STUDIES IN THE β-AMYRIN GROUP OF TRITERPENES.

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T H E S I S

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DEGREE OF DOCTOR OF PHILOSOPHY

by

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CONTENTS.

HISTORICAL.

INTRODUCTION.

Two classes of non-nitrogenous substances, containing approx--imately thirty carbon atoms, are to be found in the vast field of naturally occurring compounds. The first class contains certain sapogenins and the sterols, whose tetracyclic structure has been examined in great detail. The second class contains compounds of the polycyclic triterpenoid type which are widely distributed in the vegetable kingdom, especially in resins and plant saps, where they occur in the free state, as glycosides (saponins), and as esters. The triterpenes yield characteristic common dehydrogenation products, most of them napthalene homologues, which have furnished useful evidence concerning the basic carbon skeletons. The triterpenes, although distinct from, are related to the mono-, sesqui-, and diterpenes in that all apparently contain structures built up from isoprene, C_5H_8.

Structurally, the triterpenes may be divided into the following groups:-

(a) The straight chain compound squalene, and the tricyclic ambrein.

(b) The tetracyclic compounds such as euphol, lanosterol, agnosterol, and the elemic acids.

(c) The pentacyclic α - and β - amyrin and lupeol groups.

No mono-, or dicyclic triterpenes are known and only recently has the occurrence of a hexacyclic triterpene been reported(1). As yet no certain method is available for the rapid allocation of any new

member to a specific group, though application of the method of molecular rotation differences helps in the assignment of a compound to its correct class (2,3).

During the past 20 years, rigorous relationships have been established between a large majority of the well characterised pentacyclic triterpenes and one or other of the three parent alcohols α-amyrin, β-amyrin, and lupeol, and recently lupeol and β-amyrin have been converted into common intermediates by reactions capable of structural interpretation(4). So far no interconversion of the α - and β - amyrins has been achieved.

The work to be described in this thesis was concerned with the structural chemistry of the β-amyrin or "β-amyrin - oleanolic acid" group of triterpenes and therefore special emphasis will be laid upon the developments in this group. Comprehensive summaries on triterpene chemistry as a whole have been made by Haworth(5), Spring(6), Noller(7) and Jeger(8).

Early work on individual triterpenes was beset by a number of practical difficulties, since purification of certain compounds proved difficult molecular weight determinations were inconclusive, and methods of analyses used often failed to distinguish between homologous formulae. With improved analytical techniques of micro-combustion and micro-titration etc., it was established that these compounds contained thirty carbon atoms.

DETECTION OF FUNCTIONAL GROUPS.

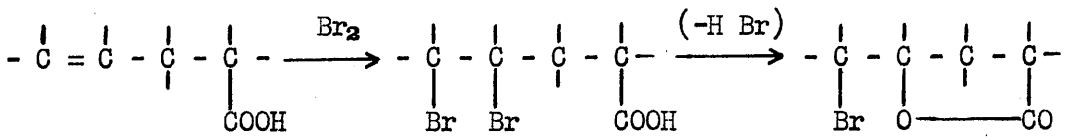
Owing to the complexity of the triterpene molecule and, in some instances, to the location of certain functional groups within that molecule, their reactivity is so reduced as to make their detection by standard methods impossible.

Carbonyl groups in the triterpenes are in general very unreactive and do not react with the usual reagents such as semicarbazide, phenyl--hydrazine, hydroxylamine etc., (9,10,11,12,13,14). Such groupings are best detected by spectroscopic methods in U.V. and I.R. regions.

The carboxyl group in some of the triterpene acids such as oleanolic acid and ursolic acid cannot be esterified by the Fischer-Spier method and esterification necessitates the use of diazomethane. The methyl esters so prepared are hydrolysed only on drastic treatment with alkali. This behaviour indicated the presence of a tertiary carboxyl group in these substances.

Inert ethylenic bonds are commonly met with in triterpene structures. Such linkages can be detected by a number of methods however, the simplest of which is the tetranitromethane colour test; the colour produced by a triterpene when a chloroform solution is treated with tetranitromethane is indicative of the number of double bonds present in the molecule and/or the extent of conjugation(15). The test fails in the case of $\alpha\beta$- unsaturated ketones which give no coloration (13,14,15).

Triterpene acids on treatment with bromine give bronolactones(16), from which the free acids are regenerated by zinc and glacial acetic acid. This indicated the presence of a double bond in such compounds as oleanolic acid and hederagenin, and that the double bond was probably $\beta\gamma$ or $\gamma\delta$ with respect to the carboxyl group. The probable mechanism of this reaction is indicated by the partial formulae shown below



Recently, use has been made of light absorption intensities in the region 2050-2250Å for investigation of isolated double bonds in the triterpene field of chemistry(17). An inspection of the ϵ values at various wavelengths within this region shows that they depend primarily upon the degree of substitution of the double bond and distinction can be made between di-, tri-, and tetra-substituted ethylenic linkages. The light absorption in this region is unreliable, however, when any other chromophore is present in the molecule.

Evidence for the presence of one double bond in lupeol and betulin was obtained by catalytic hydrogenation to dihydrolupeol(15) and dihydrobetulin(18) respectively, one mole of hydrogen being absorbed in each case. Subsequent investigations showed that the double bond in lupeol was contained in an isopropenyl side chain(19,20) this being in direct contrast to triterpenes of the α-amyrin and β-amyrin groups which contain nuclear type double bonds and cannot

be hydrogenated under similar conditions. The presence of a double bond in β -amyrin (and related compounds) was shown by the yellow colour produced with the tetranitromethane reagent and by oxidation with hydrogen peroxide(21) or perbenzoic acid(13) to give a saturated ketone, which was further reduced to give β -amyranol, a compound which is saturated to tetranitromethane(22). Chromic acid oxidation of β -amyrin acetate gave an αβ- unsaturated ketone(23), further indicating the presence of a double bond in the molecule. Similar types of experiments proved the ethenoid linkage in α -amyrin and related compounds.

Taken in conjunction with the molecular formulae, i.e., $C_{30}H_{50}O$ for the monohydric alcohols α - and β -amyrin, the presence of one double bond indicated that these substances were pentacyclic. This was confirmed by dehydrogenation experiments which will be discussed later in this section.

The secondary hydroxyl grouping, common to all triterpenes, is readily detected by acetylation, benzoylation, and oxidation to the corresponding ketone.

TRITERPENE INTER-RELATIONSHIPS.

Various inter-relationships have been established within each group, between individual members of the α- and β- amyrin and lupeol groups. Such inter-relationships have been achieved using standard methods of oxidation of primary alcohol groupings to acid groupings, reduction of carbonyl groupings, and by a method first applied by Ruzicka and Schellenberg(25), in the conversion of oleanolic acid into the alcohols, β- amyrin and erythrodiol. The method, illustrated below, consists in conversion of the acetate of the acid to its acid chloride followed by Rosenmund reduction of this to the corresponding aldehyde which is then reduced by the Wolff-Kischner method to give the required alcohol.

$$RCO_2H \longrightarrow RCOCl \longrightarrow RCHO \longrightarrow RCH_3 + RCH_2OH$$

Recently, use has been made of the versatile reagent lithium aluminium hydride which has made available the following route(64,24).

$$RCOCl \xrightarrow{LiAlH_4} RCH_2OH \begin{cases} \xrightarrow{CrO_3} RCHO \xrightarrow{W/K} RCH_3 \\ \xrightarrow[Chloride]{Tosyl} RCH_2OTs \xrightarrow{LiAlH_4} RCH_3 \end{cases}$$

A further variant depends upon the desulphurization of a thioester by Raney nickel to give the corresponding alcohol which is converted as shown (24a)

$$RCOCl \longrightarrow RCOSMe \longrightarrow RCH_2OH \xrightarrow[Chloride]{Tosyl} RCH_2OTs \xrightarrow{NaI} RCH_2I \xrightarrow{Zn} RCH_3.$$

The various transitions within the β -amyrin group are shown in Chart 1. These interconversions show that all the triterpenes involved possess the same basic carbon skeleton and differ only in the nature and position of the functional groups present.

Similar relationships have been shown to exist in the α -amyrin group and lupeol group of which excellent summaries will be found elsewhere(26,27).

The more important members of the three main groups of pentacyclic triterpenes are listed in tables I, II, and III together with the position of the various functional groups in the parent hydrocarbons oleanane(I), ursane(II), and lupane(III).

CHART I.

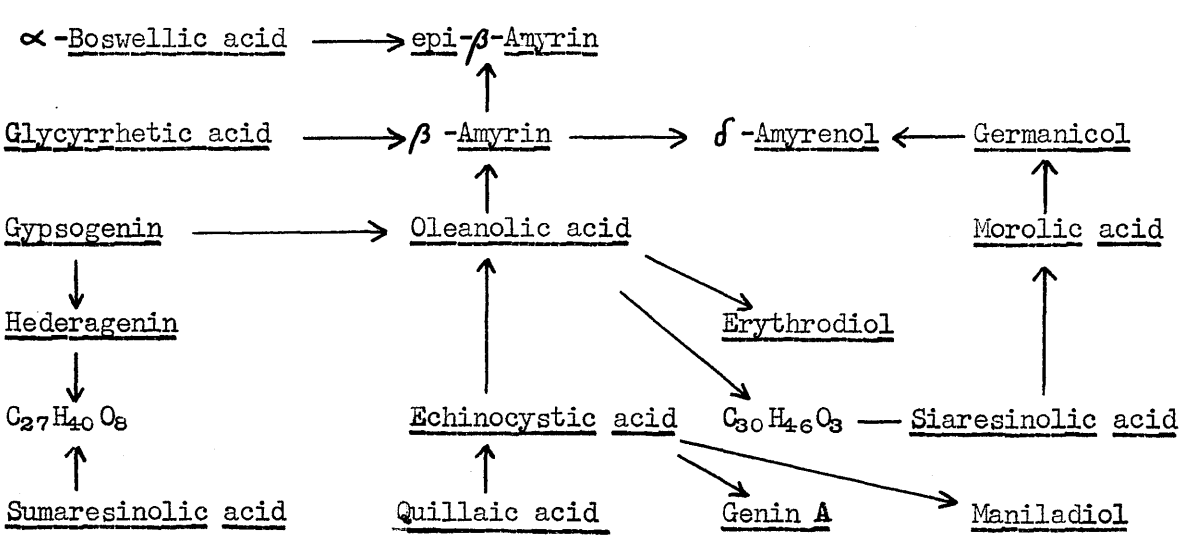
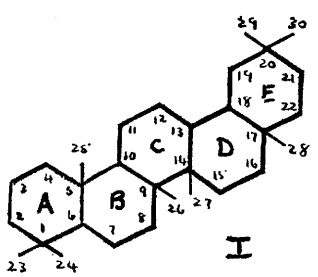


TABLE I.

β-Amyrin group.

Triterpene	Double Bond	Hydroxyl	Oxo	COOH	Occurrence.
β-Amyrin	12:13	2	-	-	Manila Elemi resin.
Erythrodiol	12:13	2.28	-	-	Fruits of Erythróxylon novogranatense.
Maniladiol	12:13	2.16(epi)	-	-	Manila Elemi resin.
Genin A	12:13	2.16.28	-	-	Primula.
α-Boswellic acid	12:13	2(epi)	-	23	Frankincense.
Oleanolic acid	12:13	2	-	28	Cloves and olives.
Hederagenin	12:13	2.23	-	28	Leaves of ivy.
Sumaresinolic acid	12:13	2.7	-	28	Sumatra gum benzoin.
Echinocystic acid	12:13	2.16	-	28	Root of Echinocystis fabacea.
Siaresinolic acid	12:13	2.19	-	28	Siam gum benzoin.
Gypsogenin	12:13	2	23	28	Soap root.
Quillaic acid	12:13	2.16	23	28	Quillaia saponin
Glycyrrhetic acid	12:13	2	11	30	Roots of liquorice.
Germanicol	18:19	2	-	-	Lactuearium germanicum.
Morolic acid	18:19	2	-	28	Heartwood of Mora excelsa Benth.



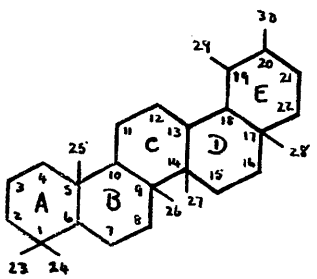
I

This group also contains the soyasapogenols whose structure is still in doubt. Soyasapogenin A is a tetrahydric alcohol, while, soyasapogenin B is a trihydric alcohol and soyasapogenin C is a diene-diol. Each contains a C_2-hydroxyl group and a 12:13 double bond(28).

TABLE II.

α -Amyrin group.

Triterpene	Double Bond	Hydroxyl	COOH	Occurrence.
α -Amyrin	12:13	2	-	Manila Elemi resin.
Uvaol	12:13	2.28(?)	-	Crataegus cuneata.
Brein	12:13	2.21(or 22)	-	Manila Elemi resin.
β -Boswellic acid	12:13	2	23	Frankincense.
Ursolic acid	12:13	2	28(?)	Wax coating of apples and pears.
Quinovic acid	12:13	2	27.28	Zygophyllum coccineum L.

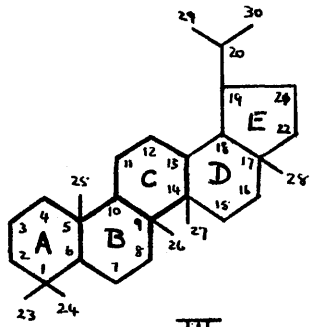


II

TABLE III.

Lupeol group.

Triterpene	Double Bond	Hydroxyl	COOH	Occurrence.
Lupeol	20:29	2	-	Shea nut fat.
Betulin	20:29	2.28	-	Birch bark.
Betulinic acid	20:29	2	28	Bark of Cornus florida L.



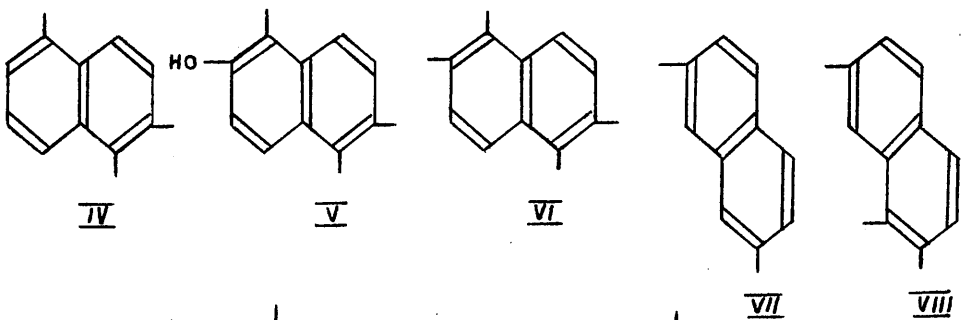
III.

The taraxasterols are closely allied to lupeol but the structure of ring-E is uncertain(29,30).

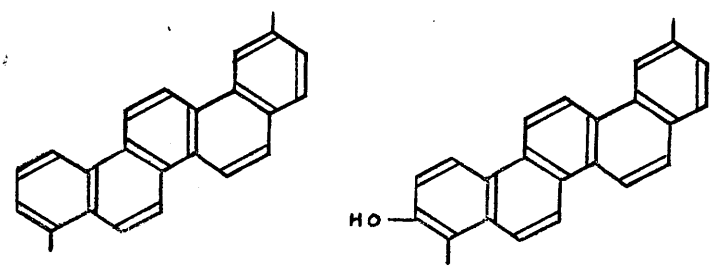
ELUCIDATION OF THE TRITERPENE STRUCTURE.

a) Dehydrogenation evidence.

Dehydrogenation studies over a number of years played a vital part in the elucidation of the structures of the pentacyclic triterpenes. Experiments using selenium, and later palladium, as dehydrogenating agents, led to the isolation of a number of products of which the most important are shown below (IV - X). (31,32,33,34,35,13).



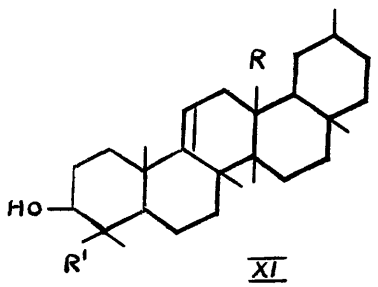
IV V VI VII VIII



IX X

The α- and β-amyrins both gave 1:5:6-trimethylnaphthalene(IV), 2-hydroxy-1:5:6 -trimethylnaphthalene(V), and 1:2:5:6-tetramethylnaphthalene(VI), which are formed from rings A and B of the pentacyclic structures, together with 2:7-dimethylnaphthalene(VII) and 1:2:7-trimethylnaphthalene(VIII) which originate from rings D and E. The isolation of 1:8-dimethylpicene(IX) and 1:8-dimethyl-

2-hydroxy-picene(X) indicated that the parent substances possessed a reduced picene structure, and led Ruzicka, Goldberg and Hofmann(36) to advance formula(XI) for the β-amyrin group of titerpenes . (e.g., R = R = CH_3 for β-amyrenol; R = COOH R = CH_3 for oleanolic acid).

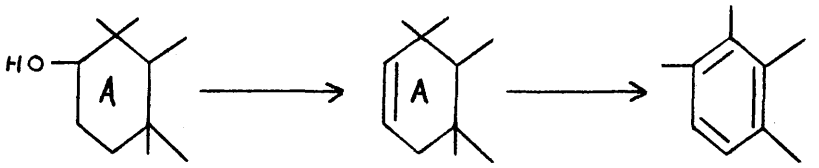


XI

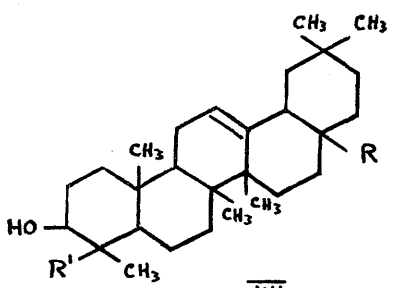
In general all triterpenes of the α - and β-amyrin groups give most of the typical dehydrogenation products, whose structures were confirmed by syntheses (37,38,39,40,41).

Lupeol, on the other hand, did not give products (VII),(VIII), (IX) and (X) on dehydrogenation(42) indicating a substantial difference in the basic carbon skeleton of lupeol from that of β-amyrin. The diol betulin, which has the same basic structure as lupeol, did give the napthalene derivatives (VII) and (VIII) on dehydrogenation but no picene derivatives were isolated(43).

Ruzicka's formula (XI) for the β-amyrin group of triterpenes afforded an adequate explanation of the dehydrogenation results and was consistent with the isoprene rule. The formation of 1:2:5:6-tetramethylnaphthalene during dehydrogenation is readily explained by



a series of changes involving dehydration of the alcohol grouping of ring A followed by a retropinacoline rearrangement as shown. The formula(XI) did not account satisfactorily for various oxidation reactions of β-amyrin and oleanolic acid, and alternative structures were suggested by Kitasato(44), Spring(23), Haworth(5), and Kon(45). Of these, the structure(XII) suggested by Haworth, is now generally accepted as being that of the β-amyrin group of triterpenes.



XII

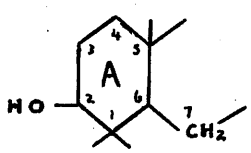
b) Oxidative Degradation Evidence.

(i) Oxidation of hydroxyl bearing ring.

The first important advances towards an elucidation of the structure of the triterpenes by oxidative methods were made on hederagenin, and finally resulted in the correct representation of ring A of the β-amyrin group.

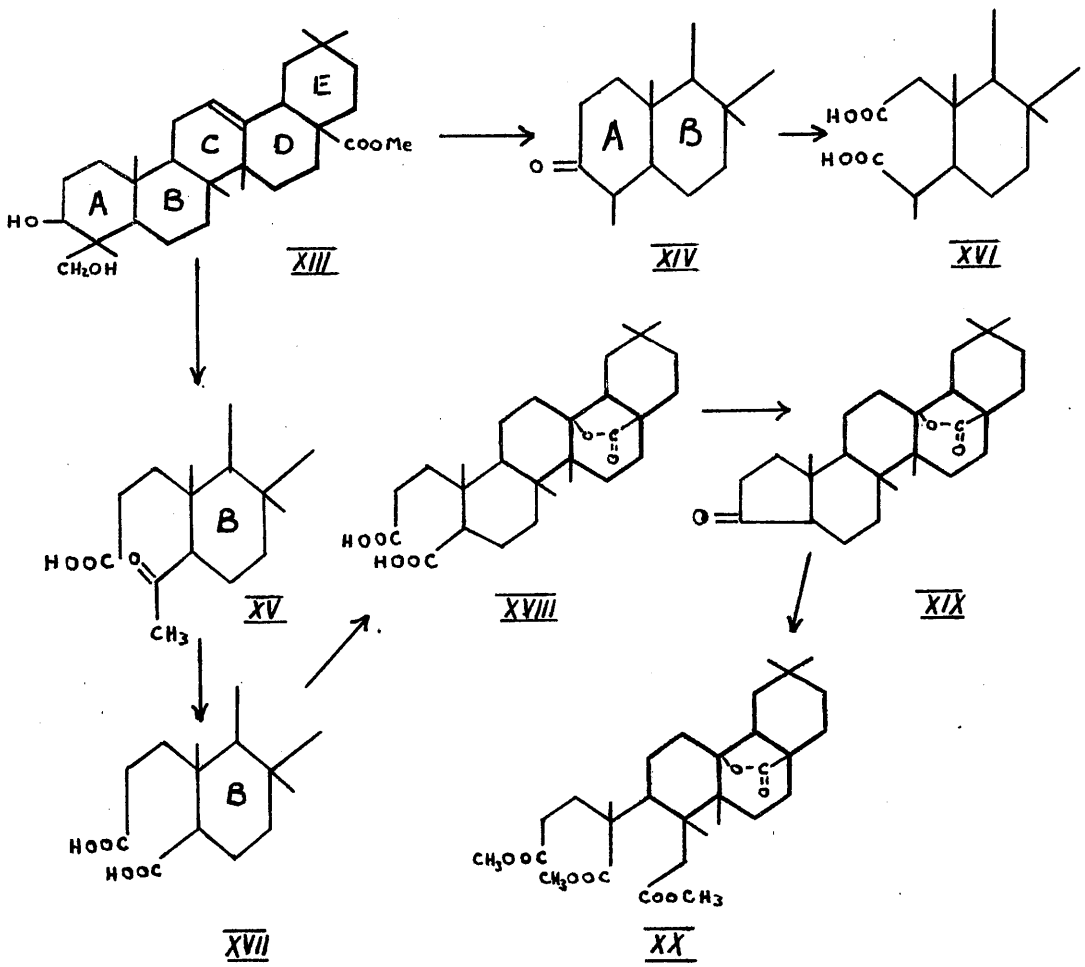
Oxidation, by Jacobs(46), of the methyl ester of hederagenin (XIII) with chromic anhydride, resulted in the formation of a mixture of the methyl esters of a C_{29}-keto acid(XIV) and a dibasic C_{29}-keto acid(XV) which were further oxidised with potassium hypobromite (47,48,11) to the monomethyl esters of a C_{29}-tricarboxylic acid(XVI) and a C_{28}-tricarboxylic acid(XVII). From the C_{28}-tricarboxylic acid was obtained a C_{28}-dicarboxylic acid lactone(XVIII) which was pyrolysed to a C_{27}-keto lactone(XIX). This keto lactone on oxidation and subsequent esterification gave the trimethyl ester lactone(XX). These results were interpreted as shown in Chart II and indicated a 1:3-diol system in hederagenin. The formation of the tricarboxylic lactone excludes the possibility of the presence of a methyl group at C_6 and led to the partial expression (XXI; R = CH_2OH) for hederagenin and hence for oleanolic acid and β-amyrenol (R = CH_3).

Further proof for this partial formula was obtained by Ruzicka and Giacomello(49) in the course of similar degradations of gypsogenin, and by oxidative attack on the unsaturated centre of oleanolic acid.



XXI

CHART II.

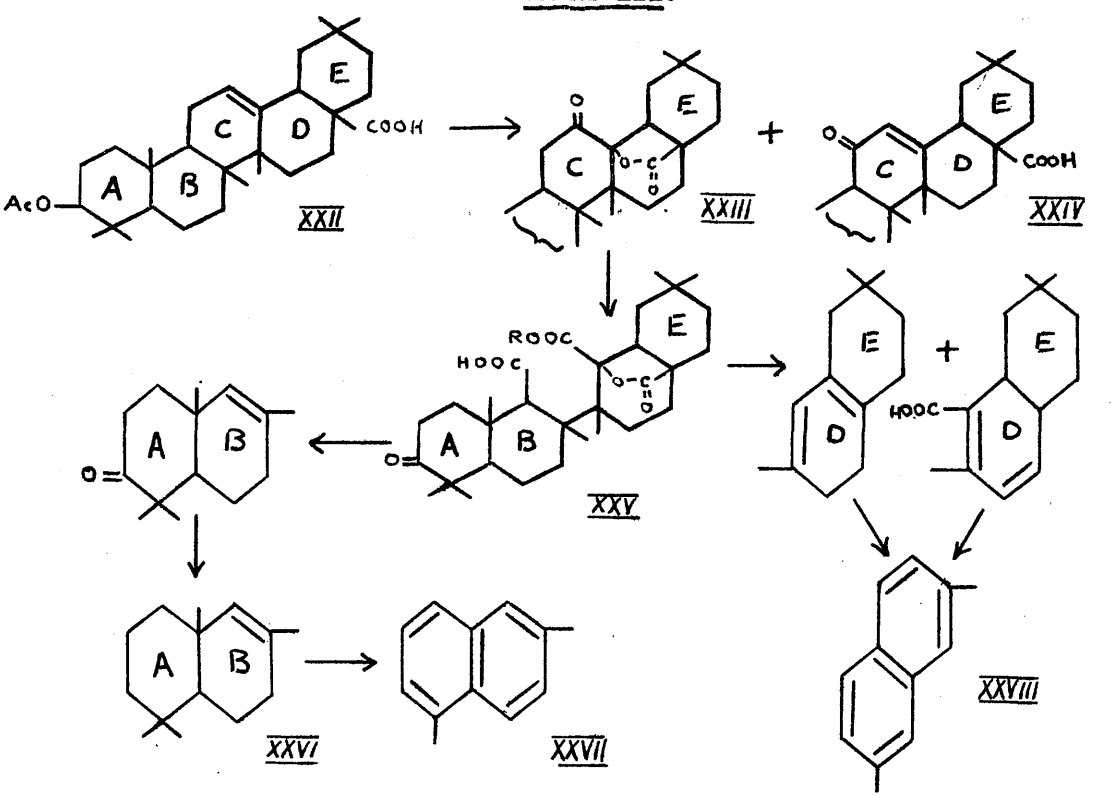


(II) Oxidation of the unsaturated centre.

The most important degradations of this type were carried out on oleanolic acid, but, as before, any information therefrom regarding the structure of this compound may be taken as evidence in the proof of the structures of the compounds of the β-amyrin group in general.

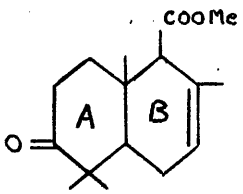
Oxidation of acetyl oleanolic acid(XXII) with chromic anhydride gave acetyl keto-oleanolic acid lactone(XXIII) and acetyl keto-oleanolic acid (XXIV)(50,51,52). The former product was further oxidised to a keto-dicarboxylic acid lactone(XXV, R = H)(34,51,52). Pyrolysis of the monomethyl ester of (XXV)(R = CH_3) by Ruzicka(53,54) gave a mixture which was separated into ketonic and non-ketonic fractions. The ketonic fragment was reduced by the Wolff-Kischner method to a hydrocarbon $C_{14}H_{24}$(XXVI) which on dehydrogenation with selenium gave 1:6-dimethylnaphthalene(XXVII). The non-ketonic fraction was hydrolysed with alkali and thereby separated into acidic and neutral fractions, each of which gave 2:7-dimethylnaphthalene (XXVIII) on selenium dehydrogenation.

CHART III.

