THESIS

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DEGREE OF DOCTOR OF PHILOSOPHY

by

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STUDIES ON TRITERPENOIDS AND STEROIDS.

CONTENTS.

SUMMARY.

An Examination of the Steroids of Sisal Juice

An examination of the steroidal sapogenin content of sisal juice resulted in the isolation of three known sapogenins, neotigogenin, neohecogenin (sisalagenin) and hecogenin, and a compound of unknown structure, compound S acetate. Compound S acetate on Wolff-Kishner reduction gave tigogenin acetate, indicating that compound S acetate is a ketotigogenin acetate. Unsuccessful attempts were made to determine the position of the carbonyl group.

4-Bromo- and 4-Chlorocholest-4-en-3-one

An unambiguous method of preparation of 4-bromo-and 4-chlorocholest-4-en-3-one has been found. This comprised in treatment of 4β:5-epoxycoprostan-3-one with hydrobromic or hydrochloric acid. The ultra-violet absorption characteristics of these compounds and derivatives were examined.

α-Amyrin

Experiments leading to a new stereoformula for α-amyrin are described, including the conversion of a simple ursane derivative to an oleanane derivative. With the nature of ring E

still in doubt, experiments with the object of introducing reactive groupings in ring E were carried out in order to obtain some direct evidence to the nature of this ring.

A thorough examination of the oxidation of ursa-9(11):12-dien-3β-yl acetate was made, four neutral compounds being isolated and identified.

The first recorded preparation of an ursane derivative with a double bond in ring E is described. Four compounds with an 18-19 double bond were obtained, these are, ursa-9(11):12:18-trien-3β-yl acetate, 3β:12-diacetoxyursa-9(11):12:18-triene, 12-oxoursa-9(11):18-dien-3β-yl acetate, and 12-oxours-18-en-3β-yl acetate.

A discussion of the merits of the two principal stereoformulae for α-amyrin (i.e. the six-membered ring E, and the five-membered ring E) is given.

AN EXAMINATION OF THE STEROIDS

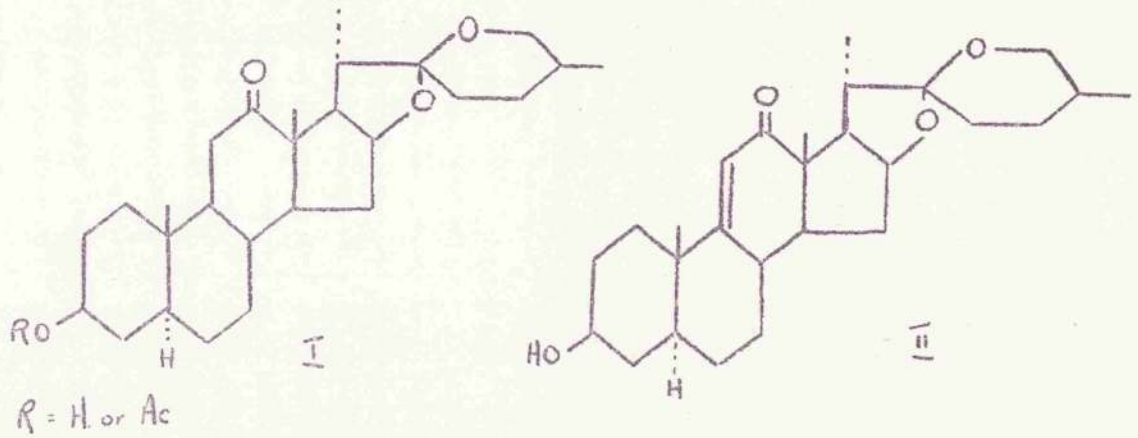
OF

SISAL JUICE.

INTRODUCTION

Steroidal sapogenins are valuable starting materials for the synthesis of steroid hormones (e.g. progesterone, androsterone and cortisone, 4,5,6). Of particular importance are the sapogenins which are oxygenated at C_{12}, since methods are available for the introduction of oxygen functions at C_{11}, starting from C_{12} oxygenated steroids. Steroid sapogenins may be isolated from Digitalis plants, Mexican sarsasparilla root, Mexican plants of the lily species and various Agaves or sisal plants, occurring in Mexico, southern United States, and Kenya.

Sisal waste has been examined by Callow, Cornforth and Spensley (1,2) who showed it to be a good source of hecogenin (I); they also isolated small amounts of an α,β-unsaturated ketone, probably 9-dehydrohecogenin (II). Recently Callow and James (3) isolated a number of other steroidal sapogenins from this source. This latter publication (3) appeared some time after the completion of the work described in this thesis.

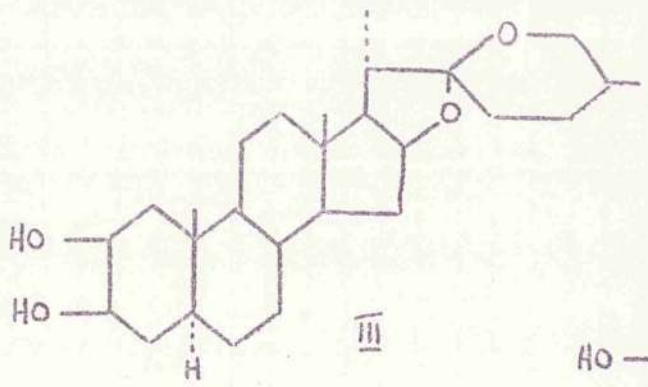


I II

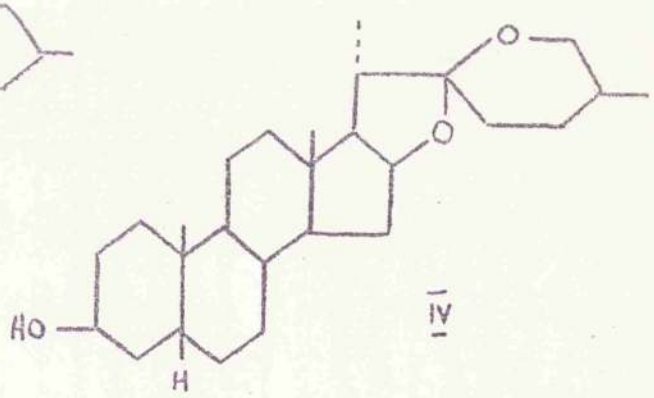
R = H or Ac

Through the kindness of T. and H. Smith, Ltd., Edinburgh, sisal juice residues from which the bulk of hecogenin (I) had been removed were made available, and an examination of their steroidal content was possible.

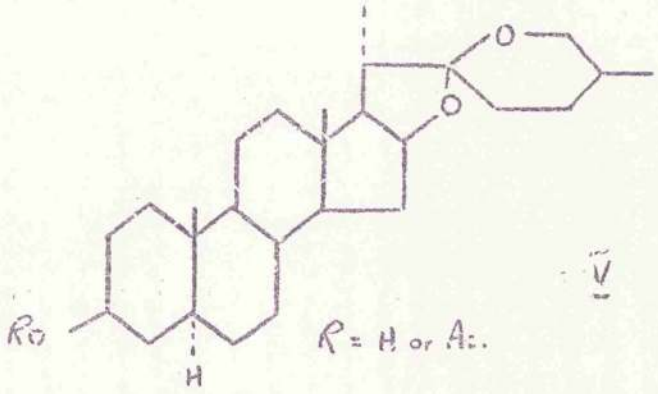
Steroidal sapogenins are the aglycones resulting on hydrolysis of glycosidic saponins. Their chemistry has been summarised by Fieser and Fieser, (7) and Marker from 1939-1947, in particular (4). The earliest known steroid sapogenins are digitogenin (III), (8 - 11), sarsasapogenin (IV), (12 - 14), and tigogenin (V), (11, 15).



III



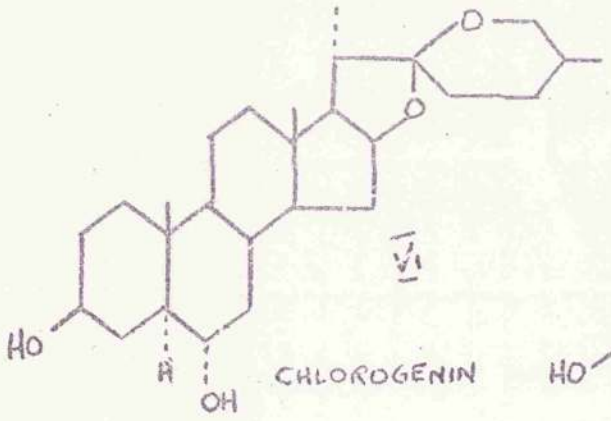
IV

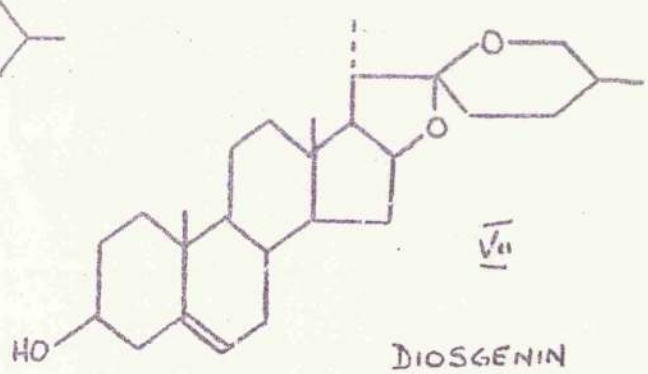


V

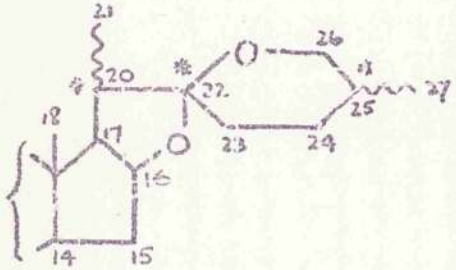
The important discovery by Jacobs and Fleck, (16, 17),

that Diels' hydrocarbon is obtained on dehydrogenation of the sapogenins suggested that the sapogenins possess the steroid ring system. Precision analysis of several sapogenins established their C-27 character (18, 19). The spiroketal formulation was proposed by Marker (20). The various sapogenins are related, numerous interconversions and correlations have been achieved, particularly by Marker and his co-workers. The structures of some sapogenins are shown in the formulae (I) to (VII).



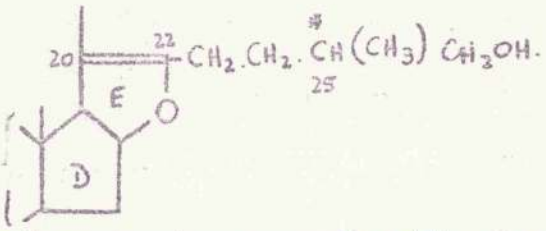


The stereochemistry of the spiroketal side chain of these substances has until recently remained obscure. The sapogenin spiroketal side chain contains three asymmetric centres as shown:-



When sapogenins are heated with acetic anhydride at 200° in a sealed tube (21), rupture of ring F occurs with the formation

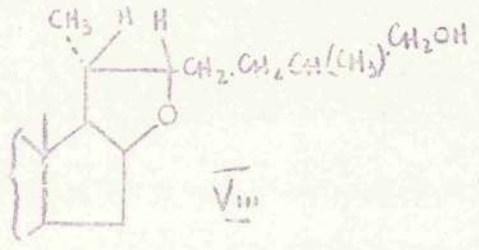
of isomeric pseudosapogenins of structure:-



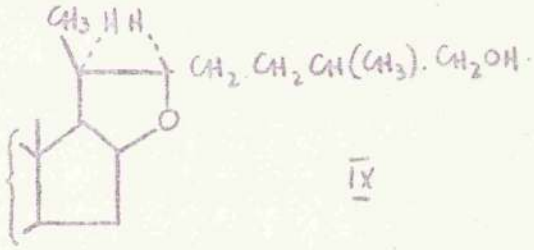
Oxidation of a pseudosapogenin with chromic trioxide gives a Δ-16-pregnen-3-one derivative, and α-methyl glutaric acid, which still contains an asymmetric centre derived from the original sapogenin. It has been found that D- and L-α-methyl glutaric acids have been obtained, depending on the nature of the starting sapogenin (22). A pseudosapogenin on treatment with ethanolic hydrochloric acid at reflux reverts to the original sapogenin. However, if weak acid conditions(1:1 acetic acid : ethanol) are used at room temperature, the pseudosapogenin is converted into an isomer, differing from the original sapogenin only in configuration at C_{20}. This isomer is known as a 20-isosapogenin. Treatment of the 20-isosapogenin with acetic anhydride regenerates the parent pseudosapogenin. Refluxing the 20-isosapogenin in ethanolic hydrochloric acid results in rapid formation of the sapogenin (23).

Catalytic hydrogenation of sarsasapogenin (IV), and of smilagenin (C_{25} isomers) results in the formation of the corresponding dihydrosapogenin, (VIII), which were shown by Scheer et al. (22) to differ only at C_{25}. This follows from the conversion of each into the same 16:22-epoxycoprostan-3β-ol, and into D- and L-α-methyl glutaric acid, respectively. Similar hydrogenation of 20-

isosarsasapogenin and of 20-isosmilagenin gives dihydro-20-isosarsasapogenin (IX) and dihydro-20-isosmilagenin which again differ only in configuration at C_{25}.



VIII

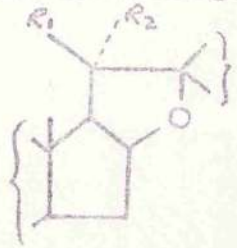


IX

Hydrogenation of pseudosarsasapogenin and pseudosmilagenin, as the 3:26-diacetates, followed by alkaline hydrolysis, yield the corresponding dihydropseudosapogenins (IX). These are identical with the dihydro-20-isosapogenins (IX) obtained as described above. The conversion of 20-isosapogenins to pseudosapogenins and dihydro-20-isosapogenins is analagous to the reactions of normal sapogenins. From this data it was concluded that the 20-isosapogenins must have the spiroketal side chain similar to that of their naturally occuring analogues (23). Oxidation of 20-isosapogenins give Δ-16-pregnen-3:20-dione, and either D- or L-α-methyl glutaric acid.

Configurations at C_{20} — Steroidal sapogenins (natural) and their 20-iso analogues must have one of the configurations A and B. Examination of models indicates that the less stable 20-isosapogenin should be formulated as B. Accordingly, naturally occurring sapogenins have the configuration shown in A.

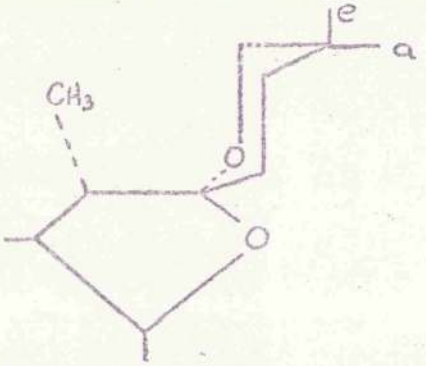
A, natural sapogenins,
$R_1 = H$; $R_2 = CH_3$.



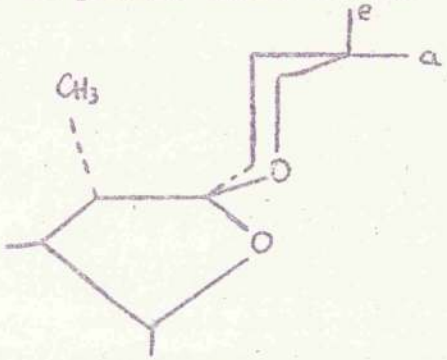
B, isosapogenins,
$R_1 = CH_3$; $R_2 = H$.

This is the same absolute configuration as the side chain of cholesterol, which has been established as (α) by Jeger (24) and Cornforth (25).

Configurations at C_{22} and C_{25} —— Ring F in steroidal sapogenins can theoretically have two configurations at C_{22}, and C_{25}. The various possibilities of rings E and F may be constituted with ring F in a variety of planar chair forms:-

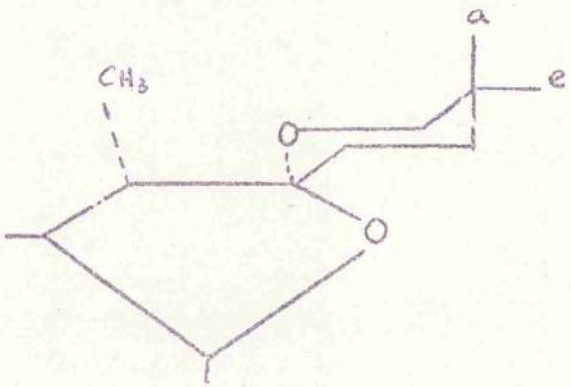


1a. Sarsasapogenin, a = H; e = CH_3.
1b. Smilagenin, a = CH_3; a = H.

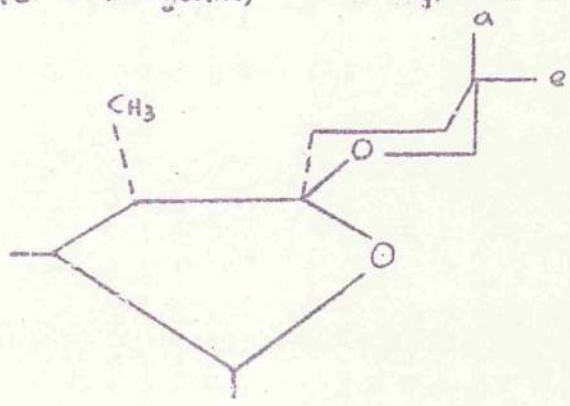


3a. Sarsasapogenin, a = CH_3; e = H.
3b. Smilagenin, a = H; e = CH_3.

2a. Sarsasapogenin, a = CH_3; e = H.
2b. Smilagenin, a = H, e = CH_3.



4a. Sarsasapogenin, a = H; e = CH_3.
4b. Smilagenin, a = CH_3; e = H.



From a study of molecular models and conformational evidence (23, 26, 27, 28), 4a, is the preferred formulation for sarsasapogenin

(IV), and accordingly smilagenin is formulated as 2b. The same conclusions have been reached by Shoppee (29) who also states that absolute configuration at C_{22} is still undetermined.

The prefix neo- in the following text does not refer to the 20-isosapogenins, but to the C_{25} isomer of a normal sapogenin e.g. neohecogenin (now given the trivial name of sisalagenin (3)) which is converted into hecogenin by mineral acid and neotigogenin which is converted into tigogenin by mineral acid.

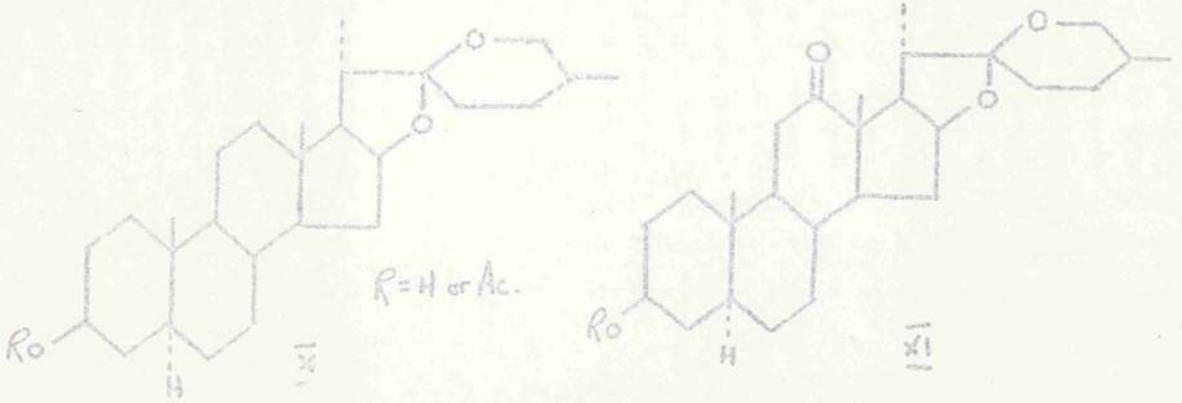
THEORETICAL

The source of the sisal juice was the cultivated sisal plant Agave sisalana Perrine grown in Kenya. After the fibre is removed from the sisal plant, the residue is compressed and the juice collected. This juice contains the sapogenins as their glycosides, and is a commercial source of hecogenin, (I), (2), (5α:22ξ:25D)-spirostan-12-one-3β-ol; nomenclature by N.R.C. sub-committee on steroid nomenclature - G. Meuller and B. Riegel). The glycosides are hydrolysed by mineral acid, and the crude sapogenins extracted with isopropyl ether. The ether is concentrated and the crude sapogenins removed by filtration. Acetylation of the crude sapogenins is carried out by refluxing with acetic anhydride. The reaction solution is then diluted with methanol. From this solution crude hecogenin acetate (I) separates. The mother liquor from this crude hecogenin acetate (I) was the starting material for the present investigation.

The mother liquor (75 l.) was evaporated to dryness, and a portion of the solid residue chromatographed on alumina. Three known compounds were isolated and identified. The most easily eluted compound was neotigogenin acetate (X), previously described by Goodson and Noller (30). Hydrolysis of the acetate gave neotigogenin (X), the physical constants of which were in good agreement with those previously reported. Further

elution of the alumina column gave a crystalline mixture, repeated recrystallisation of which gave a less soluble component identified as hecogenin acetate (I) by direct comparison with an authentic sample. The more soluble component from the fractional crystallisation is considered to be neohecogenin acetate (XI), first described by Marker, and isomerised by him to hecogenin acetate (I) (4). Hydrolysis of the acetate gave neohecogenin (XI), the constants of which were in good agreement with those reported in the literature (4). As stated earlier neohecogenin has been renamed sisalagenin to avoid confusion with the 20-isosapogenins. Callow and James (3) also obtained these three compounds in an examination of sisal juice. Their constants and those found in this work are tabulated:-

Compound	Callow and James		As Found	
	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$
neoTigogenin acetate	175-181°	-79	175-178°	-86°
neoTigogenin	197-203°	-75	198-202°	-73°
Hecogenin acetate	237-243°	+ 4	244-245°	-2.5°
neoHecogenin (Sisalagenin) acetate	228-232°	-12°	220-222°	-14°
neoHecogenin (Sisalagenin)	244-246°	-45°	238-240°	- 4°



The most strongly adsorbed fraction from the column was re-acetylated, as a precaution against hydrolysis having taken place on the alumina column. In the process of working up the acetylation mixture through ether, an insoluble fraction, was obtained. After chromatography of this insoluble fraction, and extensive crystallisation of the eluted solid, a pure compound was obtained. This compound will be referred to as compound S acetate. Crude compound S acetate i.e. as obtained from the column, and before a number of crystallisations showed light absorption (238 mμ, ε =1,200), indicative of the presence of approximately 10% of an $\alpha\beta$-unsaturated ketone, such as 9-dehydrohecogenin (II). Small amounts of the dehydro compound do not appear to depress the melting point of the parent saturated compound; a similar behaviour is shown by 9-dehydro-hecogenin (II) which does not depress the melting point of hecogenin, (I), (31).

A method for the separation of saturated ketones from $\alpha\beta$-unsaturated ketones, by application of Girard P reagent, has been developed by Meuller, (32). The separation depends upon the rapidity with which saturated ketones form the Girard addend in comparison with the time taken by the $\alpha\beta$-unsaturated ketones. When crude compound S acetate was treated by this method, pure compound S acetate was obtained, and a small quantity of an unidentified $\alpha\beta$-unsaturated ketone (238 mμ ε = 11,200) was also isolated.

Analysis of compound S acetate agrees with the molecular formula $C_{29}H_{44}O_5$. Hydrolysis of the acetate yielded the corresponding compound S alcohol $C_{27}H_{42}O_4$. Assuming that compound S alcohol is a steroid sapogenin, two of the four oxygen atoms will be present in the spiroketal side chain, and one as a hydroxyl function. The nature of the fourth oxygen atom was established by the ready formation of a crystalline 2:4-dinitrophenylhydrazone from compound S acetate. Compound S acetate gives no colour with tetranitromethane in chloroform, and does not show high intensity ultra-violet light absorption.

Wolff-Kishner reduction of compound S acetate, followed, by acetylation, gave tigogenin acetate (V), characterised both by its acetate and alcohol, the constants of which were in good agreement with those published (11, 15). The identification was confirmed by comparison with an authentic sample of tigogenin acetate(V), obtained by isomerising neotigogenin acetate (X) by the standard procedure using ethanolic hydrochloric acid, (4). The reduction of compound S acetate to tigogenin acetate (V) shows that compound S acetate is a keto-tigogenin acetate. The ultra-violet spectra of the 2:4-dinitrophenylhydrazone of compound S acetate (370 mμ, ε = 26,000) shows that the carbonyl is a ketone, rather than an aldehyde group. Aldehyde 2:4-dinitrophenylhydrazones absorb from 358 - 361 mμ, while ketone 2:4-dinitrophenylhydrazones absorb from 365 - 368 mμ, (33).

Attempts were made to determine the position of the carbonyl group. Compound S alcohol was oxidised to a diketone $C_{27}H_{40}O_4$, which does not give a colour with ferric chloride solution; consequently, the ketone group in compound S acetate cannot be at C_1, C_2, or at C_4. The ease of formation of the 2:4-dinitrophenylhydrazone excludes the C_{11} position, and furthermore, the crystalline form and other physical properties show that compound S acetate differsfrom 11-ketotigogenin acetate (XII), (supplied by Glaxo, Ltd.,). 12-Ketotigogenin is hecogenin (I), and compound S acetate gives a large depression in melting point when mixed with hecogenin acetate (I).

7-Ketotigogenin acetate (XIII) was prepared by catalytic hydrogenation of 7-ketodiosgenin acetate, (supplied by Dr. G.T. Newbold, R.T.C.), (XIV). The more stable configuration of the hydrogen at C_5, i.e. (α) forms during the hydrogenation of 7-ketodiosgenin acetate (XIV) (4, 7b). The constants of 7-ketotigogenin acetate (XIII) were again found to be different from those of compound S acetate.

