

Examination of the Fractions.

Fraction A. - The mixture of plates and needles gave a positive reaction in the Liebermann-Burchard test, a red-violet

colour being formed. Acetylation with pyridine and acetic anhydride at 100° for 90 min. yielded a product which was dissolved in light petroleum (50 c.c.) and chromatographed on alumina (25 g.). Light petroleum (1600 c.c.) eluted fractions (841 mg.) which, after many recrystallisations from chloroform-methanol, yielded α-amyrin acetate (182 mg.) as plates, m.p. and mixed m.p. 222-224°, $[\alpha]_D$ + 77° (c,1.6).

From the mother liquors was isolated a low melting solid which, on recrystallisation from aqueous acetone, yielded small plates, m.p. 59-60°, $[\alpha]_D \pm 0°$. It gave a negative reaction in the Liebermann-Burchard test.

Fraction B. - This material gave a blue-green colour in the Liebermann-Burchard test and did not depress the melting point of β-sitosterol. As the alcohol remained highly coloured after several recrystallisations, the fractions were combined, evaporated to dryness and the residue treated with pyridine and acetic anhydride on the steam-bath for 1 hr. A solution of the acetylated product (91 mg.) in light petroleum (25 c.c.) was adsorbed on a column of alumina (5 g.). Elution with light petroleum (900 c.c.) yielded fractions (43 mg.) which crystallised from chloroform-methanol to give β-sitosteryl acetate, m.p. and mixed m.p. 130-132°, $[\alpha]_D$ - 36° (c,0.9).

Fraction C. - The amorphous solid, which gave a

red-violet colour in the Liebermann-Burchard test, was treated with pyridine-acetic anhydride (1:1) at 100° for 1 hr. and the acetylated product (225 mg.) was dissolved in light petroleum (50 c.c.) and chromatographed on alumina (6 g.). Light petroleum (100 c.c.) eluted a fraction (7 mg.) which crystallised from chloroform-methanol to give β-sitosteryl acetate as blades, m.p. and mixed m.p. 129-131°.

Light petroleum-benzene (4:1, 500 c.c.) eluted fractions (21 mg.) which were crystallised from chloroform-methanol to give betulin diacetate as needles, m.p. and mixed m.p. 223-224°, $[\alpha]_D$ + 31° (c, 1.4).

Further elution with benzene and benzene-ether mixtures afforded a highly coloured intractable gum.

Aqueous Alcohol Extraction. - The bark (1.7 kg.) was extracted (6 x 6 hr.) with 80% (v/v) methanol (6 x 7 l.) in a hot extractor; the extracts were combined and evaporated to dryness to yield a dark brown tar (80.8 g.). A solution of the extract in water (4.5 l.) and methanol (1.8 l.) was refluxed with concentrated hydrochloric acid (1 l.) for 30 hr. and, after removal of the methanol by distillation, the solution was filtered free from a dark brown solid (12.5 g.) which had been precipitated. This material was identified as a lignin by colour tests.

The filtrate was made alkaline with sodium hydroxide solution and extracted with ether to yield a dark brown tar (1.7 g.) which was not examined.

III. Soyasapogenol B.

Oxidation of 3β:22β:24-Triacetoxy-12:19-dioxo-oleana-9(11):13(18)-diene (with J. M. Allison, B.Sc., A.R.C.S.T.). - To a stirred solution of the triacetoxy dioxo-dienyl derivative (2.3 g., m.p. 273-275°) in glacial acetic acid (40 c.c.) at 90° was added chromium trioxide (2 g.) in water (4 c.c.) and glacial acetic acid (46 c.c.) over a period of 10 min. Stirring was continued for 1 hr. at the same temperature then the mixture was diluted with water. The product, isolated by means of ether, was chromatographed in light petroleum-benzene (1:3, 200 c.c.) on alumina (60 g.). Fractions eluted with benzene and benzene-ether (19:1) yielded 3β:22β:24-triacetoxy-12:19-dioxo-13(18)-oxido-oleana-9(11):13(18)-diene, m.p. 220-222°, $[\alpha]_D$ + 51° (c,0.8) $\lambda_{max.}$ 2560 Å. (ε 12,000) (Found: C,68.9; H,8.2. $C_{36}H_{50}O_9$ requires C,69.0; H,8.0).