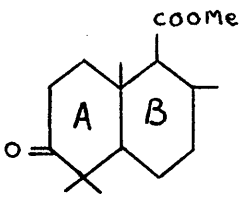


These reactions are outlined in Chart III and proved conclusively that the double bond of oleanolic acid is in fact situated in ring C, and that earlier interpretations of the dehydrogenation results were correct.

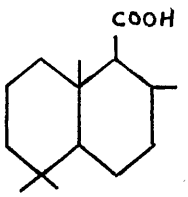
The configurations of rings A and B have been compared in different series by examining the pyrolysis fragments containing these rings. Thus, pyrolysis of the dimethyl ester of (XXV) gave two ketonic esters (XXIX) and (XXX) containing rings A and B of the original triterpene(56). Reduction of the saturated ester (XXX) by the Wolff-Kischner method, gave an acid (XXXI) which proved to be identical with an acid previously obtained from ambrein and corresponding to rings A and B of that tricyclic triterpene (XXXII), and the diterpenes manool, sclareol, and abietic acid (57,58). This correlation led to the elucidation of the stereochemistry of rings A and B in the β-amyrin group which will be discussed elsewhere.



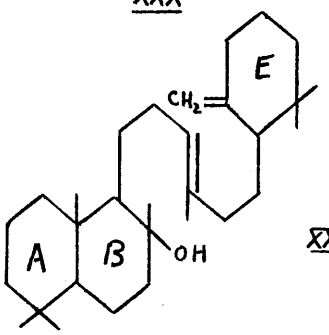
XXIX



XXX

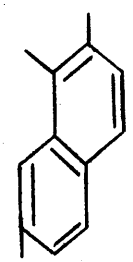


XXXI

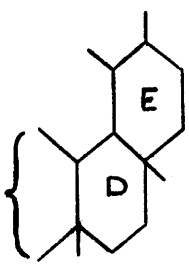


XXXII

By a similar series of reactions Meisels Jeger and Ruzicka(55) opened ring C of α -amyrin and obtained pyrolysis products corresponding to rings A and B in the β -amyrin series. But from the products corresponding to rings D and E, dehydrogenation gave 1:2:7-trimethylnaphthalene (XXXIII) instead of 2:7-dimethylnaphthalene obtained from β -amyrin. Rings D and E in α -amyrin are therefore formulated as in (XXXIV) (c.f.II).



XXXIII

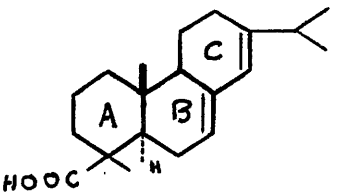


XXXIV

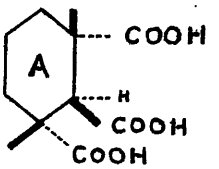
STEREOCHEMISTRY.

The complex structure of the pentacyclic triterpenes presented a difficult problem in stereochemistry on which little attack had been made until recent years, when, due mainly to the work of D.H.R.Barton and his colleagues, an almost complete stereochemical structure has been proved for the β-amyrin group.

As has been shown in the preceding section, rings A-B of oleanolic acid had been related to rings A-B of the triterpene ambrein. Ambrein had been related through the diterpene manool to abietic acid (XXXV), which by degradation to a C_{11}-tricarboxylic acid (XXXVI) was shown to have rings A-B trans fused (8,59). From this relationship it was concluded that rings A-B in oleanolic acid are trans fused.



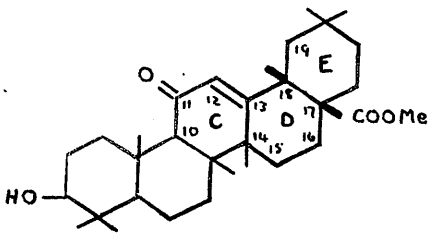
XXXV



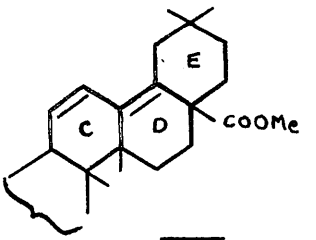
XXXVI

Treatment of methyl-11-keto-oleanolate (XXXVII) with hydrogen bromide in acetic acid was shown by Kitasato(60) to give an isomeric methyl ψ-11-keto-oleanolate. Recently, it has been proved by Barton and Holness(61) that this change can be effected with alkali and that the isomerisation involves only the hydrogen atom attached to C_{18};

possible isomerisation at C_{10} was excluded by conversion of both esters into the same diene, methyl dehydro-oleanolate(XXXVIII), by reactions not involving the C_{10}-position. The stability of the C_{10}-position in β-amyrin had been demonstrated previously by Budziarek et al(62). Rings D-E in oleanolic acid were thus shown to be fused in the unstable cis configuration, and since the properties of the carboxyl grouping indicated that this grouping was vertical (polar)(63)(64), and therefore in the β position, rings D-E are represented as in (XXXVII). That rings D-E are similarly fused in siaresinolic acid was also proved by Barton and Holness (loc.cit.).



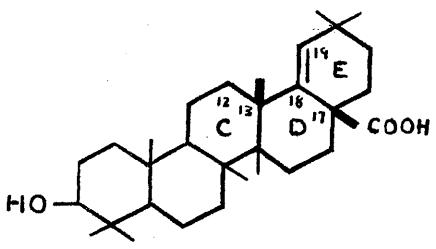
XXXVII



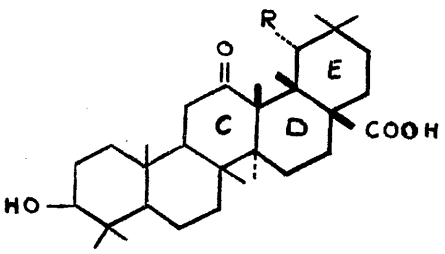
XXXVIII

During an investigation of morolic acid (olean-18-enolic acid) (XXXIX), Barton and Brooks(64) were able to prove that the C_{13}-hydrogen was vertical and on the same side of the molecule as the C_{17}-carboxyl grouping. The partial synthesis of morolic acid(65), by Wolff-Kischner reduction of dihydro-12-keto siaresinolic acid (XL;R = OH) followed by dehydration, indicated that the C_{13}-hydrogen, in morolic acid, was in the thermodynamically more stable configuration; the same being

true for 12-keto oleanolic acid (XL;R = H). The configurations at positions C_{13}, C_{18}, C_{17} having been determined, Barton and Holness(loc.cit.) by analogy with the relative stabilities of the perhydrophenanthrenes (66,67), concluded that rings C,D, and E in oleanolic acid are fused trans - syn - cis as shown in (XL).



XXXIX

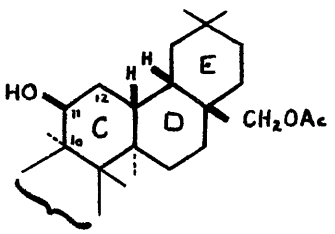


XL

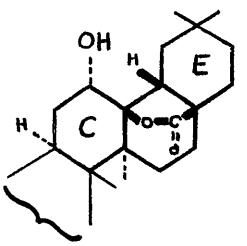
The stereochemical configuration at C_{10}, in oleanolic acid, was deduced by Barton and Holness(61) in the following way.

The properties of the C_{11}-hydroxyl group in 2:28-diacetoxy-olean-11-ol(XL1), prepared by reduction of methyl-11-keto-oleanolate acetate with lithium aluminium hydride, and the C_{12}-hydroxyl group in the hydroxylactone (XL11), prepared by oxidation of oleanolic acid acetate with perhydrol in acetic acid(66), indicated that both were vertical. The method of formation of (XL11) indicated that the C_{12}-hydroxyl must lie on the opposite side of the molecule to the lactone linkage, i.e., in the α-position, and since ring C exists in the chair form, the

vertical C_{11}-hydroxyl in (XLI) must therefore have the β configuration. Furthermore, the C_{11} vertical hydroxyl group must be <u>trans</u> with respect to the C_{10}-hydrogen, because of ease of dehydration to give olean-10-ene-2:28-diol diacetate. Consequently, the C_{10}-hydrogen must have the configuration shown in (XLI) and (XLII).

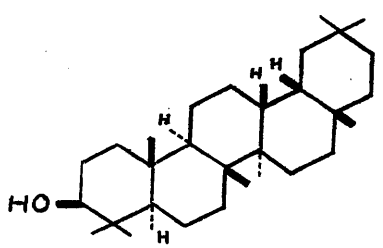


XLI

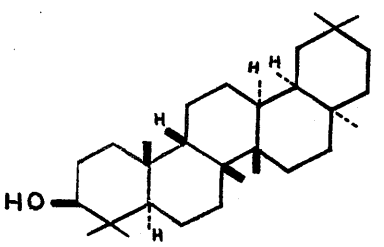


XLII

The configurations at C_2 and C_9 remain for discussion. That at C_2 is regarded as β, on elimination evidence, while the steric properties indicated an equatorial hydroxyl group (63,61). No direct chemical evidence is available by which a configuration can be assigned to the methyl group at C_9. Two possible stereochemical representations (XLIII) and XLIV) can therefore be written for β-amyranol.



XLIII



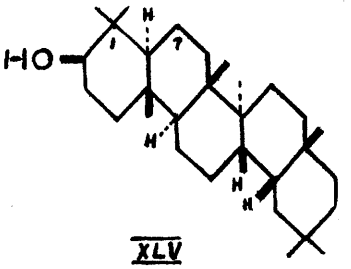
XLIV

By comparison of the molecular rotation differences of C_7 substituted triterpenes and C_6 substituted steroids, Klyne(67) has been able to decide in favour of (XLIII); a decision which is in agreement with the stereochemistry deduced from X-ray investigation of methyl oleanolate iodoacetate (c.f.68).

The identical steric configurations in rings A-B and C of β-amyrin and lupeol, and at C_{18} in lupeol and morolic acid, have been shown by the inter-relationship of lupeol and β-amyrin(4), and betulinic acid and moradiol(69). That lupeol differs from β-amyrin in having a <u>trans</u> fusion of rings D and E has been proved by Davy, Halsall Jones and Meakins(70), and confirmed by Barton and Holness(61).

NOMENCLATURE AND CONVENTIONS.

Until recently, it has been customary to write triterpene formulae as shown in (XLV). However, there is evidence(73) that the conventional representation of the C_{10}-methyl group in the steroids and the C_5-methyl group in the triterpenes - both labelled β - was used in opposite senses. With a view to reconciling these conventions, Halsall, Jones and Meakins(74) proposed that formula (XLV) should be turned through 180° about an axis through C_1 and C_7 and that the α and β prefixes of the triterpenes be retained as previously. This led to formulae of the type (XLIII) which emphasise the apparent relationships between steroids and triterpenoids. The latter type of projection formulae has been used throughout this thesis.



XLV

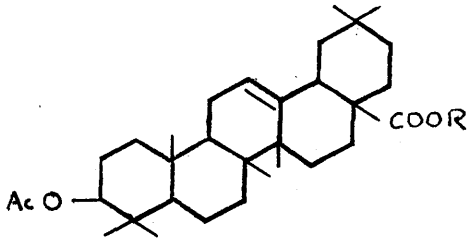
The ever increasing number of triterpene derivatives has resulted in more frequent use of the convention first proposed by Ruzicka et. al. (54) for naming such compounds. In this scheme, the triterpenes and their derivatives are represented as originating from the hypothetical hydrocarbons oleanane(I), ursane(II) and lupane(III). Hence β-amyrin becomes 2-hydroxy-12(13)-oleanene or, since the location of the C_2-hydroxyl group is generally assumed, olean-12(13)-enol.

This system is useful for naming new structures, particularly isomeric structures, and will be used in this work, wherever possible. Trivial names will be used for well known substances where common usage has excluded all possibility of ambiguity.

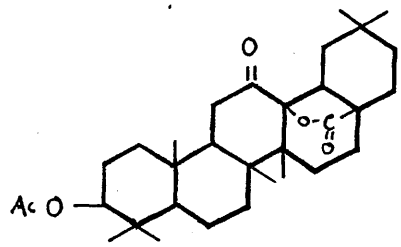
THEORETICAL.

1. Acetylketo-oleanolic Lactone and its Hydrolysis Products.

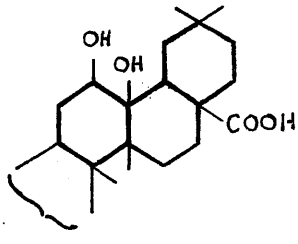
Oxidation of acetyloleanolic acid (XLVI; R = H) with chromic anhydride gives acetylketo-oleanolic acid and acetylketo-oleanolic lactone (75,12). The structure (XLVII) of this latter compound has been established unequivocally and its formation is preceeded presumably by the oxidation of the double bond of oleanolic acid to the glycol (XLVIII), followed by lactonisation of the tertiary hydroxyl group and further oxidation of the secondary hydroxyl to a carbonyl. Support for this theory of formation is to be found in the formation of the hydroxy lactone (XLIX) by milder oxidation of acetyloleanolic acid with hydrogen peroxide, and subsequent oxidation of this compound to give acetylketo-oleanolic lactone (66).



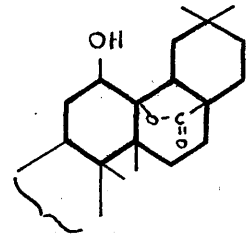
XLVI



XLVII



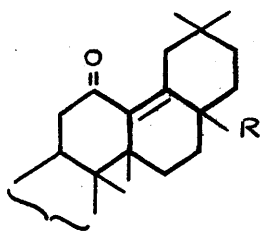
XLVIII



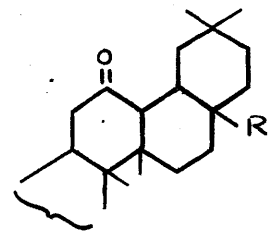
XLIX

Treatment of acetylketo-oleanolic lactone with hydrogen bromide in acetic acid (72) gives acetylketoisooleanolic acid, which was formulated as (L ; R = COOH) by Ruzicka, Cohen, Furter and Sluys Veer(71) who obtained it by treatment of the lactone with hydrogen bromide in ethanol. This formula received support from the behaviour of the acid on treatment with boiling quinoline, an isomeric neutral compound being obtained, presumably by lactonisation between the carboxyl group and the double bond.

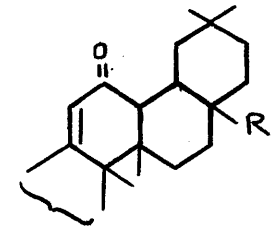
According to Picard Sharples and Spring(76) methyl acetylketo-isooleanolate can be prepared from methyl acetyloleanolate (XLVI; R = COOMe) by oxidation to methyl acetyldihydroketo-oleanolate (LI ; R = COOMe)(12) followed by bromination of the last compound. Bromination of β-amyranonyl acetate (LI ; R = CH_3), which differs from methyl acetyldihydro-oleanolate solely in the nature of the attachment at C_{17}, gives iso-β-amyrenonyl acetate which has been shown to be 12-keto-olean-10-enyl acetate (LII ; R = CH_3)(77). By analogy the structure of methyl acetylketoisooleanolate would be (LH ; R = COOMe).



L



LI



LII

Of the alternative formulae (L ; R = COOH) and (LII; R = COOH) for acetylketoisooleanolic acid, the latter is not in harmony with the behaviour of the acid when treated with quinoline. The formation, under these conditions, of an isomeric lactone requires that the carboxyl group and the ethylenic linkage of the acid be relatively close to each other, i.e., that acetyl ketoisooleanolic acid is a $\beta\gamma$ - or $\gamma\delta$ - unsaturated acid. On the other hand, comparison of the optical rotations of the corresponding oleanolic acid and β-amyrin derivatives, which are shown in Table I, supports the view that iso-β-amyrenonyl acetate (LII; R = CH_3) and acetylketoisooleanolic acid differ only in the nature of the substituent attached to C_{17}. With the object of deciding between the alternative formulae (L) and (LII) acetylketoisooleanolic acid was re-examined.

TABLE I.

β-amyrin acetate +81° (27)	Acetyl oleanolic acid +75° (27) Methyl acetyloleanolate +70° (27)
β-amyranonyl acetate -15° (77)	Methyl acetyldihydroketo-oleanolate. -11° (78)
iso-β-amyrenonyl acetate +61°(77)	Acetylketoisooleanolic acid. +61° (71).

Treatment of acetylketo-oleanolic lactone with hydrogen bromide in ethanol gave an acid which proved to be the hitherto undescribed ketoisooleanolic acid and not the acetate acid as described by Ruzicka et al(71). Acetylation of this acid gave acetylketoiso-oleanolic acid, the physical constants of which were in good agreement with those observed by these workers for the product obtained directly from the acid treatment of the lactone. The specific rotation (+61°) of the acetylketoisooleanolic acid obtained was in agreement with that recorded by the Swiss workers (+61°), but was markedly different from the value (+47°) reported by Kitasato(72). This value is more consistant with the specific rotation (+45°) of ketoisooleanolic acid isolated from the original reaction mixture. A repeat of Kitasato's experiment gave acetylketoisooleanolic acid.

Esterification of acetylketoisooleanolic acid with diazomethane gave methyl acetylketoisooleanolate, the physical constants of which were similar to those reported by Picard Sharples and Spring(76) for the ester obtained by bromination of methyl acetylketodihydro-oleanolate. Since direct comparison of the ester obtained by these workers with the ester of the acid obtained by the action of hydrogen bromide on the lactone was not made, the possibility existed that the two materials, although similar, were distinct, the ester obtained by the bormination route being methyl 12-keto-olean-10-enolate (LII; R = COOMe) and that obtained from the lactone the isomeric methyl 12-keto-olean-13(18)-enolate (L; R = COOMe).

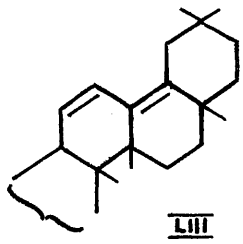
Bromination of the saturated keto-ester, methyl acetylketodihydro-oleanolate, gave the unsaturated keto-ester, methyl acetylketoiso-oleanolate, as reported by Picard Sharples and Spring, and a direct comparison by mixed melting point was made between this ester and that obtained by the route involving hydrolysis of the lactone. The two esters proved to be identical, and this was confirmed by hydrolysis of methyl acetylketoisooleanolate, obtained by the bromination route, to methyl ketoisooleanolate and comparison with a specimen obtained by methylation of ketoisooleanolic acid. The constants of the esters from both series of reactions are shown below in Table II.

TABLE II.

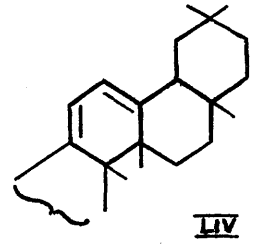
	From(LI;R=COO)		From (XLVII).	
	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$
Methyl acetylketoisooleanolate	208-209°	+ 57°	207-208°	+ 56°
Methyl ketoisooleanolate	233°	+ 42°	232-233°	+ 40°

In order to distinguish between the two formulae (L;R=COOH) and LII; R=COOH) for acetylketoisooleanolic acid, attempts were made to reduce the ketone group with formation of a diene system. Structure(L) would be expected to give the heteroanular diene system (LIII) while structure (LII) would product a homoanular diene (LIV). Both systems exhibit characteristic light absorption, dienes

of the type (LIII) at 2500Å (80,81) and dienes containing both double bonds in one ring at 2800Å (23,79).



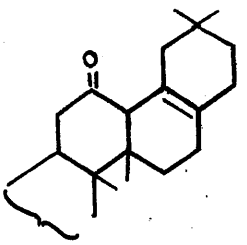
LIII



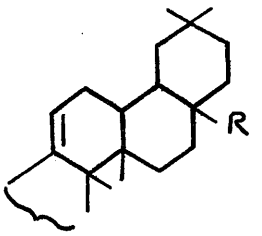
LIV

Attempts to prepare the diene from methyl acetylketoisooleanolate by reduction with sodium and amyl alcohol, and by reduction with sodium analgam were unsuccessful. However, the reduction of ketoisooleanolic acid with sodium analgam proceeded smoothly to give a hydroxy-dienoic acid which is almost certainly identical with the dehydroisooleanolic acid obtained by a similar reduction of acetylketoisooleanolic acid by Kitasato(11). The hydroxydienoic acid exhibited an intense light absorption maximum at 2830Å, which proved it to be olean-10:12-dienolic acid (LIV; R=COOH), and consequently that acetylketoisooleanolic acid is acetyl-12-keto-olean-10-enolic acid (LII; R=COOH) and not acetyl-12-keto-olean-13(18)-enolic acid (L; R = COOH). This decision was confirmed by two methods.

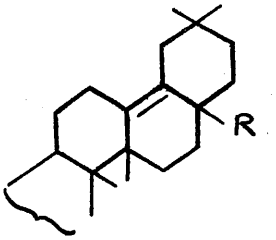
Firstly, acetylketoisooleanolic acid was sublimed unchanged in a vacuum at 250°. This pronounced stability to heat is in accord with formula (LII; R = COOH) but not with formula (L; R = COOH); the $\beta\gamma$-unsaturated acid would be expected to lose carbon dioxide when heated, to give the nor-β-amyrenonyl acetate (LV).



LV



LVI



LVII

Secondly, Clemmensen reduction of acetylketoisooleanolic acid gives acetylisooleanolic acid(71), while the analagous reduction of iso-β-amyrenonyl acetate by the same method(77) or by catalytic means(82) gives olean-10-enyl acetate (LVI; R = CH_3). If acetylketoisooleanolic acid is represented by formula (LII; R = COOH), acetylisooleanolic acid is acetyl-olean-10-enolic acid (LVI; R = COOH). If, on the other hand, acetylketoisooleanolic acid is represented by (L; R = COOH), acetylisooleanolic acid is acetyloolean-13(18)-enolic acid (LVII; R=COOH). The last formula is used without title in Elsevier's "Encyclopaedia of Organic Chemistry"(1940, Vol. XIV, p.543) and assumes not only that L; R=COOH) is a valid representation of acetylketoisooleanolic acid but that Clemmensen reduction of the $\alpha\beta$-unsaturated keto-acid has proceeded without migration of the double bond. Support for this last assumption is to be found in the stability of olean-13(18)-enyl acetate (LVII; R=CH_3) to mineral acid(83). Methyl acetylolean-13(18)-enolate (LVII; R=$COOCH_3$) has been prepared by an unambiguous method from methyl acetyloleanolate by oxidation with selenium dioxide to methyl acetyloolean-11:13(18)-dienolate (LIII; R = COCMe) followed by catalytic reduction of the latter (84,64).

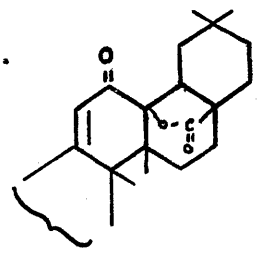
Catalytic reduction of methyl acetylketoisooleanolate gave methyl acetylisooleanolate together with a small amount of methyl acetylolean-10:12-dienolate (LIV; R=COOMe) though the quantity of the latter substance was insufficient for complete purification. Methyl acetylisooleanolate differs from methyl acetylolean-13(18)-enolate thus proving that the former compound is, in fact, methyl acetylolean-10-enolate (LVI; R=COOMe) and that acetylketoisooleanolic acid is not acetyl-12-keto-olean-13(18)-enolic acid (L; R=COOH). The reducibility of methylacetylketoisooleanolate is in contrast to that of the corresponding acid which was recovered unchanged from attempted catalytic reduction by Ruzicka et.al.(71).

Since acetylketoisooleanolic acid is acetyl-12-keto-olean-10-enolic acid (LII; R=COOH), the formation of a lactone, isomeric with acetylketo-oleanolic lactone, which has been reported by Ruzicka et. al.(71) requires comment. This lactone was prepared by treatment of acetyl ketoisooleanolic acid with quinoline . Several attempts were made to repeat this preparation, the majority of which were unsuccessful. In one case, however, a very small yield of a neutral product was obtained, the melting point and crystalline form of which were similar to those observed by Ruzicka and his co-workers.

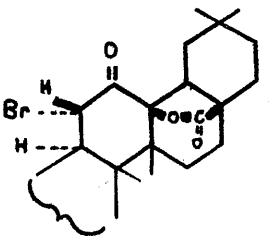
Although this neutral compound was not available in sufficient quantity for detailed study, it exhibits a light absorption maximum at 2570Å (ε = 14,000) and does not give a colour with the tetranitromethane reagent. Whatever the nature of this neutral product, the presence therein of a strongly absorbing chromophore shows that it has not been produced from acetylketoisooleanolic acid by simple

additive interaction of the carboxyl group and the double bond, and its formation does not indicate a necessarily close proximity of these two functions in this acid.

In an attempt to establish the structure of this neutral product acetylketo-oleanolic lactone (XLVII) was treated with bromine, when it was hoped that dehydrobromination would result in the formation of the lactone (LVIII). The product of the bromination was 11-bromo-12-keto-oleanolic lactone acetate (LIX). On attempted dehydrobromination, the bromo-lactone was recovered unchanged after heating in acetic acid at 100° for two days, and treatment with sodium iodide in acetone gave an amorphous solid which contained no halogen but showed no appreciable light absorption. It may be assumed that bromination of acetyketo-oleanolic lactone, unlike that of methyl acetyl-12-keto-oleanolate (LI; R=COOMe) results in replacement of C_{11}-α-hydrogen atom; the relatively large bromine atom being below the plane of the molecule while the lactone linkage lies above the plane as shown in (LIX). The stereochemical configuration of the C_{10}-hydrogen (vertical) and the C_{11}-bromine (equatorial) thus renders difficult the ionic elimination of hydrogen bromide(63).



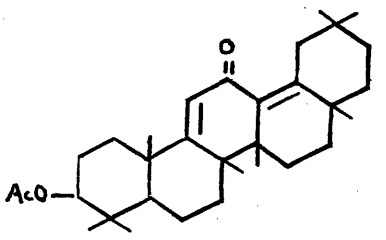
LVIII



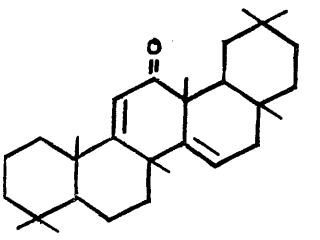
LIX

Since the completion of this work, Barton et. al.(85) have attempted to prepare the lactone (XIII) by the same method. When these workers treated the bromo-lactone with collidine, loss of hydrogen bromide was accompanied by reductive opening of the lactone ring to give acetyl-12-keto-olean-10-enolic acid (LII; R=COOH).

Following the preceeding investigation, which proved that bromination of methyl acetyl-12-keto-oleanate and hydrolysis of acetylketo-oleanolic lactone result in the formation of methyl acetyl-12-keto-olean-10-enolate, an investigation of the action of selenium dioxide on this ester was undertaken. Green, Mower, Picard and Spring(86) have shown that oxidation of the analagous 12-keto-olean-10-enyl acetate with this reagent results in the formation of iso-β-amyradienonyl acetate, which was formulated by these workers as (LX), and by Jeger and Ruzicka(82) as (LXI). The structure of iso-β-amyradienonyl acetate will be discussed in greater detail in a later section of this work, and at this juncture it is sufficient to say that a considerable weight of evidence supports structure (LXI).



LX

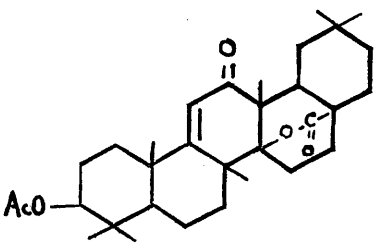


LXI

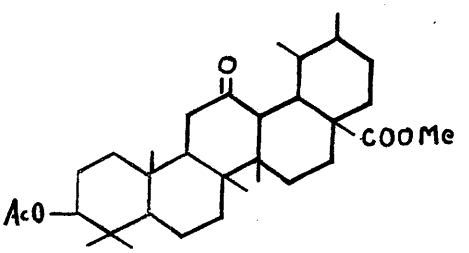
Oxidation of methyl acetyl-12-keto-olean-10-enolate gave a neutral product, $C_{32}H_{46}O_5$, which gave no colour with tetranitromethane in chloroform and showed a light absorption maximum at 2380Å, ε = 11,450, indicative of an $\alpha\beta$-unsaturated ketone grouping. Estimation of methoxyl content proved that the substance was not a methyl ester and that oxidation had resulted essentially in the loss of methane (CH_4) from the molecule. Attempted hydrolysis of the substance by treatment with ethanolic hydrogen bromide and by refluxing with ethanolic potassium hydroxide resulted in recovery of unchanged starting material, after acetylation of the initial product in each case. The substance was also resistant to catalytic reduction.

