An Investigation of the Triterpene α-Amyrin and of 6-Chloro-3-Benzoyloxy-Cholest-4-ene.

A Thesis in Two Parts

submitted by

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In fulfilment of the requirements for

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Glasgow.

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In presenting this thesis, the author wishes to express his sincere thanks to Professor F. S. Spring for his guidance during the course of the investigations, and to Dr. J. McLean for his continued interest and encouragement.

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SUMMARY.

Part I - α-Amyrin.

Investigations were carried out on the naturally occurring triterpene α-amyrin, $C_{30}H_{50}O$, with a view to elucidating its structure.

Oxidation of the hydrocarbon 1-α-amyradiene, $C_{30}H_{48}$, by various reagents, failed to give homogeneous products or reproducible results though potassium permanganate gave material analysing as $C_{30}H_{48}O$.

Both hydrogen peroxide and ozone have been shown to react with α-amyrin esters to give oxides which may be isomerised to saturated ketones. The compound described in the literature as α-amyranonyl benzoate is, in fact, α-amyrin benzoate oxide. Both the oxides and ketones may be partially dehydrogenated by bromine to give <u>iso</u>-α-amyrenonyl esters.

Reduction of α-amyrone by three different methods gave the same hydrocarbon α-amyrene, $C_{30}H_{50}$, in every case. This was oxidised to α-amyrene oxide which could be isomerised to the saturated ketone <u>iso</u>-α-amyranone (also obtained by Kishner-Wolff reduction of α-amyrane dione) or reduced to the alcohol <u>iso</u>-α-amyranol.

Attempts to chlorinate this alcohol so that the halogenated derivative might be reduced to the saturated hydrocarbon α-amyrane, resulted in every case in dehydration, and α-amyrene was formed.

In hydrogen peroxide oxidations of α-amyrin esters high melting by-products are formed together with the oxide, but

investigation of these failed to elucidate their nature.

It was hoped to prepare various α-diketones and ketols so that these could be treated with periodic acid. Ring opening might occur giving acid products. α-Amyradionol, α-amyradionyl acetate and α-amyradondiol mono-benzoate were all prepared. The first of these was made by an improvement of a published method, and the last (an α-ketol) by replacement of bromine by hydroxyl in bromo-α-amyranonyl benzoate. These, on treatment with periodic acid, gave neutral products which were apparently isomeric with the starting material, but without the original ketol, or dione, grouping.

Reduction of α-amyrin benzoate oxide gave the compound described in the literature, but the action of various reducing agents on α-amyranonyl benzoate gave non-homogeneous products.

New methods of investigation of the problem were examined, and the amyrin nucleus was found to be stable to alkali at high temperature, but completely broken down by aluminium trichloride.

Many other compounds were prepared, among them a sulphite of α-amyrin, and an examination of molecular rotation differences between some of the compounds and their esters showed that changes in the neighbourhood of the double bond appear to exert a vicinal action on the hydroxyl group.

Part II - 6-Chloro-3-Benzoyloxy-Cholest-4-ene.

This compound, formulated as above by Spring and Swain (J.(1939),1356) as 4-chloro-3-benzoyloxy-cholest-5-ene by

Petrow, Rosenheim and Starling (J.(1943),135) and as 3-chloro-4-benzoyloxy-cholest-5-ene by Berg and Wallis, (J.Biol.Chem.(1946)162(3),683) was investigated.

A synthesis of 3-chloro-4-hydroxy-cholest-5-ene was carried out by the action of selenium dioxide on cholesteryl chloride, and a new synthesis of 6-chloro-3-acetoxy-cholest-5-ene achieved by the action of phosphorus pentachloride on 6-keto-cholestanyl acetate.

Degradative experiments on Spring and Swain's compound by way of its oxide failed to give the required products because of ready loss of halogen, but some new light has been thrown on the problem.

A comparison of the optical rotations of the compound under investigation (as acetate) and 3-chloro-4-acetoxy-cholest-5-ene leads to the belief that Petrow, Rosenheim and Starling's formulation may be correct.

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Part I - An investigation of the Triterpene α-Amyrin

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Part One.

An Investigation of the Triterpene.

α-Amyrin.

HISTORICAL.

HISTORICAL.

Naturally occurring crystalline compounds of high molecular weight containing only carbon, hydrogen and oxygen generally fall into either the steroid or the triterpene group. Members of the former group give Diel's hydrocarbon (3-methyl-1:2-cyclopentenophenanthrene) on dehydrogenation, while the latter give homologues of napthalene (1.). The triterpenes include the alcohol, amyrin, which occurs in many resins, the diol, betulin, obtained from birch bark, and several hydroxy acids, which may occur free in the resins, e.g. elemolic acid in Manila-elemi resin, or combined as glucosides in the saponins, e.g. glycyrrhetic acid, from liquorice.

The formulation of triterpenes has presented many difficulties. Purification is seldom easy, combustion results frequently fail to distinguish between homologous formulae and cryoscopic determination of molecular weights are not always reliable. Investigations embodying careful micro-combustions, determination of the equivalents of acids, the acetyl value of acetates and methoxyl value of esters have shown that many of these compounds contain 30 carbon atoms. (2,3,4,5,6,7,8,9.).

Structural work on the series is further complicated by the fact that functional groups are frequently difficult to detect. Thus, carbonyl groups may not react with the usual reagents, and double bonds may be inert towards reduction in the presence of catalysts. Indeed, the presence of an ethylenic linkage is often only indicated by the yellow colour developed with tetranitromethane.

Two methods of investigation have been widely employed, namely: (a) dehydrogenation, and (b) oxidative degradation. These have enabled the triterpenes to be divided into several groups on structural grounds, the two principal being those of α- and β-amyrin. The α-amyrin series consists of four members:-

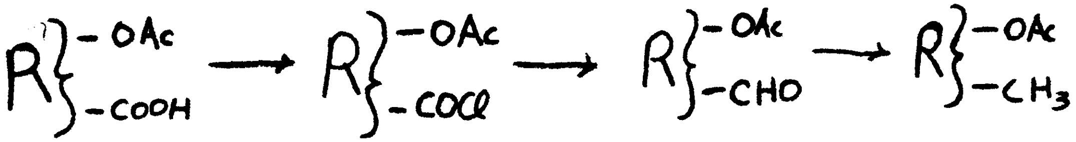
			Functional groups.
i)	α-Amyrin	$C_{30}H_{50}O$	OH
ii)	Uvaol	$C_{30}H_{50}O_2$	OH, OH.
iii)	Ursolic Acid	$C_{30}H_{48}O_3$	OH, COOH.
iv)	β-Boswellic Acid.	$C_{30}H_{48}O_3$	OH, COOH.

Occurrence and Inter-relationship.

α-Amyrin occurs naturally along with β-amyrin, and is the chief representative of the α-amyrin group. The two isomeric alcohols constitute the mixture, named Amyrin by Baup (10), which was first isolated by Rose (11.) from Manila Elemi resin, this remaining the principal source though many others are now known (12). Separation is usually achieved by methods depending on the differing solubilities of acetates, benzoates or anisates (13.).

Ursolic acid occurs in apple pomace, cranberries, saxifrage and other plants; also glycosidified with d-sorbitol in pyracantha augustofolia. Uvaol occurs with the above in arctostaphyles uva ursi, and leucothoe keiskei and β-boswellic acid in frankincense.

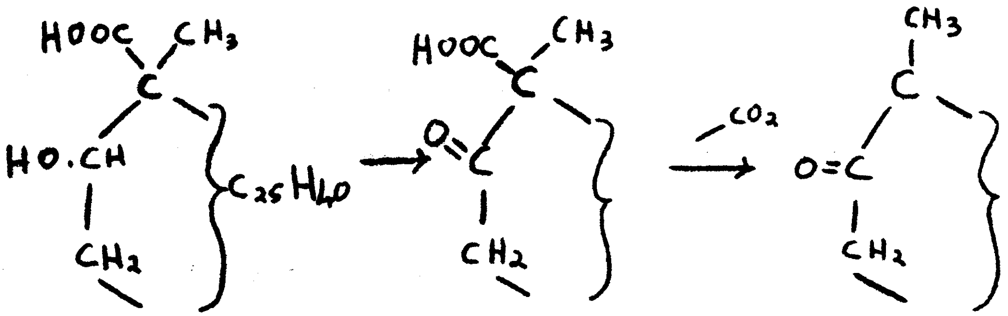
Both ursolic and β-boswellic acid have been converted to α-amyrin by conversion of the carboxylic acid grouping to a methyl group (14). The acid, the hydroxyl group having been protected, is converted to the acid chloride, Rosenmund reduction of which gives the corresponding aldehyde which is reduced by the Kishner-Wolff method, thus:-



$$R\left\{\begin{matrix}-OAc\\-COOH\end{matrix}\right. \longrightarrow R\left\{\begin{matrix}-OAc\\-COCl\end{matrix}\right. \longrightarrow R\left\{\begin{matrix}-OAc\\-CHO\end{matrix}\right. \longrightarrow R\left\{\begin{matrix}-OAc\\-CH_3\end{matrix}\right.$$

Ursolic acid (as benzoate) has also been reduced to uvaol (15.). The two acids thus differ from α-amyrin by having a carboxylic grouping in place of one angular methyl group, while uvaol has a $-CH_2OH$ in place of the carboxyl group of ursolic acid.

The carboxyl group of β-boswellic acid is situated at C_1 since mild oxidation gives a compound, $C_{29}H_{46}O$, nor-β-boswellenone, by oxidation of the C_2 hydroxyl group to a carboxyl and loss of carbon dioxide from the intermediate β-keto acid (16.).

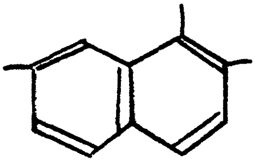


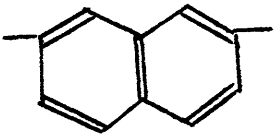
Only those reactions of the other members of the group which have a direct bearing on the structure of α-amyrin will be considered in the following pages.

Structural Evidence.

I. Dehydrogenation.

Selenium dehydrogenation of α-amyrin and of ursolic acid produced the compounds (I) to (VI) as shown in the table below. All have been synthesised.


(I)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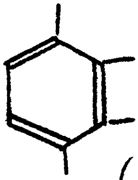
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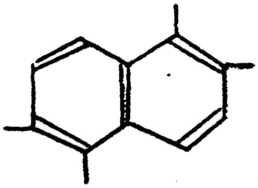
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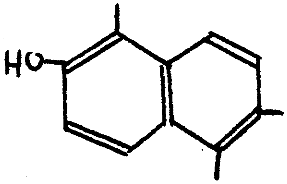
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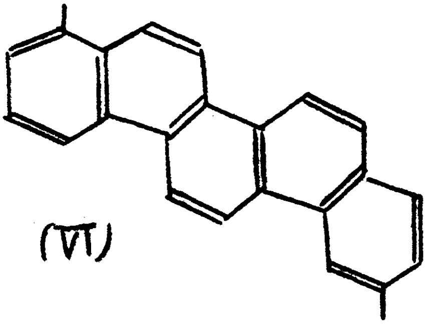
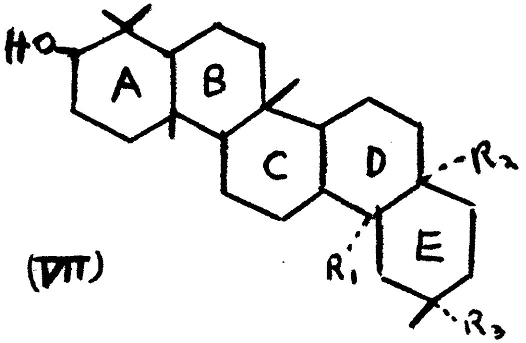

(VI)

	(I)	(II)	(III)	(IV)	(V)	(VI)
α-Amyrin	(17)	-	-	-	(17)	(17)
Ursolic Acid	(18)	(I)	(19)	(19)	(19)	(19)

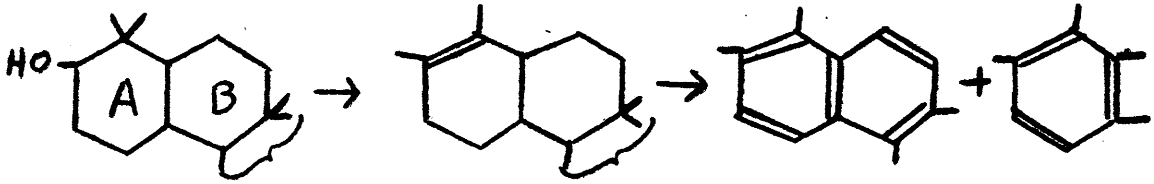
The formation of (VI) points to a pentacyclic structure. The construction of a triterpene formula to account for this, and the other dehydration products, leads to the polymethyl perhydropicene skeleton (VII). This structure consists of six isoprene (C_5H_8) units as is essential in the formulation of triterpenes.



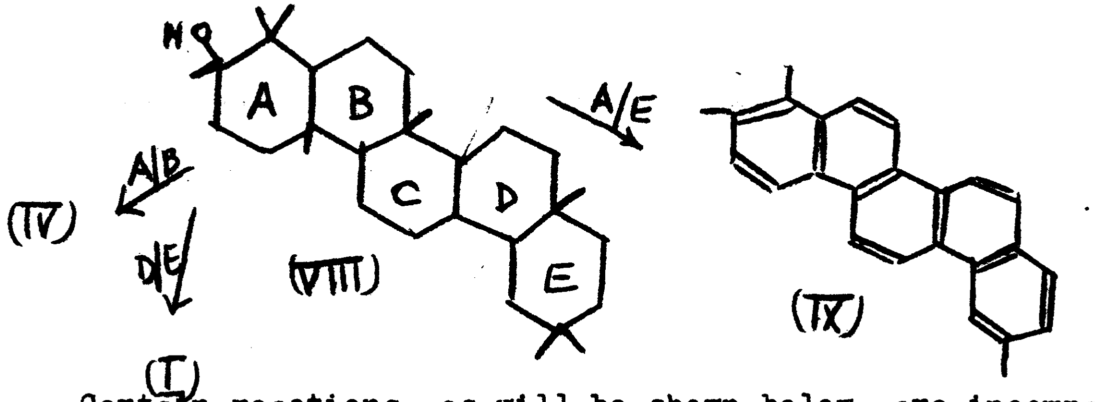
(VII)

$R_1 = CH_3$ if $R_2 = H$
$R_2 = CH_3$ if $R_1 = H$
$R_3 - CH_3$

Products (I) and (II) are thus derived from rings D/E, (III) from ring A, and (IV) and (V) from rings A/B. The formation of 1:2;5;6-tetramethylnaphthalene and 1:2;3;4-tetramethylbenzene can be explained by assuming a retro-pinacolic dehydration of the hydroxyl group to have first occurred.



This has been confirmed by dehydrogenation of methyl-α-amyrin (VIII), prepared by the action of a Grignard reagent on α-amyrenone, when only (IV), (I) and a picene homologue, almost certainly 1:2;8-trimethylpicene (IX) were isolated.



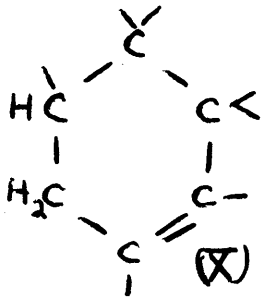
Certain reactions, as will be shown below, are incompatible with the skeleton (VII).

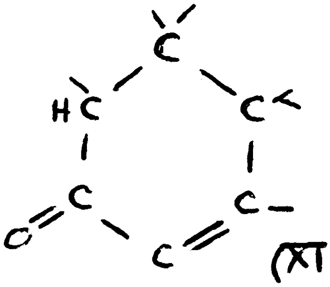
2. The Double Bond and the Hydroxyl Group.

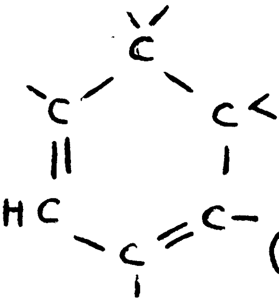
α-Amyrin is a secondary alcohol since mild oxidation gives a reactive ketone (20). The double bond is exceedingly unreactive, and proof of its presence is given by the yellow colour produced with tetranitromethane on a chloroform solution of the substance, and also by the fact that oxidation of α-amyri esters with chromic acid gives the corresponding esters of α-amyrenonol, the "oxy-α-amyrin" of Vesterberg (20), which has been shown by Spring and Vickerstaff to be an α,β-unsaturated ketone (17). Catalytic hydrogenation of α-amyrenonol gives α-amyrin (21). Reduction of the esters with sodium and amyl alcohol, however, gives either α-amyradienyl esters (17) or amyl-α-amyratriol, an addition-reduction adduct which with acetic anhydride gives α-amyradienyl acetate (22).

α-Amyradienol contains a conjugated system of double bonds located in one ring (23,24). It may also be formed by partial dehydrogenation of α-amyrin with sulphur (25), or (as acetate) by the action of N-bromsuccinimide on α-amyrin acetate (26).

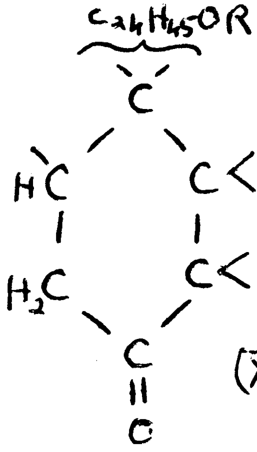
The fragment (X) in α-amyrin is thus suggested, α-amyrenonol containing (XI) and the dienol (XII).


(X)

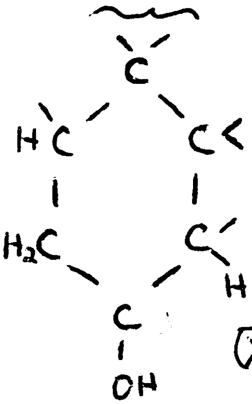

(XI)


(XII)

Hydrogen peroxide oxidation of α-amyrin benzoate gives a compound which was thought to contain an unreactive ketone group, and was described as α-amyranonyl benzoate (XIII, R = Bz). This, on reduction with sodium and amyl alcohol, gave a saturated diol, dihydroxy-α-amyrane (XIV, R = H).



(XIII)



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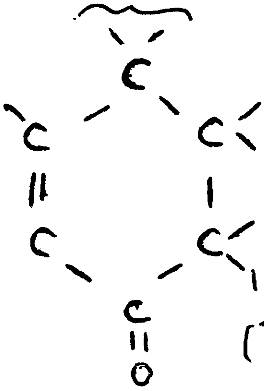

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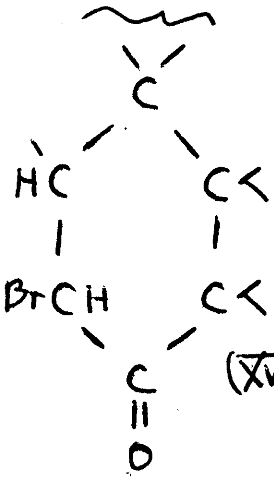

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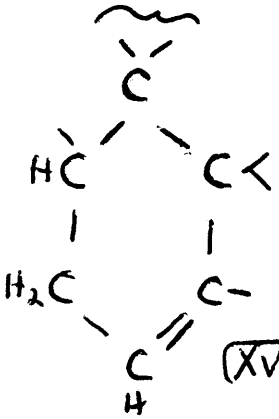
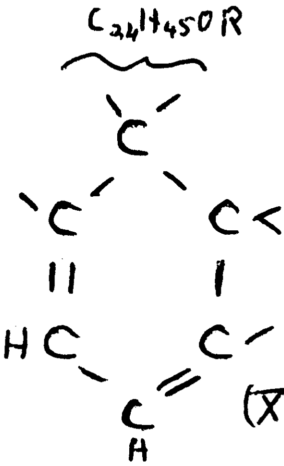


(XVII)



(XVIII)

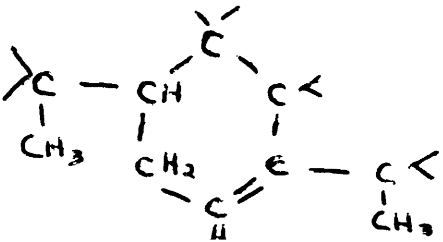
Treatment of (XIII) with bromine results in partial dehydrogenation, giving an α,β-unsaturated ketone, <u>iso</u>-α-amyrenonyl benzoate (XV). The intermediate bromo-ketone (XVI) may be isolated (27). Sodium and amyl alcohol reduction of (XV) followed by treatment with acetic anhydride gives α-amyradienyl acetate (XVIII, R = Ac) (24). Fragment (X) can therefore be extended to (XVII).

Partial dehydrogenation of β-amyrin or treatment of β-amyrin esters with N-bromsuccinimide results in the introduction of a conjugated triene system into the molecule (28). Similar treatment of α-amyrin and its esters, however, introduces one new double bond only giving a diene system (25,26). This suggests one of the positions α- to a double bond in α-amyradienol is blocked by an angular methyl group. Also, partial dehydrogenation of α-amyrenonyl esters with bromine cannot be carried out, whereas in the β-series β-amyradienonyl esters are formed. This might be due to the absence of a hydrogen atom on the γ-carbon atom of the α,β-unsaturated ketone α-amyrenonol, i.e. there is a methyl group.

Further evidence that there is at least one methyl group in the vicinity of the double bond was obtained by Ewen and Spring (30). Acetyl ursolic acid (which differs from α-amyrin acetate by having a carboxylic grouping in place of one of the methyl groups) on oxidation yields ketoacetylursolic acid, which is an α,β-unsaturated ketone. This, when heated with quinoline, loses the elements of formic acid, and gives nor-α-amyradienonyl acetate, which contains the system (XIX).

$$O=\overset{|}{C}-\overset{|}{C}=\overset{|}{C}-\overset{|}{C}=C<$$

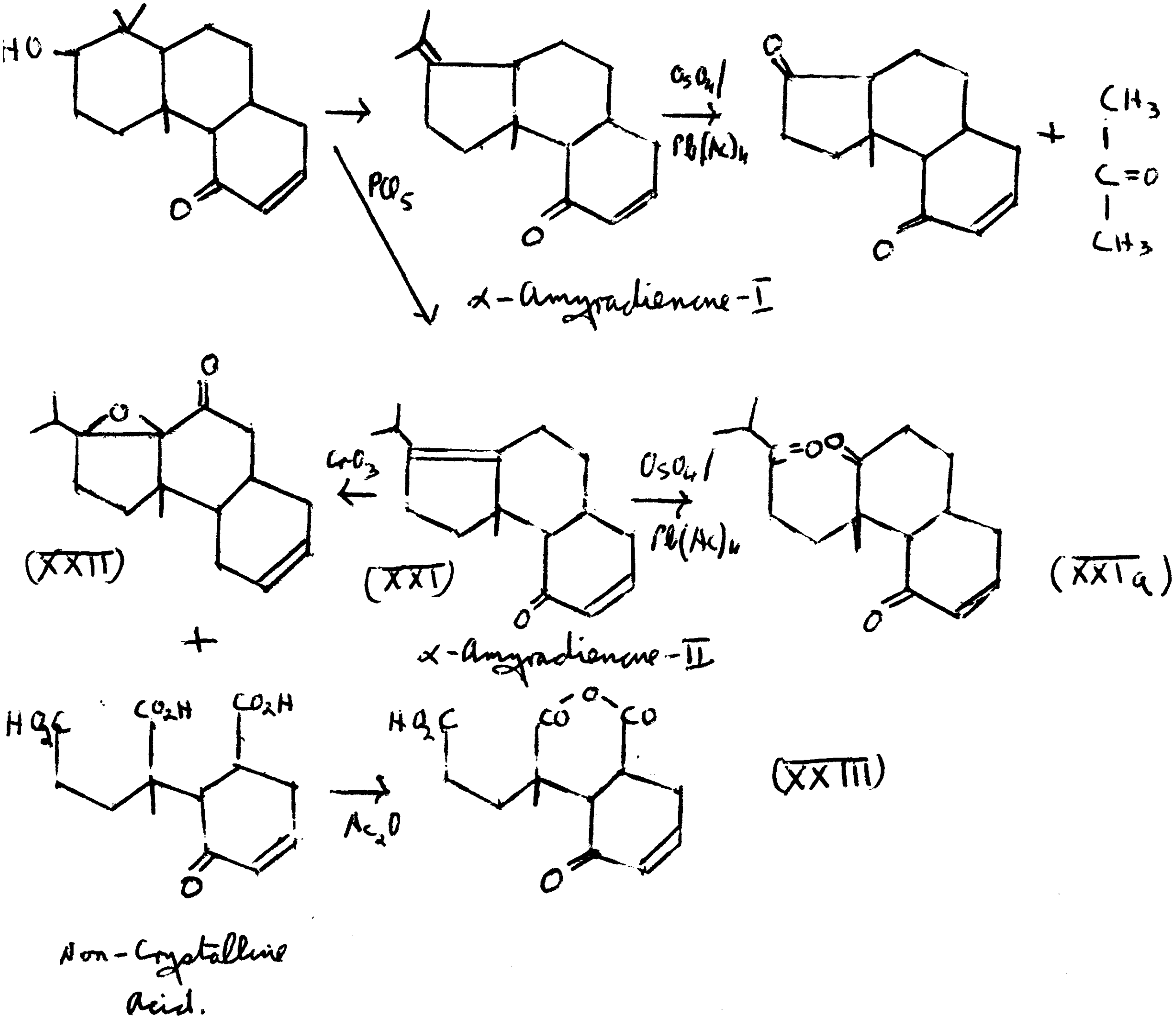
We can thus, tentatively, elaborate (XVII) to (XX).



(XX)

Dehydration of α-amyrin with phosphorus pentoxide gives l-α-amyradiene (31), which has been shown by Ewen, Gillam and Spring to contain a conjugated system of double bonds distributed between two rings (32). The same authors showed that dehydration of α-amyradienol gave l-α-amyratriene containing a conjugated system of three ethylenic linkages. These, and other similar reactions, seemed to indicate that the double bond was more closely situated to the hydroxyl group than hitherto suspected, possibly even in ring B (32). Proof that this was not so was provided as follows:-

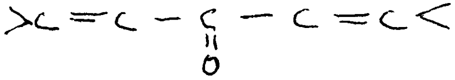
α-Amyrenonol on treatment with phosphorus pentachloride gives α-amyradienone-I and α-amyradienone-II, in neither of which are the two double bonds conjugated. The first of these on treatment with osmium tetroxide followed by lead tetracetate oxidation of the product gives a diketone $C_{27}H_{40}O_2$, together with acetone, indicating the presence of an isopropylidene ($Me_2C =$) grouping in the diene. A retropinacolic rearrangement accompanied by the conversion of ring A from a six-membered to a five-membered ring must then have occurred on treatment with phosphorus pentachloride (33).



α-Amyradienone-II on similar oxidation by osmium tetroxide and lead tetracetate gives a triketone (XXIa), and so has been formulated as (XXI). Treatment of (XXI) with chromic acid gives a diketo-oxide (XXII). More drastic oxidation of (XXI) using the same reagent gives (XXII) together with an acid fraction. The acid cannot be crystallised, but on refluxing with acetic anhydride gives the anhydride (XXIII). In all these reactions the original α,β-unsaturated ketone grouping of α-amyrenonol has been maintained, the double bond of α-amyrin must therefore lie in ring C.

