THE STRUCTURAL CHEMISTRY OF THE TRITERPENE

β-AMYRIN AND ITS DERIVATIVES

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THESIS

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by

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C O N T E N T S

HISTORICAL

INTRODUCTION

The triterpenes are a class of naturally occurring compounds forming that group of polyterpenes each member of which contains thirty carbon atoms and the majority containing a five-ring carbocyclic skeleton which, theoretically, can be built up of isoprene units linked regularly or irregularly.

Most triterpenes are obtained from plant sources, often in association with phytosterols (1), where they may occur in the free state or glycosidically linked with sugars as saponins. A few triterpenes are found in animal organisms, where they occur with sterols, which are structurally similar compounds.

Like the sterols, the triterpenes yield characteristic, common dehydrogenation products, most of which are homologues of naphthalene, indicating therefore a common basic carbon skeleton.

Apart from the pentacyclic triterpenes, which are in the majority, there are a few which contain a smaller number of alicyclic rings. For example, the tetracyclic lanosterol, agnosterol and euphol, the tricyclic ambrein and the aliphatic squalene. Mono-, and dicyclic triterpenes are unknown.

Chemical investigation of the triterpenes over the

last twenty years has shown that for reasons of structural differences they can be divided into at least four main sub-groups, viz,

1. the β-amyrin/oleanolic acid group,
2. the α-amyrin group,
3. the lupeol group,
4. the tetracyclic triterpenes.

Many of the compounds within these groups have been interrelated and some compounds of different groups have been converted into common intermediates. No compound of the α-amyrin sub-group, however, has been related to one of the β-amyrin sub-group.

The most abundant pentacyclic triterpenes are probably the amyrins, which were first isolated by Rose in 1839 (2) from Manila Elemi resin. Resolution of the α- and β-forms, which occur together, was carried out later by Vesterberg (3).

The work described in this thesis was concerned solely with the structures of β-amyrin and its derivatives, and therefore in the general historical review, which follows, emphasis has been laid almost entirely upon the structures of the compounds of the β-amyrin/oleanolic acid sub-group as revealed by the now classical work on dehydrogenation, oxidative degradation etc., and also the

very recent work on stereochemistry. The historical work pertaining directly to the author's own work is described in the theoretical section of this thesis.

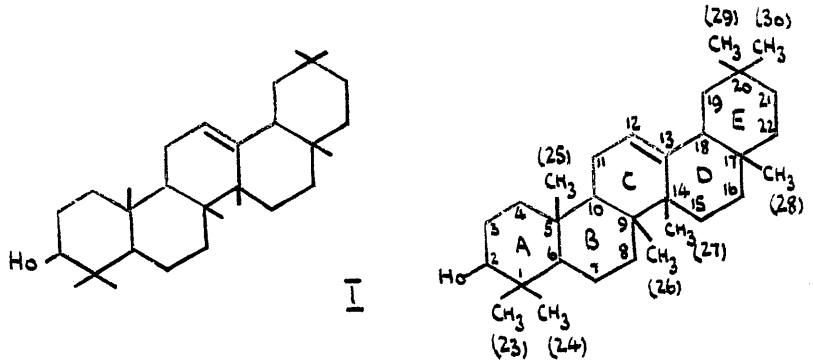
In view of the large and ever-increasing data which is available concerning the structures of the triterpenes it has been found impossible to give more than a brief, general historical outline of the subject in this thesis.

For descriptions of, and discussions on the whole triterpene family of compounds, the reader's attention is directed to the excellent reviews of Haworth (4), Spring (5), Noller (6), Jeger (7), Birch (8) and also to Elsevier's Encyclopaedia of Organic Chemistry (9).

Classification of the triterpenes.

As stated earlier, the naturally occurring triterpenes fall into a number of well-defined sub-groups which differ from each other in certain important structural respects. In this section of the "historical" part of this thesis it is proposed to deal only with the three sub-groups of the pentacyclic triterpenes.

(i) The β-amyrin/oleanolic acid group consists of the compounds named in the Table below. The positions of the functional groups in these compounds are listed and formula (I) represents the presently accepted structure of β-amyrin (or β-amyrenol).



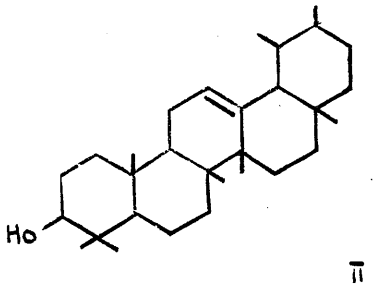
I

All the compounds in this group contain a hydroxyl group at position C_3 and all except δ-amyrin, germanical and morolic acid have a 12:13 ethylenic linkage. Of the nineteen naturally occurring compounds in this series only the basic structures of the soyasapogenols remain in doubt.

Triterpene	Double bonds	Hydroxy	Keto	COOH	Source
β-amyrin	12:13	2	-	-	M.Elemi Resin
Soy sapogenin C	12:13,15:16(?)	2.24(or 23)	-	-	Soy beans
Erythrodiol	12:13	2.28	-	-	Cocoa bush
Maniladiol	12:13	2.16 (epi)	-	-	M.Elemi Resin
Genin A	12:13	2.16.28	-	-	Primula
Soy sapogenin B	12:13	2.16(?)24(or 23)	-	-	Soy beans
Soy sapogenin A	12:13	2.15(?)16(?)24(or23)	-	-	Soy beans
α-Boswellic acid	12:13	2 (epi)	-	24(or 23)	Frankincense
Oleanolic acid	12:13	2	-	28	Cloves
Hederagenin	12:13	2.23(or 24)	-	28	Ivy
Sumaresinolic acid	12:13	2.7	-	28	Sumatra Benzoin
Echinocystic acid	12:13	2.16	-	28	Quillaia bark
Siaresinolic acid	12:13	2.19	-	28	Siam Benzoin
Gypsogenin	12:13	2	23(or 24)	28	Saponaria Officinalis
Quillaic acid	12:13	2.16	23(or 24)	28	Quillaia bark
Glycyrrhetic acid	12:13	2	11	29(or 30)	As saponins
-amyrin	13:18	2	-	-	Broom
Germanicol	18:19	2	-	-	Lactuca virosa
Lorolic acid	18:19	2	-	28	Hora excelsa

(ii) The α-amyrin group. This group comprises the naturally occurring triterpenes, listed in the following Table, with the carbon skeleton (II) for α-amyrenol.

Triterpene	Double bond	Hydroxyl	COOH	Source
α-Amyrin	12:13	2	-	M.Elemi Resin
Uvaol	12:13	2.28(?)	-	Ericaceœ Arctostaphylos
Brein	12:13	2.21(or 22)	-	M.Elemi Resin
β-Boswellic acid	12:13	2	23(or 24)	Frankinsence
Ursolic acid	12:13	2	28(?)	Apples and pears
Quinovic acid	12:13	2	27.28	Zygophyllium coccineum



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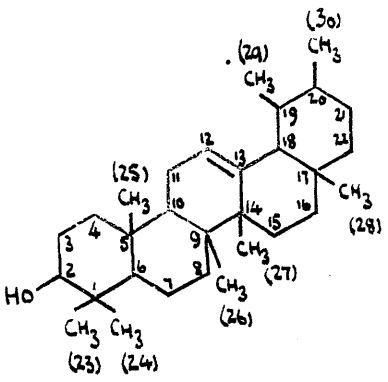
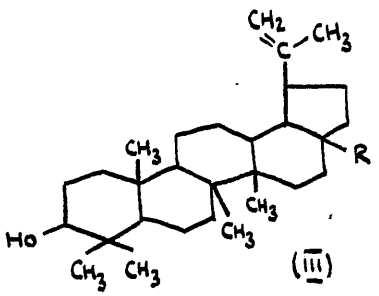


Again in this group, each member carries a hydroxyl group at C_2 and a 12:13 double bond. Quinovic acid was only recently proved to be a group member (25).

(iii) The lupeol group. This group of naturally occurring pentacyclic triterpenes comprises the compounds, lupeol (III, R=CH_3), betulin (III, R=CH_2OH) and betulic acid (III, R=COOH). The compounds of this group differ from the other triterpenes, listed above, in that they all contain an exocyclic methylene group in an isopropyl group which is attached to a five-membered ring E as shown in formula (III). Conversions of lupeol and its derivatives into β-amyrin derivatives have been carried out (31, 32) thus proving that the rings A, B, C and D skeleton is the same in both groups. Associated with this group is the heterobetulin group, consisting of heterobetulin (IV) ($\Delta^{18:20}$, or $\Delta^{20:21}$), taraxasterol, Ψ-taraxasterol, faradiol and arnidiol, the precise structures of which are not yet fully established.



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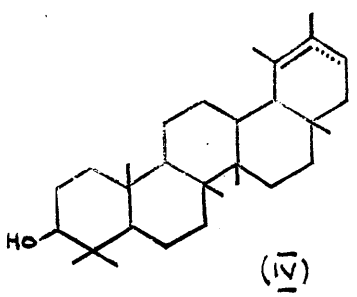
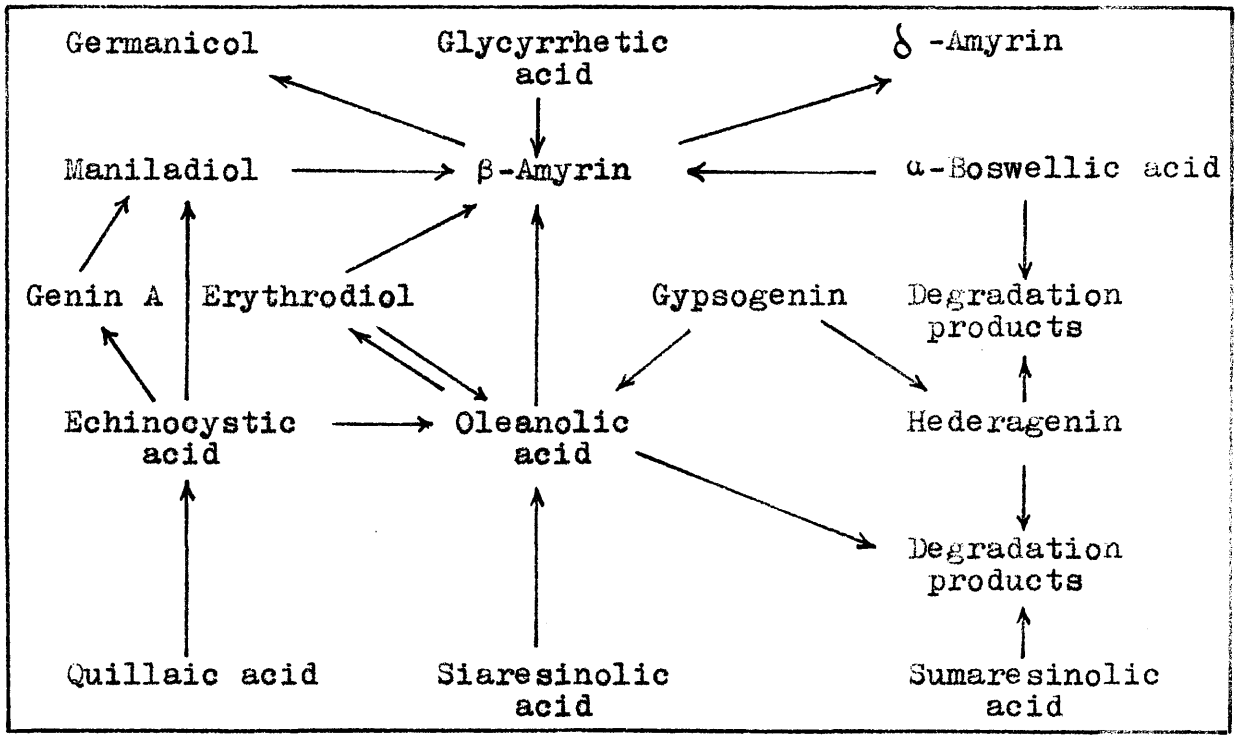


(IV)

Transitions within the Triterpene Groups.

The inter-relation of individual triterpenes has been an outstanding feature of research in this field for many years and complete intra-group relationship has now more or less been achieved. The main transitions within the β-amyrin group are given in the following Table.



Most of these transitions were established by using the method first developed by Ruzicka and Schellenberg (26) in their conversion of oleanolic acid into β-amyrin

and erythrodiol. Acetyloleanolic acid was converted through its acid chloride to the corresponding aldehyde by the Rosenmund method. The aldehyde was then reduced by the Wolff-Kishner method (27) to yield a mixture of β-amyrin and erythrodiol, as shown in the partial formulae below:

$$\underset{\text{Acetyloleanolic acid}}{AcO.C_{29}H_{46}COOH} \rightarrow AcO.C_{29}H_{46}COCl \rightarrow AcO.C_{29}H_{46}CHO \rightarrow \begin{matrix} HO.C_{29}H_{46}CH_3 \\ \beta\text{-amyrin} \\ + \\ HO.C_{29}H_{46}CH_2OH \\ \text{erythrodiol} \end{matrix}$$

The members of the α-amyrin group have likewise been inter-related by this and other methods (28,29,30) and similar relationships have been established between the members of the lupeol group.

These inter-conversions within the groups have proved that all the related compounds have the same basic carbon skeletons and that they differ from one another only in the nature and positions of the substituents which are attached to the basic carbon skeleton.

Transitions between different groups of triterpenes has proved a more difficult task and, as yet, no compound of the α-amyrin group has been converted into one of the β-amyrin group. A number of transitions from lupeol and its derivatives into β-amyrin derivatives, and other

members of the β-amyrin group have been made (31,32) and by this means the fact that the basic structural difference between the two groups lies only in ring E has been established.

Nomenclature.

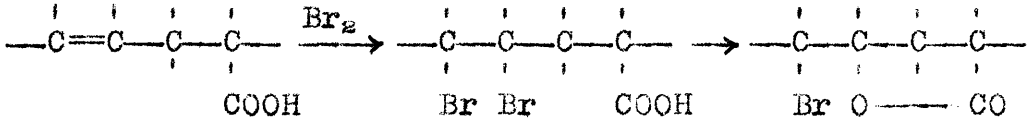
A rational nomenclature for the β-amyrin group of triterpenes was proposed by Ruzicka in 1943 (74, cf. Barton and Brooks, 84) based on the name oleanane = 1.1.8.8.16.18.19.22, octamethyl perhydropicene. Similar names, ursane (97,98) and lupane for the α-amyrin and lupeol series respectively have also been used. The trivial nomenclature, e.g. β-amyrin, oleanolic acid etc., etc., however, is in common usage and is used for well known and well defined compounds in this thesis. Where possible the rational nomenclature has been used for new derivatives, and where this has proved impossible or doubtful practice irrational nomenclature has been used.

Detection of Functional Groups.

Owing to the generally inert nature of the functional groups in the triterpenes and possibly to undeveloped techniques of purification etc., the early workers were beset with difficulties in their researches in this field. With the advent of microanalysis, however, it was proved that the triterpenes contained a thirty carbon atom skeleton (10-17) and gradually methods for the detection of the functional groups were perfected. Descriptions of the methods employed for the detection of the various functional groups are given below.

(i) Ethylenic linkages. In the triterpenes of the β-amyrin and α-amyrin groups the ethylenic linkages are extremely inert and are not susceptible to catalytic hydrogenation. The exocyclic double bond in the lupeol series, however, can be hydrogenated (22,24). In the α- and β-amyrin series much use is now made of the tetranitromethane colour test (henceforth designated T.N.M. test)(18) for isolated and conjugated double bonds. This test is limited, however, as it fails when the double bond is in conjugation with a carbonyl group (20,21,22). Double bonds conjugated with carbonyl groups are also often not susceptible to hydrogenation (18). The method involving ultra-violet light absorption is much used

qualitatively and quantitatively (19,33) but it also has its limitations as the absorption caused by a double bond is not qualitatively reliable if there is any other absorption at higher wavelengths in the ultra-violet. A useful method for the detection of ethylenic linkages in triterpene acids was discovered by Winterstein (10) who found that well defined bromo-lactones were formed by the action of bromine on triterpene acids such as oleanolic acid and hederagenin, as shown below. Using hydrobromic acid or hydrochloric acid (10,18,23), bromine free lactones were formed and in both cases the free acid could be regenerated by the action of zinc and acetic acid. Apart from providing a useful method for the



detection of double bonds in triterpene acids this method provided evidence that the double bonds in these compounds were located in positions γ-δ to the carboxyl groups.

(ii) Carboxyl groups. In many triterpene acids, e.g. oleanolic acid, hederagenin, ursolic acid, the tertiary nature of the carboxyl group is displayed in its non-reactivity with common esterifying reagents. Esterification can, however, be carried out using diazomethane

but the esters produced are hydrolysed only with difficulty.

(iii) Carbonyl groups in many derivatives show themselves to be very unreactive to ordinary carbonyl reagents and their presence can often only be established by spectrographic analysis.

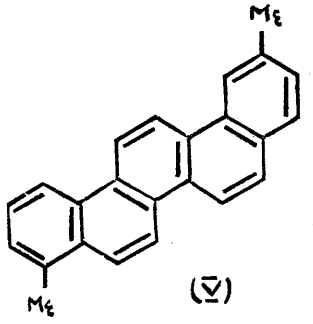
(iv) Hydroxyl groups. The ubiquitous secondary hydroxyl group at position C_3 in the triterpenes is quite reactive and undergoes acylation and oxidation under normal conditions.

Dehydrogenation Experiments.

An important contribution to the elucidation of the structure of the pentacyclic triterpenes was made as a result of studies in the dehydrogenation of various triterpenes with selenium, and palladium on charcoal.

In 1929 Ruzicka (22) isolated a hydrocarbon, $C_{13}H_{14}$, called "sapotaline" from a number of different triterpenes by dehydrogenation with selenium. This hydrocarbon was later shown by Ruzicka and his co-workers to be 1:2:7-trimethylnaphthalene (34), and a second main dehydrogenation product was shown to be 1:2:5:6-tetramethylnaphthalene (35). By using this method of dehydrogenation on a large number of pentacyclic triterpenes a number of common degradation products were obtained. These products are listed in the following Table.

Dehydrogenation Product	Reference
1:2:7-trimethylnaphthalene	22,34,36
1:2:5:6-tetramethylnaphthalene	35
6-hydroxy-1:2:5-trimethylnaphthalene	37
2:7-dimethylnaphthalene	38
1:8-dimethylpicene (V)	39,40,41
1:2:3:4-tetramethylbenzene	42,43



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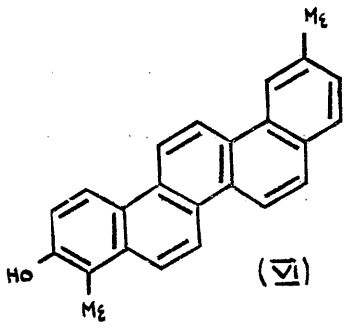


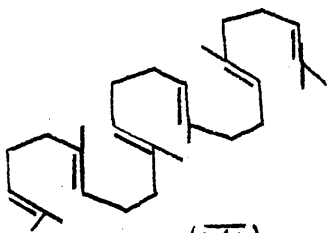
(VI)

It was found that apart from yielding the products listed above, α- and β-amyrenol yielded two new products, 2-hydroxy-1:8-dimethylpicene (VI) and a $C_{18}H_{18}$ phenanthrene derivative (20,39,40,44,45,46,47). Oleanolic acid also yielded the products listed above with the exception of (VI) (22,47,48). Lupeol, however, yielded neither sapotaline nor any of the picene derivatives on dehydrogenation (22,49), its principal product being 1:2:5-trimethylnaphthalene (agathaline).

It was concluded, therefore, from this work that all members of the α- and β-amyrin groups had the same basic carbocyclic skeleton and that the carbocyclic skeleton of lupeol differed in that part of the molecule from which sapotaline was produced on dehydrogenation.

As a result of the work on dehydrogenation the first real attempts at formulation of the amyrins were made. In 1932 Ruzicka suggested a formula based on a hypothetical pentacyclosqualene (squalene VII). Ruzicka's

formulation was only accepted for a short time, however, and since then many modifications have been made to account for new dehydrogenation products and other new information. Alternatives to Ruzicka's structure which were suggested included those of Kitasato (50), Haworth (4), Spring (51) and Kon (52). Of all the suggested



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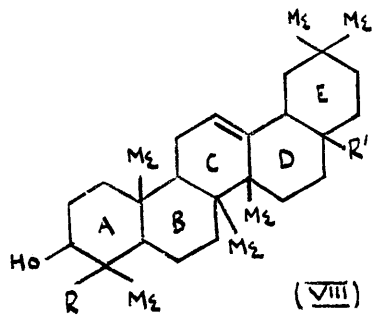
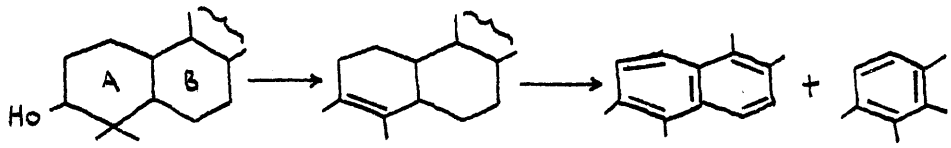


(VIII)

formulae that of Haworth (VIII) for the β-amyrin group interprets most satisfactorily the dehydrogenation and oxidation reactions of β-amyrenol and is consistent with the isoprene rule. The dehydrogenation results are readily explained if it is assumed that breaking of ring C in (VIII) produces the naphthalene derivatives, sapotaline being produced from rings D and E. The production of 1:2:5:6-tetramethylnaphthalene and 1:2:3:4-tetramethylbenzene is explained on the assumption of a retropinacoline rearrangement [cf. Ruzicka (46)] thus:-



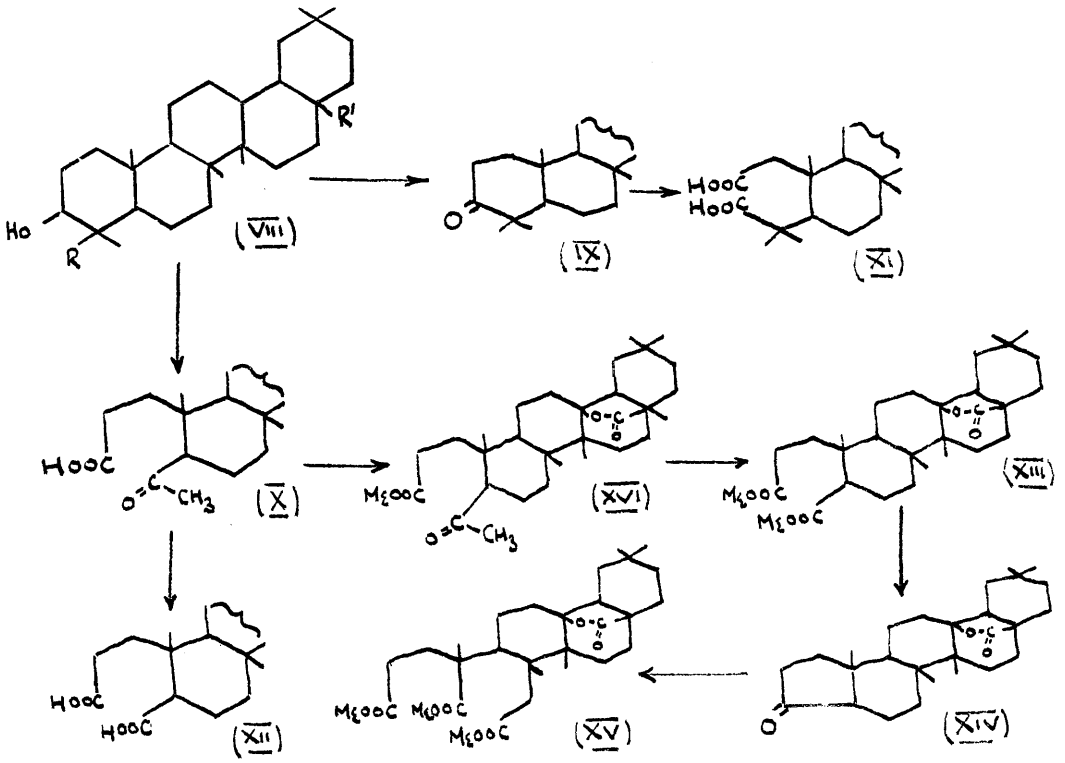
Structure (VIII) represents the more important members of the β-amyrin group, e.g. β-amyrenol (VIII; R=R'=Me); oleanolic acid (VIII; R=Me, R'=COOH); erythrodiol (VIII; R=Me, R'=CH_2OH); hederagenin (VIII; R=CH_2OH, R'=COOH); and gypsogenin (VIII; R=CHO, R'=COOH).

Oxidative Degradation.

Valuable information concerning the structure of the β-amyrin group of triterpenes has been obtained from oxidative degradion experiments on the monocarboxylic acids, oleanolic acid and heteragenin and because of the interrelationships existing in the β-amyrin group the conclusions reached in this work apply equally to β-amyrenol and the other members of the group. The presence of the hydroxyl group in ring A and the centre of unsaturation in ring C makes two distinct types of oxidative attack possible.

(i) Oxidation of ring A. Most of the work on the determination of the structure of this part of the molecule was carried out on hederagenin (VIII; R=CH_2OH, R'=COOH). Oxidation of hederagenin (VIII) with chromic acid (53) resulted in the loss of a carbon atom and the production of the C_{29} keto-acids (IX) and (X), further oxidation of which with potassium hypobromite (54,55,56) yielded the monomethyl esters of tricarboxylic acids (XI) and (XII) respectively. Pyrolysis of (XIII) which was obtained from (XII) gave the keto-lactone (XIV), oxidation and esterification of which gave the trimethyl ester lactone (XV). The trimethyl ester lactone (XV) was also obtained by oxidation of (XVI) (56,57,58). This

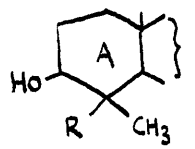
series of reactions indicates a 1:3-diol system in ring A of hederagenin and the formation of (XV) excludes the



possibility of a methyl group at C_8. The presence of a methylene group at C_3 was proved by the formation at C_3 of a formyl ester of an oleanolic acid derivative (59). The presence of a methylene group at C_4 was established by oxidative work on betulin (lupeol group) (60,61,62) in which ring A was oxidised to a cyclopentanone derivative.

The conclusions derived from this work are summed up in partial formula (XVII; R=CH_3 for β-amyrenol etc.)

for ring A of the β-amyrin group of triterpenes. Although

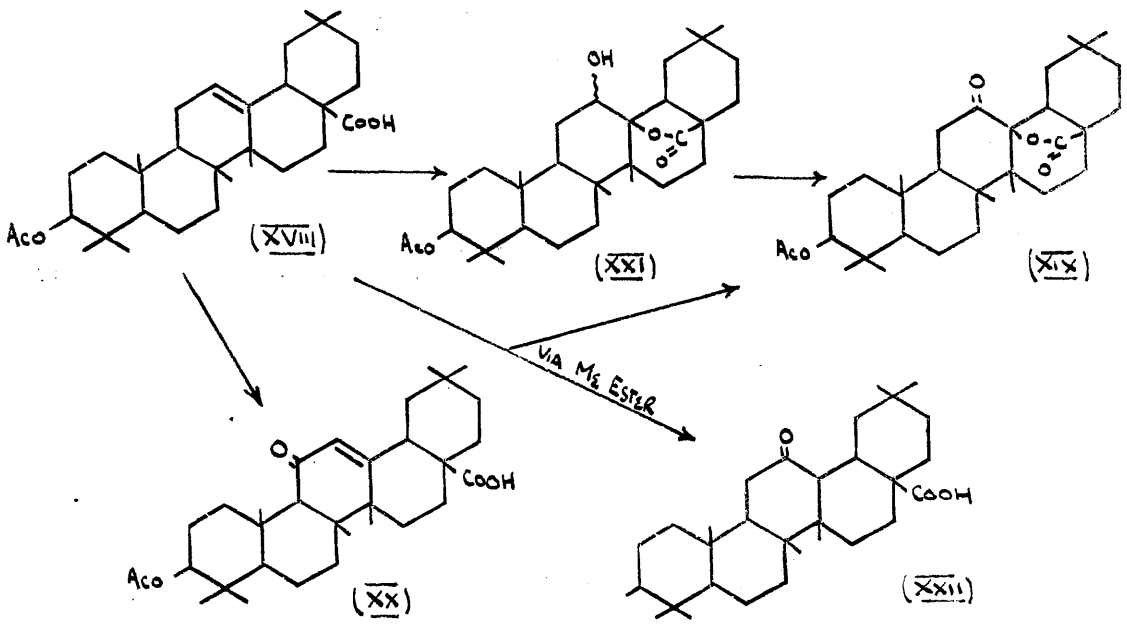


(XVII)

proof for the structure of ring A was thus provided, the final proof of the structure of rings A and B was provided by oxidative attack on the unsaturated centre and this work will be described in the next section.

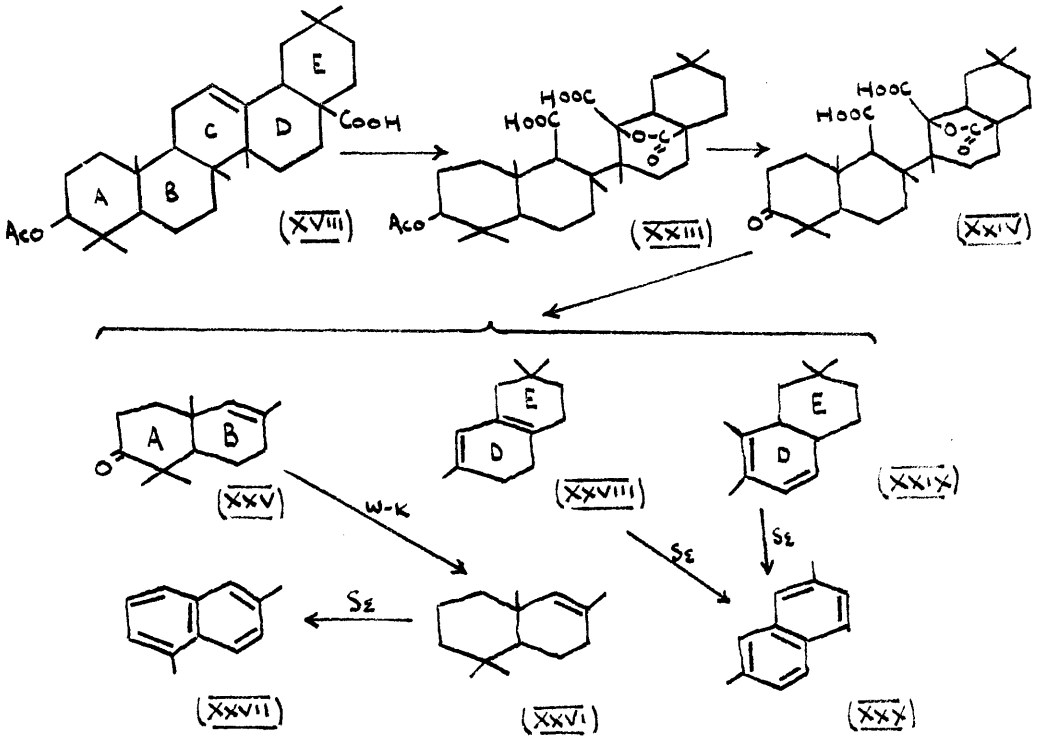
(ii) Oxidation of the unsaturated centre. The position and environment of the unsaturated centre in the β-amyrin group of triterpenes has been established by the extensive researches of Spring and Ruzicka, and their respective co-workers, on oleanolic acid and its derivatives. (Reviews 5,4,6,7,8 and 9).