6-Ketotigogenin acetate (XV) could not be readily obtained, but 6-ketotigogenone (XVI) (chlorogenone) was prepared. Mild oxidation of diosgenin (VII) with chromic acid at room temperature gave 3:6-diketodiosgen-4-ene (XVII), which on reduction by zinc in acetic acid gave 6-ketotigogenone (XVI), the hydrogen again assuming the more stable α-configuration at C_5. The diketone obtained by oxidising compound S alcohol differed in properties

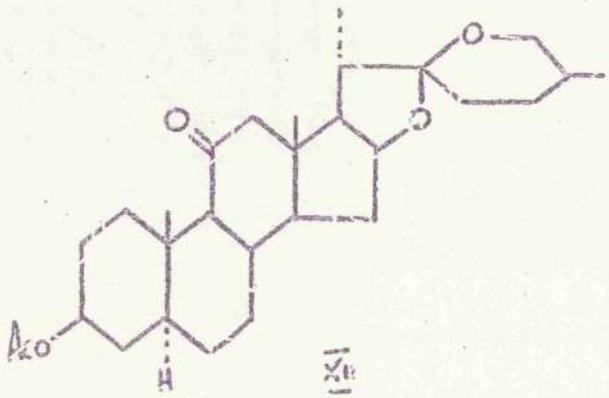
from 6-ketotigogenone (XVI).

The constants of compound S alcohol and its derivatives and other ketotigogenin acetates are tabulated:-

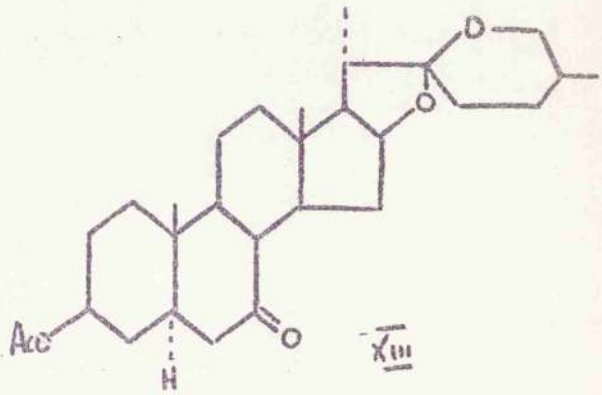
	m.p.	$[\alpha]_D$	Depression in m.p.
Compound S alcohol	240-242°	-6.0°	
Compound S acetate	238-241°	+1.0	
Compound S 3-Ketone	236°	+22.0	
12-Ketotigogenin acetate	244-245°	-2.5°	40°
11-Ketotigogenin acetate	222-224°	-40°	
7-Ketotigogenin acetate (4)	206°	-107°	20°
6-Ketotigogenone (34)	233-235°	-75.4°	7°

The infra-red absorption spectra of compound S alcohol shows a strong carbonyl band at 1706 $cm.^{-1}$, from which it is concluded that the carbonyl group is not at C_{15}.

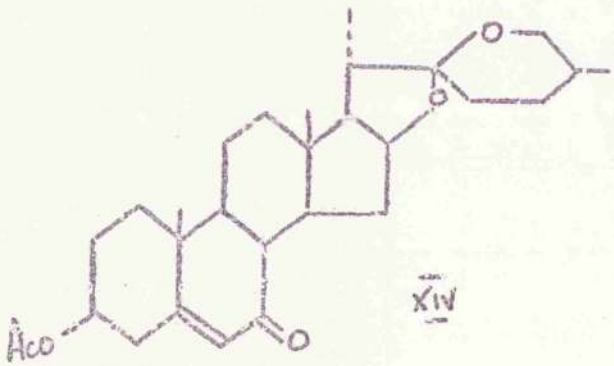
Positions 1, 2, 4, 6, 7, 11, 12 and 15 have been eliminated as the possible location of the carbonyl group, but a re-examination of positions 6 and 7 would be desirable as inversion at C_5 may have occurred during the Wolff-Kishner reduction of compound S acetate. Positions 23 and 24 have not been examined but the infra-red spectrum of the pseudosapogenin, prepared by acetolysis of compound S acetate, would indicate the presence of a carbonyl in such positions.



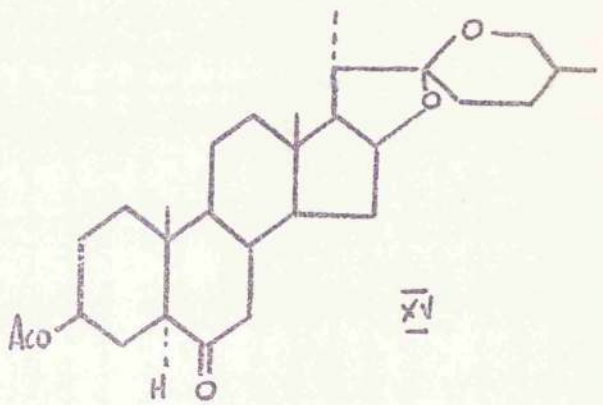
XII



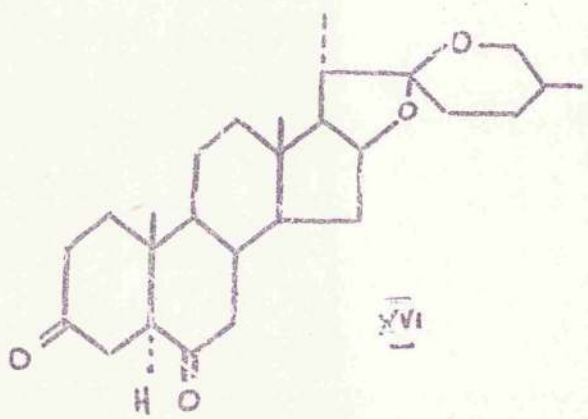
XIII



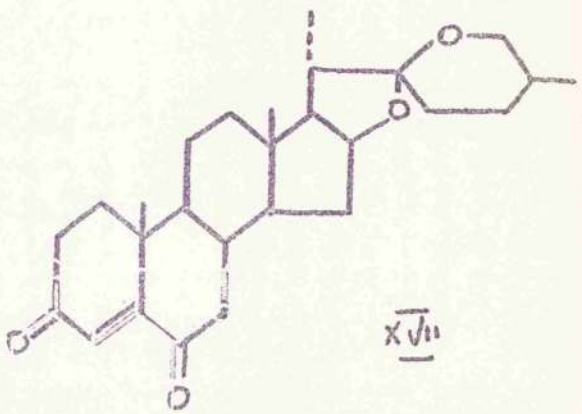
XIV



XV



XVI



XVII

EXPERIMENTAL

M.ps. are uncorrected. Specific rotations were measured in chloroform solution at room temperature with a 1 dm. tube. Ultra-violet absorption spectra were measured in ethanol solution unless otherwise stated, with an Unicam S.P. 500, and a Hilger H 700.307 spectrophotometer. GradeII alumina and a light petroleum fraction, (b.p. 60-80°) were used for chromatography.

Isolation of neoTigogenin Acetate, Hecogenin Acetate, neoHecogenin Acetate and Compound S Acetate.

The mother liquor (75 l.) from crude hecogenin acetate was evaporated to dryness, under reduced pressure. The dry solid (3 kg.) was collected, and a portion (100 g.) acetylated using pyridine (100 c.c.) and acetic anhydride (100 c.c.) at room temperature for 15 hours. The mixture was poured into water (1 l.), and the solid extracted with ether (3 x 500 c.c.) The ether extract was washed with water, hydrochloric acid and saturated sodium bicarbonate solution. The extract was dried (Na_2SO_4), and evaporated yielding a dark-brown solid. The solid was dissolved in a mixture of light petroleum (b.p. 60-80°) and benzene (3:1, 1500 c.c.), and chromatographed on alumina (2.5 kg.). Elution with benzene (1 l.) gave fraction a) (2.5 g.,

m.p. 165-170°). Benzene (17 l.) and benzene/5% ether mixture (11 l.) gave fraction b) (45 g., m.p. 190-220°). Benzene/50% ether mixture (5 l.) gave uncrystallisable gums (5 g.). Ether (5 l.) and ether/10% methanol (7 l.) gave a solid residue (46 g.)

Fraction a) crystallised from chloroform-methanol as large plates (1 g.), and further crystallisation from acetone gave neotigogenin acetate (0.6 g.), m.p. 175-178°, $[\alpha]_D$ -86°, (C, 2.03). Analysis. Found: C, 75.7; H, 10.3. Calc. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1%. It gives no colour with tetranitromethane in chloroform, and does not show high intensity ultra-violet light absorption.

Fraction b) on crystallisation from chloroform-methanol gave a first crop, which on repeated crystallisation from chloroform-methanol gave hecogenin acetate as long needles m.p. and mixed m.p. 244-45°, $[\alpha]_D$ -2.45°, (C, 3.9). Analysis. Found: C, 73.5; H, 9.4. Calc. for $C_{29}H_{44}O_5$: C, 73.7; H, 9.4%. It shows no high intensity ultra-violet light absorption. The combined mother liquors on concentration gave a crystalline solid repeated recrystallisation of which from chloroform-methanol yielded neohecogenin acetate as plates, m.p. 220-222°, $[\alpha]_D$ -14°, (C, 1.1). Analysis. Found: C, 73.4; H, 9.6. Calc. for $C_{29}H_{44}O_5$: C, 73.7; H, 9.4%. It does not show selective absorption of high intensity in the ultra-violet.

The residue from the column (46 g.) was reacetylated using pyridine and acetic anhydride at 100° for 2 hours. In the process

of working up through ether an insoluble portion was obtained (6.5 g.). This portion was dissolved in a mixture of light petroleum (b.p. 60-80°) and benzene (1:1, 200 c.c.), and chromatographed on alumina (190 g.); light petroleum/75% benzene, benzene and benzene/50% ether all eluted fractions m.p. 230-240°, light absorption λ max. 2380 Å, ϵ =1,200, (5 g.). After seven recrystallisations from chloroform-methanol compound S Acetate was obtained as large leaflets m.p. 238-241°, no high intensity light absorption. The m.p. of a mixture with hecogenin acetate (m.p. 244-245°) was 205-211°; $[\alpha]_D$ +1°, (C 1.7), it gives no colour with tetranitromethane in chloroform. Analysis. Found: C, 73.6; H, 9.7. $C_{29}H_{44}O_5$ requires: C, 73.7; H, 9.4%

neoTigogenin:- A solution of neotigogenin acetate (135 mg.) in ethanolic potassium hydroxide solution (3%, 7 c.c.) was refluxed for 2 hours. The reaction mixture was poured into water and extracted with ether. The ether was washed with water, dried (Na_2SO_4), and evaporated to yield a solid (100 mg.), which on crystallisation from methanol gave neotigogenin as needles m.p. 198-202°, $[\alpha]_D$ -73°, (C, 1.1). Analysis. Found: C, 77.8; H, 10.8. Calc. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.75%

neoHecogenin:- neoHecogenin acetate (145mg.) was hydrolysed as described above. neoHecogenin separated from aqueous acetone as fine needles (70 mg.), m.p. 238-240°, $[\alpha]_D$ -4°, (C, 1.1.)

Analysis. Found: C, 75.6; H, 10.0. Calc. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8%.

Compound S alcohol:- Alkaline hydrolysis of compound S acetate (30 mg.) yielded compound S alcohol as square prisms from methanol (17 mg.), m.p. 240-242°, $[\alpha]_D$ -6°, (C, 1.0). Analysis. Found: C, 75.2; H, 9.4. $C_{27}H_{42}O_4$ requires: C, 75.3; H, 9.8%.

Ketone from Compound S Alcohol:- Compound S alcohol (200 mg.) in glacial acetic acid solution (50 c.c.) was treated with a solution of chromium trioxide (45 mg.) in acetic acid (95%, 100 c.c.) at room temperature for 16 hours. The product isolated through ether, crystallised from acetone to give the ketone as long fine needles (75 mg.), m.p. 236°, $[\alpha]_D$ +22°, (C, 1.0). Analysis. Found: C, 75.1; H, 9.5. $C_{27}H_{40}O_4$ requires: C, 75.6; H, 9.4%.

2:4-Dinitrophenylhydrazone of Compound S Acetate:- A solution of compound S acetate (100 mg.) in ethanol (40 c.c.) was treated with Brady's reagent (acidified ethanolic solution of 2:4-dinitrophenylhydrazine). The hydrazone was collected, dried, dissolved in benzene and filtered through an alumina column. The filtrate was evaporated and the product crystallised from chloroform-ethanol as plates, m.p. 250° (d.), light absorption λ max. 3700 Å,

ε= 26,000. Analysis. Found: C, 63.3; H, 7.4.
$C_{35}H_{48}N_4O_8$ requires: C, 64.4; H, 7.4%

Wolff-Kishner Reduction of Compound S Acetate:-
Compound S acetate (500 mg.) was heated with sodium methoxide (1 g. of sodium in 10 c.c. methanol) and hydrazine hydrate (100%, 5 c.c.) for 17 hours at 200°. The reaction mixture was poured into water and extracted with ether. The ether was washed with hydrochloric acid and water, dried (Na_2SO_4) and evaporated to dryness. The residue was dissolved in pyridine (2 c.c.) and acetic anhydride (2 c.c.) and heated for 2 hours at 100°. The product (480 mg.) isolated through ether crystallise from methanol to give tigogenin acetate, as long needles m.p. 203-205°, $[\alpha]_D$ -68.5°, (C, 1.2), no high intensity light absorption. Analysis. Found: C, 75.8; H, 10.3. Calc. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1%

Tigogenin:- Tigogenin acetate (100 mg.) was hydrolysed by ethanolic potassium hydroxide in the usual way. Tigogenin crystallised as plates from methanol, m.p. 203-205°, $[\alpha]_D$ -75°, (C, 1.2). Analysis. Found: C, 74.9; H, 10.6. Calc. for $C_{27}H_{44}O_3.CH_3OH$ C, 74.9; H, 10.8%.

Isomerisation of neotigogenin Acetate:- with H.S. Watson B.Sc.

A solution of neotigogenin acetate (1 g.) in ethanol (100 c.c.)

and concentrated hydrochloric acid (20 c.c.) was refluxed for 20 hours. Water was added to the reaction mixture, and the solid collected, dried, and heated with acetic anhydride (15 c.c.) for 30 minutes. The product separated on cooling and was crystallised from acetone to yield tigogenin acetate as long needles (800 mg.), m.p. 203-205°, $[\alpha]_D$ -68°, (C, 1.2). A mixture with tigogenin acetate prepared as described above from compound S acetate, was undepressed in m.p.

Girard 'P' Separation of Crude Compound S Acetate:-

Crude compound S acetate (m.p. 230-240°, ε_{max}=1,200) (500 mg.) was refluxed for 30 minutes with Girard 'P' reagent (pyridinium -aceto-hydrazide chloride) (1.2 g.) in ethanol (20 c.c.) and glacial acetic acid (2 c.c.). The cooled reaction mixture was poured into ice-cold saturated sodium carbonate solution (500 c.c.), and the white precipitate of unsaturated ketone extracted with ether. The ether was washed with sodium carbonate solution, the washings being retained. The ether was washed with water, dried (Na_2SO_4) and evaporated to yield a yellow solid (10 mg.), which after two crystallisation from methanol had m.p. 228-235°, ultra-violet light absorption, $\lambda_{max.}$ 2380Å, ε = 11,200. The alkaline solution of the Girard 'P' addend was acidified with glacial acetic acid (75 c.c.), and then extracted with ether. The ether was washed free of acid, dried (Na_2SO_4), and evaporated to yield a white solid, (400 mg.). This solid crystallised from methanol as large leaflets

of compound S acetate, m.p. 238-241°, $[\alpha]_D$ +0.8°, (C, 1.2), mixed m.p. with hecogenin acetate (244-245°) was 205-214°. Analysis. Found: C, 73.9; H, 9.7. Required for $C_{29}H_{44}O_5$: C, 73.7; H, 9.4% .

7-Ketotigogenin Acetate:- A solution of 7-ketodiosgenin acetate (1 g.) in ether (150 c.c.) was shaken with palladium-strontium carbonate catalyst (2 g.) and hydrogen at 3 atmospheres pressure for 2 hours. The catalyst was removed and the solvent evaporated to dryness yielding a residue, which crystallised from acetone, to give 7-ketotigogenin acetate as long flat needles m.p. 206°, mixed m.p. with compound S acetate (238-241°) was 185-206°. $[\alpha]_D$ -107°, (C, 1.7). Marker gives m.p. 216-219°, (4).

6-Ketotigogenone, (Chlorogenone):- Diosgenin (2 g.) in glacial acetic acid solution (200c.c.) was treated with a solution of chromic trioxide (2 g.) in acetic acid (95%, 40 c.c.) at room temperature for 1 hour. Methanol was added and the reaction mixture poured into water and ether extracted. The ether was washed, dried (Na_2SO_4), and evaporated to yield 3:6-diketodiosgen-4-ene as a pale yellow solid. The crude ene-dione was dissolved in glacial acetic acid (160 c.c.), and zinc dust (4 g.) added. The mixture was refluxed for 4 hours, and then filtered. The filtrate was diluted with water, and ether extracted. The ether was washed with water,

dried (Na_2SO_4), and evaporated to give a white solid (800 mg.), which on crystallisation from acetone gave 6-ketotigogenone as plates, m.p. 233-235°, $[\alpha]_D$ -75.4°, (C, 1.8), a mixture with the ketone from compound S alcohol had m.p. 229-233°.

Literature constants, m.p. softens 236°, 247-248°, $[\alpha]_D$ -69.6°, and -81.4° in dioxan, (35).

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4-BROMO- AND 4-CHLOROCHOLEST-4-EN-3-ONE

INTRODUCTION.

The characteristic ultra-violet absorption spectra of steroids containing unsaturated groups are of considerable value as a guide to structure.

The extinction curve, relating wavelength of light (λ) and the intensity of absorption at these wavelengths as expressed by the molecular extinction coefficient (ε), consists of groups of narrow absorption bands each representing a transition from a particular combination of vibrational and rotational levels in the electronic ground state to the corresponding combination in an excited electron state.

Absorption of ultra-violet light by organic compounds, such as $\alpha\beta$-unsaturated ketones, involve the valency electrons. In $\alpha\beta$-unsaturated ketones the electrons involved are, the π-electrons forming the double bonds, and the p-electrons which are the unshared electron pair on the oxygen atom. The spectra due to individual groups are modified by interaction with the other groups present. Electronic interaction occurs in $\alpha\beta$-unsaturated ketones, and is particularly strong as the grouping contains highly polarisable π-and p-electrons. The classical term for the electronic interaction, where there is an arrangement of multiple bonds in adjacent positions i.e. separated by one single bond is conjugation. With a few exceptions each type of conjugation

results in a closing up of ground and excited levels i.e. decrease in transition energy and consequent band displacement to longer wavelengths, (bathochromic shift).

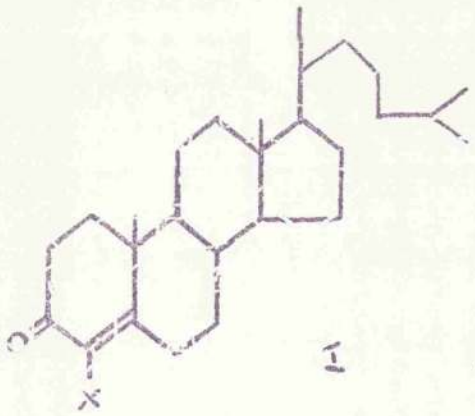
In modern usage chromophore designates π -electron groups, and auxochrome, p-electron groups. Two chromophores in conjugation produce intense bands from 230 mμ upwards, $\alpha\beta$-unsaturated ketones produce intense bands from 230mμ to 260mμ, dependent upon environment. This intense band is due to the interaction of the π -electrons of the C = C group, and the C = O group. A band of low intensity exists from 315mμ to 320 mμ, which is dependent on the interaction of the π -electrons of the chromophore C = O , and the p-electrons of the auxochrome = O:. The former band, i.e. 230 mμ to 260 mμ, is used for characterisation purposes of $\alpha\beta$-unsaturated ketones. The latter band has not so far been so widely used for structural determination purposes.

Most substituents which possess no unshared electrons have well defined bathochromic effects when directly attached to the chromophoric system. Relatively few substituents in the same position cause a hypsochromic effect (i.e. band displacement to lower wavelengths). (See reviews e.g. 1, 2).

Considerable interest has been shown in the effect on the ultra-violet spectra of $\alpha\beta$-unsaturated ketones by halogen substituted on the ethylenic linkage of the $\alpha\beta$-unsaturated ketones, especially in the steroid series (3,4,5,6). The data available

are not very extensive, and the bathochromic shifts observed have made some of the structures postulated for halogen substituted α/β-unsaturated steroidal ketones open to doubt. The hypsochromic shifts are also of value as proof of structure e.g. in the halogen substituted α/β-unsaturated ketones and the hydrazones of these ketones.

A discussion of the preparation, chemistry, and the ultraviolet absorption characteristics of 4-bromo- and 4-chlorocholest-4-en-3-one (I) and derivatives is presented.



I X = Br. or Cl.

THEORETICAL.

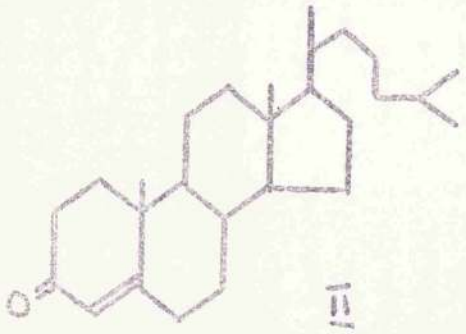
A study of the ultra-violet spectrum of a series of α-bromo-αβ-unsaturated ketones has shown that the α-bromine substitution results in a bathochromic shift of ca 25 mμ, (5-12), rather than ca 10 mμ as stated earlier (1, 13-15).

A study of 2-bromo-Δ1-3-ketosteroids was made by Djerassi, (6), who states that the structures of these compounds appear to be conclusively established by their mode of syntheses, and their reactions. 2-Bromo-Δ1-3-ketosteroids were prepared either by dehydrobromination of a 2:2-dibromo-3-ketone, or by direct bromination of a Δ1-3-ketosteroid. Debromination of the 2 bromo-Δ1-3-ketosteroid by means of zinc in ethanol gives the corresponding Δ1-3-ketosteroid. Djerassi found that 2-bromo-Δ1-3-ketosteroids exhibit a maximum at ca 255 mμ, to be compared with a maximum at 230 mμ for Δ1-3-ketosteroids, i.e. α-bromine substituent causes a bathochromic shift of ca 25 mμ. This observation in turn made untenable the structures given to a series of bromination products of Δ4-3-ketosteroids (1), which have subsequently been revised (6).

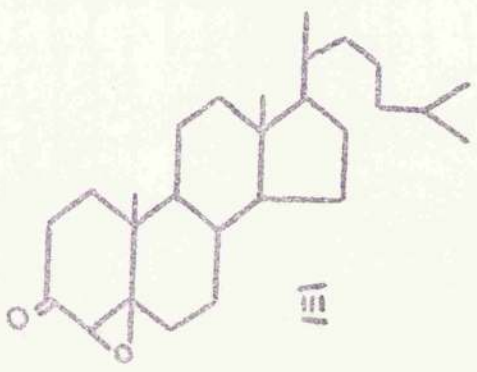
In a review of constitution and ultra-violet spectra, Fieser and Fieser (1), refer to three halogen-substituted unsaturated ketones, the structures of which are certainly incorrect. Of the three compounds, 4-bromocholest-4-en-3-one (I) is the most

important. This bromo- compound was described by Barkov (16) who prepared it by tetra- or hexa-brominating cholest-4-en-3-one (II) for 24 hours, and refluxing the solution to complete spontaneous dehydrobromination. The polyunsaturated dibromo- derivative was then refluxed with palladium-barium sulphate catalyst in amyl alcohol in an atmosphere of hydrogen for 8 hours, to give a product in small yield, characterised by a bromine analysis (2.4% too high for $C_{27}H_{43}OBr$), and by its ultra-violet maximum at 250 mμ. On the basis of the light absorption, the structure 4-bromocholest-4-en-3-one (I) was assigned to this compound. Djerassi predicted an ultra-violet absorption maximum of ca 265 mμ for 4-bromocholest-4-en-3-one (I), (6).

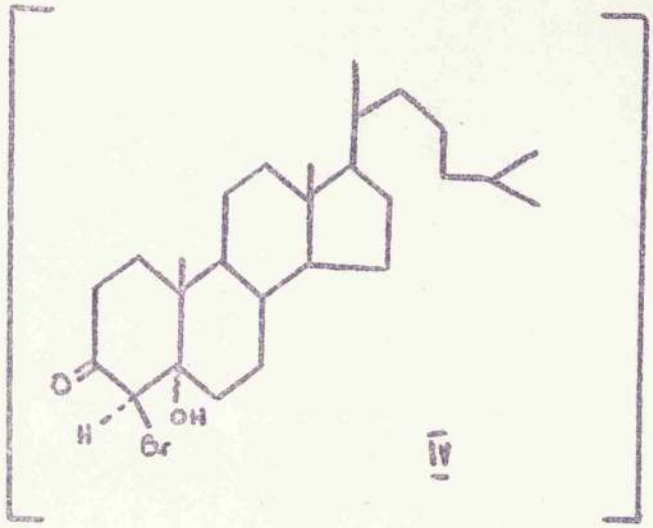
A simple unambiguous method has been discovered for the preparation of 4-bromocholest-4-en-3-one (I). This consists in treatment of 4β:5-epoxycoprostan-3-one (III) with hydrobromic acid, and involves diaxial opening of the epoxide, and spontaneous dehydration of the intermediate β-hydroxy ketone (IV) to yield the bromoketone (I). 4β:5-Epoxycoprostan-3-one (III) was prepared by the action of alkaline hydrogen peroxide on cholest-4-en-3-one (II), (17). Cholest-4-en-3-one (II) was itself prepared by an improved method developed by Fieser (18).