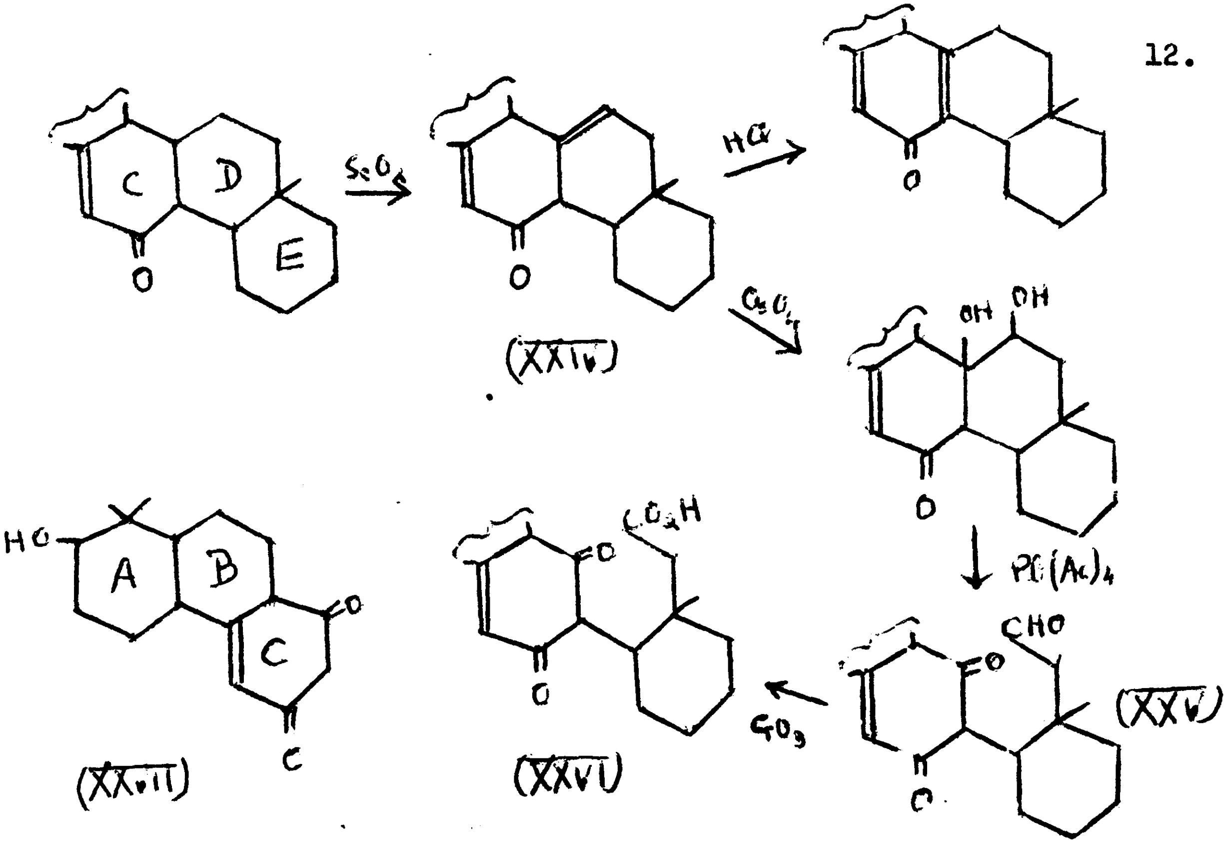
Of great importance, but conflicting in results, are two series of reactions, which have been carried out by Ruzicka and his co-workers (35,36) (37). The first of these may be summarised thus.

iso-α-Amyrenonyl acetate was treated with selenium dioxide in acetic acid solution, a new double bond being thereby introduced. This product gave a yellow colouration with tetranitromethane, and was designated iso-α-amyradienonyl acetate (XXIV). Isomerisation of this with hydrochloric acid gave a product which showed no colouration with tetranitromethane. This fact, together with its absorption spectrum (λ, 2370 Å, log. E_{max}, 4.2) suggest it contains the system

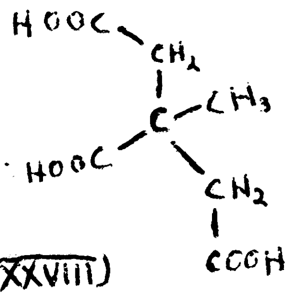


$$>C=C-\underset{\overset{\|}{O}}{C}-C=C<$$

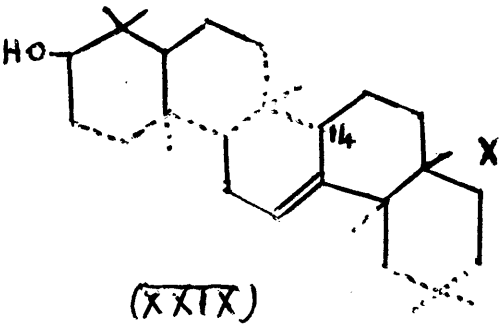
Treatment of (XXIV) with osmium tetroxide gave a mixture of two glycols, which must have been formed by attack at the new ethylenic linkage, since the absorption spectrum of the iso-α-amyradienonyl acetate was maintained. Oxidation of either of these glycols with lead tetracetate resulted in ring fission, and a tricarbonyl compound possessing one aldehyde group (XXV) was formed. The aldehyde was oxidised to the acid (XXVI). These reactions, which are believed to take place without change in the carbon skeleton, may be summarised schematically by the following partial formulation.



Pyrolysis of (XXVI) gave a volatile and a non-volatile fraction. The latter could be methylated, giving three products, two of which were hydroxy compounds $C_{20}H_{30}O_3$, and therefore consisted of rings A, B and C. The third product was considered to be an ester of one of these with the volatile acid fraction. The compounds $C_{20}H_{30}O_3$ were thought to be of type (XXVII). The volatile fraction, which must have been formed from ring E, was a monocarboxylic acid, $C_{11}H_{18}O_2$, which absorbed one mol. of hydrogen on hydrogenation. Oxidation of the acid by chromic acid, followed by chromatography on charcoal gave, as one of the products, an optically active tribasic acid $C_7 H_{10}O_6$, identified as β-methyltricarballylic acid (XXVIII).



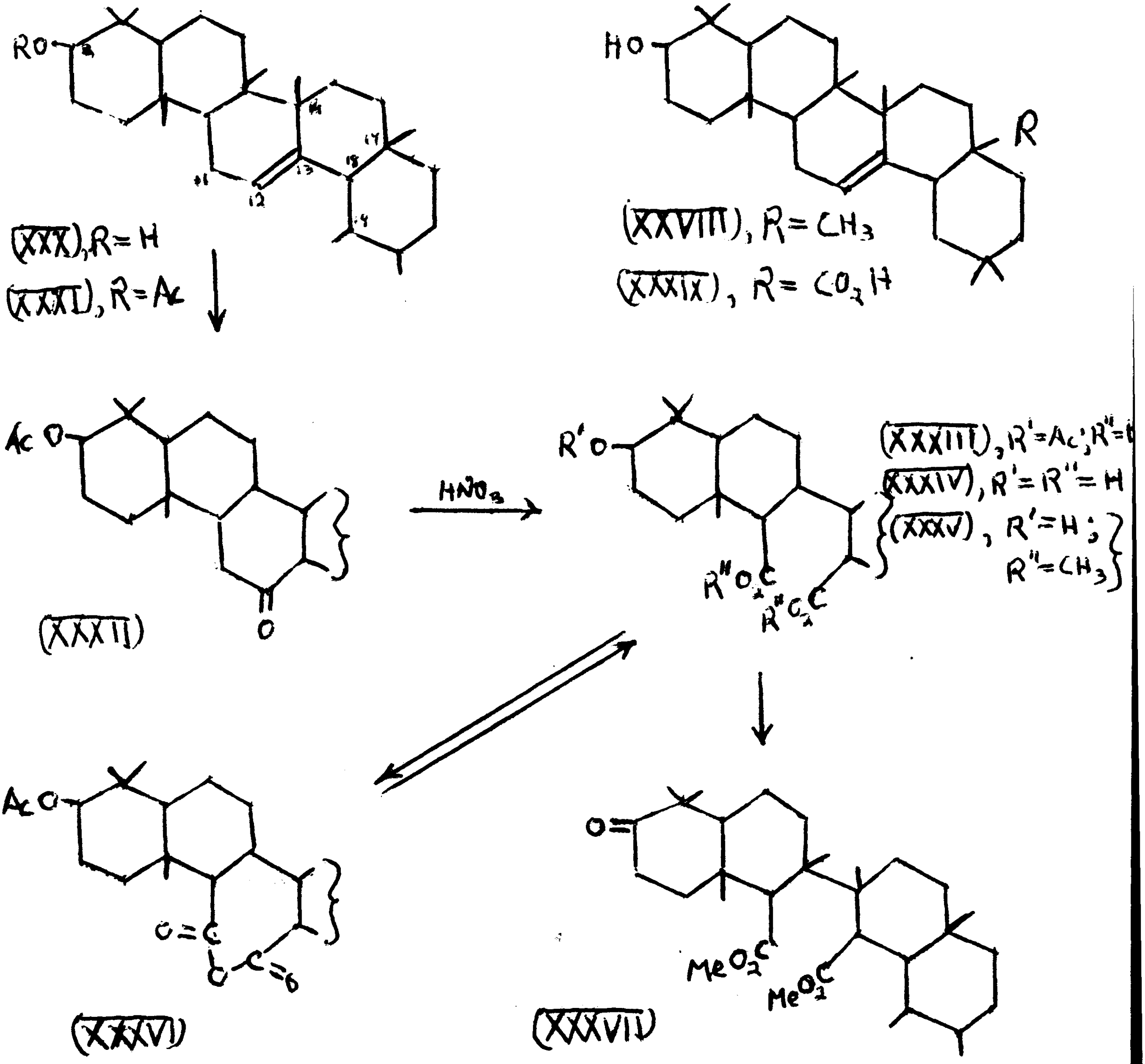
(XXVIII)



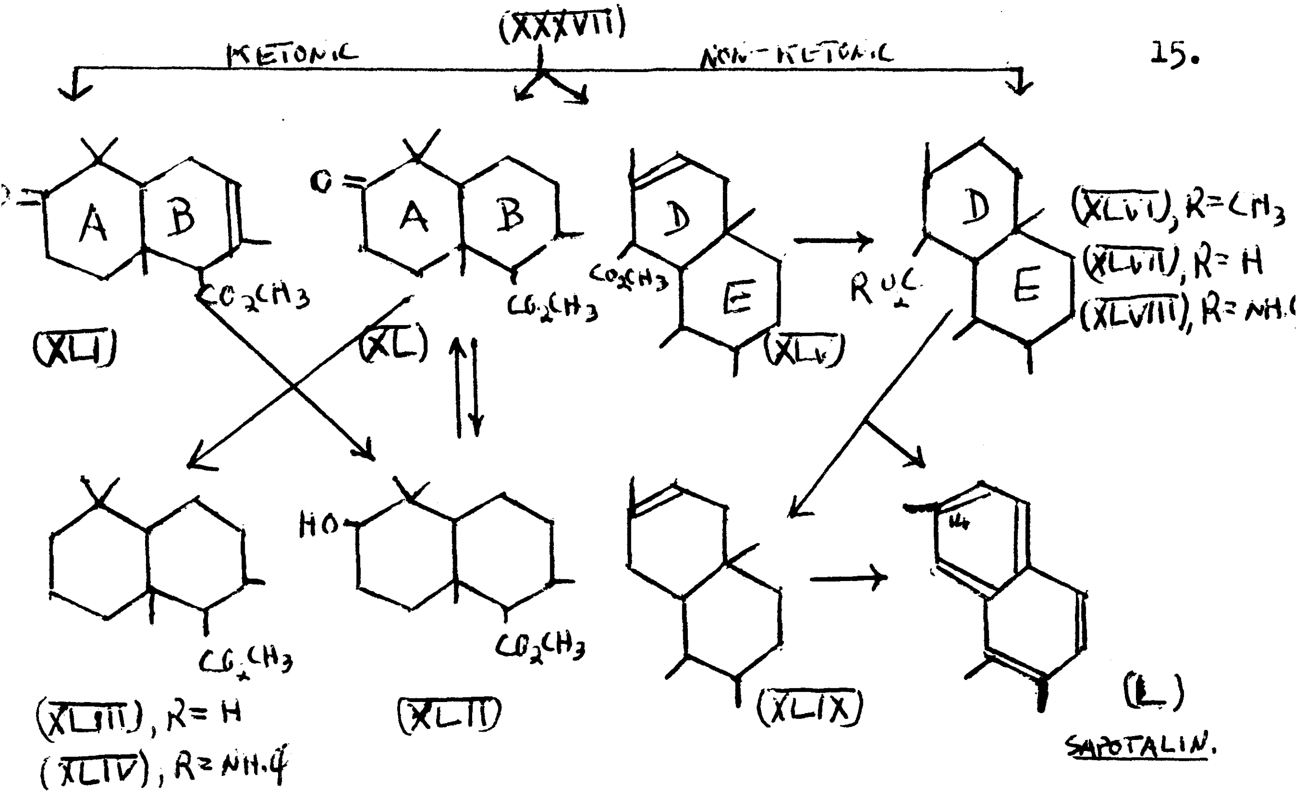
(XXIX)

On the basis of this evidence, the authors considered that the presence of the methyl group at X in (XXIX) is established together with that part of the molecule shown in heavy lines. The introduction of the new double bond by the action of selenium dioxide on iso-α-amyrenonyl acetate to give (XXIV) disallows of the possibility of a methyl group at C_{14}, unless this reaction is accompanied by migration of the methyl to C_{15}. The fact that the dienonyl ester can be reduced back to iso-α-amyrenonyl acetate, and the oxidation of the glycol gives an aldehyde and not a ketone, shows that this cannot be the case.

The structure of (XXIX) is, however, incompatible with the results obtained by these workers at a later date (37), and which, in fact, leads them to formulate α-amyrin as (XXX) on the basis of the following evidence, α-Amyranonyl acetate (XXXII) was oxidised by fuming nitric acid to give an amorphous dicarboxylic acid claimed to be (XXXIII). Though this could not be crystallised, it gave a crystallisable anhydride (XXXVI), which on saponification gave back the same non-crystalline acid.



This acid could be hydrolysed to give (XXXIV) or treated with diazomethane to give (XXXV) which was oxidised by chromic acid to the ketone (XXXVII). None of these products was obtained in a crystalline state, but (XXXV) and (XXXVII) were prepared for analysis by careful chromatography. Compound (XXXVII) was pyrolysed, and the volatile portion separated into ketonic and non-ketonic fractions.



The ketone fraction consisted of a saturated fraction (XL) and an unsaturated fraction (XLI) or an isomer with the double bond elsewhere. Catalytic reduction of (XLI) gave a compound $C_{16}H_{28}O_3$, (XLII) which was identical in all respects with a compound obtained by analogous degradation of oleanolic acid (XXXIX) (38). Whether derived from oleanolic acid or α-amyrin, (XLII) could be oxidised to give (XL) and reduced to give (XLIII) which forms the anilide (XLIV). But since oleanolic acid can be converted to β-amyrin (XXXVIII) (39), then, it is concluded, the configuration of both α- and β-amyrin in rings A and B must be identical.

Likewise, the non-ketonic fraction consisted of an unsaturated fraction, formulated as (XLV), or an isomer with the double bond in another position, and a saturated portion (XLVI). Reduction of (XLV) gave what was thought to be a mixture of stereoisomers of structure (XLVI). This, on vigorous saponification gave (XLVII) which could not be obtained crystalline, and so was characterised as the anilide (XLVIII). The non-crystalline material (XLVII) was dehydrogenated by selenium at 330°, and the mixture of hydrocarbons obtained chromatographed. Two fractions were eluted. Firstly, a mixture of isomers of formula $C_{14}H_{24}$, and possible structure (XLIX), and secondly sapotalin (L) identified by melting point and mixed melting point of trinitrobenzoate and styphnate. Further dehydrogenation of (XLIX) also gave sapotalin, seeming to indicate that in the conversion of (XLVII) to (L) the carboxyl group had not been reduced to a methyl group, but completely removed. Had reduction of the carboxyl group taken place, then (XLIX) would be expected to have five, and not four, methyl groups.

The formation of sapotalin according to the scheme suggested requires a methyl group at C_{14} in α-amyrin (XXX). This is in contradiction to the opinions expressed in the publication of 1947 (36) (v.s.).

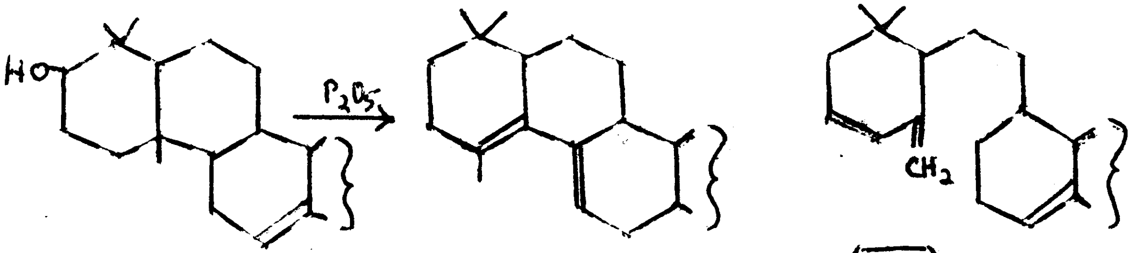
Two obvious criticisms of the work described on the previous pages are, firstly the fact that starting materials (XXXVII) and (XLVII) for the degradative work on which the conclusions are based, were not obtained in a crystalline state, and secondly, the general unsuitability of pyrolysis in structural determination since the possibility of migrations and rearrangements in such reactions is increased, and renders interpretation of results uncertain.

These conflicting results are typical of many obtained in the work on α-amyrin, and in the above summary several have been omitted, since they tend to obscure rather than illuminate the problem.

THEORETICAL.

THEORETICAL.

As reported in the previous section, the action of phosphorus pentoxide on α-amyrin produces a diene, 1 -α-amyradiene, which contains a conjugated system of double bonds distributed between two rings (32). Since the hydroxyl group of α-amyrin is in ring A, and, as shown above, there is considerable evidence that the double bond is in ring C, it is obvious that important changes have occurred in the preparation of the diene, e.g. rearrangement in ring A, accompanied by migration of the methyl group at C_5, and the double bond in ring C, thus:-



(LI)

Nor could the possibility that the compound is in fact a triene be overlooked. This might be formed by ring opening, giving (LI) (40). Decisions as to this latter point are made difficult by the inertness of the double bonds, which can neither be reduced, nor made to combine with maleic anhydride to form an adduct (40). Oxidative reactions were therefore undertaken in an attempt to introduce a reactive grouping into the molecule. Such a grouping might form a point of attack for investigation of the true nature of the diene.

It had been shown that on treatment of the diene with perbenzoic acid, 1.2 atoms of oxygen were absorbed, and a crystalline product, m.p. 204-206°, containing one oxygen atom, isolated (40).

This was repeated, using, so far as possible, the same experimental conditions. The amount of oxygen adsorbed was observed to be the same, but the product isolated was not homogeneous. When the reaction mixture was allowed to stand for a longer period, very little more oxygen was adsorbed, and constant melting material only obtained with great difficulty. This corresponded to neither of the previously obtained specimens, and analysis again pointed to the fact that mixed crystals had been isolated.

l - α-Amyradiene, when treated with hydrogen peroxide in acetic acid, gave crystals, m.p. 203-205°. Notwithstanding the fact that this had every appearance of being a homogeneous material, and had been intensively dried, the analysis corresponded to $C_{30}H_{48}\frac{1}{2}H_2O$. The ultra-violet absorption spectrum was λ, 2380Å; E_{max}, 17,000, which is almost identical with the figure of λ, 2395Å, $E_{max.}$, 15,000 obtained for the diene (32).

A compound, the analysis of which corresponded to the introduction of one oxygen atom was eventually obtained by the action of potassium permanganate on a solution of the hydrocarbon in acetic acid and carbon tetrachloride. Light absorption in alcohol, however, was not selective, and pointed once again to the fact that the product was not homogeneous. The introduction of oxygen should have produced either an α,β-unsaturated ketone showing a distinct maximum, or an oxide possessing no chromophore at all. An attempt to isomerise a specimen of the permanganate product with hydrochloric acid met with no success.

In view of the failure to obtain homogeneous products and reproducible results, (facts which have been observed by other workers (41)), investigation of this particular compound was suspended, and attention turned to more profitable fields.

<u>The Action of Hydrogen Peroxide on α-Amyrin Esters.</u>

Seymour, Sharples and Spring showed that vigorous hydrogen oxidation of α-amyrin benzoate gave a compound $C_{37}H_{54}O_3$, m.p. 205-206°, $[\alpha]_D + 113°$ (24). This was considered to be an unreactive ketone formed by dehydration of an intermediate glycol, and was named α-amyranonyl benzoate. Before commencing the projected synthesis of α-amyrane described at length in the section following this one (pp. 27 - 34), it was thought desirable to prepare the saturated ketone as a step towards the saturation of ring C.

Attempts to prepare α-amyranonyl benzoate by the published method yielded not the product described, but a crystalline solid, m.p. 217-219°, $[\alpha]_D + 131°$, the yield being always between 40% and 50% of theory. This was clearly an oxide, and not a ketone, since it was isomerised on treatment with hydrochloric acid to give a product, m.p. 227-229°, $[\alpha]_D + 24.7°$. This latter compound was undoubtedly the required α-amyranonyl benzoate, the ketone previously described being, in actual fact, a slightly impure form of the oxide.

The reaction of the oxide with bromine to give <u>iso</u>-α-amyrenonyl benzoate, as described in the literature (24, 27), can be readily explained since the reaction was carried out in the presence of hydrobromic acid. Under these conditions the oxide would first rearrange to the ketone, after which the reaction would proceed normally. To test this hypothesis, both the oxide and the ketone were subjected to bromination, followed by dehydrohalogenation. In both cases, the same product, iso-α-amyrenonyl benzoate resulted.

The yield of α-amyranonyl benzoate obtained by isomerisation of the oxide was always about 50% of theoretical. The remainder of the product crystallised as needles of indefinite melting point which on treatment with bromine yielded bromo--amyranonyl benzoate. As crystallisation and chromatography failed to produce any homogeneous material from these needles, thwy were assumed to be a mixture containing largely the ketone and possibly some unchanged oxide.

The same authors, Seymour, Sharples and Spring, reported that similar peroxide treatment of α-amyrin acetate was unsuccessful, and that oxidation was only enforced after a change in experimental conditions. The failure to oxidise the acetate had been previously reported by Spfing and Vickerstaff (42). In spite of this, it was found that treatment of the acetate under the same conditions as for the bwnzoate, proceeded smoothly, and in the same way gave α-amyrin acetate oxide, m.p. 207-209°. The oxide, on isomerisation with mineral acid, gave α-amyranonyl acetate, as plates, m.p. 282-284°.

The saturated acetoxy ketone showed light absorption in alcohol at λ, 2820A, $E_{max.}$, 225, whereas the corresponding oxide was optically transparent. This is sufficient evidence to show that the compounds were oxide and ketone respectively, and not, as might have been expected, two isomeric ketones. Spectographic examination of the benzoates was complicated by absorption by the benzoyl group, which has a masking effect on the other chromophore.

α-Amyranonyl acetate reacted with bromine in the same way as the benzoate, partial dehydrogenation taking place, and iso-α-amyrenonyl acetate being formed. Here the intermediate bromo-ketone was soluble in acetic acid, and was not isolated. iso-α-Amyrenonyl acetate melted at 284-286°, and not at 276.5° as previously reported (cf. 35 and 24). Hydrolysis of iso-α-amyrenonyl benzoate and acetylation of the product gave the acetate melting at 284-286° as before.

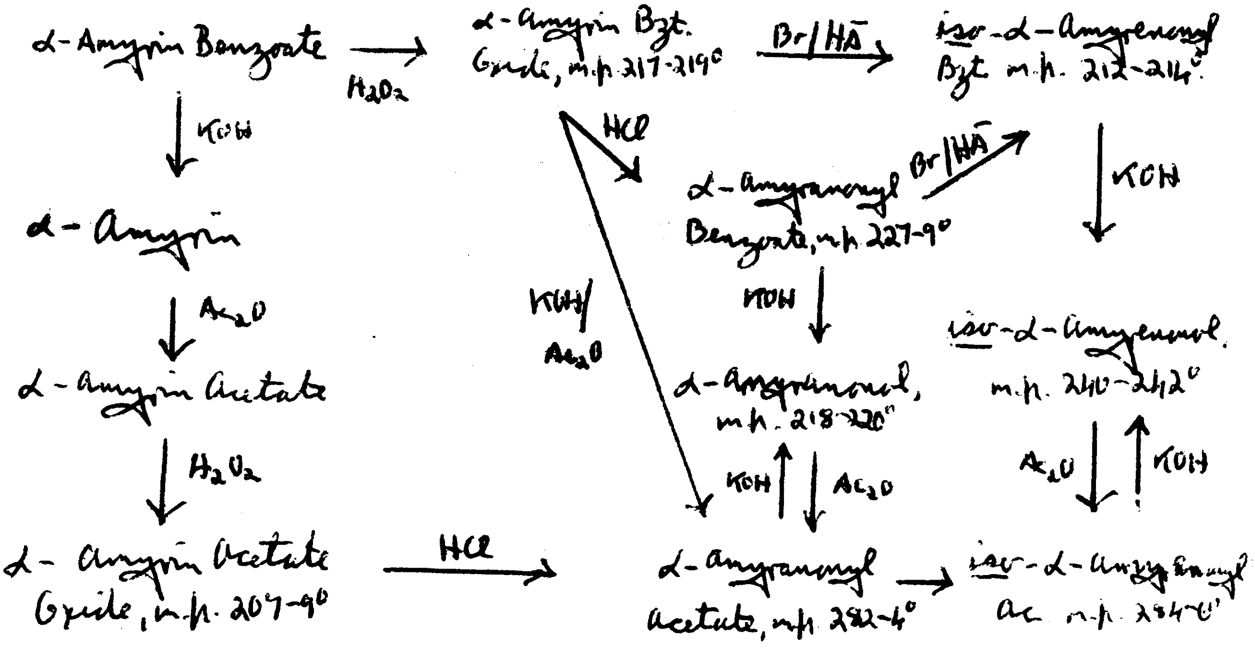
iso-α- Amyrenonol, the melting point again being slightly higher than reported in the literature, was prepared by hydrolysis of both benzoate and acetate.

iso-α-Amyrenonyl acetate and iso-α-amyrenonol were observed to show the characteristic absorption spectrum of an α,β-unsaturated ketone. The former was found to give no melting point depression on admixture with α-amyranonyl acetate, but the two ketones differ considerably in optical rotation, and only the saturated compound reacts with bromine at 40°.

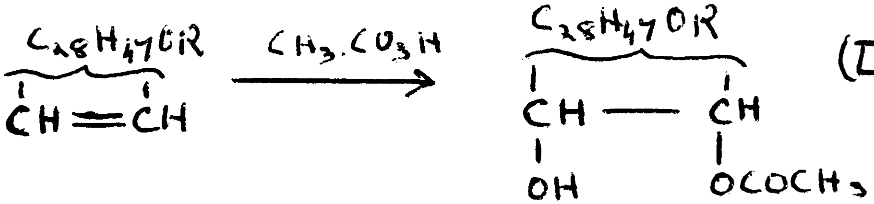
Attempts to hydrolyse the benzoate oxide, to give α-amyrin oxide, were unsuccessful. Vigorous hydrolysis gave an oil which on acetylation gave α-amyranonyl acetate. Mild hydrolysis resulted in an oil also, but this on acetylation yielded a crude form of α-amyrin acetate oxide which could be rearranged to the ketone.

Early attempts to prepare α-amyranonol also failed. In one case the resin obtained by saponification of the benzoate was divided into two parts. The first of these, on acetylation, gave α-amyranonyl acetate, together with a compound, m.p. 230-232°, of unknown constitution. The second portion was chromatographed, and a product, m.p. 218-220°, showing light absorption in alcohol at 2310A and 3070A, $E_{max.}$ 25,800 and 10,000, isolated. The presence of these chromophores indicated that considerable changes must have occurred in the molecule, the conditions having been too severe. Milder conditions enabled both acetate and benzoate to be hydrolysed to α-amyranonol, m.p. 218-220°, which could be readily re-acetylated to give the acetate.

The above results may be summarised as follows:-

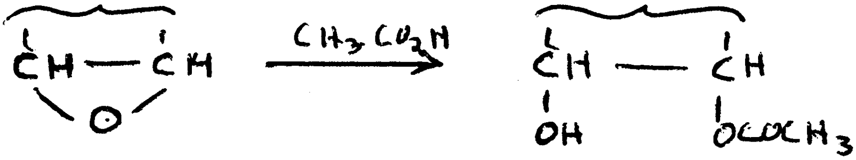


In the oxidation of both α-amyrin benzoate and acetate by hydrogen peroxide, a high melting by-product is obtained in very small yield; that from the benzoate, m.p. 302-304° has already been described (24). These compounds, which are highly laevorotatory, were thought, in view of their analyses, to be the di-esters of α-amyrane triol formed by intereaction of peracetic acid with the α-amyrin esters:-



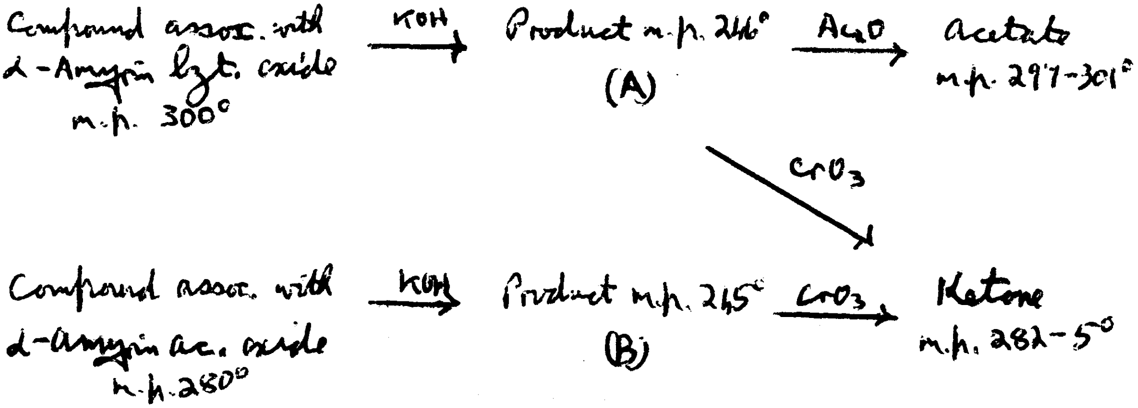
(LII)

or by the action of acetic acid on the oxide:-



Attempts to increase the yield of the by-product by carrying out the oxidation at a higher temperature, or to prepare it by refluxing the oxide with acetic acid, were without success. Also, the use of potassium permanganate as an oxidising agent gave α-amyrin benzoate oxide, and not a glycol as had been hoped.

Hydrolysis of these by-products, formulated as (LII, R = Ac and Bz), gave two different, but isomeric, compounds closely related in physical properties giving a melting point depression on admixture. The analysis of these two compounds, from acetate and benzoate, corresponded to neither α-amyrane triol nor any of its esters. The hydrolysis product of the by-product, associated with α-amyrin benzoate oxide, did not react with periodic acid, indicating the absence of an α-glycol grouping. It could be acetylated to give an acetate, m.p. 297-301°, or oxidised to a ketone, m.p. 282-285°; hydrazone, m.p. 336-338°. The hydrolysis product of the compound associated with α-amyrin acetate oxide, on oxidation, gave what was apparently the same ketone, thus:-

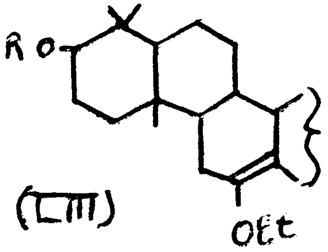


None of the analysis results for these compounds could be interpreted, though they could be reproduced. (For figures, see Experimental Section). The only conclusions that could be drawn were that since the by-products themselves contained no readily oxidisable groups, and since neither the by-products nor the ketone could be acetylated, the ketone had been formed simply by the oxidation of the ring A hydroxyl group which had been freed on hydrolysis. The compounds (A) and (B) might simply differ stereo-isomerically.