Oxidation of acetyl oleanolic acid (XVIII) with chromic acid, under mild conditions, was shown by Kitasato (18) and Ruzicka (59,63,64) to yield ketoacetyloleanolic acid lactone (XIX) and ketoacetyloleanolic acid (XX) as a minor product. The keto-lactone (XIX) was shown to be saturated and was clearly formed as a result of direct attack on the double bond. The compound (XXI) was shown to be an intermediate in this reaction and was prepared by a variety of methods (65,66,67). Oxidation of the methyl ester of acetyloleanolic acid again yielded (XX),



as its methyl ester, and also, the saturated keto-acid (XXII) which was subsequently obtained by other methods (68,56,69,70,66,63).

Under more drastic conditions it was found that acetyloleanolic acid (XVIII) could be oxidised with chromic acid to give the acetyl dicarboxylic acid lactone (XXIII) (71) which was also obtained by oxidation of (XIX) and (XXII) (71,40,64,72). The keto-dicarboxylic acid (XXIV), which was formed from (XXIII) (71) was pyrolysed by Ruzicka and his co-workers (73,74) to yield ketonic and non-ketonic fractions in almost equal amounts indicating that the molecule had been split into two halves. The

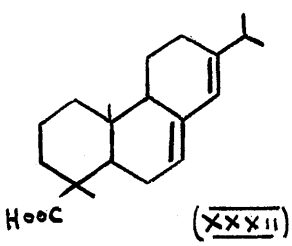


products of this fission and their derivatives are shown in formulae (XXV to XXX) above. The ketone (XXV) was clearly derived from rings A and B of the parent compound (XVIII) and its structure was proved by its conversion to (XXVII) by the steps shown. The non-ketonic fragments (XXVIII) and (XXIX) clearly had their origin in rings D and E as was shown by their dehydrogenation to 2:7-dimethylnaphthalene (XXX).

To generalise on these reactions, it may be said that they proved conclusively that the unsaturated centre in oleanolic acid, and therefore in the whole

β-amyrin group lies in ring C, and that the products obtained corroborated the conclusions drawn from the dehydrogenation experiments described above.

It is of interest to note here that this method of pyrolysis has been used with great success in the α-amyrin and lupeol groups for structural elucidation, and one notable example of the use of this method was in the correlation of the β-amyrin group with ambrein (XXXI) through a common degradation product of the latter and of oleanolic acid (75,76). These experiments provided the final proof of the structure of rings A and B in the β-amyrin group, and incidentally the stereochemistry (see later section) of ring A and B, since the constitution of ambrein had been established by inter-relationship with the diterpene abietic acid (XXXII) and the bicyclic manool group.



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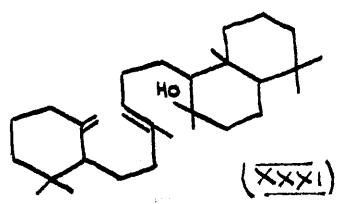
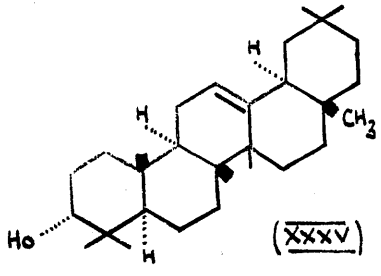


(XXXI)

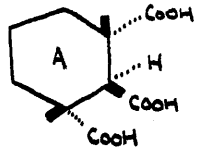
Stereochemistry of the β-Amyrin Group of Triterpenes.

Previous to the elegant researches of D.H.R. Barton, and E.R.H. Jones and, to a lesser degree, certain other workers, little was known about the stereochemistry of the pentacyclic triterpenes. The problem has now been almost completely elucidated as far as the β-amyrin and lupeol groups are concerned, and a brief outline of the work involved is given below.

In 1949 Barton showed that vigorous oxidation of abietic acid (XXXII) produced a C_{11} acid which, he concluded, had the structure represented by (XXXIV) (78), and in view of the relationship existing between abietic acid and other diterpenoids on one hand, and abietic acid and the triterpenoids on the other (76,7) he concluded that rings A and B in oleanolic acid must be trans-fused.



(XXXV)

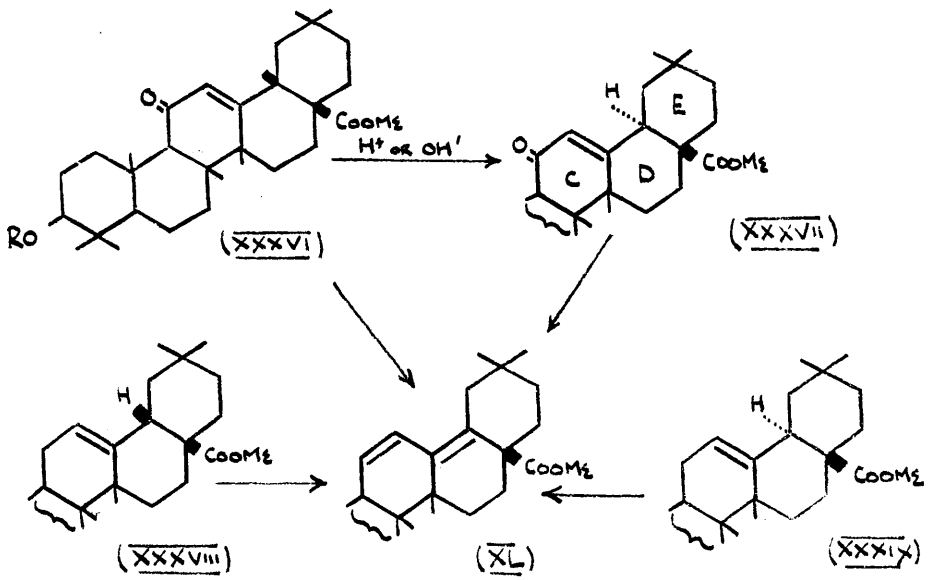


(XXXIV)

From an X-ray study of various members of the β--amyrin group, Giaconello in 1938 had concluded that all five rings were fused trans-anti-trans as shown in (XXXV) (77) for β-amyrenol, and the spacial configuration thus assigned to the C_3 hydroxyl group was tentatively confirmed by Ruzicka (79). In 1951, however, a study of the relative stabilities of β- and epi-β-amyrin, the relative degree of steric hindrance in the two alcohols and elimination evidence, all considered in the light of the theory of equatorial and vertical bonds (81) enabled Barton (80) to show that the C_3-hydroxyl group in the β-amyrin group has the opposite configuration to that assigned to it by Giacanello and Ruzicka. The C_3-hydroxyl group is therefore now regarded as "β" in configuration and has the properties of an equatorial hydroxyl group.

A study of the reactions of the carboxyl group at C_{17} in oleanolic acid, the esters of which are difficult to hydrolyse and which easily lactonise, has led Barton (84,83) and Jones (85) to the conclusion that it is vertical in the stereochemical sense. This stereochemical configuration, however, still permits rings D and E to be either cis- or trans-fused. Bilham and Kon (86) concluded from reactions of quillaic acid, a member of the

β-amyrin group, that two forms of D/E linking exist, i.e. the less stable cis- which can be converted to the more stable trans-. From their evidence they argued that the oleanolic acid D/E linkage must be cis-. Convincing proof of the correctness of this argument has been given by Barton (83) as shown in the following series of reactions. It was found that methyl-11:keto--oleanolate acetate (XXXVI, R=Ac) could be isomerised

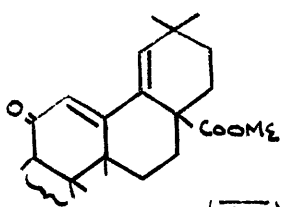


to (XXXVII) by either mineral acid (23) or alkali (83). Corresponding isomerisations have been carried out on β-amyrin and its derviatives (87,88,89,90). Catalytic hydrogenation of (XXXVI) and (XXXVII) gave the isomers (XXXVIII) and (XXXIX) respectively. Both these isomers

were oxidised to methyl dehydro-oleanolate acetate (XL) which had been previously prepared by Ruzicka et al.(91).

The epimerisation at C_{18} in oleanolic acid proves that the configuration at that point is the unstable one and the D/E ring junction must be cis-. Further proof of this fact has been given by Barton (83) from a consideration and comparison of the thermodynamic stabilities of the lactones of oleanolic acid and those of its 18-iso form.

Proof that inversion does not occur at position C_{10} in the above reactions is given by the conversion of both (XXXVI) and (XXXVII) to (XL) by sodium and alcohol reduction (23) and further by the fact that the dienone (XLI) cannot be isomerised by alkali (83). This proof

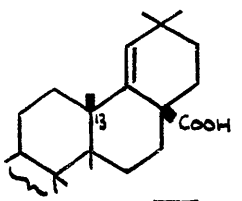


(XLI)

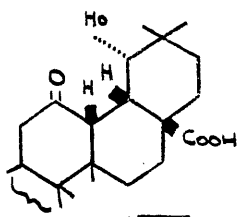
of the non-inversion at C_{10} also implies that in oleanolic acid and the other members of the β-amyrin group the orientation at C_{10} is the stable one and a convincing proof is included in the theoretical section of this thesis and by Budziarek et al. (89,92).

Thus the stereochemistry of rings A and B, and rings D and E in the β-amyrin group of triterpenes has been established and the remaining centres to be considered are C_8, C_{10}, C_{13} and C_{14}.

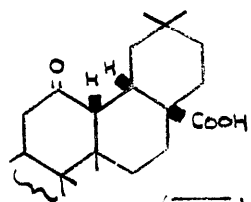
In 1951, Barton and Brooks (84) concluded that the configuration at C_{13} in morolic acid (XLII) was such that the C-H bond was stereochemically "vertical" and on the same side of the molecule as the carboxyl group. Evidence that the configuration at C_{13} is the stable one is given by the partial synthesis of morolic acid from



(XLII)



(XLIII)

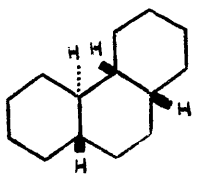


(XLIV)

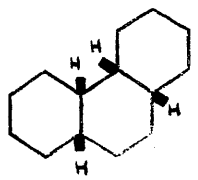
dihydro-12-ketosiaresinolic acid (XLIII)(93). The same conclusion was reached by Barton and Holness (83) from reactions on 12-ketooleanolic acid (XLIV).

A consideration of the centres C_{13} and C_{14} with respect to C_{17}, in the light of the theory of equatorial and vertical bonds, has led Barton (83) to the conclusion that rings C and D in the compounds of the β-amyrin group are fused in the more stable trans-linkage. In this work Barton found an analogy with the investigations of

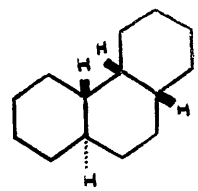
Linstead (94) and Johnson (95). In a comparison of the hypothetical pairs of hydrocarbons (XLV) and (XLVI), and (XLVII) and (XLVIII) these workers concluded that (XLV) would be more stable than (XLVI), and (XLVII) more stable than (XLVIII).



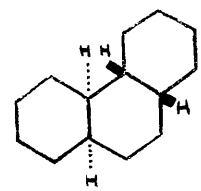
(XLV)



(XLVI)

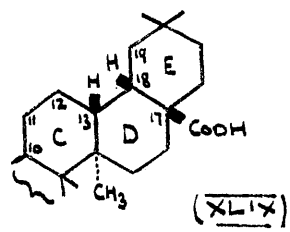


(XLVII)



(XLVIII)

The analogy between these hypothetical hydrocarbons and rings C, D and E in oleanolic acid is obvious and in view of the evidence described above, the structure of rings C, D and E in the compounds of the β-amyrin group can be summarised in partial formula (XLIX).

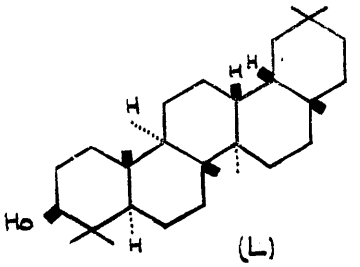


(XLIX)

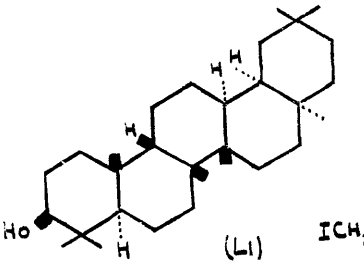
The nature of the linking of rings B and C has not yet been proved conclusively, but it has been shown by Barton (83) that the substituent at C_{10} in oleanolic acid must lie on the opposite side of the plane to the substituents

at C_{18} and C_{17}.

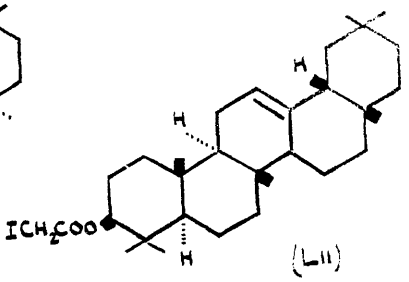
In view of the above evidence it would appear that only two structures, (L) and (LI), are possible for the β-amyrin group of triterpenes. No distinction between



(L)



(LI)



(LII)

these two compounds has so far been attainable on chemical grounds, but on the basis of molecular rotation arguments of Klyne (96) a decision in favour of structure (L) has been made. This decision has been confirmed by the work of Carlisle and (Miss) Adb El Rehin (Personal communication to Dr. D.H.R. Barton - see ref.82) who carried out an X-ray investigation of the structure of methyl oleanolate iodoacetate and chose conformation (LII) as being the correct one for that compound.

---oOo---

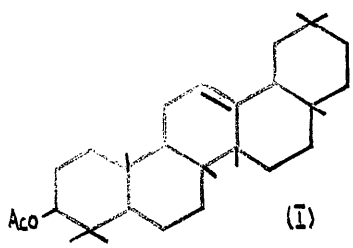
THEORETICAL.

SECTION I

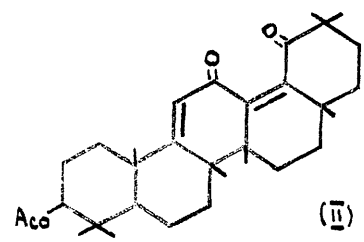
β-Amyradiendionyl acetate (12:19-diketo-oleana--10:13(18)-dienyl acetate) was reduced to oleana--10:13(18)-dien-2:19-diol with lithium aluminium hydride. The mono- and diacetates of this compound yielded oleana-10:13(18)-dienyl acetate on catalytic hydrogenolysis. By a stepwise series of reductions, β-amyradiendionyl acetate was converted into oleana--10-en-2:19α-diol, the monoacetate of which gave oleana-10:18-dienyl acetate on dehydration. Oleana--10:13(18)- and oleana-10:18-dienyl acetates were isomerised by mineral acid to oleana-11:13(18)-dienyl acetate (β-amyradienyl-II acetate).

The experiments described in this section were undertaken with the object of preparing the unconjugated dienyl acetates, oleana-10:13(18)-, oleana-10:18- and oleana-11:18-dienyl acetates, which were required for comparison with two unconjugated dienyl acetates, the preparation of which from iso-β-amyradienonyl acetate is described in the third section of the theoretical part of this thesis. A method for the preparation of oleana-11:18-dienyl acetate has not yet been discovered but methods for the preparation of the oleana-10:18- and the oleana-10:13(18)-dienyl acetates are described below.

A number of alternative methods for the preparation of β-amyradiendionyl acetate (12:19-diketo-oleana--10:13(18)-dienyl acetate) (II) from β-amyrin acetate or its derivatives are known (103,104,105,84,102,92) but the best is undoubtedly that in which β-amyrin acetate (I) is oxidised in dioxan by selenium dioxide in an



(I)



(II)

autoclave at 200° to give β-amyradiendionyl acetate in 75% yield. The diendione chromophore of this compound is characterised by its ultraviolet light absorption: Maximum at 2780 Å (ε = 12,000).

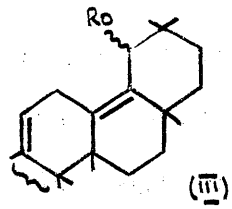
In the course of a study of the action of reducing and hydrogenating reagents on β-amyradiendionyl acetate, it was treated with lithium aluminium hydride in ether solution. When carried out at room temperature, the reaction yielded only an uncrystallisable gum but when the reaction mixture was refluxed, a $C_{30}H_{48}O_2$ diendiol was obtained in approximately 50% yield. The reaction clearly involved the replacement of the C_2 acetoxyl group with hydrogen, the normal reduction of one of the two carbonyl groups to a secondary hydroxyl, and surprisingly, the reduction or hydrogenolysis of the second carbonyl group to a methylene. Treatment of this $C_{30}H_{48}O_2$ diol with acetic anhydride and pyridine at room temperature gave a $C_{32}H_{50}O_3$ diol-monoacetate and more drastic treatment with the same reagents gave a $C_{34}H_{52}O_5$ diol-diacetate, thus indicating that the lithium aluminium hydride product did in fact possess two secondary hydroxyl groups and that one of them was sterically hindered to a certain degree. The diol, diol-monoacetate and the diol-diacetate all gave a strong

yellow colour with tetranitromethane in chloroform and showed ultra-violet light absorptions characteristic of unconjugated double bonds as is shown in the following Table A of physical constants.

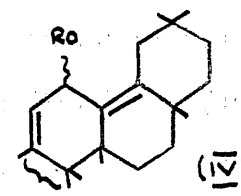
TABLE A

Compound	$[\alpha]_D$	max.	ε_{2100}	ε_{2150}	ε_{2200}	ε_{2250}
Diol	+58°	2090Å	12000	10000	6300	1200
Diol-monoacetate	+75°	2100Å	10000	8500	5600	2600
Diol-diacetate	+60°	2100Å	11800	8900	6000	3200
Oleana-10:13(18)-dienyl acetate	+59°	2130Å	-	9300	6000	3100
Oleana-10:18-dienyl acetate	+99°	2090Å	3100	1250	280	-

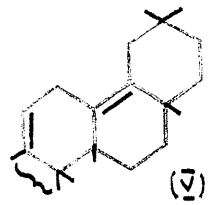
Catalytic hydrogenation of both the monoacetate and the diacetate yielded a $C_{32}H_{50}O_2$ acetate in very high yield which gave a strong yellow colour with tetranitromethane in chloroform and showed a light absorption in the region 2100Å to 2250Å similar to that of the diol, the diol-monoacetate and the diol diacetate, as shown in Table A. The acetate was readily converted into β--amyradienyl-II acetate (VI) by treatment with hydrochloric acid in acetic acid, and was thereby shown to be a dienyl acetate. The intense absorption of light between 2100 and 2250Å shown by this compound together



(III)



(IV)



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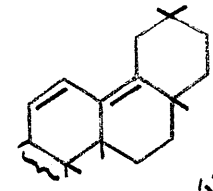
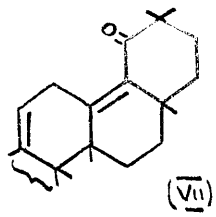


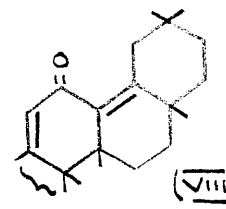
(VI)

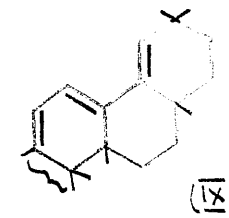
with its method of preparation suggested that one of its double bonds was between C_{13} and C_{18}, that is, a $\rangle C = C \langle$ type of ethylenic linkage (33,19). This acetate was also shown to be identical with one of the two compounds obtained from the catalytic hydrogenation of β-amyradiendionyl acetate (II) (106) and in view of the methods of preparation of this compound, its reactions and characteristics described above, it was concluded to be oleana-10:13(18)-dienyl acetate (V).

The position of the double bonds in the lithium aluminium hydride product of β-amyradiendionyl acetate was therefore established as -10:11- and -13(18)-, and in order to decide between the two structures possible for the monoacetate of this compound, oleana-10:13(18)--dien-2:19-diol-2-acetate (III, R=H) and oleana-10:13(18)-

-dien-2:12-diol-2-acetate (IV, R=H) an attempt was made to oxidise it to the corresponding ketone, (VII) or (VIII), either of which would have been readily distinguishable from the other. The ketone was not


(VII)


(VIII)


(IX)

obtained, however, and using chromic acid, equivalent to one atom of oxygen, at room temperature, the monoacetate was oxidised to yield a mixture from which β-amyradiendionyl acetate (II) and unchanged diol--monoacetate were separated. Using an excess of oxidising agent, again at room temperature, β-amyradiendionyl acetate was isolated as sole product in good yield Treatment of the diol diacetate (III, R=Ac) or (IV, R=Ac) with chromic anhydride in acetic acid also gave β-amyradiendionyl acetate.

Treatment of the monoacetate with mineral acid in acetic acid converted it, in good yield, into oleana--10:12:18-trienyl acetate (β-amyratrienyl acetate)(IX) (107), the reaction probably involving an anionatropic rearrangement and dehydration. The triene was also found as a by-product in the above lithium aluminium

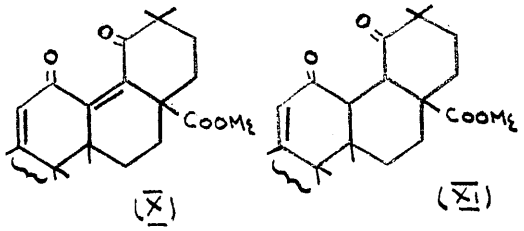
hydride reduction of β-amyradiendionyl acetate and considering the ease with which it can be converted into β-amyradiendionyl acetate (102) it seems likely that it was an intermediate in the above oxidations of the mono- and diacetates of the lithium aluminium hydride reduction product of β-amyradiendionyl acetate. An attempt to oxidise the diol-monoacetate in acetone solution with manganese dioxide (108,109,110) also gave the triene in very high yield.

Although all the experiments, described above, failed to produce compounds which would have led to a decision between structures (III) and (IV) for the lithium aluminium hydride product of β-amyradiendionyl acetate, a decision was reached as a result of the work described below.

The reduction of β-amyradiendionyl acetate (II) with zinc dust and acetic acid (106) gave, among other products, 12-keto-oleana-10:13(18)-dienyl acetate (IX) which was reduced by lithium aluminium hydride to oleana-10:13(18)-dienyl acetate (V). In this reaction, therefore, there is another example of a carbonyl group being reduced to a methylene group with lithium aluminium hydride, and the analogy with the lithium aluminium hydride reduction of β-amyradiendionyl acetate

is obvious. It seems probable, therefore, that the carbonyl group involved in each case was the one at C_{12}, and if this is true, then the diol-monoacetate obtained from β-amyradiendionyl acetate by reduction with lithium aluminium hydride, followed by partial acetylation was oleana-10:13(18)-dien-2:19-diol-2--acetate (III, R=H). Additional, though less convincing evidence in favour of this decision is found in the known relative difficulty of acetylation of C_{19}-hydroxyl groups in the β-amyrin group (see below and cf.123).

Attention was next directed to the preparation of oleana-10:18-dienyl acetate. Barton, Holness, Overton and Rosenfelder (111) observed that methyl-12:19-diketo--10:13(18)-dienolate acetate (X) is reduced to methyl--12:19-diketo-olean-10-enolate acetate (XI) by zinc

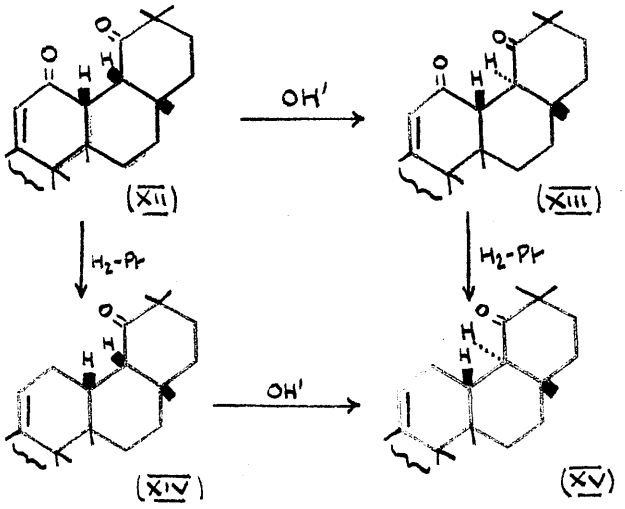


(X) (XI)

dust and acetic acid. Using their conditions, McKean (106) found that the reduction of the related 12:19--diketo-oleana-10:13(18)-dienyl acetate (β-amyradiendionyl acetate) (II) was a complex reaction giving

(a) oleana-10:13(18)-dienyl acetate (V) (see also above), (b) 12-keto-oleana-10:13(18)-dienyl acetate (VIII) (see above) and (c) an acetate, $C_{32}H_{48}O_4$ which was also obtained in excellent yield by the reduction of β--amyradiendionyl acetate with zinc dust in ethanol. This acetate was probably identical with a $C_{32}H_{48}O_4$ acetate obtained by Ruzicka and Jeger (103) by the catalytic hydrogenation of β-amyradiendionyl acetate and for which the structure, 12:19-diketo-olean-13(18)-enyl acetate was considered and rejected. It was also suggested that the compound contained a secondary hydroxyl group, produced by the reduction of a carbonyl group. Barton, Holness, Overton and Rosenfelder (112), however, preferred to regard the reduction product as 12:19-diketo-olean-10-enyl acetate (XII), which is undoubtedly correct in view of the failure of the compound to be further acetylated or oxidised under conditions in which a secondary hydroxyl group would react. The presence of an αβ-unsaturated carbonyl grouping in this compound was established by its ultraviolet absorption spectrum, maximum at 2460 Å (ξ = 12,500), and the presence of a second (isolated) carbonyl group was established by its conversion into 19-keto-olean-10-enyl acetate (XIV) by hydrogenolysis, as described below.

Consequently, the formation of the acetate, $C_{32}H_{48}O_4$ (12:19-diketo-olean-10-enyl acetate) (XII) from β--amyradiendionyl acetate involved the reduction of the -13(18)-ethylenic linkage with hydrogen as in the analogous case of the reduction of methyl-12:19-diketo--oleana-10:13(18)-dienolate acetate (X).



Hydrogenolysis of 12:19-diketo-olean-10-enyl acetate (XII), using platinum catalyst in acetic acid at room temperature, gave 19-keto-olean-10-enyl acetate (XIV), identical with one of the two products obtained by catalytic hydrogenation of β-amyradiendionyl acetate (106). It gave a yellow colour with tetranitromethane, indicative of an isolated double bond, confirmed by its light absorption (see Table B) which also showed the presence of an isolated carbonyl group. Oxidation of

19-keto-olean-10-enyl acetate (XIV) with selenium dioxide gave the parent β-amyradiendionyl acetate in good yield.

Treatment of 12:19-diketo-olean-10-enyl acetate (XII) with strong ethanolic caustic potash gave the isomeric 12:19-diketo-18α-olean-10-enyl acetate (XIII) which had similar characteristics to those of (XII). The presence of an isolated carbonyl group in (XIII) was established by its hydrogenolysis to 19-keto-18α--olean-10-enyl acetate (XV), a transition which was considerably more difficult to effect than the analogous hydrogenolysis of (XII) to (XIV), due no doubt to the changed stereo-conformation of the molecule brought about by the epimerisation at C_{18}. A similar epimerisation to the one described above was achieved by the conversion of 19-keto-olean-10-enyl acetate (XIV) to the isomer 19-keto-18α-olean-10-enyl acetate (XV).

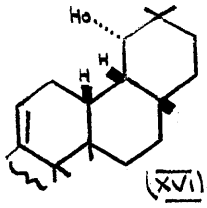
TABLE B.

Compound	$[\alpha]_D$	λ max.	ε max.
12:19-diketo-olean--10-enyl acetate	+132°	2460Å	12,400
12:19-diketo-18α--olean-10-enyl acetate	+92°	2430Å	11,700
19-keto-olean-10--enyl acetate	+117°	2060Å;3020Å	2,900;45
19-keto-18α-olean--10-enyl acetate	+139°	2060Å;2980Å	3,300;62

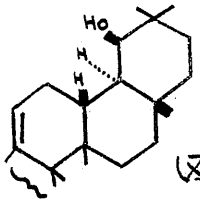
The above reactions prove that 19-keto-olean-10--enyl acetate (XIV) and its alkali stable isomer (XV) have the same configuration at C_{18}, that they differ solely in orientation at C_{18} and that a similar relationship exists between 12:19-diketo-olean-10-enyl acetate (XII) and its alkali stable isomer (XIII). The physical characteristics of these four compounds are compared in Table B.

Reduction of 19-keto-olean-10-enyl acetate (XIV) with lithium aluminium hydride yielded olean-10-en--2:19α-diol which was readily converted into its monoacetate (XVI) by partial acetylation. This indicated that the C_{19}-hydroxyl group was sterically hindered (the analogous 19β-hydroxyl group described by Beaton et al. (106) was similarly hindered) and an analogy between it and the secondary hydroxyl group in the monoacetate of the lithium aluminium hydride product of β-amyradiendionyl acetate can be drawn. Oxidation of the monoacetate (XVI) with chromic anhydride in acetic acid at room temperature, gave the parent ketone (XIV). Dehydration of the monoacetate (XVI) with phosphorous oxychloride in pyridine gave a high yield of the required oleana-10:18-dienyl acetate (XVIII), which had the ultraviolet light absorption shown in

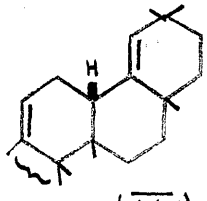
Table A, gave a yellow colour with tetranitromethane in chloroform and on treatment with hydrochloric acid



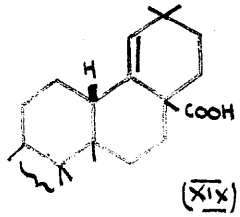
(XVI)



(XVII)



(XVIII)



(XIX)

in acetic acid gave oleana-11:13(18)-dienyl acetate (β-amyradienyl-II acetate) (VI). Reduction of 19--keto-18α-olean-10-enyl acetate (XV) with lithium aluminium hydride (106) gave 18α-olean-10-en-2:19β-diol which was readily converted into its monoacetate (XVII). Dehydration of this monoacetate with phosphorous oxychloride in pyridine again gave oleana-10:18-dienyl acetate (XVIII).

The conversions of 19-keto-olean-10-enyl acetate and its alkali stable isomer into oleana-10:18-dienyl acetate again prove that they have the same configuration at C_{18}. The configurations assigned to the 19--hydroxyl groups in olean-10-en-2:19-diol-2-acetate

(XVI) and its 18α-isomer (XVII) follow from the ease of dehydration with phosphorous oxychloride which requires a trans-arrangement of the hydrogen attached to C_{18} and the hydroxyl attached to C_{19} in each case.