II



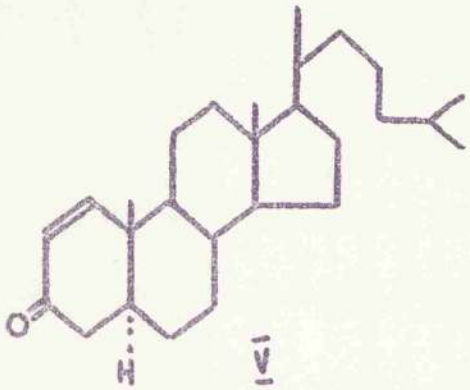
III



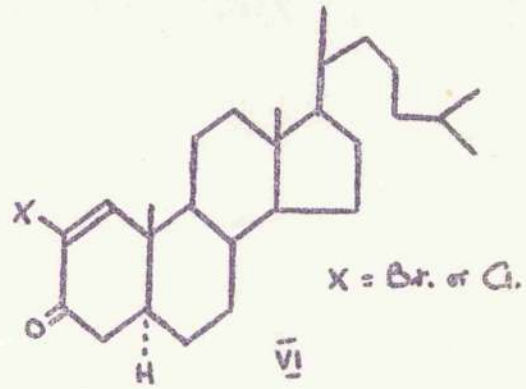
IV

4-Bromocholest-4-en-3-one (I) exhibits an ultra-violet maximum at 260 mμ (ε = 11, 500), and not at 250 mμ as in Barkov's compound. In comparison with a bathochromic shift of 25 mμ observed on substitution of a bromine atom at C_2 in cholest-1-en-3-one, (V), (12), the bromoketone (I) exhibits a shift of 20 mμ with respect to cholest-4-en-3-one(II). In the author's opinion Barkov's compound was not 4-bromocholest-4-en-3-one (I).

4-Chlorocholest-4-en-3-one (I) was obtained by brief treatment of the epoxide (III) with hydrogen chloride. The chloroketone exhibits an ultra-violet maximum at 256 mμ (ε = 11,000). Introduction of an α-chlorine atom into cholest-4-en-3-one (II), thus causes a bathochromic shift of 16 mμ, in excellent agreement with the shift of 15 mμ found for the change from cholest-1-en-3-one (V) to 2-chlorocholest-1-en-3-one(VI), (19, 20).



V

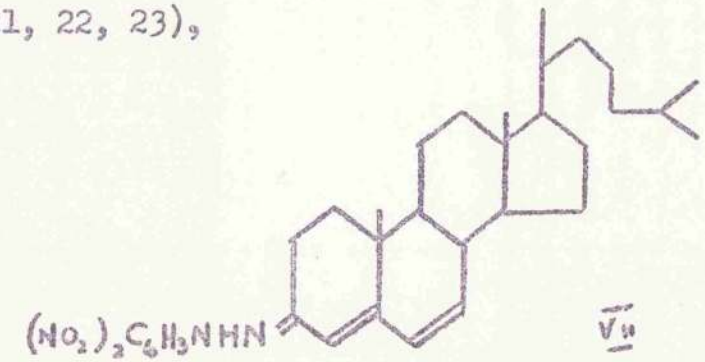


VI

Treatment of the bromoketone (I) with zinc in acetic acid gave cholest-4-en-3-one (II), but under the same conditions the chloroketone (I) was recovered unchanged. The chloroketone (I) was also recovered unchanged after refluxing in ethanol solution with Raney nickel. The bromoketone (I) was recovered unchanged after refluxing in collidine solution.

The action of hydriodic acid on 4β:5-epoxycoprostan-3-one (III) did not produce the desired 4-iodocholest-4-en-3-one, the reaction leading to cholest-4-en-3-one (II).

2α-,6α-, and 6β-Bromo- and chloro- derivatives of cholest-4-en-3-one (II) on treatment with 2:4-dinitrophenylhydrazine in hot acetic acid yield cholesta-4:6-dien-3-one 2:4-dinitrophenylhydrazone (VII) (21, 22, 23),



VII

4-Bromo- and 4-chlorocholest-4-en-3-one (I) give 2:4-dinitrophenylhydrazones with retention of the halogen atom. This parallels the behaviour of 2-bromo- and 2-chlorocholest-1-en-3-one (VI) (21, 22, 23).

The 2:4-dinitrophenylhydrazones provide further examples of the hypsochromic effect of halogen substitution on the ethylenic double bond of $\alpha\beta$-unsaturated ketone 2:4-dinitrophenylhydrazones. Cholest-4-en-3-one 2:4-dinitrophenylhydrazone has an ultra-violet maximum at 390 mμ, whereas the bromo- and chloro- compounds have maxima at 385 mμ and 384 mμ respectively, giving hypsochromic effects of 5 mμ, and 6 mμ respectively. These values are in good agreement with those found for the 2:4-dinitrophenylhydrazones of cholest-1-en-3-one, and 2-bromo- and 2-chlorocholest-1-en-3-one, i.e. the unsubstituted hydrazone has an ultra-violet maximum at 382 mμ, and the two substituted hydrazones have maxima at 375 mμ and 374 mμ respectively, giving a hypsochromic effect of 7 mμ and 8 mμ respectively.

These effects are in agreement with values given by Djerassi viz.

Δ1-3-keto-allosteroids hydrazones - 382-384 mμ, 2-bromo-Δ1-3-keto-allosteroids hydrazones-375-376 mμ (24).

After publication of these results (25), Petrov and his co-workers (26, 27) described alternative preparations of the haloketones, described above, and reported their ultra-violet

characteristics and other constants:-

Bromoketone			Chloroketone	
Author's	Petrov's		Author's	Petrov's
114-115°	113°	m.p.	124-125°	126-127°
+107°	+110°	$[\alpha]_D$	+106°	+106°
2600 Å	2610-2620Å	$\lambda_{max.}$	2560 Å	2560 Å
11,500	12,000	ε	11,000	14,000(propan-2-ol).

EXPERIMENTAL

Cholest-4-en-3-one:-

a) Cholesterol Dibromide:- A solution of cholesterol (150 g.) in absolute ether (1 l.) was treated at 25° with a solution of anhydrous sodium acetate (5 g.) and bromine (68 g.) in glacial acetic acid (600 c.c.). The mixture was cooled to 15-20°, and the separated dibromide collected and washed with glacial acetic acid (3 l.) until the filtrate was colourless.

b) 5α:6β-Dibromocholestan-3-one:- The moist dibromide from 150 g. of cholesterol was suspended in glacial acetic acid (2 l.) and the suspension stirred. A solution of sodium dichromate (80 g.) in glacial acetic acid (2 l.) preheated to 90° was poured into the stirred suspension. Stirring was continued for 30 minutes, and the solution then cooled to 20°. The product was collected, and washed with methanol (3 l.) until the filtrate was colourless.

c) Cholest-5-en-3-one:- The methanol-moist 5α:6β-dibromocholestan-3-one from 150 g. of cholesterol was covered with ether (2 l.), acetic acid was added (25 c.c.), and the suspension stirred. Zinc dust (40 g.) was added over 5 minutes, the temperature being maintained at 15° by cooling. Pyridine (70 c.c.) was added and the stirred mixture was filtered to remove the zinc complex. The filtrate was washed with water (3 x 1 l.), sodium bicarbonate

solution (5% - 600 c.c.), dried (Na_2SO_4), and concentrated to a volume of 1 litre. After addition of methanol (500 c.c.) concentration was continued until solid started to separate as white needles. The solution was then allowed to cool and cholest-5-en-3-one (98 g.) was collected, m.p. 127-129°, $[\alpha]_D$-2.5°, (C, 1.7). Light absorption λmax. 2060 Å, ε =4,000.

Cholest-4-en-3-one:- A solution of cholest-5-en-3-one (20 g.) and anhydrous oxalic acid (2 g.) in ethanol (95%, 160 c.c.) was concentrated to 100 c.c., cooled and seeded. Cholest-4-en-3-one (19 g.) separated as long needles, m.p. 81-82° $[\alpha]_D$+92°, (C, 1.3). Light absorption λmax. 2400 Å, ε =17,000. Literature constants (18), m.p. 81-82°, $[\alpha]_D$+92°, λmax. 2405 Å, ε = 18,000.

4β:5-Epoxycoprostan-3-one:- To a solution of cholest-4-en-3-one (5 g.) in methanol (500 c.c.), a solution of sodium hydroxide (20 c.c., 4N), and hydrogen peroxide (20 c.c., 30%) were added dropwise simultaneously, with stirring. The mixture was kept at 4° for 48 hours, when the large white needles of 4β:5-epoxycoprostan-3-one were filtered off, and recrystallised from aqueous methanol, (2.5 g.); it has m.p. 118-119°, $[\alpha]_D$ +128°, (C, 2.5), and no high intensity ultra-violet light absorption. Literature constants, (17), m.p. 116-117°, $[\alpha]_D$ +134°.

4-Bromocholest-4-en-3-one:- Aqueous 40% hydrobromic acid (2 c.c. was added to a solution of 4β:5-epoxycoprostan-3-one (200 mg.)

in chloroform (20 c.c.) and glacial acetic acid (2 c.c.). After 16 hours at room temperature, the mixture was diluted with water and extracted with chloroform. The chloroform was washed with water, dried (Na_2SO_4), and evaporated to give an oil which crystallised from aqueous methanol or aqueous acetone to give 4-bromocholest-4-en-3-one, as long needles (120 mg.) m.p. 114-115° $[\alpha]_D$ +107°, (C, 1.4). Analysis. Found: C, 70.2; H, 9.7. Required for $C_{27}H_{43}OBr$: C, 69.9; H, 9.4%. Light absorption: λmax. 2600 Å, ε = 11,500.

Literature constants (26), m.p. 113°, $[\alpha]_D$ +110°, λmax. 2610-2620 Å, ε = 12,000

4-Bromocholest-4-en-3-one 2:4-dinitrophenylhydrazone:-

A solution of 4-bromocholest-4-en-3-one (500 mg.) and 2:4-dinitrophenylhydrazine (250 mg.) in acetic acid (15 c.c.) was heated at 100° for 15 minutes. The solution was cooled and the product collected and recrystallised from chloroform-ethanol to yield 4-bromocholest-4-en-3-one 2:4-dinitrophenylhydrazone as red blades,, m.p. 217°(d.), light absorption ($CHCl_3$) λmax. 2620 Å, ε =17,000, 3850 Å, ε =26,800. Analysis. Found: C, 61.9; H, 7.4. Required for $C_{33}H_{47}O_4N_4Br$: C, 61.6; H, 7.4%

Action of Zinc Dust on 4-Bromocholest-4-en-3-one:-

Zinc dust (250 mg.) was added to a solution of 4-bromocholest-4-en-3-one (50 mg.) in glacial acetic acid (30 c.c.) and the mixture refluxed for 4 hours. The zinc was removed, and the filtrate

diluted with water, and extracted with ether.

The ether was washed free of acid with water, dried (Na_2SO_4), and evaporated to yield cholest-4-en-3-one as needles (20 mg.), from aqueous methanol, m.p. and mixed m.p. 81-82°, $[\alpha]_D$ +91°, (C, 1.7); light absorption, λmax 2400 Å, ε=17,000.

Action of Collidine on 4-Bromocholest-4-en-3-one:- A solution of 4-bromocholest-4-en-3-one (100 mg.) in collidine (2 c.c.) was refluxed for 1 hour. The reaction solution was diluted with ether, filtered, and the filtrate washed with hydrochloric acid (3 x 40 c.c., 3N), water, and saturated sodium bicarbonate solution. After drying (Na_2SO_4), and evaporating to dryness, a red gum was obtained, which was dissolved in light petroleum (b.p. 60-80°) and chromatographed on alumina (3 g.). Elution with petrol/benzene (6:1) gave a white gum crystallising from aqueous acetone to give 4-bromocholest-4-en-3-one (80 mg.) as needles, m.p. and mixed m.p. 114-115°; light absorption, λmax. 2600 Å, ε = 11,000.

4-Chlorocholest-4-en-3-one.— A stream of hydrogen chloride gas was passed through a solution of 4β:5-epoxycoprostan-3-one (500 mg.) in chloroform (20 c.c.) for 2 minutes. The solution was washed free from acid with water, dried (Na_2SO_4), and evaporated to yield an oil which crystallised from aqueous methanol to give 4-chlorocholest-4-en-3-one as needles (420 mg.), m.p. 124-125°, $[\alpha]_D$+106°, (C, 1.5). Analysis. Found: C, 77.65;

H, 10.6. Required for $C_{37}H_{43}OCl$: C, 77.4; H, 10.3%. Light absorption, λ max. 2560 Å, ε =11,000.

Literature constants (27), m.p. 126-127°, $[\alpha]_D$ +106° λ max. 2560 Å, ε =14,000 (in propan-2-ol).

4-Chlorocholest-4-en-3-one 2:4-dinitrophenylhydrazone:-

2:4-Dinitrophenylhydrazine (250 mg.) was added to a solution of 4-chlorocholest-4-en-3-one (500 mg.) in glacial acetic acid (15 c.c.). The mixture was heated at 100° for 15 minutes, cooled and the product collected and recrystallised from chloroform-ethanol to give 4-chlorocholest-4-en-3-one 2:4-dinitrophenylhydrazone as red blades, m.p. 255° (d.), light absorption, λ max. 2630 Å. ε = 13,700, 3840 Å, ε = 27,300 (in $CHCl_3$). Analysis. Found: C, 66.5; H, 7.8. Required for $C_{33}H_{47}ClN_4O_4$: C, 66.3; H, 7.8%.

4-Chlorocholest-4-en-3-one Semicarbazone:- A solution of semicarbazide hydrochloride (200 mg.) and sodium acetate (300 mg.) in water (1 c.c.) was added to a solution of 4-chlorocholest-4-en-3-one (200 mg.) in ethanol (50 c.c.), and the mixture refluxed for 1 hour. Water was added, and on cooling the product separated. Crystallisation from chloroform-methanol gave 4-chlorocholest-4-en-3-one semicarbazone, as needles m.p. 165° (d.), light absorption, λ max. 2700 Å, ε = 21,000. Analysis. Found: C, 70.9; H, 9.3 Required for $C_{28}H_{46}ON_3Cl$: C, 70.7; H, 9.7%.

Action of Hydriodic Acid on 4β:5-Epoxycoprostan-3-one:-

A solution of 4β:5-epoxycoprostan-3-one (500 mg.) in chloroform (25 c.c.) was refluxed with freshly distilled 48% hydriodic acid (6 c.c.), for 1 hour. The solution was washed with water, sodium thiosulphate solution (10%) and water, dried (Na_2SO_4), and evaporated. Crystallisation of the residue from aqueous methanol gave cholest-4-en-3-one (450 mg.) m.p. and mixed m.p. 81-82°, $[\alpha]_D$ +91°, (C, 1.4); light absorption, λ max. 2400 Å, ε = 17,100.

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α-AMYRIN

α-AMYRIN.

INTRODUCTION:-

The name triterpene is applied to a class of naturally occurring hydrocarbons and oxygenated hydrocarbons, containing 30 carbon atoms arranged is such a manner that six isopentane units can be recognised in the molecular skeleton. In recent years, as a result of the discovery that certain natural products with obvious triterpene characteristics are in fact C_{31} compounds (1 - 4), the more comprehensive term triterpenoid has been adopted.

Apart from the aliphatic hydrocarbon squalene, the triterpenoids are alicyclic and the majority contain hydroxyl, carboxyl or carbonyl oxygen functions. They can be conveniently subdivided into three main classes:-

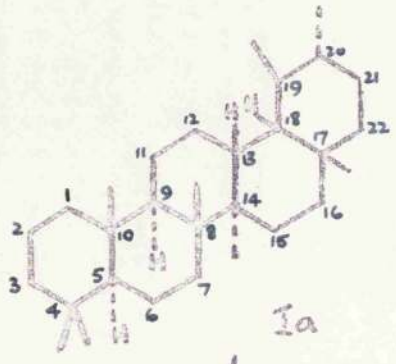
a) The aliphatic compound squalene, and the tricyclic compound, ambrein.

b) The tetracyclic compounds such as lanosterol, agnosterol, the elemic acids, the polyporenic acids, eburicoic acid, euphol, tirucallol, and butyrospermol, which all bear a structural relationship to the steroids.

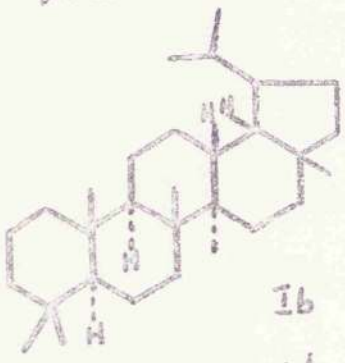
c) The pentacyclic triterpenoids which form the largest class, and include such compounds as α- and β-amyrin, lupeol, taraxasterol, and taraxerol.

No mono- or di-cyclic triterpenoids are known. The recently characterised pentacyclic triterpenoids cycloartenol, (5, 6), and cycloläudenol (3,4), are closely related to lanosterol, and are best classified with the tetracyclic triterpenoids. Similarly, the hexacyclic triterpenoid phyllanthol, (5) which is closely related to α-amyrin is best classified with the pentacyclic triterpenoids. More recently, the triterpenoid onocerin has been shown to be tetracyclic. (7).

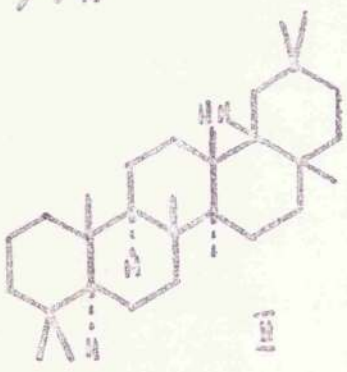
This section of the thesis is concerned with the pentacyclic triterpenoids. The majority of these are polyfunctional compounds, which can be related to simpler monohydric alcohols by fairly standard methods (8, - 11), and can be considered to be members of four main classes based on α-amyrin, β-amyrin, 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 whenever possible throughout this section.



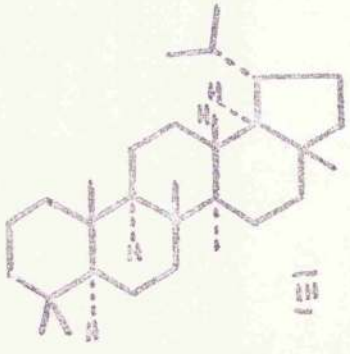
Ia



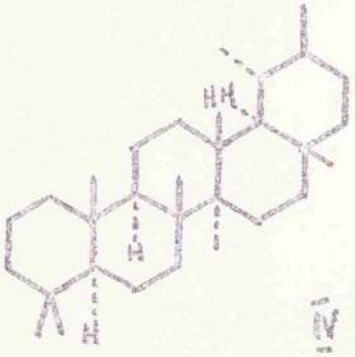
Ib



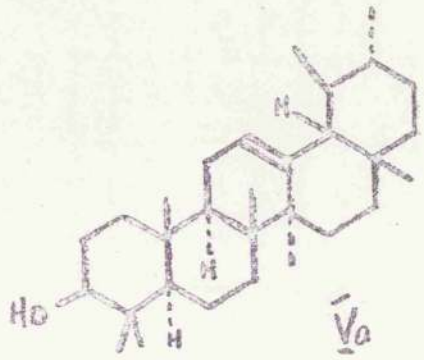
II



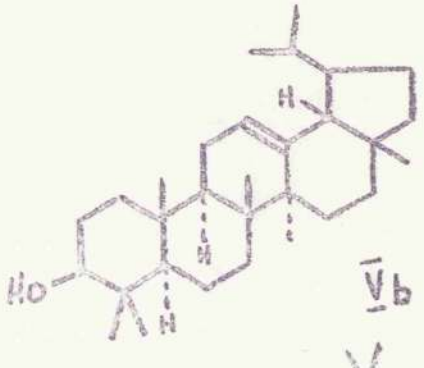
III



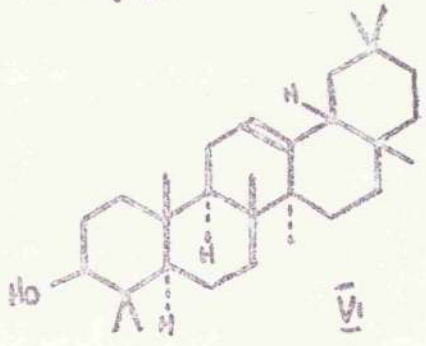
IV



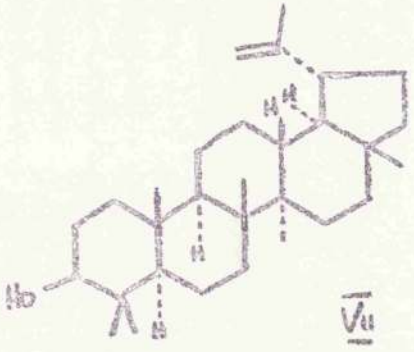
Va



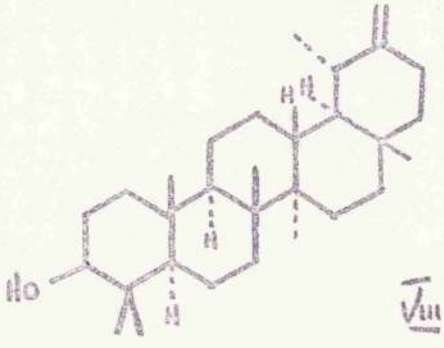
Vb



VI



VII



VIII

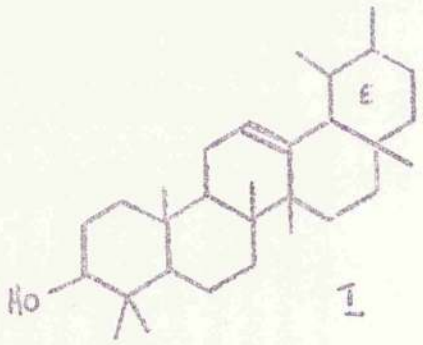
In addition to a large number of interconversions which have been achieved within each class, interconversions among the oleanane, lupane and taraxastane groups have also been effected. The first recorded instance of the conversion of an ursane derivative into a derivative of another triterpenoid group was carried out by co-workers in this laboratory, during the course of this work, and was verified by the author as reported in this section.

For comprehansive discussions of the triterpenoids as a whole and for descriptions of the general methods employed in their structural elucidation, attention is drawn to the reviews of Haworth, (12) Spring, (13), Noller (14), Jeger (15), Birch (16), and Barton (17), and to Elsevier's Encyclopaedia of Organic Chemistry (18).

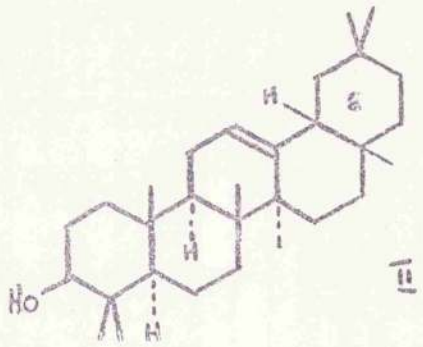
Degradation of α-Amyrin.

α-Amyrin is the parent alcohol of the ursane group of triterpenoids, and β-amyrin is the parent alcohol of the oleanane group. The present section summarises the principal degradative evidence regarding the structure of α-amyrin at the time the work described in this thesis commenced. The extensive researches of Ruzicka, Jeger et al., in Zurich led them to propose structure (I) as an adequate representation of the constitution of α-amyrin. (19).

The constitution and stereochemistry of β-amyrin (II) are supported by a wealth of experimental evidence,



I

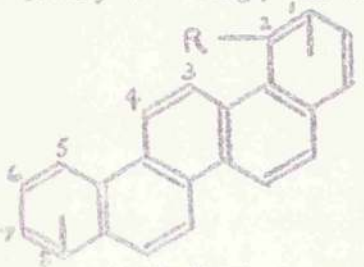


II

and are established with almost complete certainty, although a total synthesis has not been achieved (15, 20, 21).

Inspection of formulae (I) and (II) shows that the only constitutional difference between α- and β-amyrin is in ring E, i.e. in the position of two methyl groups. Jeger (15), states that both α-and β-amyrin on dehydrogenation give the same hydrocarbons, an apparent discrepancy which he did not overlook. The hydrocarbons obtained from α-amyrin (I), and related acids

included 1:8-dimethylpicene (III, R = H), rather than 1:2:8-trimethylpicene (III, R = CH_3) as would be expected.



