A T H E S I S

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DEGREE OF DOCTOR OF PHILOSOPHY

by

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

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SOME ASPECTS OF

THE CHEMISTRY OF URSOLIC ACID AND BREIN.

C O N T E N T S.

GENERAL INTRODUCTION.

The name triterpene is applied to a group of naturally occurring compounds containing thirty carbon atoms the structures of which are divisible into six isoprene units. In recent years, as a result of the discovery that certain products with obvious triterpene characteristics are in fact C_{31} compounds[1,2,3,4], the more comprehensive term triterpenoid has been adopted.

Apart from the aliphatic hydrocarbon squalene, all triterpenoids are alicyclic and fall into one of three main classes:-

(a) The aliphatic compound squalene and the tricyclic ambrein.

(b) The tetracyclic compounds such as lanosterol, agnosterol, the elemic acids, the polyporenic acids, eburicoic acid, euphol, tirucallol and butyrospermol, bearing certain structural relationships to the steroids.

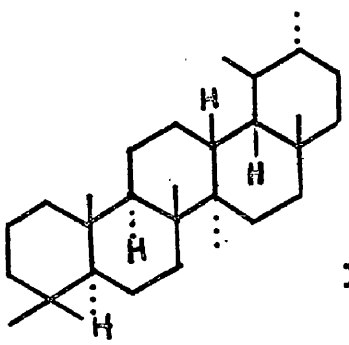
(c) The pentacyclic triterpenoids which form the largest class and include such compounds as α- and β- amyrin, lupeol and taraxasterol.

The recently characterised pentacyclic triterpenoids cycloartenol[5,6] and cycloläudenol[3,4] bear a close relationship to lanosterol and are best classified with the tetracyclic triterpenoids. Similarly the hexacyclic triterpenoid phyllanthol[5], which is closely related to α-amyrin, should be

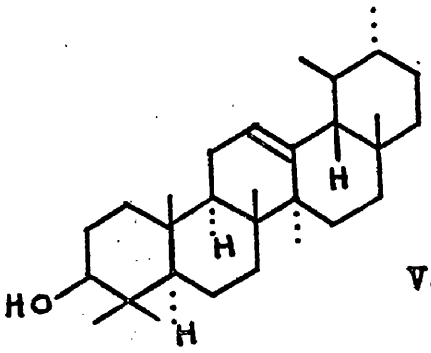
classified with the pentacyclic group. Onocerin, a tetracyclic terpenoid[7], bears a resemblance to the tricyclic ambrein.

This thesis is concerned with naturally occurring pentacyclic triterpenoids. The majority of these are polyfunctional compounds which can be related to simpler monohydric alcohols by standard methods[8,9,10,11]. They fall into four main classes based on α-amyrin, β-amyrin, lupeol and taraxasterol. The saturated hydrocarbons from which these alcohols could theoretically be derived are ursane (Ia or Ib), oleanane (II), lupane (III) and taraxastane (IV) respectively. All triterpenoids belonging to these classes can be named systematically as derivatives of the basic hydrocarbons, e.g. α-amyrin is urs-12-en-3β-ol (Va or Vb), β-amyrin is olean-12-en-3β-ol (VI), lupeol is lup-20(29)-en--3β-ol (VII) and taraxasterol is taraxast-20(30)-en-3β-ol (VIII). This rational nomenclature will be used wherever possible throughout this thesis.

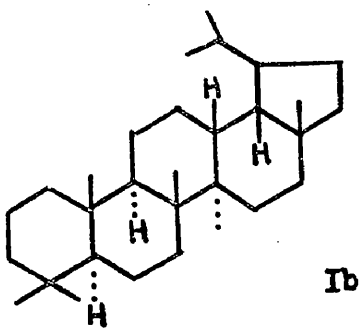
In addition to the large number of interconversions which have been achieved within each class, inter-relationships among the oleanane, lupane and taraxastane groups have also been established and more recently between the ursane and oleanane groups.



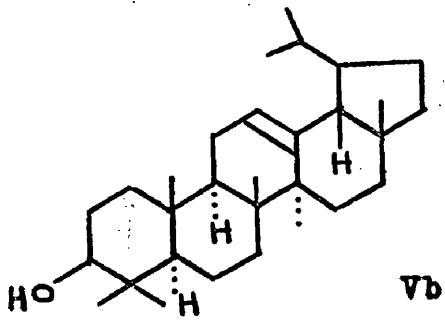
Ia



Va



Ib



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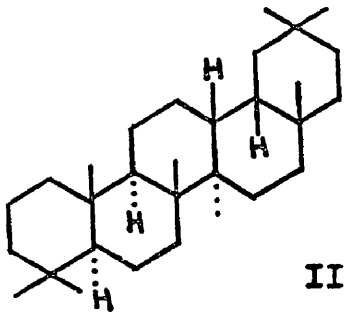

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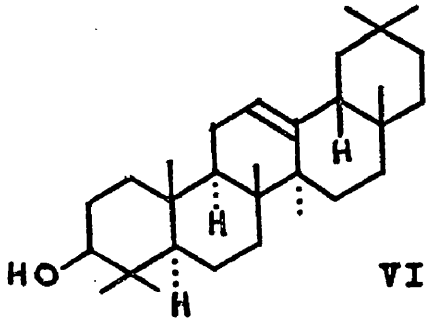

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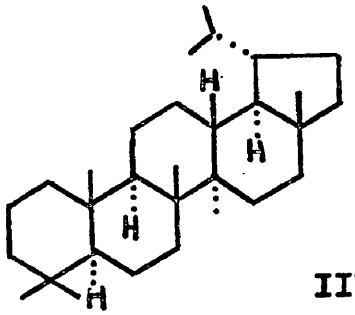

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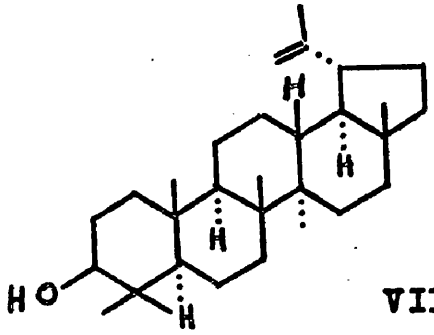

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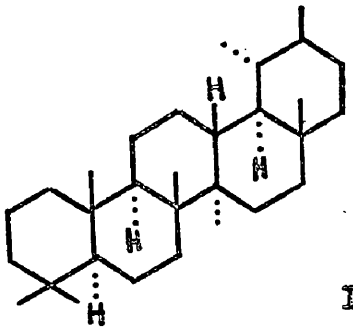

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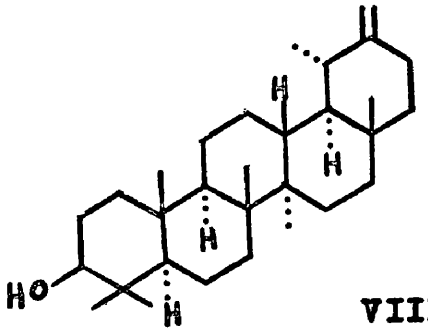


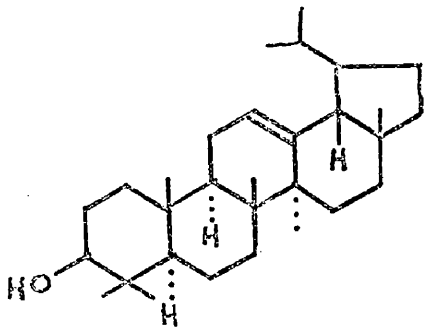
VIII

For comprehensive discussions of the chemistry of the triterpenoids, attention is directed to the reviews of Haworth[12], Spring[13], Noller[14], Jeger[15], Birch[16], Barton[17] and to Elsevier's Encyclopaedia of Organic Chemistry[18].

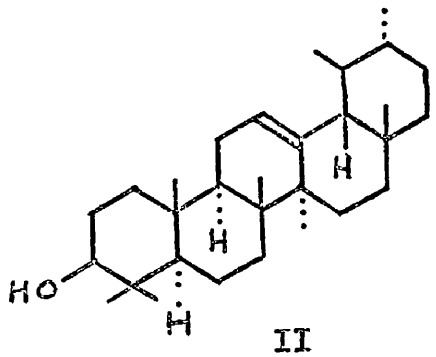
SECTION I.

The Ursane Group of Triterpenoids.

Chemical studies of the ursane group of triterpenoids and of α-amyrin in particular have failed to evolve a unique formulation which satisfactorily explains all the known reactions of α-amyrin and its relatives. At present the structure and stereochemistry of α-amyrin can be represented by (I)[19,20,21] or (II)[22,23,24] and for the purpose of this thesis structure (I) will be used.



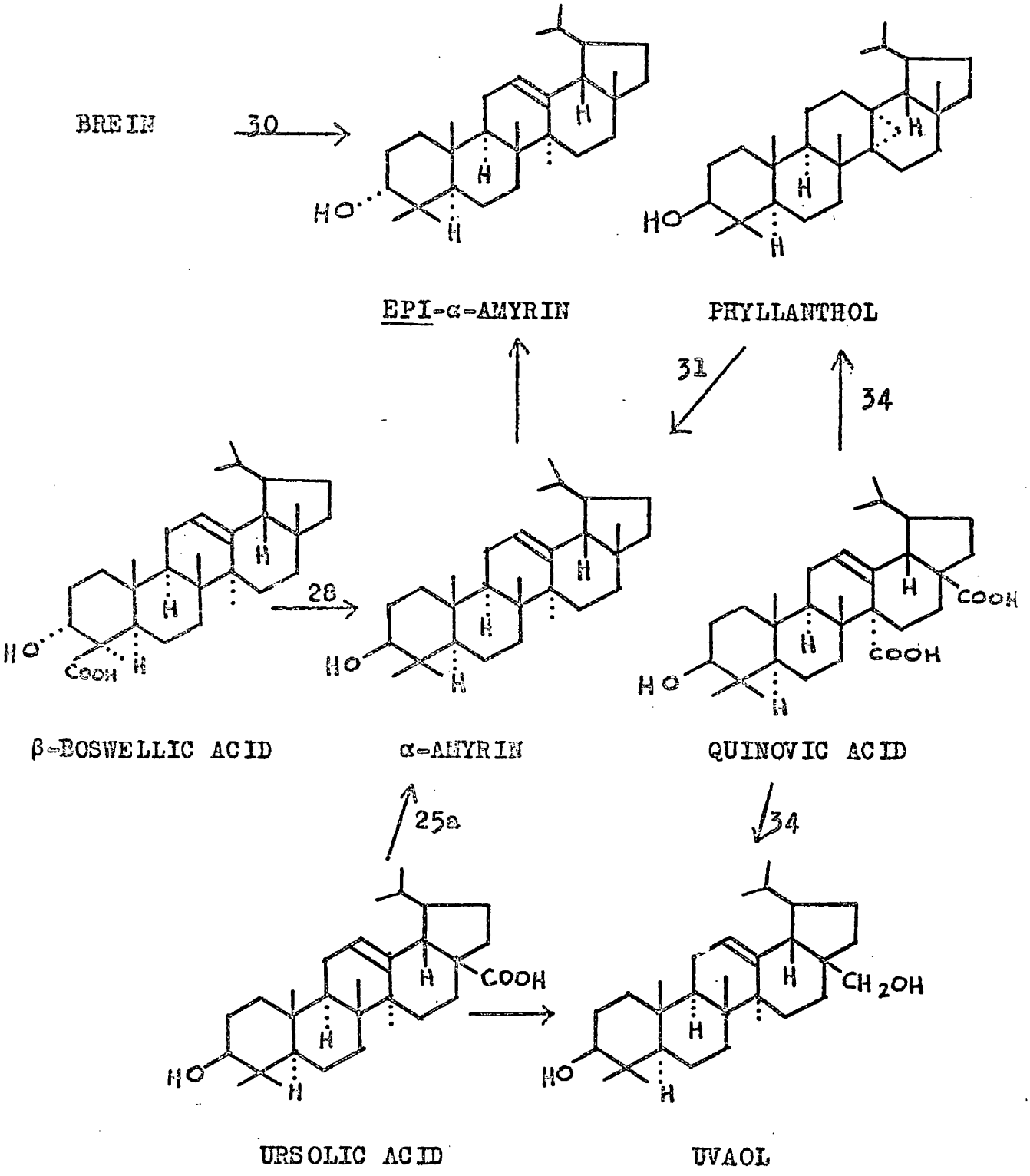
I



II

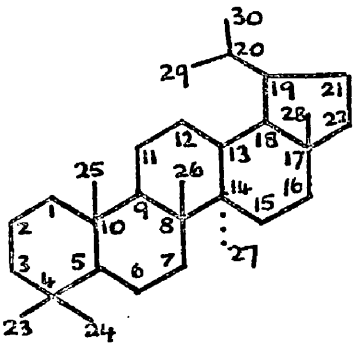
The ursane group of triterpenoids comprises of α-amyrin, uvaol, brein, β-boswellic acid, ursolic acid, quinovic acid and phyllanthol, all of which have been inter-related with α-amyrin by the transformations indicated in table I.

TABLE I.


BREIN
30
EPI-α-AMYRIN
PHYLLANTHOL
31
34
28
β-BOSWELLIC ACID
α-AMYRIN
QUINOVIC ACID
25a
34
URSOLIC ACID
UVAOL

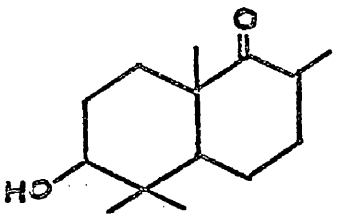
Investigations in the ursane group of the triterpenoids, by the author, have centred on certain structural aspects of ursolic acid and brein (see page55).

Ursolic acid occurs widely in the wax-like coating on the skins of fruit. The conversion of ursolic acid into α-amyrin, achieved by Goodson[26a], demonstrated their close relationship. Jacobs and Fleck[25b] suggested that the carboxyl group in ursolic acid is tertiary because of the extreme difficulty encountered in hydrolysing its esters. This result was later confirmed by Manson and Spring[26], using the Barbier-Wieland degradation method. The carboxyl group was shown by Ewen and Spring[27] to be in the vicinity of the double bond since oxidation of ursolic acid acetate with chromic acid gives an acetoxy-diketo-lactone, $C_{32}H_{46}O_6$. Jeger[13] described the degradation of ursolic acid benzoate to



a hydroxy-ketone, $C_{14}H_{44}O_2$, (III) which had previously been obtained from α-amyrin[32]. He concludes that the carboxyl

group of ursolic acid is attached to either carbon atoms 14 or 17 since the hydroxy-ketone (III) contains the methyl groups 23,24,25 and 26 of α-amyrin and dehydrogenation of ursolic acid with selenium[33] gives 2:7-dimethylnaphthalene (IV), 1:2:7-trimethylnaphthalene (V), and 1:2:5:6-tetramethylnaphthane (VI).



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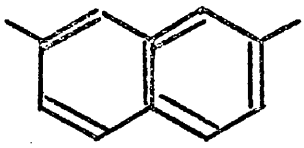

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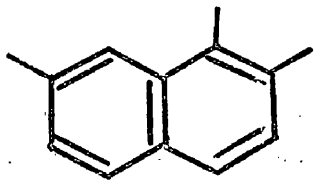

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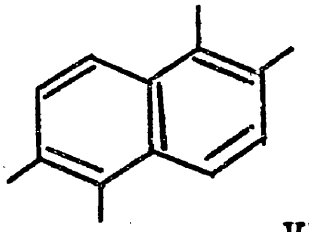
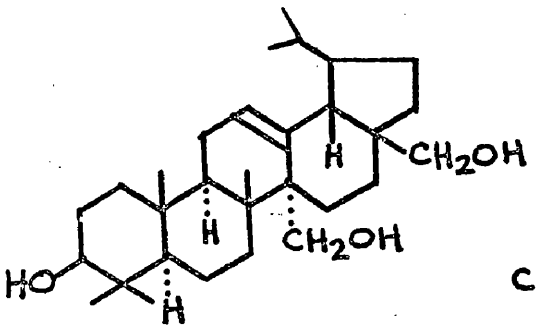


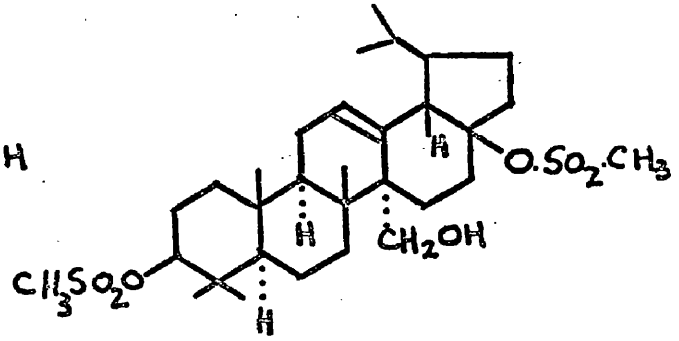
VI

Recently, ursolic acid has been inter-related with quinovic acid by Zürcher, Jeger and Ruzicka[34]. Quinovatriol (VII)[35] was converted into its dimesylate (VIII), dehydration of which gave 13:27-cyclo-3β:28-dimesyloxy-urs-11-ene (IX). Treatment of (IX) with lithium aluminium hydride followed by acetylation gave the 3β:28-diacetate which on hydrogenation gave 13:27-cyclo-3β:28-diacetoxyursane (X). Partial hydrolysis of (X) gave the 3-monoacetate which on chromic acid oxidation

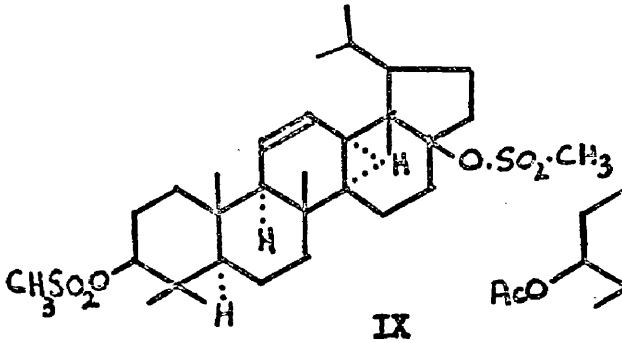
followed by Wolff-Kishner reduction gave phyllanthol (XI)[31].



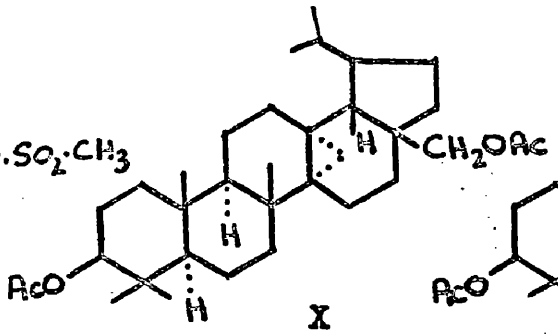
VII



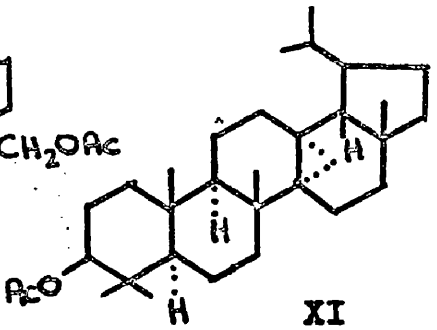
VIII



IX

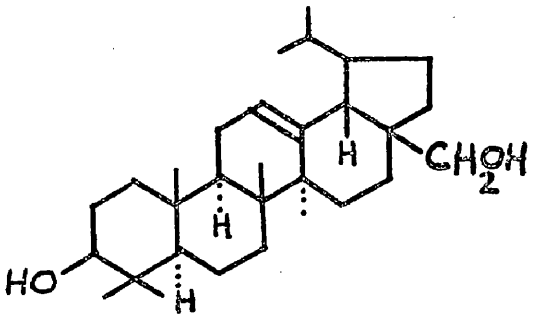


X

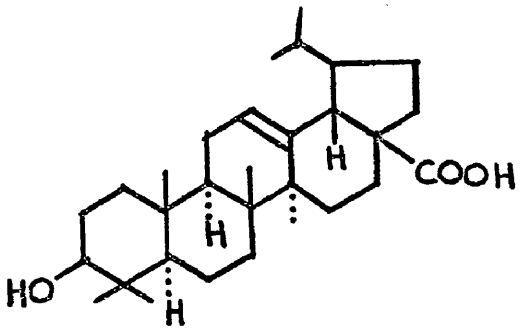


XI

Zürcher et al. also treated the diacetate (X) with hydrochloric acid and obtained the naturally occurring triterpenoid diol, uvaol (XII)[36]. The relationship between uvaol and ursolic acid has been established since reduction of methyl ursolate with sodium and alcohol gives uvaol[37]. Since the carboxyl groups in quinovic acid are attached to $C_{(14)}$ and $C_{(17)}$[38,39] it follows that the hydroxymethyl group in uvaol and the carboxyl group in ursolic acid are attached to C_{17}. Thus ursolic acid is represented by the structure (XIII).



XII



XIII

The Oxidation of Ursolic Acid with Hydrogen Peroxide.

The following section describes the products obtained by the oxidation of ursolic acid acetate with hydrogen peroxide. These are shown to be 3β-acetoxy-12β-hydroxyursano-28(13)-lactone (VI), 11:12-epoxyursano-28(13)-lactone acetate (XV) and 3β-acetoxy-12-oxo-13α-ursan-28-oic acid (XXX).

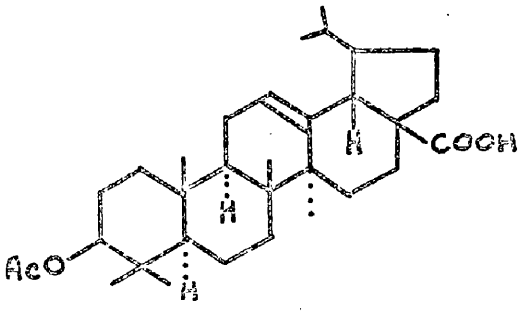
Jeger, Borth and Ruzicka[40] have reported that oxidation of ursolic acid acetate (I) with hydrogen peroxide gives two lactone acetates; acetate-lactone A, ($C_{32}H_{48}O_4$) and acetate-lactone B, ($C_{32}H_{48}O_5$) and an acid C, ($C_{32}H_{50}O_5$). The two lactones are readily separated by chromatography.

Acetate-Lactone B.

The more strongly adsorbed acetate-lactone B does not give a colour with tetranitromethane and it does not show selective absorption in the ultra-violet above 2000 Å. Jeger et al.[40] reported that the acetate-lactone was recovered unchanged after treatment with acetic anhydride-pyridine and with an ethereal solution of diazomethane. Later, however, Farquhar[41] prepared a diacetate by treatment of the acetate-lactone B with acetic anhydride-pyridine thus showing acetate-lactone B to be a diol monoacetate-lactone, and thus

accounting for all five oxygen functions. Farquhar oxidised acetate-lactone B to an oxo-lactone acetate, $C_{32}H_{48}O_5$, which readily formed a bromo-derivative. The latter could not be dehydrobrominated to an αβ-unsaturated ketone. Farquhar concluded that acetate-lactone B is 3β-acetoxy-12β-hydroxyursano--28(13)-lactone (II), that the oxidation product is 3β-acetoxy--12-oxoursano-28(13)-lactone (III) and that the bromo-derivative is (IV). The corresponding bromo-ketone in the oleanolic acid series, 3β-acetoxy-11-bromo-12-oxo-oleanolic acid lactone (V) is known to resist dehydrobromination[42].

A reinvestigation of acetate-lactone B was undertaken in order to confirm its structure (II) and to establish the configuration of the 12-hydroxyl group.