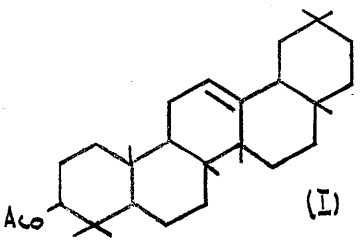
The conversion of 19-keto-18α-olean-10-enyl acetate into 18α-oleana-2:19β-diol proves that the configuration at C_{18} in 19-keto-olean-10-enyl acetate, 12:19-diketo-olean-10-enyl acetate and its alkali stable isomer is the same (β) as that in morolic acid (XIX) (83,84) and it follows that 12:19-diketo-olean-10-enyl acetate has the 13(β):18(β)-configuration shown in (XII) and that its alkali-stable isomer has the 13(β):18(α)--configuration shown in (XIII). Thus the zinc dust reduction of β-amyradiendionyl acetate involves cis--addition of hydrogen to the 13:18 double bond, as assumed by Barton et al. (loc.cit.), for the reduction of the analogous methyl 12:19-diketo-oleana-10:13(18)--dienolate acetate (X). The first case of such a cis--addition of hydrogen to an ene-1:4-dione was reported by Barton et al.(loc.cit.) who showed that reduction of methyl 12:19-diketo-olean-13(18)-enolate acetate gave methyl 12:19-diketo-oleanolate acetate and a similar cis-addition of hydrogen to a steroid ene-1:4-dione has subsequently been reported by Budziarek and Spring (113).

Recently, the experiments of Barnes and Barton (114) have led to the generalisation that selenium dioxide oxidation of ane-diones of the type -CO-CH-CH-CO- to the ene-dione -CO-C=C-CO- requires a cis-arrangement of the hydrogen atoms in the former. In agreement with this generalisation and with the stereochemical relationships deduced above, 12:19-diketo-olean-10--enyl acetate (XII) was readily oxidised to β-amyradiendionyl acetate (II) with selenium dioxide while the 18(α) isomer (XIII) was recovered unchanged after the same treatment.

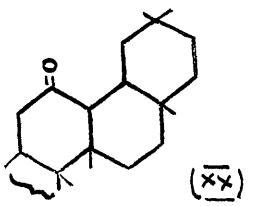
SECTION 2

The reactions of the enol acetate of iso-β-amyrenonyl acetate showed that the parent αβ-unsaturated ketone is correctly formulated as 2-acetoxyolean-10--en-12-one. iso-β-Amyrin derived from iso-β-amyrenonyl acetate by catalytic or Clemmensen reduction was shown to be 2-acetoxyolean-10-ene and it was concluded that the locking of rings B/C in β-amyrin corresponds to the more stable configuration. The chromic acid oxidation of β-amyrin acetate to produce acetone (90) was repeated and verified and the preparation of 11--keto-18α-olean-12-enyl acetate and its reactions are reported.

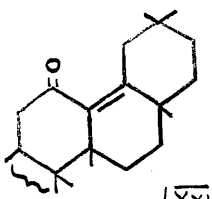
Oxidation of β-amyrin acetate (I) with hydrogen peroxide in acetic acid (115,116) or better, with hydrogen peroxide in formic acid and ethyl acetate, gives the saturated ketone β-amyranonyl acetate (2--acetoxyoleanan-12-one) (XX). Treatment of the saturated ketone with bromine gives bromo-β-amyranonyl acetate which readily looses hydrogen bromide to give iso-β-amyrenonyl acetate (116,117) for which the alternative structures (XXI) and (XXII) have been considered (5,118).



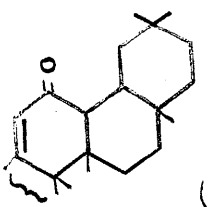
(I)



(XX)



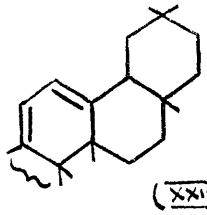
(XXI)



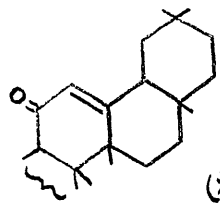
(XXII)

A decision in favour of the latter has been made since reduction of iso-β-amyrenonyl acetate with sodium and alcohol, followed by treatment of the product with acetic anhydride, gives β-amyradienyl-I acetate (XXIII) which contains a homoannular diene system and which is

identical with the product obtained by similar treatment of β-amyrenonyl acetate (XXIV) (87,119).

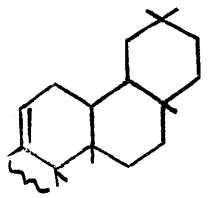


(XXIII)

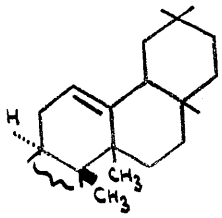


(XXIV)

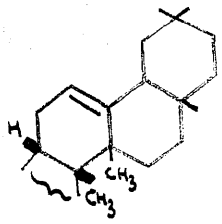
Catalytic hydrogenation (120) and Clemmensen reduction of iso-β-amyrenonyl acetate (XXII) gave an isomer of β-amyrin acetate which contained a $\rangle C{=}CH-$ ethylenic linkage since it was oxidised by hydrogen peroxide to a saturated ketone (120) isomeric with β-amyranonyl acetate (XX). The isomeric β-amyrin acetate has been formulated as 2-acetoxyolean-10-ene (XXV) (120). This structure did not appear to be rigidly established, however, since hydrogenation of (XXII) could have proceeded by the reduction of the ethylenic linkage and the simultaneous or consecutive reduction of the carbonyl group, followed by dehydration to give an isomeric β-amyrin acetate (XXVII), differing only in the configuration of the hydrogen atom at C_{10} in β-amyrin acetate (XXVI) (cf. the catalytic hydrogenation of β-amyranonyl acetate to β-amyrin acetate (103). Of the alternative structures (XXV) and (XXVII) for the isomeric β-amyrin acetate the former has been established



(XXV)

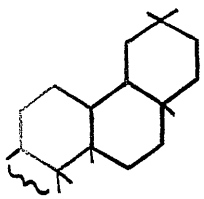


(XXVI)

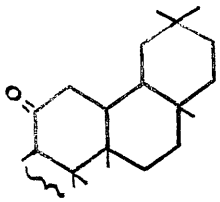


(XXVII)

by Wolff-Kishner reduction of the derived isomeric β-amyranonyl acetate to a product which, after acetylation, gave β-amyranyl acetate (XXVIII), identical with the compound obtained by a similar reduction of β-amyranonyl acetate (XX) (103). The isomeric β--amyranonyl acetate is therefore 2-acetoxyoleanan-11-one (XXIX).



(XXVIII)



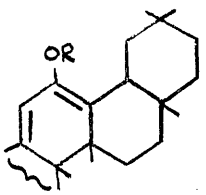
(XXIX)

An interesting point concerning the nature of the locking of rings B/C in the β-amyrin group of triterpenes emerges from the series of changes described above. The conversion of β-amyrin acetate into 11--keto-β-amyranyl acetate (XXIX) and thence into β-amyranyl acetate (XXVIII) by Wolff-Kishner reduction proves

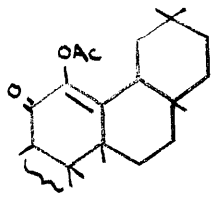
that rings B/C are locked in the more stable conformation since the introduction of a carbonyl group at C_{11} and its subsequent elimination would permit a change from a less to a more stable conformation. Since no such isomerisation takes place it is concluded that the more stable B/C locking exists in β-amyrin and therefore in the other members of the β-amyrin group. An additional interesting point arising from these reactions is that in the investigations involving epimerisation of the hydrogen atom at C_{18} in various compounds (87,88,89) there was a possibility that the changes brought about at C_{18} were accompanied by epimerisation at C_{10}. The above reactions confirm that no inversion has occurred at C_{10}.

In an attempt to characterise further iso-β-amyrenonyl acetate by the formation of its benzoate, it was treated with benzoyl chloride and pyridine. The product proved to be an enol-dibenzoate (XXX, R=B_z) which gave a brown-colour with tetranitromethane in chloroform and exhibited selective light absorption in the ultraviolet with maxima at 2300 Å (ε = 31,000), attributable to the two benzoyl groups, and 2750 Å (ε = 11,000) due to the homoannular diene system. Treatment of iso-β-amyrenonyl acetate with acetic anhydride and sodium acetate gave

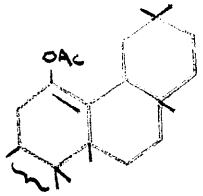
an enol acetate (XXX, R=Ac), which gave a brown colour with tetranitromethane and had a light absorption maximum at 2780 Å (ε = 8,500) attributable to the homo-annular diene system. The enol acetate was extremely



(XXX)



(XXXI)



(XXXII)

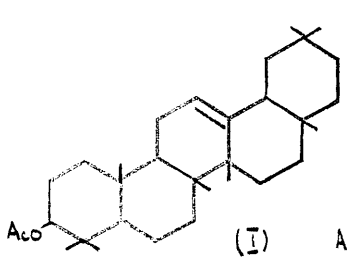
readily hydrolysed back to iso-β-amyrenonyl acetate (activated alumina) and thus the structure (XXII) for iso-β-amyrenonyl acetate was confirmed.

The structure (XXX, R=Ac) for the enol acetate of iso-β-amyrenonyl acetate was further supported by its oxidation with hydrogen peroxide in acetic acid, the 10:11-ethylenic linkage of (XXX, R=Ac) being oxidised, to yield the enol acetate of 11:12-diketo-β-amyranyl acetate (XXXI) which was previously obtained by Ruzicka and Jeger (103) by the chromic acid oxidation of the enol acetate of β-amyranonyl acetate (XXXII).

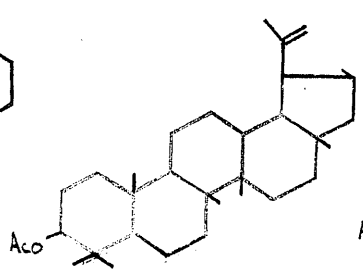
Two other oxidation products were obtained from the enol acetate (XXX, R=Ac). Oxidation with selenium dioxide in acetic acid gave iso-β-amyradienonyl acetate

(XXXVIII) and treatment with N-bromosuccinimide gave β-amyradiendionyl acetate (II). The production of iso-β-amyradienonyl acetate (XXXVIII) from the enol acetate can be readily explained on the assumption that the enol acetate was first converted in situ, into the parent αβ-unsaturated ketone (XXII) which is known to be oxidised to iso-β-amyradienonyl acetate (118) with selenium dioxide (see also section 3 of this theoretical review).

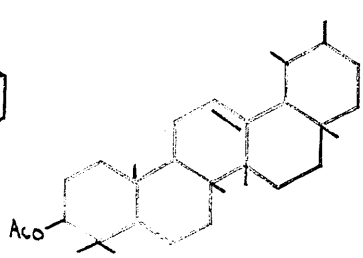
In view of the fact that the enol acetate of iso--β-amyrenonyl acetate yielded β-amyradiendionyl acetate on treatment with N-bromosuccinimide, the parent αβ--unsaturated ketone was treated with the same reagent. Starting material was, however, recovered quantitatively. Oxidation of olean-10-enyl acetate (iso-β-amyrin acetate) (XXV) with selenium dioxide, however, yielded β-amyradiendionyl acetate, analogously to β-amyrin acetate. Attempts to brominate the enol acetates of (XXX, R=Ac) and (XXXII) failed, and starting material was recovered nearly quantitatively.



(I)

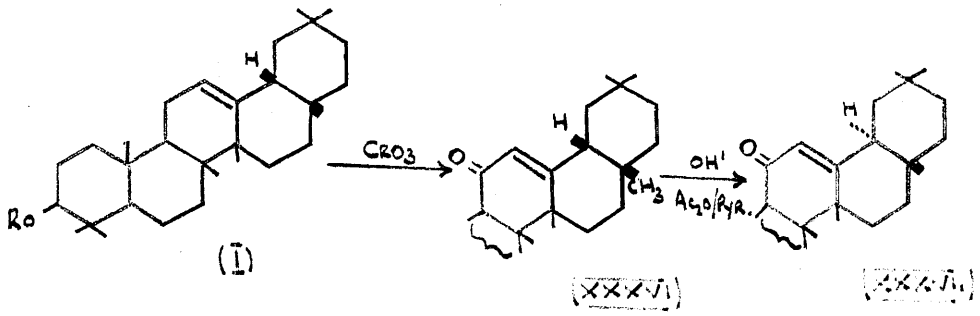


(XXXIV)



(XXXV)

The generally accepted structure ascribed to the β-amyrin oleanolic acid group of triterpenes, exemplified by that of β-amyrin acetate (I) is supported by a very large amount of evidence which has been amassed during the last twenty years. The structure assigned to the α-amyrin ursolic acid group is illustrated by that of α-amyrin acetate (XXXV). In 1949 it was reported that the chromic acid oxidation of highly purified β-amyrin acetate gives an appreciable yield (8%) of acetone whereas similar oxidation of α-amyrin acetate does not give acetone (90). If (I) is the true structure of β-amyrin acetate the acetone obtained probably originates in the geminal dimethyl group in ring E. The natural inference is, however, that β-amyrin acetate contains an <u>iso</u>propyl group as in lupenyl acetate (XXXIV). The chromic acid oxidation of β-amyrin acetate was repeated, and verified, and in order to test the hypothesis that lupeol and β-amyrin have the same carbon skeleton, the preparation of isomeric saturated alcohols of the β-amyrin group was undertaken, in order to compare them with lupanol (89, 90). In the course of this work, 11-keto-18α-olean--12-enyl acetate (XXXVII) was prepared by the stages illustrated below, in which β-amyrin benzoate (I, R=COPh)



(I)

(XXXVI)

(XXXVII)

was oxidised to β-amyranonyl benzoate (XXXVI), treatment of which with strong alkali followed by acetylation yielded 11-keto-18α-olean-12-enyl acetate (XXXVII). An attempt to brominate this compound, and an attempt to form its enol acetate with acetic anhydride and sodium acetate gave unchanged starting material.

As stated earlier, reduction of β-amyrenonyl acetate (XXIV) with sodium and alcohol, followed by acetylation, yields β-amyradienyl-I acetate (XXIII) (87,119). It is interesting to note that the reduction of 11-keto-18α--olean-12-enyl acetate under exactly the same conditions, repeatedly yielded a mixed crystal, which defied all attempts at separation, but which by virtue of the red--brown colour it gave with tetranitromethane in chloroform and also its ultraviolet light absorption: Maxima

at 2420 (ε = 19,000), 2510 (ε = 22,000) and 2600 Å (ε = 15,100), is thought to have consisted mainly of β-amyradienyl-II acetate. The minor constituent of the mixed crystal was probably the 18(α)-isomer of β-amyradienyl-I acetate.

SECTION 3.

iso-β-Amyradienonyl acetate was reduced with lithium aluminium hydride to a diol which was rearranged to a new triene (β-amyratrienyl-II acetate), a structure for which is suggested. The preparation of an isomer of β-amyrin acetate, named neo-β-amyrin acetate is described and formulations for its structure are suggested. The preparation and reactions of two new unconjugated dienyl acetates from iso-β-amyradienonyl acetate are described and the conversion of these derivatives to known β-amyrin derivatives is reported.

Oxidation of iso-β-amyradienonyl acetate with various oxidising agents produced an oxide, the reactions of which and other oxidations of iso-β-amyradienonyl acetate are described and discussed.

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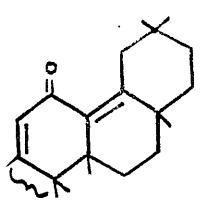
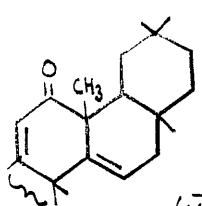
iso-β-Amyrenonyl acetate (XXII) contains half the chromophore of β-amyradiendionyl acetate (II) and accordingly, Green, Mower, Picard and Spring (118) attempted to correlate these two compounds by oxidation of the former with selenium dioxide in the expectation that β-amyradiendionyl acetate would result. Instead, the reaction gave iso-β-amyradienonyl acetate which shows absorption maxima at 2090 Å (ξ = 3,000) and 2450 Å (ξ = 10,000), and unlike iso-β-amyrenonyl acetate gives a yellow colour with tetranitromethane. iso-β-Amyradienonyl acetate was also obtained by the action of bromine on iso-β-amyrenonyl acetate by the same workers and by Jeger and Ruzicka (99); iso-β-amyradienonyl acetate can also be obtained directly from β-amyranonyl acetate by bromination (92). Green, Mower, Picard and Spring (loc. cit.) suggested the structure (VIII) for iso-β-amyradienonyl acetate, but in view of the recent synthesis of this compound (106), which has shown it to be different from iso-β-amyradienonyl acetate, their suggestion must be rejected.



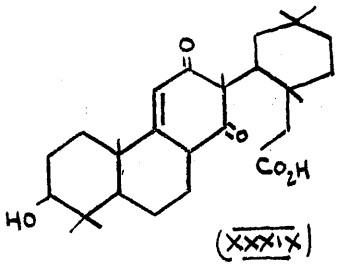
(VIII)



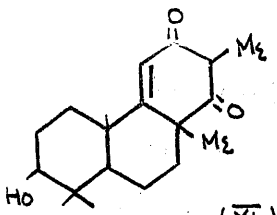
(XXXVIII)

In 1945 Jeger and Ruzicka proposed structure (XXXVIII) in which it is represented as formed from iso-β-amyrenonyl acetate by the migration of an angular methyl group from $C_{(14)}$ to $C_{(15)}$ with simultaneous introduction of a 14:15-double bond. More recently Meisels, Jeger and Ruzicka (120) have oxidised iso-β--amyradienonyl acetate to a hydroxy-diketo-acid formulated as (XXXIX) the methyl ester of which on pyrolysis gave an acidic fraction represented as the hydroxy--diketone (XL). The hydroxy diketone was characterised by methylation and acetylation which gave two isomeric compounds formulated as (XLI) and (XLII) and identical with two compounds obtained by, in all essential features, the same route starting from α-amyrin. These reactions cannot be construed as proof of migration of the angular methyl group during conversion of iso-β--amyrenonyl acetate into iso-β-amyradienonyl acetate, and the work described below was initiated with a view to proving whether the methyl group migration had taken place and also with a view to studying the general reactions of the compound.

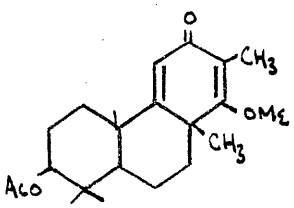
In the course of a study of the action of reducing agents on iso-β-amyradienonyl acetate it was reduced by lithium aluminium hydride in ether to a diol, characterised



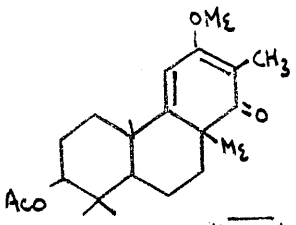
(XXXIX)



(XL)

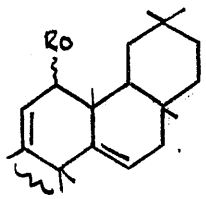


(XLI)

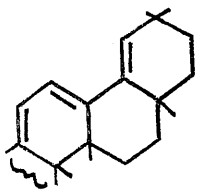


(XLII)

by the formation of its diacetate which gave a yellow colour with tetranitromethane in chloroform and exhibited strong light absorption between 2100-2250 Å indicative of unconjugated double bonds. Treatment of iso-β-amyradienonyl acetate with sodium methoxide in an autoclave at 200°, followed by acetylation yielded the same diacetate in good yield. To interpret these two reductions it is reasonable to suppose, on the basis of structure (XXXVIII) for iso-β-amyradienonyl acetate, that the C_{12} carbonyl group was reduced to a secondary hydroxyl group with the double bonds remaining at positions 10:11 and 14:15. The diacetate may therefore be represented as (XLIII, R=Ac) with an acetoxyl group at C_2. Treatment of the diacetate with hydrochloric

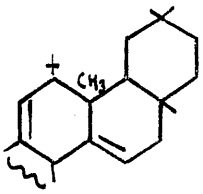


(XLIII)

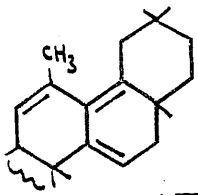


(IX)

acid in acetic acid resulted in the elimination of the elements of acetic acid to give a conjugated trienyl acetate, differing from β-amyratrienyl acetate (IX) which has a light absorption maximum at 3100 Å (ε = 14,500) and which was the only known conjugated triene in the β-amyrin group. The new triene, β-amyratrienyl--II acetate, had light absorption maxima at 2280 (ε = 18,500), 2820 (ε = 16,000) and 2940 Å (ε = 12,500) which suggested that the triene chromophore was cross-conjugated rather than continuously conjugated as in (IX). Analogous light absorption is exhibited by a cross--conjugated steroid triene recently reported by Laubach, Schreiber, Agnello, Lightfoot and Brunnings (121), and in view of this analogy and also the reactions described below, structure (XLV) is proposed for β-amyratrienyl-II acetate. A plausible mechanism for the formation of this compound can be found if it is assumed that the rearrangement of the diacetate (XLIII, R=Ac) proceeds



(XLIV)



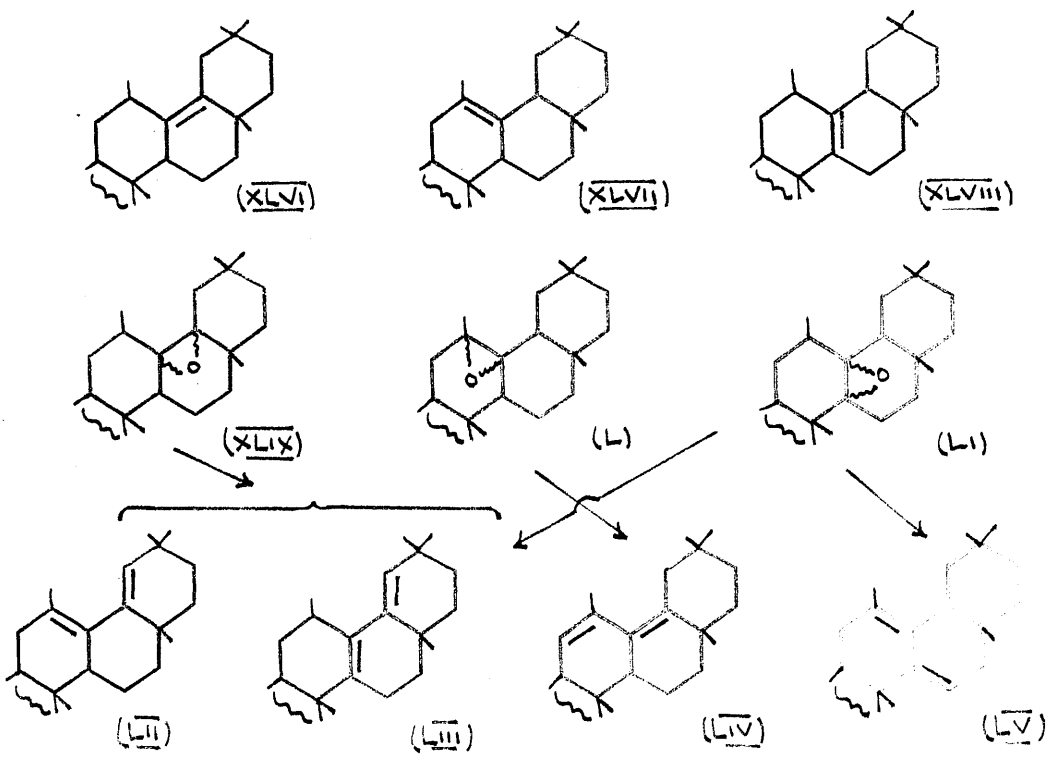
(XLV)

via the carbonium ion (XLIV) stabilisation of which is brought about by the migration of the methyl group from C_{14} to C_{13} and the subsequent introduction of a double bond at 13:18 with the conjugation of the 10:11 bond. Hydrogenation of β-amyratrienyl-3-acetate, with platinum catalyst in acetic acid proceeded with the extremely rapid absorption of two molecular equivalents of hydrogen, to yield a $C_{32}H_{52}O_2$ acetate. Neo-β-amyrin acetate (as this compound is now called after being reported as iso-β-amyradienyl acetate by Budziarek et al. (92) gave an orange-red colour with tetranitromethane and had a light absorption which is shown in Table C together with those of β-amyrin acetate, iso-β-amyrin acetate and δ--amyrin acetate (oleana-13(18)-enyl acetate) (LVI). The comparison of light absorptions in Table C established definitely that the type of double bond present in neo--β-amyrin is the type $\rangle C{=}C\langle$ present in δ-amyrin acetate rather than the $\rangle C{=}CH-$ type present in β-amyrin

TABLE C

Compound	λ max.	ε max.	ε 2100	ε 2150	ε 2200	ε 2250
β-amyrin acetate	2080Å	3,200	3,000	1,550	520	190
iso-β-amyrin acetate	2070Å	3,100	2,200	850	260	185
δ-amyrin acetate	2110Å	5,900	-	5,100	3,500	1,800
Neo-β-amyrin acetate	2110Å	5,000	-	4,450	3,100	1,800

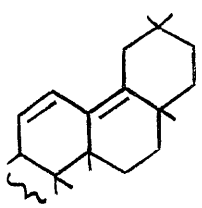
acetate or iso-β-amyrin acetate. On the basis of structure (XLV) for β-amyratrienyl-II acetate, therefore, three possible structures (XLVI), (XLVII) and (XLVIII) for neo-β-amyrin acetate suggested themselves. Oxidation



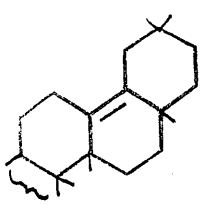
of neo-β-amyrin acetate with chromic anhydride or potassium permanganate yielded a $C_{32}H_{52}O_3$ oxide (incidentally proving that neo-β-amyrin acetate contains only one ethylenic linkage, in spite of its colour reaction with tetranitromethane, see above) which gave no colour with tetranitromethane in chloroform and showed no selective light absorption in the ultraviolet. The oxide, which on the basis of the above possible structures for neo--β-amyrin acetate may be represented by (XLIX), (L) or (LI), rearranged with hydrochloric acid to a hetero--annular diene, characterised by its light absorption, maximum at 2580 Å (ε = 21,000). Of the four possibilities for this hetero-annular diene, (LII), (LIII), (LIV) and (LV), the structure (LII) can be immediately excluded because of the inconsistency of the intensity of the light absorption of this hetero-annular diene with that of known cisoid-hetero-annular dienes (cf. Barton and Brooks (84)). Treatment of neo-β-amyrin acetate with bromine and selenium dioxide showed the compound to be extremely reactive and only by treatment with N-bromo-succinimide could any other derivative of neo-β-amyrin acetate be obtained. This reaction gave an amorphous material which could not be purified, but which showed light absorption maxima at 2520 (ε = 13,000), 2600

(ε = 12,500) and 3570Å (ε = 10,000) and gave a dark-brown colour with tetranitromethane in chloroform.

In spite of the fact that no chemical evidence which would enable a decision to be made between the above three structures is available, a tentative decision in favour of structure (XLVI) for neo-β-amyrin acetate, and therefore (XLIX) for its oxide and (LIII) for the hetero-annular diene, has been made for the following reason. Catalytic hydrogenation of β-amyradienyl-II acetate (VI) gives δ-amyrin acetate (LVI) by the

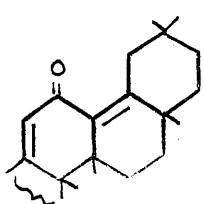


(VI)

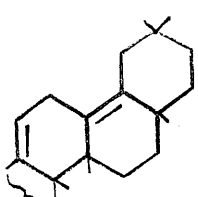


(LVI)

reduction of the 11:12-double bond, the bridgehead 13:18 double bond being inert to hydrogenation (105). The



(VIII)

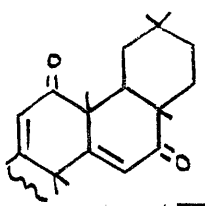


V

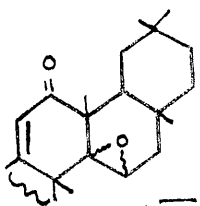
13:18 double bond in 12-keto-oleana-10:13(18)-dienyl acetate (VIII) is also not hydrogenated in the conversion

of (VIII) to (VII) (106). It is reasonable to assume, therefore, that in the catalytic hydrogenation of β-amyratrienyl-II acetate (XLV) the 13:18 (δ -amyrin) double bond remains inert while the 11:12 and 14:15 double bonds are saturated.

neo-β-Amyrin acetate was also found to be the product of direct catalytic hydrogenation of the diacetate (XLIII, R=Ac), iso-β-amyradienonyl acetate (XXXVIII), iso-β-amyradiendionyl acetate (LVII) from the selenium dioxide oxidation of iso-β-amyradienonyl acetate (99), and also by hydrogenation of the oxide of iso-β-amyradienonyl acetate (LVIII) (see below).



(LVII)



(LVIII)

In each case attempted hydrogenation in neutral solution with platinum catalyst failed, and with platinum in acetic acid was either very slow and incomplete, or did not proceed at all. With the addition of a trace of hydrochloric acid to the acetic acid solutions, however, the reactions were catalysed and the hydrogenations proceeded very rapidly to give neo-β-amyrin acetate in

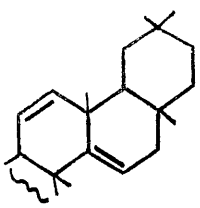
good yield. The inference drawn from this phenomenon is that before or during these hydrogenations an intramolecular rearrangement takes place, possibly due to a trace of hydrogen ion in the solution, to give a common intermediate which is then hydrogenated to neo-β-amyrin acetate. From a consideration of the mode of formation of β-amyratrienyl-II acetate (XLV), and the extreme ease with which it is hydrogenated to neo-β-amyrin acetate, it seems probable that it is the intermediate compound in all the above routes to neo-β-amyrin acetate.

Attention was next turned to the reduction of iso--β-amyradienonyl acetate (XXXVIII) by the method of Wolff and Kishner. Reduction with sodium methoxide and hydrazine in an autoclave at 200° followed by acetylation gave a mixture of two isomeric $C_{32}H_{50}O_2$ acetates, which were separated by fractional crystallisation, and each of which gave a yellow colour with tetranitromethane. The physical constants of these two acetates are compared in Table D.