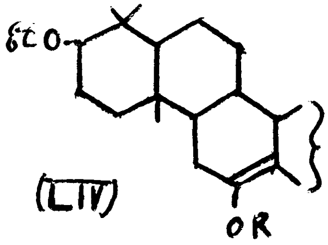
The formation of α-amyrin acetate oxide had already been described by Ruzicka, Jeger, Redel and Volli (43) who obtained it by the action of ozone on α-amyrin acetate. This they isomerised to α-amyranonyl acetate by hydrochloric acid. The melting point was observed by these authors to be 259-260° for the ketone. Treatment of both α-amyrin acetate and benzoate with ozone, using the same conditions as described by Ruzicka et al. gave the same oxides as had been obtained by the action of hydrogen peroxide. α-Amyrin acetate oxide prepared by ozonolysis gave, when isomerised, α-amyranonyl acetate, m.p. 282-284°. In one case, when a 7% ozone stream was used, the ketone itself was isolated from the reaction mixture.

There can be no doubt that the ketone m.p. 282° and that described as melting at 259° are identical. The melting point, when taken in a sealed tube in vacuo, was 294-296°, this approximating to the figure obtained by Ruzicka and his co-workers using the same technique. These workers also obtained a higher figure for the optical rotation of α-amyrin acetate oxide (43) than the present writer.

It was reported (43) that Kishner-Wolff and Clemmenson reduction of the ketone, m.p. 259-260°, were unsuccessful, but no mention was made of any hydrolysis or other product being isolated from the reaction mixture. A specimen of α-amyranonyl acetate was therefore subjected to Kishner-Wolff reduction. A product, m.p. 237-239°, was isolated in very small yield, together with much resin which, when acetylated, gave a small amount of starting material, together with an acetate, m.p. 240-242°. The analysis of both of these compounds suggested that hydrolysis had occurred, and had been accompanied by ether formation, giving (LIII) or (LIV). The latter could be formed by interaction of sodium ethoxide with the acetoxy group, sodium acetate also resulting.



(LIII)



(LIV)

Cpd. m.p. 237-9°
R=H

Cpd. m.p. 240-242°
R=Ac

The very small yields obtained prevented further investigation, while an attempt to ethylate α-amyranonol was unsuccessful.

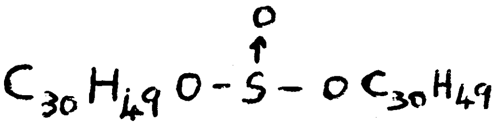
The above results stress the difference between α- and β-amyrin as regards reactivity of the double bond, and of the saturated ketone. In the β-amyrin series, the action of hydrogen peroxide gives the ketone directly without intermediate oxide formation (44), and the carbonyl group can be reduced to methylene by the usual methods (45).

A Projected Synthesis of α-Amyrane.

Though no product of the α-amyrin series has proved to be identical with any obtained from the β-series, it was thought of interest to attempt to prepare the saturated hydrocarbon α-amyrane in order to compare its physical characteristics with the already known β-amyrane. The latter compound was prepared by Kishner-Wolff reduction of a β-amyranonyl ester, followed by oxidation and further reduction of the saturated alcohol thereby obtained. As the carbonyl group of α-amyranonyl acetate could not be reduced to methylene (v.s.) this route could not be applied to α-amyrin.

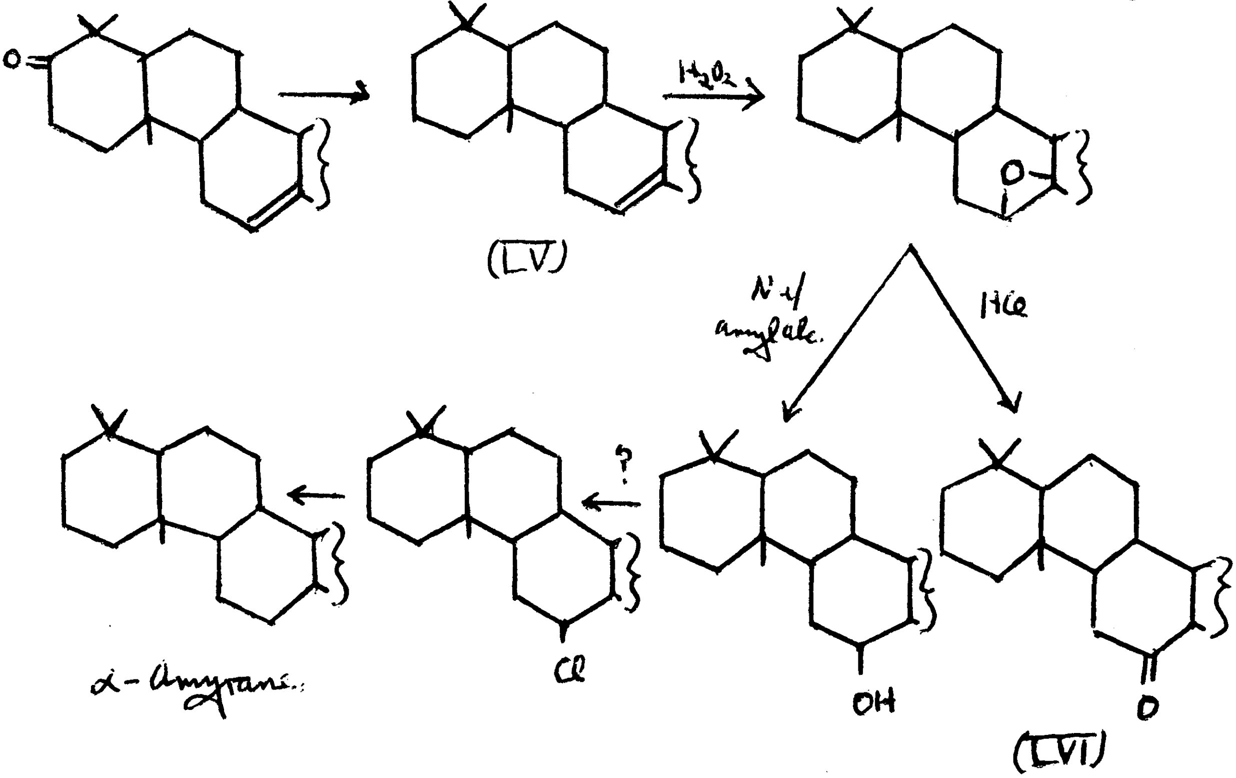
It was therefore decided to use as a starting point in the synthesis the unsaturated hydrocarbon α-amyrene (LV). This, on treatment with hydrogen peroxide should give an oxide. Since treatment of α-amyranonyl benzoate oxide with sodium and amyl alcohol gives α-amyranediol (24), then reduction of α-amyrene oxide in this way should also give a saturated alcohol. The next stage would involve replacement of the hydroxyl group by halogen and subsequent reduction. It was unlikely that the ketone (LVI) formed by rearrangement of the oxide would be reduced any more readily than α-amyranonyl acetate. (See page 29).

With a view to the possibility of chlorination, the action of thionyl chloride on α-amyrin itself was investigated. The only product isolated contained sulphur, and was probably the sulphite (LVII) with which the analysis agrees.



$$C_{30}H_{49}O-\overset{\overset{O}{\uparrow}}{S}-OC_{30}H_{49}$$

(LVII)



Winterstein and Stein (46) have shown the existence of three forms of α-amyrene. The first of these, α-amyrene-I, m.p. 96-97,5°, was obtained by dehydration of α-amyrin with phosphorus pentoxide followed by reduction of the diene thereby obtained. The other two forms were obtained as a mixture, α-amyrene-II, when α-amyrenone was subjected to a Clemmenson reduction. One of these melted at 120-122°, $[\alpha]_D$ + 98.9°, the other at 110-112°, $[\alpha]_D$ + 88.8°. Neither was isolated in a completely pure state. α-Amyrene-II was also prepared by reduction of the diene obtained on pyrolysis of α-amyrin benzoate. Ruzicka, Muller and Schellenberg (47) carried out a Kishner-Wolff reduction of α-amyrenone by way of the semicarbazone, and obtained a hydrocarbon, m.p. 124°, $[\alpha]_D$ +95°, which they claim to be identical with the highest melting form obtained by Winterstein and Stein.

When a Kishner-Wolff reduction of α-amyrenone was carried out using hydrazine hydrate as the reducing agent, the product isolated in good yield, melted at 109-111°, $[\alpha]_D + 92.6^\circ$, and analysed as $C_{30}H_{50}$. This same product was also obtained by Clemmenson reduction as described by Winterstein and Stein, or Kishner-Wolff reduction, according to the method of Ruzicka et al. In no case was any material m.p. 120-122° or m.p. 124° isolated.

Reduction of α-amyrenone using unamalgamated zinc in hydrochloric acid and acetic acid gave a specimen of 1 -α - amyradiene in poor yield. This had probably been formed by reduction of the carbonyl to a hydroxyl group followed by dehydration.

The α-amyrene, m.p. 109-111°, could not be reduced by hydrogen in the presence of a catalyst, and azonolysis gave a resinous prdduct. By modifying the method used for the oxidation of α-amyrin esters, it was found possible to oxidise the hydrocarbon to α-amyrene oxide, $C_{30}H_{50}O$, m.p. 116-118°, using hydrogen peroxide, and isolating the product chromatographically.

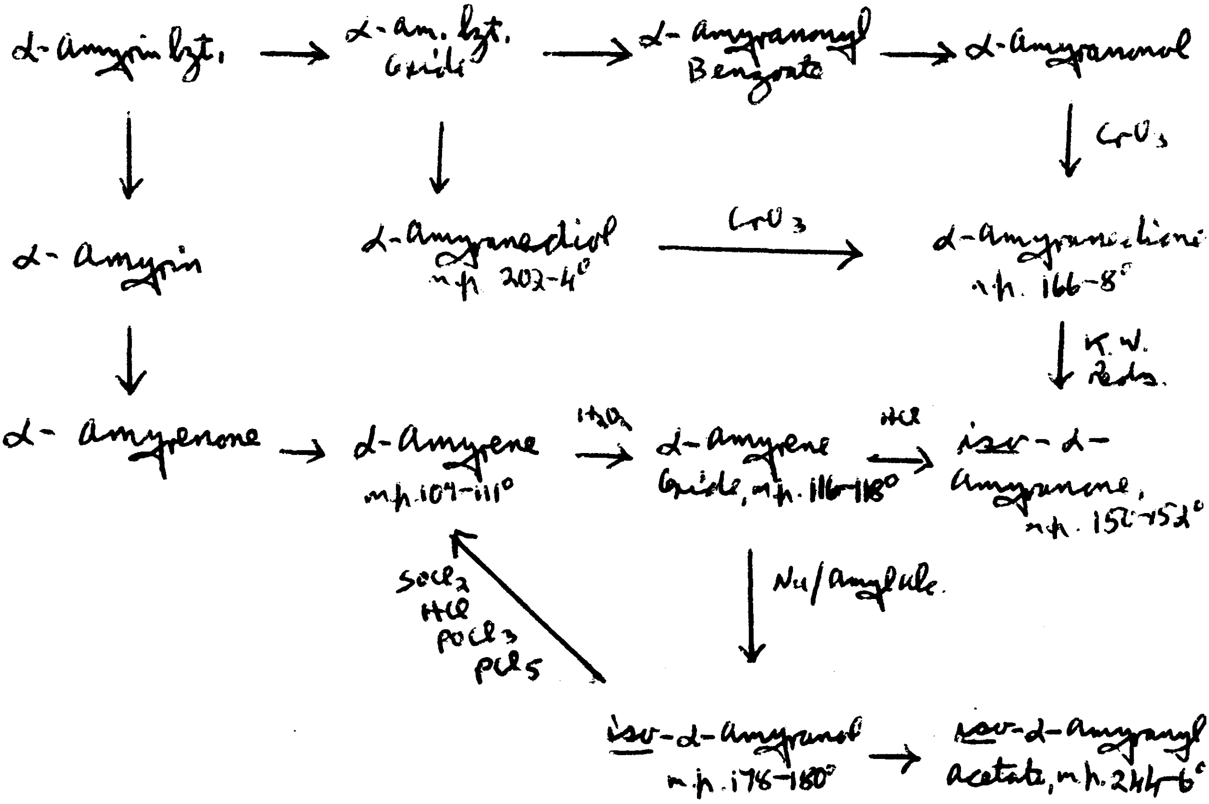
Isomerisation of the oxide to the ketone was successfully carried out by treatment with hydrochloric acid. The product, designated iso-α-amyranone, exists in two crystalline forms. The pure form, m.p. 150-152°, and a solvated form, m.p. 141-143°, rising to 150-152° on drying. The prefix *iso* is used to distinguish it from the hypothetical α-amyranone formed by saturating the double bond of α-amyrenone.

iso-α-Amyranone was also obtained, in low yield, by Kishner-Wolff reduction of α-amyranedione. This latter compound was prepared by chromic acid oxidation of either α-amyranonol or α-amyranediol obtained on reduction of α-amyrin benzoate oxide. Oxidation of α-amyrenone by hydrogen peroxide did not provide a successful alternative route to α-amyranedione, no crystalline oxide being obtained.

The isolation of the mono-ketone from Kishner-Wolff reduction of the di-ketone demonstrated the inactivity of the ring C carbonyl group, and the impossibility of obtaining α-amyrane by attempting its reduction.

The saturated alcohol, *iso*-α-amyranol, m.p. 178-180°, was obtained by sodium and amyl alcohol reduction of α-amyrene oxide. It was characterised as the acetate, m.p. 244-246°. Attempts to replace the hydroxyl group by chlorine were made using thionyl chloride, hydrochloric acid, phosphorus oxychloride, and phosphorus pentachloride as chlorinating agents. In each case dehydration resulted, and α-amyrene, m.p. 109-111° was the only product. No trace of material containing halogen was observed. This ease of dehydration of the alcohol is thought to have accounted for initial difficulty in its acetylation.

The above results may be indicated schematically as follows:-



It being found impossible to replace the hydroxyl group by halogen, the synthesis was abandoned at this stage.

Attempts to open ring C of α-amyrin by the use of the Malaprade Reagent.

Periodic acid was introduced by Malaprade as a reagent for the cleavage of carbon to carbon bonds in 1928 (48). α-Glycols, he observed, were split to give two molecules of aldehyde, thus:-

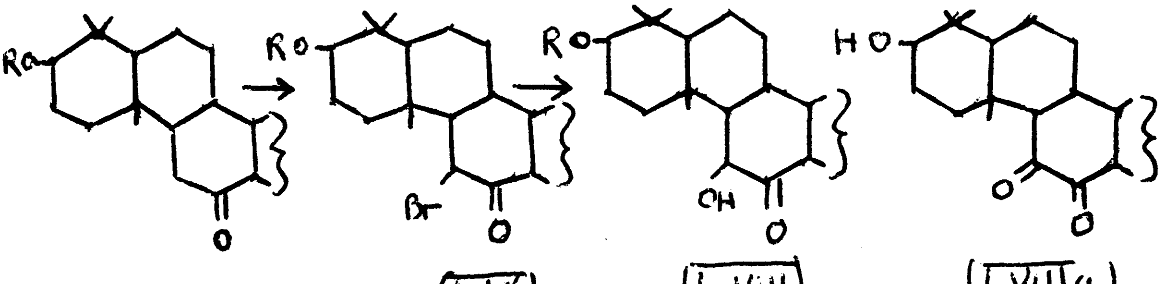
$$R.CH(OH).CH(OH).R' \longrightarrow R.CHO + R'.CHO$$

Later workers showed that a carbonyl group adjacent to a hydroxyl or another carbonyl group also reacted. Thus α-ketols (49, 50, 51) reacted to give one molecule of aldehyde, and one of acid, and α-diketones (50) to give two of acid.

$$R.C(O).CH(OH).R' \longrightarrow R.COOH + R'.CHO$$

$$R.C(O).C(O).R' \longrightarrow R.COOH + R'.COOH$$

Though much literature is available on the fission of straight chain compounds, steroid side chains, carbohydrates and some carbocyclic polyhydroxy compounds (52), no work appears to have been described on ring fission in polycyclic compounds, though lead tetra-acetate has been used with success (e.g. 33, 36). It was proposed, therefore, to attempt to prepare α-amyranonediol (LVIII, R = H) and α-amyradionol (LVIIIa) using α-amyranonyl benzoate as starting material, and to treat these with the reggent, whereby ring C should be opened and acid products obtained.

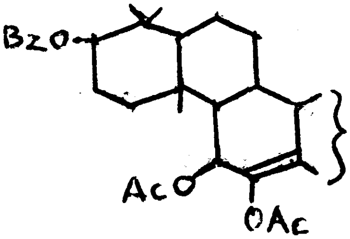


(LIX) (LVIII) (LVIIIa)

Bromo-α-amyranonyl benzoate (LIX) is readily prepared, and on heating with acetic acid loses the elements of hydrobromic acid to give iso-α-amyrenonyl benzoate (v.s.). The possibility that heating with alkali might replace bromine by hydroxyl, and at the same time hydrolyse the benzoyloxy group, giving the required ketol, was envisaged.

The bromo-ketone proved to be considerably more stable than had been expected, and could be heated with alkali for long periods with apparently no effect. When conditions were finally found to enforce the replacement of bromine, the experiment was found to be uncertain as to reproducibility and to give poor yields. Attempts to replace halogen by using silver hydroxide, silver acetate, or sodium acetate were without effect.

The product obtained using potassium hydroxide analysed as the monobenzoate of α-amyranonediol, the presence of bromine having apparently hindered the hydrolysis of the benzoyl group. The product was associated with iso-α-amyrenonol formed by dehydrohalogenation, and by hydrolysis. The ketol gave the blue colouration in alcoholic ferric chloride solution typical of such compounds, and on acetylation gave a diacetate, presumably (LX).



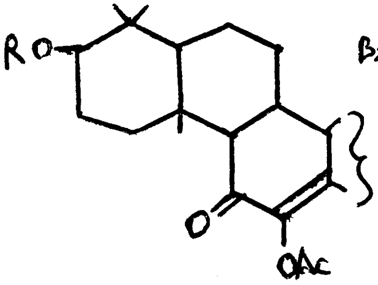
(LX)

Treatment of (LVIII, R = Bz) with periodic acid was found to give a neutral product very close in analysis to the starting material, but giving no colouration with ferric chloride solution.

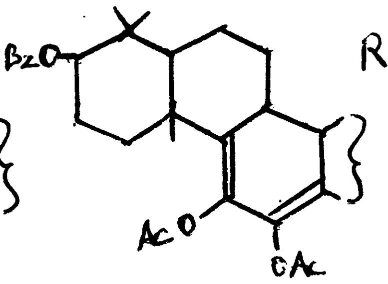
The failure to reproduce satisfactorily the preparation of the ketol, due no doubt to the ease of dehydrohalogenation and hydrolysis, though as stated above, the latter seemed hindered by the presence of the bromine, led to attention being directed to the diketone (LVIIIa).

α-Amyradionol and its acetyl ester had previously been described by Ruzicka and his co-workers (43) who obtained them by the oxidation of α-amyranonyl esters. A compound believed to be α-amyradionyl acetate had also been prepared by Ewen and Spring (60) by the ozonolysis of α-amyradienyl acetate. It was hoped to make the benzoate, α-amyrin benzoate being more readily accessible than the acetate, and try and improve on the yields of the original workers.

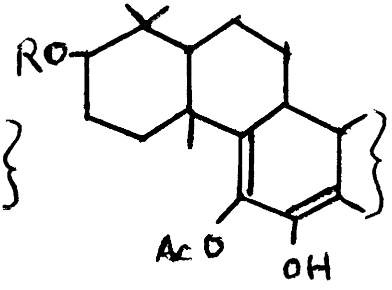
Oxidation of α-amyranonyl esters by chromic acid in acetic acid gave initially bright yellow products, undoubtedly mixtures containing the required diketone contaminated by starting material. Hydrolysis of the yellow products gave an unhomogeneous product, but giving a strong colour reaction with ferric chloride. Further oxidation of the yellow products gave new compounds, which could also be obtained by more vigorous initial oxidation of the α-amyranonyl esters. These were of type (LXI). One specimen analysed as (LXII).



(LXI)



(LXII)



(LXIII)
(Enolic Form)

Hydrolysis, in the hope of obtaining α-amyradionol, again gave a mixture of indistinct melting point, though the analysis approximated to the required $C_{30}H_{48}O_3$. When acetylated, it gave a well defined acetate, m.p. 346-348°, and on treatment with periodic acid, a small, non-crystalline acid fraction, together with a neutral portion, m.p. 301-303°, with the same analysis as the starting material, but no ultra-violet absorption spectrum.

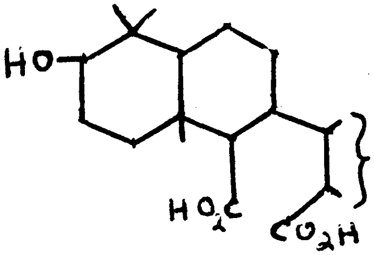
To limit the possibility of formation of compounds of type (LXII) and (LXIII) by reaction with the solvent in the initial oxidative reactions, α-amyranonyl benzoate, and acetate, enol acetates were used as starting materials. These gave (LXI, R = Ac and R = Bz) in good yield as compounds with well defined physical constants. A specimen of the acetate prepared, in poor yield, according to the published method, was shown to be identical with that prepared by the new technique. The benzoate, on hydrolysis, gave pure α-amyradionol, differing only slightly in physical constants from the published figures.

It is worth noting that compounds of type (LXI), containing a modified α,β -unsaturated ketone grouping, show an absorption maximum at 2530A. in the ultra violet. The diketone has high intensity absorption at 2900A.

Acetylation of the α-amyradionol could not be satisfactorily accomplished, though a published method is given (43). This may be due to the possibility of forming enol acetates, or mixtures of these.

Periodic acid oxidation of pure α-amyradionol gave no acid product. The neutral fraction differed markedly from the dione in properties, but was clearly a mixture. Chromatography and acetylation both failed to give a pure specimen. Likewise, a specimen of α-amyradionyl acetate prepared according to Ruzicka et al. (43) on periodic acid oxidation gave a similar mixture of neutral products.

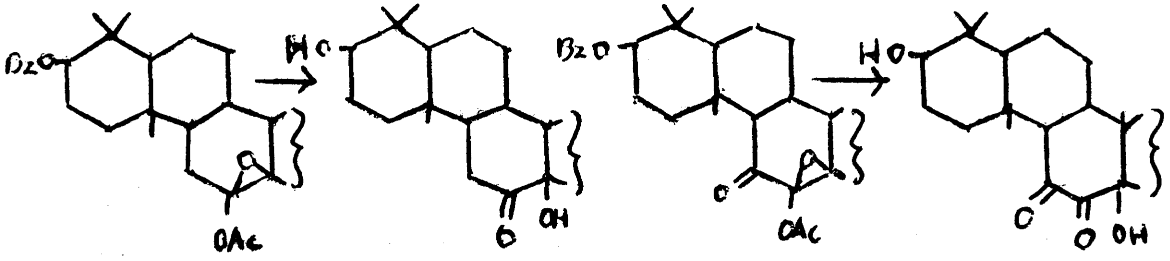
No conclusions as to the action of the Malaprade reagent can be made from the above results. Reaction certainly takes place, the product being close in analysis to the original ketol or dione, but is optically transparent, and apparently does not contain the original grouping. The acid that was on one occasion obtained might have been the required (LXIV), but the quantity was too small for further investigation.



(LXIV)

A specimen of α-amyradionol was oxidised by chromic acid in an attempt to prepare the ketone corresponding to (LXIV), but no crystalline material was isolated from the reaction mixture.

The work of Kritchevsky and Gallagher (53) in the steroid field seemed to point to two new points of attack in ring C. If α-amyranonyl benzoate enol acetate and α-amyradionyl benzoate enol acetate were treated with perbenzoic acid, then the double bonds might be attacked to give (LXV) and (LXVI) respectively, which on hydrolysis might give (LXVII) and LXVIII).



(LXV) (LXVII) (LXVI) (LXVIII)

Both α-amyranonyl benzoate enol acetate and its chromic acid oxidation product were given prolonged treatment with perbenzoic acid in chloroform, but with no success, starting material being recovered almost quantitatively in each case.

The Reduction of α-Amyranonyl Esters.

The reduction (by sodium in amyl alcohol) of α-amyrin benzoate oxide, at the time thought to be α-amyranonyl benzoate, was shown by Seymour, Sharples and Spring to give the saturated diol, α-amyranediol (24). This was carried out, and α-amyranediol corresponding in properties to that described in the literature obtained. It could be oxidised to α-amyranedione (v.s.) or acetylated, though the di-acetyl derivative formed melted considerably higher than that previously reported.

When α-amyranonyl benzoate itself was treated with sodium and amyl alcohol, the product melted at 198-200°, and the optical rotation in four successive experiments was +32.7°, +41.9°, +44.4°, and +30°. α-Amyranonyl acetate on similar treatment gave material, m.p. 198-200°, $[\alpha]_D + 33^\circ$.

The analyses of these two compounds was the required $C_{30}H_{52}O_2$, but if any of the starting material, $C_{30}H_{50}O_2$, were present, the analysis would not have been affected sufficiently to be noticeable.

It seems probable that reduction of the ketone is more difficult than that of the oxide, and this seems confirmed by the fact that acetylation of the material, m.p. 198-200°, obtained by reduction of the benzoate, gave a di-acetate, m.p. 242-244°, apparently identical with that obtained by acetylation of the α-amyranediol from reduction of the oxide.

The action of lithium aluminium hydride in dry ether was also investigated; α-amyranonyl benzoate gave two products. The first of these, m.p. 250-252°, $[\alpha]_D + 12.3^\circ$, must have contained a large amount of α-amyranonol, since it gave, on acetylation, a product indistinguishable from α-amyranonyl acetate. The second product, m.p. 170-172° $[\alpha]_D + 12.9°$, analysed as the diol, and gave, on acetylation, a monoacetate, m.p. 189-191°. Acetylation at a higher temperature gave a di-acetate of the same melting point. If the monoacetate was a derivative of α-amyranediol, or one of its possible isomers, it should have been possible to oxidise it to α-amyranonyl acetate, or an isomeric ketone. This was attempted without success. These results point to imcomplete reduction by the lithium aluminium hydride, accompanied by hydrolysis, giving non-homogeneous products.

The same was found of red phosphorus and hydriodic acid as a means of reducing the benzoyloxy ketone. This was attempted with a view of reducing the carbonyl to methylene group. Here the experiment was complicated by the possibility of dehydration. In view of this, and the fact that two successive experiments gave two different products, again possibly mixed crystals, no further reductions were attempted.

The Action of Alkali and of Aluminium Trichloride on the Amyrin Nucleus.

To investigate the possibility of new methods of degradation of the amyrin nucleus, specimens of α-amyrenone and β-amyrin benzoate were heated with strong alkaline solutions in an autoclave to temperature as high as 290°. The β-amyrin benzoate was chosen to test the reaction so as to conserve valuable stocks of α-amyrin benzoate.

In the first instance, starting material was recovered unchanged, while in the second only hydrolysis occurred, benzoic acid and β-amyrin being the only products. These results are indicative of the stability of the amyrin nucleus.

When β-amyrin benzoate was heated with anhydrous aluminium chlride in benzene, considerable degradation occurred, hydrochloric acid was evolved, and the products consisted of benzoic acid and a red oil. This red oil yielded a steam volatile fraction of about 13% of the total weight. This was fractionally distilled, and analysis of one sample revealed it to be a mixture of hydrocarbons. The non steam volatile portion did not give any fraction of constant boiling point on distillation.

The nature of the changes occurring is suggested in a paper by Zelinsky and Koslow (54). These workers, seeking to find the precursors of petrol, distilled betulin and abietic acid, both naturally occurring compounds, with aluminium trichloride, and obtained similar products to the above. The molecule, they say, is split by the reaction. The larger portion undergoes progressive dehydrogenation, giving finally a carbonaceous mass, the fractions of lower molecular weight become saturated at the expense of the hydrogen from the higher, and mixtures of paraffins are formed.

It is clear that if degradation had occurred in this fashion, then, even if any recognisable product was obtained, no valid conclusions could be drawn, because of the drastic nature of the reaction which might have caused rearrangements and migrations in the molecule.

Molecular Rotation Differences.

The relationship between optical rotatory power, and structure in steroids, was examined by Wallis and his co-workers (55) who improved on the work of Callow and Strain (56). It was then shown that if a certain change be effected in two different steroid molecules differing from one another in a part of the molecule removed from the reacting centre, then the variations in molecular rotation is approximately the same.

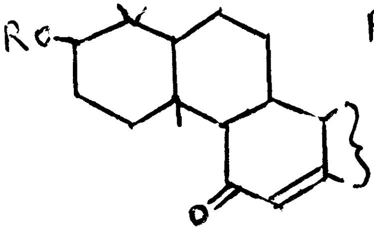
This was applied by Barton and Jones to the triterpenes with good results (57). Thus, the molecular rotation differences between β-amyrin and its acetate, glycyrrhetic acid and its acetate, oleanolic acid and its acetate, and so on for the other members of the β-amyrin group, were approximately the same. The difference between various acids and their esters, within any one group, was also constant.

Applying these results to some of the compounds described above, in which the configuration in the neighbourhood of the double bond has been effected, we obtain the following figures. Mean values have been taken for $[\alpha]_D$, Δ, represents the molecular rotation difference between alcohol and acetate, Δ_2 between alcohol and benzoate, and Δ_3 between acetate and benzoate. If Δ_1 and Δ_2 are constant, then Δ_3 must be also.