III

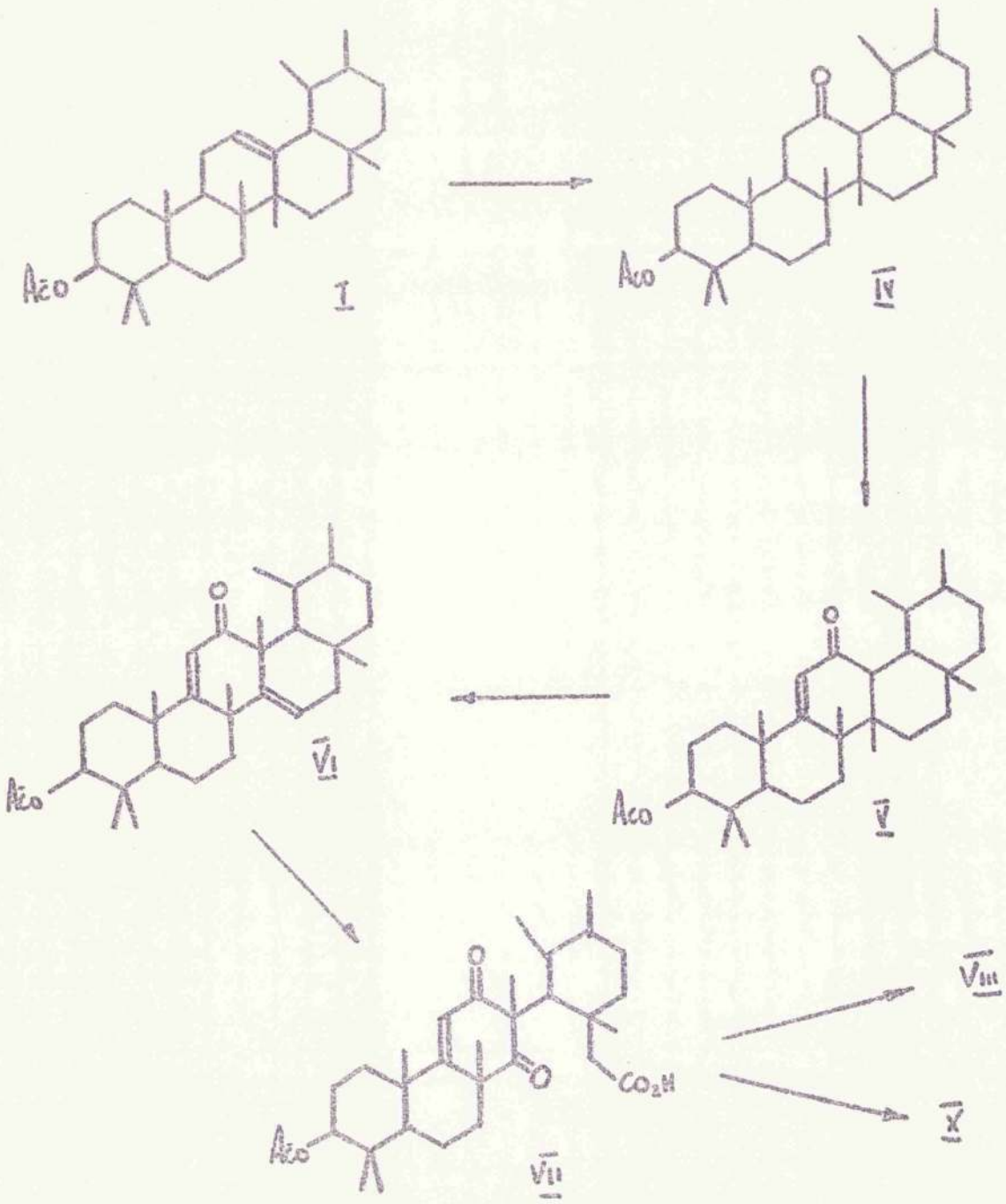
Jeger (15) has suggested that an unidentified hydrocarbon, m.p. 306° obtained in the dehydrogenation of α-amyrin (22, 23) may be 1:2:8-trimethylpicene (III, R = CH_3).*

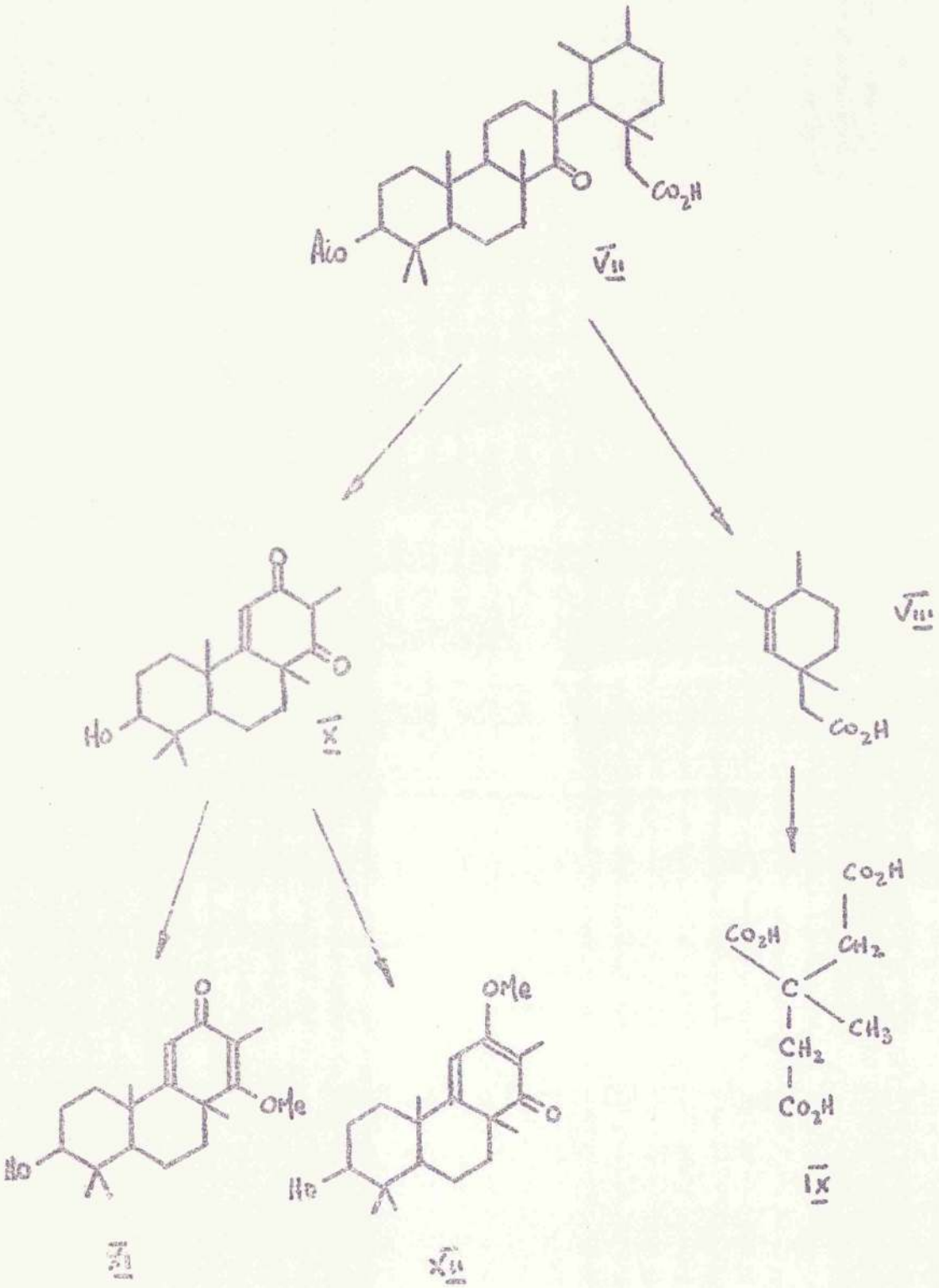
Ruzicka (25, 26) and his co-workers have shown by the following series of reactions that rings A, B, and C in α- and β-amyrin are similarly constituted and that the configurations at C_3, C_5, C_8, and C_{10} are the same. Peracid oxidation of α-amyrin acetate to 12-oxoursan-3β-yl acetate (IV), followed by bromination at C_{11} and subsequent dehydrobromination gives 12-oxours-9(11)-en-3β-yl acetate (V). This compound (V) was oxidised to 12-oxoisoursa-9(11):14-dien-3β-yl acetate (VI) with selenium dioxide. Osmium tetroxide oxidation of (VI) gave the 14:15-diol which on cleavage gave a diketo-monocarboxylic acid (VII). Pyrolysis at 300° of (VII) gave two fractions. The volatile fraction was a monobasic acid (VIII) considered to arise

*

Footnote 1:2:8-Trimethylpicene (III, R = CH_3) has recently been prepared (24), and although no direct comparison has been made, its m.p. 252-254° is so different from that of the hydrocarbon (m.p. 306°) obtained from α-amyrin that it is highly unlikely that the two compounds are identical.

from ring E in α-amyrin. Further oxidation of (VIII) gave β-methyltricarballylic acid (IX). The non-volatile fraction, a diketone (X), was converted by diazomethane into two enol ethers (XI, XII), considered to originate in rings A, B, and C of α-amyrin (I). A series of parallel reactions on β-amyrin (II) gave the same two enol ethers (XI, XII).



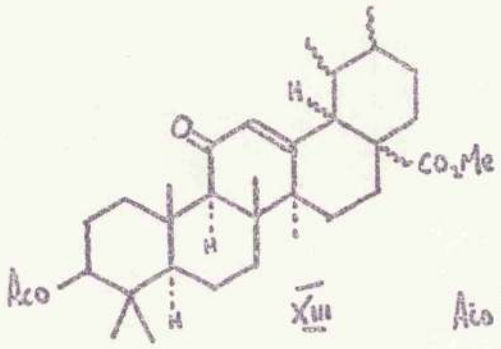

AcO
CO2H
O
VII
HO
X
VIII
CO2H
CO2H
CH2
C
CH3
CH2
CO2H
IX
MeO
MeO
HO
XI
OMe
O
HO

The Structure and Stereochemistry of α-Amyrin.

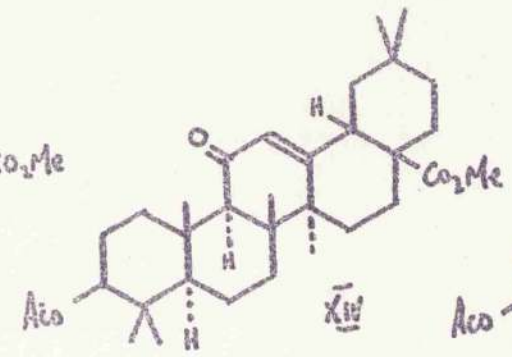
As outlined earlier (p.49), the configurations at C_3, C_5, C_8, and C_{10} in α-amyrin were established by degradation into two enol ethers, obtained by a parallel series of reactions from β-amyrin whose stereochemistry has been fully elucidated. In addition comparative studies on ursane and oleanane derivatives suggested that configurations at C_9 and C_{14} in both groups are identical (19, 26) although, these did not constitute an absolute proof. Later studies (27, 28) proved the correctness of these latter configurational assignments.

Apart from the configuration of alkyl substituents in ring E, the remaining asymmetric centres to be considered are at C_{17}, and C_{18}. During the last three years, each of the four theoretically possible arrangements for the locking of rings D and E have been included in representations of the stereochemistry of α-amyrin (27, 29, 30, 31, 32, 33).

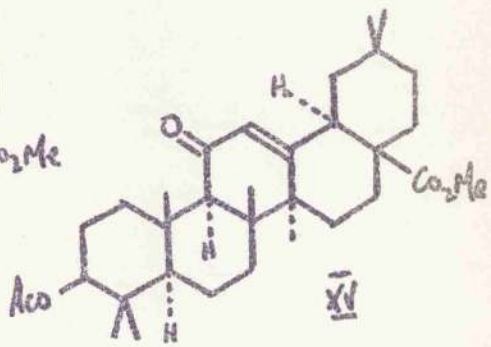
An early observation of importance with regard to the stereochemistry at these positions was made by Barton and Holness (20) who observed that methyl 11-oxoursolate acetate (XIII) was not isomerised on treatment with acid or base in contrast to methyl 11-oxo-oleanate acetate (XIV) which was isomerised to its 18α-isomer (XV). This clearly indicated that the hydrogen



XIII



XIV

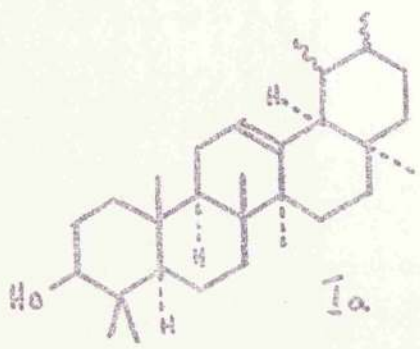


XV

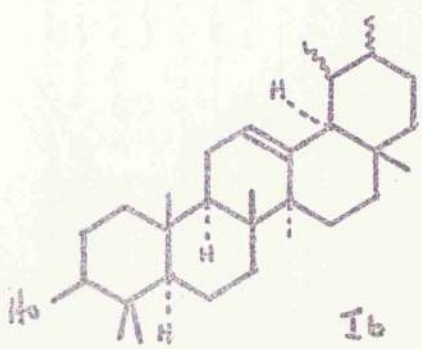
atom at C_{18} in the α-amyrin series has the more stable configuration, or that no hydrogen is present at this position.

In 1951, Jeger (30) outlined a series of degradations on α- and β-amyrin leading to two tricarboxylic esters containing the original C_{17} of the isomeric amyrins as the only asymmetric centre. It was claimed that these esters were enantiomorphs, and that the methyl group attached to C_{17} in α-amyrin must have the opposite configuration to that in β-amyrin, i.e. α-amyrin has a 17α-methyl group. Experimental details of these degradations have not been revealed. In apparant agreement, but again lacking any theoretical discussion or experimental, Jeger and Ruzicka (33) more recently stated that rings D and E in α-amyrin are cis-α-fused. (Structure Ia).

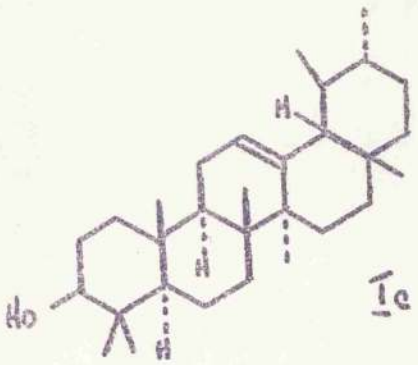
It has also been suggested by Ruzicka (29), on consideration of the biogenesis of triterpenoids that α-amyrin should be represented as Ib.



Ia

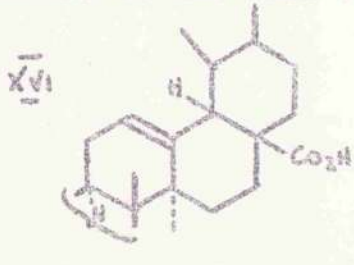


Ib

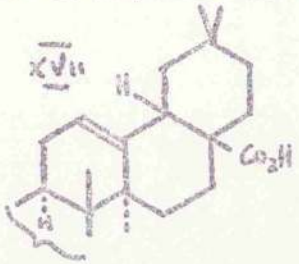


Ic

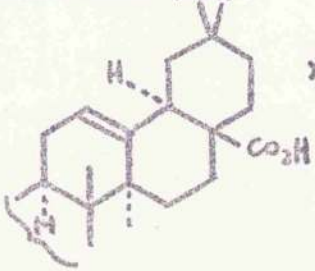
Corey and Ursprung (27, 31) present reasons favouring a third formula (Ic) for α-amyrin. They state that the hydrogen attached to C_9, which will not epimerise when adjacent to an 11-carbonyl function (20), must have the stable α-configuration as in β-amyrin, and this conclusion is supported by a comparison of the dehydrohalogenation of 11-bromo-12-ketones derived from both ursane and oleanane series (20, 34). With the configurations at C_3, C_5, C_8, C_9, and C_{10} determined, there are eight possible formulae for α-amyrin which differ at the three remaining asymmetric centres i.e. C_{14}, C_{17}, and C_{18}. Corey and Ursprung reject six of these formulae on steric considerations, leaving two structures (Ib) and (Ic) which correspond to 18α-olean-12-en-3β-ol and olean-12-en-3β-ol respectively. A consideration of the stabilities of lactones derived from ursolic acid (XVI), oleanolic acid (XVII), and 18α-oleanolic acid (XVIII), and of molecular rotation relations and conformational theory led these authors to the view that α-amyrin has the structure (Ic).



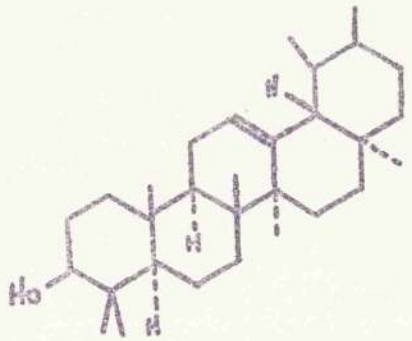
XVI



XVII

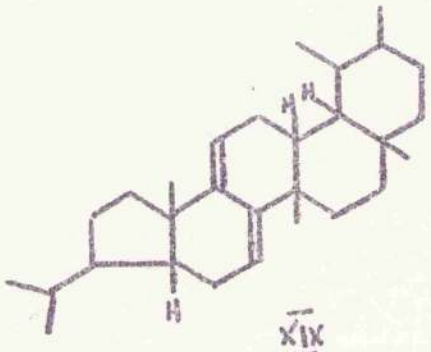


XVIII

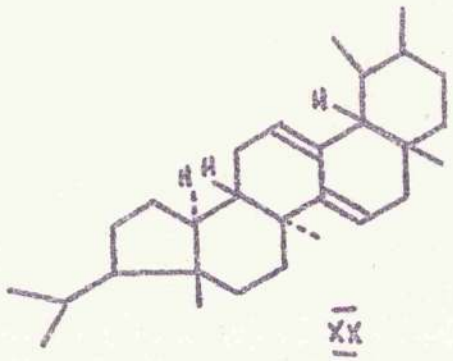


Id

Thr fourth D/E ring locking for α-amyrin, shown in (Id) was proposed by Beton and Halsall (32). Dehydration of α-amyrin with phosphorus pentoxide in benzene gives the hydrocarbon 1-α-amyradiene. This compound contains a heteroannular conjugated system, and a satisfactory mechanism for the formation of such a diene was hitherto difficult to devise. A particular difficulty was the reason for this reaction being characteristic of α-amyrin, but not of β-amyrin. According to Beton and Halsall (32), this difference is ascribed to a conformational driving force supplied by the specific nature of the locking of rings D and E in α-amyrin as represented in (Id); a driving force not supplied by the D/E fusion in β-amyrin. The structure (XIX) was proposed by Beton and Halsall for 1-α- amyradiene, but this structure (XIX) has since been shown to be unacceptable (35,36). The true structure of 1-α-amyradiene is shown in (XX). It has also been found



XIX



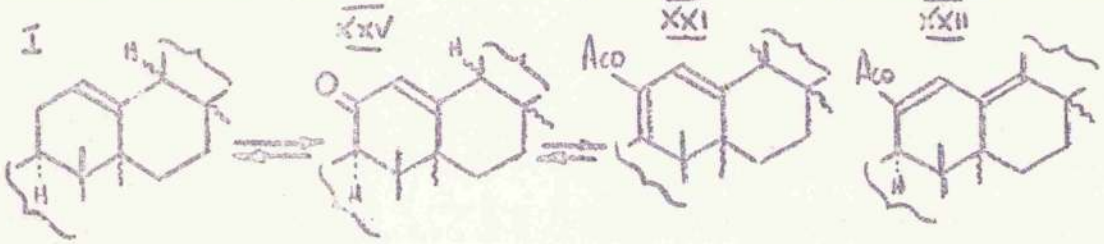
XX

that β-amyrin derivatives undergo a change comparable with the α-amyrin ⟶ 1-α-amyradiene reaction. For these reasons structure (Id) for α-amyrin is untenable.

Recently, a new stereoformula for α-amyrin has been proposed (28, 37), and a short description of the arguments which led to this proposal will now be given. As stated earlier (p.49) the configurations at C_3, C_5, C_8, and C_{10} in α-amyrin have been established.

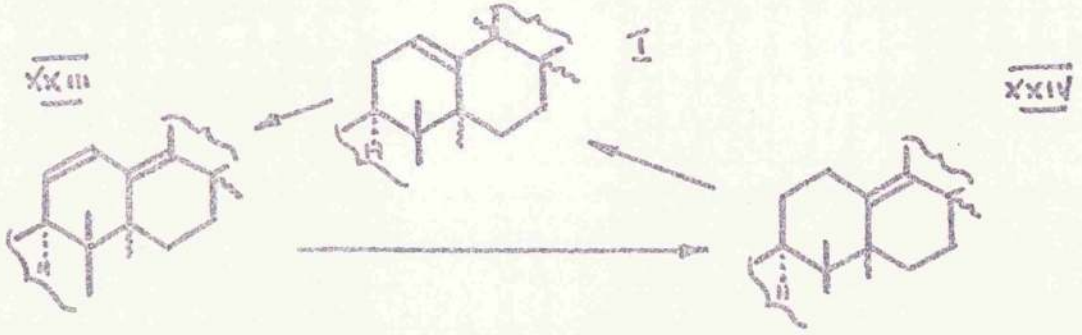
Evidence that the configuration at C_9 in α-amyrin is the more stable arrangement has already been indicated (p.52, 54) A proof that this view is correct was obtained in the following way. 11-Oxours-12-en-3β-yl acetate (XXV), which is obtained by oxidation of α-amyrin acetate (I) with chromic acid (22), and which is reduced to α-amyrin acetate (I) by catalytic hydrogenolysis (38), forms an enol acetate, which is strongly dextrorotatory ($[\alpha]_D$ +275°), and which shows an absorption maximum at 2760 Å (ε 8,000). All known 9(11):12-dienes derived

from ursane and oleanane show a similarly strong dextro-rotation, and an absorption maximum at ca 2800 Å. In contrast, the isomeric 11:13(18)-dienes are laevorotatory, and are characterised by an intense triplet absorption curve, the major peak of which is at ca 2500 Å (ε = 30,000). These facts show that the enol acetate is 3β:11-diacetoxyursa-9(11):12-diene (XXI) and not the hetero-annular isomer (XXII). The observation that hydrolysis of the

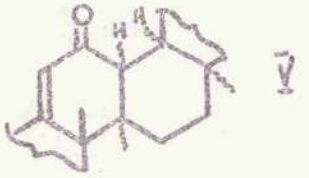


enol acetate (XXI), with either acid or alkali, followed by reacetylation, regenerates 11-oxours-12-en-3β-yl acetate (XXV) in excellent yield established that the hydrogen at C_9 in α-amyrin has the more stable configuration (37), and consequently has a 9α-hydrogen atom.

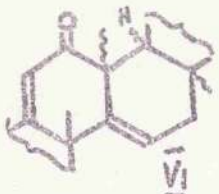
The configuration at C_{18} will now be considered. Vigorous oxidation of α-amyrin acetate (I) with selenium dioxide gives ursa-11:13(18)-dien-3β-yl acetate (XXIII) (39), catalytic hydrogenation of which yields urs-13(18)-en-3β-yl acetate (XXIV). This latter compound (XXIV) on treatment with mineral acid isomerises to α-amyrin acetate (I) (39), from which it follows that the hydrogen atom attached to C_{18} has the more stable configuration.



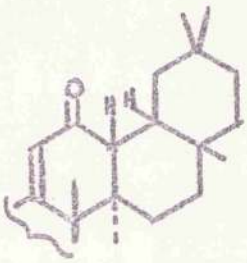
Oxidation of 12-oxours-9(11)-en-3β-yl acetate (V) with selenium dioxide gives 12-oxoisoursa-9(11):14-dien-3β-yl acetate (VI) (40), and similar oxidation of the corresponding 12-oxo-olean-9(11)-en-3β-yl acetate (XXVI) gives 12-oxoisoöleana-9(11):14-dien-3β-yl acetate (XXVII) (25). It has been noted (41), that the same treatment of 12-oxo-18α-olean-9(11)-en-3β-yl acetate (XXVIII) does not yield an analogously constituted dienone.



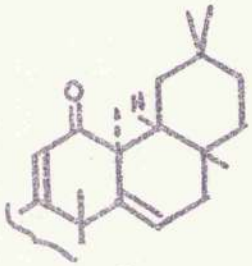
V



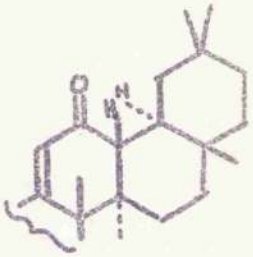
VI



XXVI



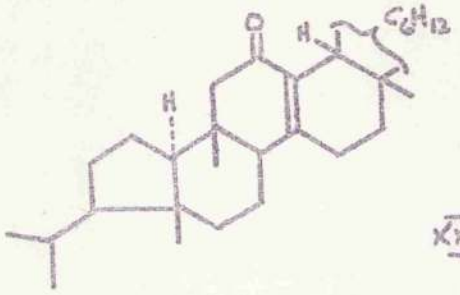
XXVII



XXVIII

This suggests that the hydrogen atom attached to C_{18} in α-amyrin and in β-amyrin have the same (i,e,β) configuration.

Treatment of the saturated 12-ketones derived from α-amyrin, β-amyrin, and 18α-β-amyrin with hydroiodic acid in acetic acid (35, 36), under the same conditions, causes dehydration to give different products in each case. The unsaturated ketones obtained from α- and β- amyrin have the partial structure shown in (XXIX), whereas a similar product is not obtained from the 18 α-isomer. This experiment also provides strong support for the view that the hydrogen atom at C_{18} has the same configuration (i,e,β) in both α- and β- amyrin.