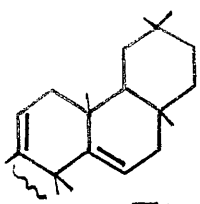
TABLE D

Compound	m.p.	$[\alpha]_D$	λmax.	ε max	ε_{2150}	ε_{2200}	ε_{2250}	Yield
$C_{32}H_{50}O_2$ acetate	231°	-9°	2120Å	4970	4180	1700	320	50%
$C_{32}H_{50}O_2$ acetate	203°	-95°	2100Å	5900	3700	1460	430	25%

The acetate, m.p.231°, which was obtained in about 50% yield was characterised by hydrolysis to the corresponding alcohol which gave the parent acetate on reacetylation. On treatment with hydrochloric acid in acetic acid, the acetate, m.p.231°, was converted into β-amyradienyl-I acetate (XXIII) in good yield. Similar treatment of the acetate, m.p.203°, converted it into β-amyradienyl-II acetate (V) also in good yield. In consequence of these two transitions into well known and defined dienyl acetates (XXIII) and (V), which undoubtedly possess the same basic structure as β-amyrin, and in consideration of the mode of formation of the two acetates, m.p.231° and m.p.203°, the structures (LIX) and (LX) are respectively proposed for them. The



(LIX)



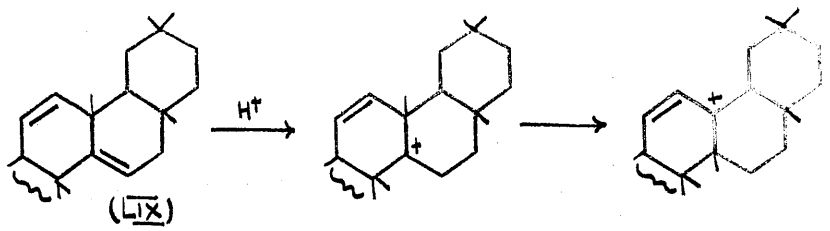
(LX)

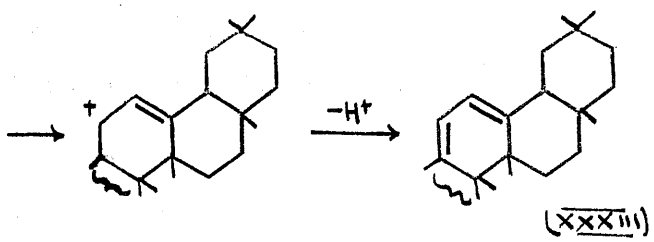
hydrochloric acid rearrangement, the ultraviolet absorption spectra and the colour reaction with tetranitromethane established that these acetates were both unconjugated dienes and it should be noted that they were

both different from oleana-10:13(18)-dienyl acetate (V) and oleana-10:18-dienyl acetate (XVIII). Furthermore the ready conversion of the acetate, m.p.231°, into oleana-10:12-dienyl acetate (β-amyradienyl-I acetate) (XXIII) showed that it was not the unknown oleana-11:18--dienyl acetate since in analogy with oleana-10:18-dienyl acetate, oleana-11:18-dienyl acetate would be expected to yield the stable olean-11:13(18)-dienyl acetate on treatment with mineral acid. Thus as the acetate, m.p.231° is not one of the three possible non-conjugated dienes in the oleanane series in which the double bonds are located in the C_{10}-C_{19} system (10:13(18), 10:18, 11:18) it is probable that it is not a simple oleanane derivative and that it has a different carbon skeleton from that of β-amyrin, i.e. that of iso-β-amyradienonyl acetate.

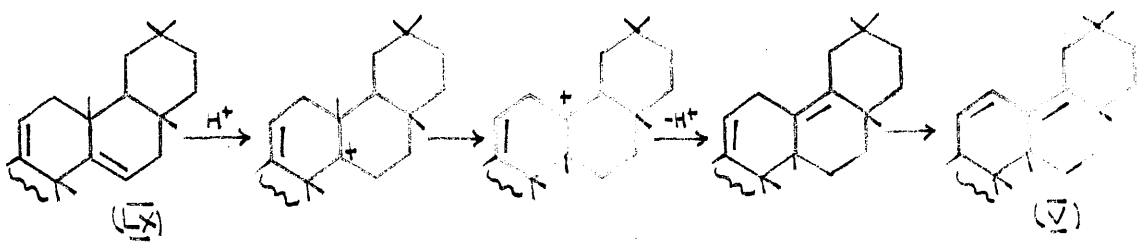
The Wolff-Kishner reduction of iso-β-amyradienonyl acetate is therefore thought to have proceeded simultaneously in two ways; firstly, by the reduction of the C_{12} carbonyl group to a methylene with an accompanying shift of the 10:11 double bond to the 11:12 position to give the acetate, m.p.231° (LIX) (For similar double bond migration in Wolff-Kishner reductions cf. 124, 125), and secondly, by the normal reduction of the C_{12} carbonyl group to give the acetate, m.p.203° (LX).

If the structures (LIX) and (LX) are correct then treatment with mineral acid in each case causes a migration of the methyl group at C_{13} to C_{14} and a movement of the 14:15 double bond to the most stable position. The following mechanism for the isomerisation of the acetate, m.p.231° (LIX) is suggested.





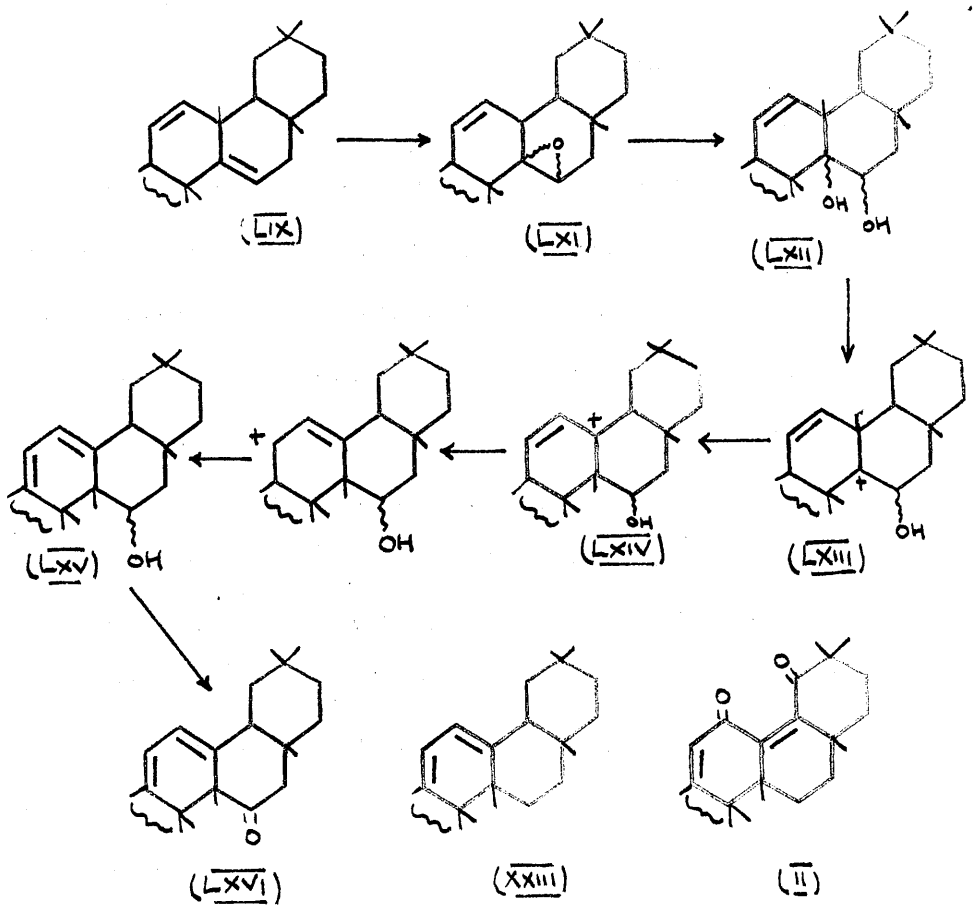
As stated above the minor product of the Wolff-Kishner reduction of iso-β-amyradienonyl acetate, the acetate, m.p.203° (LX) was isomerised to β-amyradienyl-II acetate, and a suggested mechanism for the transformation is given below.



It is noteworthy that although both non-conjugated dienes obtained from iso-β-amyradienonyl acetate were susceptible to mineral acid rearrangement, the parent iso-β-amyradienonyl acetate resisted all attempts at isomerisation with mineral acid. Thus the presence of a carbonyl at C_{12} inhibits the carbonium ion reactions depicted above for the two dienyl acetates. It is also significant that the conversion of the non-conjugated dienyl acetate, m.p.231°, into oleana-10:12-dienyl acetate (unstable configuration at C_{18}) shows that C_{18} is not involved in the conversion of iso-β-amyrenonyl acetate (12-keto-olean-10-enyl acetate) (XXII) into iso-β-amyradienonyl acetate.

Treatment of (LIX) with perbenzoic acid (1 mol.) yielded a $C_{32}H_{50}O_3$ acetate, formulated as (LXV), which gave a brown colour with tetranitromethane in chloroform and had an ultraviolet light absorption maximum at 2820Å (ε = 9,100) indicative of a homo-annular diene chromophore and exactly similar to that of β-amyradienyl-I acetate (XXIII). This compound was shown to possess a secondary hydroxyl group by its oxidation with chromic anhydride at room temperature to the corresponding ketone (LXVI) which had a light absorption maximum at 2820Å (ε = 8,700). In the light of the above information,

it is suggested that the perbenzoic acid oxidation proceeded by the oxidation of the 14:15 double bond in (LIX) to give an unstable oxide (LXI) a fission of which occurred either in the reaction solution (chloroform) or in the working up to give the intermediates (LXII), (LXIII) and (LXIV) which led to the product (LXV).



The assumption that the molecule has reverted to the original β-amyrin structure is borne out by the comparison of the physical constants in Table E below.

TABLE E

Compound	$[\alpha]_D$	λmax.	εmax.	T.N.M. colour
β-amyradienyl-I acetate (XXIII)	+337°	2820Å	9000	Brown
The alcohol (LXV)	+293°	2820Å	9100	Brown
The ketone (LXVI)	+253°	2820Å	8700	Brown

Oxidation of the acetate, m.p.231° (LIX) with selenium dioxide in acetic acid gave β-amyradiendionyl acetate (II) in good yield, and in view of the fact that β-amyradienyl-I acetate is known to be oxidised to the diendione (II) with selenium dioxide (104) it is assumed that in the conditions of the reaction the acetate, m.p.231° (LIX) first rearranged to β-amyradienyl-I acetate (XXIII) which was then oxidised to (II).

Attempts which were made to hydrogenate the unconjugated dienes (LIX) and (LX) with platinum in acetic acid resulted in the quantitative recovery of starting material.

Another conversion of iso-β-amyradienonyl acetate into a known β-amyrin derivative was achieved when it

was reduced by the Clemmensen method to β-amyradienyl-II acetate (V) in 50% yield. An intramolecular rearrangement obviously took place under the strong acid conditions of this reaction and it is possible that the acetate, m.p.203°, formulated as (LX) was intermediate in the conversion.

Reagents which failed to react with iso-β-amyradienonyl acetate were zinc dust in acetic acid, ethanolic caustic potash and concentrated hydrochloric acid in acetic acid.

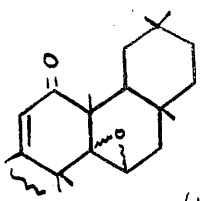
To summarise this work: the study of the action of reducing agents on iso-β-amyradienonyl acetate would seem to substantiate Ruzicka's postulation (99) that iso-β-amyradienonyl acetate does not have the same basic carbon skeleton as β-amyrin. The failure to convert neo-β-amyrin acetate into any known β-amyrin derivative suggests that in neo-β-amyrin acetate the molecule has adopted a very stable conformation, possible formulations for which have been suggested, and it seems likely that the difference between the β-amyrin and the neo-β--amyrin structures lies in the placing of the methyl group which was originally at C_{14} rather than any more profound molecular difference.

The production of the unconjugated dienes (LIX)

and (LX) by the Wolff-Kishner reduction of iso-β-amyradienonyl acetate, the fact that they are different from the known unconjugated diene derivatives of β-amyrin (see section 1 of the theoretical part of this thesis), and their conversion into known conjugated dienes possessing the β-amyrin structure also tend, in the author's opinion, to support the view that in the preparation of iso-β-amyradienonyl acetate a methyl group migrates from C_{14} to C_{13}. It is also apparent from the above work that the methyl group at C_{13} in iso-β--amyradienonyl acetate is only stable in that position as long as a carbonyl group is present at C_{12}, and it would appear from the above reactions that when the carbonyl group is converted directly into a methylene at C_{12} (e.g. Wolff-Kishner reduction and presumably Clemmensen reduction) potential β-amyrin derivatives are produced, but when the carbonyl group is reduced to a secondary hydroxyl group (e.g. lithium aluminium hydride reduction and presumably catalytic hydrogenation) further rearrangements take place and neo-β-amyrin or potential neo-β-amyrin compounds (e.g. β-amyratrienyl-II acetate) are formed.

As a second approach to the problem, a study of the action of oxidising agents on iso-β-amyradienonyl acetate was undertaken.

Oxidation of iso-β-amyradienonyl acetate (XXXVIII) with perbenzoic acid in chloroform gave a $C_{32}H_{48}O_4$ acetate, which unlike the starting material gave no colour with tetranitromethane in chloroform and showed an ultraviolet light absorption maximum at 2400 Å (ε = 10,800) indicating that the αβ-unsaturated ketone grouping was unaffected by the oxidation. In view of the above facts and also the behaviour of the compound with acidic reagents (see below) it was concluded that it is an oxide of iso-β-amyradienonyl acetate and it is formulated as (LVIII). This oxide was also prepared,



(LVIII)

in good yield, by the oxidation of iso-β-amyradienonyl acetate with potassium permanganate in stabilised glacial acetic acid at room temperature (95% yield) and by oxidation with chromic anhydride in stabilised glacial acetic acid at 60°. Hydrolysis of the oxide

with ethanolic caustic potash gave the corresponding alcohol which was reacetylated to the parent oxide acetate (LVIII).

Oxidation of iso-β-amyradienonyl acetate with hydrogen peroxide in unstabilised glacial acetic acid gave a mixture of two products which were easily separated by chromatography. The main product was a $C_{32}H_{48}O_4$ acetate, m.p.318° which gave no colour with tetranitromethane in chloroform and showed a light absorption maximum at 2360 Å (ε = 13,400). The minor product was an acetate, m.p.226° which showed no light absorption in the region 2000-4000 Å and gave no colour with tetranitromethane in chloroform or with ferric chloride in ethanol or dioxan. Starting material was recovered quantitatively from an attempted acetylation of this material. The major product, the acetate, m.p.318°, was shown to contain a secondary hydroxyl group by oxidation to the corresponding $C_{32}H_{46}O_4$ ketone, m.p.315°, and also by acetylation to the corresponding $C_{34}H_{50}O_5$ diacetate, m.p.196°. Hydrolysis of the acetate, m.p.318°, gave the $C_{30}H_{46}O_3$ diol as expected, and acetylation of this diol gave the $C_{34}H_{50}O_5$ diacetate, m.p.196° also obtained as stated above.

The $C_{32}H_{46}O_4$ ketone, m.p.315°, was also obtained

by chromic acid oxidation of the oxide of iso-β-amyradienonyl acetate (LVIII) in stabilised glacial acetic acid at 100° and by the chromic acid oxidation of iso--β-amyradienonyl acetate in boiling acetic acid. The ketone was hydrolysed to the corresponding alcohol with ethanolic caustic potash and the alcohol was acetylated to the parent ketone acetate.

Treatment of the oxide of iso-β-amyradienonyl acetate (LVIII) with concentrated hydrochloric acid in acetic acid, brought about a fission of the oxide linkage to give a compound containing one atom of chlorine, analysing for $C_{32}H_{47}O_3Cl$ and showing a light absorption maximum at 2360 Å (ε = 11,000). Alkaline hydrolysis of the chloro-compound gave a chlorine-free diol identical with the diol obtained, as stated above, from the hydrolysis of the acetate, m.p.318°, from the hydrogen peroxide oxidation of iso-β-amyradienonyl acetate. Treatment of the chloro-compound with activated zinc in acetic acid effected a replacement of the chlorine atom by an acetoxyl group to give the diacetate, m.p.196°, which was previously obtained, as stated above, by acetylation of the acetate, m.p.318°.

Finally, in this series of reactions, the chlorine atom in the chloro-compound was replaced with hydrogen

by treatment with activated zinc dust in ether and methanol and by unactivated zinc dust in acetic acid, to give a $C_{32}H_{48}O_3$ acetate, m.p.275°. The constants of all the above products are compared in Table F.

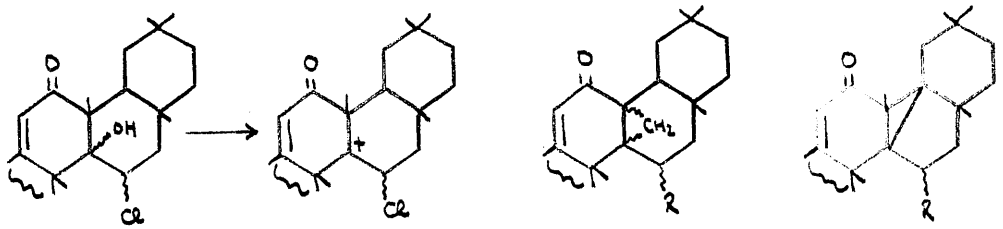
TABLE F

Compound	m.p.	$[\alpha]_D$	λmax.	ε max.
iso-β-Amyradienonyl acetate	220-221°	-40°	2450Å	10,100
Oxide of iso-β-amyrdienonyl acetate	280-281°	-12.5°	2400Å	10,800
Alcohol of oxide	249-250°	-26°	2420Å	14,000
Acetate, $C_{32}H_{46}O_4$ from H_2O_2 oxidation	318-319°	+155°	2360Å	13,400
Diol from hydrolysis of the acetate	290-291°	+167°	2360Å	12,500
Diacetate from acetylation of the acetate	194-194.5°	+141°	2360Å	11,500
Ketone from CrO_3 oxidation of the acetate	314-315°	+94°	2360Å	13,000
Alcohol from the ketone	298-299°	+71°	2360Å	13,000
Chloro-compound	227-228°	+117°	2360Å	11,100
$C_{32}H_{48}O_3$ acetate from the chloro-compound	274-275°	+130°	2340Å	11,500

For reasons which will become obvious, the formulation of the above oxidation products of iso-β-amyradienonyl acetate is very difficult, but at this juncture

the tentative formulations given below give a reasonable interpretation of the modes of formation and the reactions of the above compounds.

From a comparison of the physical constants of the compounds listed in Table F it was concluded that they all had the same basic structure and that they differed only in the nature of a substituent placed at a certain point, probably C_{15}, in the molecule. Examination of the modes of formation of these compounds makes it clear that each oxidation probably proceeds firstly with the formation of the oxide (LVIII) and secondly by oxidative fission of the oxide. The key to the modus operandi of the above oxidations clearly lies, then, in fission of the oxide linkage and this has been exemplified by fission with hydrochloric acid in acetic acid to give the chloro-compound. The intermediate in this reaction is thought to be an unstable trans-chlorohydrin (LXVII) which proceeds through the carbonium ion (LXVIII) to

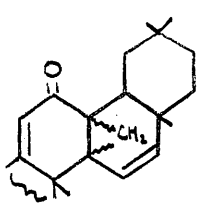


(LXVII) (LXVIII) (LXIX) (LXX)

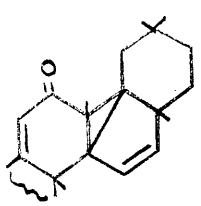
give the chloro-compound, tentatively formulated as (LXIX, R=Cl) or (LXX, R=Cl), the merits of which are discussed below.

On the basis of structures (LXIX, R=Cl) or (LXX, R=Cl) for the chloro-compound, therefore, the acetate, m.p.318° can be tentatively formulated as (LXIX, R=OH) or (LXX, R=OH), the ketone, m.p.315° as (LXIX, R=O) or (LXX, R=O) and the $C_{32}H_{46}O_3$ acetate from the zinc/ether/ /methanol reduction of the chloro-compound as (LXIX, R=H) or (LXX, R=H).

A further reaction of the chloro-compound which is pertinent to note at this stage, is that on treatment with collidine in a sealed tube at 200° it was dehydrochlorinated to give a $C_{32}H_{46}O_3$ acetate which gave a yellow colour with tetranitromethane and had light absorption maxima at 2090 (ε = 3,000) and 2320 Å (ε = 12,500). On the basis of structures (LXIX) and (LXX) it is formulated as (LXXI) or (LXXII). It is significant that the isolated double bond present in this



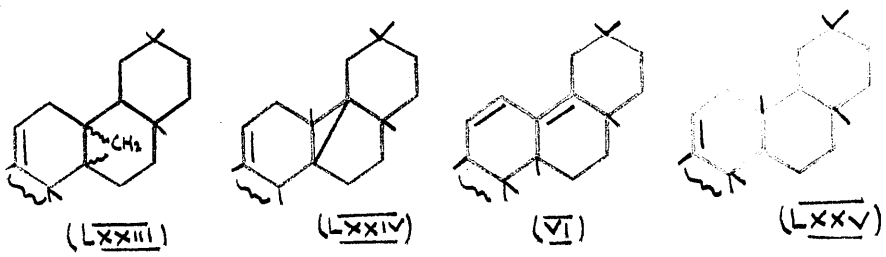
(LXXI)



(LXXII)

compound, presumably at 15:16 did not conjugate with the remainder of the chromophore on treatment with hydrochloric acid in acetic acid, starting material being recovered unchanged.

Reduction of the $C_{32}H_{48}O_3$ acetate (LXIX, R=H) or (LXX, R=H) by the Wolff-Kishner method yielded a $C_{32}H_{50}O_2$ acetate, m.p.230°, which gave a yellow colour with tetranitromethane in chloroform and had the light absorption shown in Table G. Treatment of this acetate with hydrochloric acid in acetic acid gave β-amyradienyl--II acetate (VI) and it was recovered unchanged after attempted hydrogenation with platinum catalyst in acetic acid. On the basis of the above formulations this $C_{32}H_{50}O_2$ acetate, m.p.230°, may therefore be represented by (LXXIII) or (LXXIV).



(LXXIII) (LXXIV) (VI) (LXXV)

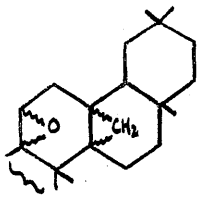
The rearrangement of the acetate, (LXXIII) or (LXXIV), to β-amyradienyl-II acetate has therefore involved the breaking of the postulated cyclopropane bridge to produce a double bond in the stable 13:18 position with the simultaneous reproduction of the

methyl group which reverted to its former C_{14} position, and the conjugation of the 10:11 double bond. It may also be stated here that the non-hydrogenability of this compound is consistent with the known inert nature of the 10:11 double bond and also with the properties of a cyclopropane bridge. The acetate, m.p.230° (LXXIII) or (LXXIV) was also produced as one of the two products of catalytic hydrogenation of the ketone, m.p. 315°, from the chromic acid oxidation of iso-β-amyradienonyl acetate (LXIX, R=O) or (LXX, R=O) and the second product was a $C_{32}H_{52}O_2$ acetate, isomeric with β--amyrin acetate, formulated as (LXXV). This compound was recovered unchanged after treatment with hydrochloric acid in acetic acid and the non-hydrogenability of the first product (see above), the $C_{32}H_{50}O_2$ acetate, m.p.230° (LXXIII) or (LXXIV) proves that the hydrogenation of the ketone acetate, m.p.315° (LXIX, R=O) or (LXX, R=O) was a bilateral reaction and that the first product was not a precurser of the second (LXXV).

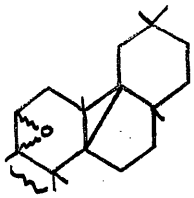
In order to find whether a cyclopropane bridge did exist in the acetate, m.p.230° (LXXIII) or (LXXIV), it was treated with perbenzoic acid. This reaction yielded a compound which analysed for $C_{32}H_{50}O_3$, one atom of oxygen more than the starting material, gave no tetra-

nitromethane colour reaction and showed no light absorption in the region 2000-4000 Å. This compound is formulated as (LXXVI) or (LXXVII). These facts are consistent with a structure containing one ethylenic linkage and one cyclopropane bridge because if the compound had contained two ethylenic linkages then either they would both have been oxidised, in which case the analysis would have shown the oxidation product to contain four atoms of oxygen and not three, or if only one of the double bonds had been attacked the other would still have given a yellow colour with tetranitromethane in chloroform. As already stated, the compound (LXXVI) or (LXXVII) gives no colour reaction with tetranitromethane and it is therefore assumed that it does contain a cyclopropane bridge together with an oxide linkage and, therefore, the starting material, the acetate, m.p.230° contains a cyclopropane bridge and one ethylenic linkage.

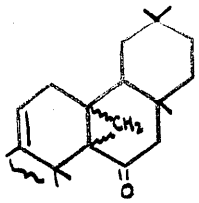
A final reaction which was carried out in this series was the reduction of the acetate (LXIX, R=O) or (LXX, R=O) by the Wolff-Kishner method. The product from this reaction was a $C_{32}H_{48}O_3$ acetate which gave a yellow colour with tetranitromethane in chloroform, had light absorption shown in Table G and was recovered unchanged after treatment with hydrochloric acid in acetic acid. The compound may be formulated as (LXXVIII) or (LXXIX).



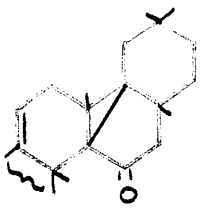
LXXVI



LXXVII



LXXVIII



LXXIX

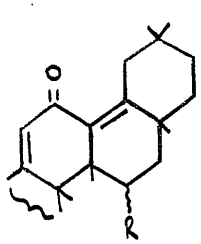
TABLE G

Compound	$[\alpha]_D$	λmax.	εmax	ε_{2100}	ε_{2150}	ε_{2200}	ε_{2250}
iso-β-amyrin acetate	+77°	2070Å	3,100	2,200	850	260	185
$C_{32}H_{50}O_2$ acetate, m.p.230°	+87°	2080Å	3,000	2,800	1,800	860	320
Second product (LXXV) of Hydrogenation of ketone, m.p.315°	+13°	2060Å	1,520	1,070	650	560	280
W.-K. product of ketone, m.p.315°	-2°	2090Å	3,400	-	2000	1100	550

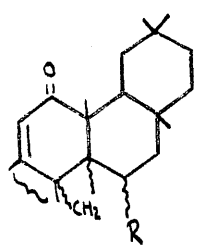
Although the weight of evidence provided by the above work seems to be in favour of the existence of a cyclopropane bridge in the oxidation products of iso-β--amyradienonyl acetate two experiments were carried out which could be taken as evidence against such a structure. These were, the treatment of the acetate,

$C_{32}H_{48}O_3$ (LXIX, R=H) or (LXX, R=H) with hydrochloric acid in acetic acid under the strongest possible conditions and the treatment of the acetate, m.p.315°, with sulphuric acid in acetic acid. In both cases, starting material was recovered unchanged. Now, cyclopropane bridges would be expected to break under the influence of strong protein denoting reagents to produce an ethylenic linkage and theoretically a rearrangement would be expected, under these conditions, in compounds with structures (LXIX) or (LXX). In this case, however, it may be true to say that when the cyclopropane bridge is in conjugation with a carbonyl group as in (LXIX) the bridge is stable to acid and whenever the carbonyl group is removed, as in the acetate (LXXIII) or (LXXIV) rearrangement takes place under the influence of a strong acid.

Other structures which havebeen considered for the oxidation products are (VIII, R=H) and (LXXX).



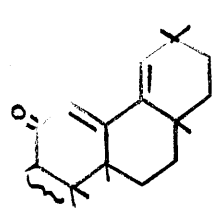
(VIII)



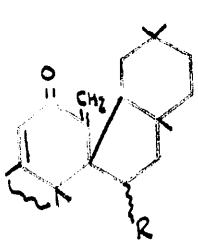
(LXXX)

Structure (VIII) (see section 1) had to be rejected because its ultraviolet light absorption with maxima at 2080, 2600 and 2950 Å was different from the common light absorption (maximum at 2360 Å) shown by the oxidation products of iso-β-amyradienonyl acetate described above.

No such sound argument can be found against structure (LXXX) at present although if the cyclopropane bridge is considered to have, to a certain extent, the properties of a double bond, then the wavelength at which maximum light absorption took place should have been increased from 2450 Å as in iso-β-amyradienonyl acetate rather than decreased to 2360 Å as in the oxidation products of iso-β-amyradienonyl acetate. The chromophore of (LXXX) in fact bears some resemblance to that of β-amyradienonyl acetate (LXXXI) which shows maximum light absorption at 2800 Å. The cyclopropane bridge



(LXXXI)



(LXXXII)

in (LXXX) would also seem to be very susceptible to hydrogen ion rearrangement which, if true, would preclude its formation by the hydrogen chloride fission of the oxide (LVIII).

The structure (LXXXII) was also considered but in view of the fact that oxidation of the chloro-compound (LXXXII, R=Cl) with ozone, under conditions which would have given formaldehyde from an exocyclic methylene group, and also its non-hydrogenability in neutral solution, both of which resulted in the complete recovery of starting material, the formulation (LXXXII) was rejected.

By a process of elimination, therefore, rather than any weight of direct chemical evidence it is concluded that the oxidation products of iso-β-amyradienonyl acetate possess cyclopropane bridges and the basic structures which best fit the reactions of these compounds are (LXIX) and (LXX). On the chemical evidence available no decision between these structures can be made but a tentative decision can perhaps be made from a consideration of the light absorption of these compounds.

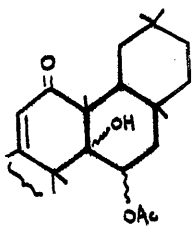
The location of the maximum light absorption of the oxidation products (Table F) at 2360Å indicates that the introduction of a cyclopropane bridge into the molecule

caused a decrease of 900Å in the location of the maximum shown by the αβ-unsaturated ketone group of iso-β--amyradienonyl acetate and it would therefore appear that the cyclopropane bridge must be in conjugation with the αβ-unsaturated ketone chromophore as in (LXIX) rather than out of conjugation as in (LXX).

Treatment of the oxide (LVIII) with sulphuric acid in acetic acid yielded a $C_{34}H_{50}O_5$ diacetate which was hydrolysed to the corresponding diol, acetylation of which produced the parent diacetate. The diacetate gave no colour with tetranitromethane in chloroform and had a light absorption maximum at 2500 Å (ε = 13,500).

An attempt to oxidise this rearrangement product of the oxide with selenium dioxide resulted in the quantitative recovery of starting material but catalytic hydrogenation yielded a $C_{34}H_{52}O_4$ acetate in 80% yield, which gave a yellow colour with tetranitromethane and had the light absorption shown in Table H. This compound was recovered unchanged after treatment with hydrochloric acid in acetic acid. The fission of the oxide linkage by sulphuric acid is assumed to have proceeded via an unstable intermediate (LXXXIII) as in the case of the hydrochloric acid fission reaction described above in which the unstable intermediate

stabilised itself by the loss of the elements of water. The hydroxyl group at C_{15} in this compound was obviously acetylated *in situ*.



(LXXXIII)

Two alternative structures each containing a cyclopropane bridge for the oxidation products and the hydrochloric acid fission product of the oxide of *iso*--β-amyradienonyl acetate have been suggested. As no definite choice between the two structures for the oxidation products can be made at this juncture and in view of the similarity between the modes of formation of the hydrochloric acid fission product and the sulphuric acid fission product of the oxide of *iso*-β-amyradienonyl acetate, it is suggested that the latter product may also contain a cyclopropane bridge and may possess one of the two structures (LXIX, R=OAc) or (LXX, R=OAc) suggested for the oxidation products of *iso*-β-amyradienonyl acetate.