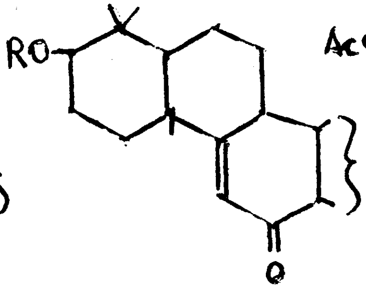
	$10^{-2} \times M.[\alpha]_D$			$\times 10^{-2}$		
	Alcohol	**Acetate**	**Benzoate**	Δ_1	Δ_2	Δ_3
α-Amyrin	358	370	498	12	140	128
α-Amyrin Oxide	-	552	721	-	-	169
α-Amyranonol	-22	53	137	75	159	84
iso-α-Amyrenonol	326	410	495	84	169	85
Epi-iso-α-Amyrenonol (58)	-48	77	114	125	162	37
α-Amyranonol Enol Acetate	-	258	432	-	-	174
α-Amyradionol Enol Acetate	-	494	518	-	-	24
α-Amyradionol	657	578 (43)	-	-79	-	-

No degree of constancy is shown, except apparently between the third and fourth of the compounds listed. This could be taken to mean that whereas the change involving the introduction of the carbonyl group has been sufficiently close to the hydroxyl to effect the molecular rotation differences, the introduction of the new double bond has been far enough away to cause no change. In other words, that the double bond of iso-α-amyrenonol is more removed from the hydroxyl group than is the carbonyl group.

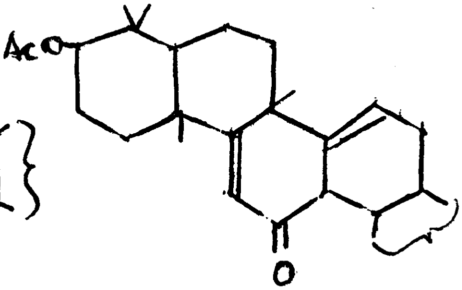
This requires a formulation such as (LXIX) for iso-α-amyrenonol, making α-amyrenonol (LXX).



(LXIX)

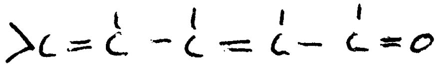


(LXX)



(LXXI)

In this respect, it should be remembered that Ewen, Gillam and Spring dehydrated α-amyrenonol with hydriodic acid, and obtained a product, α-amyradienone-III, which showed an absorption maximum at 2920A, indicating the system (LXXII) (32).


$$>C=\overset{|}{C}-\overset{|}{C}=\overset{|}{C}-\overset{|}{C}=O$$ (LXXII)

While this might be formed from (LXX), it is unlikely to occur by dehydration of (LXIX).

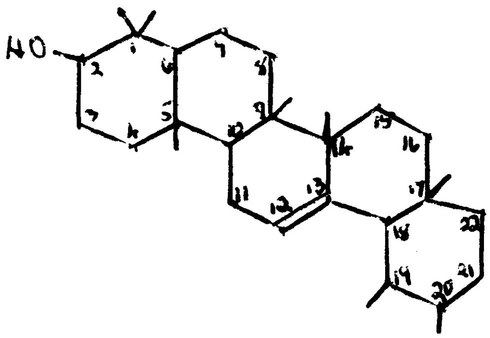
It is as yet unknown how far the point of difference and reacting centre of the molecule have to be apart before the law of Barton and Jones is obeyed, but the above figures for α-amyrin do show that the double bond and hydroxyl groups are sufficiently close for a change in the former to affect molecular rotation differences concerning the latter.

The recent investigations by Ruzicka and his co-workers whereby iso-α-amyrenonyl acetate was treated with selenium dioxide (35) also require re-examining. The product obtained by these workers, originally formulated as (LXXI), was claimed to be formed by the introduction of an isolated double bond into (LXX). *iso*-α-Amyrenonyl acetate shows an ultra violet absorption maximum at 2520A, the compound obtained after treatment with selenium dioxide exhibits selective absorption at 2550A. That is, the chromophore has been altered, although this is denied by Ruzicka et al. (35).

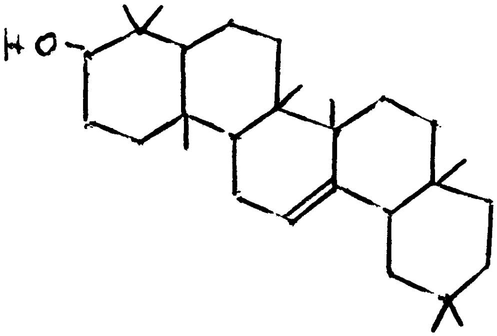
It is interesting to note that when *iso*-β-amyrenonyl acetate is similarly oxidised, the product shows light absorption at 2450A, this being in agreement with the figure of 2470A obtained for the starting material (59).

iso-α-Amyradienonyl acetate (obtained by selenium dioxide oxidation of *iso*-α-amyrenonyl acetate) may be reduced catalytically to give *iso*-α-amyrenonyl acetate or isomerised by acid to give a new compound. *iso*-β-Amyradienonyl acetate, it was found, is neither reduced nor isomerised when submitted to the same treatment as the α-compound. That α- and β-amyrin differ considerably in some reactions is thus again demonstrated.

As mentioned in the Historical Section, Ruzicka et al. (37) now suggest the structure (LXXIII) for α- and (LXXIV) for β-amyrin. This renders the introduction of a double bond at C_{14} impossible unless accompanied by migration of the methyl group to C_{15}, but this has been shown to be unlikely in view of subsequent degradation of the molecule.



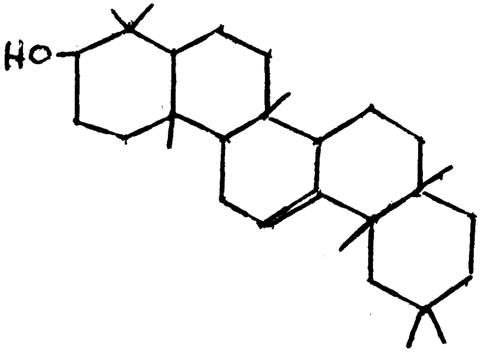
(LXXIII)



(LXXIV)

The presence of a methyl group at C_{19} in α-amyrin does not seem sufficient to account for the reactivity differences in the double bond of α- and β-amyrin, as well as for other differences of reactivity in the two molecules as exemplified above.

The carbonyl group of β-amyranonyl esters can be reduced to methylene, but not of α-amyranonyl. Oxidation by peroxide gives ketones in the case of β-amyrin, but, as has been shown above, oxides in α-amyrin esters. The previous suggestion (LXXV) is more satisfactory, but fails to account for the formation of sapotalin as described on page 16.



(LXXV)

Only methods of degradation which enable the intermediates to be characterised, and which preclude rearrangements, will allow of a solution. Until such methods are evolved, no single formulation for α-amyrin can account for all the evidence available.

EXPERIMENTAL.

EXPERIMENTAL.

All melting points are uncorrected for exposed stem. Optical rotations were observed in chloroform solution, using a one decimetre tube.

α- and β-Amyrin Benzoates. - Elemi resin was steam distilled to remove volatile oils, sesquiterpenes, etc., and the solid mass remaining dissolved in ether. Acid constituents were removed by twice washing the solution with about one third its volume of 10% caustic soda solution, and once with the same volume of water. Washing was completed with dilute hydrochloric acid, and 2% salt solution, until neutral to litmus. The ether was dried over sodium sulphate. On removal of the solvent, a crystalline solid remained, which was crystallised from alcohol or industrial methylated spirits, collected, washed with more of the solvent, pressed thoroughly, and dried at 105°.

Crude mixed amyrins (250 g.), obtained as above, were dissolved in pyridine (150 c.c.), and redistilled benzoyl chloride (175 c.c.) slowly added with stirring at 100°. Heating and stirring were continued for a further six hours after all the reagent had been added. On cooling, the reddish brown semi-solid mass was diluted with benzene (750 c.c.), and washed with hydrochloric acid (10%), caustic soda (10%), and twice with dilute salt solution before being dried over sodium sulphate. The benzene solution was concentrated to ca. 400 c.c., and alcohol added at the boil until the solution was turbid. A crystalline mass separated on cooling, and was collected and dried at 105°.

The crude mixed benzoates were transferred to a wide necked bottle fitted with a rubber stopper containing a tap for relief of pressure. Ether (250 c.c.) was added, and the bottle shaken for ten minutes, and the undissolved solid collected. As the melting point of mixed amyrins is indefinite, the clearing point is used to test purity, and this operation must be repeated until this is above 210°. Three such washings left material of clearing point 220°. This was crystallised from benzene-acetone to give pure *β-amyrin benzoate*, m.p. 234-235° (40 g.).

The ethereal washings were evaporated to dryness, and the solid residue crystallised from benzene-alcohol. Five crystallisations gave pure *α-amyrin benzoate* as stout needles, m.p. 195-196°. The yield was 55 g. Subsequent preparations gave yields as high as 90 g.

α- and β-Amyrin - The benzoate (50 g.) was dissolved in benzene (300 c.c.) and a solution of caustic potash (50 g.) in water (66 c.c.) and alcohol (1150 c.c. refluxed and distilled over caustic soda) added, and refluxing carried out for 24 hours. The solution was concentrated until solid began to appear, and then poured into water, collected, washed well with water, and oven dried. The yield was 42 g. (91%) in both cases.

α-Amyrin melted at 170-174°, β-amyrin at 180-182°.

When prepared by this method, both possess a yellow tinge, but are sufficiently pure for subsequent reactions.

α- and β-Amyrin Acetate - The alcohol (40 g.) in pyridine (120 c.c.) and acetic anhydride (180 c.c.) was heated on a steam bath. Acetate began to separate almost at once. After 2 hours, the mixture was cooled and filtered, and the solid washed with a little cold acetic acid and crystallised from chloroform-methanol. The yield was 40 g. (90%) of α-amyrin acetate, m.p. 225°, or β-amyrin acetate m.p. 235°.

l-α-Amyradiene - α-Amyrin (27 g.) was dissolved in dry benzene (700 c.c.) and phosphorus pentoxide (54 g.) added with shaking. The mixture was shaken on a reciprocal shaker for 24 hours, then poured into water. The benzene solution was twice washed with water, and twice with sodium sulphate solution before drying (sodium sulphate). Concentration of the benzene solution to about 50-60 c.c. was carried out, when on addition of ether the product separated. Recrystallisation from benzene-alcohol gave l-α-amyradiene (12 g. 46%) as needles, m.p. 194-196°.

Action of Perbenzoic Acid on l-α-Amyradiene - i) l-α-Amyradiene (1.78 g.) in chloroform (40 c.c.) was treated with a solution of perbenzoic acid (1.62 g. 2.7 atoms oxygen) in chloroform (60 c.c.) and the mixture left at 0°. A blank of perbenzoic acid in chloroform was prepared also, and left at the same temperature. From time to time 1 c.c. samples were taken and titrated against 0.98 N thiosulphate solution.

Time.	Blank (c.c.)	Reac.Mix. (c.c.)	Atoms O adsorbed.
18 hours	2.42	1.40	1.20
90 hours	2.41	1.28	1.27

The chloroform solution was twice washed with 2% caustic soda, twice with water, and the solvent removed after drying over sodium sulphate, leaving a pale yellow resin, which was crystallised from chloroform-methanol. Six crystallisations gave colourless blades, m.p. 201-203°, giving no depression with a specimen, m.p. 204-206°, prepared by G.T.Newbold. The product developed no apparent colour with tetranitromethane in chloroform solution. (Found: C, 82.4; H, 10.8% $C_{30}H_{48}O_2$ requires: C,81.7; H,10.9% $C_{30}H_{48}O$ requires: C,84.8; H,10.8%).

Further quantities of solid obtained from the mother liquors were chromatographed, but no separation of any mixture was achieved.

ii) The experiment was repeated as before using l-α-amyradiene (5 g.), chloroform (320 c.c.) and perbenzoic acid (4.25 g. 2.5 atoms). After 168 hours titration indicated that 1.35 atoms of oxygen had been adsorbed. Working up as before gave a yellow resin which crystallised as yellow plates, m.p. ca. 170°. Continued crystallisation from chloroform-methanol failed to remove the colour or give material of constant melting point. Chromatography eventually gave a low yield of material crystallising from aqueous alcohol as colourless blades, m.p. 206-210°.

Two specimens were prepared for analysis and spectographic examination, but, on drying in vacuo, at the temperature of boiling xylene, one of these turned yellow, and the melting point dropped to 140-150°. No selective adsorption was shown in the ultra-violet range, however. (Found: C,80.3; H,10.8% $C_{30}H_{48}O_2$ requires: C,81.8; H,10.9%).

Action of Hydrogen Peroxide on 1-α-Amyradiene - The hydrocarbon (1 g.) in benzene (50 c.c.) was treated with hydrogen peroxide (0.21 g. 2.5 atoms) in acetic acid (50 c.c.) on a steam bath for 3 hours, poured into water, and the benzene layer separated and worked up in the usual manner. A solid was obtained which crystallised from benzene-alcohol as blades, m.p. 203-205°. (Found: C,86.3; H,11.5% $C_{30}H_{48}O$ requires: C,84.9; H,11.3%: $C_{30}H_{48}$ requires: C,88.2; H,11.8%) λ = 2380A, $E_{max.}$ = 17,000.

The product gave no m.p. depression on admixture with starting material, or woth the product m.p. 201-203° obtained by Mr. S.H.Ruff, using potassium permanganate as the oxidising agent.

Action of Potassium Permanganate on 1-α-Amyradiene.

i) In pyridine solution - 1-α-Amyradiene (1 g.) in pyridine (100 c.c.) was treated with a solution of potassium permanganate (0.8 g.) in water (10 c.c.) over a period of

1 hour on a steam bath. Decolourisation occurred, and manganese dioxide separated. After heating for a further hour with addition of more pyridine (20 c.c.), the solution was filtered hot. On cooling, crystals separated, and were collected. Crystallisation from benzene-alcohol gave pure starting material, m.p. 197-199°. The mother liquors were poured into water, and extracted with benzene, when a further small quantity of 1-α-amyradiene was recovered.

Pyridine alone, when treated in the same way, was found to decompose the permanganate solution.

ii) In Acetic Acid Solution - 1-α-Amyradiene (1 g.) in acetic acid (stabilised, 150 c.c.) was refluxed, and permanganate (0.6 g., $4\bar{O}$) in water (10 c.c.) added dropwise. At first decolourisation was instantaneous, but it took place more slowly subsequently. After 2 hours, the solution was filtered hot, and the volume reduced in vacuo. It was then largely diluted with water, and extracted with benzene. Removal of the benzene left a resinous mass, part of which crystallised when left in alcoholic solution at 0°, giving yellow needles, m.p. 198-204°. Two subsequent crystallisations gave almost colourless needles, m.p. 210-212°.

iii) In Carbon Tetrachloride-Acetic Acid Solution - 1-α-Amyradiene (1 g.) in carbon tetrachloride (50 c.c.) and acetic acid (100 c.c.) was refluxed on a water bath, and potassium permanganate (0.6 g., $4\bar{O}$) in water (7 c.c.) added dropwise over a period of 45 minutes, and refluxing continued for a further hour. The solution was filtered hot, cooled, poured into water, and the carbon tetrachloride layer separated and washed. It was evaporated in vacuo after drying over sodium carbonate. The yellow solid which remained was crystallised five times from benzene-methanol giving colourless needles, m.p. 214-215°. $[\alpha]_D^{17}$ - 60.2° (c = 0.6) (Found: C,84.6; H,11.1% $C_{30}H_{48}O$ requires: C,84.9; H,11.3%).

The product showed U.V. absorption over a considerable range, but this was not selective.

Attempted isomerisation of the product $C_{30}H_{48}O$ - The product (0.8 g.) in chloroform (10 c.c.) and acetic acid (20 c.c.) was treated with hydrochloric acid (conc. 1 c.c.) for 30 minutes at 40°, poured into water, and extracted with ether. The product crystallised as blades, m.p. 214-216°, from chloroform-methanol (Found:C,84.4; H,11.2 $C_{30}H_{48}O$ requires: C,84.9; H,11.3%).

There was no selective adsorption in the U.V. range.

Action of Hydrogen Peroxide on α-Amyrin Esters.

a) α-Amyrin Benzoate - α-Amyrin benzoate (10 g.) in acetic acid (400 c.c.) was stirred at 100°. A solution of hydrogen peroxide (30%, 60 c.c.) in acetic acid (60 c.c.) was prepared, and a portion (80 c.c.) was added dropwise over a period of 20 minutes, and conditions maintained for a further 2 hours, during which all precipitated solid redissolved. The remainder of the peroxide solution was added, and after a further hour, hot water was dropped in until the solution remained turbid. The stirrer was stopped, and the solution allowed to cool. The collected solid was recrystallised from benzene-methanol giving α-amyrin benzoate oxide (5 g., 48%) as fine needles, m.p. 217-219°. $[\alpha]_D^{16}$ + 131.8° (c = 2.96). Found: C,81.7; H,9.6% $C_{37}H_{54}O_3$ requires: C,81.3; H,10.0%).

Light absorption in alcohol, λ = 2300A, $E_{max.}$ = 13,400.

The acetic acid mother liquors were largely diluted with water and extracted with benzene, giving in very low yield (2-5%) a product as plates from chloroform-methanol, m.p. 305-310° (softens ca. 300°). $[\alpha]_D^{19}$ - 107° (C = 2.24). (Found: C,77.6, 77.2; H,9.3,9.4% $C_{39}H_{58}O_5$ requires: C,77.2: H,9.6%).

The ultra violet ansorption spectrum showed no selective absorption.

b) α-Amyrin Acetate - The acetate (10 g.) in acetic acid (400 c.c.) was treated with hydrogen peroxide as described for the benzoate under a). The crude oxide formed plates from chloroform methanol, m.p. 207-209°, $[\alpha]_D^{17}$ +105° (C = 1.56) (Found: C,79.7; H,10.9%. $C_{32}H_{52}O_3$ requires: C,79.3; H,10.7%) (Yield 5 g.).

This, on fractional crystallisation from alcohol, gave three fractions of constant melting point:-

A. m.p. 208-210°, $[\alpha]_D^{17}$ + 100° (C = 2.12)
B. m.p. 203-205°, $[\alpha]_D^{19}$ + 117.6° (C = 3.46]
C. m.p. 203-205°, $[\alpha]_D^{16}$ + 114.5° (C = 3.19)

Fraction B, (2 g.), having the highest optical rotation was chromatographed, giving a further three fractions. The first (0.27 g.) was unchanged α-amyrin acetate, m.p. 227-229°, $[\alpha]_D^{17}$+77°. The second (1.50 g.) was pure α-amyrin acetate oxide, m.p. 207-209°, $[\alpha]_D^{22}$+114° (C = 2.29) (Found: C,79.3, H,10.5% $C_{32}H_{52}O_3$ requires: C,79.3, H,10.7%). The third (0.18 g.) melted at 217-219° (Found: C,78.9; H,10.6%) and was probably a mixture.

The by-product obtained from the acetic acid mother liquors forms very small fine needles from chloroform-methanol decomposing 270-280°. The melting point in a sealed, evacuated tube was 284-290°, softening at 280-284°. $[\alpha]_D^{19}$ - 129° (C = 2.04).

Neither the oxide nor the by-product show selective adsorption in the ultra-violet.

Action of Ozone on α-Amyrin Esters. (cf. Ruzicka, Jeger, Redel and Volli, Helv. (1944), 28, 199).

a) α-Amyrin Benzoate - The benzoate (2.5 g.) in dry redistilled carbon tetrachloride (50 c.c.) was subjected to a stream of ozone (c.2.5%) in oxygen for a period of 7 hours after which a specimen of the solution gave no yellow colour with tetranitromethane. The carbon tetrachloride was removed by distillation, and the ozonide boiled with water for 1 hour, and the product extracted with benzene. The small acid fraction was extracted by washing the benzene with dilute caustic soda solution. It proved too small for investigation. The neutral portion was recrystallised from chloroform-methanol, giving *α-amyrin benzoate oxide* as needles, m.p. 215-217°, $[\alpha]_D^{14} + 130.3^\circ$ (C = 2.97). (Yield 1.5 g.).

b) *α-Amyrin Acetate* when treated exactly as described above for the benzoate gave *α-amyrin acetate oxide* as small blades from aqueous acetone, m.p. 208-210°, $[\alpha]_D^{17} + 111^\circ$ (C = 3.10). (Found: C,79.6; H,10.7% $C_{32}H_{52}O_3$ requires: C,79.3; H,10.7%).

ii) When subjected to a stream of 7% ozone in oxygen, the acetate completely reacted in just under 2 hours, and the product crystallised from benzene-methanol, giving *α-amyranonyl acetate* (1 g.) as plates, m.p. 280-282°d. (Sintered at 278°) $[\alpha]_D^{15} + 11.5^\circ$ (C = 1.57). The m.p. in a sealed tube in vacuo was 294-296°.

Action of potassium permanganate on α-Amyrin Benzoate -

α-Amyrin benzoate (1 g.) in acetic acid (stabilised, 75 c.c.) was stirred at 100°, and potassium permanganate (0.24 g.) in water (8 c.c.) dropped in over a period of 30 minutes. The conditions were maintained for 2 hours after which the brown solution was poured into water, and extracted with ether. The solid product on crystallisation from alcohol gave some unchanged starting material, while from the mother liquors was obtained α-amyrin benzoate oxide (200 mg.) m.p. 217-219°, undepressed on admixture with an authentic specimen.

<u>Attempted Hydrolysis of α-Amyrin Benzoate Oxide</u> - i) The oxide (1 g.) in alcohol (50 c.c.) and benzene (10 c.c.) was refluxed on a water bath with potassium hydroxide solution (10% 10 c.c.) for 6 hours, and allowed to stand overnight. The solution was then poured into water, and extracted with ether. A resinous product was obtained which could not be crystallised, and so was acetylated by acetic anhydride (5 c.c.) in pyridine (5 c.c.) for 15 minutes at 100°. Many crystallisations of the product gave α-amyranonyl acetate, m.p. 280-282° (d.) (Sintered at 278°) undepressed on admixture with a specimen obtained by the action of ozone on α-amyrin acetate.

ii) The oxide (2 g.) in methanol (100 c.c.) containing potassium hydroxide (1 g.) was refluxed. After 1 hour, benzene (20 c.c.) was added to aid solution of the material, and refluxing continued for 2 hours more. The solution was poured into water, and extracted with banzene. Crystallisation of the product gave unchanged starting material (0.85 g.) while from the mother liquors was obtained a non-crystallisable resin. This was acetylated by acetic anhydride (2 c.c.) in pyridine (5 c.c.) for 18 hours at room temperature. The acetylated product crystallised from aqueous acetone to give very small blades, m.p. 198-200°. $[\alpha]_D^{15}$ +113.9° (C = 1.58)

(Found: C,79.3; H,10.5% $C_{32}H_{52}O_3$ requires: C,79.3; H,10.7%).

This, on isomerisation, as described below, gave α-amyranonyl acetate, m.p. 280-282° (d.).

Rearrangement of the Oxides was carried out in chloroform-acetic acid solution, using concentrated hydrochloric acid (see Ruzicka, Jeger, Redel and Volli, Helv., (1944), 28, 199).

The benzoate oxide gives α-amyranonyl benzoate in 50% yield as plates from chloroform-methanol, m.p. 227-229°, $[\alpha]_D^{17}$ + 24.7° (C = 2.02). (Found: C,81.0; H,10.2% $C_{37}H_{54}O_3$ requires: C,81.3; H,10.0%). Light absorption in alcohol, λ = 2290A, $E_{max.}$ = 13,000. From the mother liquors are obtained a mixture, as needles, m.p. 223-225°, (Sinters 216-223°), $[\alpha]_D^{17}$ + 92.3° (C = 2.2) which was investigated as described below.

α-Amyranonyl acetate is obtained in 60% yield as plates from chloroform-methanol, m.p. 280-282° (d.). Sintering at 278° (m.p. in a sealed tube in vacuo, 294-296°) $[\alpha]_D^{15}$ + 11.4° (C = 1.57) (Found: C,79.1; H,10.7% $C_{32}H_{52}O_3$ requires: C,79.3; H,10.7%) Light absorption in alcohol, λ = 2820A, $E_{max.}$ =225.

Attempted Oxime Formation - α-Amyranonyl benzoate (0.5 g.) in alcohol (25 c.c.) was treated with hydroxylamine hydrochloride (1 g.) and sodium acetate (1 g.), both dissolved in the minimum of water, and refluxed for 1 hour. Water was added, and the collected solid crystallised as unchanged starting material.

Investigation of the needles isolated from the mother liquors of benzoate after rearrangement of the oxide -

i) α-Amyrin benzoate oxide (4.7 g.) was isomerised giving the ketone (2 g.) and a quantity of solid (2.7 g.) from the mother liquors which was chromatographed, 17 fractions being taken. Fractions 1) - 8) (0.9 g.) on recrystallisation, behaved like the crude ketone, giving some of this, together with the mixture under investigation. Fractions 9) - 12) (0.29 g.) gave needles m.p. 218-220°. The remainder (1.3 g.) gave m.p. 232-236° (sintering above 228°), $[\alpha]_D^{17}$ + 88.4° (C = 2.25) (Found: C,81.7; H,10.2% $C_{37}H_{54}O_3$ requires: C,81.3; H,10.0%. This was rechromatographed, but no separation was achieved.

This last fraction (100 mg.) in acetic acid (3 c.c.) was treated at 60° with bromine in acetic acid (13% 2 c.c.), allowed to stand for 10 minutes, poured into water, and extracted with ether. The product was bromo-α-amyranonyl benzoate, m.p. 186-188°, crystallising as plates from chloroform-methanol. (Found: Br. 13.1% $C_{37}H_{54}O_3$ Br. requires Br, 12.8%).

ii) The crude material associated with the ketone (0.5 g.) in acetic acid (20 c.c.) was treated at 60° with bromine in acetic acid (13% 8 c,c.) and allowed to cool. The bromo-compound which separated was recrystallised from chloroform-methanol to give bromo-α-amyranonyl benzoate (250 mg) as plates, m.p. 184-186°, $[\alpha]_D^{16}$ + 24.1° (C = 2.28).

Hydrolysis of α-Amyranonyl Benzoate - The ketone (3 g.) was dissolved in benzene (20 c.c.) and refluxed with alcohol (100 c.c.) containing aqueous potassium hydroxide (10%, 30 c.c.) for 7 hours, and allowed to stand overnight at room temperature. After reduction of the volume by distillation in vacuo, the solution was poured into water, and extracted with ether. An exceedingly soluble resinous product was obtained, which was divided into two portions.

The first of these was absorbed on alumina, and fractionally eluted, benzene removing 0.36 g. of a crystalline solid, which was taken up in methanol (aq.). Colourless cubes separated on long standing, and on recrystallisation from the same solvent gave needles, m.p. 218-220°. (Found: C,80.0, 79.2; H,10.6, 10.6%). Light absorption in alcohol showed two maxima, λ = 2510, 3070A $\varepsilon_{max.}$ = 25,800, 10,000.

The second portion was heated on a steam bath for 20 minutes with pyridine (10 c.c.) and acetic anhydride (20 c.c.) poured into water, and extracted with ether. Crystallisation of the product from chloroform-methanol gave α-amyranonyl acetate as faintly yellow plates, m.p. 282-284° (d. Sintered at 278°) undepressed on admixture with an authentic specimen. $[\alpha]_D^{16}$ + 11.6° (C = 1.63).

From the mother liquors were obtained, in equal quantity, a compound forming needles from chloroform-methanol, m.p. 230-232°, $[\alpha]_D^{16}$ + 37.9° (C = 1.95). (Found: C,78.4, 78.7; H, 11.1, 11.4%).

α-Amyranonol - α-Amyranonyl benzoate or acetate (1 g.) was refluxed with 1% potassium hydroxide in methylated spirits (50 c.c.) for 2 hours, poured into water and extracted with benzene. The product crystallised from 60-80° light petroleum as fern-like growths of exceedingly fine needles, m.p. 218-220°, $[\alpha]_D^{16}$ - 4.96° (C = 1.51) (Found: C,81.1; H,10.9% $C_{30}H_{50}O_2$ requires: C,81.4; H,11.3%).

Acetylation of α-Amyranonol - The alcohol (ex α-amyranonyl benzoate, 0.5 g.) in pyridine (5 c.c.) and acetic anhydride 2 c.c.) was left overnight at room temperature. The acetate began to separate after a few hours, and was finally filtered off, and recrystallised from chloroform-methanol to give α-amyranonyl acetate as plates, m.p. 280-282° (d. Sintered at 278°) undepressed on admixture with an authentic specimen. $[\alpha]_D^{15}$ +9.4° (C = 2.86).

α-Amyranonyl Benzoate Enol Acetate - α-Amuranonyl benzoate (3 g.) in acetic anhydride (60 c.c.) containing fused sodium acetate (3 g.) was refluxed for 24 hours, poured into water, and extracted with ether. The product was crystallised from aqueous acetone to give the enol acetate (2 g.) as small colourless blades, m.p. 220-222°. $[\alpha]_D^{14}$ + 65.3° (C = 7.37) (Found: C,79.7; H,9.29%, $C_{39}H_{58}O_4$ requires: C, 79.6; H,9.52%).

α-Amyranonyl Acetate Enol Acetate may be prepared by treatment of α-amyranonyl acetate as described for the benzoate. The product crystallises as needles from alcohol, m.p. 255-257°, $[\alpha]_D^{17}$ +49.2° (C = 2.58) (Found: C, 77.6; H, 10.1%

$C_{34}H_{54}O_4$ requires: C, 77.6; H, 10.25%).