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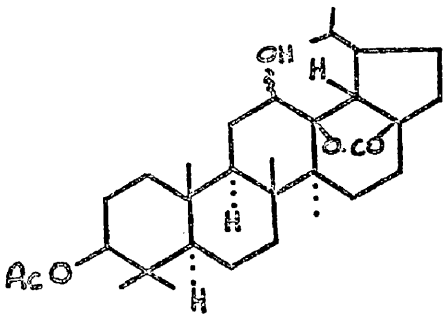
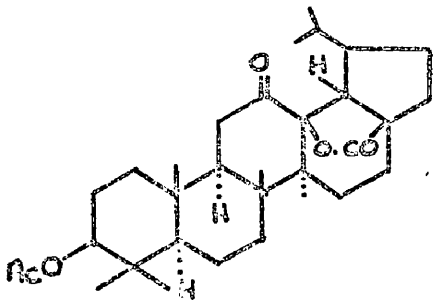


II



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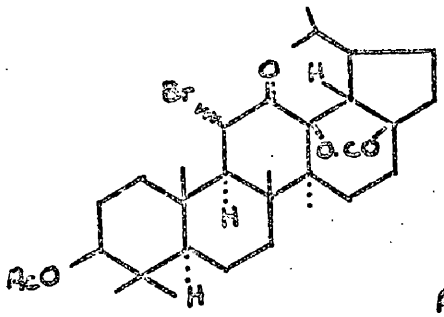

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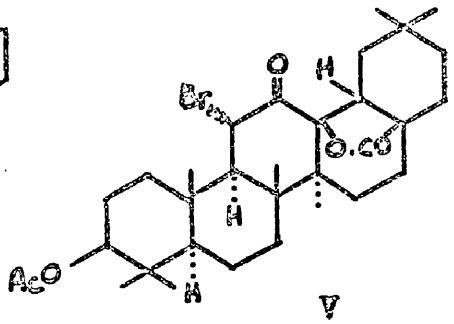


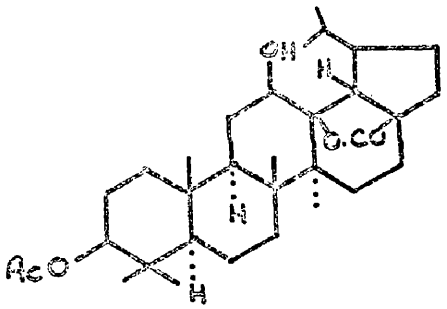
V

As a first consideration, if the lactone group is linked between positions 13 and 28 then acetate-lactone B should show the light absorption properties of a γ-lactone. This was confirmed, the infra-red absorption spectrum of acetate-lactone B, in carbon tetrachloride, including bands at 1730 cm.$^{-1}$ (acetate), 3550 cm.$^{-1}$ (hydroxyl) and 1772 cm.$^{-1}$ (γ-lactone).

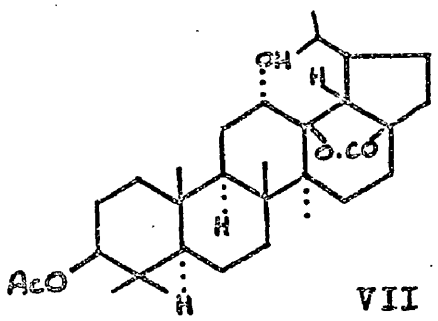
When the oxo-lactone acetate (III), derived from acetate-lactone B by chromic acid oxidation, was treated with sodium borohydride at room temperature, a compound isomeric with acetate-lactone B was obtained in good yield. It was recovered unchanged when heated with acetic anhydride and pyridine. This compound must differ from acetate-lactone B only in the configuration of the 12-hydroxyl group, since it was readily converted to the oxo-lactone (III) on chromic acid oxidation. A distinction, with regard to the configuration of the 12-hydroxyl groups in the two epimers, was made in the following way.

It is well established[44,49] that an equatorial hydroxyl group is more easily acetylated and less readily oxidised than the axial epimer. It is, in fact, found that acetate-lactone B forms a diacetate and is oxidised at temperatures greater than 35° to the oxo-lactone (III) while

its epimer is resistant to further acetylation but is readily oxidised to (III) at room temperature. It follows, therefore, that acetate-lactone B contains a 12-equatorial hydroxyl group and its epimer a 12-axial hydroxyl group. Acetate-lactone B is fully described as 3β-acetoxy-12β-hydroxyursano-28(13)--lactone (VI) and its epimer as 3β-acetoxy-12-α-hydroxyursano--28(13)-lactone (VII).



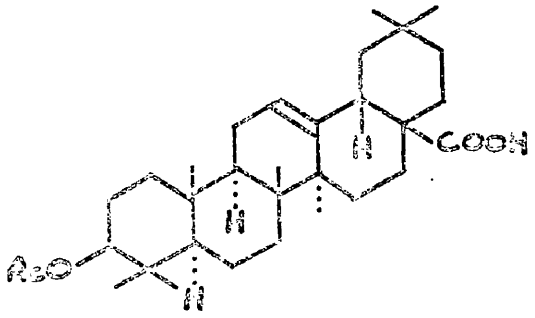
VI



VII

In view of the conclusions regarding the stereochemistry of these 12-substituted ursolic acid derivatives it was of interest to compare similar derivatives derived from oleanolic acid. Barton and Holness[43] have formulated the product of oxidation of oleanolic acid acetate (VIII) with hydrogen peroxide[43], perbenzoic acid[46] and potassium permanganate[47] as 3β-acetoxy-12α-hydroxyoleanano-28(13)-lactone (IX), i.e. the 12-axial epimer, because of its method of formation. Barton et al.[43] report a double melting point, 294-295° and

325-328° (decomp.) for this compound, in this work the compound was found to have a single melting point 324-326°.



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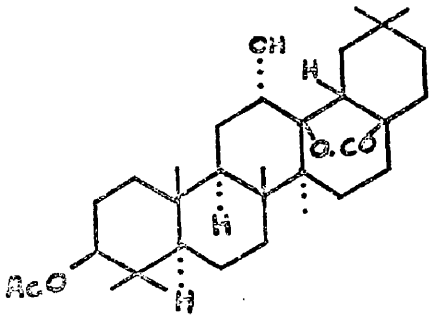
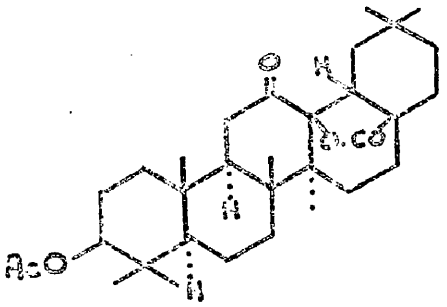


IX



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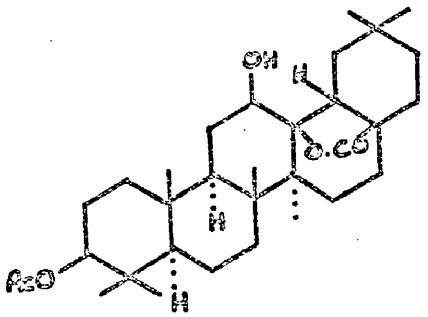


XI

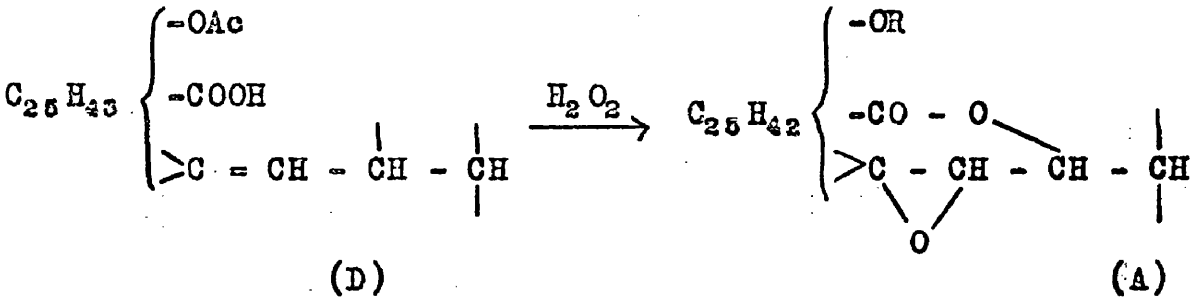
Oxidation of the hydroxylactone acetate from oleanolic acid acetate with chromic-acetic acid gives the oxo-lactone acetate (X) which on reduction with sodium borohydride gives the original hydroxylactone acetate. If the ketonic function in the oxo-lactone (X) is regarded as unhindered, since it forms an oxime m.p. 220-221°[48], then by a general rule propounded by Barton[49] that reduction of an unhindered ketone with sodium borohydride should afford the equatorial epimer;

the 12-hydroxyl group in the hydroxylactone acetate is likely to have the β-configuration (equatorial) and can be formulated as 3β-acetoxy-12β-hydroxyoleanano-28(13)-lactone (XI). It must be borne in mind, however, that there are many known exceptions to this rule and it is also reasonable to assume that the 14α-methyl group (1:3 to the ketone) is the controlling influence, hence attack from the β-face would give the 12α-isomer in agreement with the configuration chosen by Barton et al.[43]

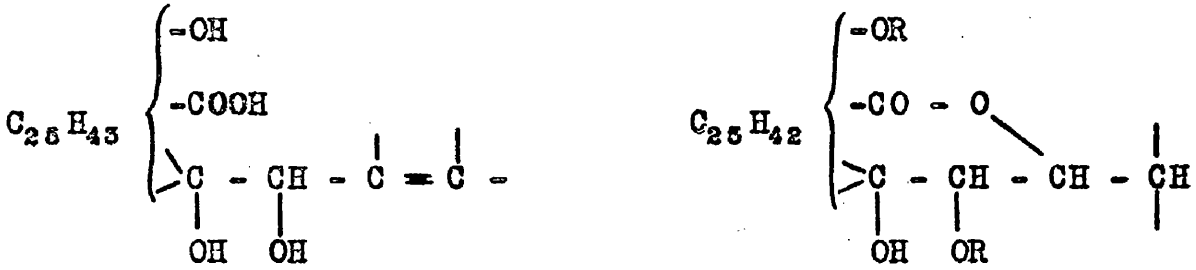
Acetone-Lactone A.

Acetate-lactone A, $C_{32}H_{48}O_5$, was examined in some detail by Jeger et al.[40] who proposed the partial formulae shown below to illustrate the reactions of this compound. It was shown that acetate-lactone A (R = Ac) obtained by oxidation of ursolic acid acetate (D) with hydrogen peroxide, is hydrolysed by mineral acid to the hydroxy-lactone oxide (A; R = H), acetylation of which regenerates the acetate-lactone A (R = Ac). Treatment of acetate-lactone A with alkali gives a mixture from which an unsaturated acid, $C_{30}H_{48}O_3$ (E) and an isomeric saturated trihydroxy-lactone (F; R = H) were isolated. The trihydroxy--lactone forms a diacetate (F; R = Ac) which is also obtained by treatment of the unsaturated acid (E) with acetic annydride and pyridine. The presence of an α-glycol group in the trihydroxy-lactone (F; R = H) was established by its oxidation with lead tetra-acetate to a dicarbonyl compound (G; R = H) which forms a mono-acetate (G; R = Ac). These partial formulae

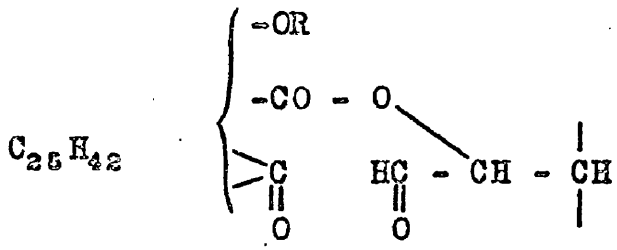
have been translated into full structural formulae (A') - (G') based on the structure (D') for ursolic acid[18], generally accepted at that time. A reinvestigation of the acetate--lactone A has now shown that the partial formulae (A) - (G) and (A') - (G') do not correctly represent the relationship between ursolic acid acetate and acetate-lactone A and its derivatives.



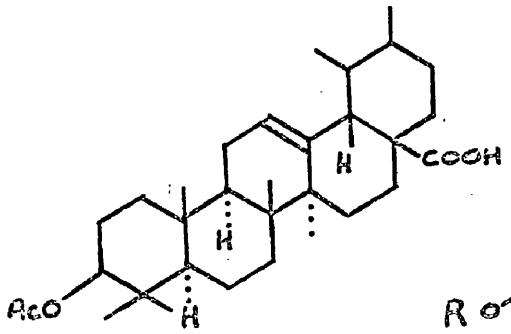
(D) (A)



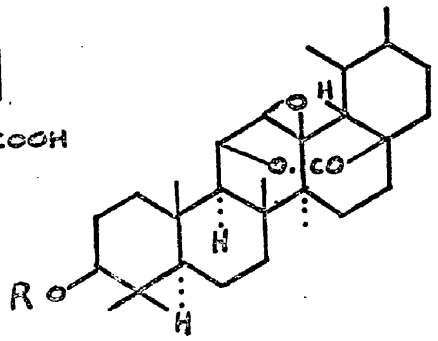
(E) (F)



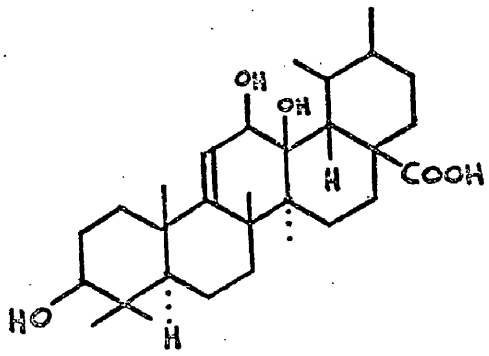
(G)



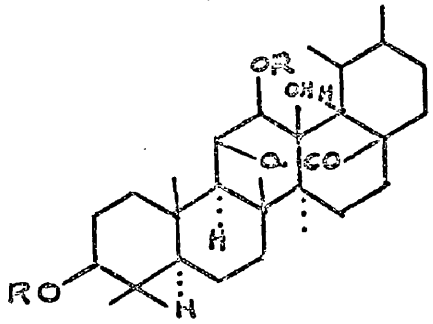
D'



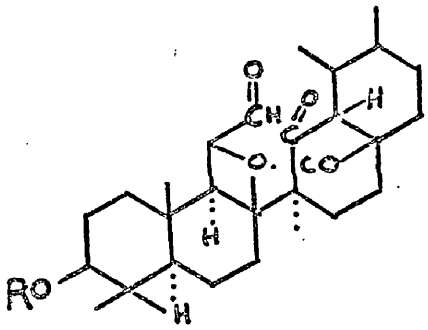
A'



E'



E'

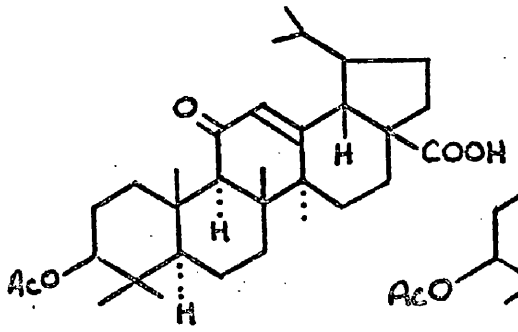


G'

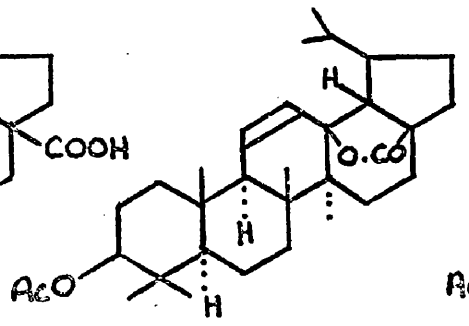
It is convenient at this stage to discuss the structure of an unsaturated lactone which is converted into acetate-lactone A by hydrogen peroxide oxidation. Oxidation of ursolic acid acetate (I) with potassium permanganate gives an acid and a neutral fraction. From the former, 11-oxoursolic acid acetate (XII) was isolated[41]. Chromatography of the neutral oxidation product gave two homogeneous products, the structure of the less readily eluted component of this mixture is discussed later. The more easily eluted neutral product is an unsaturated lactone acetate, $C_{32}H_{48}O_4$. Measurement of its infra-red spectrum includes a strong band at 1770 $cm.^{-1}$ in carbon disulphide and at

1753 cm.$^{-1}$ in Nujol and consequently the compound is characterised as a γ-lactone. Other authors[41,50] have reported that this compound gives a positive test with tetranitromethane but this could not be confirmed during this investigation. The presence, however, of an isolated double bond was established by its typical ultra-violet absorption spectra. The unsaturated lactone acetate can be represented as urs-11-eno-28(13)-lactone acetate (XIII) and the negative tetranitromethane test may be attributed to the fact that it is an allylic ester. In an effort to confirm the position of the lactone bridge in (XIII), it was proposed to reduce the Δ^{11} double bond with platinum and hydrogen and to show that the product was identical with ursolic lactone acetate (XIV) prepared in 9% yield by isomerisation of ursolic acid acetate (I) with dry hydrochloric acid gas in chloroform[43]. When the reduction was carried out either in acetic acid or ethyl acetate the reaction product was identified as ursolic acid acetate (I). Under the same conditions of hydrogenation, ursolic lactone acetate (XIV) was recovered unchanged and it is therefore concluded that the unsaturated lactone (XIII) suffers hydrogenolysis with simultaneous movement of the cis-ethylenic bond to the 12-position. The unsaturated lactone can also be prepared by reduction of 11-oxoursolic acid acetate (XII) with sodium

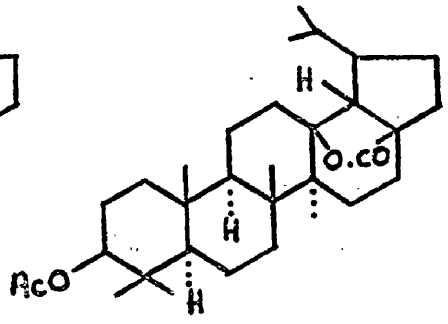
and alcohol followed by acetylation[50].



XII

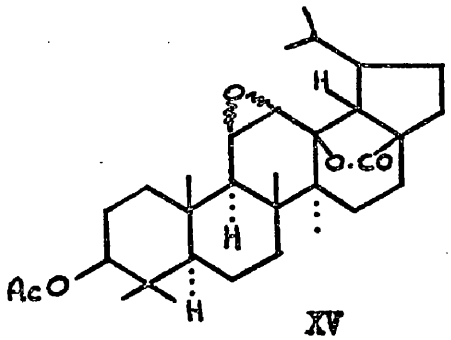


XIII



XIV

As mentioned earlier, oxidation of the unsaturated lactone (XIII) with hydrogen peroxide in acetic acid gives a lactone which is identical with the product obtained by oxidation of ursolic acid acetate (I) with hydrogen peroxide, as described by Jeger et al.[40], and this compound, acetate--lactone A is consequently formulated as 11:12-epoxyursano--28(13)-lactone acetate (XV).

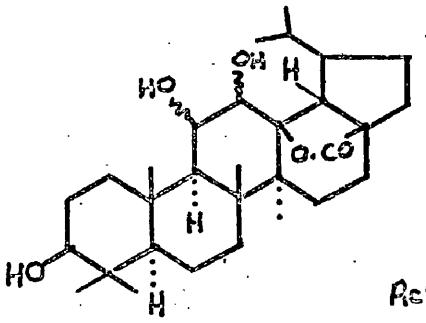


XV

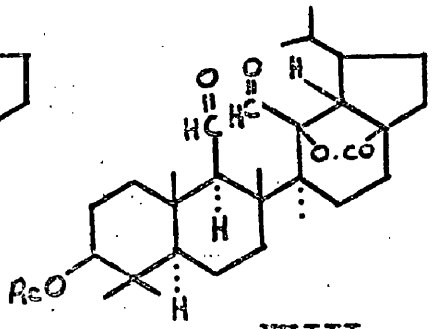
When acetate-lactone A (XV) is treated with alkali and the reaction mixture acidified with mineral acid, the saturated trihydroxy-lactone, $C_{30}H_{48}O_5$ and the isomeric unsaturated trihydroxy-acid, described by Jeger et al.[40]

were isolated if the critical conditions described in the experimental section were rigidly applied. Slight deviations from this procedure resulted in the formation of the trihydroxy-lactone as sole product.

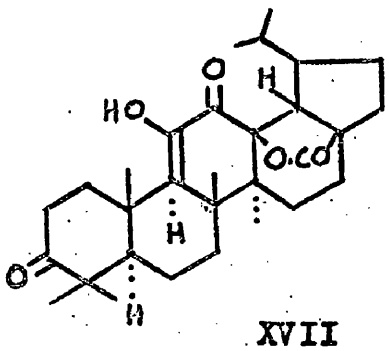
Treatment of the trihydroxy-lactone with acetic anhydride and pyridine at room temperature gives the previously described diacetate[40]. Acetylation of the trihydroxy-lactone at 100°, however, gives a triacetate which is also obtained from the diacetate by treatment with acetic anhydride and pyridine at 100°. Oxidation of the trihydroxy--lactone with chromic acid gives a triketolactone, which, in addition to the 3-carbonyl group, contains an α-diketone group since it gives a positive enol test with ferric chloride and shows an absorption maximum at 3100 Å. (ϵ = 8,200). The infra-red absorption spectrum of the trihydroxy-lactone includes a strong band at 1778 $cm.^{-1}$ which is characteristic of a γ-lactone group. It follows that the trihydroxy-lactone must have three secondary hydroxyl groups and is 3:11:12--trihydroxyursano-28(13)-lactone (XVI); consequently, the triketolactone is 3:11:12-triketoursano-28(13)-lactone (XVII) and the dicarbonyl compound obtained by oxidation of the trihydroxy-lactone with lead tetra-acetate is identified as (XVIII).



XVI

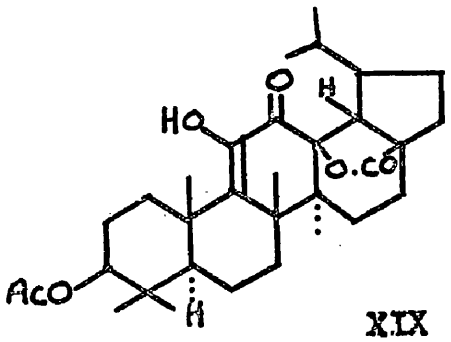


XVIII



XVII

As stated above (page 18), oxidation of ursolic acid acetate with potassium permanganate gives a neutral product, chromatography of which gives, as the more easily eluted component urs-11-eno-28(13)-lactone acetate (XIII). The more strongly adsorbed component is believed to be 11:12--dioxoursano-28(13)-lactone acetate (XIX) since it was characterised as the enol form of an α-diketone by its ultra-violet absorption spectrum (ϵ_{3120} = 9,000) and by its positive ferric chloride test. Confirmation of this structure was obtained by hydrolysis of (XIX) and oxidation of the product with chromic-acetic acid to give the triketo-lactone (XVII). The diketone (XIX) has also been isolated, in small yield, from the chromic-acetic acid oxidation of ursolic acid acetate (I)[27].