The product of catalytic hydrogenation of the sulphuric acid fission product, the $C_{34}H_{52}O_4$ acetate is assumed to have been produced by the hydrogenolysis of the C_{12} carbonyl group to a methylene and this is borne out by its light absorption and colour reaction with tetranitromethane (see Table H).

Finally, in this series of reactions, the oxide of iso-β-amyradienonyl acetate (LVIII) was treated with boron trifluoride etherate in benzene, and from this reaction two isomeric $C_{32}H_{48}O_4$ acetates, easily separated by fractional crystallisation, were produced. The first, and minor product, m.p.314°, gave no colour with tetranitromethane and showed a light absorption maximum at 2380 Å (ε = 11,700) and the second, m.p.253°, also gave no colour with tetranitromethane in chloroform, but had a light absorption maximum at 2500 Å (ε = 13,500). The constants of these and other compounds are compared in Table H.

Infra-red light absorption of these compounds showed that neither contained a hydroxyl group and this was confirmed by their non-acylability and resistance to chromic acid oxidation.

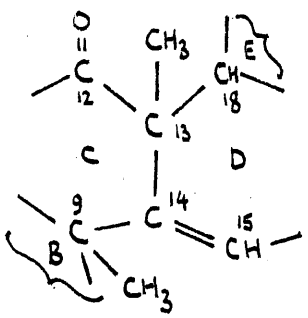
TABLE H

Compound	$[\alpha]_D$	λ max.	ε max.
H_2SO_4 rearrangement product	-78°	2500Å	13,500
Hydrogenation product of H_2SO_4 product	-30°	2070Å	1,200
Acetate, m.p.253° from BF_3 rearrangement	-76°	2500Å	13,500
Acetate, m.p.314° from BF_3 rearrangement	-180°	2380Å	11,700

Although there is a similarity in ultraviolet light absorption between the main product of this boron trifluoride rearrangement and the sulphuric rearrangement product on one hand, and between the minor product and the hydrochloric acid rearrangement product on the other, not enough data concerning these boron trifluoride rearrangement products is available to formulate them.

To summarise this work, and to conclude the third section of the theoretical part of this thesis, it may be said that the exceedingly complex reactions of iso--β-amyradienonyl acetate with oxidising and reducing agents have shown that it does not possess the same basic structure as β-amyrin, because many of the reactions described above defy interpretation on the basis of the β-amyrin structure, and it seems likely, in the author's

view, that the structure (XXXVIII) proposed by Ruzicka for this compound is correct in every respect. The extraordinary migration and back migration of the methyl group between C_{13} and C_{14} would now appear to be an established fact, and the further migration of the methyl group, as in the formation of <u>neo</u>-β-amyrin acetate, and the formation of cyclopropane bridges, as postulated in the above pages, would seem, from hypothetical mechanistic and factual considerations, to be the property of the eight carbon system (LXXXVIII) which is present in <u>iso</u>-β-amyradienonyl acetate.



(LXXXVIII)

EXPERIMENTAL

Melting points are uncorrected.

"(K)" denotes Köfler melting point apparatus.

Specific rotations were determined in chloroform solution in a 1 dm. tube at room temperature.

"Stabilised acetic acid" denotes acetic acid which has been refluxed over and distilled from chromic anhydride.

Ultraviolet absorption spectra were measured in absolute ethanol solution with a Unicam SP.500 spectrophotometer.

The analysts were Dr. A. C. Syme and Mr. Wm. McCorkindale of The Royal Technical College, Glasgow.

Isolation of β-amyrin from Manila Elemi Resin.- Elemi resin (5 kilos), from which the steam-volatile constituents had been previously removed, was stirred for 4 hours with 80% aqueous methylated spirit (12 l.). After standing overnight at room temperature, the suspension was filtered and the residue was washed with aqueous methylated spirit and dried at 80°. The melting point of the crude mixed amyrins at this stage was 155-167°. The crude mixed amyrins (1800 g.) were dissolved in pyridine (1 l.) at 100° and benzoyl chloride (1.2 l.) was added slowly during one hour. The solution was then heated on the steam-bath for a further 6 hours, cooled and diluted with chloroform (4 l.). The chloroform extract was washed with 5% hydrochloric acid solution, 5% potassium hydroxide solution, 2% sodium chloride solution, water, and was concentrated to approximately 2 litres. Addition of hot methanol (1.5 l.) and standing overnight yielded crystals of mixed amyrin benzoates (900 g.). Concentration of the mother liquors yielded a further quantity (550 g.) of mixed benzoates. The combined crops were then washed with ether until a clearing point of 214° was reached. The residue (600 g.) of crude β-amyrin benzoate was then crystallised from chloroform/methanol to yield prismatic needles of β-amyrin

benzoate (318 g.), m.p.232-234°. Concentration of mother liquors yielded solid material (260 g.), m.p.190--195°.

β-Amyrin Acetate.- β-Amyrin benzoate (100 g., m.p.232--234°) in benzene (700 c.c.) and ethanolic potassium hydroxide (ethanol, 2000 c.c.; water, 150 c.c.; potassium hydroxide, 90 g.) was refluxed for 8 hours. Concentration and cooling of the solution yielded long needles of β-amyrin (87 g.), m.p.186-187°. The β-amyrin was then dissolved in pyridine (250 c.c.), acetic anhydride (90 c.c.), and heated on the steam-bath for 2 hours. The solution was then allowed to cool and the crystalline product which separated was filtered and washed with cold methanol to yield prismatic needles of β-amyrin acetate (77 g.), m.p.240-241°, $[\alpha]_D$ +77° (c,2.1).

β-Amyradiendionyl Acetate.- β-Amyrin acetate (30 g.) and selenium dioxide (48 g.) were heated with purified dioxan (700 c.c.) in an autoclave for 22 hours at 200--210°. The crude mixture was treated with water, ether extracted and the extract was washed with water, dried (Na_2SO_4), shaken with charcoal, filtered and evaporated. The crude product was then treated with light petroleum (40-60°) in which the selenium was preferentially

dissolved and the solid residue was crystallised from ether/light petroleum (40-60°) to yield β-amyradiendionyl acetate as square plates (24 g.), m.p.236-238°. Two recrystallisations from aqueous methanol yielded square plates, m.p.240-241°, $[\alpha]_D$ -95° (c, 2.0). No colour with T.N.M. in chloroform. Light absorption in ethanol: Maximum at 2780 Å (ε = 12,500). No depression in melting point on admixture with an authentic sample of β-amyradiendionyl acetate.

Reduction of β-Amyradiendionyl Acetate with Lithium Aluminium Hydride.- A solution of β-amyradiendionyl acetate (2 g.) in dry ether (250 c.c.) was added to a suspension of lithium aluminium hydride (3 g.) in ether (400 c.c.) and the mixture heated under reflux for 4 hours. The product was isolated in the usual manner, avoiding mineral acid. A solution of the product in pyridine (50 c.c.) and acetic anhydride (25 c.c.) was kept overnight at room temperature and then heated on the water-bath for one hour. The crude product was probably contaminated with β-amyratrienyl acetate since it gave a red-brown colour with tetranitromethane and a broad absorption maximum at 3110 Å (ε = 1100). Seven crystallisations of the acetylated product from methanol

gave olean-10:13(18)-dien-2:19-diol 2-acetate as needles, m.p.223-224°; the $[\alpha]_D$ +75° (c, 0.7), did not change during the last three crystallisations.

Found: C,79.35; H,10.4.

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

It gave a yellow colour with tetranitromethane in chloroform and did not selectively absorb in the ultraviolet above 2200 Å.

A solution of the monoacetate (300 mg.) in pyridine (10 c.c.) and acetic anhydride (10 c.c.) was kept at room temperature for 6 days. Crystallisation of the product from methanol gave olean-10:13(18)-dien-2:19-diol diacetate as needles, m.p.231-231.5°, $[\alpha]_D$ +60° (c, 0.8).

Found: C,77.6; H,10.1.

$C_{34}H_{52}O_4$ requires: C,77.8; H,10.0%.

Light absorption: ε_{2100} = 11,800, ε_{2150} = 8900, ε_{2200} = 6000, ε_{2250} = 3200, ε_{2300} = 1100. The diacetate gave a yellow colour with tetranitromethane.

Oleana-10:13(18)-dien-2:19-diol.- A solution of the diol diacetate (200 mg.) in dry ether (200 c.c.) was treated with lithium aluminium hydride (200 mg.). After one hour the product was isolated (avoiding the use of mineral acid) and crystallised from aqueous methanol from which oleana-10:13(18)-dien-2:19-diol separated as plates,

m.p.240-243°. Purification by recrystallisation from the same solvent was accompanied by a drop in m.p. to 230-231° unaltered by further recrystallisation or by drying in a vacuum; $[\alpha]_D$ +58° (c, 0.5, 0.6).

Found: C,81.5; H,11.2.

$C_{30}H_{48}O_2$ requires: C,81.8; H,11.0%.

Light absorption: $\varepsilon_{2090} = 12,500$, $\varepsilon_{2150} = 10,000$, $\varepsilon_{2200} = 6300$, $\varepsilon_{2300} = 1200$.

Oleana-10:12:18-trienyl Acetate from Olean-10:13(18)--dien-2:19-diol 2-Acetate.- (a) A solution of the monoacetate (210 mg.) in acetic acid (25 c.c.) was treated with 3 drops of concentrated hydrochloric acid and the solution kept at room temperature for 16 hours. It was then heated on a steam bath for 30 minutes. The product was isolated by means of ether and crystallised from chloroform-methanol to yield oleana-10:12:18-trienyl acetate (175 mg.) as plates, m.p.180-181°, $[\alpha]_D$ +570° (c, 1.0); it gives a deep brown colour with T.N.M. in chloroform. Light absorption: Maximum at 3100 Å (ε = 14,800). Newbold and Spring (102) give m.p.184-185°, $[\alpha]_D$ +560°, maximum at 3100 Å (ε = 14,000).

(b) The monoacetate (310 mg.) in acetone (100 c.c.) was shaken with freshly prepared manganese dioxide (10 g.) for 9 hours. The product isolated in the usual manner

was crystallised from methanol to give oleana-10:12:18--trienyl acetate (198 mg.), m.p.179-183° and 180-181.5° after recrystallisation from the same solvent; $[\alpha]_D$ +545° (c, 1.5); maximum at 3100 Å (ε = 14,000); a mixture with an authentic specimen was undepressed in melting point.

Catalytic Hydrogenolysis of Olean-10:13(18)-dien-2:19--diol diacetate.- A solution of the diacetate (150 mg.) in glacial acetic acid (50 c.c.) was added to a suspension of platinum (from 100 mg. PtO_2) in glacial acetic acid (20 c.c.) and the mixture shaken with hydrogen for 18 hours. The product crystallised from chloroform--methanol to yield oleana-10:13(18)-dienyl acetate (100 mg.) as plates, m.p.199-200°, $[\alpha]_D$ +58°, +59° (c,0.8, 0.5).

Found: C,82.2; H,10.9.

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

Light absorption: ε_{2160} = 6200, ε_{2200} = 5600, ε_{2250} = 3300, ε_{2300} = 950. A mixture with a specimen prepared by the hydrogenolysis of β-amyradiendionyl acetate was undepressed in melting point.

Similar hydrogenolysis of olean-10:13(18)-dien-2:19--diol 2-acetate gave oleana-10:13(18)-dienyl acetate as plates from chloroform-methanol, m.p.198-200°, $[\alpha]_D$ +59°

(c, 1.2) undepressed in m.p. when mixed with an authentic specimen.

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 c.c.) containing concentrated hydrochloric acid (1.5 c.c.) was heated for 3 hours on the steam bath. The product, isolated by means of ether, was treated with acetic anhydride (1 c.c.) and pyridine (1 c.c.). Crystallisation of the acetylated product from methanol-chloroform gave oleana-11:13(18)-dienyl acetate (50 mg.) as plates, m.p. 223-226°, $[\alpha]_D$ -63° (c, 1.2) undepressed in m.p. when mixed with an authentic specimen Light absorption: Maxima at 2420 (ε = 23,500), 2500 (ε = 27,000) and 2600 Å (ε = 17,600).

Oxidation of Oleana-10:13(18)-2:19-diol 2-Acetate with Chromic Acid.- (a) The diol monoacetate (500 mg.) in glacial acetic acid (100 c.c.) was treated with chromic acid (77 mg.) in water (1 c.c.) and acetic acid (24 c.c.) at room temperature and the solution kept at room temperature for 16 hours. It was heated on the water-bath for 15 minutes and the product isolated in the usual manner. Crystallisation from methanol gave a less-soluble fraction

(210 mg.) as needles, m.p.215-218°, recrystallisation of which gave the diol monoacetate, m.p.221-223°, $[\alpha]_D$ +70° (c, 1.3) undepressed in m.p. when mixed with starting material. A more-soluble fraction (160 mg.) separated as plates, m.p.222-225°, $[\alpha]_D$ -62° recrystallisation of which gave β-amyradiendionyl acetate, m.p.234-236° undepressed when mixed with an authentic specimen and showing an absorption maximum at 2800 Å (ε = 10,000).

(b) Repetition of the experiment using chromic anhydride equivalent to 4 O gave β-amyradiendionyl acetate, m.p.239-240°, $[\alpha]_D$ -86° (c, 1.8), maximum at 2780 Å (ε = 12,000) in nearly quantitative yield

A similar oxidation of olean-10:13(18)-2:19-diol diacetate (325 mg.) using chromic acid equivalent to 2 O in acetic acid at room temperature gave a mixture which was separated by chromatography on alumina into unchanged diol diacetate (23 mg.), needles from methanol, m.p.218-224° undepressed when mixed with starting material, and the more strongly absorbed β-amyradiendionyl acetate (250 mg.) which separated from aqueous methanol as plates, m.p.236-238°, $[\alpha]_D$ -87° (c, 0.9). Light absorption: Maximum at 2760 Å (ε = 12,600); a mixture with an authentic specimen was undepressed in m.p.

Reduction of β-Amyradiendionyl Acetate with Zinc Dust in Ethanol.- A solution of β-amyradiendionyl acetate (m.p.240-241°, 5 g.) in boiling ethanol (500 c.c.) was refluxed with freshly activated zinc dust (25 g.) for 5 hours. The product was crystallised from chloroform/ /methanol to yield 12:19-diketo-olean-10-enyl acetate as blades, m.p.287-290° (decomp.), $[\alpha]_D$ +135° (c, 1.4). No colour with T.N.M. in chloroform. Three recrystallisations from chloroform/methanol yielded 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: Maximum at 2460 Å (ε = 12,400).

Hydrogenolysis of 12:19-Diketo-olean-10-enyl Acetate.- A solution of 12:19-diketo-olean-10-enyl acetate (537 mg.) in acetic acid (200 c.c.) was shaken with hydrogen and platinum (from 190 mg. PtO_2). Absorption of hydrogen was slow and ceased after 21 hours. The product was crystallised from chloroform-methanol to give 19-keto--olean-10-enyl acetate (413 mg.) as plates, m.p.256-258°, $[\alpha]_D$ +117°, +116° (c, 1.4, 0.8).

Found: C,79.6; H,10.4.

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

It gave a yellow coloration with T.N.M. Light absorption: $\varepsilon_{2060} = 2900$, $\varepsilon_{2100} = 2200$, $\varepsilon_{2150} = 1100$, $\varepsilon_{2200} = 280$.

Oxidation of 19-Keto-olean-10-enyl Acetate with Selenium Dioxide.- The acetate (83 mg.) in glacial acetic acid was refluxed with selenium dioxide (83 mg.) for 6 hours. The product was isolated by means of ether and treated with methanol. A relatively insoluble fraction (12 mg.), m.p.283-286°, $[\alpha]_D$ +97° (c, 0.4) was not investigated further. The mother liquor was evaporated to dryness and the residue crystallised from light petroleum from which β-amyradiendionyl acetate (30 mg.) separated as prisms, m.p.232-234°. Recrystallised from aqueous methanol it separated as plates, m.p.236-238°, undepressed when mixed with an authentic specimen; $[\alpha]_D$ -91° (c, 0.5). Light absorption: Maximum at 2780 Å (ε = 11,000).

12:19-Diketo-18α-olean-10-enyl Acetate.- A solution of 12:19-diketo-olean-10-enyl acetate (400 mg.) in 10% methanolic potassium hydroxide was refluxed for 3 hours. The product was acetylated using acetic anhydride and pyridine and the acetate crystallised from methanol to yield 12:19-diketo-18α-olean-10-enyl acetate as plates, m.p.279-281°, $[\alpha]_D$ +91°, +92°, +89.5° (c, 0.5, 1.2, 1.3).

Found: C,77.6; H,9.7.

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

Light absorption: Maximum at 2430 Å (ε = 11,700).

19-Keto-18α-olean-10-enyl Acetate.- (a) A solution of 19-keto-olean-10-enyl acetate (1.3 g.) in 10% ethanolic potassium hydroxide (500 c.c.) was heated under reflux for 3 hours. The product was isolated by means of ether and acetylated using pyridine and acetic anhydride. Crystallisation of the acetylated product from methanol--chloroform gave 19-keto-18α-olean-10-enyl acetate (1.14 g.) as blades or needles, m.p.254-256°, $[\alpha]_D$ +139° (c, 1.2).

Found: C,79.8; H,10.4.

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

(b) The catalytic hydrogenolysis of 12:19-diketo-18α--olean-10-enyl acetate was more difficult to effect than that of the corresponding 18β-isomer. A solution of the 18α-diketone (1 g.) in glacial acetic acid (150 c.c.) was shaken with hydrogen and platinum (from 0.5 g. PtO_2) for 48 hours. The filtered solution was added to freshly prepared platinum (from 0.25 g. PtO_2) in glacial acetic acid (20 c.c.) and shaken with hydrogen for 24 hours. The product obtained by filtration and evaporation of the solution gave a pale yellow colour with T.N.M. in chloroform and showed absorption maxima at 2070 (ε = 2100) and 2440 Å (ε = 3700). A solution of the reaction product

in light petroleum-benzene (2:1, 150 c.c.) was chromatographed on a column of alumina (Grade II/III, 15 x 2 cm.). The same solvent mixture (250 c.c.) eluted a solid (260 mg.) crystallisation of which from chloroform--methanol yielded 19-keto-18α-olean-10-enyl acetate as blades, m.p.257-259°, undepressed when mixed with the specimen described under (a); $[\alpha]_D$ +136° (c, 1.2). Light absorption: $\varepsilon_{2080} = 3300$, $\varepsilon_{2100} = 2200$, $\varepsilon_{2150} = 670$. It gives a yellow coloration with the T N M. reagent. Continued washing of the alumina column with the same solvent mixture (200 c.c.) gave intermediate fractions (total, 250 mg.) of 19-keto-18α-olean-10-enyl acetate of decreasing purity, whereafter benzene-ether (1:1, 100 c.c.) eluted a fraction (360 mg.) crystallisation of which from chloroform-methanol yielded 12:19--diketo-18α-olean-10-enyl acetate as plates, m.p.282-284°, $[\alpha]_D$ +86° (c, 2.2) showing an absorption maximum at 2440 Å (ε = 9800) and undepressed in m.p. when mixed with the starting material.

Reduction of 19-Keto-olean-10-enyl Acetate with Lithium Aluminium Hydride.- The ketone (0.5 g.) in dry ether (200 c.c.) was treated with lithium aluminium hydride (0.5 g.) in ether (200 c.c.) and the mixture kept at

room temperature for 4 hours. The product was isolated (avoiding mineral acid) and crystallised from aqueous methanol from which olean-10-en-2:19α-diol separated as prismatic needles or plates according to the concentration, m.p.204-205°, $[\alpha]_D$ +78°, +79° (c, 0.7; 1.3).

Found: C,81.3; H,11.5.

$C_{30}H_{50}O_2$ requires: C,81.4; H,11.4%.

Light absorption: $\varepsilon_{2100} = 2000$, $\varepsilon_{2150} = 1400$, $\varepsilon_{2200} = 470$. It gives a pale yellow colour with the T.N.M. reagent.

Partial acetylation of the diol (125 mg.) using acetic anhydride (2 c.c.) and pyridine (2 c.c.) at room temperature for 16 hours yielded olean-10-en-2:19α-diol 2-acetate separating as prismatic needles from chloroform--methanol, m.p.262-263°, $[\alpha]_D$ +88°, +89° (c, 1.5, 0.9).

Found: C,79.1; H,10.9.

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

Light absorption: $\varepsilon_{2090} = 2000$, $\varepsilon_{2150} = 820$, $\varepsilon_{2200} = 200$; it gave a pale yellow colour with the T.N.M. reagent.

Olean-10-en-2:19α-diol 2-acetate (150 mg.) in glacial acetic acid (50 c.c.) was treated at room temperature with chromic anhydride (≡ 1.2 O) in acetic acid (11.5 c.c.) added dropwise over 30 minutes and the

solution was kept at room temperature for 20 hours. The product was isolated in the usual manner and crystallised from chloroform-methanol to yield 19-keto--olean-10-enyl acetate as plates, m.p.253-255°, $[\alpha]_D$ +118° (c, 0.8) undepressed in m.p. when mixed with an authentic sample; a mixture with 19-keto-18α-olean-10--enyl acetate, m.p.254-256°, $[\alpha]_D$ +139° had m.p.232-238°.

Oleana-10:18-dienyl Acetate.- A solution of olean-10--en-2:19α-diol 2-acetate (m.p.263-264°, 1.37 g.) in pyridine (75 c.c.) and phosphorous oxychloride (25 c.c.) was heated on the steam bath for 7 hours. After standing overnight at room temperature the product was carefully precipitated with water, filtered, dissolved in chloroform (300 c.c.) and the solution dried (Na_2SO_4). Evaporation yielded a solid residue which crystallised from chloroform/methanol as plates (1 g.), m.p.256-258°. Concentration of the mother liquors yielded plates (150 mg.), m.p.247-251°. Three recrystallisations of the first crop yielded plates, m.p.256-257°, $[\alpha]_D$ +99° (c, 2.2) giving a yellow colour with T.N.M. in chloroform. Light absorption in ethanol: ε_{3000} = 3200, ε_{2150} = 1250; ε_{2200} = 280.

Found: C,82.59; H,10.99.

$C_{32}H_{50}O_2$ requires: C,82.34; H,10.80%.

Oxidation of 12:19-diketo-olean-10-enyl Acetate with Selenium Dioxide.- 12:19-Diketo-olean-10-enyl acetate (m.p.292-294°, 250 mg.) was refluxed with selenium dioxide (250 mg.) and stabilised glacial acetic acid (100 c.c.) for 5 hours. The pale yellow solution was filtered, poured into water, treated with ether and the extract was washed with sodium bicarbonate solution, water and dried (Na_2SO_4). The extract was then treated with charcoal, and evaporated to yield a solid residue which crystallised from aqueous methanol as square plates (170 mg.), m.p.235--238°. Concentration of the mother liquors yielded a second crop of plates (50 mg.), m.p.225-232°. The first crop recrystallised from aqueous methanol as square plates, m.p.237-239°, $[\alpha]_D$ -87°, showing no colour with T.N.M. in chloroform. Light absorption in ethanol: maximum at 2800 Å (ε = 11,200). No depression in m.p. on admixture with an authentic specimen of β-amyradien-dionyl acetate.

Oxidation of 12:19-diketo-18α-olean-10-enyl Acetate with Selenium Dioxide.- 12:19-Diketo-18α-olean-10-enyl acetate (m.p.284-286°, 250 mg.) was refluxed with selenium dioxide (250 mg.) and stabilised glacial acetic acid for 5 hours. Treatment of the solution in the manner

described in the previous experiment yielded a solid which crystallised from chloroform/methanol as plates (196 mg.), m.p.282-285°. Concentration of the mother liquors yielded a second crop of plates (30 mg.), m.p. 276-281°. The first crop recrystallised from chloroform/methanol as plates, m.p.283-285°, $[\alpha]_D$ +81° (c, 1.5) showing no colour with T.N.M. in chloroform. Light absorption in ethanol: Maximum at 2440 Å (ε = 10,500). There was no depression in m.p. on admixture with starting material.

β-Amyranonyl Acetate.- (a) β-Amyrin acetate (20 g., m.p. 240-241°) in glacial acetic acid (1800 c.c.) was treated at 100° with a mixture of hydrogen peroxide (100 vol.; 150 c.c.) in glacial acetic acid (150 c.c.) added dropwise during 30 minutes with stirring. Stirring was continued for 2 hours at 100° and the solution again treated with hydrogen peroxide (100 vol.; 50 c.c.) in acetic acid (50 c.c.) during 15 minutes. The solution was then kept at 95° for one hour and hot water was added until it became faintly opalescent. The crystalline solid which separated on standing overnight at room temperature was collected by filtration (m.p.288-291°; 8.7 g.). The mother liquor was heated to 100° then treated with hot water until opalescent, and a second crop of crystals (m.p.286-287°; 2.0 g.) was isolated. The two crops were combined, dissolved in light petroleum (b.p.60-80°)-benzene (1:2; 300 c.c.), and chromatographed on a column of activated alumina (Grade I/II, 42 x 3.5cm.). Washing with the same solvent mixture (3450 c.c.) gave an eluate (7.05 g.) which when crystallised from chloroform--methanol yielded β-amyranonyl acetate as plates, m.p.300--301° (K) (299-300° in an open capillary), $[\alpha]_D$ -15°, (c, 2.82, 5.83). Light absorption in ethanol: Maximum at 2780 Å ($\varepsilon \div 280$).

Found: C,79.0; H,10.7.

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

Continued washing of the column with light petroleum (60-80)-benzene (1:4; 600 c.c.), benzene (600 c.c.), and benzene-ether (19:1; 200 c.c.) yielded material (total 1.3 g.) which on crystallisation from chloroform--methanol gave β-amyranonyl acetate as plates, m.p.299--300°, $[\alpha]_D$ -15° (550 mg.).

(b) β-Amyrin acetate (m.p.240-241°, 3 g.) in ethyl acetate (175 c.c.) at 54° was treated with a solution of hydrogen peroxide (100 vol ; 5 c.c.) in formic acid (98-100%, 25 c.c.) added dropwise during three hours at 54° with stirring. Stirring at 54° was continued for 36 hours and the solution was concentrated to 30 c.c. On cooling, plates of β-amyranonyl acetate (2.20 g.), m.p.298-300°, $[\alpha]_D$ -12° (c, 1.4) separated.

Concentration of the mother liquor yielded an amorphous material.

Recrystallisation of the above crystals from chloroform-methanol yielded plates, m.p.299-301°, $[\alpha]_D$ -14° (c, 1.0) showing no colour with T.N.M., and no depression in melting point on admixture with authentic β-amyranonyl acetate.

Note. In using this method on a large scale (20 g.

β-amyrin acetate) it was found necessary to use four times as much hydrogen peroxide as in the above method in order to ensure complete oxidation. The temperature at which the preparation is carried out is not critical and six hours is sufficient for complete reaction.

iso-β-Amyrenonyl Acetate.- β-Amyranonyl acetate (m.p.299--300°; 5 g.) in glacial acetic acid (450 c.c.) was treated, at 45-50°, with a solution of bromine (2 g., 1.2 mols.) in glacial acetic acid (27 c.c.) added dropwise during 30 minutes with stirring. Stirring at 45--50° was continued for a further two hours and the solution was allowed to stand overnight at room temperature. It was then heated to 80° and hot water was added until it became faintly opalescent. The crystalline solid (m.p.288-289°, 2.92 g.) which separated on cooling was collected by filtration and more water was added to the hot mother liquor until a faint opalescence appeared. On cooling, a second crop of crystalline material (m.p. 281-283°, 1.0 g.) was deposited and a third crop (m.p.240--250°, 0.2 g.) was obtained in a similar manner.

Recrystallisation of the combined first and second crops yielded hexagonal plates of iso-β-amyrenonyl acetate, m.p.290-291° (K), $[\alpha]_D$ +62° (c, 1.9). Other preparations had m.p.289-290°, $[\alpha]_D$ +61° (c, 1.2); m.p.287-289°,

$[\alpha]_D$ +60° (c, 2.2). Light absorption in ethanol: Maximum at 2450 Å (ε = 10,000).

Found: C,79.6; H,10.6.

Calc. for $C_{32}H_{50}O_3$: C,79.6; H,10.45%.

iso-β-Amyrin Acetate.- Following the method of Jeger and Ruzicka (99), iso-β-amyrenonyl acetate (m.p.288-289°, 1.3 g.) in stabilised glacial acetic acid (200 c.c.) was added to platinum catalyst (from 0.2 g. PtO_2) in acetic acid (10 c.c.). Shaking in hydrogen was then carried out for 48 hours at slightly greater than atmospheric pressure. The solution was warmed, filtered and poured into water (500 c.c.). The ether extract was neutralised with sodium bicarbonate solution, dried (Na_2SO_4) and evaporated. The crystalline residue was recrystallised from chloroform/methanol to yield plates, m.p.250--251°, $[\alpha]_D$ +77° (c, 1.6), (0.83 g.). Yellow colour with T.N.M. in chloroform. Light absorption in ethanol: Maximum at 2070 Å (ε = 3,500).

iso-β-Amyrin Acetate.- iso-β-Amyrenonyl acetate (m.p.288--289°, 0.3 g.) in acetic acid (15 c.c.) containing concentrated hydrochloric acid (15 c.c.) was added to amalgamated zinc (9 g.) and maintained at 100° for 6 hours. During this period additions of concentrated hydrochloric

acid (5 c.c.) were made at intervals of two hours. The hot solution was decanted into water, and the precipitated solid was collected, washed, dried and crystallised from methanol/chloroform to yield plates, m.p.240-255° (155 mg.). This product was readily separated by chromatography of a light petroleum (60-80)-benzene solution (4:1, 50 c.c.)on activated alumina (Grade II, 14 x 1.5 cm.) into a fraction I (94 mg.) eluted by light petroleum (60-80)-benzene (4:1, 200 c.c.), and a fraction II (53 mg.) eluted by benzene (150 c.c.). Crystallisation of fraction I from chloroform/methanol gave plates, m.p.250-251°, $[\alpha]_D$ +17° (c, 0.3). Yellow colour with T.N.M. in chloroform. Light absorption in ethanol: ε_{2070} = 3,100, ε_{2100} = 2,200, ε_{2150} = 850, ε_{2200} = 260, ε_{2250} = 185.

Found: C,82.1; H,11.4.

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

Admixture with an authentic specimen of <u>iso</u>-β-amyrin acetate prepared by the catalytic hydrogenation of <u>iso</u>--β-amyrenonyl acetate produced no depression in melting point.

Crystallisation of fraction II from chloroform/ /methanol gave <u>iso</u>-β-amyrenonyl acetate as plates, m.p.285-286°. Light absorption in ethanol: Maximum

at 2450 Å (ε = 9,500). No depression in melting point on admixture with starting material and no colour with T.N.M.