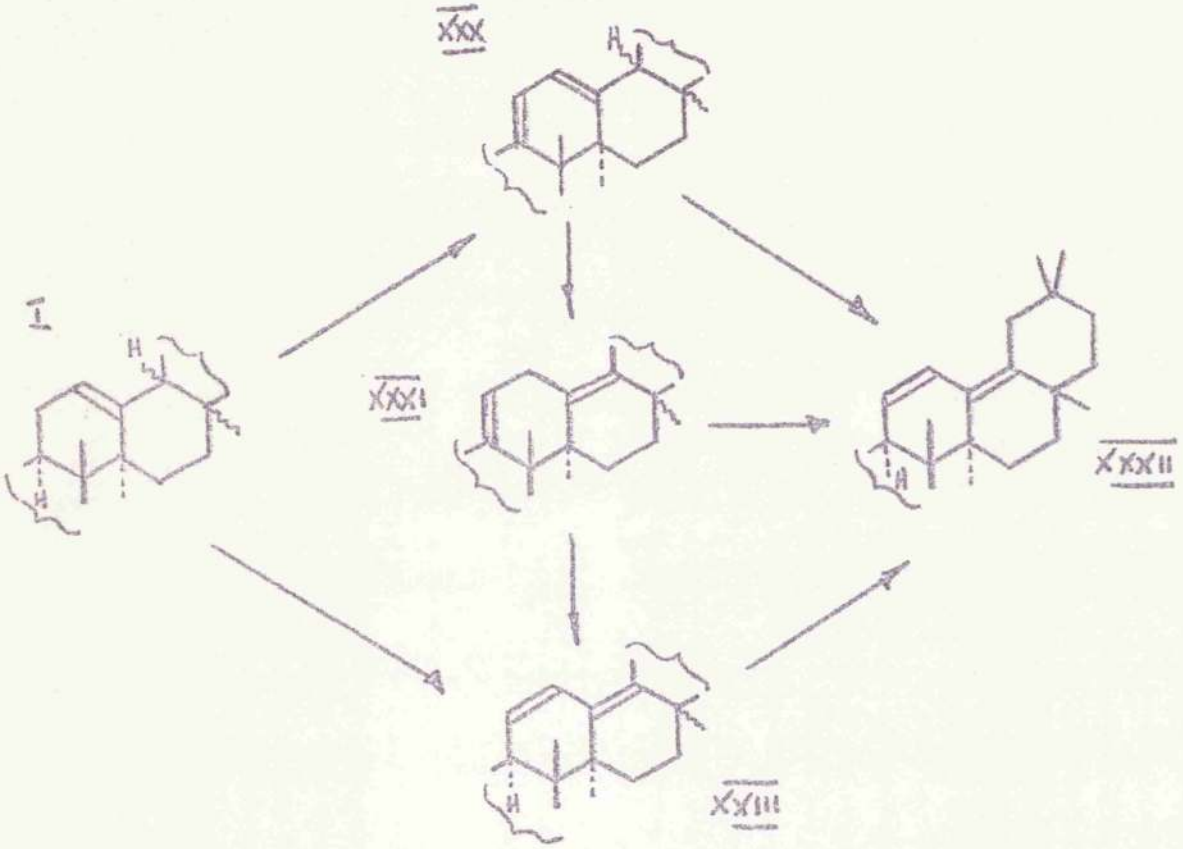


XXIX

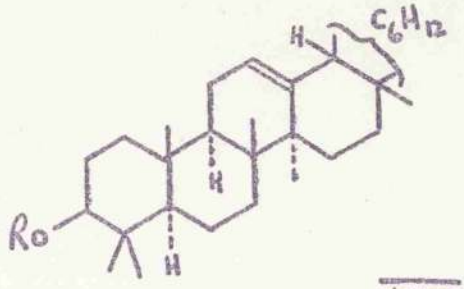
Three ursadien-3β-yl acetates are known. These are the homoannular ursa-9(11):12-dien-3β-yl acetate (XXX) (42), the heteroannular ursa-11:13(18)-dien-3β-yl acetate (XXIII) (see p. 57) (39), and the nonconjugated ursa-9(11):13(18)-dien-3β-yl acetate (XXXI) (37). The three corresponding oleanadien-3β-yl acetates are known, and, of these the 11:13(18)-dien-3β-yl acetate is thermodynamically the most stable since it is obtained from the isomeric 9(11):13(18)- and 9(11):12-dien-3β-yl acetates by treatment with mineral acid (43, 44). A study of the action of mineral acid (37) on the three ursadien-3β-yl acetates has recently revealed that ursa-9(11):13(18)-dien-3β-yl acetate (XXXI) is isomerised to oleana-11:13(18)-dien-3β-yl acetate (XXXII) in 10% pure yield with hydrochloric-acetic acid.

This conversion of ursa-9(11):13(18)-dien-3β-yl acetate (XXXI) into oleana-11:13(18)-dien-3β-yl acetate (XXXII) proceeds through ursa-11:13(18)-dien-3β-yl acetate (XXIII) since the latter compound (XXIII) is obtained from (XXXI) under milder conditions, and treatment of (XXIII) with hydrochloric-acetic acid also gives oleana-11:13(18)-dien-3β-yl acetate (XXXII) in 10% pure

yield. Again, the same treatment of the homoannular ursa-9(11):12-dien-3β-yl acetate (XXX) gives oleana-11:13(18)-dien-3β-yl acetate (XXXII) in similar yield (37).



These experiments are the first recorded instances of the conversion of a simple ursane derivative into an isomer belonging to another triterpenoid group, and proves that the stereochemistry of α-amyrin at C_3, C_5, C_8, C_9, C_{10}, C_{14} and C_{17} is identical with that in β-amyrin. As stated earlier (p. 57) the hydrogen atom at C_{18} in α-amyrin has almost certainly the β-configuration, and α-amyrin must be represented by the partial formula (XXXIII). Any complete representation of the constitution and stereochemistry of α-amyrin must include this partial formula (XXXIII), and again, in the opinion of



R = Ac. or H

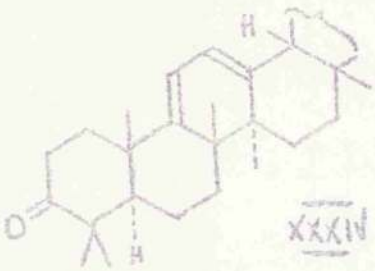
XXXIII

the author, proposals that the methyl group attached to C_{17} is α-orientated (30, 32, 33,) are invalidated.

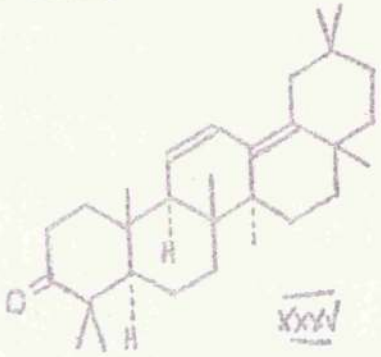
In view of the importance of the conclusions drawn from this conversion of ursa-9(11):12-dien-3β-yl acetate (XXX) to oleana-11:13(18)-dien-3β-yl acetate (XXXII), which was in very low yield (10%), it was considered necessary to verify this important ursane ⟶ oleanane conversion. In the author's opinion, this low yield was probably due to the instability of the 3β-acetoxy group under strongly acid conditions, where secondary reactions such as contraction of ring A may occur.
To overcome this difficulty ursa-9(11):12-dien-3-one (XXXIV) was chosen as starting material, since ring A contraction would then be avoided.

Ursa-9(11):12-dien-3-one (XXXIV) was prepared, by an improved method, by oxidation of ursa-9(11):12-dien-3β-ol with chromic trioxide-pyridine complex (45), thus preventing oxidation of the conjugated diene system. As anticipated treatment of ursa-

9(11):12-dien-3-one (XXXIV) with hydrochloric-acetic acid gave olean-11:13(18)-dien-3-one (XXXV) in a crude yield of 65%, and a pure yield of 30%. An authentic sample of olean-11:13(18)-dien-3-one (XXXV) was prepared by oxidation of the corresponding alcohol with chromic trioxide in acetic acid.

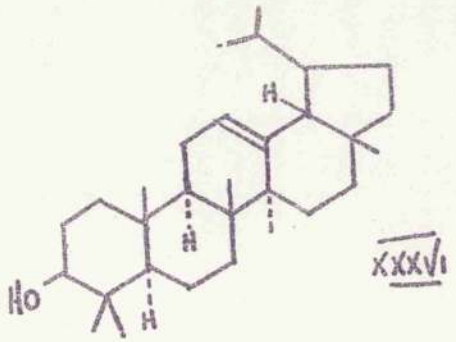


XXXIV

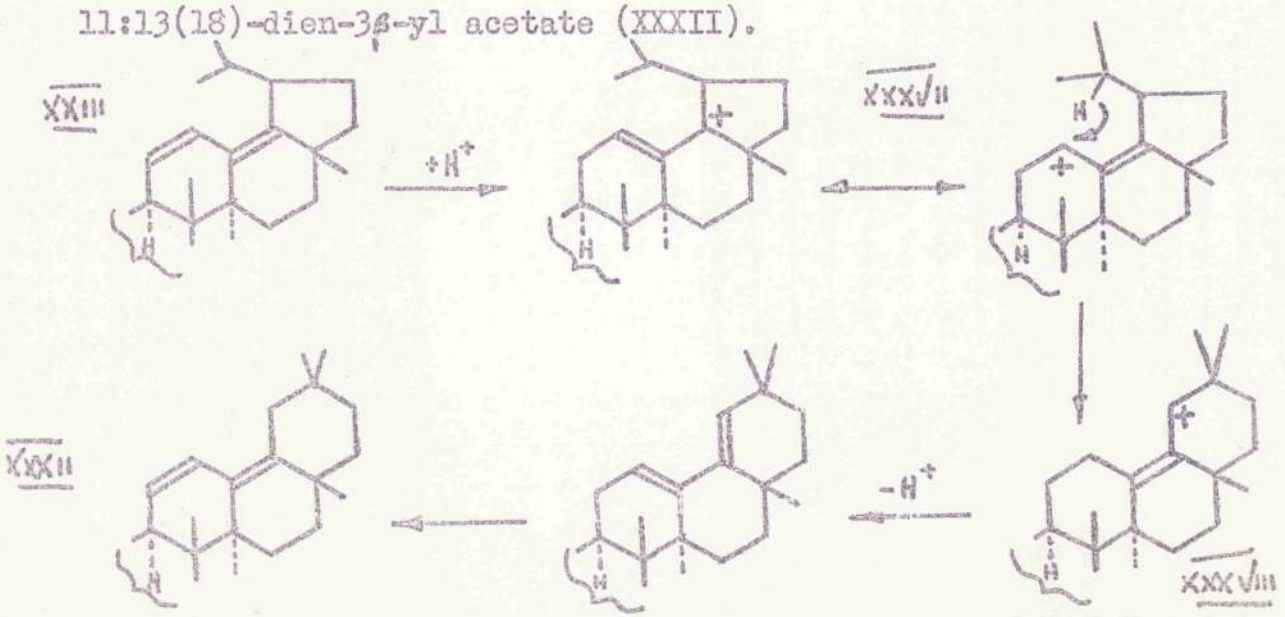


XXXV

Since, as has been shown, α- and β-amyrin are identical in rings A,B,C, and D, the conformational and structural nature of ring E must be responsible for the stability of the cis-fusion of this ring with ring D. The nature of ring E must also explain the greater stability of urs-12-en-3β-yl acetate (XXXIII) relative to urs-13(18)-en-3β-yl acetate (XXIV), and the substantial hinderance upon the double bond in α-amyrin, and upon the ketone group in 12-oxoursan-3β-yl acetate (IV). Again ring E of the ursane group must be so constituted that it can rearrange, under suitable conditions, to the oleanane system. These considerations are best reflected and explained, in the author's opinion, if ring E of α-amyrin is 5- membered with an isopropyl group attached to C_{19}. The constitution and stereochemistry of α-amyrin is therefore represented by (XXXVI).



The isopropyl group is given the β- configuration for two reasons. Firstly, an α-isopropyl group gives a molecular structure in which severe interaction between this group and the C_{14} methyl group takes place. Secondly, the chosen configuration leads to a conformation in which the isopropyl group protects the double bond in α-amyrin, and the carbonyl group in 12-oxoursan-3β-yl acetate (IV), thus affording a satisfying explanation, of the inert character of these two functions. The acid catalysed rearrangement of the ursadien-3β-yl acetates probably includes, as a first phase, isomerisation to ursa-11:13(18)-dien-3β-yl acetate (XXIII) protonation of which gives the ion (XXXVII). The proximity of the C_{20} hydrogen and C_{12} carbonium ion permits a transannular hydride exchange with synchronous ring enlargement, thus leading to the ion (XXXVIII), which rearranges to oleana - 11:13(18)-dien-3β-yl acetate (XXXII).

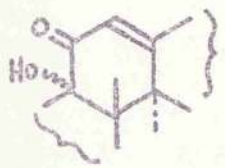
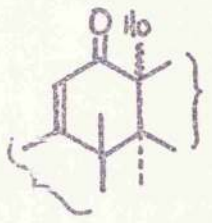


With a view to obtaining more conclusive evidence regarding the structure of ring E in α-amyrin, wherein lies the reasons for the difference in reactivity as compared to β-amyrin (see 27, 46, 47), the oxidation of the readily available urs-9(11):12-dien-3β-yl acetate (XXX) was undertaken with the hope of introducing functional groups in ring E.

Oxidation of Ursa-9(11):12-dien-3β-yl Acetate

Methods available for the preparation of ursa-9(11):12-dien-3β-yl acetate (XXX) from α-amyrin acetate, include dehydrogenation by sulphur at 210° (48), oxidation of α-amyrin acetate by chromic trioxide to 11-oxours-12-en-3β-yl acetate (XXV), followed by reduction of the ketone by sodium in amyl alcohol, and subsequent dehydration to the homoannular diene (XXX) (22). The easiest method and the one giving the best yield is oxidation of α-amyrin acetate or benzoate with N-bromosuccinimide to give ursa-9(11):12-dien-3β-yl acetate or benzoate (XXX) (49).

The oxidation of ursa-9(11):12-dien-3β-yl acetate (XXX) by chromic trioxide in acetic acid was first examined by Spring et al., (50), who isolated an acetate, $C_{32}H_{50}O_4$, in which the presence of an α,β-unsaturated ketone and a tertiary hydroxyl was established. This acetate, was formulated as (XXXIX) or (XL).

XXXIX   XL

The oxidation of the diene was repeated by Jeger, Ruzicka, et al., (51) who obtained, in addition to the acetate ($C_{32}H_{50}O_4$)

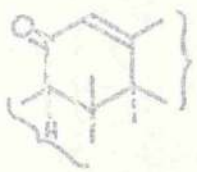
(XXXIX or XL), a second acetate $C_{32}H_{48}O_4$, which they suggested contains the group $-\underset{\overset{\cdot\cdot}{O}}{C} - C = C - \underset{\overset{\cdot\cdot}{O}}{C}-$. In terms of the generally accepted formula or the new stereoformula for α-amyrin acetate, it is impossible to formulate such an ene-dione system unless molecular rearrangement is assumed during the oxidation of the homoannular diene (XXX).

Repetition of the oxidation showed that no less than four neutral oxidation products are formed. In addition to the acetates $C_{32}H_{50}O_4$ (Acetate A) and $C_{32}H_{48}O_4$ (Acetate B) previously described, two new oxidation products were isolated (Acetates C, and D). An examination of the structure of these four products was therefore undertaken.

Structure of Acetate A.

Reduction of Acetate A (XXXIX or XL) with zinc in acetic acid gave 11-oxours-12-en-3β-yl acetate (XXV)

XXV



in excellent yield, from which it follows that the parent acetate is 11-oxours-12-ene-3β:9ξ-diol 3-acetate (XXXIX).

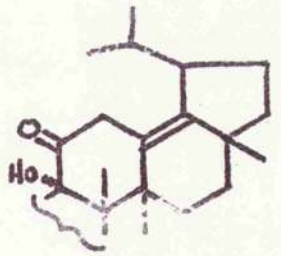
Ruzicka et al., (51) found that treatment of Acetate A (XXXIX) with strong alkali at 200° followed by acetylation gave a mixture of two compounds, one of which was formulated as $C_{32}H_{48}O_3$

i.e. as a dehydration product of the Acetate A, $C_{32}H_{50}O_4$. The author noted that the properties of this compound (m.p. 285-286°, $[\alpha]_D$ +93°; $\lambda_{max.}$ 2480 Å ($\log.\varepsilon$ 4.05); no colour with tetranitromethane in chloroform) are very similar to those of 11-oxours-12-en-3β-yl acetate (XXV) (m.p. 284-285°; $[\alpha]_D$ +98°; $\lambda_{max.}$ 2480 Å ($\log.\varepsilon$ 4.08); no colour with tetranitromethane in chloroform). Repetition of the experiment showed that this product, which is obtained as the more soluble component of the reaction mixture, is indeed 11-oxours-12-en-3β-yl acetate (XXV), and its formation is to be ascribed to reduction and not to a dehydration of Acetate A.

The less soluble component obtained from the strong alkali treatment of Acetate A differed considerably from the second compound isolated by Ruzicka. In contrast to the compound described by Ruzicka, which had m.p. 190 - 191°, $[\alpha]_D$ -20°, $\lambda_{max.}$ 3150 Å ($\log.\varepsilon$ 1.45) and empirical formula $C_{32}H_{50}O_4$, the product $C_{32}H_{50}O_4$, obtained in this work had m.p. 169 - 170°, $[\alpha]_D$ -60°, and exhibited end absorption (ε 9,800 at 2080 Å) in the ultra-violet region. The infra-red spectrum of the product isolated in this work includes bands attributable to a hydrogen-bonded hydroxyl group, a hydrogen-bonded six-ring carbonyl group, and an acetate group. The product gives a pale yellow colour with tetranitromethane in chloroform. The intensity of absorption in the 2,000 - 2250 Å region is compatible with the view that the product contains a 13:18-double-bond (44).

Product $C_{32}H_{50}O_4$	β-Amyrin derivatives.			
	Δ13-18	neo-β-amyrin	Δ9-11	Δ12-13
$\varepsilon_{208} = 10,800$			$\varepsilon_{208} = 3200$	$\varepsilon_{207} = 3100$
$\varepsilon_{210} = 10,000$	$\varepsilon_{211} = 5900$	$\varepsilon_{211} = 5000$	$\varepsilon_{210} = 3000$	$\varepsilon_{210} = 2200$
$\varepsilon_{215} = 6,800$	$\varepsilon_{215} = 5100$	$\varepsilon_{215} = 4500$	$\varepsilon_{215} = 1600$	$\varepsilon_{215} = 850$
$\varepsilon_{220} = 3,800$	$\varepsilon_{220} = 3500$	$\varepsilon_{220} = 3100$	$\varepsilon_{220} = 500$	$\varepsilon_{220} = 260$
$\varepsilon_{225} = 1,800$	$\varepsilon_{225} = 1800$	$\varepsilon_{225} = 1800$	$\varepsilon_{225} = 190$	$\varepsilon_{223} = 185$

On the basis of these physical properties, the most likely structure for the product $C_{32}H_{50}O_4$ is (XLI)



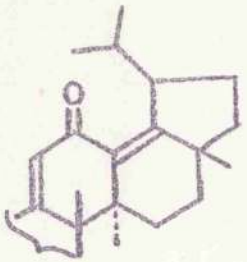
XLI

Acetate A (XXXIX) contains an α-ketol group. Attempts made to cleave this system using lead tetra-acetate or periodic acid were unsuccessful, Acetate A being recovered unchanged. Attempts were also made to form the enol acetate of Acetate A (XXXIX). 11-Oxours-12-en-3β-yl acetate (XXV) under acid conditions forms 11-acetoxyursa-9(11):12-dien-3β-yl acetate (XXI) (see p.57). The enol acetate of Acetate A (XXXIX) was not obtained, however, 11-oxours-12-en-3β-yl acetate (XXV) being the only product isolated from this reaction.

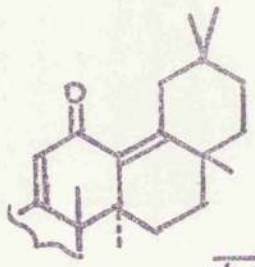
Structure of Acetate B

The acetate, $C_{32}H_{48}O_4$, obtained by oxidation of ursa-9(11):12-dien-3β-yl acetate (XXX) has been identified as 13:18ε-epoxy-12-oxours-9(11)-en-3β-yl acetate (XLII). This compound gives an infra-red spectrum which includes bands attributable to the acetate group, and the α,β-unsaturated ketone (ultra-violet light absorption $\lambda_{max.}$ 2560 Å (log ε 4.08), but it does not include bands due to the presence of either a hydroxyl or an isolated ketone group.

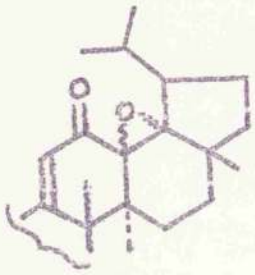
Reduction of Acetate B with zinc in ethanol gives 12-oxoursa-9(11):13(18)-dien-3β-yl acetate (XLIII), a yellow coloured compound which shows a highly characteristic ultra-violet absorption curve, similar to that of 12-oxo-oleana-9(11):13(18)-dien-3β-yl acetate (XLIV) (43.) The characteristic absorption spectrum of these two compounds is attributed to the cisoid-transoid geometry of its C = C - C(=O) - C = C chromophore; in contrast, compounds containing the same chromophore in a six-membered ring show an absorption at ca 2400 Å. The cisoid - transoid dienone, 12-oxoursa-9(11):13(18)-dien-3β-yl acetate (XLIII), differs from its oleanane analogue (XLIV) in possessing a yellow colour, confirmation that this colour was constitutional was obtained by hydrolysis to the corresponding almost colourless alcohol, followed by reacetylation to the yellow acetate.



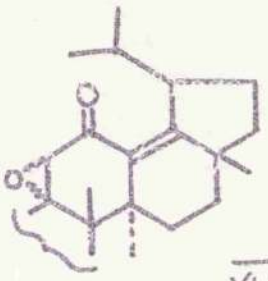
XLIII



XLIV



XLII



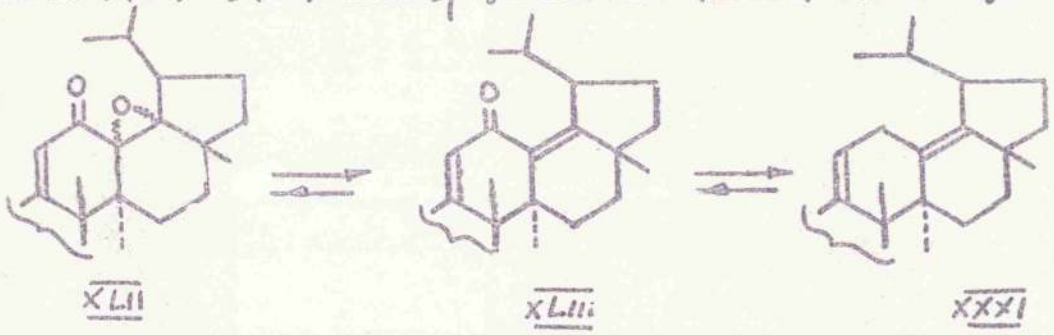
XLV

Oxidation of the yellow dienone, 12-oxoursa-9(11):13(18)-dien-3β-yl acetate (XLIII) with chromic trioxide in acetic acid regenerates Acetate B. On this evidence and that from the ultra-violet and infra-red spectra, Acetate B must possess an oxide ring. The oxygen atom removed from Acetate B by zinc must be the oxidic function, and therefore two constitutions are possible for the Acetate B, viz. (XLII) and (XLV).

Of the alternatives for Acetate B, the former (XLII) is preferred for two reasons. Firstly, the epoxide is recovered unchanged after treatment with hydrochloric-acetic acid. In the author's opinion, the secondary-tertiary oxide in (XLV) would not survive this treatment. Secondly, the intensity of

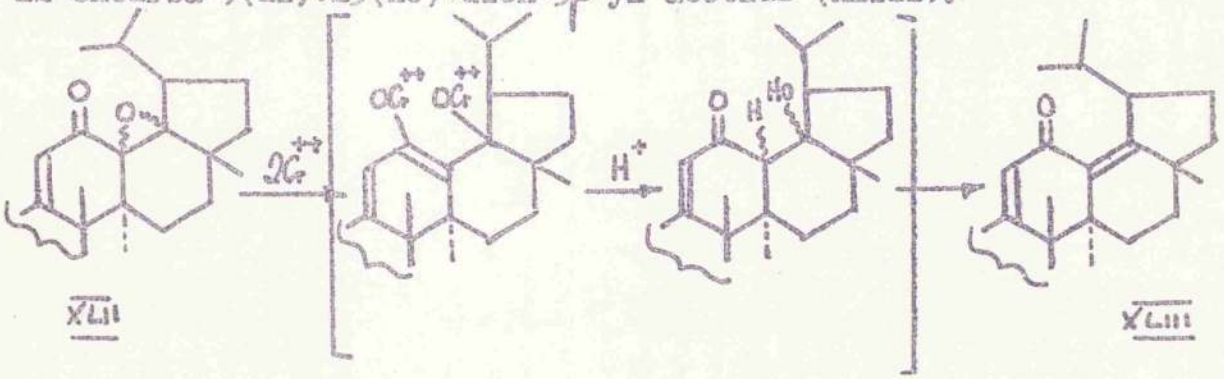
ultra-violet absorption maximum of Acetate B is not compatible with the presence of a cisoid $\alpha\beta$-unsaturated ketone as in (XLV)

In contrast to zinc-ethanol reduction, reduction of Acetate B (XLII) with zinc in acetic acid yielded a mixture which was separated by chromatography into 12-oxoursa-9(11):13(18)-dien-3β-yl acetate (XLIII), and ursa-9(11):13(18)-dien-3β-yl acetate (XXXI). Oxidation of ursa-9(11):13(18)-dien-3β-yl acetate (XXXI) with ozone gave 12-oxoursa-9(11):13(18)-dien-3β-yl acetate (XLIII). 12-Oxoursa-9(11):13(18)-dien-3β-yl acetate (XLIII) can be hydrogen-



XLII XLIII XXXI

olysed to ursa-9(11):13(18)-dien-3β-yl acetate (XXXI) (52). Reduction of Acetate B (XLII) with chromous chloride also gives 12-oxoursa-9(11):13(18)-dien-3β-yl acetate (XLIII).



XLII XLIII

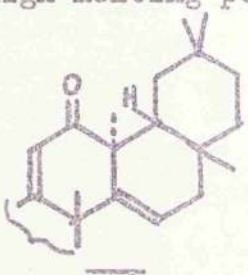
Other aspects of the chemistry of Acetate B, 13:18ξ-epoxy-12-oxours-9(11)-en-3β-yl acetate (XLII) will be discussed later.

Isolation of Acetates C and D.

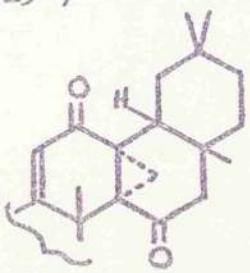
After removal of Acetates A and B from the mixture obtained by oxidation of ursa-9(11):12-dien-3β-yl acetate (XXX), the residues were combined and chromatographed on alumina. Elution of the column with benzene gave a high melting compound, Acetate C, $C_{32}H_{48}O_4$, with a characteristic ultra-violet light absorption at 2360 Å ($\log\varepsilon$ 4.04). It does not give a colour with tetranitromethane in chloroform. Further elution of the column with benzene gave successively, Acetate B (XLII), and an Acetate D, readily identified by direct comparison as 12-oxours-9(11)-en-3β-yl acetate (V). Further elution with benzene-ether gave Acetate A (XXXIX).