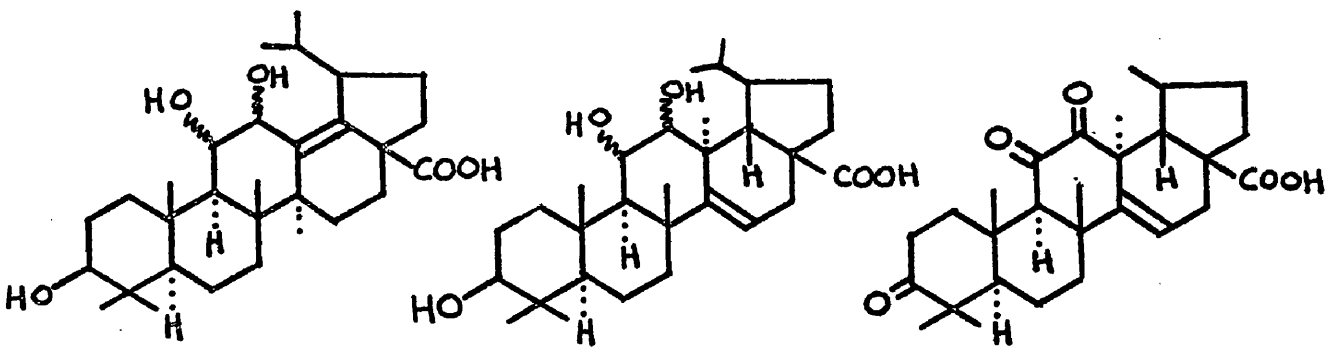


XIX

Having assigned a structure to the trihydroxy-lactone (XVI), it becomes necessary to consider possible formulations for the isomeric unsaturated acid, mentioned above, which was partially formulated by Jeger et al.[40] as (E). According to these workers, this acid was converted into the diacetate of the trihydroxy-lactone (XVI) by treatment with acetic anhydride and pyridine at room temperature, and consequently a possible structure would appear to be (XX). Doubt concerning the correctness of this formula was aroused by measurement of its ultra-violet absorption spectrum which did not correspond with the presence of a tetrasubstituted double bond but was more in agreement with a trisubstituted double bond.

Acetylation of the unsaturated acid was repeated, and in our hands, an acid was obtained which could not be crystallised. Esterification of this product with diazomethane, however, gave the same diacetate-methyl ester as was obtained by cold acetylation of the methyl ester of the unsaturated acid. Futher evidence was forthcoming to support the view that the

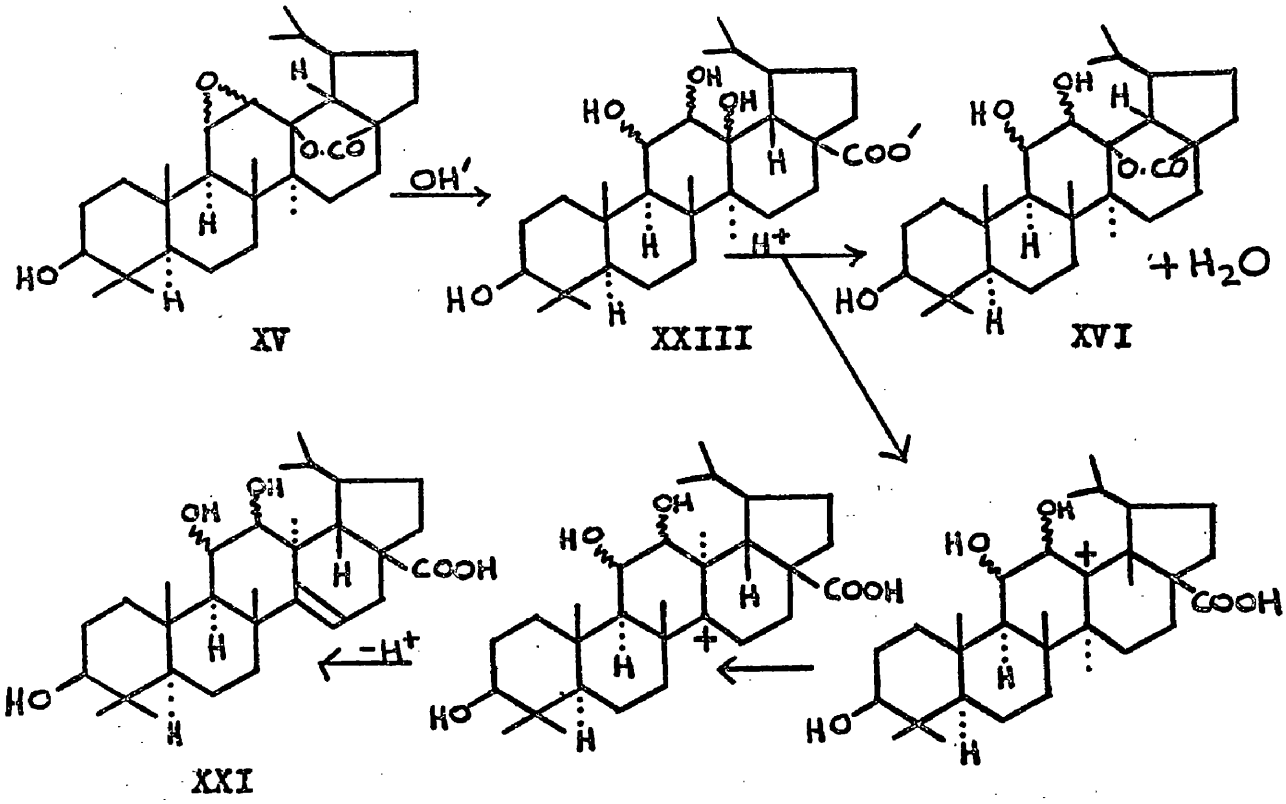
double bond in the unsaturated acid was not in the 13:18 position. Oxidation of the methyl ester of the unsaturated acid with chromium trioxide-pyridine complex gave a yellow product, $C_{31}H_{44}O_5$. This product contains an isolated double bond and its infra-red spectrum shows that it does not contain a hydroxyl group. Although the product does not give a colour with ferric chloride, after being refluxed for 1 hour with 10% potassium hydroxide a positive ferric test is obtained. The product, $C_{31}H_{44}O_5$, is therefore formulated as methyl 3:11:12-trioxo<u>iso</u>urs-14-en-28-oate (XXII) and the unsaturated acid as 3:11:12-trihydroxy<u>iso</u>urs-14-en-28-oic acid (XXI), in which a methyl group migration from $C_{(13)}$ to $C_{(14)}$ has taken place.



XX XXI XXII

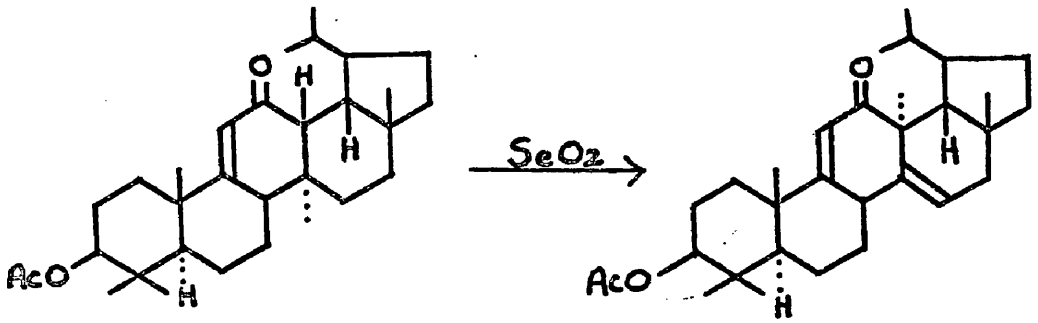
It is now possible to suggest a mechanism for the alkaline hydrolysis of acetate-lactone A (XV) to the trihydroxy-lactone (XVI) and the trihydroxy-acid (XXI). It

has been established that acetate-lactone A (XV) is recovered unchanged when treated with mineral acid followed by acetylation[40,41], therefore the opening of the oxide linkage may be base catalysed i.e. attack is by a nucleophilic reagent (OH^-). The reaction can be envisaged as proceeding by hydrolysis to give the unstable intermediate (XXIII). When the reaction



mixture is acidified with mineral acid, there are two possible means of stabilisation: either direct lactonisation can occur with the elimination of the elements of water to give (XVI) or the $C_{(14)}$ methyl group can migrate to $C_{(13)}$ under the influence of H^+ followed by loss of a proton to give (XXI). Methyl group migration of this type is well established in the ursane series; oxidation of 12-oxours-9(11)-en-3β-yl

acetate (XXIV) with selenium dioxide gives 12-oxoisoursa-9(11):14-dien-3β-yl acetate (XXV)[51] and the conditions of the reaction are such that the methyl group migration is acid (H^+) catalysed. Similar results have been reported in the oleanane series[52].



XXIV XXV

The configurations of the 11 and 12-hydroxyl groups in the lactone (XVI) and the acid (XXI) can be deduced since hydrolytic opening of oxides generally affords the diaxial product[48,49,53]. Thus the trihydroxy-lactone (XVI) can be fully formulated as 3β:11β:12α-trihydroxyursano-28(13)-lactone (XXVI) and the trihydroxy-acid as 3β:11β:12α-trihydroxyisours--14-en-28-oic acid (XXVII)[x]. The diacetates of (XXVI) and (XXVII) are formulated as the 3:11-diacetates since it has been shown that the 12α-hydroxylactone (VII) is non-acylable at room temperature.

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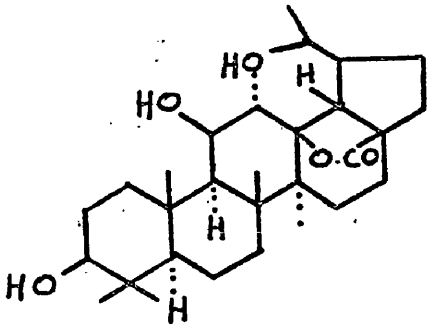
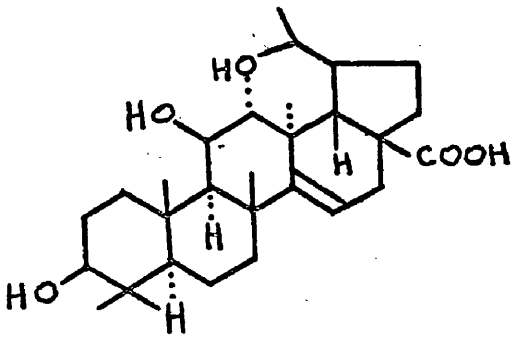
x It is appreciated that the opening of the oxide linkage in acetate-lactone A, with alkali, must be more complex than has been outlined. The configurations for the 11 and 12-hydroxyl groups in (XXVI) and (XXVII) are, therefore, proposed with considerable reservation.



XXVI

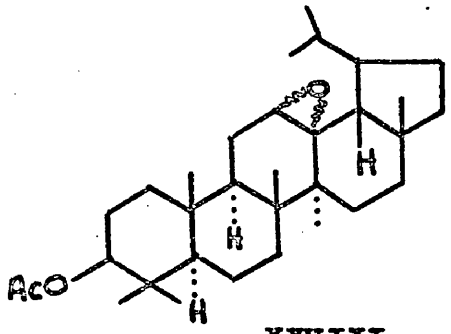


XXVII

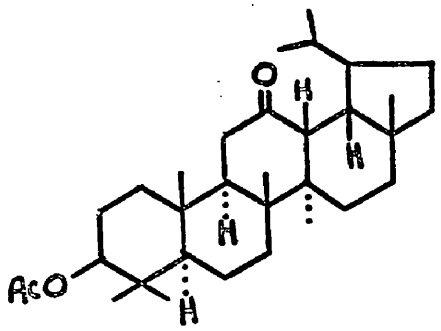
Acid C.

The acid, $C_{32}H_{50}O_3$, resulting from the oxidation of ursolic acid acetate (I) with hydrogen peroxide has been investigated by Jeger et al.[40] and as a result of an examination of the ultra-violet absorption spectrum of the compound, they suggested that the acid contained an isolated keto group. Later Manson and Spring[55], working with the methyl ester of the acid, converted it to an isomeric methyl ester and proposed that both these acids contained saturated ketonic functions and that they were epimers. This conclusion they based on two facts. Firstly, oxidation of α-amyrin acetate with hydrogen peroxide gives 12:13-epoxyursanyl acetate (XXVIII)($[\alpha]_D$ + 114°) and this compound on treatment with mineral acid isomerises to 12-oxoursanyl acetate (XXIX)

($[\alpha]_D$ + 11°)[54]. The corresponding reactions on methyl acetyl ursolate, however, gives isomeric esters with specific rotations of + 27° and + 32° respectively and from this it was suggested that the reactions were not analogous. Secondly, each of the isomeric oxo-ursolic esters when treated



XXVIII

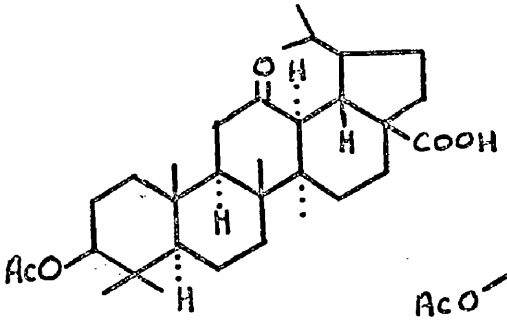


XXIX

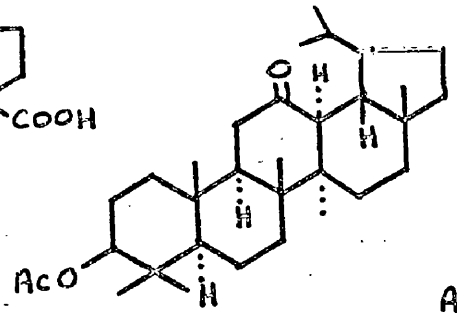
with bromine in the presence of hydrogen bromide gave the same αβ-unsaturated ketone. Farquhar[41] later proposed that the conversion of the acid C into its isomer was analogous to the corresponding change of (XXVIII) into (XXIX) and that the molecular rotation change evidence was invalid because of vicinal action with the carboxyl group with the $C_{(13)}$ substituent. It has recently been shown[56], however, that the oxidation product of α-amyrin acetate, formerly formulated as the epoxide (XXVIII) is in fact 12-oxo-13α-ursan-3β-yl acetate (XXXI) and that on isomerisation with mineral acid it gives 12-oxoursan-3β-yl acetate (XXIX). Kaye, Fieser and Fieser[57] have since prepared 12:13-epoxyursan-3β-yl acetate (XXVIII) by

ozonolysis of α-amyrin acetate and found it to be readily isomerised under very mild acid treatment to 12-oxo-13α-ursan--3β-yl acetate (XXXI).

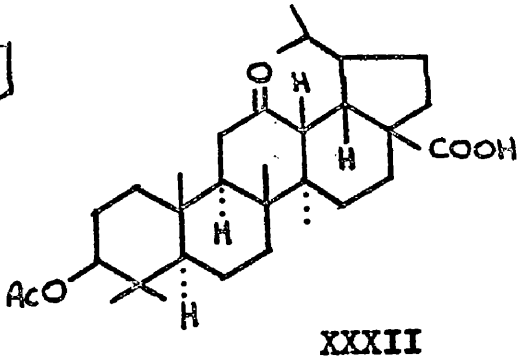
A reinvestigation of acid C and measurement of its infra-red spectrum (peak at 1686 $cm.^{-1}$) has shown it to contain an isolated ketone group. Thus, this acid, can be formulated as 3β-acetoxy-12-oxo-13α-ursan-28-oic acid (XXX) and its isomer as 3β-acetoxy-12-oxoursan-28-oic acid (XXXII).



XXX



XXXI

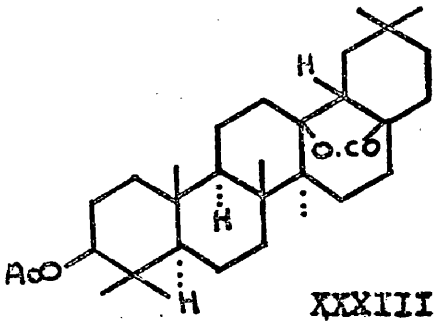


XXXII

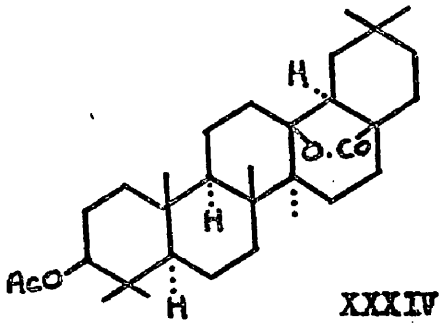
Ursanyl Lactone Acetate.

Barton and Holness[43], in their investigations of the stereochemistry of β-amyrin, prepared oleanolic lactone acetate by treating a solution of oleanolic acid acetate (VIII) in

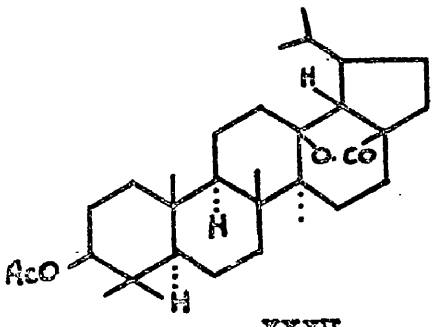
chloroform with a stream of dry hydrogen chloride and they proposed that the lactone had the structure (XXXIII). Previously a different oleanolic lactone acetate had been prepared[38] by treating (VIII) with hydrochloric-acetic acid under reflux and Barton et al.[43] formulated this compound as 18α-oleanolic lactone acetate (XXXIV). They also prepared the analogous ursolic lactone acetate from ursolic acid acetate (I) using the same method and proposed the structure (XXXV) for the lactone. It is possible, however, that under the influence of protons, methyl group migration has occurred to give a δ-lactone system (XXXVI) in both oleanolic and ursolic lactone acetates.



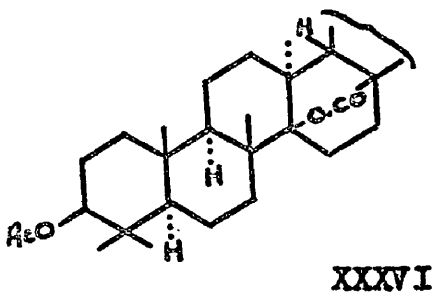
XXXIII



XXXIV

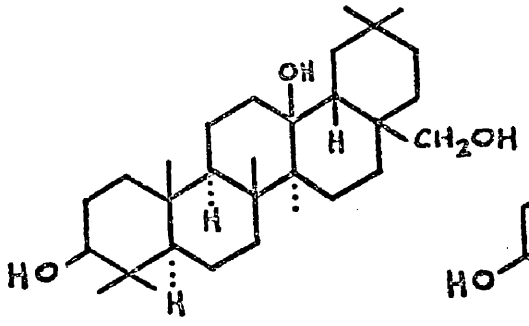


XXXV

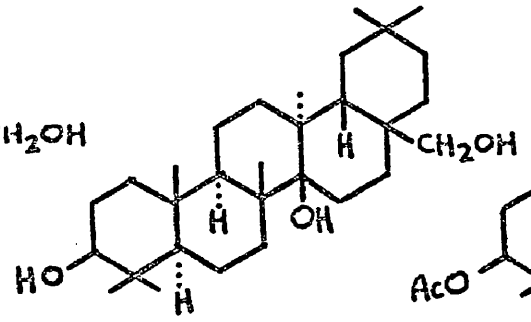


XXXVI

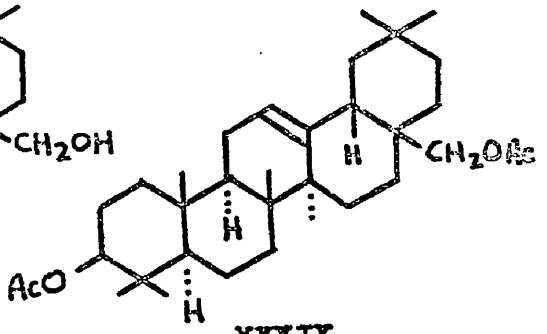
Reduction of oleanolic lactone acetate with lithium aluminium hydride gives a saturated triol which can be formulated as either 3:13:28-trihydroxyoleanane (XXXVII) or 3:14:28-trihydroxy*iso*oleanane (XXXVIII). Treatment of the triol with acetic anhydride and sodium acetate resulted in smooth dehydration to give erythrodiol diacetate (XXXIX), identity being confirmed by direct comparison with an authentic specimen. The non-acid conditions used in the dehydration excludes the possibility of methyl group migration and it is concluded that oleanolic lactone acetate is correctly represented by (XXXIII) and ursolic lactone acetate as (XXXV).



XXXVII



XXXVIII



XXXIX

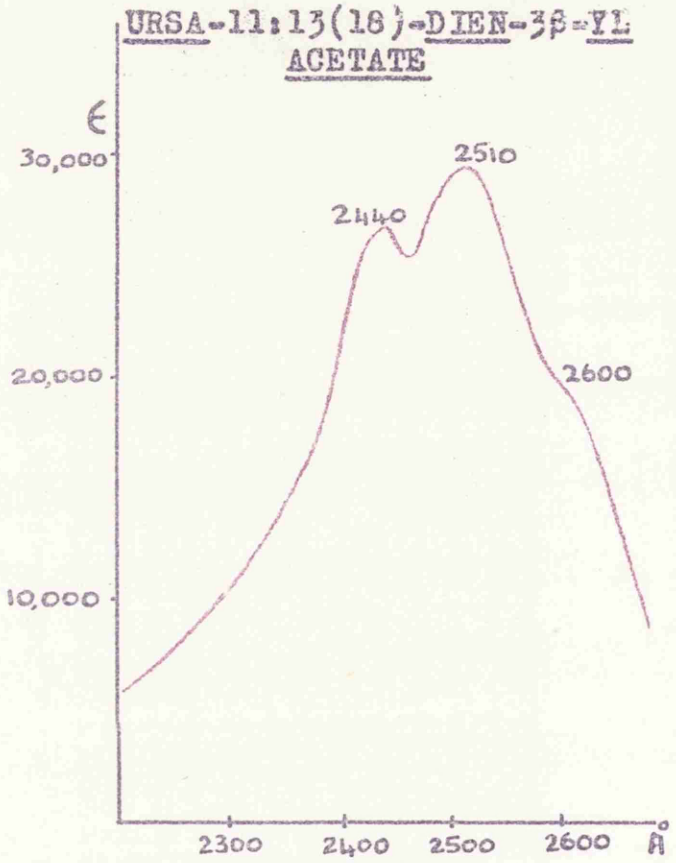
Ursa-11:13(18)-Diene-28-Oic Acid Derivatives.

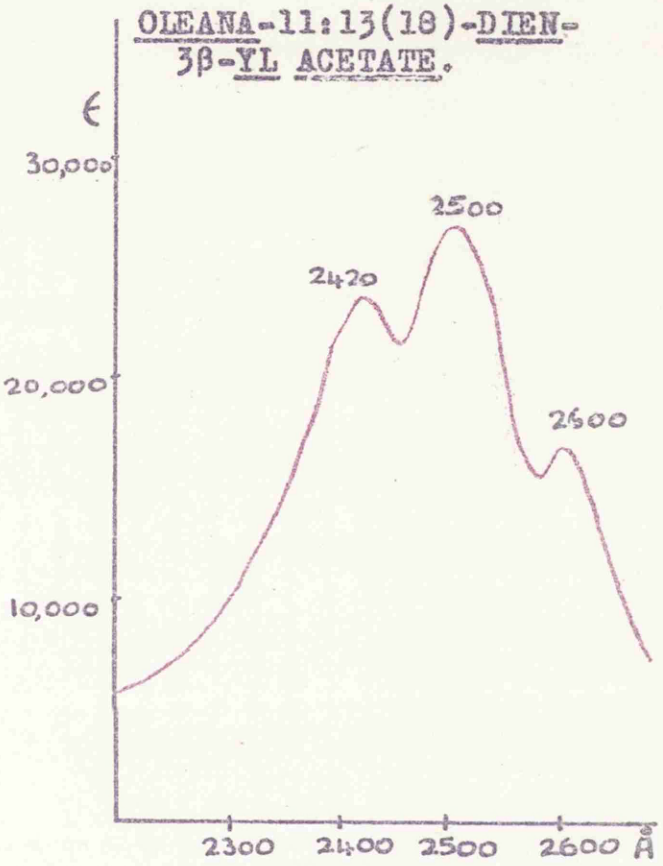
Recently[19], derivatives of α-amyrin have been converted to derivatives of β-amyrin by isomerisation with hydrochloric acid and it was of interest to attempt similar isomerisations

of ursolic acid derivatives to oleanolic acid derivatives.

