THE STRUCTURAL CHEMISTRY OF THE TRITERPENE α-AMYRIN

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T H E S I S

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by

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C O N T E N T S

HISTORICAL

HISTORICAL

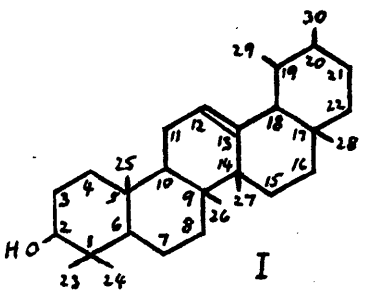
Introduction

Most non-nitrogenous natural products of high molecular weight may be allocated to one of two classes of compounds, the steroids or the triterpenoids. In general, members of the latter class are characterized by having a molecule containing thirty carbon atoms, theoretically arranged in six iso-pentane units, and by yielding naphthalene homologues on vigorous dehydrogenation. The vast majority of such compounds occur in the vegetable kingdom, although triterpenes of animal origin are known.

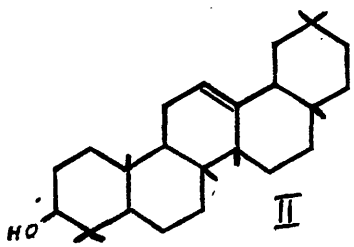
With the exception of squalene and ambrein, which have aliphatic and tricyclic structures respectively, all triterpenes whose constitutions have been elucidated are either tetra- or pentacyclic. It is of interest to note that lanosterol, the best known of the tetracyclic triterpenes, has recently been shown to possess the same fused ring system as the steroids (1, 2) and has thus affinities with both groups of compounds.

The class of pentacyclic triterpenes is, however, by far the larger, and it is in this field that the most thorough investigation has been made. As a result of the researches of many workers, all pentacyclic triterpenes about whose structure sufficient is known may

be allocated to one of four series, and related to one of the compounds α-amyrin (I), β-amyrin (II), lupeol (III) and taraxasterol (IV) (3). A structure has only recently been allocated to taraxasterol (4). Members of the same series differ, in general, only in the arrangement of functional groups about the same carbon skeleton.



I



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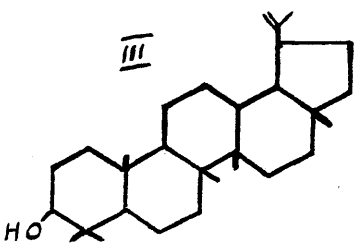
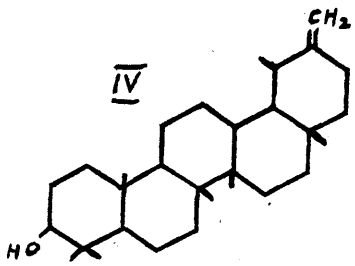




Three of the four groups of triterpenoid compounds have been inter-related. Thus lupeol has been converted to δ-amyrin, a known derivative of β-amyrin (5), and betulin, a triterpene with the basic carbon skeleton of lupeol, is converted on acid treatment (6) to allobetulin in the β-amyrin series (7). In addition, heterobetulin in the taraxasterol series may be obtained by treatment of allobetulin with benzoyl chloride (8). As yet no attempt

to convert a compound of the α-amyrin series to a member of any of the other series has succeeded, nor has the converse operation been performed.

At the time of writing, six other naturally occurring pentacyclic triterpenes have been related to α-amyrin (9), viz. uvaol, brein, ursolic acid, asiatic acid, β-boswellic acid, and quinovic acid. The relation-ships which members of the series bear to α-amyrin are shown in Table I.

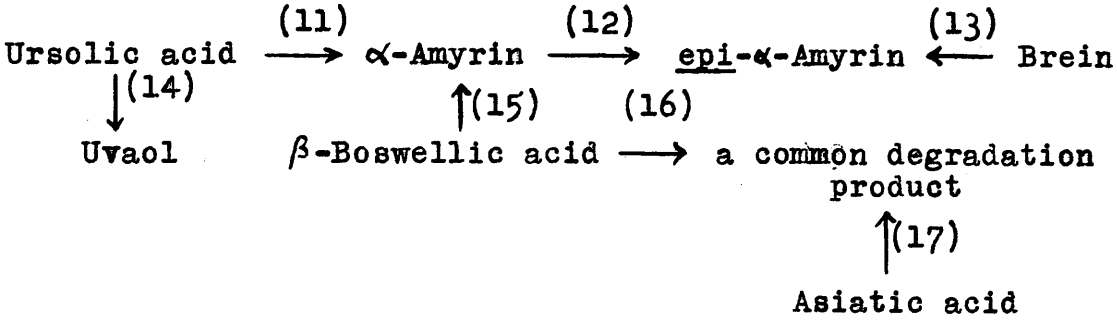
Table I

Triterpene	Functional Groups			Source
	C=C	-OH	-COOH	
α-Amyrin	12:13	2	-	Manila Elemi resin
Uvaol	12:13	2, 28 (?)	-	Arctostaphylos uva-ursi
Brein	12:13	2(epi), 21 or 22	-	Manila Elemi resin
β-Boswellic acid	12:13	2	23 or 24	Frankincense
Ursolic acid	12:13	2	28 (?)	Bear-berry, apples, pears
Quinovic acid	12:13	2	27, 28	Zygophyllum coccineum
Asiatic acid	12:13	2,3,23 or 24	28	Hydrocotyle asiatica

With the exception of quinovic acid (10), these compounds have been linked with α-amyrin by interconversion, as shown in Table II. In epi-α-amyrin, which has not been found in nature, the hydroxyl group is epimeric with that

in α-amyrin; the same configuration occurs in brein. A recently discovered hexacyclic triterpene, phyllanthol, has also been converted into α-amyrin (18).

Table II



Since the work described in this thesis is concerned with α-amyrin only, the chemistry of the other triterpenes will not be discussed further except where relevant. Several recent reviews of general triterpene chemistry are available (19, 20, 21, 22).

The structure assigned above to α-amyrin is at present generally accepted. It has been built up by painstaking degradative work which will be summarized in the following pages, but it cannot be said to be proved beyond all doubt. Objections to the current formulation may be made on several counts, which will be mentioned below, but no formula which gives more convincing expression to the reactions of α-amyrin has been suggested.

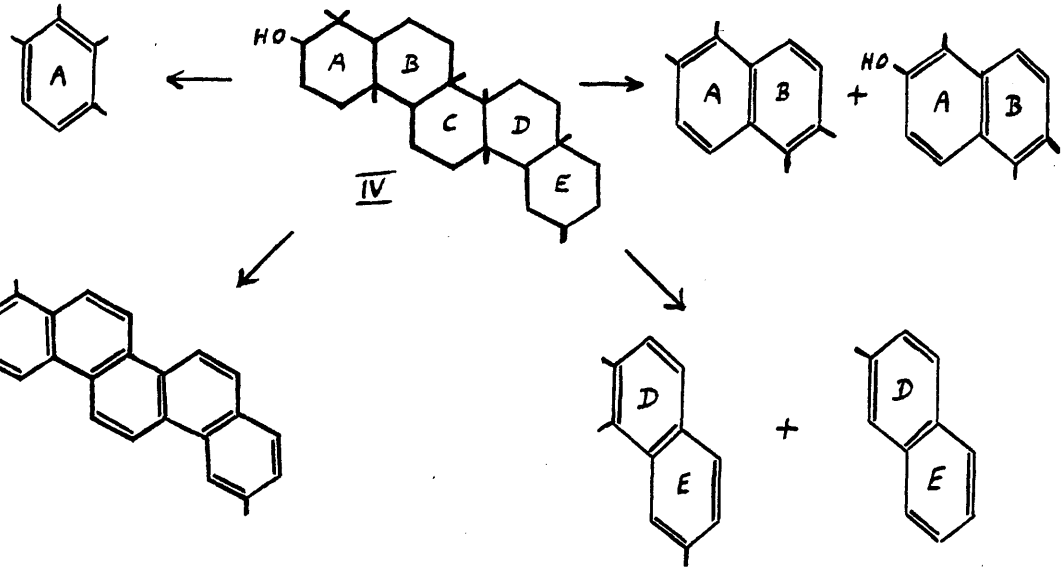
The Structure of α-Amyrin

α-Amyrin occurs in many vegetable products, but is usually isolated along with β-amyrin from Manila Elemi resin. The mixed amyrins were first isolated from this by Rose in 1839 (23), and were separated by Vesterberg in 1887 (24), but it is only in the last thirty years or so that any real progress in the structural problem presented by these compounds has been made. This had to await reagents and techniques capable of detecting and reacting with the extraordinarily inert functional groups which are characteristic of the triterpenes. Selenium dehydrogenation, tetranitromethane colour tests, and spectrographic analysis, all comparatively recent developments in organic chemistry, have been indispensable in advancing our knowledge to its present state.

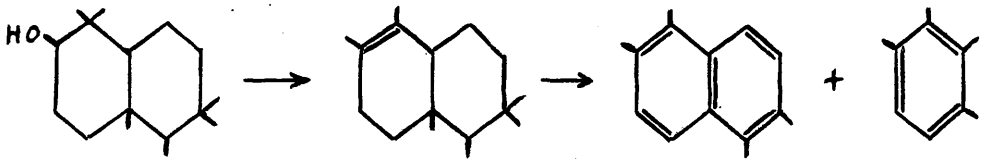
The first significant steps in the structural investigation of α-amyrin were derived from selenium dehydrogenation experiments carried out by various workers (25-28). The products shown in Chart I were obtained, and from these Ruzicka of Zurich postulated the basic hydropicene structure IV.

Chart I (overleaf)

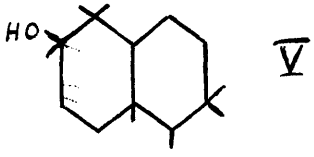
Chart I



The formation of 1:2:5:6 tetramethylnaphthalene and 1:2:3:4 tetramethylbenzene was explained by assuming a retropinacolinic rearrangement in ring A on dehydration, thus:



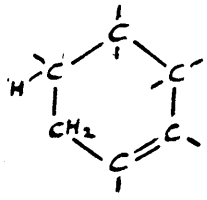
This was confirmed by a study of the products of dehydrogenation of methyl-α-amyrin, V (29).



V

The location of the angular methyl group in IV was largely arbitrary, since allowance had to be made for possible migration during the reaction; it was later modified, and a position assigned to the double bond.

The existence of the double bond of α-amyrin was demonstrated by Ruzicka, who showed that while it could not be hydrogenated, it was attacked by ozone (30). Spring and Vickerstaff (25) showed that the product of chromium trioxide oxidation of α-amyrin esters contained an αβ-unsaturated ketone grouping, detectable by spectroscopic methods. This, on catalytic hydrogenation, was reconverted to the original α-amyrin ester (31), showing that the double bond was in the same position in both compounds. It follows that α-amyrin must contain a methylene group adjacent to its double bond, as originally suggested by Spring (25). Reduction of α-amyrenonol, the unsaturated ketone, with sodium and amyl alcohol, gave α-amyradienol (25) which from spectroscopic evidence appeared to be a homoannular diene. Thus the environment of the double bond is as shown in VI. α-Amyradienol has also been obtained

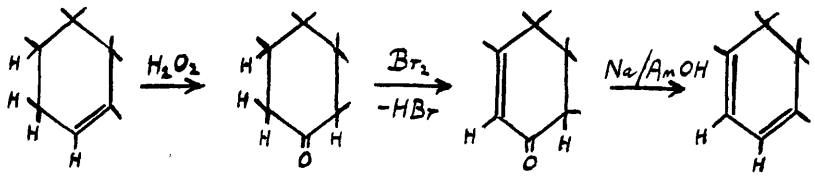


VI

by mild sulphur dehydrogenation of α-amyrin (32) and - as acetate - by the action of N-bromsuccinimide on α-amyrin acetate (33).

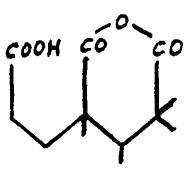
Hydrogen peroxide oxidation of α-amyrin benzoate gives a compound which on bromination and dehydrobromination yields an αβ-unsaturated ketone, isomeric with α-amyrenonyl

benzoate, but not identical with it (34). On sodium and amyl alcohol reduction, this too gives α-amyradienol. These changes were formulated as shown, leading to fragment VII as representing the location of the unsaturated centre of α-amyrin.

VII 

It has since been proved (35) that the product of peroxide oxidation of the double bond of α-amyrin is in fact an oxide, which is easily isomerized by mineral acid to the ketone shown; this, of course, occurs in the course of the above bromination.

Further light was thrown on the problem of double bond position by the dehydration reactions of α-amyrenonol. By the action of dehydrating agents, and subsequent oxidation, rings A and B in this compound were degraded extensively, to give a product partially formulated as in VIII (36). From its ultra-violet



VIII

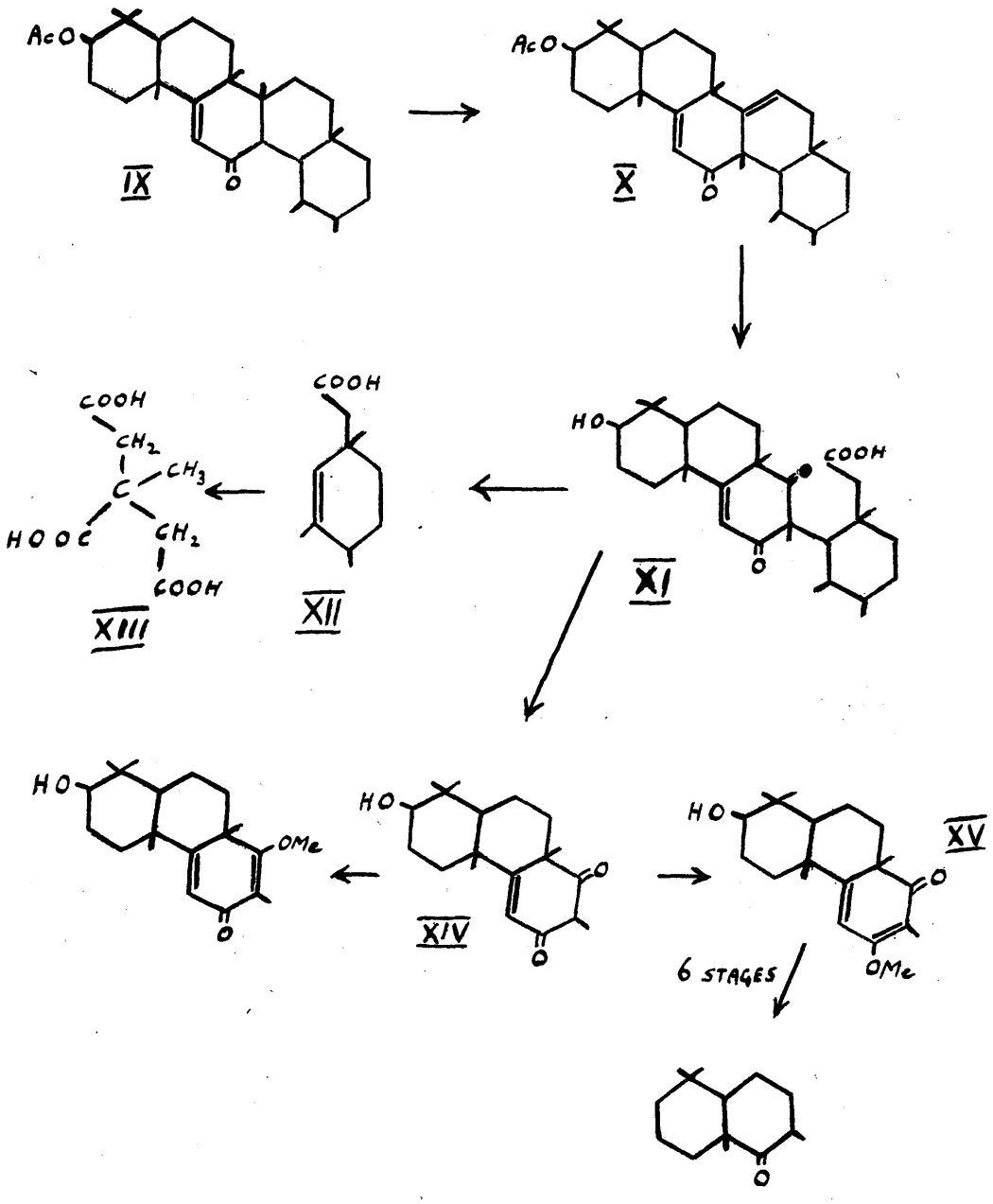
spectrum, it is evident that this acid still retains unchanged the original αβ-unsaturated ketone chromophore of α-amyrenonol, thus excluding rings A and B as possible sites of the unsaturated centre.

Two series of reactions carried out by Ruzicka and his co-workers at Zurich have assumed great importance in the structural study of α-amyrin. In both cases, oxidative degradation leads to the opening of a ring, and subsequent pyrolytic fission breaks the molecule into two fragments which are more readily investigated.

The first of these reaction series starts from iso-α-amyrenonyl acetate (IX), which on treatment with selenium dioxide gave a product containing one more double bond (37); by spectroscopic and other evidence this was shown to be unconjugated with the original chromophore. These reactions are shown, as finally formulated, in Chart II. This new compound, iso-α-amyradienonyl acetate (X), was oxidised to a glycol by osmium tetroxide, the ring opened with lead tetra-acetate, and the product oxidised to a diketo-monocarboxylic acid (XI). This acid was then subjected to pyrolysis, and two fractions, volatile and non-volatile, obtained (38). The former consisted of a monobasic acid (XII) which could be further oxidised to β-methyl-tricarballylic acid (XIII), and was considered to arise from ring E of α-amyrin. The non-volatile portion was treated with diazomethane, and two main products isolated. Since these still contained a hydroxyl group, they were considered to come from rings A, B and C of α-amyrin,

though the parent compound XIV, by methylation, in turn, of both enolized ketone groups. The ether XV was later further degraded to 1:1:6:10-tetramethyl-5-keto-*trans*-decalin (39).

Chart II

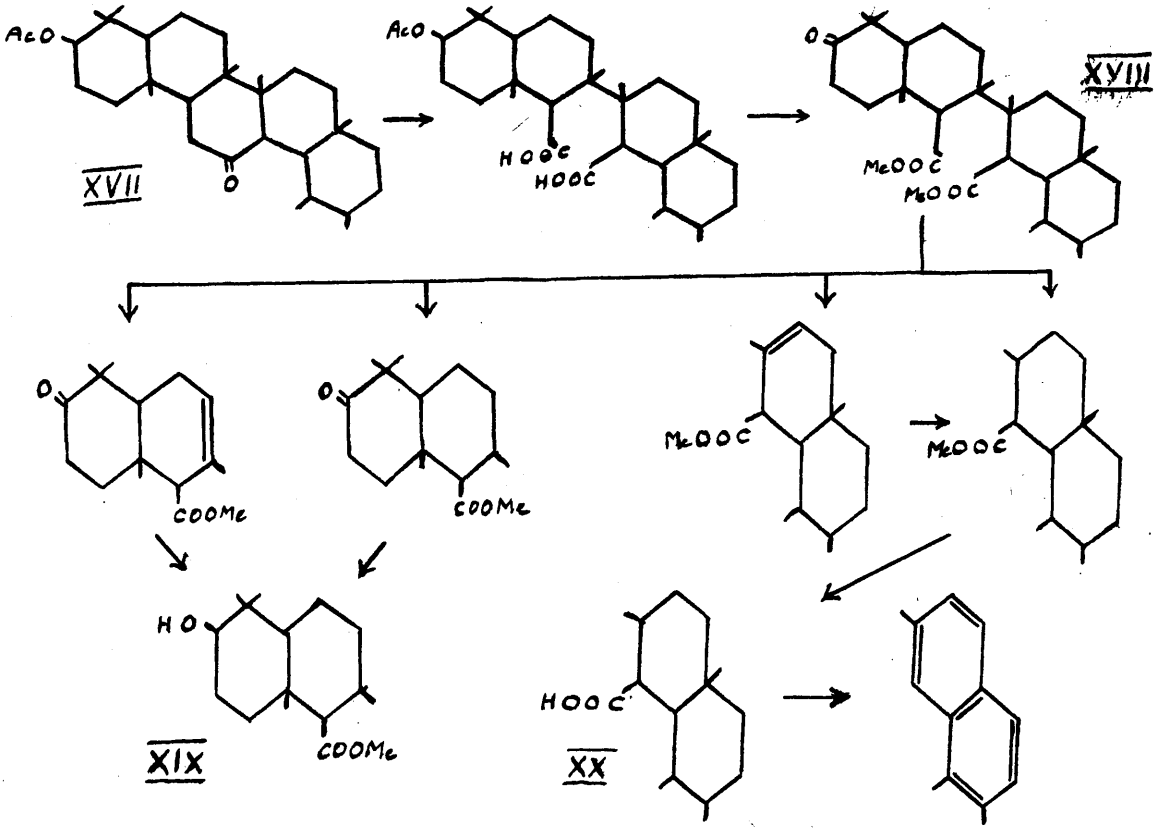


A similar series of experiments was then carried out on iso-β-amyradienonyl acetate, analogous to X above; in this case pyrolysis gave a volatile acid, to which the structure XVI was ascribed, and the same pair of tricyclic compounds as obtained previously in the α-amyrin series(40).

XVI

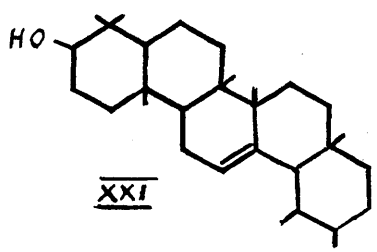
The second series of oxidative degradations starts from α-amyranonyl acetate (XVII), and is shown as finally formulated in Chart III.

Chart III

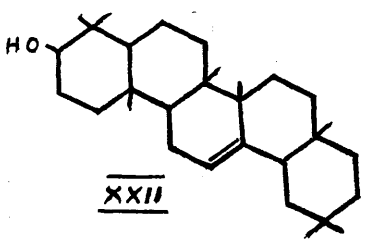


On treatment with fuming nitric acid, the carbonyl group of α-amyranonyl acetate was attacked, ring opening took place, and a dicarboxylic acid was produced (41). This acid was estified with diazomethane, and its secondary hydroxyl group oxidised to the corresponding ketone. Pyrolysis of this compound (XVIII) gave a set of products separable into ketonic and non-ketonic fractions. The former consisted of two compounds, differing only in degree of saturation, which could both be hydrogenated to a compound XIX, already obtained by pyrolysis from oleanolic acid in the β-amyrin series (42). The non-ketonic fraction also contained two compounds, a saturated and an unsaturated ester. These, after hydrogenation and hydrolysis, gave a saturated acid (XX), from which was obtained, on selenium dehydrogenation, 1:2:7-trimethylnaphthalene, one of the products supposed to result from the dehydrogenation of rings D and E of α-amyrin itself.

From a consideration of the above evidence, and especially that in the last section, the hypothetical structure XXI has been accepted as representing the molecule of α-amyrin. On a basis of similar, but rather more



XXI



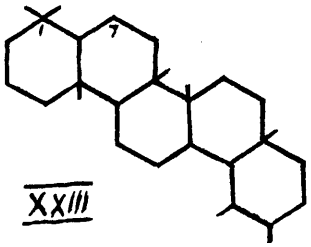
XXII

detailed and conclusive work, β-amyrin has been assigned the structure XXII. While no real doubt has been cast on formula XXII for β-amyrin, XXI for α-amyrin does not give an entirely satisfactory explanation of its reactions, chiefly in so far as its differences from β-amyrin are concerned. This matter will be discussed more fully in the Theoretical Section.

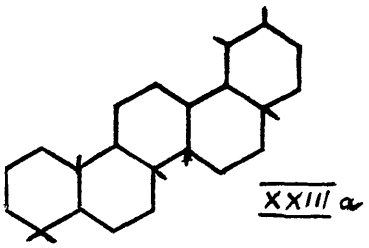
Nomenclature and Conventions

As the chemistry of the amyrins became more complex, and more new compounds were prepared, the existing nomenclature as used in the previous section became exceedingly clumsy. In consequence, Dreiding, Jeger and Ruzicka (43) have recently suggested a rational nomenclature for α-amyrin derivatives, similar to that used in steroid chemistry. It is based on the parent saturated hydrocarbon, ursane (XXIII) (1:1:7:8:16:18:19:22-octamethylperhydropicene), although this compound has not itself been prepared. The conventional numbering of carbon atoms remains unchanged, and functional groups are indicated by prefixes or suffixes; their position is denoted numerically, with the exception of the 2-hydroxyl group, whose location is generally assumed. Thus α-amyrenonyl acetate and α-amyradienol become 11-keto-urs-12-enyl acetate and ursa-10:12-dienol respectively, and α-amyrin itself is urs-12-enol, although in this case it

is still customary to use the trivial name. A similar system, based on the hydrocarbon oleanane, was earlier applied to β-amyrin derivatives (44).



XXIII



XXIIIa

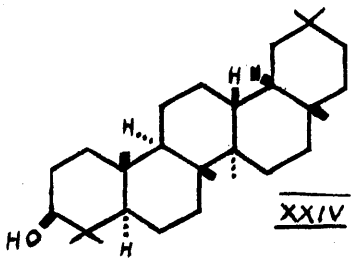
This nomenclature will be used in subsequent pages, except in cases where it is manifestly inapplicable. These cases include derivatives of iso-α-amyradienonal, where there is a possibility that a change in the carbon skeleton has occurred, and stereoisomeric compounds in which the stereochemical relationship is not clear.

