Thesis being presented in Two Parts:

PART I: THE STRUCTURAL CHEMISTRY OF THE TRITERPENE β-AMYRIN.

PART II: ROUTES TO 11-OXYGENATED STEROIDS FROM ERGOSTEROL.

THESIS

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by

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SUMMARY.

SUMMARY.

PART I: The Structural Chemistry of the Triterpene β-Amyrin.

The reactions of the enol acetate of iso-β-amyrenonyl acetate show that the parent αβ-unsaturated ketone is correctly formulated as 2-acetoxyolean-10-en-12-one. iso-β-Amyrin acetate, derived from iso-β-amyrenonyl acetate by catalytic or Clemmensen reduction, is shown to be 2-acetoxyolean-10-ene and it is concluded that the locking of rings B/C in β-amyrin corresponds to the more stable configuration. Clemmensen reduction of iso-β-amyradienonyl acetate gives β-amyradienyl-II acetate which contains the same carbon skeleton as β-amyrin. In contrast to the behaviour of iso-α-amyradienonyl acetate, iso-β-amyradienonyl acetate is reduced catalytically to neo-β-amyrin acetate, a monoene, which differs from each of the previously described isomers.

Treatment of β-amyrenonyl benzoate with strong alkali gives 18-iso-β-amyrenonol. Catalytic hydrogenation of 18-iso-β-amyrenonyl acetate yields 18-iso-β-amyrin acetate, oxidation of which with hydrogen peroxide yields a saturated ketone, 18-iso-β-amyranonyl acetate. The orientation at C_{13} in the last compound is shown to represent the sterically stable configuration. Bromo-18-iso-β-amyranonyl acetate is considerably more stable than the isomeric bromo-β-amyranonyl acetate, and yields the corresponding αβ-unsaturated ketone on heating in pyridine. Reduction of 18-iso-β-amyranonyl a acetate by the Kishner-Wolff procedure gives 18-iso-β-amyranol which differs from the saturated pentacyclic triterpenoid alcohols hitherto described.

PART II: Routes to 11-Oxygenated Steroids from Ergosterol.

Ergosterol has been converted by various procedures into 11α-hydroxy and 11-keto-steroids. The action of oxidising agents on ergosteryl-D acetate has been investigated: treatment with chromic acid giving 3β-acetoxyergosta-9(11):22-dien-7-one and 3β-acetoxyergosta-8:22-dien-7-one, with one mol. of performic acid giving 3β-acetoxy-8α-ergosta-9(11):22-dien-7-one, with two mols. of performic acid giving 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one, and with perbenzoic acid giving 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate. By using mild alkaline conditions, hydrolysis of the ketoxide is accompanied by rearrangement to give 3β:11α-dihydroxyergosta-8:22-dien-7-one, whereas treatment with strong alkali also effects a rearrangement, to give in this case, after acetylation, 7:11-diketoergost-22-en-3β-yl acetate. 9α:11α-Epoxyergosta-7:22-dien-3β-yl acetate has been converted into 3β-acetoxy-9α:11α-dihydroxyergost-22-en-7-one characterised by its conversion into 3β:11α-diacetoxyergosta-8:22-dien-7-one on treatment with strong alkali followed by acetylation.

Oxidation of ergosteryl-D acetate 22:23-dibromide with one mol. of perbenzoic acid gives 22:23-dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate, whereas with two mols. a corresponding 7ξ:8ξ,9α:11α-diepoxide is obtained. Treatment of the former compound with sulphuric acid gives 22:23-dibromo-7ξ:11α-dihydroxyergost-8-en-3β-yl acetate. Oxidation of this diol with chromic acid yields 22:23-dibromo-7:11-diketoergost-8-en-3β-yl acetate and 22:23-dibromo-8α:9α-epoxy-7:11-diketoergostan-3β-yl acetate, treatment of which with zinc and acetic acid gives in each case 7:11-diketoergost-22-en-3β-yl acetate. 22:23-Dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate has been

rearranged to 3β-acetoxy-22-23-dibromoergost-8-en-7-one by treatment with dilute hydrochloric acid and to 3β-acetoxy-22:23-dibromoergost-8-en-11-one by treatment with boron trifluoride.

Oxidation of 22:23-dibromoergosta-7:9(11)-dien-3β-yl acet-ate with performic acid gives 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one, debromination of which with zinc yields 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one characterised by its conversion by relatively mild alkaline hydrolysis followed by acetylation into 3β:11α-diacetoxyergosta-8:22-dien-7-one. 3β-Acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one is converted by alkali into 22:23-dibromo-3β:11α-dihydroxyergost -8-en-7-one and is isomerised by filtration of its benzene solution through alumina into 3β-acetoxy-22:23-dibromo-11α-hydroxyergost-8-en-7-one. The last compound is smoothly oxidised by chromic acid to 22:23-dibromo-7:11-diketoergost-8-en-3β-yl acetate. Catalytic reduction of 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one in the presence of alkali is accompanied by debromination to 3β:11α-dihydroxyergost-22-en-7-one.

Oxidation of 22:23-dibromo-7ξ:11α-dihydroxyergost-8-en-3β-yl acetate with perbenzoic acid gives 22:23-dibromo-8α:9α-epoxy-7ξ:11α-dihydroxyergostan-3β-yl acetate, which has been converted into 22:23-dibromo-8α:9α-epoxy-7:11-diketoergostan-3β-yl acetate by treatment with chromic acid and 22:23-dibromo-9α:11α-dihydroxy-7-ketoergostan-3β-yl,acetate with hydrogen bromide. The behaviour of the last compound with alkali has been examined. With dilute alkali simple hydrolysis of the 3β-acetoxy group occurs, but, with stroger alkali, dehydration also occurs with formation of 22:23-dibromo-3β:11α-dihydroxy-ergost-8-en-7-one. Oxidation of 22:23-dibromo-9α:11α-dihydroxy-7-ketoergostan-3β-yl acetate with chromium trioxide

gives 22:23-dibromo-9α-hydroxy-7:11-diketoergostan-3β-yl acetate, vigorous treatment of which with alkali followed by acetylation gives the known 22:23-dibromo-7:11-diketoergost-8-en-3β-yl acetate. Whereas treatment of the last compound with zinc dust and acetic acid gives 7:11-diketoergost-22-en-3β-yl acetate, with zinc dust in ether-methanol it gives 7:11-diketo-8α-ergost-22-en-3β-yl acetate, readily isomerised to the more stable form on being heated with acetic acid. The 8α-isomer is also obtained by treatment of 7:11-diketo-ergost-8:22-dien-3β-yl acetate , 22:23-dibromo-8α:9α-epoxy-7:11-diketoergostan-3β-yl acetate, or 8α:9α-epoxy-7:11-diketo-ergost-22-en-3 -yl acetate with zinc dust in a neutral solvent.

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PART I.

THE STRUCTURAL CHEMISTRY OF THE TRITERPENE β-AMYRIN.

CONTENTS.

HISTORICAL

Introduction.

The triterpene class of compounds consists of non-nitrogenous natural products containing basically thirty carbon atoms, the fundamental skeleton of which consists of six isoprene (CH_2:CH.CMe:CH_2) units. On dehydrogenation with selenium the triterpenes give a mixture of aromatic hydrocarbons, particularly homologues of naphthalene. This is in contrast to a somewhat similar class of natural products, the sterols, which give the characteristic Diels hydrocarbon (3-methyl-1:2-cyclopentenophenanthrene) on dehydrogenation.

The triterpenes are widely distributed in the plant kingdom and may occur in all parts of the plant, free or glycosidically linked with sugars as saponins. The most abundant triterpenes are probably the amyrins which were first isolated by Rose (1), in 1839, from Manila elemi resin, and in 1887 separated into the α- and β-forms by Vesterberg (2). Since then the amyrins have been found in numerous resins and saponins, the latex of many other plants (3) and shea nut oil (4).

The triterpenes, like the steroids, are interesting because of the complicated alicyclic structures to which they can give rise, which are not easily accessible by

synthesis. The ease of forming complex unsaturated and oxygenated systems depends on the presence of angular methyl groups which prevent aromatisation, and on the location of some of the double bonds which causes additions to be hindered and allylic reactions to predominate.

Owing to the immense amount of work on the subject, only fundamental advances and certain features of the β-amyrin structure will be described in the historical part, especially as excellent reviews of this field are already available (5-10).

Evolution of the General Triterpene Formula.

Two methods have been widely employed for the determination of the structures of triterpenes, namely, (a) dehydrogenation with selenium, and (b) oxidative degradation.

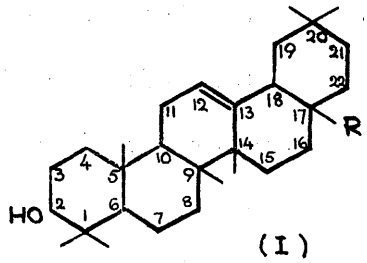
In 1929 Ruzicka (11) subjected a mixture of α- and β-amyrin to dehydrogenation with selenium at relatively high temperatures (320 to 350°) and isolated various homologues of naphthalene, regarded as typical dehydrogenation products of the pentacyclic triterpenes. The structural problem resolved itself into the construction of a triterpene skeleton consistent with the isoprene rule and capable of accounting for the products. The

isolation of 1:8-dimethylpicene led to the important conclusion that the pentacyclic system of the triterpenes was a reduced picene nucleus (12).

Recently, increasing use has been made of the pyrolysis of oxidation products in which one ring is open, giving rise to fragments containing two or three rings which are readily investigated. This procedure was also originated by Ruzicka (13) and was at first confined to oleanolic acid (I; R = CO_2H) which has been finally degraded to two sets of products representing rings A-B, and D-E (14). The configuration of rings A and B have been recently compared in different series of triterpenes by examining the pyrolysis fragments containing these rings, and in all cases they have been found to be the same. α-Amyrin and β-amyrin have been degraded to common products containing rings A, B and C (15) which proves steric identity in this region.

The formulation of the skeleton of β-amyrin has been modified many times to account for increasing knowledge, but the modifications have usually been confined to rings C, D and E, and particularly to the location of the two methyl groups placed finally at C_{14} and C_{17}. Structure (I) suggested by Haworth in 1937 (5,6) is compatible with the reactions of the members of β-amyrin group, and is now

considered to be almost certainly correct.



(I)

Interconversion of Triterpenes.

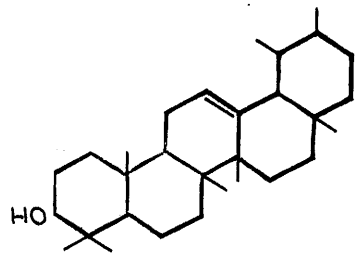
Probably the most important advance in the field of triterpene chemistry has been the establishment of the suspected relationship between various individual compounds and their assignment to different groups, so that the reactions of each compound in a given group have a bearing on the structure of all members in the group and the complete determination of structure for one member will establish the constitution of most members of the group.

The inter-relationships have, for the most part, been determined by the application of a method developed by Ruzicka in which monocarboxylic acid members of the group are converted into the corresponding aldehyde by the Rosenmund method (the hydroxyl group being protected), and the aldehyde then reduced to the corresponding deoxocompound

by the Wolff-Kishner method:

$$RCO_2H \xrightarrow{SOCl_2} RCOCl \xrightarrow[(Rosenmund)]{Cat.\ red.} RCHO \xrightarrow{W\text{-}K} RCH_3 + RCH_2OH$$

Polycyclic triterpenes can now be classified into at least three different groups: the β-amyrin group (I), the α-amyrin group (II), and the lupeol group (III). The first two groups have basic structures consisting of five six-membered rings, while the third has a structure composed of four six-membered rings and one five-membered ring.



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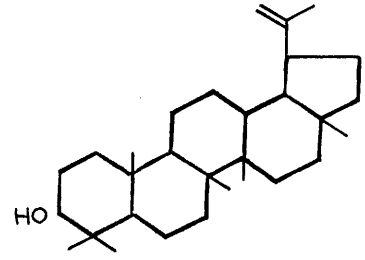
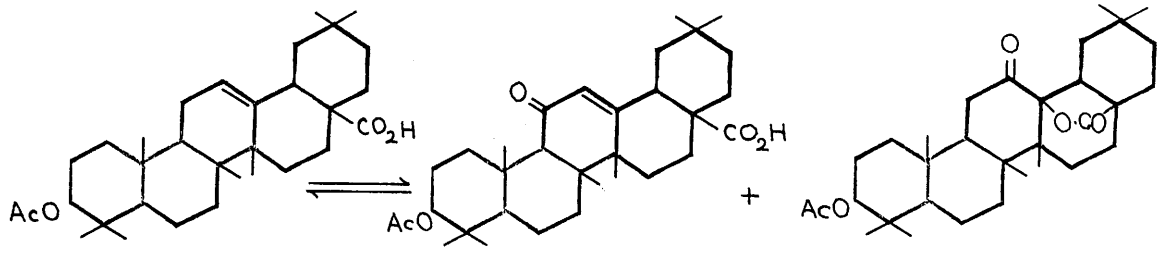


(III)

The most important interconversions that have been accomplished so far (cf. 7,10) indicate that not only has a relationship been established between the functional groups of the different compounds within a group but that all have the same carbon skeleton, that the unreactive double bond occupies the same position in all, and that the same stereochemical configurations exist about the numerous asymmetric carbon atoms.

The Ethylenic Linkage and its Environment.

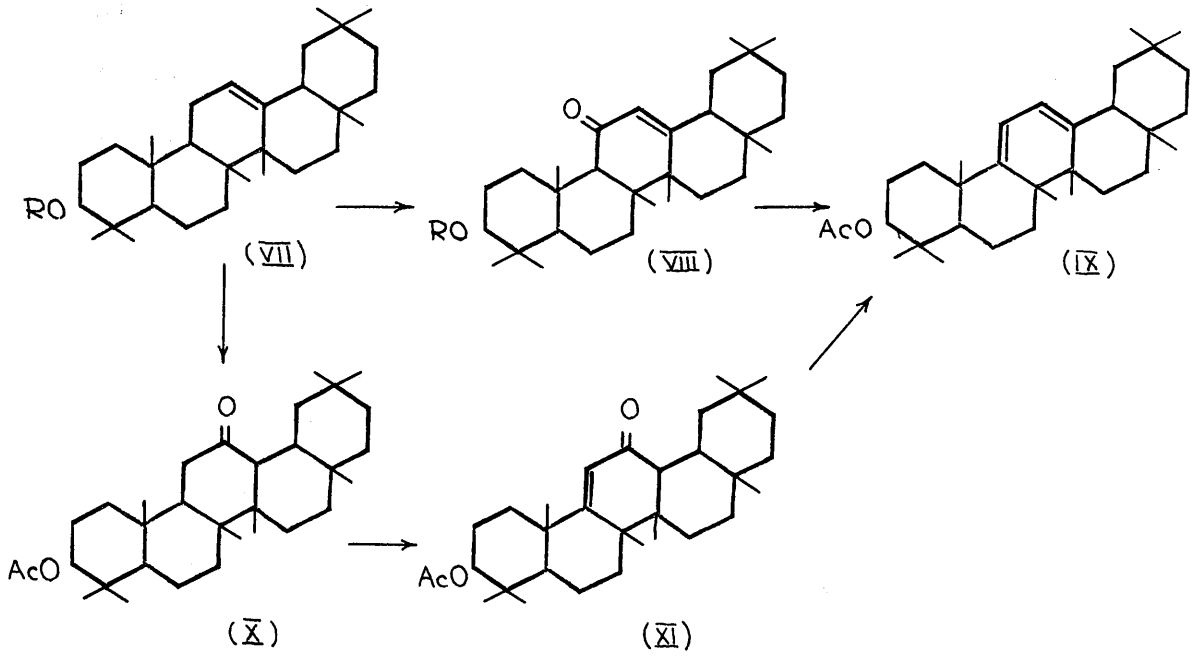
The location of the unsaturated centre of the β-amyrin group in ring C (which is not revealed by catalytic reduction) was indicated by pyrolysis experiments (14,16). Further information was obtained by experiments on oleanolic acid. Oxidation of acetyloleanolic acid (IV) with chromic acid gives an αβ-unsaturated ketone (V) and a keto-lactone (VI) (16,17). The former compound can be reduced by standard methods to oleanolic acid, which indicates the presence of a methylene group adjacent to the ethylenic linkage. On the other hand, in the keto--lactone, the double-bond has been oxidised, so that the carbonyl and the lactone groupings mark, therefore, the position of this linkage.



(IV) (V) (VI)

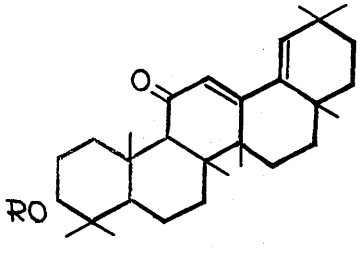
β-Amyrin benzoate (VII; R = Bz) is likewise oxidised to an αβ-unsaturated ketone, β-amyrenonyl benzoate (VIII; R = Bz) formed by oxidation of the methylene group immediately adjacent to the ethylenic linkage (18,19,20).

Catalytic reduction of β-amyrenonyl benzoate (VIII; R = Bz) led to complete reduction of the carbonyl group, the ethenoid linkage being unaffected, with formation of β-amyrin benzoate (VII; R = Bz). Reduction of β-amyrenonol (VIII; R = H) with sodium and alcohol, followed by acetylation, has led to valuable information concerning the environment of the ethylenic linkage of β-amyrin. The product, β-amyradienyl-I acetate (IX), presumably formed by reduction of the carbonyl group of β-amyrenonol to a secondary alcohol and dehydration of the latter, contains a conjugated system of two ethylenic linkages located in a single ring, as shown by the absorption spectrum.

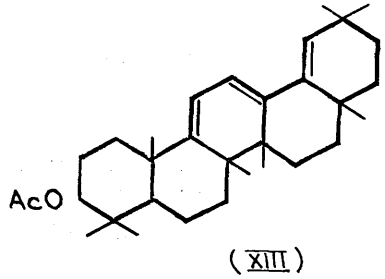


Oxidation of β-amyrin acetate (VII; R = Ac) with hydrogen peroxide (21, cf. 22) gives a saturated ketone, β-amyranonyl acetate (X). [This compound was previously described as an oxide (21)]. Bromination of β-amyranonyl acetate in acetic acid with one mol. of bromine yields an αβ-unsaturated ketone, iso-β-amyrenonyl acetate (XI), reduction of which with sodium and alcohol, followed by acetylation, yields β-amyradienyl-I acetate (IX).

The nature of the unsaturated centre in β-amyrin was further defined by Picard and Spring (23) who have shown that β-amyrenonyl esters are partially dehydrogenated when treated with bromine to yield β-amyradienonyl esters (XII), which contain a conjugated dienone system -CO-C=C-C=C- spectroscopically established. Treatment of β-amyrin acetate with N-bromosuccinimide yields β-amyratrienyl acetate (XIII) (24) containing a conjugated triene chromophore.

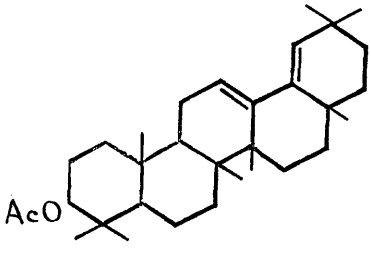


(XII)



(XIII)

When β-amyrenonyl acetate (VIII; R = Ac) is reduced to β-amyradienyl-I acetate (IX) there is also produced at the same time an isomeric compound named by Picard and Spring (23) β-amyradienyl-II acetate, containing a conjugated diene system distributed between two rings. This compound had previously been obtained by Ruzicka and co-workers (19, 25) by the oxidation of β-amyrin acetate with selenium dioxide. Spring and co-workers (26, 27) prepared later the same compound by treatment of β-amyrenonyl acetate (VIII; R = Ac) and β-amyradienonyl acetate (XII; R = Ac) with selenium dioxide. β-Amyradienyl-II acetate was formerly (19, 23, 25-28) represented by formula (XIV), although (XV) was not excluded. The corresponding oleanolic acid-compound has been shown, however, to have the structure (XV; R = CO_2H) by Barton and Brooks (29), so that (XV; R = CH_3) is now the accepted structure for β-amyradienyl-II acetate.



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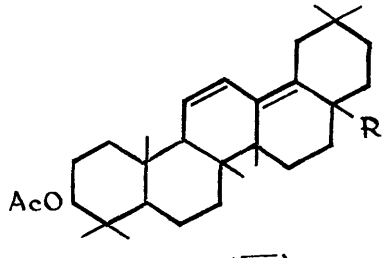

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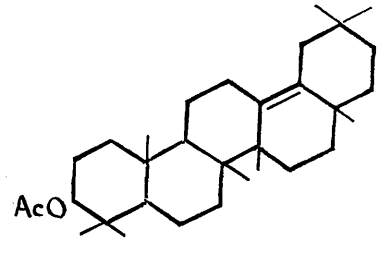
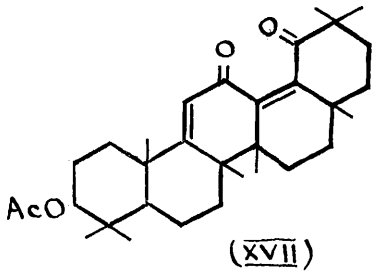


(XVI)

Both representations involve bond migration during their formation from β-amyrenonyl acetate. Catalytic hydrogenation of β-amyradienyl-II acetate yields a compound isomeric with β-amyrin acetate, termed δ-amyrin acetate (XVI), containing a 13(18)-double bond.

A compound which has given much useful information regarding the C-D-E portion of the β-amyrin molecule (cf. 31) is β-amyradiendionyl acetate (XVII). This compound (as an alcohol) was first prepared by Jacobs and Fleck in 1930 (32) by a method involving treatment of β-amyrin benzoate with sulphur, followed by saponification, oxidation of the sulphur-compound with potassium permanganate and hydrolysis. Since then it has been prepared by a number of other methods: Spring and co-workers (23, 34) prepared it by the action of selenium dioxide on β-amyradienyl-I acetate (IX) and β-amyratrienyl acetate (XIII). Ruzicka and co-workers (25, 30) prepared it by the treatment of β-amyrin acetate, β-amyradienyl-II



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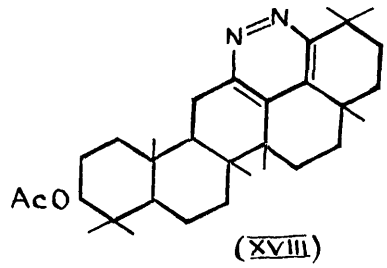
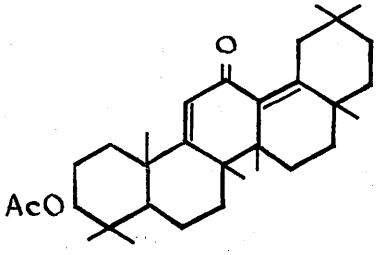


(XVIII)

acetate (XV) and δ-amyrin acetate (XVI) with selenium dioxide. Jacob's compound was shown by Ruzicka and Jeger (25,33) to be a hydroxy-dione containing -CO-C=C-CO- grouping; on treatment with hydrazine it gave a pyridazine--derivative, formulated as (XVIII). The researches of the last authors have provided much evidence in favour of the structure (XVII) for β-amyradiendionyl acetate, although it has been criticised (cf. 35) on the grounds that it cannot fully account for the properties of certain derivatives.

It has not been possible to prepare β-amyradiendionyl acetate by stepwise reaction from either β-amyrin acetate or iso-β-amyrenonyl acetate (XI), although the latter compound contains half of the chromophore of β-amyradien-dionyl acetate (XVII). Barton and co-workers (31) put forward a hypothesis that all the reactions leading to the diketo-diene system proceed via the 10:12:18-triene (XIII) (cf. 34).

Treatment of iso-β-amyrenonyl acetate (XI) with selenium dioxide gives iso-β-amyradienonyl acetate (27), originally formulated by Spring (27) as (XIX), and later by Ruzicka (45) as (XX), which involved the migration of the angular methyl group from C_{14} to C_{18}. Further treatment of iso-β-amyradienonyl acetate with selenium dioxide gives



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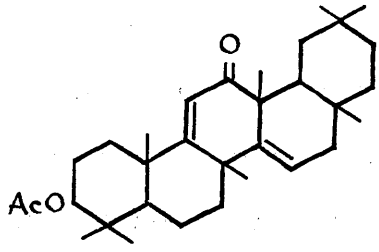
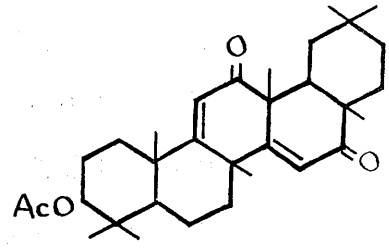


(XX)



(XXI)

an isomeric β-amyradiendionyl acetate, formulated by Ruzicka and Jeger (46) as (XXI).