11-Keto-β-Amyranyl Acetate.- Following the method of Jeger and Ruzicka (99), iso-β-amyrin acetate (m.p.250--251°, 0.7 g.) was dissolved in glacial acetic acid (100 c.c.) at 110° and while refluxing gently, a solution of hydrogen peroxide (100 vols., 7 c.c.) in glacial acetic acid (7 c.c.) was added dropwise during 10 minutes. The solution was then refluxed gently for one hour and a further quantity (10 c.c.) of hydrogen peroxide solution in glacial acetic acid was added. The solution was then refluxed for a further two hours and on cooling the product crystallised as plates, m.p.327-332° (decomp.) (0.2 g.). Recrystallisation from chloroform/methanol yielded plates, m.p.338-339° (in vacuo), $[\alpha]_D$ +6.4° (c,0.3). Light absorption in ethanol: Maximum at 2800 Å ($\varepsilon \doteqdot 230$). Negative T.N.M. test. Addition of water to the hot mother liquor yielded a second crop of plates (0.04 g.), m.p.310-315°.

β-Amyranyl Acetate.- Following the method of Jeger and Ruzicka (100), β-amyranonyl acetate (m.p.299-300°, 0.2 g.), hydrazine hydrate (100%, 2 c.c.) and sodium amylate (5%,

10 c.c.) were heated in an autoclave for 18 hours at 200°. The crude product was treated with water and extracted with ether. The extract was washed with water, dried (Na_2SO_4) and evaporated. The residue was dissolved in pyridine (3 c.c.) and acetic anhydride (3 c.c.) and allowed to stand at 20° for 48 hours. The solution was worked up in the usual manner and yielded a residual gum which crystallised from chloroform/methanol as plates (0.1 g.), m.p.276-279°.

Recrystallisation from chloroform/methanol yielded plates, m.p.283-284°, $[\alpha]_D$ +24° (c, 0.5). The product showed no selective light absorption in the region 2000--4000 Å and showed no colour reaction with T.N.M. in chloroform.

β-Amyranyl Acetate.- 11-Keto-β-amyranyl acetate (m.p.338--339°, 0.25 g.), hydrazine hydrate (100%, 2c.c.) and sodium ethoxide (7.5%, 10 c.c.) were heated in an autoclave for 18 hours at 200-210°. The ether extract of the crude product was washed with 2% hydrochloric acid, water, dried (Na_2SO_4) and evaporated. The residue was acetylated with acetic anhydride and pyridine and the crude acetylated product (185 mg.) was dissolved in a mixture of light petroleum (60-80°)-benzene (2:1; 50 c.c.)

and chromatographed on a column of activated alumina (Grade II, 10 x 1.25 cm.). Elution with the same solvent mixture gave a fraction I (30 mg.), crystallisation of which from chloroform/methanol yielded plates, m.p.282-284°, $[\alpha]_D$ +24° (c, 0.46) showing no selective light absorption in the region 2000-4000 Å, and no colour reaction with T.N.M. in chloroform. Admixture with an authentic sample of β-amyranyl acetate produced no depression in melting point. Further elution with the same solvent mixture gave a fraction II (40 mg.) which crystallised from chloroform/methanol as plates, m.p.297-303° (decomp.), undepressed in melting point on admixture with starting material.

Enol Acetate of iso-β-Amyrenonyl Acetate.- A solution of iso-β-amyrenonyl acetate (m.p.289-290°, 1 g.) in acetic anhydride (60 c.c.), containing freshly fused sodium acetate (0.5 g.) was refluxed for 80 hours. The reaction mixture was treated with water and extracted with ether. The extract was washed with water, shaken with charcoal, filtered, dried (Na_2SO_4) and evaporated. The residue crystallised from chloroform/methanol to give the enol acetate of iso-β-amyrenonyl acetate as long prismatic needles (0.85 g.), m.p.216.5-217°, $[\alpha]_D$ +201° (c, 1.6)

Light absorption in ethanol: Maximum at 2780 Å (ε = 8,500). The enol acetate gave a brown colour with T.N.M. in chloroform.

Found: C,77.5; H,9.9.

$C_{34}H_{52}O_4$ requires: C,77.8; H,9.9%.

Attempted purification of the enol acetate by chromatography on alumina yielded iso-β-amyrenonyl acetate quantitatively.

Enol Acetate of β-Amyranonyl Acetate.- Following the method of Ruzicka and Jeger (100), a solution of β-amyranonyl acetate (m.p.299-300°, 1 g.) in acetic anhydride (40 c.c.) containing freshly fused sodium acetate (1 g.) was refluxed for 48 hours. The reaction mixture was treated with water and extracted with ether. The extract was washed with water, shaken with charcoal, filtered, dried (Na_2SO_4) and evaporated. The residue crystallised from chloroform/methanol to give the enol acetate of β-amyranonyl acetate as needles,(0.7 g.), m.p.232-233°, $[\alpha]_D$ +60° (c, 1.3). Yellow colour with T.N.M. in chloroform. Light absorption in ethanol: Maximum at 2080 Å. (ε = 2,500).

Enol Acetate of 11:12-Diketo-β-Amyranyl Acetate.- Following the method of Ruzicka and Jeger (100), the enol acetate

of β-amyranonyl acetate (m.p.232-233°, 0.15 g.) in acetic acid (6 c.c.), benzene (20 c.c.) and concentrated sulphuric acid (0.1 c.c.) was treated with a solution of chromic oxide (0.1 g.) in acetic acid (5 c.c., 90%) during 30 minutes at 20°. After standing at room temperature overnight the solution was treated with water and ether, and the ether extract was washed with saturated sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated. The residue crystallised from chloroform/methanol as flat prismatic needles (0.1 g.), m.p.233-234° (K), $[\alpha]_D$ +78° (c, 1.2). No colour with T.N.M. in chloroform. Light absorption in ethanol: Maximum at 2550 Å (ε = 11,200).

Enol Acetate of 11:12-Diketo-β-Amyranyl Acetate.- A solution of the enol acetate of iso-β-amyrenonyl acetate (m.p.216-217°, 0.3 g.) in acetic acid (20 c.c.) was treated with a solution of hydrogen peroxide (100 vols.; 25 c.c.) in acetic acid (25 c.c.) during 30 minutes at 100° with stirring. After one hour a second addition of the hydrogen peroxide-acetic acid mixture (20 c.c.) was made and stirring was continued for two hours, when the remainder of the peroxide solution was added. After a further hour the solution was diluted with water, the

solid precipitate collected, dried and crystallised from chloroform/methanol to yield the enol acetate of 11:12--diketo-β-amyranyl acetate as flat prismatic needles, m.p.228-229°, $[\alpha]_D$ +80° (c, 1.5). Negative T.N.M. test. Light absorption in ethanol: Maximum at 2550 Å (ε = 10,900). This product was undepressed in melting point on admixture with an authentic specimen of the enol acetate of 11:12-diketo-β-amyranyl acetate as prepared above.

Acid Hydrolysis of the Enol Acetate of β-Amyranonyl Acetate.- The enol acetate of β-amyranonyl acetate (m.p.232-233°, 0.15 g.) was refluxed for two hours in methanol-hydrochloric acid solution (19:1, 30 c.c.). A further quantity of methanol-hydrochloric solution (20 c.c.) was then added and refluxing was carried out for a further 3 hours. The solution was treated with water and ether, and the ether extract was washed with dilute sodium bicarbonate, water, dried (Na_2SO_4), and evaporated. The residue was dissolved in pyridine (4 c.c.), acetic anhydride (5 c.c.), heated on the steam--bath for two hours, and the acetylating solution worked up in the usual manner. The residue crystallised from chloroform/methanol as plates (90 mg.), m.p.295-296°,

$[\alpha]_D$ -14° (c, 0.5), negative T.N.M. test, and was undepressed in melting point on admixture with β-amyranonyl acetate. A second crop of plates, m.p.294-296° (30 mg.), also undepressed in melting point on admixture with β-amyranonyl acetate, was obtained by concentration of the mother liquors.

Attempted Bromination of the Enol Acetate of β-Amyranonyl Acetate.- The enol acetate of β-amyranonyl acetate (m.p.233-234°, 0.35 g.) in glacial acetic acid (50 c.c.) was treated, at 60° and constant stirring, with two drops of aqueous 48% HBr solution immediately followed by the dropwise addition of a solution of bromine in acetic acid (4.8 c.c., 2.5%, 1.1 mols.). Stirring was continued for two hours at 60° and the solution was allowed to stand at room temperature for a further five hours. It was then heated to 80° and hot water was added until it became opalescent. The product which separated was recrystallised from chloroform/methanol to yield needles (0.14 g.), m.p.233-234°, $[\alpha]_D$ +60° (c, 0.33). Yellow colour with T.N.M. in chloroform. Admixture of the product with starting material produced no depression in melting point. Concentration of the mother liquors yielded an uncrystallisable gum.

Attempted Bromination of the Enol Acetate of iso-β-Amyrenonyl Acetate.- The enol acetate of iso-β-amyrenonyl acetate (m.p.216-217°, 0.3 g.) in glacial acetic acid (125 c.c.) was treated at 60° with a solution of bromine in acetic acid (2.4 c.c., 5%, 1.1 mols.) added dropwise during 15 minutes with stirring. Stirring was continued at 60° for two hours, during which the bromine colour disappeared and the solution became pale yellow in colour. After standing for five hours the solution was heated to 80° and hot water was added until a faint opalescence appeared. On cooling, crystalline material separated and filtration yielded prismatic needles, m.p.213-215°, $[\alpha]_D$ +180° (c, 1.1) (0.21 g.), which gave a brown colour with T.N.M. in chloroform. Light absorption in ethanol: Maximum at 2800 Å (ε = 8,000). Admixture with starting material produced no depression in melting point. Further investigation of the mother liquors produced only low melting amorphous material.

Treatment of the Enol Acetate of iso-β-Amyrenonyl Acetate with N-Bromosuccinimide.- A solution of the enol acetate of iso-β-amyrenonyl acetate (0.4 g.) and N-bromosuccinimide (0.5 g.) in carbon tetrachloride (100 c.c.) was refluxed for 8 hours during which the solution became deep yellow in colour and hydrogen bromide was evolved.

The filtered solution was evaporated to dryness under vacuum and the residue was twice recrystallised from aqueous methanol to yield square plates, m.p.237-238° (K), $[\alpha]_D$ -95° (c, 0.25), negative T.N.M. reaction, and showing a light absorption maximum at 2780 Å (ε = 10,000). When mixed with a specimen of β-amyradiendionyl acetate from the selenium dioxide oxidation of β-amyrin acetate, there was no depression in melting point.

Oxidation of iso-β-Amyrin Acetate with Selenium Dioxide.- iso-β-Amyrin acetate (m.p.250-251°, 0.4 g.) was refluxed in acetic acid (30 c.c.) with selenium dioxide (0.4 g.) for 40 hours. The solution was filtered, poured into water, ether extracted and the extract was washed with sodium bicarbonate solution, dried (Na_2SO_4) and evaporated. The residue was dissolved in benzene (50 c.c.) and filtered through a short column of activated alumina (Grade II, 5 x 1.5 cm.) to remove the most of the colloidal selenium. The residue obtained on evaporation of the benzene (0.3 g.) crystallised from chloroform/ /methanol as plates (0.2 g.), m.p.248-249°, $[\alpha]_D$ +76° (c, 0.5), yellow colour with T.N.M. in chloroform. There was no depression in melting point on admixture with starting material. The residue obtained by evaporation of the mother liquor crystallised from aqueous methanol

as square plates, m.p.233-234° (K), $[\alpha]_D$ -87° (c, 0.33). Light absorption in ethanol: Maximum at 2780 Å (ε = 10,700). Negative T.N.M. reaction. Admixture with a sample of β-amyradiendionyl acetate from the selenium dioxide oxidation of β-amyrin acetate produced no depression in melting point.

Treatment of iso-β-Amyrenonyl Acetate with N-Bromosuccinimide.- iso-β-Amyrenonyl acetate (m.p.290-291°, 0.3 g.) and N-bromosuccinimide (0.5 g.) were refluxed in carbon tetrachloride (100 c.c.) for 6 hours. The solution was filtered and the filtrate was washed with sodium thiosulphate solution, water, dried (Na_2SO_4) and evaporated under vacuum. The residue crystallised from chloroform/methanol as plates (0.2 g.), m.p.284-286°, showing no depression in melting point on admixture with starting material. Concentration of the mother liquors produced a second crop of plates (0.05 g.), m.p.279-282°, also showing no depression in melting point on admixture with starting material.

Treatment of iso-β-Amyrenonyl Acetate with Aqueous Ethanolic Caustic Potash.- iso-β-Amyrenonyl acetate (m.p. 290-291°, 0.67 g.) was refluxed in 15% aqueous ethanolic caustic potash solution (130 c.c. ethanol, 20 c.c. water,

22.5 g. potassium hydroxide) for 70 hours. The solution was treated with water and ether, and the ether extract was washed with water, dried (Na_2SO_4) and evaporated The residue was dissolved in pyridine (6 c.c.) and acetic anhydride (10 c.c.) and after heating on the steam-bath for 3 hours the solution was worked up in the normal manner. Crystallisation from chloroform/methanol yielded plates (0.43 g.), m.p.288-290°. There was no depression in melting point on admixture with starting material. Concentration of the mother liquors yielded plates (0.15g.) m.p.287-289°. No depression in melting point on admixture with starting material.

Treatment of β-Amyranonyl Acetate with Aqueous Ethanolic Caustic Potash.- β-Amyranonyl acetate (m.p.299-300°, 0.2 g.) was refluxed in an aqueous ethanolic solution of potassium hydroxide (25 c.c. 15%) for 60 hours. The solution was treated with water and ether, and the ether extract was washed with water, dried (Na_2SO_4) and evaporated. The residue was acetylated on the steam-bath with pyridine and acetic anhydride and the acetylating solution was worked up in the usual manner. The residue (0.16 g.) was dissolved in light petroleum (b.p.60-80°)-benzene 4:1, 25 c.c.) mixture and chromatographed on a column of activated alumina (Grade II, 14 x 1.5 cm.).

Fractions	Eluant		Wt.	m.p.
1-2	Light Petroleum/Benzene (4:1)	50 c.c.	-	-
3-10	- do. -	200	0.100 g.	255-265°
11-14	- do. -	100	0.006	-
15-18	Light Petroleum/Benzene (2:1)	100	0.002	-
19-20	Light Petroleum/Benzene (1:1)	50	0.024	274-277
21-22	Benzene	50	0.029	284-287
23-24	do.	50	-	-

Fractions (3-10) were combined and twice recrystallised from chloroform/methanol to give flat needles, m.p.269--271° which on further recrystallisation gave plates, m.p.269-271°, $[\alpha]_D$ +92° (c, 1.28). No colour with T.N.M. in chloroform. Depression of 30-35° in melting point on admixture with β-amyranonyl acetate.

Found: C,77.64; H,10.15.

$C_{34}H_{54}O_4$ requires: C,77.72; H,10.32%.

Fractions (11-22) were combined and crystallised from chloroform as plates, m.p.296-298°. No depression in melting point on admixture with starting material.

Chromic Acid Oxidation of β-Amyrin Acetate.- β-Amyrin acetate (m.p.240-241°, 10 g.) was dissolved in hot stabilised glacial acetic acid (650 c.c.). The solution was gently boiled to effect slow distillation, and a solution

of chromic oxide (30 g.) in aqueous stabilised acetic acid (100 c.c.; 80%) was added dropwise. The distillate was collected in 40 c.c. fractions, each being neutralised with caustic soda solution (30%) and redistilled. The first 10 c.c. of the redistillate from each fraction was treated with a solution of 2:4-dinitrophenyl hydrazine in aqueous hydrochloric acid and the yellow 2:4-dinitrophenyl hydrazone was collected. After eight such distillations the reaction mixture was diluted with stabilised glacial acetic acid (100 c.c.) and a solution of chromic oxide (10 g.) in water (10 c.c.) and acetic acid (20 c.c.) was added. A total of twelve fractions was taken and the 2:4-dinitrophenyl hydrazone (80 mg.), m.p.122-124°, was collected. The product was recrystallised twice from methanol to yield yellow needles, m.p.124-126°, undepressed in melting point on admixture with authentic acetone 2:4-dinitrophenylhydrazone.

Chromic Acid Oxidation of β-Amyrin Benzoate.- Following the method of Ruzicka, Müller and Schellenberg (101), β-amyrin benzoate (m.p.231-233°, 30 g.) in acetic acid (1800 c.c.) at 100° was treated with a solution of chromic oxide (30 g.) in acetic acid (200 c.c.) and water (10 c.c.), added dropwise during one hour and the solution

was refluxed for a further hour. Water was then added until a faint opalescence appeared and on cooling, plates (11.4 g.), m.p.261-264°, separated. Recrystallisation from chloroform/methanol gave β-amyrenonyl benzoate as plates, m.p.271-272°, $[\alpha]_D$ +113° (c, 2.9) showing no colour with T.N.M. in chloroform and exhibiting light absorption maxima in ethanol at 2300 Å (ε = 21,500) and 2520 Å (ε = 13,800).

Epimerisation of β-Amyrenonyl Benzoate.- Following the method of Ruzicka et al. (101), β-amyranonyl benzoate (m.p.271-272°, 7.4 g.) was dissolved in ethanolic potassium hydroxide solution (600 c.c., 15%) and refluxing was carried out for 70 hours. The solution was then treated with water, filtered and the dried product was acetylated on the steam-bath with pyridine and acetic anhydride. The product was isolated in the usual manner and was crystallised from chloroform/methanol as prismatic plates of 18-iso-β-amyrenonyl acetate (3 g.), m.p.276-278°, $[\alpha]_D$ +73° (c, 0.75), undepressed in melting point on admixture with an authentic sample of 18-iso-β-amyrenonyl acetate.

Attempted Bromination of 18-iso-β-Amyrenonyl Acetate.- 18-iso-β-Amyrenonyl acetate (m.p.275-277°, 0.5 g.) in

glacial acetic acid (200 c.c.) was treated at 60°, first with a few drops of aqueous hydrogen bromide solution (48%) and then with a solution of bromine in acetic acid (4.4 c.c.; 1.2 mols., 2.5%) added dropwise with stirring. Stirring was continued for three hours at 60° and after standing overnight at room temperature the solution was heated to 80° and water was added until faint opalescence appeared. On cooling, plates (0.4 g.), m.p.273-275°, separated and showed no depression in melting point on admixture with starting material. A second crop of plates, m.p.269-272°, (0.05 g.) was obtained from the mother liquors in the same way and this crop was also undepressed in melting point on admixture with starting material.

Attempted Formation of the Enol Acetate of 18-iso-β--Amyrenonyl Acetate.- 18-iso-β-Amyrenonyl acetate (m.p. 276-278°, 0.5 g.) in acetic anhydride (50 c.c.) was treated with freshly fused sodium acetate (1 g.) and refluxing was carried out for 80 hours. The solution was filtered, treated with water and ether, and the extract was washed with water, dried (Na_2SO_4) and decolorised with charcoal. The residue obtained on evaporation of the ether crystallised from chloroform/methanol as plates (0.3 g.), m.p.275-277°. Concentration yielded

a second crop of plates (0.14 g.), m.p.270-273°. Neither crop was depressed in melting point on admixture with starting material.

Sodium/Amyl Alcohol Reduction of 18-iso-β-Amyrenonyl Acetate.- 18-iso-β-Amyrenonyl acetate (m.p.276-278°, $[\alpha]_D$ +79°, 500 mg.) in boiling amyl alcohol (20 c.c.) was treated with sodium metal (900 mg.) added in small amounts with frequent shaking during one hour. The solution was then refluxed for a further hour, cooled and steam-distilled to yield a white solid which gave a yellow colour with T.N.M. in chloroform. Light absorption in ethanol: Maxima at 2080 (ε = 1,200), 2450 Å (ε = 350). The product was heated for 1½ hours on the steam-bath with freshly fused sodium acetate (0.5 g.) in acetic anhydride (10 c.c.). The product crystallised from chloroform/methanol as plates, m.p.213-216° (370 mg.). Red-brown colour with T.N.M. in chloroform. Light absorption in ethanol: Maxima at 2420 (ε = 19,000), 2510 (ε = 22,000) and 2600 Å (ε = 15,100). Four recrystallisations from the same solvent, using various crystallisation techniques yielded plates, m.p. 216-217°, $[\alpha]_D$ +21° (c, 1.1). Red-brown colour with T.N.M. and no depression in melting point on admixture with β-amyradienyl-II acetate, m.p.227-229°, $[\alpha]_D$ -63°.

Fractional crystallisation, hydrolysis and double chromatography all failed to purify the β-amyradienyl-II acetate which appeared to have formed a mixed crystal with some other compound, possibly 18-iso-β-amyradienyl-I acetate.

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iso-β-Amyradienonyl Acetate.- iso-β-Amyrenonyl acetate (m.p.290-291°, 15 g.) was refluxed in glacial acetic acid (500 c.c.) with selenium dioxide (16 g.) for 48 hours. The solution was treated with water and ether and the ether extract was washed with potassium cyanide solution (5%), water, and dried (Na_2SO_4). The extract was then treated with charcoal, filtered and evaporated to yield a solid residue which was dissolved in benzene (100 c.c.) and filtered through a column of activated alumina (Grade II, 14 x 3 cm.). The pale yellow residue crystallised from chloroform/methanol as prisms (8 g.), m.p.222-223°, $[\alpha]_D$ -38° (c, 2.6) and gave a yellow colour with T.N.M. in chloroform. Light absorption in ethanol: Maxima at 2090 (ξ = 3,000) and 2450 Å (ξ = 10,000). Admixture with an authentic specimen of iso-β-amyradienonyl acetate produced no depression in melting point.

Oxidation of the Enol Acetate of iso-β-Amyrenonyl Acetate with Selenium Dioxide.- The enol acetate (m.p.216-217°, 0.45 g.) was refluxed in stabilised glacial acetic acid (100 c.c.) with selenium dioxide (0.45 g.) for 72 hours. Solid selenium was removed by filtration and the solution was worked up as described in the previous experiment to yield a residue (0.43 g.) which was fractionally crystallised from chloroform-methanol. The first two crops

(0.34 g.) separated as needles, m.p.215-216°, undepressed when mixed with the enol acetate of iso-β-amyrenonyl acetate. The third crop (0.06 g.) separated as needles, m.p.190-196°, and it was thrice recrystallised from the same solvent to give prisms, m.p.218-219°, $[\alpha]_D$ -39° (c, 0.7), yellow colour with T.N.M. in chloroform. Light absorption in ethanol: Maxima at 2090 (ϵ = 2,000) and 2450 Å (ϵ = 11,200). It was depressed to m.p.180-195° when mixed with the enol acetate of iso-β-amyrenonyl acetate, and undepressed when mixed with a specimen of iso-β-amyradienonyl acetate prepared by the method described above.

Clemmensen Reduction of iso-β-Amyradienonyl Acetate.- A hot solution of iso-β-amyradienonyl acetate (m.p.220-221°, 0.5 g.) in stabilised acetic acid (50 c.c.) was treated with concentrated hydrochloric acid (10 c.c.) and added to freshly amalgamated zinc (from 15 g. of zinc). The solution was refluxed for two hours, filtered, treated with water and ether, and the extract was washed with dilute sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated. The residue crystallised from chloroform--methanol as plates (0.15 g.), m.p.215-218°, showing a red-brown colour with T.N.M. in chloroform. Six re-crystallisations from the same solvent gave plates,

m.p.230.5-231.5°, $[\alpha]_D$ -65° (c, 1.1). Light absorption in ethanol: Maxima at 2430 (ε = 23,600), 2510 (ε = 28,000) and 2600 Å (ε = 17,400).

Found: C,82.1; H,10.8.

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

There was no depression in melting point on admixture with authentic β-amyradienyl-II acetate.

A second crop of plates (0.1 g.), m.p.222-225°, giving a red-brown colour with T.N.M. in chloroform and showing no depression in melting point on admixture with β-amyradienyl-II acetate was obtained by concentration of the mother liquors of the first crop.

Lithium Aluminium Hydride Reduction of iso-β-Amyradienonyl Acetate.- A solution of iso-β-amyradienonyl acetate (m.p.220-221°, 0.4 g.) in dry ether (50 c.c.) was added dropwise to lithium aluminium hydride (0.3 g.) in dry ether (40 c.c.). The mixture was refluxed for four hours, treated with water, acidified with dilute sulphuric acid and ether extracted. The ethereal solution was washed with water, dried (Na_2SO_4) and evaporated. The product was acetylated by heating with pyridine (3 c.c.) and acetic anhydride (3 c.c.) on the steam-bath for three hours and the solution was worked up in the usual manner. Crystallisation of the product from methanol gave a

diacetate as square plates (0.36 g.), m.p.167-168°, $[\alpha]_D$ +25° (c, 1.0) which produced a yellow colour with T.N.M. in chloroform. Light absorption in ethanol: ε_{2900} = 9,200, ε_{2150} = 5,800, ε_{2200} = 2,300, ε_{2250} = 280.

Found: C,78.0; H,10.05.

$C_{34}H_{52}O_4$ requires: C,77.8; H,10.0%.

Treatment of iso-β-Amyradienonyl Acetate with Sodium Methoxide.- iso-β-Amyradienonyl acetate (m.p.221-222°, 1 g.) was heated with sodium methoxide (1.25 g. sodium in 15 c.c. methanol) for 6 hours at 200°. The crude product was treated with water and ether, and the extract was washed with water, dried (Na_2SO_4) and evaporated. The residue was heated on the steam-bath for one hour with pyridine and acetic anhydride and the solution was worked up in the usual manner to yield a residue (0.95 g.) which was dissolved in light petroleum (60-80°) (50 c.c.) and chromatographed on a column of activated alumina (Grade II; 18 x 2 cm.).

Fractions	Eluant	Volume	Weight	
1-18	L.P.	900 c.c.	486 mg.	Solid from MeOH
19-22	L.P./B.(9:1)	200 c.c.	82 mg.	Gum.
23-24	" (4:1)	100 c.c.	58 mg.	"
25-29	" (3:2)	250 c.c.	121 mg.	Solid from MeOH
30-31	" (2:3)	100 c.c.	28 mg.	Gum.
32-36	Benzene	250 c.c.	88 mg.	Solid from MeOH
37-38	B/E(3:2)	100 c.c.	19 mg.	Gum

Fractions (1-18) and (25-27) were combined and crystallised from methanol as square plates (400 mg.), m.p.165--167°, $[\alpha]_D$ +24° (c, 1.4) showing a yellow colour with T.N.M. in chloroform. Light absorption in ethanol: $\varepsilon_{2090} = 9,000$, $\varepsilon_{2150} = 5,200$, $\varepsilon_{2200} = 1,400$, $\varepsilon_{2250} = 320$. There was no depression in melting point on admixture with the product of the lithium aluminium hydride reduction of iso-β-amyradienonyl acetate. Fractions (32-36) were combined and acetylated on the steam-bath with acetic anhydride and pyridine to yield square plates from methanol (55 mg.), m.p.163-165°, $[\alpha]_D$ +22° (c, 0.5) giving a yellow colour with T.N.M. in chloroform. Admixture with the product obtained from fractions (1-18) and (25-27) produced no depression in melting point.

Attempted Zinc/Acetic Acid Reduction of iso-β-Amyradienonyl Acetate.- iso-β-Amyradienonyl acetate (m.p.220--222°, 1 g.) in stabilised acetic acid (100 c.c.) was treated with freshly activated zinc (1.8 g.) and the solution was refluxed for two hours. A further quantity of zinc (1 g.) was then added and refluxing was continued for a further four hours. The solution was filtered, poured into water and extracted with ether. The residue from the ether solution crystallised from chloroform/

/methanol as prisms (0.55 g.), m.p.219-222°, giving a yellow colour with T.N.M. in chloroform. Light absorption in ethanol: Maxima at 2090 (ε = 3,700) and 2440 Å (ε = 10,500). There was no depression in melting point on admixture with starting material. Concentration of the mother liquors yielded prisms (0.3 g.), m.p.217-220° and a third crop of prisms (0.08 g.) m.p.190-210°. Both these crops gave a yellow colour with T.N.M. in chloroform and were undepressed in melting point on admixture with starting material.

iso-β-Amyradiendionyl Acetate.- Following the method of Jeger and Ruzicka (99), iso-β-amyradienonyl acetate (m.p.221-222°, 2.4 g.) was heated in a sealed tube with dioxan (160 c.c.) and selenium dioxide (8 g.) for 24 hours at 200°. The solution was treated with water and ether, and the ether extract was washed with potassium cyanide solution (10%), water, dried (Na_2SO_4) and evaporated. The residue was dissolved in light petroleum (60-80°)-benzene (1:1, 100 c.c.) and filtered through a short column of activated alumina (Grade II, 30 g.). The residue crystallised from chloroform/methanol to give needles of iso-β-amyradiendionyl acetate (0.7 g.), m.p. 256-259°. Concentration of the mother liquors yielded a second crop of needles (0.14 g.), m.p.247-257°.

Repeated recrystallisation of the first crop gave needles, m.p.261.5-262° (K), $[\alpha]_D$ -122° (c, 1.2), which produced no colour with T.N.M. in chloroform. Light absorption in ethanol: Maximum at 2520 Å (ε = 17,300).

Found: C,77.7; H,9.4.

Calc. for $C_{32}H_{46}O_4$: C,77.5; H,9.5%.

Treatment of iso-β-Amyradienonyl Acetate with Ethanolic Caustic Potash Solution.- iso-β-Amyradienonyl acetate (m.p.218-221°, 0.5 g.) was refluxed for 40 hours in ethanol potassium hydroxide solution (18 g. KOH, 20 c.c. water, 100 c.c. ethanol). The solution was treated with water and ether and worked up in the usual manner to yield a residue which was acetylated with acetic anhydride and pyridine on the steam bath. The acetylated product crystallised from chloroform/methanol as prismatic needles (0.38 g.), m.p.217-220°. Two recrystallisations from the same solvent yielded prisms, m.p.220-221°, $[\alpha]_D$ -36° (c, 3.1), giving a yellow colour with T.N.M. in chloroform and showing no depression in melting point when mixed with starting material.

Concentration of the mother liquors yielded amorphous material (0.06 g.).

Neo-β-Amyrin Acetate.- (a) From iso-β-Amyradienonyl Acetate. A solution of iso-β-amyradienonyl acetate (m.p.220-221°, 1 g.) in stabilised glacial acetic acid (150 c.c.) was added to a suspension of platinum (from 0.5 g. PtO_2) in glacial acetic acid (10 c.c.) and the solution was shaken in hydrogen for 48 hours with an apparent absorption of approx. 3 mols. of hydrogen. The solution was filtered and treated with water and ether. The extract was washed with sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated. The residue crystallised from chloroform-methanol as blades (0.6 g.), m.p.222-224°. After three recrystallisations from the same solvent, neo-β-amyrin acetate was obtained as blades, m.p.225-226°, $[\alpha]_D$ +5° (c, 2.0) showing an orange-red colour with T.N.M. in chloroform. Light absorption in ethanol: Maximum at 2140 Å (ε = 3,500).

Found: C,82.0; H,11.2.