Bromo-α-Amyranonyl Benzoate - i) The ketone (0.5 g.) in glacial acetic acid (20 c.c.) containing a trace of hydrobromic acid, was treated with a solution of bromine in acetic acid (7%, 5 c.c.) at 60° with shaking. Decolourisation of the solution was accompanied by separation of the product which was collected after 10 minutes, and crystallised from chloroform-methanol as blades, m.p. 186-188° (d.). $[\alpha]_D^{17} + 27.1°$ (C = 2.36). The yield was 0.5 g. (Found: C, 71.1; H, 8.77% $C_{37}H_{53}O_3$ Br. req. C, 71.0; H, 8.48%).
ii) α-Amyrenyl benzoate oxide when treated in an analogeous manner gave the same bromo-compound as blades, m.p.186-188° (d.). (Found: C, 71.47; H, 8.56% $C_{37}H_{53}O_3$ Br. requires: C, 71.0; H, 8.48%).

iso-α-Amyrenonyl Benzoate - i) The bromo-compound (200 mg.) obtained as above, was heated on a steam bath with acetic acid (4 c.c.) containing a trace of hydrobromic acid, for 3 hours, during which darkening occurred, and hydrobromic acid was evolved. After standing overnight at 0°, the mixture was diluted with water, and the collected solid crystallised from benzene-methanol (norite), giving the required product as small prisms, m.p. 212-214°.
ii) α-Amyrenyl benzoate oxide (5 g.) in acetic acid (150 c.c.) containing a trace of hydrobromic acid was treated at 60° with a solution of bromine in acetic acid (12.8%, 15 c.c.)

over a period of 10 minutes during which the bromo-ketone separated. The mixture was then heated on a steam bath for 2 hours, when the solid dissolved, darkening occurred, and hydrobromic acid was evolved. After standigg overnight at 0°, the frozen mass was diluted with water, and the collected solid crystallised from benzene-alcohol (norite) giving *iso*-α-amyrenonyl benzoate (3 g.) as small prisms, m.p. 212-214°, undepressed on admixture with the previously obtained specimen. $[\alpha]_D^{15}$ + 90.8° (C = 1.96) (Found: C, 81.7; H, 9.7% $C_{37}H_{52}O_3$ requires: C, 81.6; H, 9.6%).

iso-α-Amyrenonyl Acetate - i) α-Amyranonyl acetate (1 g.) in acetic acid (50 c.c.) containing a trace of hydrobromic acid was treated with a solution of bromine in acetic acid (8%, 8 c.c.) over a period of 10 minutes at 50-60°. The bromine was decolourised, but no solid separated. The solution was heated on a steam bath for 2 hours, and left overnight at 0°. It was worked up as in the case of the benzoate. The product crystallised as plates, m.p. 284-286° (d.) from benzene-methanol, and gave no m.p. depression on admixture with α-amyranonyl acetate. The specific rotation of the product was $[\alpha]_D^{16}$ +87.4° (C = 2.07) (Found: C,79.4: H,10.6% $C_{32}H_{50}O_3$ requires: C,79.6; H,10.5%). Light absorption in alcohol, λ = 2520A, $E_{max.}$ = 7,500.

ii) *iso*-α-Amyrenonol (0.5 g., obtained by hydrolysis of the benzoate, see below) was refluxed for 15 minutes with acetic anhydride (5 c.c.), and a little fused sodium acetate.

On cooling *iso*-α-amyrenonyl acetate separated, and was collected and recrystallised from benzene-methanol as plates, m.p. 284-286° (d.). $[\alpha]_D^{18}$ + 82.8° (C = 1.92). There was no m.p. depression on admixture with a specimen obtained by method i).

iso-α-Amyrenonol - i) *iso*-α-amyrenonyl benzoate (2 g.) in alcoholic potassium hydroxide (12%, 150 c.c.) was refluxed for 4 hours, and left overnight at room temperature. The solution was poured into water, and the precipitated solid collected and crystallised from aqueous alcohol (norite) giving *iso*-α-amyrenonol as fine needles, m.p. 241-242°, $[\alpha]_D^{15}$ + 71.6° (C = 1.99).
ii) *iso*-α-Amyrenonyl acetate (1.3 g.) in alcohol (50 c.c.) and aqueous potassium hydroxide (40%, 5 c.c.) was refluxed for 2 hours on a steam bath, and worked up as above. The product formed long fine needles, $[\alpha]_D^{18}$ + 75.1°, (C = 2.15), m.p. 241-242° undepressed with a previous specimen. (Found: C, 82.0; H, 11.1% $C_{30}H_{48}O_2$ requires: C, 81.8; H, 10.9%). Light absorption in alcohol, λ = 2500A, $E_{max.}$ = 9,300.

Attempted Kishner-Wolff Reduction of α-Amyranonyl Acetate - The ketone (1 g.), sodium ethoxide (1.2 g. sodium and 30 c.c. alcohol) and hydrazine hydrate (98%, 3.4 c.c.) were heated together in an autoclave for 18 hours at 190°. The contents of the autoclave were poured into water, and extracted with ether, yielding a resin which crystallised from aqueous alcohol to give, in very small yield, very fine needles, m.p. 237-239°. (Found: C,81.9; 82.1; H, 12.5, 12.6% and from a subsequent preparation, C, 81.6; H, 12.1% $C_{32}H_{54}O_2$ requires: C, 81.7; H, 11.5%).

The mother liquors yielded much resinous material, which was acetylated by heating with acetic anhydride (5 c.c.) and pyridine (5 c.c.) for 15 minutes at 100°. Crystallisation of the product gave a little α-amyranonyl acetate, and an unknown compound crystallising as needles from aqueous alcohol, m.p 240-242°. (Found: C, 79.9; H, 10.8% $C_{34}H_{56}O_3$ requires: C, 79.7; H, 10.9%).

Attempted Ethylation of α-Amyranonol - The alcohol (0.7 g.) was added to dry toluene (10 c.c.) containing finely divided sodium (0.04 g., 1 atom), and refluxed for 1 hour. Some sodium remained unreacted. Excess of ethyl iodide (0.5 c.c.) was then added, and the solution refluxed for a further hour, during which sodium iodide separated. The solution was poured into water, and the toluene layer washed and dried (sodium sulphate). Evaporation to dryness left a resin from which no crystalline material could be obtained.

α-Amyranediol was prepared by reduction of α-amyrin benzoate oxide with sodium in amyl alcohol as described by Seymour, Sharples and Spring, (J. (1941), 319). The product was crystallised first from light petroleum (60-80°), then from aqueous acetone, giving fine needles, m.p. 200-202°, $[\alpha]_D^{16}$ + 73.5° (C = 1.66), $[\alpha]_D^{19}$ + 70° (C = 2.44).

α-Amyranedione - i) α-Amyranonol (1 g.) in acetic acid (17 c.c.) was treated with a solution of chromic acid (0.22 g.) in the minimum volume of water and acetic acid (3 c.c.) After standing overnight, the mixture was poured into water, and extracted with ether. The small acid fraction, isolated by washing of the ether with dilute caustic soda solution, was neglected. The neutral product crystallised from aqueous alcohol as needles, m.p. 166-168°, $[\alpha]_D^{20}$ +1.6° (C = 2.42). Found: C, 82.0; H, 10.9% $C_{30}H_{48}O_2$ requires: C, 81.7; H, 11.0%).

ii) α-Amyranediol (1 g.) in acetic acid (17 c.c.) was treated, as above, with chromic acid (0.44 g.) in a little water plus acetic acid (3 c.c.). α-Amyranedione, m.p. 166-168°, undepressed on admixture with a specimen prepared as above, was obtained in low yield (200 mg.).

Hydrazone of α-Amyranedione - The dione (200 mg.) in alcohol (10 c.c.) was treated with hydrazine hydrate (90%, 1 c.c.) at room temperature, and left overnight. The solution was then poured into water, and extracted with ether. The product crystallised as small needles from benzene-alcohol, m.p. 314-316°. (Found: C, 78.5; H, 10.9% $C_{30}H_{52}O_2$ requires: C, 78.9; H, 11.4%).

α-Amyrenone was prepared in 70% yield according to the method of Ruzicka et al. (Helv. (1939), 22, 758). The product crystallised as tiny needles from acetone, m.p. 125°.

α-Amyrene - i) α-Amyrenone (6 g.) sodium ethoxide (6 g. sodium in 150 c.c. ethanol), and hydrazine hydrate (90% 18 c.c.) were heated together in an autoclave at 180° for 18 hours. On cooling, the contents were poured into water, and extracted with ether. The extract was washed with water, and dilute mineral acid, then dried over sodium sulphate. Removal of the solvent left a yellow oil which crystallised from ethanol to give colourless plates, m.p. 109-111°, $[\alpha]_D^{20}$ + 92.6° (C = 1.75) (Found: C, 88.1; H, 12.4% $C_{30}H_{50}$ requires: C, 87.7; H, 12.3%). The yield was 3.5 g. (60%).

ii) α-Amyrenone was reduced by amalgamated zinc in hydrochloric/acetic acid solution as described by Winterstein and Stein (*Ann.* (1933), **502**, 223). The only product isolated was α-amyrene, $[\alpha]_D^{20}$ +92.4° (C = 1.98), m.p. 109-111°, undepressed on admixture with a specimen prepared as described above.

iii) α-Amyrenone semicarbazone (1 g.) was treated with sodium ethoxide in an autoclave as described by Ruzicka et al. (*Helv.* (1939), 22, 758). The product was -amyrene, m.p. 109-111°, $[\alpha]_D^{19}$ + 92.6° (C = 2.44), the yield being ca. 400 mg.

Reduction of α-Amyrenone with Zinc and Hydrochloric Acid was carried out using the method described by Winterstein and Stein (*Ibid.*), but using unamalgamated zinc. A small quantity of material, crystallising from aqueous acetone as needles, m.p. 192-4° was obtained. (Found: C, 88.3; H, 11.6% $C_{30}H_{48}$ requires: C, 88.2; H, 11.8%. This gave no m.p. depression on admixture with 1-α-amyradiene.

Attempted Hydrogenation of α-Amyrene - i) α-Amyrene (1 g.) was dissolved in ethyl acetate (100 c.c.) and shaken in an atmosphere of hydrogen in the presence of a platinum catalyst for 12 hours. No hydrogen was adsorbed, and on filtering free from platinum, and removing the solvent, the starting material was recovered.

ii) α-Amyrene (1.3 g.) in ethyl acetate (150 c.c.) in the presence of platinum oxide (0.1 g.) was treated with hydrogen under 50 atmospheres pressure at a temperature of 75° for 6 hours. After allowing the autoclave to cool overnight, the pressufe was released and the solvent filtered and removed in vacuo. Unchanged α-amyrene was recovered.

α-Amyrene Oxide - α-Amyrene (5 g.) was treated with hydrogen peroxide in acetic acid as in the preparation of α-amyrin benzoate oxide (v.s.). The reaction mixture was diluted only very slightly after cooling, however, to aid crystallisation of some solid which separated and was collected. This, on crystallisation, gave unchanged hydrocarbon (0.5 g.). The acetic acid mother liquors were poured into water, and extracted with benzene. Removal of the benzene left a non-crystallisable gum (3.8 g.) which was dissolved in 50/50:benzene/petrol, and filtered through alumina. A colourless oil (2.0 g.) passed straight through the column, and was crystallised from alcohol, giving α-amyrene oxide as plates, m.p. 116-118°, $[\alpha]_D^{21}$ + 135.2° (C = 1.96). (Found: C, 84.5; H, 11.9% $C_{30}H_{50}O$ requires: C, 84.5; H, 11.7%).

A second chromatographic fraction was obtained crystallising from alcohol to give, in low yield, needles, m.p. indistinct between 268-274°, sintering above 250°. (Found: C, 79.3; H, 10.7% $C_{32}H_{54}O_3$ requires: C, 79.1; H, 11.1%).

iso-α-Amyranone - i) α-Amyrene oxide (0.9 g.) was dissolved in chloroform (5 c.c.) and acetic acid (20 c.c.) and concentrated hydrochloric (1 c.c.)madded. The solution was maintained at 40° for 30 minutes, poured into water, and extracted with ether. The washed and dried extract, on evaporation, left a resin which crystallised from aqueous alcohol as clusters of microscopic blades, m.p. 150-152°. $[\alpha]_D^{24} \pm 0°$ (C = 0.67) (Found: C, 84.1; H, 11.6% $C_{30}H_{50}O$ requires: C, 84.5; H, 11.7%).

This product has been found sometimes to crystallise in a solvated form as small blades, m.p. 141-143°, rising to 150-152° after drying in vacuo at 120°.

ii) α-Amyranedione (0.75 g.), sodium ethoxide (0.75 g. sodium in 30 c.c. ethanol) and hydrazine hydrate (90%, 3.5 c.c.) were heated in an autoclave for 18 hours at 180°. The contents were poured into water and extracted with ether. A resinous product was obtained, which was purified chromatographically, giving, in very low yield, iso-α-amyranone, m.p. 141-143°, undepressed on admixture with authentic specimens of either the solvated or dried form.

iso-α-Amyranol - α-Amyrene oxide (2.85 g.) in amyl alcohol (38 c.c.) was treated under reflux with sodium (2.85 g.) and boiled for 20 minutes after which more sodium (2.85 g.) was added. Refluxing was carried out for 2½ hours, during which small quantities of amyl alcohol were added. The amyloxide was decomposed by boiling with water, and the alcohol removed by steam distillation after which extraction with ether was carried out. The resinous product crystallised 178-180°, $[\alpha]_D^{20}$ + 48.1° (C = 2.08). Yield, 1.5 g. (53%).

17(Found: C, 84.0; H, 12.25% $C_{30}H_{52}O$ requires: C, 84.1; H, 12.45

(Found: C, 84.0; H, 12.25% $C_{30}H_{52}O$ requires: C, 84.1; H, 12.45%)

Action of Acetic Anhydride on iso-α-Amyranol - i) The alcohol (0.5 g.) in pyridine (5 c.c.) was treated with acetic anhydride (5 c.c.) and heated on a steam bath for 15 minutes, then poured into water and extracted with ether. A resinous product was obtained, which would not crystallise.

ii) The alcohol (200 mg.) in pyridine (4 c.c.) and acetic anhydride (2 c.c.) was allowed to stand for 20 hours at room temperature, and worked up as above. The resinous product was taken up in alcohol, and after long standing crystallised, and was recrystallised from the same solvent giving iso-α-amyranyl acetate as blades, m.p. 244-246° (Found: C, 81.1; H, 11.9% $C_{32}H_{54}O_2$ requires: C, 81.7; H, 11.5%).

Attempts to chlorinate iso-α-Amyranol - i) The alcohol (300 mg.) in pyridine (5 c.c.) was treated at 0° with a solution of thionyl chloride (0.5 c.c.) in pyridine (2.5 c.c.)

and left overnight at room temperature.

ii) The alcohol (600 mg.) was treated with thionyl chloride (5 c.c.), and the solution heated for 30 minutes on a steam bath.

iii) The alcohol (300 mg.) in dry ether (20 c.c.) was cooled in ice-salt, and a stream of dry hydrochloric acid slowly bubbled in, the temperature being maintained below 5°. When the solution was saturated, the flask was tightly stoppered, and left overnight in the refrigerator.

iv) The alcohol (500 mg.) was dissolved in ice cold phosphorus oxychloride (5 c.c.) and allowed to stand overnight at room temperature. The solution developed a pink colour.

v) The alcohol (500 mg.) and phosphorus pentachloride (500 mg.) were heated together on a steam bath for 15 minutes, when they melted. Before working up, the mixture was diluted with a little phosphorus trichloride, so that it could be poured.

In each of the above cases, working up was achieved by pouring into ice water, followed by extraction of the product with ether.

ii), iii), iv) and v) gave, as the only product, α-emyrene, m.p. 109-111°, undepressed on admixture with an authentic specimen. Analysis of the product was carried out in case ii) only. (Found: C, 87.9; H, 12.3% $C_{30}H_{50}$ requires: C 87.7; H, 12.3%).

In i) above, no crystalline product was isolated, but the low melting resin obtained was free of halogen.

Attempted Reduction of iso-α-Amyranone by Red Phosphorus and Hydriodic Acid - Iodine (0.02 g.) and red phosphorus (0.05 g.) were allowed to stand in contact with one another in acetic acid (10 c.c.) for 20 minutes; the ketone (200 mg.) and water (0.2 c.c.) were then added, and refluxing carried out for 2½ hours. The solution was filtered through asbestos, and poured into sodium bisulphite solution. The precipitated solid was collected and crystallised from aqueous acetone, giving unchanged ketone, m.p. 150-152°, in low yield. There was no trace of any product.

Action of Ozone on α-Amyrene - α-Amyrene (1 g.) in dry carbon tetrachloride (25 c.c.) was subjected to a stream of oxygen containing ozone, until a sample gave no further yellow colouration with tetranitromethane. The solvent was removed in vacuo, and the remaining resin boiled with water for 1 hour to decompose the ozonide which was then extracted with ether. Washing of the extract with 2N caustic soda gave a small acid fraction which was taken up in methanol, but did not crystallise. The neutral resinous product failed to give any crystalline material.

Action of Hydrogen Peroxide on α-Amyrenone - α-Amyrenone (5 g.) in glacial acetic acid (150 c.c.) was treated on a steam bath, with stirring, with 40 c.c. of a solution of hydrogen peroxide (30% 30 c.c.) in acetic acid (30 c.c.) over a period of 10 minutes. After 2 hours, the remainder of the peroxide solution was added, and conditions maintained for a further hour, after which the solution was poured into water, and the precipitated solid collected, and taken up in alcohol. No solid was obtained. The alcohol was evaporated to dryness, and the oil dissolved in chloroform-acetic acid solution, and treated with hydrochloric acid to isomerise any oxide present. Again, however, no crystalline product could be isolated.

Action of Thionyl Chloride on α-Amyrin - α-Amyrin (1 g.) in pyridine (5 c.c.) was treated with thionyl chloride (2 c.c.) in pyridine (2 c.c.) at room temperature. Slight warming occurred. After 18 hours, the mixture was poured into water, and extracted with ether. The extract was washed with dilute hydrochloric acid and water, then dried over sodium sulphate. From the extract was obtained a dark resin, which was crystallised from benzene-methanol (norite) to give small colourless plates, m.p. 260-261°, $[\alpha]_D^{18} + 35.1^\circ$ (C = 2.05), containing sulphur. Found: C, 79.4; H, 10.5; S, 3.09% $C_{60}H_{98}O_3$ requires: C, 80.2; H, 10.9; S, 3.56%).

Action of Acetic Acid on α-Amyrenyl Benzoate Oxide - The oxide (1 g.) was refluxed with acetic acid (25 c.c.) containing a trace of hydrogen peroxide, for 3 hours. The solution became yellow, and was poured into water, and extracted with ether, giving a yellow resin which was crystallised with difficulty from chloroform-methanol as unchanged starting material.

Hydrolysis of Product, m.p. 305°, associated with α-Amyrin Benzoate Oxide - i) The compound (0.7 g.) in alcoholic potassium hydroxide (2%, 50 c.c.) and benzene (5 c.c.) was refluxed for 2 hours. The solution was then concentrated by distillation, poured into water, and the product extracted with ether. The solid thus obtained crystallised from aqueous alcohol as needles, m.p. 246-248°, $[\alpha]_D^{20}$ - 180° (C = 2.14). (Found: C, 76.2; H, 9.87%).

ii) The compound (1 g.) in benzene (5 c.c.) and alcoholic potassium hydroxide (5%, 100 c.c.) was refluxed for 2 hours, and worked up as above. The product crystallised as needles, m.p. 247-249°, $[\alpha]_D^{24}$ - 182.2° (C = 2.76). (Found: C, 75.9; H, 9.5%).

Acetylation of the Hydrolysis Product - The hydrolysis product (0.1 g.) in pyridine (2 c.c.) and acetic anhydride (2 c.c.) was heated on a steam bath for 15 minutes, poured into water, and extracted with ether. The product crystallised from benzene-alcohol as blades, sintering 285-295°, m.p. 297-301° (d.). $[\alpha]_D^{20}$ - 64° (C = 0.78). (Found: C, 74.9; H, 9.26%).

Oxidation of the Hydrolysis Product - The hydrolysis product (0.5 g.) in acetic acid (8.5 c.c.) and benzene (2 c.c.) was treated with chromic acid (0.22 g. $2\bar{O}$) in water (1 c.c.) and acetic acid (2 c.c.) at room temperature, and left overnight. The solution was poured into water, and extracted with ether. The neutral product crystallised from chloroform-methanol giving a ketone as glistening plates, m.p. 282-285° (d.) sintering above 276°, $[\alpha]_D^{15}$ - 210.2° (C = 1.76). (Found: C, 76.2, 76.6, H, 9.4; 9.45%).

Hydrazone of the Ketone - The ketone (420 mg.) in alcohol (60 c.c.) and chloroform (5 c.c.) was treated with hydrazine hydrate (90%, 5 c.c.) and left overnight. The solution was poured into water, and extracted with ether. The derivative crystallised from chloroform-methanol as needles, m.p. 336-338° (d.). Found: C, 75.7; H, 8.97; N, 3.57, 3.72%).

Action of Periodic Acid on Benzoate Hydrolysis Product - The hydrolysis product (100 mg.) in alcohol (50 c.c.) was treated with sodium periodate (200 mg.) dissolved in sulphuric acid solution (10%, 2 c.c.) and left overnight at room temperature. On working up, only unchanged starting material was recovered.

Hydrolysis of Product, m.p. 280°, associated with α-Amyrin Acetate Oxide - The by-product (100 mg.) in methylated spirits containing potassium hydroxide (2%, 10 c.c.) was refluxed for 2 hours, and the solution concentrated by distillation. On cooling, the product crystallised, and was collected and recrystallised from aqueous alcohol as needles, m.p. 245-247°, (depressed to below 240° on admixture with a specimen of the benzoate hydrolysis product). $[\alpha]_D^{16}$ - 134.7° (C = 0.49) (Found: C, 76.4; H, 9.62%).

Oxidation of the Acetate Hydrolysis Product - The hydrolysis product (420 mg.) in benzene (2 c.c.) and acetic acid (7 c.c.)

was treated with a solution of chromic acid (0.18 g.) in water (1 c.c.) and acetic acid (2 c.c.), left overnight, and worked up as usual. The product crystallised as blades from chloroform-methanol, m.p. 286-290° (d.), sintered above 278°. The m.p. was undepressed on admixture with a specimen of the ketone previously obtained (v.s.). $[\alpha]_D^{20}$ - 206.2° (C = 1.79) (Found: C, 76.8; H, 9.98%).

Action of various reagents on the by-product associated with α-Amyrin Benzoate Oxide.

a) Acetic Anhydride - i) The by-product (100 mg.) in pyridine (5 c.c.) and acetic anhydride (2 c.c.) was heated for 15 minutes at 100°.

ii) The compound (500 mg.) was refluxed for 2 hours in acetic anhydride (20 c.c.).

In both cases, only unchanged starting material was recovered.

b) Chromic Acid - The material (0.5 g.) was dissolved in benzene (8 c.c.) and acetic acid (8 c.c.) and a solution of chromic acid (0.22 g.) in water (0.5 c.c.) and acetic acid (2 c.c.) added. After standing at room temperature overnight, the solution was worked up, when it was found that no reaction had taken place.

c) Thionyl Chloride - i) The by-product (200 mg.) in pyridine (10 c.c.) was treated with thionyl chloride (1 c.c.) in pyridine (4 c.c.). The solution was left overnight at 0°, poured into water, and extracted with ether. Unchanged starting material was recovered.

ii) The by-product (500 mg.) in thionyl chloride (12 c.c.) was refluxed for $1\frac{1}{2}$ hours, poured into water, and the granular solid obtained collected. This material appeared to contain sulphur, but could not be obtained in a crystalline form.

Attempted Acetylation of the Ketone, m.p. 282° - The ketone (250 mg.) was treated with acetic anhydride (3 c.c.) in pyridine (3 c.c.) for 15 minutes at 100°. On working up, starting material was recovered quantitavely.

Action of Alkali on Bromo-α-Amyranonyl Benzoate - i) The bromo-ketone (0.7 g.) was refluxed with alcoholic potassium hydroxide (4%, 50 c.c.) for 10 minutes.

ii) The bromo-ketone (300 mg.) was dissolved in benzene (5 c.c.) and alcoholic aqueous potassium hydroxide (50 c.c.) containing 1 g. alkali) added. The mixture was allowed to stand for 5 days.

iii) The bromo-ketone (1 g.) was refluxed with alcoholic potassium hydroxide (1%, 100 c.c.) for 2 hours.

In each of these three cases, on working up by the usual methods, only unchanged starting material was recovered.

iv) The bromo-ketone (1 g.) in benzene (25 c.c.) was refluxed with methylated spirits (50 c.c.) containing water (5 c.c.) and potassium hydroxide (1 g.). After 2 hours, the solution was poured into water, and extracted with benzene. Some starting material crystallised first, but from the mother liquors was obtained a product crystallising as needles from aqueous alcohol, m.p. 209-211°, and free of halogen. (Found: C, 79.6; H, 9.91% $C_{37}H_{54}O_4$ requires: C, 79.0; H, 9.6%).

v) The bromo-ketone (2.5 g.) in benzene (62 c.c.) methylated spirits (125 c.c.) and water (12 c.c.) containing potassium hydroxide (2.5 g.) was refluxed until all the solid had dissolved (ca. 20 minutes), and then for a further 2 hours. The solution was concentrated, poured into water, and extracted with benzene. A resin was obtained which crystallised from benzene-alcohol, giving, in small yield, α-amyranon~~e~~diol mono-benzoate, as fine needles from benzene-alcohol, m.p. 243-245° (Indistinct) $[\alpha]_D^{20}$ 64.4° (C = 1.32). (Found: C, 78.7; H, 9.34% $C_{37}H_{54}O_4$ requires: C, 79,0; H, 9.6%). The product gives a strong colour reaction with alcoholic ferric chloride.

The mother liquors were evaporated to dryness, and the residual oil crystallised, first from light petroleum (60-80°), then from aqueous alcohol, giving iso- α-amyrenonol, m.p. 240-242°, light absorption in alcohol λ = 2490Å, $E_{max.}$ = 9,800. (Found: C, 81.6; H, 11.0% $C_{30}H_{48}O_2$ requires: C, 81.8; H, 10.9%).

vi) The bromo-ketone (11.75 g.) in benzene (247.5 c.c.) was treated with potassium hydroxide (11.75 g.) in water (55.5 c.c.) and methylated spirits (555 c.c.) exactly as described under v) above. The resinous product crystallised from benzene-alcohol giving in low yield (ca. 1.5 g.) a product, m.p. 218-220°. $[\alpha]_D^{17}$ +48.1° (C = 2.75). (Found: C, 76.7; H, 10.6%)

Acetylation of α-Amyranonediol Monobenzoate - The ketol (100 mg.) in pyridine (2 c.c.) was treated with acetic anhydride (2 c.c.) on a steam bath for 15 minutes, poured into water, and extracted with ether. A resin was obtained, which crystallised from alcohol, giving, in small yield, the di-acetoxy compound, as tiny prisms, m.p. 247-249° (Indistinct). (Found: C, 75.8; H, 8.98% $C_{41}H_{58}O_6$ requires: C, 76.2; H, 8.98%).

Action of Silver Oxide on Bromo-α-Amyranonyl Benzoate -
i) The bromo-ketone (0.5 g.) in alcohol (50 c.c.) and benzene (30 c.c.) containing water (0.5 c.c.) was shaken with silver oxide (0.5 g.) for 18 hours at room temperature in a flask protected from light.
ii) The bromo-ketone (0.5 g.) in benzene (30 c.c.), alcohol (50 c.c.) and water (0.5 c.c.) was refluxed for 2 hours in a flask protected from light.

In both cases, the solution was filtered and evaporated to dryness, when starting material was recovered quantitatively.

Action of Silver Acetate on the Bromo-ketone - The bromo-ketone (1 g.) in pyridine (30 c.c.) was treated with silver acetate (1 g.) in pyridine (20 c.c.) and left overnight at room temperature. As no silver bromide had separated, the solution was heated on a steam bath for 1½ hours before pouring into water, and extracting with ether. Only some unchanged bromo-compound was recovered.

Action of Sodium Acetate on the Bromo-ketone - The bromoketone (1 g.) in dry benzene (10 c.c.) was refluxed with dry alcohol (50 c.c.) containing fused sodium acetate (0.5 g.) for 4 hours. On cooling, the unchanged bromocompound, not all of which had dissolved, separated, and was collected. No product was obtained.

Action of periodic Acid on α-Amyranonediol Monobenzoate - i) The ketol (250 mg.) was dissolved in alcohol (150 c.c.) and sodium periodate (500 mg.) in sulphuric acid (N, 5 c.c.) added to the cold solution. After 24 hours, water was added, and the alcohol removed by vacuum distillation. The product was dissolved in ether, and the solution washed with dilute sodium carbonate. No acid fraction resulted, however. The neutral product was crystallised from aqueous alcohol as small, fine needles, m.p. 195-197°, clearing at 207°, $[\alpha]_D^{19}$ + 64.2° (C = 1.06).)Found: C, 78.8, 79.2; H, 8.9, 9.6% $C_{37}H_{54}O_4$ requires: C, 79.0, H, 9.6%).

ii) The ketol (200 mg.) was treated with sodium periodate (400 mg.) as described above. The solution became slightly yellow at first, but the colour then faded. Again there was no acid fraction, the neutral product crystallising as fine needles, m.p. 206-208° (d.) from aqueous alcohol, and giving no colouration with alcoholic ferric chloride solution. (Found: C, 78.9; H, 9.6%).