Of the two possible structures (LVIII) and (LXII) for this compound, the former is rejected for two reasons. Firstly, introduction of the lactone grouping as in acetylketo-oleanolic lactone (XLVII), and introduction of the C_{10} : C_{11} double bond as in acetyl-12-keto-olean-10-enolic acid (LII; R=COOH) produce a positive change in the specific rotation of the saturated ketone, e.g. methyl acetyl-12-keto-oleanolate (LI; R=COOMe), and therefore a compound of structure (LVIII) would be expected to be dextrarotatory. The product obtained had a pronounced negative rotation (-73°), which indicates that lactonisation has not taken place at the C_{13} position. Secondly, Dreiding, Jeger and Ruzicka(37) recently showed that a similar oxidation of methyl acetyl-12-keto-ursa-10-

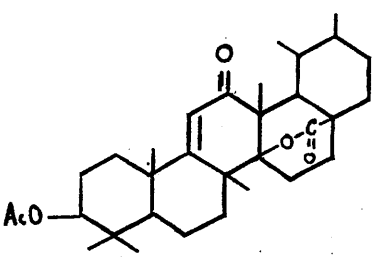
enolate (LXIII) results in the formation of two lactones, formulated as (LXIV) and (LXV). The lactone (LXIV) exhibits light absorption at 2380Å, ε = 10,700, and lactone (LXV) at 2570Å, ε = 11,700. The former compound, which would seem to be the analogue of the product obtained from the oxidation of methyl acetyl-12-keto-olean-10-enolate, was obtained by Manson(88) who recovered it unchanged from attempted catalytic reduction and after treatment with alkali.



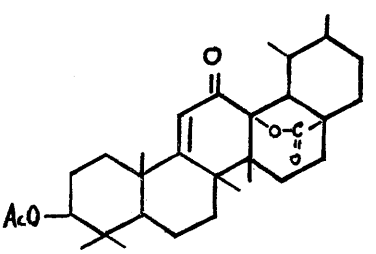
LXII



LXIII

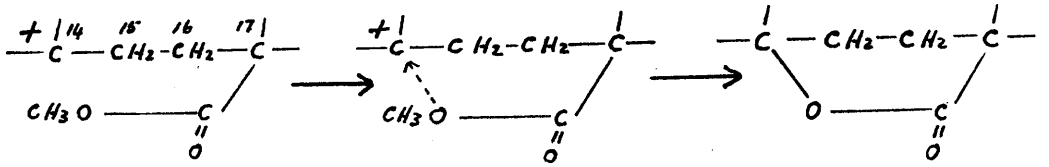


LXIV

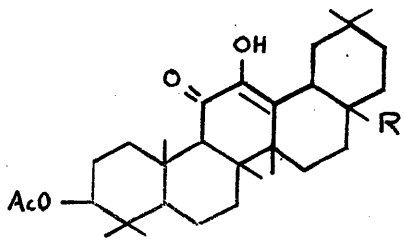


LXV

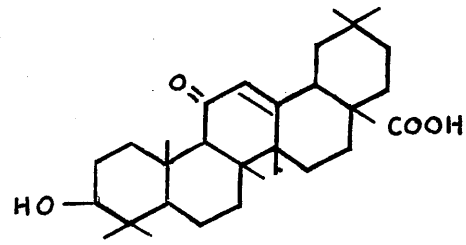
Both lactones (LXII) and (LXIV) are formed presumably through the carbonium ion at C_{14}. In the case of iso-α- or iso-β-amyrenonyl acetate this results in the introduction of the C_{14} : C_{15} ethylenic bond of iso-α-amyradienonyl acetate or iso-β-amyradienonyl acetate, while in the acid series this intermediate is stabilized through the lone pair of electrons on the oxygen of the carbomethoxyl grouping as illustrated below.



The above lactone (LXII) was obtained also by selenium dioxide oxidation of methyl acetyl-12-keto-oleanolate (LI; R=COOMe) together with the methyl ester of a diosphenol-carboxylic acid (LXVI; R=COOMe). Ruzicka and Jeger(22) have provided substantial evidence in support of (LXVI; R=CH_3) for the diosphenol obtained by a similar oxidation of 12-keto-oleanyl acetate. The presence of the diosphenol grouping was proved by the strong ferric chloride reaction and the light absorption maximum at 2380Å, ε = 10,900. This reaction has since been investigated by Barton et.al(85) who also isolated the diosphenol (LXVI; R=COOMe) but not the lactone (LXII). This is probably due to the milder conditions used by these workers.



LXVI



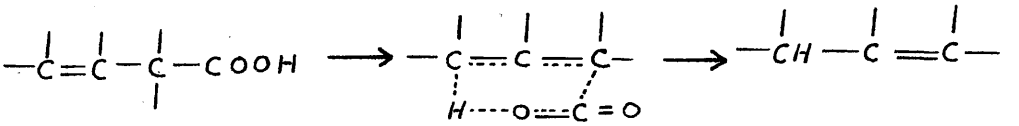
LXVII

In view of the results obtained by treatment of acetylketo-oleanolic lactone with hydrogen bromide, a reinvestigation was made of the alkaline hydrolysis of this lactone, which according to Kitasato(48) gives 11-keto-olean-10-enolic acid (LXVII).

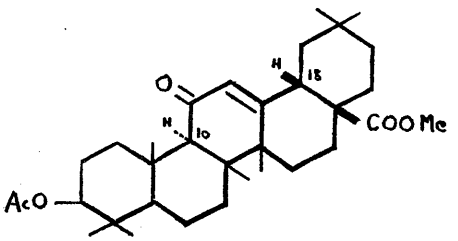
A hydrolysis of the lactone with ethanolic ptoassium hydroxide gave an acid fraction which, however, relactonised, presumably in the presence of hydrogen ion during the ensuing process of working up, to give unchanged lactone. A repeat hydrolysis, using the method of Kitasato, in which the potassium salt of the acid was treated with dimethyl sulphate, gave a netural product which was acetylated. The resultant acetate could not be purified adequately, but showed no appreciable light absorption and therefore was not methyl acetyl-11-keto-olean-12-enolate. Ruzicka and Sluys Veer(89) also have been unable to repeat Kitasato's results.

II. Decarboxylation of Acetyldehydro-oleanolic Acid.

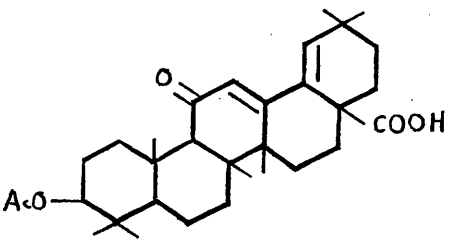
Kitasato(72) has shown that treatment of methyl acetyl-11-keto-olean-12-enolate (LXVIII) with mineral acid gives a ψ-isomer. This isomerisation could involve either C_{10} or C_{18} or both centres similtaneously, but the fact that bromination of the corresponding acid gives the conjugated acetyldehydro-oleanolic acid, formulated by Ruzicka, Jeger and Winter(90) as (LXIX), indicates that enolisation of (LXVIII) involves C_{18}. That acetyldehydro-oleanolic acid is formulated correctly as acetyl-11-keto-olean-12:18-dienolic acid (LXIX) was confirmed by decarboxylation of the acid, prepared according to Ruzicka et.al.(90) to give nor-β-amyradienonyl acetate (LXX) identical with the compound obtained originally from prolonged treatment of acetyl-11-keto-olean-12-enolic acid with boiling quinoline(71). The ease of decarboxylation of the dienone-acid indicates that it is a $\beta\gamma$-unsaturated acid and this affords additional proof for the proposed structure. It is noteworthy that this decarboxylation provides an additional example of the shift of the double bond from the $\beta\gamma$- to the $\alpha\beta$-position during decarboxylation of acids of this type and according to Barton and Brooks(64) is to be interpreted as proceeding through the transition state as shown.



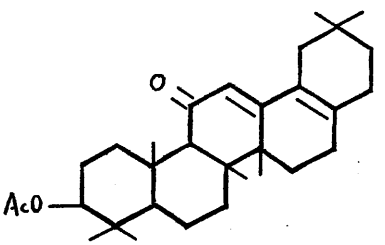
Proof of isomerisation at C_{18} in methyl ψ-11-keto-oleanolate has been obtained by Barton and Holness(61) and is paralleled by isomerisation of β-amyrenonyl acetate to 18-iso-β-amyrenonyl acetate (70).



LXVIII



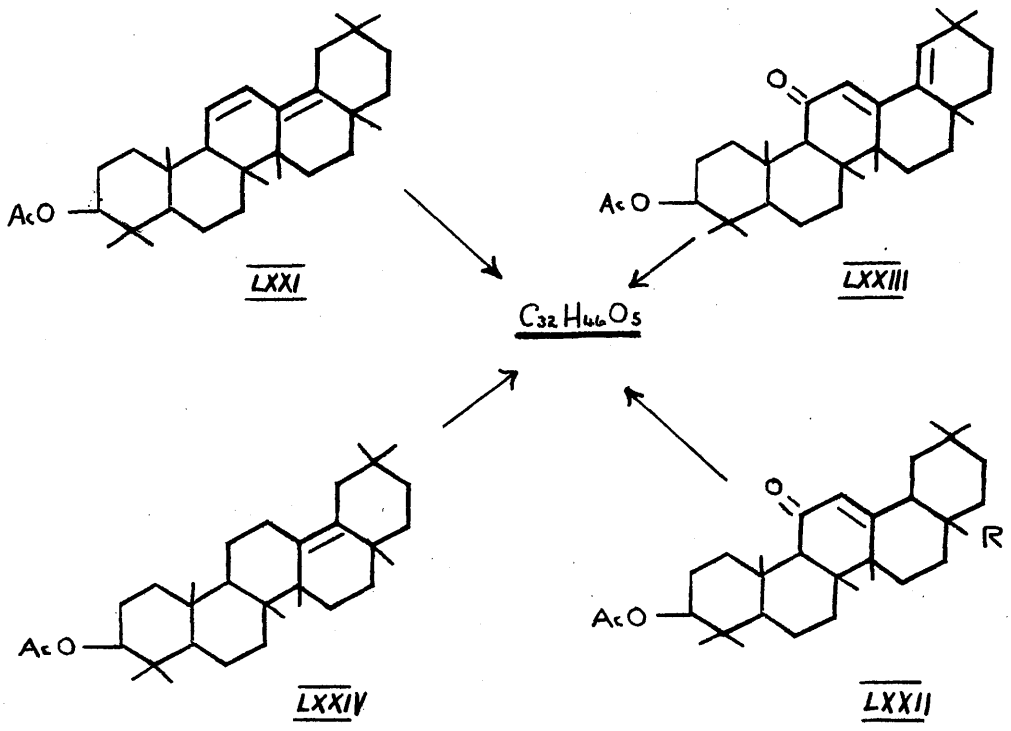
LXIX



LXX

III. The "O_5-Acetate" of β-Amyrin.

In 1941, Ruzicka and Jeger(83) oxidised oleana-11:13(18)-dienyl acetate (LXXI) with chromic acid and obtained an acetate, $C_{32}H_{46}O_5$. This substance was not formulated by these workers and was obtained later by Mower, Green and Spring(91) by oxidation of 11-keto-olean-12-enyl acetate (LXXII; R=CH_3) and 11-keto-oleana-12:18-dienyl acetate (LXXIII) with selenium dioxide in acetic acid. The corresponding benzoate was prepared by similar oxidations with selenium dioxide. By a slight modification of the method used by the Swiss workers, Mower et.al. isolated a mixed crystal of this "O_5-acetate" and 11-keto-oleana-12:18-dienyl acetate by chromic acid oxidation of the -11:13(18)-diene (LXXI). The conversion of (LXXI) into (LXXIII) had been effected previously (92) by oxidation with lead tetra-acetate. The analagous "O_7-acetate" was prepared by Green et.al. by oxidation of methyl acetyl-11-keto-olean-12-enolate (LXXII; R=COOMe) with selenium dioxide, thus proving that the angular methyl group attached to C_{17} is not involved in the formation of the "O_5-acetate". The "O_5-acetate" was also obtained by Jeger, Norymberski and Ruzicka(84) by oxidation of olean-13(18)-enyl acetate (LXXIV) with chromic acid. The "O_5-acetate" is thus a major oxidation product in the β-amyrin series and the work described in this section was carried out with a view to establishing its structure.



Chromic acid oxidation of oleana-11:13(18)-dienyl acetate in the presence of water proceeded smoothly to give the "O_5-acetate" as described by Mower, Green and Spring(91). It was found, however, that the neutral fraction from which the "O_5-acetate" was isolated also contained a substantial quantity of the mixed crystal, m.p.213-217°, of the "O_5-acetate" and 11-keto-oleana-12:18-dienyl acetate described by these workers. Since the "O_5-acetate" is formed by oxidation of 11-keto-oleana-12:18-dienyl acetate with selenium dioxide, the mixed crystal was resolved by a treatment with selenium dioxide which gave a further quantity of pure "O_5-acetate". It was shown that during the initial chromic acid oxidation no volatile fragment containing a carbonyl group is produced. This fact supports

the $C_{32}H_{46}O_5$ formula for the "O_5-acetate".

The "O_5-acetate" gives a pale yellow colour with tetranitromethane in chloroform and shows light absorption in the ultra-violet at 2280Å, ε =4,100, with a low intensity inflection at 3,000Å. It does not give a colour indicative of a 1:2- or 1:3-diketone system with ferric chloride in alcohol or dioxan solution. Previous investigations by Mower, Green and Spring have shown that it is unaffected by boiling acetic anhydride and does not contain an active hydrogen as estimated by the Zeriwitinoff method and therefore does not contain a hydroxyl group; furthermore, these workers reported that the "O_5-acetate" does not give a positive Legal test, is unreactive towards hydrochloric acid and hydrogen bromide in hot acetic acid, and is recovered unchanged after heating with hydroxylamine acetate and from attempted reduction by the Clemmensen method, and catalytic hydrogenation in ethyl acetate. During the course of the present work the "O_5-acetate" was found also to be resistant to catalytic reduction in acetic acid.

On treatment with methanolic hydrogen chloride, the acetate was converted into the corresponding alcohol, $C_{30}H_{44}O_4$, by Mower et.al. The alcohol exhibited the same light absorption as the acetate and was reacetylated to this compound on treatment with acetic anhydride and pyridine.

In contrast to the stability of the O_5-acetate" with hydrochloric acid, treatment with alcoholic potassium hydroxide results in a more profound change than simple hydrolysis of the acetate grouping at the C_2 position. Thus, Mower et.al. obtained a substance which

could not be obtained crystalline but gave a well-defined acetate on acetylation. According to these workers, this acetate is to be formulated as $C_{32}H_{46}O_5$ or a near homologue, does not give a colour with tetranitromethane or ferric chloride, and it does not show intense selective absorption in the ultra-violet above 2200Å. From a similar alkaline hydrolysis of the "O_5-acetate" Ruzicka, Jeger and Norymberski (92) also obtained the same amorphous compound characterised as its crystalline acetate. The acetate was saturated to tetranitromethane and showed a low intensity absorption maximum at 2,900Å, ε = 80, indicative of an isolated carbonyl group. Ruzicka formulated the acetate as $C_{30}H_{46}O_4$, and recovered it unchanged after an attempted catalytic reduction. The same compound was obtained after acetylation of the product from an attempted reduction of the "O_5-acetate" by the Wolff-Kischner method.

Both groups of workers report the formation of an acid during the alkaline hydrolysis of the "O_5-acetate". Mower et.al. give m.p. 292-293° for this compound, while Ruzicka et.al. report m.p. 274-275° (in vacuo). The acid was formulated by the Swiss workers as $C_{29}H_{46}O_5$; it shows light absorption at 2270Å (ε = 18,000), it is saturated to tetranitromethane and it gives no colour with ferric chloride. In view of these varying results the alkaline hydrolysis of the "O_5-acetate" was re-examined.

The "O_5-acetate" was hydrolysed with potassium hydroxide in aqueous methanol to give an acidic and a neutral product. The neutral product, which was amorphous, was acetylated with acetic anhydride and pyridine at 100° to give a well-defined acetate, $C_{31}H_{48}O_4$, which gives

no colour with tetranitromethane in chloroform and shows light absorption at 2100Å, $\varepsilon = 1,768$. The specific rotation (+158°) of this acetate is in good agreement with the value (+158°) recorded by Ruzicka et.al. for the compound obtained from hydrolysis of the "O_5-acetate."

Consideration of the light absorption of this acetate, $C_{31}H_{48}O_4$, suggests that this substance contains an isolated double bond. This possibility was excluded when the acetate was recovered unchanged after treatment with chromic acid, potassium permanganate and perbenzoic acid. Furthermore, the absence of any absorption in the hydroxyl stretching region of the infra-red proves that the compound does not contain a hydroxyl group.

The acidic product $C_{30}H_{46}O_5$, m.p. 272-274°, gives no colour with tetranitromethane in chloroform and is identical with the acid $C_{29}H_{46}O_5$ isolated by Ruzicka et.al., but different from the acid, m.p. 292-293°, isolated by Mower et.al. No analytical data is given by Mower et.al. for this compound. The identity was confirmed by light absorption of the acid prepared by a modified hydrolysis of the "O_5-acetate".

The modified method of hydrolysis of the O_5-acetate" using potassium hydroxide in anhydrous methyl alcohol resulted in the formation of three products. Firstly, an acid, m.p. 272-274°, which exhibits light absorption in the ultra-violet at 2260Å, $\varepsilon = 12,700$ and is identical with the acid obtained from the hydrolysis of the "O_5-acetate" using an aqueous solvent. Secondly, purification of the crystalline

neutral fraction gave an alcohol, $C_{31}H_{48}O_5$, which exhibits light absorption in the ultra-violet with a maximum at 2220Å, ε = 15,500. This alcohol does not give a colour with tetranitromethane in chloroform, but it gives a positive Legal test, although somewhat more slowly than simple $\alpha\beta$-unsaturated lactones. Acetylation of the alcohol, $C_{31}H_{48}O_5$ gave a well-definied acetate, $C_{33}H_{50}O_6$, showing essentially the same light absorption properties as the parent alcohol. While acetylation of an amorphous fraction obtained from the crystallisation of this crystalline alcohol, $C_{31}H_{48}O_5$, gave, as the third product from the hydrolysis, the acetate, $C_{31}H_{48}O_4$, identical with that described above.

The similarity of the light absorption of the crystalline alcohol, $C_{31}H_{48}O_5$, and that of the acid obtained in these hydrolysis experiments suggested that the former compound might be a methyl ester. This was confirmed by esterification of the acid with diazomethane to give a product which was identical with the alcohol, $C_{31}H_{48}O_5$, and by estimation of the methoxyl content of the acid itself.

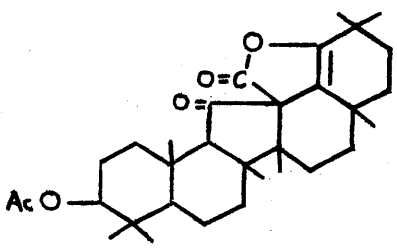
Attempts to characterise further the alcohol methyl ester, $C_{31}H_{48}O_5$, by treatment with bromine, and by oxidation of the corresponding acetate with potassium permanganate, were fruitless and resulted in each case in the recovery of unchanged starting material. The ultra-violet absorption maximum at 2220Å exhibited by the methyl ester, $C_{31}H_{48}O_5$, corresponds to that shown by an $\alpha\beta$-unsaturated lactone system (93,94) and therefore an attempt was made to hydrolyse this compound.

After treatment with 5% methanolic potassium hydroxide, the ester was recovered unchanged, but employing more drastic hydrolysis conditions, acid was obtained which was amorphous, but gave a crystalline dimethyl ester, $C_{32}H_{52}O_6$, on treatment with diazomethane. This dimethyl ester gave a crystalline acetate dimethyl ester, $C_{34}H_{54}O_7$, on acetylation with acetic anhydride and pyridine. None of these compounds gives a colour with the tetranitromethane reagent and the acetate dimethyl ester, $C_{32}H_{52}O_6$, exhibits light absorption at 2800Å, ε = 340, indicative of an isolated carbonyl grouping. The acetate dimethyl ester was recovered unchanged after attempted bromination and the suspected carbonyl group could not be reduced by the Clemmensen method. The presence of a carbonyl group, other than those of the ester groupings, in the dimethyl ester, $C_{32}H_{52}O_6$, was confirmed, however, by the infra-red light absorption of this compound. Two intense bands of diagnostic significance at 1734 cm^{-1} and 1723 cm^{-1} were apparent in the carbonyl stretching region; the band at 1735 cm^{-1} is attributed to the carbonyls of the ester groupings, while the band at 1723 cm^{-1} is indicative of a ketone in a six-membered ring.

Since hydrolysis of the monomethyl ester, $C_{31}H_{48}O_5$, obtained from the "O_5-acetate", has resulted in the formation of a second carboxyl grouping and a carbonyl group, the ester probably contains a lactone grouping as well as the ester grouping. By virtue of the ultra-violet light absorption, the lactone must be unsaturated in the position α to the carbonyl and since the dimethyl ester, $C_{32}H_{52}O_6$, does not contain a double bond, this unsaturated centre must be capable of migration during hydrolysis to form part of an enolised ketone which is stable as the

keto form in the dimethyl ester $C_{32}H_{50}O_6$.

A consideration of the experiments which have been described so far in this work, indicates that the "O_5-acetate" must have a lactone type of structure and must also contain an ethylenic bond which is not in conjugation with a carbonyl group, but, which can readily migrate into such a position to form part of an -unsaturated lactone system shown to be present in the methyl ester, $C_{31}H_{48}O_5$. A survey of the methods of preparation of the "O_5-acetate" shows that it is formed under certain conditions from compounds containing functional groups at the C_{10}, C_{11}, C_{12}, C_{13}, C_{18} and C_{19}, and is produced by a change in the C_{10}-C_{19} chain of carbons. From the evidence presented, structure (LXXV) is proposed as a reasonable formulation for the "O_5-acetate". This structure satisfies a large part of the chemical evidence, and the mechanism of formation from the various precursors of the "O_5-acetate" will be discussed later in this section.

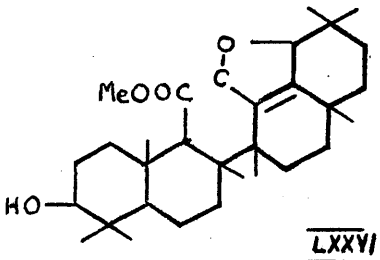


LXXV

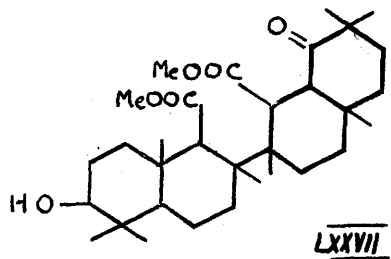
Some measure of support has been obtained for this proposed formulatiom, by investigation of the infra-red absorption spectrum of the "O_4-alcohol" corresponding to the "O_5-acetate". This compound exhibits intense absorption in the hydroxyl stretching region at 3450 cm^{-1}, in the

carbonyl stretching region at 1742 cm^{-1} and 1728 cm^{-1}, and in the ethylenic bond stretching region at 1686 cm^{-1}. The carbonyl band at 1728 cm^{-1} may be attributed to the five-membered ring ketone, and therefore the absorption at 1742 cm^{-1} must be considered to be that of the lactone carbonyl, though this value is somewhat lower than expected for such a grouping(98). The band at 1686 cm^{-1} is significant in that the intensity (0.63) calculated in the manner described by Rosenkrantz and Gut(98) indicates that the terminal carbon of the double bond is directly attached to an oxygen atom. This band is comparable with the intense band at 1696 cm^{-1} exhibited by cholestanone enol-acetate which has been attributed by Rosenkrants and Gut to the $-O-\overset{|}{C}=C\diagup$ grouping.

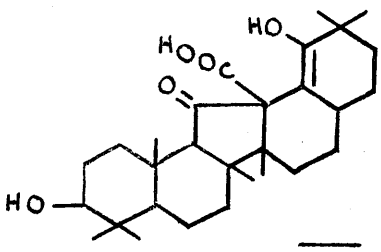
From structure (LXXV) it is theoretically possible to obtain two compounds by alkaline hydrolysis. Thus, hydrolysis with methanolic potassium hydroxide results in cleavage of the $C_{11}:C_{13}$ bond followed by migration of the $C_{18}:C_{19}$ double bond to the $C_{13}:C_{18}$ position to give the enol-lactone ester (LXXVI). This structure provides an admirable explanation of the properties of the monomethyl ester, $C_{31}H_{48}O_5$. Derived from this structural formula the dimethyl ester, $C_{32}H_{52}O_6$, is represented as (LXXVII). The position of the ketone group of this compound at the sterically hindered C_{19} position, affords a reasonable explanation why this carbonyl could not be reduced by the Clemmensen method, and doubtless a similar steric effect explains the unreactivity of the acetate dimethyl ester, $C_{34}H_{54}O_7$, towards bromine.



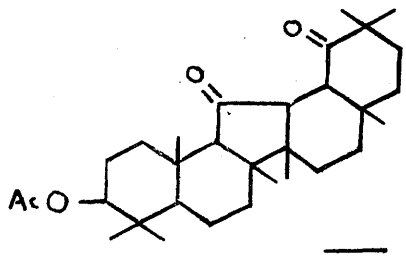
LXXVI



LXXVII



LXXVIII



LXXIX

Hydrolysis of the "O_5-acetate" using aqueous methanolic potassium hydroxide is visualised as proceeding through hydrolysis of the lactone ring of (LXXV) to give the intermediate β-keto acid (LXXVIII) which on decarboxylation and subsequent acetylation would give the 1:4-diketone (LXXIX). This latter formulation is proposed for the acetate, $C_{31}H_{48}O_4$, obtained from the "O_5-acetate".

In order to confirm the structure (LXXIX) a further study was made of the acetate, $C_{31}H_{48}O_4$. Although the alcohol corresponding to this acetate had never been obtained crystalline by direct hydrolysis of the "O_5-acetate", an alcohol was obtained by hydrolysis of the acetate itself. This alcohol crystallises from aqueous methanol with one molecule of methanol of crystallisation and is readily reacetylated to parent acetate. The infra-red absorption spectrum of this alcohol was kindly recorded by Dr. G.D. Meakins of Manchester

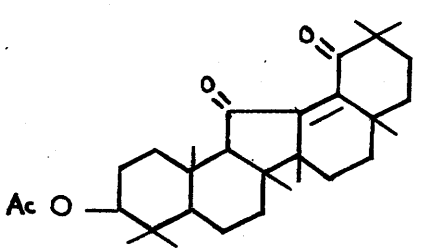
University with the object of detecting the presence of a ketone group in a five-membered ring. The spectrum showed two bands of diagnostic significance in the carbonyl stretching region at 1725 cm^{-1} and 1706 cm^{-1} and also the hydroxyl stretching band at 3690 cm^{-1}. In all probability these figures indicate that one ketone group is in a five-membered ring and the other in a six-membered ring. The only unexpected feature is the position (1725 cm^{-1}) of the five-membered ring ketone. This value is decidedly low by about 10 cm^{-1}. This shift to the longer wavelength could be caused by such a special feature as intramolecular hydrogen bonding of the carbonyl with a hydroxyl group, and, when it is considered that this compound contains a molecule of methanol of crystallisation, this would seem to be extremely probable and would not necessarily result in displacement of the hydroxyl stretching band which might be expected to absorb at a lower frequency under these conditions. It may be noted, however, that the possibility cannot be overlooked that the 1725 cm^{-1} absorption does represent a six-membered ring ketone which has been displaced to a higher frequency. This type of effect is possible in compounds having two carbonyl groups in close proximity, e.g. 1:2-, 1:3-, 1:4-diketones (95,96).