Until recently, it has been customary to write triterpene formulae as in the foregoing pages. On investigation of stereochemistry, however, it was found that rings A and B of the triterpenes bear a close relationship to those of the steroids, but that the conventional representation of reference methyl groups was used in opposite senses (see next section). In order to reconcile these conventions, Halsall, Jones and Meakins (45), with the concurrence of the other principal triterpene chemists in Britain, suggested that the formulae of triterpenoids should be turned through 180°

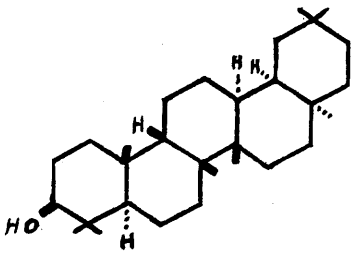
about an axis through $C_{(1)}$ and $C_{(7)}$, and the other conventions retained unchanged (XXIIIa). This emphasises the apparent relationships between steroids and triterpenoids; however the realities behind these appearances in many cases remain to be established.

Stereochemistry

Most of our knowledge of the stereochemistry of α-amyrin is not derived directly, but has been deduced by comparison with β-amyrin, the configuration of which has been elucidated with almost complete certainty. This is due, in the main, to the elegant theoretical work of Barton (46), who made great use of the concept of vertical and equatorial bonds, and of W.S. Johnson's interpretation (47) of the researches of Linstead and his co-workers (48) on perhydrophenanthrenes. Barton applied this theoretical knowledge to his own experimental results, and to those of other workers, and concluded that there were only two possible expressions (XXIV, XXV) of the configuration of the β-amyrin nucleus. In these formulae, substituents above and below the plane of the paper are indicated by thick and broken bonds respectively, and, as in the steroid field, are denoted by the prefixes "β" and "α". All configurations are referred to the methyl group at $C_{(5)}$.



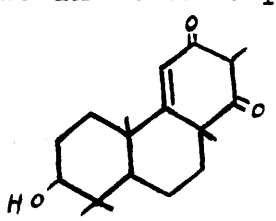
XXIV



XXV

A decision between these on chemical grounds seemed impossible, and physical methods have been applied to the problem. Klyne, by a consideration of molecular rotation differences (49) has decided in favour of XXIV, a conclusion supported by X-ray studies of methyl oleanolate iodo-acetate (50). An excellent summary of the evidence leading to XXIV is available in the Tilden Lecture, 1953, delivered by Barton (50).

Before the completion of these studies, it had been recognised that there are stereochemical similar-ities between α- and β-amyrin. The tricyclic pyrolysis fragment, XXVI, obtained from both α- and β-amyrin (see p. 10) showed that configurations at carbon atoms 2, 5, 6 and 9 were the same in both compounds (40).



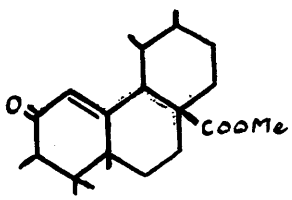
XXVI

In the same paper, it was suggested that substituents at $C_{(10)}$ and $C_{(14)}$ might also be similarly oriented in both triterpene series; in the former case from a consideration

of molecular rotation differences, and in the latter because of a retropinacolinic rearrangement affecting $C_{(14)}$, postulated in both series to explain the formation of the iso-amyradienonols.

It has been found that groups similarly situated relative to the D/E ring junction in α- and β-amyrin show considerable differences in reactivity in the two series, α-amyrin derivatives being markedly less active (35, 51). In particular, reaction of methyl 11-keto-ursolate acetate (XXVII) with either acid or base, unlike the behaviour of the corresponding methyl 11-keto-oleanolic acid compound, failed to cause isomerisation at $C_{(18)}$ (46). To explain this it was

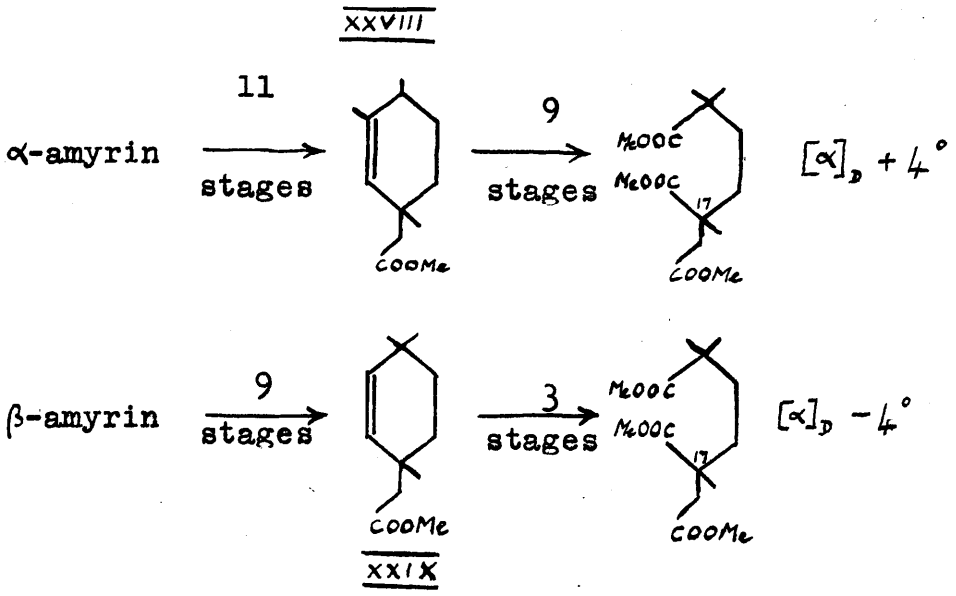
XXVII



suggested that either the $C_{(18)}$ configuration in the α-amyrin series, unlike that in the β-amyrin series, was in the more stable arrangement, or that there was no hydrogen attached at that point.

Some reactions carried out by Jeger of Zürich (52) suggest that the configurations at $C_{(17)}$ in α- and β-amyrin are enantiomorphous. Degradation of α- and β-amyrin as in Chart II, p. 10, yielded the monocyclic pyrolysis products XXVIII and XXIX respectively, which were further degraded to give two esters of equal and

opposite optical rotation, and containing the original $C_{(17)}$ of the amyrins as the only asymmetric centre.

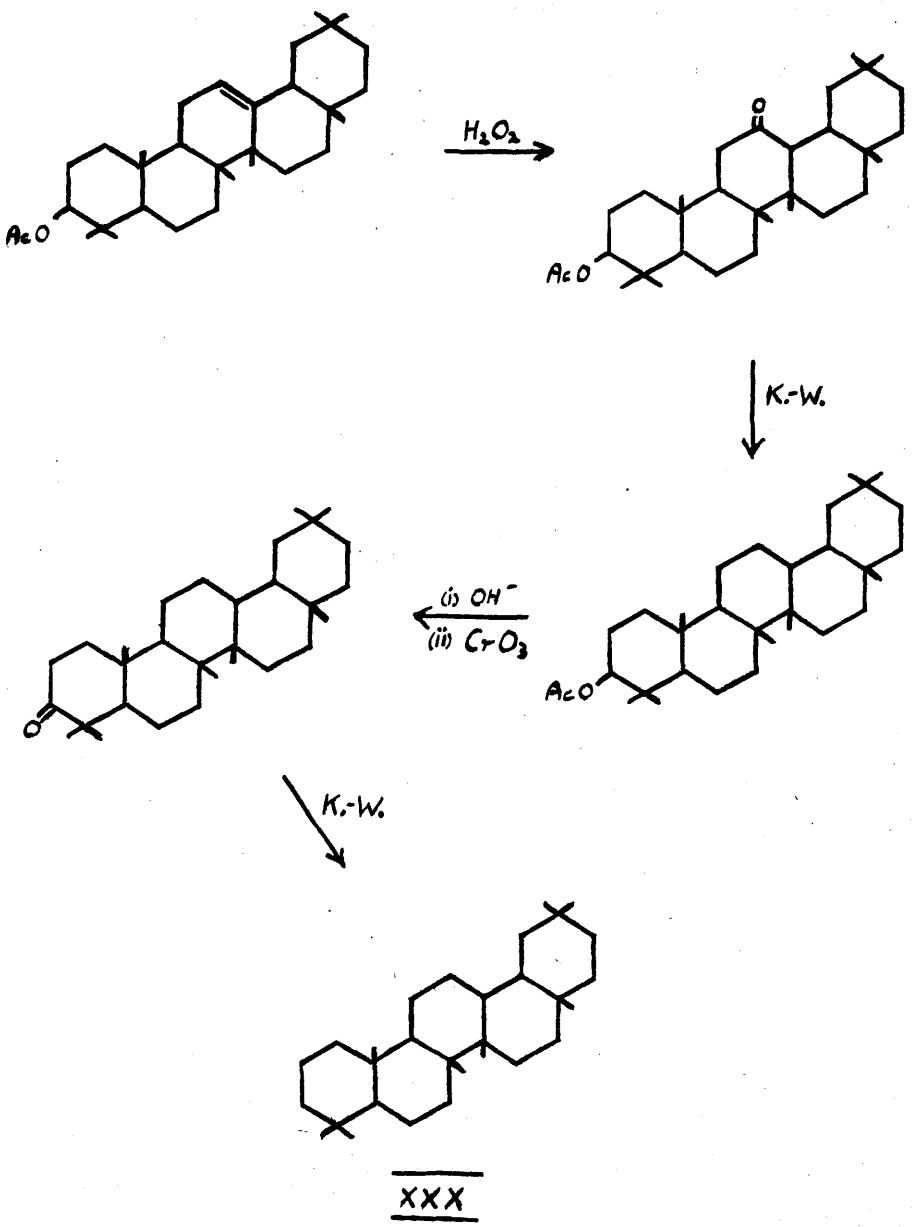


Triterpenoid Saturated Hydrocarbons

It has been considered of interest in triterpene chemistry to attempt to prepare the parent saturated hydrocarbons of each of the four series, viz. oleanane (β-amyrane), ursane (α-amyrane), lupane and taraxastane for comparison purposes.

Oleanane (XXX) was first prepared by Ruzicka and Jeger (53), by the reaction series shown. (See overleaf).

Lupeol, having no unreactive unsaturated centre, was easily converted to lupane (54). Catalytic reduction of the exocyclic double bond, followed by chromic acid oxidation of the hydroxyl group at $C_{(2)}$, gave a saturated ketone whose carbonyl group was reduced by the Kishner-



XXX

Wolff method.

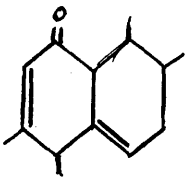
Taraxastane has been prepared from several members of its series by similar methods involving catalytic reduction of double bonds and Kishner-Wolff reduction of carbonyl groups (55, 56, 57). The formulation for taraxastane (4) suggests that in fact it is the same compound as ursane, unless stereochemical differences occur.

Owing to the extreme unreactivity of substituents in ring C of α-amyrin, it has not yet been found possible to prepare ursane. The hydroxyl group at $C_{(2)}$ may be removed (58, 51) by chromic acid oxidation followed by Kishner-Wolff reduction, to give urs-12-ene (α-amyrene). However, the removal of functional groups from ring C has not yet been attained. Attempts to reduce the carbonyl group of 12-keto-ursanyl acetate by Kishner-Wolff or Clemmensen methods have proved unsuccessful (59, 60).

iso-α-Amyradienonyl Acetate

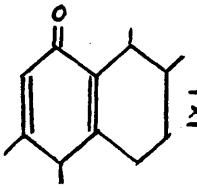
iso-α-Amyradienonyl acetate, the product obtained when 12-keto-urs-10-enyl acetate is oxidised with selenium dioxide, is a compound whose structure has assumed some importance in α-amyrin chemistry, since it is a vital link in one of the important degradations described above (Chart II, p. 10).

Ruzicka and his co-workers, when they originally prepared this material (37), observed that it gave a yellow colour reaction with tetranitromethane, characteristic of a single double bond unconjugated with a carbonyl group, and that it showed maximum ultra-violet absorption at 2350Å , a result not inconsistent with the presence of an $\alpha\beta$-unsaturated ketone chromophore. iso-α-Amyradienonyl acetate was therefore formulated as in the partial structure XXXI. On subjecting it to catalytic

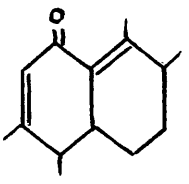


XXXI

hydrogenation, two compounds were isolated from the product, ursa-10:12-dienyl acetate and 12-keto-urs-10-enyl acetate. When iso-α-amyradienonyl acetate was treated with hydrogen chloride, an isomeric compound was obtained, which possessed substantially the same ultra-violet absorption spectrum as the starting material, but gave no colour reaction with tetranitromethane. This has been partially formulated as XXXII or XXXIII.



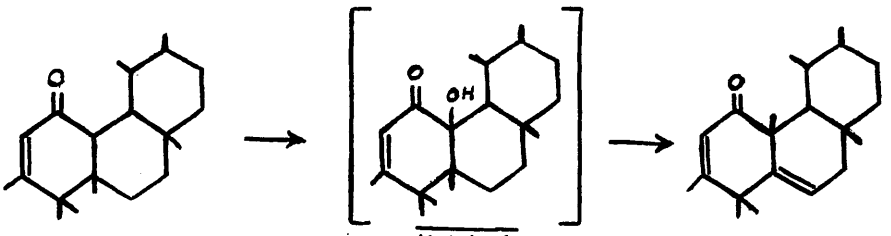
XXXII



XXXIII

After the two series of degradation reactions described above (Charts II and III, pp. 10, 11) had been carried out, it was found that they were mutually

inconsistent as regards $C_{(27)}$. This inconsistency was resolved by assuming a methyl group migration in the formation of iso-α-amyradienonyl acetate, on dehydration of an intermediate hydroxyl compound (XXXIV). A similar change of carbon skeleton was postulated in the formation of the analogous iso-β-amyradienonyl acetate.



XXXIV

It was then pointed out by McLean, Ruff and Spring (61) that a difference in carbon skeleton between 12-keto-urs-10-enyl acetate and iso-α-amyradienonyl acetate was extremely unlikely, since the latter could be converted to the former by catalytic hydrogenation. They then critically re-examined the relationship between 12-keto-urs-10-enyl acetate and α-amyrin itself, and concluded that iso-α-amyradienonyl acetate retained the carbon skeleton of α-amyrin. A similar criticism of the postulated structure of iso-β-amyradienonyl acetate was later made (62), although this is not such a clear-cut case, since on catalytic reduction this compound gives a product hitherto unknown in the β-amyrin series.

The obvious escape from this impasse is to suppose that $C_{(27)}$ in the amyrins is not, in fact, attached to $C_{(14)}$, but is located elsewhere in the molecule,

perhaps in a seven-membered ring. This last can easily be reconciled with the dehydrogenation evidence (p. 5), since under the reaction conditions used, methyl group migration is quite possible. However, Meyer, Jeger, Prelog and Ruzicka (63) examined the infra-red spectra of many triterpenoid saturated ketones, and concluded that in both amyrins ring C was six-membered.

At the time of commencement of the research described in this thesis, the above was one of the outstanding problems of α-amyrin chemistry still unsolved, and it will be discussed in greater detail in the subsequent Theoretical Section.

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THEORETICAL

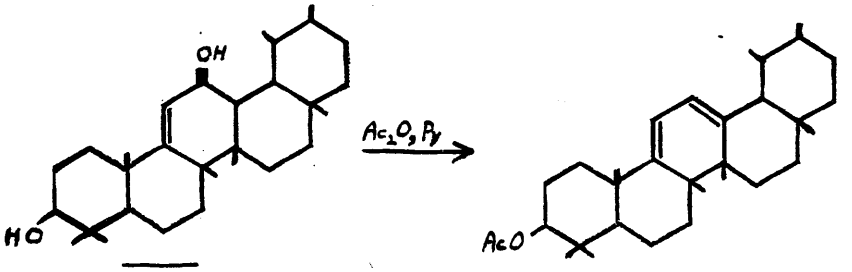
THEORETICAL

Experiments relating to the Synthesis of Ursane

As mentioned in the Historical Section, the preparation of ursane, the parent saturated hydrocarbon of the α-amyrin series of triterpenes, might of of great value in relating that series to the triterpenes based on taraxastane, but this had not been achieved in the past. A projected synthesis analogous to that which leads to oleanane failed since the carbonyl group of 12-keto-ursanyl acetate could not be reduced by Kishner-Wolff or Clemmensen methods (59, 60). Attempts were also made to convert 12-hydroxy-ursane into the corresponding chloride with the object of reducing the latter to ursane. These attempts were unsuccessful, treatment of 12-hydroxy-ursane with a variety of chlorinating agents giving urs-12-ene in each case (35).

In 1951, Budziarek, Johnston, Manson and Spring (62) showed that oleananyl acetate, which is easily converted into oleanane by classical methods, could be prepared by Kishner-Wolff reduction of 11-keto-oleananyl acetate (64). Thus the first attack on the problem of ursane in this work was directed towards the preparation of urs-10-enyl acetate, which might be oxidised to 11-keto-ursanyl acetate. Attempted catalytic hydrogenation of 12-keto-urs-10-enyl acetate

and 12-keto-urs-10-enol left both compounds unchanged. Kishner-Wolff reduction of 12-keto-urs-10-enyl acetate was then attempted; when carried out in amyl alcohol, this reaction gave no crystallisable product, and in ethanol, gave mainly unchanged starting material in addition to a small amount of a crystalline mixture, whose physical properties indicated the presence of ursa-10:12-dienyl acetate. The formation of the latter is easily explained by the dehydration of an intermediate 12-hydroxy-urs-10-enol (XXXV) during re-acetylation. No further attempts to prepare urs-10-enyl acetate were made.

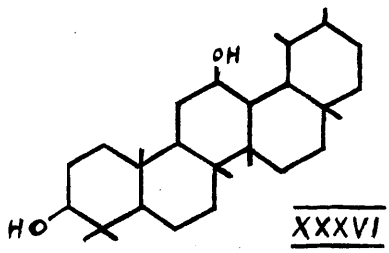


XXXV

In this connection, it is interesting to note that reduction of 11-keto-urs-12-enyl acetate with lithium in liquid ammonia gave, instead of the expected 11-keto-ursanol, 11-hydroxy-urs-12-enol (65).

Lithium aluminium hydride reduction of the p-toluenesulphonates of certain steroids, e.g. 7-hydroxy-cholestane, is known to give the parent saturated hydrocarbon (66). As a first step to the formation of

its p-toluenesulphonyl ester, which might be reduced to ursane, the preparation of 12-hydroxy-ursanol (XXXVI) was examined. This compound was first reported by



XXXVI

Seymour, Sharples and Spring (34), who prepared it by reduction of 12:13-epoxy-ursanyl benzoate with sodium in amyl alcohol. Subsequent preparations by Silverstone (60), using the same reducing agents on 12:13-epoxy-ursanyl benzoate, and on 12-keto-ursanyl esters, gave products whose physical properties were widely different from those of the previous workers, as also were those of the material obtained similarly in this work. These differences are best exemplified by specific rotations, as shown in Table III.

Table III

	Seymour et al	Silverstone		This work
		12:13-epoxy-	12-keto-	
12-Hydroxy-ursanol	+70°	+70°	+44°, +33°*	+50°
12-Acetoxy-ursanyl acetate	+90°	+50°	-	+77°

* Prepared from benzoate and acetate respectively.

Barton (65) also repeated this preparation,

under vigorous equilibrating conditions, and obtained a diol and di-acetate of $[\alpha]_D$ +15° and -4° respectively. The dibenzoyl derivative of the diol agreed in all respects with a sample of 12-benzoxy-ursanyl benzoate prepared by lithium aluminium hydride reduction. Lithium aluminium hydride reduction of 12-keto-ursanyl benzoate was also carried out by Silverstone (60) who obtained a diol of $[\alpha]_D$ +13°, and a di-acetate of $[\alpha]_D$ ±0°.

Drastic reduction of 12-keto-urs-10-enyl acetate with lithium in liquid ammonia gave a 12-hydroxy-ursanol of $[\alpha]_D$ +10°, whose di-acetate had $[\alpha]_D$ -4°, and these, having physical properties in reasonable agreement with the reproducible results of Barton and Silverstone, were taken to be pure diol and di-acetate respectively.

The materials of higher specific rotation obtained previously might be either mixtures of stereoisomers or of starting material and product. The latter hypothesis is unlikely, since Silverstone obtained a material of $[\alpha]_D$ +33° from 12-keto-ursanyl acetate, and both that compound and the pure diol are less dextro-rotatory. The production of a mixture of stereoisomers seems more likely, since reduction under equilibrating conditions gives the pure compound. In an attempt to prepare the missing epimeric 12-hydroxy-ursanol, 12-keto-ursanyl acetate and 12:13-epoxy-ursanyl

acetate were subjected to catalytic hydrogenation techniques, but in neither case was a product obtained. Chromatography of a sample of "12-acetoxy-ursanyl acetate" of $[\alpha]_D$ +65° gave a separation into fractions of higher and lower specific rotation, but no homogeneous material was isolated.

12-Hydroxy-ursanol of $[\alpha]_D$ +10° was treated with p-toluenesulphonyl chloride, but gave a compound which showed a yellow colour with tetranitromethane, suggesting that dehydration had taken place. This was confirmed by preparation of α-amyrin p-toluenesulphonate, which gave no melting point depression when mixed with the above product, and by the observation that 12-hydroxy-ursanol was converted into α-amyrin by treatment with hydrochloric acid. In default of the epimeric 12-hydroxy-ursanol, which might be expected to dehydrate less easily, a sample of material of $[\alpha]_D$ +80°, obtained by chromatography of the sodium/amyl alcohol reduction product from 12:13-epoxy-ursanyl benzoate, was treated with p-toluenesulphonyl chloride. The only compound which could be isolated from the reaction, however, was shown to be α-amyrin p-toluenesulphonate.

Ursa-11:13(18)-dienol

In contrast to the unsaturated centre in β-amyrin, that in α-amyrin is unreactive, and in particular there is a lack of reactivity at the adjacent $C_{(18)}$-atom in α-amyrin. Whereas β-amyrin can be smoothly converted into oleana-10:12-dienol (67), oleana-10:12:18-trienol (33), 11-keto-olean-12-enol (68), and 11-keto-oleana-12:18-dienol (67), corresponding reactions with α-amyrin have been limited to the formation of ursa-10:12-dienol and 11-keto-urs-12-enol (25). Again, β-amyrin acetate is readily oxidised by selenium dioxide to oleana-11:13(18)-dienyl acetate (69, 70) as initial product, further oxidation of which with the same reagent gives 12:19-diketo-oleana-10:13(18)-dienyl acetate (71). Hitherto, attempts to duplicate the selenium dioxide reactions with α-amyrin have been unsuccessful. It has been considered that these results suggest the presence of a methyl group at the $C_{(18)}$-atom in α-amyrin, a hypothesis which is not specifically dismissed by any of the structural evidence available (46, 72). To test this theory, α-amyrin acetate was subjected to selenium dioxide oxidation under conditions more drastic than had been previously employed.

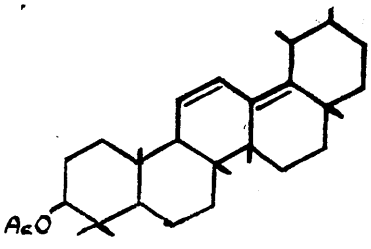
Treatment of α-amyrin acetate with selenium dioxide in dioxan at 200°, or in boiling benzyl acetate (216°) gave a mixture from which, in addition to much

unchanged α-amyrin acetate, a dienyl acetate was isolated in 2-3% yield. The same product was obtained in lower yield by oxidation in boiling glacial acetic acid. That the dienyl acetate originates in α-amyrin, and not in a difficultly separable impurity follows from the observation that similar oxidation of α-amyrin acetate, recovered from a previous treatment with selenium dioxide, gave the same product in the same yield.

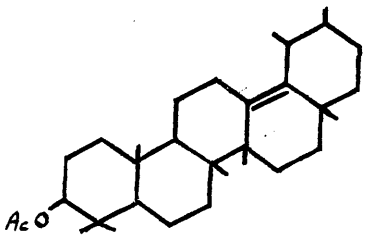
A number of variations in the experimental conditions did not lead to an increase in the yield of the dienyl acetate; in one experiment using boiling diphenyl as solvent, ursa-10:12-dienyl acetate was isolated as sole product. In general, the conclusion could be drawn from these experiments that oxidation below about 220° gives the new dienyl acetate, and above that temperature ursa-10:12-dienyl acetate is formed. Mild sulphur dehydrogenation of α-amyrin acetate has been reported to give ursa-10:12-dienyl acetate (32).

Analytical data agreed with the formula $C_{32}H_{50}O_2$ for the new dienyl acetate. Its properties closely simulated those of oleana-11:13(18)-dienyl acetate, but a mixture of the two compounds showed a marked depression in melting point. In particular, both dienyl acetates show the characteristic ultra-violet spectrum of a transoid hetero-annular diene. The new compound was provisionally formulated as ursa-11:13(18)-dienyl acetate (XXXVII).