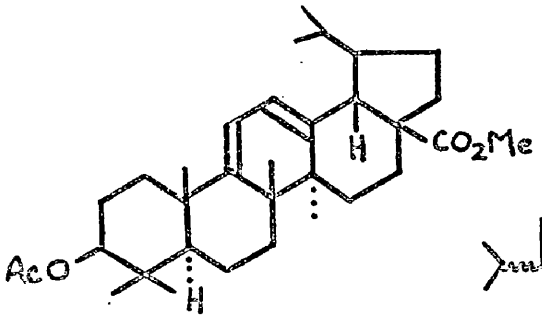
Methyl 3β-acetoxyursa-9(11):12-dien-28-oate (XL), prepared by the treatment of methyl acetylursolate with N-bromosuccinimide[59], was heated at 100° for 20 hours with hydrochloric-acetic acid. Removal of the solvent, followed by chromatography, gave in small yield two products. The first, $C_{31}H_{46}O_2$ was characterised as a conjugated triene (ϵ_{2970} = 32,400) and from the analysis and the appearance of a third ethylenic bond it is obvious that acetic acid has been eliminated. The compound, $C_{31}H_{46}O_2$, is consequently formulated as[Ξ] methyl 5:8β:14α-trimethylnovursa-9:11:13(18)-trien-28-oate (XLI). The second product, $C_{33}H_{50}O_4$, has the characteristic absorption spectrum of a conjugated heteroannular diene which is similar to that of ursa-11:13(18)-dienes. The compound $C_{33}H_{50}O_4$ is therefore identified as methyl 3β-acetoxyursa-11:13(18)-dien-28-oate (XLII). The corresponding compound in the oleanane series, 3β-acetoxyoleana-11:13(18)-dien-28-oate has m.p. 225°, $[\alpha]_D$ - 133°[60,61], as compared with m.p. 186-188°, $[\alpha]_D$ - 77° for the diene (XLII). The following graphs shows the ultra-violet absorption spectra of a typical ursa-11:13(18)-diene and an oleana-11:13(18)-diene.

Ξ The nomenclature for the hydrocarbon novursane has recently been proposed by Allan, Spring, Stevenson and Strachan[62].

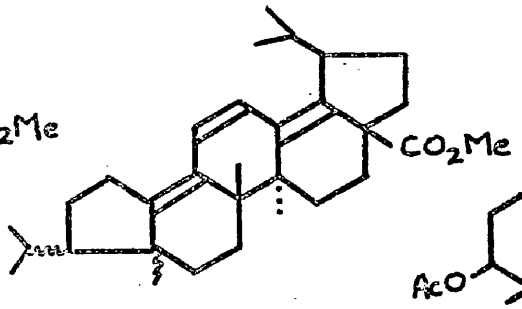




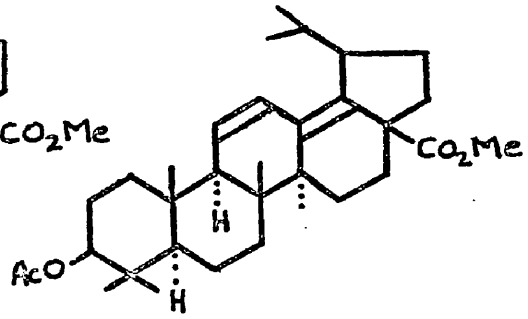
The action of hydrochloric acid on methyl 3-oxoursa--9(11):12-dien-28-oate (XLIII) was next investigated since Shaw, Spring and Stevenson[63] have reported that isomerisation of ursa-9(11):12-diene-3-one to oleana-11:13(18)-dien-3-one is effected in high yield. When this reaction was carried out for a period of 70 hours, the resulting gum showed no selective absorption in the ultra-violet above 2200 Å.; for a shorter period of 30 hours a compound $C_{31}H_{46}O_3$ was isolated, which showed the characteristic absorption spectrum of ursa--11:13(18)-dienes and it is therefore formulated as methyl 3-oxoursa-11:13(18)-dien-28-oate (XLIV).



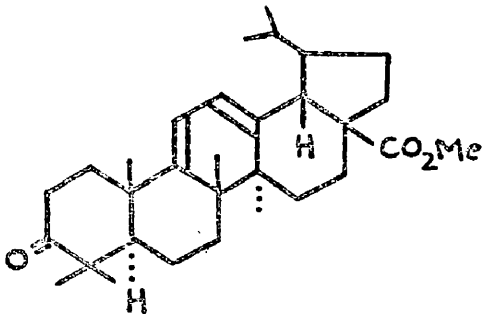
XL



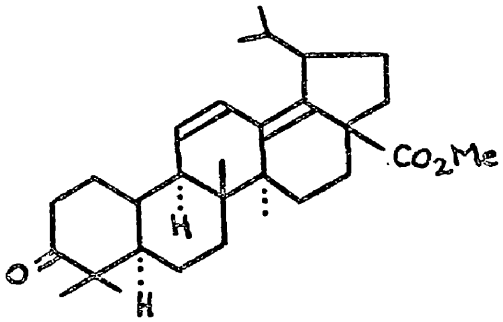
XLI



XLII



XLIII



XLIV

The failure of these ursolic acid derivatives to isomerise to oleanolic acid derivatives can only be accounted for by the presence in the molecule of the carboxyl group.

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

SECTION I.

Melting points were determined using a standard N.P.L. thermometer.

Specific rotations were measured in chloroform solution in a 1 dm. tube at approximately 15°.

Ultra violet absorption spectra were determined in ethanol solution (unless otherwise stated) with a Unicam SP. 500. Spectrophotometer and (ϵ) denotes intensity of absorption.

Colour reactions with tetranitromethane were done in chloroform solution (unless otherwise stated).

The phrase 'in the usual way' implies, in general, dilution with water, extraction with ether, washing consecutively with aqueous sodium hydroxide, water, aqueous hydrochloric acid and aqueous sodium bicarbonate, followed by drying of the ethereal extract over anhydrous sodium sulphate, filtration and evaporation to dryness under reduced pressure.

For chromatography, Brockman Grade II alumina and a light petroleum fraction b.p. 60-80° were used.

Isolation of Ursolic and Oleanolic Acids from Apple Peel. - Dry apple peel (1.5 kg.) was continuously extracted with light petroleum (15 l.) for 3 days and then with ether for 3 days. From the ether extract, ursolic acid acetate, m.p. 285-286°, $[\alpha]_D$ + 63° (c,1.2) was isolated and purified using the method of Drake and Duvall[64].

After concentrating the light petroleum extract to half bulk a solid (55 g.) separated, from which a crystalline acid (12 g.) was obtained. Acetylation of this acid and crystallisation of the product from methanol gave oleanolic acid acetate as needles m.p. and mixed m.p. 263-265°, $[\alpha]_D$ + 76° (c,1.4).

Oxidation of Ursolic Acid Acetate with Hydrogen Peroxide. - Ursolic acid acetate was oxidised with hydrogen peroxide and the reaction mixture separated into acid and neutral fractions as described by Jeger, Borth and Ruzicka[40].

The acid fraction was methylated using diazomethane, the product was isolated by means of ether and purified by crystallisation from chloroform-methanol to give methyl 3β-acetoxy-12-oxo-13α-ursan-28-oate as needles, m.p.253-255°, $[\alpha]_D$ + 27° (c,1.2).

(Found: C,78.8; H,10.2. Calc. for $C_{32}H_{50}O_5$. C,79.0; H,9.9%).

The ester gives no colour with tetranitromethane and shows no selective absorption of high intensity above 2000 Å. in the ultra-violet.

The neutral fraction was chromatographed on alumina. The less strongly adsorbed lactone, 11:12--epoxyursano-28(13)-lactone acetate separated from ethanol as needles, m.p. 283-285°, $[\alpha]_D$ + 49° (c,1.1). The literature[40] gives m.p. 280-282°, $[\alpha]_D$ + 51° for this compound.
(Found: C,74.7; H,9.5. $C_{32}H_{48}O_5$ requires C,75.0; H,9.4%). It was recovered unchanged after heating for 2 hr. with pyridine and acetic anhydride.

The more strongly adsorbed lactone was crystallised from ethanol to give 3β-acetoxy-12β-hydroxyursano-28(13)-lactone as plates, m.p. 283-285°, $[\alpha]_D$ + 30° (c,1.0). The literature[40] gives m.p. 279°, $[\alpha]_D$ + 30° for this lactone. (Found: C,74.4; H,9.5. $C_{32}H_{50}O_5$ requires C,74.7; H,9.8%).

3β:12β-Diacetoxyursano-28(13)-lactone. - 3β-Acetoxy--12β-hydroxyursano-28(13)-lactone was heated with acetic anhydride and pyridine at 100° for 2 hours. Crystallisation of the product from ethanol gave 3β:12β-diacetoxyursano-28(13)--lactone as needles, m.p. 318-319°, $[\alpha]_D$ + 37° (c,1.3). (Found: C,73.1; H,9.6. $C_{34}H_{52}O_6$ requires C,73.3; H,9.4%). The compound does not give a colour with tetranitromethane in chloroform.

3β-Acetoxy-12-oxoursano-28(13)-lactone. - A solution of 3β-acetoxy-12β-hydroxyursano-28(13)-lactone (1.5 g.) in glacial acetic acid (100 c.c.) was treated at 30-35° with chromic anhydride (400 mg.) in acetic acid (10 c.c.) over 2 hr. The mixture was kept at 35° for 7 hr. and the neutral product isolated in the usual way; it was purified by chromatography on alumina and by crystallisation from ethanol from which 3β-acetoxy-12-oxoursano-28(13)-lactone (1.2 g.) separated as plates, m.p. 281-283°, $[\alpha]_D$ - 5° (c,1.2). The literature[41] gives m.p. 287-288°, $[\alpha]_D$ - 2° for this compound. Light absorption: ϵ_{2900} = 160.
(Found: C,75.2; H,9.7. Calc. for $C_{32}H_{48}O_5$ C,75.0; H,9.4%).
This oxidation was attempted at room temperature unsuccessfully. In Nujol mull, the oxolactone acetate shows bands at 1774 $cm.^{-1}$ (γ-lactone) and at 1722 $cm.^{-1}$ (six-ring ketone and acetate).

3β-Acetoxy-12α-hydroxyursano-28(13)-lactone. - A solution of 3β-acetoxy-12-oxoursano-28(13)-lactone (200 mg.) in ethanol (40 c.c.) containing sodium borohydride (21 mg.) was kept at room temperature for 4 hr. The product was isolated by means of ether and crystallised from methanol to yield 3β-acetoxy-12α-hydroxyursano-28(13)-lactone (120 mg.) as needles, m.p. 299-301°, (323-325° in vac.), $[\alpha]_D$ + 32° (c,1.4).

(Found: C,75.0; H,9.8. $C_{32}H_{50}O_5$ requires C,74.7; H,9.8%). A mixture with the 12β-hydroxyisomer (m.p. 283-285°) had m.p. 268-274°. It does not give a colour with tetranitromethane and it was recovered unchanged after heating at 100° for 1 hr. with acetic anhydride and pyridine.

A solution of the 12α-hydroxylactone acetate (60 mg.) in acetic acid (20 c.c.) was treated with chromium trioxide (8.5 mg.) and the mixture kept at room temperature for 18 hr. The product was isolated by means of ether in the usual manner and crystallised from ethanol to give 3β-acetoxy-12-oxoursano-28(13)-lactone as plates, m.p. and mixed m.p. 279-280°, $[\alpha]_D$ - 5° (c,1.7).

3β-Acetoxy-12β-hydroxyoleanano-28(13)-lactone. - (a) A solution of oleanolic acid acetate (3 g.) in acetic acid (120 c.c.) was treated with a solution of hydrogen peroxide (36 c.c.) in acetic acid (36 c.c.) for 1 hr. when hot water (60 c.c.) was added. The product crystallised from chloroform-methanol as needles, m.p. 324-326° and 341-343° in vac., $[\alpha]_D$ + 47° (c,1.1). The literature[46] gives m.p. 292-294° for this lactone.

(Found: C,74.4; H,9.9. Calc. for $C_{32}H_{50}O_5$. C,74.7; H,9.8%).

(b) A solution of oleanolic acid acetate (3 g.) in chloroform (20 c.c.) was kept at 0° for 14 days with a solution of

perbenzoic acid in chloroform (84 c.c.; 0.3N). The product was isolated in the usual manner by means of chloroform and the residue crystallised drom chloroform-methanol to give 3β-acetoxy-12β-hydroxyoleanano-28(13)-lactone as needles, m.p. and mixed m.p. 324-326°, $[\alpha]_D$ + 48° (c,1.7). The literature[46] gives m.p. 333° for this lactone.

3β-Acetoxy-12-oxo-oleanano-28(13)-lactone. - A solution of 3β-acetoxy-12β-hydroxyoleanano-28(13)-lactone (10.0 g.) in glacial acetic acid (400 c.c.) was treated at room temperature with a solution of chromic anhydride (2.6 g.) in acetic acid (100 c.c.) over a period of 3 hr. The solution was allowed to stand overnight, after which the product was isolated in the usual manner. Crystallisation from chloroform-methanol gave 3β-acetoxy-12-oxo-oleanano--28(13)-lactone as needles, m.p. 283-286°, $[\alpha]_D$ + 9° (c,2.5) The literature[65] gives m.p. 286-288°, $[\alpha]_D$ + 9° to + 12° for this lactone.

(Found: C,75.0; H,9.7. Calc. for $C_{32}H_{48}O_5$. C,75.0; H,9.4%).

Reduction of 3β-Acetoxy-12-oxo-oleanano-28(13)-lactone with Sodium Borohydride. - A solution of 3β-acetoxy-12-oxo--oleanano-28(13)-lactone (1.0 g.) in ethanol (175 c.c.) containing sodium borohydride (100 mg.) was kept at room temperature for 1 hr. The product was isolated by means of

ether and acetylated on the steam bath for 1 hr. Isolation of the diacetate by means of ether was followed by chromatography on a column of alumina (35 g.). Elution of the column with benzene (2 x 100 c.c.) gave 3β:12β-diacetoxyoleano-28(13)--lactone (700 mg.) from chloroform-methanol as needles, m.p. and mixed m.p. 294-295°, $[\alpha]_D$ + 58° (c,1.5).

Further elution of the column with ether gave 3β--acetoxy-12β-hydroxyoleanano-28(13)-lactone (150 mg.) crystallising from chloroform-methanol as needles, m.p. and mixed m.p. 317-321°, $[\alpha]_D$ + 45° (c,1.0).

Oxidation of Ursolic Acid Acetate with Potassium Permanganate. - A solution of ursolic acid acetate (5 g.) in acetic acid (500 c.c.) at 90° was treated over 10 min. with a solution of potassium permanganate (3.3 g.) in water (100 c.c.) with stirring. After stirring at 90° for 2 hr. the excess oxidising agent was reduced with sodium metabisulphite. The product was isolated by means of ether, the ethereal solution shaken with aqueous sodium hydroxide and the insolube sodium salt separated. A solution of the sodium salt in ethanol (10 c.c.) was acidified (Congo Red) with dilute hydrochloric acid, the precipitated acid isolated in the usual manner and crystallised from ethanol to give 11-oxours-12-en-28-oic acid acetate (2.6 g.), m.p. 325-326°, $[\alpha]_D$ + 92° (c,1.0).

Light absorption: $\lambda_{max.}$ 2500 Å. (ϵ = 13,000).

(Found: C,74.9; H,9.5. Calc for $C_{32}H_{48}O_5$: C,75.0; H,9.4%).

The methyl ester acetate separated as needles from aqueous methanol, m.p. 241-243° (no depression).

A solution of the dry neutral oxidation product (1.2 g.) in benzene-light petroleum (1:1; 100 c.c.) was filtered through alumina (16 x 1.2 cm.). The same solvent mixture (250 c.c.) eluted a crystalline fraction A (240 mg.) and benzene eluted a fraction B (470 mg.) which is described later (page 48). Recrystallisation of fraction A from ethanol gave urs-11-eno--28(13)-lactone acetate (250 mg.) as fine needles, m.p. 276-278°, $[\alpha]_D$ + 47° (c,1.0).

(Found: C,77.2; H,9.7. $C_{32}H_{48}O_4$ requires C,77.4; H,9.7%).

Hydrolysis of the lactone acetate (500 mg.), by refluxing with 5% ethanolic potassium hydroxide for 2 hr., gave a neutral product, crystallisation of which gave 3β-hydroxy-urs-11-eno--28(13)-lactone (380 mg.) as needles, m.p. 275-277°, $[\alpha]_D$ + 43° (c,0.9).

Ursanyl Lactone Acetate. - Dry hydrochloric acid gas was passed into a solution of acetyl ursolic acid (3 g.) in chloroform (150 c.c.) for 1 hour. The solution was evaporated to dryness, dissolved in ether and separated into acidic and neutral fractions by means of aqueous sodium hydroxide (10%).

The acidic fraction crystallised from methanol to give acetyl ursolic acid.

The neutral fraction crystallised from methanol to give ursanyl lactone acetate (340 mg.), m.p. 252-254°, $[\alpha]_D$ + 13° (c,2.4). The literature[43] gives m.p. 252-254°, $[\alpha]_D$ + 14° for this lactone.

(Found: C,76.8; H,10.3. Calc. for $C_{32}H_{50}O_4$. C,77.1; H,10.1%).

The lactone shows no selective absorption in the ultra-violet above 2000 Å. and gives no colour with tetranitromethane.

Oxidation of Ursolic Acid Acetate with Chromic Acid. - To a refluxing solution of acetyl ursolic acid (5 g.) in acetic acid (125 c.c.) was added a solution of chromium trioxide (4 g.) in acetic acid (30 c.c.) over a period of 15 min. Refluxing was continued for a further 2 hr. when the solution was cooled, diluted with water, extracted with ether and separated into neutral and acid fractions. The sodium salt of the acid fraction was insoluble in water and was isolated by filtration. Regeneration of the free acid and crystallisation from methanol gave 11-oxours-12-en-28-oic acid acetate (2.4 g.) as stout needles, m.p. 323-325°, $[\alpha]_D$ + 87° (c,1.1).

Light absorption: $\lambda_{max.}$ 2500 Å. (ϵ = 12,200).

(Found: C,74.7; H,9.5. Calc. for $C_{32}H_{50}O_3$. C,74.7; H,9.8%)
The compound gives no colour with tetranitromethane.

The neutral fraction (270 mg.) was filtered through a column of alumina in benzene (200 c.c.) and benzene-ether (1:1; 300 c.c.). The latter fraction was crystallised from methanol to give 11:12-dioxoursano-28(13)-lactone acetate (60 mg.) as colourless needles, m.p. 325-327°, $[\alpha]_D$ + 117° (c, 0.7).
Light absorption: $\lambda_{max.}$ 3120 Å. (ϵ = 7,800).
(Found: C,72.9; H,8.9. Calc. for $C_{32}H_{46}O_6$. C,73.0; H,8.8%)

Urs-11-eno-28(13)-lactone acetate. - Sodium (5 g.) was added in portions to a boiling solution of 11-oxours-12--en-28-oic acid acetate (1 g.) in ethanol (100 c.c.) and the mixture was refluxed for 30 minutes. The mixture was acidified with dilute hydrochloric acid and the product extracted with ether. The ethereal solution was washed with 5% sodium hydroxide solution and the neutral product acetylated on the steam bath with acetic anhydride and pyridine. The acetylated material was isolated by means of ether and crystallisation from ethanol gave urs-11-eno-28(13)--lactone acetate (600 mg.) m.p. 268-269°, $[\alpha]_D$ + 46° (c,1.3).
Light absorption: ϵ_{2060} = 6,200
(Found: C,77.6; H,10.0. Calc. for $C_{32}H_{48}O_4$. C,77.4; H,9.7%).
The compound gives no colour with tetranitromethane in chloroform.

Hydrogenolysis of Urs-11-eno-28(13)-lactone acetate. - A solution of the lactone acetate (100 mg.) in acetic acid (75 c.c.) was shaken with platinum (100 mg.) and hydrogen for 2 hours. The filtered solution was evaporated under reduced pressure and the residue crystallised from ethanol to give ursolic acid acetate, m.p. and mixed m.p. 286-287°, $[\alpha]_D$ + 68° (c,1.0).

Light absorption: ϵ_{2060} = 3,800.

The same result was obtained using ethyl acetate as solvent. Ursanyl lactone acetate was recovered unchanged when treated under the same conditions.

11:12-Epoxyursano-28(13)-lactone acetate. - A solution of urs-11-eno-28(13)-lactone acetate (1.2 g.) in glacial acetic acid (80 c.c.) was treated at 100° with a solution of hydrogen peroxide (100 vol.; 25 c.c.) in acetic acid (25 c.c.) added over 30 min. with stirring. The mixture was kept at 100° for 2 hr. and the reaction product isolated by the addition of water and extraction with ether. Crystallisation of the product from ethanol gave 11:12-epoxyursano-28(13)--lactone (800 mg.) as needles, m.p. 286-287°, $[\alpha]_D$ + 51° (c,0.8) undepressed in m.p. when mixed with a specimen, m.p. 283-285°, $[\alpha]_D$ + 49° (c,1.1) obtained by oxidation of ursolic acid acetate with peracetic acid followed by chromatography of the neutral product as described on page 37 .

Hydrolysis of 11:12-Epoxyursano-28(13)-lactone acetate with 10% Ethanolic Potassium Hydroxide. - A solution of the epoxylactone acetate (1 g.) in ethanol (40 c.c.) containing potassium hydroxide (4 g.) and water (5 c.c.) was refluxed for 5 hours. The solution was cooled to 0° and acidified (Congo Red) by adding a solution of concentrated hydrochloric acid (3.3 c.c.) in ethanol (6.6 c.c.). The mixture was kept at room temperature overnight and then separated into acid and neutral fractions in the usual way. The neutral fraction was crystallised from chloroform-methanol to give 3:11:12--trihydroxyursano-28(13)-lactone as large prisms, m.p. 328-330°, $[\alpha]_D$ + 24° (c,0.8). The literature[40] gives m.p. 316-318°, $[\alpha]_D$ + 25° for this lactone.
(Found: C,73.9; H,10.1. $C_{30}H_{48}O_5$ requires C,73.7; H,9.9%).
It does not give a colour with tetranitromethane in chloroform and it does not show selective absorption of high intensity in the ultra-violet region.

The acidic fraction was crystallised from aqueous methanol to give 3:11:12-trihydroxyisours-14-en-28-oic acid as needles, m.p. 281-284°, $[\alpha]_D$ - 36° (c,1.2)(in methanol). The literature[40] gives m.p. 281-285°, $[\alpha]_D$ - 37° for this acid. Light absorption: ϵ_{2050} = 4,100, ϵ_{2100} = 2,800, ϵ_{2150} = 1250, ϵ_{2200} = 140.

(Found: C,73.9; H,10.1. $C_{30}H_{48}O_3$ requires C,73.7; H,9.9%). It gives a yellow colour with tetranitromethane in methanol and a negative reaction with this reagent in chloroform.