An attempt was made to reduce the acetate, $C_{31}H_{48}O_4$, by the Clemmensen method. This resulted in recovery of unchanged starting material. The acetate was also recovered unaltered after prolonged treatment with acetic anhydride and sodium acetate in an attempt to form an enol-acetate. In the hope that a pyridazine derivative would be obtained from the 1:4-diketone structure, the acetate was

heated at 200° with hydrazine. This resulted only in the hydrolysis of the C_2-acetoxy grouping. Reduction of the acetate with lithium aluminium hydride in ether gave an uncrystallisable gum which was not purified by chromatography, while treatment with the milder reducing agent sodium borohydride again resulted in the recovery of the starting material after acetylation.

An attempt to introduce a double bond between the carbonyl groups of the acetate by treatment with selenium dioxide resulted in the formation of a neutral substance of unknown constitution containing an additional oxygen atom and showing light absorption in the ultraviolet at 2570Å, ε = 6,800. This substance gives a pale yellow colour with tetranitromethane in chloroform but no colour with ferric chloride indicative of a 1:2-diketone system.

Ruzicka, Jeger and Norymberski(92) reported previously that bromination of the acetate, obtained by hydrolysis of the "O_5-acetate", with two moles of bromine results in the loss of two atoms of hydrogen from the molecule with formation of a yellow substance which absorbs in the ultraviolet at 2500Å, ε = 10,000, and 3500Å, ε = 4,000. Since this bromination would be expected to result in the formation of the dionene (LXXX) from the acetate (LXXIX), this reaction was reinvestigated.



LXXX

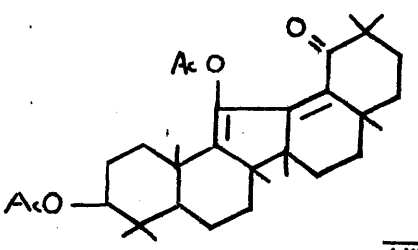
Bromination according to the method of Ruzicka, Jeger and Norymberski did not give a homogeneous compound but gave what was apparently a mixture of two substances. The first product isolated was a coloured substance exhibiting light absorption in the ultra-violet at 2300Å, ε = 7,120, and 3500Å, ε = 8,240 and the second product, which was also coloured, absorbed at 2500Å, ε = 9,700 and 3500Å, ε = 2,100. In view of this result, this method was abandoned in favour of that using one mole of bromine and subsequent results prove that the substance isolated by the Swiss workers was a mixture.

Treatment of the acetate, $C_{31}H_{48}O_4$ with one mole of bromine at 50° followed by heating at 100° for one hour gave an orange coloured substance, $C_{31}H_{46}O_4$, m.p. 211-213°, $[\alpha]_D$ + 91°, showing light absorption in the ultra-violet at 2280Å, ε = 6,700 and 3500Å, ε = 9,300. This substance contains no bromine and gives a red-brown colour with tetranitromethane in chloroform. The methanolic mother liquors from the crystallisation of this substance, which were yellow in colour, became almost colourless on standing for some days and deposited very pale yellow crystals which were recrystallised unchanged to give a compound $C_{31}H_{46}O_4$, m.p. 211-213°, $[\alpha]_D$ - 119° showing light absorption in the ultra-violet at 2500Å, ε = 10,000, and giving no colour with tetranitromethane in chloroform. A synthetic mixture of these two compounds exhibits light absorption at 2500Å and 3500Å, which is comparable with that reported by Ruzicka et. al. for the bromination product.

In a further variation of the method of bromination, the acetate, $C_{31}H_{48}O_4$, was treated at 50° with two moles of bromine which were added in two stages. The product proved to be a colourless bromo-compound

showing light absorption in the ultra-violet at 2120Å, ε = 4,800, and 2,700Å, ε = 5,400, with a low intensity inflection at 3,500Å. This bromo-compound, $C_{31}H_{47}O_4Br$, gives no colour with tetranitromethane and is dehydrobrominated by refluxing with collidine to give the orange coloured substance described above and exhibiting light absorption at 2280Å and 3,500Å.

On the basis of the proposed structure (LXXIX) for the acetate $C_{31}H_{48}O_4$, obtained from the "O_5-acetate", the above bromination results are difficult to interpret. However, the ultraviolet absorption spectrum of the compound, $C_{31}H_{46}O_4$, absorbing at 2500Å is consistent with the light absorption of low intensity shown by cisoid 1:4-diketo-enes (64,85) and suggests therefore that this product is to be formulated as (LXXX)



LXXXI

This formulation was supported by the formation of a well-defined colourless enol-acetate on treatment of the acetate, $C_{31}H_{46}O_4$, with acetic anhydride and fused sodium acetate. The enol-acetate, $C_{33}H_{48}O_5$, gives a yellow colour with tetranitromethane and exhibits selective absorption in the ultra-violet at 2280Å, ε = 7,400, and

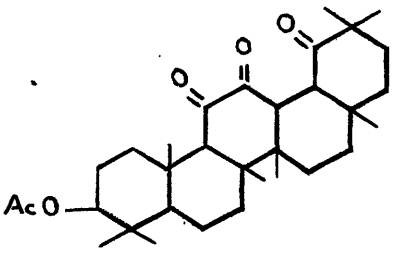
2850Å, ε = 13,690. The intense band at 2850Å closely resembles that shown by compounds containing the chromophore $\overset{\backslash}{C} = \overset{|}{C} - \overset{|}{C} = \overset{|}{C} - \overset{|}{C} = O$ (97) and would be expected to be exhibited by the enol-acetate (LXXXI) derived from (LXXX). The appearance of the band at 2280Å in the enol-acetate absorption spectrum is significant in that this absorption band is shown also by the orange coloured bromination product.

Assuming that the orange product is produced from the compound showing light absorption at 2500Å by an enolisation mechanism, the latter compound was treated with alkali. The product, which was a mixture, showed light absorption at 2500Å, ε = 9,980, and 3500Å, ε = 1,872, and was not examined further.

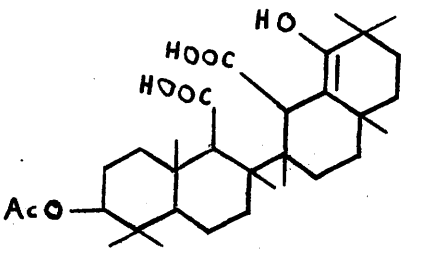
The formulation of the orange bromination product on the basis of (LXXIX) for the acetate, $C_{31}H_{48}O_4$, from which it was obtained, is rendered difficult when account is taken of the properties of the bromination product and in particular of its remarkable ultra-violet absorption spectrum. The compound must, however, possess the same basic structure as the acetate, $C_{31}H_{48}O_4$, since it is smoothly converted to this compound by simple reduction with zinc and acetic acid. In contrast to this, the coloured product is catalytically reduced to give a colourless compound which is not identical with the acetate, $C_{31}H_{48}O_4$ and exhibits light absorption at 2060Å, ε = 1,800, and 2800Å, ε = 650. This product is formulated as $C_{31}H_{50}O_4$, and since the absorption at 2800Å indicates the presence of a carbonyl group, the compound is probably formed by reduction of one of the carbonyls of the bromination product to an alcohol grouping and saturation of a double bond. Insufficient material prevented further investigation of

this product.

It is obvious from the work described above that, while structure (LXXV) affords an adequate explanation of the properties of the "O_5-acetate", some reactions are not easily formulated on the proposed structures of its derivatives. Such anomalies cannot be taken as definite proof that the proposed structure is incorrect and indeed it is difficult, with the evidence available, to otherwise formulate the "O_5-acetate". Moreover, the proposed structure is also supported by consideration of the methods of preparation of the "O_5-acetate". As pointed out previously, the "O_5-acetate" must be produced by a breaking of the C_{10} to C_{19} chain of carbons. From each of the compounds from which the "O_5-acetate" has been obtained (c.f. pp.42) it is theoretically possible to derive structure (LXXXII) by oxidation. This hypothetical compound provides a suitable intermediate preceeding the formation of the "O_5-acetate", since further oxidation would result in the formation of the acid (LXXXIII) which is visualised as forming the keto-lactone (LXXV) by lactonisation of the C_{13}-carboxyl with the enolised C_{19}-ketone and by interaction of the C_{10}-carboxyl with the C_{13}-hydrogen atom. The formation of the "O_5-acetate" through an acid intermediate is confirmed to some extent by the presence of an acidic fraction in the reaction mixture obtained from oxidation of oleana-11:13(18)-dienyl acetate.

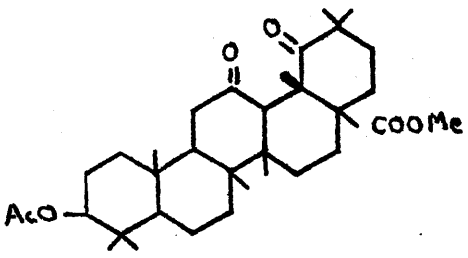


LXXXII

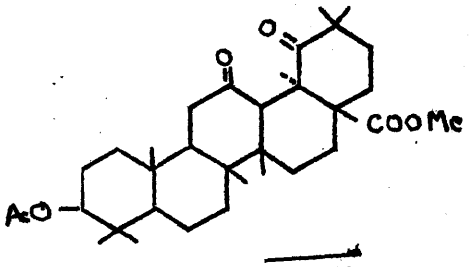


LXXXII

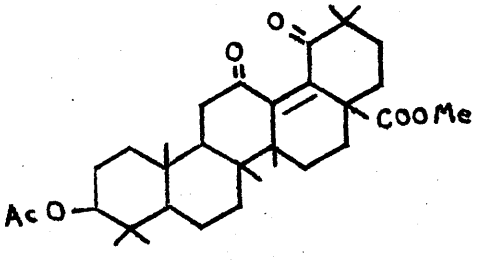
Recently Barton, Holness, Overton and Rosenfelder(85) have shown that treatment of methyl acetyl-12:19-diketo-oleanolate (LXXXIV) with selenium dioxide results in the formation of the "O_7-acetate", whereas a similar oxidation of methyl acetyl-12:19-diketo-18α-oleanolate (LXXXV) results in the recovery of unchanged ester. These results would seem to indicate that the formation of the "O_7-acetate" from (LXXXIV) is preceeded by introduction of the C_{13}:C_{18} double bond to give methyl acetyl-12:19-diketo-olean-13(18)-enolate (LXXXVI) which is then oxidised further to the "O_7-acetate". If this is the case, then the analagous "O_5-acetate" cannot be formed through the intermediate (LXXXII). However, Barton et.al. have also shown that methyl acetyl-12:19-diketo-olean-13:18-enolate (LXXXVI) does not react with selenium dioxide. This is taken as being proof that this compound is not an intermediate in the formation of the "O_7-acetate" from (LXXXIV), and that oxidation of this compound at C_{11} to give the trione type intermediate with subsequent formation of the "O_7-acetate" is possible.



LXXXIV



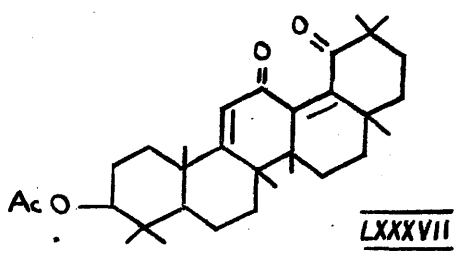
LXXXV



LXXXVI

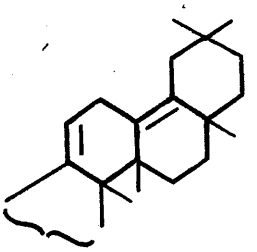
IV. β-Amyradiendionyl Acetate and its Derivatives.

β-Amyradiendionol, initially designated as "Jacob's keto-diol" was first prepared by Jacobs and Fleck(100). The corresponding acetate was subsequently prepared by Ruzicka and Jeger(83), by oxidation of β-amyrin acetate with selenium dioxide, and formulated as (LXXXVII). This formulation has recently been substantiated for the corresponding dienone of oleanolic acid, methyl acetyl-12:19-diketo-oleana-10:13(18)-dienolate, by Barton, Holness, Overton and Rosenfelder(85) who achieved a partial synthesis of this compound from siaresinolic acid. β-Amyradiendionyl acetate (12:19-diketo-oleana-10:13(18)-dienyl acetate) is one of the two major oxidation products which are obtained from compounds of the β-amyrin group (the second being the "O_5-acetate"), and it was from this compound that a series of experiments was undertaken with the object of preparing the non-conjugated dienyl acetates, oleana-10:13(18)-, -10:18-, and -11:18-dienyl acetates, which were required for comparison with a non-conjugated dienyl acetate obtained from iso-β-amyradienonyl acetate(101). The work to be described in this section constitutes part of this series.

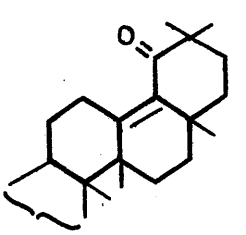


LXXXVII

As a first approach to oleana-10:13(18)-dienyl acetate(LXXXVIII), the hydrogenolysis of β-amyradiendionyl acetate was examined. This reduction had been examined previously by Ruzicka and Jeger(83) who reported the formation of two products, an acetate, $C_{32}H_{48}O_4$, and an acetate, $C_{32}H_{48}O_3$. The former substance showed an ultra-violet absorption maximum at 2420Å, ε = 10,100, and was considered to have been formed by reduction of a carbonyl group to an alcohol group. The second product gave a brown colour with tetranitromethane and was further catalytically reduced to an acetate, $C_{32}H_{50}O_3$, which showed no colour with tetranitromethane and absorbed in the ultra-violet at 2590Å, ε = 6,300; this substance was provisionally formulated as (LXXXIX).



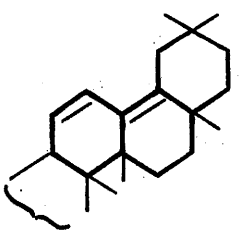
LXXXVIII



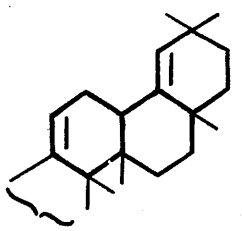
LXXXIX

In the reinvestigation of this reaction, using platinum as a catalyst and acetic acid as solvent two products, $C_{32}H_{50}O_2$, were isolated. The former compound gives a deep yellow colour with tetranitromethane in chloroform and shows an apparent absorption maximum at 2100Å, ε = 10,000, but does not selectively absorb in the ultra-violet above 2200Å. These facts indicated that the

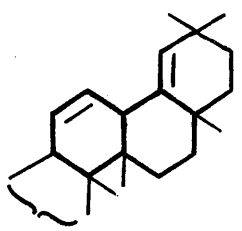
substance was a non-conjugated diene, and this was confirmed when, on treatment with mineral acid, the substance was isomerised to oleana-11:13(18)-dienyl acetate (XC) in high yield. Of the three possible structures, (LXXXVIII)(XCI) and (XCII), for this compound, the first is the most probable; its itense absorption of light between 2100Å and 2250Å suggests that the substance contains a tetra-substituted double bond (17,102), and this with the method of preparation indicates a C_{13}:C_{18} ethylenic linkage. It is therefore concluded that this product is oleana-10:13(18)-dienyl acetate (LXXXVIII), a decision confirmed by its formation by another route to be described. The corresponding alcohol was obtained by hydrolysis, and reacetylation afforded the parent acetate with unaltered physical characteristics.



XC



XCI

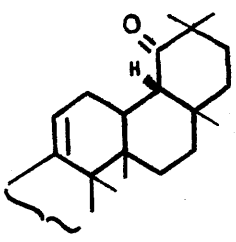


XCII

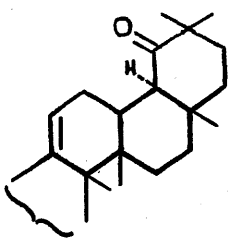
The second product of the catalytic hydrogenolysis of β-amyradiendionyl acetate was 19-keto-olean-10-enyl acetate (XCIII). In support of this view, the compound gives a yellow colour with tetranitromethane and its ultra-violet absorption spectrum indicates the presence of a

triply substituted bond and an isolated carbonyl group. The proposed structure again was confirmed by an unambiguous stepwise synthesis from β-amyradiendionyl acetate. The method will be described later in this section.

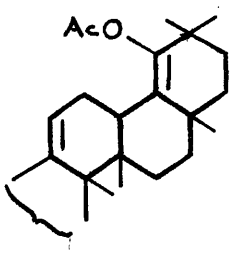
In an attempt to effect conjugation of the double bond with the ketone group, 19-keto-olean-10-enyl acetate was treated with hydrochloric acid in acetic acid. The major product proved to be the isomeric 19-keto-18α-olean-10-enyl acetate (XCIV) which had been obtained previously by an isomerisation using alkali (103). As a by-product in this reaction as small amount of a substance showing light absorption maxima at 2370Å, ε = 20,000, 2450Å, ε = 23,700, 2550Å, ε = 15,500, was isolated. This substance gives a red brown colour with tetranitromethane and the m.p. is depressed on admixture with oleana-11,13(18)-dienyl acetate (XC). Insufficient material prevented further investigation.



XCIII



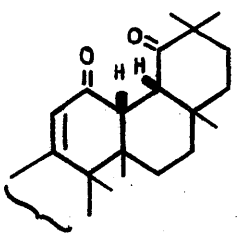
XCIV



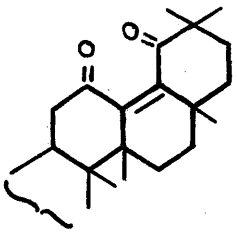
XCV

Two attempts to prepare the enol acetate (XCV) of 19-keto-olean-10-enyl acetate by treatment with isopropenyl acetate and by treatment with a mixture of perchloric acid and acetic anhydride, resulted in the recovery of unchanged starting material.

Attention was next directed to the preparation of oleana-10:18-dienyl acetate (XCI). Barton, Holness, Overton and Rosenfelder(85) observed that methyl acetyl-12:19-diketo-oleana-10:13(18)-dienolate is reduced smoothly by zinc and acetic acid to methyl acetyl-12:19-diketo-olean-10-enolate. By analogy, reduction of the related 12:19-diketo-oleana-10:13(18)-dienyl acetate(LXXXVII) was expected to give 12:19-diketo-olean-10-enyl acetate (XCVI) which would afford a convenient starting material for the preparation of the required diene.



XCVI



XCVII

However, using the conditions of Barton et.al., it was found that reduction of β-amyradiendionyl acetate, unlike the corresponding reduction in the oleanolic acid series, gave a mixture separated by chromatography into three components. The first product was the unconjugated oleana-10:13(18)-dienyl acetate(LXXXVIII) identical with

the product obtained by catalytic hydrogenation of the diendione. The second product was an acetate, $C_{32}H_{48}O_3$ and the third an acetate $C_{32}H_{48}O_4$.

Dealing with the latter product first; this compound showed a characteristic light absorption maximum at 2460Å, ε = 12,400, indicative of an $\alpha\beta$-unsaturated ketone grouping and is probably identical with the acetate $C_{32}H_{48}O_4$ obtained by Ruzicka and Jeger(83) from the catalytic reduction of β-amyradiendionyl acetate and for which the structure(XCVII) was considered and rejected. Ruzicka and Jeger suggested that the acetate may have been produced by reduction of one of the carbonyl groups to a secondary hydroxyl. Barton et.al.(85) however, suggested that this reduction product is 12:19-diketo-olean-10-enyl acetate (XCVI). That this view is undoubtedly correct was shown by the following facts. The acetate was recovered unchanged after treatment with acetic anhydride and was stable to chromic anhydride at room temperature and therefore does not contain a secondary hydroxyl group. The presence of an $\alpha\beta$-unsaturated ketone group having been established by light absorption, the presence of a second carbonyl (at C_{19}) was proved by catalytic reduction of the acetate which gave 19-keto-olean-10-enyl acetate identical with the product obtained by hydrogenolysis of β-amyradiendionyl acetate. Furthermore, the acetate, $C_{32}H_{48}O_4$, was obtained as the sole product in excellent yield by reduction of β-amyradiendionyl acetate with zinc dust in ethanol. This latter reaction provides confirmation of the proposed structure, since, the relatively mild reducing conditions make it unlikely that any part of the molecule other than the double bond between the two carbonyl groups is reduced. This evidence, in toto,

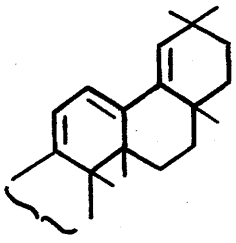
affords adequate proof that the acetate, $C_{32}H_{48}O_4$, is formulated correctly by (XCVI).

The differences between the products obtained from the catayltic reduction of β-amyradiendionyl acetate by this author and those obtained by Ruzicka and Jeger are to be attributed either to difference in the activity of the catalysts used or to the incompleteness of the latter reaction, since, as mentioned above, 12:19-diketo-olean-10-enyl acetate (XCVI) can be further reduced to 19-keto-olean-10-enyl acetate(XCIII) under conditions similar to those used in the hydrogenolysis of β-amyradiendionyl acetate.

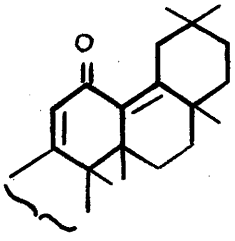
Following from the work described above, it would seem that the two products are formed by concurrent reactions in the hydrogenation of β-amyradiendionyl acetate; the keto-ene (XCIII) as a result of saturation of the $C_{13}:C_{18}$ ethylenic bond followed by hydrogenolysis of the C_{12}-carbonyl group, and the diene (LXXXVIII) by initial hydrogenolysis of one of the carbonyl groups to give 19- and/or 12-keto-oleana-10:13(18)-dienyl acetates, which are then further reduced to the 10:13(18)-dienyl acetate.

The acetate, $C_{32}H_{48}O_3$, obtained as the third product from the reduction of β-amyradiendionyl acetate with zinc and acetic acid, gives a pale yellow colour with tetranitromethane and its ultra-violet absorption spectrum shows intense maxima at 2600Å, ε = 9,250, and 2950Å, ε = 8,450. As a first attempt to establish the structure of this compound it was subjected to catalytic reduction and was converted thereby into oleana-10:13(18)-dienyl acetate (LXXXVIII).

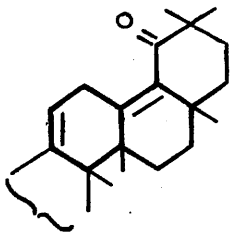
On oxidation with selenium dioxide in dioxan in a sealed tube it was converted into the parent β-amyradiendionyl acetate (LXXXVII). Furthermore, during an attempt to convert it into an enol-acetate by prolonged refluxing with acetic anhydride and potassium acetate, it was oxidised to β-amyradiendionyl acetate, presumably by air oxidation. In view of this remarkable ease of oxidation the substance was again treated with selenium dioxide, under less drastic conditions. β-Amyradiendionyl acetate was again isolated. Reduction of the acetate $C_{32}H_{48}O_3$ with lithium aluminium hydride produced oleana-10:13(18)-dienyl acetate (LXXXVIII), with a small quantity of oleana-10:12:18-trienyl acetate (XCVIII). Because of the remarkable ultra-violet absorption spectrum of this compound, considerable pains were taken to ensure its homogeneity; on alkaline hydrolysis it gave a well-defined alcohol, $C_{30}H_{46}O_2$, showing essentially the same light absorption as the parent acetate. Reacetylation of the alcohol yielded the acetate with unaltered physical characteristics.



XCVIII

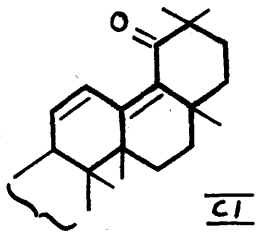


XCIX



C

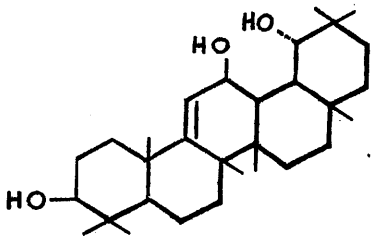
This acetate is formed from β-amyradiendionyl acetate by the reduction of one of the carbonyl groups to a methylene and consequently is to be formulated as ether (XCIX) or (C). It was realised, however, that the nature of the absorption spectrum is roughly consistant with the view that the substance is an inseparable mixture of (C) and the conjugated dienone (CI). This being so, the maximum at 2950Å is attributable to the dienone chromophore of (CI) and the maximum at 2600Å is due to the αβ-unsaturated ketone chromophore of (C).



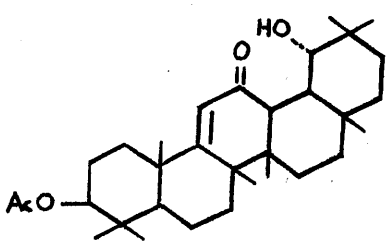
CI

This view became improbable when the acetate was found to be unchanged after treatment with strong mineral acid, and in particular, when the ultra-violet absorption spectrum of the acetate was found to be unchanged; if the mixed crystal view were correct, mineral acid treatment would yield the homogenous conjugated dienone (CI) with a consequent change in light absorption. This indicated that the acetate $C_{32}H_{48}O_3$, is 12-keto-oleana-10:13(18)-dienyl acetate (XCIX) and this was confirmed by an unambiguous partial synthesis carried out by Beaton (103)

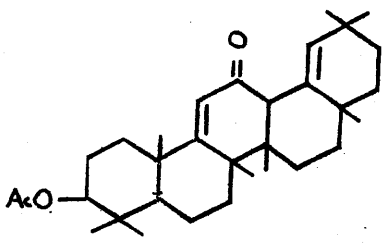
Briefly, this synthesis involved reduction of 12:19-diketo-olean-10-enyl acetate (XCVI) with lithium aluminium hydride to the triol (CII) which was oxidised with manganese dioxide followed by partial acetylation to give 12-keto-olean-10-en-2:19α-diol-2-acetate (CIII). Dehydration of the latter gave 12-keto-oleana-10:13-dienyl acetate (CIV) which on treatment with mineral acid readily gave 12-keto-oleana-10:13(18)-dienyl acetate (XCIX) identical with the acetate $C_{32}H_{48}O_3$.



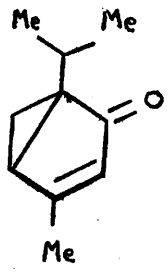
CII



CIII



CIV

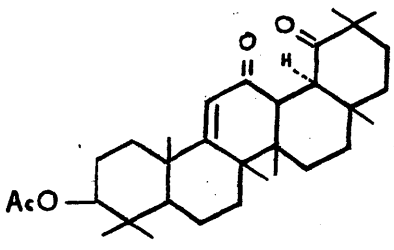


CV

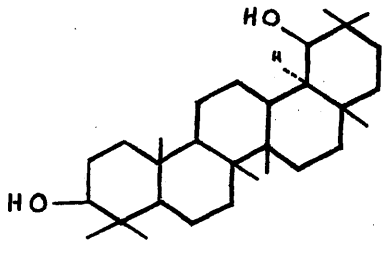
The establishment of the structure of the acetate $C_{32}H_{48}O_3$ as (XCIX) appears to provide the first example in either the steriod or triterpene fields of a dienone in which the chromophore is spread over two rings. The ultra-violet absorption spectra of a number of conjugated dienones containing the chromophoric group $\overset{|}{C}=\overset{|}{C}-\overset{||}{\underset{O}{C}}-\overset{|}{C}=\overset{|}{C}$ have been recorded (71). The acyclic phorone shows a single absorption band at 2650Å in alcohol whilst cyclic dienones of that type which contain the chromophore in a single six-membered ring (e.g. steroid 1:4-dien-3 ones) show an intense absorption maximum at 2400Å. It is probable that the abnormal absorption spectrum of 12-keto-oleana-10:13(18)-dienyl acetate is to be attributed to the distorted geometry of the chromophoric group. There is a noteworthy resemblance in the shape of the absorption curves of (XCIX) and umbellulone (CV) which shows anomolous absorption at 2200Å, ε = 5,000, and 2650Å, ε = 2,900, (104) which has been associated with the distorted geometry of the total chromophone.