Nevertheless, the conversion of α-amyrin



XXXVII



XXXVIII

into an 11:13(18)-dienol supplies no proof of the absence of a $C_{(18)}$-methyl group in the former, since it is possible that the formation of the dienyl acetate involves elimination of such a group, in which case that compound would be a nor-ursa-11:13(18)-dienyl acetate. A decision between the formula $C_{32}H_{50}O_2$ and its lower homologue from micro-analysis is impossible. However, the structure XXXVII may be supported on the ground that more drastic reaction conditions yield ursa-10:12-dienyl acetate, with no loss of a methyl group, and the less drastic conditions giving ursa-11:13(18)-dienyl acetate are hence unlikely to effect such a change.

Ursa-11:13(18)-dienyl acetate was hydrolysed to the parent alcohol, and its physical constants checked by re-acetylation. Catalytic hydrogenation of ursa-11:13(18)-dienyl acetate gave an isomer of α-amyrin, to be formulated as urs-13(18)-enyl acetate (XXXVIII). This is analagous to the formation of olean-13(18)-enyl

acetate by hydrogenation of oleana-11:13(18)-dienyl acetate (73). Halsall (74) has shown that ultra-violet absorption in the region 2000-2250Å gives insight into the state of substitution of an isolated double bond in the triterpene molecule. The ultra-violet spectrum of urs-13(18)-enyl acetate suggests a tetra-substituted double bond similar to that in olean-13(18)-enyl acetate.

Chromium trioxide oxidation of ursa-11:13(18)-dienyl acetate was attempted, using first 4 mols and then 1.05 mols of reagent. In the first case, the amounts of crystalline products obtained were too small for any useful investigation. Using 1.05 mols of oxidising agent, about 40% of the starting material was recovered unchanged, and only a very low yield of crystalline product, probably containing the tetra-substituted double bond and one additional oxygen atom, was isolated. The only conclusion to be drawn from these experiments is that ursa-11:13(18)-dienyl acetate is very easily oxidised to a variety of products. This may explain the low yield in the initial preparation, where the slowly formed dienyl acetate would be rapidly oxidised further, leaving only a small equilibrium concentration.

Treatment of oleana-11:13(18)-dienyl acetate with N-bromsuccinimide gives oleana-10:12:18-trienyl acetate (33). From an attempt to reproduce this

reaction in the α-amyrin series, no such product was obtained; a large amount of unchanged starting material was recovered.

Other methods for the preparation of ursa-11:13(18)-dienyl acetate, not involving possible loss of a methyl group, suggested themselves. Clemmensen reduction of iso-β-amyradienonyl acetate gives oleana-11:13(18)-dienyl acetate in good yield (62); while iso-α-amyradienonyl acetate is known to undergo isomerisation in presence of hydrogen chloride (37), it was decided to attempt its reduction under Clemmensen conditions chosen to minimise isomerisation. However, no identifiable product could be isolated from this reaction.

From the theory of hyperconjugation, first applied to triterpenes by Barton and Brooks (70), it appears that ursa-10:12-dienyl acetate, having only one hyperconjugating carbon-hydrogen bond, should be less stable than ursa-11:13(18)-dienyl acetate, which has two, and might be converted into it by acid treatment. Such reactions involving conversion of a homoannular to a heteroannular diene are well known in steroid chemistry (75). Nevertheless, ursa-10:12-dienyl acetate was unaffected by either sulphuric or hydrochloric acid. It was also shown that ursa-11:13(18)-dienyl acetate was not isomerised by hydrogen chloride.

Acid conditions cause isomerisation of 2-keto-

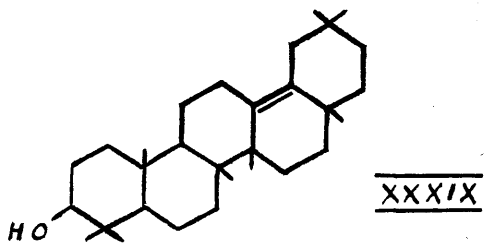
olean-12-ene to 2-keto-olean-13(18)-ene (51), and of β-amyrin acetate to olean-13(18)-enyl acetate (76). Although a similar attempt to rearrange 2-keto-urs-12-ene had failed (51), α-amyrin acetate was treated with sulphuric acid under different conditions. However, only unchanged starting material was recovered.

Since chemical methods had failed to show that ursa-11:13(18)-dienyl acetate retained the carbon skeleton of α-amyrin, physical methods for attaining this object were considered. The only one of these which appeared to be practicable was determination of the equivalent of the half-succinate. A model experiment was carried out on α-amyrin, using the method of Heusser and Werthier (77) for the preparation of succinyl derivatives of steroids. α-Amyrin half-succinate ($C_{30}H_{49}O.OC(CH_2)_2COOH$) was prepared and characterised, and its equivalent determined by micro-titration against 0.1N sodium hydroxide solution. The margin of error, however, was found to be rather greater than the 14 parts in 500 required to distinguish between ursa-11:13(18)-dienyl half-succinate and its lower homologue. Accordingly, ursa-11:13(18)-dienyl half-succinate was not prepared.

In conclusion, it may be said that while it was not possible to prove that the new dienyl acetate was in fact ursa-11:13(18)-dienyl acetate, and not a

nor-ursa-11:13(18)-dienyl acetate, the similar formation of ursa-10:12-dienyl acetate is in favour of the former formulation, and hence of the absence of a $C_{(18)}$-methyl group in α-amyrin.

Whichever of these formulations is true, the results obtained are of interest in the light of the structural proposals of Tschesche and Fugmann (78), who sought to explain the differences in reactivity between α- and β-amyrin by postulating the existence of a di-tertiary double bond in the former compound. The proposed structure for α-amyrin is that shown in XXXIX,



XXXIX

which would then differ from olean-13(18)-enol only in the configuration at $C_{(17)}$, as shown by Jeger (52). This structure was supported by new interpretations of the reactions of α-amyrin, but these are not altogether satisfactory, especially in the case of peroxide oxidation, where a 13:18-epoxide was postulated, and thought to rearrange to a 12-ketone; the mechanism adduced to account for this is open to doubt.

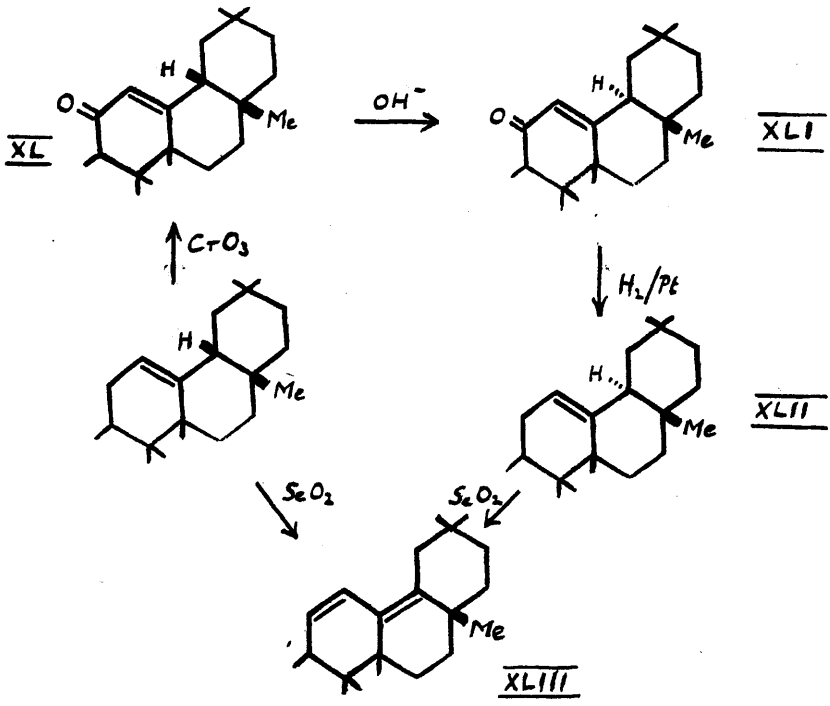
The new formulation is not easy to reconcile with the spectroscopic results of Halsall (74), and with

the production of acetone on chromium trioxide oxidation of β-amyrin acetate only (79). In addition, while a compound of the structure XXXIX could be oxidised to a transoid hetero-annular diene, the hydrogenation product from that diene, being different from α-amyrin, would have to possess either a $C_{(11)}$-$C_{(12)}$ or a $C_{(12)}$-$C_{(13)}$ double bond. Neither of these is likely, in view of the close correspondence in physical properties between α-and β-amyrin, and between urs-13(18)-enyl acetate and olean-13(18)-enyl acetate; also, catalytic reduction to give a mono-ene of the type required is unknown in triterpene chemistry, and seems precluded by steric considerations.

An account of the salient features of the above work has already been published (80).

The Stereochemistry of α-Amyrin Derivatives

It has been shown that in β-amyrin the junction of rings D and E is in the less stable cis-configuration, since 11-keto-olean-12-enol (XL) is epimerised by alkali (79), as also is methyl 11-keto-olean-12-enolate acetate (46). The epimeric 11-keto-olean-12-enyl acetate (XLI) may be hydrogenated to give an isomer (XLII)



of β-amyrin, which, on selenium dioxide oxidation, is converted into the known oleana-11:13(18)-dienyl acetate (XLIII). This chain of reactions, and a similar one in the oleanolic acid series, show that epimerisation occurs at C only.
(18)

11-Keto-urs-12-enyl acetate was prepared by chromium trioxide oxidation of α-amyrin acetate (25).

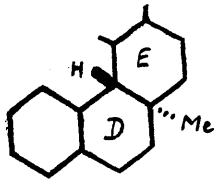
It was confirmed that this compound could not be brominated under normal conditions, a finding explained on grounds of steric hindrance by the methyl group attached to $C_{(19)}$. 11-Keto-urs-12-enyl acetate was then subjected to prolonged treatment with strong alkali, and was recovered unchanged; this observation indicates that the junction of rings D and E is in the more stable configuration in this case. In order to confirm that enolisation of 11-keto-urs-12-enyl acetate affects $C_{(18)}$, its enol acetate was sought, but it was not formed, even after refluxing for 72 hours with sodium acetate and acetic anhydride.

Evidence of the stability of the D/E ring junction in ursolic acid was given by Barton and Holness (46), who found that reaction of methyl 11-keto-urs-12-enolate acetate with either acid or base failed to cause isomerisation. They concluded that either the $C_{(18)}$ configuration was in the more stable arrangement, or that there was no hydrogen attached to $C_{(18)}$ in ursolic acid. The possibility that a methyl group is attached to $C_{(18)}$ in α-amyrin has already been discussed (see above) and this seems less likely than the presence of a hydrogen atom at that point.

Jeger (52) had already suggested that the methyl group attached to $C_{(17)}$ was opposite in configuration in the two amyrins, from degradations leading to two analogous

compounds of equal and opposite optical rotation, and apparently containing the original $C_{(17)}$ of the amyrins as the only asymmetric centre. While this work was not described in sufficient detail for a critical analysis to be made, there is at present no reason to doubt the conclusion.

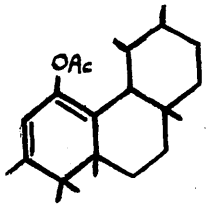
On applying this in the light of Barton's stereochemical formula for β-amyrin (50), the D/E ring junction in α-amyrin may be assigned the configuration



XLIV

shown in XLIV, in which the hydrogen atom attached to $C_{(18)}$ is on the same side of the molecule as the methyl group at $C_{(5)}$.

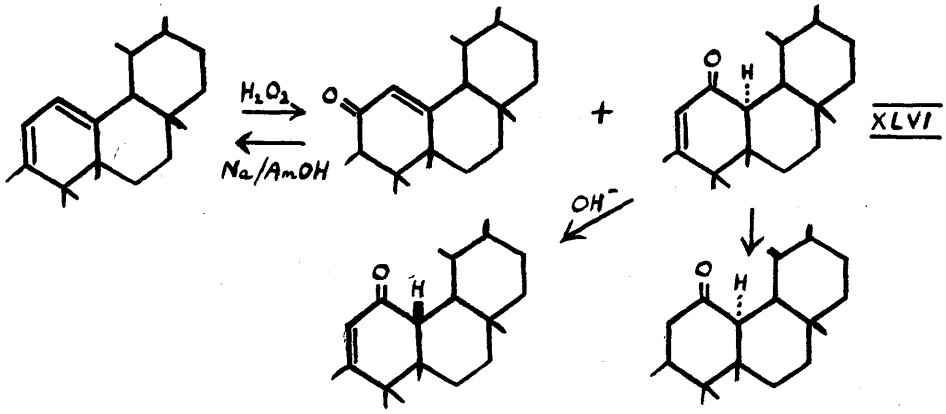
Attention was then turned to the configuration at $C_{(13)}$ in 12-keto-urs-10-enyl acetate, and it was found that this compound was also unaffected by strong alkali. Its enol acetate was formed, and shown to possess the 10:12-diene structure (XLV) as its ultra-violet absorption spectrum was that characteristic of a



XLV

homoannular diene. The stability at $C_{(13)}$ was confirmed by the recovery of 12-keto-urs-10-enyl acetate on hydrolysis of its enol acetate.

Oxidation of ursa-10:12-dienyl benzoate with hydrogen peroxide gives a mixture of 11-keto-urs-12-enyl-benzoate and another $\alpha\beta$-unsaturated ketone which may be converted into 12-keto-urs-10-enol by alkali treatment. Reduction of this new $\alpha\beta$-unsaturated ketone with sodium and amyl alcohol yields ursa-10:12-dienol, and catalytic hydrogenation of its acetate gives a saturated ketone, isomeric with 12-keto-ursanyl acetate, but not identical with it. This work was carried out by McLean, Ruff and Spring (61), who suggested that the



new $\alpha\beta$-unsaturated ketone (XLVI) differed from 12-keto-urs-10-enol only in the orientation of the hydrogen atom at $C_{(13)}$, and referred to it as <u>iso</u>-α-amyrenonol II.

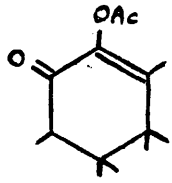
The peroxide oxidation of ursa-10:12-dienyl benzoate was repeated and substantially similar results

obtained, although iso-α-amyrenonyl II benzoate isolated in much lower yield than previously reported. iso-α-Amyrenonyl II benzoate was smoothly converted into 12-keto-urs-10-enol on vigorous alkaline hydrolysis, and on mild hydrolysis gave an alcohol which was acetylated to give iso-α-amyrenonyl II acetate. The enol acetate of iso-α-amyrenonyl II acetate was prepared, and found to be identical with that of 12-keto-urs-10-enyl acetate. This appears to confirm that the new αβ-unsaturated ketone is the $C_{(13)}$-epimer of 12-keto-urs-10-enol, and the name 13-iso-12-keto-urs-10-enol is suggested.

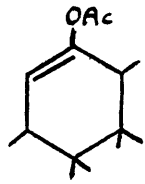
13-iso-12-Keto-urs-10-enyl acetate was hydrogenated, and gave α-amyranonyl II acetate, which will be referred to as iso-12-keto-ursanyl acetate. This was found to differ somewhat in melting point from that published. The presence of a strong band at 1696 $cm.^{-1}$ in its infra-red absorption spectrum confirmed that this compound contained a carbonyl group. iso-12-Keto-ursanyl acetate was brominated and dehydrobrominated under the conditions used for the introduction of the $C_{(10)}$-$C_{(11)}$ double bond into 12-keto-ursanyl acetate. 12-Keto-urs-10-enyl acetate was obtained as sole product; this is not unexpected, since epimerisation at $C_{(13)}$ in either starting material or initially formed 13-iso-12-keto-urs-10-enyl acetate could occur in the presence of hydrogen bromide.

The enol acetate of iso-12-keto-ursanyl acetate was then prepared, and, surprisingly, found to be different from the known enol acetate of 12-keto-ursanyl acetate (35). On hydrolysis, the parent saturated ketone was recovered unchanged. 12-Keto-ursanyl acetate, with the opposite configuration at $C_{(13)}$ was also obtained by hydrolysis of its enol acetate.

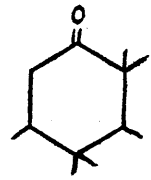
Two explanations of this seeming anomaly are possible. Enolisation of the ketone unstably oriented at $C_{(13)}$, or of both ketones, may lead to the formation of a $C_{(11)}$-$C_{(12)}$ double bond, rather than one between $C_{(12)}$ and $C_{(13)}$. However, chromium trioxide oxidation of the enol acetate of 12-keto-ursanyl acetate gives a



XLVII



XLVIII

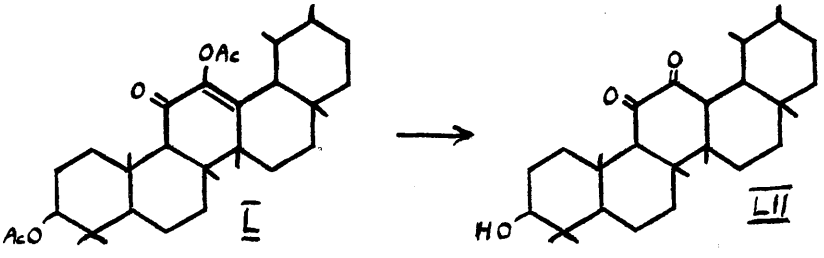


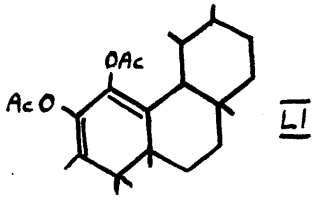
IL

compound formulated as in XLVII (60, 59), which is unlikely to arise if the starting material has the structure XLVIII. It will be later shown that a saturated ketone of the structure IL fails to form an enol acetate, an improbable result if enolisation towards $C_{(11)}$ is possible. Finally, the ultra-violet absorption spectra of the enol acetates of both 12-keto-ursanyl acetate and iso-12-keto-ursanyl acetate are very similar, and suggestive of tri-substituted double bonds (74, 81).

The alternative possibility is that saturation of the $C_{(10)}$-$C_{(11)}$ double bond of 13-iso-12-keto-urs-10-enyl acetate leads to the opposite configuration at $C_{(10)}$ from that in 12-keto-ursanyl acetate, and that that difference reverses the stability of the orientation at $C_{(13)}$.

This second hypothesis, although less simple, appeared the more probable, and directed attention to $C_{(10)}$ of α-amyrin derivatives. 12-Acetoxy-11-keto-urs-12-enyl acetate (L) was prepared by chromium trioxide oxidation of the enol acetate of 12-keto-ursanyl acetate (60). An attempt to form the di-enol di-acetate (LI)

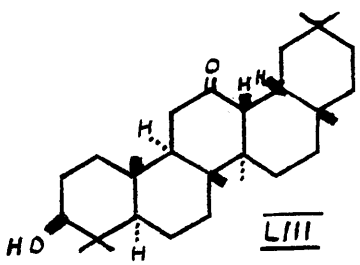




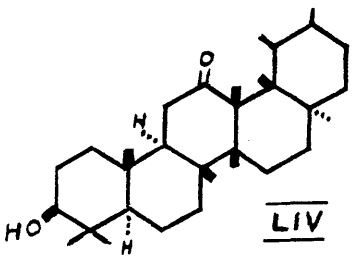
of this was unsuccessful, but prolonged alkali treatment of L gave 11:12-diketo-ursanol (LII) identical with that obtained on mild hydrolysis (60), thus indicating stability of configuration at $C_{(10)}$. The enol acetate of iso-12-keto-ursanyl acetate was oxidised with chromium trioxide,

with the object of preparing an iso-12-acetoxy-11-keto-urs-12-enyl acetate. The only crystalline product obtained, however, was a small amount of 13-iso-12-keto-urs-10-enyl acetate, whose identity was confirmed by conversion into 12-keto-urs-10-enol.

If the hypothesis that 12-keto-ursanyl acetate and iso-12-keto-ursanyl acetate differ both at $C_{(10)}$ and $C_{(13)}$ is correct, the fact that both are stable is significant. On application of the rules formulated by Johnson (47) for perhydrophenanthrenes, based on the work of Linstead and his collaborators (48), it appears that this common stability can only be explained by postulating a cis-relationship between the substituents at $C_{(9)}$ and $C_{(14)}$. As was mentioned in the Historical Section, configurations at $C_{(2)}$, $C_{(5)}$, $C_{(6)}$ and $C_{(9)}$ have been shown to be the same in both amyrins, with a possibility that this similarity extends to $C_{(10)}$ and $C_{(14)}$. By applying all these data, in the light of the known stereochemistry of 12-keto-oleananol (LIII), and making the necessary assumption that the two compounds differ in configuration at $C_{(14)}$, the hypothetical stereochemical

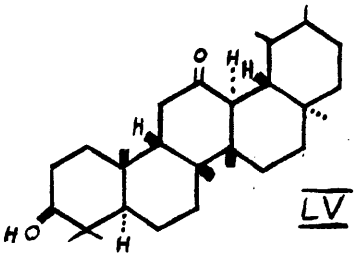


LIII



LIV

formula LIV may be put forward for 12-keto-ursanyl acetate. The situation at the junction of rings D and E has been discussed earlier. The hypothetical $C_{(13)}$ epimer of 12-keto-ursanol would then possess the thermodynamically unstable cyclohexane boat form in ring C, as this would be linked trans-syn-trans with its neighbours. iso-12-Keto-ursanol would be represented by LV, and epimerisation at $C_{(13)}$ would again



LV

lead to an unfavoured form in ring C.

The view that configurations at $C_{(14)}$ in both amyrins are the same is based on the retropinacolinic rearrangement thought to be common to the formation of both iso-amyradienonyl acetates (40). However, similarity of configuration at $C_{(10)}$ in α- and β-amyrin was inferred from comparison of optical rotations, and it is the contention of the author of this thesis that the application of this method to $C_{(14)}$ leads to the opposite conclusion (see Table 3). If α- and β-amyrin differ stereochemically at two asymmetric centres, and have similar optical rotations, then the rotational contribution of these two centres must be approximately

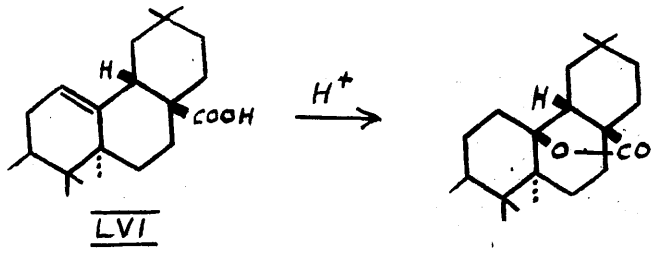
equal and opposite, and removal of one of them will lead to a difference in optical rotation between the two series. Such differences are postulated in formulae LIII and LIV, and appear to be demonstrated in Table 3.

Table 3

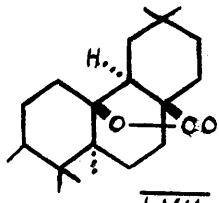
Functional Groups (in addition to 2-acetoxy)	Specific Rotations	
	α	β
Δ 12-13	+79° (a)	+83° (c)
12-keto-Δ10-11	+84° (a)	+61° (b)
Δ10-11, 12-13	+315° (a)	+331° (d)
12-acetoxy	-4° (a)	+11° (e)
Δ11-12, 13-18	-79° (a)	-62° (b)
12-keto-Δ10-11, 14-15	+7° (a)	-40° (b)

References: (a) this work; (b) (62); (c) (79); (d) (82); (e) (46).

Barton and Holness have succeeded in equilibrating ursolic acid with its hitherto unknown lactone by the action of hydrogen chloride; this is similar to the behaviour of oleanolic acid (LVI), but is in contrast to that of the more thermodynamically stable 18-iso-oleanolic acid, which is converted irreversibly into its lactone (LVII) (46). This suggests that while ursolic acid itself possesses a stable

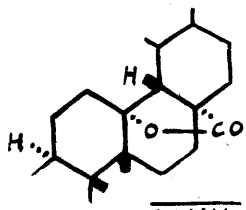


LVI

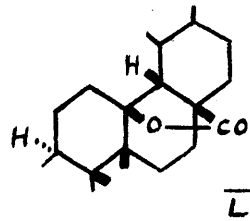


LVII

configuration, its lactone is in some way unstably orientated, unlike the lactone of 18-iso- oleanolic acid. If structure LIV is accepted, it will be seen that lactonisation requires that the substituent at either $C_{(13)}$ or $C_{(17)}$ should have the opposite configuration from that shown, in either case leading to an unstable arrangement, as in LVIII or LIX.



LVIII



LIX

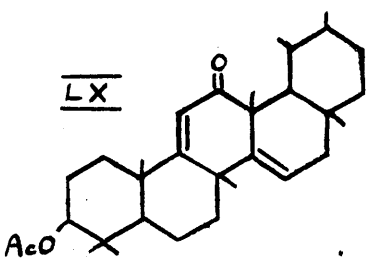
The weakness of the argument leading to LIV lies in the application of rules deduced from perhydrophenanthrenes to the central rings of triterpenes, which is perhaps unjustified. In addition, structure LIV

suggests that a cis-syn-trans system in rings C,D and E of 12-keto-urs-10-enol is more stable than a similar trans-anti-trans system in 13-iso-12-keto-urs-10-enol, although this might be rendered possible by the presence of the double bond. However, the hypothesis of structure LIV is put forward by the author as a possible basis for further investigation.