Alkaline Treatment of 3β:22β:24-Triacetoxy-12:19-dioxo-13(18)-oxido-oleana-9(11):13(18)-diene. - (a) The 13(18)-oxido derivative (407 mg.) was treated with 10% methanolic potassium hydroxide solution (10 c.c.) in an autoclave at 150° for 7 hr. The resulting mixture was diluted with water and partitioned between aqueous potassium hydroxide and ether. Acidification of the alkaline extract and extraction with ether gave an acidic fraction (91 mg.) which could not be obtained crystalline. Chromatography on silica gel afforded only an intractable gum.

The neutral fraction (193 mg.) was treated with pyridine and acetic anhydride (1:1, 2 c.c.) for 1 hr. at 100° and the acetylated product chromatographed on alumina. No crystalline material could be isolated.

(b) The 13(18)-oxido derivative (192 mg.) in amyl alcohol (5 c.c.) was added to freshly prepared sodium amyloxide (from 250 mg. sodium and 5 c.c. amyl alcohol) and the mixture gently refluxed for 90 min. Water was added and the mixture was steam-distilled to remove the amyl alcohol. Extraction of the product with aqueous base, followed by acidification and ether extraction, gave the acidic fraction (59 mg.) which proved to be an intractable gum. Ether extraction yielded the neutral fraction (145 mg.) which could not be obtained crystalline after acetylation and chromatography.

22β-Hydroxy-3β:24-isopropylidenedioxy-olean-12-ene. - A solution of soyasapogenol B (370 mg.) in dry acetone (60 c.c.) and dry ether (300 c.c.) was treated with concentrated sulphuric acid (2 c.c.). After standing at room temperature for 17 hr., the mixture was diluted with ether, washed with saturated aqueous sodium bicarbonate solution, water and the solvent removed to yield a yellow resinous solid (418 mg.). The product was purified by filtration through a column of alumina and crystallised from light petroleum to yield the isopropylidene-dioxy derivative (350 mg.) as needles, m.p. 199-201°, $[\alpha]_D$

+ 76° (c,0.8).

22-Oxo-3β:24-isopropylidenedioxy-olean-12-ene. - The hydroxyisopropylidenedioxy derivative (340 mg.) in dry pyridine (1 c.c.) was added to a suspension of chromium trioxide (350 mg.) in pyridine (0.5 c.c.) and the mixture allowed to stand at room temperature for 18 hr. The product, isolated in the usual way, was chromatographed on alumina (10 g.) and elution with light petroleum (750 c.c.) yielded fractions which crystallised from chloroform-light petroleum to give 22-oxo-3β:24-isopropylidenedioxy-olean-12-ene as rods, m.p. 209-210°, $[\alpha]_D$ + 15° (c,1.2).

22α-Hydroxy-3β:24-isopropylidenedioxy-olean-12-ene. - A solution of the 22-oxo-isopropylidenedioxy derivative (1.0 g.) in dry ether (250 c.c.) was treated with lithium aluminium hydride (0.5 g.) and the mixture refluxed for 90 min. The product was isolated in the usual way and its solution in light petroleum-benzene (1:1, 50 c.c.) adsorbed on a column of alumina (100 g.). Elution with benzene (3.5 l.) yielded fractions (299 mg.) which were recrystallised from methanol to yield 22β-hydroxy-3β:24-isopropylidenedioxy-olean-12-ene as needles, m.p. 200-202°, $[\alpha]_D$ + 75° (c,1.3). Continued elution with the same solvent (3 l.) gave mixtures (352 mg.) whereafter, it afforded fractions (262 mg.) which were recrystallised from methanol to yield 22α-hydroxy-3β:24-

isopropylidenedioxy-olean-12-ene as needles, m.p. 128-130°, $[\alpha]_D$ + 56° (c,0.9).

3β:22α:24-Triacetoxy-olean-12-ene. - The 22α-hydroxy-isopropylidenedioxy derivative (210 mg.) was refluxed with methanol (75 c.c.) and concentrated hydrochloric acid (19 c.c.) for 20 min. The mixture was diluted with water and the product, isolated by means of ether, was treated with pyridine and acetic anhydride (1:1, 10 c.c.) for 1 hr. on the steam-bath. The acetylated product was crystallised from methanol to yield 3β:22α:24-triacetoxy-olean-12-ene (160 mg.) as prismatic blades, m.p. 214-216°, $[\alpha]_D$ + 66° (c,1.0).