The reduction of β-amyradiendionyl acetate with zinc and acetic acid proceeds in two ways. Firstly, reduction of the $C_{13}:C_{18}$ double band to give (XCVI) and secondly, reduction of the C_{19}-carbonyl to give the dienone (XCIX). This latter compound is an intermediate in the formation of oleana-10:13(18)-dienyl acetate (LXXXVIII), since, on further treatment with zinc and acetic acid the diene was obtained from a pure specimen of the dienone. The reduction of the $C_{13}:C_{18}$ ethylenic bond of β-amyradiendionyl acetate in this reaction and that using zinc andethanol involves cis-addition of hydrogen as assumed by Barton

et.al. (85) for the analgous reduction of methyl acetyl-12:19-diketo-oleana-10:13(18)-dienolate. This was proved in the following way. Treatment of 12:19-diketo-olean-10-enyl acetate (XCVI) with alkali followed by reacetylation of the product gave the isomeric 12:19-diketo-18α-olean-10-enyl acetate (CVI) which was catalytically reduced by Johnston(103) to give 19-keto-18α-olean-10-enyl acetate (XCIV), identical with that obtained by acid or alkaline treatment of 19-keto-olean-10-enyl acetate (XCIII).



CVI



CVII

The conversion of 19-keto-18α-olean-10-enyl acetate by Beaton (103) into 18α-oleana-2:19β-diol (CVII) which was first described by Ames, Davy, Halsall and Jones (105), proves that the configuration at C_{13} in 19-keto-olean-10-enyl acetate, 12:19-diketo-olean-10-enyl acetate, and its alkali-stable isomer 12:19-diketo-18α-olean-10-enyl acetate, is the same (β) as that in morolic acid (64,61). It follows therefore that the configurations at C_{13} and C_{18} in 12:19-diketo-olean-10-enyl acetate are as shown in (XCVI) and that reduction of

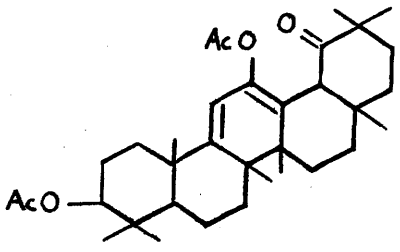
β-amyradiendionyl acetate has involved cis-addition . The first case of such a cis-addition of hydrogen to an ene-1:4-dione was that reported by Barton et.al. (85) and a similar addition of hydrogen to a steriod-ene-1:4-dione has since been reported (106).

In a further investigation of the properties of 12:19-diketo-olean-10-enyl acetate, attempts were made to enolise one or both ketone groupings in the formation of an enol-acetate by treatment with sodium acetate and acetic anhydride, and by treatment with isopropenyl acetate. The former method gave 19-keto-12-acetyoxy-oleana-10:12-dienyl acetate (CVIII) which shows selective absorption in the ultra-violet at 2800Å, ε = 9,150, characteristic of a homoamular diene system. This enol-acetate was recovered unchanged from attempted catalytic hydrogenolysis. The second method of enol-acetate preparation using isopropenyl acetate gave a product, $C_{32}H_{46}O_3$, which gives a yellow colour with tetranitromethane and exhibits light absorption at 2200Å, ε = 5,850, and 3240Å, ε = 15,400.

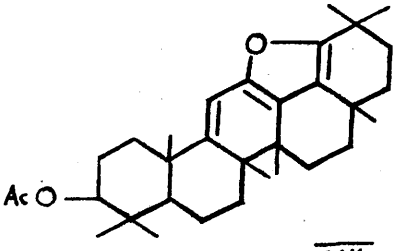
Consideration of the method of preparation of this compound indicates that it is 12:19-epoxy-oleana-10:12:18-trienyl acetate(CIX) formed by enolisation of the C_{12}- and C_{19}-carbonyl groups and subsequent loss of the elements of water from the intermediate diol. This is confirmed to some extent by comparison with the analagous sulphur compound prepared by Jacobs and Fleck (100) and formulated as (CX) by Ruzicka and Jeger(83). This thio-derivative shows light absorption at 3200Å, ε = 12,000. The substantial difference in the position of the light absorption maximum of the compound (CIX) from that of furyl ethylene (CXI) at 2600Å, ε = 16,600, (107), is to be

attributed to the degree of substitution in the chromophoric grouping; a similar discrepancy has been noted (83) between the absorption spectra of (CX) and α-vinyl thiophene. The light absorbing properties of both (CIX) and (CX) bear closer resemblance to that of oleana-10:12:18-trienyl acetate at 3100Å (108).

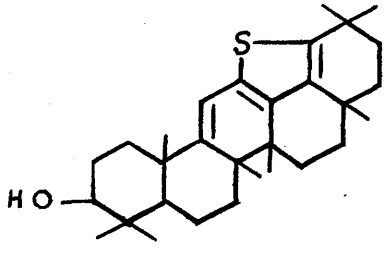
The absorption maximum at 3240Å indicates that the compound (CIX) is related to the substance of unknown constitution isolated by Barton et.al. (85) during the conversion of methyl 19-hydroxy-12-keto-oleanolate acetate to methyl 12:19-diketo-18α-olean-10-enolate; this substance showed an absorption maximum at 3190Å, ε = 15,000.



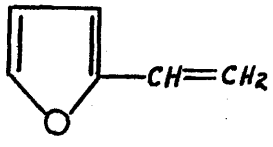
CVIII



CIX



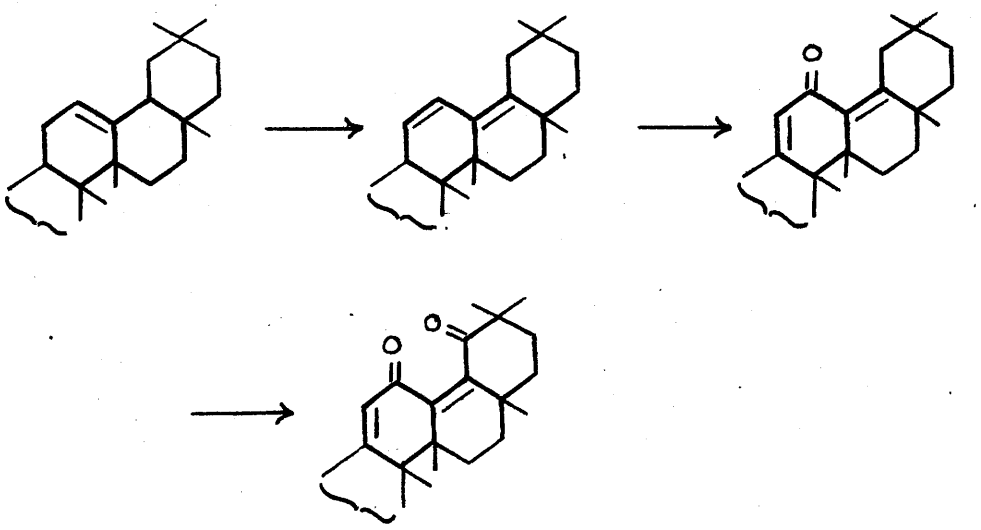
CX



CXI

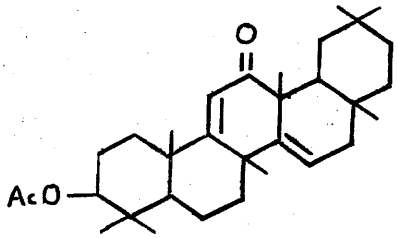
As a conclusion to this part of the work, comment may be made on the formation of β-amyradiendionyl acetate. In a recent discussion, Barton et.al. (85) stated that the diendione is never produced if oxygen substituents are already present in the molecule, even if they are at positions 12 and/or 19.

By virtue of the work on 12-keto-oleana-10:13(18)-dienyl acetate which has been detailed herein, this can not be true. Moreover, while these authors' hypothesis, that selenium dioxide oxidations of -enes and -dienes to the β-amyradiendionyl structure proceed via the 10:12:18-triene, may still be true, the 12-keto-10:13(18)-diene does offer an alternative intermediate for some of these oxidations. For example the oxidation of β-amyrin acetate may proceed through the stages shown below. The fact that the dienone has not been isolated as an intermediate is to be explained by the relative ease of oxidation of this compound to the diendione.

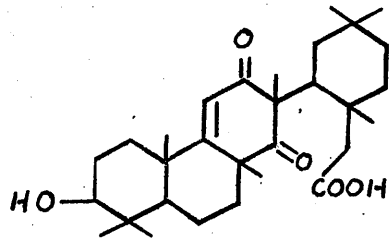


V. iso-β-Amyradiendionyl Acetate and its Derivatives.

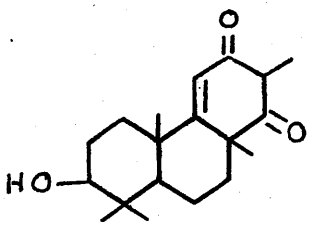
In an attempt to correlate 12-keto-olean-10-enyl acetate with 12:19-diketo-oleana-10:13(18)-dienyl acetate, Green, Mower, Picard and Spring(86) obtained iso-β-amyradiendionyl acetate by selenium dioxide oxidation of the former. This compound gives a yellow colour with tetranitromethane and shows light absorption at 2450Å, ε = 10,000. iso-β-Amyradienonyl acetate was obtained also by the action of bromine on 12-keto-olean-10-enyl acetate by these workers and by Jeger and Ruzicka(82). The formula proposed by Green et.al. has been shown to be in error by subsequent work on the iso-β-amyradienonyl structure and by the preparation of the dienone (XCIX) reported in this thesis (Section IV). The structure (CXII) proposed by Jeger and Ruzicka, in which iso-β-amyradienonyl acetate is represented as being formed from 12-keto-olean-10-enyl acetate by migration of the C_{14}-methyl group and introduction of the C_{14}:C_{15}-double bond, has been substantiated to some extent by recent work of Meisels Jeger and Ruzicka (109). These workers succeeded in oxidising iso-β-amyradienonyl acetate to the hydroxy diketo-acid (CXIII), the methyl ester of which on pyrolysis gave an acid fraction represented as the hydroxy diketone (CXIV). This hydroxy diketone, by subsequent methylation and acetylation, gave two isomeric methyl ethers which were formulated as (CXV) and (CXVI), and were identical with two products obtained from α-amyrin by a similar series of reactions.



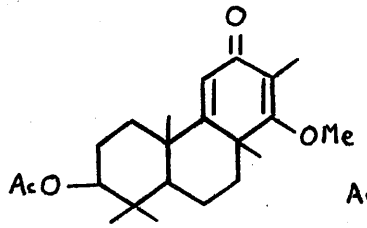
CXII



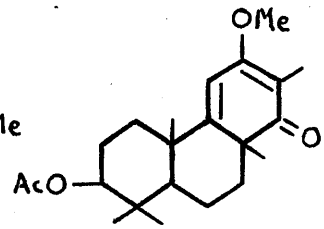
CXIII



CXIV

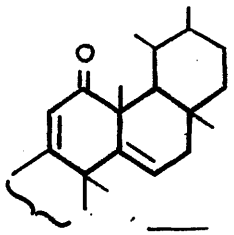


CXV

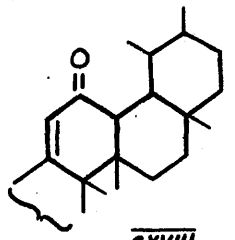


CXVI

In a recent discussion, Budziarek, Johnston, Manson and Spring (77) pointed out that these reactions cannot be constructed as proof of migration of the angular methyl group, and moreover, that certain reactions of iso-β-amyradienonyl acetate and the analagous iso-α-amyradienonyl acetate indicate that such a migration has not taken place. In particular, the catalytic reduction of iso-α-amyradienonyl acetate (CXVII) into 12-keto-urs-10-enyl acetate (CXVIII) has been offered as proof against such a hypothesis (110). The work detailed herein was undertaken with a view to establishing the correct structure of iso-β-amyradienonyl acetate, and as a first approach, the catalytic hydrogenation of this compound was re-examined.



CXVII



CXVIII

As mentioned above, the reduction of iso-α-amyradienonyl acetate by catalytic hydrogenation results in the formation of a known α-amyrin derivative. On the other hand, hydrogenolysis of iso-β-amyradienonyl acetate, according to Budziarek et.al.(77), gives a compound which is designated as iso-β-amyradienyl acetate and for which no structural formula is given. This compound is unique in that it did not show selective absorption in the ultra-violet yet gave a red colouration with the tetranitromethane reagent, indicating a conjugated diene system. The same product was obtained by hydrogenolysis of the diacetate of the diene-diol obtained by reduction of iso-β-amyradienonyl acetate with lithium aluminium hydride.

Reduction of iso-β-amyradienonyl acetate gave a product which was identical with the iso-β-amyradienyl acetate obtained by Budziarek et.al. The compound, although exhibiting no selective absorption in the ultra-violet above 2200Å, is characterised by an absorption maximum at 2120Å, ε = 4,450.

As a first attempt to establish the structure of this compound, it was treated under drastic conditions with hydrochloric acid, when it was hoped that conjugation of the unsaturated centres would result.

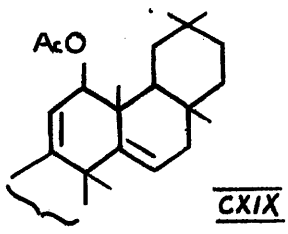
The starting material was recovered unchanged. On oxidation with potassium permanganate in acetic acid, it gives as the main product, a substance which contains one atom of oxygen more than the starting material and shows no appreciable absorption in the ultra-violet and gives no colour with tetranitromethane. The second product from the oxidation contains two atoms of oxygen more than the starting material and likewise gives no colour with the tetranitromethane reagent.

The remarkable stability of the hydrogenation product of iso-β-amyradienonyl acetate to mineral acid indicated that this substance might be a mono-ene. Moreover, except for the colour produced with tetranitromethane, the compound had the properties of a mono-ene; being apparently oxidised to a compound which was saturated to tetranitromethane and transparent in the ultra-violet. This latter product was shown to be an oxide by the formation of a diene exhibiting light absorption at 2580Å, ε = 21,000, on treatment with hydrochloric acid. The formation of this diene and not a triene in this reaction is further proof of the saturation of the oxide and consequently that the product obtained by the hydrogenation of iso-β-amyradienonyl acetate is not a diene. This product is designated neo-β-amyrin acetate.

Following the proof that neo-β-amyrin acetate contains one double bond, it was realised, by consideration of the light absorption, that this double bond must be tetra-substituted and therefore not one of those originally present in the Ruzicka formulation of iso-β-amyradienonyl acetate. The mechanism of the hydrogenation and subsequent formulation of the structure of neo-β-amyrin acetate was

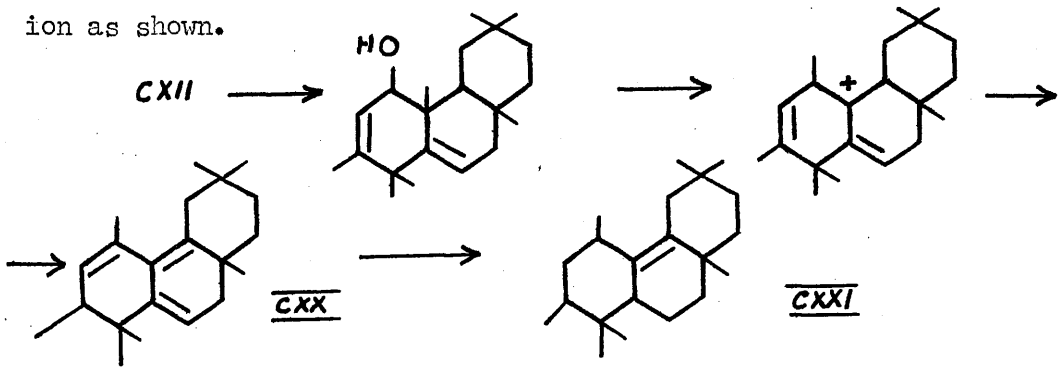
elucidated as follows.

It was found (111) that treatment of the diacetate of the lithium aluminium hydride reduction product of iso-β-amyradienonyl acetate with hydrochloric acid gives a cross-conjugated triene which on catalytic reduction rapidly absorbs two moles of hydrogen to form neo-β-amyrin acetate. Also, the same diacetate (CXIX) was found to be stable to catalytic reduction except in the presence of a trace of mineral acid. This stepwise reduction of iso-β-amyradienonyl acetate suggested that the catalytic hydrogenation proceeds via the triene, and indeed it was found that the rate of hydrogenation was increased by a factor of four by addition of one drop of hydrochloric acid to the reaction mixture.

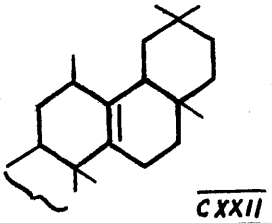


CXIX

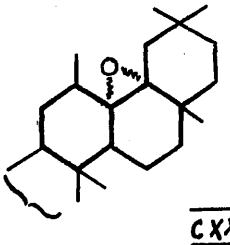
On the basis of the Ruzicka formulation, the reduction of iso-β-amyradienonyl acetate is formulated as proceeding through the carbonium ion as shown.



The structure (CXXI) for neo-β-amyrin acetate is preferred to the alternative (CXXII) on the basis of the known stability of the $C_{13}:C_{18}$ bond to catalytic reduction and the distinct similarity of the absorption spectrum of neo-β-amyrin acetate below 2200Å to that of olean-13:(18)-enyl acetate (17). The oxide of neo-β-amyrin acetate is formulated as (CXXIII).

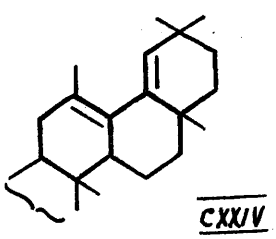


CXXII

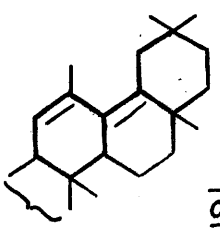


CXXIII

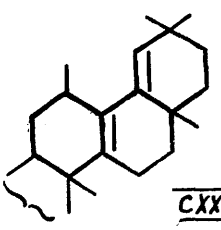
Three conjugated dienes (CXXIV), (CXXV), and (CXXVI) may be theoretically derived from the oxide (CXXIII). However, transoid chromophores, in general, absorb with higher intensity than cisoid chromophores (64,112), and therefore structure (CXXIV) is rejected for the diene derived from the oxide of neo-β-amyrin acetate. On the evidence available, no distinction can be made with certainty between the remaining two alternatives, but since the diene (CXXV) would be expected to exhibit light absorption properties similar to oleana-11:13(18)-dienyl acetate, structure (CXXVI) is preferred.



CXXIV

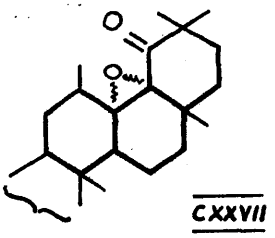


CXXV



CXXVI

The minor product from the oxidation of neo-β-amyrin acetate with potassium permanganate was at first thought to be a 1:2-glycol. This was rejected when the infra-red absorption spectrum showed that it does not contain a hydroxyl group. The compound shows an intense absorption at 1691 cm^{-1}, and this with the low intensity inflection at 2950Å in the ultra-violet is taken as being indicative of an isolated carbonyl group, and therefore structure (CXXVII) is proposed for this derivative.



CXXVII

Since the work on neo-β-amyrin acetate outlined in this section is explained adequately on the basis of the Ruzicka formulation for iso-β-amyradienonyl acetate, this may be offered as additional evidence in favour of such a structure.

EXPERIMENTAL.

All melting points are uncorrected. Specific rotations were estimated at 15-25°C in chloroform solution using a 1 dcm. tube, unless otherwise stated.

"Stabilized acetic acid" refers to glacial acetic acid which has been refluxed and distilled over chromic anhydride.

Extraction of Oleanolic Acid from Spent Cloves.

Finely ground cloves (2.7 kg.) were heated under reflux with ether (7 l.) for twenty four hours, after which more ether (7 l.) was added and heating continued for twenty four hours. The solution was filtered, washed with aqueous alkali (N.,4) and the potassium salt filtered, using a "Hyflo" filter pad on the paper. The dried salt was dissolved in methylated spirits and the hot solution filtered and acidified (congo red) with hydrochloric acid. Crude oleanolic acid (120 g) was collected, dried in air (m.p. 290°) and purified by acetylation.

Acetyl Oleanolic Acid.

Crude oleanolic acid (20g) was dissolved in boiling acetic anhydride (140 ml.) and the solution was heated under reflux for three hours. The diacetyl compound which crystallised from the solution was collected and dissolved in boiling methanol and the solution refluxed for two hours and allowed to cool. Recrystallisation of the product from methanol gave acetyloleanolic acid (15g) as needles m.p. 260-261°C. Wedekind and Schicke (Z.Physiol.Chem.195, 132) give m.p.268° for this acid.

Oxidation of Acetyloleanolic Acid with Chromic Anhydride.

Acetyloleanolic acid (10g) was dissolved in stabilized acetic acid (550 ml.) and to this solution was added, dropwise over a period of two hours, chromic anhydride (6.7g) dissolved in a little water and glacial acetic acid (385 ml.) , according to the method of Ruzicka and Cohen(12). The well stirred mixture was kept at room temperature throughout the addition. Stirring was continued for a further thirty minutes and then the mixture was allowed to stand at room temperature for two days. After decomposing the excess anhydride with methanol, the solvent was removed under reduced pressure and the dark green residue dissolved in a mixture of chloroform and water, to which was added enough ether to give two separable layers. The ether-chloroform extract was washed with water and then with sodium hydroxide solution (200 ml;, 2N). The emulsion was filtered under slightly reduced pressure and the two layers of the filtrate were separated.

The ether-chloroform layer was washed with sodium hydroxide solution (100 ml;, 2N), and with water till the washings were neutral, and dried over sodium sulphate. The residue (4.4g.) obtained on evaporation of the ether-chloroform, was further purified by filtration through a short column of alumina, using a 1:1 mixture of benzene and light petroleum (b.p.60-80°) as eluant. Crystallisation from methanol-chloroform gave acetylketo-oleanolic lactone as needles, m.p. 283-285°, $[\alpha]_D$ + 10°(C, 1.0).

Found :	C, 75·1 ;	H, 9·6 %
Calculated for $C_{32}H_{48}O_5$:	C, 75·0 ;	H, 9·4 %

The impure sodium salt remaining on the filter was dried and dissolved in ethanol. The filtered solution was made acid to congo red, and the oxide acetylketo-oleanolic acid (2-acetoxy-11-keto-olean-12-en-17-oic acid) (3.5g) which crystallised on cooling was recrystallised many times from methanol-chloroform. The acid crystallises as fine needles, m.p. 267-268°.

Light absorption in ethanol ; maximum at 2,500 Å., ε = 11,900.

Treatment of Acetylketo-oleanolic Lactone with Hydrogen Bromide.

(a) In ethanol.

A solution of acetylketo-oleanolic lactone (1 g) in dry ethanol (50 ml.) was saturated with dry hydrogen bromide, and refluxed for thirty minutes. The mixture was again saturated with hydrogen bromide and refluxed for thirty minutes. After cooling to room temperature the mixture was saturated with hydrogen bromide, kept overnight, and then diluted with water. The precipitate was extracted with ether, the extract washed with aqueous sodium hydroxide (3%) and the alkaline solution washed with ether before acidification with dilute hydrochloric acid. The precipitated solid was collected by means of ether, and the ethereal solution washed with water, dried over magnesium sulphate and evaporated. The solid residue (0.8g.) was crystallised from methanol-chloroform to give keto-iso-oleanolic acid (12-keto-olean-10-enolic acid) as prisms, m.p. 320°(decomp.), $[\alpha]_D$ + 45° (C, 1·14).

Found :	C, 76.8 ;	H, 10.2 %
$C_{30}H_{46}O_4$ requires :	C, 76.55 ;	H, 9.9 %

Light absorption in ethanol; max. at 2,490Å, ϵ = 10,000.

Acetyl-12-keto-olean-10-enolic Acid.

12-Keto-olean-10-enolic acid (o.1 g) in pyridine (1 ml.) and acetic anhydride (3 ml.) was heated on the steam bath for one hour. The mixture was poured into water, extracted with ether, and the ether layer washed with dilute hydrochloric acid and aqueous sodium hydroxide (3%).

Acidification of the alkaline extract gave the free acid which was collected with ether, and the ethereal solution washed with water, dried over magnesium sulphate and evaporated. The residue crystallised from methanol-chloroform as prisms, m.p. 328°(decomp.), $[\alpha]_D$ + 61°(C, 1.0).

Found : C, 74.6 ; H, 9.7 %

Calculated for $C_{32}H_{48}O_5$: C, 74.9 ; H, 9.5 %

Ruzicka, Cohen, Furter and Sluys-Veer(71) give m.p. 328-30°, $[\alpha]_D$ + 61°, and Kitasato(72) gives m.p.324-330° $[\alpha]_D$ + 47°, for this acid.

The acid was recovered unchanged after sublimation in vacuum at 250°.

(b) In acetic acid.

Acetylketo-oleanolic lactone (0.9g) was dissolved in acetic acid (7 ml.); the solution was saturated with dry hydrogen bromide and allowed to stand at room temperature for a week, according to the method of Kitasato(72). The solution was poured into water and the product collected by means of ether. The ethereal extract was then worked up in a manner similar to that described under method(a).

The acid fraction crystallised from methanol-chloroform to give acetyl-12-keto-olean-10-enolic acid as prisms, m.p.326°(decomp.), undepressed on admixture with that described above under method(a).

Methyl 12-Keto-olean-10-enolate (Methyl iso-Keto-oleanolate) - 12-Keto-olean-10-enolic acid (0.2g) was dissolved in dry ether (50 ml.) and to this solution was added an ethereal solution of diazomethane (50 ml.) The misture was allowed to stand for two hours and the excess of diazomethane was then evaporated off. The ether solution was washed with sodium bicarbonate solution(3%), water, and dried over sodium sulphate. The residue on evaporation of the ether gave methyl-12-keto-olean-10-enolate as fine needles from methanol-chloroform, m.p. 232-233°, $[\alpha]_D$ + 40°(C, 0.53).

Found : C, 77.0 ; H, 10.2 %

Calculated for $C_{31}H_{48}O_4$: C, 76.8 ; H, 10.0 %

Light absorption in ethanol : max. at 2,500 Å, ϵ = 10,900.

Kitasato(72) gives m.p. 216°, $[\alpha]_D$ + 25.7°, for this ester.

Methyl Acetyl-12-keto-olean-10-enolate (Methyl Acetylketo-iso-oleanolate) Methyl Acetyl

Methyl-12-keto-olean-10-enolate(0.12g.) was acetylated with acetic anhydride (3 ml.) and pyridine(1 ml.) on the steam bath for one hour. The mixture was poured into water, extracted with ether, and the ether layer washed with dilute hydrochloric acid, aqueous sodium hydroxide(3 %), and water. After drying over sodium sulphate, the ether was evaporated and the residue crystallised from methanol as plates, m.p. 207-208°, $[\alpha]_D$ + 56° (C, 0.9).

Found : C, 75.5 ; H, 9.7 %

Calculated for $C_{33}H_{50}O_5$: C, 75.2 ; H, 9.6 %.

Methyl Acetyloleanolate.

Acetyl oleanolic acid (10 g.) in dry ether (500 ml.) was treated with an excess of diazomethane in ether. The mixture was allowed to stand at room temperature for two hours and the excess of diazomethane was destroyed with acetic acid. The ethereal solution was washed with aqueous sodium hydroxide (200 ml; 3%) and then with water. The residue obtained on evaporation of the dried (Na_2SO_4) ethereal solution was crystallised from ethanol to give methyl acetyl oleanolate (9.6g) as prismatic needles, m.p. 219-221°, $[\alpha]_D$ + 67° (C, 0.67).

Methyl Acetyl-12-keto-oleanolate (Methyl Acetyldihydroketo-oleanolate).