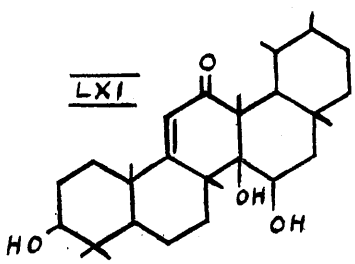
iso-α-Amyradienonyl Acetate

iso-α-Amyradienonyl acetate may be catalytically hydrogenated to give 12-keto-urs-10-enyl acetate, which is known to contain the carbon skeleton of α-amyrin (37). This suggests that iso-α-amyradienonyl acetate also possesses the carbon skeleton of α-amyrin (61), a conclusion which is in direct contradiction to the formulation LX for iso-α-amyradienonyl acetate, deduced from other chemical evidence (see Historical Section).

LX



LXI



At the time of commencement of this work, published details of the investigation of the chemistry of iso-α-amyradienonyl acetate had been confined to the

catalytic reduction quoted above, its rearrangement to iso-α-amyradienonyl II acetate with hydrogen chloride, and the formation of the triol LXI by oxidation with osmium tetroxide. (In the interests of clarity, the postulated structure LX for iso-α-amyradienonyl acetate will be used for formulation of its derivatives). This triol had been further degraded to the products of pyrolytic fission described in the Historical Section. Since these products had been used as evidence in favour of the commonly accepted structure of α-amyrin, any doubt thrown on the formulation of iso-α-amyradienonyl acetate was reflected in uncertainty about that of α-amyrin itself. Accordingly, it was considered that a further investigation into the chemistry of iso-α-amyradienonyl acetate was necessary, especially as regards its behaviour towards reducing agents.

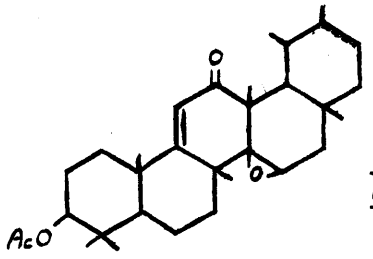
12-Keto-urs-10-enyl acetate was oxidised with selenium dioxide by a method slightly different from that of the original preparation (37), but more suitable for large-scale work. The product obtained showed no reproducibility in melting point over successive preparations, and was chromatographed to give a compound, unchanged on further chromatography, and having m.p. 221-222°, $[\alpha]_D$+7°, which appears to be pure iso-α-amyradienonyl acetate. The original publication reports that iso-α-amyradienonyl acetate has m.p. 217-218°, $[\alpha]_D$+14°. In

addition to this, a mixture having considerably higher melting point and dextro-rotation was obtained from the column. This is thought to be a mixture of iso-α-amyradienonyl acetate and its isomer, iso-α-amyradienonyl II acetate, a hypothesis supported by its ultra-violet spectrum, and by the fact that the two isomers do not give a mixed melting point depression. Furthermore, pure iso-α-amyradienonyl II acetate was obtained by subjecting the mixture to the action of hydrogen chloride. Confirmation of the production of this mixture on selenium dioxide oxidation of 12-keto-urs-10-enyl acetate was later provided independently by Vogel, working at Zürich. (83).

The parent alcohols of both iso-α-amyradienonyl acetate and its isomer were prepared and characterised, as this did not appear to have been done before. The physical constants of the acetates were confirmed by re-acetylation of the alcohols.

The existence of the isolated double bond of iso-α-amyradienonyl acetate, which is suggested by its colour reaction with tetranitromethane was then examined. It was discovered that the ultra-violet absorption spectrum of iso-α-amyradienonyl acetate shows, in addition to the maximum at 2370Å (ε = 9,500), another at 2100Å (ε = 6,100), indicative of a double bond; such a maximum is absent from the ultra-violet absorption

spectrum of iso-α-amyradienonyl II acetate. Since this double bond absorption is superimposed on that due to the αβ-unsaturated ketone group, its intensity gives no proof of the state of substitution of the double bond. However, an attempt to allocate the absorption at 2100Å between the two chromophores showed that the increment apparently due to the isolated ethylenic linkage was not inconsistent with its tri-substituted state, as shown in LX. Oxidation of iso-α-amyradienonyl acetate with potassium permanganate gave a compound containing one additional oxygen atom. Whether the additional oxygen is present as oxide or ketone is not knwwn, but an analogous permanganate oxidation product from iso-β-amyradienonyl acetate, which is undoubtedly an oxide (84), has similar physical properties, and there is no reason to believe that structure LXII does not represent the product from iso-α-amyradienonyl acetate. This compound



LXII

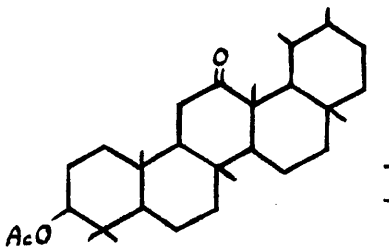
gives no colour reaction with tetranitromethane, and its ultra-violet absorption spectrum shows a maximum at 2400Å only, confirming that the maximum at 2100Å in the spectrum of iso-α-amyradienonyl acetate is due to

the presence of an isolated double bond, which may be removed by oxidation.

It will be noted that the absorption maximum due to the $\alpha\beta$-unsaturated ketone grouping in iso-α-amyradienonyl acetate is at 2370Å, 130Å lower than that of 12-keto-urs-10-enyl acetate, which would appear to contain the same chrompophore. However, if the theoretical position of maximum ultra-violet absorption for that chromophore, containing two β-substituents is calculated according to Woodward's rules (85), it is found to be at 2390Å. Consequently, while this shift cannot yet be explained, it is not evidence against the postulated formulation of iso-α-amyradienonyl acetate.

The catalytic hydrogenation of iso-α-amyradienonyl acetate was next studied, since this reaction is the crux of the evidence against the accepted structure LX. In the published work of Ruzicka and his collaborators, it is reported that catalytic hydrogenation of iso-α-amyradienonyl acetate yields both 12-keto-urs-10-enyl acetate and ursa-10:12-dienyl acetate (37). This experiment was later repeated in Zürich (83), and rather different results obtained. Very carefully purified iso-α-amyradienonyl acetate, whose physical contents agree well with those determined in this work, was dissolved in glacial acetic acid,

shaken with hydrogen over platinum, the the product chromatographed. A low yield of 12-keto-urs-10-enyl acetate was obtained, in addition to a new saturated ketone different from any previously prepared from α-amyrin. This ketone, which was also obtained in

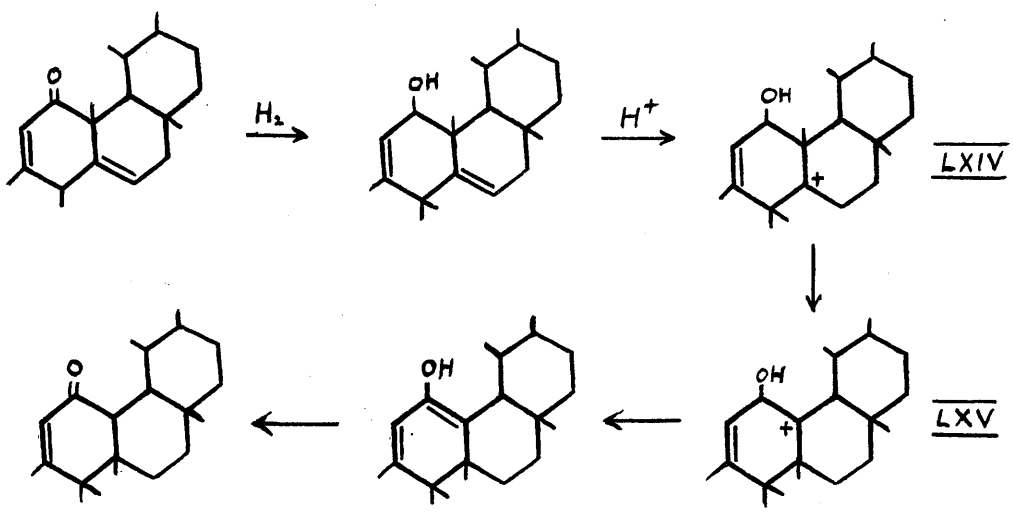


LXIII

very low yield, was assigned the structure LXIII.

This reaction was repeated with carefully purified materials, and similar results obtained. The hydrogenation product gave a yield of 6% of 12-keto-urs-10-enyl acetate, and after three successive chromatographic separations, 3% of the new saturated ketone. The remainder of the product was an intractable mixture. Attempts to hydrogenate iso-α-amyradienohyl acetate in neutral and in alkaline solution left the molecule unattacked. iso-α-Amyradienonyl acetate was then hydrogenated in glacial acetic acid containing a trace of hydrochloric acid, and, after chromatography, a 15% yield of 12-keto-urs-10-enyl acetate was isolated, in addition to material containing a high proportion of ursa-10:12-dienyl acetate, as shown by ultra-violet spectroscopy.

These results suggest that the conversion of iso-α-amyradienonyl acetate into 12-keto-urs-10-enyl acetate is dependant on the presence of hydrogen ions, a theory which is quite consistent with methyl group migration, as in the reaction mechanism shown.



The intermediate LXIV undergoes a carbonium ion rearrangement to LXV, which then rearranges to the enolic form of 12-keto-urs-10-enyl acetate.

The observation that iso-α-amyradienonyl acetate was recovered unchanged after shaking with platinum catalyst in glacial acetic acid without hydrogen (61) is not inconsistent with the above mechanism, since rearrangement only takes place after reduction of the carbonyl group.

It was later shown (see below) that iso-α-amyradienonyl II acetate, formed by acid treatment of iso-α-amyradienonyl acetate, may be catalytically

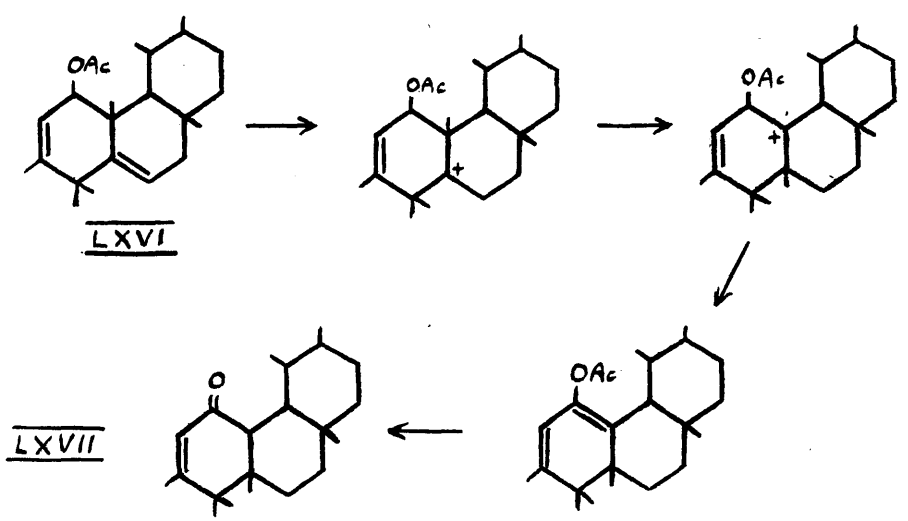
hydrogenated to a diene-type compound, which easily rearranges to ursa-10:12-dienyl acetate. This provides an explanation of the appearance of that compound among the products of catalytic hydrogenation of iso-α-amyradienonyl acetate. In the case of the first published experiment, the iso-α-amyradienonyl II acetate may have been either produced in the course of the reaction, or present in the starting material, as it had not then been recognised as a product of selenium dioxide oxidation of 12-keto-urs-10-enyl acetate.

Attention was then turned to experiments connected with the presence of a methyl group at C in iso-α-amyradienonyl acetate. It was discovered that (13) although 12-keto-urs-10-enyl acetate formed an enol acetate with comparitive ease (see above), iso-α-amyradienonyl acetate was recovered unchanged from prolonged treatment with both acetic anhydride and sodium acetate, and iso-propenyl acetate. The latter is a reagent comparatively recently introduced, which has been found more effective for forming enol acetates than classical methods (86), and which in some cases gives products differing in double bond position from those obtained by these methods (87). When the enol acetate of 12-keto-urs-10-enyl acetate was treated with selenium dioxide, again with the object of forming iso-α-amyradienonyl acetate enol acetate, the only

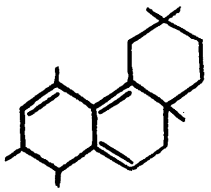
product isolated was iso-α-amyradienonyl acetate itself; hydrolysis of the original enol acetate, followed by oxidation, would account for this. iso-β-Amyradienonyl acetate has been prepared in an analogous way (62).

The presence of a carbonyl group in the saturated ketone LXIII was confirmed by its strong infra-red absorption at 1703 $cm.^{-1}$, but this compound also was recovered unchanged from prolonged treatment with sodium acetate and acetic anhydride; the enol acetate of 12-keto-ursanyl acetate is well known (35).

Reduction of iso-α-amyradienonyl acetate with lithium aluminium hydride, followed by acetylation of the product, gave a compound possessing two isolated double bonds, as shown by the tetranitromethane test and ultra-violet absorption. This compound was assigned the structure LXVI. The analogous compound formed by



similar reduction of iso-β-amyradienonyl acetate gives a triene on treatment with acid, which has been formulated as in LXVIII (84). On treating LXVI with



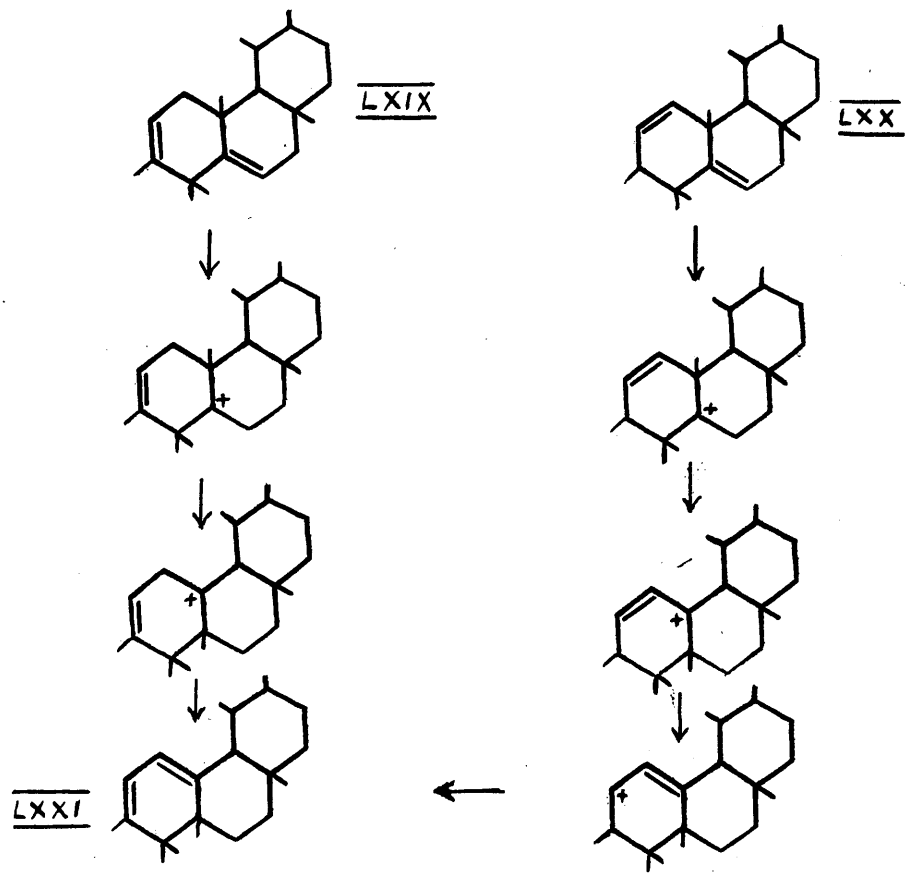
LXVIII

acid, however, the only product isolated was 12-keto-urs-10-enyl acetate (LXVII), in very low yield. This result is not unexpected if the reaction mechanism put forward for the catalytic reduction and rearrangement of iso-α-amyradienonyl acetate (p. 54) is correct, and may be achieved through similar stages, as shown above. The di-acetate LXVI was found to be identical with a compound prepared by reduction of iso-α-amyradienonyl acetate with sodium in amyl alcohol, and subsequent acetylation. This preparation had been earlier reported by Ruff (88), who, however, considered that the product had only one double bond.

Clemmensen reduction of iso-β-amyradienonyl acetate goes smoothly to produce oleana-11:13(18)-dienyl acetate (62), but similar treatment of iso-α-amyradienonyl acetate gave no identifiable product (see above).

Kishner-Wolff reduction of iso-α-amyradienonyl acetate gave a compound which showed a yellow colour

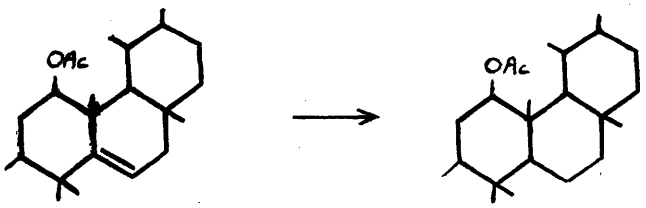
with tetranitromethane, and ultra-violet absorption consonant with the presence of two unconjugated ethylenic linkages. This compound was resistant to catalytic hydrogenation, but rearranged in the presence of hydrogen chloride to give ursa-10:12-dienyl acetate.



Two structures, LXIX and LXX are possible for the Kishner-Wolff product, either of which could give the homoannular diene by carbonium ion rearrangement, as shown. A decision between these is not possible at present, although on grounds of simplicity LXIX appears preferable.

It has been reported that reduction of αβ-unsaturated ketones with lithium in liquid ammonia can lead to one of two products, either the correspond -ing saturated ketone, or, if alcohol is present in the reaction mixture, the saturated hydroxy-compound being formed (89). It was found in this work that the hydroxy-compound could be produced, in the absence of alcohol, merely by prolonging the reaction (cf. p.27). Such reductions were carried out on iso-α-amyradienonyl acetate to give both possible products.

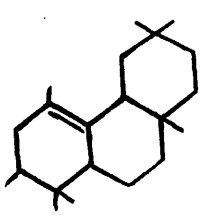
Prolonged reduction of iso-α-amyradienonyl acetate with lithium in liquid ammonia, followed by acetylation, gave a product whose ultra-violet spectrum and reaction with tetranitromethane were suggestive of an isolated double bond. To this compound the structure LXXII was ascribed, a conclusion supported by analysis,



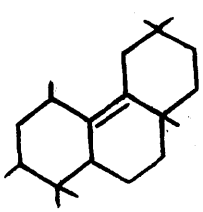
LXXII LXXIII

and by its infra-red spectrum. Catalytic hydrogenation of LXXII gave a saturated di-acetate, presumably LXXIII, which was shown to be different from 12-acetoxy-ursanyl acetate. This difference cannot be explained on a basis of the stereochemistry at C(12) alone, since

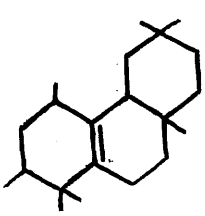
mixtures of 12-acetoxy-ursanyl acetate, and its unknown $C_{(12)}$ epimer having a higher dextro-rotation than both 12-acetoxy-ursanyl acetate and the di-acetate LXXIII are known (see p. 26). The di-acetate LXXIII was treated with hydrochloric acid and gave a compound isomeric with α-amyrin acetate, but not identical with it, nor with urs-13(18)-enyl acetate. This compound is notable in that it gives an orange-red colour with tetranitromethane, but has the ultra-violet absorption characteristic of an isolated tetra-substituted double bond. The only known compound which has these properties is the product of catalytic hydrogenation of iso-β-amyradienonyl acetate, neo-β-amyrin. The structure of neo-β-amyrin has been closely studied, and there is evidence that it possesses one of the structures LXXIV, LXXV or LXXVI, most probably the latter.



LXXIV

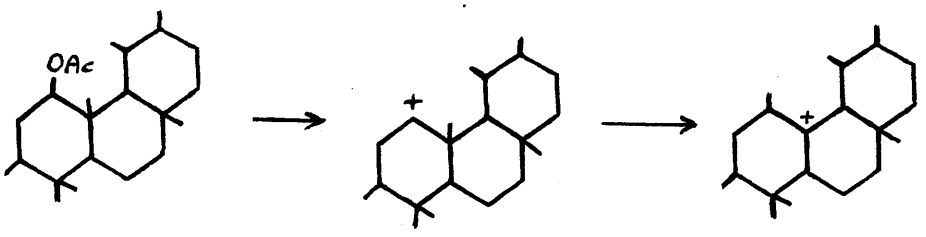


LXXV



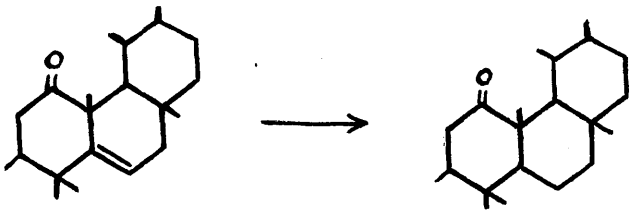
LXXVI

Whichever of these formulations is correct, the formation of an analogous neo-α-amyrin from the di-acetate LXXIII would proceed as shown to give the carbonium ion LXXVII, which could give rise to any of them.



LXXIII LXXVII

Limited reduction of iso-α-amyradienonyl acetate with lithium in liquid ammonia, followed by hydrolysis, gave a product which showed an infra-red absorption maximum at 1690 $cm.^{-1}$, indicative of a carbonyl group. Its ultra-violet absorption spectrum was that of a mono-ethylenic compound, in which the double bond was unconjugated with any other group, and the structure LXXVIII was considered probable. Catalytic hydrogenation of LXXVIII, after acetylation,



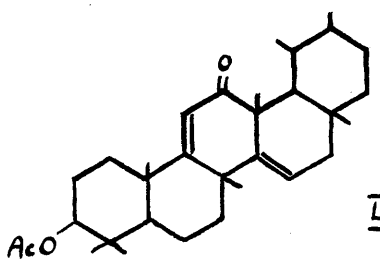
LXXVIII LXXIX

gave a saturated ketone, (LXXIX) identical with that obtained by catalytic hydrogenation of iso-α-amyradienonyl acetate.

This saturated ketone, and the di-acetate LXXIII, are compounds in which the functional groups are similarly

placed to those in known derivatives of α-amyrin; hence the two new compounds cannot have the carbon skeleton of α-amyrin. This difference in structure might be stereochemical, at $C_{(14)}$ and/or $C_{(10)}$, in which case iso-α-amyradienonyl acetate could still have the carbon skeleton of α-amyrin, but this is not in agreement with the formation of 'neo-α-amyrin' from the di-acetate, nor with the resistance of the ketone to enol acetylation.

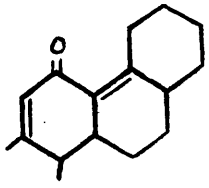
Thus the three lines of attack on the problem, hydrogenation, other reduction reactions, and enol acetylation, all suggest that iso-α-amyradienonyl acetate does in fact possess a methyl group at $C_{(13)}$, a postulate originally necessary to explain its degradation reactions. While incontrovertible proof of the position of this methyl gro up has not been found, it is the opinion of the author that the weight of evidence is overwhelmingly in favour of the original structure LXXX.



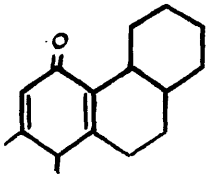
LXXX

iso-α-Amyradienonyl II Acetate

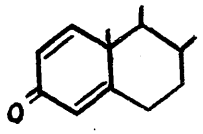
When iso-α-amyradienonyl acetate was first prepared, and the presence of an isolated double bond suspected, it was realised that that double bond would tend to move into conjugation with the αβ-unsaturated ketone group also present, under the action of acidic reagents. On subjecting iso-α-amyradienonyl acetate in glacial acetic acid solution, to treatment with dry hydrogen chloride, a new compound was formed, isomeric with the starting material, but which did not give a colour reaction with tetranitromethane (37). This product, referred to as iso-α-amyradienonyl II acetate, was taken to be a conjugated dienone, and partial formulations LXXXI or LXXXII tentatively suggested.



LXXXI



LXXXII



LXXXIII

The ultra-violet absorption spectrum of this compound, showing a maximum at 2370Å (ε=11,000) only is notably similar to that of $\Delta^{1,4}$ cholestadiene-3-one (LXXXIII), which has maximum absorption at 2360Å (ε=16,000) (85). It is interesting to note that iso-β-amyradienonyl acetate is unaffected by acidic reagents (62).

In view of the problem discussed in the

previous section, regarding the presence or absence of a methyl group at $C_{(13)}$ in iso-α-amyradienonyl acetate, it was considered that the structural chemistry of iso-α-amyradienonyl II acetate required further investigation. The only work known to have been carried out on this compound since the original preparation was that of Ruff (88). This worker reduced iso-α-amyradienonyl II acetate with sodium in amyl alcohol, acetylated the product by refluxing in acetic anhydride, and obtained a compound which he called deoxy-iso-α-amyradienonyl II acetate, and which appeared to contain a homoannular diene chromophore, with an additional double bond. Catalytic hydrogenation of this gave what was apparently an unconjugated diene, referred to as iso-α-amyradienyl acetate.