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

(b) From iso-β-Amyradiendionyl Acetate. A solution of iso-β-amyradiendionyl acetate (m.p.261-262°, 0.2 g.) in stabilised glacial acetic acid (50 c.c.) was added to a suspension of platinum (from 0.2 g. of PtO_2) in acetic acid (10 c.c.) and the mixture was shaken in hydrogen at room temperature for 12 hours. The solution was

filtered, treated with water and ether, and the ether extract was washed with sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated. The residue was dissolved in light petroleum (60-80°, 50 c.c.) and chromatographed on a column of activated alumina (Grade II, 12 x 1.5 cm.). Elution with light petroleum (60--80°)-benzene (9:1; 100 c.c.) gave a major fraction I (0.093 g.) and further elution with the same eluant gave a fraction II (0.044 g.). Fraction I crystallised from chloroform-methanol as blades, m.p.222-224°. Three recrystallisations from the same solvent yielded blades, m.p.224-225° (K), $[\alpha]_D$ +5° (c, 1.5) showing an orange-red colour with T.N.M. in chloroform. Light absorption in ethanol: $\varepsilon_{2110} = 5,000$, $\varepsilon_{2150} = 4450$, $\varepsilon_{2200} = 3,100$, $\varepsilon_{2250} = 1,800$, $\varepsilon_{2300} = 550$. No depression in melting point on admixture with neo-β-amyrin acetate obtained by the catalytic hydrogenation of iso-β-amyradienonyl acetate.

Found: C,81.8; H,11.2.

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

(c) From the Oxide of iso-β-Amyradienonyl Acetate. A solution of the oxide of iso-β-amyradienonyl acetate (m.p.279-281°, 1 g.) in stabilised glacial acetic acid (200 c.c.) was added to a suspension of platinum (from

0.5 g. of PtO_2) in glacial acetic acid (10 c.c.) and the mixture was shaken in hydrogen at room temperature for 40 hours. The solution was worked up in the manner described in the previous experiment to yield a residue which crystallised from chloroform-methanol as needles (0.35 g.), m.p.215-221°. Concentration of the mother liquors yielded a second crop of needles (0.2 g.), m.p. 209-214°. Both crops gave an orange-red colour with T.N.M. in chloroform. Six recrystallisations of the first crop from chloroform-methanol produced blades, m.p.226-227°, $[\alpha]_D$ +5° (c, 2.0). Light absorption in ethanol: Maximum at 2140 Å (ε = 3,600). There was no depression in melting point with a sample of neo-β-amyrin acetate prepared by the above two methods.

Found: C,81.9; H,11.5.

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

Chromic Acid Oxidation of neo-β-Amyrin Acetate.- neo-β--Amyrin acetate (m.p.223-225°, 0.55 g.) in stabilised acetic acid (50 c.c.) was treated at 100° with a solution of chromic oxide (0.25 g.; 2.5 atoms O) in stabilised acetic acid (80 c.c.) and water (5 c.c.), added dropwise during 30 minutes. The solution was heated on the steam--bath for 90 minutes and treated with methanol, water and filtered. The residue was dissolved in ether and washed

with an aqueous solution of sodium hydroxide (50 c.c., 5%). Acidification of the alkaline solution produced no acid fraction. The ether extract was then washed with water, dried (Na_2SO_4), and evaporated to yield a residue which was dissolved in light petroleum (60-80°)--benzene (5:2; 70 c.c.) and chromatographed on a column of activated alumina (Grade II, 15 x 2 cm.). Elution with light petroleum (60-80°)-benzene (3:2; 200 c.c.) gave a main fraction (0.175 g.) which crystallised from methanol as prismatic needles, m.p.203-205° and after five recrystallisations from the same solvent, m.p.203.5--204.5° (K), $[\alpha]_D$ -4° (c, 1.0), giving no colour with T.N.M. in chloroform. There was no light absorption in the region 2000-4000 Å.

Found: C,79.4; H,10.9.

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

Oxidation of neo-β-Amyrin Acetate with Potassium Permanganate.- neo-β-Amyrin acetate (m.p.225-227°, 0.4 g.) in stabilised glacial acetic acid (150 c.c.) was treated with a solution of potassium permanganate (100 mg. $KMnO_4$ in 25 c.c. of acetic acid) in acetic acid, added dropwise during 30 minutes at room temperature. The solution was stirred for one hour and the excess permanganate was destroyed with 10% bisulphite solution. The solution was

then worked up through water and ether in the usual manner to yield a residue which crystallised from methanol as prisms (0.22 g.), m.p.197-204°. Concentration of the mother liquors yielded a second crop of prisms (0.05 g.), m.p.190-202°. Five recrystallisations of the first crop yielded prisms, m.p.207-208°, $[\alpha]_D$ -6° (c, 1.5), showing no light absorption in the region 2000--4000 Å and no colour with T.N.M. in chloroform.

Found: C,79.3; H,11.2.

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

Admixture with the chromic acid oxidation product of neo-β-amyrin acetate produced no depression in melting point.

Treatment of neo-β-Amyrin Acetate with N-Bromosuccinimide.- neo-β-Amyrin acetate (0.3 g.) in dry carbon tetrachloride (50 c.c.) was treated with N-bromosuccinimide (1 g.; 95%) and the solution was refluxed gently for one hour. The solution became red in colour immediately after the addition of the N-bromosuccinimide and hydrogen bromide was evolved during the reaction. After being filtered, washed with water, and dried (Na_2SO_4), the solution was evaporated under vacuum to yield a brown gum which was dissolved in light petroleum (60-80°, 50 c.c.) and

chromatographed on a column of activated alumina (Grade II, 14 x 1.75 cm.). Elution with light-petroleum (60--80°)-benzene (4:1; 250 c.c.) produced a main fraction (0.25 g.) which was deposited from aqueous methanol as a semi-crystalline material which was extremely soluble in all the normal crystallisation reagents. After two such crystallisations the material had m.p.205-210°, $[\alpha]_D$ -28° (±2) (c, 0.9) and showed a deep red-brown colour with T.N.M. in chloroform. Light absorption in ethanol: inflection at 2250 Å, maxima at 2520 (ε = 13,000), 2600 (ε = 12,500) and 3570 Å (ε = 10,000).

Attempted Catalytic Hydrogenation of iso-β-Amyradienonyl Acetate in Neutral Solution.- iso-β-Amyradienonyl acetate (m.p.219-221°, 0.25 g.) in ethyl acetate (250 c.c.) was added to platinum catalyst (from 100 mg. PtO_2) in ethyl acetate (25 c.c.) and shaking in hydrogen was carried out for 12 hours. Platinum oxide (100 mg.) was then added to the solution and shaking in hydrogen was carried out for a further 60 hours. The solution was filtered, evaporated to dryness, and the residue crystallised from methanol as plates (0.19 g.), m.p.212-215°. Recrystallisation from methanol yielded prismatic rods, m.p.218-221°, $[\alpha]_D$ -36° (c, 1.2) giving a yellow colour with T.N.M. in

chloroform. Light absorption in ethanol: Maxima at 2090 (ε = 3,100) and 2450 Å (ε = 10,000).

There was no depression in melting point on admixture with starting material.

Concentration of the mother liquors yielded a further crop of iso-β-amyradienonyl acetate (0.04 g.), m.p.210-215°.

Wolff-Kishner Reduction of iso-β-Amyradienonyl Acetate.- iso-β-Amyradienonyl acetate (m.p.221-222°, 2 g.) was heated with sodium methoxide (2 g. sodium in 25 c.c. methanol) and hydrazine hydrate (10 c.c., 100%) in an autoclave at 200° for 13 hours. The crude product was acetylated with pyridine (20 c.c.) and acetic anhydride (20 c.c.) on the steam-bath for 2 hours and the solution was worked up in the usual manner to yield a solid residue. The residue crystallised from chloroform--methanol as plates (1.0 g.), m.p.228-231°. Four recrystallisations from the same solvent yielded plates, m.p.230.5-231°, $[\alpha]_D$ -9° (c, 2.0) giving a yellow colour with T.N.M. in chloroform. Light absorption in ethanol: ε_{2180} = 4,970, ε_{2150} = 4,180, ε_{2200} = 1,700, ε_{2250} = 320.

Found: C,82.2; H,10.9.

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

Concentration of the mother liquors yielded a second crop of plates (0.5 g.), m.p.175-195° which after two recrystallisations yielded plates (0.16 g.), m.p.230-231° showing no depression in melting point on admixture with the first crop. Concentration of the mother liquors of the second crop yielded plates (0.27 g.), m.p.180-186° which, after five recrystallisations from the same solvent, yielded a second compound as plates, m.p.202-203°, $[\alpha]_D$ -95°, -93° (c, 1.0, 0.75) giving a yellow colour with T.N.M. in chloroform. Light absorption in ethanol: $\varepsilon_{2100} = 5,900$, $\varepsilon_{2150} = 3,700$, $\varepsilon_{2200} = 1,460$, $\varepsilon_{2250} = 430$.

Found: C,82.6; H,10.8.

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

Hydrolysis of the $C_{32}H_{50}O_2$ Acetate (m.p.230°) from the Wolff-Kishner Reduction of iso-β-Amyradienonyl Acetate.- The acetate (m.p.230-231°, 0.2 g.) was refluxed in ethanolic potassium hydroxide solution (3%, 15 c.c.) for 3 hours and the solution was poured into water and worked up through ether in the usual way. The residue crystallised from methanol as needles, m.p.193-195°. Four recrystallisations from the same solvent yielded needles, m.p.195-196°, $[\alpha]_D$ -12°, -12° (c, 0.8, 0.8) giving a yellow colour with T.N.M. in chloroform. Light absorption

in ethanol: Maximum at 2110 Å (ε = 4,500).

Found: C,84.8; H,11.4.

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

The above alcohol (m.p.195-196°, 100 mg.) was acetylated on the steam-bath with pyridine (3 c.c.) and acetic anhydride (3 c.c.) and the solution was worked up in the usual manner to yield plates from chloroform/methanol, m.p.229-231°. Four recrystallisations from the same solvent yielded plates, m.p.230.5-231°, $[\alpha]_D$ -8° (c,2.5). This material showed no depression in melting point on admixture with the $C_{32}H_{50}O_2$ acetate (m.p.230°) from which the reacetylated alcohol was obtained.

Treatment of the $C_{32}H_{50}O_2$ Acetate (m.p.230°) with Hydrochloric Acid.- The acetate (m.p.230°, 0.15 g.) in stabilised glacial acetic acid (25 c.c.) and concentrated hydrochloric acid (1 c.c.) was heated on the steam-bath for four hours. The solution was treated with water and ether, and the ether extract was washed with sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated. The residue was heated with pyridine (5 c.c.) and acetic anhydride (5 c.c.) on the steam-bath for one hour and the solution was worked up in the usual manner to yield a residue which crystallised from chloroform/methanol as needles, m.p.214-217°. Five recrystallis-

ations from the same solvent yielded fine needles, m.p.219.5-220°, $[\alpha]_D$ +337° (c, 1.5), giving a brown colour with T.N.M. in chloroform. Light absorption in ethanol: Maximum at 2820 Å (ε = 9,000).

Found: C,82.6; H,11.1.

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

Admixture of this product with β-amyradienyl-I acetate produced no depression in melting point.

Treatment of the $C_{32}H_{50}O_2$ Acetate (m.p.203°) with Hydrochloric Acid.- The acetate (m.p.203°, 0.06 g.) in stabilised glacial acetic acid (50 c.c.) and concentrated hydrochloric acid (1 c.c.) was heated on the steam-bath for seven hours. The yellow solution was poured into water, ether extracted, and the ether extract was washed with sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated to yield a solid residue which gave a red-brown colour with T.N.M. in chloroform and showed light absorption maxima at 2430, 2500 and 2600 Å. Crystallisation from chloroform/methanol yielded plates (0.035 g.) m.p.223-255°. Two recrystallisations from the same solvent yielded plates, m.p.227-228°, $[\alpha]_D$ -62° (c, 0.6) giving a red-brown colour with T.N.M. in chloroform. Light absorption in ethanol: Maxima at 2420 (ε = 22,000), 2510 (ε = 27,200) and 2600 Å (ε = 17,000). There was no

depression in melting point on admixture with β-amyradienyl-II acetate.

Selenium Dioxide Oxidation of the $C_{32}H_{52}O_2$ Acetate (m.p.230°).- The acetate (m.p.230-231°, 0.5 g.) in stabilised glacial acetic acid (100 c.c.) was refluxed with selenium dioxide (0.5 g.) for 24 hours. The deep red solution was filtered, poured into water, ether extracted, and the extract was washed with sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated. The residue was dissolved in benzene and filtered through a short column of activated alumina. The residue obtained on evaporation of the benzene crystallised from chloroform-methanol as plates (0.05 g.) m.p.225-227°. Two recrystallisations from the same solvent yielded plates, m.p.230-231°, undepressed in melting point on admixture with starting material.

The mother liquors were evaporated to dryness and the residue crystallised from light-petroleum (60-80°) as prisms (0.2 g.), m.p.239-242°. Two recrystallisations from aqueous methanol yielded square plates, m.p.240-241°, $[\alpha]_D$ -91° (c, 1.5) giving no colour with T.N.M. in chloroform. Light absorption in ethanol: Maximum at 2780 Å (ε = 11,700). Admixture of this product with

β-amyradiendionyl acetate produced no depression in melting point.

Oxidation of the $C_{32}H_{50}O_2$ Acetate (m.p.230°) with Perbenzoic Acid.- The acetate (m.p.228-230°, 0.75 g.) in chloroform (35 c.c.) was treated with perbenzoic acid in chloroform (4.6 c.c., 1.3 mols.) at 0° and the solution was kept at 0° for four days. It was then washed with sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated to yield a gum which was dissolved in light petroleum (60-80°)-benzene (3:1, 120 c.c.) and chromatographed on a column of activated alumina (Grade I, 14 x 2 cm.).

Fractions	Eluant	Volume	Weight	
1-10	LP/B (3:1)	500 c.c.	197 mg.	Gum
11-13	" (9:4)	150	Trace	Gum
14-16	" (3:2)	150	65 mg.	Solid
17-19	" (9:7)	150	53	"
20-28	" (1:1)	450	119	"
29-30	" "	100	9	"
31-33	" (2:3)	150	12	"
34-35	" (1:4)	100	17	"
36-38	Benzene	150	14	"
39-40	B/E (9:1)	100	110	Gum
41-44	" (3:2)	200	30	"
45-46	Ether	100	—	
47-50	Acetone	200	—	

Fractions 14-38 were combined and crystallised from light petroleum (60-80°) to give plates, m.p.257-260° giving a red-brown colour with T.N.M. in chloroform. Five

recrystallisations from aqueous methanol gave plates, m.p.270-270.5°, $[\alpha]_D$ +293° (c, 0.5) giving a brown colour with T.N.M. in chloroform. Light absorption in ethanol: Maximum at 2820 Å (ε = 9,100).

Found: C,79.6; H,10.5.

$C_{52}H_{50}O_3$ requires: C,79.6; H,10.4%.

Chromic Acid Oxidation of the Perbenzoic Acid Product of the $C_{52}H_{50}O_2$ Acetate (m.p.230°).- The perbenzoic acid oxidation product of the acetate (m.p.230°) (m.p.269-271°, 0.1 g.) in stabilised glacial acetic acid (50 c.c.) was treated at room temperature with a solution of chromic oxide in acetic acid (12.5 c.c.; 15.4 mg. CrO_3; 1.1 atoms of O) added dropwise during 30 minutes. After standing at room temperature for two hours the green solution was poured into water, extracted with ether, and the extract was washed with sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated. The residue crystallised from aqueous methanol as prismatic needles (0.033 g.), m.p. 246-248°, giving a red-brown colour with T.N.M. in chloroform. Six recrystallisations from the same solvent yielded prismatic needles, m.p.249-250°, $[\alpha]_D$ +252°, +253° (c, 0.5, 0.6) giving a red-brown colour with T.N.M. in chloroform. Light absorption in ethanol: Maximum at 2820 Å (ε = 8,700).

Found: C,79.9; H,10.3.

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

Attempted Catalytic Hydrogenation of the $C_{32}H_{50}O_2$ Acetate (m.p.230°).- The acetate (m.p.229-230°, 0.25 g.) in stabilised glacial acetic acid (150 c.c.) was added to a suspension of platinum (from 0.5 g. PtO_2) in acetic acid (20 c.c.) and shaking in hydrogen was carried out for 48 hours. The solution was filtered and evaporated to dryness. The residue crystallised from chloroform/ /methanol as plates (0.19 g.), m.p.226-228°, $[\alpha]_D$ -5° (c, 1.5) giving a yellow colour with T.N.M. in chloroform. Concentration of the mother liquors yielded a second crop of plates (0.03 g.), m.p.224-227° giving a yellow colour with T.N.M. in chloroform. Neither crop was depressed in melting point on admixture with starting material.

Attempted Catalytic Hydrogenation of the $C_{32}H_{50}O_2$ Acetate (m.p.203°) from the Wolff-Kishner Reduction of iso-β--Amyradienonyl Acetate.- The acetate (m.p.203-204°, 0.15 g.) in stabilised glacial acetic acid (100 c.c.) was added to a suspension of platinum (from 0.25 g. of PtO_2) in acetic acid (10 c.c.) and shaking in hydrogen was carried out at room temperature for 48 hours. The solution was filtered and evaporated to dryness to yield a residue which

crystallised from chloroform/methanol as blades (0.1 g.), m.p.200-202°, $[\alpha]_D$ -89° (c, 1.0) which produced a yellow colour with T.N M. in chloroform. Concentration of the mother liquors produced a second crop as blades (0.025 g.), m.p.197-202° giving a yellow colour with T.N.M. in chloroform.

Neither crop was depressed in melting point on admixture with starting material.

Oxide of iso-β-Amyradienonyl Acetate.- (a) iso-β-Amyradienonyl acetate (m.p.221-222°, 1 g.) in dry chloroform (10 c.c.) at 0° was treated with a solution of perbenzoic acid in chloroform (9 c.c.; 1.2 mols.) also at 0°. The solution was kept at this temperature for 8 days, treated with ether (100 c.c.) and washed with sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated. The residue crystallised from chloroform/methanol as plates (0.64 g.), m.p.277-279° giving no colour with T.N.M. in chloroform. Four recrystallisations from the same solvent yielded plates, m.p.280-281° (K), $[\alpha]_D$ -12° (c,4.9) giving no colour with T.N.M. in chloroform. Light absorption in ethanol: Maximum at 2400 Å (ε = 10,800).

Found: C,77.3; H,9.9.

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

Concentration of the mother liquors produced a second crop

of plates (0.1 g.), m.p.273-276°, showing no colour with T.N.M. in chloroform and undepressed in melting point on admixture with the first crop.

(b) iso-β-Amyradienonyl acetate (m.p.221-222°, 1 g.) in stabilised glacial acetic acid (150 c.c.) was treated with a solution of potassium permanganate (0.75 g.) in water (75 c.c.) added dropwise with stirring at room temperature during 30 minutes. Stirring was continued for two hours and the solution was treated with sodium metabisulphite solution (10%), water and ether. The extract was washed with sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated. The residue crystallised from methanol as rhombic plates (0.95 g.), m.p.276-280°, producing no colour with T.N.M. in chloroform. Four recrystallisations from the same solvent yielded rhombic plates, m.p.281-282°, $[\alpha]_D$ -12.5° (c,2.1). Light absorption in ethanol: Maximum at 2420 Å (ε = 10,500). Admixture with the product from the perbenzoic acid oxidation of iso-β-amyradienonyl acetate produced no depression in melting point.

(c) iso-β-Amyradienonyl acetate (m.p.221-222°, 1 g.) in stabilised glacial acetic acid (80 c.c.) at 60° was treated with a solution of chromic oxide in stabilised glacial acetic acid (16 c.c., 1.5 atoms O) added dropwise

with stirring at 60° during one hour. Stirring was continued for a further 30 minutes and the solution was poured into water and extracted with ether. The extract was washed with sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated, to yield rhombic plates (0.56 g.), m.p.270-274° from chloroform/methanol. Concentration of the mother liquors yielded plates (0.21 g.), m.p.257-264°, and further concentration yielded a third crop of plates (0.085 g.), m.p.225-234°. Four recrystallisations of the first crop from the same solvent gave rhombic plates, m.p.280-281°, $[\alpha]_D$ -12° (c, 1.0) showing no colour with T.N.M. in chloroform. Light absorption in ethanol: Maximum at 2400 Å (ε = 10,500). Admixture of this product with the perbenzoic acid and permanganate oxidation products of iso-β-amyradienonyl acetate produced no depression in melting point.

Oxide of iso-β-Amyradienonol.- The oxide of iso-β-amyradienonyl acetate (m.p.280-281°, 0.1 g.) was refluxed in aqueous ethanolic potassium hydroxide solution (5 c.c., 3%) for three hours. The solution was treated with water and ether, and the extract was washed with water, dried (Na_2SO_4), and evaporated. The residue crystallised from methanol as prismatic rods, m.p.244-246°. Three recrystallisations from the same solvent yielded prismatic needles,

m.p.249.5-250°, $[\alpha]_D$ -26° (c, 1.4) giving no colour with T.N.M. in chloroform. Light absorption in ethanol: Maximum at 2420 Å (ε = 14,000).

Found: C,79.15; H,10.1.

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

The alcohol obtained by the above hydrolysis (14 mg.) was heated on the steam-bath with pyridine (0.5 c.c.) and acetic anhydride (0.5 c.c.) for two hours. The solution was worked up through water and ether in the usual manner and the residue crystallised from methanol as rhombic plates, m.p.275-277°. Admixture of this material with the original acetate (oxide of iso-β-amyradienonyl acetate) produced no depression in melting point.

Oxidation of iso-β-amyradienonyl Acetate with Hydrogen Peroxide.- iso-β-Amyradienonyl acetate (m.p.220-222°, 1g.) in unstabilised glacial acetic acid (150 c.c.) was treated with a solution of hydrogen peroxide (100 vols.) in acetic acid (1:1, 200 c.c.) added dropwise during two hours at 95° with stirring. The solution was stirred at this temperature for a further three hours, poured into water, and ether extracted. The extract was washed with sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated to yield a residue which was dissolved in light petroleum (60-80°)-benzene (7:3, 100 c.c.) and chromatographed on

activated alumina (Grade II, 14 x 1.75 cm.).

Fractions	Eluant	Volume	Weight	
1-2	LP/B (7:3)	100 c.c.	57 mg.	Solid
3-6	LP/B (3:2)	200	317	"
7-12	Benzene	300	284	"
13-14	B/E (9:1)	100	31	gum
15-16	B/E (4:1)	100	46	"
17-18	B/E (1:1)	100	33	"
19-20	Ether	100	38	"
21-23	E/Methanol(1:1)	150	180	"
24	Methanol	50	34	"

Fractions 1-14 were combined (0.69 g.), dissolved in light petroleum (60-80°)-benzene (4:5, 135 c.c.) and rechromatographed on neutral activated alumina (Grade II, 18 x 2 cm.).

Fractions	Eluant	Volume	Weight	
1-6	LP/B (4:5)	300 c.c.	-	
7-12	LP/B (2:3)	300	107 mg.	Solid
13-16	LP/B (1:2)	200	27	"
17-18	LP/B (1:3)	100	Trace	
19-20	LP/B (1:4)	100	5 mg.	gum
21-25	Benzene	250	-	
26-37	B/E (9:1)	600	238 mg.	solid
38-41	B/E (1:1)	200	19 mg.	"

Fractions 7-16 were combined and crystallised from chloroform-methanol as plates (0.08 g.), m.p.213-220°, giving no colour with T.N.M. in chloroform. Five recrystallisations from the same solvent yielded plates, m.p.225-226°, $[\alpha]_D$ +78° (c, 0.7) giving no colour with T.N.M. in chloroform and no light absorption in the region 2200-4000 Å.

Found: C,74.4; H,10.2.

$C_{32}H_{52}O_5$ requires: C,74.4; H,10.1%.

$C_{32}H_{50}O_5$ requires: C,74.7; H, 9.8%.

Fractions 26-37 were combined and crystallised from methanol as plates, m.p.318-319°, $[\alpha]_D$ +155° (c, 1.3) giving no colour with T.N.M. in chloroform. Light absorption in ethanol: Maximum at 2360 Å (ε = 13,400).

Found: C,77.7; H,9.9.

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

Hydrolysis of the Acetate (m.p.318°) from the Hydrogen Peroxide Oxidation of iso-β-Amyradienonyl Acetate.- The acetate (m.p.318-319°, 0.1 g.) was refluxed in aqueous ethanolic potassium hydroxide (3%, 15 c.c.) for four hours. The solution was poured into water, treated with ether, and the extract was washed with water, dried (Na_2SO_4) and evaporated. Crystallisation of the residue from aqueous methanol, yielded needles, m.p.264-270°. Five recryst-

allisations from the same solvent yielded prisms, m.p. 290-291°, $[\alpha]_D$ +167° (c, 1.0) giving no colour with T.N.M. in chloroform. Light absorption in ethanol: Max. at 2360 Å (ε = 12,500).

Found: C,79.4; H,10.4

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

Acetylation of the Acetate (m.p.318°) from the Hydrogen Peroxide Oxidation of iso-β-Amyradienonyl Acetate.- The acetate (m.p.318-319°, 0.1 g.) in pyridine (20 c.c.) and acetic anhydride (10 c.c.) was heated on the steam-bath for two hours. The solution was worked up through water and ether in the usual manner to yield a residue which crystallised from aqueous methanol as needles (0.08 g.), m.p.190-193°. Four recrystallisations from the same solvent yielded needles, m.p.194-194.5°, $[\alpha]_D$ +141° (c,0.6) giving no colour with T.N.M. in chloroform. Light absorption in ethanol: Max. at 2360 Å (ε = 11,500).

Found: C,75.7; H,9.6

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

Chromic Acid Oxidation of the Acetate (m.p.318°) from the Hydrogen Peroxide Oxidation of iso-β-Amyradienonyl Acetate.- The acetate (m.p.318°, 0.12 g.) in stabilised glacial acetic acid (40 c.c.) was treated with a solution of chromic anhydride in water and acetic acid (10 c.c.,

1.3 atoms of O) added dropwise at room temperature during 30 minutes. The solution was allowed to stand overnight at room temperature, and then heated on the steam-bath for 30 minutes. It was poured into water, treated with ether, and the extract was washed with sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated. The residue crystallised from chloroform--methanol as needles (0.09 g.), m.p.308-314°. Five recrystallisations from the same solvent yielded needles, m.p.314-315°, $[\alpha]_D$ +94° (c, 1.0) giving no colour with T.N.M. in chloroform. Light absorption in ethanol: Max. at 2360 Å (ε = 13,000).

Found: C,78.0; H,9.6.

$C_{32}H_{46}O_4$ requires: C,77.7; H,9.4%.

Attempted Acetylation of the Acetate (m.p.226°) from the Hydrogen Peroxide Oxidation of iso-β-Amyradienonyl Acetate.- The acetate (m.p.226-228°, 0.15 g.) in pyridine (5 c.c.) and acetic anhydride (5 c.c.) was heated on the steam-bath for two hours. The solution was worked up in the usual way to yield a residue which crystallised from chloroform-methanol as plates (0.13 g.), m.p.227-229°, $[\alpha]_D$ +75° (c, 1.5) giving no colour with T.N.M. in chloroform. Concentration of the mother liquors yielded a second crop of plates (0.015 g.), m.p.215-221°, giving no

colour with T.N.M. in chloroform. Neither crop was depressed in melting point on admixture with the starting material.

Oxidation of iso-β-Amyradienonyl Acetate with Chromic Acid.- A gently refluxing solution of iso-β-amyradienonyl acetate (m.p.221-222°, 5 g.) in stabilised glacial acetic acid (400 c.c.) was treated with a solution of chromic oxide (5 g.) in stabilised glacial acetic acid (150 c.c.) added dropwise during one hour. The solution was then refluxed for a further two hours, poured into water and ether extracted. The extract was washed with sodium hydroxide solution (5%), water, dried (Na_2SO_4) and evaporated. Acidification of the alkali washings yielded an acid fraction (0.26 g.) which was not investigated further. The residue obtained by evaporation of the ether, crystallised from chloroform-methanol as needles (2.1 g.), m.p.293-298° Concentration yielded a second crop (0.2 g.), m.p.267-275°. Five recrystallisations of the first crop from the same solvent yielded needles, m.p.315-316°, $[\alpha]_D$ +57° (c, 2.0) giving no colour with T.N.M. in chloroform. Light absorption in ethanol: Max. at 2360 Å (ε = 12,000). Admixture with the product from the chromic acid oxidation of the acetate (m.p.318°) from the hydrogen peroxide oxidation of iso-β-amyradienonyl

acetate produced no depression in melting point.

Chromic Acid Oxidation of the Oxide of iso-β-Amyradienonyl Acetate.- (a) The oxide of iso-β-amyradienonyl acetate (m.p.278-280°, 0.5 g.) in stabilised glacial acetic acid (100 c.c.) at 80° was treated with a solution of chromic oxide (0.1 g., 1.5 mols.) in stabilised glacial acetic acid (20 c.c.) and a few drops of water, added dropwise with stirring during two hours. The solution was then stirred at 80° for a further 30 minutes, poured into water and ether extracted. The extract was washed with sodium hydroxide solution (3%) (acidification produced no acid fraction), water, dried (Na_2SO_4) and evaporated. The residue crystallised from chloroform--methanol as a mixture of plates and needles (0.35 g.), $[\alpha]_D$ +33° (c, 1.0). Concentration produced a second crop of mixed plates and needles (0.055 g.).
(b) The oxide of iso-β-amyradienonyl acetate (m.p.278--280°, 0.5 g.) in stabilised glacial acetic acid (100 c.c.) at 80° was treated with a solution of chromic oxide in acetic acid and water (22 c.c., 2.5 mols.) added dropwise during four hours with stirring. The solution was then stirred at 80° for a further one hour, poured into water and ether extracted. The extract was worked up in the usual manner to yield a residue which crystallised from

chloroform-methanol as needles (0.35 g.), m.p.313-316°, $[\alpha]_D$ +58° (c, 1.0) giving no colour with T.N.M. in chloroform. Light absorption in ethanol: Max. at 2360 Å (ε = 11,900). Admixture with the chromic acid oxidation product of iso-β-amyradienonyl acetate produced no depression in melting point.

Alkaline Hydrolysis of the Chromic Acid Oxidation Product of iso-β-Amyradienonyl Acetate.- The chromic acid oxidation product (m.p.312-314°, 0.15 g.) was refluxed in aqueous ethanolic potassium hydroxide solution (3%, 15 c.c.) for three hours. The solution was poured into water, treated with ether, and the extract was washed with water, dried (Na_2SO_4) and evaporated. The residue crystallised from chloroform-methanol as long needles, m.p.293-297°. Four recrystallisations from methanol yielded long needles, m.p.298-299°, $[\alpha]_D$ +71° (c, 0.5) giving no colour with T.N.M. in chloroform. Light absorption in ethanol: Max. at 2360 Å (ε = 13,000).

Found: C,79.8; H,9.9.

$C_{30}H_{44}O_3$ requires: C,79.6; H,9.8%.

Acetylation.- The above alcohol (m.p.297-298°, 0.05 g.) in pyridine (2 c.c.) and acetic anhydride (2 c.c.) was heated on the steam-bath for one hour. The solution, which was worked up in the usual manner yielded a residue

which crystallised from chloroform-methanol as needles, m.p.311-313°. Three recrystallisations from the same solvent yielded needles, m.p.313-314°, $[\alpha]_D$ +90° (c,0.06) giving no colour with T.N.M. in chloroform. Light absorption in ethanol: Max. at 2360 Å (ε = 13,000). There was no depression in melting point on admixture with the chromic acid oxidation products ($[\alpha]_D$ +57°) of iso-β-amyradienonyl acetate and the oxide of iso-β-amyradienonyl acetate nor the chromic acid oxidation product ($[\alpha]_D$ +94°) of the acetate (m.p.318°) from the hydrogen peroxide oxidation of iso-β-amyradienonyl acetate.