In view of various reactions performed on iso-β--amyradienonyl acetate, the validity of the formulation (XX) is questioned, and this, together with later work carried out by Ruzicka and co-workers (15) having a direct bearing on the problem, is further discussed in the theoretical section of this thesis.

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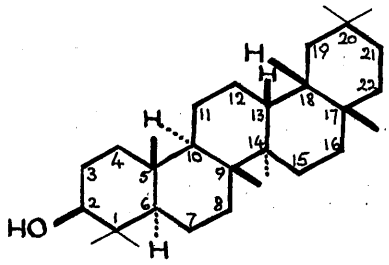
Stereochemistry

Giacomello in 1938 (36) concluded from X-ray analysis that the β-amyrin molecule was flat and the asymmetric centres were in the alternating trans-configurations.

Recently Barton (37,38) has extended the concept of equatorial and polar bonds (39) to the difficult problem of the stereochemistry of the pentacyclic triterpenes of the β-amyranol group. The basic assumption was made that in the more stable configurations all five rings would adopt chair conformations.

Rings A and B had been shown to be trans-fused from the known relation (40) to the diterpene abietic acid. Rings D and E were shown to be cis-fused with the less stable orientation at C_{18} (19,27,37,63), while the configuration at C_{18} in β-amyranol derivatives was shown to be the more stable one and to have the hydrogen on the same side of the molecule as the C_{17}-methyl group (41). Correlation with the stereochemistry of perhydrophenanthrene (38) showed that rings C and D must be trans-fused, and conclusive evidence has been produced (37,61) showing that C_{18} has also the more stable configuration. The configuration at C_2 is regarded as β (The symbols α and β are used with the same significance as in steroid



(XXII)

chemistry), on elimination evidence (38) and on steric properties which are those of an equatorial hydroxyl group (37,39). The assignment of configuration at C_9 (hence the fusion of ring B to C) is on a less certain basis. Molecular rotation arguments (42) and X-ray investigation (38) decided in favour of the stereochemical representation (XXII), which requires B/C-*trans* (C_9:C_{14}--*anti*)C/D-*trans*.

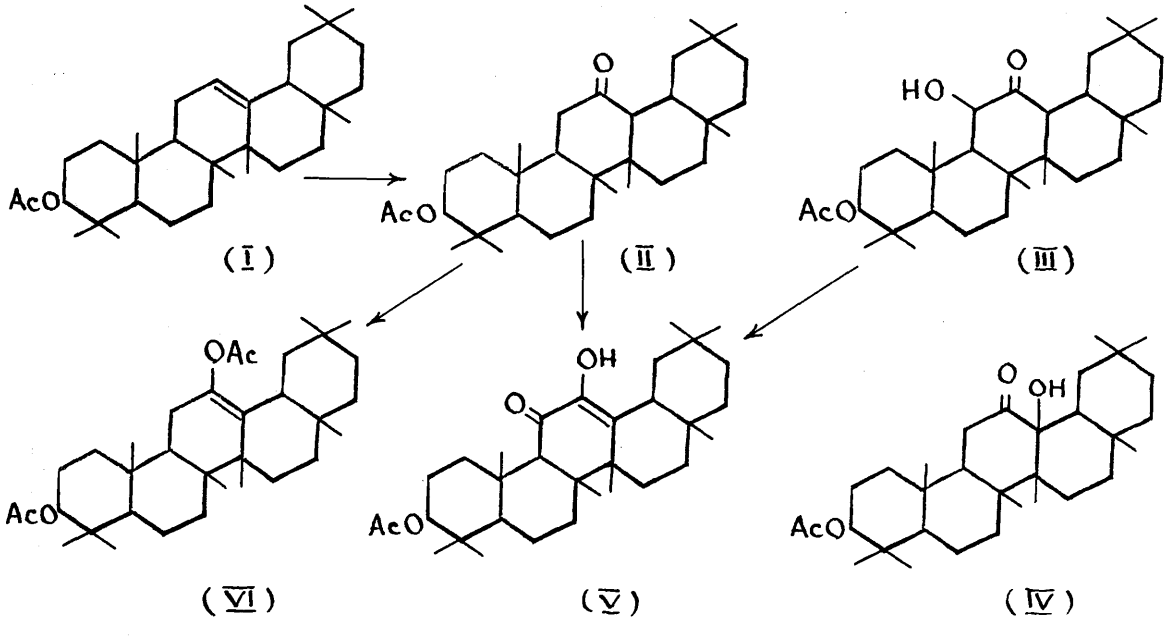
THEORETICAL

SECTION I: iso-β-Amyrenonol and iso-β-Amyradienonol.

The work described in this section was commenced in July, 1950, and had as its object the study of reactions of certain derivatives of β-amyrin with particular reference to iso-β-amyradienonol and the structural problem of the latter compound.

Oxidation of β-amyrin acetate (2-acetoxyolean-12-ene) (I) with hydrogen peroxide gives the saturated ketone β-amyranonyl acetate (2-acetoxyoleanan-12-one) (II)(21,43). The amorphous solid obtained from the reaction mixture after the removal of β-amyranonyl acetate was chromatographed on alumina, yielding a further quantity of β-amyranonyl acetate and a new acetate, $C_{32}H_{52}O_4$, which does not exhibit selective absorption of high intensity in the ultra-violet region and does not show a colour with tetranitromethane in chloroform or ferric chloride in alcohol. It cannot be acetylated under normal conditions but is hydrolysed by alkali to the corresponding alcohol $C_{30}H_{50}O_3$. The hydrolysed product does not precipitate on addition of water to its alcoholic potassium hydroxide solution but readily separates when the diluted solution is acidified. The alcohol was recovered unchanged after treatment with diazomethane and dimethyl sulphate. Of the structures

(III) and (IV) which appear probable for this compound, the former (III) was excluded since the acetate is stable to chromic anhydride at room temperature whereas (III) should give the enol of 2-acetoxyoleanane-11:12-dione (V) first prepared by Ruzicka and Jeger by selenium dioxide oxidation of β-amyranonyl acetate (44).

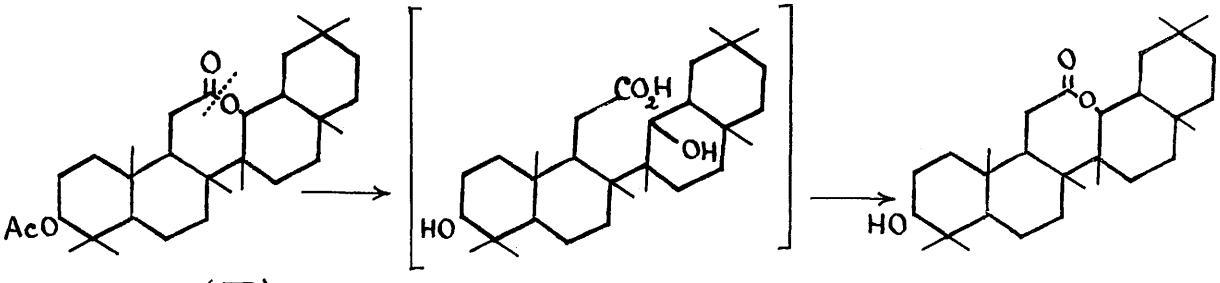


(I) (II) (III)

(VI) (V) (IV)

Another structure (VII) has been considered for the new acetate. This, however, seems unlikely as hydrolysis with potassium hydroxide would give the salt of the acid (VIII), which presumably would be insoluble in ether, whereas the alcohol was recovered from alkaline media by

ether extraction.

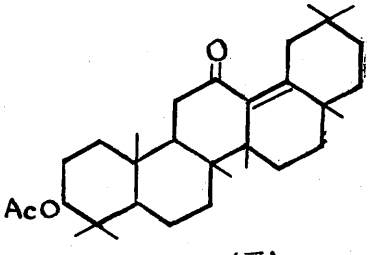


(VII) (VIII) (IX)

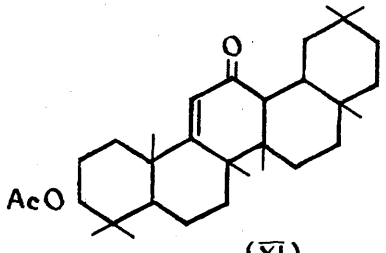
Alkaline hydrolysis of β-amyranonyl acetate (II) gave β-amyranonol. The acetate was further characterised by the formation of its enol acetate (VI) using acetic anhydride and sodium acetate (cf. 44).

Treatment of the saturated ketone (II) with bromine in acetic acid gives bromo-β-amyranonyl acetate which readily loses hydrogen bromide to give iso-β-amyrenonyl acetate (43,45) for which the alternative structures (X) and (XI) have been considered (6,27). A decision in favour of the latter has been made since reduction of iso--β-amyrenonyl acetate with sodium and alcohol, followed by treatment of the product with acetic anhydride, gives β-amyradienyl-I acetate (2-acetoxyoleane-10:12-diene)(XII) containing a conjugated diene system in a single ring and identical with a product obtained by similar treatment of β-amyrenonyl acetate (2-acetoxyolean-12-en-11-one) (XIII).

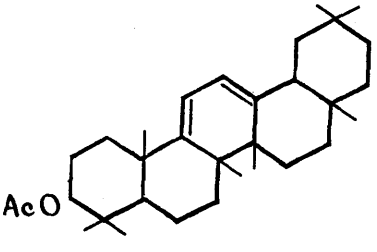
Catalytic reduction of iso-β-amyrenonyl acetate gives an



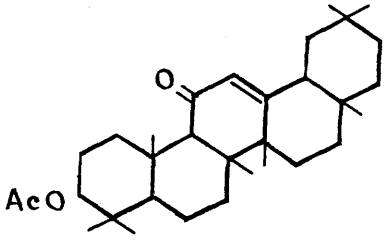
(X)



(XI)



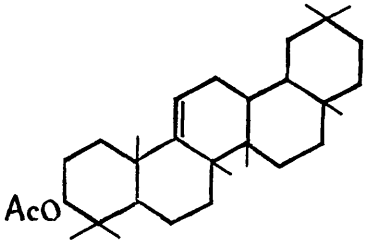
(XII)



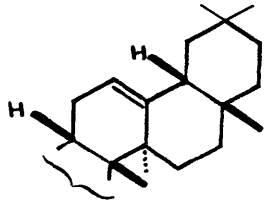
(XIII)

isomer of β-amyrin acetate which contains a >C=CH- linkage since it is oxidised by hydrogen peroxide to a saturated ketone isomeric with β-amyranonyl acetate. The isomeric β-amyrin acetate has been formulated as 2-acetoxyolean-10-ene (XIV) by Jeger and Ruzicka (46). This structure did not appear to be rigidly established, since hydrogenation of iso-β-amyrenonyl acetate (XI) could proceed by reduction of the ethylenic linkage and simultaneous or consecutive reduction of the carbonyl group to a secondary alcohol, followed by dehydration to

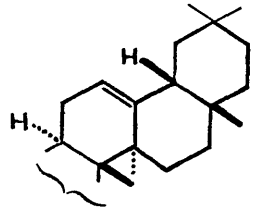
give an isomeric β-amyrin acetate (XVI) differing from β-amyrin (XV) solely in the orientation around $C_{(10)}$*. [cf. the catalytic reduction of β-amyranonol to β-amyrin described by Ruzicka and Jeger (44)]. Of the alternative structures (XIV) and (XVI) for the isomeric β-amyrin acetate, the former has been established by Wolff-Kishner reduction of the derived isomeric β-amyranonyl acetate to a product which, after acetylation, gave β-amyranyl acetate (XVII) identical with the compound obtained by



(XIV)



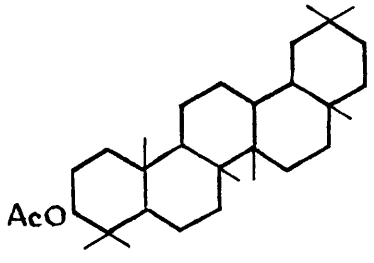
(XV)



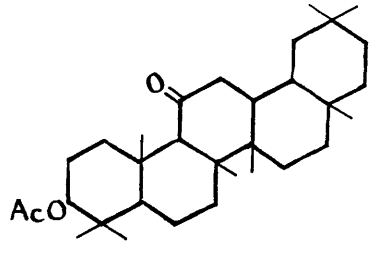
(XVI)

* The configurations shown in (XV) and (XVI) are for the most part arbitrary and are used for comparative purposes only. The cis-locking of rings D/E, however, has been established by Barton and Holness (37) [cf. Davy, Halsall and Jones (47)].

similar treatment of β-amyranonyl acetate (44). The isomeric β-amyranonyl acetate is therefore 2-acetoxy-oleanan-11-one (XVIII). An interesting point concerning the nature of the locking of rings B/C in the β-amyrin group of triterpenoids emerges from this series of changes. The conversion of β-amyrin into 2-acetoxyoleanan-11-one (XVIII) and thence into β-amyranyl acetate (XVII) proves that these rings are locked in the more stable configuration since the introduction of a carbonyl group at the 11-position and the subsequent treatment of this ketone



(XVII)

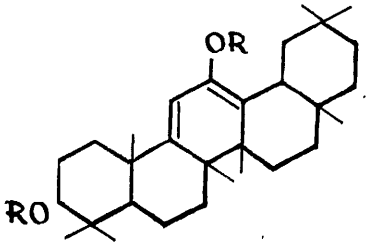


(XVIII)

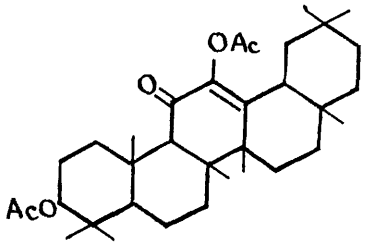
would permit the change from a less to a more stable configuration at this junction. Since no such isomerisation occurs it is concluded that the more stable configuration exists in β-amyrin. Whether the more stable configuration is <u>cis</u> or <u>trans</u> remains to be established.

In an attempt to further characterise <u>iso</u>-β-amyrenonol by the formation of its benzoate, it was treated with

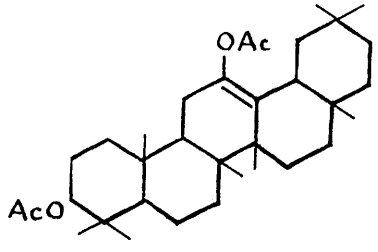
benzoyl chloride in pyridine. The product proved to be an enol dibenzoate which gives a brown colour with tetranitromethane in chloroform and exhibits selective light absorption in the ultra-violet with maxima at 2300 Å (ε = 31,000), attributable to the two benzoyl groups and at 2750 Å (ε = 11,000) attributable to a diene system present in a single ring. The enol dibenzoate is therefore (XIX; R = COC_6H_5), and the structure of iso--β-amyrenonyl acetate as 2-acetoxyolean-10-en-12-one (XI) is confirmed. Treatment of iso-β-amyrenonyl acetate



(XIX)



(XX)

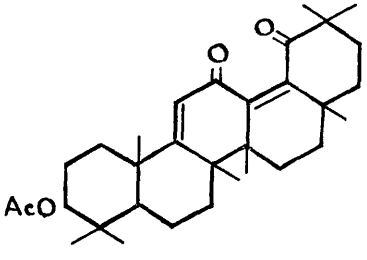


(XXI)

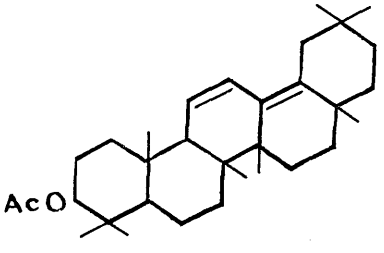
with acetic anhydride and sodium acetate gives an enol acetate (XIX; R = Ac) which exhibits an absorption maximum at 2780 Å characteristic of a conjugated diene system present in a single ring. The enol acetate grouping is easily hydrolysed with the re-formation of iso-β-amyrenonyl acetate. The structure (XIX; R = Ac) was supported by oxidation of the 10-ethylenic linkage

of the enol acetate with hydrogen peroxide, with formation of 2-acetoxyoleanane-11:12-dione enol acetate (XX) previously obtained by Ruzicka and Jeger (44) by chromic acid oxidation of the enol acetate of β-amyranonyl acetate (XXI).

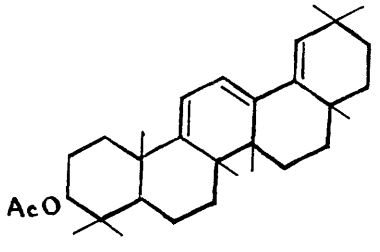
The interpretation of the complex reactions of β-amyradienedionyl acetate and of analogous compounds obtained from other members of the β-amyrin group of triterpenoids by Ruzicka and Jeger has provided weighty evidence in favour of part of the detail of the formula (I) for β-amyrin acetate.



(XXII)



(XXIII)

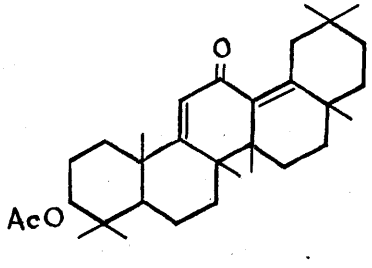


(XXIV)

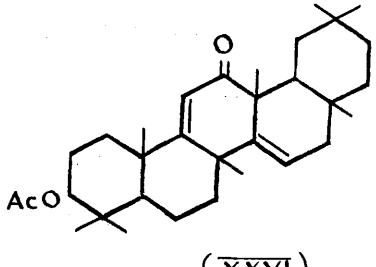
β-Amyradienedionyl acetate (XXII) is obtained by the oxidation of β-amyrin acetate (I) (25), β-amyradienyl-I acetate (XII) (23), β-amyradienyl-II acetate (XXIII)(35,41) and β-amyratrienyl acetate (XXIV)(34) with selenium dioxide. Oxidation of iso-β-amyrin acetate (2-acetoxyolean-10-ene) (XIV) with selenium dioxide also gives β-amyradienedionyl

acetate (XXII). It is found that Clemmensen reduction of β-amyradienedionyl acetate gives β-amyradienyl-II acetate (XXIII).

iso-β-Amyrenonyl acetate (XI) contains half of the chromophore of β-amyradienedionyl acetate (XXII) and accordingly Green, Mower, Picard and Spring (27) attempted to correlate these two compounds by oxidation of the former with selenium dioxide in the expectation that β-amyradienedionyl acetate would result. Instead, the reaction gave iso-β-amyradienonyl acetate which shows an absorption maximum at 2450 Å (ε = 11,000) and unlike iso-β-amyrenonyl acetate gives a yellow colour with tetranitromethane in chloroform. iso-β-Amyradienonyl acetate was also obtained by the action of bromine on iso-β-amyrenonyl acetate by the same workers and later by Jeger and Ruzicka (46). It is found that iso-β-amyradienonyl acetate can also be obtained directly from β-amyranonyl acetate (II) by bromination. Green, Mower, Picard and Spring (27) suggested

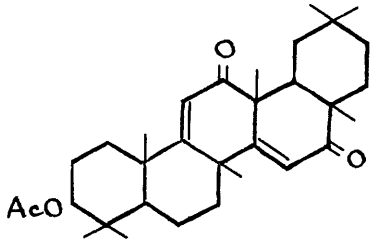


(XXV)

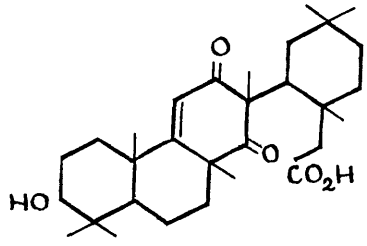


(XXVI)

the structure (XXV) for iso-β-amyradienonyl acetate, whereas Jeger and Ruzicka (46) proposed the structure (XXVI) in which it is represented as formed from iso-β--amyrenonyl acetate by the migration of the angular methyl group from $C_{(14)}$ to $C_{(15)}$ with simultaneous introduction of a 14:15-double bond. This formulation (XXVI) would explain the light absorption, the colour with tetranitromethane and the formation of a new compound, isomeric with β-amyradienedionyl acetate, by the action of selenium dioxide on iso-β-amyradienonyl acetate (46). Jeger and Ruzicka (46) suggested the structure (XXVII) for this new



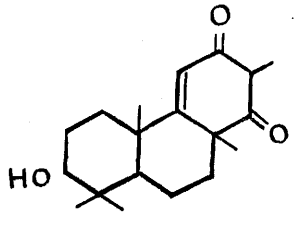
(XXVII)



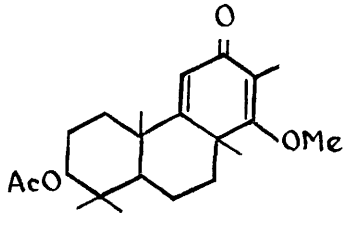
(XXVIII)

isomeric β-amyradienedionyl acetate. More recently, Meisels, Jeger and Ruzicka (48) have oxidised iso-β-amyradienonyl acetate to a hydroxy-diketo-acid formulated as (XXVIII), the methyl ester of which on pyrolysis gave an acidic fraction represented as the hydroxy-diketone (XXIX). The hydroxy-diketone was characterised by methylation and

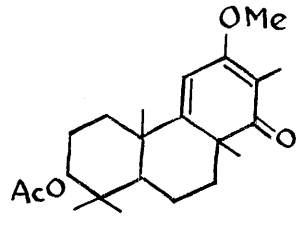
acetylation which gave two isomeric compounds formulated as (XXX) and (XXXI) and identical with two compounds obtained by, in all essential features, the same route starting from α-amyrin. These reactions, however, cannot be construed as proof of migration of the angular methyl group during conversion of iso-β-amyrenonyl acetate into iso-β-amyradienonyl acetate.



(XXIX)



(XXX)



(XXXI)

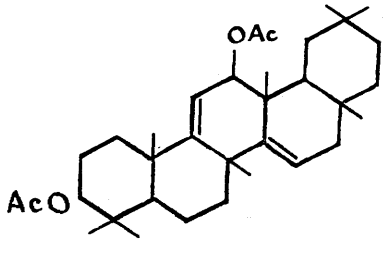
In order to further investigate the structural problem of iso-β-amyradienonyl acetate, it was prepared by the selenium dioxide and bromine method previously described (27,46) and also by direct bromination (2 mols) of β-amyranonyl acetate. Alkaline hydrolysis gives the corresponding alcohol, iso-β-amyradienonol, acetylation of which yields the parent acetate. Benzoylation of the alcohol gives iso-β-amyradienonyl benzoate. Pure iso-β--amyradienonyl acetate crystallises in prisms melting just over 220°, whereas the acetate was previously described

as forming plates melting below 210°. The plates can readily be converted into prisms by further crystallisation or by chromatography. It is found that Clemmensen reduction of iso-β-amyradienonyl acetate gives β-amyradienyl-II acetate (XXIII) which contains the same carbon skeleton as β-amyrin. In itself this reaction can be reconciled with the structure (XXVI) for iso-β-amyradienonyl acetate by assuming that the strongly acid medium induces a second migration of the angular methyl group at C_{18} to its original position at C_{14}, thus giving the dienone (XXV), Clemmensen reduction of which would yield β-amyradienyl-II acetate. It was noted, however, that iso-β-amyradienonyl acetate was recovered unchanged after prolonged treatment with hydrochloric acid in acetic acid. On the other hand, the conversion of iso-β-amyradienonyl acetate into β-amyradienyl-II acetate could be considered to be proof that the carbon skeleton of both compounds is the same and that a methyl group has not changed its place during the conversion of the one into the other. In favour of such a view are the following very substantial facts: first, that iso-α-amyrenonyl acetate is oxidised by selenium dioxide to iso-α-amyradienonyl acetate which when oxidised and pyrolysed gives a product from which

are isolated compounds (XXX) and (XXXI) identical with those obtained from iso-β-amyradienonyl acetate; and, secondly, that catalytic hydrogenation of iso-α-amyradienonyl acetate in acetic acid at room temperature gives iso-α-amyrenonyl acetate. The latter observation proves that iso-α-amyradienonyl acetate has the same carbon skeleton as α-amyrin and that iso-α-amyradienonyl acetate does not carry a methyl group at C_{13} (49). The conversion of both iso-α-amyradienonyl acetate and iso-β-amyradienonyl acetate into the compounds (XXX) and (XXXI) requires that iso-β-amyradienonyl acetate likewise does not carry such a methyl group. In marked contrast to the behaviour of iso-α-amyradienonyl acetate, iso-β-amyradienonyl acetate is reduced catalytically in acetic acid at room temperature with absorption of three molecular proportions of hydrogen and formation of a compound apparently containing one ethylenic linkage. This compound, named neo-β-amyrin acetate was previously reported by Budziarek, Johnston, Manson and Spring (61) as iso-β-amyradienyl acetate. It differs from each of the previously discovered isomers, does not show selective absorption of high intensity above 2200 Å (Maximum at 2140 Å, ε = 3500) and, anomalously, gives an intense red colour with tetranitro-

methane in chloroform. This is the first example of a triterpene which does not contain a conjugated system of ethylenic bonds giving an intense red or brown colour with this reagent. This acetate is not isomerised by mineral acid. Catalytic hydrogenation of iso-β-amyradienonol followed by acetylation also gives neo-β-amyrin acetate.