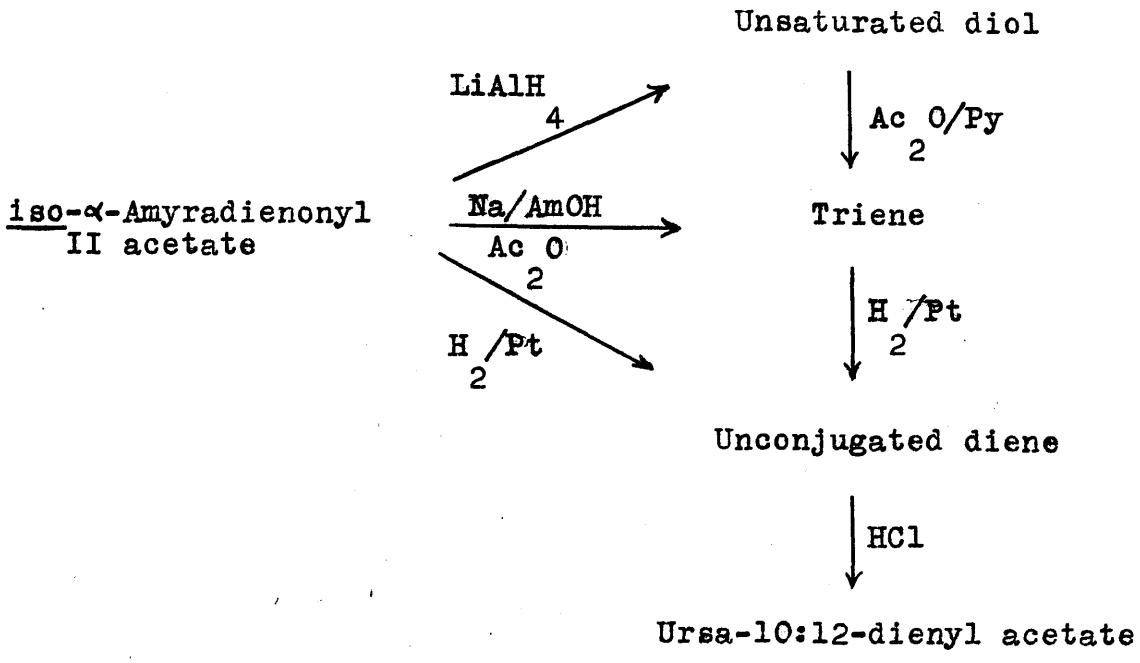
The sodium and amyl alcohol reduction was repeated, and substantially similar results obtained. The product was very strongly laevo-rotatory, showed maximum ultra-violet absorption at 2220 (ε=10,000) and 2800Å (ε=7,700), and gave a deep red colour with tetranitromethane. Catalytic hydrogenation of this compound reduced the conjugated system, and gave a product showing yellow tetranitromethane colour, and having the ultra-violet absorption spectrum expected of an unconjugated diene.

Lithium aluminium hydride reduction of iso-

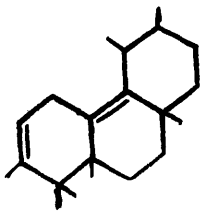
α-amyradienonyl II acetate, and subsequent hydrolysis, gave a diol apparently formed by reduction of the original carbonyl to a hydroxyl group, and showing ultra-violet absorption in agreement with this. Acetylation of this diol in acetic anhydride and pyridine on the steam bath effected dehydration, and the same triene acetate as obtained previously was produced. It appeared that this triene grouping might be reproduced in the unknown enol acetate of iso-α-amyradienonyl II acetate, but that compound could not be formed by sodium acetate and acetic anhydride, nor by iso-propenyl acetate treatment.

Catalytic hydrogenation of iso-α-amyradienonyl II acetate was then carried out, and it was found that hydrogenolysis occurred, to give the same apparently unconjugated diene as described above. On treatment of this diene with hydrogen chloride, ursa-10:12-dienyl acetate was formed.

The inter-relationship of these reactions is shown below.



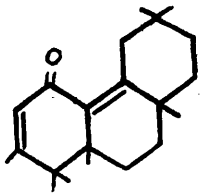
The fact that the unconjugated diene rearranges to ursa-10:12-dienyl acetate renders it very unlikely that iso-α-amyradienonyl II acetate is correctly formulated as in LXXXI. In this case, the compound formed on hydrogenolysis would have the structure LXXXIV, which would be expected to rearrange to ursa-11:13(18)-dienyl acetate. Other evidence against this formulation



LXXXIV

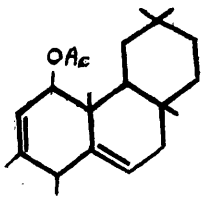
arises from the work of Beaton, Johnston, McKean and Spring (90). These workers obtained, by zinc and acetic acid reduction of 12:19-diketo-oleana-10:13(18)-dienyl

acetate (β-amyradiendionyl acetate), a compound formulated as in LXXXV, a structure then confirmed



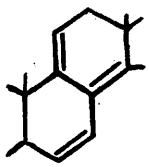
by partial synthesis. This compound shows ultra-violet absorption maxima at 2,080 (ε=9,000), 2,600 (ε=9,250), and 2,950Å (ε=8,450); in addition, on catalytic hydrogenation it yields oleana-10:13(18)-dienyl acetate, which is rearranged under acid conditions to oleana-11:13(18)-dienyl acetate. The properties of 12-keto-oleana-10:13(18)-dienyl acetate are thus significantly different from those of iso-α-amyradienonyl II acetate, and preclude the formulation of the latter as 12-keto-ursa-10:13(18)-dienyl acetate.

The structure of the sodium and amyl alcohol reduction product was examined; the fact that it absorbs one mol of hydrogen to give a diene confirms that it contains three double bonds in the molecule. Three compounds containing a cross-conjugated triene chromophore are known, viz. $\Delta^{6,8(14),9(11)}$ cholestatriene -3-ol acetate (85), $\Delta^{6,8(14),9(11),22}$ ergostatetraene-3-ol acetate (91), and a triene obtained by hydrochloric acid treatment of a reduction product LXXXVI from iso-β-amyradienonyl acetate (84). The two steroid trienes

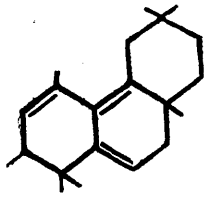


LXXXVI

possess the chromophore LXXXVII, and the triterpenoid triene has been formulated as in LXXXVIII.



LXXXVII



LXXXVIII

The ultra-violet absorption maxima for these compounds are shown in Table 4.

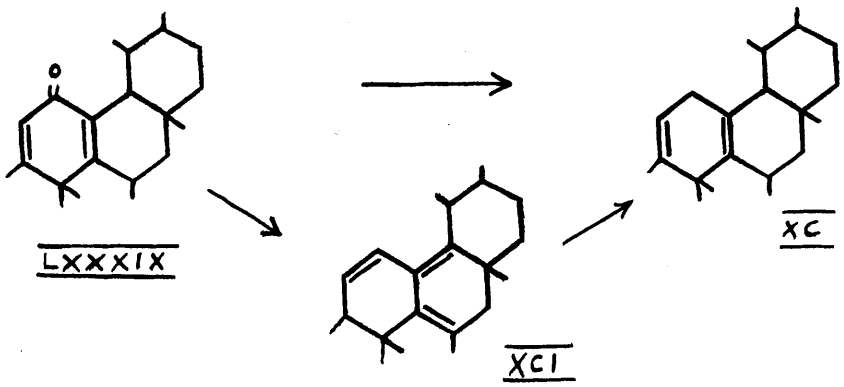
Table 4

Compound	Ultra-violet absorption	
	λ max.(Å)	ε max.
△6,8(14),9(11) cholesta-triene-3-ol Ac.	2840 (end absorption at 2200)	8000
△6,8(14),9(11),22 ergosta-tetraene-3-ol-Ac.	2875 2325	6300 18000
triene LXXXVIII from β-amyrin	2280 2820 2940	18500 16000 12500
triene from iso-α-amyradienonyl II Ac.	2220 2800	10000 7700

The similarity of the spectrum of the triene obtained by reduction of iso-α-amyradienonyl II acetate to these suggests that it possesses the cross-conjugated chromophore. The linear triene chromophore in oleana-10:12:18-trienyl acetate (33) gives ultra-violet absorption maxima at 2300 (ε=2,600) and 3110Å (ε=11,600).

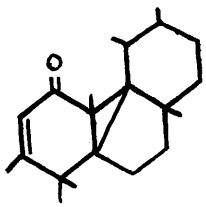
Any formulation of iso-α-amyradienonyl II acetate must account for the disappearance of the isolated double bond of iso-α-amyradienonyl acetate, and this may be done by assuming either a conjugated dienone structure or the formation of an additional ring. With the object of making a decision between these possibilities, an attempt was made to estimate the number of double bonds proper in the unconjugated 'diene' by oxidation with perbenzoic acid; however, the only crystalline product which could be isolated proved to be unchanged starting material.

It has not been found possible to write a structure for iso-α-amyradienonyl II acetate which will represent all the facts. The structure LXXXIX, derived from LXXXII, has been considered, and it appears

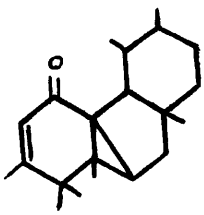


LXXXIX XCI XC

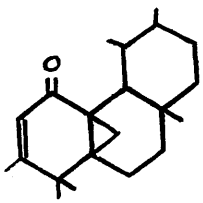
to be possible to formulate the diene (XC) and the triene (XCI) reduction products on this basis. However, mechanisms to account for the formation of LXXXIX from iso-α-amyradienonyl acetate, and of ursa-10:12-dienyl acetate from XC are lacking. If a cyclopropane ring is assumed (XCII, XCIII or XCIV), the formulation of the 'triene', which would then contain



XCII

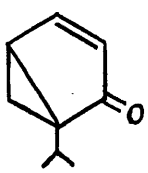


XCIII



XCIV

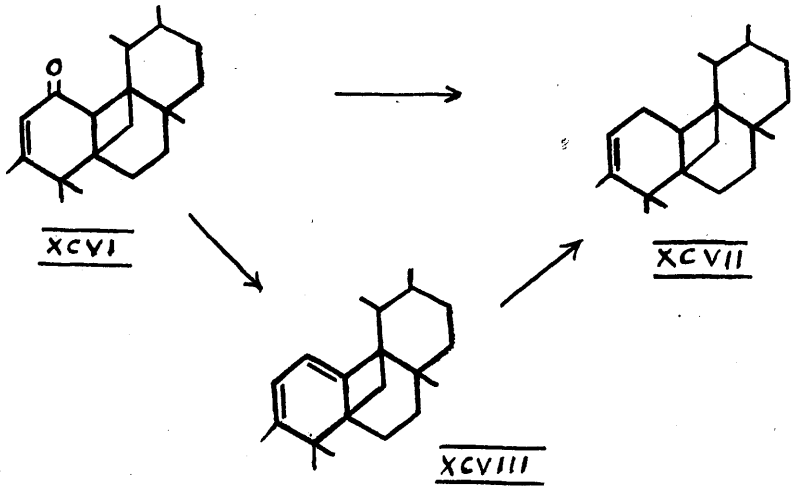
two double bonds and one cyclopropane ring, becomes extremely difficult, and furthermore any mechanism to account for the original formation of that ring seems unlikely. However, the reactive group in any of these structures is similar to that in umbellulone (XCV) (92), and formation of the reduction products of iso-α-amyradienonyl II acetate may be accompanied by complex



XCV

molecular rearrangements such as are known in the chemistry of that compound. The introduction of a cyclobutane ring, as in XCVI, XCVII and XCVIII is also

difficult to justify on mechanistic grounds, and the formation of ursa-10:12-dienyl acetate presents difficulties.



Until further knowledge of the chemistry of iso-α-amyradienonyl II acetate becomes available, any formulation must lie in the realms of speculation, since it seems probable that the formation and reactions of this compound involve structures and mechanisms of types hitherto unknown in triterpene chemistry.

EXPERIMENTAL

EXPERIMENTAL

Melting points are corrected. They were determined in open capillaries in the usual way unless specified to the contrary. Rotations were determined in chloroform solution, and values of $[\alpha]_D$ have been approximated to the nearest degree. Ultra-violet sbsorption spectra were measured in absolute ethanol solution.

Grade II alumina was used for chromatography, and light petroleum refers to the fraction of b.p. 60-80°.

The phrase "in the usual way" implies, in general, dilution with water, extraction with ether, washing successively with aqueous hydrochloric acid, aqueous potassium hydroxide and water as necessary, followed by sodium sulphate drying and evaporation of the ethereal solution *in vacuo*.

Acetylations, unless specified to the contrary, were carried out in pyridine and acetic anhydride on the steam bath, and the product obtained in the usual way, as described for the acetylation of 12-keto-urs-10-enol (see below).

Isolation of α-Amyrin Benzoate

The solid material obtained from Manila Elemi resin after removal of volatile oils by steam-distillation was ground to a fine powder. This powder (1,640 g.) was shaken for one hour with methylated spirit (1,300 cc., 95%), the suspension filtered, and the filtrate discarded. The residue, consisting mainly of α- and β-amyrin, was dried in air at 80°.

The crude mixed amyrins (600 g.) were dissolved in pyridine (360 cc.), and benzoyl chloride (420 c.c.) added dropwise over one hour to the stirred solution on the steam-bath. Heating and stirring were continued for six hours. The mixture was diluted with benzene (2 l.), allowed to cool, and the solution washed with water (twice), 2% hydrochloric acid (twice), 2% sodium hydroxide (once) and 2% sodium chloride. The dried (sodium sulphate) solution was concentrated to a bulk of 900 c.c. Hot ethanol was added to the boiling solution until it was faintly turbid, and on cooling a crystalline mass separated. This was filtered and air-dried.

The crude mixed benzoates (300 g.) were shaken with ether for ten minutes, and filtered. The clearing-point of the undissolved solid was then determined, and the ether extraction process repeated

until that clearing-point was above 210°. The residue was crystallised several times from benzene-acetone, to give crude β-amyrin benzoate (75g.), melting point 228-230 . The combined ethereal extracts, on evaporation to dryness, gave a solid residue which after several crystallisations from benzene-ethanol gave pure α-amyrin benzoate (150 g.), m.p. 197-198°, $[\alpha]_D$ +94° (c, 1.2).

α-Amyrin

α-Amyrin benzoate (50 g.) was dissolved in boiling benzene (300 c.c.), and a solution of potassium hydroxide (50 g.) in aqueous ethanol (65 c.c. water, 1150 c.c. ethanol) added. The solution was refluxed for 24 hours, concentrated until solid began to appear, and poured into water. The precipitated solid was collected, and after crystallisation from aqueous methanol, α-amyrin (40 g.) was obtained as needles of m.p. 176-178°, $[\alpha]_D$ = +81° (c, 0.77).

α-Amyrin Acetate

α-Amyrin (10 g.) was dissolved in pyridine (30 c.c.) and acetic anhydride (45 c.c.) and the solution heated on the steam-bath for two hours. It was then cooled, and the crystals which separated were filtered off. After washing with cold glacial acetic acid, they were crystallised from chloroform-methanol to give

α-amyrin acetate (8.9 g.), m.p. 226-227°, $[\alpha]_D$ +79° (c, 0.41).

12:13-Epoxy-ursanyl Acetate

(cf. McLean, Silverstone and Spring, J., 1951, 935).

α-Amyrin acetate (5.0 g.) in glacial acetic acid (250 c.c.) was treated at 100° with a mixture of hydrogen peroxide (100 vol.; 30 c.c.) and glacial acetic acid (30 c.c.) added dropwise during thirty minutes with stirring. Stirring was continued for two hours at 100° and the solution again treated with hydrogen peroxide (100 vol.; 20 c.c.) in glacial acetic acid (20 c.c.) during 15 minutes. The solution was kept at 100° for one hour, and hot water was added until the mixture became faintly opalescent. The crystalline solid separating overnight was collected and, after drying and recrystallisation from chloroform-methanol, yielded 12:13-epoxy-ursanyl acetate (1.9 g.), m.p. 209-211°, $[\alpha]_D$ +107° (c, 0.61).

12:13-Epoxy-ursanyl Benzoate

(cf. idem, loc. cit.)

α-Amyrin benzoate (5g.) was oxidised as described for the acetate. Crystallisation of the product from chloroform-methanol gave 12:13-epoxy-ursanyl benzoate (2.6 g.), m.p. 218-219°, $[\alpha]_D$ +132° (c, 0.59).

12-Keto-urs-10-enyl Benzoate

(cf. Seymour, Sharples and Spring, J., 1939, 1075).

12:13-Epoxy-ursanyl benzoate (5.0 g.) in glacial acetic acid (150 c.c.) containing a trace of hydrobromic acid was treated at 60° with a solution of bromine in glacial acetic acid (12.8% w/v; 15 c.c.) over a period of ten minutes, during which time the bromo-ketone separated. The mixture was then heated on the steam-bath for two hours, and allowed to cool overnight. It was then poured into excess water, and the precipitated solid collected and dried. After crystallisation from benzene-methanol, 12-keto-urs-10-enyl benzoate (3.0 g.) was obtained as small prisms, m.p. 211-212°, $[\alpha]_D$ +94° (c, 0.73).

12-Keto-urs-10-enol

12-Keto-urs-10-enyl benzoate (500 mgm.) was dissolved in ethanolic potassium hydroxide (3%; 25 c.c.) and the solution refluxed for six hours. It was then poured into excess water, and the precipitated solid taken up in ether; the ethereal solution was washed free of alkali with water, dried and evaporated to dryness. Crystallisation of the residue from aqueous ethanol gave 12-keto-urs-10-enol as needles, of m.p. 240-241°, $[\alpha]_D$ +72° (c, 1.6). Light absorption: Max. at 2500Å (ε = 10,500).

12-Keto-urs-10-enyl Acetate

(cf. idem, loc. cit.)

(i) 12:13-Epoxy-ursanyl acetate (5.0 g.) was brominated and dehydrobrominated as described for the benzoate. Crystallisation of the product from chloroform-methanol gave 12-keto-urs-10-enyl acetate (3.6 g.) as plates, of m.p. 286-288°, $[\alpha]_D$ +84.3° (c, 0.98). Light absorption: Max. at 2510Å (ε =10,900).

(ii) 12-Keto-urs-10-enol (3.0 g.) was dissolved in pyridine (6.0 c.c.) and acetic anhydride (6.0 c.c.), and the solution heated on the steam-bath for 15 minutes. It was then poured into excess water, and the product obtained by ether extraction in the usual manner. This was crystallised from chloroform-methanol to give 12-keto-urs-10-enyl acetate (2.5 g.) as plates of m.p. 286-288°, $[\alpha]_D$ +83.0° (c, 1.10).

Attempted Catalytic Hydrogenation of 12-Keto-urs-10-enyl Acetate

12-Keto-urs-10-enyl acetate (300 mgm.), dissolved in stabilised glacial acetic acid (70 c.c.), was shaken with hydrogen over previously reduced platinum catalyst (100 mgm.) for 72 hours. The filtered solution was evaporated to dryness, and the residue crystallised from methanol-chloroform. This gave plates (240 mgm.) of m.p. 286-288°, undepressed on mixing with starting material, and $[\alpha]_D$+85° (c, 1.58). Light

absorption: Max. at 2500Å (ε = 10,700).

Attampted Catalytic Hydrogenation of 12-Keto-urs-10-enol

A solution of 12-keto-urs-10-enol (230 mgm.) in redistilled ethanol (70 c.c.) was shaken under hydrogen over previously reduced platinum catalyst (100 mgm.) for 72 hours. The filtered solution was evaporated to dryness, and the residue crystallised from light petrol. This gave needles (200 mgm.) of m.p. 236-240°, $[\alpha]_D$ +68° (c, 0.94). Light absorption: Max. at 2490Å (ε =10,300). A mixture of product with starting material showed no melting point depression.

Attempted Kishner-Wolff Reduction of 12-Keto-urs-10-enyl Acetate

(i) 12-Keto-urs-10-enyl acetate (900 mgm.), hydrazine (100%; 6.0 c.c.), and a solution of sodium ethoxide (2.25 g. sodium in 30 c.c. dry ethanol) were heated together in an autoclave for 17 hours at 200°. The product was washed out with hot ethanol, poured into excess water, and worked up as usual. The gum obtained was acetylated with pyridine and acetic anhydride on the steam bath. The acetate was crystallised from chloroform -methanol, to give plates (425 mgm.), m.p. 281-284°, undepressed on mixing with starting material, and having $[\alpha]_D$ +82.4° (c, 0.92). From the mother-liquor a small amount of material of m.p. 220-230°, $[\alpha]_D$ +219° (c, 0.56)

was obtained. This had maximum light absorption at 2750Å (ε = 6,000), and gave a red-brown tetranitromethane colour.

(ii) 12-Keto-urs-10-enyl acetate (1.0 g.), hydrazine (100%; 3.0 c.c.), and a solution of sodium amyloxide (1.0 g. sodium in 20 c.c. redistilled amyl alcohol) were heated together in an autoclave for 17 hours at 240°. The product, after removal of traces of amyl alcohol by steam-distillation, was obtained and acetylated as in the previous experiment, but yielded only a gum which could not be crystallised.

12-Hydroxy-ursanol

Liquid ammonia (150 c.c.) was added to a suspension of lithium (400 mgm.) in dry ether (60 c.c.), and stirred to give a deep blue solution. A solution of 12-keto-urs-10-enyl acetate (300 mgm.) in dry ether (500 c.c.) was added to this, with stirring over 20 minutes, and stirring continued for a further two hours. These operations were carried out an an atmosphere of nitrogen. Ammonia was allowed to evaporate overnight, and excess lithium destroyed with water. The ethereal layer was washed free of alkali, dried, and evaporated to dryness. The residue was crystallised from n-hexane to give 12-hydroxy-ursanol (150 mgm.) as prismatic needles, m.p. 176-177° and 199-200° (double melt), $[\alpha]_D$ + 10°, + 9° (c, 1.4, 2.2).

12-Acetoxy-ursanyl Acetate

12-Hydroxy-ursanol (100 mgm.) was dissolved in acetic anhydride and pyridine, and heated for 2½ hours on the steam-bath. The product was obtained in the usual manner, and after several crystallisations from aqueous methanol gave 12-acetoxy-ursanyl acetate (60 mgm.) as plates of m.p. 205-206°, $[\alpha]_D$ -4°, -4° (c, 1.8, 1.4). Found: C, 77.4; H, 10.8. Calc. for $C_{34}H_{56}O_4$: C, 77.2; H, 10.7%.

Reduction of 12:13-Epoxy-ursanyl Benzoate with Sodium in Amyl Alcohol

(cf. Seymour, Sharples and Spring, J, 1939, 1075)

12:13-Epoxy-ursanyl benzoate (4.0 g.) was dissolved in boiling amyl alcohol (redistilled; 100 c.c.), and sodium (4.0 g.) added portionwise to the solution. After boiling under reflux for 1 hour, a further addition of sodium (4.0 g.) was made, and refluxing continued for 30 minutes. The solution was then cooled, excess sodium destroyed with water, and amyl alcohol removed by steam-distillation after alkali had been washed out. The solid residue remaining was dried and crystallised from n-hexane-chloroform, to give prismatic needles (0.54 g.) of m.p. 206-208°, $[\alpha]_D$ + 50°, + 50°, (c, 2.1, 2.2).

The above material (m.p. 206-208°; 3.0 g.) was acetylated in the usual manner, and the product crystallised from aqueous methanol as needles, of m.p.

194-196°, $[\alpha]_D$ +79°, +77°, (c, 1.1, 1.6).

Some of the crude acetate obtained above (m.p. 165-172°, $[\alpha]_D$ +65° (c, 1.5); 2.44 g.) was dissolved in light petroleum (100 c.c.) and chromatographed on alumina (15.5 x 3 cm.). The first fraction (280 mgm.), eluted with benzene-light petroleum (1:2; 500 c.c., and 1:1, 500 c.c.), crystallised from aqueous methanol as needles of m.p. 180-188°, $[\alpha]_D$ +83° (c, 1.2). Elution with benzene (750 c.c.) gave a second fraction (300 mgm.), obtained from aqueous methanol as needles, m.p. 171-175°, $[\alpha]_D$ +87° (c, 1.1). The third fraction (1,180 mgm.), eluted with benzene-ether (19:1; 1,000 c.c.) gave needles of m.p. 164-170°, $[\alpha]_D$ +69° (c, 0.93), from aqueous methanol. Finally, benzene-ether (4:1; 750 c.c. and 1:1; 250 c.c.) and ether (250 c.c.) gave material (85 mgm.) which crystallised from aqueous methanol as needles of m.p. 102-110°, $[\alpha]_D$ +22° (c, 1.1). The remaining material, eluted with ether-methanol and methanol, could not be crystallised. None of the solid fractions obtained gave a pure substance on crystallisation.

Attampted Catalytic Hydrogenation of 12:13-Epoxy-ursanyl Acetate

A solution of 12-13-epoxy-ursanyl acetate (300 mgm.) in stabilized glacial acetic acid (90 c.c.) was shaken under hydrogen over previously reduced platinum

catalyst (200 mgm.) for 20 hours. The filtered solution was evaporated to dryness, and the residue crystallised from chloroform-methanol, to give plates (210 mgm.) of m.p. 208-210°, $[\alpha]_D$ +118° (c, 1.1). A mixture of product and starting material showed no melting-point depression.