Acetylation of 3:11:12-Trihydroxyursano-28(13)-lactone. - (a) A solution of the trihydroxylactone (270 mg.) in pyridine (2 c.c.) and acetic anhydride (2 c.c.) was kept at room temperature for 3 hours. The product was isolated by means of ether and crystallised from aqueous methanol to give 3:11:12--trihydroxyursano-28(13)-lactone diacetate as needles, m.p. 306-307°, $[\alpha]_D$ + 60° (c,1.1). The literature[40] gives m.p. 296-298°, $[\alpha]_D$ + 61° for this lactone.
(Found: C,71.4; H,9.4. $C_{34}H_{52}O_7$ requires C,71.3; H,9.2%). It does not exhibit absorption of high intensity in the ultra-violet region.
(b) The trihydroxy-lactone (200 mg.) in pyridine (2 c.c.) and acetic anhydride (2 c.c.) was heated at 100° for 3 hr. The product was isolated in the usual manner and crystallised from ethanol to give 3:11:12-triacetoxyursano-28(13)-lactone (155 mg.) as needles, m.p. 318-320°, $[\alpha]_D$ + 55° (c,1.2).
(Found: C,70.3; H,8.9. $C_{36}H_{54}O_8$ requires C,70.4; H,8.8%). Acetylation of the trihydroxy-lactone-diacetate using these conditions gave the triacetoxy-lactone m.p. and mixed m.p. 318-320°, $[\alpha]_D$ + 55° (c,1.2).

3:11:12-Trioxoursano-28(13)-lactone. - (a) A solution of the trihydroxy-lactone (200 mg.) in glacial acetic acid

(25 c.c.) was treated with a solution of chromic anhydride (30 mg.) in acetic acid (8 c.c.) added over 10 min. When the addition was complete, the mixture was gradually heated to 100°. The neutral product was isolated in the usual manner by chromatography on alumina, crystallisation from acetone-light petroleum giving 3:11:12-trioxoursano-28(13)--lactone as needles, m.p. 292-294°, $[\alpha]_D$ + 81° (c,0.9). Light absorption: $\lambda_{max.}$ 3100 (ϵ = 8,200)

(Found: C,74.4; H,8.9. $C_{30}H_{42}O_5$ requires C,74.7; H,8.8%). Its solution in dioxan gives a green colour with aqueous ferric chloride.

(b) The fraction B obtained as described previously (page 42) by chromatography of the neutral product from the oxidation of ursolic acid acetate with potassium permanganate separated from methanol as needles which, after repeated crystallisation from the same solvent yielded 11:12-dioxoursano-28(13)-lactone acetate (55 mg.), m.p. 328-330°, $[\alpha]_D$ + 120° (c,0.5). Light absorption: $\lambda_{max.}$ 3120 Å. (ϵ = 9,000).

(Found: C,73.1; H,9.0. $C_{32}H_{46}O_6$ requires C,73.0; H,8.8%). Its solution in dioxan gives a green colour with aqueous ferric chloride.

The 11:12-dioxolactone acetate was hydrolysed by refluxing its solution in 5% aqueous ethanolic potassium

hydroxide for 1 hour. The crude product (31 mg.) was isolated in the usual way and its solution in acetic acid (20 c.c.) treated with a solution of chromium trioxide (4.9 mg.) in acetic acid (5 c.c.) added dropwise with stirring. The mixture was gradually heated to 100° and the product isolated in the usual manner and crystallised from acetone-light petroleum to yield 3:11:12-trioxoursano-28(13)--lactone (12 mg.) as needles, m.p. and mixed m.p. 293-294°, $[\alpha]_D$ + 80° (c,0.6).

Methyl 3:11:12-Trihydroxyisours-14-en-28-oate. - A solution of the acid (200 mg.) in ether (60 c.c.) was treated with a solution of diazomethane in ether. Crystallisation of the product from acetone-petrol gave methyl 3:11:12-trihydroxy-isours-14-en-28-oate as prisms, m.p. 161-163°, $[\alpha]_D$ - 34° (c,0.6). The literature[40] gives m.p. 154-158°, $[\alpha]_D$ - 35° for this compound.

Light absorption: ϵ_{2050} = 5,750, ϵ_{2100} = 4,400, ϵ_{2150} = 1830 ϵ_{2250} = 120.

(Found: C,74.4; H,10.0. $C_{31}H_{50}O_5$ requires C,74.1; H,10.0%).

Methyl 3:11:12-Trioxoisours-14-en-28-oate. - A solution of methyl 3:11:12-trihydroxyisours-14-en-28-oate (250 mg.) in pyridine (10 c.c.) was allowed to stand at room temperature for 3 hr. with a solution of chromium trioxide (1 g.) in pyridine (10 c.c.). The product was isolated in the usual manner by

means of ether, dissolved in benzene (300 c.c.) and filtered through a column of alumina (10 g.). Evaporation of the filtrate gave a yellow gum (150 mg.) which crystallised from petroleum ether as yellow needles giving methyl 3:11:12-trioxoisours-14-en-28-oate as yellow needles (80 mg.), m.p. 187-189°, $[\alpha]_D$ - 82° (c,1.6).

Light absorption: ϵ_{2060} = 4,300.

(Found: C,75.0; H,9.0 $C_{31}H_{44}O_5$ requires C,75.0; H,9.3%).

Methyl 3:11:12-Trihydroxyisours-14-en-28-oate diacetate.- (a) A solution of the trihydroxy unsaturated acid (200 mg.) in pyridine (3 c.c.) and acetic anhydride (3 c.c.) was kept at room temperature for 3 hr. The product was isolated as an acid fraction by means of ether and sodium hydroxide (10%). The resulting gum failed to crystallise and was methylated with diazomethane. The methylated product crystallised from aqueous methanol as plates to give methyl 3:11:12-trihydroxyisours-14-en-28-oate diacetate (130 mg.) m.p. 231-233°, $[\alpha]_D$ - 6° (c,0.7).

Light absorption: ϵ_{2040} = 4,700.

(Found: C,71.4; H,9.3. $C_{35}H_{54}O_7$ requires C,71.6; H,9.3%).

(b) A solution of the trihydroxy unsaturated acid methyl ester (100 mg.) in pyridine (2 c.c.) and acetic anhydride (2 c.c.) was allowed to stand at room temperature for 3 hr. The

product was isolated by means of ether and crystallised from aqueous methanol to give methyl 3:11:12-trihydroxyiscurs-14--en-28-oate diacetate (70 mg.), m.p. and mixed m.p. 229-231°, $[\alpha]_D$ - 7° (c,1.4). The literature[40] gives m.p. 231°, $[\alpha]_D$ - 9° for this compound.

Erythrodiol diacetate. - A solution of 18β-oleanane--3β:13β:28-triol (200 mg.), m.p. 245-247°, $[\alpha]_D$ + 12° (c,1.2), prepared according to Djerassi et al.[66], in acetic anhydride (25 c.c.) and sodium acetate (250 mg.) was refluxed for 18 hr. The product was isolated by means of ether and crystallised from methanol to give erythrodiol diacetate as needles, m.p. and mixed m.p. 167-169°, $[\alpha]_D$ + 58° (c,1.2).
Light absorption: ϵ_{2050} = 2,700.

Treatment of Methyl 3β-Acetoxyursa-9(11):12-dien-28--oate with Hydrochloric Acid. - A solution of the methyl ester (1.0 g.) in acetic acid (180 c.c.) was heated at 100° for 2 hr. with concentrated hydrochloric acid (15 c.c.) after which a further quantity of concentrated hydrochloric acid (5 c.c.) was added. The solution was then allowed to stand at 100° overnight. The product was isolated in the usual manner by means of ether, dissolved in light petroleum (5 c.c.) and chromatographed on alumina, to give the following fractions.

1. Petrol (400 c.c.) - 64 mg. yellow gum

2. Petrol-benzene (9:1; 400 c.c.) - 122 mg. semi-solid

3. Petrol-benzene (4:1; 400 c.c.) - 100 mg. yellow gum.

4. Petrol-benzene (7:3; 500 c.c.) - 124 mg. yellow gum.

5. Petrol-benzene (2:1; 400 c.c.) - 186 mg. yellow gum.

Further elution of the column gave dark brown uncrystallisable gums.

Fraction 2 crystallised from methanol to give methyl 5:8β:14α-trimethylnoursa-9:11:13(18)-trien-28-oate (35 mg.) as plates m.p. 151-152°, $[\alpha]_D$ - 546° (c,1.0).

Light absorption: $\lambda_{max.}$ 2970 Å. (ϵ = 32,400)

(Found: C,82.2; H,10.0. $C_{31}H_{46}O_2$ requires C,82.6; H,10.3%).

Fraction 3 crystallised from methanol to give starting material (60 mg.), m.p. and mixed m.p. 227-228°, $[\alpha]_D$ + 245° (c,1.2).

Fraction 5 crystallised from methanol as needles of methyl 3β-acetoxyursa-11:13(18)-dien-28-oate (55 mg.), m.p. 186-188°, $[\alpha]_D$ - 77° (c,1.4). $\lambda\lambda_{max.}$ 2430 and 2510 Å. (ϵ = 25,100 and 27,800).

(Found: C,77.7; H,10.1 $C_{33}H_{50}O_4$ requires C,77.6; H,9.9%).

The compound gives a red brown colour with tetranitromethane.

Methyl 3β-hydroxyursa-9(11):12-dien-28-oate. - Methyl 3β-Acetoxyursa-9(11):12-dien-28-oate (1.2 g) was hydrolysed with methanolic potassium hydroxide (135 c.c.; 3%). The

product crystallised from aqueous methanol to give the hydroxy-ester (1.05 g.) as needles, m.p. 192-194°, $[\alpha]_D$ + 306° (c,0.6).

Light absorption: $\lambda_{max.}$ 2820 Å. (ϵ = 8,300).

(Found: C,79.4; H,10.3. $C_{31}H_{48}O_3$ requires C,79.4; H,10.3%).

Methyl 3-oxoursa-9(11):12-dien-28-oate. - A solution of methyl 3β-hydroxyursa-9(11):12-dien-28-oate (950 mg.) in pyridine (20 c.c.) was allowed to stand at room temperature overnight with chromic anhydride-pyridine complex (950 mg.; 20 c.c.). The product was isolated in the usual manner and crystallisation from aqueous methanol gave methyl 3-oxoursa-9(11):12-dien-28-oate as as needles, m.p. 163-164°, $[\alpha]_D$ + 334° (c,0.9)

Light absorption: $\lambda_{max.}$ 2840 Å. (ϵ = 9,200)

(Found: C,79.5; H,10.1. $C_{31}H_{46}O_3$ requires C,79.8; H,9.9%).

Treatment of methyl 3-oxoursa-9(11):12-dien-28-oate with hydrochloric acid. - A solution of the methyl ester (400 mg.) in acetic acid (50 c.c.) was heated on the steam bath for 30 hr. with concentrated hydrochloric acid (5 c.c.). The product, isolated in the usual manner by means of ether, was dissolved in light petrol (50 c.c.) and adsorbed on a column of alumina (12 g.). Elution of the column with petrol-benzene (4:1; 400 c.c.) and crystallisation of the

residue from aqueous methanol gave methyl 3-oxoursa-11:13(18)--dien-28-oate (20 mg.) as needles, m.p. 163-164°, $[\alpha]_D$ - 126° (c,0.5).

Light absorption: $\lambda\lambda_{max.}$ 2440 and 2510 Å. (ϵ = 19,000 and 21,200).

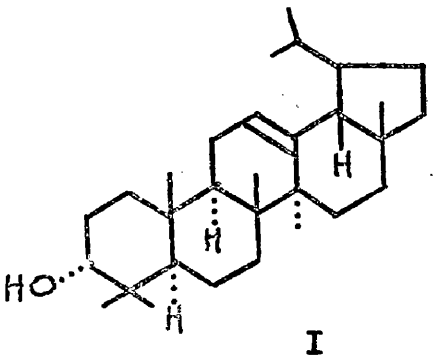
(Found: C,79.7; H,9.7. $C_{31}H_{46}O_3$ requires C,79.8; H, 9.9%).

SECTION II

INTRODUCTION.

The triterpenoid brein occurs in close association with maniladiol in Manila-elemi resin. A possible relationship between brein and the amyrins was recognised, on biogenetic grounds, by Vesterberg[67], who characterised the compound as a dihydric alcohol, $C_{30}H_{50}O_2$, a formula confirmed by Rollet[68]. Oxidation of brein with chromic acid[68,69] gives a dicarbonyl compound, breindione, thus indicating that both hydroxyl groups are either primary or secondary. Morice and Simpson[69] showed that brein contains a double bond which resembles the double bond in α-amyrin, in so far as it does not react with perbenzoic acid. They also showed that this is the only ethenoid linkage in brein (of the type $>C = CH - CH_2-$) since oxidation of brein diacetate with chromic acid gives, in good yield, an αβ-unsaturated ketone which gives no colour with tetranitromethane. Büchi, Jeger and Ruzicka[50] confirmed the results of Morice and Simpson[69] by converting a brein derivative to epi-α-amyrin. On this basis, brein may be formulated as 3α:x-dihydroxyurs-12-ene (I)[Ξ]

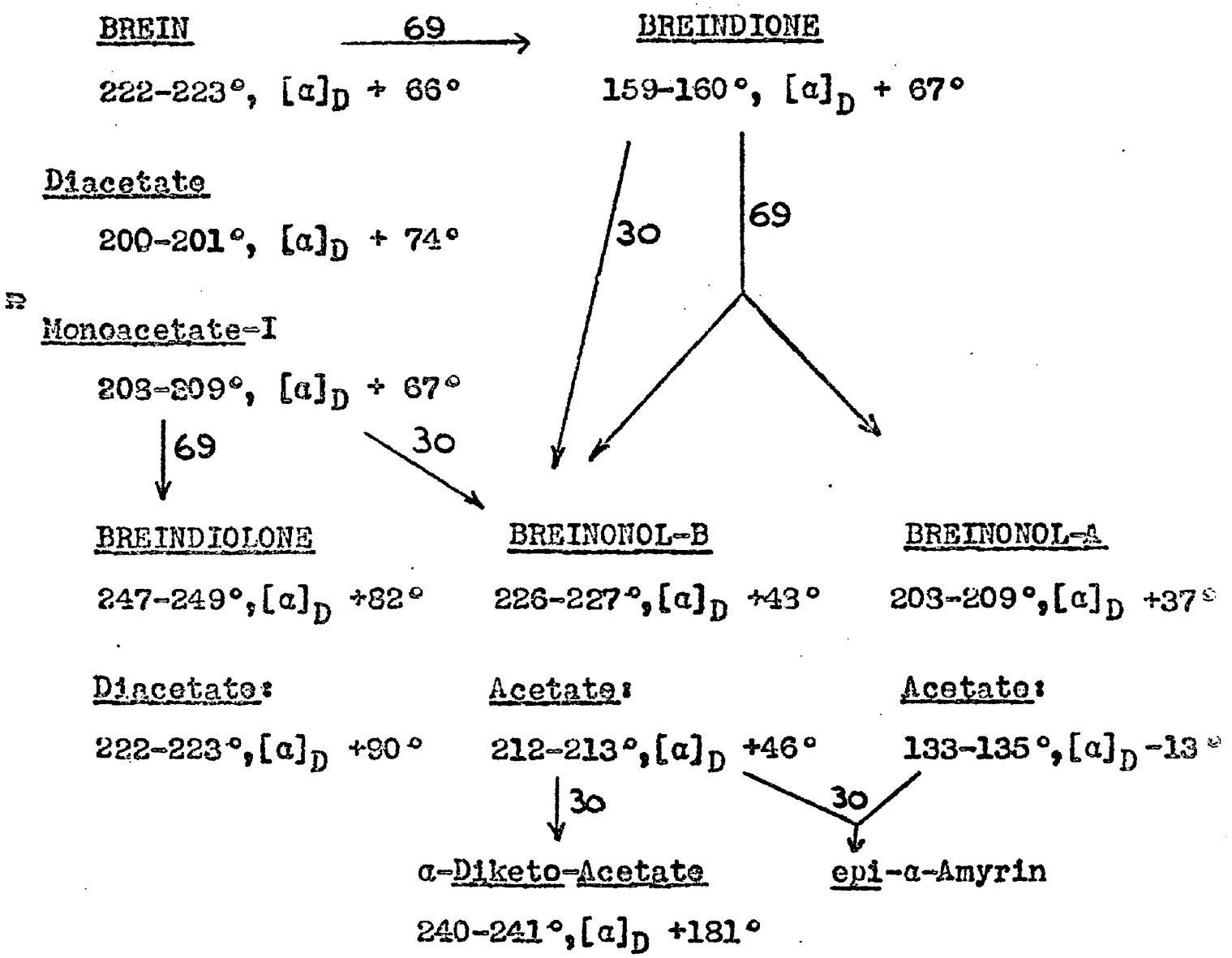
Ξ As is shown later in this section, the conclusion reached by Büchi et al.[50] regarding the configuration of the 3-hydroxyl group is erroneous; brein has a 3β-hydroxyl group and is shown as such in all formulae.



+ 1 hydroxyl group

I

Table II gives a brief outline of the principle reactions of brein and its derivatives known at the outset of these investigations. The nomenclature used is that existent in the literature.



[R] Brein monoacetate, prepared by treatment of brein with acetyl chloride (30) will hereafter be designated as brein monoacetate-I, as opposed to an isomeric brein monoacetate-II, the formation and structure of which will be discussed later.

THE STRUCTURE AND STEREOCHEMISTRY OF BREIN.

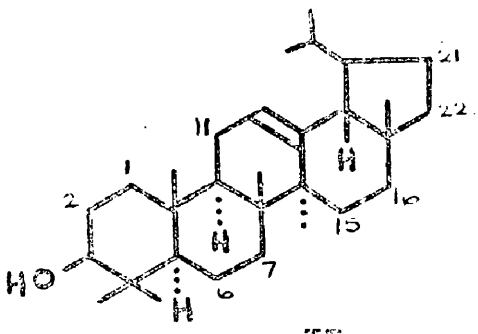
This section describes experiments designed to establish the structure of the triterpenoid diol, brein. Evidence is produced to show that brein is 3β:16β-dihydroxyurs-12-ene (XXI) (the conformation of both hydroxyl groups being equatorial). An attempted partial synthesis of a brein derivative, from α-amyrin is also described, together with the synthesis of isoursenol (XXX) the ursane analogue of taraxerol.

The Position of the x-hydroxyl group in brein.

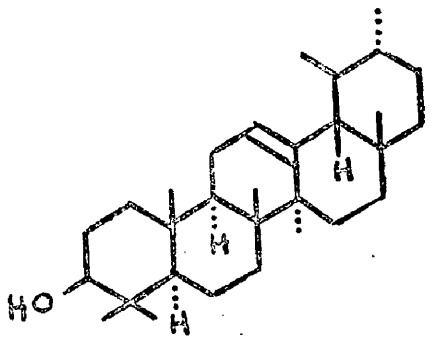
The possible positions for the x-hydroxyl group with reference to the α-amyrin molecule (II) are 1, 2, 6, 7, 11, 15, 16, 21 and 22. Positions 1, 2 and 11 can be eliminated since brein is not an α-glycol and breindione is neither a β-diketone nor an αβ-unsaturated ketone[69]. Büchi, Jeger and Ruzicka[30,70] have prepared an α-diketone by selenium dioxide oxidation of breinonol-B acetate (x-oxours-12-en-3-acetate), which only gives a ferric chloride test on standing for several months. These authors, on this evidence, decided that the x-oxygen function could not be at position 15 or 16, since there are no adjacent hydrogen atoms to produce enolisation. (The formation of this

α-diketone, also excludes the possibility of the x-hydroxyl group in brein being primary). Further, Jeger[15] has suggested that since the 6:7-diketone derived from sumaresinolic acid[71] enolises very readily, then the x-hydroxyl group is not at position 6 or 7; consequently, he proposes that it is situated in ring E at position 21 or 22.

Recently, a proposed constitution for α-amyrin (II)[19] includes a five-membered ring; examination of the infra-red absorption spectrum of breindione excludes the possibility of the presence of a ketone group in a five-membered ring. The proposed location, therefore, of a hydroxyl group in ring E at positions 21 or 22 is incorrect if α-amyrin has structure (II). Although there is no definite proof regarding the nature of ring E (see also 20-24), it now appears probable that α-amyrin is represented constitutionally and stereochemically as (II) or (III). In this thesis it is formulated as II.



II

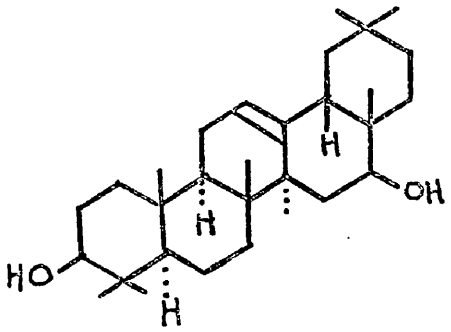


III

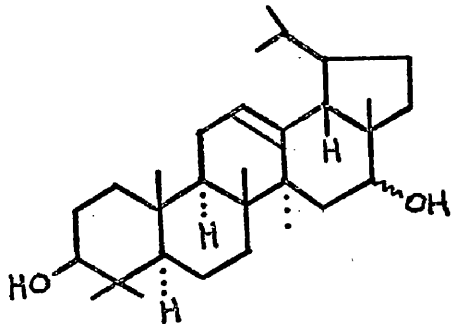
Maniladiol which occurs with brein in Manila elimi resin has been shown to be 3β:16β-dihydroxyolean-12-ene (IV)[72] and it has been suggested[69] that the two triterpenoids were closely related since their physical constants are strikingly similar. These facts would suggest that the x-hydroxyl group in brein is also situated at position 16 of the ursane skeleton. A comparison of brein derivatives with 15 and 16-hydroxyoleanan--3β-ol derivatives does not exclude either possibility.

Brein		3:16-Dihydroxyoleanane		3:15-Dihydroxyoleanane	
diol	+64°	16β-OH	+ 68°	15β-OH	+ 66°
		16α-OH	-	15α-OH	+ 82°
diketone	+ 66°		+ 48°		-
diolmonoacetate	+ 67°	16β-OH	+ 84°	15β-OH	+ 64°
		16α-OH	-	15α-OH	+ 73°
diacetate	+ 70°	16β-OH	+ 80°	15β-OH	-
		16α-OH	-	15α-OH	+ 50°

Since the presence of the x-hydroxyl group at position 15 or 16 seemed probable, experimental evidence in support of this postulate was sought. As a working hypothesis brein was assumed to be 3β:16-dihydroxyurs-12-ene(V).