Structure of Acetate C.

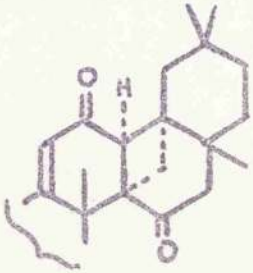
A β-amyrin derivative is known which has similar physical characteristics to Acetate C. The β-amyrin compound, $C_{32}H_{48}O_4$, formulated as (XLVI) or (XLVII), is obtained by vigorous oxidation of 12-oxoiso-oleana-9(11):14-dien-3β-yl acetate (XXVII) (53), and has an ultra-violet maximum at 2360 Å ($\log\varepsilon$ 4.11) and a high melting point (315°)



XXVII



XLVI

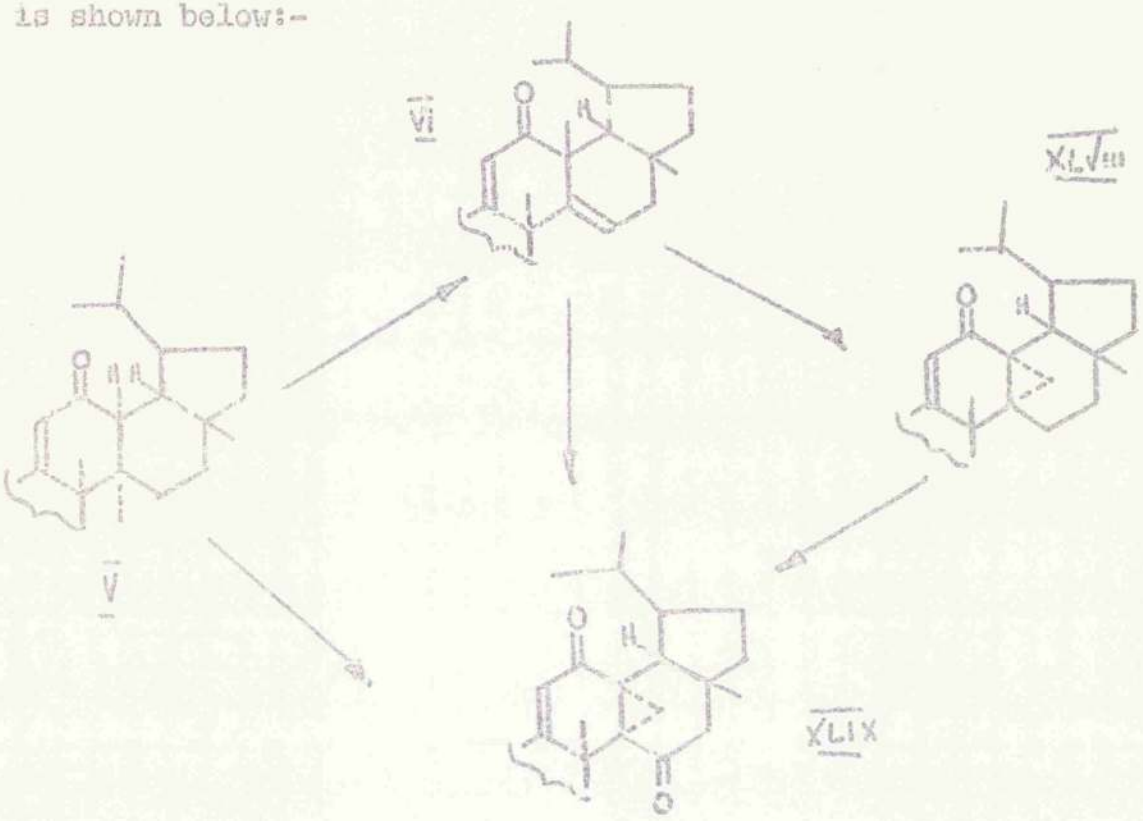


XLVII

Since 12-oxours-9(11)-en-3β-yl acetate, (Acetate D) (V), and Acetate C, $C_{32}H_{48}O_4$, were obtained by the oxidation of ursa-9(11):12-dien-3β-yl acetate (XXX), the probability that Acetate C was an oxidation product of Acetate D (V) was considered likely, and verified experimentally. Although Acetate D (V) is stable to cold chromic acid, vigorous oxidation yielded Acetate C in good yield (54). In order to account for the ultra-violet spectrum of Acetate C, the possibility of molecular re-arrangement during its formation was considered. In this connection 12-oxoisoursa-9(11):14-dien-3β-yl acetate (VI) suggested itself as a likely intermediate, which under the influence of acid would form a second intermediate , 12-oxophyllanth-9(11)-en-3β-yl acetate (XLVIII) (55). In agreement with this postulate, 12-oxoisoursa-9(11):14-dien-3β-yl acetate (VI), prepared by the standard method of selenium dioxide oxidation of 12-oxours-9(11)-en-3β-yl acetate (V) (40), gave 12-oxophyllanth-9(11)-en-3β-yl acetate (XLVIII) on treatment with cold chromic acid. Moreover, vigorous chromic acid oxidation of either intermediate (VI) or (XLVIII) gave Acetate C. On the basis of these syntheses, the structure of Acetate C is considered to be 12:15-dioxophyllanth-9(11)-en-3β-yl acetate (XLIX). The infra-red spectrum of Acetate C (XLIX) exhibits bands at 1648 and 1686 cm^{-1} which may be attributed to the cyclopropanedione system.

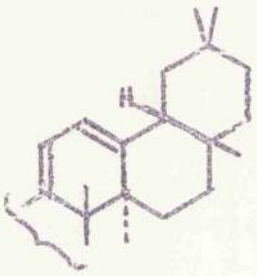
The course of the oxidation of Acetate D (V), to Acetate C (XLIX)

is shown below:-



Acetates A,B,C and D have therefore been identified and characterised; although their structures gave no direct evidence regarding the nature of ring E, Acetate B (XLII) with its 13:18 epoxide function proved to be a useful starting material for the introduction of unsaturation in ring E, as described later.

An attempted oxidation of ursa-9(11):12-dien-3β-yl acetate (XXX) with selenium dioxide was unsuccessful, the diene being recovered unchanged. Under similar conditions oleana-9(11):12-dien-3β-yl acetate (L) gives 12:19-dioxo-oleana-9(11):13(18)-dien-3β-yl acetate (LI) (56, 57). In the author's opinion, this difference is indicative of the presence of an alkyl group at C_{19}.



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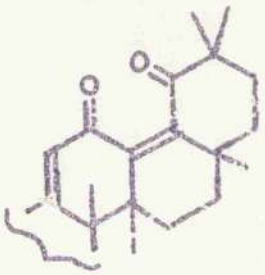
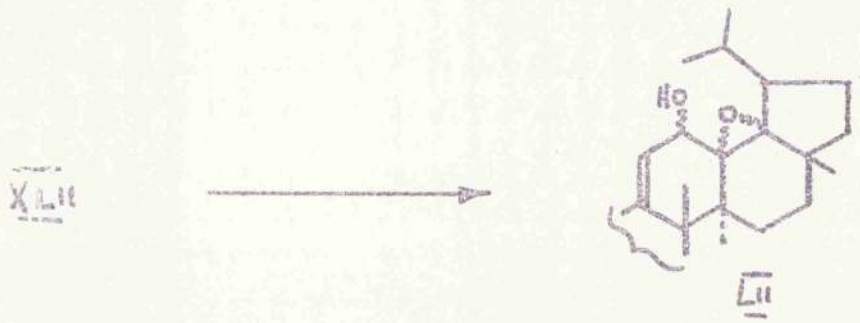


8

Structure of Ring E in α-Amyrin.

Consideration will now be given to experiments designed to obtain more conclusive evidence regarding the nature of ring E in α-amyrin and to a discussion of the relative merits of the alternative formulae for α-amyrin, i.e. the structures involving the five-membered ring E, and the six- membered ring E.

Reduction of Acetate B, 13:18ξ-epoxy-12-oxours-9(11)-en-3β-yl acetate (XLII) with lithium aluminium hydride gave, 13:18ξ-epoxyurs-9(11)-ene-3β:12ξ-diol 3-acetate (LII). The ultra-violet spectrum of (LII) showed double bond absorption only (2080 Å log ε 3.61) and the infra-red spectrum confirmed the presence of a hydrogen-bonded hydroxyl group. The compound (LII) gave a yellow colour with tetranitromethane in chloroform solution. On acetylation it gave the diol monoacetate, 13:18ξ-epoxyurs-9(11)-ene-3β:12ξ-diol 3-acetate (LII), the 12-hydroxyl group being sterically hindered. The diol monoacetate (LII) was readily oxidised by chromic trioxide-pyridine complex (45) to the 13:18ξ-epoxide (XLII), indicating that no molecular rearrangement had occurred during the reduction stage.

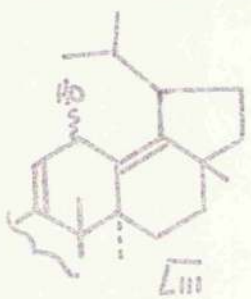


XLII → LII

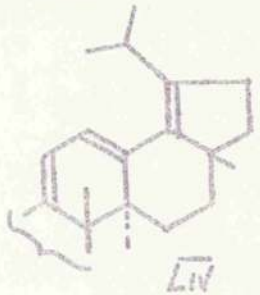
Treatment of the diolmonoacetate (LII) with mineral acid gave a compound $C_{32}H_{52}O_4$ which gives a light yellow colour with tetranitromethane in chloroform solution, and showed ultra-violet end absorption at 2040 Å ($\log\varepsilon 3.66$). Its structure has not been elucidated. The infra-red spectrum of this compound showed strong absorption attributable to a six-ring carbonyl group. The compound was recovered unchanged after treatment with pyridine and acetic anhydride, zinc dust in ethanol or acetic acid, and chromous chloride reagent. Reduction of the compound with lithium aluminium hydride followed by acetylation gave a gum.

Reduction of 12-oxoursa-9(11):13(18)-dien-3β-yl acetate (XLIII) with lithium aluminium hydride gave the expected ursa-9(11):13(18)-diene-3β-12ξ-diol (LIII), which on acetylation gave a mixture of two products, readily separable by chromatography. The more strongly adsorbed compound, $C_{32}H_{50}O_3$, is the corresponding diol monoacetate (LIII), 3β-acetoxyursa-9(11):13(18)-dien-12ξ-ol, which was readily dehydrated by treatment with acetic anhydride at 100° to yield a product, $C_{32}H_{48}O_2$, identical with the second (less strongly adsorbed) compound isolated by chromatography. On the basis of its method of formation, the structure, ursa-9(11):12:18-trien-3β-yl acetate (LIV) can be attributed to this compound. The conjugated triene structure is supported by the ultra-violet maximum at 3060 Å ($\log\varepsilon 4.15$); this slightly lower absorption

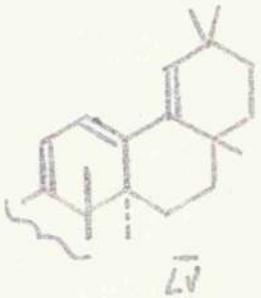
wavelength, as compared to oleana-9(11):12:18-trien-3β-yl acetate (LV) (3100 Å log ε 4.1)(43), which has one less alkyl substituent on the triene system, is surprising, and may be attributable to the difference in size of ring E.



LIII

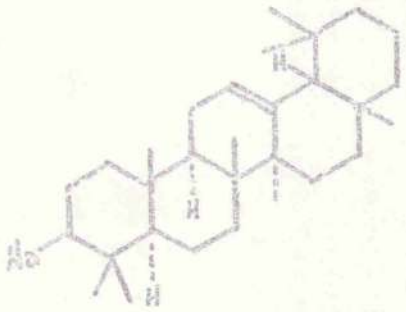


LIV



LV

This ursatriene (LIV) in which the parent ursane skeleton has not undergone re-arrangement (see later) is the first example of an ursane derivative with an ethylenic linkage in ring E. It demonstrates conclusively the existance of a hydrogen atom at C_{18} in α-amyrin, and consequently excludes formulae for α-amyrin such as (LVI), tentatively suggested by Meakins (58).



LVI

The first attempt to establish that the triene (LIV) still retained the ursane skeleton, consisted of catalytic hydrogenation, in the hope of isolating a known ursadienyl acetate or ursenyl acetate. This was unsuccessful, however, since the only isolated product having high intensity end absorption in the ultra-violet region, is possibly an unknown non-conjugated diene. The intensity of the absorption in the 2000 - 2250 Å region is compatible

with the view that the compound contains a 13:18-double bond, i.e.

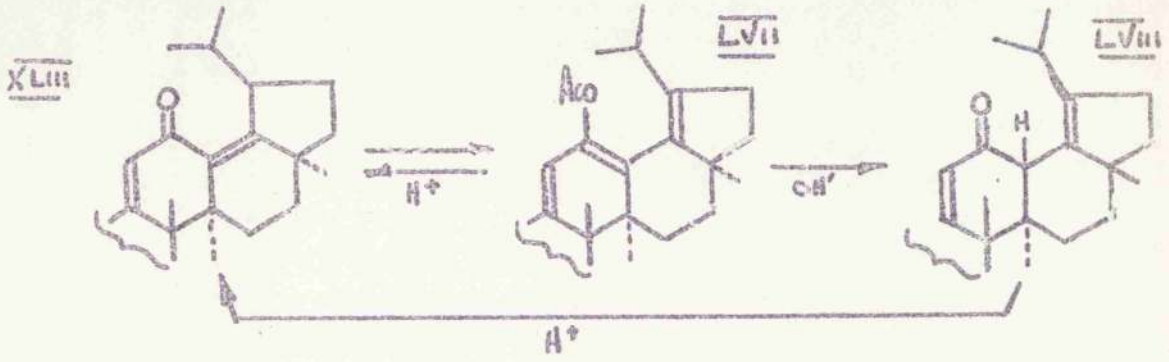
Hydrogenation Product	Urs-13(18)-en-3β-yl acetate
210 = 8,400	210 = 4,000
215 = 7,200	215 = 5,100
220 = 4,900	222 = 2,800
225 = 3,000	223 = 1,100
230 = 1,100	

This hydrogenation product was recovered unchanged on mineral acid treatment, in marked contrast to the triene (LIV) from which no crystalline products could be isolated on similar acid treatment.

The conversion of ursa-9(11):12:18-trien-3β-yl acetate (LIV) to a known ursadienyl acetate was readily achieved however, by employing lithium metal in liquid ammonia as the reducing agent, ursa-9(11):13(18)-dien-3β-yl acetate (XXXI) was readily isolated and identified.

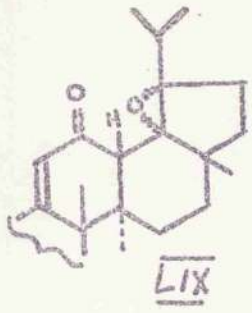
A second ursatrienyl acetate, 3β:12-diacetoxyursa-9(11):12:18-triene (LVII), was obtained in excellent yield by subjecting 12-oxoursa-9(11):13(18)-dien-3β-yl acetate (XLIII) to forcing enol acetylating conditions. When hydrolysed with methanolic hydrochloric acid, the enol acetate (LVII) gave 12-oxoursa-9(11):13(18)-dien-3β-ol, characterised by formation of its acetate (XLIII). In contrast, base hydrolysis of the enol acetate (LVII), followed by acetylation, gave a mixture, from which

two isomeric compounds were isolated by crystallisation and chromatography.

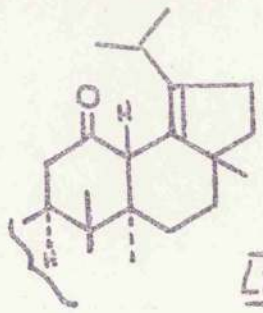


The first compound, $C_{32}H_{48}O_3$, was an $\alpha\beta$-unsaturated ketone (λmax. 2500Å) which gave a yellow colour with tetranitromethane in chloroform solution, indicating the presence of an isolated double bond. Accordingly the compound is formulated as 12-oxoursa-9(11):18-dien-3β-yl acetate (LVIII), in agreement with this structural assignment, treatment of (LVIII) with mineral acid, followed by acetylation gave 12-oxoursa-9(11):13(18)-dien-3β-yl acetate (XLIII).

The isolated Δ-18 bond in (LVIII) is not strongly hindered, since treatment with monoperphthalic acid yielded the mono-epoxide still possessing the typical ultra-violet absorption spectrum of an $\alpha\beta$-unsaturated ketone. This epoxide is therefore considered to be 18:19ξ-epoxy-12-oxours-9(11)-en-3β-yl acetate (LIX). Reduction of 12-oxoursa-9(11):18-dien-3β-yl acetate (LVIII) with lithium metal in liquid ammonia gave 12-oxours-18-en-3β-yl acetate (LX). An attempt to convert the dienone (LVIII) to a diene by catalytic hydrogenolysis failed, starting material being recovered.

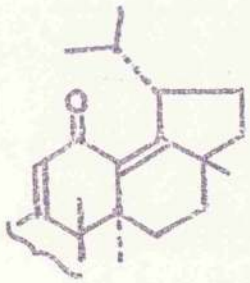


LIX



LX

The second isomer isolated from base hydrolysis of the enol acetate (LVII) exhibits the typical ultra-violet absorption spectrum (maxima at 2050, 2620, and 2940 Å) of a cisoid-transoid "en-one-ene" system. Since it differs from 12-oxoursa-9(11):13(18)-dien-3β-yl acetate (XLIII), the most likely formulation is that of the 19α-alkyl isomer (LXI). This compound, unfortunately, was extremely labile, simple recrystallisations causing change



LXI

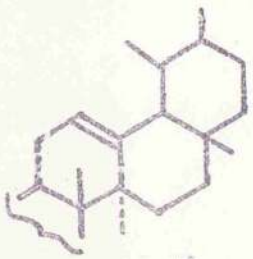
of melting point range and alteration of specific rotation.

An attempt to gain experimental support for the supposition that acid hydrolysis of the enol acetate was giving the thermodynamically more stable isomer with a 19β- alkyl group (XLIII), and alkaline hydrolysis yielded the less stable isomer with the 19α-alkyl group (LXI), failed because no crystalline product could be isolated from alkaline hydrolysis of 12-oxours-18-en-3β-yl acetate (LX).

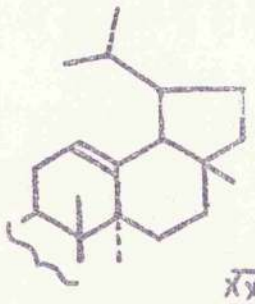
Reduction of the enol acetate (LVII) with lithium metal in

liquid ammonia, followed by reacetylation yielded a substance, $C_{34}H_{50}O_3$, exhibiting an ultra-violet absorption maximum at 2620 Å (log 4.28), whose structure has not been elucidated.

Budziarik, Manson, and Spring (34) reported that vigorous chromic acid oxidation of β-amyrin acetate yielded acetone; under identical conditions, no acetone was isolated from α-amyrin acetate. In view of the identity of rings A,B,C, and D in these compounds, it seems likely that the acetone from β-amyrin acetate, is derived from ring E. In view of the known ease of oxidative attack in ring E of β-amyrin (e.g. preparation of 12:19-dioxo-oleana-9(11):13(18)-dien-3β-yl acetate (LI) see p.74), the acetone might well be derived from the gem- dimethyl group at C_{20}. The failure to isolate acetone from α-amyrin acetate, might at first sight, appear to support the Ruzicka - Jeger formulation (I), and be construed as evidence against the five-membered ring E structure (XXXVI). It should



I



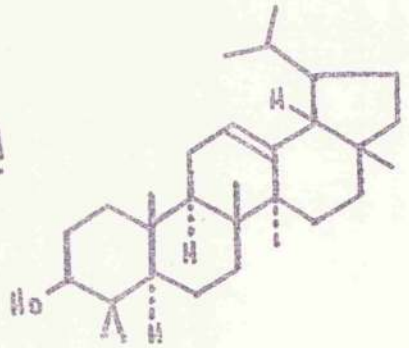
XXXVI

be borne in mind, however, that the C_{12}, C_{13}, C_{18} and C_{19} region in α-amyrin is subject to severe hinderance, and prior to this work, no ursane derivatives with unsaturation or oxygen functions in ring E had been synthesised. Accordingly, the non-appearance of acetone in the oxidation products of α-amyrin acetate could

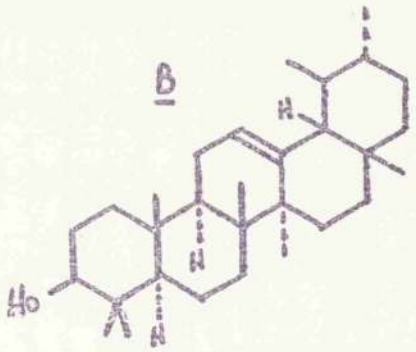
be attributed to the hinderance to oxidative attack in the environment of ring E. The availability of an ursatriene with unsaturation at C_{18} permitted the testing of this hypothesis. Vigorous oxidation of 3β:12-diacetoxyursa-9(11):12:18-triene (LVII) yielded acetone, isolated as its 2:4-dinitrophenylhydrazone. These observations may therefore be interpreted as evidence in favour of the α-amyrin formulation (XXXVI) possessing an isopropyl group.

Summarising the state of knowledge concerning the structure of α-amyrin two formulae (A) and (B) exist from which a definite choice remains to be made.

A



B



The formulation (A) was proposed (28, 37) on the basis of experiments performed in these laboratories, some of which are the subject matter of this thesis. The principal points of interest are:-

1. The stereochemistry at all asymmetric centres has been elucidated on the basis of chemical evidence.
2. The stability of the ring D/E cis-fused system is readily explicable on the basis of a hydrindane system.
3. The steric hinderance observed on reactions of the α-amyrin double bond, or 12-keto- derivatives is explained by the

protecting influence of the bulky isopropyl group.

4. A satisfactory mechanism for the ursadienyl acetate ——→ oleanadienyl acetate is given.

5. The differences in behaviour of α- and β-amyrin acetates on vigorous oxidation have been rationalised.

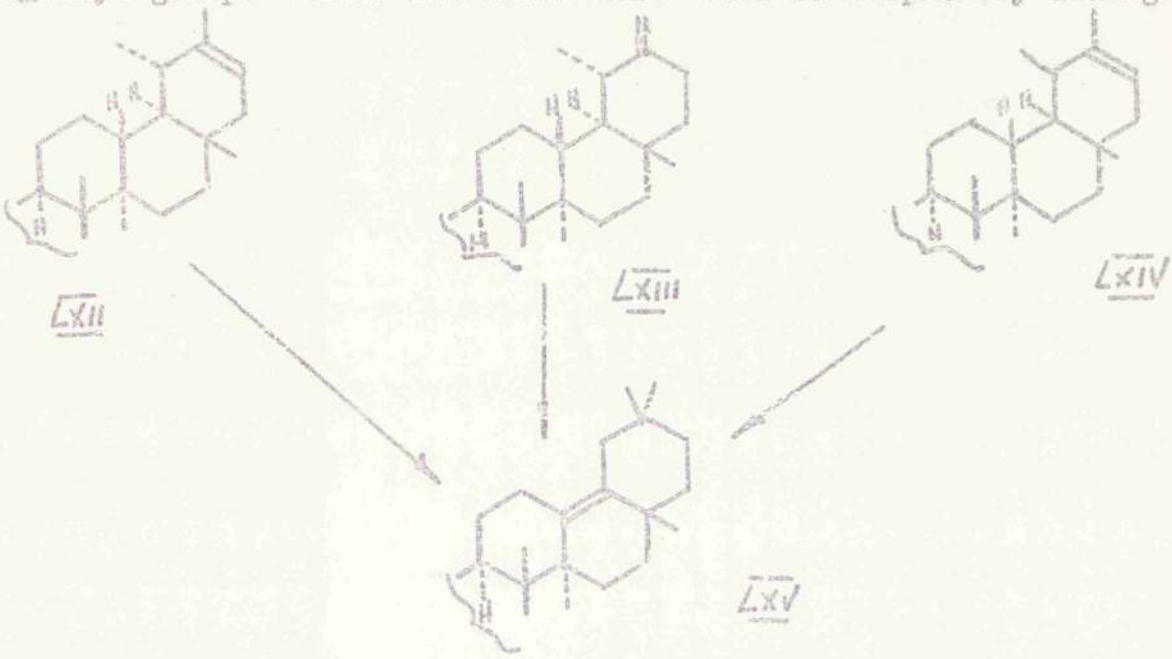
After publication of the new stereoformula (A) several papers have appeared presenting new (and in some cases conflicting) evidence, some of which has been interpreted as favouring (A), and some favouring (B), the structure proposed originally by Ruzicka, Jeger and co-workers (19) and elaborated by Corey and Ursprung (27, 31).

The objections to formula (A) made by Corey and Ursprung (31) are :-

1. The five membered ring E is in apparent disagreement with several degradative studies carried out by Ruzicka et al. (see p.49and p53). It should be borne in mind, that these are mostly pyrolytic reactions in which the possibility of re-arrangement has not been exaluded, non-crystalline intermediates only have been isolated and in some instances full experimental descriptions have not been given.

2. It is unnecessary to adopt a five-membered ring E to explain the ursane→oleanane interconversion, since this could be expected on the basis of a six-membered ring E. They quote (31) that ψ-taraxastene (LXII), taraxastene (LXIII), and lupene-I (LXIV) isomerises to olean-13(18)-en-3β-ol (LXV), which involves the transformation of a 19:20 dimethyl group to a 20:20

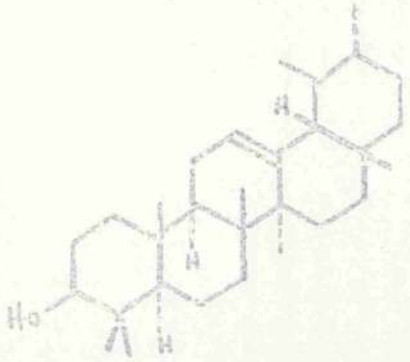
dimethyl group. Their statement that "this is completely analogous



LXII LXIII LXIV LXV

to that which is required if (B) is the structure of α-amyrin" is somewhat superficial. If, indeed, the mechanism involves the migration of the unsaturated system into ring E to effect the re-arrangement, it must involve the diene system losing its conjugation. Moreover no examples of conversion of an ursene derivative to an oleanene derivative are known.