12-Keto-ursanyl Acetate

(cf. Ruzicka, Jeger, Redel and Volli, Helv. Chim. Acta, 1945, 28, 199)

12:13-Epoxy-ursanyl acetate (6.0 g.) was dissolved in a mixture of chloroform (30 c.c.) and glacial acetic acid (120 c.c.), and conc. aqueous hydrochloric acid (6.0 c.c.) added. The solution was heated to 40° for 30 minutes, and the product then obtained in the usual manner. On crystallization from chloroform-methanol, 12-keto-ursanyl acetate (2.45 g.) was obtained as plates of m.p. 280-282°, $[\alpha]_D$ +12° (c, 0.80). Light absorption: Max. at 2080 (ε = 285) and 2870Å (ε = 30).

Attempted Catalytic Reduction of 12-Keto-ursanyl Acetate

A solution of 12-keto-ursanyl acetate (300 mgm.) in ethyl acetate (100 c.c.) and stabilised glacial acetic acid (150 c.c.) was shaken under hydrogen over previously reduced platinum catalyst (200 mgm.) for 18 hours. The filtered solution was evaporated to dryness, and the

residue crystallised from chloroform-methanol, to give plates (220 mgm.) of m.p. 277-279°, $[\alpha]_D$ + 9° (c, 1.5). A mixture of product and starting material showed no melting-point depression.

α-Amyrin p-Toluenesulphonate

p-Toluenesulphonyl chloride (200 mgm.) was added to a solution of α-amyrin (200 mgm.) in pyridine (redistilled; 1.5 c.c.), and the solution allowed to stand at room temperature for 24 hours. The reaction mixture was worked up in the usual manner, and after repeated crystallisation from acetone-methanol, α-amyrin p-toluenesulphonate (190 mgm.) was obtained as a white amorphous powder, of m.p. 132°, $[\alpha]_D$ +62°, +60° (c, 0.75, 1.1), giving a yellow colour reaction with tetranitromethane.

Found: C, 76.6; H 9.7. $C_{37}H_{56}O_3S$ requires C, 76.5; H, 9.7%.

The Action of p-Toluenesulphonyl Chloride on 12-Hydroxy-ursanol.

p-Toluenesulphonyl chloride (500 mgm.) was added to a solution of 12-hydroxy-ursanol, (500 mgm.), in pyridine (redistilled, 2.0 c.c.), and the solution allowed to stand at room temperature for 70 hours. The reaction mixture was worked up in the usual manner, and a white amorphous material (250 mgm.) obtained on

crystallisation from acetone-methanol. This showed a yellow colour with tetranitromethane, and had m.p. 132°, $[\alpha]_D$ + 57° (c, 0.86). A mixture of this material and α-amyrin p-toluenesulphonate prepared as above showed no melting-point depression.

The Action of p-Toluenesulphonyl Chloride on the Product of Sodium/Amyl Alcohol Reduction of 12:13-Epoxy-ursanyl Benzoate.

The product (m.p. 197-200°, $[\alpha]_D$ + 80°; 65 mgm.) of sodium/amyl alcohol reduction of 12:13-epoxy-ursanyl benzoate (see above) was hydrolysed by refluxing for 2 hours in 2% ethanolic potassium hydroxide (15 c.c.). The alcohol was obtained as usual, and proved to be a gum. p-Toluenesulphonyl chloride (60 mgm.) was added to a solution of this gum in pyridine (redistilled; 0.5 c.c.), and the solution allowed to stand at room temperature for 70 hours. The product was obtained as usual, and crystallised from aqueous methanol as an amorphous material (40 mgm.) of m.p. 120-123°, $[\alpha]_D$ + 67° (c, 0.47). This material gave a yellow colour with tetranitromethane, and its melting point was undepressed on mixing with α-amyrin p-toluenesulphonate, prepared as above.

The Action of Aqueous Hydrochloric Acid on 12-Hydroxy-ursanol

Conc. aqueous hydrochloric acid (5.0 c.c.) was

added to a solution of 12-hydroxy-ursanol (200 mgm.) in ethanol (30 c.c.), and the mixture refluxed for 3 hours, then allowed to stand at room temperature for 3 days. The product was obtained as usual, and acetylated in the normal manner. The acetate was crystallised from chloroform-methanol, to give -amyrin acetate (40 mgm.) of m.p. 225-227°, $[\alpha]_D$ + 75° (c, 0.93), melting point undepressed on mixing with an authentic specimen.

Ursa-10:12-dienyl Benzoate

(cf. Ruzicka, Jeger and Redl, Helv. Chim. Acta. 1943, 26, 1235).

α-Amyrin benzoate (15 g.) was dissolved in dry carbon tetrachloride (375 c.c.), and the solution refluxed with N-bromsuccinimide (10 g.) for 3 hours. The succinimide produced was removed by filtration, and the cold filtrate washed with dilute alkali, sodium thiosulphate solution, and water. After drying over sodium sulphate, carbon tetrachloride was removed under reduced pressure, and the reddish oil remaining was crystallised once from acetone-methanol. The crystals thus obtained were dissolved in dry ether, and treated with active charcoal. After removal of ether, the residue was crystallised from methanol-chloroform, to give ursa-10:12-dienyl benzoate (9.5 g.) as plates of m.p. 176-177°.

Ursa-10:12-dienyl Acetate

(i) Ursa-10:12-dienyl benzoate (1.0 g.) was hydrolysed by refluxing in ethanolic potassium hydroxide (10%; 25 c.c.) for 8 hours. The alcohol was obtained as usual, and acetylated in the normal manner. Ursa-10:12-dienyl acetate (700 mgm.) was deposited as needles from chloroform-methanol, having m.p. 168-169°, $[\alpha]_D$ + 317° (c, 1.3). Light absorption: Max. at 2800Å (ε = 10,100).

(ii) A solution of α-amyrin acetate (2.0 g.) in molten diphenyl (15 g.) was treated with selenium dioxide (2.4 g.) and refluxed for 40 hours. The solvent was removed by steam-distillation and the residue extracted with ether (Soxhlet). The ether solution was washed with aqueous potassium cyanide (3%) and the product crystallised repeatedly from chloroform-methanol from which ursa-10:12-dienyl acetate (200 mgm.) separated as needles, m.p. 166-167°, $[\alpha]_D$ + 311° (c, 0.9), undepressed in melting-point when mixed with an authentic specimen. Light absorption: Max. at 2800Å (ε = 11,700).

Ursa-11:13(18)-dienyl Acetate

(i) A mixture of α-amyrin acetate (18 g.), dioxan (750 c.c.), and selenium dioxide (21.6 g.) was heated in an autoclave for 10 hours at 200°. The cold mixture was filtered, and the filtrate diluted with water and extracted with ether. The ethereal solution was

washed with aqueous potassium cyanide (5%), and the dried solution treated with charcoal. After removal of the ether, the red product was crystallised from chloroform-methanol, from which two crops of α-amyrin acetate (8.5 g.; m.p. and mixed m.p. 218-221) were removed. The mother-liquor on evaporation gave a partly crystalline residue (2.9 g.), a solution of which in benzene-light petroleum (1:10; 250 c.c.) was chromatographed on alumina (15 x 3.5 cm.). Washing the column with the same solvent mixture (500 c.c.) gave a fraction (352 mgm.) which could not be crystallised. Continued washing with the same solvent mixture (625 c.c.) gave a crystalline eluate (550 mgm.), recrystallisation of which from methanol-chloroform gave α-amyrin acetate, m.p. 220-221 , undepressed in m.p. when mixed with an authentic specimen. A third fraction (511 mgm.) obtained by continued washing with the same solvent mixture (1125 c.c.), crystallised from methanol-chloroform as needles, m.p. 202-204°, and gave a red-brown colour with tetranitromethane. Several recrystallisations of this fraction from methanol-chloroform gave ursa-11:13(18)-dienyl acetate as needles, m.p. 206-207°, $[\alpha]_D$ -77°, -79° (c, 1.2, 0.35). Found: C, 82.3; H, 10.9. $C_{32}H_{50}O_2$ requires C, 82.3; H, 10.8%. Light absorption: Max. at 2440 (ε = 29,000), 2520 (ε = 31,200), and 2600Å (ε = 21,400). A mixture with oleana-11:13(18)-dienyl acetate (m.p. 230-231°) had m.p. 183-190°.

(ii) A solution of ∝-amyrin acetate (40 g.) in boiling benzyl acetate (redistilled; 500 c.c.) was treated with selenium dioxide (48 g.) and the mixture refluxed for 24 hours. The filtered solution was then evaporated to dryness in vacuo, the residue taken up in ether, washed with potassium cyanide solution (3%) and water, treated with charcoal after drying, and a crystalline solid was obtained on removal of the ether. This was crystallised from methanol-chloroform and two crops (22 g.) of ∝-amyrin acetate (m.p. and mixed m.p. 216-9°) were recovered. The mother-liquor on evaporation gave a partly crystalline residue (15 g.), a solution of which in benzene-light petroleum (1:3; 1,000 c.c.) was chromatographed on alumina (27 x 4.5 cm.). Washing the column with the same solvent (1,000 c.c.) gave a fraction (0.09 g.) which could not be crystallised. Continued washing with the same solvent mixture (750 c.c.) gave a crystalline eluate (3.5 g.), recrystallisation of which from methanol-chloroform gave ∝-amyrin acetate (m.p. and mixed m.p. 223-225°), A third fraction (3.0 g.) obtained by wontinued washing with the same solvent mixture (2,000 c.c.) crystallised as needles, m.p. 190-200°, from methanol-chloroform. This third fraction was combined with the residue from the mother-liquors of the second fraction, and the whole (3.5 g.) dissolved in light petroleum (125 c.c.) and rechromatographed on alumina (17 x 3.5 cm.). Washing with the same solvent (450 c.c.)

gave a fraction (0.11 g.) which could not be crystallised. A second fraction (2.05 g.) eluted with light petroleum (1,500 c.c.) crystallised from methanol-chloroform to give α-amyrin acetate, and continued washing with the same solvent (450 c.c.) gave a material (0.24 g.) which crystallised from chloroform-methanol as needles of m.p. 180-190°. Washing with benzene-light petroleum (1:1; 300 c.c.) gave a fraction (1.23 g.) which after several crystallisations from chloroform-methanol gave ursa-11:13(18)-dienyl acetate (700 mgm.) as needles of m.p. 205-206°, undepressed by a sample prepared as described above.

(iii) A solution of α-amyrin acetate (40 g.) in boiling glacial acetic acid (1,600 c.c.) was treated over one hour with a solution of selenium dioxide (20 g.) in water (10 c.c.) and glacial acetic acid (400 c.c.). Fused sodium acetate (160 g.) was then added, and the mixture refluxed for 15 minutes. The hot solution was filtered, then largely diluted with water. The precipitated solid was collected, taken up in ether, and the ethereal solution washed with dilute alkali, potassium cyanide solution (3%), and water. After drying and treatment with charcoal, the solution was evaporated to dryness, and the residue crystallised with chloroform-methanol. This gave two crops of unchanged α-amyrin acetate (34 g.). The mother-liquor on evaporation gave a gummy residue

(1.67g.) a solution of which in light petroleum (100 c.c.) was chromatographed on alumina (15 x 2 cm.). Washing the column with the same solvent (1,200 c.c.) gave a fraction (0.78 g.) which crystallised from methanol-chloroform to give α-amyrin acetate. Continued washing with the same solvent (200 c.c.) gave a fraction (0.10 g.) obtained as needles, m.p. 165-180°, from chloroform-methanol. The third fraction (0.33 g.), eluted on washing with benzene-light petroleum (1:10; 400 c.c.), after several crystallisations from chloroform-methanol, gave ursa-11:13(18)-dienyl acetate (260 mgm.) as needles of m.p. 205-206°, undepressed on mixing with a sample prepared as described above.

Ursa-11:13(18)-dienol

Ursa-11:13(18)-dienyl acetate (90 mgm.) was hydrolysed by refluxing in ethanolic potassium hydroxide solution (5%; 10 c.c.) for 3 hours, and the alcohol obtained in the usual manner. Ursa-11:13(18)-dienol (60 mgm.) separated from aqueous methanol as needles, m.p. 194-195°, $[\alpha]_D$ -83°, -86° (c, 1.2, 1.0). Found: C, 84.65; H, 11.5. $C_{30}H_{48}O$ requires C, 84.8; H, 11.4%.

Acetylation of the alcohol, with pyridine and acetic anhydride, gave ursa-11:13(18)-dienyl acetate, m.p. 205-206°, $[\alpha]_D$ -76° (c, 0.44), undepressed in m.p. when mixed with a specimen prepared as described above.

Urs-13(18)-enol

A solution of ursa-11:13(18)-dienyl acetate (300 mgm.) in ethyl acetate (70 c.c.) and stabilised glacial acetic acid (80 c.c.) was shaken with hydrogen over previously reduced platinum catalyst (150 mgm.) for 21 hours. The filtered solution was evaporated and the residue hydrolysed by refluxing it for 2 hours with ethanolic potassium hydroxide (3%; 25 c.c.). The product was isolated in the usual manner and crystallised from methanol, giving urs-13(18)-enol as plates, m.p. 204-205°, $[\alpha]_D$ -35°, -36.5°, (c, 1.2, 0.6).

Found: C, 84.1; H, 12.0. $C_{30}H_{50}O$ requires C, 84.4; H, 11.8%.

Urs-13(18)-enol gave a yellow colour with tetranitromethane.

Urs-13(18)-enyl Acetate

Urs-13(18)-enol was acetylated with acetic anhydride and pyridine, and the product obtained in the usual manner. On crystallisation from chloroform-methanol, urs-13(18)-enyl acetate separated as needles, m.p. 214-216°, $[\alpha]_D$-23°, -22° (c, 1.4, 0.4).

Found: C, 82.3; H, 11.4. $C_{32}H_{52}O_2$ requires C, 82.0; H, 11.2%.

Light absorption: ε_{2100} = 4,000, ε_{2150} = 5,100, ε_{2200} = 2,800, and ε_{2230} = 1,100. Urs-13(18)-enyl acetate gave a yellow colour with tetranitromethane, and a mixture with olean-13(18)-enyl acetate (m.p. 209-210°) had m.p. 190-195°.

A mixture with ursa-11:13(18)-dienyl acetate showed no melting-point depression.

Oxidation of Ursa-11:13(18)-dienyl Acetate with Chromium Trioxide

A solution of chromium trioxide in stabilised glacial acetic acid (5.8 c.c., containing 1.84 mgm. active oxygen/c.c., i.e. 1.05 mols) was added dropwise with shaking to a solution of ursa-11:13(18)-dienyl acetate (300 mgm.) in stabilised glacial acetic acid (30 c.c.) at 65°. An immediate green colour was produced. After all the oxidant had been added, the solution was kept at 65° for 15 minutes, and the product was then obtained in the usual manner. It proved to be a gummy yellow solid (270 mgm.), which was dissolved in light petroleum (50 c.c.) and chromatographed on alumina (6 x 1.5 cm.). On washing the columa with light petroleum (500 c.c.), a fraction (120 mgm.) was obtained, and this was crystallised from chloroform-methanol to give ursa-11:13(18)-dienyl acetate, m.p. 205-206°, undepressed on mixing with an authentic specimen. Benzene (50 c.c.) then eluted a small fraction (25 mgm.) which crystallised from methanol as needles, m.p. 197-9°, depressed on mixing with starting material, which gave a yellow colour with tetranitromethane.

Found: C, 80.0; H, 10.5. $C_{32}H_{50}O_3$ requires C, 79.6;

H, 10.4%.

Light absorption: ε_{2100} = 8,100, ε_{2150} = 5,200, ε_{2200} = 2,800, and ε_{2230} = 2,100.

Subsequent fractions, obtained by washing with various solvents, (benzene; 150 c.c.: 1:2 ether-benzene; 100 c.c.; ether; 100 c.c.) could not be crystallised. Washing with methanol-ether (1:2; 50 c.c.) gave a fraction (6 mgm.) which crystallised from aqueous methanol as plates of m.p. 226-238°. Light absorption: Max. at 2080 (ε = 3,900) and 3100Å (ε = 8,700).

The Action of N-Bromsuccinimide on Ursa-11:13(18)-dienyl Acetate

A solution of ursa-11:13(18)-dienyl acetate (170 mgm.) in dry carbon tetrachloride (8.5 c.c.) was refluxed with N-bromsuccinimide (100 mgm.) for 3 hours. After cooling and filtration from succinimide, the solution was washed with dilute alkali, acid and water, dried, and evaporated *in vacuo*. The residue was crystallised from methanol-chloroform, to give needles (80 mgm.) of m.p. 180-186°, undepressed on mixing with starting material, and having $[\alpha]_D$ -66° (*c*, 0.33). The residue (60 mgm.) obtained on evaporation of the mother liquor was dissolved in light petroleum (25 c.c.) and chromatographed on alumina (0.5 x 4.5 cm.). The only fraction which could be crystallised was that eluted with light petroleum (50 c.c.), and this (27 mgm.) gave,

from methanol, needles of m.p. 184-187°, $[\alpha]_D$ + 67° (c, 0.28). Light absorption: Max. at 2420 (ε = 16,000), 2520 (ε = 19,300), and 2600Å (ε = 16,600).

The Action of Sulphuric Acid on Ursa-10:12-dienyl Acetate

Conc. sulphuric acid (4.8 c.c.) was added to a solution of ursa-10:12-dienyl acetate (300 mgm.) in benzene (5.0 c.c.) and glacial acetic acid (28 c.c.), and the mixture allowed to stand at room temperature for thirteen days. The product was isolated in the usual manner, and proved to be a gummy solid, which crystallised from chloroform-methanol to give needles (180 mgm.) of m.p. 158-160°, undepressed on mixing with starting material. The gum obtained on evaporation of the mother-liquor (100 mgm.) was acetylated in the usual manner, and the acetate dissolved in light petroleum (25 c.c.) and chromatographed on alumina (4.5 x 1 cm.). The only fraction which could be crystallised was that eluted with light petroleum (125 c.c.), and this (43 mgm.) was crystallised from chloroform-methanol to give needles of m.p. 146-151°, undepressed on mixing with starting material.

The Action of Hydrochloric Acid on Ursa-10:12-dienyl Acetate

Conc. aqueous hydrochloric acid (10 c.c.) was added to a boiling solution of ursa-10:12-dienyl acetate (200 mgm.) in ethanol (40 c.c.), and the mixture refluxed

for 5 hours. The product was obtained in the normal manner, and acetylated as usual with acetic anhydride and pyridine. The product crystallised from chloroform -methanol as needles (95 mgm.) of m.p. 163-165°, undepressed on mixing with starting material. Light absorption: Max. at 2800Å (ε = 11,200).

The Action of Hydrogen Chloride on Ursa-11:13(18)-dienyl Acetate

Dry hydrogen chloride was passed through a solution of ursa-11:13(18)-dienyl acetate (12.5 mgm.) in chloroform (redistilled; 2.0 c.c.) for 15 minutes. The solution was then allowed to stand at room temperature in a stoppered flask for 6 days. On evaporation of solvent, a gummy solid remained, and this was crystallised from chloroform-methanol to give needles of m.p. 195-198°, undepressed on mixing with starting material. Light absorption: Max. at 2440 (ε = 17,000), 2500 (ε = 17,900), and 2600Å (ε = 11,600), and ε_{2800} = 765, with no inflexion.

The Action of Sulphuric Acid on α-Amyrin Acetate

Conc. sulphuric acid (20 c.c.) was added to a solution of α-amyrin acetate (2.5 g.) in dry benzene (70 c.c.) and stabilised glacial acetic acid (300 c.c.) and the mixture allowed to stand at room temperature for 8 weeks. The product was obtained as usual, and

crystallised from chloroform-methanol to give α-amyrin acetate (2.15 g.), m.p. 225-226°, undepressed on mixing with an authentic specimen.

α-Amyrin Half-succinate

Succinic anhydride (0.37 g., i.e. 1.3 mols) was added to a solution of α-amyrin (1.56 g.) in pyridine (12 c.c.), and the mixture heated to 100° for 17 hours. The reaction mixture was then largely diluted with water and the precipitate formed taken up in ether, after which the acid fraction was extracted by washing with potassium hydroxide solution (3%). The ether layer, containing the neutral fraction, was washed with water, dried, and the solvent removed. The residue was crystallised from methanol to give needles (0.80 g.) of m.p. 177-179°, undepressed on mixing with an authentic sample of α-amyrin. The acid fraction was precipitated by acidification of the alkaline washings with dilute hydrochloric acid, and was then taken up in ether, washed with water, and dried. The residue after removal of ether was crystallised from n-hexane-acetone, to give α-amyrin half-succinate (100 mgm.) as prisms of m.p. 220-223°.

Found: C, 77.8; H, 10.3. $C_{34}H_{54}O_4$ requires C, 77.5; H, 10.6%.

Results of micro-equivalent titrations: Equivalent weight of α-amyrin half-succinate = 520, 533, 520, 521.

Calculated value of equivalent weight = 527.

11-Keto-urs-12-enyl Acetate

(cf. Spring and Vickerstaff, J., 1937, 249)

A solution of chromium trioxide (8.4 g.) in acetic acid (90%; 110 c.c.) was added dropwise over 15 minutes to a boiling solution of α-amyrin acetate (10 g.) in glacial acetic acid (300 c.c.), and the mixture then refluxed for one hour. The product was obtained in the usual manner, and crystallised from chloroform-methanol to give 11-keto-urs-12-enyl acetate as plates of m.p. 286-288°, $[\alpha]_D$ +96° (c, 1.3). Light absorption: Max. at 2480Å (ε = 13,100).

Attempted Bromination of 11-Keto-urs-12-enyl Acetate

A solution of bromine in glacial acetic acid (5.9% w/v; 2.0 c.c., i.e.1.1 mols) was added dropwise with shaking to a solution of 11-keto-urs-12-enyl acetate (300 mgm.) in glacial acetic acid (40 c.c.) containing a trace of hydrogen bromide. During the addition, and for 22 hours thereafter, the temperature of the solution was maintained at 60-70°. The product was obtained in the usual manner, and crystallised from chloroform-methanol to give plates (200 mgm.) of m.p. 280-283°, undepressed on mixing with starting material.

Attempted Formation of the Enol Acetate of 11-Keto-urs-12-enyl Acetate

11-Keto-urs-12-enyl acetate (2.0 g.) was refluxed for 72 hours in acetic anhydride (100 c.c.), with freshly fused sodium acetate (3.0 g.). The product was obtained in the usual manner, and crystallised from chloroform-methanol as plates (1.69 g.) of m.p. 284-287°, $[\alpha]_D$ +98° (c, 2.3). A mixture with an authentic specimen of 11-keto-urs-12-enyl acetate showed no melting-point depression.

The Action of Strong Alkali on 11-Keto-urs-12-enyl Acetate

11-Keto-urs-12-enyl acetate (5.0 g.) was refluxed for 50 hours in ethanolic potassium hydroxide (15%; 400 c.c.). The product was obtained as usual, and acetylated in the normal manner. The acetate was crystallised from chloroform-methanol, and gave plates (4.5 g.) of m.p. 283-286°, $[\alpha]_D$ +98° (c, 1.6). A mixture of product and starting material showed no melting-point depression.

The Action of Strong Alkali on 12-Keto-urs-10-enyl Acetate

12-Keto-urs-10-enyl acetate (500 mgm.) was refluxed for 65 hours in ethanolic potassium hydroxide (15%; 40 c.c.). The product was obtained as usual, and acetylated in the normal manner. The acetate was crystallised from chloroform-methanol, and gave plates (470 mgm.) of m.p. 277-281°, $[\alpha]_D$ +82° (c, 1.1). A

mixture of product and starting material showed no melting-point depression.

The Enol Acetate of 12-Keto-urs-10-enyl Acetate

(i) 12-Keto-urs-10-enyl acetate (790 mgm.) was refluxed for 60 hours in acetic anhydride (60 c.c.) with freshly fused sodium acetate (1.2 g.). The product was obtained as usual, and crystallised from methanol-benzene to give the enol acetate (500 mgm.) as fine needles of m.p. 257-258°, $[\alpha]_D$ +231° (c, 0.46). Found: C, 77.9; H, 9.9. $C_{34}H_{52}O_4$ requires C, 77.8; H, 9.9%.

Light absorption: Max. at 2780Å (ε = 8,900). The enol acetate gives a red brown colour with tetranitro-methane.

(ii) 12-Keto-urs-10-enol (280 mgm.) was acetylated as described above. The product crystallised from chloroform-methanol as needles (190 mgm.) of m.p. 256-257°, undepressed by a sample of the enol acetate prepared as described above.

Hydrolysis of the Enol Acetate of 12-Keto-urs-10-enyl Acetate

The enol acetate of 12-keto-urs-10-enyl acetate (77 mgm.) was hydrolysed by refluxing for two hours in ethanolic potassium hydroxide (3%; 10 c.c.), and the product obtained in the usual way. This was crystallised

from aqueous methanol to give needles (47 mgm.) of m.p. 238-240°, $[\alpha]_D$ +77° (c, 0.62). The melting point was undepressed on mixing with an authentic sample of 12-keto-urs-10-enol.