Action of Chromic Acid on α-Amyranonyl Benzoate - i) α-Amyranonyl benzoate (1 g.) was dissolved in acetic acid

(12 c.c.) by heating, and cooled rapidly, so that the ketone separated in a finely divided state. Solid chromic acid (0.4 g.) was added, and the mixture refluxed for 30 minutes, cooled, poured into water, and the solid collected. The resinous product could not be crystallised.

ii) The ketone (4 g.) was dissolved in acetic acid (200 c.c.) and treated at 70° with a solution of chromic acid (1.6 g.) dissolved in the minimum of water and acetic acid (40 c.c.) added over a period of 20 minutes with intermittent shaking. The solution was kept at 70° for 1 hour. The reaction mixture was poured into water, and the product extracted with ether. The extract was washed with carbonate to remove the minute acid fraction which was neglected. The neutral yellow ethereal extract was dried over sodium sulphate, and evaporated to dryness. The yellow product was crystallised from aqueous acetone to give a solid, m.p. 233-235° as light yellow plates. $[\alpha]_{D}^{14}$ +30.1° (C = 3.02). (Found: C, 80.1, 79.4; H, 9.5, 9.4% $C_{37}H_{54}O_4$ requires: C, 79.0; H, 9.4%).

The product gave a slight, but positive, blue colouration with alcoholic ferric chloride solution.

iii) The ketone (0.5 g.) in acetic acid (30 c.c.) was refluxed with a solution of chromic acid (0.2 g.) in water (0.5 c.c.) and acetic acid (10 c.c.) for 1 hour, poured into water, and extracted with ether. The neutral product was taken up in alcohol. A very low yield of material crystallising as small prisms, m.p. 270-272° as obtained. There was too little for investigation.

iv) α-Amyranonyl Benzoate (1 g.) in acetic acid (50 c.c.) was treated with a solution of chromic acid (0.8 g.) in water (1 c.c.) and acetic acid (20 c.c.) added over a period of 20 minutes, with shaking, at 70°. After 2 hours at this temperature, the solution was poured into water, and extracted with ether. Washing of the extract with dilute potassium hydroxide yielded a small acid fraction, which crystallised from chloroform-light petroleum (60-80°), m.p. indistinct between 220 and 240° (d.). The neutral portion of the product crystallised from aqueous acetone to give pale yellow needles, m.p. 264-266°, $[\alpha]_D^{15}$ + 13.1° (C = 1.67) (Found: C, 78.18, 78.02; H, 8.87, 9.05% $C_{41}H_{58}O_5$ requires: C, 78.1; H, 9.21%).

Hydrolysis of Oxidation Product, m.p. 233° - The benzoate (o.8 g.) was refluxed with alcoholic potassium hydroxide (1%, 50 c.c.) for 2 hours, poured into water, and extracted with ether. The product crystallised from chloroform-light petroleum (60-80°) as fine needles. The melting point was not sharp, the material decomposing above 250°, and melting without clearing. It developed a blue colouration with ferric chloride in alcohol. (Found: C, 78.9; H, 11.0% $C_{30}H_{46}O_3$ requires: C, 79.2; H, 10.2%).

Action of Periodic Acid on Product, m.p. 233° - The oxidation product (480 mg.) in alcohol (300 c.c.) was treated with sodium periodate (1 g.) in sulphuric acid (10%, 7 c.c.) and allowed to stand for 48 hours at room temperature. On working up, only unchanged starting material was recovered.

Further Oxidation of the Product, m.p. 233° - The yellow oxidation product (320 mg.) in acetic acid (16 c.c.) was treated, at 70°, with a solution of chromic acid (130 mg.) in a little water and acetic acid (4 c.c.) added over a period of 15 minutes. After 1 hour, at 70°, the solution was poured into water, and worked up as before. The tiny acid fraction isolated in the usual fashion was ignored. The neutral product crystallised from aqueous acetone as pale yellow needles, m.p. 269-271°. (d.) softening about 265°. The melting point was undepressed on admixture with a specimen of the material previously obtained melting at this temperature (v.s.). The product gave no colouration with ferric chloride in alcohol.

Hydrolysis of Product, m.p. 264-266° - The oxidation product (0.5 g.) in ethanolic potassium hydroxide (1%, 50 c.c.) was refluxed for 2 hours, poured into dilute hydrochloric acid solution, and extracted with ether. The solid product crystallised from aqueous alcohol as very fine needles, m.p. uncertain, decomposing between 250 and 270°, without clearing or forming a pool. The ferric chloride test was positive. (Found: C, 78.1; H, 10.1% $C_{30}H_{48}O_3$ requires: C, 78.9; H, 10.5%).

Acetylation of this Hydrolysis Product - The hydrolysis product (0.5 g.) in pyridine (5 c.c.) and acetic anhydride

(5 c.c.) was heated at 100° for 15 minutes, and worked up as usual. The product crystallised as fine colourless needles from chloroform-methanol, m.p. 346-348°, $[\alpha]_D^{11}$ + 39.2°, (C = 1.43). (Found: C, 76.3; H, 9.69%).

Action of Periodic Acid on the Hydrolysis Product - The product (0.5 g.) in alcohol (300 c.c.) was treated with sodium periodate (1 g.) in sulphuric acid (10%, 7 c.c.) and left overnight at room temperature. The solution was diluted with water, and the volume reduced in vacuo before the product was extracted with ether. Washing of the extract with sodium carbonate solution gave a small acid fraction, but there was too little for investigation. The neutral portion crystallised from chloroform-methanol as fine needles, m.p. 301-303° (d.) giving no colouration with ferric chloride solution. (Found: C, 78.9; H, 10.1% $C_{30}H_{48}O_3$ requires: C, 78.9; H, 10.5%).

Action of Chromic Acid on α-Amyranonyl Acetate - i) The acetate (1.7 g.) in acetic acid (85 c.c.) at 70° was treated with a solution of chromic acid (0.74 g.) in water (1 c.c.) and acetic acid (17 c.c.) as described for the benzoate under number ii). The product crystallised as yellow plates from chloroform-methanol, m.p. 287-288° (d.). $[\alpha]_D^{13}$ + 5.6° (C = 2.67). The product gave a positive colour reaction with alcoholic ferric chloride, but no m.p. depression on admixture with starting material. (Found: C, 76.5; H, 10.2% $C_{32}H_{50}O_4$ requires: C, 77.1; H, 10.0%).

ii) (cf. Ruzicka, Jeger, Redel and Volli, Helv. (1944), 28,199) α-Amyranonyl acetate (2 g.) in boiling acetic acid (150 c.c.) was refluxed with a solution of chromic acid (0.80 g.) in water (2.5 c.c.) and acetic acid (50 c.c.) for 1 hour. The solution was then poured into water, and extracted with ether. The neutral product crystallised from chloroform-methanol as pale yellow plates, m.p. 282-284°, $[\alpha]_D^{14} + 95.9°$ (C = 1.42). Ferric chloride test positive. (Found: C, 77.7; H, 9.8% $C_{32}H_{50}O_4$ requires: C, 77.1; H, 10.0%).

Further Oxidation of the Product, m.p. 287° - The yellow acetate oxidation product from i) (400 mg.) in acetic acid (30 c.c.) was oxidised at 70° by chromic acid (0.2 g.) in a little water and acetic acid (10 c.c.) using exactly the same procedure as before. The neutral product crystallised from aqueous alcohol as pale yellow plates, m.p. 263-265°. (Found: C, 76.5; 76.1; H, 10.1, 10.3%).

Action of Periodic Acid on product m.p. 282, from ii) above - The "dionyl acetate" (0.5 g.) in alcohol (300 c.c.) was treated at room temperature with sodium periodate (1 g.) in sulphuric acid (10%, 7 c.c.) and allowed to stand for 48 hours. The solution was poured into water, and some of the alcohol removed by distillation in vacuo after which the product was dissolved in ether. Washing of the ether extract with carbonate solution gave no acid fraction. The neutral portion was dried over sodium sulphate. Removal of the solvent left a solid which on crystallisation gave what appeared to be starting material, m.p. 280-282°, ferric chloride test positive, $[\alpha]_D^{16} + 64.5^\circ$ (C = 1.21). From the mother liquors was obtained a product as very fine pale yellow needles from methanol or aqueous acetone, m.p. 216-220°, sintering 212-216°. Ferric chloride test negative. (Found: C, 75.8, 77.7; H, 10.6; 10.7%).

α-Amyradionyl Acetate Enol Acetate - i) α-Amyranonyl acetate enol acetate (1.9 g.) in acetic acid (40 c.c.) was treated with a solution of chromic acid (1.44 g.) in the minimum volume of water and acetic acid (40 c.c.) added over a period of 20 minutes at a temperature of 70° with occasional shaking. After a further 2 hours, the solution was poured into water, and extracted with ether as usual. The product crystallised from aqueous acetone as needles, m.p. 244-246°, $[\alpha]_D^{15} + 72.9^\circ$ (C = 2.55). Light absorption in alcohol at λ = 2540A, $E_{max.}$ = 14,900. (Found: C, 75.9; H, 9.77% $C_{34}H_{52}O_5$ requires: C, 75.6; H, 9.63%). (Yield 50%).

ii) α-Amyranonyl acetate enol acetate (1.3 g.) was oxidised exactly as described by Ruzicka et al. (Helv.(1944),23,199) and the product isolated chromatographically in low yield. It crystallised as needles, m.p. 244-246° (undepressed on admixture with a specimen of the above prepared product). $[\alpha]_D^{15} + 73.2^\circ$ (C = 1.42).

The material associated with it in this preparation is described as α-amyradionyl acetate by the above authors, and was assumed to be this. It crystallised from aqueous ethanol as plates, m.p. 280-282°.

Action of Periodic Acid on α-Amyradionyl Acetate - The acetate (120 mg.) in alcohol (75 c.c.) was treated with sodium periodate (240 mg.) in sulphuric acid (10%, 2 c.c.) overnight at room temperature. The solution was poured into water, and extracted with ether. There was no acid fraction. The neutral product crystallised from aqueous alcohol as fine, pale yellow needles, m.p. 206-212°, giving a positive ferric chloride test. (Found: C, 79,0; H, 9.78%).

Hydrolysis of α-Amyradionyl Acetate Enol Acetate - The ester (0.5 g.) was refluxed for 2 hours in ethanolic potassium hydroxide (2%, 25 c.c.), poured into dilute hydrochloric acid and extracted with ether. The product crystallised from aqueous acetone as fine needles, m.p. 226-228°, $[\alpha]_D^{16}$ + 184.1° (C = 1.89). (Found: C, 78.7; H, 10.7% $C_{30}H_{48}O_3$ requires: C, 78.9; H, 10.5%.

α-Amyradionyl Benaoate Enol Acetate - α-Amyranonyl benzoate enol acetate (1 g.) in acetic acid (50 c.c.) was treated, at 70 ° with a solution of chromic acid (0.68 g.) in a little water, and acetic acid (20 c.c.) added over a period of 20 minutes with occasional shaking. The temperature was maintained for 2 hours more, after which the reaction mixture was poured into water, and the product extracted with ether. Crystallisation of the solid thus obtained, from chloroform-methanol, gave small colourless needles, m.p. 262-264°.

$[\alpha]_D^{12}$ + 85.8°. The yield was 50% of theory. (Found: C, 77.5; H, 8.93% $C_{39}H_{54}O_5$ requires: C, 77.7; H, 9.27%). Light absorption in alcohol showed a maximum at λ = 2530A, $E_{max.}$ = 12,000.

<u>α-Amyradionol</u> - α-Amyradionyl benzoate enol acetate (1.4 g.) was refluxed in alcoholic potassium hydroxide (2%, 70 c.c.) for 2 hours, poured into water, and the product extracted with ether. The alcohol crystallised from aqueous acetone (norite) as very fine colourless needles, m.p. 233-235°, $[\alpha]_D^{14}$ + 145.6° (C = 3.11), $[\alpha]_D^{16}$ + 144.2° (C = 1.56), giving a strong colour reaction with ferric chloride in alcohol. (Found: C, 78.4; H, 10.1% $C_{30}H_{48}O_3$ requires: C, 78.9; H, 10.5%). Light absorption in alcohol showed a maximum at λ = 2900A, $E_{max.}$ = 9,000.

<u>Attempted Further Hydrolysis by Hydrochloric Acid of the α-Amyradionol obtained as above</u> - The above dionol (0.5 g.) was refluxed in ethanol (50 c.c.) containing hydrochloric acid (conc. 10 c.c.) for 2 hours. More acid (5 c.c.) was then added, and after another 1 hour's refluxing, the solution was evaporated to dryness, and the residue crystallised from aqueous alcohol, giving starting material, m.p. 232-233°, $[\alpha]_D^{18}$ + 142.5° (C = 1.2). (Found: 78.5; H, 10.7% $C_{30}H_{48}O_3$ requires: C, 78.9; H, 10.5%).

<u>Action of Hydrazine Hydrate on α-Amyradionol</u> - i) The diketone (100 mg.) in alcohol (10 c.c.) was treated with

hydrazine hydrate (90%, 1 c.c.) and left overnight at room temperature.

ii) The diketone (100 mg.) in alcohol (10 c.c.) plus hydrazine hydrate (90% 2 c.c.) was refluxed for 2 hours.

In both cases, when the solutions were poured into water and extracted with ether, unchanged starting material was recovered.

Action of Acetic Anhydride on α-Amyradionol - i) The alcohol (300 mg.) in pyridine (2 c.c.) was treated with acetic anhydride (2 c.c.), and left overnight at room temperature.

ii) The alcohol (400 mg.) in pyridine (5 c.c.) and acetic anhydride (5 c.c.) was heated on a steam bath for 15 minutes.

In both cases, the reaction mixture was poured into water, and the product extracted with ether. The material obtained crystallised from aqueous acetone or alcohol as needles, m.p. 224-226°, giving a colour reaction with ferric chloride. (Found: C, 79.8; H, 9.7%).

Action of Periodic Acid on α-Amyradionol - i) The dione (1 g.) in methanol (400 c.c.) was treated with sodium periodate (2 g.) in sulphuric acid (10%, 15 c.c.) and left overnight at room temperature. The solution was largely diluted with water, and the alcohol removed by vacuum distillation, after which the product was dissolved in ether. The ether solution was washed with caustic soda (2%), and a small acid fraction obtained, which could not be crystallised. The neutral portion of the product was crystallised from aqueous acetone as fine, pale yellow needles, m.p. 226-230° (d.) sintering above 224°. $[\alpha]_D^{15}$ - 91.1 (C = 2.24). This gave no colouration with alcoholic ferric chloride. (Found: C, 79.6 79.6, H, 9.4; 9.4%).

The material (700 mg.) was absorbed on Grade III Brockmann Alumina, from 200 c.c. of benzene-petrol (50:50) and fractionally eluted.

	Solvent.	Vol. (c.c.)	Wt. Eluted (mg.)
1)	Benzene-Petrol (50:50)	300	-
2)	" " (72:25)	300	-
3)	Benzene alone	200	50
4)	" "	200	80
5)	" "	400	100
6)	" "	400	140
7)	" "	600	50
8)	Benzene-Acetone (95:5)	400	100
9)	" "	400	200
10)	Acetone	100	60
		Total	780 mg.

Fractions 1) to 6) and 7) to 10) were combined, the first giving what seemed to be unchanged starting material, m.p. 220-240°, ferric chloride test negative. The second fraction, however, again melted over a range, 160-180°, but gave a blue colouration with ferric chloride in alcohol solution. No homogeneous material was isolated.

ii) The reaction was repeated as described above, the temperature, however, was maintained at 40-60° for the first

4 hours. Considerable darkening of the solution occurred, but the product was as before.

Action of Acetic Anhydride on the Periodate Product - The neutral yellow product obtained by the action of periodic acid on α-amyradionol (250 mg.) in pyridine (5 c.c.) and acetic anhydride (5 c.c.) was heated for 15 minutes at 100°.

The solution, on pouring into water, and extracting with ether, yielded a solid crystallising as fine pale yellow needles from aqueous alcohol, m.p. indistinct, ca. 216-220°. (Found: C, 76.7; H, 9.4%).

Action of Chromic Acid on α-Amyradionol - α- Amyradionol (0.5 g.) in acetic acid (20 c.c.) was treated at 70° with a solution of chromic acid (0.44 g.) dissolved in the minimum volume of water, plus acetic acid (20 c.c.) added over a period of 20 minutes. Conditions were maintained for 2 hours, and the solution poured into water, and extracted with ether. Washing of the extract with dilute caustic soda removed an acid fraction, which could not be crystallised. The neutral fraction of the product also failed to yield any crystalline material.

Action of Perbenzoic Acid on α-Amyranonyl Acetate Enol Acetate - The enol acetate (1 g.) was dissolved in a solution of perbenzoic acid (0.75 g.) in chloroform (10 c.c.) and left for 7 days at room temperature. After being washed with sodium carbonate solution, and dried over sodium sulphate, the solvent was removed, and the residue crystallised from alcohol, giving unchanged starting material, m.p. 255-257°, $[\alpha]_D^{18}$ + 46.9° (C = 1.23).

From the mother liquors was obtained a very small amount of product, crystallising as needles, m,p. 242-244° from alcohol. (Found: C, 77.2; H, 11.0% $C_{34}H_{54}O_5$ requires: C, 75.3; H, 9.96%).

Action of Perbenzoic Acid on α-Amyradionyl Benzoate Enol Acetate - The enol acetate (1 g.) was dissolved in a solution of perbenzoic acid (0.63 g.) in chloroform (10 c.c.) and left for 12 days at 0°. The solution was worked up as in the above experiment, only unchanged starting material, m.p. 262-264°, $[\alpha]_D^{20}$ + 84.8° (C = 2.51) was recovered, however.

Action of Hydriodic Acid and Red Phosphorus on α-Amyranonyl Benzoate - i) Iodine (0.07 g.) and red phosphorus (0.21 g.) were added to acetic acid (75 c.c.) and allowed to stand for 20 minutes. The ketone (1 g.) and water (0.5 c.c.) were then added, and the mixture refluxed for 3 hours. The solution was filtered through asbestos, and poured into sodium bisulphite solution The precipitated solid was extracted with benzene, and the extract washed and dried (sodium sulphate). Removal of the solvent left a resin which was crystallised from benzene-methanol as needles, m.p. 218-220°. (Found: C, 82.02; H, 10.15%).

ii) The reaction was repeated on twice the above scale. The product this time crystallised as blades from alcohol, m.p. 214-216°, and giving a depression on admixture with the previous product, m.p. 218-220°. (Found: C, 83.1; H, 10.52% $C_{37}H_{56}O_2$ requires: C, 83.5; H, 10.5%).

The product showed no low intensity absorption in the ultra-violet range.

Action of Lithium Aluminium Hydride on α-Amyranonyl Benzoate - The ketone (2 g.) in dry ether (120 c.c.) was added to a suspension of the reagent (0.5 g.) in dry ether (50 c.c.), then refluxed for 3 hours. After cooling, water was cautiously added to destroy excess hydride, then 2N sulphuric acid to dissolve metallic hydroxides. The ether layer was separated, washed with water, and dried over sodium sulphate. Evaporation of the solvent left a solid which crystallised from chloroform-methanol as needles, m.p. 250-252°, $[\alpha]_D^{16}$ +12.3° (C = 2.35). Yield, 300 mg. (Found: C, 81.1; H, 11.9% $C_{30}H_{52}O_2$ requires: C, 81.0; H, 11.7%.

The mother liquors, on evaporation, yielded a resin, which on treatment with light petroleum (60-80°) gave crystals, which were recrystallised from chloroform-light petroleum (60-80°) as small prisms, m.p. 170-172°, $[\alpha]_D^{16}$ + 12.9° (C = 4.03). Yield, 500 mg. (Found: C, 81.0; H, 11.8% $C_{30}H_{52}O_2$ requires: C, 81.0; H, 11.7%.

Acetylation of the Product, m.p. 250-252° was carried out by dissolving the material (200 mg.) in pyridine (5 c.c.) by warming, adding acetic anhydride (3 c.c.) and letting stand overnight. The solution, which deposited crystals, was poured into water, and the product extracted with ether. The acetate crystallised from chloroform-methanol, or aqueous ethanol, as plates, m.p. 279-281°. (Found: C, 78.9; H, 10.9% $C_{32}H_{52}O_3$ requires: C, 79.3; H, 10.7%).

Acetylation of the Product, m.p. 170-172° - i) The material (200 mg.) in pyridine (3 c.c.) and acetic anhydride (3 c.c.) was allowed to stand overnight, and worked up as above. The acetate crystallised from aqueous alcohol as blades, m.p. 189-191°, $[\alpha]_D^{20} \pm 0°$ (C = 2.12) (Found: C, 78.9; H, 11.2% $C_{32}H_{54}O_3$ requires: C, 79.0; H, 11.1%).
ii) The acetylation was repeated, heating the reaction mixture for 1 hour at 100°. The product melted, as before, at 189-191°. (Found: C, 77.0; H, 10.4% $C_{34}H_{56}O_4$ requires: C, 77.3; H, 10.6%).

Action of Chromic Acid on the Mono-Acetate m.p. 189-191° - The acetate (150 mg.) in benzene (3 c.c.) and acetic acid (10 c.c.) was treated with a solution of chromic acid (0.1 g.) in a little water and acetic acid (5 c.c.), left overnight at room temperature, and worked up in the usual manner. No crystalline material was obtained.

Sodium and Amyl Alcohol Reduction of α-Amyranonyl Benzoate - The ketone (2 g.) in amyl alcohol (24 c.c.) under reflux, was treated with sodium (2 g.), and after 30 minutes more sodium (2 g.) added. Refluxing was continued for another 2 hours. Unreacted sodium was destroyed by addition of a little ethanol. Steam distillation decomposed the amyloxide, and removed the amyl alcohol, after which the product was extracted with ether, and the extract washed, and dried over sodium sulphate. Removal of the ether left a resin which was crystallised first from light petroleum (60 - 80°), then from aqueous acetone to give fine needles, m.p. 198-200°.

In four successive preparations, the optical rotations were $[\alpha]_D^{15}$ +32.7° (C = 1.71), $[\alpha]_D^{15}$ + 41.9° (C = 1.29), $[\alpha]_D^{17}$ + 44.4° (C = 2.41) and $[\alpha]_D^{19}$ + 30° (C = 1.50). (Found: C, 81.5; .5; H, 11.8% $C_{30}H_{52}O_2$ requires: C, 81.1; H, 11.7%).

<u>Acetylation of the Product, m.p. 198-200°</u> - The compound (200 mg.) in pyridine (3 c.c.) and acetic anhydride (3 c.c.) was heated on a steam bath for 15 minutes, poured into water, and the product extracted with ether. It crystallised from aqueous acetone as needles, m.p. 242-244°. (Found: C, 77.2; H, 10.5% $C_{34}H_{56}O_4$ requires: C, 77.3; H, 10.6%).

<u>Diacetoxy-α-Amyrane</u> - α-Amyranediol (v.s. 500 mg.) in pyridine (5 c.c.) and acetic anhydride (5 c.c.) was heated for 1 hour at 100°, and worked up in the usual manner. The product crystallised from aqueous alcohol as needles, m.p. 242-244°, undepressed on admixture with a specimen of the above acetate. $[\alpha]_D^{19}$ + 50° (C = 1.72). (Found: C, 77.7; H, 10.6%. $C_{34}H_{56}O_4$ requires: C, 77.3; H, 10.6%).

<u>Sodium and Amyl Alcohol Reduction of α-Amyranonyl Acetate</u> -

α-Amyranonyl aceate (1 g.) in amyl alcohol (12 c.c.) was treated, under reflux, with sodium (1 g.). After 30 minutes, more sodium (1 g.) was added, and refluxing continued for 2 hours. Unreacted sodium was destroyed by addition of a little ethanol, and the amyl alcohol removed by steam distillation, after which the product was extracted with ether. The product crystallised from aqueous acetone as needles, m.p. 198-200°, $[\alpha]_D^{18}$ +32.8° (C = 1.25). (Found: C, 79.9; H, 11.3% $C_{30}H_{52}O_2$ requires: C, 81.8; H, 11.9%).

Action of Caustic Soda on α-Amyrenone - α-Amyrenone (14 g.) in caustic soda (40%, 100 c.c.) was heated in an autoclave. After 1½ hours, when the temperature had reached 120°, stirring was commenced. Heating was continued for another 4 hours, when the temperature rose to 250°, and the pressure to 37 atmospheres. After 8 more hours, during which the temperature rose to 270°, and the pressure to 50 atmospheres, heating and stirring were discontinued, and the autoclave cooled overnight. The contents were extracted with ether, and the alkaline layer acidified to see if there was any acid fraction, but there was none. The ether, on evaporation to dryness, yielded unchanged starting material.

Action of Caustic Soda on β-Amyrin Benzoate - β-Amyrin benzoate (20 g.) in caustic soda (40%, 100 c.c.) was heated in an autoclave. After 4½ hours, the temperature had reached 250°, and the pressure 25 atmospheres, so stirring was commenced. Heating was continued for 8 more hours, during which the temperature rose to 290°, and the pressure to 42 atmospheres. On cooling overnight, the pressure dropped to atmospheric, and the contents of the autoclave were diluted with water, and extracted with ether. The caustic soda, on acidification, yielded benzoic acid, crystallising from water as needles, m.p. 121°. The neutral product was crystallised from aqueous alcohol, and gave β-amyrin (15 g.) as needles, m.p. 184-186°, $[\alpha]_D^{14}$ + 88.9° (c = 3.09).

Action of Aluminium Trichloride on β-Amyrin Benzoate

i) Aluminium trichloride (5 g.) was stirred in dry benzene (50 c.c.) and a solution of β-amyrin benzoate (2 g.) in benzene (10 c.c.) added. The mixture was stirred on a steam bath for 1 hour, during which darkening occurred, and hydrochloric acid was evolved. The mixture was cooled, ice added, and the benzene layer separated, washed with dilute mineral acid to remove aluminium hydroxide, water, then sodium carbonate, which extracted an acid fraction, crystallising from water as benzoic acid, m.p. 121°. The neutral product was a red oil.

ii) When the experiment was repeated on five times the above scale, the yield of benzoic acid was 0.7 g. The oily fraction was distilled in vacuo to give the following fractions:-

1. Mobile yellow oil, B.P. 60-120°/1 mm. c. 2g.
2. More viscous oil, B.P. 120-150°/1 mm. c. 1g.
3. Viscous yellow oil, B.P. 140-160°/6 x 10^{-3} mm c. 1g.
4. Small dark residue.

Attempts were made to hydrogenate fractions 1. and 3., thus:-

a) The oil first collected (1.8 g.) was dissolved in ethyl acetate (100 c.c.) and shaken with hydrogen in the presence of a platinum catalyst. There was no absorption after initial reduction of the platinum oxide.

b) Fraction 3. (0.78 g.) was treated in the same way. Again there was no absorption.

In both cases, on filtering the solution and removing the solvent, the oil was recovered.

iii) 50 g. of β-amyrin benzoate were treated as before. The neutral product was stwam distilled, giving 6.2 g. of a yellow oil (s.g. $<$ 1). This fraction was dissolved in ether, dried over sodium sulphate, and the solvent removed. The oil was distilled, and the following fractions collected:-

1. Pale yellow oil, B.P. 48-54°/0.4 mm. c. 3 c.c.
2. Darker " " B.P. 52-60°/0.4 mm. c. 1 c.c.
3. " " " B.P. 60-70°/0.4 mm. c. 2 c.c.
4. " " " B.P. 70-100°/0.5 mm. c. 0.5 c.c.
5. Small residue, B.P.$>$100°/0.5 mm.

The first fraction redistilled between 50-60°/0.5 mm. (c. 150° at A.P.), $[\alpha]_D^{15}$ ±0 (C = 1.55). (Found: C, 87.5; H, 12.4% M = 468).

The non steam volatile portion (39 g.) was freeze dried before being subjected to distillation. A fraction (8 - 10 g.) was collected over the range 70 - 180°/0.1 mm. Most of this came over between 160° and 180°/0.1 mm., but there was no constant boiling fraction. A tar-like residue was left in the distillation flask.

β-Amyranonyl Acetate - β-Amyrin acetate (10 g.) in acetic acid (500 c.c.) was heated on a steam bath with stirring. Hydrogen peroxide (30%, 30 c.c.) was added, and the sttrring continued for 2 hours. More hydrogen peroxide (10 c.c.) was then added, and after a further hour, boiling water was added dropwise, until the solution was milky. The product separated on cooling, was collected, and crystallised from chloroform-methanol as plates, m.p. 292-294°. The yield was 6 g.

iso-β-Amyrenonyl Acetate was prepared according to the method of Picard, Sharples and Spring, (J. (1939), 1047). It crystallised from chloroform-methanol, in 50% yield, as plates, m.p. 286-288°.

iso-β-Amyradienonyl Acetate - was made from the above by the action of selenium dioxide in acetic acid. (Green, Mower, Picard and Spring, J. (1944), 527). It crystallised from aqueous alcohol as blades, m.p. 205-207°, $[\alpha]_D^{16}$ -31.9° (C = 3.23), in 50% yield.

Attempted Isomerisation of iso-*β-Amyradienonyl Acetate* - (cf. Ruzicka, Ruegg and Jeger, (*Helv.* (1949), *30*, 1294) - The acetate (200 mg.) in acetic acid (50 c.c.) was saturated with dry hydrochloric acid, and allowed to remain at room temperature overnight. On evaporation to dryness in vacuo, unchanged starting material was recovered.

iso-*β-Amyradienonol* - The method of Green, Maver, Picard and Spring (*ibid.*), gave the alcohol as needles from aqueous ethanol, m.p. 240-241°, $[\alpha]_D^{19}$ - 51.4° (C = 2.22),

Re-acetylation of iso-*β-Amyradienonol* - The alcohol (250 mg.) in pyridine (5 c.c.) and acetic anhydride (5 c.c.) was heated at 100° for 15 minutes, and worked up as usual. *iso*- -Amyradienonyl acetate crystallised from aqueous alcohol as plates, m.p. 205-207°, giving a yellow colour with tetranitromethane in chloroform solution.