The carbonyl group of iso-β-amyradienonyl acetate is reduced by lithium aluminium hydride and the resulting diene-diol is characterised as its diacetate (XXXII) assuming the structure (XXVI) proposed by Jeger and Ruzicka.* Catalytic reduction of this diacetate gives neo-β-amyrin acetate by hydrogenolysis of the acetoxy-group



(XXXII)

* The structures (XXXII)-(XXXIX) are used provisionally for comparative purposes only.

in ring C and saturation of one double bond.

Proof that neo-β-amyrin acetate contains only one ethylenic linkage was found later (51) by chromic acid oxidation which gives a compound containing one atom of oxygen more than neo-β-amyrin acetate. The oxidation product does not exhibit light absorption of high intensity in the region 2000-4000 Å and does not give a colour with tetranitromethane in chloroform.

Oxidation of iso-β-amyradienonyl acetate with excess chromic acid under rigorous conditions gives a compound, $C_{32}H_{48}O_4$, needles, m.p.308-309°, $[\alpha]_D$ +57°, which exhibits an absorption maximum at 2350 Å (ε = 12,100) and does not show a colour with tetranitromethane in chloroform. Treatment of this compound with bromine in acetic acid yields a bromo-compound, $C_{32}H_{45}O_4Br$, which is recovered unchanged after prolonged heating in acetic acid solution.

Oxidation of iso-β-amyradienonyl acetate with excess hydrogen peroxide gives two products which are readily separated by the chromatographic method. The minor product, $C_{32}H_{48}O_5$, m.p.225-227°, $[\alpha]_D$ +80° appears to be formed by a simple addition of two atoms of oxygen to iso-β-amyradienonyl acetate. It does not exhibit selective light absorption of high intensity in the ultra-violet

region and does not show a colour with tetranitromethane in chloroform. The major product, $C_{32}H_{48}O_4$ ($C_{32}H_{46}O_4$ not excluded), plates, m.p.308-309°, $[\alpha]_D$ +179°, exhibits selective absorption in the ultra-violet with maximum at 2380 Å (ε = 12,500), does not give a colour with tetranitromethane and contains a secondary hydroxyl group as shown later (51) by chromic acid oxidation to the compound, $C_{32}H_{46}O_4$, needles, m.p.308-309°, $[\alpha]_D$ +57°, first obtained by chromic acid oxidation of iso-β-amyradienonyl acetate; the plates and needles give practically no depression of mixed melting point. Treatment of the hydrogen peroxide compound, m.p.308-309°, with hydrochloric acid in acetic acid yielded a new product containing chlorine, exhibiting light absorption maximum at 2340 Å (ε = 13,000) and showing no colour with tetranitromethane.

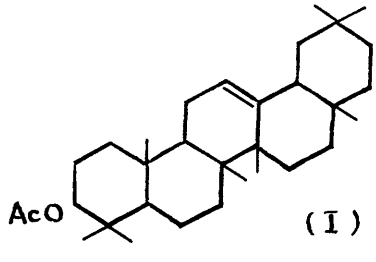
Catalytic hydrogenation of the chromic acid oxidation product of iso-β-amyradienonyl acetate gives three distinct compounds separated by chromatography. The first, $C_{32}H_{50}O_2$ ($C_{32}H_{52}O_2$ not excluded), m.p.230.5-232°, $[\alpha]_D$ +84°, does not exhibit light absorption of high intensity above 2200 Å, and shows a pale yellow colour with tetranitromethane in chloroform. The second, $C_{32}H_{52}O_3$, m.p.324-325.5°, $[\alpha]_D$ -94°, does not exhibit

selective absorption in the ultra-violet region and does not give a colour with tetranitromethane in chloroform. The third compound, $C_{52}H_{52}O_2$, m.p.172-173°, $[\alpha]_D$ +4° does not exhibit light-intensity absorption above 2200 Å and gives a dark yellow colour with tetranitromethane in chloroform. The compounds were not obtained in sufficient amount to allow a detailed investigation of their structures.

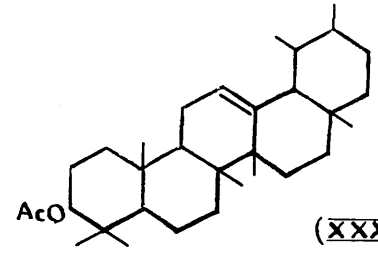
This work was discontinued in June, 1951, and further study of the oxidation and reduction products described above was carried out in this laboratory by J. D. Johnston (Ph.D. Thesis, 1953).

Conclusion

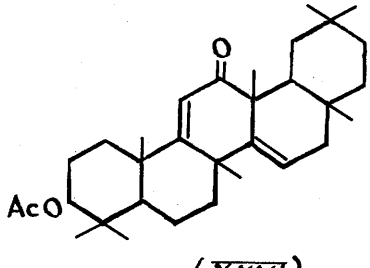
The conclusions from this work may be summarised as follows. A series of well-defined reactions of the α- and β-amyrin groups has been interpreted in terms of the formulations (I) for β-amyrin acetate and (XXXVII) for α-amyrin acetate, by the assumption of migration of a methyl group in the conversion of iso-amyrenonol to iso-amyradienonol. In the α-amyrin series the postulated migration is demonstrably incorrect since simple catalytic reduction of iso-α-amyradienonyl acetate yields the parent iso-α-amyrenonyl acetate. In the β-amyrin series, the postulated migration of a methyl group appears unlikely (but not excluded) since iso-β-amyradienonol can be converted by Clemmensen reduction into a well-known



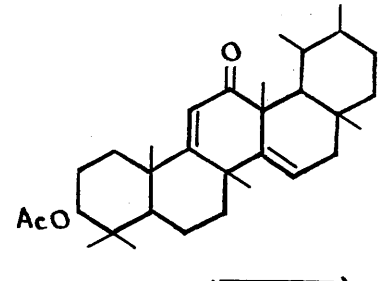
(I)



(XXXVII)



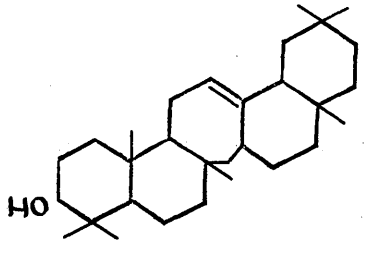
(XXVI)



(XXXVIII)

derivative of β-amyrin which possesses the same carbon skeleton as the latter. The conversion of the iso-α- and the iso-β-amyradienonyl acetates into two common degradation products which apparently include the rings A-C of the triterpenes, suggests that in so far as these rings are concerned, the α- and β-amyradienonyl acetates have identical carbon skeletons; since (XXXVIII) is not an acceptable formulation for iso-α-amyradienonyl acetate, (XXVI) for iso-β-amyradienonyl acetate is suspect.

An attractive hypothesis to circumvent the anomalies described above is that neither α- nor β-amyrin carries a methyl group attachment at C_{14} [cf. Jeger, Rüegg, and Ruzicka (50)]; in such an event the accommodation of the displaced carbon atom becomes a matter for speculation. The possibility that ring C is 7-membered (β-amyrin = XXXIX) [cf. Meyer, Jeger, Prelog and Ruzicka (15)] has



(XXXIX)

been considered, together with alternative methods for the accommodation of the carbon atom displaced from C_{14}. Such speculations are premature since the views expressed above are to a large measure dependent on the validity of the interpretation of the reactions leading from the iso--amyradienonyl acetates to the compounds (XXX) and (XXXI) and, in particular, on the validity of the structures ascribed to the last two compounds.

---oOo---

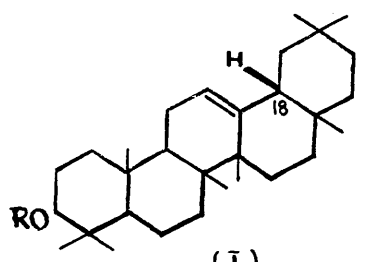
SECTION II: 18-iso-β-Amyranol.

The generally accepted structure ascribed to the β-amyrin-oleanolic acid group of triterpenoids, exemplified by that of β-amyrin (I; R = H)* has been supported by a formidable and, in most cases, convincing array of evidence, much of which has emerged from the scholarly researches of Ruzicka. The structure ascribed to the α-amyrin-ursolic acid group of triterpenoids is illustrated by that of α-amyrin (II; R = H).

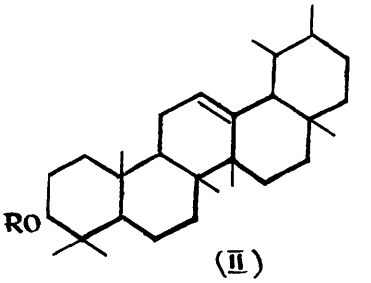
Oxidation of β-amyrin acetate with chromic anhydride in acetic acid gives an 8% yield of acetone, isolated as its 2:4-dinitrophenylhydrazone. Similar oxidation of α-amyrin acetate does not give acetone. The triterpenoid acetates employed were purified to constant optical rotation, and were rigorously dried; a common batch of purified acetic acid was employed and the oxidations were effected under as far as possible identical conditions. If (I; R = H) is the true structure of β-amyrin, the acetone obtained from β-amyrin acetate probably originates in the geminal dimethyl group in ring E. While this has

* In order to avoid the use of high Roman numerals, the formulae in this section are again numbered from (I) upwards.

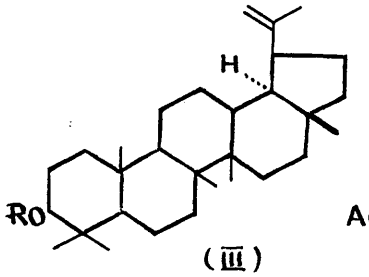
not been considered to be unlikely, a more natural inference was that β-amyrin contains an <u>iso</u>propyl group attached to ring E.



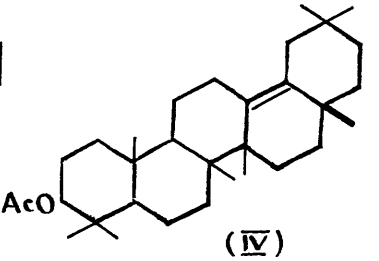
(I)



(II)



(III)



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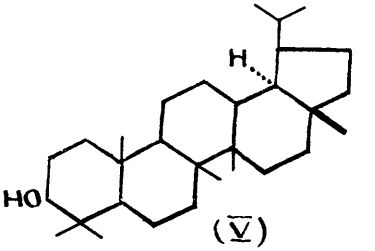
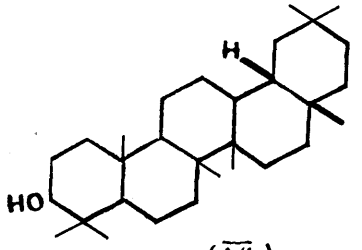
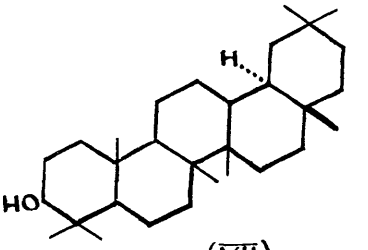


(V)



(VI)



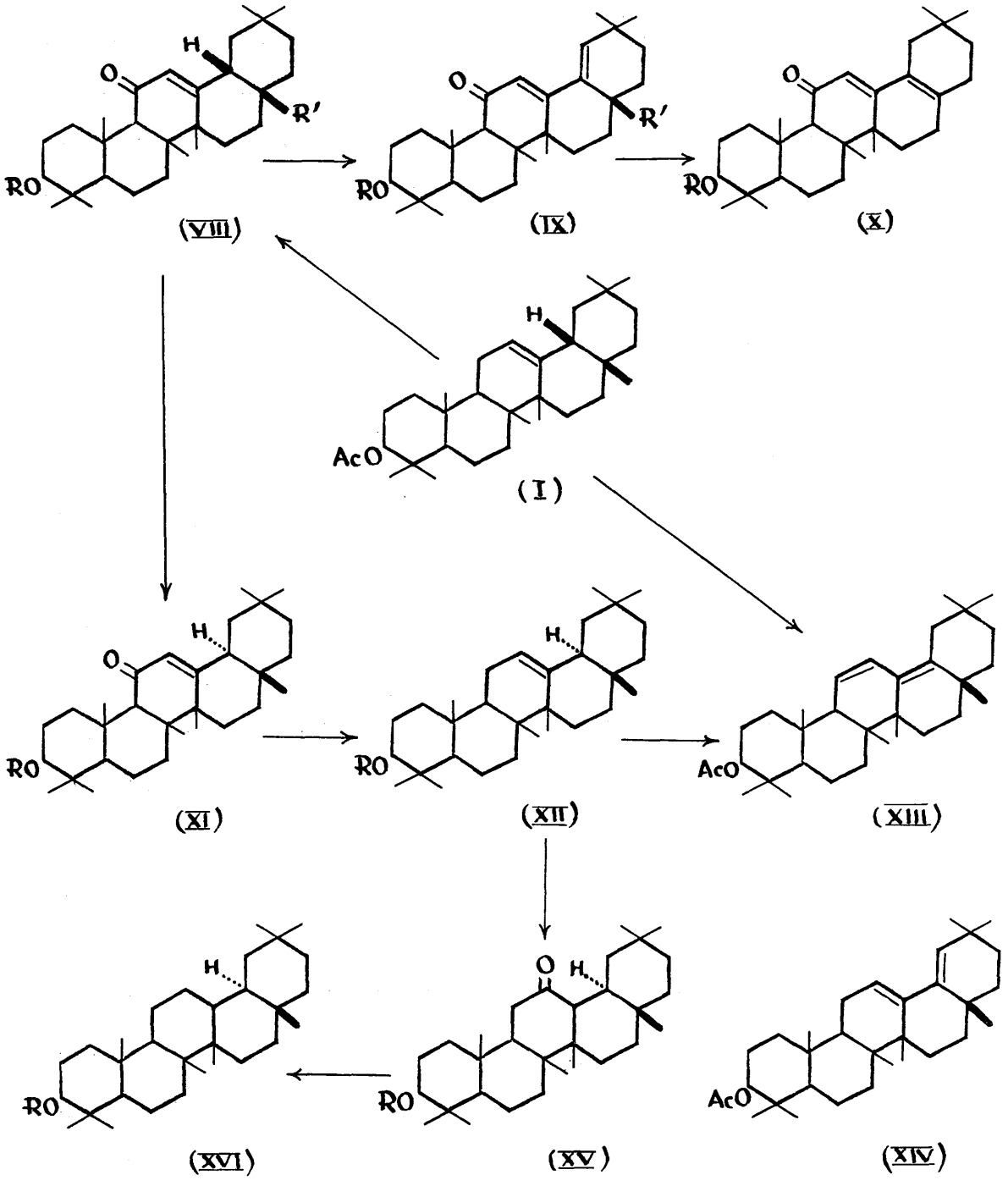
(VII)

The structures ascribed to the α- (II; R = Ac) and the β- (I; R = Ac) acetate differ only in the nature of ring E, and there is evidence that they are sterically identical at C_3, C_5, C_9, C_{10} and possibly at C_{14}. Ames,

Halsall, and Jones (52) have shown that lupenyl acetate (III; R = Ac), which contains an isopropenyl group attached to ring E, is isomerised by mineral acid to δ-amyrin acetate (IV), itself obtained from β-amyrin acetate by a two-stage process. It has been established [Davy, Halsall, and Jones (47); Barton and Holness (37)] that rings D/E in lupeol (III; R = H) are trans-fused and that the rings D/E in β-amyrin (I; R = H) are cis-fused. Consequently, structural differences apart, lupanol (dihydrolupeol) (V) and β-amyranol (dihydro-β-amyrin)(VI) differ in orientation around C_{18}. Before attempting to assess the significance of the formation of acetone from β-amyrin acetate (to test the hypothesis that both lupeol and β-amyrin have the same carbon skeleton) it was considered essential to embark upon the preparation of the isomeric saturated alcohol of the β-amyrin group, 18-iso--β-amyranol (VII) in order to compare it with lupanol and other saturated pentacyclic triterpenoid alcohols.

A simple route to 18-iso-β-amyranol appeared to be available since indications existed in the literature that epimerisation at C_{18} in β-amyrenonol (VIII; R = H) could be achieved. Thus in a discussion concerning the physical constants of β-amyrenonol and its esters,

Ruzicka, Müller, and Schellenberg (19) reported that treatment of β-amyrenonol (m.p.230-231°, $[\alpha]_D$ +102°) (VIII; R = H, R' = Me) for a prolonged period with 10% alcoholic alkali gave an isomer, m.p.247-248°, $[\alpha]_D$ +81.5°. This isomerisation could involve either C_{10} or C_{18} or both centres simultaneously, but the fact that bromination of β-amyrenonyl acetate (VIII; R = Ac, R' = Me) proceeds smoothly to give β-amyradienonyl acetate [Picard and Spring (23)] (IX; R = Ac, R' = Me) suggests that enolisation of β-amyrenonyl acetate involves C_{18}. Furthermore, reduction of β-amyradienonyl acetate by sodium ethoxide and hydrazine gave a complex mixture from which an allo--β-amyrenonyl acetate was obtained; this probably differs from β-amyrenonyl acetate in the orientation around C_{18} [Green, Mower, Picard and Spring (27)]. Kitasato (53) has shown that methyl acetylketo-oleanolate (VIII; R = Ac, R' = CO_2Me) is isomerised by mineral acid to a ψ-isomer; that this isomerisation also involves C_{18} is indicated by the fact that bromination of acetylketo-oleanolic acid (VIII; R = Ac, R' = CO_2H) gives the conjugated acetyloleanan--12:18-dien-11-onolic acid (IX; R = Ac, R' = CO_2H)[Ruzicka, Jeger, and Winter (54)]. It was found (63, cf. 55) that when melted, the dienone-acid loses carbon dioxide rapidly


H
O
R'
RO
(VIII)
(IX)
(X)
AcO
(I)
(XI)
(XII)
(XIII)
(XIV)
(XV)
(XVI)

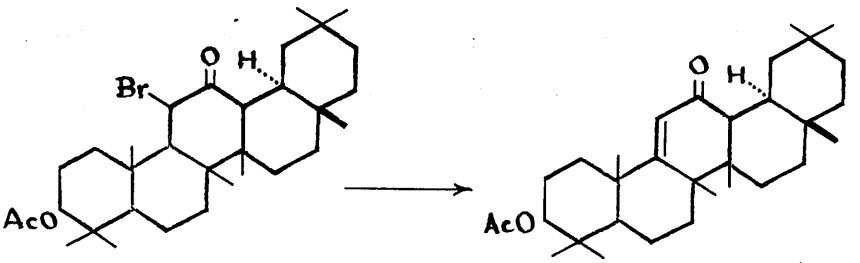
to give nor-β-amyradienonyl acetate (X; R = Ac) identical with the compound obtained originally by Ruzicka, Cohen, Furter, and Sluys-Veer (56) by prolonged treatment of acetylketo-oleanolic acid (VIII; R = Ac, R' = CO_2H) with boiling quinoline. The ease of decarboxylation of the dienone-acid indicates that it is a βγ-unsaturated acid and that it is correctly formulated as (IX; R = Ac, R' = = CO_2H). Barton and Holness (37) have recently reported that the alkali isomerisation of methyl acetylketo-oleanolate involves inversion at C_{18}.

It was found that treatment of β-amyrenonyl benzoate with strong alcoholic potassium hydroxide gives in high yield an isomeric β-amyrenonol which shows the characteristic light-absorption properties of an αβ-unsaturated ketone and was characterised by the formation of its acetate. The reactions described below established that this αβ-unsaturated keto-acetate is 18-<u>iso</u>-β-amyrenonyl acetate (XI; R = Ac). 18-<u>iso</u>-β-Amyrenonyl acetate was recovered unchanged after treatment with bromine in acetic acid under conditions which led to the conversion of β-amyrenonyl acetate or acetylketo-oleanolic acid into the corresponding conjugated dienones (IX). Catalytic reduction of 18-<u>iso</u>-β-amyrenonyl acetate at room temperature gives

in high yield an isomeric β-amyrin acetate, the relationship of which to β-amyrin acetate (I; R = Ac) was established by its easy oxidation with selenium dioxide to β-amyradienyl-II acetate. Of the two possible structures (XIII) and (XIV) previously considered for the last compound, first prepared by Ruzicka, Müller and Schellenberg (19) by the oxidation of β-amyrin acetate (I; R = Ac) with selenium dioxide, Barton and Brooks (41) have shown that the former is correct. The formation of β-amyradienyl-II acetate (XIII) by oxidation of both β-amyrin acetate and the isomeric β-amyrin acetate described above proves that the last compound is 18-iso--β-amyrin acetate (XII; R = Ac).

Oxidation of 18-iso-β-amyrin acetate (XII; R = Ac) with hydrogen peroxide in acetic acid gives a saturated ketone, 18-iso-β-amyranonyl acetate (XV; R = Ac), alkaline hydrolysis of which gives the corresponding alcohol, 18-iso-β-amyranonol (XV; R = H). 18-iso-β-Amyranonyl acetate (XV; R = Ac) is not isomerised by either mineral acid or strong alkali, and represents the sterically stable isomer in so far as the orientation at C_{18} is concerned. Treatment of 18-iso-β-amyranonyl acetate with bromine gives bromo-18-iso-β-amyranonyl acetate (probably XVII).

This is considerably more stable than the isomeric bromo--β-amyranonyl acetate, obtained by similar bromination of

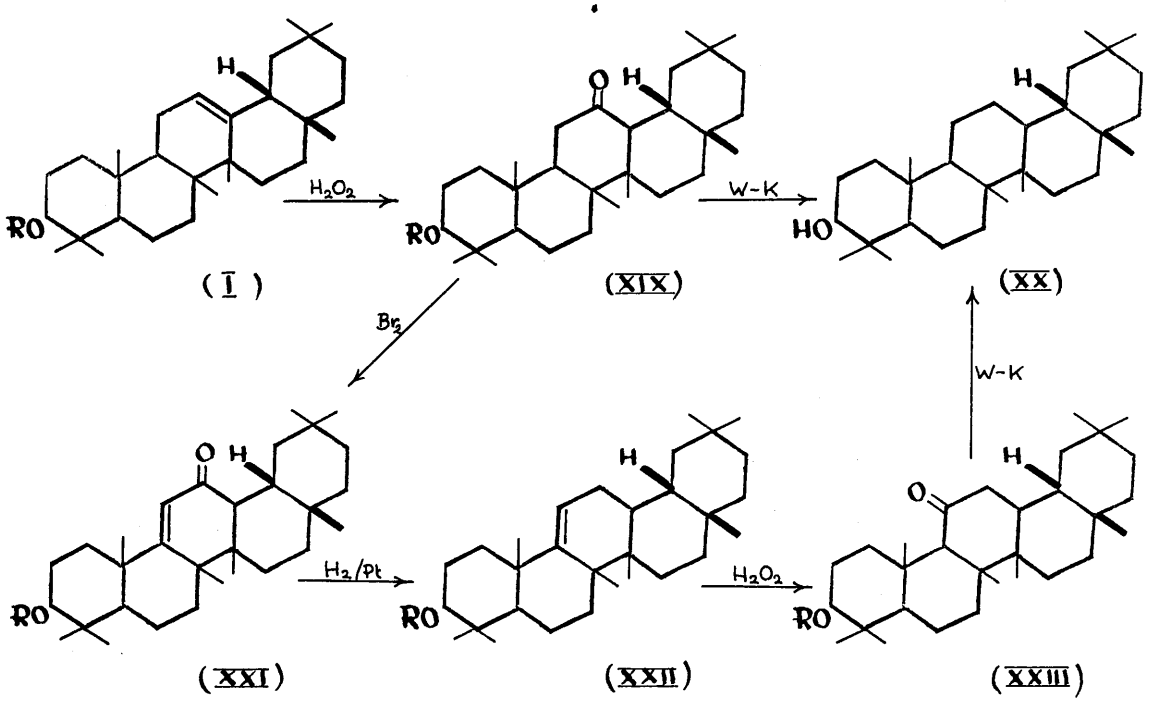


(XVII) (XVIII)

β-amyranonyl acetate, which readily loses hydrogen bromide when warmed with acetic acid, giving iso-β-amyrenonyl acetate (45); bromo-18-iso-β-amyranonyl acetate is recovered unchanged after prolonged heating in glacial acetic acid. Slow dehydrobromination, however, takes place on prolonged heating in pyridine, with the formation of an αβ-ketone showing a light-absorption maximum at 2410 Å (XVIII).

Reduction of 18-iso-β-amyranonyl acetate (XV; R = Ac) by the Wolff-Kishner method, followed by acetylation, gives 18-iso-β-amyranyl acetate (XVI; R = Ac), hydrolysis of which gives 18-iso-β-amyranol (XVI; R = H) which is different from lupanol and also from β-amyranol (XX) prepared as described by Ruzicka by the steps (I) → (XIX) → (XX) or by Budziarek, Johnston, Manson and Spring (61)

by the steps (I) → (XIX) → (XXI) → (XXII) → (XXIII) → (XX).