13-iso-12-Keto-urs-10-enyl Benzoate

(cf. McLean, Ruff and Spring, J., 1951, 1093)

A solution of ursa-10:12-dienyl benzoate (9.0 g.) in glacial acetic acid (400 c.c.) was treated for 20 minutes at steam-bath temperature with a mixture of hydrogen peroxide (30%; 36 c.c.) and glacial acetic acid (36 c.c.) with vigorous stirring. Stirring and heating were continued until the precipitated solid redissolved (2 hours). More peroxide-acetic acid (72 c.c.) was added, and the solution heated and stirred for one hour, after which it was diluted with hot water until it was permanently turbid. The crystalline solid separating on cooling was collected, washed with a little methanol and then with water, and dried in air at 100°. It was then dissolved in chloroform (5 c.c.) and ethanol (200 c.c.) and the solution evaporated until crystallisation occurred in the boiling mixture. This was filtered at the boiling-point and the filtrate again concentrated until crystallisation occurred. Two crops were obtained thus, and were combined and recrystallised from chloroform -methanol to give 13-iso-12-keto-urs-10-enyl benzoate (1.0 g.) as needles of m.p. 243-244°, $[\alpha]_D$ +24° (c, 0.70).

The Action of Strong Alkali on 13-iso-12-Keto-urs-10-enyl Benzoate

(cf. idem, loc. cit.)

13-iso-12-Keto-urs-10-enyl benzoate (200 mgm.) was refluxed for 6 hours in ethanolic potassium hydroxide (6.6%; 12 c.c.). After the addition of potassium hydroxide (0.5 g.) in water (0.4 c.c.) and ethanol (1.6 c.c.) refluxing was continued for 8 hours. The product was obtained in the usual manner, and crystallised from aqueous methanol as needles (85 mgm.) of m.p. 239-240°, $[\alpha]_D$ +75° (c, 1.2). A mixture with an authentic sample of 12-keto-urs-10-enol showed no melting-point depression.

13-iso-12-Keto-urs-10-enyl Acetate

(cf. idem, loc. cit.)

A solution of 13-iso-12-keto-urs-10-enyl benzoate (1.0 g.) in benzene (20 c.c.) was treated with a solution of potassium hydroxide (1.0 g.) in water (8 c.c.) and ethanol (80 c.c.). The mixture was refluxed for two hours, and the product obtained in the usual manner. The alcohol was not purified, but was acetylated in the usual way. The acetate was crystallised from aqueous methanol to give 13-iso-12-keto-urs-10-enyl acetate (600 mgm.) as plates of m.p. 200-201°, $[\alpha]_D$ +11° (c, 2.2). Light absorption: Max. at 2500Å (ε = 10,500).

iso-12-Keto-ursanyl Acetate

(cf. idem, loc. cit.)

13-iso-12-Keto-urs-10-enyl acetate (500 mgm.) in stabilised glacial acetic acid (420 c.c.) was shaken with hydrogen in the presence of previously reduced platinum catalyst (350 mgm.) for 16 hours. The filtered solution was evaporated to dryness in vacuo, and crystallised repeatedly from chloroform-methanol, to give iso-12-keto-ursanyl acetate (360 mgm.) as needles of m.p. 206-217°, (m.p. 233-235° in a sealed, evacuated tube), $[\alpha]_D$ +65°, +62° (c, 1.4, 0.98).

Found: C, 79.1; H, 10.6. Calc. for $C_{32}H_{52}O_3$ C, 79.3; H, 10.8%.

Light absorption: Max. at 2100 (ε = 360) and 2880-2990Å (ε = 50). iso-12-Keto-ursanyl acetate was recovered unchanged in physical properties after chromatography on alumina.

iso-12-Keto-ursanol

iso-12-Keto-ursanyl acetate (44 mgm.) was refluxed for 2 hours in ethanolic potassium hydroxide (3%; 15 c.c.), and the product obtained in the usual way. This crystallised from chloroform-methanol as prisms of m.p. 255-266° (m.p. 276-278° in a sealed, evacuated tube), $[\alpha]_D$ +60° (c, 0.66).

The Enol Acetate of 13-iso-12-Keto-urs-10-enyl Acetate

13-iso-12-Keto-urs-10-enyl acetate (200 mgm.) was refluxed for 72 hours in acetic anhydride (15 c.c.) with freshly fused sodium acetate (300 mgm.). The product was obtained as usual, and crystallised from chloroform-methanol as needles (130 mgm.), showing a red-brown colour with tetranitromethane, and having m.p. 257-258°, $[\alpha]_D$ +243° (c, 1.0). Light absorption: Max. at 2760Å (ε = 10,100). A mixture of the product and the enol acetate of 12-keto-urs-10-enyl acetate showed no melting-point depression.

The Enol Acetate of iso-12-Keto-ursanyl Acetate

iso-12-Keto-ursanyl acetate (1.0 g.) was refluxed for 70 hours in acetic anhydride (25 c.c.) with freshly fused sodium acetate (1.0 g.). The product was obtained as usual, and crystallised from chloroform-methanol to give the enol acetate (480 mgm.) as flattened needles of m.p. 260-262°, $[\alpha]_D$ -73°, -74° (c, 1.3, 1.6), giving a yellow colour with tetranitromethane.

Found: C, 77.9; H, 10.2. $C_{34}H_{54}O_4$ requires C, 77.6; H, 10.3%.

Light absorption: ε_{2080} = 3,300, ε_{2100} = 3,000, ε_{2150} = 1,750, ε_{2200} = 1,100 and ε_{2230} = 750.

Acid Hydrolysis of the Enol Acetate of iso-12-Keto-ursanyl Acetate

Conc. aqueous hydrochloric acid (5 c.c.) was added to a solution of the enol acetate of iso-12-keto-ursanyl acetate (100 mgm.) in benzene (6.0 c.c.) and methanol (25 c.c.), and the mixture refluxed for 4 hours. The product was obtained as usual, and, without further purification, was acetylated in acetic anhydride and pyridine in the normal manner. The acetate was crystallised from methanol to give plates (40 mgm.) of m.p. 211-215° (m.p. 227-229°, undepressed on mixing with an authentic specimen of iso-12-keto-ursanyl acetate, in a sealed, evacuated tube), $[\alpha]_D$ +60° (c, 1.4).

The Enol Acetate of 12-Keto-ursanyl Acetate

(cf. McLean, Silverstone and Spring, J. 1951, 935)

12-Keto-ursanyl acetate (1.0 g.) was refluxed for 24 hours in acetic anhydride (20 c.c.) with freshly fused sodium acetate (1.0 g.). The product was obtained as usual, and crystallised from chloroform-methanol to give the enol acetate (780 mgm.) as needles of m.p. 257-259°, $[\alpha]_D$ +49° (c, 0.86), giving a yellow colour with tetranitromethane.

Found: C, 77.6; H, 10.7. Calc. for $C_{34}H_{54}O_4$, C, 77.6; H, 10.3%.

Light absorption: ε_{2080} = 2,400, ε_{2100} = 2,000, ε_{2150} = 1,150, ε_{2200} = 820, ε_{2230} = 720.

Acid Hydrolysis of the Enol Acetate of 12-Keto-ursanyl Acetate

Conc. aqueous hydrochloric acid (10 c.c.) was added to a solution of the enol acetate of 12-keto-ursanyl acetate (200 mgm.) in benzene (20 c.c.) and methanol (60 c.c.), and the mixture refluxed for 4 hours. The product was obtained as usual, and, without further purification, was acetylated in acetic anhydride and pyridine in the normal manner. The acetate was crystallised from chloroform-methanol to give flattened needles (90 mgm.) of m.p. 273-275°, $[\alpha]_D$ +14° (c, 1.4). A mixture with 12-keto-ursanyl acetate showed no melting-point depression.

Bromination of iso-12-Keto-ursanyl Acetate

iso-12-Keto-ursanyl acetate (270 mgm.) in glacial acetic acid (15 c.c.) containing a trace of hydrobromic acid was treated at 60° with a solution of bromine in glacial acetic acid (11.7% w/v; 0.92 c.c.) over a period of 10 minutes. The mixture was then heated to 100° for one hour, and allowed to cool overnight. The product was obtained in the usual way, and crystallised from chloroform-methanol as plates (190 mgm.) of m.p. 282-285°, $[\alpha]_D$ +83° (c, 1.3). A mixture with 12-keto-urs-10-enyl acetate showed no melting-point depression.

Found: C, 79.5; H, 10.7. Calc. for $C_{32}H_{50}O_3$; C, 79.6; H, 10.4%. Light absorption: Max. at 2460Å (ε = 10,900).

12-Acetoxy-11-keto-urs-12-enyl Acetate

(cf. Silverstone, Ph.D. Thesis, Glasgow, 1949)

A solution of the enol acetate of 12-keto-ursanyl acetate (530 mgm.) in glacial acetic acid (13 c.c.) at 70° was treated over 20 minutes with a solution of chromium trioxide (400 mgm.) in the minimum volume of water and glacial acetic acid (11 c.c.). The temperature was maintained at 70°, and the reaction mixture stirred, for further 2 hours, and the product was then obtained as usual. This proved to be a gum, and was crystallised several times from aqueous acetone, to give 12-acetoxy-11-keto-urs-12-enyl acetate (270 mgm.) as needles of m.p. 249-251°, $[\alpha]_D$ +72° (c, 2.0). Light absorption: Max. at 2530Å (ε = 8,700). The material gave no colour with tetranitromethane.

Attempted Formation of the Enol Acetate of 12-Acetoxy-11-keto-urs-12-enyl Acetate

12-Acetoxy-11-keto-urs-12-enyl acetate (240 mgm.) was refluxed in acetic anhydride (12 c.c.) with freshly fused sodium acetate (360 mgm.) for 66 hours. The product was obtained in the usual way, and crystallised from aqueous acetone as needles (190 mgm.) of m.p. 248-250°, $[\alpha]_D$ +72° (c, 1.4), showing no colour reaction with tetranitromethane. Light absorption: Max. at 2540Å (ε = 10,500). A mixture of product and starting material showed no melting-point depression.

The Action of Strong Alkali on 12-Acetoxy-11-keto-urs-12-enyl Acetate

12-Acetoxy-11-keto-urs-12-enyl acetate (160 mgm.) was refluxed for 24 hours in ethanolic potassium hydroxide (10%; 20 c.c.). The product was obtained in the usual manner, and crystallised from aqueous acetone to give 11:12-diketo-ursanyl acetate (100 mgm.) as needles, m.p. 231-233°, $[\alpha]_D$ +131° (c, 0.54). Light absorption: Max. at 2900Å (ε = 9,000). 11:12-Diketo-ursanyl acetate gave a strong dark green colour with ferric chloride.

Chromium Trioxide Oxidation of the Enol Acetate of iso-12-Keto-ursanyl Acetate

A solution of the enol acetate of iso-12-keto-ursanyl acetate (420 mgm.) in glacial acetic acid (11 c.c.) at 70° was treated over 15 minutes with a solution of chromium trioxide (320 mgm.) in the minimum volume of water and glacial acetic acid (10 c.c.). The temperature was maintained at 70°, and the reaction mixture stirred, for further 2 hours, and the product was then obtained as usual. This proved to be a gum, but after five crystallisations from aqueous methanol, plates (40 mgm.) of m.p. 196-199°, $[\alpha]_D$ +22° (c, 1.09), were obtained. Light absorption: Max. at 2520Å (ε = 11,700). Found: C, 79.6; H, 10.6. Calc. for $C_{32}H_{50}O_3$: C, 79.6; H, 10.4%.

A mixture with 13-iso-12-keto-urs-10-enyl acetate showed

no melting-point depression.

This material (36 mgm.) was refluxed for 40 hours in ethanolic potassium hydroxide (12%; 25 c.c.), and the product obtained as usual. This crystallised from aqueous acetone to give needles of m.p. 237-240°, $[\alpha]_D$ +77° (c, 0.72). Light absorption: Max. at 2500Å (ε = 11,800). A mixture with 12-keto-urs-10-enol showed no melting-point depression.

iso-α-Amyradienonyl Acetate

(cf. Ruzicka, Rüegg, Volli and Jeger, Helv. Chim. Acta, 1947, 30, 140)

A solution of 12-keto-urs-10-enyl acetate (2.0 g.) in glacial acetic acid (65 c.c.) was refluxed for 24 hours, with selenium dioxide (3.0 g.). The cold solution was filtered from selenium, poured into water, and the precipitated solid taken up in ether. The ethereal solution was washed with dilute alkali, potassium cyanide solution (3%), and water, dried, and treated with charcoal. After removal of the ether, the gum remaining was crystallised once from methanol, to give needles (600 mgm.) of m.p. 226-229°, $[\alpha]_D$ +26° (c, 2.0), which gave a yellow colour with tetranitromethane. This material was dissolved in benzene-light petroleum (1:4; 100 c.c.) and chromatographed on alumina (8 x 2 cm.). The first fraction (300 mgm.) was eluted by washing with the same solvent mixture (1:4; 500 c.c., 1:2; 200 c.c.) and with

benzene (300 c.c.). Washing the column with ether-benzene (1:19; 300 c.c.) gave a second fraction (150 mgm.) which was crystallised from chloroform-methanol to give needles of m.p. 228-230°, $[\alpha]_D$ +19° (c, 0.88). The third fraction (70 mgm.), obtained by washing with ether -benzene (1:1; 100 c.c.) and ether (100 c.c.), crystallised from chloroform-methanol as needles of m.p. 250-253°, $[\alpha]_D$ +99° (c, 2.9). All three fractions gave yellow colours with tetranitromethane. The first fraction was crystallised from chloroform-methanol, and gave iso-α-amyradienonyl acetate (270 mgm.) as needles, m.p. 221-222°, $[\alpha]_D$ +7°, +8° (c, 0.53, 1.10).

Found: C, 79.8; H, 10.3. Calc. for $C_{32}H_{48}O_3$: C, 79.95; H, 10.1%.

Light absorption: Max. at 2100 (ε = 6,100) and 2370Å (ε = 9,500).

iso-α-Amyradienonol

iso-α-Amyradienonyl acetate (200 mgm.) was refluxed for 2 hours in ethanolic potassium hydroxide (3%; 40 c.c.), and the product obtained in the usual way. This was crystallised from n-hexane-acetone, to give iso-α-amyradienonol as needles of m.p. 116-119°, $[\alpha]_D$ -6°, -6°, (c, 1.6, 1.8).

Found: C, 81.9; H, 10.7. $C_{30}H_{46}O_2$ requires C, 82.1; H, 10.6%.

Acetylation of this alcohol with acetic anhydride

and pyridine in the usual way gave iso-α-amyradienonyl acetate, m.p. 220-221°, $[\alpha]_D$ +7° (c, 1.4), undepressed in melting-point when mixed with the specimen described above.

iso-α-Amyradienonyl II Acetate

(cf. idem, loc. cit.)

(i) Dry hydrogen chloride was passed through a solution of iso-α-amyradienonyl acetate (3.0 g.) in glacial acetic acid (600 c.c.) for 40 minutes. The solution was then allowed to stand at room temperature in a stoppered flask for 4 days. At the end of this time the solvent was removed by distillation in vacuo, and the residue crystallised several times from benzene-n-hexane to give iso-α-amyradienonyl II acetate (1.20 g.) as needles of m.p. 269-270°, $[\alpha]_D$ +160°, +157° (c, 0.79, 1.3), giving a very faint yellow colour with tetranitromethane.

Found: C, 80.2; H, 10.3. Calc. for $C_{32}H_{48}O_3$: C, 79.95; H, 10.1%.

Light absorption: Max. at 2370Å (ε = 11,000).

(ii) Material from the second and third fraction of the chromatography of the selenium dioxide oxidation product of 12-keto-urs-10-enyl acetate (see above) (680 mgm.) was treated with hydrogen chloride as described above. The product crystallised from benzene-n-hexane as needles (300 mgm.) of m.p. 269-271°, $[\alpha]_D$

+157° (c, 2.0) undepressed in melting-point when mixed with a specimen prepared as described in (i) above.

iso-α-Amyradienonol II

iso-α-Amyradienonyl II acetate (200 mgm.) was refluxed for 2 hours in ethanolic potassium hydroxide (3%; 40 c.c.), and the product obtained in the usual way. This was crystallised from n-hexane-acetone to give iso-α--amyradienonol II as needles of m.p. 215-216°, $[\alpha]_D$ +154°, +157° (c, 0.60, 1.0).

Found: C, 82.0; H, 10.6. $C_{30}H_{46}O_2$ requires C, 82.1; H, 10.6%.

Acetylation of this alcohol with acetic anhydride and pyridine in the usual way gave iso-α-amyradienonyl II acetate, m.p. 268-269°, $[\alpha]_D$+158° (c, 1.7), undepressed in melting-point when mixed with the specimen described above.

Oxidation of iso-α-Amyradienonyl Acetate with Potassium Permanganate

A stirred solution of iso-α-amyradienonyl acetate (500 mgm.) in stabilised glacial acetic acid (200 c.c.) was treated, over 30 minutes, at room temperature with a solution of potassium permanganate (132 mgm.) in water (20 c.c.). Stirring was continued for a further 30 minutes, and the reaction mixture was then worked up in the usual manner. On recrystallisation from chloroform

-methanol, the oxidation product was obtained as flattened needles (300 mgm.), m.p. 280-283°, $[\alpha]_D$ +56°, +55° (c, 1.2, 0.92).

Found: C, 77.5; H, 9.8. $C_{32}H_{48}O_4$ requires C, 77.4; H, 9.7%.

Light absorption: Max. at 2400Å (ε = 8,100).

Attempted Formation of the Enol Acetate of iso-α-Amyradienonyl Acetate

A solution of iso-α-amyradienonyl acetate (164 mgm.) in iso-propenyl acetate (15 c.c.), containing a trace of conc. sulphuric acid, was refluxed for 5 hours and allowed to cool overnight. It was then diluted with ether, the ethereal solution washed free of acid with water, dried, and solvent removed in vacuo. The residue was crystallised from chloroform-methanol to give needles (80 mgm.) showing a yellow colour with tetranitromethane, and having m.p. 215-217°, $[\alpha]_D$ +8° (c, 1.8). A mixture with starting material showed no melting-point depression.

The Action of Selenium Dioxide on the Enol Acetate of 12-Keto-urs-10-enyl Acetate

A solution of the enol acetate of 12-keto-urs-10-enyl acetate (500 mgm.) in stabilised glacial acetic acid (60 c.c.) was refluxed for 24 hours with selenium dioxide (1.5 g.). After cooling and filtration from

selenium, the mixture was poured into water, and the precipitate taken up in ether. The ethereal solution was washed free of acid with water, dried, and the solvent removed. The product was then crystallized from chloroform-methanol to give five fractions. The first three fractions, which gave red-brown colour reaction with tetranitromethane, were combined, and when recrystallised from chloroform-methanol gave needles (250 mgm.) of m.p. 256°, undepressed on mixing with starting material. The fourth and fifth crops, which gave yellow colour reactions with tetranitromethane, were combined and crystallised from chloroform-methanol. After four crystallisations, needles (10 mgm.) were obtained, which had m.p. 219-220°, undepressed on mixing with iso-α-amyradienonyl acetate.

Catalytic Hydrogenation of iso-α-Amyradienonyl Acetate

(i) With platinum catalyst in glacial acetic acid. (cf. Vogel, Diss., E.T.H., Zürich, 1952)

iso-α-Amyradienonyl acetate (1.0 g.) dissolved in stabilised glacial acetic acid (200 c.c.) was shaken under hydrogen for 20 hours with previously reduced platinum catalyst (300 mgm.). After filtration from platinum, solvent was removed in vacuo, and the residue taken up in benzene, washed free of acid, and the benzene distilled off. The crystalline solid remaining (1.0 g.) was dissolved in benzene-light petroleum (1:2; 225 c.c.)

and chromatographed on alumina (12 x 1.5 cm.). Washing with the same solvent mixture (800 c.c.) gave a fraction (750 mgm.) which crystallised from chloroform -methanol to give material of m.p. 207-240°. Washing with benzene-light petroleum (2:1; 200 c.c.) and benzene (200 c.c.) gave a fraction (100 mgm.) which crystallised from chloroform-methanol as plates of m.p. 275-279°. After several further crystallisations this material had m.p. 285-288°, $[\alpha]_D$ +82° (c, 1.3) (59 mgm.). A mixture with 12-keto-urs-10-enyl acetate showed no melting-point depression. Light absorption: Max. at 2520Å (ε = 10,300). A third fraction (80 mgm.) obtained on washing with benzene-ether (19:1; 400 c.c.) crystallised from chloroform-methanol as plates of m.p. 222-251°, but was not further investigated.

The first fraction from the above chromatography was dissolved in benzene-light petroleum (1:5; 220 c.c.) and chromatographed again on alumina (10 x 2 cm.). Washing with the same solvent mixture (1:5; 800 c.c., 1:2; 400 c.c.) gave a fraction (120 mgm.) which, on crystallisation from chloroform-methanol gave material of m.p. 176-190°, which was not further investigated. Continued washing with the same solvent mixture (2:1; 800 c.c.), with benzene (400 c.c.) and with ether-benzene (1:19; 200 c.c.) gave a fraction (270 mgm.) which crystallised from chloroform-methanol as plates of m.p.

207-210°. A third fraction (170 mgm.), eluted with more polar solvent mixtures, crystallised from chloroform-methanol to give material of m.p. 201-255°, and was ignored.

The second fraction from the second chromatogram was dissolved in light petroleum (170 c.c.) and chromatographed on alumina (11 x 1 cm.). Washing with petrol (400 c.c.) gave a fraction (36 mgm.) which could not be crystallised. The second fraction (70 mgm.) was obtained by washing with benzene-light petroleum (1:9; 300 c.c.), and crystallised from chloroform-methanol as plates of m.p. 220-222°. Later fractions, obtained by washing with more polar solvents, crystallised to give material of m.p. 206-210°, which was ignored. The second fraction was recrystallised several times from chloroform-methanol, and gave a saturated ketone (30 mgm.) as plates of m.p. 222-223°, $[\alpha]_D$ +93°, +91° (c, 0.91, 1.1).

Found: C, 79.3; H, 10.9. Calc. for $C_{32}H_{52}O_3$: C, 79.3; H, 10.8%.

Light absorption: Max at 2890 (ε = 63) and 2950Å (ε = 66)

The compound gave no colour reaction with tetranitromethane.

(ii) With platinum catalyst in glacial acetic acid containing a trace of hydrochloric acid.

iso-α-Amyradienonyl acetate (1.0 g.) was hydrogenated precisely as in the previous experiment,

except that one drop of conc. aqueous hydrochloric acid was added to the solution before hydrogenation. The product was obtained as described above, and, after drying, was dissolved in benzene-light petroleum (1:4; 100 c.c.) and chromatographed on alumina (12 x 2 cm.). The first fraction (421 mgm.) was obtained by washing with benzene-light petroleum (1:4; 600 c.c. 1:1; 450 c.c.) and benzene (300 c.c.), and after several crystallisations from chloroform-methanol gave needles of m.p. 178-179°, $[\alpha]_D$ +262° (c, 0.76).
Light absorption: Max. at 2060 (ε = 3,100) and 2800Å (ε = 6,900). The material gave a red-brown colouration with tetranitromethane. Washing with ether-benzene (1:19; 450 c.c. 1:2; 300 c.c. and 2:1; 150 c.c.) gave a second fraction (360 mgm.). A third fraction (124 mgm.) eluted with methanol-ether (1:19; 150 c.c.) could not be crystallised. The second fraction was crystallised from chloroform-methanol to give plates (156 mgm.) of m.p. 280-283°, $[\alpha]_D$ +89° (c, 1.0). A mixture with 12-keto-urs-10-enyl acetate showed no melting-point depression. Light absorption: Max. at 2480Å (ε = 9,600).

(iii) With platinum catalyst in ethanol.

iso-α-Amyradienonyl acetate (300 mgm.) dissolved in ethanol (redistilled; 120 c.c.) was shaken under hydrogen for 22 hours with previously reduced platinum

catalyst (100 mgm.). The filtered solution was evaporated to dryness *in vacuo*. The residue was crystallised from chloroform-methanol to give needles (270 mgm.) which gave a yellow colour with tetranitromethane, and had m.p. 216-218°, $[\alpha]_D$ +7° (*c*, 1.4). A mixture of product and starting material showed no melting-point depression.

(iv) With palladium catalyst in ethanolic potassium hydroxide solution.

iso-α-Amyradienonyl acetate (250 mgm.) dissolved in ethanol (redistilled; 100 c.c.) was added to a solution of potassium hydroxide (1.5g.) in ethanol (redistilled; 25 c.c.), and the mixed solution added to a suspension of 10% palladium on charcoal catalyst (200 mgm.) in ethanol (redistilled; 25 c.c.). The mixture was then shaken with hydrogen for 23 hours. The filtered solution was then poured into excess water, and the product obtained by ether extraction in the usual way. It was acetylated in the normal way, and the acetate crystallised from chloroform-methanol as needles (180 mgm.) of m.p. 215-216°, $[\alpha]_D$ +9° (*c*, 1.0), which gave a yellow colour with tetranitromethane. A mixture of starting material and product gave no melting-point depression.