Attempted Hydrogenation of iso-*β-Amyradienonyl Acetate* - The acetate (0.5 g. specially purified from selenium by solution in benzene and filtration through alumina), in acetic acid (50 c.c.) was shaken with platinum oxide (100 mg.) in an atmosphere of hydrogen for 3 days. The solution was filtered and evaporated in vacuo, giving unchanged starting material. This material (470 mg.) was treated again as before, but no product was obtained.

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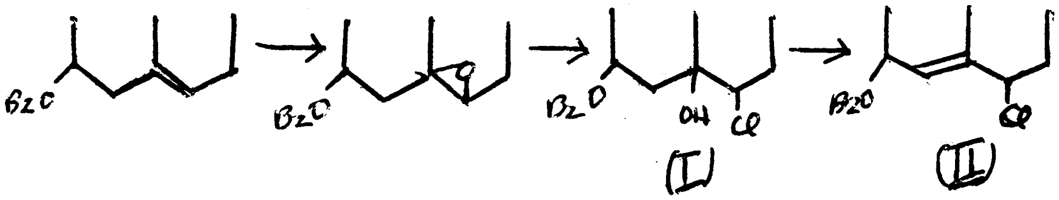
Part Two

An Investigation of 6-Chloro-3-Benzoyloxy-Cholest-4-ene.

HISTORICAL.

HISTORICAL.

As a preliminary study of the dehydration of the oxides of cholesteryl esters, Spring and Swain (1) prepared α- and β-cholesteryl benzoate oxides. By the action of hydrochloric acid on the former, or of benzoyl chloride on the α-oxide of cholesterol itself, a compound $C_{34}H_{51}O_3Cl$ m.p. 202-203°, was obtained. This was subsequently characterised as 6-chloro-5-hydroxy-3-benzoyloxy cholestane (I). Treatment of (I) with thionyl chloride in pyridine at 0° resulted in dehydration, and a crystalline product, m.p. 127-128°, considered to be 6-chloro-3-benzoyloxy-cholest-4-ene (II) was isolated in good yield. This chain of reactions may be represented by the following partial formulation (BzO = C_6H_5OCO):-



In order further to characterise (II), it was reduced with aluminium amalgam in moist ether, when it was expected to give allo-cholesteryl benzoate (3-benzoyloxy-cholest-4-ene), but a cholestadiene, $C_{27}H_{44}$, m.p. 80-81° was obtained, and shown to possess an intense absorption maximum at 2350A, indicating it to be cholesta-3:5-diene. (2).

Treatment with methanol and potassium hydroxide or with sodium ethoxide gave unworkable oils, while heating with pyridine gave halogen free resinous products which could not be crystallised. Halogen was successfully removed by heating with potassium acetate in alcohol, giving:

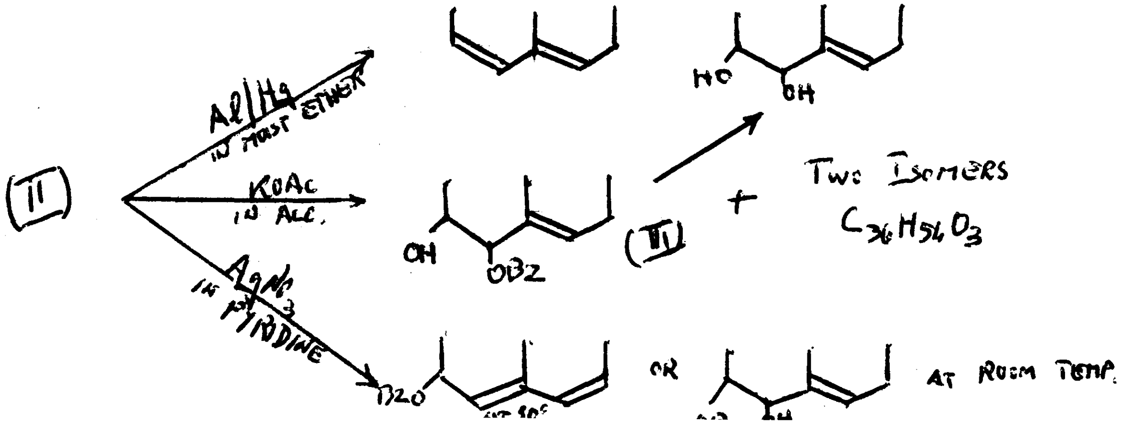
(A) a solid m.p. 166-167°, $[\alpha]_D - 47.2^\circ$
(B) " " m.p. 131-132°, $[\alpha]_D - 29.4^\circ$
(C) " " m.p. 153-154°, $[\alpha]_D - 27.8^\circ$

(C) was shown to be $C_{34}H_{50}O_3$ formed by replacement of chlorine by hydroxyl, and should therefore be 6-hydroxy-3-benzoyloxy-cholest-4-ene or 4-hydroxy-3-benzoyloxy-cholest-5-ene, the latter requiring an allylic rearrangement for its formation. It differed from the ester prepared by Rosenheim and Starling (3), but on hydrolysis gave 3:4-dihydroxy-cholest-5-ene, and not the expected 3:6-dihydroxy-cholest-4-ene.

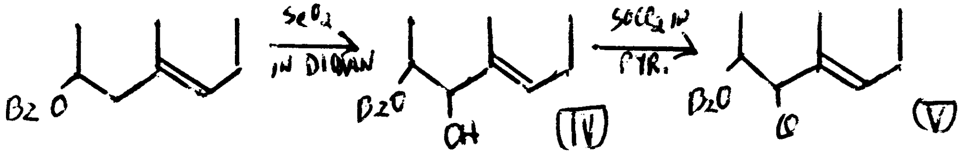
Benzoylation of (C) gave cis - 3:4-dibenzoyloxy-cholest-5-ene and acetylation gave an ester, m.p. 130-131°, differing from the cis-3-benzoyloxy-4-acetoxy-cholest-5-ene described by Rosenheim and Starling. (C) was then the corresponding 4-ester, a migration having occurred during formation. The 3:4-diol of cholest-5-ene was partly acetylated, and then benzoylated, giving an identical product, showing (C) to be 4-benzoyloxy-3-hydroxy-cholest-5-ene (III).

(A) and (B) were isomeric, having as formula $C_{36}H_{54}O_3$, and containing one ethoxyl group. They were probably ester-ethers of either cis-3:4-dihydroxy-cholest-5-ene, or 3:6-dihydroxy-cholest-4-ene, and in view of the possibility of migration of the benzoyl group, there were four possibilities for (A) and (B). Hydrolysis of (A) gave a diol monoethyl ether m.p. 123-124°, and of (B) gave a similar compound m.p. 122-123°, both of which gave mono-acetates. The similarity of (B), regarding physical properties, to 3-benzoyloxy-6-acetoxy-cholest-4-ene (3,4) was accentuated by the fact that the two gave no melting point depression on admixture. This suggested that (B) was 3-benzoyloxy-6-ethoxy-cholest-4-ene formed by direct replacement of halogen by an ethoxyl grouping, but the failure of its hydrolysis product to form a digitonide was not in accordance with this.

In further attempts to convert (II) into either cholesta 5:7-dienyl or 4:6-dienyl benzoate, its behaviour with silver nitrate in pyridine was examined, (5). At room temperature, a pyridinium salt and 3-benzoyloxy-4-hydroxy-cholest-5-ene were formed, and at 90° the same salt and a compound m.p. 128-129°, whose colour reactions and origin indicated it to be 3-benzoyloxy-cholest-4:6-diene. This was subsequently proved by the ultra-violet absorption spectrum which showed a single maximum at 2390A.

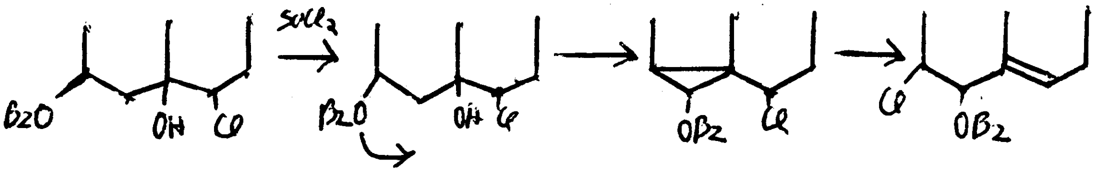


An alternative suggestion fpr the structure of (II) was offered by Petrow, Rosenheim and Starling (6), who obtained an identical product by treatment of 3-benzoyloxy-4-hydroxy-cholest-5-ene (IV) with thionyl chloride in pyridine, this time a chlorinating reaction, and formulated it as 4-chloro-3-benzoyloxy-cholest-5-ene (V).

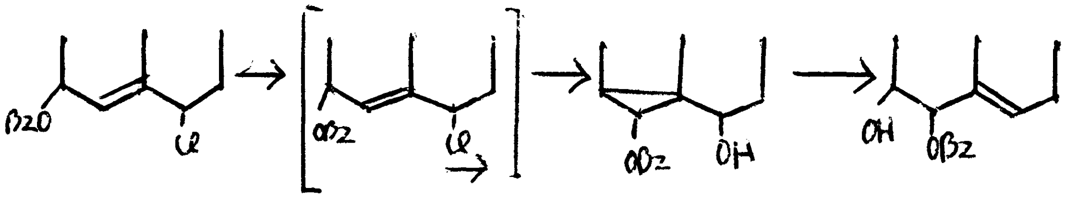


The structure of (IV) had previously been established by oxidation of the hydroxyl to a carbonyl group, whereby an α,β -unsaturated ketone was obtained, and by comparison of (V) with the products of Darzen dehydration of the 3-monoesters of 6-chloro-3:5-dihydroxy-cholestane. It is claimed that this reaction is accompanied by allylic rearrangement. (4). This series of observations explains the formation of (III) by the action of potassium acetate by allowing for the occurrence of simple rearrangement during hydrolysis.

Berg and Wallis (7.) on purely theoretical grounds, suggest a further alternative whereby (II) is, in fact, 3-chloro-4-benzoyloxy-cholest-5-ene (VI), having been formed by the following mechanism:-

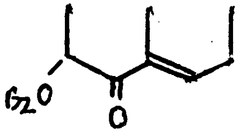


The formation of (III) on hydrolysis would then be expected, and the above explanation becomes unnecessary, together with Rosenheim's original suggestion, (8), viz:-

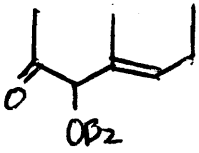


Considering the method of formation of (II) and (V.) respectively, it would seem that an allylic rearrangement would be just as, or even more, likely to occur in the latter case, as not only is a hydroxyl group being replaced by halogen, but the reaction is carried out at a much higher temperature. Thus, if allylic rearrangement did occur (II) would result, or if simple acyl migration only, (VI) would follow.

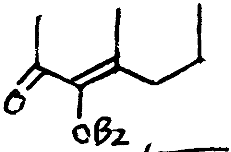
On the other hand, Berg and Wallis could be correct if the 3-benzoyloxy-4-hydroxy-cholest-5-ene of Petrow, Rosenheim and Starling was in fact the corresponding 4-ester of the 3:4 diol, and vice versa. The only tangible proof of the structure of (IV.) was the production of the unsaturated (α, β) ketone claimed to be (VII) (v.s.).



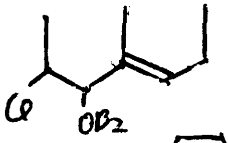
(VII)



(VIII)



(VIIIa)



(IX)

The 4-ester should on similar treatment give (VIII) though it is feasible that conjugation might occur to give (VIIIa).

THEORETICAL.

THEORETICAL.

With the observations made in the previous section in mind, it was decided to commence by verifying the absorption spectrum of (VII) and to chlorinate the 4-ester of the 3:4-diol to (IX) and oxidise it to (VIII). For convenience, it was decided to prepare, not the benzoyloxy compounds which have been described in the Historical section, but the acetyl derivatives which are also known.

3-Acetoxy-4-hydroxy-cholest-5-ene was prepared by partial acetylation of the 3:4-diol, and oxidised to the ketone by the published method. This was shown to possess the ultra-violet absorption spectrum typical of an α,β -unsaturated ketone.

Work was carried out on the corresponding 4-ester by Mr. S. U. Ruff, but without success, no successful method being found for either replacement of the hydroxyl by chlorine, or oxidation to the ketone (9).

The main problem, viz., the structure of the so-called 6-chloro-3-benzoyloxy-cholest-4-ene, offered two lines of attac

i) synthesis of the principal isomers of 6-chloro-3-benzoyloxy (or acetoxy)-cholest-4-ene by methods which would leave no doubt as to the structure of the products,

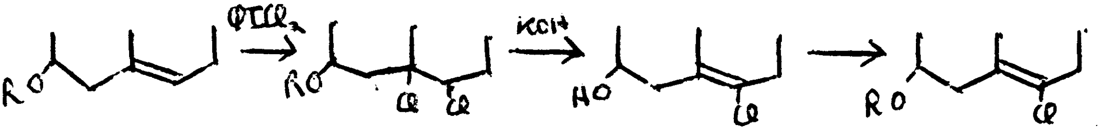
ii) degradative work on Spring and Swain's compound itself.

These will be considered separately.

Synthesis of two Mono-Acetoxy-Mono-Chloro-Cholestenes.

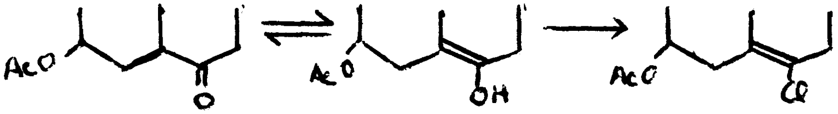
i) 6-Chloro-3-Acetoxy-Cholest-5-ene.

Berg and Wallis (7) claim to have prepared the 3-esters of 6-chloro-3-hydroxy-cholest-5-ene by refluxing cholesteryl benzoate dichloride with alcoholic potash and esterifying the product.



(X)

It was though of interest to prepare (X, R = Ac) by a new route, the chlorination of the enolic form of 6-keto-cholestanyl acetate.



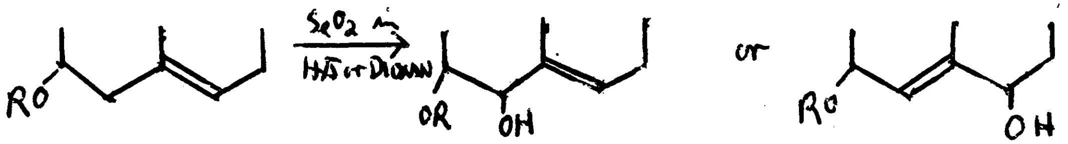
6-Keto-cholestanyl acetate was readily prepared according to the method of Heilbron, Jackson, Jones and Spring (10). At first it was assumed that this compound might exist in its enolic form in a suitable polar solvent, and the action of thionyl chloride, in the cold, in ether and in pyridine, was investigated. It soon became clear that more vigorous conditions would be required, and as the same reagent, in large excess under reflux, has been successfully used for replacement of hydroxyl by chlorine in a number of cases with or without small amounts of pyridine present (11), it was decided to apply this method. In the presence of pyridine, no noticeable darkening occurred for some hours, and only a tar could be obtained from the reaction mixture. Without pyridine, however, darkening of the solution was more rapid, and sulphur dioxide and hydrochloric acid were evolved. A resinous solid was obtained on working up, and chromatographic analysis of this indicated that up to 5% of the material might have been chlorinated, though 30% of the ketone was returned unchanged.

Phosphorus oxychloride was even less successful, and unchanged starting material could be recovered almost quantitavely after refluxing with the reagent for 24 hours at 120°.

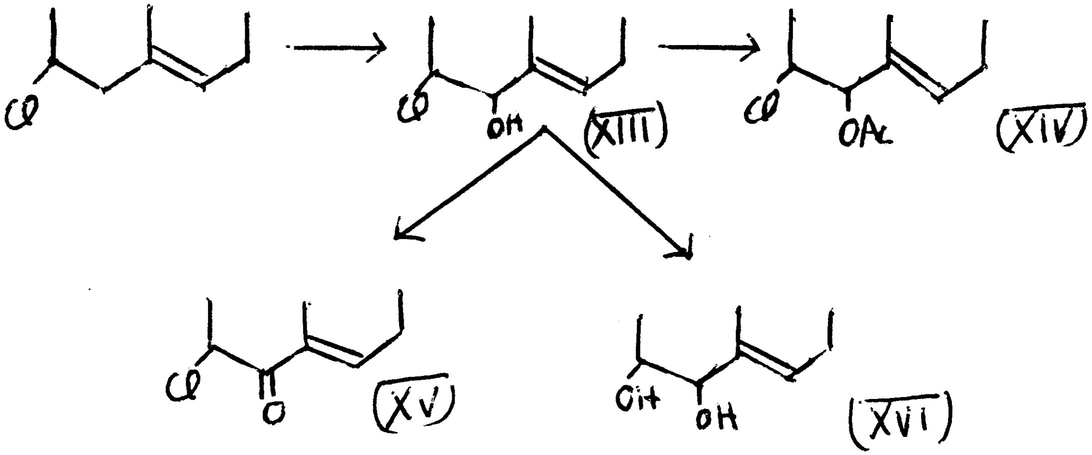
Chlorination was finally achieved by the action of phosphorus pentachloride in molar proportions, giving, in small yield, a crystalline product, the analysis of which corresponds to 6-chloro-3-acetoxy-cholest-5-ene. The melting point, 128-130° is in agreement with that reported by Berg and Wallis, though the optical rotation appears to be somewhat greater, $[\alpha]_D$ -53.8° as compared with the $[\alpha]_D$ -41.2° previously reported.

ii) <u>3-Chloro-4-Acetoxy-Cholest-5-ene</u>.

Selenium dioxide oxidation of cholesterol and its esters is known to give a hydroxyl group in the 4th, or under different conditions, in the 6th, position (3, 12).



and so it seemed probable that a similar oxidation of cholesteryl chloride under the correct conditions would give 3-chloro-4-hydroxy-cholest-5-ene (XIII) which could then be acetylated to 3-chloro-4-acetoxy-cholest-5-ene (XIV).



The position of the double bond and hydroxyl in (XIII) would then be established by oxidation to the α,β-unsaturated ketone (XV), and by hydrolysis to the known 3:4-diol (XVI).

Cholesteryl chloride was treated with selenium dioxide in acetic acid solution, using a method similar to that employed in the preparation of the 3:4-diol. (3). Approximately half of the starting material was in every case returned unchanged by crystallisation of the crude product from alcohol. Evaporation of the mother liquors followed by crystallisation from acetone gave mixed crystals of the product with cholesteryl chloride. Separation was finally achieved by chromatography, and pure hydroxy compound obtained in yields of up to 10%, together with traces of high melting material which was not investigated.

The product crystallises from alcohol as colourless needles, m.p. 125-127°, and gives the blue colouration with antimony trichloride in chloroform typical of an ethylene linkage in the α,β- position with respect to the hydroxyl group. This fact, together with the high negative rotation, $[\alpha]_D$ - 53.2°, indicates a double bond at the fifth carbon atom, and so the compound has been formulated as 3-chloro-4-hydroxy-cholest-5-ene (XIII).

More concrete proof of its structure, however, has not been forthcoming, as an attempt to replace halogen by hydroxyl was a failure, while attempted oxidation of the hydroxyl to a keto-group, by way of the dibromide, gave high melting crystals, analysis of which indicated that bromine was probably still retained within the molecule, though the required absorption spectrum was obtained. Lack of success in the latter case was probably due to the hydroxyl group having been attacked initially, or to incomplete debromination. The small stock of material available did not permit of a repetition.

Acetylation was carried out rather more readily, the product forming very fine colourless needles from alcohol, m.p. 96-98°, $[\alpha]_D$ - 71.4°. This has been formulated as 3-chloro-4-acetoxy-cholest-5-ene (XIV) to which the analysis corresponds.

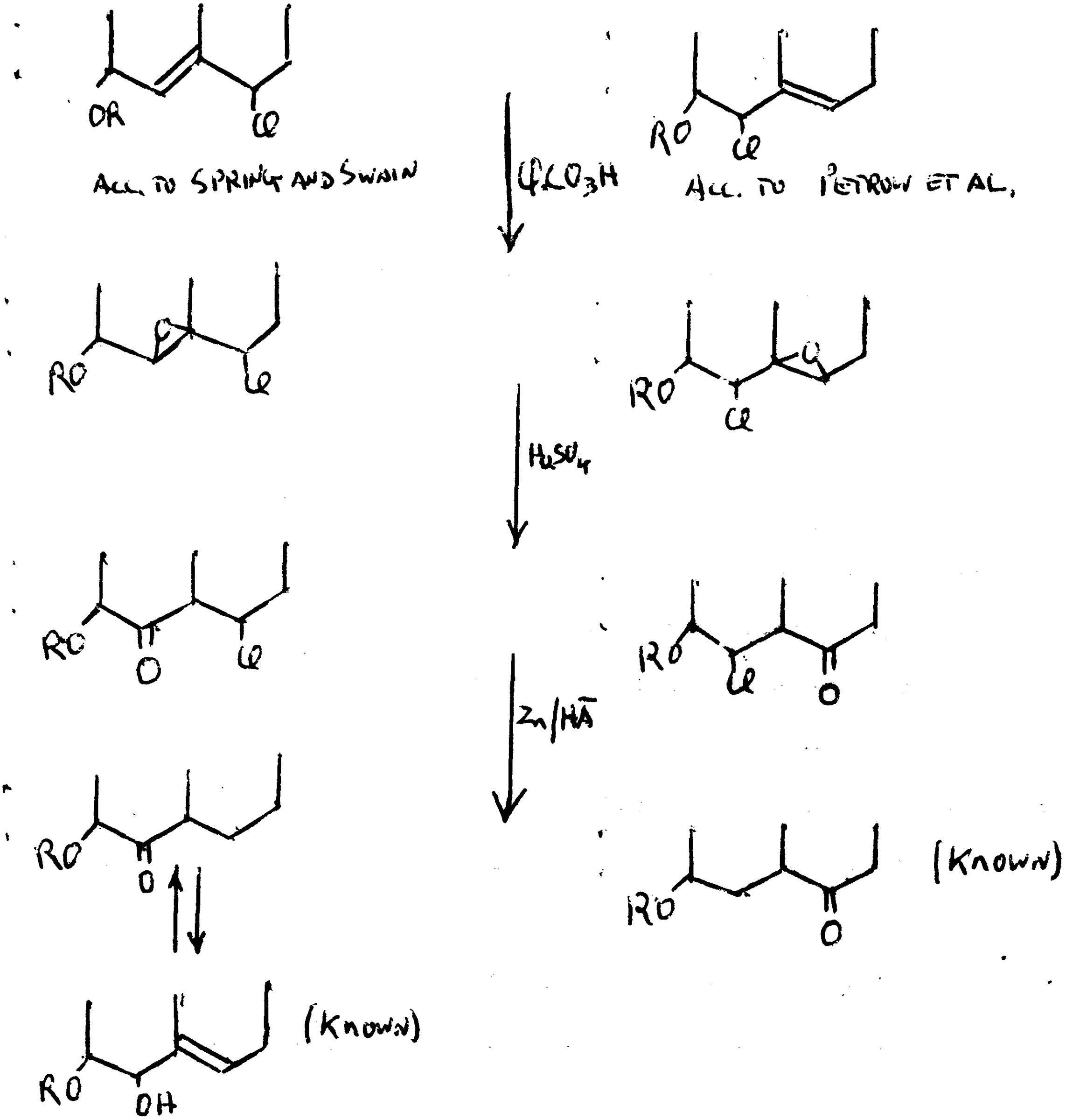
3-Chloro-4-acetoxy-cholest-5-ene gave a melting point depression on admixture with a specimen of 6-chloro-3-acetoxy-cholest-4-ene prepared by Spring and Swain, thus eliminating Berg and Wallis's suggestion that (II) may be represented by the formulation (VI).

Degradative Evidence.

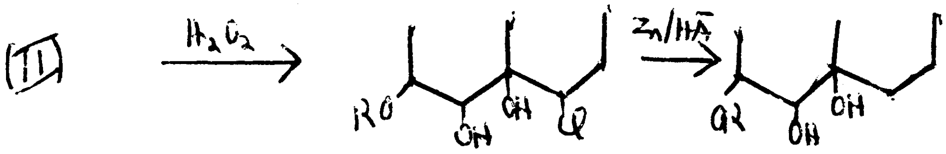
It seemed that the only satisfactory method of deciding the position of the double bond in relation to the chlorine in the molecule would be by degradative work on 6-chloro-3-benzoyloxy-cholest-4-ene itself, and so a stock of this was prepared, using the method adopted by Spring and Swain (v.s.).

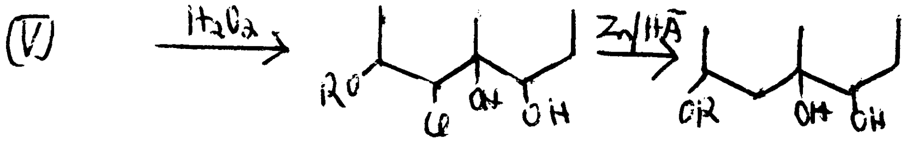
Hydrolysis of the compound by alcoholic potassium carbonate was found to give 3:4-dihydroxy-cholest-5-ene, and this forms fresh evidence in favour of Petrow, Rosenheim and Starling's alternative structure (V), but while the possibility of migration or rearrangement remained, nothing could be established. It was therefore decided to immobilise the double bond by formation of an oxide, and attempt the following chain of reactions, whereby a known product might be obtained.

If the structure of the chloro-ester is (II), then oxide formation followed by isomerisation to the ketone and dehalogenation should produce (XVII), whereas if the structure is (V), the same series of reactions should give the 6-keto-3-ester which is known.



Alternatively, the starting material might give a glycol on treatment with hydrogen peroxide. The glycol could then be reduced in the same way, thus:-





(XVIII)

the compound (XVIII) being identifiable.

The action of monoperphthalic acid on the compound (II) was without effect, but perbenzoic acid formed slowly, and in low yield (34%), a crystalline oxide, m.p. 121-122°. Small amount of a substance m.p. 160° were in one case isolated from the mother liquors, but as halogen was absent it was felt that this was a product of alcoholysis caused by concentration of the selvent by boiling, and it was therefore not investigated.

Treatment of the oxide by acid gave a crystalline solid, m.p. 231°, containing no halogen, but which could not be identified.

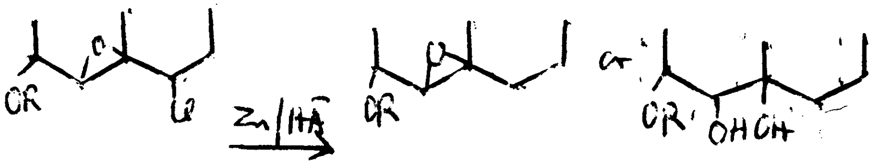
The action of hydrogen peroxide on 6-chloro-3-benzoyloxy-cholest-4-ene in tert.-butonal was tested under both aqueous and anhydrous conditions. In the former case, an unidentifiable product was again obtained, halogen once more being absent.

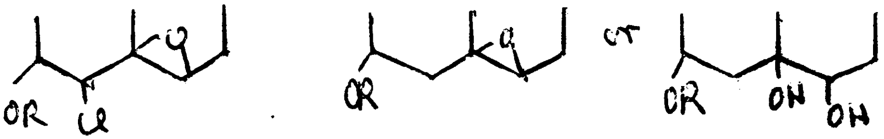
The most remarkable feature of the compound under consideration, and by far the greatest source of trouble in the above reactions, was the ease with which the chlorine was removed from the molecule. This was most marked when it was heated with glacial acetic acid, a green colour developing, and unworkable oils being formed with the liberation of hydrochloric acid. This phenomenon was also obsevred when samples of 6-chloro-3-acetoxy-cholest-4-ene and 3-chloro-4-acetoxy-cholest-5-ene were similarly treated. It will be remembered in this connection that Spring and Swain (2) found that heating with pyridine gave halogen free resinous products.

This ready loss of hydrochloric acid might account for the product formed when 6-chloro-3-benzoyloxy-cholest-4-ene was heated with aqueous hydrogen peroxide in <u>tert</u>.-butanol, the product being one of dehydrohalogenation rather than oxidation.

When (II) was heated with water in <u>tert</u>.-butanol, the reaction mixture became acid, and resinous products were formed. Indeed, when heated with water alone, the liquid was found to quickly develop an acid reaction to congo red paper. It seems this ready loss of halogen is shared by the oxide resulting in the breakdown of the suggested scheme.

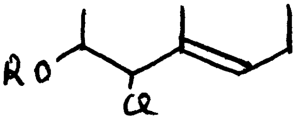
An attempt to reduce the oxide by zinc and acetic acid whereby it was hoped to form one of the products indicated below, failed to produce any crystalline material.





Thus, while no definite proof has been forthcoming as to the structure of (II), we can, in the light of the above results, state that Berg and Wallis's alternative structure (VI) is most probably incorrect.

Again, in view of the large negative rotation of the Spring and Swain compound (II) (Acetoxy derivative) $[\alpha]_D$ - 70.4°, and the great similarity between this and the figure obtained for the 3-chloro-4-acetoxy-cholest-5-ene above described, $[\alpha]_D$ - 71.4°, it might be deduced that both have a double bond in position five, and differ only in the positions of ester and halogen groupings. If this is so, then Petrow, Rosenheim and Starling's suggestion that the structure is (V) is correct.