(I) (XIX) (XX) (XXI) (XXII) (XXIII)

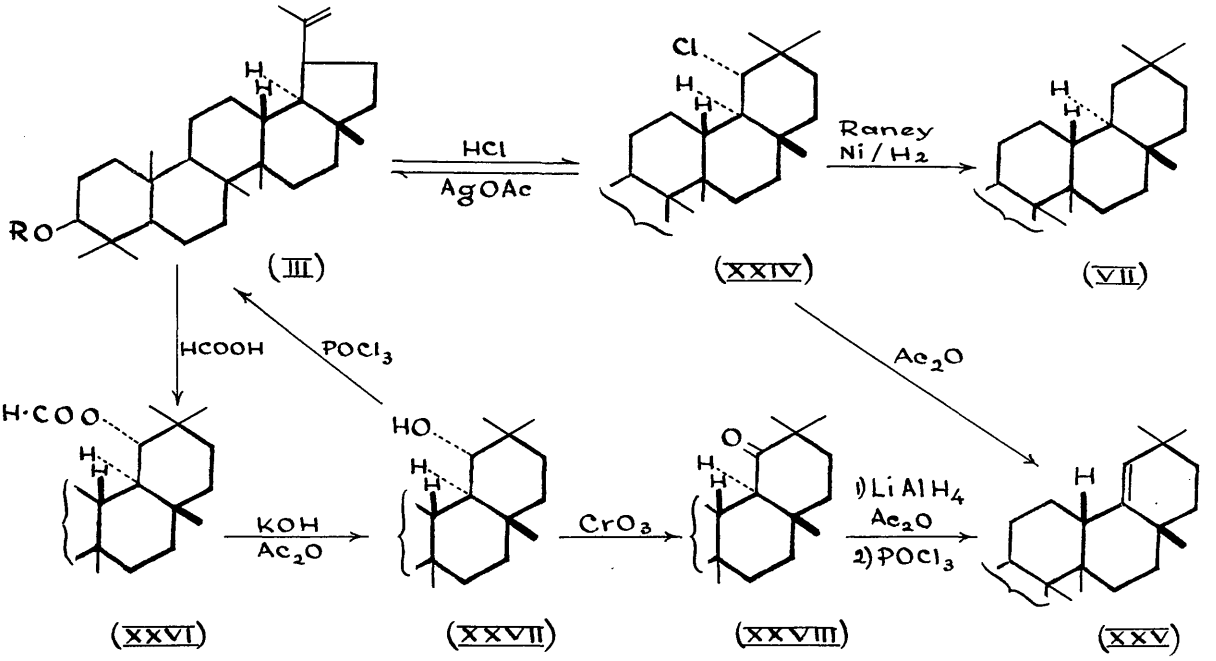
The constants of β-amyranol (44), lupanol (57), and taraxastanol (heterolupanol) (58) are shown below, together with those of 18-iso-β-amyranol (this work).

	Alcohol		Acetate	
	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$
β-Amyranol	186-186.5°	+18.5°	284.5-285°	+21°
Lupanol	201-202	-17.8	245 - 246	-1.8
Taraxastanol	218-220	+11	262 - 263	+23
18-iso-β-Amyranol	229-230	+36	280 - 282	+44

The orientation at C_{18} in the two isomers, β-amyranol and 18-*iso*-β-amyranol, in each case represents the sterically stable configuration since neither β-amyranol nor 18-*iso*-β-amyranonol is isomerised at C_{18} under strongly acid or alkaline conditions. There is furthermore a strong *prima facie* case for a common C_{18}-configuration in β-amyranol, 18-*iso*-β-amyranol, germanicol [cf. the conversion of siaresinolic acid into morolic acid [Barton, Brooks and Holness (41)], and lupeol [cf. the conversion of betulin into moradiol diacetate by Davy, Halsall and Jones (47)]. This was later confirmed by Ames, Davy, Halsall, Jones and Meakins (59) who obtained 18-*iso*-β-amyranol (VII) from lupeol (III), and a further relationship between β-amyrin and lupeol, confirming the stereochemistry of rings D and E, is thus established.

Duerden, Heilbron, McMeeking and Spring (60) described in 1939 the formation of a hydrochloride from lupeol and its conversion into the acetate of an isomeric alcohol - the so-called *iso*-lupeol. Re-investigation of this reaction by Jones and his co-workers (59) has shown that *iso*-lupeol is, in fact, germanicol (XXV); the hydrochloride can be represented by (XXIV) from a consideration of the reactions indicated in the chart. [Formic acid adds on to lupeol in

the same manner as hydrogen chloride and the addition product (XXVI) has been converted into lupenyl acetate and germanicyl acetate (59). The infra-red spectrum of the intermediate ketone (XXVIII) shows a band at 1700 cm.$^{-1}$ characteristic of a carbonyl group on a six--membered ring. This proves that addition of formic acid to lupeol is accompanied by ring enlargement analogous to that postulated during the addition of hydrogen chloride].



This formulation (XXIV) for the hydrochloride implies structure (VII) for its reduction product, which should be 18-iso-β-amyranol provided β-amyrin is (I). The constants of the alcohol and its acetate were identical with those reported by Budziarek, Manson, and Spring (63).

Mixed m.p. determinations of 18-iso-β-amyranol and its acetate with the specimens supplied by Professor E.R.H. Jones confirmed the identity of the compounds. The hydrogen atom at C_{18} in 18-iso-β-amyranol must therefore be cis to the methyl group at C_{17} as in lupeol.

The method of formation of 18-iso-β-amyranol from β-amyrin indicates a trans D/E ring fusion. Hence lupeol hydrochloride and, in turn, lupeol, which can be regenerated from the hydrochloride, have a trans D/E ring junction, which confirms the conclusions reached by Davy, Halsall and Jones (47). A consideration of the mechanism of the formation of lupeol from its hydrochloride also indicates that its iso-propenyl group is trans to the methyl group at C_{17}.

The non-identity of 18-iso-β-amyranol and lupanol leads to the conclusion that the acetone obtained by oxidation of β-amyrin acetate originates in the gem-dimethyl group in ring E.

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EXPERIMENTAL

Melting Points are corrected.

Specific rotations were determined in chloroform solutions (unless otherwise stated) in a 1-dm. tube at room temperature.

Ultra-violet absorption spectra were measured in absolute ethanol solution (except where otherwise stated) with a Unicam SP. 500 spectrophotometer.

Micro- analyses were by Dr. A.C. Syme and Mr. Wm. McCorkindale, to whom grateful acknowladgements are due.

For chromatography, activated alumina (supplied by Savory and Moore), Grade II (except where stated) standardised according to Brockmann, was employed.

SECTION I.

Isolation of β-Amyrin Benzoate from Manila Elemi Resin.

The solid material obtained from Manila Elemi resin (350 g.) after steam distillation to remove volatile oils, was dried and dissolved in ether (1000 c.c.). This ethereal solution was then washed with sodium hydroxide solution (10%) until all the acids were removed, with hydrochloric acid (10%), and finally with water. It was dried over sodium sulphate. The solid residue obtained after removal of ether was crystallised once from ethanol, dried at 100°, and finally powdered giving a crude mixture of α- and β-amyrin (200 g.; m.p.160-170°).

The crude mixed amyrins (200 g.) were dissolved in pyridine (120 c.c.) and benzoyl chloride (140 c.c.) was added dropwise to the stirred solution over 30 minutes at 100°. After the addition, the reaction mixture was heated on the steam bath with stirring for six hours. The dark red mixture was cooled, diluted with benzene (600 c.c.), and washed twice with hydrochloric acid (5%), once with sodium hydroxide (5%) and twice with salt solution (2%). The benzene solution was then dried over sodium sulphate and concentrated to 300 c.c. Hot ethanol was added to the boiling solution until faintly turbid, and on cooling

a crystalline solid separated. This was washed with cold ethanol and dried at 100°.

The crude mixed benzoates were then extracted with ether (four times) until the melting point of the undissolved solid was above 210°. This residue, after repeated crystallisation from methanol-chloroform gave pure β-amyrin (30 g.) as plates, m.p.233-235°, $[\alpha]_D$ +100° (c, 2.5).

The ethereal washings, on evaporation to dryness, gave a solid residue which after repeated crystallisations from methanol-chloroform gave α-amyrin benzoate (70 g.) as prismatic needles, m.p.195-196°, $[\alpha]_D$ +95° (c, 2.1).

β-Amyrin (2-Hydroxyolean-12-ene).

A solution of β-amyrin benzoate (40 g.; m.p.230-232°) in benzene (200 c.c.) and ethanolic potassium hydroxide (4%; 1000 c.c.) was heated under reflux for 12 hours. It was then concentrated until solid began to separate and poured into water (2 l.). The crystalline solid was collected, washed with water and dried (32 g.; fine needles, m.p.184-187°). A sample was crystallised three times from methanol-chloroform to give β-amyrin as prismatic needles, m.p.197-198°, $[\alpha]_D$ +88° (c, 2.3).

β-Amyrin Acetate (2-Acetoxyolean-12-one).

A solution of β-amyrin (32 g.; m.p.184-187°) in pyridine (100 c.c.) and acetic anhydride (150 c.c.) was heated on the steam bath for 2 hours during which time the acetate separated as prismatic needles. The mixture was cooled, the crystals collected, washed with methanol and dried (32 g.; m.p.238-239°). Recrystallisation from methanol-chloroform gave β-amyrin acetate as prismatic needles, m.p.241-242°, $[\alpha]_D$ +80° (c, 3.2). It gives a yellow colour with tetranitromethane in chloroform.

β-Amyranonyl Acetate (2-Acetoxyoleanan-12-one).

A solution of β-amyrin acetate (30 g.; m.p.240-241°) in glacial acetic acid (1500 c.c.) was treated at 100° with a mixture of hydrogen peroxide (100 vol.; 200 c.c.) in glacial acetic acid (200 c.c.) added dropwise during 30 minutes with stirring. Stirring was continued for 2 hours at 100° and the solution again treated with hydrogen peroxide (100 vol.; 50 c.c.) in acetic acid (50 c.c.) during 15 minutes. The solution was kept at 100° for 1 hour and then boiling water was added with vigorous stirring until the mixture became opalescent. The crystalline solid separating overnight was collected, washed with methanol and dried (plates, m.p.293-294°; 15.5 g.). Two

crystallisations from methanol-chloroform gave β-amyranonyl acetate as plates, m.p.299-300°, $[\alpha]_D$ -15° (c, 1.3). The mother-liquor was heated to 100°, then treated with hot water until opalescent and a second crop isolated (plates, m.p.280-282°; 4.0 g.). This was dissolved in benzene (100 c.c.) and purified by chromatography on an alumina column (Grade II, 10 x 2 cm.). Washing with the same solvent (300 c.c.) gave an eluate (2.5 g.) which when recrystallised from methanol-chloroform yielded β-amyranonyl acetate as plates, m.p.300-301° (K) (299-300° in an open capillary), $[\alpha]_D$ -15° (c, 2.2) (Found: C,79.2; H,10.9. Calc. for $C_{32}H_{52}O_3$: C,79.3; H,10.8%). Light absorption: Maximum at 2900 Å ($\mathcal{E}$ = 63; log ε = 1.8). It does not give a colour with tetranitromethane in chloroform.

β-Amyranonol (2-Hydroxyoleanan-12-one).

A solution of β-amyranonyl acetate (840 mg.) in benzene (10 c.c.) and alcoholic potassium hydroxide (3%; 25 c.c.) was refluxed for 5 hours. The solution was concentrated and the product precipitated with water. It was collected (0.77 g.) and crystallised three times from acetone giving β-amyranonol(500 mg.) as prisms, m.p.207-208°, $[\alpha]_D$ -26° (c, 2.8) (Found: C,81.3; H,11.6. Calc. for

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

Reacetylation of the alcohol (400 mg.) using pyridine (3 c.c.) and acetic anhydride (2 c.c.) at 100°, gave β-amyranonyl acetate which crystallised from methanol--chloroform as plates (320 mg.), m.p.300-301°, $[\alpha]_D$ -15.3°, -15.1° (c, 5.8, 2.5).

Investigation of the amorphous solid obtained from the H_2O_2 oxidation of β-amyrin acetate (Acetate, $C_{32}H_{52}O_4$).

After removal of the two crystalline crops of β-amyranonyl acetate from the hydrogen peroxide oxidation mixture, further dilution with water gave an amorphous solid. The liquor was poured into water and the solid collected. Attempted crystallisation of this solid was not successful. A solution of the dry amorphous solid (19 g.; obtained from the oxidation of 50 g. of β-amyrin acetate) in light petroleum (b.p. 60-80°)-benzene (3:1; 500 c.c.) was filtered through a column of activated alumina (Grade II/III, 40 x 3.5 cm.), and the column eluted with light petroleum-benzene (3:1):

Chromatogram I

Fr.	Solvent		Eluate			M.p.
1.	petrol-benzene, (3:1)	400 c.c.	0.15g.	cryst.	solid	200-260
2.	"	250	3.44	"	"	220-250
3.	"	150	0.65	"	"	220-245
4.	"	150	0.38	"	"	220-255
5.	"	200	0.32	"	"	220-240
6.	"	200	0.32	"	"	230-255
7.	"	250	0.29	"	"	230-245
8.	"	250	0.25	"	"	230-250
9.	"	300	0.24	"	"	232-245
10.	"	450	0.19	"	"	235-240
11.	"	550	0.21	"	"	225-235
12.	"	800	0.28	"	"	200-220
13.	(1:1)	400	0.24	"	"	180-210
14.	"	600	0.21	"	"	180-200
15.	"	600	0.17	"	"	180-200
16.	benzene	500	0.25	yellow	resin	
17.	"	550	0.18	"	"	
18.	"	600	0.09	"	"	
19.	"	800	0.08	"	"	
20.	"	800	0.07	"	"	
			7.91 g.			

Further elution of the column with benzene-ether, ether, and ether-alcohol gave a yellow resin which did not crystallise.

Fraction 1 was discarded.

Fraction 2, thrice recrystallised from ethanol, gave β-amyranonyl acetate (1.0 g.), m.p.290-292°, $[\alpha]_D$ -15° (c, 1.8), showing no depression of m.p. when mixed with an authentic specimen. The mother liquors were evaporated to dryness and the solid re-chromatographed as described under "Chromatogram II".

Fractions 3-6, crystallised individually from ethanol, gave prismatic needles, m.p.270-275°. The crystals were combined and recrystallised three times from methanol, giving an acetate as prismatic needles (0.7 g.), m.p.289-290°, $[\alpha]_D$ +29° (c, 0.6) (Found: C,76.5, 76.4; H,10.5, 10.4. $C_{32}H_{52}O_4$ requires C,76.75; H,10.5%). A mixture with β-amyranonyl acetate had m.p.263-273°. This acetate does not give a colour with tetranitromethane in chloroform or with aqueous alcoholic ferric chloride. It does not exhibit selective absorption of high intensity in the ultra-violet region above 2200 Å.

The acetate was recovered unchanged after treatment (a) with acetic acid - dry hydrogen bromide at room

temperature for 3 days,

(b) in benzene-acetic acid (1:9) with chromic anhydride (1.5 atoms of O) in acetic acid for 20 hours at room temperature, or

(c) with bromine in acetic acid at 80°.

Fractions 7-11, treated individually, crystallised from ethanol as needles, m.p.240-260°. Further purification by crystallisation was not successful. This was re-chromatographed as described under "Chromatogram III".

Fractions 12-15 gave white amorphous solid from methanol.

Attempted purification of other fractions from the chromatogram did not give homogeneous material.

Chromatogram II.

A solution of the dry solid (2.4 g.) from fraction 2 (after removal of β-amyranonyl acetate) in light petroleum-benzene (10:1; 100 c.c.) was filtered through a column of activated alumina (Grade II; 20 x 2 cm.), the column being eluted as follows:

Fr.	Solvent		Eluate	
1.	light petrol-benzene	(10:1) 200 c.c.	0.31 g.	crystalline solid
2.	"	250	0.22	" "
3.	"	250	0.12	" "
4.	"	250	0.06	" "
5.	"	300	0.05	" "
6.	"	300	0.12	" "
7.	"	300	0.12	" "
8.	"	300	0.10	" "
9.	"	(3:1) 250	0.18	" "
10.	"	250	0.07	" "
11.	"	250	0.05	" "
12.	benzene	250	0.32	" "
13.	"	250	0.11	" "
14.	"	250	0.04	" "
15.	benzene-ethanol	(10:1) 250	0.30	yellow resin
16.	"	800	0.10	" "
			2.31 g.	

Fractions 1-5 gave mixed crystals from ethanol (m.p. 192-206°) and were discarded.

Fractions 6-14 crystallised from methanol as needles, m.p. < 280°. Two recrystallisations from methanol gave prismatic needles, m.p.288-289°, showing no depression of

m.p. when mixed with the acetate, m.p.289-290°, isolated from chromatogram I.

Fractions 15 and 16 gave white amorphous solid from methanol.

Chromatogram III

The mother-liquors from fractions 3-6 were evaporated to dryness and the solid combined with the mixed crystals from fractions 7-11. A solution of this material (1.8 g.) in light petroleum-benzene (3:1; 150 c.c.) was filtered through a column of activated alumina (Grade I/II; 28 x 2.5 cm.), the column being eluted as follows:

Fr.	Solvent		Eluate	
1-5	light petrol-benzene	(3:1) 1500 c.c.	Nil	
6-12	"	(1:1) 2300	Nil	
13.	benzene	300	Trace	crystalline solid
14.	"	300	0.02 g.	" "
15.	"	300	0.05	" "
16.	"	300	0.09	" "
17.	"	300	0.12	" "
18.	"	300	0.07	" "
19.	"	300	0.05	" "
20.	"	300	0.05	" "
21.	"	300	0.03	" "

Fr.	Solvent		Eluate	
22.	benzene	300 c.c.	0.02 g.	crystalline solid
23.	benzene-ether (3:1)	300 c.c.	0.32 g.	crystalline solid
24.	"	300	0.26	" "
25.	"	300	0.02	" "
			1.10 g.	

Further elution of the column with ether and ether--alcohol gave a yellow resin which did not crystallise.

Fractions 14-22 crystallised from methanol as large, prismatic needles, m.p.291-292°, undepressed in m.p. when mixed with the acetate m.p.289-290° from chromatogram I.

Fractions 23-25 gave mixed crystals from methanol, m.p.236-240°, showing no colour with tetranitromethane in chloroform.

Alcohol, $C_{30}H_{50}O_3$.

(a) A solution of the acetate m.p.288-289°, $[\alpha]_D$ +29° (200 mg.) in ethanolic potassium hydroxide (3%; 10 c.c.) was refluxed for 3 hours. It was poured into water (50 c.c.) but no precipitate appeared. The product separated from the solution on addition of a few drops of dilute hydrochloric acid. It was extracted with ether, and the ethereal solution washed with water, dried (alkali free Na_2SO_4) and ether removed. Crystallisation of the

product from methanol gave prisms (170 mg.), m.p.247-249°, which after two recrystallisations from the same solvent had m.p.249-250°, $[\alpha]_D$ +20° (c, 1.4) (Found: C,75.65; 75.7; H,11.3, 11.4. $C_{30}H_{50}O_3.H_2O$ requires C,75.6; H,11.0%. $C_{30}H_{50}O_3.MeOH$ requires C,75.9; H,11.1%). The alcohol was sublimed at 180-200°, 10^{-3} mm. press. (m.p. 249-250°) (Found: C,77.4, 77.55; H,11.3, 11.2. $C_{30}H_{50}O_3$ requires C,78.4; H,11.0%). It was resublimed twice at (a) 210-230°, 10^{-2} mm. press., and (b) 220-240°, 10^{-2} mm. press. (m.p.249-250°) (Found: C,77.7; H,11.1. $C_{30}H_{50}O_3$ requires C,78.4; H,11.0%). When heated in open capillary (at about 190°) or in a vacuum, the substance disintegrates with loss of solvent which is complete only after three sublimations). The alcohol does not exhibit selective absorption of high intensity in the ultra-violet region and does not show a colour with tetranitromethane in chloroform.

It does not dissolve in cold or hot aqueous potassium hydroxide (20%). It dissolves in boiling alcoholic potassium hydroxide (3%) but precipitates on addition of water (m.p. being unchanged). The alcohol was recovered unchanged after treatment (a) with diazomethane in ether at room temperature for 2 hours, (b) with dimethyl sulphate in alkaline solution.

(b) A solution of the acetate (70 mg.) in ethanolic potassium hydroxide (3%; 4 c.c.) was refluxed for 4 hours. It was poured into water (80 c.c.; no precipitate) and the clear alkaline solution extracted with ether (4 times). The ethereal solution was washed with water, dried (Na_2SO_4) and evaporated. The residue (25 mg.) crystallised from methanol, giving the alcohol as prisms, m.p.246-248°, undepressed in m.p. when mixed with the alcohol m.p.249-250° from experiment (a). Working up the aqueous solution as under (a) gave a further quantity of the alcohol (30 mg.).

(c) A solution of the acetate (50 mg.) in ethanolic potassium hydroxide (3%; 3 c.c.) was boiled for 2 minutes. On addition of water, a white precipitate appeared which was collected, washed and dried. Crystallisation from methanol gave the alcohol as prisms, m.p.245-247°, undepressed in m.p. when mixed with an authentic specimen.

Reacetylation of the alcohol (50 mg.) using pyridine (0.5 c.c.) and acetic anhydride (0.5 c.c.) (at 100°) gave the acetate $C_{32}H_{52}O_4$ as prismatic needles (40 mg.) (from methanol), m.p.292-293°, $[\alpha]_D$ +28° (c, 1.5), undepressed in m.p. when mixed with the acetate described above. (Test for acid: The ethereal solution of the pure acetate was shaken twice with sodium carbonate solution (3%), which

was then acidified with dilute hydrochloric acid. No precipitate was obtained).

iso-β-Amyrenonyl Acetate (2-Acetoxyolean-10-ene-12-one).

(a) A solution of β-amyranonyl acetate (3 g.) in glacial acetic acid (250 c.c.) was treated with two drops of 40% aqueous hydrogen bromide and a mixture of bromine in glacial acetic acid (8%; 1.1 mols) added dropwise during 15 minutes at 50° with stirring. Stirring was continued for 2 hours at 50-60°, the mixture was then heated to 90° and hot water added (with stirring) until a permanent separation of a crystalline solid occurred. The crystalline solid which separated on cooling was collected, washed with methanol and dried (plates (2.2g.), m.p.284-286°). Recrystallisation from acetone (or methanol) gave iso-β-amyrenonyl acetate as hexagonal plates, m.p.289-290°, $[\alpha]_D$ +61°, +60° (c, 1.2, 2.2) (Found: C,79.6; H,10.4. Calc. for $C_{32}H_{50}O_3$: C,79.6; H,10.45%). Light absorption: Maximum at 2470 Å (ε = 10,600). It does not show a colour with tetranitromethane in chloroform and the m.p. is undepressed when mixed with β-amyranonyl acetate.

A solution of a sample of iso-β-amyrenonyl acetate (1.0 g.) in light petroleum-benzene (2:1; 100 c.c.) was

filtered through a column of activated alumina (Grade II; 2 x 12 cm.) and the column eluted with the same mixture of solvents. Evaporation of the solvent (350 c.c.) gave a crystalline solid (0.94 g.; m.p.289-290°) which crystallised from acetone as hexagonal plates, m.p.290-291°, $[\alpha]_D$ +61° (c, 2.3), undepressed in m.p. with the sample described above.

(b) A solution of β-amyranonyl acetate (17 g.) in glacial acetic acid (1.5 l.) was treated with a mixture of bromine in acetic acid (8%; 1.2 mols.) added dropwise during 30 minutes at 55-60° with stirring as before. The solution was maintained at the same temperature for 4 hours. Working up gave bromo-β-amyranonyl acetate (13 g.), m.p.270-272° (decomp.). The product was dissolved in glacial acetic acid and heated on the steam bath for 3 hours with stirring, hydrogen bromide being evolved. Working up as in experiment (a) gave iso-β-amyrenonyl acetate (11 g.), m.p.289-290°, $[\alpha]_D$ +62° (c, 1.4).

iso-β-Amyrenonyl acetate was recovered unchanged after refluxing its solution in 15% ethanolic potassium hydroxide for 70 hours.

iso-β-Amyrenonol (2-Hydroxyolean-10-ene-12-one).

A solution of iso-β-amyrenonyl acetate (800 mg.) in

benzene (5 c.c.) and ethanolic potassium hydroxide (3%; 30 c.c.) was heated under reflux for 5 hours. The solution was concentrated and diluted with water. Isolation by means of ether gave a product, which crystallised from methanol to give iso-β-amyrenonol (660 mg.) as prisms, m.p.249-250°, $[\alpha]_D$ +57° (c, 1.6) (Found: C,82.2; H,10.7. Calc. for $C_{30}H_{48}O_2$: C,81.75; H,11.0%). Light absorption: Maximum at 2460 Å (ε = 10,000). It does not show a colour with tetranitromethane in chloroform.