Acid Hydrolysis of the Chromic Acid Oxidation Product of iso-β-Amyradienonyl Acetate.- A solution of the chromic acid oxidation product (m.p.312-314°, $[\alpha]_D$ +58°, 0.25 g.) in ethanol (60 c.c.) and concentrated hydrochloric acid (8 c.c.) was refluxed for three hours, poured into water and ether extracted. The extract was washed with sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated. The residue crystallised from chloroform-methanol as needles, m.p.296-300°. Three recrystallisations from the same solvent yielded long needles, m.p.298-299°, $[\alpha]_D$ +71° (c, 0.7) giving no colour with T.N.M. in chloroform. Light absorption in ethanol: Max. at 2360 Å (ε = 12,200). Admixture with the alcohol from the alkaline hydrolysis

of the chromic acid oxidation product of iso-β-amyradienonyl acetate produced no depression in melting point.

Wolff-Kishner Reduction of the Chromic Acid Oxidation Product ($[\alpha]_D$ +90°) of iso-β-Amyradienonyl Acetate.- Chromic acid oxidation product (m.p.313-314°, $[\alpha]_D$ +90°, 1 g.) was heated with sodium methoxide (30 c.c. methanol; 2.5 g. sodium) and hydrazine hydrate (100%, 10 c.c.) in an autoclave at 200° for 16 hours. The crude product was treated with water and ether, and the extract was washed with water, dried (Na_2SO_4) and evaporated. The residue was heated with pyridine (10 c.c.) and acetic anhydride (10 c.c.) on the steam-bath for one hour and the solution was worked up in the usual manner. The residual gum was dissolved in light petroleum (60-80°, 50 c.c.) and chromatographed on a column of activated alumina (Grade II/III, 14 x 2 cm.).

Fraction	Eluant	Volume	Weight	
1-7	LP (60-80°)	350 c.c.	-	
8-11	LP/B (9:1)	200	47 mg.	solid
12-15	" (17:3)	200	127	"
16-17	" (3:1)	100	81	"
18-24	" (1:1)	350	324	"

Further elution with benzene yielded gum.

Fractions (10-22) were combined and crystallised from chloroform-methanol to yield plates and prisms (0.26 g.), m.p.223-235°.

Five recrystallisations from methanol yielded plates, m.p.227-229°, $[\alpha]_D$ -2.5°, -2° (c, 2.0, 2.8) giving a yellow colour with T.N.M. in chloroform. Light absorption in ethanol: ε_{2090} = 3,400, ε_{2150} = 2,000, ε_{2200} = 1,100, ε_{2250} = 550.

Found: C,79.6; H,10.1.

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

Treatment of the Wolff-Kishner Product of the Chromic Acid Oxidation Product of iso-β-Amyradienonyl Acetate with Hydrochloric Acid.- The Wolff-Kishner product (m.p.225-227°, 0.05 g.) in stabilised glacial acetic acid (25 c.c.) and concentrated hydrochloric acid (3.5 c.c.) was heated on the steam-bath for 3 hours. On working up through water and ether in the usual manner the solution yielded a residue which was dissolved in pyridine (2 c.c.) and acetic anhydride (1 c.c.) and the solution was heated on the steam-bath for one hour. On working up in the usual manner, a residue which crystallised from methanol as plates (0.038 g.), m.p.221-224° was obtained. Recrystallisation from the same solvent yielded plates, m.p.223-225°, $[\alpha]_D$ -5° (c, 0.5) giving a yellow colour with T.N.M.

in chloroform.

Admixture with starting material produced no depression in melting point.

Concentration of the mother liquors gave an uncrystallisable brown gum.

Treatment of the Chromic Acid Oxidation Product of iso-β-Amyradienonyl Acetate with Sulphuric Acid.- The chromic acid oxidation product (m.p.313-314°, $[\alpha]_D$ +90°, 0.25 g.) in acetic acid (200 c.c.) and sulphuric acid (16N, 15 c.c.) was heated on the steam-bath for 6 hours. The yellow solution was treated with water and ether, and the extract was washed with sodium bicarbomate solution, water, dried (Na_2SO_4) and evaporated. The residue crystallised from chloroform-methanol as needles (0.16 g.), m.p.310-314°, $[\alpha]_D$ +87°. Recrystallisation from the same solvent yielded needles, m.p.312-315°, $[\alpha]_D$ +90° (c, 1.3). Light absorption in ethanol: Max. at 2380 Å (ε = 12,500). Admixture with starting material produced no depression in melting point. Concentration of the mother liquors yielded needles (0.043 g.), m.p. 308-312°, $[\alpha]_D$ +85° (c, 0.5). Admixture with starting material produced no depression in melting point.

Catalytic Hydrogenation of the Chromic Acid Oxidation Product of iso-β-Amyradienonyl Acetate.- The chromic acid oxidation product (m.p.313-315°, $[\alpha]_D$ +90°, 1.6 g.) in stabilised glacial acetic acid (250 c.c.) was added to platinum catalyst (from 0.5 g. of PtO_2) in acetic acid (20 c.c.) and shaking in hydrogen was carried out for 48 hours. The solution was then filtered and the filtrate was added to fresh platinum catalyst (from 0.25 g. of PtO_2) and shaking in hydrogen was carried out for a further 24 hours. The solution was then filtered and evaporated to dryness. The residue was dissolved in light petroleum (60-80°)-benzene (2:1, 150 c.c.) and chromatographed (alumina - Grade II/III, 14 x 2 cm.).

Fractions	Eluant	Volume	Weight	
1-8	LP/B (2:1)	400 c.c.	1.34 g.	solid
9-10	" "	100	-	
11-17	Benzene	350	0.106	solid
18-19	Ether	100	0.073	gum

Fractions (1-8) were dissolved in light petroleum (60-80°) but an attempt which was made to separate the components by rechromatography failed. Crystallisation of the combined fractions (1-8) from methanol-ether yielded plates (0.25 g.), m.p.220-230°, giving a yellow colour

with T.N.M. in chloroform. Four recrystallisations from chloroform-methanol yielded hexagonal plates, m.p.231-232°, $[\alpha]_D$ +86°, +87° (c, 1.0, 1.5) giving a yellow colour with T.N.M. in chloroform. Light absorption in ethanol: ε_{2080} = 3,000; ε_{2100} = 2,800; ε_{2150} = 1,800; ε_{2200} = 860; ε_{2250} = 320.

Found: C,82.5; H,10.8.

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

Concentration of the mother liquors yielded fine needles, (0.6 g.), m.p.164-166°. Four recrystallisations from methanol gave fine needles, m.p.168-170°, $[\alpha]_D$ +12°, +13° (c, 1.3, 1.3) giving a yellow colour with T.N.M. in chloroform. Light absorption in ethanol: ε_{2060} = 1,520, ε_{2100} = 1,070; ε_{2150} = 650, ε_{2200} = 560, ε_{2250} = 280, ε_{2300} = 140.

Found: C,81.95; H,11.4.

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

Fractions (11-17) were not investigated further.

Treatment of the $C_{32}H_{50}O_2$ Acetate (m.p.230°) from the Catalytic Hydrogenation of the Chromic Acid Oxidation Product with Hydrochloric Acid. - The acetate (m.p.227--229°, 0.065 g.) in acetic acid (35 c.c.) and concentrated hydrochloric acid (3 c.c.) was heated on the steam-bath for 5 hours. The solution was then allowed

to stand overnight at room temperature, treated with water and ether, and the extract was washed with sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated. The residue was dissolved in pyridine (5 c.c.) and acetic anhydride (3 c.c.) and heated on the steam-bath for one hour. The solution was worked up in the usual manner and the residue crystallised from chloroform-methanol as plates (0.038 g.), m.p.224-226°, giving a red-brown colour with T.N.M. in chloroform. Recrystallisation from the same solvent yielded plates, m.p.226-228°, $[\alpha]_D$ -61° (c, 0.7) giving a red-brown colour with T.N.M. in chloroform. Light absorption in ethanol: Max. at 2430 (ε = 26,000), 2510 (ε = 28,000), and 2600 Å (ε = 20,000). Admixture with a sample of β-amyradienyl-II acetate produced no depression in melting point.

Treatment of the $C_{32}H_{52}O_2$ Acetate (m.p.170°) from the Catalytic Hydrogenation with Hydrochloric Acid. - The acetate (m.p.168-170°, 0.12 g.) in acetic acid (50 c.c.) and concentrated hydrochloric acid (3 c.c.) was heated on the steam-bath for 4 hours. The solution was worked up in the usual manner to yield a residue which was dissolved in pyridine (2 c.c.) and acetic anhydride (2 c.c.) and heated on the steam-bath for one hour. The

residue from the acetylation crystallised from aqueous methanol as fine needles (0.026 g.), m.p.155-165°. Recrystallisation from the same solvent gave fine needles, m.p.163-167° giving a yellow colour with T.N.M. in chloroform. Admixture with the starting material produced no depression in melting point. Concentration of the mother liquors gave an uncrystallisable brown gum.

Attempted Catalytic Hydrogenation of the Acetate (m.p. 230°) from the Hydrogenation of the Chromic Acid Oxidation Product. - The acetate (m.p.227-229°, 0.15 g.) in stabilised glacial acetic acid (150 c.c.) was added to freshly prepared platinum catalyst (from 0.25 g. of PtO_2) and shaking in hydrogen was carried out for 24 hours. The solution was filtered, evaporated to dryness, and the residue crystallised from chloroform-methanol as plates (0.12 g.), m.p.223-226°, $[\alpha]_D$ +82° (c, 0.5) giving a yellow colour with T.N.M. in chloroform.

Concentration of the mother liquors yielded plates (0.02 g.), m.p.220-223°, giving a yellow colour with T.N.M. in chloroform. Neither crop was depressed in melting point on admixture with the starting material.

Oxidation of the Acetate (m.p.230°) from the Hydrogenation of the Chromic Acid Oxidation Product with Perbenzoic Acid. - The acetate (m.p.227-228°; 0.34 g.) in chloroform (25 c.c.) at 0° was treated with perbenzoic acid in chloroform (2 c.c.; 105 mg./c.c.; 2.1 mols.) and the solution was allowed to stand at 0° for 4 days. It was then treated with ether and washed with sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated. The residue crystallised from chloroform-methanol as plates (0.1 g.), m.p.219-223°, giving a yellow colour with T.N.M. in chloroform.

Recrystallisation from chloroform-methanol yielded hexagonal plates, m.p.225-227°, $[\alpha]_D$ +81° (c, 1.5) giving no depression in melting point on admixture with starting material.

Concentration of the original mother liquors yielded plates (0.11 g.), m.p.176-185°. Six recrystallisations from the same solvent yielded plates, m.p.194-195°, $[\alpha]_D$ +23° (c, 1.0), giving no colour with T.N.M. in chloroform and showing no light absorption in the region 2000-4000 Å.

Found: C,79.4; H,10.7%.

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

Treatment of the Oxide of iso-β-Amyradienonyl Acetate with Hydrochloric Acid. - The oxide (m.p.279-280°)(1.2 g.) was heated at 60° for 2 hours in chloroform (20 c.c.), acetic acid (50 c.c.) and concentrated hydrochloric acid (5 c.c.). The greenish-yellow solution was treated with water and ether and the extract was washed with sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated. The residue crystallised from chloroform--methanol as needles (0.6 g.), m.p.220-223°. Five recrystallisations from the same solvent gave needles, m.p.227-228° (decomp., acid vapours evolved) (m.p.218--219°, in vacuo), $[\alpha]_D$ +117°, +118° (c, 1.3, 0.7) giving no colour reaction with T.N.M. in chloroform. Positive 'Beilstein' test for halogen. Light absorption in ethanol: Max. at 2360 Å (ε = 11,000).

Found: C,74.8; H,9.3; Cl,6.5.

$C_{32}H_{47}O_5Cl$ requires: C,74.6; H,9.2; Cl,6.9%.

Hydrolysis of the Chloro-compound (m.p.227°) from the Hydrochloric Acid Fission of the Oxide of iso-β-Amyradienonyl Acetate. - The chloro-compound (m.p.225-227°, 0.15 g.) was dissolved in aqueous ethanolic potassium hydroxide (1 g. of KOH; 5 c.c. of water; 25 c.c. of ethanol) and refluxing was carried out for four hours. The solution was worked up through water and ether in

the normal manner and the residue crystallised from aqueous methanol as needles, m.p.287-290°, $[\alpha]_D$ +165° (c, 0.6). Two recrystallisations from the same solvent yielded prisms, m.p.289.5-291°, $[\alpha]_D$ +167° (c, 0.7). Light absorption in ethanol: Max. at 2380 Å (ε = 13,500). Admixture of this product with the alcohol obtained by hydrolysis of the acetate (m.p.318°), from the hydrogen peroxide oxidation of iso-β-amyradienonyl acetate, produced no depression in melting point.

Treatment of the Chloro-Compound with Activated Zinc in Acetic Acid. - The chloro-compound (m.p.227-229°, 0.25 g.) in stabilised glacial acetic acid (70 c.c.) was refluxed with freshly activated zinc (1.5 g.) for five hours. The solution was filtered, poured into water, extracted with ether and the extract was washed with sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated. The residual gum crystallised from light petroleum (40-60°) as prisms (0.1 g.), m.p.194-196°. Three recrystallisations from the same solvent yielded prisms, m.p.197-198°, $[\alpha]_D$ +134° (c, 0.5) giving no colour with T.N.M. in chloroform and a negative 'Beilstein' test. Light absorption in ethanol: Max. at 2360 Å (ε = 12,800).

Found: C,75.8; H,9.7.

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

Admixture with the diacetate obtained by acetylation of the acetate (m.p.318°) from the hydrogen peroxide oxidation product of iso-β-amyradienonyl acetate produced no depression in melting point.

Treatment of the Chloro-Compound with Unactivated Zinc in Acetic Acid. - The chloro-compound (m.p.227-229°, 1 g.) in stabilised glacial acetic acid (300 c.c.) was refluxed with unactivated zinc (6 g.) for 5 hours. The solution was filtered and when worked up through water and ether in the usual manner gave a residue which was dissolved in light petroleum (60-80°)-benzene (3:2; 50 c.c.) and chromatographed. (Alumina, grade II, 14 x 2 cm.).

Fractions	Eluant	Volume	Weight
1-11	LP/B (3:2)	550 c.c.	361 mg.
12-13	LP/B (2:3)	100	33
14-19	Benzene	300	110
20-23	B/E (1:1)	200	170

Combined fractions (1-19) crystallised from aqueous acetone as prismatic needles, m.p.265-272°. Five re-crystallisations from the same solvent yielded prismatic needles, m.p.274-275°, $[\alpha]_D$ +13° (c, 1.4) giving no colour with T.N.M. in chloroform. Light absorption in

ethanol: Max. at 2340 Å (ε = 11,500).

Found: C,79.6; H,10.3.

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

Fractions (20-23) were not investigated further.

Treatment of the Chloro-Compound with Activated Zinc in Ether and Methanol. - The chloro-compound (m.p.226--228°, 0.5 g.) in ether (75 c.c.) and methanol (75 c.c.) was refluxed with activated zinc (5 g.) for 5 hours. The solution was filtered and when worked up through water and ether in the usual way yielded a residue which was dissolved in light petroleum(60-80°)-benzene (2:3, 50 c.c.) and chromatographed on neutral alumina (Grade I, 9 x 2 cm.).

Fractions	Eluant	Volume	Weight
1-9	LP/B (2:3)	450 c.c.	175 mg.
10-15	LP/B (1:2)	300	53
16-18	LP/B (1:5)	150	30
19-38	Benzene	1000	166
39-41	Ether	150	106

Combined fractions (1-38) crystallised from aqueous acetone as prismatic needles, m.p.268-271°. Two re-crystallisations from the same solvent gave prismatic needles, m.p.273-275°, $[\alpha]_D$ +139° (c, 1.1) having no

colour reaction with T.N.M. in chloroform and a negative 'Beilstein' test. Light absorption in ethanol: Max. at 2350 Å (ε = 11,900).

There was no depression in melting point on admixture with the acetate (m.p.275°) from the unactivated zinc/acetic acid reduction of the chloro-compound.

Attempted Rearrangement of the Product of the Unactivated Zinc/Acetic Acid Reduction of the Chloro-Compound. - The acetate (m.p.270-272°, 0.1 g.) was dissolved in stabilised glacial acetic acid (35 c.c.) and concentrated hydrochloric acid (10 c.c.) and the solution was heated on the steam-bath for 24 hours and allowed to stand overnight. The solution was worked up through water and ether in the usual way and the residue was dissolved in pyridine (5 c.c.) and acetic anhydride (5 c.c.) and heated on the steam-bath for one hour. The material obtained from the acetylating solution crystallised from aqueous acetone as needles (0.073 g.), m.p.268-271° giving no colour with T.N.M. in chloroform. Light absorption in ethanol: Max. at 2370 Å (ε = 10,300).

Concentration of the mother liquors yielded a second crop of needles (0.015 g.), m.p.262-266°, giving no colour with T.N.M. in chloroform. Neither crop was depressed in melting point on admixture with starting material.

Wolff-Kishner Reduction of the Unactivated Zinc/Acetic Acid Reduction of the Chloro-Compound. - The acetate (m.p.270-272°, 0.5 g.) was heated with sodium methoxide (2 g. of sodium, 25 c.c. of methanol) and hydrazine hydrate (100%, 5 c.c.) in an autoclave at 200° for 10 hours. The crude product was treated with water and ether and the ether extract was washed with water, dried (Na_2SO_4) and evaporated. The residue was dissolved in pyridine (10 c.c.) and acetic anhydride (10 c.c.) and the solution was heated on the steam-bath for one hour. The residue, which was obtained on working up the acetylating solution in the usual manner, was dissolved in light petroleum (60-80°, 50 c.c.) and chromatographed on a column of activated alumina (Grade II, 14 x 2 cm.).

Fractions	Eluant	Volume	Weight
1-10	L.P.(60-80°)	500 c.c.	239 mg.
11-13	Benzene	150	30
14-16	Ether	150	166

Combined fractions (1-10) crystallised from chloroform--methanol as plates (0.2 g.), m.p.220-224°, giving a yellow colour with T.N.M. in chloroform. Three recrystallisations from chloroform-methanol yielded plates, m.p.226-228°, $[\alpha]_D$ +84° (c, 1.0) giving a yellow colour with T.N.M. in chloroform. Light absorption in ethanol:

ε_{2100} = 2,600, ε_{2150} = 2,000, ε_{2200} = 850, ε_{2250} = 110. There was no depression in melting point on admixture with the acetate (m.p.230°) from the catalytic hydrogenation of the chromic acid oxidation product of iso-β--amyradienonyl acetate.

Treatment of the Chloro-Compound with Collidine - The chloro-compound (m.p.226-228°, 0.25 g.) was heated with redistilled collidine (25 c.c.) in an autoclave at 200° for 3 hours. The crude product was treated with water, extracted with ether, and the extract was washed with dilute hydrochloric acid (5N), water, sodium bicarbonate solution, water, dried (Na_2SO_4), and evaporated. The residue crystallised from chloroform-methanol as plates (0.15 g.), m.p.278-281°, giving a yellow colour with T.N.M. in chloroform, and a negative 'Beilstein' test. Five recrystallisations from the same solvent yielded plates, m.p.286-287°, $[\alpha]_D$ +117° (c, 1.25) which gave a yellow colour with T.N.M. in chloroform and a negative 'Beilstein' test for halogen. Light absorption in ethanol: Max. at 2090 (ε = 3,000) and 2320 Å (ε = 12,800).

Found: C,82.3; H,9.9.

$C_{32}H_{48}O_2$ requires: C,82.3; H,9.7%.

Attempted Rearrangement of the Product of Collidine Treatment of the Chloro-Compound. - The collidine dehydrochlorination product (m.p.284-286°, 0.03 g.) was dissolved in dry benzene (3 c.c.), stabilised glacial acetic acid (10 c.c.) and concentrated hydrochloric acid (0.5 c.c.), and the solution was allowed to stand at room temperature for five days and was heated at 60° for three hours. After being worked up through water and ether in the usual manner, the residue crystallised from chloroform-methanol as plates (0.025 g.), m.p.270--278°. Recrystallisation from the same solvent yielded plates, m.p.283-285°, $[\alpha]_D$ +115° (c, 0.5) which gave a yellow colour with T.N.M. in chloroform. Admixture with starting material produced no depression in melting point.

Attempted Catalytic Hydrogenation of the Chloro-Compound in Ethyl Acetate. - The chloro-compound (m.p.226-228°, 1 g.) in ethyl acetate (200 c.c.) was added to freshly prepared platinum catalyst (from 0.35 g. of PtO_2) in ethyl acetate (25 c.c.) and shaking in hydrogen was carried out for 24 hours. The solution was filtered and evaporated to dryness to yield a residue which crystallised from chloroform-methanol as needles (0.66 g.),

m.p.228-230°, $[\alpha]_D$ +115° (c, 2.0). Light absorption in ethanol: Max. at 2360 Å (ε = 10,500).

Concentration of the mother liquors yielded a second crop of needles (0.2 g.), m.p.224-227°. Light absorption in ethanol: Max. at 2360 Å (ε = 10,000). Neither crop was depressed in melting point on admixture with starting material.

Attempted Ozonolysis of the Chloro-Compound. - The chloro-compound (m.p.226-228°, 1 g.) in purified chloroform (50 c.c.) was treated with a stream of ozone in oxygen (approx. 7%) passed at 0° for three hours. The blue solution was then evaporated to dryness under vacuum at 25° and the solid residue was treated with distilled water (25 c.c.) and slowly heated to 100° during one hour. The water was then distilled until only 5 c.c. remained and the distillate was collected in a saturated solution of dimedene (10 c.c.). The dimedone solution was heated on the steam-bath for 10 minutes and then allowed to stand at 0° for 24 hours. No dimedene derivative crystallised. The residue from the distillation was dissolved in chloroform, dried (Na_2SO_4), and crystallised from chloroform-methanol to yield needles (0.82 g.), m.p.224-227°, $[\alpha]_D$ +114° (c,1.7). Concentration of the mother liquors yielded a second crop

of needles (0.11 g.), m.p.221-225°. Neither crop was depressed in melting point on admixture with starting material.

Treatment of the Oxide of iso-β-Amyradienonyl Acetate with Boron Trifluoride. - The oxide (m.p.280-281°, 2.5 g.) in dry benzene (250 c.c.) was treated with freshly distilled boron trifluoride etherate (4 c.c.) and the solution was allowed to stand at room temperature for 60 hours. The greenish-yellow solution was then treated with water and ether, and the ether-benzene solution was washed with sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated. The residue crystallised from chloroform-methanol as needles (0.43g.) m.p.308-312°. Four recrystallisations from the same solvent yielded needles, m.p.314-315°, $[\alpha]_D$ -180° (c,0.7) giving no colour with T.N.M. in chloroform. Light absorption in ethanol: Max. at 2380 Å (ε = 11,700).

Found: C,77.2; H,10.0.

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

Infra-red spectroscopic investigation showed that the compound did not contain a hydroxyl group.

Concentration of the mother liquors yielded a second product as small cubes (1.3 g.), m.p.237-245°. Six recrystallisations from methanol gave prisms, m.p.253-253.5°, $[\alpha]_D$ -76° (c, 1.2) giving no colour with T.N.M.

in chloroform. Light absorption in ethanol: Max. at 2500 Å (ε = 13,500).

Found: C,77.3; H,9.7.

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

Infra-red spectroscopic investigation showed that the compound did not contain a hydroxyl group.

Attempted Acetylation of the Acetate (m.p.253°) from the Boron Trifluoride Rearrangement of the Oxide of iso-β-Amyradienonyl Acetate. - The acetate (m.p.250-252°, 0.1 g.) in pyridine (5 c.c.) and acetic anhydride (5 c.c.) was heated on the steam-bath for $3\frac{1}{2}$ hours. The solution was worked up in the usual manner to give a residue which crystallised from methanol as cubes (0.06 g.), m.p.248-251°, $[\alpha]_D$ -72° (c, 0.5) giving no colour with T.N.M. in chloroform. Light absorption in ethanol: Max. at 2500 Å (ε = 12,000). Recrystallisation from the same solvent yielded cubes, m.p.251-252.5°, $[\alpha]_D$ -74°.

Concentration of the mother liquors gave a second crop of cubes (0.02 g.), m.p.246-249°.

Neither crop was depressed in melting point on admixture with the starting material.

Attempted Chromic Acid Oxidation of the Acetate (m.p.253°) from the Boron Trifluoride Rearrangement of the Oxide. - The acetate (m.p.250-252°, 0.25 g.) in stabilised glacial

acetic acid (200 c.c.) was treated, at room temperature, with a solution of chromic anhydride (1.25 atoms O), in acetic acid (15 c.c.) added dropwise. The solution was allowed to stand at room temperature overnight and was heated at 80° for 5 hours. The solution was worked up in the usual way, giving no acid fraction, and the residue obtained crystallised from methanol as cubes (0.17 g.), m.p.248-251°, $[\alpha]_D$ -20° (c, 1.0) giving no colour with T.N.M. in chloroform and showing no depression in melting point on admixture with starting material.

Concentration of the mother liquors yielded amorphous material (0.025 g.).

Treatment of the Oxide of iso-β-Amyradienonyl Acetate with Sulphuric Acid. - The oxide (m.p.279-281°, 0.8 g.) in glacial acetic acid (500 c.c.) and dilute sulphuric acid (12N, 20 c.c.) was heated on the steam-bath for four hours. The solution was treated with water and ether, and the extract was washed with sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated. The residue was dissolved in light petroleum (60-80°)-benzene (1:1; 50 c.c.) and chromatographed on activated alumina (Grade II, 14 x 1.25 cm.).

Fractions	Eluant	Volume	Weight
1	LP/B (1:1)	50 c.c.	-
2-9	" (1:4)	400	465 mg.
10-12	Benzene	150	27
13-15	B/E (9:1)	150	89
16-17	" (1:1)	100	20
18-19	Ether	100	5
20-21	E/Methanol	100	126
22	Methanol	50	-

Combined fractions (2-9) crystallised from light petroleum as prismatic needles, m.p.241-246°. Six recrystallisations from the same solvent gave prismatic needles, m.p.251.5-252°, $[\alpha]_D$ -78° (c, 1.1), giving no colour with T.N.M. in chloroform. Light absorption in ethanol: Max. at 2500 Å (ε = 13,500).

Found: C,75.9; H,9.65; Acetoxyl, 16.9.

$C_{34}H_{50}O_5$ requires: C,75.8; H,9.4; 2(Acetoxyl),17.5%.

Fractions (13-22) yielded uncrystallisable gums.

Alkaline Hydrolysis of the Product from the Sulphuric Acid Treatment of the Oxide of iso-β-Amyradienonyl Acetate. - The sulphuric acid product (m.p.253-254°, 0.5 g.) was dissolved in aqueous ethanolic potassium hydroxide (3%; 30 c.c.) and the solution was refluxed

for 3 hours. The residue obtained by working up the solution through water and ether in the usual manner, crystallised from aqueous acetone as needles (0.39 g.), m.p.285-288°. Four recrystallisations from the same solvent yielded needles, m.p.288-289°, $[\alpha]_D$ -110° (c,1.0) giving no colour with T.N.M. in chloroform. Light absorption in ethanol: Max. at 2500 Å (ξ = 14,050).

Found: C,79.0; H,10.2.

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

The alcohol (m.p.286-288°, 0.1 g.) was dissolved in pyridine (5 c.c.) and acetic anhydride (5 c.c.) and the solution was heated on the steam-bath for two hours. The residue which was obtained after working up the solution in the usual manner crystallised from light petroleum (60-80°) as prismatic needles, m.p.249-252°. Recrystallisation from the same solvent gave prismatic needles, m.p.251-253°, $[\alpha]_D$ -75° (c, 1.4). Light absorption in ethanol: Max. at 2500 Å (ξ = 13,600). Admixture with the diacetate from which the alcohol (m.p.289°) was obtained produced no depression in melting point.

Treatment of the Sulphuric Acid Product with Selenium Dioxide. - The sulphuric acid product (m.p.251-253°, 0.5 g.) in stabilised glacial acetic acid (100 c.c.) was refluxed with selenium dioxide (0.5 g.) for 24 hours.

The yellow solution was filtered, poured into water and extracted with ether. The extract was washed with sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated to yield a residue which crystallised from light petroleum (60-80°) as needles (0.3 g.), m.p.246--248°. Light absorption in ethanol: Max. at 2500 Å (ε = 14,200). Concentration of the mother liquors yielded a second crop of prismatic needles (0.12 g.), m.p.244-251°. Recrystallisation of the second crop from the same solvent yielded prismatic needles, m.p. 249-252°. Neither crop was depressed in melting point on admixture with starting material.

Catalytic Hydrogenation of the Sulphuric Acid Fission Product of the Oxide of iso-β-Amyradienonyl Acetate. - The sulphuric acid fission product (m.p.250-251°; 0.75 g.) in stabilised glacial acetic acid (100 c.c.) was added to freshly prepared platinum catalyst (from 0.5 g. of PtO_2) in acetic acid (20 c.c.) and shaking in hydrogen was carried out for 24 hours. The solution was filtered and evaporated to dryness to yield a residue which crystallised from chloroform-methanol as needles (0.57 g.), m.p.248-254°, giving a yellow colour with T.N.M. in chloroform. Concentration of the mother liquors yielded a second crop of needles (0.1 g.), m.p.

m.p.245-250°, giving a yellow colour with T.N.M. in chloroform. Six recrystallisations of the first crop from chloroform-methanol yielded needles, m.p.251-253°, $[\alpha]_D$ -29°, -30° (c, 1.0, 1.2) giving a yellow colour with T.N.M. in chloroform. Light absorption in ethanol: $\varepsilon_{2070} = 1,200$, $\varepsilon_{2100} = 980$, $\varepsilon_{2150} = 440$, $\varepsilon_{2200} = 75$.

Found: C,77.5; H,10.5.

$C_{54}H_{52}O_4$ requires: C,77.8; H,10.0%.

Treatment of the Catalytic Hydrogenation Product with Hydrochloric Acid. - The catalytic hydrogenation product (m.p.250-253°, 0.25 g.) in glacial acetic acid (100 c.c.) and concentrated hydrochloric acid (3 c.c.) was heated on the steam-bath for 4 hours. The solution was poured into water and worked up through ether in the usual manner. The residue was dissolved in pyridine and acetic anhydride and the solution was heated on the steam-bath for one hour. The acetylating solution yielded a gum which crystallised from chloroform-methanol as needles (0.09 g.), m.p.230-240°. Recrystallisation from the same solvent gave needles, m.p.249-252°, yellow colour with T.N.M. in chloroform and showing no depression in melting point on admixture with starting material.

Concentration of the mother liquors yielded amorphous material and gum which showed no light absorption in ethanol in the region 2200-4000 Å.

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