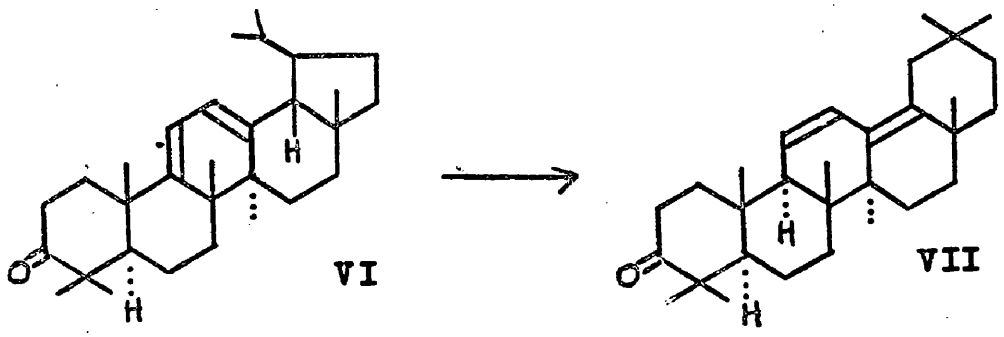


IV

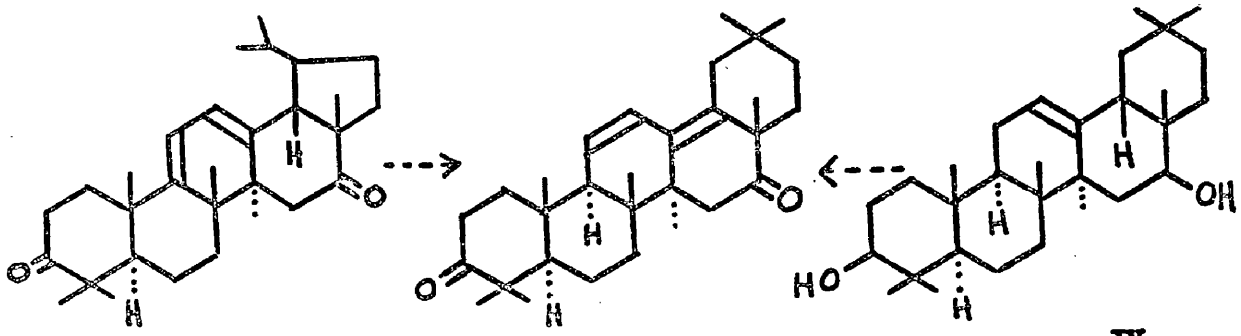


V

Recently, Shaw, Spring and Stevenson[63] have isomerised ursa-9(11):12-dien-3-one (VI) with hydrochloric acid to oleana-11:13(18)-dien-3-one (VII). As a first approach to the problem, it was proposed to subject the diketo-homoannular diene (VIII) (3:16-dioxoursa-9(11):12-diene, synthesised from brein, to similar treatment. It was hoped that the product might be identified as 3:16-dioxo-oleana-11:13(18)-diene (IX), obtainable from maniladiol, and thus establish the oxygen function unequivocally at $C_{(16)}$.

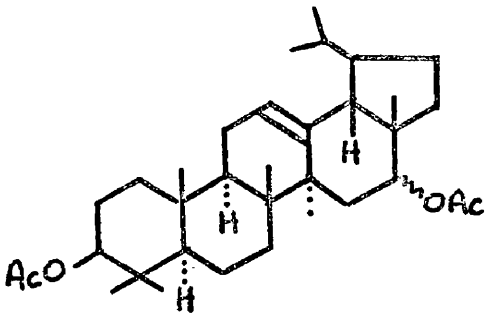


VI VII

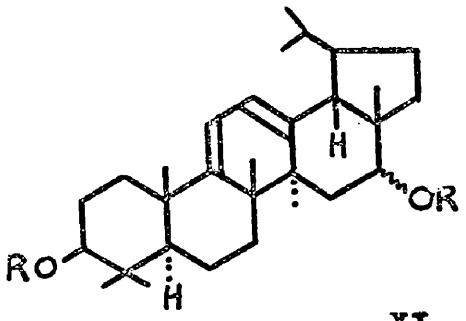


VIII IX IV

Oxidation of brein diacetate (X) with N-bromosuccinimide yields a homoannular diene, which in analogy with α-amyrin has the structure 3:16-diacetoxyursa-9(11):12-diene (XI; R = Ac), hydrolysis of which gives the corresponding diol (XI; R = H). Oxidation of the diol (XI; R = H) with chromium trioxide in pyridine gives the diketone, 3:16-dioxoursa-9(11):12-diene (VIII). The diketone (VIII) has a slight yellow colour which could not be removed by crystallisation or chromatography - this colour may be of structural significance. Treatment of the diketone (VIII) with hydrochloric-acetic acid under various conditions, however, gave only impure starting material or uncrystallisable gums which showed no light absorption properties of a hetero-annular diene system.



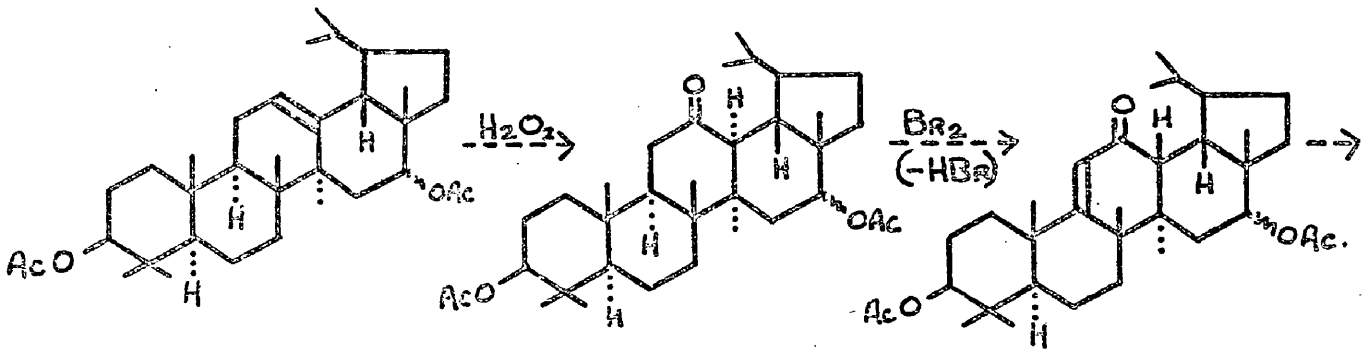
X

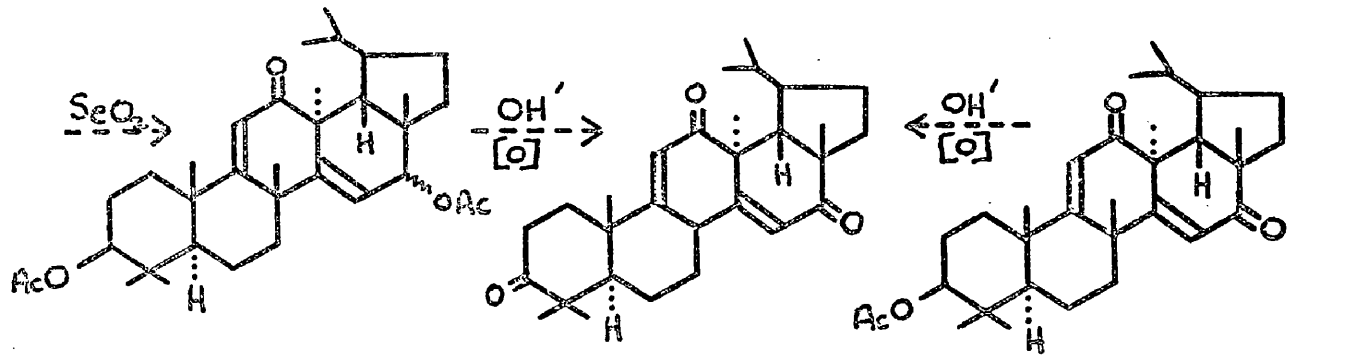


XI

As an alternative approach to confirm the postulated structure of brein as 3:16-dihydroxyurs-12-ene, the synthetic scheme, shown below, (which had previously been successfully applied to α-amyrin) was envisaged. Oxidation of brein diacetate (X) with peracetic acid should give 12-oxo-13α-ursan-

-3:16-diol diacetate (XII), which on bromination and dehydrobromination was expected to yield 12-oxours-9(11)-en-3:16-diol diacetate (XIII). Oxidation of (XIII) with selenium dioxide should yield 12-oxoisoursa-9(11):14-dien-3:16-diol diacetate (XIV) which on hydrolysis and oxidation would give a triketone (XV) which is also obtainable from the known iso-α-amyradienedionyl acetate (XVI)[73]. This would prove the presence of a $C_{(16)}$ hydroxyl group in brein .

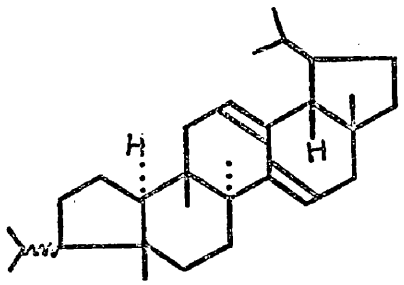




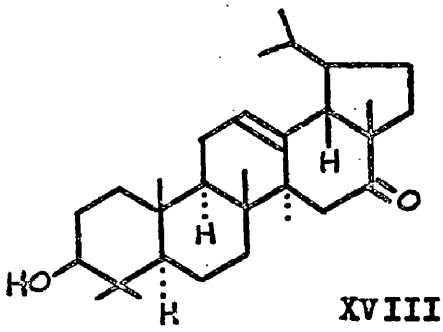
This approach failed at the first stage since repeated attempts to oxidise brein diacetate (X) with hydrogen peroxide in acetic acid were unsuccessful. Similar oxidations of brein, breindione and brein monoacetate-II were also unsuccessful. In a comparative experiment, α-amyrin acetate and brein diacetate were subjected simultaneously to identical oxidising conditions: α-amyrin acetate was smoothly oxidised while brein diacetate was recovered unchanged. An attempt was also made to obtain the saturated ketone (XII) by ozonolysis of brein diacetate (X) but only a very small neutral non-crystallisable fraction was obtained (cf. 74). The inference to be drawn from this lack of reactivity of the double bond is that the α-hydroxyl group in brein exercises a hindering effect, absent in α-amyrin. Examination of molecular models with the hydroxyl group situated at carbon atoms 6, 7, 15, 16, 21 or 22 of the ursane skeleton does not provide a satisfying explanation for this lack of reactivity.

A third approach to the problem was based on the fact that the structure of "1-α-amyradiene", a dehydration product of α-amyrin, has recently[76] been shown to be (XVII). If brein has a hydroxyl group at $C_{(16)}$, then ring contraction of the

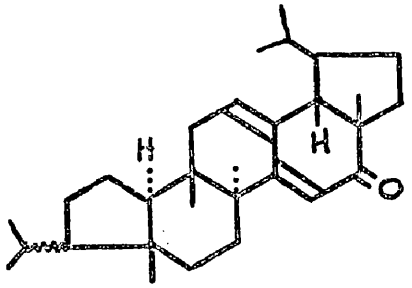
hydroxy-ketone, breinonol-B[x] (XVIII) should yield a conjugated dienone (XIX). Dehydrations of brein itself, using phosphorus pentachloride and phosphoric oxide have already been attempted without success[74].



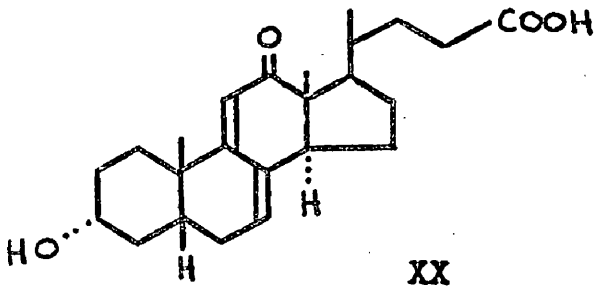
XVII



XVIII



XIX



XX

Dehydration of breinonol-B (XVIII) with hydriodic-acetic acid mixture gave an uncrystallisable gum with no characteristic light absorption properties. It has been observed[75] that

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[x] The nomenclature breinonol-B, first used by Morice and Simpson[69] is misleading since it might indicate a trihydroxy-ketone. This is erroneous and any reference made to either breinonol-A or B is meant to represent an unsaturated compound containing a 3-hydroxyl group and an x-ketone group.

hydriodic acid can act as a reducing agent under ring contraction conditions. Dehydration of breinonol-B (XVIII) with phosphoric oxide also gave an uncrystallisable gum which, however, showed low intensity absorption at 2400 Å. and 2930 Å. Repeated chromatographic purification of this gum, gave finally, in 8%, yield, a fraction showing high intensity absorption at 2400 Å. and 2930 Å. ($\epsilon_{2400} = 4,950$; $\epsilon_{2930} = 9,100$). The absorption spectrum of 3α-hydroxy-12-keto-7:9(11)-choladienic acid (XX) which contains the same dienone chromaphore as that formulated in (XIX), also shows maxima at 2400 Å. and 2930 Å. ($\epsilon_{2400} = 3,700$; $\epsilon_{2930} = 12,900$)[77]. Although this fraction could not be obtained crystalline, it is our contention that the conjugated dienone was formed as expected, thus indicating that the x-hydroxyl group in brein is situated at position 16.

The Configuration of the hydroxyl groups in brein.

At the outset of these investigations there was some confusion regarding the configuration of the $C_{(3)}$ hydroxyl group in brein. Two epimeric hydroxy-x-ketones (breinonol-A and B)[69] had been obtained by Meerwein-Ponndorf reduction of the 3:x-dione, breindione. Of these epimers breinonol-B must have the same configuration at $C_{(3)}$ as brein, because it can also be obtained by oxidation of brein-3-monoacetate with chromic acid[30]. Büchi et al.[30] have reported, however, that

both breinonol-A and B acetates on Wolff-Kishner reduction gave epi-α-amyrin (urs-12-en-3α-ol), in poor yield, and consequently infer that brein has a 3α-hydroxyl group. An apparent anomaly is that catalytic reduction of breindione with platinum oxide in glacial acetic acid yields breinonol-B[30], whereas urs-12-en-3-one in similar circumstances gives the 3β-hydroxy compound.

Klyne and Stokes[73], using the method of molecular rotation differences, have questioned the assignment of the 3α-configuration to brein and prefer a 3β-configuration.

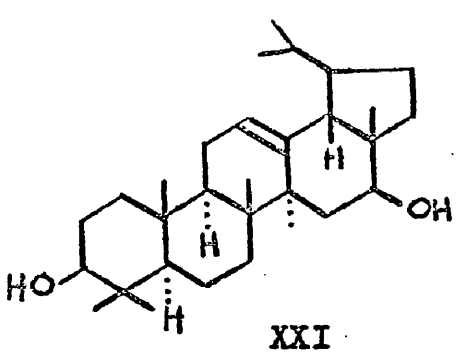
As a first consideration, it appeared that the Wolff-Kishner reduction of breinonol-B to give epi-α-amyrin, as reported by Büchi et al.[30] merited further examination, since this is the only evidence contrary to the assignment of a 3β-(equatorial)-configuration to the 3-hydroxyl group in brein. This reaction was therefore repeated and in marked contrast, α-amyrin (urs-12-en-3β-ol) was obtained in good yield. Since Büchi et al.[30] obtained epi-α-amyrin as the only isolated reduction product, it would appear that their starting material was highly impure. Thus, it is now evident that brein contains a 3β-(equatorial) group; further evidence was forthcoming in support of this.

The 3:x-dione, breindione, has an unhindered ketone group at position 3 and a hindered ketone group at position x, since breindione forms only 3-monocarbonyl derivatives[68,69]. The reduction of hindered and unhindered ketones with lithium aluminium hydride and sodium borohydride has been extensively investigated and a generalisation, that unhindered ketones are reduced to the equatorial isomer and hindered ketones to the axial isomer, has been propounded[49]. Consequently, reduction of breindione with sodium borohydride or lithium aluminium hydride would be expected to give a 3β (equatorial) :x(axial)-diol.

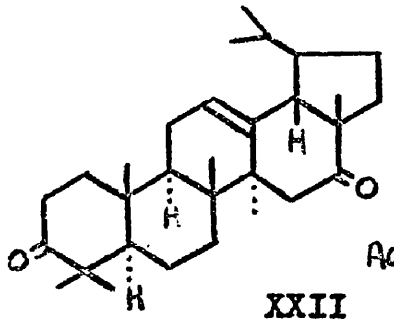
Reduction of breindione with sodium borohydride, followed by room temperature acetylation, gave two products readily separated by chromatography. The least strongly adsorbed compound, $C_{32}H_{50}O_3$, was identified as breinonol-B acetate by direct comparison with an authentic specimen prepared by catalytic hydrogenation of breindione[30]. The more strongly adsorbed compound, $C_{32}H_{52}O_3$, was identified as a diol monoacetate (brein monoacetate-II) since it was oxidised by chromium trioxide in acetic acid to breinonol-B acetate. The other known brein monoacetate-I, prepared in very poor yield by treatment of brein with acetyl chloride[30] is also readily oxidised to breinonol-B acetate. These two diol

monoacetates must therefore differ in the configuration of the x-hydroxyl group. Since brein monoacetate-I is readily acetylated at room temperature to brein diacetate, whereas brein monoacetate-II is recovered unchanged when heated on the steam bath with acetic anhydride and pyridine, then the x-hydroxyl group in the monoacetate-I can be assigned the equatorial conformation and the x-hydroxyl group in the monoacetate-II the axial conformation[49]. Since brein monoacetate-I contains an x-equatorial hydroxyl group then brein itself must also contain an x-equatorial hydroxyl group. Thus the formation of the 3(eq.):x(ax.)-diol and the 3(eq.)-hydroxyl:x-ketone conforms to the above generalisation concerning the reduction of ketones. Similarly, reduction of breindione with lithium aluminium hydride, followed by room temperature acetylation also gives the diol monoacetate-II.

From the above evidence it is proposed that brein has the structure 3β:16β-dihydroxyurs-12-ene (XXI). Accordingly, breindione is described as 3:16-dioxours-12-ene (XXII), brein monoacetate-I as 3β:16β-dihydroxyurs-12-en-3 acetate (XXIII), brein monoacetate-II as 3β:16α-dihydroxyurs-12-en-3 acetate (XXIV), breinonol-A acetate as 16-oxours-12-en-3α-yl acetate (XXV) and breinonol-B acetate as 16-oxours-12-en-3β-yl acetate (XXVI).



XXI



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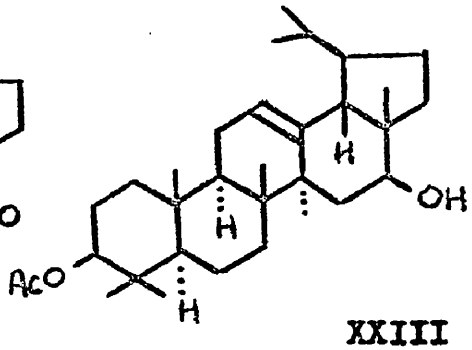

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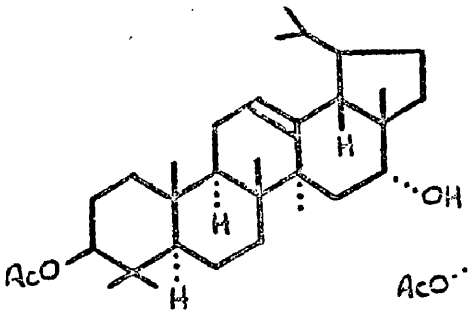
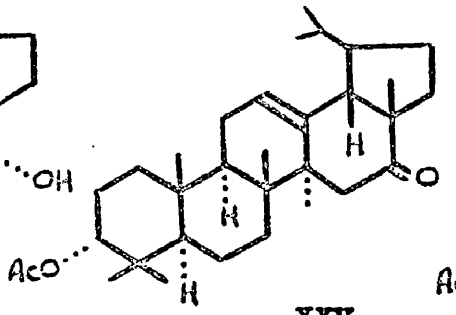
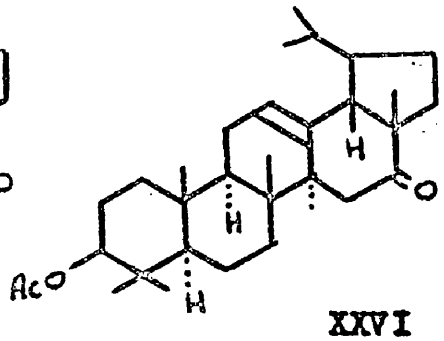


XXIV

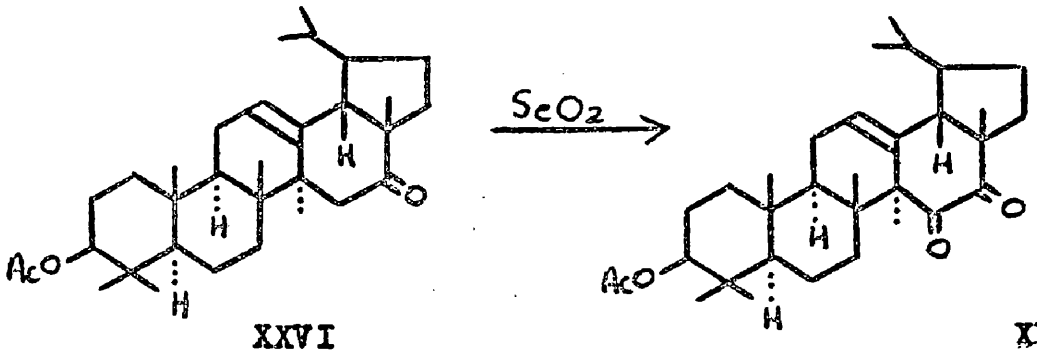


XXV



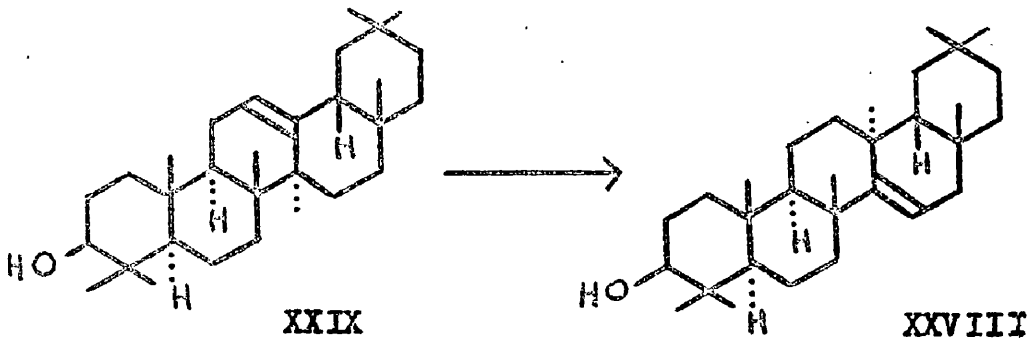
XXVI

To obtain further confirmation for the structure (XXI) for brein, an attempt to synthesise 15:16-dioxours-12-en-3β-yl acetate (XXVII), from α-amyrin acetate, was attempted. This compound should be identical with a dioxo-acetate prepared by the action of selenium dioxide on breinonol-B acetate (XXVI)[80,70].

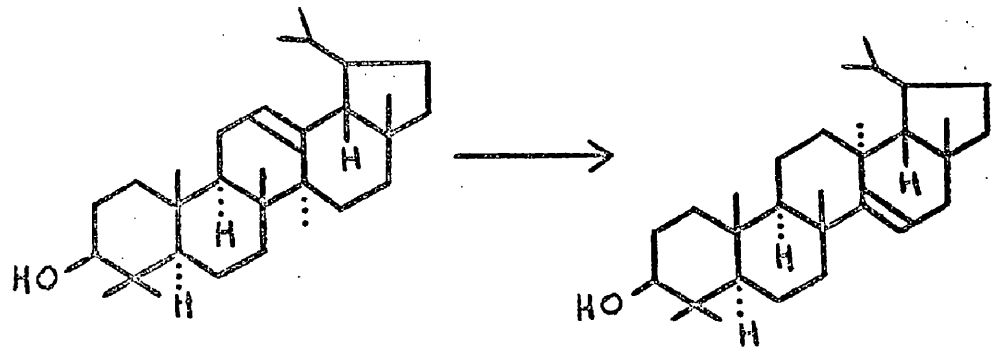


XXVI XXVII

The initial stages of this project were concerned with the synthesis of an alcohol, for which the name isoursenol is proposed and which is the ursane analogue of taraxerol (XXVIII), a naturally occurring triterpenoid related to the oleanane group. Recently, the synthesis of taraxerol (XXVIII), from β-amyrin (XXIX), has been described[79] and an analogous synthesis of isoursenol (XXX), from α-amyrin (XXXI), was desired and has been achieved.