Methyl acetyloleanolate (5.2g.) was dissolved in glacial acetic acid (178 ml) at 80°. To this solution, hydrogen peroxide (22.3 ml; 40 vols.) in glacial acetic acid (45 ml.) was added dropwise, with stirring, according to the method of Ruzicka and Cohen(12). The addition was made over a period of one hour. A small amount of unchanged ester, which crystallised on cooling the solution, was removed by filtration. The filtrate was heated on the steam bath and the product was precipitated by addition of water, collected, washed with water, and dried in vacuum over caustic potash. Crystallisation from methanol gave methylacetyl-12-keto-oleanolate (4.8g.) as needles m.p.194-195°. The product gave nocolouration with tetranitromethane in chloroform.

Bromination of Methyl Acetyl-12-keto-oleanolate.

Methyl acetyl-12-keto-oleanolate (13 g.) in acetic acid (850 ml.) was treated with a few drops of 48% aqueous hydrobromic acid, and then with a solution of bromine in acetic acid (85.5 ml; 5%) at 40°. The solution was kept at room temperature overnight before being heated on the steam bath for thirty minutes. The mixture was then poured into water, and the precipitated solid collected, washed with water, dried *in vacuo* and crystallised from methanol, to give methyl acetyl-12-keto-olean-10-enolate (8.5 g.) as plates, m.p. 208-209°, $[\alpha]_D$ + 57° (*C*, 1.06).

Found : C, 75.2 ; H, 9.7 %

Calculated for $C_{33}H_{50}O_5$: C, 75.2 ; H, 9.6 %

Light absorption in ethanol : max. at 2,500 Å, ε = 10,000.

A mixture with the specimen (m.p.207-208°) obtained from acetylketo-oleanolic lactone had a m.p. 207.5 - 209°. Methyl acetyl-12-keto-olean-10-enolate crystallises from methanol as either plates or prismatic needles according to the concentration of the solution, the two forms being interchangeable.

Methyl 12-Keto-olean-10-enolate.

Methyl acetyl-12-keto-olean-10-enolate (0.2g.), prepared as above, was dissolved in ethanolic potassium hydroxide solution (10 ml.; 5%) and the solution heated under reflux for two hours. The mixture was poured into water and the solid collected with ether. The ethereal solution was washed with water, dried (Na_2SO_4) and evaporated. The residue crystallised from aqueous methanol to give methyl 12-keto-olean-10-enolate as needles, m.p. 233°, $[\alpha]_D$ + 42° (*C*, 0.95).

Found	:	C, 77.1	;	H, 10.1 %
Calculated for $C_{31}H_{48}O_4$	:	C, 76.8	;	H, 10.0 %.

A mixture of this ester with the hydroxy-ester (m.p.232-233°) from acetylketo-oleanolic lactone had a m.p. 233°.

Methyl Acetylolean-10-enolate.

Methyl acetyl-12-keto-olean-10-enolate (1 g.) in stabilized glacial acetic acid (75ml.) was shaken with hydrogen in the presence of platinum (0.4g.) at room temperature and atmospheric pressure for two days, during which the equivalent of approximately 2 mols. of hydrogen was absorbed. The solution was filtered, the filtrate diluted with water, and the crystalline solid, m.p. 172-177°, collected. After two recrystallisations from methanol-chloroform the product had m.p. 172-177° and showed an absorption maximum in ethanol at 2830Å (ε =880). The solid (0.47g.) was dissolved in light petroleum (b.p.60-80°; 100 ml.) and chromatographed on activated alumina (Grade II; 15 x 2 cm.). Light petroleum (b.p. 40-60°; 620 ml.) and light petroleum-benzene (4:1 ; 100 ml.) eluted a solid (235mg.), m.p. 184-187°, crystallisation of which from methanol-chloroform gave methyl acetylolean-10-enolate as needles, m.p. 191.5-192.5°, $[\alpha]_D$ + 52°, + 52° (C, 0.84; 0.40.).

Found	:	C, 77.4	;	H, 10.4 %.
$C_{33}H_{52}O_4$ requires	:	C, 77.3	;	H, 10.2 %

Methyl acetylolean-10-enolate gives a yellow colour with titranitromethane in chloroform; it does not show selective absorption in the ultra-violet above 2200 Å.

After the column had been washed with light petroleum-benzene (4:1 ; 500 ml.), benzene (200 ml.) eluted a solid (42 mg.) m.p. 191-196°, λ max. 2830Å ($\epsilon \simeq 1500$). This fraction was not obtained pure but probably contained an appreciable quantity of methyl acetyloleana-10:12-dienolate.

Methyl Olean-10-enolate.

Methyl acetylolean-10-enolate, (0.1g.) was treated with boiling ethanolic potassium hydroxide (10 ml.; 5%) for two hours. The product, methyl olean-10-enolate, was isolated by means of ether in the usual way and crystallised from methanol-chloroform as needles m.p. 213-214°, $[\alpha]_D$ + 41° (C, 0.93).

Found :	C, 79.1	;	H, 11.0 %
$C_{31}H_{50}O_3$ requires :	C, 79.1	;	H, 10.7 %

Treatment of Acetyl-12-keto-olean-10-enolic Acid with Quinoline.

Acetyl-12-keto-olean-10-enolic acid (0.3g) in quinoline(50 ml.) was heated for two hours at 250-255°. The cooled solution was poured into dilute hydrochloric acid (250 ml.; 7%), and the mixture extracted with ether. The ethereal extract was washed successively with dilute hydrochloric acid, sodium hydroxide solution and water, and dried over magnesium sulphate. Removal of the solvent gave a dark brown residue (46 mg.) which after being washed with a little methanol was crystallised from methanol, yielding needles, m.p. 278-281°; a mixture with acetylketo-oleanolic lactone (m.p. 280-282°) had m.p. 262-270°.

Light absorption in ethanol ; max. at 2570Å, ϵ = 14,200.

Acidification of the alkaline washings of the ethereal extract followed by crystallisation of the product from methanol-chloroform, gave acetyl-12-keto-olean-10-enolic acid (0.24g.) as prisms m.p.316-319° (decomp.), undepressed on mixed melting with starting material.

Reduction of Methyl Acetyl-12-keto-olean-10-enolate with Sodium and Amyl Alcohol.

To a boiling solution of methyl acetyl-12-keto-olean-10-enolate (0.75g.) in Technical amyl alcohol(20 ml.), sodium (0.75g.) was added over a period of 2-3 minutes. After the initial reaction had subsided, a further quantity of sodium (0.75g.) was added and the mixture was refluxed for forty minutes. Amyl alcohol (8 ml.) was added and heating continued for a further hour. To the cooled mixture, hot water was added to destroy the sodium amyl oxide, and the amyl alcohol was distilled off in steam. A brown resinous solid was precipitated as the distillation proceeded, which was filtered off and dissolved in a mixture of chloroform and ether. The ether-chloroform solution was washed with water and evaporated to dryness after being dried over magnesium sulphate. The residue was dissolved in acetic anhydride (25 ml.) and fused sodium acetate (0.3g.) was added and the solution was heated under reflux for one hour. The cooled reaction mixture was poured into water and the product isolated by means of ether in the usual way. The resultant gum was dissolved in petrol (150 ml.) and chromatographed on activated alumina. Only two crystallisable fractions were obtained from the chromatogram. Both fractions crystallised from methanol, m.p. 115°(approx.) and neither gave a colouration with tetranitromethane in chloroform, or showed any

appreciable absorption above 2,200Å.

Reduction of Methyl Acetyl-12-keto-olean-10-enolate with Sodium Amalgam.

Sodium amalgam (50g.; 3%) was added to a boiling solution of methyl acetyl-12-keto-olean-10-enolate (1g.) in ethyl alcohol (250 ml.; 90%). The mixture was heated under reflux for three hours, cooled, filtered acidified with dilute hydrochloric acid and the precipitated solid extracted with ether. The ether extract was washed with water, dried ($MgSO_4$), and evaporated to dryness. The residue was redissolved in ethyl alcohol and the above procedure repeated. The product obtained gave a yellow colour with tetranitromethane and was dissolved in acetic anhydride (50 ml.), fused sodium acetate (0.5g.) was added and the solution was refluxed for one hour.

Working up the acetylation mixture as before gave a product which crystallised from methanol-chloroform as needles, m.p.180°(range), which gave a brown colour with tetranitromethane in chloroform.

The product was heated under reflux with ethanolic potassium hydroxide (100 ml.; 4%) for one hour and the resulting alcohol was reacetylated as described above. Crystallisation of the product many times from methanol-chloroform gave needles, m.p. 217-220°, $[\alpha]_D$ + 77.5°, (C, 2.3). The product gave a yellow colour with tetranitromethane, which indicated that it was not the required methyl acetyl-olean-10:12-dienolate.

Reduction of 12-Keto-olean-10-enolic Acid with Sodium Amalgam.

Oleana-10:12-dienolic Acid.

12-Keto-olean-10-enolic acid (1.2g.) was heated under reflux in ethyl alcohol (100 ml.; 90%) with sodium amalgam (70g.; 3%) for three hours. The cold mixture was filtered, then acidified with dilute hydrochloric acid, and the precipitated solid extracted with ether. The extract was washed with water, dried over magnesium sulphate, and evaporated. The residue was crystallised from methanol, to give oleana-10:12-dienolic acid as prisms, m.p. 293-295°, $[\alpha]_D$ + 202° (C, 0.78).

Found : C, 79.2 ; H, 10.2 %

Calculated for $C_{30}H_{46}O_3$: C, 79.2 ; H, 10.2 %

Light absorption in ethanol ; max. at 2830Å, ε = 7,000.

Kitasato(11) gives m.p. 295-300°, $[\alpha]_D$ + 206.8° for the sodium amalgam reduction product from acetylketo-iso-oleanolic acid.

Purification of the acid was extremely difficult and, although the m.p. remained unaltered after each crystallisation, the specific rotation and intensity of the ultra-violet absorption maximum increased slightly.

Methyl Oleana-10:12-dienolate was prepared by estrification of the acid with ethereal diazomethane. It separates from methanol as needles, m.p. 194-196°, $[\alpha]_D$ + 203° (C, 0.92).

Light absorption in ethanol : max. at 2840 Å, ε = 6,800.

Kitasato(11) gives m.p. 198° for the methyl ester of the acid obtained by sodium amalgam reduction of acetylketo-iso-oleanolic acid.

Bromination of Acetylketo-oleanolic Lactone (12-Keto-Oleanolic Lactone Acetate).

The keto-lactone acetate (0.55g.) in glacial acetic acid (50 ml.) was treated at 50° with a 48% aqueous solution of hydrogen bromide (3-4 drops) and then with bromine in acetic acid (3.5 ml.; 5%), added dropwise with stirring. The solution was heated on the steam bath for five minutes and then allowed to stand overnight at room temperature. The product obtained by addition of water to the hot solution was collected and recrystallised from acetic acid to give 11-bromo-12-keto-oleanolic lactone acetate as needles, m.p.320° (decomp.) with darkening over 200°, $[\alpha]_D$ + 5° (C, 1.p).

Found :	C, 64.8	;	H, 8.1 %
$C_{32}H_{47}O_5$ Br requires :	C, 65.0	;	H, 8.0 %

The compound gave a positive Beilstein test for halogen and was recovered unchanged after heating at 100° for two days in glacial acetic acid.

Treatment of 11-Bromo-12-Keto-Oleanolic Lactone Acetate with Sodium Iodide and Acetone.

The bromo-ketone(0.17g.) in acetone(60 ml.) was treated with sodium iodide (0.5g.) and the solution was heated under reflux for 4½ hours. On cooling, the dark brown colour which had developed was destroyed with sodium thiosulphate solution and the precipitation of the solid was completed by addition of water. The product was isolated by means of ether in the usual manner. The residue obtained on evaporation of the ether was dissolved in methanol-chloroform, from which an amorphous solid was deposited. The substance gave

a negative Beilstein test and could not be purified by further crystallisation or by chromatography.

Light absorption in ethanol ; max. at 2,200 Å, ε = 800.

Oxidation of Methyl Acetyl-12-keto-olean-10-enolate with Selenium Dioxide.

Selenium dioxide (0.84g.) was added to a boiling solution of methyl acetyl-12-keto-olean-10-enolate (0.84g.) in acetic acid (50 ml.) and the mixture was heated under reflux for 24 hours. The cooled solution was filtered and the filtrate diluted with water. The precipitated solid was extracted with ether and the ether layer was washed with potassium hydroxide solution (3%) till the washings were slightly alkaline, then with potassium cyanide solution (100 ml.; 3%) and finally with water and dried over sodium sulphate. On evaporation of the ether, the residue was dissolved in light petroleum (b.p.60-80°; 50 ml.) and benzene (40 ml.) and chromatographed on a column of activated alumina [Grade II; 13.5 x 1.5 cm.). Light petroleum-benzene (1:1 ; 400 ml.) eluted a solid, (127mg.) m.p. 291-297°. Benzene (1000 ml.) eluted a fraction (285 mg.) m.p.294-296°, and benzene-ether (4:1 ; 300 ml.) gave a further 138 mg., m.p.296-299°. These three fractions were combined and crystallised twice from methanol-chloroform to give an α-β-unsaturated keto lactone, as needles, m.p. 300-302°, $[\alpha]_D$ - 73° (C, 1.43).

Found : C, 75.2 ; H, 9.15% $-OCH_3$, 0.0%

$C_{32}H_{46}O_5$ requires : C, 75.25 ; H, 9.1 %

Light absorption in ethanol ; max. at 2,380 Å, ε = 11,450.

This compound gave no colouration with tetranitromethane in chloroform.

Attempted Hydrolysis of the Lactone Obtained by the Action of Selenium Dioxide on Methyl Acetyl-12-keto-olean-10-enolate.

a) Using hydrogen bromide.

The lactone (0.55g.), prepared as above, was dissolved in dry ethanol (50 ml.) and the solution was saturated four times with hydrogen bromide, with intermittant half hour refluxing after each saturation. The cold solution was poured into water and the solid extracted with a mixture of ether and chloroform. The ether-chloroform extract was washed with potassium hydroxide solution (3%) and water and dried over sodium sulphate. Acidification of the alkaline washings gave no acid fraction. After evaporation of the solvent, the neutral fraction crystallised from methanol as small prismatic needles, m.p.360-365° (decomp.) The product gave a marked depression on mixed melting with the starting material and showed no tetranithromethane colouration.

Acetylation of the above product with acetic anhydride and pyridine on the steam bath and working up in the usual way gave an acetate which crystallised from methanol-chloroform as plates, m.p. 307-309°, $[\alpha]_D$ - 75° (C, 1.09). This acetate showed no depression on admixture with the original lactone.

The compound proved to be dimorphous and can be crystallised as needles or plates according to the concentration of the solution and/or the crystalline form of the seed crystal. The acetate in the form of needles, m.p. 300-302° was undepressed by starting material.

b) Using alcoholic potassium hydroxide.

The lactone (0.3g.) in ethanolic potassium hydroxide (40 ml.; 10%) was heated under reflux for five hours. The solution was cooled, acidified with dilute hydrochloric acid, and the solid extracted with ether. The ether extract was worked up in the usual way and the product acetylated on the steam bath with acetic anhydride and pyridine for one hour. The acetylation mixture was worked up in the usual way and the product crystallised from methanol-chloroform as needles m.p. 300-302°, undepressed on admixture with starting material.

Attempted Catalytic Hydrogenation of the Lactone Obtained by the Action of Selenium Dioxide on Methyl Acetyl-12-keto-olean-10-enolate.

The lactone (0.24g.) in stabilized acetic acid (150 ml.) was shaken with platinum (0.1g.) in an atmosphere of hydrogen, at room temperature for twenty four hours. The solution was filtered and the product precipitated from the filtrate by addition of water, was collected, dried, and crystallised from methanol-chloroform to give needles, m.p. 300-302°, undepressed by starting material, $[\alpha]_D$-73° (C, 1.0).

Oxidation of Methyl Acetyl-12-keto-oleanolate with Selenium Dioxide.

Methyl Acetyl-12-keto-oleanolate (1g.) was dissolved in stabilized acetic acid (50 ml.) and the solution was refluxed with selenium dioxide (1g.) for twenty four hours. The cold solution was filtered and the filtrate diluted with water. The precipitate was extracted with ether and the ether extract was worked up as described in the oxidation of methyl acetyl-12-keto-olean-10-enolate (p. 95).

The residue remaining after removal of the solvent from the dried ether extract, was dissolved in light petroleum (b.p. 60-80°; 100 ml.) and benzene (50 ml.) and chromatographed on a column of activated alumina (Grade II ; 14 x 15 cm.)

Fraction	Solvent	Eluate
1	400 ml. petrol-benzene (1:1)	70 mg. m.p. Wide range.
2	100 ml. " " (2:3)	25 mg. Oil
3	200 ml. " " (3:7)	65 mg. m.p. 288-292°
4	100 ml. " " (1:4)	30 mg. m.p. 288-292°
5	400 ml. benzene	76 mg. m.p. 284-290°
6	400 ml. benzene-ether (9:1)	93 mg. m.p. Wide range.
7	100 ml. " " (9:1)	130 mg. m.p. 219-221°
8	100 ml. " " (1:1)	10 mg. m.p. 219-221°
9	200 ml. ether.	Trace.
10	200 ml. methanol.	153 mg. m.p. 218-221°

Fractions 3,4 and 5, being undepressed in melting point when mixed, were combined and crystallised twice from methanol to give needles, m.p.297-300°, $[\alpha]_D$ - 71°(C, 1.13). This substance gave no depression on mixed melting with the lactone obtained by selenium dioxide oxidation of methyl acetyl-12-keto-olean-10-enolate, and an alcoholic or dioxan solution gave no colour with ferric chloride.

Fraction 7 was crystallised from methanol to give methyl-11-keto-12-hydroxyolean-12-enolate acetate as needles, m.p.224-225°, $[\alpha]_D$ + 123° (C, 0.3).

Found: C, 72.65 ; H, 9.5 %

$C_{33}H_{50}O_6$ requires : C, 73.0 ; H, 9.3 %

Light absorption in ethanol; max. at 2,880 Å, ε = 10,900.

This substance gave a bluish green colour with alcoholic ferric chloride and a yellow colour with tetranitromethane.

Fraction 10 after many crystallisations from methanol was obtained as needles, m.p.218-221°, undepressed on admixture with the substance isolated from fraction 7.

Found: C, 72.65 ; H, 9.2 %

$C_{33}H_{50}O_6$ requires : C, 73.0 ; H, 9.3 %

Alkaline Hydrolysis of Acetylketo-oleanolic Lactone.

Method (a).

Acetylketo-oleanolic lactone(1g.) was heated under reflux for one hour with ethanolic potassium hydroxide (50 ml.; IN). The solution was poured into water and the mixture twice washed with ether. The ether washings were discarded and the alkaline extract was acidified with dilute hydrochloric acid. The resultant precipitate from acidification of the alkaline layer was extract with ether and the ethereal solution was washed with water and dried over sodium sulphate. The residue obtained on evaporation of the ether was treated with an ethereal solution of diazomethane. After destroying the excess of diazomethane with acetic acid, the ethereal solution was washed with potassium hydroxide solution (3%) and water and dried over sodium

sulphate. No acid fraction was obtained on acidification of the alkali wash. The neutral fraction was acetylated with acetic anhydride and pyridine on the steam bath for one hour. The acetylation mixture was worked up in the usual way and the product (0.9g.) was crystallised from methanol-chloroform as needles, m.p. 255-260°.

The substance was dissolved in light petroleum (b.p.60-80°; 100 ml.) and passed through a column of activated alumina (20x2 cm.). Washing the column with mixtures of light petrol-benzene and benzene gave 0.6 g. of solid which crystallised from methanol as needles, m.p. 280-282°, $[\alpha]_D$ + 10°(C, 1.3). This product gave no depression on melting with acetylketo-oleanolic lactone.

Further washing of the column with benzene-ether, and ether gave fractions which were gummy solids and could not be purified further.

Method (b).

(c.f. Kitasato, Acta.Phytochim., 1933, 7, 1.)

Acetylketo-oleanolic lactone (225 mg.) was hydrolysed with methanolic potassium hydroxide (0.5%) to give keto-oleanolic lactone as needles from methanol, m.p. 299-301°.

Keto-oleanolic lactone (0.2 g.) was treated with boiling ethanolic potassium hydroxide (16 ml.; IN) for two hours. The mixture was poured into water and filtered. The small residue was neglected. The alkaline solution was shaken with dimethyl sulphate (1 ml.) and allowed to stand overnight. The mixture was twice extracted with ether and the alkaline liquor was again shaken with

dimethyl sulphate and thenwashed with ether. The ether extracts were combined and worked up in the usual way. The product was acetylated with acetic anhydride and sodium acetate in the usual way. The acetate crystallised from methanol to give an impure solid, m.p. 300° which could not be purified further and showed no appreciable light absorption.

Acidification of the original alkaline extract gave a solid which was isolated through ether in the usual manner. The product was crystallised with difficulty from methanol to give prisms, m.p. 380°, showing no appreciable light absorption above 2,200Å. This acid was not purified any further, since it obviously did not contain any α - β unsaturated ketone grouping.

Bromination of Acetyl-11-keto-olean-12-enolic Acid.

(c.f. Ruzicka, Jeger and Winter Helv.Chim.Acta. 1943,75,265).

Acetyl-11-keto-olean-12-enolic acid (1g.) in glacial acetic acid (200 ml.) was treated at 80° with hydrobromic acid (48% aqueous), and then with bromine in acetic acid (3.5 ml.; 10%) added dropwise with stirring. The reaction was very sluggish, and the orange coloured solution was left standing overnight and then heated on the steam bath for one hour. The mixture was poured into water and the product was collected, washed with water and dried. Crystallisation from methanol-chloroform (thrice) gave acetyl-11-keto-olean-12:18(19)-dienolic acid (acetylketodehydro-oleanolic acid) as needles, m.p. 287-288°. Yield = 25%.

The product gave a yellow colour with tetranitromethane. Ruzicka et al. give m.p. 288-289° for this acid.

Decarboxylation of Acetyl-11-keto-oleana-12:18(19)-dienolic Acid.

The acid (0.25 g.) was heated in an atmosphere of nitrogen at 285-295°. After five minutes, when the evolution of carbon dioxide had ceased, the mixture was cooled and dissolved in ether. The ether solution was washed with sodium hydroxide solution (5%), water and dried over magnesium sulphate. The residue on evaporation of the ether was dissolved in light petroleum (b.p.60-80°; 100 ml.) and filtered through a column of activated alumina (Grade II).

After the column had been washed with light petroleum (200 ml.) and light petroleum-benzene (7:1; 100 ml.), eluates were obtained by washing with light petroleum-benzene (4:1; 100 ml.), light petroleum-benzene (1:1; 500 ml.) and ether (100 ml.); these were combined (75 mg.) and crystallised three times to give nor-β-amyradienonyl acetate as prisms from methanol, m.p. 203-205°, $[\alpha]_D$ + 144° (C, 1.23). The product gave a bright yellow colour with tetranitromethane.

Found : C, 80.0 ; H, 10.1 %

Calculated for $C_{31}H_{46}O_3$: C, 79.8 ; H, 9.9 %

Light absorption in ethanol ; max. at 2,970 Å, ϵ = 22,000.

Ruzicka Jeger and Winter (loc.cit.) give m.p. 202°, $[\alpha]_D$ + 150° and light absorption maximum at 2,970Å, (ϵ = 22,400) for nor-β- amyradienonyl acetate obtained by treatment of acetyl-11-keto olean-12-enolic acid with boiling quinoline.

Isolation of β-Amyrenyl Benzoate.

Manila Elemi resin (5000 g.) from which the steam volatile constituents had been previously removed, was stirred for four hours with 80% aqueous methylated spirit (12:1). The mixture was allowed to stand overnight and the solid was collected, washed with aqueous methylated spirit, and dried at 80°. The melting point of the mixed amyrins at this stage was 155-157°.

The mixed amyrins (1800g.) were dissolved in pyridine (1.2 l.) at 100°, and benzoyl chloride (792 ml.) was added dropwise with stirring. After stirring for 6 hours, the mixture was cooled, and diluted with chloroform (3.5 l.). The chloroform solution was washed five times with hydrochloric acid (5%; 1 l.), thrice with sodium hydroxide solution (5%; 1.5 l.), once with sodium chloride solution (2%; 1 l.), and once with water (1 l.). The solution was then concentrated to ca. 1.5 litres and hot methanol (1.2 l.) was added. The mixture was allowed to stand overnight and the crystallised mixed benzoates (885g.) were collected by filtration. Concentration of the filtrate gave a further quantity (582g.) of benzoates.

The crude mixed benzoates were shaken with ether (1.5 l.) and the clearing point of the undissolved material was determined. This ether washing was repeated till a clearing point of 214° was obtained, when the residue was crystallised for methanol-chloroform to give β-amyrenyl benzoate (320g.) m.p. 232-234°.

β -Amyrenol.

β -Amyrenyl benzoate (44.5g.) was dissolved in boiling benzene (268 ml.) and a solution of potassium hydroxide (22.4g.) in ethanol (334 ml.) and water (57 ml.) was added. The solution was refluxed for 24 hours, concentrated till solid began to appear, and finally poured into water. The solid was extracted with ether and the ether extract was washed with water and dried over sodium sulphate. β -Amyrenol (44g.), m.p. 186-187°, was obtained on evaporation of the ether.

β -Amyrenyl Acetate.

Crude β -amyrenol (44g.) was heated on the steam bath for four hours with a mixture of benzene (84 ml.), pyridine (150 ml.) and acetic anhydride (176 ml.). On cooling the solution, β -amyrin acetate (35.5g.) crystallised as needles, m.p. 240-242°, $[\alpha]_D$ + 80° (C, 1.5).

β -Amyradienyl-II-Acetate (Oleana-11:13(18)-dienyl Acetate).

(c.f. Ruzicka, Muller and Schellenberg. Helv. Chim. Acta. 1939, 22, 767.).

β -Amyrenyl acetate (30g.) in boiling glacial acetic acid (1,200 ml.) was treated with a solution of selenium dioxide (15g.) in water (15 ml.) and acetic acid (600 ml.) added dropwise over a period of one hour. The mixture was refluxed for a further hour after the addition was completed, and fused sodium acetate (120g.) was then added and refluxing continued for 15 mins. The solution was filtered and allowed to cool. The product which crystallised out was

collected and recrystallised from acetone to give β-amyradienyl-II-acetate (15g.) as plates, m.p. 229-231°, $[\alpha]_D$ - 66° (C, 1.88).

The original acetic acid mother liquor was poured into water and the solid collected, washed with water and dried. Crystallisation from acetone gave a further quantity (7g.) of the diene, m.p.227.5-229.5°.

The diene gave a red brown colour with tetranitromethane in chloroform.

Light absorption in ethanol; max. at 2420Å, ε = 29,000 ;
2500Å, ε = 30,000 ;
2600Å , ε = 21,000.

Oxidation of β-Amyradienyl-II Acetate with Chromic Anhydride.

(c.f. Mower, Green and Spring J., 1944, 256.)

β-Amyradienyl-II acetate (15g.) was dissolved in boiling stabilized acetic acid (1200 ml.) and a solution of chromic anhydride (15g.) in water (15 ml.) and glacial acetic acid (450 ml.) was added over a period of thirty minutes. During the addition of the chromic anhydride three fractions (20 ml. each) were distilled off from the reaction mixture. After the addition was completed, the solution was boiled for two hours, the excess of chromic anhydride was destroyed with methanol, and the mixture was evaporated to dryness under reduced pressure. The dark green residue was dissolved in a mixture of ether and dilute sulphuric acid. The ether layer was separated and washed with potassium hydroxide solution (3%), water, and dried (Na_2SO_4). Crystallisation of the ether residue from methanol-chloroform gave the "O_5-acetate"(4.8g.) as needles, m.p. 259.5-260.5°, $[\alpha]_D$ + 38° (C, 0.91).

Found : C, 75.5 ; H, 9.5 %.

Calculated for $C_{32}H_{46}O_5$: C, 75.3 ; H, 9.1 %

The "O_5-acetate" gave a yellow colour with tetranitromethane and no colour with ferric chloride in dioxan or ethanol.

Light absorption in ethanol ; max. at 2280Å, ε = 3,400 with low intensity inflection at 3,000Å.

The second crop of crystals m.p. 213-217° corresponded to the mixed crystal of "O_5-acetate" and 11-keto-oleana-12:18-dienyl acetate described by Mower, Green and Spring (loc.cit.). This was treated as described below.