3. If α-amyrin has structure (A), ursolic lactone (trans-pentalane) would be more strained than oleanolic lactone (trans-hydrindane), contrary to observation. Corey and Ursprung (31) formulate the structure of α-amyrin as having a β- C_{10} methyl group, and an α-C_{20} methyl group as shown in (LXVI).



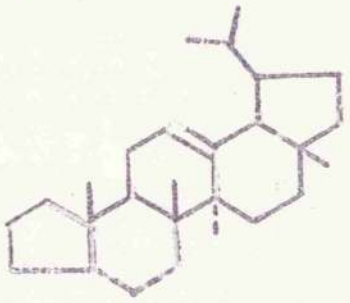
LXVI

They state that direct elucidation of the configurations at C_{19}, and C_{20} is not a simple matter, because of the difficulty encountered in carrying out stereochemically meaningful reactions in ring E. However, many of the reactions in the accessible rings C and D of the α-amyrins are strongly affected by the configurations at C_{19} and C_{20}. The configurations at C_{19} and C_{20} are responsible for the cis-fusion, since there is only one orientation of the two methyl groups which is consistent with these findings. This orientation, 19β-equatorial, and 20-α equatorial, is such that the cis-locking of ring E is anchored by the two methyl groups, which are equatorial only as long as rings D and E are cis-locked. The methyl groups would be forced into the unfavourable axial orientation if the ring junction D/E was trans-fused. If the C_{19}-methyl group was axial i.e. had the α-configuration, severe interaction would occur with the C_{14}-methyl group, causing the cis-β-fusion to be so destabilised that trans- fusion of rings D and E would be the more stable. The inertness of the 12:13-double bond is due to the hinderance by the 19β-methyl group.

Attempts have been made to distinguish between formulae (A) and (B) by means of infra-red spectrographic determinations. Meakins (58) drew attention to the fact that formula (A) has two and formula (B) has only one gem-dimethyl group. Since this structural system exhibits a characteristic peak near 1367 cm^{-1} (62),

a physical method of distinguishing between the two formulae was available.

To accentuate the difference, α-amyrin was converted to the trisnorhydrocarbon viz.

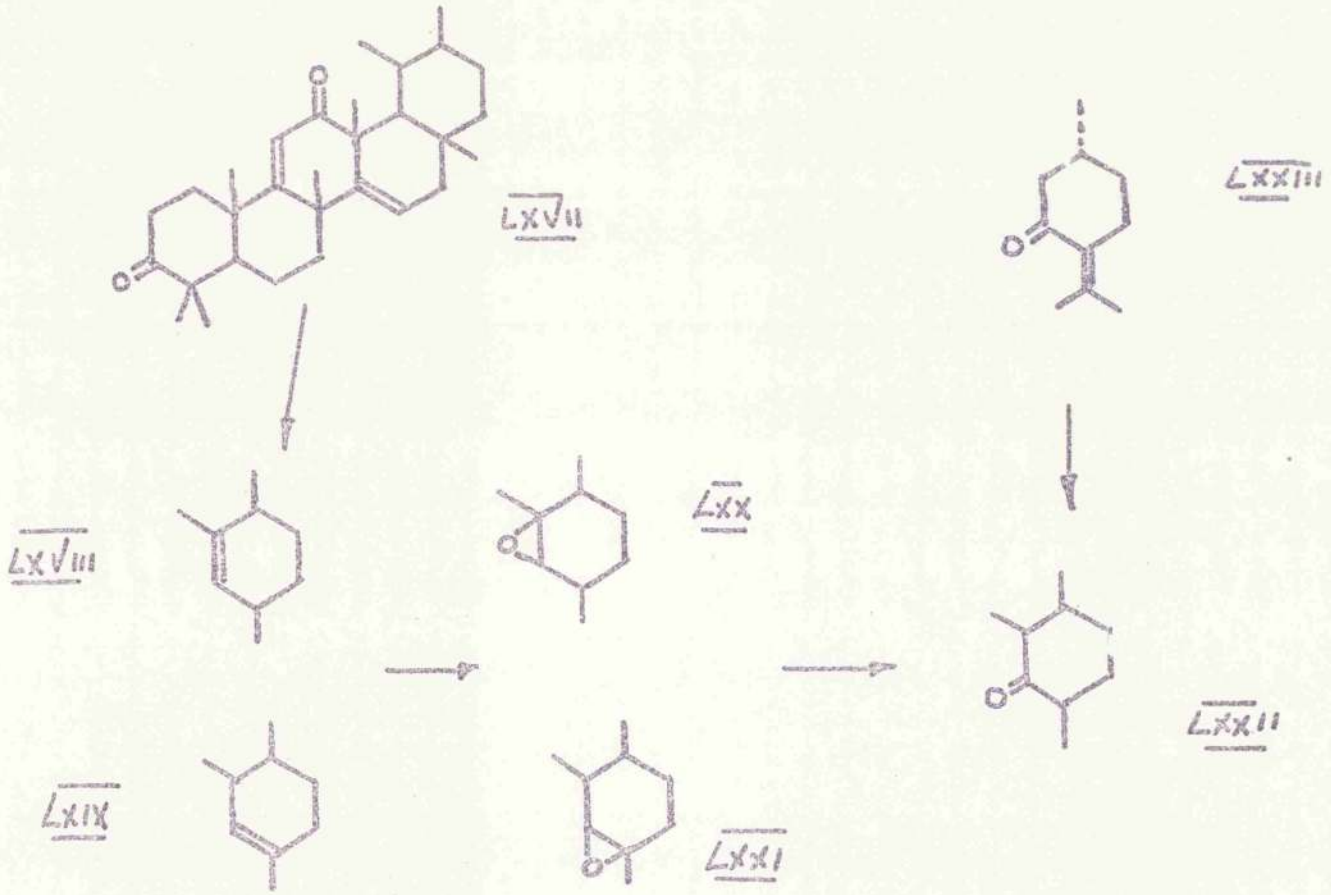


thus removing the gem-dimethyl group from ring A. β-Amyrin and lupanol were likewise converted to the corresponding trisnorhydrocarbons. In all three derivatives, the intensity at 1367 $cm.^{-1}$ was the same, indicating that in all three hydrocarbons there was one gem-dimethyl group present. It was therefore concluded that α-amyrin possessed two gem-dimethyl groups, thus excluding formula (B). An alternative structure for α-amyrin, other than (A), was proposed by Meakins but this is excluded by experimental evidence discussed in this thesis (p.78).

Meakins conclusions have very recently been challenged, by Cole, Thornton, and White (63), who claim that the absorption at 1365 $cm.^{-1}$ in β-amyrin and lupane is much more intense than that of α-amyrin.

Jeger and co-workers (59) dismiss the formula (A) for

α-amyrin in the light of recent degradation reactions which they have carried out. Pyrolysis of 3:12-dioxoisoursa-9(11):14-diene (LXVII) at 320 - 340° yielded a hydrocarbon, C_9H_{16}, to which structure (LXVIII) or (LXIX)is assigned, treatment of which with monoperphthalic acid gave an oxide (LXX) or (LXXI). Treatment of this oxide with Lewis acid yielded a ketone (-)-2:3:6-trimethyl-cyclohexanone (LXXII), identified by synthesis from D(+)-pulegone (LXXIII). On this evidence Jeger claims that the formation of ketone



(LXXII) cannot be interpreted on the basis of the five- membered

ring E structure (A), and also that on their structure the methyl group at C_{20} possesses the α-configuration.

It should be noted, however, that the possibility of molecular re-arrangement either during the pyrolysis or acid treatment stages, has not been excluded.

Jeger (see p.48, 49) has pointed out a discrepancy in his structure for α-amyrin (I), in that the dehydrogenation products from α-amyrin are the same as those from β-amyrin, and include 1:8-dimethylpicene (III , R = H) rather than the expected 1:2:8-trimethylpicene (III R = CH_3). Because of the difficulty in making comparisons among the high-melting picene derivatives, Jeger has suggested that the hydrocarbon m.p. 306°, obtained in the dehydrogenation of α-amyrin (22, 23) might well be the expected trimethylpicene, which was unknown at the time.

1:2:8-Trimethylpicene has recently been synthesised by Phillips and Tuites (24), and differs from the 306° hydrocarbon. These authors state "Since there seems to be no well established example of the elimination of non-quaternary methyl groups during dehydrogenation (60), we find it difficult to rationalise our observations on the basis of Jeger's structure for α-amyrin. Moreover, well authenticated examples of 5-membered rings expanding to 6-membered rings during dehydrogenation are on record (61), so that there is some precedent for the formation of 1:8-dimethylpicene (III R = H). Thus although more definite degradations are

highly desirable, our observations are compatible with (A) as the structure of α-amyrin and not with (B)".

At the present time, the formulae of the family of compounds based on α-amyrin must be regarded as unsettled.

For general instructions see p.15. Colour tests with tetranitromethane were done in chloroform solution.

EXPERIMENTAL

Ursa-9(11):12-dien-3-one.—

Chromium trioxide (2 g.) in pyridine (20 c.c.) was added to a solution of ursa-9(11):12-dien-3β-ol (2 g) in pyridine (20 c.c.), and the mixture kept at room temperature for 18 hours with occasional shaking. The suspension was filtered through kieselguhr, the filtrate diluted with water and extracted with ether. The ether extract was washed with dilute hydrochloric acid, water, sodium bicarbonate solution, dried (Na_2SO_4) and evaporated. A solution of the product in benzene was filtered through an alumina column, the filtrate evaporated, and the residue crystallised from chloroform-methanol to give ursa-9(11):12-dien-3-one as fine needles (1.3 g.), m.p 164-166°, $[\alpha]_D$ +411°, (C, 1.7), +414° (C, 1.0); $\lambda_{max.}$ 2820 Å (ε 10,200). It gives a red-brown colour with tetranitromethane. Analysis. Found: C, 85.1; H, 11.15. Calc. for $C_{30}H_{48}O$: C, 85.2; H, 11.0 %.

Jacobs and Fleck, (48) give m.p. 133-134°, $[\alpha]_D$ +412° (pyridine). Spring and Vickerstaff, (22) give m.p. 135-137°.

Ursa-9(11):12-dien-3-one Oxime.—

A solution of ursa-9(11):12-dien-3-one (100 mg.) in ethanol (40 c.c.)

was refluxed with a mixture of hydroxylamine hydrochloride (100 mg.) and crystalline sodium acetate (100 mg) dissolved in minimum of water, for 90 minutes. The solution was concentrated and cooled. The solid which separated was collected and crystallised from chloroform-methanol to yield ursa-9(11):12-dien-3-one oxime as needles, m.p. 249-250°(d.), λ max. 2060, 2820 Å (ε 4,700; 9, 400) Analysis. Found: C, 82.6; H, 10.9. Calc. for $C_{30}H_{47}NO$: C, 82.3; H, 10.8%.

Jacobs and Fleck (48), give m.p. 233-235°, and Spring and Vickerstaff (22) give m.p. 236°.

Oleana-11:13(18)-dien-3β-yl Acetate.——— A solution of olean-12-en-3β-yl acetate (15 g.) and selenium dioxide (15 g.) in acetic acid (1 l.) was refluxed for 3 hours. The solution was filtered, and the filtrate concentrated until solid began to separate. The solid was collected, dried, and a solution in benzene was filtered through a column of alumina (200 g.). The eluates on evaporation gave the product which crystallised from chloroform-methanol to give oleana-11:13(18)-dien-3β-yl acetate as plates (5 g.), m.p. 225-226°. $[\alpha]_D$ -63° (C, 1.1) λmax. 2,420, 2500, 2600 Å. (ε 26,500; 30,000; 19,300).

Oleana-11:13(18)-dien-3β-ol.———

A solution of oleana-11:13(18)-dien-3β-yl acetate (5 g.) in methanolic potassium hydroxide (3%, 600 c.c.) was refluxed for 3 hours. The product, isolated by means of ether, was crystallised from acetone or methanol to give oleana-11:13(18)-dien-

3β-ol as plates (4.5 g.), m.p. 230-231°.

Oleana-11:13(18)-dien-3-one.——

A solution of chromium trioxide (280 mg.) in acetic acid (5 c.c.) was added to a solution of oleana-11:13(18)-dien-3β-ol (1 g.) in acetic acid (600 c.c.) at 30°, and the mixture kept at room temperature for 50 hours. The product, isolated through ether, and a solution of it in benzene filtered through a column of alumina (20 g.), crystallised from chloroform-methanol to give oleana-11:13(18)-dien-3-one as fine needles, m.p. 236-240°, $[\alpha]_D$ -48.5°, (C, 0.83) it gives a reddish-brown colour with tetranitromethane; λmax. 2420, 2500, 2600 Å, (ε, 28,200; 32,400; 21,100).

Analysis. Found: C, 85.2; H, 10.8. $C_{30}H_{48}O$ requires C, 85.2; H, 11.0%.

Oleana-11:13(18)-dien-3-one Oxime.——

A solution of oleana-11:13(18)-dien-3-one (100 mg.) in ethanol (40 c.c.) was refluxed for 90 minutes with a solution of hydroxylamine hydrochloride (100 mg.) and crystalline sodium acetate (100 mg.) in water (1 c.c.). Water was added, and the solution cooled. The solid was collected, dried, and crystallised from chloroform-methanol to give oleana-11:13(18)-dien-3-one oxime as needles, m.p. 279-280 (d.) λmax. 2420, 2510, 2600 Å (ε, 29,200; 32,000; 20,700).

Analysis. Found C, 82.1; H, 10.9. $C_{30}H_{47}NO$ requires C, 82.3; H, 10.8%.

Conversion of Ursa-9(11):12-dien-3-one into Oleana-11:13(18)-dien-3-one.——

Ursa-9(11):12-dien-3-one (360 mg.) was heated with concentrated hydrochloric acid (5 c.c.) and acetic acid (30 c.c.) at 100°

for 60 hours, with addition of hydrochloric acid (5 c.c.) after 24 hours and 48 hours. Concentration of the solution yielded needles (340 mg.); a solution of which in benzene was filtered through a column of alumina (5 g.). The filtrate was evaporated, and a solution of the solid (280 mg.) in light petroleum filtered through alumina (5 g.). The product (240 mg.) was crystallised twice from chloroform-methanol to give oleana-11:13(18)-dien-3-one as needles (110 mg.), m.p. and mixed m.p. 236-238°, $[\alpha]_D$ -48° (C, 1.0); λ max. 2430, 2510, 2600 Å (ε, 25,400; 28,500; 18,400).

Oxidation of Ursa-9(11):12-dien-3β-yl Acetate.

To a stirred solution of ursa-9(11):12-dien-3β-yl acetate (7.65 g.) in acetic acid (200 c.c.) was added, over 15 minutes, a solution of chromic trioxide (7.5 g.) in acetic acid (90%, 80 c.c.). The mixture was refluxed for 30 minutes, diluted with water, cooled, and ether added. A crystalline solid remained undissolved in the ether. This solid was collected and crystallised from chloroform-methanol to give 11-oxours-12-ene-3β:9ξ-diol 3-acetate (Acetate A) as blades (1.5 g.), m.p. 316-317° (high-vac.), $[\alpha]_D$ +52°, (C, 1.4), light absorption, λ max. 2500 Å (ε 13,500). The compound gives an orange-red colour with concentrated sulphuric acid, but gives no colour with tetranitromethane in chloroform solution. Analysis. Found: C, 77.3; H, 10.3. Calc. for $C_{32}H_{50}O_4$: C, 77.1; H, 10.1%

Beynon, Sharples, and Spring, (50) give m.p. 312°, $[\alpha]_D$ +61°.

Ruzicka, Jeger, Redel, and Volli, (51) give m.p. 316° (high-vac.) $[\alpha]_D$ +62°. Acetate A was recovered unchanged after refluxing with periodic acid in ethanol, or lead tetra-acetate in acetic acid solution.

The ether extract was evaporated and the residue crystallised from chloroform-methanol to give 13:18β-epoxy-12-oxours-9(11)-en-3β-yl acetate (Acetate B) as plates (2.65 g.), m.p. 269-271°, $[\alpha]_D$+70° (C, 1.4); λ max. 2560 Å (ε 11,900). It does not give a colour with tetranitromethane. Analysis. Found: C, 77.7; H, 9.6. $C_{33}H_{48}O_4$ requires C, 77.4; H, 9.7%. Infra-red absorption, 1250 and 1722 cm.$^{-1}$ (acetate group), 1603 and 1652 cm.$^{-1}$ (α/β-unsaturated ketone).

Ruzicka, Jeger, Redel, and Volli, (51) give m.p. 258°, $[\alpha]_D$+70°, and λ max. 2590 Å (log.ε4.1).

The mother liquors, after removal of above two compounds, were collected and evaporated to dryness yielding a yellow gum (4 g.). A solution of this gum in light petroleum/benzene (3:2) was chromatographed on a column of alumina (120 g.). Elution of the column with benzene gave a substance (400 mg.), crystallised from chloroform-methanol to give 12:15-dioxophyllanth-9(11)-en-3β-yl acetate (Acetate C) as needles, m.p. 321-324°, $[\alpha]_D$+99°, (C, 1.5); +100°(C, 0.7). The compound gives no colour with tetranitromethane; λ max. 2360 Å (ε 11,000), infra-red light absorption, 1255 and 1728 cm.$^{-1}$ (acetate group), 1648 and 1686 cm.$^{-1}$ (cyclopropane-dione system) Analysis. Found: C, 77.8; 77.4; H, 9.5; 9.6. $C_{32}H_{46}O_4$ requires C, 77.7; H, 9.4%.

Acetate C was recovered unchanged after heating at 100° for 24 hours with acetic acid and concentrated hydrochloric acid (30:1).

Further elution of the alumina column with benzene gave 13:18ξ-epoxy-12-oxours-9(11)-en-3β-yl acetate (Acetate B) (700 mg.), followed by 12-oxours-9(11)-en-3β-yl acetate (Acetate D) (150 mg.), m.p. and mixed m.p. 286-289°, crystallising from chloroform-methanol as needles, $[\alpha]_D$ +85°, (C, 1.4); λ max. 2480 Å (ε 12,000).

Continued elution with benzene/ether (9:1 to 1:1) gave 11-oxours-12-ene-3β:9ξ-diol 3-acetate (Acetate A), (800 mg.).

Reduction of 11-oxours-12-ene-3β:9ξ-diol 3-acetate with Zinc in Acetic Acid.——

Zinc dust (4.3 g.- activated by warming in a 10% ammonium chloride solution) was added over 1 hour (cf. Barton and Robinson, (64)) to a refluxing solution of 11-oxours-12-ene-3β:9ξ-diol 3-acetate (600 mg.) in acetic acid (50 c.c.). The zinc was removed, and the filtrate diluted with water. The product, isolated through ether, crystallised from chloroform-methanol to give 11-oxours-12-en-3β-yl acetate as plates (520 mg.), m.p. and mixed m.p. 284-285°, $[\alpha]_D$ +98°, (C 1.2); λ max. 2490 Å (ε 12,500).

Action of Methanolic Potassium Hydroxide upon 11-Oxours-12-ene-3β:9ξ-diol 3-acetate.——

A solution of 11-oxours-12-ene-3β:9ξ-diol 3-acetate (3 g.) and potassium hydroxide (12 g.) in methanol (120 c.c.) was heated at 175° for 6 hours. The product isolated through ether, was acetylated using pyridine and acetic anhydride at 100° for 2 hours. The acetylated product, again isolated through ether, crystallised from

chloroform-methanol to yield a substance as needles (900 mg.), m.p. 169-170°, $[\alpha]_D$ -60°, (C, 1.1); λ max. 2080 Å (ε 9,800); infra-red light absorption, 3430 cm.$^{-1}$ (hydrogen-bonded hydroxyl group), 1738 and 1245 cm.$^{-1}$ (acetate group), 1662 cm.$^{-1}$ (hydrogen-bonded six-ring carbonyl group), 1624 cm.$^{-1}$ (double bond), 1035 and 1005 cm.$^{-1}$ (C - O stretch, hydroxyl group). The substance gives a pale-yellow colour with tetranitromethane. Analysis. Found: C, 76.64; H, 10.36. $C_{32}H_{50}O_4$ requires C, 77.06; H, 10.11%.

Concentration of the mother liquor gave 11-oxours-12-en-3β-yl acetate as plates (1.4 g.), m.p. and mixed m.p. 285°, $[\alpha]_D$ +98°, (C, 0.9), λ max. 2480 Å (ε 12,250), it gives no colour with tetra-nitromethane.

Ruzicka, Jeger, Redel and Volli, (51) obtained a substance m.p. 190-191°, $[\alpha]_D$ -20°, λ max. 3150 Å (log.ε 1.45).

The substance, above, gave uncrystallisable gums on treatment with hydrochloric acid in acetic acid. Reduction using zinc dust in acetic acid gave the same result.

Attempted Enol Acetylation of 11-Oxours-12-ene-3β:9α-diol 3-Acetate.

A solution of the diol-monoacetate (5 g.) p-toluene sulphonic acid (2.5 g.), concentrated sulphuric acid (0.25 c.c.) in acetic anhydride (300 c.c.) was refluxed for 100 hours. Potassium acetate was added, and the mixture evaporated to dryness. The residue, in light petroleum solution, was chromatographed on a column of alumina (150 g.). Elution of the column with benzene gave the only crystalline material, recrystallised from chloroform-

methanol to give 11-oxours-12-en-3β-yl acetate as plates (1.7 g.) m.p. and mixed m.p. 286-288°, $[\alpha]_D$ +99° (C, 0.9); λ max. 2480 Å (ε 12,000).

Reduction of 13:18ξ-Epoxy-12-oxours-9(11)-en-3β-yl Acetate with Zinc Dust in Ethanol.—

A solution of 13:18ξ-epoxy-12-oxours-9(11)-en-3β-yl acetate (500 mg.) in ethanol (150 c.c.) was refluxed with zinc dust (10 g., activated with a hot solution of 10% ammonium chloride) for 5 hours. The mixture was filtered, and the filtrate evaporated to give a yellow solid. The solid crystallised from methanol to give 12-oxoursa-9(11):13(18)-dien-3β-yl acetate as yellow plates (340 mg.) m.p. 202-204°, $[\alpha]_D$ -40°, (C, 0.8); λ max. 2080, 2630, 2950 Å (ε 7,900; 9,100; 7,300).

Oxidation of 12-Oxoursa-9(11):13(18)-dien-3β-yl Acetate.—

To a solution of 12-oxoursa-9(11):13(18)-dien-3β-yl acetate (75 mg.) in stabilised acetic acid (15 c.c.) was added a solution of chromic trioxide (19 mg.) in stabilised acetic acid (4 c.c.) at room temperature. The mixture was allowed to stand at room temperature for 18 hours, then heated at 100° for 1 hour. The product, isolated through ether, was dissolved in light petroleum and chromatographed on a column of alumina (3 g.). Elution with benzene gave a product crystallising from methanol to give 13:18ξ-epoxy-12-oxours-9(11)-en-3β-yl acetate at plates (35 mg.), m.p. and mixed m.p. 266-269°, $[\alpha]_D$ +69.5° (C, 0.7); λ max. 2580 Å (ε 11,400).

Mineral Acid Treatment of 13:18ξ-Epoxy-12-oxours-9(11)-en-3β-yl Acetate.—

A solution of 13:18ξ-epoxy-12-oxours-9(11)-en-3β-yl acetate (250 mg.)

in acetic acid (30 c.c.) and concentrated hydrochloric acid (1 c.c.) was heated at 100° for 24 hours. The solvent was removed under reduced pressure and the residue after three crystallisations from methanol gave unchanged 13:18ξ-epoxy-12-oxours-9(11)-en-3β-yl acetate as plates (150 mg.), m.p. 267-270°, $[\alpha]_D$ +74° (C, 1.4), λ max. 2560 Å (ε 12, 200).

Reduction of 13:18ξ-Epoxy-12-oxours-9(11)-en-3β-yl Acetate with Zinc Dust in Acetic Acid.—

To a refluxing solution of 13:18ξ-epoxy-12-oxours-9(11)-en-3β-yl acetate (520 mg.) in Analar acetic acid (50 c.c.) was added zinc dust (4.3 g.) over 1 hour. The zinc was removed, and the filtrate diluted with water and extracted with ether. The residue obtained crystallised from methanol as plates m.p. 208°, $[\alpha]_D$ +23.7°, (C, 1.2), it gave a yellow colour with tetranitromethane; λ max. 2080, 2530, 2940 Å (ε 8,500; 7,100; 4,500). The residue was dissolved in light petroleum (75 c.c.) and chromatographed on a column of alumina (10 g.). Elution of the column with light petroleum gave a product crystallising from methanol to give ursa-9(11):13(18)-dien-3β-yl acetate as plates (70 mg.), m.p. 192-194°, $[\alpha]_D$ +74° (C, 1.0); λmax. 2120 Å (ε, 11,000). The compound gives a deep-yellow colour with tetranitromethane. Elution of the column with light petroleum/benzene (1:2) gave a product crystallising from methanol to give 12-oxoursa-9(11):13(18)-dien-3β-yl acetate as yellow plates, (30 mg.), m.p. 202-204°, $[\alpha]_D$ -44°(C, 1.1); λ max. 2060,

2600, 2950 Å (ε 8,300; 8,800; 6,900). Analysis. Found: C, 79.8; H, 10.0. Calc. for $C_{32}H_{48}O_3$: C, 79.95; H, 10.1%.