Acetylation of the alcohol (100 mg.) using pyridine (1 c.c.) and acetic anhydride (0.5 c.c.) at 100° (2 hours) gave iso-β-amyrenonyl acetate (100 mg.) as hexagonal plates from acetone, m.p.290-291°, $[\alpha]_D$ +61.5° (c, 1.4).

Enol Acetate of β-Amyranonyl Acetate (Enol Acetate of 2-Acetoxyoleanan-12-one).

Following the method of Ruzicka and Jeger (44), a solution of β-amyranonyl acetate (500 mg.) in acetic anhydride (25 c.c.) containing freshly fused sodium acetate (500 mg.) was heated under reflux for 92 hours. The reaction mixture was poured into water and extracted with ether. The ethereal solution was washed with water and dried (Na_2SO_4). Removal of the ether gave a cyrstalline solid, which crystallised from methanol-chloroform

to give the enol acetate of β-amyranonyl acetate (450 mg.) as needles, m.p.240-241°, $[\alpha]_D$ +58°, +60° (c, 0.8, 1.4) (Found: C,77.5; H,10.4. Calc. for $C_{34}H_{54}O_4$: C,77.7; H,10.3%). It shows a yellow colour with tetranitromethane in chloroform and does not exhibit light absorption of high intensity above 2200 Å.

Enol Acetate of iso-β-Amyrenonyl Acetate (Enol Acetate of 2-Acetoxyolean-10-ene-12-one).

A solution of iso-β-amyrenonyl acetate (2.0 g.) in acetic anhydride (100 c.c.) containing freshly fused sodium acetate (1 g.) was heated under reflux for 72 hours. The reaction mixture was treated with water and extracted with ether. The extract was washed with water and dried (Na_2SO_4). After removal of the ether, the residue crystallised from methanol-chloroform to give the enol acetate of iso-β-amyrenonyl acetate (1.8 g.) as prismatic needles, m.p.217-218°, $[\alpha]_D$ +202° (c, 1.2) (Found: C,77.6; H,9.9. $C_{34}H_{52}O_4$ requires C,77.8; H,9.9%). Light absorption: Maximum at 2780 Å (ε = 8600). The enol acetate gives a red-brown colour with tetranitromethane in chloroform.

The enol ester group is easily hydrolysed, attempted purification by chromatography on alumina giving

iso-β-amyrenonyl acetate in quantitative yield.

Enol Benzoate of iso-β-Amyrenonyl Benzoate.

A solution of iso-β-amyrenonol (90 mg.) in pyridine (1 c.c.) was treated with 3 drops of benzoyl chloride, and the mixture heated at 100° for 4 hours. The product, isolated by means of ether in the usual manner, crystallised from acetone-methanol (1:1) as plates (65 mg.), m.p.225.5--227°. After four recrystallisations from methanol the enol benzoate was obtained as plates, m.p.235-235.5°, $[\alpha]_D$ +246° (c, 1.2) (Found: C,81.1; H,8.4. $C_{44}H_{66}O_4$ requires C,81.4; H,8.6%). Light absorption: Maxima at 2300 (ε = 31,000) and 2750 Å (ε = 11,000). The enol benzoate gives a red-brown colour with tetranitromethane.

iso-β-Amyrin Acetate (2-Acetoxyolean-10-ene).

Following the method of Jeger and Ruzicka (46) a solution of iso-β-amyrenonyl acetate (2.0 g.) in stabilised glacial acetic acid (300 c.c.) was added to a suspension of freshly reduced platinum oxide catalyst (200 mg.) in glacial acetic acid (10 c.c.) and the mixture shaken with hydrogen at 16° for 44 hours. The solution was filtered and poured into water. The product, isolated by means of ether, crystallised from methanol-chloroform as plates (2.4 g.), m.p.250-251°, $[\alpha]_D$ +77° (c, 1.6) (Found: C,82.1;

H,11.4. Calc. for $C_{32}H_{52}O_2$: C,82.0; H,11.2%). Light absorption: Maximum at 2070 Å (ε = 3500). It gives a yellow colour with tetranitromethane in chloroform.

iso-β-Amyranonyl Acetate (2-Acetoxyoleanan-11-one).

Following the method of Jeger and Ruzicka (46) a solution of iso-β-amyrin acetate (0.7 g.) in glacial acetic acid (100 c.c.) was heated under reflux and treated with a solution of hydrogen peroxide (7 c.c.; 100 vols.) in glacial acetic acid (7 c.c.) added dropwise during 10 minutes. The solution was then refluxed gently for 1 hour when a further quantity of hydrogen peroxide-acetic acid solution (10 c.c.) was added. The solution was refluxed for a further 2 hours and on cooling the product crystallised from the solution as plates (0.25 g.), m.p. 327-332°. Recrystallisation from methanol-chloroform gave plates, m.p.338-339°, $[\alpha]_D$ +7° (c, 1.3) (Found: C,79.0; H,10.7. Calc. for $C_{32}H_{52}O_3$: C,79.3; H,10.8%). Light absorption: Maximum at 2800 Å (ε = 200). It does not show a colour with tetranitromethane in chloroform.

β-Amyranyl Acetate (2-Acetoxyoleanan). (With J.D. Johnston)

A solution of iso-β-amyranonyl acetate (250 mg.) and hydrazine hydrate (100%; 2 c.c.) in ethanolic sodium ethoxide (7.5%; 10 c.c.) was kept at 200-210° for 18 hours

in an autoclave. The acetylated reaction product (85 mg.) was dissolved in light petroleum (b.p. 60-80°)-benzene (2:1; 50 c.c.) and filtered through a column of activated alumina (Grade II, 10 x 1.25 cm.). Washing with the same solvent mixture (50 c.c.) gave a fraction (30 mg.), crystallisation of which from methanol-chloroform yielded β-amyranyl acetate as plates, m.p.282-284°, $[\alpha]_D$ +24° (c, 0.5), undepressed when mixed with a specimen prepared by Wolff-Kishner reduction of β-amyranonyl acetate as described by Ruzicka and Jeger (44).

β-Amyradiendionyl Acetate (2-Acetoxyolean-10:13(18)-dien--12:19-dione).

(a) Three sealed glass tubes, each containing β-amyrin acetate (500 mg.), selenium dioxide (800 mg.) and dioxan (25 c.c.), were heated in a furnace at 200-210° for 22 hours. The mixture was filtered through sintered glass, diluted with water and the product extracted with ether. The extract was shaken several times with potassium cyanide solution (3%), water and dried (Na_2SO_4). Removal of the ether gave a residue, which was taken up in methanol and the solution decolorised by boiling with charcoal. Crystallisation from methanol gave β-amyradiendionyl acetate as square plates (1.2 g.), m.p.237-238°, $[\alpha]_D$ -94° (c, 1.7)

(Found: C,77.5; H,9.5. Calc. for $C_{32}H_{46}O_4$: C,77.7; H,9.4%). Light absorption: Maximum at 2760 Å (ε = 11,200). It gives no colour with tetranitromethane in chloroform.

(b) A solution of β-amyrin acetate (1.0 g.) in benzyl acetate (5 c.c.; b.p.216°) was refluxed with selenium dioxide (1.6 g.) for 19 hours. Benzyl acetate was removed by steam distillation, and the dark-brown residue dissolved in ether. Working up as before gave a product, which crystallised from methanol to give β-amyradiendionyl acetate as square plates (250 mg.), m.p.237-238°, $[\alpha]_D$ -93° (c, 1.5), undepressed in m.p. when mixed with the specimen described under (a). Light absorption: Maximum at 2760 Å (ε = 11,000).

Clemmensen Reduction of β-Amyradiendionyl Acetate.

A hot solution of β-amyradiendionyl acetate (500 mg.) in glacial acetic acid (50 c.c.) was treated with concentrated hydrochloric acid (10 c.c.) and added to freshly amalgamated zinc (from 15 g. of zinc), and the mixture heated under reflux for 90 minutes. The product, isolated by means of ether, crystallised from methanol as elongated plates (130 mg.), m.p.190-200°. Four recrystallisations from the same solvent gave β-amyradienyl-II

acetate as plates, m.p.217-220°, $[\alpha]_D$ -62° (c, 1.3), undepressed in m.p. when mixed with an authentic specimen (m.p.227-228°) prepared by selenium dioxide oxidation of β-amyrin acetate. Light absorption: Maxima at 2510 Å (ε = 26,000), 2430 Å (ε = 23,000), 2600 Å (ε = 16,000). It gives a red-brown colour with tetranitromethane in chloroform.

Chromatography of the residue (360 mg; obtained by evaporation of the mother liquors) on alumina gave a further quantity (170 mg.) of β-amyradienyl-II acetate (from light petroleum).

iso-β-Amyradienonyl Acetate.

Selenium dioxide method. (cf. Green, Mower, Picard, and Spring, J.,1944,527).

(a) iso-β-Amyrenonyl acetate (7.5 g.) in glacial acetic acid (200 c.c.) was refluxed with powdered selenium dioxide (8 g.) for 24 hours. The hot, pale yellow mixture was filtered (sintered glass) and the filtrate diluted with water. The solid was collected, washed with water and dried. A solution of the solid in ether was washed with 5% aqueous potassium cyanide, then with water, dried, and freed from ether, and the solid crystallised from methanol (or acetone) as long prisms (5.0 g.) m.p.218-219°. Recrystallisation gave iso-β-amyradienonyl

acetate as prisms, m.p.220-221°, $[\alpha]_D$ -39°, -40° (c, 1.1, 1.3), not altered in crystalline form or in m.p. by further crystallisation. After sublimation at 180-190°/ /10^{-3} mm. press., iso-β-amyradienonyl acetate had m.p. 221-222°, $[\alpha]_D$ -39° (c, 1.1) (Found: C,80.0; H,9.9. Calc. for $C_{32}H_{48}O_2$: C,79.95; H,10.1%). Light absorption: Maximum at 2450 Å (ε = 11,200). It gives a yellow colour with tetranitromethane in chloroform.

(b) In another experiment, the reaction product separated as plates, m.p.212-213°, from alcohol. When this material was recrystallised from acetone, large, hexagonal plates separated which were kept overnight in contact with its mother-liquor. The plates had then changed into prisms, m.p.215-217°. Recrystallisation from the same solvent gave iso-β-amyradienonyl acetate as prisms, which when dried for a week in a vacuum over phosphoric oxide at 135° had m.p.220-221°, $[\alpha]_D$ -40° (c, 1.4). Light absorption: Maximum at 2450 Å (ε = 11,100).

After chromatography of its benzene solution on alumina, the m.p. and the rotation were unchanged.

Bromine method.

(a) iso-β-Amyrenonyl acetate (1.0 g.) in glacial acetic acid (60 c.c.) was treated dropwise with a solution

of bromine in acetic acid (8%; 1.1 mols.) and 2 drops of a solution of hydrogen bromide in acetic acid during 15 minutes at 85° with stirring. The solution was maintained at 80° for 3 hours, and then hot water was added with stirring until the mixture became faintly opalescent. The crystalline solid which separated on cooling was unchanged starting material (0.47 g.; m.p.286-288°, $[\alpha]_D$ +59° (c,1.7) undepressed in m.p. when mixed with iso-β-amyrenonyl acetate). The mother-liquor was diluted with water and the precipitate extracted with ether. Removal of the ether gave a crystalline solid which crystallised from acetone to give iso-β-amyradienonyl acetate (0.22 g.) as prisms, m.p.215-217°, $[\alpha]_D$ -38° (c, 1.0), showing no depression of m.p. when mixed with an authentic sample. Light absorption: Maximum at 2450 Å (ε = 10,100).

(b) β-Amyranonyl acetate (1.9 g.) in glacial acetic acid (150 c.c.) was treated with a solution of bromine in glacial acetic acid (8%; 2.1 mols.) during 5 minutes at 90°. The solution was kept at 90° for 1 hour and then overnight at room temperature. The mixture was diluted with water, and the solid collected, washed, dried, and crystallised from acetone. The first crop (0.34 g.) separated as plates, m.p.278-280°, which did not give a colour with tetranitromethane in chloroform and proved to

be iso-β-amyrenonyl acetate. The second crop consisted of prisms contaminated with a small number of plates. The two forms were separated mechanically into plates (0.03 g.), m.p.215-255°, giving no colour with tetranitromethane in chloroform, and prisms (0.69 g.), m.p.207-209°, $[\alpha]_D$ -25° (c, 2.1), which gave a yellow colour with tetranitromethane. Recrystallisation of the prisms from acetone gave prisms (0.56 g.), m.p.214-217°. A solution of this solid (0.53 g.), in light petroleum-benzene (5:1; 100 c.c.) was filtered through a column of activated alumina (Grade II; 15 x 2 cm.). Light petroleum-benzene (4:1; 500 c.c.) eluted iso-β-amyradienonyl acetate (400 mg.) which after crystallisation from acetone (prisms) followed by sublimation, had m.p.219-220°, $[\alpha]_D$ -40° (c, 2.0) (Found: C,80.0; H,9.7. Calc. for $C_{32}H_{48}O_3$: C,79.95; H,10.1%). Light absorption: Maximum at 2450 Å (ε = 11,000).

iso-β-Amyradienonol.

A solution of iso-β-amyradienonyl acetate (500 mg.) in ethanolic potassium hydroxide (3%; 50 c.c.) was heated under reflux for 3 hours. Isolation by means of ether gave a product which crystallised from methanol to yield iso-β-amyradienonol (410 mg.) as large, hard prisms,

m.p.241-242°, $[\alpha]_D$ -51° (c, 1.2) (Found: C,82.0; H,10.7. Calc. for $C_{30}H_{46}O_2$: C,82.1; H,10.6%). Light absorption: Maximum at 2450 Å (ε = 11,000). It gives a yellow colour with tetranitromethane in chloroform.

Reacetylation of iso-β-amyradienonol (50 mg.) using pyridine (1 c.c.) and acetic anhydride (0.3 c.c.) at 100° (5 hours) gave iso-β-amyradienonyl acetate (45 mg.) as prisms (from methanol), m.p.220-221°, $[\alpha]_D$ -40° (c, 1.0). Light absorption. Maximum at 2450 Å (ε = 12,100).

iso-β-Amyradienonyl Benzoate.

iso-β-Amyradienonol (80 mg.) in pyridine (1 c.c.) and benzoyl chloride (0.2 c.c.) was heated at 100° for 3 hours. The product, isolated by means of ether, crystallised from methanol to give iso-β-amyradienonyl benzoate (75 mg.) as prismatic needles, m.p.207-208°, $[\alpha]_D$ -23° (c, 0.8) (Found: C,81.5; H,9.0. $C_{37}H_{50}O_2$ requires C,81.9; H,9.3%). Light absorption: Maximum at 2320 Å (ε = 31,000). It gives a yellow colour with tetranitromethane in chloroform.

iso-β-Amyradienonyl Acetate was recovered unchanged after treatment with:

(a) dry hydrogen chloride at room temperature for 14 hours in chloroform, followed by acetylation;

(b) concentrated hydrochloric acid in glacial acetic acid at room temperature for 2 weeks;

(c) concentrated hydrochloric acid in glacial acetic acid under reflux for 1 hour;

(d) acetic anhydride and sodium acetate under reflux for 41 hours, and for 172 hours;

(e) acetic anhydride and sodium acetate at 180-200° for 68 hours;

(f) benzoyl chloride and pyridine at 100° for 10 hours;

(g) 15% ethanolic potassium hydroxide under reflux for 48 hours, followed by acetylation.

Clemmensen Reduction of iso-β-Amyradienonyl Acetate.

A hot solution of iso-β-amyradienonyl acetate (0.5 g.) in glacial acetic acid (40 c.c.) was treated with concentrated hydrochloric acid (10 c.c.) and added to freshly amalgamated zinc (from 15 g. of zinc), and the mixture heated under reflux for 30 minutes. The product, isolated in the usual manner, separated from acetic acid as plates (220 mg.), m.p.204-210°. It was recrystallised from acetone and then thrice from alcohol, to yield β-amyradienyl-II acetate as plates, m.p.227-228°, $[\alpha]_D$ -62° (c, 2.1) (Found: C,82.6; H,10.6. Calc. for $C_{32}H_{50}O_2$:

C,82.3; H,10.8%). It gives a red-brown colour with tetranitromethane in chloroform. Light absorption: Maximum at 2515 (ε = 28,200), 2435 (ε = 24,700), and 2600 Å (ε = 18,000). A mixture with a specimen (m.p. 227-228°) prepared by selenium dioxide oxidation of β--amyrin acetate had m.p.227-228°.

Catalytic Hydrogenation of iso-β-Amyradienonyl Acetate.

A solution of iso-β-amyradienonyl acetate (200 mg.) in glacial acetic acid (50 c.c.) was added to a suspension of freshly reduced platinum catalyst (from 100 mg. of platinum oxide) in glacial acetic acid (10 c.c.) and the mixture shaken with hydrogen at 16°. Absorption of hydrogen was at first rapid, and after 45 minutes, approximated to 1 mol. After 44 hours the apparent absorption of hydrogen was 3 mols. The product, isolated in the usual manner and crystallised repeatedly from methanol, gave neo-β-amyrin acetate (150 mg.) as blades, m.p.225-226°, $[\alpha]_D$ +5° (c, 2.0) (Found: C,82.0; H,11.2. $C_{32}H_{52}O_2$ requires C,82.0; H,11.2%). It gives an intense red-brown colour with tetranitromethane in chloroform and does not exhibit selective absorption above 2200 Å (Maximum at 2140 Å, ε = 3500).

Catalytic Hydrogenation of iso-β-Amyradienonol.

A solution of iso-β-amyradienonol (140 mg.) in glacial acetic acid (40 c.c.) was shaken with hydrogen over freshly pre-reduced platinum catalyst as described before. Isolation by means of ether gave a product which did not crystallise and showed a red-brown colour with tetranitromethane in chloroform. It was acetylated using pyridine and acetic anhydride at 100°. Isolation by means of ether gave a product which crystallised from methanol as blades (70 mg.), m.p.210-216°. A solution of this solid in light petroleum (10 c.c.) was filtered through a column of alumina (Grade II; 1 x 6 cm.) and the column washed with the same solvent. Evaporation of the first fraction (50 c.c.) gave a crystalline solid which crystallised from methanol to give neo-β-amyrin acetate as blades (40 mg.), m.p.224-225°, $[\alpha]_D$ +5° (c,1.1), undepressed in m.p. when mixed with a specimen described in the previous experiment. It gave a red-brown colour with tetranitromethane in chloroform.

neo-β-Amyrin acetate was recovered unchanged after treatment with:

(a) concentrated hydrochloric acid in glacial acetic acid under reflux for 1½ hours;

(b) dry hydrogen chloride in chloroform for 3 hours at room temperature;

(c) tetranitromethane in chloroform for 30 minutes at room temperature.

Lithium Aluminium Hydride Reduction of iso-β-Amyradienonyl Acetate.

A solution of iso-β-amyradienonyl acetate (400 mg.) in dry ether (50 c.c.) was added dropwise to a suspension of lithium aluminium hydride (300 mg.) in ether (40 c.c.). The mixture was gently refluxed for 4 hours after which time excess of water was cautiously added to decompose the reduction adduct and the excess of lithium aluminium hydride. Dilute sulphuric acid (10%) was then added until the mixture was just acid and the water layer clear. The ethereal solution was separated, washed with water till neutral to litmus, dried ($MgSO_4$), and evaporated. The product was acetylated by warming with acetic anhydride (0.5 c.c.) in pyridine (3 c.c.) for 3 hours. Isolation by means of ether gave a product, which crystallised from aqueous methanol to give the diacetate (360 mg.) as square plates, m.p.167-168°, $[\alpha]_D$ +25° (c, 1.0) (Found: C,78.0; H,10.05. $C_{34}H_{52}O_4$ requires C,77.8; H,10.0%). The diacetate gives a yellow colour with tetranitromethane in

chloroform and does not exhibit selective absorption of appreciable intensity in the ultra-violet region above 2200 Å. (Maximum at 2100 Å, ε = 6600).

Catalytic Hydrogenation of the Diacetate $C_{34}H_{52}O_4$.

A solution of the diacetate, m.p.167-168° (200 mg.) in glacial acetic acid (20 c.c.) was added to a suspension of a freshly reduced platinum catalyst (from 100 mg. of platinum oxide) in glacial acetic acid (100 c.c.) and the mixture shaken with hydrogen for 20 hours. The product was isolated in the usual manner and crystallised from methanol to give neo-β-amyrin acetate as blades (100 mg.), m.p.223-224°, $[\alpha]_D$ +5° (c, 1.0) (Found: C,81.7; H,11.0. $C_{32}H_{52}O_2$ requires C,82.0; H,11.2%), giving a red colour with tetranitromethane in chloroform and showing no selective absorption of high intensity above 2200 Å. A mixture with a specimen m.p.225° prepared by previous methods had m.p. 224-225°.

Hydrogen Peroxide Oxidation of iso-β-Amyradienonyl Acetate.

A solution of iso-β-amyradienonyl acetate (500 mg.) in glacial acetic acid (30 c.c.) was treated at 100° with a mixture of hydrogen peroxide (100 vol.; 30 c.c.) in glacial acetic acid (30 c.c.) added dropwise during 1 hour

with stirring. The solution was kept at 100° for 4 hours, diluted with water and the solid extracted with ether. Removal of the ether gave a residue which crystallised from methanol in plates (90 mg.), m.p.303-305°. Recrystallisation from methanol gave plates, m.p.304-305°, $[\alpha]_D$ +147° (c, 0.8), showing no colour with tetranitromethane in chloroform. Light absorption: Maximum at 2380 Å (ε = 10,800).

The mother liquors were evaporated to dryness, the residue (250 mg.) dissolved in benzene (30 c.c.) and the solution filtered through a column of alumina (Grade II; 1.5 x 10 cm.), the column being eluted as follows:

Fr.	Solvent		Eluate		M.p.
1.	benzene	50 c.c.	20 mg.	colourless resin	190°
2.	"	60	25	" "	190
3.	"	50	5	" "	190
4.	"	50	3	" "	
5.	"	60	2	" "	
6.	"	70	Trace	" "	
7.	benzene-ether (1:1)	50	70	" "	290
8.	"	50	7	" "	290
9.	"	50	3	" "	
10.	ether	50	Trace	" "	
11.	ethanol	50	8	" "	
12.	"	100	6	" "	
			150 mg.		

Fractions 1, 2 and 3 were crystallised from methanol four times giving plates, m.p.225-227°, $[\alpha]_D$ +80° (c, 0.5) (Found: C,74.7; H,9.5. $C_{32}H_{50}O_3$ requires C,74.7; H,9.8%. $C_{32}H_{48}O_3$ requires C,75.0; H,9.4%). The product does not exhibit light absorption of high intensity above 2200 Å. It does not show a colour with tetranitromethane in chloroform, or ferric chloride in alcohol.

Fraction 7 was crystallised twice from methanol to give hexagonal plates, m.p.308-309°, $[\alpha]_D$ +179° (c, 0.4) (Found: C,77.6; H,9.8. $C_{32}H_{48}O_4$ requires C,77.7; H,9.4%. $C_{32}H_{48}O_4$ requires C,77.4; H,9.7%) undepressed in m.p. when mixed with the first crop, m.p.304-305° described above. Light absorption: Maximum at 2380 Å (ε = 12,500). The product does not show a colour with tetranitromethane in chloroform.

Treatment of the Hydrogen Peroxide Oxidation Product of iso-β-Amyradienonyl Acetate with Hydrochloric Acid.

A solution of the acetate m.p.308-309° (12 mg.) in chloroform (0.5 c.c.) and glacial acetic acid (2 c.c.) was treated with concentrated hydrochloric acid (2 drops) and kept at 45-50° for 1 hour. Isolation by means of ether gave a product which crystallised from methanol as prisms (6 mg.), m.p.234-235° (decomp.), $[\alpha]_D$ +126° (c, 0.4).

Light absorption: Maximum at 2340 Å (ε = 13,000). It does not show a colour with tetranitromethane in chloroform and gives a positive Beilstein test for halogen.

Chromic Acid Oxidation of iso-β-Amyradienonyl Acetate.

A solution of iso-β-amyradienonyl acetate (2.0 g.) in stabilised glacial acetic acid (150 c.c.) was treated dropwise with a solution of chromic anhydride (2.0 g.) in water (1 c.c.) and acetic acid (80 c.c.) over 30 minutes, the solution being heated under reflux. It was refluxed for a further 1½ hours, and diluted with water. Isolation by means of ether (small acid fraction) gave a product which crystallised from methanol-chloroform as prismatic needles (1.0 g.), m.p.306-307°. A sample was recrystallised twice from methanol-chloroform to yield prismatic needles, m.p.308-309°, $[\alpha]_D$ +57° (c, 1.0) (Found: C,77.1; H,9.6. $C_{32}H_{48}O_4$ requires C,77.4; H,9.7. $C_{32}H_{48}O_4$ requires C,77.7; H,9.4%). Light absorption: Maximum at 2350 Å (ε = 12,100). The product does not show a colour with tetranitromethane in chloroform nor with ferric chloride in alcohol.