The precipitate obtained on acidification of the original alkali washings was worked up through ether in the usual way and esterified with ethereal diazomethane. The product of the esterification was a gum and could not be crystallised.

The three fractions which were distilled off from the original reaction mixture were made alkaline to phenolphthalein with potassium hydroxide solution (50%) and distilled. The distillate in each case was treated with a solution of 2:4-dinitrophenylhydrazine (1% in HCl). No precipitate was obtained from any fraction, indicating that no volatile carbonyl compounds had been produced during oxidation.

The second crop from the crystallisation of the "O_5-acetate" was combined with the mother liquor material and dissolved in glacial acetic acid (300 ml.). The solution was refluxed for nine hours with selenium dioxide (10g.), filtered and diluted with water. The solid was collected, washed with water and dried. Crystallisation from methanol-chloroform gave needles (3g.), m.p. 258.5-259.5°, undepressed with the above specimen of "O_5-acetate".

Attempted Catalytic Reduction of the "O_5-Acetate"

The "O_5-acetate" (1g.) in stabilized acetic acid (100 ml.) was shaken with platinum (0.25g.) in an atmosphere of hydrogen at room temperature and pressure for 65 hours. There was no appreciable uptake of hydrogen during this time. The solution was filtered and the filtrate taken to dryness under reduced pressure. The residue was crystallised from methanol-chloroform as needles m.p. 259-260°, undepressed with starting material.

Hydrolysis of the "O_5-Acetate".

Method (a).

The "O_5-acetate" (1.1g.) was heated under reflux for three hours with a solution of potassium hydroxide (5g.) in water (10 ml.) and methanol (90 ml.). The mixture was poured into water, extracted with ether, and the ether extract was washed with water, and dried (Na_2SO_4). The residue of neutral material obtained on evaporation of the ether could not be crystallised and was acetylated with acetic anhydride and pyridine at 100°. The acetylation mixture was worked up in the usual way and the product crystallised from methanol-acetone as needles, m.p. 259.5-260.5°, $[\alpha]_D$ + 158° (C, 0.93). Yield 660 mg. (60%).

This product gave a marked depression on mixed melting with the "O_5-acetate" and no colour with tetranitromethane.

Found : C, 76.6 ; H, 9.85% - OCH_3, 0.0%.

Calculated for $C_{30}H_{46}O_4$: C, 76.55 ; H, 9.85%

Calculated for $C_{31}H_{48}O_4$: C, 76.8 ; H, 10.0%

Light absorption in ethanol : max. at 2,100Å, ε = 1,760.

Acidification of the original alkaline solution gave an acid fraction which was isolated through ether in the usual way. The acid (50 mg.) crystallised from acetone-petrol as needles, m.p. 272-274°, (279-281° in vacuo), $[\alpha]_D$ - 36° (C, 1.26).

Found : C, 74.2 ; H, 9.6%

$C_{30}H_{46}O_5$ requires : C, 74.0 ; H, 9.5%

Method (b).

The "O_5-acetate" (1.78g.) was heated under reflux with a solution of potassium hydroxide (1.7g.) in methanol (34 ml.) for two hours. The cooled solution was poured into water and the solid extracted with ether. The ethereal solution was washed with water and dried over sodium sulphate. The residue obtained on evaporation of the ether was crystallised from aqueous methanol to give an ester as cubes, m.p. 234-236°, $[\alpha]_D$ - 46° (C, 1.63). Yield 765 mg. (43%).

The compound gave no colour with tetranitromethane in chloroform nor with ferric chloride in dioxan. A colouration was produced from a Legal test after standing for some time.

Found : C, 74.6 ; H, 9.6% $-OCH_3$, 6.0%.

$C_{31}H_{48}O_5$ requires : C, 74.7 ; H, 9.7% $-OCH_3$, 6.4%.

Light absorption in ethanol : max. at 2250Å, ε = 15,500.

The acetyl ester was obtained by acetylation with acetic anhydride and pyridine at 100°.

The mixture was worked up in the usual way and the product crystallised from aqueous methanol as elongated prisms, m.p. 192 - 194°, $[\alpha]_D$ - 33° (C, 1.19).

Found : C, 73.3 ; H, 9.3 %

$C_{33}H_{50}O_6$ requires : C, 73.0 ; H, 9.3 %

Light absorption in ethanol : max. at 2240Å, ε = 15,700.

Acetylation of the mother liquor material from the neutral fraction with acetic anhydride and pyridine at 100°, gave a product which crystallised from methanol-acetone as needles, m.p. 253-256.5°, undepressed with acetate described under method (a).

Acidification of the original alkaline solution of the hydrolysis with dilute hydrochloric acid gave an acid fraction which was isolated through ether in the usual way. The acid crystallised from acetone-petrol as needles, m.p. 272-273°$[\alpha]_D$ -37° (C, 0.74), and showed no depression on mixed melting with the acid described under method (a). Yield 40 mg. (2.2%).

Found : C, 74.05 ; H, 9.63%

$C_{30}H_{46}O_5$ requires: C, 74.0 ; H, 9.5 %

Light absorption in ethanol ; max. at 2,260Å, ε = 12,720.

The corresponding ester was obtained by esterification with ethereal diazomethane and crystallised from aqueous methanol as prisms, m.p. 231-232.5° undepressed on admixture with the methyl ester described above.

Attempted Catalytic Reduction of the Methyl Ester Obtained from the "O_5-Acetate".

The methyl ester (m.p.234-235°)(0.35g.) in stabilized acetic acid (100 ml.) was shaken with platinum (0.1g.) in an atmosphere of hydrogen for twenty four hours at room temperature and pressure.

The platinum was removed by filtration and the filtrate was taken to dryness under reduced pressure. The residue was crystallised once from aqueous methanol as cubes, m.p. 230-231°, undepressed on admixture with starting material.

Attempted Hydrolysis of the Methyl Ester $C_{31}H_{48}O_5$.

Method (a).

The ester (0.25g.) was heated under reflux for three hours with a solution of potassium hydroxide (1g.) in methanol (18 ml.) and water (2 ml.). The solution was poured into water and the solid isolated by means of ether in the usual way. The material crystallised from aqueous methanol as cubes m.p. 232-234°, undepressed with starting material. Recovered yield, 0.2g.

Method (b).

The methyl ester (0.35g.) was dissolved in ethanolic potassium hydroxide (40 ml. ; 20%) and the solution was heated in a tube autoclave at 200° for eight hours. No solid was precipitated on pouring the cold solution into water. Acidification of the alkaline solution with dilute hydrochloric acid gave an acid fraction which was extracted with ether. The ethereal solution was washed with water and dried over magnesium sulphate and evaporated. The acid was obtained as an amorphous solid from methanol-acetone, m.p. 332-333°(decomp.), $[\alpha]_D$ -28° in pyridine (C, 1.25).

Found : C, 70.8, 70.8, 70.4, 70.6, ; H, 9.6, 9.7, 9.7, 9.6 %

$C_{31}H_{50}O_6$ requires : C, 71.8 ; H, 9.7 %

$C_{30}H_{48}O_6$ requires : C, 71.4 ; H, 9.6 %

$C_{29}H_{46}O_6$ requires : C, 71.0 ; H, 9.4 %

$-OCH_3$ found : 4.3 %

$C_{31}H_{50}O_6$ requires : 6.0 %

$C_{30}H_{48}O_6$ requires : 6.1 %

$C_{29}H_{46}O_6$ requires : 6.2 %

The acid gave no colour with tetranitromethane.

Light absorption in ethanol ; max. at 2,800 Å, ε = 390.

The dimethyl ester was prepared by esterification with ethereal diazomethane. The ester crystallised from acetone-n-hexane as hexagonal plates m.p. 228°, $[\alpha]_D$ - 35° (C, 1.0).

Found : C, 72.3 ; H, 10.0%, $-OCH_3$, 11.1%

$C_{32}H_{52}O_6$ requires: C,72.1 ; H, 9.8%, $-OCH_3$, 11.7%.

This ester gave no colour with tetranitromethane and gave a marked depression on mixed melting with the methyl ester obtained by hydrolysis of the "O_5-acetate".

The acetate dimethyl ester was obtained by acetylation with acetic anhydride and pyridine at room temperature for sixteen hours. It crystallised from n-hexane as prisms m.p. 202.5-203.5°, $[\alpha]_D$ - 28.5° (C, 1.27).

Found : C, 71.0, 71.6, 71.2, 71.3, 70.8, ; H, 9.8, 9.7, 9.8, 9.7, 9.5 %

$C_{34}H_{54}O_7$ requires : C, 71.0 ; H, 9.5 %

Light absorption in ethanol ; max. at 2,800Å, ε = 340.

Hydrolysis of the Acetate $C_{31}H_{48}O_4$ Obtained by Hydrolysis of the "O_5-acetate".

The acetate (m.p.259.5-260.5°)(80mg.) prepared as described on page was dissolved in ethanolic potassium hydroxide (25 ml., 5%) and the solution was heated on the steam bath for three hours. The mixture was worked up through ether in the usual way. The alcohol crystallised from aqueous methanol as needles m.p.102-103°, $[\alpha]_D$ + 147° (C, 1.46).

Found :	C, 76.3	;	H, 10.7%
$C_{29}H_{46}O_3.CH_3OH$ requires:	C, 75.9	;	H, 10.6%

Light absorption in ethanol ; max. at 2090Å, ε = 1,700.

Acetylation of this product with acetic anhydride and pyridine at 100° gave a quantitative yield of the original acetate , m.p. 259.5-260.5°, $[\alpha]_D$ + 154° (C, 1.04).

Reduction of the Acetate $C_{31}H_{48}O_4$ with Lithium Aluminium Hydride.

The acetate (m.p.259.5-260.5°)(0.3g.) was dissolved in dry ether (50 ml.) and the solution was added dropwise to a suspension of lithium aluminium hydride (0.7g.) in dry ether (25 ml.) at room temperature. The solution was refluxed for 3½ hours and the excess of hydride was then destroyed with water. The mixture was diluted with dilute sulphuric acid and the ether layer was separated, washed with water and dried over sodium sulphate. The residue obtained on evaporation of the ether was acetylated with acetic anhydride (5 ml.) and pyridine (2 ml.) at room temperature for 2 hours and at 100° for ¾ hour. The

mixture was worked up in the usual way. The product which was a gum, could not be crystallised from any of the usual solvents. The gum was dissolved in light petroleum (b.p. 60-80°; 50 ml.) and chromatographed on a column of activated alumina. No crystalline fraction was obtained from the chromatogram.

Attempted Formation of An Enol Acetate of the Acetate $C_{31}H_{48}O_4$.

The acetate (0.19g.) was heated under reflux for 72 hours with acetic anhydride (10 ml.) and freshly fused sodium acetate (0.2g.). The cooled mixture was poured into water and heated for 15 minutes on the steam bath. The solution was then extracted with ether and the ether layer separated and washed repeatedly with water. The residue obtained on evaporation of the dried ($MgSO_4$) ether extract, crystallised from methanol as needles, m.p. 257.5-260.5° undepressed on admixture with starting material. Recovered yield 0.1g.

Attempted Clemmensen Reduction of the Acetate $C_{31}H_{48}O_4$.

The acetate (0.3g.) in glacial acetic acid (100 ml.) was heated at 100° for three hours with amalgamated zinc (10.2g.) and concentrated hydrochloric acid (30 ml.). Hydrochloric acid (10 ml.) was then added and heating continued for one hour, when a further 10 ml. of hydrochloric acid were added and the heating continued for a further hour. The hot solution was filtered and the product precipitated with water and isolated through ether in the usual way. After acetylation with acetic anhydride and pyridine at 100° the product crystallised from methanol as needles, m.p. 259.5-260.5°, which showed no depression

on mixed melting with starting material . Investigation of the mother liquor material yielded further quantities of unchanged acetate.

Treatment of the Acetate $C_{31}H_{48}O_4$ with Chromic Anhydride.

The acetate (0.34g.) in boiling stabilized acetic acid (20 ml.) was treated with a solution of chromic anhydride (0.18g.) in water (1 ml.) and acetic acid (10 ml.), added over a period of 10 minutes. The solution was refluxed for 2¼ hours. Excess of chromic anhydride was destroyed with methanol, and the product was precipitated by addition of water, collected by filtration, washed with water and dissolved in ether. The ethereal solution was worked up in the usual way and the residue on evaporation of the solvent was crystallised from methanol to give needles m.p. 257.5° - 259.5°, undepressed with starting material. Recovered yield 0.27g.

Treatment of the Acetate $C_{31}H_{48}O_4$ with Potassium Permanganate.

To a solution of the acetate (m.p.259.5-260.5°) (0.25g.) in stabilized acetic acid (110 ml.), potassium permanganate (0.2g.) in water (20 ml.) was added dropwise, at room temperature. The solution was left standing overnight and then heated on the water bath for 45 minutes. There was no immediate colour change at room temperature but the solution went brown on heating. The hot solution was decolourised by addition of aqueous sodium metabisulphite solution. The product (0.2g.) crystallised from the reaction mixture on cooling, as needles, m.p. 259-260°. This material gave no melting point depression with starting material.

Treatment of the Acetate $C_{31}H_{48}O_4$ with Perbenzoic Acid.

The acetate (0.33g.) in dry chloroform (5ml.) was treated at 0° with a solution of perbenzoic acid in chloroform (2 ml.; 73.5 mg./ml). The solution was allowed to stand at 0° for 10 days. Titration of a sample of the solution, with sodium thiosulphate solution, compared with that of a blank solution indicated no uptake of oxygen.

The chloroform solution was washed with saturated sodium bicarbonate and water and dried over magnesium sulphate. The residue, on evaporation, crystallised from methanol, m.p. 258-260°, undepressed with starting material. That the substance was starting material was confirmed by light absorption.

Treatment of the Acetate $C_{31}H_{48}O_4$ with Sodium Borohydride.

The acetate (0.3g.) was dissolved in dry methanol (50 ml.). Sodium borohydride (0.29g.) in methanol (10 ml.) was added to the boiling solution and refluxing continued for $2\frac{1}{2}$ hours. The solution was left standing overnight and finally refluxed for a further hour. Concentrated hydrochloric acid (15 ml.) was added and the solution was heated for an hour, poured into water and ether extracted. The ether extract was worked up in the usual way. After acetylation with acetic anhydride and pyridine at room temperature for 16 hours the product was crystallised from methanol, as needles m.p. 260-261°, showing no depression on admixture with starting material. Recovered yield 85%.

Treatment of the Acetate $C_{31}H_{48}O_4$ with Hydrazine.

The acetate (0.4g.) in ethanol (25 ml.) was heated in a tube autoclave at 200° for 8 hours with anhydrous hydrazine (6 ml.). The cooled solution was poured into water and the solid extracted with ether in the usual way. The residue on evaporation of the ether could not be crystallised from any of the usual solvents. Acetylation with acetic anhydride and pyridine at 100° gave an acetate which crystallised from methanol as needles, m.p. 256-259°, and gave no colour with tetranitromethane and no depression with starting material on mixed melting point determination. Recovered yield 75%.

Treatment of the Acetate $C_{31}H_{48}O_4$ with Selenium Dioxide.

The acetate (0.5g.) in acetic acid (25 ml.) was heated under reflux for 24 hours with selenium dioxide (0.5g.). The cold solution was filtered and the product precipitated with water, collected, dried, and crystallised from methanol giving needles, m.p. 160°, which were recovered unchanged from treatment with acetic anhydride and pyridine at 100°. The product was dissolved in light petroleum (b.p.60-80°; 55 ml.) and chromatographed on a column of alumina (10.5 x 1.5 cm.).

Washing the column with benzene (250 ml.), benzene-ether (9:1 ; 250 ml.) and benzene-ether (1:1 ; 150 ml.) gave a solid (320 mg.) which was crystallised from aqueous methanol to give a compound as needles, m.p. 165-166°, $[\alpha]_D$ - 73°, -72° (C, 1.4, 1.3). After drying at 80° this compound had a m.p. 245-246°.

Found :	C, 75.1	;	H, 9.65 %
$C_{31}H_{46}O_5$ requires:	C, 74.7	;	H, 9.3 %.

The compound gave a pale yellow colour with tetranitromethane and no ferric chloride colouration.

Light absorption in ethanol ; max. at 2570Å, ε = 6,800.

Bromination of the Acetate $C_{31}H_{48}O_4$.

Method (a).

(c.f. Ruzicka, Jeger and Norymberski Helv.Chim.Acta. 1944, 27, 1532).

The acetate (0.3g.) was dissolved in boiling glacial acetic acid (120 ml.) and the solution was treated with a few drops of aqueous hydrobromic acid (48%). Bromine in acetic acid (2.1 ml. ; 9.6% ≡ 2 moles) was added dropwise to the boiling solution and refluxing was continued for 5 minutes, when the solution turned yellow in colour. The mixture was poured into water and the solid extracted with ether. The ether extract was washed with potassium hydroxide solution (3%), water, and dried (Na_2SO_4). The residue on evaporation of the solvent was taken up in light petroleum (b.p. 60-80°; 50 ml.) and the solution filtered through activated alumina (12 g.). The yellow gum obtained after washing the column with light petroleum-benzene (2:1) was crystallised from methanol as yellow needles, m.p. 208-210°.

Light absorption in ethanol: max. at 2,300Å, ε = 7,120;
3,520Å, ε = 8,240.

A second crop of needles from the above crystallisation had m.p. 203-207°.

Light absorption in ethanol; max. at 2,500Å, ε = 9,700;
3,500Å, ε = 2,100.

This method was abandoned in favour of that using one mole of bromine.

Method (b).

The acetate (0.63g.) was dissolved in acetic acid (30 ml.) at 50° and a few drops of aqueous hydrobromic acid (48%) added. Bromine in acetic acid (4.2 ml. ; 5.5% ≡ 1.1 moles.) was added dropwise and the mixture was kept at 50° for 30 mins. and then heated on the steam bath for one hour. The solution changed in colour from red to yellow and finally black. The mixture was worked up as described above and a solution of the product in light petroleum (b.p.60-80°) was filtered through a short column of alumina. The yellow gum obtained on evaporation of the filtrate, was crystallised many times for methanol to give orange rods, m.p. 211-213°, $[\alpha]_D$ + 91° (C, 1.2). The compound gave a red brown colour with tetranitromethane in chloroform.

Found : C, 77.2 ; H, 9.7%

$C_{31}H_{46}O_4$ Requires : C, 77.1 ; H, 9.6%

Light absorption in ethanol: max. at 2,280 Å, ϵ = 6,700;
3,500 Å, ϵ = 9,300.

The mother liquors from the crystallisation of this substance became almost colourless and deposited very pale yellow needles after standing for some time. Recrystallisation from methanol gave needles, m.p. 211-213°, $[\alpha]_D$ - 119° (C, 0.98) which gave no colour with tetranitromethane.

Found : C, 76.8 ; H, 9.7 %

$C_{31}H_{46}O_4$ requires : C, 77.1 ; H, 9.5 %

Light absorption in ethanol; max. at 2,500 Å , ϵ = 10,800.

A synthetic mixture of this compound (6 mg) and the orange coloured product (1 mg) showed light absorption in ethanol; max. at

2,500 Å, ε = 10,400; 3,500 Å, ε = 1,750. (c.f. product from method (a)).

Method (c).

The acetate (0.96g.) was dissolved in glacial acetic acid (60 ml.) at 50° and the solution was treated with a solution of bromine in acetic acid (6.3 ml.; 5.5% = 1.1 mole.). The mixture was kept at 50° for 30 minutes and then heated on the steam bath till the solution just turned yellow in colour. The mixture was worked up as before, and the product on crystallisation from methanol was mixture of yellow and white substances.

The mixture was redissolved in acetic acid (60 ml.) and treated as above with another 1 mole of bromine. The mixture was worked up through ether as before and the product was crystallised from methanol as colourless needles, m.p. 185°, with darkening and decomposition at the melting point, $[\alpha]_D$ −175° (C, 1.13). The substance gave no colour with tetranitromethane in chloroform and a positive Beilstein test indicated the presence of halogen.

Found : C, 66.3 ; H, 8.3%

$C_{31}H_{47}O_4Br$ requires: C, 66.1 ; H, 8.4%

Light absorption in ethanol; max. at 2,120Å, ε = 4,800 ;
2,700Å, ε = 5,400 ;
3,500Å, ε = 170.

Dehydrobromination of the Bromo Compound Prepared by Method(c).

The bromo compound (0.1g.) in collidine (5 ml.) was heated under reflux for two hours. The cold mixture was poured into dilute hydrochloric acid and then extracted with ether. The ether extract was

washed with water, dried (Na_2SO_4) and evaporated. The brown gum was crystallised twice from methanol to give orange coloured rods, m.p. 211-213°, $[\alpha]_D$ + 93° (C, 0.99). This product gave a red-brown colour with tetranitromethane and no depression on mixed melting with the substance described under method(b).

Light absorption in ethanol; max. at 2,280Å, ε = 6,700;
3,520Å, ε = 9,500.

Reduction of the Coloured Bromination Production with Zinc and Acetic Acid.

The orange compound ($[\alpha]_D$ + 91°)(0.1g.) was dissolved in boiling acetic acid (10 ml.). Powdered zinc (0.6g.) was added to the boiling solution over a period of ten minutes and refluxing continued for 5½ hours, during which time the solution went colourless. The solution was filtered hot and the product was isolated by means of ether and water in the usual way. The product cyrstallised from methanol as colourless needles, m.p. 258.5-260.5°, $[\alpha]_D$ + 150° (C, 0.9). This substance gave no depression on mixed melting with the acetate $C_{31}H_{48}O_4$ (m.p.259.5-260.5°) previously obtained from hydrolysis of the "O_5-acetate". Investigation of the mother liquors from the crystallisation yielded further quantities of the same substance.

Catalytic Reduction of the Coloured Bromination Product.

The compound (70 mg.) was shaken with platinum (50 mg.) in acetic acid (50 ml.) at room temperature in an atmosphere of hydrogen for 24 hours. The colourless solution was filtered free of platinum and the filtrate was taken to dryness under reduced pressure. The

residue was twice crystallised from methanol to give plates, m.p. 272-273° $[\alpha]_D$ + 83° (C, 1.24).

Found :	C, 76.3	;	H, 10.5%
$C_{31}H_{50}O_4$ requires :	C, 76.5	;	H, 10.3%

This compound showed a marked depression with the acetate $C_{31}H_{48}O_4$ used in the original bromination.

Light absorption in ethanol: max. at 2,060Å, ε = 1,800;
2,800Å, ε = 650.

Treatment of the Colourless Bromination Product with Alkali.

The substance($[\alpha]_D$ - 119°) (60 mg.) was heated under reflux with ethanolic potassium hydroxide (6 ml.; 3%) for 3 hours. The mixture was worked up through ether in the usual way and the product crystallised from aqueous methanol as needles, m.p. 225-228°.

Light absorption in ethanol; max. at 2,500Å, ε = 9,980;
3,500Å, ε = 1,872.

This indicated that the substance was a mixture and it was not investigated further.

Preparation of the Enol Acetate of the Colourless Bromination Product.

The substance $C_{31}H_{46}O_4$ ($[\alpha]_D$ - 119°) (99.2 mg.) was dissolved in acetic anhydride (10 ml.) and fused sodium acetate (99 mg.) was added. The mixture was refluxed for 24 hours, poured into water, and the solution warmed slightly to facilitate decomposition of the excess of anhydride. The cold mixture was then extracted and the ether washed many times with water and dried (Mg.SO_4). Evaporation of the solvent gave a brown gum which was crystallised from methanol as prisms, m.p. 225-226.5°, $[\alpha]_D$ - 56° (C, 0.95).

The enol acetate gave a yellow colour with tetranitromethane.

Found : C, 75.5 ; H, 9.22%

$C_{33}H_{48}O_5$ requires: C, 75.5 ; H, 9.22%

Light absorption in ethanol; max. at 2850Å, ε = 13,690;
2280Å, ε = 7,408.

Attempted Bromination of the Methyl Ester $C_{31}H_{48}O_5$ Obtained by Hydrolysis of the "O_5-Acetate".

The ester (0.3g.) was dissolved in acetic acid (25 ml.) and a few drops of aqueous hydrobormic acid (48%) were added. Bromine in acetic acid (5.9 ml. of 1.9% ≡ 1.1 moles.) was added dropwise to the solution at 100°. The solution was refluxed for 5 minutes, and a further quantity of bromine was added to the cooled solution, to make the quantity added equivalent to 2 moles. The solution was allowed to stand overnight, and then refluxed for a further 5 minutes, cooled and poured into water. The product, which was a gum, was isolated by means of ether in the usual way, and was acetylated with acetic anhydride and pyridine at 100°. The product from the acetylation (0.28g.) was dissolved in light petroleum (b.p. 60-80°; 100 ml.) and chromatographed on a column of activated alumina (Grade II; 6.5x2 cm.). There was total adsorption of the dissolved solid on the column. Washing the column with benzene (350 ml.) and benzene-ether(9:1;100 ml.) eluted a solid (230 mg.) which was recrystallised from aqueous methanol as blades, m.p. 192-194°. This substance gave no depression on mixed melting with the acetate of the starting material.

Further washing of the column with benzene-ether and ether gave only traces of material which could not be crystallised.

Treatment of the Acetate Methyl Ester $C_{33}H_{50}O_6$ with Potassium Permanganate.

The ester (0.22g.)(m.p.190-192°) dissolved in acetone (15 ml. distilled from $KMnO_4$) was shaken at room temperature for 22 hours with powdered potassium permanganate (0.22g.). The excess of permanganate was destroyed by addition of aqueous sodium metabisulphite solution and the resultant mixture was made acid to congo red with dilute hydrochloric acid. Precipitation of the solid was completed with water and the mixture was extracted with ether. The ether extract was washed with potassium hydroxide solution (3%), water, and dried (Na_2SO_4). No acid fraction was obtained from the alkaline wash. The neutral residue was crystallised from aqueous methanol as blades, m.p. 190-192°, undepressed on admixture with the starting material. Recovered yield 168 mg.(75%).

Treatment of the Acetate Dimethyl Ester $C_{34}H_{54}O_7$ with Bromine.

The dimethyl ester (m.p.202.5-203.5°)(0.44g.) in acetic acid (50 ml.) was treated at 50° with a few drops of hydrobromic acid (48%), and bromine in acetic acid (2.8 ml. of 5.12% ≡ 1.1 moles) was added dropwise with stirring. After the addition was completed the solution was stirred for 30 minutes at 50° and then slowly heated to 100° and kept at this temperature for one hour. The colour of the solution indicated that there was no uptake of bromine. The mixture was worked up in the usual way. Crystallisation of the product from n-hexane gave prisms, m.p. 201-203°, which gave no depression on mixed melting with the starting material. Recovered yield 370 mg.(84%).

Light absorption in ethanol ; max. at 2800Å, ϵ = 380.

Attempted Clemmensen Reduction of the Acetate Dimethyl Ester $C_{34}H_{54}O_7$.

The ester (0.35g.) was dissolved in glacial acetic acid (100 ml.). The solution was heated at 100° with analganated zinc (12 g.) and concentrated hydrochloric acid (25 ml.) for 2 hours, when a further quantity of concentrated hydrochloric acid (5 ml.) was added and heating continued for 1 hour; addition of concentrated hydrochloric acid (10 ml.) and heating for 30 minutes completed the reaction. The hot solution was filtered and the product was precipitated with water and isolated by ether extraction in the usual way. After acetylation with acetic anhydride and pyridine at 100°, the substance crystallised for n-hexane as prisms, m.p. 202.5-203.5°, and gave no depression on admixture with the starting material. Recovered yield 240 mg.(69%).

12:19-Diketo-oleana-10:13(18)-dienyl Acetate.

(c.f. Ruzicka, Jeger and Norymberski Helv.Chim.Acta. 1942, 25, 451.)