Ozonolysis of Ursa-9(11):13(18)-dien-3β-yl Acetate.——

A solution of ursa-9(11):13(18)-dien-3β-yl acetate (150 mg.) in chloroform was cooled (ether - carbon dioxide bath), and a stream of ozone passed through for 2 hours. The chloroform solution was washed with water, dried (Na_2SO_4), and evaporated to yield a yellow gum crystallising from methanol to give 12-oxoursa-9(11):13(18)-dien-3β-yl acetate as yellow plates (135 mg.), m.p. 201-203°, $[\alpha]_D$ -40° (C, 0.5); $\lambda_{max.}$ 2070; 2640, 2940 Å (ε 9,300; 9,500; 7,500).

Chromous Chloride.——

cf. Conant and Cutter, (65); Cole and Julien, (66); and Organic Reactions, Vol VII, p. 163.

Zinc dust (100 g.) was activated to the mossy form by washing three times with a hot hydrochloric acid solution (2 N). The mossy zinc, mercuric chloride (10 g.), concentrated hydrochloric acid (5 c.c.) and water (150 c.c.) were stirred together for 5 minutes to amalgamate the zinc. The aqueous solution was decanted, and the zinc amalgam covered with water (75 c.c.) and concentrated hydrochloric acid (100 c.c.). Chromic chloride hexahydrate (40 g.) was added to the zinc amalgam. The green solution soon turned to the reduced blue chromous state. The solution was left for 4 hours to complete reduction. The chromous chloride solution was stored over zinc amalgam.

Reduction of 13:18-Epoxy-12-oxours-9(11)-en-3β-yl Acetate with Chromous Chloride.——

A solution of 13:18-epoxy-12-oxours-9(11)-en-3β-yl acetate (100 mg.) in stabilised acetic acid (25 c.c.) was covered with carbon dioxide gas, and a solution of chromous chloride (1 g. in 5 c.c. as from previous experiment) added. The solution was allowed to stand at room temperature for 30 minutes, then poured into water. The product, isolated through ether, crystallised from methanol to give 12-oxoursa-9(11):13(18)-dien-3β-yl acetate as yellow plates, (90 mg.) m.p. and mixed m.p. 201 -203°, $[\alpha]_D$ -41° (C, 1.2); λ max. 2080, 2620, 2940 Å (ε 8,000; 8,900; 7,300).

Oxidation of 12-Oxours-9(11)-en-3β-yl Acetate (Acetate D).——

A solution of chromic trioxide (1.5 g.) in acetic acid (30 c.c.) was added, over 30 minutes, to a refluxing solution of 12-oxours-9(11)-en-3β-yl acetate (1.5 g.) in acetic acid (200 c.c.). The mixture was refluxed for a further 1 hour, diluted with water, and extracted with ether. The product crystallised from chloroform-methanol to give 12:15-dioxophyllanth-9(11)-en-3β-yl acetate (Acetate C) as needles (1.1 g.), m.p. and mixed m.p. 321-324°, $[\alpha]_D$ +103° (C, 1.0); λ max. 2360 Å (ε 10,800).

Oxidation of 12-Oxoisoursa-9(11):14-dien-3β-yl Acetate.——

a) A solution of 12-oxoisoursa-9(11):14-dien-3β-yl acetate (350 mg.) in acetic acid (100 c.c.) was treated at room temperature for 16 hrs. with a solution of chromic trioxide (100 mg.) in acetic acid (90%, 2 c.c.). Isolation of the product through ether gave a solid

which crystallised from chloroform- methanol to give 12-oxophyllanth-9(11)-en-3β-yl acetate as needles, (300 mg.), m.p. and mixed m.p. 268-272°, $[\alpha]_D$ +157° (C, 2.0), $\lambda_{max.}$ 2360 Å (ε 9,200), it gives no colour with tetranitromethane.

with Dr. G.G. Allan.

b) A solution of 12-oxoisoursa-9(11):14-dien-3β-yl acetate (350 mg.) in acetic acid (100 c.c.) was treated at room temperature with a solution of chromic trioxide (350 mg.) in acetic acid (90%, 7 c.c.) for 16 hours, then heated at 100° for 3 hours. The product, isolated through ether, crystallised from chloroform-methanol to give 12:15-dioxophyllanth-9(11)-en-3β-yl acetate as needles (250 mg.) m.p. and mixed m.p. 320-323°, $[\alpha]_D$ +103° (C, 1.8).

Oxidation of 12-Oxophyllanth-9(11)-en-3β-yl Acetate.--(with Dr. G.G.Allan

To a refluxing solution of 12-oxophyllanth-9(11)-en-3β-yl acetate (480 mg.) in acetic acid (75 c.c.) was added a solution of chromic trioxide (480 mg.) in acetic acid (90%, 40 c.c.) over 20 minutes. Refluxing was continued for 1 hour, then the product, isolated through ether, crystallised from chloroform-methanol to give 12:15-dioxophyllanth-9(11)-en-3β-yl acetate as needles, (300 mg.), m.p. and mixed m.p. 320-322°, $[\alpha]_D$ +100°, (C, 0.6).

Action of Selenium Dioxide on Ursa-9(11):12-dien-3β-yl Acetate.

A solution of ursa-9(11):12-dien-3β-yl acetate (1 g.) in acetic acid (200 c.c.) was refluxed with a solution of selenium dioxide (1 g.) in water (1 c.c.) for 2 hours. The selenium was removed

the filtrate evaporated to dryness, and the product crystallised from methanol to give unchanged ursa-9(11):12-dien-3β-yl acetate as needles m.p. and mixed m.p. 165-166°, $[\alpha]_D$ +334° (C, 0.91); λ max. 2800 Å (ε 8,500).

Reduction of 13:18ξ-Epoxy-12-oxours-9(11)-en-3β-yl Acetate with Lithium Aluminium Hydride.——

Lithium aluminium hydride (500 mg.) was added to a solution of 13:18ξ-epoxy-12-oxours-9(11)-en-3β-yl acetate (550 mg.) in dry ether (100 c.c.). The mixture was allowed to stand at room temperature for 20 hours. Water was added, and the product, isolated through ether, crystallised from chloroform-methanol to give 13:18ξ-epoxyurs-9(11)-ene-3β:12ξ-diol as plates, (420 mg.) m.p. 250-253° $[\alpha]_D$ +83°, (C, 1.4) λ max. 2080 Å (ε 4,200). The compound gives a light-yellow colour with tetranitromethane. Analysis. Found: C, 78.84; H, 10.61. $C_{30}H_{48}O_3$ requires C, 78.89; H, 10.59%.

13:18ξ-Epoxyurs-9(11)-ene-3β:12ξ-diol 3-acetate.——

A solution of 13:18ξ-epoxyurs-9(11)-ene-3β:12ξ-diol (125 mg.) in pyridine (8 c.c.) and acetic anhydride (4 c.c.) stood at room temperature for 46 hours. The product isolated by means of ether, crystallised from methanol to give 13:18ξ-epoxyurs-9(11)-ene-3β:12ξ-diol 3-acetate as blades, (103 mg.), m.p. 276-279°, $[\alpha]_D$ +93° (C, 0.8); λ max. 2060 Å (ε 5,000). Infra-red spectrum shows a band at 3440 cm.$^{-1}$ (nujol), 3455 cm.$^{-1}$ ($CHCl_3$) —internally-bonded hydroxyl group. The compound gives a light yellow colour

with tetranitromethane. Analysis. Found: C, 76.6; 77.6; 76.6. H, 10.2; 10,3; 10.2. $C_{32}H_{50}O_4$ requires C, 77.1; H, 10.1%.

Oxidation of 13:18ξ-Epoxyurs-9(11)-ene-3β:12ξ-diol 3-acetate.—

A solution of 13:18ξ-epoxyurs-9(11)-ene-3β:12ξ-diol 3-acetate (100 mg.) in dry pyridine (10 c.c.) was added to chromic trioxide-pyridine complex (100 mg. CrO_3 in 10 c.c. dry pyridine) and the mixture allowed to stand at room temperature for 20 hours. The mixture was filtered through kieselguhr, and the filtrate poured into water and extracted with ether. The ether extract was washed with dilute hydrochloric acid solution, and water, dried (Na_2SO_4), and evaporated to dryness. The residue crystallised from chloroform-methanol to give 13:18ξ-epoxy-12-oxours-9(11)-en-3β-yl acetate as plates, (75 mg.) m.p. and mixed m.p. 266-269°, $[\alpha]_D$ +71°, (C, 1.4); λmax. 2580 Å (ξ 10,800).

Action of Mineral Acid on 13:18ξ-Epoxyurs-9(11)-ene-3β:12ξ-diol 3-acetate.—

A solution of 13:18ξ-epoxyurs-9(11)-ene-3β:12ξ-diol 3-acetate (1.4 g.) in acetic acid (180 c.c.) and concentrated hydrochloric acid (3 c.c.) stood at room temperature for 16 hours. Water was added, and the product was isolated through ether. A solution of the product in light petroleum was chromatographed on a column of alumina (40 g.). Elution of the column with light petroleum/benzene (4:1) to benzene gave material crystallising from chloroform-methanol to give a substance as prisms, (975 mg.), m.p. 167-170°, $[\alpha]_D$ +139°, (C, 0.85) light absorption λ max. 2040 Å

($\leq$ 4,600). Infra-red spectrum shows a very strong six-ring carbonyl at 1703 cm.$^{-1}$ (nuj ol) and 1708 cm.$^{-1}$ ($CHCl_3$). The compound gives a light-yellow colour with tetranitromethane in chloroform solution. Analysis. Found: C, 76.9; 77.4; 76.7. H, 10.2; 10.2; 10.1. $C_{32}H_{50}O_4$ requires, C, 77.1; H, 10.1%.

The substance was recovered unchanged after treatment with pyridine-acetic anhydride at 100° for 2 hours. The substance was also recovered unchanged after attempted reduction with zinc in ethanol or acetic acid, or chromous chloride (specific for epoxy-α-ketones).

Reduction of the substance with lithium aluminium hydride, followed by acetylation, gave an intractable gum.

Reduction of 12-Oxoursa-9(11):13(18)-dien-3β-yl Acetate with Lithium Aluminium Hydride.—

A solution of 12-oxoursa-9(11):13(18)-dien-3β-yl acetate (100 mg.) in dry ether (50 c.c.) was cooled to 0°, and lithium aluminium hydride (115 mg.) added. The mixture was kept at 0° for 72 hours. Water was added and the product isolated by means of ether. The product crystallised from aqueous methanol (1:1) to give ursa-9(11):13(18)-diene-3β:12β-diol as needles, (70 mg.), m.p. 168-169°, $[\alpha]_D$ +107°, (C, 0.8); λmax. 20 60 Å ($\leq$ 12,600). The compound gives a yellow colour with tetranitromethane. Analysis. Found: C, 81.9; H, 11.0. $C_{30}H_{48}O_3$ requires C, 81.8; H, 11.0%.

Acetylation of Ursa-9(11):13(18)-diene-3β:12ξ-diol.

A solution of ursa-9(11):13(18)-diene-3β:12ξ-diol(1.8 g.) in pyridine (20 c.c.) and acetic anhydride (20 c.c.) stood at room temperature for 48 hours. Water was added, and the product was isolated by means of ether. The product (1.7 g.) in a light petroleum solution was chromatographed on a column of alumina (45 g.). Elution with light petroleum/benzene (1:3) gave a compound crystallising from chloroform-methanol to give ursa-9(11):12:18-trien-3β-yl acetate as prismatic plates, (250 mg.), m.p.184-185°, $[\alpha]_D$ +784° (C, 1.4); + 806° (C, 0.9); λmax. 3060 Å (ε 14,000), it gives a dark red-brown colour with tetranitromethane. Analysis. Found: C, 82.5; H, 10.4. $C_{32}H_{48}O_2$ requires C, 82.7; H, 10.4%.

Elution of the column with benzene/ether (1:1) gave a compound crystallising from chloroform-methanol to give ursa-9(11):13(18)-diene-3β:12ξ-diol 3-acetate as long plates, (400 mg.), m.p. 190-193°. $[\alpha]_D$ + 136° (c, 1.1); λmax. 2140 Å (ε 9,000), it gives a yellow colour with tetranitromethane. Analysis. Found: C, 79.3; H, 10.3. $C_{32}H_{50}O_3$ requires C, 79.6; H, 10.4 %.

Acetic Anhydride Dehydration of Ursa-9(11):13(18)-diene-3β:12ξ-diol 3-acetate.

A solution of ursa-9(11):13(18)-diene-3β:12ξ-diol 3-acetate (80 mg.) in acetic anhydride (8 c.c.) was heated at 100 ° for 2 hours. The solution was cooled and the crystalline solid collected and washed with methanol. The product on recrystallisation from methanol gave

ursa-9(11):12:18-trien-3β-yl acetate as prisms, (60 mg.), m.p. and mixed m.p. 183-184°, $[\alpha]_D$ +792° (C, 1.1); λ max. 3060 Å (ε 13,500).

Hydrogenation of Ursa-9(11):12:18-trien-3β-yl Acetate.—

A solution of ursa-9(11):12:18-trien-3β-yl acetate (85 mg.) in glacial acetic acid (85 c.c.) was shaken with platinum catalyst (from 85 mg. PtO_2) over hydrogen at atmospheric pressure for 24 hours. The platinum was removed, and the filtrate evaporated to dryness. The residue crystallised from methanol to give a substance as blades, (75 mg.), m.p. 231-233°, $[\alpha]_D$ +34° (C 0.9); λ max. 2100 Å (ε 9,000), it gives an orange-brown colour with tetranitromethane.

Analysis. Found: C, 82,31; H, 10.73. $C_{32}H_{50}O_2$ requires C, 82.34; H, 10.80%.

The substance was recovered unchanged after treatment with hydrochloric - acetic acid.

Reduction of Ursa-9(11):12:18-trien-3β-yl Acetate with Lithium in Liquid Ammonia.—

A solution of ursa-9(11):12:18-trien-3β-yl acetate (400 mg.) in dry ether (50 c.c.) was stirred with liquid ammonia (200 c.c.). Lithium metal was added over 15 minutes, and the reaction allowed to continue for a further 30 minutes, with continuous stirring. Acetone was added, and the ammonia was allowed to evaporate. The residue was extracted with ether, and the ether extract washed with dilute hydrochloric acid and water, dried (Na_2SO_4), and evaporated to dryness. The residue (λ max. 2140 Å (ε11,600) was

acetylated using pyridine and acetic anhydride for 2 hours at 100°. The product, isolated by means of ether, crystallised from chloroform-methanol to give ursa-9(11):13(18)-dien-3β-yl acetate as plates (310 mg.), m.p. and mixed m.p. 192-193°, $[\alpha]_D$ +72.4° (C, 0.97); $\lambda_{max.}$ 2100 Å (ε 12,300), it gives a strong yellow colour with tetranitromethane.

Attempted Enol Acetylation of 12-Oxoursa-9(11):13(18)-dien-3β-yl Acetate.—

A solution of 12-oxoursa-9(11):13(18)-dien-3β-yl acetate (200 mg.) and freshly fused sodium acetate (350 mg.) in acetic anhydride (40 c.c.) was refluxed for 66 hours. Water was added, and the product isolated by means of ether, crystallised from methanol to give unchanged 12-oxoursa-9(11):13(18)-dien-3β-yl acetate as yellow plates (160 mg.), m.p. and mixed m.p. 201 -203°, $[\alpha]_D$ -41° (C, 1.6); $\lambda_{max.}$ 2040, 2620, 2940 Å (ε 8,500; 9,700; 8,300).

Enol Acetylation of 12-Oxoursa-9(11):13(18)-dien-3β-yl Acetate.—

A solution of 12-oxoursa-9(11):13(18)-dien-3β-yl acetate (500 mg.) p-toluene sulphonic acid (250 mg.), concentrated sulphuric acid (one drop) in acetic anhydride (80 c.c.) was refluxed for 100 hours. Water was added, and the product was isolated by means of ether. A solution of the product (a black gum) in light petroleum was filtered through a column of alumina (30 g.). A colourless gum was obtained which crystallised from methanol to give 3-β:12-diacetoxyursa-9(11):12:18-triene as long needles, (195 mg.), m.p.

242-243°, $[\alpha]_D$ +478°, (C, 1.5); λ max. 2940 Å (ε 13,000), it gives a brown colour with tetranitromethane. Analysis. Found: C, 77.9; H, 9.8. $C_{34}H_{50}O_4$ requires C, 78;1; H, 9.6%. This experiment was repeated in order to improve the yield.

i. 12-Oxoursa-9(11):13(18)-dien-3β-yl acetate (250 mg.), p-toluene sulphonic acid (125 mg.), acetic anhydride (40 c.c.) refluxed for 24 hours. Yield, 175 mg. - 70%.

ii. 12-Oxoursa-9(11):13(18)-dien-3β-yl acetate (250 mg.), p-toluene sulphonic acid (100 mg.), concentrated sulphuric acid (one drop) acetic anhydride (40 c.c.), heated at 100° for 24 hours. Yield, 195 mg. - 80%.

iii. 12-Oxoursa-9(11):13(18)-dien-3β-yl acetate (250 mg.), p-toluene sulphonic acid (125 mg.), acetic anhydride (40 c.c.), heated at 100° for 24 hours. Yield, 130 mg. - 50%.

In all these cases the reaction solution was poured into a mixture of pyridine and water. This mixture decomposes the acetic anhydride much more rapidly than water. The products were isolated by means of ether, and filtered through alumina. An alternative working up process was to add potassium acetate, and evaporate reaction solution to dryness, followed by filtration of a solution of the residue in light petroleum through alumina. Employing this method and condition ii above, a yield of 80% was obtained i.e. 5.55 g. enol acetate was obtained from 6.5 g. 12-oxoursa-9(11):13(18)-dien-3β-yl acetate.

Acid Hydrolysis of 3β:12-Diacetoxyursa-9(11):12:18-triene.——

A solution of 3β:12-diacetoxyursa-9(11):12:18-triene (165 mg.) in methanol (150 c.c.) and concentrated hydrochloric acid (9c.c.)was refluxed for 2 hours. Water was added, and the product, isolated through ether, crystallised from aqueous methanol to give 12-oxoursa-9(11):13(18)-dien-3β-ol as faint yellow blades (120 mg.) m.p. 220-222°, $[\alpha]_D$ -70° (C, 1.1); $\lambda_{max.}$ 2060, 2630, 2940 Å (ε 9,300; 9,500; 7,500). Acetylation gave 12-oxoursa-9(11):13(18)-dien-3β-yl acetate, m.p. 200-202°, $[\alpha]_D$ -40° (C, 1.3); $\lambda_{max.}$ 2060, 2620, 2950 Å. (ε 9,600; 9,500; 7,900).

Alkaline Hydrolysis of 3β:12-Diacetoxyursa-9(11):12:18-triene.——

A solution of 3β:12-diacetoxyursa-9(11):12:18-triene (2.2 g.) in methanolic potassium hydroxide (3% - 800 c.c.) was refluxed for 2½ hours. Water was added, and the solid extracted with ether. The ether extract was washed with water, dried (Na_2SO_4) and evaporated to dryness. The residue was acetylated using pyridine and acetic anhydride for 16 hours at 18°. The product isolated by means of ether crystallised from chloroform- methanol to give as a first crop 12-oxoursa-9(11):18-dien-3β-yl acetate as plates (400 mg.) m.p. 264-265°, $[\alpha]_D$ +122°(C, 1.0); $\lambda_{max.}$ 2040, 2500 Å, (ε 8,900; 9,300), it gives a yellow colour with tetranitromethane. Analysis. Found: C, 80.17; H, 10.18. $C_{32}H_{48}O_3$ requires C, 79.95; H, 10.07%.

The mother liquors were evaporated to dryness, and a solution of

the residue in benzene was filtered through alumina. The product crystallised from aqueous acetone to give 19α-(isopropyl)-12-oxoursa-9(11):13(18)-dien-3β-yl acetate as needles, (950 mg.), m.p. 193-195° $[\alpha]_D$ +6°, (C, 0.9); λmax. 2050, 2620, 2940 Å (ε 10,300; 9,300; 8,700). Analysis. Found: C, 79.5; 79.45; H, 10.2; 10.2. $C_{32}H_{48}O_3$ requires C, 79.95; H, 10.1%.

The more this latter compound was recrystallised the less pure it seemed to become, variations in m.p. and rotation becoming marked.

Acid Hydrolysis of 12-Oxoursa-9(11):18-dien-3β-yl Acetate.——

A solution of 12-oxoursa-9(11):18-dien-3β-yl acetate (70 mg.) in methanol (50 c.c.) and concentrated hydrochloric acid (3 c.c.) was refluxed for 2 hours, then evaporated to dryness under reduced pressure. The residue crystallised from aqueous methanol to give 12-oxoursa-9(11):13(18)-dien-3β-ol as pale yellow blades, (50 mg.), m.p. 220-222°, $[\alpha]_D$ -70° (C, 1.2); λmax. 2050, 2620, 2940 Å, (ε 10,500; 10,700; 8,300). Acetylation gave 12-oxoursa-9(11):13(18)-dien-3β-yl acetate m.p. 201-203°, $[\alpha]_D$ -41°, (C, 0.73); λmax. 2040, 2630, 2940 Å (ε 9,100; 9,400; 8,100).

Monoperphthalic Acid Oxidation of 12-Oxoursa-9(11):18-dien-3β-yl Acetate.——

A solution of 12-oxoursa-9(11):18-dien-3β-yl acetate (500 mg.) in dry ether (250 c.c.) was treated with an ethereal solution of monoperphthalic acid (285 mg. - 1.5 molar) for 10 days at 20°. The ethereal solution was washed with saturated sodium bicarbonate

solution, water, dried (Na_2SO_4) and evaporated to dryness. The residue crystallised from methanol to give 18:19ξ-epoxy-12-oxours-9(11)-en-3β-yl acetate as blades, (380 mg.), m.p. 240-241°, $[\alpha]_D$ +117°, (C, 0.96); λ max. 2480 Å (≤ 10,000), it gives no colour with tetranitromethane. Analysis. Found: C, 77.9; H, 9.7. $C_{32}H_{48}O_4$ requires C, 77.4; H, 9.7%

Reduction of 12-Oxoursa-9(11):18-dien-3β-yl Acetate with Lithium in Liquid Ammonia.—

To a stirred solution of lithium metal (150 mg.) in liquid ammonia (100 c.c.) was added over 1 minute a solution of 12-oxoursa-9(11):18-dien-3β-yl acetate (245 mg.) in dry ether (50 c.c.). The reaction continued for 2 minutes, then acetone was added. The ammonia was allowed to evaporate and the product, isolated through ether, acetylated using pyridine and acetic anhydride at 100° for 2 hours. The product, isolated through ether, crystallised from methanol to give 12-oxours-18-en-3β-yl acetate as blades, (50 mg.), m.p. 234-237°, $[\alpha]_D$ +60°, (C, 1.1); λ max. 2040 Å (ε 8,900), it gives a yellow colour with tetranitromethane. Analysis. Found: C, 79.8; H, 10.6. $C_{32}H_{50}O_3$ requires C, 79.6; H, 10.4%. With alkali (3% and 15% methanolic potassium hydroxide), 12-oxours-18-en-3β-yl acetate gave intractable gums.

Attempted Hydrogenolysis of 12-Oxoursa-9(11):18-dien-3β-yl Acetate.—

A solution of 12-oxoursa-9(11):18-dien-3β-yl acetate (150 mg.) in acetic acid (45 c.c.) was shaken with platinum catalyst (from 100 mg. PtO_2) over hydrogen at atmospheric pressure for

18 hours. The catalyst was removed, and the filtrate evaporated to dryness yielding the product crystallising from methanol as unchanged 12-oxoursa-9(11):18-dien-3β-yl acetate as blades, (135 mg.), m.p. and mixed m.p. 263-265°, $[\alpha]_D$ +122° (C, 1.0).

Reduction of 3β:12-Diacetoxyursa-9(11):12:18-triene with Lithium in Liquid Ammonia.

A solution of 3β:12-diacetoxyursa-9(11):12:18-triene (500 mg.) in dry ether (100 c.c.) was dropped into a solution of lithium metal (150 mg.) in liquid ammonia (100 c.c.) over a period of 1 minute. The reaction continued for a further 2 minutes then acetone was added. The ammonia was allowed to evaporate, and the product, isolated through ether, was refluxed for 3 hours in a solution of methanolic potassium hydroxide (3% - 150 c.c.). The product, isolated through ether, was acetylated using pyridine and acetic anhydride at 18° for 16 hours. The product, isolated by means of ether, was dissolved in light petroleum and chromatographed on a column of alumina. Light petroleum/benzene eluted the only crystalline material, which on recrystallisation from chloroform-methanol gave the product as blades, (190 mg.), m.p. 248-249°, $[\alpha]_D$ +159° (C, 1.0); λ max. 2620 Å (ε 19,500), it gives a red-brown colour with tetranitromethane. Analysis. Found: C, 80.24 C, 80.98; H, 10.00; 10.07. $C_{34}H_{50}O_3$ requires C, 80.58; H, 9.95%.

Oxidation of 3β:12-Diacetoxyursa-9(11):12:18-triene.—

A solution of 3β:12-diacetoxyursa-9(11):12:18-triene (1.41g.) in stabilised acetic acid (200 c.c.) was gently boiled to effect slow distillation of the solvent. A solution of chromic trioxide (4.2 g., 15 molar excess) in stabilised acetic acid (80%, 50 c.c.) was added dropwise. The distillate was collected in fractions of approximately 25 c.c.. The fractions were neutralized with sodium hydroxide solution to phenolphthalein and the resulting neutral fractions distilled. Distillates of about 10 c.c. were collected and treated with a hydrochloric acid solution of 2:4-dinitrophenylhydrazine. The fractions which gave a positive carbonyl reaction were filtered and the products recrystallised from chloroform-methanol to give acetone 2:4-dinitrophenylhydrazone (105 mg.) as needles, m.p. and mixed m.p. 123-125°.

The residue from distillation was poured into water and extracted with ether. No insoluble acid fraction was obtained. The neutral fraction (a brown gum) was chromatographed on a column of alumina, but failed to produce any crystalline material.

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