(V)

but controversy must continue until a satisfactory degradation can be accomplished, in which the possibility of rearrangement is eliminated.

EXPERIMENTAL.

EXPERIMENTAL.

All melting points are uncorrected for exposed stem. Optical rotations were measured in chloroform solution, using a one decimetre tube.

Cholesteryl Benzoate - i) Cholesterol (10 g.) and benzoyl chloride (20 c.c.) were refluxed for 1 hour at 150-165°C., and then for 30 minutes at 190-195°, cooled and 2-3 vols. of methanol added with ice cooling. The crystals which separated were dried on porous plate, and redrystallised from ethyl acetate. The yield was 55% of theory, and melted at 150-151°.

ii) An improved yield (80%) was obtained by the action of excess of benzoyl chloride in pyridine solution at 100°.

Cholesteryl Acetate - Cholesterol (50 g.), and acetic anhydride (75 c.c.) and fused sodium acetate (2 g.) were refluxed together with 1 hour, cooled and filtered. Crystallisation from ethyl acetate gave 49 g. of product (94% of theory) m.p. 141°.

Purification of Dioxan - Dioxan (250 c.c.), pyrogallol (40 g.) and caustic potash (20%, 50 c.c.) were shaken together for 3 hours, and after settling, the dark liquid was decanted and dried over caustic soda. After separatinn from the solid, excess of sodium was slowly added, and the mixture refluxed for 6 - 7 hours. Pure, peroxide-free dioxan was then separated by distillation, and stored in dark bottles over ca. 6

inches of clean copper wire.

3-Benzoyloxy-4-Hydroxy-Cholest-5-ene - i) The method of Petrow, Rosenheim and Starling (J.(1943),135) gave, not the required product, m.p. 208°, but colourless needles, m.p. 176-178° in good yield. (Found: C, 81.8; H, 9.59% $C_{29}H_{48}O_3$ requires: C, 80.6, H, 9.88%).
ii) (Rosenheim and Starling, J(1937),377) - Oxidation of cholesteryl benzoate by selenium dioxide in acetic acid solution gave a product melting at 198°.

cis - 3:4 - Dihydroxy-Cholest-5-ene - The method of Rosenheim and Starling (J.(1937),377) was found to be the most successful, and gave the required product as colourless needles, m.p. 174-176° crystallising from alcohol, but in yields no greater than 20% of theory.

3-Acetoxy-4-Hydroxy-Cholest-5-ene - i) Oxidation of cholesteryl acetate by selenium dioxide in dioxan solution (Petrow, Rosenheim and Starling (J.(1943),135) gave yields only 5% of theoretical. The product crystallised as nacreous plates from alcohol, and melted at 192-193°.
ii) Acetylation of the diol by acetic anhydride in pyridine at room temperature (Petrow and Starling, J.(1940),60) gave the ester in 20-30% yield.

Oxidation of 3-Acetoxy-4-Hydroxy-Cholest-5-ene - is carried out by the action of chromic acid on the dibromide (Petrow and Starling, Ibid.). 3-Acetoxy-4-keto-cholest-5-ene was obtained as colourless crystals from alcohol, m.p. 118-120°. Absorption maximum at 2400A in U.V.

6-Nitro-Cholesteryl-Nitrate - i) (cf. Windaus, Ber.(1903), 36,3752, and Beynon, Heilbron and Spring, J.(1937),406) - Cholesterol (10 g.) was slowly added with mechanical stirring over a period of 30 minutes to a mixture of fuming nitric acid (100 c.c.) and glacial acetic acid (150 c.c.) cooled to 0° in ice-salt. Stirring was continued at the same temperature for a further half hour, and then until the material had warmed to room temperature, when it was poured over 750 g. of crushed ice, the solid collected, washed with water, and dried in vacuo. It was crystallised from glacial acetic acid, when it gave material m.p. 126-128°. (5 g. 41% of theory).
ii) A 68% yield was obtained, using the method of Heilbron, Jackson, Jones and Spring (J.(1938),104).

6-Keto-Cholestanol - i) (Windaus,Ibid.) 5 g. of 6-nitrocholesteryl nitrate gave 2 g. of the ketone (46% of theory) crystallised from 75% alcohol, m.p. 140°.
ii) The method of Heilbron, Jackson, Jones and Spring (Ibid.) gave a 64% yield.

6-Keto-Cholestanyl Acetate - Acetylation of the above in pyridine solution (according to the same authors) gave the ester in 82% yield as colourless plates from alcohol, m.p. 124-126°.

Action of Chlorinating Agents on 6-Keto-Cholestanyl Acetate.

(a) Action of Thionyl Chloride. - i) The ketone (0.5 g.) in pyridine (2.5 c.c.) was treated with thionyl chloride (0.15 c.c.) at 0°. After 5 minutes the mixture was poured into ice-water, and the solid collected and recrystallised from alcohol as unchanged starting material.

ii) The ketone (0.5 g.) in pyridine (2 c.c.) was treated with the reagent (0.2 c.c.) in pyridine (1.8 c.c.) at room temperature, and allowed to stand for 18 hours before working up. Unchanged starting material was again recovered.

iii) Thionyl chloride (0.1 c.c.) in ether (0.9 c.c.) was added to a solution of the ketone (0.5 g.) in ether (20 c.c.) containing pyridine (0.1 c.c.) and left for 24 hours, The ethereal solution was decanted from pyridine hydrochloride, washed with sodium carbonate and water, then dried over sodium sulphate. Removal of the ether returned unchanged ketone.

iv) The ketone (0.5 g.) together with pyridine (0.5 c.c.) and thionyl chloride (5 c.c.) was refluxed for 24 hours, during which darkening occurred, and hydrochloric acid was evolved. The solution was poured into water, and extracted with ether, giving an oil which could not be solidified.

v) The ketone (2 g.) was dissolved in excess of the reagent (20 c.c.) and refluxed for 6 hours. Darkening was more rapid than in iv), and sulphur dioxide was evolved. After standing overnight, the solution was poured into water, and the granular resinous solid collected. As this could not be crystallised, it was absorbed on alumina, and fractionally eluted. (Weight of resin was 0.98 g.)

	Solvent.	Vol. (c.c.)	Wt. of Material (mg.)	Nature.
1)	Light petroleum	100	-	-
2)	" "	100	40	Colourless oil.
3)	" "	100	Trace	" "
4)	Bz./Pet: 40/60	100	"	Brown oil.
5)	" "	100	80	" "
6)	Bz.Pet: 50/50	100	20	Yellow oil.
7)	Benzene	100	140	Resin.
8)	"	100	240	Yellow solid
9)	Ac/Bz: 10/90	100	300	" "
10)	" "	100	40	Brown oil.
11)	Ac./Bz: 50/50	100	100	" "

Total = 0.96 g.

Fractions 7) - 9) were crystallised from alcohol as unchanged starting material. The first fractions contained halogen, but could not be crystallised. It is therefore probable that up to 5% of the material had reacted.

(b) Action of Phosphorus Oxychloride.

i) 6-Keto-cholestanyl acetate (0.5 g.) was refluxed for 2 hours between 120 and 140° in phosphorus oxychloride (6 c.c.) which was subsequently removed in vacuo, leaving a yellow oil which crystallised from alcohol as unchanged ketone.

ii) In a repetition of i) refluxing was carried out for 24 hours. A non-crystallisable oil was obtained.

iii) The ketone (1 g.) in phosphorus oxychloride (10 c.c.) was refluxed for 24 hours at 120°, cooled, poured into water, and extracted with ether. Removal of the solvent left a yellow oil, which solidified, and was recrystallised from alcohol as unchanged starting material.

(c) Action of Phosphorus Pentachloride.

6-Chloro-3-Acetoxy-Cholest-5-ene - i) The ketone (1 g.) and an equimolecular weight of phosphorus pentachloride (0.5 g.) were heated on a steam bath for 1½ hours. After standing overnight, excess of water was added, and extraction with ether carried out. From the ethereal extract only an unworkable red oil could be obtained (0.95 g.). This was chromatographed, thus:-

	Solvent.	Vol. (c.c.)	Wt. of Material. (mg.)	Nature.
1)	Light Petroleum.	100	-	-
2)	" "	100	Trace	Colourless oil.
3)	Bz./Pet: 20/80	100	130	ctg. halogen.
4)	"	100	250	"
5)	Bz./Pet: 50/50	100	50	"
6)	Benzene	100	50	"
7)	"	100	40	semi-solid.
8)	Ac./Bz: 10/90	100	120	oil.
9)	Ac./Bz: 50/50	100	130	yellow oil.
10)	Acetone.	100	60	" "
11)	Acetone.	100	60	" "

Fractions 3) → 7) were crystallised from alcohol, with some difficulty, to give a product, m.p. 122-124°, with positive Beilstein test for halogen. In view of this, the experiment was repeated.

ii) The ketone (3 g.) together with phosphorus pentachloride (1·5g., 1 mol.) was heated on a steam bath for 2 hours, then allowed to stand at room temperature for 20 hours. As the mass was not mobile, it was dissolved in a little phosphorus trichloride, and poured slowly, with stirring, into water. The granular solid which separated was collected, washed with water, pressed well and dissolved in ether, which was, in turn, washed with sodium bicarbonate solution, water, and dried (sodium sulphate). Removal of the ether in vacuo left an oil which partly crystallised from ether-ethanol-methanol, to give colourless needles, m.p. 123-125°, containing halogen. Several further recrystallisations from alcohol brought the melting point to 128-130°. $[\alpha]_D^{19}$ - 53.8° (C = 0.74). (Found: C, 75.9; H, 10.1: Cl, 7.2% $C_{29}H_{47}O_2Cl$ requires: C, 75.2; H, 10.2; Cl, 7.6%). The yield was ca. 0.5 g. (ca. 16% of theory).

Cholesteryl Chloride was prepared after the method of Daughenbaugh and Allison (J.A.C.S. (1929), 51, 3665) by the action of thionyl chloride on cholesterol. Working up the reaction mixture was facilitated by collecting and washing the solid obtained on pouring the reaction mixture into water, then dissolving this in ether and proceeding as described. It crystallised from alcohol as needles, m.p. 96°.

Action of Selenium Dioxide on Cholesteryl Chloride.

3-Chloro-4-Hydroxy-Cholest-5-ene - i) The chloride (5 g.) in acetic acid (100 c.c.) was heated on a steam bath, and a solution of selenium dioxide (1.4 g.) in water (0.7 c.c.) and acetic acid (30 c.c.) warmed slightly, and rapidly added (1 mol. selenium dioxide to 1 mol. chloride). Heating was continued for 15 minutes, during which darkening occurred, and selenium separated. Crystalline sodium acetate (4 g.) was added, and heating continued for a further 5 minutes, after which the solution was filtered free from selenium, and allowed to cool, when unchanged starting material (1.2 g.) separated, and was collected. To the acetic acid solution benzene (50 c.c.) was added, and the whole slowly poured into brine (10% solution , 250 c.c.). The benzene layer was separated, washed and dried (sodium sulphate). Removal of the solvent left a dark tar. Since no solid material could be obtained, acetylation was attempted, using acetic anhydride (10 c.c.) in pyridine (20 c.c.). Hydrogen selenide was evolved, due to the presence of colloidal selenium. The solution was poured into 50% brine, when an oil again separated. The aqueous phase was decanted, and the oil dissolved in boiling alcohol, the solvent being subsequently removed in vacuo, and the residue chromatographed.

Weight of resin = 1.8 g.

	Solvent.	Vol. (c.c.)	Wt. of Material (mg.)	Nature.
1)	Light Petroleum	100	Trace	Almost colourless oil
2)	" "	100	200	" "
3)	" "	100	-	-
4)	Bz./Petrol:40/60	100	400	Yellow resin.
5)	Bz./Petrol:40/60	100	150	" "
6)	"	100	90	Yellow semi-solid
7)	Bz./Petrol:50/50	100	100	" "
8)	"	100	120	Yellow resin.
9)	Benzene	100	150	Resinous solid
10)	Acetone	100	510	Dark red resin.

Total weight = 1.72 g.

The solid obtained (in very small yield) from fractions 6) and 7) gave a negative Beilstein test, and melted at ca. 240°.

Fraction 9) was crystallised from alcohol as colourless needles, giving 3-chloro-4-hydroxy-cholest-5-ene, m.p. 122-124°. (Found: C, 76.7; H, 10.7% $C_{27}H_{45}O$ Cl requires: C, 76.9; H, 10.7%).

ii) The chloride (8 g.) was dissolved in boiling benzene (30 c.c.) and a solution of selenium dioxide (2.22 g. 1 mol.) in water (1.2 c.c.) plus acetic acid (stabilised, 60 c.c.) added, and the mixture refluxed for 30 minutes on a steam bath. Sodium acetate (10 g.) was added, and refluxing continued for a further 10 minutes. The mixture was then cooled, filtered free from black selenium, poured into half-saturated brine, and the benzene layer separated, washed with salt solution, and dried. On distillation of the solution in vacuo, a semi-crystalline mass remained. This was taken up in boiling alcohol unchanged cholesteryl chloride crystallising out on cooling. This was collected and crystallised once more.

The combined alcoholic mother liquors were taken to dryness, and the tar-like residue dissolved in the minimum volume of boiling acetone, which was cooled and left in the refrigerator, when a sticky solid separated, and was collected. Crystallisation from acetone (norite) gave colourless needles, m.p. 110°, but further crystallisation caused a drop in melting point, indicating the formation of mixed crystals with cholesteryl chloride, m.p. 96°, which was finally isolated from a sample, in a pure state. The acetone mother liquors yielded only a tar.

The mixed crystals (540 mg.) were chromatographed as before, benzene eluting crystalline material (450 mg.) m.p. 126°.

iii) In subsequent preparations of the product m.p. 126°, triple the above quantities were used (i.e. 24 g. of cholesteryl chloride), and heating carried out for 50 minutes. In every case, almost half the chloride was recovered unchanged, while from the acetone separated 3.5 g. to 10.0 g. of solid, which was absorbed on alumina, and eluted with benzene. Yields of up to 5 g. of crude material were obtained, but seldom more than 2.5 g. (10%) of pure crystals. For the sake of completeness, a typical chromatography is shown below.

Weight of material = 3.64 g.

	Solvent.	Vol. (c.c.)	Wt. of Material (mg.)	Nature.
1)	Light Petroleum	200	950	Starting material.
2)	" "	100	670	Yellow oil, plus some product.
3)	" "	100	300	Colourless solid, m.p.126-128°
4)	Bz./Pet.50/50	100	630	Pale yellow solid, m.p.125-6°
5)	" "	100	580	Reddish solid, m.p.122-3°
6)	" "	100	40	Reddish yellow oil.
7)	" "	100	50	(Reddish yellow oil
8)	Benzene	100	20	(Plus colloidal sol.
9)	"	100	70	(" " "
10)	Bz./Ac:50/50	75	220	Red oil.
11)	" "	25	150	"

Total weight = 3.68 g.

Fractions 3) - 5) gave 1.51 g. (6%) of product, which when pure, formed colourless needles, m.p. 125-127° from alcohol. $[\alpha]_D^{21}$ - 53.2° (C = 0.99). (Found: C, 76.3; H, 10.8; Cl, 10.0% 3-chloro-4-hydroxy-cholest-5-ene requires: C, 76.9; H, 10.7; Cl, 8.44%). The material develops a blue colour with the antimony chloride reagent.

Later fractions of the chromatography sometimes produced small amounts of a brown solid, m.p. ca. 250°, and containing no halogen,. Owing to the low yield, however, this was not investigated.

Action of Selenium Dioxide in Dioxan Solution on Cholesteryl Chloride - Cholesteryl chloride (2 g.) in dioxan (5 c.c.) and selenium dioxide (0.26 g. 0.5 mol.) in water (0.3 c.c.) and dioxan (2 c.c.) were heated on a steam bath for 4 hours, separated from selenium, cooled and poured slowly, with stirring, into half saturated brine, and the solid collected, washed, taken up in ether, and after further washing, dried over sodium sulphate. Removal of the ether left a semi-crystalline mass, which was taken up in alcohol, cholesteryl chloride (1 g.) separating on cooling. Evaporation of the mother liquors gave a reddish oil from which no solid could be obtained. The oil was refluxed with acetic anhydride (15 c.c.) for 1 hour, and the reagent removed in vacuo, leaving again an unworkable oil. As no solid matter could be separated, and in view of the success of the oxidation in acetic acid-benzene, the oil was discarded.

Action of Excess of Selenium Dioxide in Absence of Water - Cholesteryl chloride (1 g.) was dissolved in boiling glacial acetic acid (25 c.c.) and selenium dioxide (1 g.) added, and boiling continued (cf. Mower, Green and Spring, J. (1944),258. Oxidation of α-amyrin benzoate). Rapid darkening occurred, selenium was deposited, and shortly hydrogen selenide began to be evolved. It was apparent that conditions were too severe, and that deposited selenium was causing dehydrogenation. The experiment was abandoned.

3-Chloro-4-Acetoxy-Cholest-5-ene - 3-Chloro-4-hydroxy-cholest-5-ene (obtained by the action of selenium dioxide on cholesteryl chloride, 0.5 g.) in pyridine (5 c.c.) and acetic anhydride (5 c.c.) was heated on a steam bath for 2 hours, cooled, poured into water, and extracted with ether, as usual. The product, on one crystallisation from alcohol, gave a yellow solid (0.4 g.) m.p. 82-84°. This was purified chromatographically, giving colourless needles (o.27 g.)50%), m.p. 96-98°. $[\alpha]_D^{15}$ - 71.4° (C = 0.33). (Found: C, 75.2; H, 10.0; Cl, 7.5% $C_{29}H_{47}O_2Cl$ requires: C, 75.2; H, 10.2; Cl, 7.7%).

It gave no colouration with antimony trichloride in chloroform, and gave a melting point depression on admixture with a specimen of Spring and Swain's 6-chloro-3-acetoxy-cholest-4-ene.

Attempted Dehalogenation of 3-Chloro-4-Hydroxy-Cholest-5-ene - The chloro-compound (0.75 g.) in alcohol (30 c.c.) plus potassium carbonate (4 g.) and water (15 c.c.) were refluxed together for 5 hours on a steam bath, cooled, filtered to remove a little undissolved solid, poured into water, and extracted with chloroform. A gum was thereby obtained from which only small amounts of unchanged starting material could be recovered. No product was obtained.

Oxidation by Chromic Acid of 3-Chloro-4-Hydroxy-Cholest-5-ene was attempted through the dibromide as follows:-

The chloro-compound (1 g.) was dissolved in chloroform (3 c.c.) and bromine (0.15 c.c.) in chloroform (3 c.c.) slowly added. After standing for 10 minutes, acetic acid (10 c.c.) was added, and the mixture cooled to 0°, when the dibromide separated as almost colourless plates, m.p. 98°. The bulk of the yield, however, was obtained by reduction of the volume of solution in vacuo at room temperature, when a colourless solid, m.p. 90-95° was obtained, and dried in vacuo over caustic soda.

The dibromide (1 g.) in benzene (14 c.c.) and chromic acid (0.66 g.) in water (6 c.c.) and acetic acid (14 c.c.) were shaken together for 6 hours, and the benzene layer separated, washed with water, and dried (sodium sulphate), after which debromination was attempted by refluxing for 10 minutes with sodium iodide in alcohol (10% 10 c.c.), cooling and shaking with dilute aqueous sodium sulphite, followed by caustic soda, water, and drying once more. The benzene was removed, leaving a yellowish solid, which was recrystallised from alcohol (norite) to give very fine colourless needles, m.p. 162-164° (d.). The yield was ca. 0.2 g. (Found: C, 66.8; H, 8.7; Cl, 23.7(!)% 3-chloro-4-keto-cholest-5-ene requires: C, 77.8; H, 10.3; Cl, 8.48%).

The compound showed on ultra-violet absorption maximum at 2540A, $E^{1cm}_{1\%}$ = 103.7.

6-Chloro-3-Benzoyloxy-Cholest-4-ene was prepared in good yield from both α-cholesteryl benzoate oxide and α-cholesteryl oxide, using the published method. (Spring and Swain, J. (1939), 1356). It forms needles from ethyl acetate-methanol, m.p. 126-127°.

Action of Perbenzoic Acid on 6-Chloro-3-Benzoyloxy-Cholest-4-ene - i) The ester (10 g.) in dry chloroform (50 c.c.) was treated with perbenzoic acid (2.6 g. 1 atom oxygen) in chloroform (84 c.c.) at 0°, and left overnight in a refrigerator. Titration of a 1 c.c. sample against standard thiosulphate indicated that no reaction had taken place, and so the micture was left at room temperature. Titration of a further sample, after 24 hours, again gave a negative result. After standing at room temperature for 6 days, it was found that a little over half of the perbenzoic acid remained. The solution was shaken with aqueous dilute sodium carbonate, and dried, and the chloroform removed in vacuo. The residual colourless oil, on crystallisation from ethyl acetate-methanol gave some unchanged starting material, while from the mother liquors were obtained very fine needles, m.p. 86-94°. Chromatography of this latter fraction yielded colourless needles, m.p. 121°. $[\alpha]_{D}^{18}$ - 11.7° (l = 2, C = 0.34) (Found: C, 75.3; H, 9.03; Cl, 6.62%. Monoxide of 6-chloro-3-benzoyloxy-cholest-4-ene, $C_{34}H_{49}O_{3}Cl$ requires: C, 75.5; H, 9.07; Cl, 6.57%).

Continued crystallisation of the crude material first obtained gave a very small amount of solid, melting at 159-161°.

ii) The ester (1 g.) in chloroform (10 c.c.) plus perbenzoic acid (0.6 g. 2 atoms O) in chloroform (10 c.c.) were mixed, and left at room temperature. A blank was prepared of berbenzoic acid (0.6 g.) in chloroform (20 c.c.). Titration of 1 c.c. samples against 0.045 N sodium thiosulphate was as follows:-

Reaction Mixture Blank %ge

	Reaction Mixture (c.c.)	Blank (c.c.)	%ge Reaction
Initially	5.90	5.86	-
After 7 days at room temperature	3.60	5.15	36
" 11 " " "	2.90	4.77	42
" 14 " " "	2.46	4.59	50

On working up as before, a colourless oil was obtained, which was crystallised from ethyl acetate-methanol. Seven recrystallisations failed to raise the melting point above 110°. There was, however, no melting point depression below this temperature on admixture with the pure oxide, m.p. 121°. The crude material was used in the experiment with 2N sulphuric acid described below.

iii) The ester (10 g.) was dissolved in a solution of perbenzoic acid (5.6 g. 2 atoms O) in chloroform (135 c.c.), and left at 0°, then at room temperature, as indicated.

Titrations as before showed the following results:-

	Reaction Mixt. (c.c.)	Blank (c.c.)	Normality of thio.	%ge Reaction.
Initially	8.20	8.6	0.045	-
After 4 days at 0°	9.62	8.6	"	-
" 11 " " R.T.	1.99	3.33	0.10	47.4
" 14 " " "	1.70	3.09	"	49

The mixture was worked up as before, giving, after 3 crystallisations, 3.5 g. of product (34% of theory) m.p. 121°.

Action of Monoperphthalic Acid on 6-Chloro-3-Benzoyloxy-Cholest-4-ene - The ester (1 g.) was dissolved in dry ether (11 c.c.) and monoperphthalic acid (0.42 g.) in ether (11 c.c.) added. A blank of monoperphthalic acid (0.42)g.) in ether (22 c.c.) was prepared. 1 c.c. samples were titrated against 0.045 N thiosulphate, as shown:-

	Reaction Mixture (c.c.)	Blank (c.c.)
After 24 hours at room temperature.	4.30	4.70
" 1 hour under reflux.	4.10	4.67
" 20 hours under reflux.	2.07	2.30

The mixture was washed with dilute sodium carbonate and dried. Unchanged starting material was recovered on removal of the ether.

Action of Hydrogen Peroxide on 6-Chloro-3-Benzoyloxy-Cholest-4-ene.

(a) In Presence of Water - The ester (1 g.) in *tert*.-butyl alcohol (35 c.c.) was treated with hydrogen peroxide (30%, 1 c.c.) and refluxed on a steam bath for 2 hours. Nothing separated on cooling, and so the solution was poured into water, extracted with ether, and the extract washed with aqueous sodium carbonate, water, and dried. Removal of the ether gave an unworkable gum, yellow in colour. This, on chromatography, gave, in the first volumes of the eluate, an oily fraction, which crystallised from ethyl acetate-methanol to give a small amount of solid, m.p. 116-118°. (Found: C, 80.1; H, 10.4% $C_{34}H_{52}O_3$ requires: C, 80.3; H, 10.2%).

A repetition failed to give crystalline products, but the final fractions of the chromatography were also observed to be free of halogen.

(b) In Absence of Water - The ester (0.5 g.) was treated with a solution of hydrogen peroxide (0.096 g.) in *tert*.-butanol (2 c.c.) prepared after the manner of Miles and Sussman (*J.A.C.S.*, (1936), *58*, 1302), and refluxed on a water bath for 2 hours. The yellow solution, on being worked up as before, gave a resinous product, which would not crystallise.

Action of Sulphuric Acid on the Oxide m.p. 121° - i) The crude oxide (0.6 g.) obtained from the small scale preparations (ii) above in ethanol (35 c.c.) and sulphuric acid (2N, 5 c.c.) was refluxed on a water bath for 24 hours, during which the solution became orange coloured. Hot filtration removed a little undissolved matter, and, on cooling, the solution was poured into water, and extracted with ether. An orange resinous material was obtained, which gave small amounts of solid, m.p. 155°, from ethyl acetate-methanol. This gave no m.p. depression with the solid m.p. 159-161° obtained during the first oxide preparation, and gave an intense blue colouration with the antimony trichloride reagent. The mother liquors, on treatment with 2:4 dinitrophenylhydrazine deposited a derivative, m.p. 224-226°, and containing no halogen. (Found: N,15.4%. Calc. for cholestenone 2:4 dinitrophenylhydrazone, $C_{33}H_{48}O_5N_4$: N, 9.66%).

ii) The pure oxide (1 g.) was refluxed in benzene (20 c.c.) containing sulphuric acid (66%, 0.5 c.c.) for 2 hours. The solution acquired a pink colouration, and on cooling was diluted with water, extracted with ether, and the extract washed with sodium carbonate solution, and dried. Removal of the solvent left a resinous product which was crystallised from ethyl acetate to give very tiny colourless needles, m.p. 231°. This product contained no halogen, and gave no colouration with antimony trichloride or tetranitromethane in chloroform solution. (Yield ca. 0.5 g.). (Found: C, 79.8; H, 9.5%).

Attempted Reduction of the Oxide by Zinc and Acetic Acid -

i) The oxide (1 g.) was dissolved in acetic acid (25 c.c.) with heating, and activated zinc dust (2 g.) added. The mixture was heated on a steam bath for 1 hour, filtered hot, poured into water, and extracted with ether. The extract was washed with sodium bicarbonate solution, and dried. A resinous product was obtained on removal of the solvent.

ii) The experiment was repeated as before, only, after heating for 1 hour, refluxing was carried out for 15 minutes. On working up as before, an oil was obtained. This partly crystallised from alcohol to give fine needles, m.p. 152-156° (forming a pool ca. 170°). Recrystallisation raised the melting point to 196-200°, but no homogeneous product was obtained.

Action of Glacial Acetic Acid on 6-Chloro-3-Benzoyloxy-Cholest-4-ene - i) The ester (1 g.) was dissolved in acetic acid (10 c.c.) with warming. A green colour developed, and hydrochloric acid was evolved. On standing overnight, a brown oil separated. The reaction mixture was poured into water, and extracted with ether, which was washed, and dried, over sodium carbonate. Removal of the ether left an oil, which failed to give a product, even on chromatography.

ii) The ester (0.5 g.) was heated on a steam bath for 30 minutes with glacial acetic acid (5 c.c.). The solution became bright green, after which an oil separated, On cooling, the solution was yellowish. Working up as before gave an oil, which was taken up in acetone, a small quantity of brownish solid, m.p. ca. 230°, separating on cooling. This gave a negative Beilstein test for halogen, an intense blue colour with antimony trichloride in chloroform, and a yellow tint with tetranitromethane in the same solvent. Further purification of the small amount could not be carried out.

Action of Water on 6-Chloro-3-Benzoyloxy-Cholest-4-ene was investigated by refluxing the chloro-compound (0.5 g.) in *tert*.-butanol (3.5 c.c.) with water (0.25 c.c.). The solution very soon became acid to congo-red paper, probably due to the slow evolution of hydrochloric acid, and slowly became yellow. After 4 hours, the solution was cooled, when an oil separated. Working up was carried out as in the experiment with hydrogen peroxide (v.s.)., but no crystalline product could be obtained.

Hydrolysis of 6-Chloro-3-Benzoyloxy-Cholest-4-ene - The ester (1 g.), ethanol (40 c.c.), water (20 c.c.), and potassium carbonate (6 g.) were refluxed together on a water bath for 3 hours, and filtered hot, when some undissolved ester was removed. The reaction mixture, which smelled of ethyl benzoate, was diluted with water, and extracted with ether, the extract washed and dried (sodium sulphate). Removal of the ether left a sticky solid which crystallised in part from methanol. Recrystallisation from alcohol, then acetone, gave needles, m.p. 171-173°. These gave a negative Beilstein test, and a blue colour with the antimony trichloride reagent. On admixture with an authentic specimen of 3:4 dihydroxy-cholest-5-ene, there was no melting point depression. The yield was 0.25 g. (33%).

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INDEX OF COMPOUNDS.

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This index contains only those compounds which have been characterised, or to which nomenclature has been applied. In general, experiments concerning the various compounds follow immediately after the page number given, which refers only to their preparation in the Experimental sections.