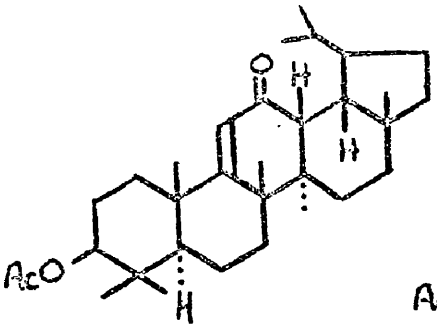


XXIX XXVIII

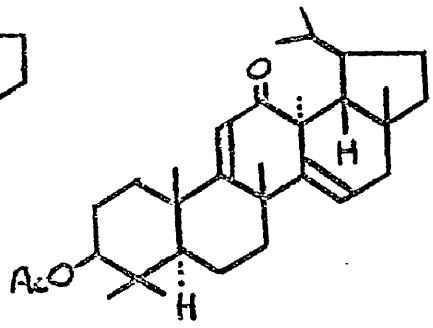


XXXI XXX

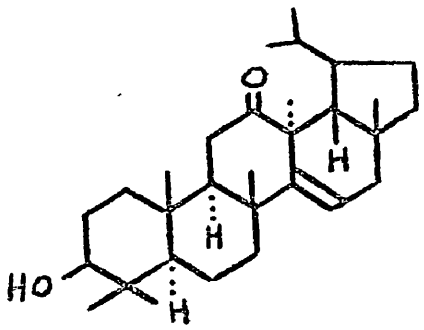
12-Oxoisoursa-9(11):14-dien-3β-yl acetate (XXXIII)[51,80], the starting point in the synthesis, was obtained by selenium dioxide oxidation of 12-oxours-9(11)-en-3β-yl acetate (XXXII) in improved yield by curtailing the time of reaction from 24 to 2 hours. The conversion of (XXXIII) to 12-oxoisours--14-en-3β-ol (XXXIV) by reduction with lithium in liquid ammonia[81] has also been considerably improved by reducing the total reaction time from 17 to 5 minutes. Reduction of (XXXIV) by the Barton modification[82] of the Huang-Minlon reaction, followed by acetylation and chromatography, gave in 25% yield, isoursenyl acetate (XXXV; R = Ac). Treatment of this acetate with mineral acid gave α-amyrin acetate (XXXVII) in analogy with the known conversion of taraxeryl acetate to β-amyrin acetate[79]. The corresponding alcohol (XXXV; R = H) and benzoate (XXXV; R = Bz) were prepared in the usual way. The conversion of isoursenyl acetate (XXXV; R = Ac) to α-amyrin acetate (XXXVII), with mineral acid involves movement of the $C_{(13)}$ methyl group to $C_{(14)}$ and an obvious intermediate in this reaction may be phyllanthyl acetate (XXXVI) which contains a cyclopropane bridge attached to $C_{(13)}$ and $C_{(14)}$[51] and which has also been converted to α-amyrin acetate (XXXVII) with mineral acid.



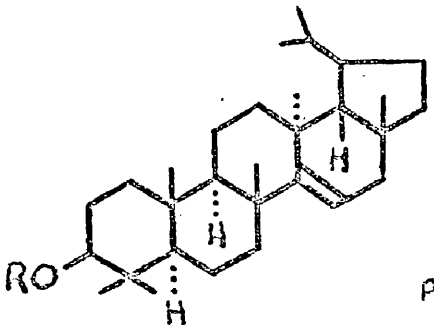
XXXII



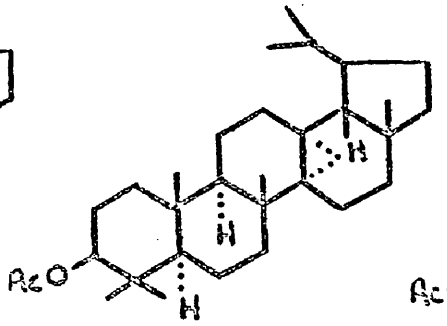
XXXIII



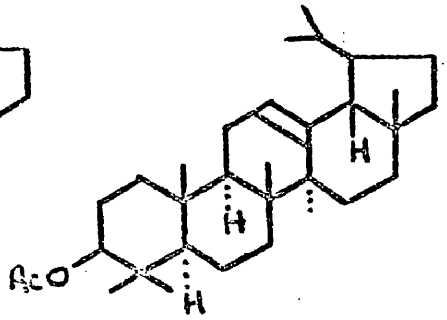
XXXIV



XXXV



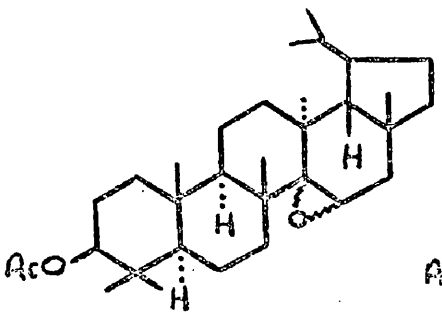
XXXVI



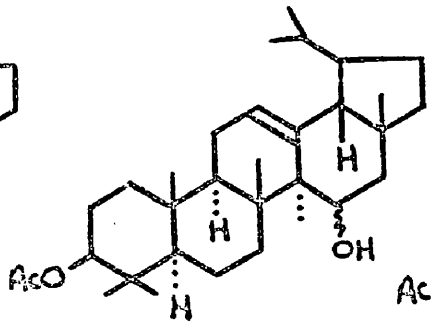
XXXVII

Treatment of isoursenyl acetate (XXXV; R = Ac) with perbenzoic acid yielded two isomeric acetates, $C_{32}H_{50}O_3$, which were readily separated by chromatography. The less adsorbed product shows no high intensity absorption in the ultra-violet region and is saturated to tetranitromethane. It is consequently considered to be 14α:15α-epoxyisoursan-3β-yl acetate (XXXVIII). The second product, however, possessed a double bond (unsaturated to tetranitromethane; double bond absorption in the ultra-violet below 2200 Å.) and a hydroxyl group (since it was oxidised to a ketone with the chromium trioxide-pyridine reagent). In analogy

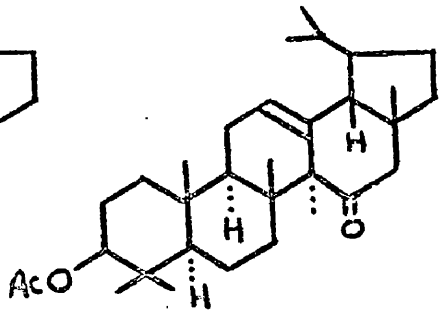
with the behaviour of taraxeryl acetate, for which the structures of the peracid oxidation products were rigorously established[79], the second product can be assigned the structure, urs-12-en-3β:15β-diol-3 acetate (XXXIX) and the derived ketone is 15-oxours-12-en-3β-yl acetate (XL). The direct rearrangement



XXXVIII

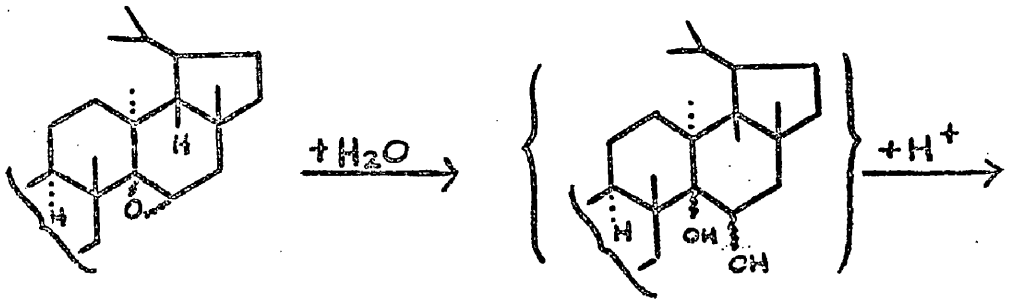


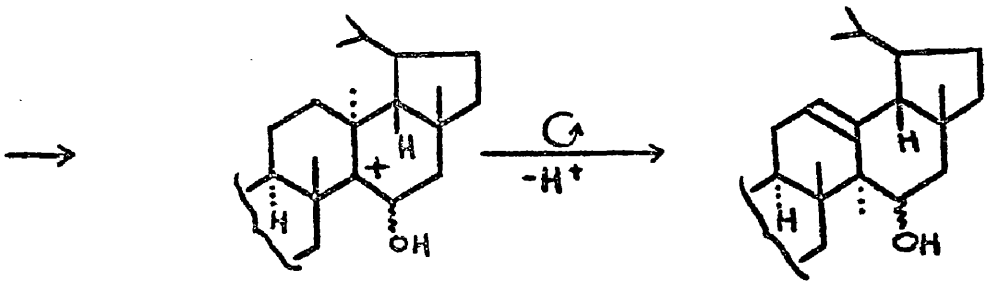
XXXIX



XL

of (XXXVIII) to (XXXIX) by mineral acid followed the analogous taraxeryl acetate oxide to olean-12-en-3β:15α-diol-3 acetate rearrangement i.e. methyl group migration with synchronous elimination of a proton from $C_{(12)}$, as shown below, but differed in that the product (XXXIX) failed to yield a diacetato under normal acetylating conditions. For this reason, no configuration is assigned to the hydroxy group in (XXXIX).





The final stage in the synthesis, the oxidation of the keto-acetate (XL) with selenium dioxide, was carried out using the conditions described by Büchi *et al.*[30] for the oxidation of brainonol-B acetate. A yellow product was obtained, in low yield, which melted at 190-240° (in vacuo) and which resisted further purification. Measurement of the infra-red absorption spectra of this product showed a band at 1712 $cm.^{-1}$, thus indicating that the oxidation of (XL) had been incomplete. Lack of material prevented further oxidation attempts.

It is of interest to note that a comparison of the physical constants of *isoursenol* (XXX) and its derivatives with those of an alcohol, $C_{30}H_{50}O$, isolated by Kasprzyk[83] from the dried flowers of *Calendula Officinalis* bear a remarkable similarity.

	KASPRZYK	EXPERIMENTAL.
Alcohol.	needles m.p.193-194°	needles, m.p.195-197°
Acetate	m.p.216-217°	m.p.214-216°
Benzoate	m.p.240-241°	m.p.237-239°.

Unfortunately, the specific rotations of the naturally occurring alcohol and its derivatives are not reported, and a direct comparison of specimens has not yet been possible.

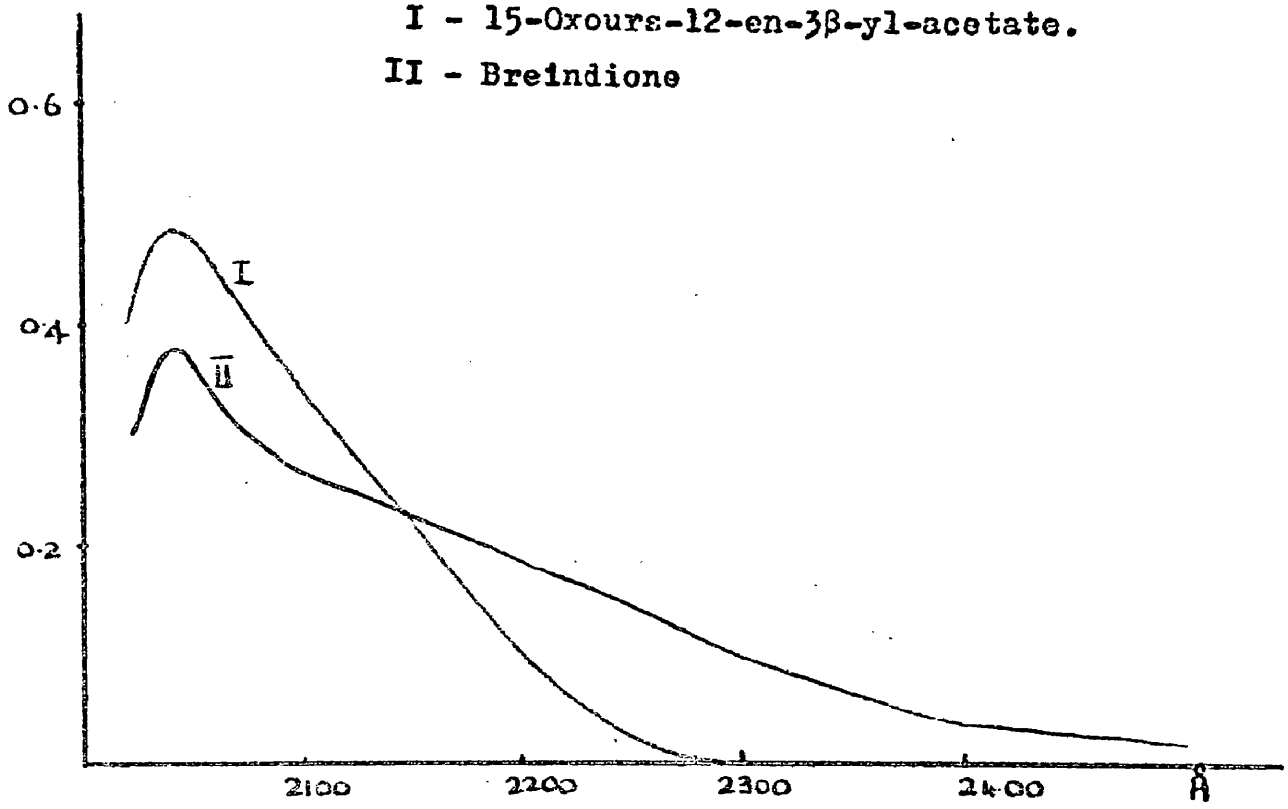
Appendix.

The author has presented evidence to show that the x-hydroxyl group in brein is at $C_{(16)}$ but there are two further factors concerning the chemistry of brein which merit further discussion.

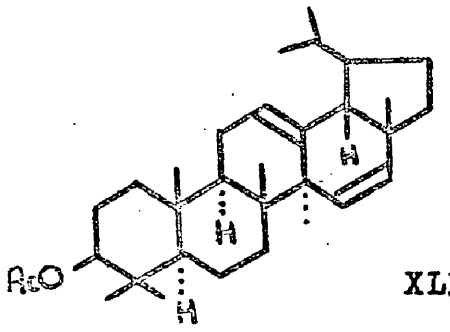
The ultra-violet absorption spectra of breindione and breinonol-B acetate show normal maxima in the double bond region, as would be expected, but it is of interest to note that the shape of these curves are suggestive of the close proximity of the x-ketone group to the double bond. Table III shows the typical spectra of breindione and breinonol-B acetate. This shape of curve is not shown by 15-oxours-12-en-3β-yl acetate.

I - 15-Oxours-12-en-3β-yl-acetate.

II - Breindione



The second point of interest is that treatment of brein monoacetate-II (3β:16α-dihydroxyurs-12-en-3 acetate) on refluxing with phosphorus oxychloride and pyridine gives in low yield a compound, $C_{32}H_{50}O_2$, which gives a strong yellow colour with tetranitromethane. This compound, because of its method of formation, can be formulated as ursa-12:15-dien-3β-yl acetate (XLI). Büchi *et al.*[70]



XLI

have also prepared an unconjugated diene by the treatment of brein monoacetate-I with methane-sulphonyl chloride followed by the treatment of the mesylate with sodium iodide in acetone. These dienes are not identical. An examination of the mother liquors of the phosphorus oxychloride experiment has shown them to contain material which gives a red-brown colour with tetranitromethane and whose ultra-violet absorption spectra shows maxima at 2050 Å. and 2460 Å. (ϵ = 6,300 and 6,900). This is typical of a heteroannular diene. Complete purification and characterisation of this compound was not achieved. The formation of this diene cannot be reconciled with brein having the x-hydroxyl group at $C_{(16)}$ unless methyl group migration has occurred during the reaction.

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

SECTION II.

Melting points were determined using a standard N.P.L. thermometer.

Specific rotations were measured in chloroform solution in a 1 dm. tube at approximately 15°.

Ultra violet absorption spectra were determined in ethanol solution (unless otherwise stated) with a Unicam SP. 500. Spectrophotometer and (ϵ) denotes intensity of absorption.

Colour reactions with tetranitromethane were done in chloroform solution.

The phrase 'in the usual way' implies, in general, dilution with water, extraction with ether, washing consecutively with aqueous sodium hydroxide, water, aqueous hydrochloric acid and aqueous sodium bicarbonate, followed by drying of the ethereal extract over anhydrous sodium sulphate, filtration and evaporation to dryness under reduced pressure.

For chromatography, Brockman Grade II alumina and a light petroleum fraction b.p. 60-80° were used.

The Isolation of Brein from Manila Elemi Resin. - Manila elemi resin (14 lb.) was steam distilled for 12 hours, the residual solid extracted with ether (10 l.) and the ethereal solution washed with sodium hydroxide (10%; 3 x 5 l.), water (2 x 2 l.), hydrochloric acid (5%; 2 x 2 l.) and water (3 x 2 l.). Evaporation of the dried (Na_2SO_4) ethereal solution yielded a solid which was dissolved in ethanol (4 l.) and the solution concentrated to yield two crops of mixed α and β-amyrins (1050 g.), m.p. 135-155°. The mother liquors were taken to dryness, dissolved in petrol (4 l.), and the petrol solution was washed with aqueous ethanol (3 x 6 l.). Concentration of the ethanolic solution yielded a gummy solid isolated by means of ether. Crystallisation of the gum from aqueous acetone gave the brein-elemol complex (39.4 g.) as needles m.p. 169-170°, $[\alpha]_D$ + 40° (c,5.0). The literature[30] gives m.p. 180-181°, $[\alpha]_D$ + 47°.

(Found: C,81.2; H,11.6. Calc. for $C_{45}H_{76}O_3$. C,81.3; H,11.5%).

A solution of brein-elemol complex (4.7 g.) in acetic anhydride (50 c.c.) was refluxed for 2 hr. and the product isolated in the usual manner by means of ether. Crystallisation from chloroform-methanol gave brein diacetate as prisms (3.14 g.), m.p. 195-197°, $[\alpha]_D$ + 74° (c,1.3). The literature[30]

gives m.p. 200-201°, $[\alpha]_D$ + 74°.

(Found: C,77.7; H,10.5. Calc. for $C_{34}H_{54}O_4$. C,77.7; H,10.3%)

Hydrolysis of the diacetate with 5% methanolic potassium hydroxide solution and crystallisation of the product from aqueous acetone gave brein as plates, m.p. 221-222°, $[\alpha]_D$ + 64° (c,1.3). The literature[80] gives m.p. 222-223°, $[\alpha]_D$ + 66°.

Breindione. - (a) To a solution of brein (900 mg.) in acetic acid (200 c.c.; 95%) was added dropwise over 3 hr. a solution of chromium trioxide (400 mg.) in acetic acid (39 c.c.). The solution was allowed to stand at room temperature overnight, diluted with water (ca. 750 c.c.) and filtered. The product was dissolved in ether and the product isolated in the usual manner. Crystallisation from aqueous methanol gave breindione (360 mg.), m.p. and mixed m.p. 155-157°, $[\alpha]_D$ + 63° (c,1.2). (b) A solution of brein (3 g.) in pyridine (20 c.c.) was allowed to stand at room temperature for 2½ hr. with a solution of the chromium trioxide (3 g.) - pyridine (30 c.c.) reagent. the product was isolated in the usual manner by means of ether and crystallisation from aqueous methanol gave breindione (1.9 g.), m.p. 159-160°, $[\alpha]_D$ + 65° (c,1.7). The literature[89] gives m.p. 159-160° $[\alpha]_D$ + 67°.

3:16-Diacetoxyursa-9(11):12-diene. - A solution of brein diacetate (2 g.) in carbon tetrachloride (200 c.c.) was refluxed with N-bromosuccinimide (2 g.) for 2 hr. Isolation of the product in the usual way and crystallisation from methanol gave 3:16-diacetoxyursa-9(11):12-diene as prisms, m.p. 167-168°, $[\alpha]_D$ + 314° (c,1.2).
Light absorption: $\lambda_{max.}$ 2800 Å. (ϵ = 8,600)
(Found: C,77.8; H,10.1. $C_{34}H_{52}O_4$ requires C,77.8; H,10.0%)

Hydrolysis of the diacetate with 5% methanolic potassium hydroxide solution and crystallisation of the product from methanol gave 3:16-dihydroxyursa-9(11):12-diene as needles, m.p. 222-223°, $[\alpha]_D$ + 320° (c,3.9).
(Found: C,81.5; H,11.1. $C_{30}H_{48}O_2$ requires C,81.8; H,10.0%).

3:16-Dioxoursa-9(11):12-diene.- A solution of 3:16-dihydroxyursa-9(11):12-diene (1.39 g.) in pyridine (15 c.c.) was treated with a solution of the chromium trioxide (2.8 g.) - pyridine (20 c.c.) reagent and allowed to stand overnight at room temperature. The product was isolated in the usual manner by means of ether, dissolved in benzene (100 c.c.) and filtered through a column of alumina (20 g.). The column was washed with benzene (500 c.c.) and the combined eluates evaporated to dryness. The residue crystallised from methanol to give 3:16-dioxoursa-9(11):12-diene as yellow plates (500 mg.), m.p.

133-134°, $[\alpha]_D$ + 360° (c,1.5).

Light absorption: $\lambda_{max.}$ 2820 Å. (ϵ = 11,400).

(Found: C,82.7; H,10.3. $C_{30}H_{44}O_2$ requires C,82.5; H,10.2%).

Treatment of 3:16-Dioxoursa-9(11):12-diene with hydrochloric acid. - A solution of 3:16-dioxoursa-9(11):12-diene (400 mg.) in acetic acid (120 c.c.) was heated on the steam bath for 24 hr. with concentrated hydrochloric acid (4 c.c.). The product was isolated in the usual manner as a gum which showed no selective absorption in the ultra-violet above 2200 Å.

Treatment of Brein Diacetate with peracetic acid. - A solution of brein diacetate (500 mg.) in acetic acid (25 c.c.) was treated at 100° with a mixture of hydrogen peroxide (5 c.c.) in acetic acid (5 c.c.) and the solution stirred for 2 hr. after which a further quantity of hydrogen peroxide (5 c.c.) in acetic acid (5 c.c.) was added and stirring continued for a further 2 hr. The product was isolated in the usual manner and crystallisation from methanol gave brein diacetate (320 mg.), m.p. and mixed m.p. 196-197°. This experiment was carried out simultaneously with α-amyrin acetate (500 mg.) under identical conditions when 12-oxo-13α-ursan-3β-yl acetate (300 mg.) was isolated, m.p. and mixed m.p. 210-212°, $[\alpha]_D$ + 114°.

16-Oxours-12-en-3β-yl acetate (Breinonol-B acetate). - A solution of breindione (420 mg.) in acetic acid (20 c.c.) was

shaken in an atmosphere of hydrogen with prereduced platinum catalyst (50 mg.) in acetic acid (5 c.c.) for 3 hr. The solution was filtered, evaporated to dryness and the residue acetylated on the steam bath with acetic anhydride and pyridine. Crystallisation of the product from aqueous methanol gave 16-oxours-12-en-3β-yl acetate (220 mg.) as plates, m.p. 210-212°, $[\alpha]_D$ + 47° (c,0.9). The literature[30] gives m.p. 212-213°, $[\alpha]_D$ + 46°.

Light absorption: ϵ_{2040} = 4,100.

(Found: C,79.2; H,10.4. Calc. for $C_{32}H_{50}O_3$. C,79.6; H,10.4%).

Hydrolysis of the acetate with 5% ethanolic potassium hydroxide gave 16-oxours-12-en-3β-ol (breinonol-B) which separates from methanol as prisms, m.p. 226-227°, $[\alpha]_D$ + 49° (c,1.2). The literature[69] gives m.p. 226-227°, $[\alpha]_D$ + 48°.