Attempted Formation of the Enol Acetate of the Saturated Ketone, m.p. 222-223°

The saturated ketone (m.p. 222-223°, prepared as described in (i) above; 87 mgm.) was refluxed for 65 hours in acetic anhydride (5 c.c.) with freshly fused sodium acetate (150 mgm.). The product was obtained in the usual manner, and after several crystallisations from methanol gave plates (25 mgm.) of m.p. 219-220°, $[\alpha]_D$ +102° (c, 0.92). A mixture of product and starting material showed no melting-point depression. The product gave no colour reaction with tetranitromethane, and no material giving a yellow colour with that reagent was obtained.

Clemmensen Reduction of iso-α-Amyradienonyl Acetate

A hot solution of iso-α-amyradienonyl acetate (500 mgm.) in glacial acetic acid (75 c.c.) was treated with freshly amalgamated zinc (from 15 g. of zinc) and conc. hydrochloric acid (15 c.c.), and the mixture heated on the steam-bath for 1½ hours. A mixture of glacial acetic acid (35 c.c.) and conc. hydrochloric acid (12.5 c.c.) was added, heating continued for 1½ hours. The reaction mixture was immediately poured into water, and worked up in the usual manner. A gum was obtained which, even after acetylation by the customary method, could not be crystallised. The gum was then chromatographed on alumina

but only minute amounts of crystalline material, which could not be further investigated, were obtained.

Lithium Aluminium Hydride Reduction of iso-α-Amyradienonyl Acetate

A solution of iso-α-amyradienonyl acetate (500 mgm.) in dry ether (120 c.c.) was added dropwise to a suspension of powdered lithium aluminium hydride (500 mgm.) in dry ether (50 c.c.), and the mixture refluxed for 4 hours. After cooling, excess lithium aluminium hydride was destroyed with water. The mixture was then transferred to a separating funnel, and the ethereal solution washed free of alkali and suspended alumina with water. It was then dried, and ether removed in vacuo; the residue was acetylated with acetic anhydride and pyridine for 3 hours on the steam bath, and the acetate obtained as usual. This was crystallised from methanol to give the product (350 mgm.) as plates of m.p. 194-195°, $[\alpha]_D$ +59°, +60° (c, 1.9, 1.5).

Found: C, 78.2; H, 10.1. $C_{34}H_{52}O_4$ requires C, 77.8; H, 10.0%.

Light absorption: $\varepsilon_{2100} = 7{,}000$, $\varepsilon_{2150} = 5{,}200$, $\varepsilon_{2200} = 2{,}400$, and $\varepsilon_{2230} = 1{,}400$. The compound shows a yellow colour reaction with tetranitromethane.

Reduction of iso-α-Amyradienonyl Acetate with Sodium in Amyl Alcohol

(cf. Ruff, Ph.D. Thesis, Glasgow, 1949)

iso-α-Amyradienonyl acetate (500 mgm.) was dissolved in boiling amyl alcohol (redistilled; 20 c.c.) and sodium (1.2 g.) added portionwise to the solution. After boiling under reflux for 30 minutes, a further addition of amyl alcohol (3.3 c.c.) was made, and refluxing continued for 40 minutes. The solution was then poured into water, and the mixture extracted with ether. The ethereal solution was washed free of alkali with water, and ether and amyl alcohol removed, the latter by steam-distillation. The residue was again taken up in ether, washed and dried, and the gum remaining on removal of solvent acetylated by refluxing for 2 hours in acetic anhydride (20 c.c.). The acetate was obtained in the usual manner, and proved to be a gum (413 mgm.) which was dissolved in light petroleum (50 c.c.) and chromatographed on alumina (10 x 1 cm.). Washing with light petroleum (300 c.c.) and benzene-light petroleum (1:9; 100 c.c. 1:1; 100 c.c.) gave a fraction (290 mgm.) which crystallised from methanol as plates of m.p. 187-190°. Washing with benzene (200 c.c.) gave a fraction (50 mgm.) which crystallised from methanol as needles of m.p. 218-222°, which were not further investigated. The first fraction was recrystallised several

times from chloroform-methanol, and gave plates (142 mgm.) of m.p. 193-194°, $[\alpha]_D$ +58° (c, 2.0). The melting point was undepressed on mixing with a sample of the product obtained as described immediately above.

Found: C, 78.1; H, 10.0%.

Light absorption: ε_{2100} = 7,900, ε_{2150} = 5,000, ε_{2200} = 2,000, ε_{2230} = 600.

Treatment of the Di-acetate, m.p. 194-195°, with Hydrogen Chloride

(i) The di-acetate (m.p. 194-195°, prepared as described above; 300 mgm.) was dissolved in stabilised glacial acetic acid (30 c.c.), and treated with conc. aqueous hydrochloric acid (1.5 c.c.). The mixture was heated on the steam-bath for 15 minutes, then allowed to stand at room temperature overnight, and the product obtained by distillation of solvent *in vacuo*. This was acetylated in the normal manner, and the acetate obtained as usual. The acetate (244 mgm.) could not be crystallised, and was dissolved in benzene-light petroleum (1:9; 50 c.c.) and chromatographed on alumina (11 x 1 cm.). Washing with the same solvent mixture (400 c.c.) gave a fraction (180 mgm.) which could not be crystallised. Washing with benzene (100 c.c.) also gave uncrystallisable material (10 mgm.). Washing with ether-benzene (1:19; 100 c.c.) gave a fraction (20 mgm.)

which crystallised from chloroform-methanol as plates of m.p. 286-289°, $[\alpha]_D$ +83° (c, 0.74). Light absorption: Max. at 2480Å (ε = 10,700). A mixture of this product with 12-keto-urs-10-enyl acetate showed no melting-point depression. Further washing with the same solvent (100 c.c.), and with ether (100 c.c.) gave fractions (10 mgm., 20 mgm.) which could not be crystallised.

(ii) A solution of the di-acetate (260 mgm.) in chloroform (redistilled: 30 c.c.) was treated with dry hydrogen chloride for 1 hour, and allowed to stand at room temperature in a stoppered flask for 4 days. The product was obtained by removal of solvent in vacuo, and acetylated in the usual way. The acetate (210 mgm.), obtained as usual, could not be crystallised, and was dissolved in benzene-light petroleum (1:9; 100 c.c.) and chromatographed on alumina (10 x 1 cm.). Washing with the same solvent mixture (400 c.c.) and with benzene (100 c.c.) gave fractions (160 mgm., 10 mgm.) which could not be crystallised. Washing with ether-benzene (1:19; 100 c.c.) gave a fraction (10 mgm.) which crystallised from chloroform-methanol as plates of m.p. 283-286°, $[\alpha]_D$ +87° (c, 0.24). Light absorption: Max. at 2480Å (ε = 10,800). A mixture of this product with 12-keto-urs-10-enyl acetate showed no melting-point depression.

Kishner-Wolff Reduction of iso-α-Amyradienonyl Acetate

iso-α-Amyradienonyl acetate (2.0 g.) 100% hydrazine hydrate (10 c.c.) and sodium methoxide solution (2.0 g. sodium in 25 c.c. methanol) were heated together

in an autoclave for 13 hours at 200°. The product was obtained in the usual way, and acetylated in the normal manner. The acetate was obtained as usual, and was crystallised once from methanol and several times from chloroform-methanol. This gave the product (312 mgm.) as needles of m.p. 171-172°, $[\alpha]_D$ +43°, +42° (c, 1.3, 1.0).

Found: C, 82.6; H, 11.0. $C_{32}H_{50}O_2$ requires C, 82.3; H, 10.8%.

Light absorption: ε_{2100} = 6,900, ε_{2150} = 4,100, ε_{2200} = 1,500, and ε_{2230} = 700. The product gives a strong yellow colour with tetranitromethane. No homogeneous material was obtained by chromatography of the residue left on evaporation of the mother-liquor.

Attempted Catalytic Hydrogenation of the Kishner-Wolff Product, m.p. 171-172°

The product obtained as described immediately above (150 mgm.) was dissolved in ethyl acetate (35 c.c.) and stabilized glacial acetic acid (45 c.c.) and shaken with hydrogen for 17 hours over previously reduced platinum catalyst (100 mgm.). The product was obtained by removal of solvent in vacuo after filtration and crystallised from chloroform-methanol to give needles (128 mgm.) of m.p. 170-171°, $[\alpha]_D$ +44° (c, 0.96). A mixture of product and starting material showed no melting-point depression.

Treatment of the Kishner-Wolff Product, m.p. 171-172°, with Hydrogen Chloride

Dry hydrogen chloride was passed for 45 minutes into a solution of the product (m.p. 171-172°, obtained as described above; 100 mgm.) in chloroform (redistilled; 20 c.c.), and the mixture allowed to stand at room temperature in a stoppered flask for 5 days. Solvent was then removed *in vacuo*, and the residue crystallised from methanol, and subsequently chloroform-methanol, to give needles (40 mgm.) of m.p. 168-169°, $[\alpha]_D$ +320° (*c*, 1.0).

Found: C, 82.2; H, 11.2. Calc. for $C_{32}H_{50}O_2$: C, 82.3; H, 10.8%.

Light absorption: Max. at 2820Å (ε = 9,200). A mixture with ursa-10:12-dienyl acetate showed no melting-point depression.

Prolonged Reduction of iso-α-Amyradienonyl Acetate with Lithium in Liquid Ammonia

Liquid ammonia (250 c.c.) was added to a suspension of lithium (1.0 g.) in dry ether (100 c.c.), and stirred to give a deep blue solution. A solution of *iso*-α-amyradienonyl acetate (1.0 g.) in dry ether (300 c.c.) was added to this, with stirring, over 20 minutes, and stirring continued for a further 2 hours. These operations were carried out in an atmosphere of nitrogen. Ammonia was allowed to evaporate overnight, and excess

lithium destroyed with water. The ethereal layer was washed free of alkali, dried, and evaporated to dryness. The gum thus obtained was acetylated in acetic anhydride and pyridine for 2 hours on the steam-bath, and the acetate obtained in the usual way. The acetate (893 mgm.) was dissolved in light petroleum (100 c.c.) and chromatographed on alumina (14 x 1.5 cm.). Washing with benzene-light petroleum (1:9; 800 c.c.) gave a fraction (236 mgm.) which crystallised from methanol as needles of m.p. 217-218°. Subsequent washing gave no further homogeneous material. The first fraction was recrystallised from methanol to give the product as needles (140 mgm.) of m.p. 222-223°, $[\alpha]_D$ +60°, +60° (c, 0.92, 1.2).

Found: C, 77.6; H, 10.0. $C_{34}H_{54}O_4$ requires C, 77.5; H, 10.3%.

Light absorption: ε_{2100} = 3,400, ε_{2150} = 1,200, ε_{2200} = 0, and ε_{2230} = 0. The compound shows a yellow colour with tetranitromethane.

Catalytic Hydrogenation of the Lithium Reduction Product, m.p. 222-223°.

The product obtained as described immediately above (380 mgm.) was dissolved in stabilised glacial acetic acid (360 c.c.) and shaken with hydrogen for 18 hours over previously reduced platinum catalyst (200 mgm.) The filtered solution was evaporated to dryness, and the

residue crystallised from chloroform-methanol. This gave the product as felted needles (180 mgm.) of m.p. 229-230°, $[\alpha]_D$ +55°, +56° (c, 0.64, 1.2).

Found: C, 77.2; H, 10.9. $C_{34}H_{56}O_4$ requires C, 77.2; H, 10.7%.

The compound gives no colour reaction with tetranitromethane. Mixtures with starting material, with 12-acetoxy-ursanyl acetate, and with the product ($[\alpha]_D$ +80°) of sodium/amyl alcohol reduction of 12:13-epoxy-ursanyl benzoate (see p. 80), all showed pronounced melting-point depressions.

The Action of Hydrochloric Acid on the Catalytic Hydrogenation Product, m.p. 229-230°.

A solution of the product prepared as described immediately above (126 mgm.) in ethanol (20 c.c.) was treated with conc. aqueous hydrochloric acid (5 c.c.) and the mixture refluxed for 7 hours. After cooling overnight, the reaction mixture was worked up as usual, and acetylated in the normal manner. This gave a gum, which after one crystallisation from methanol, and several from chloroform-methanol gave the product as needles (10 mgm.) of m.p. 221-222°, $[\alpha]_D$ -18° (c, 0.74).

Found: C, 82.1; H, 11.6. $C_{32}H_{52}O_2$ requires C, 82.0; H, 11.2%.

Light absorption: ε_{2100} = 7,700, ε_{2150} = 6,900, ε_{2200} = 5,000, and ε_{2230} = 3,800. The compound gives

an orange-red colour with tetranitromethane. A mixture with urs-13(18)-enyl acetate showed a pronounced melting-point depression.

Limited Reduction of iso-α-Amyradienonyl Acetate with Lithium in Liquid Ammonia.

Liquid ammonia (1,500 c.c.) was added to a suspension of lithium (750 mgm.) in dry ether (100 c.c.), and stirred to give a deep blue solution. A solution of iso-α-amyradienonyl acetate (8.0 g.) in dry ether (1,500 c.c.) was added to this, with stirring, over 7 minutes, and stirring continued for further 10 minutes. The reaction was then stopped by addition of ammonium chloride. These operations were carried out in an atmosphere of nitrogen. Ammonia was allowed to evaporate overnight, and water added. The ethereal layer was washed free of alkali, dried, and evaporated to dryness. The residue thus obtained was hydrolysed by refluxing for 2 hours in ethanolic potassium hydroxide (3%; 1,000 c.c.), and the hydrolysate obtained as usual. This was crystallised from methanol to give a product as needles (1.07 g.) of m.p. 233-234°, $[\alpha]_D$ -39°, -40° (c, 0.79, 0.93).

Found: C, 81.3; H, 11.1. $C_{30}H_{48}O_2$ requires C, 81.7; H, 11.0%.

Light absorption: ε_{2100} = 2,900, ε_{2150} = 1,600, ε_{2200} = 1,000, and ε_{2230} = 600. The compound gave a

yellow colour with tetranitromethane.

The residue obtained on evaporation of the mother-liquors was acetylated in acetic anhydride and pyridine for 2 hours on the steam-bath, and the acetate obtained as usual. This acetate (3.9 g.) was dissolved in light petroleum (200 c.c.) and chromatographed on alumina (24 x 2.4 cm.). Only the first fraction eluted (66 mgm.), that obtained by washing with benzene-light petroleum (1:3, 200 c.c.), gave homogeneous material. This crystallised from chloroform-methanol to give needles of m.p. 218-220°, $[\alpha]_D$ +59° (c, 1.4), whose melting-point was undepressed on mixing with a sample of the product obtained by prolonged lithium in liquid ammonia reduction of iso-α-amyradienonyl acetate (see p. 124).

Acetylation of the Lithium Reduction Product, m.p. 233-234°.

The product obtained as described immediately above (m.p. 233-234°; 100 mgm.) was acetylated in the normal manner and the reaction mixture worked up as usual. The product was crystallised from chloroform-methanol to give the acetate as needles of m.p. 227-228°, $[\alpha]_D$ -27°, -28° (c, 1.3, 1.1).

Found: C, 79.3; H, 10.4. $C_{32}H_{50}O_3$ requires C, 79.6; H. 10.4%.

Light absorption: ε_{2100} = 2,700, ε_{2150} = 1,300,

ε_{2200} = 800, and ε_{2230} = 600. The compound gives a yellow colour reaction with tetranitromethane.

Catalytic Hydrogenation of the Acetate, m.p. 227-228°.

The acetate obtained as described immediately above (450 mgm.) was dissolved in stabilized glacial acetic acid (170 c.c.) and shaken with hydrogen for 18 hours over previously reduced platinum catalyst (300 mgm.). The filtered solution was evaporated to dryness, and the residue crystallised from chloroform-methanol to give plates of m.p. 214-216°. After repeated crystallisation from the same solvent mixture, the product was obtained as plates (120 mgm.) of m.p. 221-222°, $[\alpha]_D$ +88°, +87° (c, 1.6, 1.3).

Found: C, 79.2; H, 11.1. Calc. for $C_{32}H_{52}O_3$: C, 79.3; H, 10.8%.

A mixture with the saturated ketone, m.p. 222-223°, obtained by catalytic hydrogenation of iso-α-amyradienonyl acetate (see p. 115), showed no melting-point depression.

Catalytic Hydrogenation of iso-α-Amyradienonyl II Acetate

A solution of iso-α-amyradienonyl II acetate (500 mgm.) in stabilised glacial acetic acid (170 c.c.) was shaken with hydrogen for 24 hours over previously reduced platinum catalyst (250 mgm.); towards the end of this time needle-like crystals appeared. The solution

was then diluted with chloroform to dissolve these crystals, filtered from platinum, and evaporated to dryness. The residue, after several crystallisations from chloroform-methanol, gave the product (150 mgm.) as flattened needles of m.p. 253-256°, $[\alpha]_D$ +88°, +87° (c, 1.9, 1.6).

Found: C, 82.7; H, 10.9. Calc. for $C_{32}H_{50}O_2$: C, 82.3; H, 10.8%.

Light absorption: ε_{2100} = 5,800, ε_{2150} = 5,000, ε_{2200} = 3,900, and ε_{2230} = 3,200. The compound gives a yellow colour with tetranitromethane.

Hydrolysis of the Catalytic Hydrogenation Product, m.p. 253-256°.

The product obtained as described immediately above (140 mgm.) was hydrolysed by refluxing for 2 hours in ethanolic potassium hydroxide (3%; 25 c.c.), and the alcohol obtained in the usual way. After several crystallisations from acetone, the alcohol (70 mgm.) was obtained as needles of m.p. 199-200°, $[\alpha]_D$ +84°, +83°, (c, 1.4, 0.77).

Found: C, 85.1; H, 11.2. $C_{30}H_{48}O$ requires C, 84.8; H, 11.4%.

Acetylation of this alcohol with acetic anhydride and pyridine in the usual way gave the acetate, m.p. 247-250°, $[\alpha]_D$ +89° (c, 0.68), undepressed in melting-point when mixed with the specimen described above.

Treatment of the Catalytic Hydrogenation Product, m.p. 253-256°, with Hydrogen Chloride

Dry hydrogen chloride was passed for 45 minutes through a solution of the catalytic hydrogenation product (m.p. 253-256°, prepared as described above; 100 mgm.) in dry chloroform (20 c.c.), and the solution allowed to stand at room temperature in a stoppered flask for 5 days. After removal of solvent *in vacuo*, the residue was crystallised several times from chloroform-methanol to give needles (43 mgm.) of m.p. 169-170°, $[\alpha]_D$ +323° (*c*, 0.55).

Found: C, 82.2; H, 10.8. Calc. for $C_{32}H_{50}O_2$: C, 82.3; H, 10.8%.

Light absorption: Max. at 2800Å (ε = 8,500). The product gives a red-brown colour with tetranitromethane. A mixture with ursa-10:12-dienyl acetate showed no melting-point depression.

Reduction of iso-α-Amyradienonyl II Acetate with Sodium in Amyl Alcohol

(cf. Ruff, Ph.D. Thesis, Glasgow, 1949.)

iso-α-Amyradienonyl II Acetate (1.0 g.) was dissolved in boiling amyl alcohol (redistilled; 40 c.c.), and sodium (2.5 g.) added portionwise to the solution. After boiling under reflux for 30 minutes, a further addition of amyl alcohol (7 c.c.) was made, and refluxing continued for 40 minutes. The solution was then poured

into water, and the mixture extracted with ether. The ethereal solution was washed free of alkali with water, and ether and amyl alcohol removed, the latter by steam-distillation. The residue was again taken up in ether, washed and dried, and the gum remaining on removal of solvent acetylated by refluxing for 2 hours in acetic anhydride (20 c.c.). The acetate was obtained in the usual manner, and proved to be a gum (810 mgm.) which was dissolved in light petroleum (50 c.c.) and chromatographed on alumina (11 x 1.5 cm.) Washing with light petroleum (600 c.c.) gave a fraction (300 mgm.) which crystallised from chloroform-methanol to give material of m.p. 162-165°. Subsequent washing with more polar solvents gave only uncrystallisable fractions. The first fraction was recrystallised several times from chloroform-methanol to give the product (100 mgm.) as flattened needles of m.p. 179-181°, $[\alpha]_D$ -687°, -694° (c, 1.4, 0.79).

Found: C, 83.0; H, 10.5. Calc. for $C_{32}H_{48}O_2$: C, 82.7; H, 10.4%.

Light absorption: Max. at 2220 (ε = 7,500) and 2800Å (ε = 6,700). The compound gives an intense dark red colour with tetranitromethane.

Reduction of iso-α-Amyradienonyl II Acetate with Lithium Aluminium Hydride

A solution of iso-α-amyradienonyl II acetate (500 mgm.) in dry ether (120 c.c.) was added dropwise to a suspension of lithium aluminium hydride (500 mgm.) in dry ether (80 c.c.), and the mixture refluxed for 4 hours. After cooling, excess lithium aluminium hydride was destroyed with water. The mixture was then transferred to a separating funnel, and the ethereal solution washed free of alkali and suspended alumina with water. It was then dried and solvent removed in vacuo. The residue was crystallised from n-hexane-acetone, to give the product as needles (153 mgm.) of m.p. 218-219°, $[\alpha]_D$ +76°, +76°, (c, 0.96, 0.87).

Found: C, 82.0; H, 11.3. $C_{30}H_{48}O_2$ requires C, 81.8; H, 11.0%.

Light absorption: ε_{2100} = 3,500, ε_{2150} = 1,900, ε_{2200} = 600 and ε_{2230} = 300. The compound gives a yellow colour with tetranitromethane.

Acetylation of the Lithium Aluminium Hydride Reduction Product, m.p. 218-219°.

The product obtained as described immediately above (200 mgm.) was acetylated in acetic anhydride and pyridine on the steam-bath for 2½ hours, and the reaction mixture worked up in the usual way. The product was crystallised several times from chloroform-methanol to

give flattened needles (110 mgm.) of m.p. 180-181°, $[\alpha]_D$ -706° (c, 1.0). Light absorption: Max. at 2220 (ε = 10,000) and 2820Å (ε = 7,700). A mixture with the product of sodium and amyl alcohol reduction of iso-α-amyradienonyl II acetate (see p. 131) showed no melting-point depression. The compound gives a dark red colour with tetranitromethane.

Catalytic Hydrogenation of the Acetate, m.p. 180-181°.
(cf. Ruff, Ph.D. Thesis, Glasgow, 1949)

The acetate obtained as described immediately above (100 mgm.) was dissolved in stabilised glacial acetic acid (30 c.c.) and shaken with hydrogen for 1 hour over previously reduced platinum catalyst (50 mgm.) In the course of the reaction, needle-like crystals appeared. After these had been dissolved by addition of chloroform, the solution was filtered from platinum and evaporated to dryness in vacuo. The residue was crystallised from chloroform-methanol to give the product (46 mgm.) as flattened needles of m.p. 250-252°, $[\alpha]_D$ +87° (c, 1.4). Light absorption: ε_{2100} = 3,500, ε_{2150} = 2,400, ε_{2200} = 1,400, and ε_{2230} = 800.
Found: C, 82.4; H, 11.0. Calc. for $C_{32}H_{50}O_2$: C, 82.3; H, 10.8%.
The compound gives a yellow colour with tetranitromethane.
A mixture with the catalytic hydrogenation product from iso-α-amyradienonyl II acetate (m.p. 253-256°) showed no

melting-point depression.

Attempted Formation of the Enol Acetate of iso-α-Amyradienonyl II Acetate

(i) iso-α-Amyradienonyl II acetate (300 mgm.) was refluxed for 68 hours in acetic anhydride (12 c.c.) with freshly fused sodium acetate (400 mgm.). The product was obtained in the usual way, and crystallised from chloroform-methanol to give needles (220 mgm.) of m.p. 264-265°, $[\alpha]_D$ +156°(c, 0.78). The compound gives no colour with tetranitromethane. A mixture with an authentic sample of iso-α-amyradienonyl II acetate showed no melting-point depression.

(ii) A solution of iso-α-amyradienonyl II acetate (200 mgm.) in iso-propenyl acetate (15 c.c.), containing a trace of conc. sulphuric acid, was refluxed for 5 hours and allowed to cool overnight. It was then diluted with ether, the ethereal solution washed free of acid with water, dried, and the solvent removed in vacuo. The residue was crystallised from chloroform-methanol to give needles (90 mgm.), giving no colour with tetranitromethane, and having m.p. 265-267°, $[\alpha]_D$ +157° (c, 0.28). A mixture with starting material showed no melting-point depression.

Attempted Perbenzoic Acid Oxidation of the Catalytic Reduction Product, m.p. 253-256°.

A solution of perbenzoic acid in chloroform (70 mgm./c.c.; 1.9 c.c., 2.1 mols) was added to a solution of the product (m.p. 253-256°; 215 mgm.) of catalytic hydrogenation of iso-α-amyradienonyl II acetate in chloroform (redistilled ; 25 c.c.) at 0°. The mixture was allowed to stand at that temperature for 70 hours. It was then diluted with chloroform (redistilled; 50 c.c.) and washed thoroughly with saturated sodium bicarbonate solution and with water. After drying over anhydrous sodium sulphate, the solution was evaporated to dryness in vacuo. The residue crystallised from chloroform-methanol to give plates (116 mgm.) of m.p. 250-252°, $[\alpha]_D$ +94° (c, 0.54). This material gave a yellow colour with tetranitromethane, and a mixture with starting material showed no melting-point depression. No further crystalline material could be obtained from the mother-liquor.

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