β-Amyrenyl acetate (5g.) was suspended in dioxan (250 ml.) with powdered selenium dioxide (8g.). The mixture was heated in an autoclave at 200° for 22 hours. The cooled solution was filtered through sintered glass and the product was precipitated from the filtrate by addition of water. The precipitated solid was extracted with ether and the ether extract was washed with water, dried (Na_2SO_4) and boiled with animal charcoal for a few minutes, filtered and evaporated to dryness. The residue was dissolved in benzene (100 ml.) and the solution was filtered through a short column of activated alumina. The column was washed with benzene and benzene-ether (1:1). The washings from the column were evaporated and the residue was redissolved in benzene (100 ml.) and again filtered through a short column of alumina, followed by washing with benzene. The residual solid, after evaporation of the benzene solution was crystallised five times from aqueous methanol to give 12:19-diketo-oleana-10:13(18)-dienyl acetate as plates, m.p. 242-43°, $[\alpha]_D$ - 90° (C, 1.88).

The product gave no colouration with tetranitromethane.

Light absorption in ethanol; max. at 2100Å, ε = 6,840, and 2760Å, ε = 11,850.

Concentration of the mother liquors from the above crystallisations gave a further quantity (1.7g.) of 12:19-diketo-oleana-10:13(18)-dienyl acetate, m.p. 237-241°.

12:19-Diketo-oleana-10:13(18)-dienyl acetate can also be crystallised

from light petroleum (b.p. 60-80°) as prisms, m.p. 242-243°.

Catalytic Hydrogenation of 12:19-Diketo-oleana-10:13(18)-dienyl Acetate.

A solution of 12:19-diketo-oleana-10:13(18)-dienyl acetate (1.27g.) in glacial acetic acid (120 ml.) was shaken with hydrogen and platinum (from 0.5g $P+O_2$) for 48 hours. Hydrogen absorption (approx. 4 moles) had then ceased and crystalline solid had separated. The reaction product was isolated in the usual way, dissolved in light petroleum (b.p. 60-80°; 100 ml.) and chromatographed on a column of activated alumina (12x2 cm).

Light petroleum (600 ml.) and light petroleum-benzene (1:1 ; 100 ml.) eluted a solid (400 mg.) which after four crystallisations from methanol-chloroform gave oleana-10:13(18)-dienyl acetate as plates, m.p. 198.5-200.5°, $[\alpha]_D$ + 60°, + 59.5° (C, 1.3, 1.0).

Found :	C, 82.6	;	H, 11.1 %
$C_{32}H_{50}O_2$ requires:	C, 82.3	;	H, 10.8 %

Light absorption in ethanol; ε 2100 = 10,000, ε 2150 = 9,000, ε 2220 = 5,600.

Oleana-10:13(18)-dienyl acetate gives an intense yellow colour with tetranitromethane in chloroform.

Oleana-10.13(18)-dienol was obtained by hydrolysis of the above acetate with ethanolic potassium hydroxide (4%). The alcohol crystallised from methanol as needles, m.p. 204.5-206.5°, $[\alpha]_D$ + 53.5°, + 52° (C, 0.5, 0.9).

Found:	C, 84.9	;	H, 11.5 %
$C_{30}H_{48}O$ requires:	C, 84.8	;	H, 11.4 %.

Re-acetylation of oleana-10:13(18)-dienol with acetic anhydride and pyridine at 100° gave oleana-10:13(18)-dienyl acetate, which was crystallised once from methanol-chloroform as plates, m.p. 196-198° $[\alpha]_D$ + 57° (C, 1.18). This specimen showed no depression on mixed melting with the specimen described above.

Continued elution of the alumina with benzene (300 ml.) gave a crystalline solid (250 mg.) which after five crystallisations from methanol-chloroform gave 19-keto-olean-10 enyl acetate as plates, m.p. 254-256°, $[\alpha]_D$ + 117.5, + 116° (C, 1.15, 1.0).

Found: C, 79.9 ; H, 10.4 %

$C_{32}H_{50}O_3$ requires: C, 79.6 ; H, 10.4 %

19-Keto-olean-10-enyl acetate gives a pale yellow colour with chloroformic tetranitromethane.

Light absorption in ethanol ; max. at 3020Å, $\varepsilon = 44$;

$\varepsilon_{2060} = 3,400$, $\varepsilon_{2100} = 2,400$, $\varepsilon_{2150} = 920$.

Further elution of the alumina column gave fractions which could not be adequately purified but which did not show intense selective absorption in the ultra-violet above 2200Å.

Conversion of Oleana-10:13(18)-dienyl Acetate into Oleana-11:13(18)-dienyl Acetate.

A solution of oleana-10:13(18)-dienyl acetate (87 mg.) in acetic acid (15 ml.) containing concentrated hydrochloric acid (1.5 ml.) was heated for three hours on the steam bath. The mixture was poured into water and the product, isolated by means of ether, was treated with acetic anhydride (1 ml.) and pyridine (1 ml.). The acetylated product was isolated in the usual way and crystallised from methanol-

chloroform as plates, m.p. 223-226°, $[\alpha]_D$ - 63° (C, 1.2). The substance gave a red brown colour with tetranitromethane and was undepressed in m.p. when mixed with an authentic specimen of oleana-11:13(18)-dienyl acetate.

Light absorption in ethanol; max. at 2420Å, ε = 23,500,
2500Å, ε = 27,000, and
2600Å, ε = 17,600.

Reduction of 12:19-Diketo-oleana-10.13(18)-dienyl Acetate with Zinc and Acetic Acid.

A solution of 12:19 -diketo-oleana-10:13(18)-dienyl acetate (0.95g.) in glacial acetic acid (100 ml.) was gently refluxed for 4 hours with zinc dust (1.8g.). The hot solution was filtered free of zinc and the product was precipitated with water, collected, washed with water and dissolved in ether. The ether solution was worked up in the usual way.

A solution of the product in light petroleum (b.p.60-80°; 100 ml.) and benzene (40 ml.) was chromatographed on a column of alumina (15 x 1.5 cm.). The fraction (229 mg., m.p.190°) eluted with light petroleum-benzene (2:1 ; 200 ml.) and light petroleum-benzene (1:1 ; 300 ml.) was crystallised thrice from methanol-chloroform to oleana-10:13(18)-dienyl acetate as plates, m.p. 195-197°, $[\alpha]_D$ + 60°(C, 1.0) undepressed in m.p. when mixed with the specimens obtained by catalytic hydrogenation of β-amyradiendiomyl acetate.

A fraction (28 mg.) obtained by continued washing of the alumina column with light petroleum-benzene(2:3; 100 ml.) was not examined. The fractions obtained from the column by washing with benzene (300 ml.), benzene-ether (9:1 ; 200 ml.) and benzene-ether

(4:1 ; 50 ml.) were combined (314 mg. ; m.p. 204-207°) and crystallised from aqueous methanol from which 12-keto-oleana-10:13(18)-dienyl acetate separates as plates from concentrated solution and as prisms from dilute solution, m.p. 205-207°, $[\alpha]_D$ - 79°, -78° (C, 1.0, 0.9).

Found: C, 79.95 ; H, 10.3 %

$C_{32}H_{48}O_3$ requires : C, 79.95 ; H, 10.1 %

12-Keto-oleana-10:13(18)-dienyl acetate gives a pale yellow colour with chloroformic tetranitromethane.

Light absorption in ethanol; max. at 2080Å, ε = 9,000;
2600Å, ε = 9,250;
2950Å, ε = 8,450.

A fraction (157 mg. ; m.p. 280-287°) obtained by washing the alumina column with benzene-ether (3:2 ; 50 ml.), benzene-ether (1:1 ; 100 ml.), ether-benzene (3:2 ; 50 ml.) and ether (200 ml.) crystallised from aqueous methanol to yield 12:19-diketo-olean-10 enyl acetate as plates, m.p. 285-287°, $[\alpha]_D$ + 132° (C, 1.0).

Found : C, 77.6 ; H, 9.9 %

$C_{32}H_{48}O_4$ requires: C, 77.4 ; H, 9.7 %

Light absorption in ethanol ; max. at 2460Å , ε = 12,400.

Ruzicka and Jeger (Helv.Chim.Acta.1941, 24, 1236) give m.p. 290-292°(corr.), λ max. at 2400Å (log ε = 4.05) for a compound $C_{32}H_{48}O_4$ obtained by catalytic reduction of β-amyradiendienyl acetate.

12-Keto-oleana-10:13(18)-dienol was obtained from the corresponding acetate by refluxing with ethanolic potassium hydroxide (3%) for 3 hours. It separates from aqueous methanol as needles, m.p. 267-269°, $[\alpha]_D$ - 99.5°, - 100°, - 99° (C, 1.1, 0.75, 0.8).

Found : C, 82.3 ; H, 10.7 %

$C_{30}H_{46}O_2$ requires: C, 82.1 ; H, 10.6 %

Light absorption in ethanol; max. at 2100Å, ε = 7,600;
2620Å, ε = 8,600, and
2950Å, ε = 7,900.

Reacetylation of this alcohol using acetic anhydride and pyridine gave 12-keto-oleana-10:13(18)-dienyl acetate as prisms or plates from aqueous methanol, m.p. 205-206°, $[\alpha]_D$ - 78° (C, 1.35).

Treatment of 12-keto-oleana-10.13(18)-dienyl Acetate with Mineral Acid.

The dienone (150mg.) was heated for 3 hours at 100° in glacial acetic acid (25 ml.) containing concentrated hydrochloric acid (3 ml.). The mixture was worked up in the usual way and the product was acetylated with acetic anhydride and pyridine. The product crystallised from aqueous methanol as plates, m.p. 204-205°, $[\alpha]_D$ -78.5° (C. 1.1) undepressed on mixed melting with the starting material.

Light absorption in ethanol; max. at 2080Å, ε = 7,250;
2600Å, ε = 8,950;
2950Å, ε = 8,100.

Catalytic Hydrogenolysis of 12-Keto-oleana-10:13(18)-dienyl Acetate.

A solution of the acetate (0.3g.) in acetic acid (50 ml.) was shaken with platinum (from 0.2 g. P O_2) and hydrogen for 7 hours. Towards the end of the reaction the product separated from the solution. The product isolated by means of ether, crystallised from methanol-chloroform to give oleana-10:13(18)-dienyl acetate (250 mg.) as plates, m.p. 197-199°, $[\alpha]_D$ + 59° (C, 0.9); the m.p. of a

mixture with a previously prepared specimen was undepressed.

Reduction of 12-Keto-oleana-10:13(18)-dienyl Acetate with Zinc and Acetic Acid.

A solution of the acetate (250 mg.) in glacial acetic acid (50 ml.) was refluxed with zinc (0.5g.) for 4 hours. The product was isolated by means of ether and crystallised from methanol-chloroform to yield the relatively insoluble oleana-10:13(18)-dienyl acetate (50 mg.) as plates, m.p. 190-191°. The mother liquor was evaporated to dryness, the residue dissolved in glacial acetic acid and the solution again refluxed with zinc (0.5g.) for 4 hours. The product was isolated by means of ether and crystallised from methanol-chloroform to yield oleana-10:13(18)-dienyl acetate (58 mg.) as plates, m.p. 188-190°. Recrystallisation of the combined crops from methanol-chloroform yielded the dienyl acetate as plates, m.p. 196-198° undepressed when mixed with an authentic specimen.

Reduction of 12-Keto-oleana-10:13(18)-dienyl Acetate with Lithium Aluminium Hydride.

A solution of the acetate (1.0g.) in dry ether (250 ml.) containing lithium aluminium hydride (1.0g.) was refluxed for 3 hours. The excess of hydride was destroyed with water and the product was isolated by means of ether, avoiding the use of mineral acid. Acetylation at room temperature with acetic anhydride and pyridine, and crystallisation from methanol-chloroform gave a mixture (approx. 500 mg.) m.p. 192-194°. This mixture gave a brown colour with

tetranitromethane and showed light absorption in ethanol at 2950Å (ε = 440) probably due to the presence of oleana-10:12:18-trienyl acetate. Recrystallisation of this solid from methanol-chloroform which was accompnaied by large losses, gave oleana-10:13(18)-dienyl acetate (50 mg.) as plates, m.p. 199-200°, undepressed when mixed with an authentic specimen, and giving a yellow colour with tetranitromethane in chloroform; $[\alpha]_D$ + 59° (C, 0.9, 1.0)

Light absorption; max. at 2100Å, ε = 8,000.

12:19-Diketo-oleana-10:13(18)-dienyl Acetate from 12-Keto-oleana-10:13(18)-dienyl Acetate.

Method (a).

The acetate (86 mg.) was refluxed for 72 hours with acetic anhydride (10 ml.) containing freshly fused potassium acetate (0.1g.). The product was isolated by dilution with a large excess of water and ether extraction in the usual way. Crystallisation from methanol gave 12:19-diketo-oleana-10:13(18)-dienyl acetate (50 mg.) as plates, m.p. 241-242°, $[\alpha]_D$ -89° (C, 0.6). A mixture with a reference specimen was undepressed in m.p.

Light absorption in ethanol ; max. at 2780Å, ε = 11,000.

Method (b).

The acetate (0.37g.) in dioxan (50 ml.) was heated in a sealed tube at 200° for 20 hours with selenium dioxide (0.6g.). The cold solution was filtered through sintered glass and the product was worked up in the usual way through ether. The ether extract was treated with animal charcoal before evaporation. Crystallisation of the

product from aqueous methanol gave 12:19-diketo-oleana-10:13(18)-dienyl acetate as plates, m.p. 240-41°, $[\alpha]_D$ -91.5° (C, 1.3), undepressed on mixed melting with an authentic specimen.

Light absorption in ethanol; max. at 2760Å, ε = 12,500.

Method (c).

The acetate (250mg.) in glacial acetic acid (50 ml.) was refluxed with selenium dioxide (250 mg.) for 6 hours. The mixture was worked up as described above and the product was crystallised from aqueous methanol to give 12:19-diketo-oleana-10:13(18)-dienyl acetate (130mg.) as large square plates, m.p. 240-241°, $[\alpha]_D$ - 91° (C, 1.3) and giving no depression on mixed melting with a reference sample.

Light absorption in ethanol; max. at 2760Å, ε = 12,500.

Reduction of 12:19-Diketo-oleana-10:13(18)-dienyl Acetate with Zinc Dust in Ethanol.

12:19-Diketo-oleana-10:13(18)-dienyl acetate (1g.) dissolved in ethanol (100 ml.) was refluxed for 5 hours with activated zinc dust (5g.). The solution was filtered free of zinc and the product was isolated from the filtrate in the usual way and crystallised from methanol to yield 12:19-diketo-olean-10-enyl acetate (0.9g.) as blades m.p. 283-286°, $[\alpha]_D$ + 132° (C, 1.28) undepressed in m.p. when mixed with a specimen prepared by zinc and acetic acid reduction of β-amyradiendienyl acetate.

12:19-Diketo-olean-10-enyl acetate was recovered unchanged after heating on the water bath for 1 hour with acetic anhydride and pyridine. It was also recovered unchanged after standing overnight

in glacial acetic acid containing chromic acid (1.0 atom O).

12.19-Diketo-18α-olean-10-enyl acetate.

A solution of 12:19-diketo-olean-10-enyl acetate (400 mg.) in methanolic potassium hydroxide (10%) was refluxed for 3 hours. The product was acetylated using acetic anhydride and pyridine at 100°. After working up the mixture in the usual manner the acetate was crystallised from methanol to give 12:19-diketo-18α-olean-10-enyl acetate as plates m.p. 279-281°, $[\alpha]_D$ + 91°, + 92° (C, 0.5, 1.2).

Found : C, 77.6 ; H, 9.7 %

$C_{32}H_{48}O_4$ requires: C, 77.4 ; H, 9.7 %

Light absorption in ethanol ; max. at 2430Å, ε = 11,700.

Hydrogenolysis of 12:19-Diketo-olean-10-enyl Acetate.

A solution of 12:19-diketo-olean-10-enyl acetate (0.8g.) in stabilized acetic acid (250 ml.) was shaken with hydrogen and platinum (from 0.3g P_4O_2). Absorption of hydrogen was slow and ceased after 48 hours. The solution was filtered and the filtrate was evaporated to dryness. The residue was crystallised from methanol-chloroform to give 19-keto-olean-10-enyl acetate (0.66g.) as plates, m.p. 254-256° $[\alpha]_D$ + 117° (C, 2.0). This product gave a yellow colour with the tetranitromethane reagent and was undepressed on mixed melting with a specimen prepared by hydrogenolysis of 12:19-diketo-olean-10:13(18)-dienyl acetate.

Treatment of 19-Keto-olean-10-enyl Acetate with Hydrochloric Acid.

A solution of the acetate (0.27g.) in acetic acid (50 ml.) containing concentrated hydrochloric acid (4 ml.) was allowed to stand

overnight at room temperature and was then heated at 100° for 14 hours. The mixture was worked up in the usual way and the product was acetylated with acetic anhydride and pyridine at 100°. The resultant product was dissolved in light petroleum (b.p.60-80°)(50 ml.) and chromatographed on a column of alumina (8 x 2 cm.).

Washing the column with light petroleum (400 ml.) gave traces of gum which could not be crystallised. The fraction (30 mg.) eluted with light petroleum-benzene (9:1 ; 250 ml.) was crystallised with difficulty from methanol-chloroform to give plates m.p.250-251°. This substance gave a red brown colour with tetranitromethane in chloroform and showed light absorption in ethanol; max. at 2370Å, ε = 20,000; 2450Å , ε = 23,700; 2550Å, ε = 15,500. The compound gave a marked depression on mixed melting with oleana-11:13(18)-dienyl acetate. Lack of material prevented further investigation.

Further elution of the column with light-petroleum-benzene (1:1 ; 350 ml.) gave a solid (100 mg.) which crystallised from methanol-chloroform as blades, m.p. 252-254°, $[\alpha]_D$ + 137° (C, 0.89). This substance gave a yellow colour with the tetranitromethane reagent and a marked depression on mixed m.p. with the starting material. The product gave no depression on admixture with 19-keto-18α-olean-10-enyl acetate.

Found: C, 79.6 ; H, 10.6 %

Calculated for $C_{32}H_{50}O_3$: C, 79.6 ; H, 10.4 %

A further quantity (30mg.) of 19-keto-18α-olean-10-enyl acetate was obtained by washing the column with benzene-light petroleum (3:1 ; 150 ml.) and benzene (150 ml.).

Continued washing of the column with solvents of increasing eluting powers gave very small fractions which could not be crystallised.

Treatment of 19-Keto-olean -10-enyl Acetate with Isopropenyl Acetate.

To a boiling solution of the acetate (250 mg.) in isopropenyl acetate (10 ml.) one drop of concentrated sulphuric acid was added, and the solution was refluxed for 3 hours. The product was isolated by means of ether in the usual way and crystallised from methanol-chloroform as plates, m.p. 252-254°, which gave a yellow colour with tetranitromethane in chloroform, and no depression on mixed m.p. with the starting material. Recovered yield 200 mg. (80%).

Attempted Formation of the Enol Acetate of 19-Keto-olean-10-enyl Acetate.

The acetate (0.3g.) in dry carbon tetrachloride (6 ml.) was treated with perchloric acid (1 drop of 50% aqueous) in acetic anhydride (0.5 ml.) and the solution was allowed to stand at room temperature for 2 hours. The product was isolated in the usual way and crystallised from methanol-ether as plates, m.p. 250-253°, undepressed by starting material. Recovered yield 240 mg.(80%).

Treatment of 12:19-Diketo-olean-10-enyl Acetate with Isopropenyl Acetate.

A solution of 12:19 -diketo-olean-10-enyl acetate (0.6 g.) in isopropenyl acetate (27 ml.) was treated with one drop of concentrated sulphuric acid and the mixture was heated under reflux for 3 hours. The dark brown solution was poured into water and the mixture was extracted with ether. The ether extract was washed many times with water and the ether evaporated. The solid was dried by azeotropic distillation of the water with benzene. The residue was crystallised from methanol to give 12:19-epoxy-oleana-10:12:18-trienyl acetate as plates, m.p. 180-181°, $[\alpha]_D$ + 170° (C, 1.5; 1.45).

Found : C, 80.1 ; H, 9.7%

$C_{32}H_{46}O_3$ requires: C, 80.3 ; H, 9.7%

The compound gave a yellow colour with tetranitromethane in chloroform. Light absorption in ethanol; max. at 3240Å, ϵ = 15,400 ; 2200Å, ϵ = 5,850.

Enol Acetate of 12:19-Diketo-olean-10-enyl Acetate.

A solution of 12:19-diketo-olean-10-enyl acetate (0.4g.) in acetic anhydride (50 ml.) was refluxed for 4½ hours in an atmosphere of nitrogen with freshly fused sodium acetate (0.4g.). The product was precipitated with a large excess of water and isolated by means of ether in the usual way. The residue from evaporation of the ether extract was crystallised from methanol-ether to give (0.2g.) plates, m.p. 283-285°, undepressed on mixed melting with starting material.

The mother liquor from the above crystallisation was concentrated and needles separated out. Recrystallisation from aqueous methanol gave 19-keto-12-acetoxy-oleana-10:12 dienyl acetate as needles, m.p. 251-252°, $[\alpha]_D$ + 285° (C, 0.57).

Found: C, 75.8 ; H, 9.3%

$C_{34}H_{50}O_5$ requires: C,75.8 ; H, 9.4%

The compound gave a brown colour with tetranitromethane in chloroform and showed light absorption in ethanol; max. at 2060Å, ε = 5,900; 2800Å, ε = 9,150.

This compound was recovered unchanged on attempted catalytic hydrogenolysis in acetic acid.

12-Keto-oleananyl Acetate.

A mixture of hydrogen peroxide (100 vol.; 100 mls.) in formic acid (98%; 300 mls.) was added dropwise over a period of 3 hours to a well stirred solution of β-amyrin acetate (40g.) in ethyl acetate (2800 mls.) at 55-60°. The mixture was stirred for 6 hours at 60° and concentrated under reduced pressure to a volume of about 500 mls. The solid which had separated out on cooling was collected, washed with water and dried at 100°. Recrystallisation from methanol-chloroform gave 12-keto-oleananyl acetate (27g.) as plates, m.p. 298-299°, $[\alpha]_D$ - 11° (C, 1.12).

12-Keto-olean-10-enyl Acetate.

12-Keto-oleananyl acetate (36g.) in glacial acetic acid (2500 ml.) was treated at 60° with a few drops of hydrobromic acid (50%), and

bromine (4 ml.) in acetic acid (150 ml.) was added dropwise over a period of 1½ hours. The solution was stirred for 3 hours at 60-70° and then diluted with hot water (1500 ml.). The product which crystallised on cooling was recrystallised from methanol-chloroform to give 12-keto-olean-10-enyl acetate (24g.) as plates, m.p. 287-289°, $[\alpha]_D$ + 62° (C, 1.0).

Light absorption in ethanol; max. at 2450Å, ε = 10,000.

iso-β-Amyradienonyl Acetate.

A solution of 12-keto-olean-10-enyl acetate (23g.) in glacial acetic acid (900 ml.) was refluxed for 25 hours with selenium dioxide (23g.). The cold solution was filtered and the product was isolated from the filtrate by dilution with water and ether extraction. The ether extract was worked up in the usual way. The product was dissolved in benzene and filtered through a short column of alumina. The filtrate was evaporated and the residue was twice crystallised from light petroleum-ether and finally from methanol to give iso-β-amyradienonyl acetate (13g.) as prisms, m.p. 223-224.5°, $[\alpha]_D$ − 36° (C, 1.33), undepressed in m.p. when mixed with an authentic specimen. iso-β-Amyradienonyl acetate gives a yellow colour with tetranitromethane in chloroform.

Light absorption in ethanol; max. at 2440Å, ε = 10,400.

Catalytic Hydrogenolysis of iso-β-Amyradienonyl Acetate.

A solution of iso-β-amyradienonyl acetate (1g.) in acetic acid (150 ml.) was shaken with platinum (from 0.5g. PtO_2) in an atmosphere of hydrogen for 4 days. The apparent absorption of hydrogen was

3-3.5 moles. The solution was filtered and the product was precipitated from solution by addition of water, collected, washed with water and dried. Crystallisation from methanol-chloroform gave neo-β-amyrin acetate (0.5g.) as needles, m.p. 227-229°, $[\alpha]_D$ + 4° (C, 1.0).

Found: C, 82.2 ; H, 11.4%

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

neo-β-Amyrin acetate gives a red brown colour with chloroformic tetranitromethane.

Light absorption in ethanol; max. at 2120Å, ε = 4,450;

ε_{220} = 2860, ε_{225} = 730.

In a subsequent experiment it was found that the rate of hydrogenolysis of iso-β-amyradienonyl acetate was increased by a factor of 4 by addition of one drop of concentrated hydrochloric acid to the reaction mixture.

Treatment of neo-β-Amyrin Acetate with Hydrochloric Acid.

The acetate (100 mg.) in acetic acid (16 ml.) containing concentrated hydrochloric acid (1.5 ml.) was heated on the steam bath for 4 hours, when further quantities of hydrochloric acid (1.5 ml.) and acetic acid (10 ml.) were added and heating continued for a further 3 hours. Concentrated hydrochloric (1 ml.) was added and the solution was again heated for 3 hours. The product was then isolated in the usual way and acetylated with acetic anhydride and pyridine at 100°. After working up the acetylation in the usual way, the product was dissolved in light petroleum (b.p. 60 - 80°; 50 ml.)

and the solution was filtered through a short column of alumina. The product was then crystallised from methanol-chloroform to give needles, m.p. 225-226°, which gave a red brown colour with the tetranitromethane reagent and no depression on mixed melting with starting material. Recovered yield 50 mg. (50%).

Oxidation of neo-β-Amyrin Acetate with Potassium Permanganate.

A solution of potassium permanganate (0.15g.) in distilled water (39 ml.) and acetic acid (39 ml.) was added dropwise to a well stirred solution of neo-β-amyrin acetate (0.62g.) in acetic acid (230 ml.) at room temperature. The addition was made over a period of 30 minutes, after which the solution was stirred for one hour. The solution was decolourised by addition of a solution of sodium metabisulphite and precipitation of the solid was completed by addition of water. The solid was extracted with ether and the ether extract was worked up in the usual way. The product crystallised from methanol to give neo-β-amyrin oxide as prisms, m.p. 207-208°, $[\alpha]_D$ _ 45° (C, 1.13).

Found: C, 79.4 ; H, 10.9 %

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

The oxide gave no colour with chloroformic tetranitromethane.

The mother liquor from the above crystallisation was concentrated to yield a compound which was recrystallised from aqueous methanol as needles, m.p. 230-232°, $[\alpha]_D$ - 63°,-64.5° (C, 1.0, 0.88).

Found: C, 76.8 ; H, 10.6%

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

This compound gave no colour with tetranitromethane in chloroform.

Light absorption in ethanol; max. at 2060Å, ε = 400 with low intensity inflection at 2950Å.

Treatment of neo-β-Amyrin Oxide with Hydrochloric Acid.

The oxide (150 mg.) in chloroform (3ml.) and acetic acid (20 ml.) was treated with hydrochloric acid (2 ml.) and the solution was allowed to stand overnight at room temperature. The dark green solution was diluted with water and the product was isolated by means of ether in the usual way and acetylated at 100° with acetic anhydride and pyridine. The product obtained from the acetylation was dissolved in light petroleum (b.p. 60-80°) and the solution was filtered through a short column of alumina in order to decolourise. The filtrate was evaporated and the residue was crystallised from aqueous acetic acid to give a diene as plates, m.p. 192-194°, $[\alpha]_D$ - 34°, [C, 0.85).

Found : C, 82.3 ; H, 10.7 %

$C_{32}H_{50}O_2$ requires: C, 82.3 ; H, 10.8 %

The diene gave a brown colour with tetranitromethane in chloroform.

Light absorption in ethanol; max. at 2580Å, ε = 21,000.

Bromination of neo-β-Amyrin Acetate.

neo-β-Amyrin acetate (0.3g.) in glacial acetic acid (50 ml.) was treated with bromine in acetic acid (2.6 ml. of 5% ≡ 1.1 moles). The bromine was rapidly absorbed at room temperature and the solution was allowed to stand overnight. The product was precipitated by addition of water and isolated by means of ether. The ether extract was worked up in the usual way. Crystallisation from methanol gave

needles, m.p. 226-228°, undepressed by starting material and giving a red brown colour with chloroformic tetranitromethane. Recovered yield 200 mg. (66%).

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