Treatment of 16-Oxours-12-en-3β-ol (breinonol-B) with phosphoric oxide. - A solution of 16-oxours-12-en-3β-ol (500 mg.) in dry benzene (50 c.c.) was shaken with phosphoric oxide (1.0 g.) for 20 hr. The product was isolated in the usual manner by means of ether. The residual gum was dissolved in petrol (40 c.c.) and adsorbed on a column of alumina (25 g.). Elution of the column with petrol (400 c.c.) petrol-benzene (600 c.c.; 19:1), petrol-benzene (400 c.c.; 9:1) gave a gum (385 mg.) which showed no high intensity absorption

in the ultra-violet above 2000 Å. Further elution of the column with petrol-benzene (100 c.c.; 7:3) gave a gum (43 mg.) which failed to crystallise from the normal solvents, even on further chromatographic purification. The light absorption properties of this gum were, $\lambda\lambda_{max.}$ 2400 Å. and 2930 Å. (ϵ = 4,950 and 9,100).

Wolff-Kishner reduction of 16-Oxours-12-en-3β-yl acetate (Breinonol-B acetate). - 16-Oxours-12-en-3β-yl acetate (250 mg.) was added to a solution of sodium methoxide (from 0.5 g. sodium) in methanol (20 c.c.) and the mixture heated at 260° for 3 hr. with hydrazine hydrate (5 c.c.; 100%); heating was continued for a further 15 hr. at 180°. Isolation of the product by means of ether and crystallisation of the product from methanol gave α-amyrin (urs-12-en-3β-ol)(155 mg.) as felted needles, m.p. and mixed m.p. 177-179°, $[\alpha]_D$ + 87° (c,1.7).

Reduction of Breindione with Sodium borohydride. - A solution of breindione (500 mg.) in absolute ethanol (15 c.c.) containing sodium borohydride (250 mg.) was kept at room temperature for 2 hr. The product was isolated by means of ether and acetylated overnight at room temperature with acetic anhydride and pyridine. The acetylated material was dissolved

in petrol (50 c.c.) and adsorbed on a column of alumina (15 g.) Elution of the column with petrol-benzene (400 c.c.; 7:3) yielded a gum (330 mg.) which crystallised from aqueous methanol to give 16-oxours-12-en-3β-yl acetate (breinonol-B acetate) (153 mg.) as blades, m.p. 210-212°, $[\alpha]_D$ + 44° (c,1.2).

Light absorption: ϵ_{2040} = 3,800.

(Found: C,79.2; H,10.5. Calc for $C_{32}H_{50}O_3$. C,79.6; H,10.4%).

The compound gives no depression on admixture with a specimen of 16-oxours-12-en-3β-yl acetate prepared by catalytic hydrogenation of breindione[30].

Further elution of the column with benzene-ether(200 c.c.;1·1) yielded a gum (160 mg.) which crystallised from aqueous methanol to give 3β:16α-dihydroxyurs-12-en-3 acetate (breinmonoacetate-II) as needles (120 mg.), m.p. 202-205°, $[\alpha]_D$ + 47° (c,0.9).

Light absorption: ϵ_{2080} = 3,900.

(Found: C,79.2; H,10.8. $C_{32}H_{52}O_3$ requires C,79.3; H,10.8%).

A solution of the monoacetate (200 mg.) in acetic acid (25 c.c.) was treated with a solution of chromium trioxide (30.4 mg.) in acetic acid (10 c.c.) and the mixture kept at room temperature for 18 hr. The product was isolated in the usual manner and crystallisation from aqueous methanol gave

16-oxours-12-en-3β-yl acetate (130 mg.) as plates, m.p and mixed m.p. 209-211°, $[\alpha]_D$ + 45° (c,1.2).

Light absorption: ϵ_{2080} = 3,700

Reduction of Breindione with lithium aluminium hydride. - A solution of breindione (500 mg.) in dry ether (30 c.c.) containing lithium aluminium hydride (250 mg.) was kept at room temperature for 30 min. The product was isolated by means of ether and acetylated at room temperature overnight. The product was dissolved in petrol (50 c.c.) and adsorbed on a column of alumina (15 g.). Elution of the column with petrol-benzene (500 c.c.; 4:1) gave an uncrystallisable gum (129 mg.); elution with petrol-benzene (600 c.c.; 7:3) gave a gum (250 mg.) which crystallised from aqueous methanol to give 16-oxours-12-en-3β-yl acetate (breinonol-B acetate) (140 mg.) as plates m.p. and mixed m.p. 210-212°, $[\alpha]_D$ + 46° (c,1.2).

Light absorption: ϵ_{2040} = 3,700

Further elution of the column with benzene-ether (200 c.c.; 1:1) yielded a gum (130 mg.) which crystallised from aqueous methanol to give 3β:16α-dihydroxyurs-12-en-3 acetate as needles (85 mg.), m.p. and mixed m.p. 202-205°, $[\alpha]_D$ + 48° (c,1.0)

12-Oxoisoursa-9(11):14-dien-3β-yl acetate. - A solution of 12-oxours-9(11)-en-3β-yl acetate (36 g.) in glacial acetic acid (580 c.c.) was refluxed for 2 hr. with selenium dioxide

(54 g.). The crude product, isolated in the usual manner, crystallised from methanol to give needles (23 g.), m.p. 220-221°, $[\alpha]_D$ + 14.5° (c,6.1). A solution of the solid in benzene-light petroleum (1 l.; 1:4) was adsorbed on a column of alumina (1 kg.). Elution of the column with petrol-benzene (4 l.; 3:2), petrol-benzene (2.5 l.; 1:1), and petrol-benzene (3.5 l.; 2:3) gave 12-oxoisoursa-9(11):14-dien-3β-yl acetate (17.5 g.) as needles from methanol, m.p. and mixed m.p. 218-220°, $[\alpha]_D$ + 8.5° (c,3.3). Literature[80] gives m.p. 221-22°, $[\alpha]_D$ + 7°. Light absorption: $\lambda\lambda_{max.}$ 2110 and 2370 Å. (ϵ = 8,400 and 10,000).

12-Oxoisours-14-en-3β-ol. - A solution of 12-oxoisoursa--9(11):14-dien-3β-yl acetate (2.0 g.) in dry ether (100 c.c.) was added over 2 min., with stirring, to a solution obtained by adding lithium (600 mg.) to liquid ammonia (400 c.c.) and the mixture stirred for 3 min. After the addition of acetone, the product was isolated in the usual manner by means of ether. Crystallisation of the product from methanol gave 12-oxoisours--14-en-3β-ol (0.8 g.) as felted needles, m.p. 230-231°, $[\alpha]_D$ - 39° (c,1.4).

Light absorption: ϵ_{2030} = 5,500.

(Found: C,81.0; H,10.9. Calc. for $C_{30}H_{48}O_2$:$\frac{1}{2}CH_3OH$. C,81.1; H,11.1%).

The compound gives a yellow colour with tetranitromethane.

isoUrsenyl Acetate. - 12-Oxoisours-14-en-3β-ol (1.0 g.) was added to a solution obtained by the addition of sodium (2.5 g.) to freshly distilled diethylene glycol (125 c.c.) and the mixture heated to 180°. Anhydrous hydrazine was distilled into the mixture until the solution refluxed gently at 180°. After refluxing at this temperature for 18 hr., the mixture was distilled until the temperature rose to 210°, whereafter refluxing was continued for 24 hr. The product, isolated by means of ether, was acetylated on the steam bath with acetic anhydride and pyridine for 1 hr. The acetylated product was dissolved in petrol and adsorbed on a column of alumina (40 g.). Elution with petrol gave a solid, which, on crystallisation from methanol, gave isoursenyl acetate (250 mg.) as plates, m.p. 214-216°, $[\alpha]_D$ + 36° (c,1.3). Light absorption: ϵ_{2060} = 7,100.

(Found: C,81.9; H,11.4. $C_{32}H_{52}O_2$ requires C,82.0; H,11.2%).

The compound gives a yellow colour with tetranitromethane.

Elution of the column with petrol-benzene (200 c.c.; 9:1) gave 12-oxoisours-14-en-3β-yl acetate (300 mg.), m.p. and mixed m.p. 227-228°, $[\alpha]_D$ - 27° (c,0.8).

The column was stripped with methanol-benzene and the residue crystallised from methanol to give a substance as felted needles (350 mg.), m.p. 245-247°, $[\alpha]_D$ + 19° (c,1.2).

Light absorption: $\lambda\lambda_{max.}$ 2080 and 2380 (ϵ = 13,500 and 10,000).
Found: C,76.9; 77.3; H,10.4; 10.1%).
The compound gives a yellow colour with tetranitromethane.
Infra-red shows bands at 1672 cm.$^{-1}$ ($\alpha\beta$-ketone) and 1724 cm.$^{-1}$ (acetate).

This substance was recovered unchanged on refluxing with lithium aluminium hydride followed by acetylation and also when treated, overnight, with the chromium trioxide-pyridine complex at room temperature. When the substance (250 mg.) was refluxed with acetic anhydride (2 c.c.) for 2 hr. a compound was isolated, crystallising from methanol as prisms, m.p. 201-202°, $[\alpha]_D$ + 35° (c,1.4)
Light absorption: $\lambda\lambda_{max.}$ 2060 and 2380 (ϵ = 6,250 and 4,100)
(Found: C,75.6; H,10.1%).
Infra-red shows a band at 1712 cm.$^{-1}$ (ketone in six-membered ring) but no separate acetate band.

isoUrsenol. - A solution of isoursenyl acetate (250 mg.) in ether (150 c.c.) was refluxed for 30 min. with lithium aluminium hydride (250 mg.). The product was isolated in the usual manner and crystallisation from methanol gave isoursenol as needles, m.p. 195-197°, $[\alpha]_D$ + 30° (c,1.6).
Light absorption: ϵ_{2060} = 5,500.
(Found: C,84.1; H,11.6. $C_{30}H_{50}O$ requires C,84.4; H,11.8%).

The compound gives a yellow colour with tetranitromethane. Reacetylation of isoursenol gave isoursenyl acetate as plates, m.p. and mixed m.p. 214-216°, $[\alpha]_D$ + 35° (c,1.1).

isoUrsenyl benzoate separates from chloroform-methanol as small blades, m.p. 237-239°, $[\alpha]_D$ + 55° (c,2.0) (Found: C,83.3; H,10.3. $C_{37}H_{54}O_2$ requires C,83.7; H,10.3%).

Conversion of isoUrsenyl acetate into α-Amyrin acetate. - To a suspension of isoursenyl acetate (25 mg.) in glacial acetic acid (15 c.c.) at 100° was added concentrated hydrochloric acid (2 c.c.). After the mixture had been heated for a further 25 min., the solvent was removed under reduced pressure. Crystallisation of the residue from methanol gave α-amyrin acetate as plates (12 mg.), m.p. and mixed m.p. 221-223°, $[\alpha]_D$ + 77° (c,0.7).

Treatment of isoUrsenyl acetate with perbenzoic acid. - A solution of isoursenyl acetate (750 mg.) in chloroform (10 c.c.) was treated with a freshly prepared solution of perbenzoic acid (2.0 mol.) in chloroform and the solution kept at 0° for 18 hr. The solution was washed with sodium hydrogen carbonate solution, water, dried (Na_2SO_4) and the solvent removed below 15°. The residue was dissolved in petrol-benzene (100 c.c.; 9:1) and adsorbed on a column of

alumina (25 gm.). Elution with petrol-benzene (100 c.c.; 9:1) yielded a solid which crystallised from chloroform-methanol to give 14$\frac{3}{4}$:15$\frac{3}{4}$-epoxyisoursan-3β-yl acetate (320 mg.) as plates, m.p. 249-251°, $[\alpha]_D$ + 57° (c,1.0).

(Found: C,79.5; H,10.8. $C_{32}H_{50}O_3$ requires C,79.3; H,10.8%).

The compound gives no colour with tetranitromethane and shows no selective absorption of high intensity above 2000 Å.

Further elution of the column with benzene (300 c.c.) yielded a solid which crystallised from aqueous methanol to give urs-12-ene-3β:15$\frac{3}{4}$-diol-3-acetate (270 mg.) as needles, m.p. 223-225°, $[\alpha]_D$ + 75° (c,1.5)

Light absorption: ϵ_{2040} = 6,000

(Found: C,79.0; H,11.1. $C_{32}H_{50}O_3$ requires C,79.3; H,10.8%).

The compound gives a yellow colour with tetranitromethane, but does not acetylate with acetic anhydride-pyridine, either at room temperature or on the steam bath.

Treatment of 14$\frac{3}{4}$:15$\frac{3}{4}$-epoxyisoursan-3β-yl acetate with mineral acid. - (a) Sulphuric acid (2N; 5 c.c.) was added to a solution of 14$\frac{3}{4}$-15$\frac{3}{4}$epoxyisoursan-3β-yl acetate (100 mg.) in glacial acetic acid (100 c.c.) and the mixture heated on the steam bath for 30 mins. A solution of the product in petrol (10 c.c.) was chromatographed on alumina (3 g.). Elution

with benzene-ether (9:1; 300 c.c.) gave a gum which crystallised from aqueous methanol to give urs-12-ene-3β:15β-diol-3β-yl acetate (45 mg.), m.p. and mixed m.p. 222-225°, $[\alpha]_D$ + 73° (c, 0.8).

(b) Concentrated hydrochloric acid (4 c.c.) and water (4 c.c.) were added to a solution of 14:15-epoxyisoursan-3β-yl acetate (200 mg.) in methanol (100 c.c.) and chloroform (30 c.c.). The mixture was kept at room temperature for 18 hours and the product isolated in the usual manner. Crystallisation of the product from aqueous methanol gave urs-12-ene-3β:15-diol-3β-yl acetate (125 mg.), m.p. and mixed m.p. 222-225°, $[\alpha]_D$ + 75° (c, 1.1).

15-Oxours-12-en-3β-yl acetate. - A solution of urs-12-en-3β:15-diol-3β-yl acetate (275 mg.) in pyridine (10 c.c.) was added to a solution of chromium trioxide (1.0 g.) in pyridine (10 c.c.) and allowed to stand overnight at room temperature. The product was isolated in the usual manner by means of ether. Crystallisation from aqueous methanol gave 15-oxours-12-en-3β-yl acetate as needles, m.p. 220-222°, $[\alpha]_D$ + 128° (c, 0.8).

Light absorption: $\epsilon_{2030} = 5,000$.

(Found: C, 79.4; H, 10.4. $C_{32}H_{50}O_3$ requires C, 79.6; H, 10.4%).

The compound gives a yellow colour with tetranitromethane.

Treatment of 15-oxours-12-en-3β-yl acetate with selenium dioxide in dioxan. - A solution of 15-oxours-12-en -3β-yl acetate (175 mg.) in dioxan (30 c.c.) was heated at 200° for 16 hours with selenium dioxide (450 mg.) in a sealed tube. The product was isolated in the usual manner by means of ether as a gum. The gum was dissolved in benzene and filtered through a column of alumina. The product crystallised as clusters of yellow needles from aqueous methanol, m.p. (in vacuo) 190-240°, $[\alpha]_D$ + 131°

Light absorption: ϵ_{2040} = 3800, ϵ_{2600} = 1,000, ϵ_{3000} = 950 ϵ_{3600} = 400.

Treatment of Brein Monoacetate-II with Phosphorus Oxychloride. - A solution of brein monoacetate-II (340 mg.) in pyridine (3 c.c.) was refluxed with phosphorus oxychloride (3 c.c.) for 1 hr. The product was isolated in the usual manner by means of ether and filtration through a column of alumina in petrol-benzene (50:50; 100 c.c) gave a gum (250 mg.) which crystallised from chloroform-methanol to give ursa-12:15--dien-3β-yl acetate as needles (50 mg.), m.p. 228-229°, $[\alpha]_D$ + 40° (c,0.7)

Light absorption: ϵ_{2040} = 3,760)

(Found: C,82.5; H,10.9. $C_{32}H_{50}O_2$ requires C,82.3; H,10.8%).

This compound gives a strong yellow colour with tetranitromethane.

Crystallisation of the mother liquors gave an amorphous solid, m.p. 140-150°, $[\alpha]_D$ + 56° (c,1.5)

Light absorption: $\lambda\lambda_{max.}$ 2050 and 2460 Å. (ϵ = 6,300 and 6,900).

This material gives a red-brown colour with tetranitromethane.

REFERENCES.

1. Halsall, Jones et al.	J., 1954, 2385,3070,3234 and previous papers
2. Holker, Powell, Robertson, Simes, Wright, and Gascoigne.	J., 1953, 2422.
3. Bentley, Henry, Irvine, Mukerji, and Spring.	J., 1955, 596
4. Henry, Irvine and Spring.	J., 1955, 1607.
5. Barton, Pago and Warnhoff	J., 1954, 2715.
6. Irvine, Henry and Spring.	J., 1955, 1316
7. Barton and Overton.	Chem. and Ind., 1955, 654.
8. Ruzicka and Marmor.	Helv. Chim. Acta., 1939, 22, 195
9. Prelog, Norymberski and Jeger	Helv. Chim. Acta., 1946, 29, 360
10. Diener, Jeger, and Ruzicka,	Helv. Chim. Acta., 1950, 33, 896
11. Barton and Brooks,	J., 1951, 257.
12. Haworth,	Ann. Reports, 1937, 34, 327
13. Spring,	Ann. Reports, 1941, 38, 187
14. Noller	Ann. Rev. Biochem. 1945, 14, 383.
15. Jeger,	"Fortshritte der Chemie der Organisher Naturstoffe" Springer-Verlag, 1950, 7, 1.
16. Birch,	Ann. Reports, 1950, 47, 199; 1951, 48, 196.
17. Barton	"Progress in Organic Chem." 1953, 2, 67.

18. Elsevier's Encyclopaedia of Organic Chem., 14S, 939 ff.

19. Beaton, Spring, Stevenson and Strachan. — J., 1955, 2610

20. Meakins, — Chem. and Ind., 1955, 1353.

21. Phillips and Tuites, — Chem. and Ind., 1956, B.I.F. Review R.29.

22. Molera, Arigoni, Eschenmoser, Jeger, and Ruzicka, — Helv. Chim. Acta., 1956, 39, 441.

23. Corey and Ursprung — J. Amer. Chem. Soc., 1956, 183.

24. Cole, Thornton, and White, — Chem. and Ind., 1956, 795.

25a Goodson, — J., 1938, 999

25b Jacobs and Flock, — J. Biol. Chem., 1931, 92, 487

26. Manson, — Ph.D.Thesis (Glasgow) 1950.

27. Ewen and Spring — J., 1943, 523

28. Ruzicka and Wirz, — Helv. Chim. Acta, 1939, 22, 948.

29. Huzii and Ōsumi, — J. Pharm. Soc. Japan, 1940, 60, 178.

30. Büchi, Jeger and Ruzicka, — Helv. Chim. Acta., 1946, 29, 442.

31. Barton and de Mayo, — J., 1953, 2178
Barton, Page and Warnhoff, — J., 1954, 2715.

32. Ruegg, Dreiding, Jeger and Ruzicka. — Helv. Chim. Acta., 1950, 33, 889.

33. Huzii and Ōsumi — J. Pharm. Soc. Japan, 1939, 59, 716.

34. Zürcher, Jeger and Ruzicka	Helv. Chim. Acta., 1954, 37, 2145.
35. Diener, Jeger and Ruzicka	Helv. Chim. Acta., 1950, 33, 896.
36. Huzii and Ōsumi	J. Pharm. Soc. Japan, 1939, 59 716.
37. Orr, Parks, Dunker and Uhl,	J. Amer. Pharm. Assoc. Sci. Ed. 1945, 34, 39.
38. Brossi, Bischof, Jeger and Ruzicka,	Helv. Chim. Acta, 1951, 34, 244.
39. Barton and de Mayo,	J., 1953, 3111
40. Jeger, Borth and Ruzicka,	Helv. Chim. Acta, 1946, 29, 1999.
41. Farquhar,	Ph.D. Thesis (London) 1954.
42. McKean,	Ph.D. Thesis (Glasgow) 1953.
43. Barton and Holness	J., 1952, 78.
44. Winstein and Holness	J. Amer. Chem. Soc., 1955, 77, 5563.
45. Ruzicka, Hösli, and Hofmann,	Helv. Chim. Acta, 1936, 19, 109.
46. Picard and Spring,	J., 1940, 1387.
47. Aumüller, Wedekind and Schicke,	Annalen, 1935, 517, 211
48. Barton and Cookson,	Quarterly Reviews, 1956, No.1, 44.
49. Barton,	J., 1953, 1027.

50. Huzii and Osumi	J. Pharm. Soc. Japan, 1940, 60, 117.
51. Ruzicka, Rüegg, Volli and Jeger,	Helv. Chim. Acta, 1947, 30, 140.
52. Jeger and Ruzicka,	Helv. Chim. Acta, 1945, 28, 209.
Allan, Johnston and Spring,	J., 1954, 1546.
Johnston and Spring	J., 1954, 1556.
53. Fürst and Plattner,	Abs. Papers 12th Int. Congr. Pure and Appl. Chem., N.Y.
54. Ruzicka, Jeger, Redel, and Volli,	Helv. Chim. Acta, 1945, 28, 199.
55. Manson and Spring,	J., 1951, 3332.
56. Allan, Spring, and Stevenson,	J., 1955, 3072.
57. Kaye, Fieser and Fieser,	J. Amer. Chem. Soc. 1955, 77, 5936.
58. Winterstein and Stein,	Z. physiol. Chem., 1931, 199 64.
59. Ruzicka, Jeger and Redel,	Helv. Chim. Acta, 1943, 26, 1235.
60. Ruzicka, Grob, and van der Sluys-Veer,	Helv. Chim. Acta, 1939, 22, 788.
61. Bilham, Kon, and Ross,	J., 1942, 535.
62. Allan, Spring, Stevenson and Strachan,	J., 1955, 3371.
63. Shaw, Spring and Stevenson,	J., 1956, 465.

64. Drake and Duvall,	J. Amer. Chem. Soc. 1936, 58, 1687.
65. Kuwada and Takeda,	J. Pharm. Soc. Japan, 1940, 60, 636.
66. Djerassi, Farkas, Lemin Collins and Walls,	J. Amer. Chem. Soc., 1954, 76, 2969.
67. Vesterberg,	Ber., 1906, 39, 2467.
68. Rollet,	Monatsh. 1929, 53-54, 231.
69. Morice and Simpson,	J., 1942, 198.
70. Büchi, Jeger and Ruzicka,	Helv. Chim. Acta, 1948, 31, 139.
71. Ruzicka, Jeger, Grob and Hösli,	Helv. Chim. Acta, 1943, 26, 2283
72. Bischof, Jeger and Ruzicka	Helv. Chim. Acta, 1949, 32, 1911.
73. Allan and Shaw,	Personal Communication this Department.
74. Mladenović and Hoffmann	Monatsh., 1940, 73, 200
Lieb, Mladenović and Hoffmann	Monatsh., 1940, 73, 219.
75. Allan,	Ph.D. Thesis (Glasgow) 1955.
76. Fayez, Grigor, Spring and Stevenson,	J., 1955, 3378
77. Heusser, Eichenberger, Kurath, Dallenbach and Jeger	Helv. Chim. Acta, 1951, 34, 2106.
78. Klyne and Stokes,	J., 1954, 1979.

79. Beaton, Spring, Stevenson and Stewart. J., 1955, 2131

80. Easton and Spring J., 1955, 2120.

81. Boaton,Easton, Macarthur Spring and Stevenson J., 1955, 3992

82. Barton, Ives and Thomas, J., 1955, 2056.

83. Kasprzyk Prace Glownego Inst. Chem. Przemyst, 1951, No.2, 39.