A solution of the product (0.9 g.) in benzene (50 c.c.) was filtered through a column of alumina (Grade II; 2 x 10 cm.). Evaporation of benzene fraction (120 c.c.)

gave a solid (0.8 g.) which crystallised from methanol-chloroform as prismatic needles, m.p.308-309°, $[\alpha]_D$ +57.5° (c, 0.4), undepressed in m.p. with the specimen described above. Mixed m.p. with the "hydrogen peroxide oxidation product, m.p.308-309°" was 304-307°.

The product was recovered unchanged after refluxing its solution in acetic anhydride with sodium acetate for 72 hours.

Bromination of the Chromic Acid Oxidation Product of iso-β-Amyradienonyl Acetate.

A solution of the acetate, m.p.308-309°, $[\alpha]_D$ +57° (prismatic needles; 100 mg.) in glacial acetic acid (40 c.c.) was treated dropwise with a solution of bromine (1.1 mols.) in glacial acetic acid (8%) over 30 minutes at 60-70°. The solution was kept at 60-70° for 2 hours and diluted with water. Isolation by means of ether gave a product which crystallised from methanol as plates (60 mg.), m.p.290-294° (decomp.). Three recrystallisations from the same solvent gave plates, m.p.298-299° (decomp.), $[\alpha]_D$ +85° (c, 0.4) (Found: C,66.7; H,7.8. $C_{32}H_{47}O_4Br_2$ requires: C,66.8; H,8.2. $C_{32}H_{45}O_4Br_2$ requires C,67.0; H,7.9%). Light absorption: Maximum at 2350 Å (ϵ = 12,000). The product does not show a colour with tetranitromethane in chloroform.

The product was recovered unchanged after heating its solution in acetic acid on the steam bath for 4 hours.

Clemmensen Reduction of the Chromic Acid Oxidation Product of iso-β-Amyradienonyl Acetate.

A hot solution of the acetate, m.p.308-309°, $[\alpha]_D$ +57° (270 mg.) in glacial acetic acid (30 c.c.) was treated with concentrated hydrochloric acid (8 c.c.) and added to freshly amalgamated zinc (from 12 g. of zinc), and the mixture heated under reflux for 3 hours. Isolation by means of ether gave a gum which did not crystallise. It was dissolved in light petroleum-benzene (3:1; 50 c.c.), filtered through a column of alumina (Grade I/II; 1.5 x 13 cm.), and the column washed with the same solvent mixture. Evaporation of the first fraction (100 c.c.) gave a colourless resin (130 mg.), which on standing in contact with methanol gave an amorphous solid, m.p. ca.200°. The solid showed a red-brown colour with tetranitromethane in chloroform, and did not exhibit light absorption of high intensity above 2200 Å.

Catalytic hydrogenation of the Chromic Acid Oxidation Product of iso-β-Amyradienonyl Acetate.

A solution of the acetate, m.p.308-309°, $[\alpha]_D$ +57° (300 mg.) in stabilised glacial acetic acid (70 c.c.) was

added to a suspension of freshly reduced platinum catalyst (from 120 mg. of platinum oxide) in glacial acetic acid (10 c.c.) and the mixture shaken with hydrogen at 16° for 20 hours (the apparent absorption of hydrogen was 4 mols.). The product, isolated in the usual manner, was dissolved in benzene (25 c.c.) and the solution filtered through a column of alumina (Grade I/II; 1.5 x 10 cm.).

Fr.	Solvent		Eluate	
1.	benzene	30 c.c.	180 mg.	colourless resin
2.	"	25	25	" "
3.	"	30	10	" "
4.	"	30	2	" "
5.	benzene-ether (1:1)	30	6	" "
6.	"	30	Trace	" "
7.	ether	30	Trace	" "
8.	methanol	30	30	crystalline solid
9.	"	30	20	" "
10.	"	30	7	" "
11.	"	30	2	gum
			282 mg.	

Fraction 1 crystallised from methanol as elongated plates m.p.193-195°. Four recrystallisations from the same solvent gave elongated plates, m.p.230.5-232°,

$[\alpha]_D$ +84° (c, 0.3) (Found: C,82.1; H,11.1. $C_{32}H_{52}O_2$ requires C,82.0; H,11.2%. $C_{32}H_{50}O_2$ requires C,82.3; H,10.8%). The product does not exhibit light absorption of high intensity above 2200 Å. It shows a pale yellow colour with tetranitromethane in chloroform. Mixed m.p. with β-amyrin acetate (m.p.240-241°) was 224-232°. Mixed m.p. with iso-β-amyrin acetate (m.p.250-251°) was 218-225°.

Fractions 2, 3 and the mother liquor from fraction 1 were evaporated to dryness, the solid (70 mg.) dissolved in light petroleum (20 c.c.) and the solution re-chromatographed using alumina Grade I/II (1.5 x 10 cm.). Evaporation of 150 c.c. of light petroleum gave a product (40 mg.) which crystallised from methanol as prismatic needles, m.p.172-173°, $[\alpha]_D$ +4° (c, 0.25) (Found: C,81.7; H,11.3. $C_{32}H_{52}O_2$ requires C,82.0; H,11.2%). The product does not exhibit light absorption of high intensity above 2200 Å. It shows a dark-yellow colour with tetranitromethane.

Later fractions (light petroleum-benzene; 100 c.c.) gave a product (28 mg.) which crystallised from methanol as flat needles, m.p.226-230°, undepressed in m.p. with the product m.p.230.5-232° from fraction 1.

Fractions 8, 9 and 10 were crystallised twice from methanol to give prismatic needles, m.p.324-235.5°, $[\alpha]_D$ -94° (c, 0.5) (Found: C,78.9; H,10.8. $C_{32}H_{52}O_3$ requires C,79.3; H,10.8%). The product does not exhibit light absorption of high intensity above 2200 Å and does not show a colour with tetranitromethane in chloroform (sparingly soluble).

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SECTION II.

β-Amyrenonyl Benzoate.

A solution of β-amyrin benzoate (40 g.) in boiling stabilised glacial acetic acid (2 l.) was treated with a solution of chromic anhydride (40 g.) in water (10 c.c.) and glacial acetic acid (800 c.c.) added dropwise during 1 hour. The solution was refluxed for 1½ hours, after which time it was treated with boiling water (1200 c.c.) with vigorous stirring, and kept overnight at room temperature.* The crystalline solid was collected, washed with aqueous methanol, and dried (21 g.; m.p.261-263°).

* The mother-liquor was further diluted with water. Working up using ether gave a crystalline solid (1.0 g.) which separated from the ethereal solution. Crystallisation from methanol-chloroform gave a product as fine needles, m.p.298-300°, $[\alpha]_D$ -72° (c, 0.5) (Found: C,81.3; H,9.5. $C_{37}H_{52}O_3$ requires C,81.6; H,9.6%. $C_{37}H_{54}O_3$ requires C,81.2; H,9.9%). Light absorption: Maximum at 2300 Å (ε = 21,500). (No inflection). The product does not give a colour with tetranitromethane in chloroform.

Crystallisation from chloroform-methanol gave β-amyrenonyl benzoate as prismatic needles, m.p.269-270.5°, $[\alpha]_D$ +112° (c, 1.9) (Found: C,81.5; H,9.7. Calc. for $C_{37}H_{52}O_3$: C,81.6; H,9.6%). Light absorption: Maximum at 2300 Å (ε = 21,500) and an inflection at 2520 Å (ε = 13,800). β-Amyrenonyl benzoate does not give a coloration with tetranitromethane in chloroform.

18-iso-β-Amyrenonol.

A solution of β-amyrenonyl benzoate (14.7 g.) in 15% ethanolic potassium hydroxide (1200 c.c.) was heated under reflux for 52 hours. The pale yellow solution was concentrated to half-bulk and diluted with water. The solid was collected, washed with water until the washings were neutral (litmus) and dried (11.8 g.). Crystallisation from methanol gave 18-iso-β-amyrenonol as long plates, m.p.254-255°, $[\alpha]_D$ +84° (c, 0.7) (Found: C,81.3; H,11.0. $C_{30}H_{48}O_2$ requires C,81.8; H,11.0%). Light absorption: Maximum at 2440 Å (ε = 12,300).

The alcohol, m.p.247-248°, $[\alpha]_D$ +81.5° obtained by Ruzicka, Müller and Schellenberg (19) by the action of alkali on β-amyrenonol is probably 18-iso-β-amyrenonol.

18-iso-β-Amyrenonyl Acetate.

Acetylation of 18-iso-β-amyrenonol (11 g.) by heating it with pyridine (50 c.c.) and acetic anhydride (70 c.c.) on the steam-bath for $2\frac{1}{2}$ hours gave 18-iso-β-amyrenonyl acetate which separated from chloroform-methanol as hard, square, squat prisms, m.p.277.5-279° (9.0 g.), $[\alpha]_D$ +75° (c, 1.8) (Found: C,79.8; H,10.8. $C_{32}H_{50}O_3$ requires C,79.6; H,10.4%). Light absorption: Maximum at 2450 Å (ε = 10,100). A mixture of 18-iso-β-amyrenonyl acetate and β-amyrenonyl acetate (m.p.265-266°) had m.p.254-256°, and a mixture of 18-iso-β-amyranonyl acetate and α-amyrenonyl acetate (m.p.275°) had m.p.228-230°.

18-iso-β-Amyrenonyl acetate was recovered unchanged after treatment with bromine (1.2 mols.) in acetic acid at 60°.

The allo-β-amyrenonyl acetate [m.p.262-265°, $[\alpha]_D$ +67° (pyridine), max. 2460 Å (ε = 11,000)] described by Green, Mower, Picard and Spring (27) is almost certainly a somewhat impure specimen of 18-iso-β-amyrenonyl acetate. Like the latter, it was recovered unchanged after treatment with bromine in acetic acid.

18-iso-β-Amyrin Acetate.

A solution of 18-iso-β-amyrenonyl acetate (1.0 g.)

in glacial acetic acid (180 c.c.) was added to a suspension of freshly reduced platinum (from 300 mg. of platinic oxide) in acetic acid (15 c.c.), and the mixture shaken with hydrogen at room temperature. After 24 hours the reaction product separated as plates, and after 40 hours the absorption of hydrogen was complete (approximately 2 mols.). The mixture was heated to dissolve the product and filtered to remove platinum. The filtrate was concentrated under reduced pressure to approximately 60 c.c., and after cooling, the separated solid was collected, washed with cold methanol, and dried. Recrystallisation from methanol-chloroform gave 18-iso-β-amyrin acetate as plates (740 mg.), m.p.245-246.5°, $[\alpha]_D$ +53° (c, 1.0) (Found: C,82.25; H,11.2. $C_{32}H_{52}O_2$ requires C,82.0; H,11.2%). 18-iso-β-Amyrin acetate gives a bright yellow colour with the tetranitromethane reagent and does not exhibit selective absorption in the ultra-violet. A mixture of 18-iso-β-amyrin acetate with β-amyrin acetate (m.p.241-242°) had m.p.218-222°, and a mixture of 18-iso--β-amyrin acetate with iso-β-amyrin acetate (2-acetoxy-olean-10-ene) (m.p.250-251°) had m.p.212-220°.

β-Amyradienyl-II Acetate.

A solution of 18-iso-β-amyrin acetate (220 mg.) in

boiling glacial acetic acid (20 c.c.) was treated with a solution of selenium dioxide (200 mg.) in water (0.5 c.c.) and glacial acetic acid (10 c.c.) added dropwise during 20 minutes, and the mixture refluxed for 1 hour. Freshly fused sodium acetate (1 g.) was added and the refluxing continued for 20 minutes. The hot mixture was filtered through sintered glass, the filtrate diluted with water, and the precipitated solid collected, washed with water, and dried. A solution of the solid in ether was washed with 3% aqueous potassium cyanide, and with water, and dried. The residue obtained after removal of the solvent was twice crystallised from methanol-chloroform, giving β-amyradienyl-II acetate (150 mg.) as plates, m.p.226-227°, $[\alpha]_D$ -63° (c, 1.0); the m.p. was not depressed when the acetate was mixed with a specimen (m.p.228°) obtained by the same method starting from β-amyrin acetate (Found: C,82.4; H,10.8. Calc. for $C_{32}H_{50}O_2$: C,82.3; H,10.8%). Light absorption: Maxima at 2510 (ε = 29,100), 2430 (ε = 27,300), and 2600 Å (ε = 19,500). Ruzicka, Müller and Schellenberg give m.p.228-229°, $[\alpha]_D$ -62°, for β-amyradienyl-II acetate.(19).

18-iso-β-Amyrenonyl Acetate.

A solution of 18-iso-β-amyrin acetate (0.7 g.) in

glacial acetic acid (200 c.c.), heated on a boiling-water bath with stirring, was treated with a solution of hydrogen peroxide (30%; 20 c.c.) in glacial acetic acid (20 c.c.) added dropwise during 15 minutes. The solution was maintained at the same temperature for 2 hours and again treated with a solution of hydrogen peroxide (30%; 15 c.c.) in acetic acid (15 c.c.) added during 15 minutes. After being stirred for 1 hour on the boiling-water bath, the solution was treated dropwise with boiling water with vigorous stirring until crystallisation commenced. Next morning the solid was collected, washed with aqueous methanol, and dried (m.p.276-283°; 250 mg.). A solution of the solid in benzene (20 c.c.) was filtered through a column of alumina (Grade I/II; 1.5 x 5 cm.) and the column washed with benzene (60 c.c.). The benzene filtrate was evaporated to dryness and the solid residue (180 mg.), after crystallisation from methanol-chloroform and then from methanol, gave 18-iso-β-amyranonyl acetate as plates, m.p.286-287°, $[\alpha]_D$ +77° (c, 1.2) (Found: C,79.5; H,10.8. $C_{32}H_{52}O_3$ requires C,79.3; H,10.8%). Light absorption: Maximum at 3000Å (ε = 126). A mixture of 18-iso-β-amyranonyl acetate (m.p.286-287°) with β-amyranonyl acetate (m.p.300°) did not show a marked depression in m.p. (284-290°).

In four different oxidations of 18-iso-β-amyrin acetate the yield of pure 18-iso-β-amyranonyl acetate varied between 26% and 30%.

18-iso-β-Amyranonyl acetate was recovered unchanged after treatment with concentrated hydrochloric acid in glacial acetic acid at 35-45° for 1 hour.

18-iso-β-Amyranonol.

A solution of 18-iso-β-amyranonyl acetate (m.p.284--285°; 300 mg.) in 15% ethanolic potassium hydroxide (60 c.c.) was refluxed for 50 hours. The prismatic needles separating on cooling were collected, washed with methanol, and dried (180 mg.; m.p.308-310°). Recrystallisation from methanol containing a trace of acetic acid and then from methanol gave 18-iso-β-amyranonol (110 mg.) as prismatic rods, m.p.309-310°, $[\alpha]_D$ +91° (c, 1.1) (Found: C,81.3; H,11.45. $C_{30}H_{50}O_2$ requires: C,81.4; H,11.4%). Light absorption: Maximum at 3000 Å (ε = 40).

The alkaline mother-liquor was diluted with water and the precipitated solid collected, dried, and acetylated using pyridine (2 c.c.) and acetic anhydride (1 c.c.). Crystallisation of the product from methanol-chloroform gave 18-iso-β-amyranonyl acetate (90 mg.) as elongated plates, m.p.284-285°, $[\alpha]_D$ +77° (c, 0.7), undepressed in

m.p. when mixed with the starting material.

Acetylation of 18-iso-β-amyranonol (m.p.309-310°; 60 mg.), by using acetic anhydride and pyridine in the usual manner, gave 18-iso-β-amyranonyl acetate (50 mg.) as elongated plates, m.p.287-288.5°, $[\alpha]_D$ +78° (c, 0.8), unchanged by a recrystallisation from methanol-chloroform, and undepressed in m.p. when mixed with a specimen of the starting material. Light absorption: Maximum at 3000 Å (ε = 140).

Bromo-18-iso-β-Amyranonyl Acetate.

A solution of 18-iso-β-amyranonyl acetate (250 mg.; m.p.284.5-286°) in glacial acetic acid (20 c.c.) was treated with a solution of bromine in glacial acetic acid (5%; 1.2 mols.) added during 1 hour at 65-80°. The solution was maintained at 80° for 3 hours and then diluted with water, and the solid collected, washed with water, dried, and crystallised from methanol-chloroform. The first crop (160 mg.) separated as plates, m.p.245-246° (decomp.), $[\alpha]_D$ +17.5° (c, 1.2), which did not give a coloration with tetranitromethane in chloroform and gave a positive halogen test. From the mother-liquors a second crop (65 mg.) of flat needles, m.p.260-265° (decomp.), separated. The first crop was heated on the steam-bath

for $3\frac{1}{2}$ hours with glacial acetic acid. On concentration and cooling of the solution, bromo-18-iso-β-amyranonyl acetate separated as plates, m.p.249-250° (decomp.) unchanged by two recrystallisations from methanol-chloroform, $[\alpha]_D$ +18° (c, 1.1) (Found: C,68.4; H,9.1. $C_{32}H_{51}O_3Br$ requires C,68.2; H,9.1%). Light absorption: Maximum at 3100 Å (ε = 155). Similar treatment (heating with acetic acid) of the second crop gave elongated plates, m.p.276-278°, undepressed in m.p. when mixed with 18-iso--β-amyranonyl acetate, $[\alpha]_D$ +73° (c, 0.9) (Found: C,77.6; H,10.9%). Light absorption: Maximum at 2900 Å (ε = 50). The physical properties of this fraction show that it is essentially 18-iso-β-amyranonyl acetate contaminated with bromo-ketone described above.

Attempted dehydrobromination of bromo-18-iso-β--amyranonyl acetate (60 mg.) by heating its solution in pyridine under reflux for 3 hours and working up using ether, gave elongated plates (40 mg.) from methanol, m.p.242-244°. Light absorption: Maximum at 2400 Å (ε = 7400). Repeated recrystallisation of the product from methanol gave elongated plates, m.p.253-256°, $[\alpha]_D$ +113° (c, 0.5). Light absorption: Maximum at 2410 Å (ε = 9000). It shows no colour with tetranitromethane in chloroform. Mixed m.p. with starting material

(m.p.249-250°) was 229-238°.

18-iso-β-Amyranyl Acetate.

A mixture of 18-iso-β-amyranonyl acetate (m.p.284--285.5°; 300 mg.), alcoholic sodium ethoxide (from 750 mg. of sodium and 10 c.c. of ethanol), and hydrazine hydrate (2 c.c.; 100%) was heated in an autoclave at 200° for 17 hours. The cooled mixture was diluted with water and extracted with ether. The extract was washed successively with hydrochloric acid (3%) and water and dried. After removal of the solvent, the solid residue was heated on the steam-bath for 2 hours with pyridine (3 c.c.) and acetic anhydride (2 c.c.). The crystalline solid separating on cooling was collected, washed with methanol, and dried (155 mg.; m.p.277-279°). A second crop (m.p.263--266°; 25 mg.) was undepressed in m.p. when mixed with the first crop. The first crop was twice recrystallised from methanol-chloroform, giving 18-iso-β-amyranyl acetate as plates, m.p.280-282°, $[\alpha]_D$ +43° (c, 0.9) (Found: C,81.6; H,11.7. $C_{32}H_{54}O_2$ requires C,81.6; H,11.6%). 18-iso-β--Amyranyl acetate does not give a colour with tetranitromethane in chloroform, and does not exhibit selective light absorption of high intensity in the ultra-violet region. A mixture of 18-iso-β-amyranyl acetate with

β-amyranyl acetate (m.p.284°) had m.p.256-262°, and a mixture with lupanyl acetate (m.p.246-248°) had m.p.233--237°.

The original mother-liquors from 18-iso-β-amyranyl acetate deposited on long storage a crop of large prisms (40 mg.), m.p.179-180°, recrystallisation of which from methanol gave prismatic needles, m.p.180-181°, $[\alpha]_D$ -5° (c, 1.1) (Found: C,81.1; H,11.6. $C_{32}H_{54}O_2$ requires C,81.6; H,11.6. $C_{32}H_{52}O_2$ requires C,82.0; H,11.2%). The substance gives a very faint yellow coloration with the tetranitromethane reagent, and it does not show selective absorption in the ultra-violet region of the spectrum.

18-iso-β-Amyranol.

Hydrolysis of 18-iso-β-amyranyl acetate (90 mg.) was effected by heating it under reflux with 3% alcoholic potassium hydroxide for 6 hours. The product, isolated in the usual manner, separated from ethanol as plates (50 mg.), m.p.228-229°, which after two crystallisations from methanol-chloroform, gave 18-iso-β-amyranol as flat prisms (thick plates), m.p.229-230°, $[\alpha]_D$ +36° (c, 1.2) (Found: C,83.85; H,12.4. $C_{30}H_{52}O$ requires C,84.0; H,12.2%). It does not give a colour with tetranitromethane

in chloroform and does not exhibit selective absorption of high intensity in the ultra-violet region.

Acetylation of 18-iso-β-amyranol (30 mg.) by warming it on the steam-bath for 3 hours with pyridine (1 c.c.) and acetic anhydride (1 c.c.) gave 18-iso-β-amyranyl acetate (25 mg.) as plates (from methanol), m.p.281-282°, $[\alpha]_D$ +44° (c, 1.1), undepressed in m.p. when mixed with the specimen described above.

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PART II.

ROUTES TO 11-OXYGENATED STEROIDS FROM ERGOSTEROL.

CONTENTS.

CONTENTS continued: Page

* Experiments corresponding to the section mentioned in THEORETICAL (Contents).

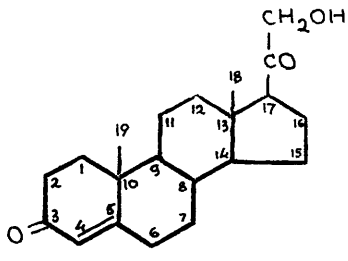
INTRODUCTION

INTRODUCTION

The unique role of the steroid hormones in nearly all phases of life has been established. With the discovery of the sex hormones and the steroid hormones of the adrenal gland, it was soon found that these compounds had a profound influence on metabolism. The small adrenal gland secretes a mixture of steroids into the blood stream. The full physiological significance of these steroids is not known. The two main physiological functions of the adrenal cortex are regulation of the electrolyte balance in serum and of carbohydrate and protein metabolism in the liver and in muscles. Removal of the adrenal gland from an animal leads to death within a few days. The discovery in 1929 that the life span of adrenalectomized dogs can be prolonged by administration of extracts of the adrenal cortex, initiated extensive investigations of the constituents of this gland (1). The active adrenal cortical agent was originally referred to as "cortin" or the "life-maintenance hormone". Chemical studies of glandular extracts undertaken in 1935 by Reichstein, by Kendall, by Wintersteiner and Pfiffner led to the isolation of twenty-eight crystalline steroids, six of which are able to maintain life in adrenalectomized animals (I-VI). The residual amorphous fractions still possess physiological

activity but the nature of the active components is unknown.

No major improvements have been reported in the methods used for the partition of steroids. Acetone or alcohol extraction from whole beef glands is made (even though the active principles are present only in the cortex), which precipitates protein constituents. Advantage is taken of the relatively high water-solubility of the hormones (2,5). The Girard procedure is one of the most successful separation methods for the isolation of keto-steroids from nonketonic or inert ketonic material (3). The most efficient method for separation of individual



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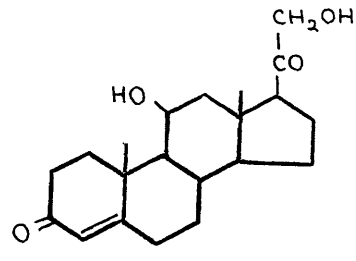

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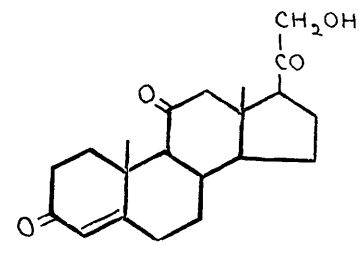

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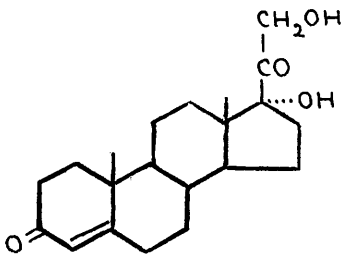

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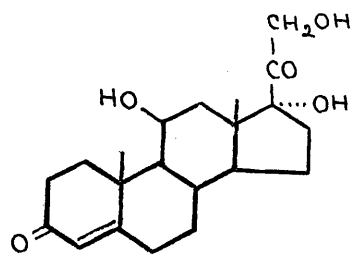

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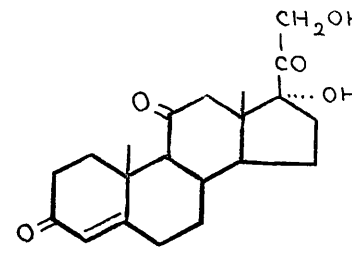


(VI)

components is chromatography of the more stable acetates (4).