3β:22α:24-Triacetoxy-12:19-dioxo-oleana-9(11):13(18)-diene. - Selenium dioxide (150 mg.) was added to a solution of the triacetate (160 mg.) in benzyl acetate (7 c.c.) and the mixture refluxed for 18 hr. The solvent was removed under vacuum from the filtered solution and the residue chromatographed in light petroleum-benzene (4:1, 15 c.c.) on alumina (6 g.). Benzene (1400 c.c.) eluted fractions which crystallised from aqueous methanol to give 3β:22α:24-triacetoxy-12:19-dioxo-oleana-9(11):13(18)-diene (76 mg.) as plates, m.p. 241-243°, $[\alpha]_D$ - 55° (c,1.1) $\lambda_{max.}$ 2790 Å. (ε 13,350).

3β:22α:24-Triacetoxy-12:19-dioxo-olean-9(11)-ene. - The triacetoxy-dioxodiene (74 mg.) in ethanol (15 c.c.) was

refluxed with freshly activated zinc dust (1.4 g.) for 5 hr. and a solution of the product in light petroleum-benzene (2:1, 15 c.c.) chromatographed on alumina (15 g.). Elution with benzene-ether (9:1, 900 c.c.) yielded fractions (47 mg.) which crystallised from chloroform-light petroleum to give 3β:22α:24-triacetoxy-12:19-dioxo-olean-9(11)-ene as plates m.p. 234-236°, $[\alpha]_D$ + 90° (c, 1.0) $\lambda_{max.}$ 2450 Å. (ε 13,000) (Found: C, 70.85; H, 8.8. $C_{36}H_{52}O_8$ requires C, 70.6; H, 8.6%).

Hydrolysis of 3β:22α:24-Triacetoxy-12:19-dioxo-olean-9(11)-ene. - The dihydro-dioxodienyl triacetate (42 mg.) was refluxed with 3% methanolic potassium hydroxide (50 c.c.) for 2½ hr. under an atmosphere of nitrogen. The mixture was diluted with water and the product, isolated in the usual way, crystallised from methanol-light petroleum to give 3β:22α:24-trihydroxy-12:19-dioxo-18α-olean-9(11)-ene (35 mg.) as plates, m.p. 350-352°, $[\alpha]_D$ + 86° (c, 1.0) (Found: C, 73.9; H, 9.4. $C_{30}H_{46}O_5$ requires C, 74.0; H, 9.5%).

3β:22α:24-Triacetoxy-12:19-dioxo-18α-olean-9(11)-ene. - The trihydroxy compound (31 mg.) was treated with pyridine and acetic anhydride (1:1, 4 c.c.) for 1 hr. at 100° and a solution of the acetylated product in light petroleum-benzene (1:1, 10 c.c.) was chromatographed on alumina (10 g.). Benzene-ether (9:1, 700 c.c.) eluted fractions which were recrystallised from

chloroform-light petroleum to yield 3β:22α:24-triacetoxy-12:19--dioxo-18α-olean-9(11)-ene as fine needles, m.p. 215-217°, $[\alpha]_D$ + 74.5° (c,1.2), $\lambda_{max.}$ 2420 Å. (ε 11,200). (Found: C,70.3; H,8.7. $C_{36}H_{52}O_8$ requires C,70.6; H,8.6%).

Hydrolysis of 3β:22β:24-Triacetoxy-12:19-dioxo-olean--9(11)-ene (with J. M. Allison, B.Sc., A.R.C.S.T.). - A solution of the triacetate (250 mg.; m.p. 265-267°, $[\alpha]_D$ + 125°) in methanolic potassium hydroxide (3%; 50 c.c.) was refluxed in an atmosphere of nitrogen for 3 hr. Methanol was removed by distillation and the volume kept constant by the addition of water. After further dilution, the precipitated product was collected and recrystallised from acetone-light petroleum to yield 3β:22β:24-trihydroxy-12:19-dioxo-18α-olean-9(11)-ene as needles, m.p. 315-316°, $[\alpha]_D$ + 54° (c,0.8) $\lambda_{max.}$ 2420 Å. (ε10,200) (Found: C,73.8; H,9.55. $C_{30}H_{46}O_6$ requires C,74.0; H,9.5%).

3β:22β:24-Triacetoxy-12:19-dioxo-18α-olean-9(11)-ene. - The triol (75 mg.) in pyridine (2.5 c.c.) was treated with acetic anhydride (2.5 c.c.) at 100° for 2 hr. Isolation of the product in the usual way gave 3β:22β:24-triacetoxy-12:19-dioxo-18α-olean-9(11)-ene, m.p. 305-307°, $[\alpha]_D$ + 53° (c,0.9) $\lambda_{max.}$ 2420 Å. (ε 12,800) (Found: C,70.3; H,8.7. $C_{36}H_{52}O_8$ requires C,70.6; H,8.6%).

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