Nearly all the substances isolated are C_{21}-steroids, and those that exhibit cortin activity all have the αβ--unsaturated ketonic grouping in ring A characteristic of testosterone and progesterone and possess a ketol grouping in the side chain, which is highly sensitive to both acids and alkalies. At C_{11}, where substitution can exist, the ketone or the hydroxyl group (oriented in the β-configuration (6)) are subjected to very pronounced steric hindrance from the angular methyl groups at C_{10} and C_{13}, a feature which is reflected in chemical behaviour. The carbonyl group at C_{11} is inert to phenylhydrazine, hydrazine under Wolff-Kishner conditions, Girard's reagents, and is resistant to catalytic hydrogenation in a neutral medium. Hydrogenation can be accomplished in acetic acid solution, or by means of lithium aluminium hydride to give exclusively the hindered 11β-hydroxy derivative (i.e. hydrogen attacks the molecule at the unhindered rear face and opens the rear bond of the carbonyl group). The 11β-hydroxyl group resists acetylation and is susceptible to dehydration, even by dilute mineral acids.

Comparisons of the six active hormones (I-VI) indicate that oxygen functions at C_{11} and C_{17} are not essential to life-maintenance activity but contribute importantly to physiological actions of still greater significance.

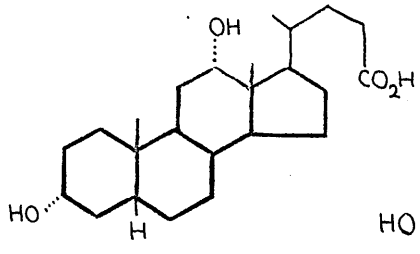
17-Hydroxy-11-dehydrocorticosterone (or "Cortisone")(VI), isolated by Kendall (5) and often designated as Kendall's Compound E, appears to be a particularly important member of the series from the point of view of physiological actions, matched only in part by compounds lacking either the tertiary hydroxyl group at C_{17} or an oxygen function at C_{11}.

Progress in a steadily expanding area of investigation in biology, biochemistry, and medicine was handicapped by the lack of sufficient quantities of the adrenal hormones. The amounts obtainable from the adrenal glands of cattle, in the form of either extracts or isolated products, are much too limited to meet the demands (e.g. from approximately 1 ton of beef adrenals, 400 mg. - 1 g. of cortisone can be isolated by a most complex and careful procedure). Since the supply of glands in any case is inadequate, synthetic methods have been investigated far beyond the requirements of structure elucidation and in particular with the objective of accomplishing the difficult and important feat of introduction of oxygen functions of proper steric orientation at C_{11} and C_{17}. The last feature was a great stumbling-block in the way of partial synthesis, because most of the steroid materials available in large quantity have a saturated C-ring.

In 1943 Reichstein (7) achieved the important feat of introducing oxygen at the 11-position and succeeded in synthesising a substance identical with natural 11-dehydrocorticosterone (III). In 1946 Sarett achieved the synthesis of cortisone (VI) starting from deoxycholic acid (8). Sufficient amounts of cortisone were made available for clinical testing. Within an extremely short time the important discovery of Hench and Kendall (9) of the beneficial effect of cortisone and of the adrenocorticotropic hormone (ACTH; the adrenal-stimulating hormone of the pituitary) on rheumatoid arthritis and rheumatic fever, was reported, which has enormously stimulated studies of the chemistry of adrenocortical steroids. The anti-arthritic effect of cortisone appears to be highly specific (10), no other known compound apart, possibly, from 17-hydroxycorticosterone (V) having comparable potency.

It was apparent by 1949, that in order to provide adequate supplies of cortisone, the partial synthesis from available naturally occurring steroids was the most rational approach. Various starting materials have been considered. Until 1952, most of the cortisone available was prepared from the bile acid deoxycholic acid (VII)(or the more abundant cholic acid). The difficulties attending this route, the limited supply of starting material,

and the large number of stages involved, some of which proceed in low yield (10), have been appreciated for a considerable time. It is natural, therefore, that attention should be turned towards alternative routes starting from steroids other than bile acids. These investigations have been successful and cortisone can now



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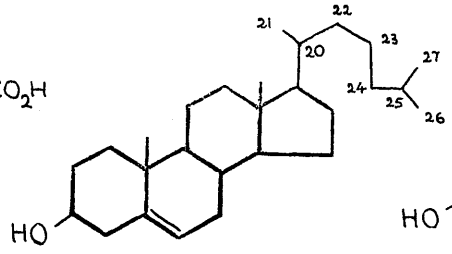

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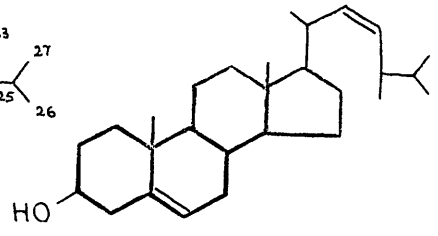

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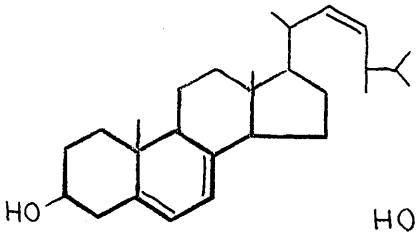

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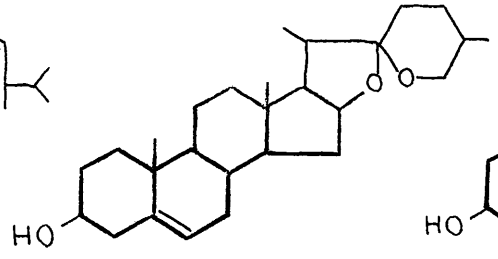

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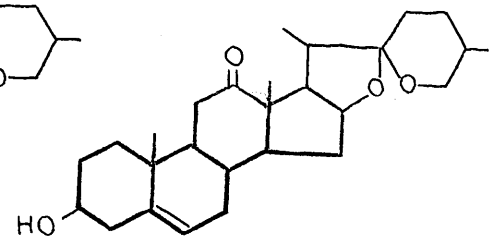
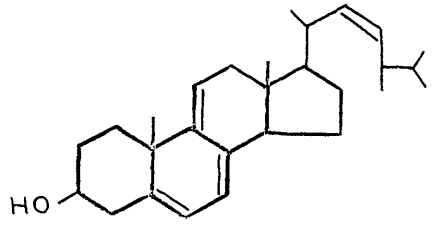


(XII)

be prepared from a number of steroids, in particular, from ergosterol, cholesterol, stigmasterol and the sapogenins, diosgenin and hecogenin. The principal disadvantage in the use of cholesterol (VIII) is the difficulty of side chain degradation.

The specific problem of devising a method for the

introduction of an 11-oxygen function into steroids devoid of oxygen function in ring C arises from the need to bring some of the more widely occurring steroids into use as starting materials for the partial synthesis of cortisone. Ergosterol (X), the characteristic sterol of yeasts, can be converted into two derivatives, dehydroergosterol (ergosta-5:7:9(11):22-tetraen-3β-ol) (XIII) and ergosterol-D (ergosta-7:9(11):22-trien-3β-ol) (XIV) each of which contains a 9:11-ethylenic linkage and so constitutes possible intermediates in the desired partial synthesis.



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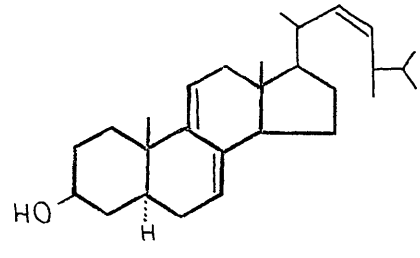


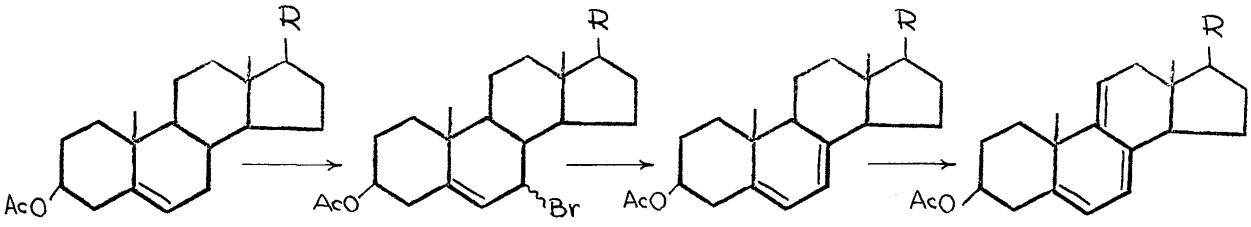
(XIV)

Although little success has as yet attended efforts to convert dehydroergosterol (XIII) into an 11-oxygenated steroid,* ergosterol-D (XIV) has been successfully converted into 11-oxygenated derivatives, and thence into cortisone. Ergosterol (X) also appears particularly attractive since the side-chain double bond should facilitate degradation (also a feature of stigmasterol).

The sterols, cholesterol (VIII) and stigmasterol (IX)

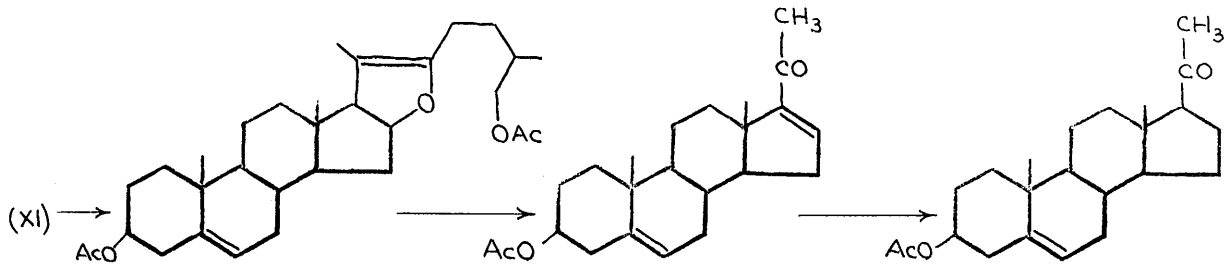
* See, however, Laubach et al. (102).

and the related sapogenin, diosgenin (XI), are capable of similar treatment with the added complication that their conversion into 7:9(11)-diene derivatives is a lengthier procedure than in the case of ergosterol. The introduction of the 11-oxygen function into these molecules can be envisaged by the general procedures of allylic bromination at C_7, dehydrobromination, and mercuric acid oxidation to introduce the 9(11)-ethylenic bond, which would serve as the necessary point of attack.



Diosgenin (XI) is an attractive steroid as regards the synthesis of intermediate pregnane derivatives. Treatment with acetic anhydride gives pseudodiosgenin acetate (XV), which on oxidation yields the pregna-5:16--diene derivative (XVI) which can be converted to pregnenolone acetate (XVII) by hydrogenation (11).

The other sapogenin, hecogenin (XII), may well assume considerable importance if the harvesting of the starting material can be economically effected. The 12-keto group



(XV) (XVI) (XVII)

has been moved to the 11-position (12) and the side-chain degraded to an acetyl group by standard methods, thus providing another route from a common natural product to cortisone.

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HISTORICAL

11-OXYGENATED STEROIDS FROM 7:9(11)-DIENES WITH PARTICULAR REFERENCE TO ERGOSTEROL-D.

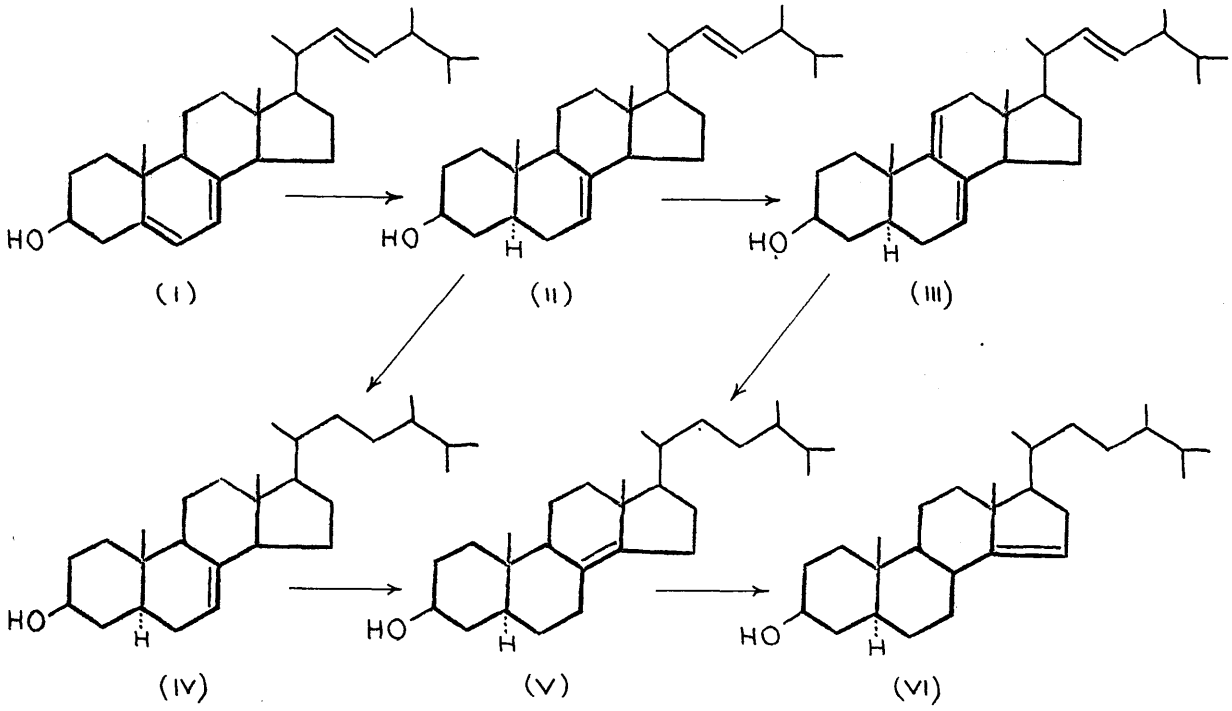
The past two years have witnessed the publication of many papers in this field. The experiments performed mainly by the American and Swiss workers are described in this section. The experiments described in the theoretical section of this thesis, performed during the same time, have in part been reported in a series of publications (107-114).

Aryl Peracid Route.

A. From Ergosteryl-D Acetate.

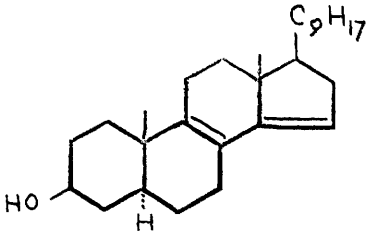
Ergosterol (I), which was investigated particularly extensively because of its relationship to the vitamin D problem, was first isolated from ergot and is now prepared in considerable quantity from the nonsaponifiable fraction from yeast. In 1932, Windaus (13) established the empirical formula $C_{28}H_{44}O$, and soon afterwards ergosterol was fully characterised by various workers. It can be converted by partial catalytic hydrogenation in a neutral solvent (14,15,16,107) into a dihydro derivative (5-dihydro-ergosterol; II) that retains the double bonds at C_7 and C_{22}. The $\triangle^{7,8}$-structure was confirmed by Barton from M_D data (17). On further hydrogenation with platinum in a

neutral solvent, the double bond in the side chain is saturated (15) and the product is Δ^{7}-ergostenol (IV). This substance is isomerised to $\Delta^{8(14)}$-ergostenol (V) by platinum saturated with hydrogen in acetic acid. The 8(14)-double bond migrates to 14(15) position (VI) under the influence of hydrogen chloride (18). In 1929,

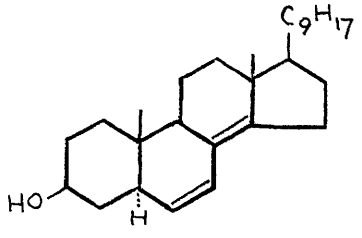


Heilbron, Johnstone and Spring (19, 20) obtained ergosterol-D (III) by the oxidation of 5-dihydroergosterol (II) with mercuric acetate and the structure ergosta-7:9(11):22--trien-3β-ol (III) was finally confirmed by Barton in 1946 (17). Ergosterol (as acetate) is isomerised with dry hydrogen chloride in chloroform giving a separable mixture

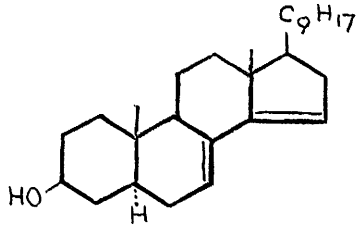
of ergosterols-B_1, B_2 and B_3 which are all capable of interrelationship (21).



B_1



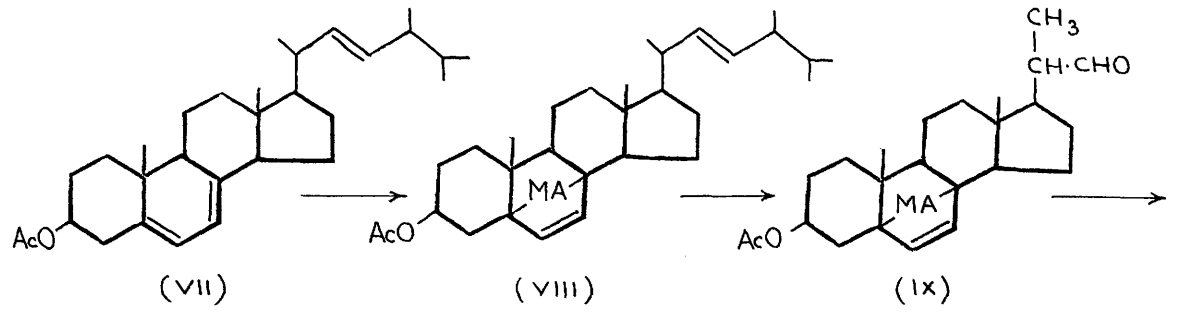
B_2



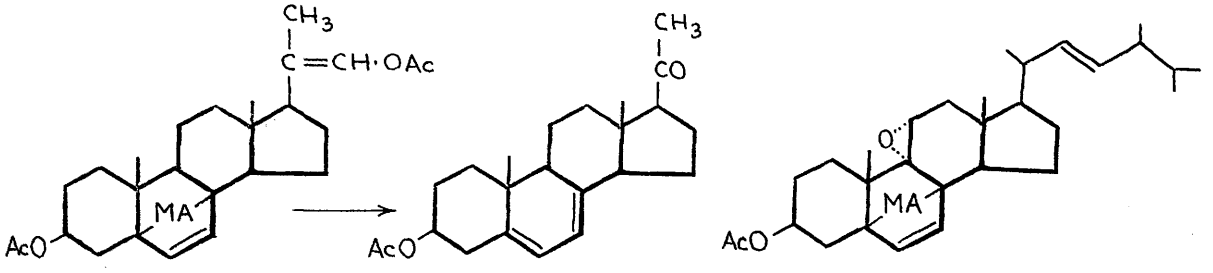
B_3

Bergmann and Stevens (22) suggested in 1948 that ergosterol (I) can be used as a starting material for the partial synthesis of adrenal cortical steroids "because of the comparative ease with which it may be converted to derivatives like dehydroergosterol which possess unsaturation at C_{11} and which might lend themselves to the introduction of oxygen at this point. In addition the 22:23-double bond was expected to facilitate removal of the side-chain to permit its replacement by one of the typical side-chains of adrenal cortical hormones." In a series of experiments they made considerable progress in the last direction, showing that protection of the conjugated nuclear double bonds of ergosteryl acetate (VII) by means of maleic anhydride allowed the 22:23-double bond to be preferentially oxidised. Thus treatment of the maleic anhydride adduct (VIII) with ozone

gave an aldehyde (IX) which was converted into the enol acetate (X), ozonolysis of which, followed by pyrolysis of the product, gave 3β-acetoxypregna-5:7--dien-20-one (XI). This procedure has been substantiated by later workers (23). Less successful were attempts to introduce either a hydroxyl or a ketone group



(VII) (VIII) (IX)



(X) (XI) (XII)

-MA- = -HC-CH-
OC CO
O

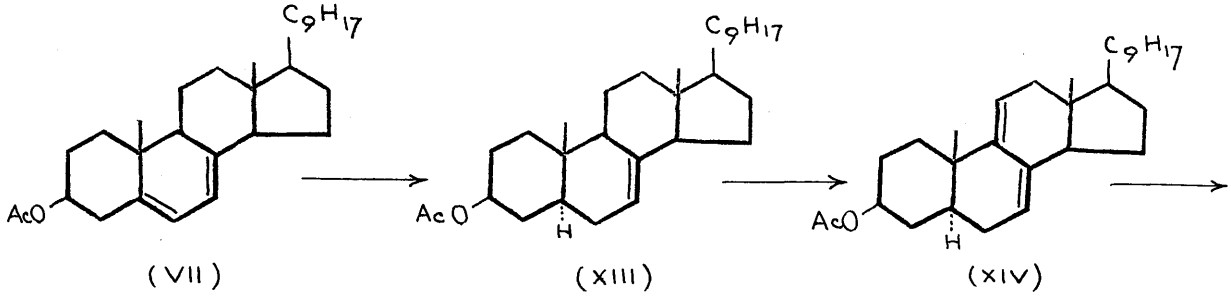
at the 11-position starting from dehydroergosteryl acetate-maleic anhydride 22:23-dibromide. Although the epoxide (XII) was obtained, pyrolysis of this was

accompanied by aromatisation of ring B.

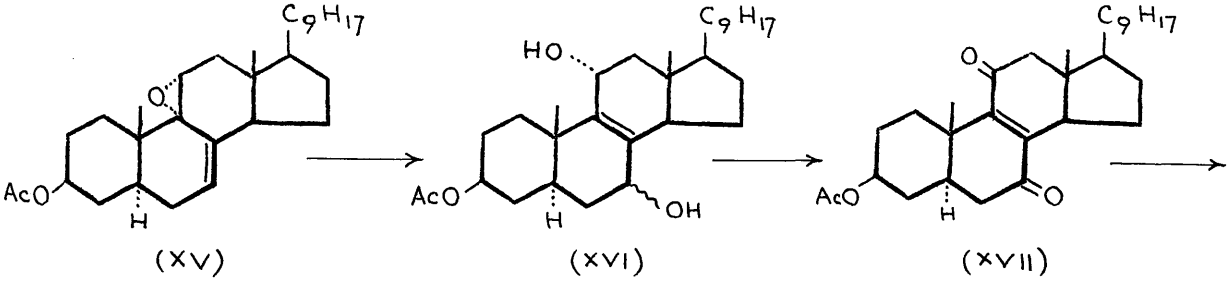
The production of the 9(11)-double bond is a development of the long known (24) dehydrogenation of Δ^7--steroids to the 7:9(11)-unsaturated compounds. The 7-double bond, if not already present as in ergosterol (I), may be introduced into a Δ^5-steroid by the action of N-bromosuccinimide followed by dehydrobromination (27); a process involving the shift of a double-bond from position 6 has also been used (28). The 7:9(11)-dienes are valuable intermediate products for the synthesis of 11-ketosteroids.

A procedure, developed in the laboratories of Merck and Company for the synthesis of 11-keto-steroids from steroids containing a 5-ethylenic linkage such as ergosterol, diosgenin and stigmasterol, has been reported in May 1951 by Tishler and co-workers in a preliminary note (29). Ergosteryl acetate (VII) is partially reduced to 5-dihydroergosteryl acetate (XIII) (14, 15, 16) oxidation of which with mercuric acetate gives ergosteryl-D acetate (ergosta-7:9(11):22-trien-3β-yl acetate (XIV) (19, 20). Treatment of ergosteryl-D acetate with one equivalent of perbenzoic acid gives a monoepoxide, believed to be Δ^7-9α:11α-epoxide (XV) (30), hydrolytic rearrangement of which yields 7ξ:11α-dihydroxyergosta-8:22-dien-3β-yl

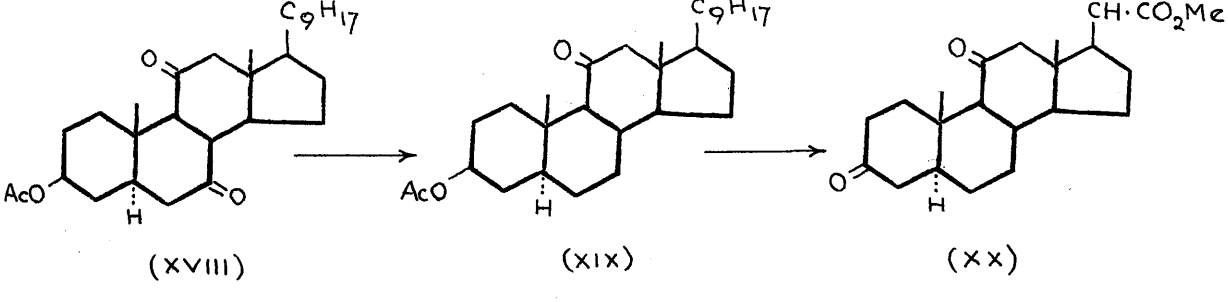
acetate (XVI). The latter compound is oxidised with chromic acid to 7:11-diketoergosta-8:22-dien-3β-yl acetate (XVII), which is converted into 7:11-diketoergost-22-en--3β-yl acetate (XVIII) on treatment with zinc and acetic acid. The last compound loses the 7-oxygen atom



(VII) (XIII) (XIV)



(XV) (XVI) (XVII)

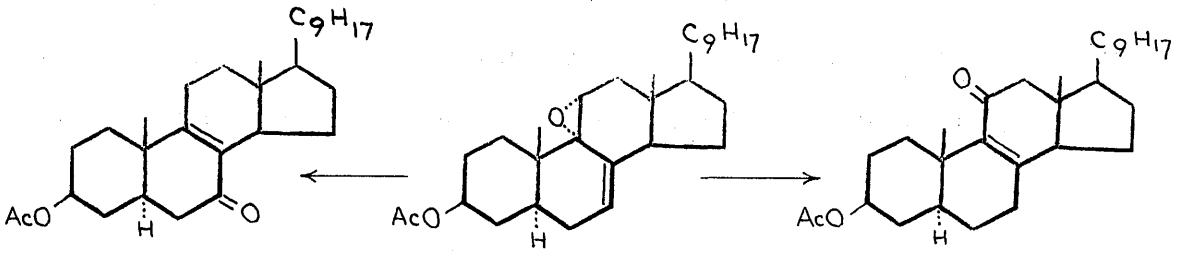


(XVIII) (XIX) (XX)

preferentially on Wolff-Kishner reduction modified by Huang-Minlon (31) to give 11-ketoergost-22-en-3β-yl acetate. This is converted to the known compound (XX).

Immediately a number of preliminary announcements were made describing the conversion of 7:9(11)-dienic steroids into 11-oxygenated steroids (32-35, 107), and more recently an independent paper on the same subject has been published by Heusser and co-workers (36) describing with experimental details the same route (VII) — (XIX). The epoxide of ergosteryl-D acetate is ascribed the structure 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate (XV) since it can be isomerised to two different αβ-unsaturated ketones, according to the experimental conditions used. Firstly, by treatment with aqueous mineral acid, it is converted into 7-ketoergosta-8:22-dien-3β-yl acetate (XXI) previously obtained (39) by chromic acid oxidation of 5-dihydroergosteryl acetate (XIII). Secondly, treatment of the epoxide (XV) with boron trifluoride etherate in absolute benzene (or with ferric chloride, 37) gives in high yield an isomeric αβ-unsaturated ketone to which was ascribed the structure 11-ketoergosta-8:22-dien-3β-yl acetate (XXII). Similar transformations are also described by Heusser and co-workers (38) in the androstane and cholestane series.

The two isomers (XXI) and (XXII) exhibit an ultraviolet absorption maximum at 2530 Å and neither is converted into

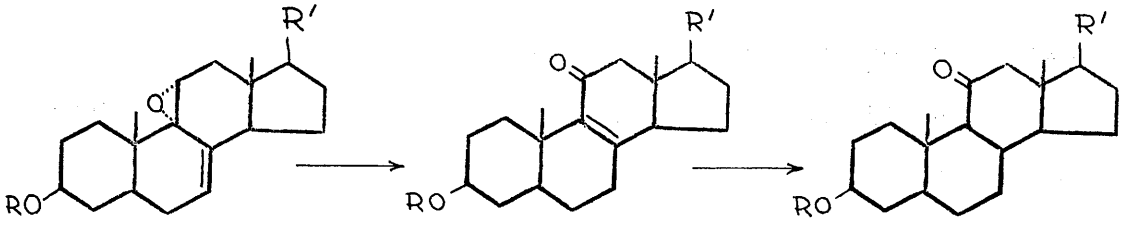


(XXI) (XV) (XXII)

the other on treatment with mineral acid. Mechanistically (36), the 9:11-structure for the epoxide is very probable; the α-configuration is ascribed to the epoxy-group since attack at the 9:11-positions will be at the rear of the molecule (cf. 40).

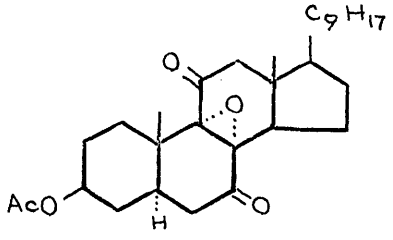
The strucutre assigned to (XXII), apart from its non-identity with (XXI) and $\Delta^{8(14)}$-7-ketone and the non--reactivity of the carbonyl group, is supported by the observation made by Tishler and co-workers (30), that the 8-ethylenic linkage of (XXII) can be selectively reduced by the action of lithium and liquid ammonia to give 11-ketoergost-22-en-3β-yl acetate (XIX) in high yield. The general availability of this most convenient route is demonstrated by its application to 7:9(11)-dienic esters derived from sapogenins (30, 41). Djerassi and co-workers (41) have shown that reduction of the Δ^{8}-11-ketones by

lithium and liquid ammonia can also produce 11α-hydroxy steroids under certain conditions.

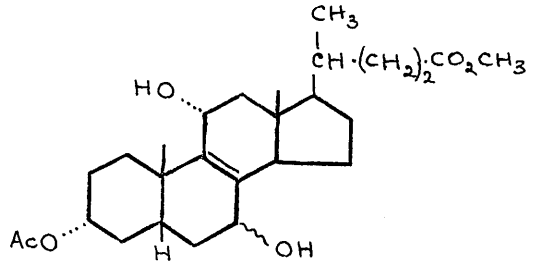


It was found by Heusser et al., (36) that oxidation of the triol-monoacetate (XVI) (which was given the 11α-configuration in accordance with the mechanistic considerations) with chromic acid gives, in addition to 7:11-diketoergosta-8:22-dien-3β-yl acetate (XVII), the corresponding 8α:9α-epoxy-7:11-diketoergost-22-en-3β-yl acetate (XXIII), which is obtained as major product when an excess of oxidising agent is employed. The 8α:9α--configuration is given to the epoxide-group in (XXIII) since a similar oxidation of the related methyl 3α-acetoxy--7ε:11α-dihydroxychol-8-enate (XXIV), even with an excess of chromic acid, gives only the corresponding unsaturated diketone and not a diketo-epoxide. A ready explanation for this marked difference is to be found if the epoxide group in (XXIII) is α-orientated since addition of an α-epoxide group to the chol-8-ene derivative is considerably

hindered. Like the diketone (XVII), the epoxide (XXIII) gives 7:11-diketoergost-22-en-3β-yl acetate (XVIII) on reduction with zinc and acetic acid.



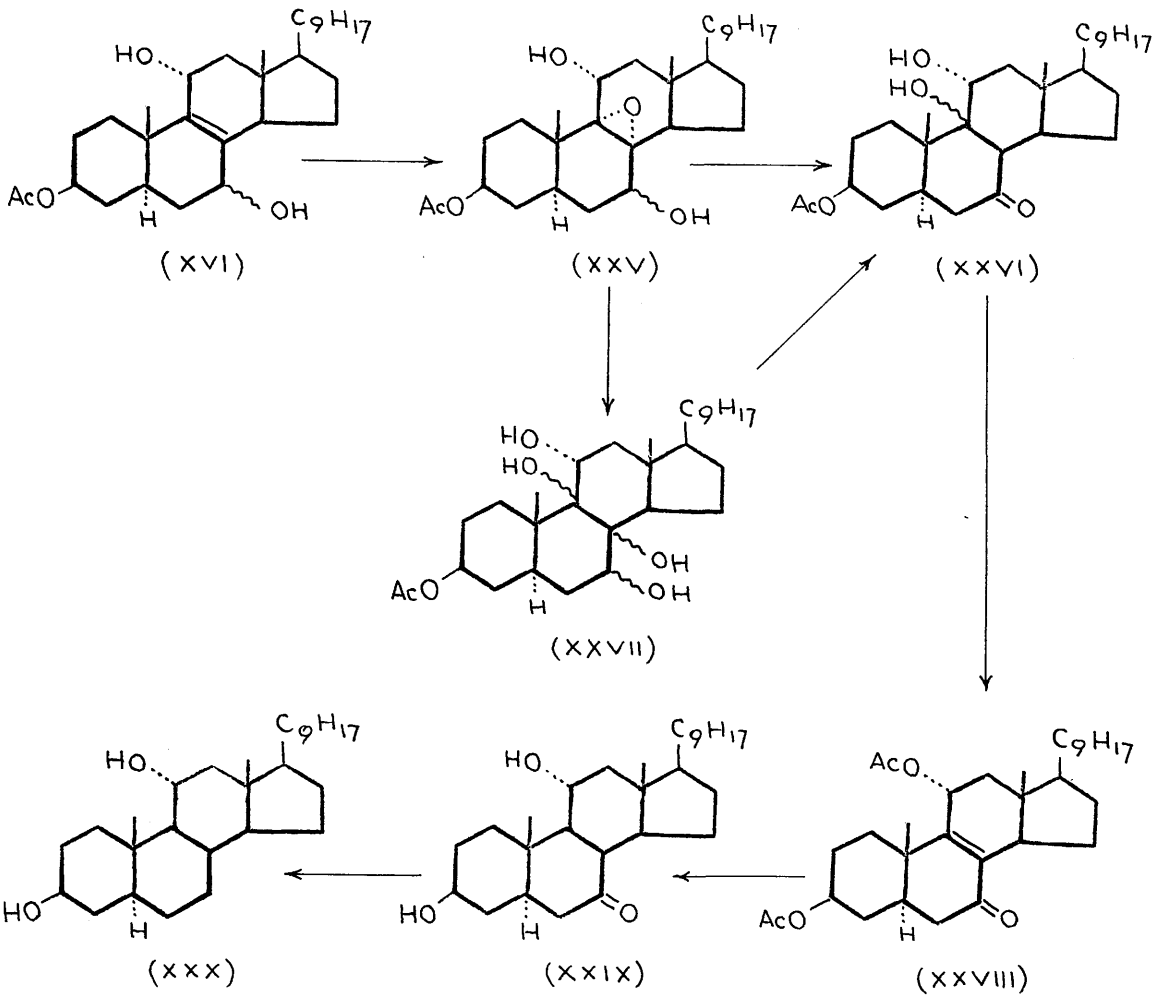
(XXIII)



(XXIV)

In a later communication, Heusser, Anliker, Eichenberger and Jeger (37) describe variations in their original route (36) to 11-oxygenated ergosterol derivatives. Starting from 7ξ:11α-dihydroxyergosta-8:22--dien-3β-yl acetate (XVI), this is partially oxidised with monoperphthalic acid to give the corresponding epoxide (XXV). The α-configuration of the 8:9-epoxide bridge follows from the considerations used in the case of the corresponding diketone (XXIII). The epoxide (XXV) is isomerised with hydrogen bromide in acetic acid, or with boron trifluoride etherate in benzene, to 3β--acetoxy-9ξ:11α-dihydroxyergost-22-en-7-one (XXVI). Treatment of the epoxide (XXV) with dilute aqueous sulphuric acid converts it into the intermediate 7:8:9:11--tetrol (XXVII) which is dehydrated to (XXVI) on treatment with hydrogen bromide in acetic acid. Treatment of

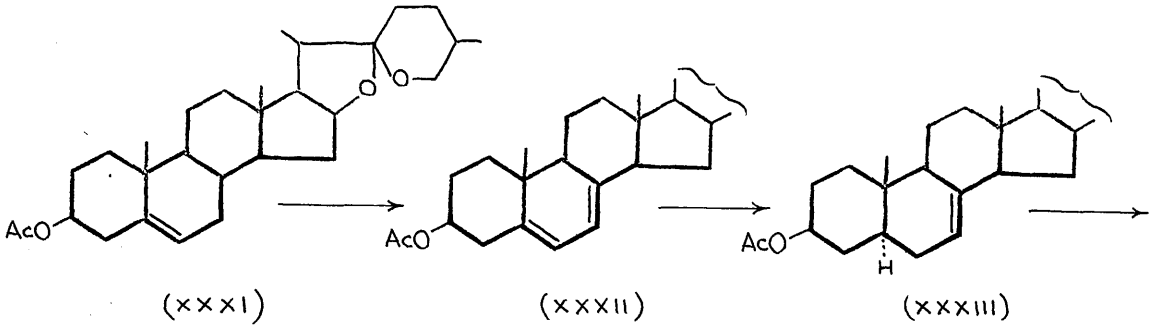
(XXVI) with strong alkali (cf. 107, 108) followed by acetylation gives 3β:11α-diacetoxyergosta-8:22-dien--7-one (XXVIII). Catalytic hydrogenation of the



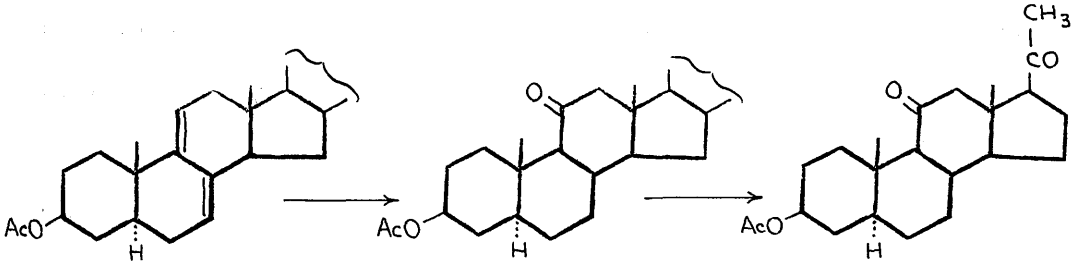
diacetate (XXVIII) in the presence of alkali effects saturation of the 8-ethylenic linkage and formation of 3β:11α-dihydroxyergost-22-en-7-one (XXIX), Wolff-Kishner reduction of which yields 3β:11α-dihydroxyergost-22-ene (XXX).

B. From Other Steroids.

The peraromatic acid route to 11-oxygenated steroids from 7:9(11)-diene derivatives has been applied to a number of other steroids. Diosgenin acetate (XXXI) is converted into the 5:7-diene (XXXII) by the standard method (29, 42), and thence via (XXXIII) to 22a-allo-spirostan-3β-yl acetate (XXXV) using the sequence of reactions described above for ergosteryl-D acetate (XIV).



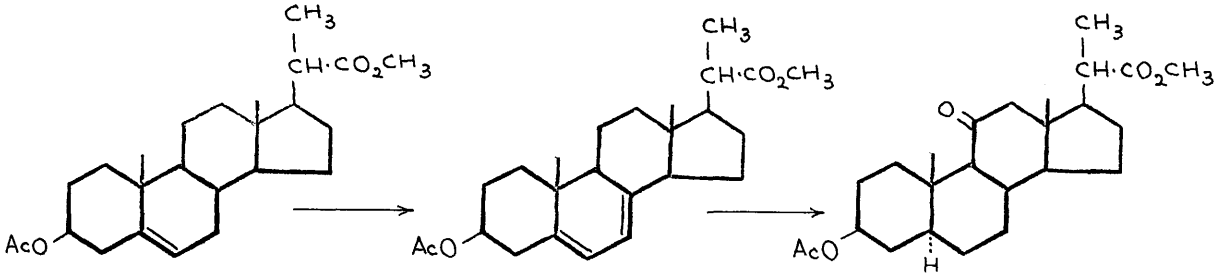
(XXXI) (XXXII) (XXXIII)



(XXXIV) (XXXV) (XXXVI)

The ketone (XXXV) is converted into 11:20-diketoallo-pregnan-3β-yl acetate (XXXVI) using standard sapogenin reactions (29, 44) for the degradation of the "side chain".

Methyl 3β-acetoxybisnorchol-5-enate (XXXVII), which may be obtained from either cholesterol (VIII)[*] or stigmasterol (IX)[*], was converted by the standard method into 5:7-diene (XXXVIII) (45). This was converted into 3β-acetoxy-11-ketobisnorallocholanate (XXXIX) using the general series of reactions described above (29).

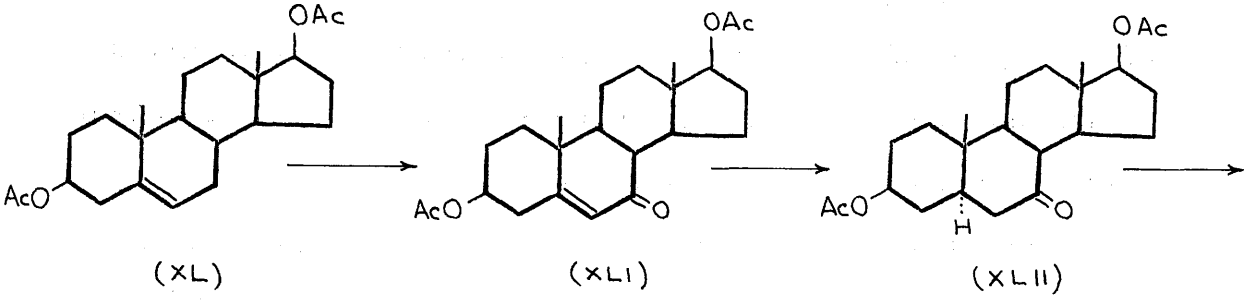


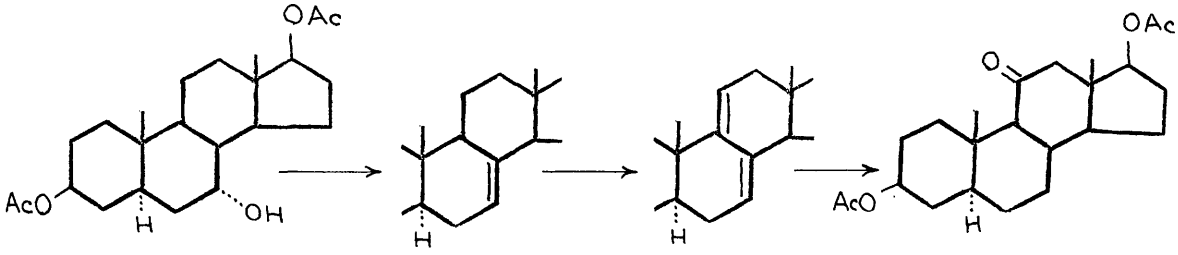
(XXXVII) (XXXVIII) (XXXIX)

In the androstane series, 3β:17β-diacetoxyandrost--5-ene (XL) (46) was converted by a novel route (47) into a 7:9(11)-diene (XLV). The diacetate (XL) was oxidised with butyl chromate (48) to 3β:17β-diacetoxyandrost-5-ene--7-one (XLI). Catalytic hydrogenation of (XLI) in ethyl acetate gives 3β:17β-diacetoxyandrostan-7-one (XLII), and catalytic hydrogenation of the latter over platinum in acetic acid gives 3β:17β-diacetoxy-7α-hydroxyandrostane (XLIII), dehydrated to 3β:17β-diacetoxyandrost-7-ene (XLIV). Oxidation of (XLIV) with mercuric acetate gives the 7:9(11)-diene (XLV), which was converted into 3β:17β--diacetoxy-11-ketoandrostane (XLVI) (38), using the same

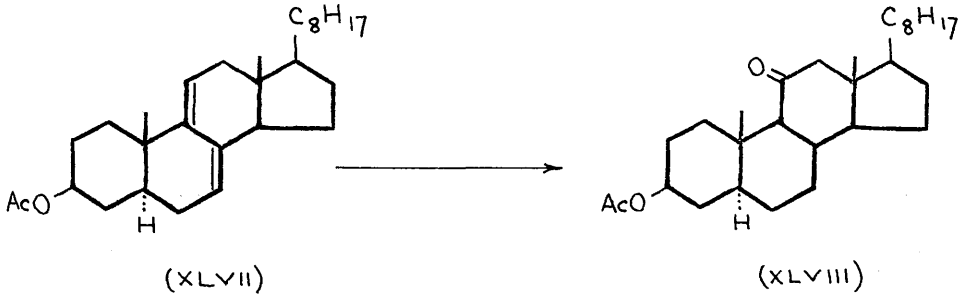
[*] Introduction.

method as that described in the case of ergosteryl-D acetate.

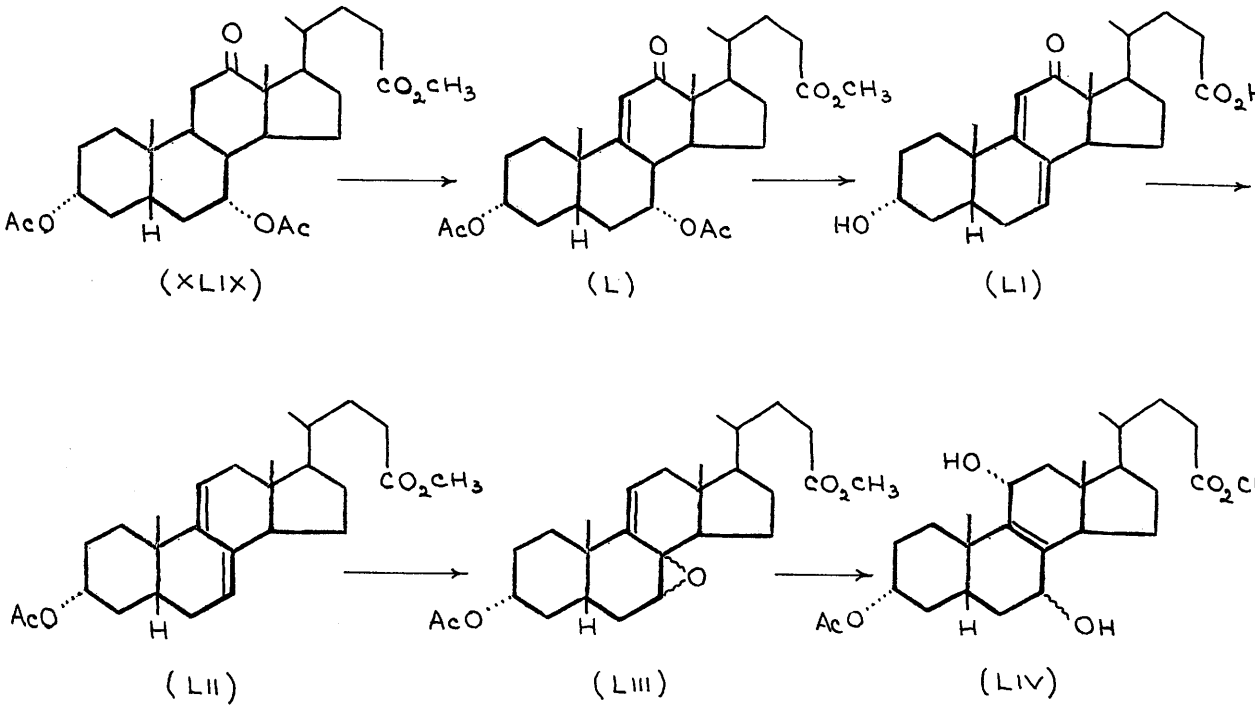




In a similar manner, Heusser and co-workers (38) converted cholesta-7:9(11)-dien-3β-yl acetate (XLVII) into 11-ketocholestanyl acetate (XLVIII).



Cholic acid was converted into a 7:9(11)-diene as follows (36, 49): Oxidation of methyl 3α:7α-diacetoxy--12-ketocholanate (XLIX) with selenium dioxide gives the 12-ketochol-9(11)-enate (L) which on treatment with alkali gives 3α-hydroxy-12-ketochola-7:9(11)-dienic acid (LI). Wolff-Kishner reduction removes the ketone group (36, 49), and the 7:9(11)-diene (LII) on treatment with monoperphthalic acid gives a monoepoxide, formulated as a 7:8-epoxide (37) (LIII) (when treated with boron trifluoride in absence of water it is isomerised to 7-ketochol-8-enate and not to 11-keto isomer). Treatment of (LIII) with mineral acid gives methyl 3α-acetoxy-

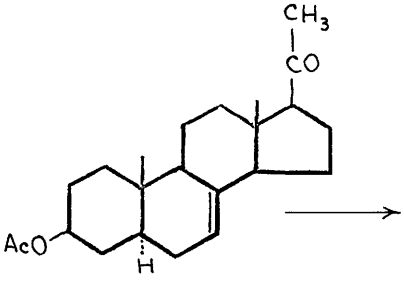


-7ξ:11α-dihydroxychol-8-enate (LIV) which is converted to the known (50) 11-ketocholanate by chromic acid oxidation, zinc dust reduction of the double bond, and Raney nickel reduction of the 7-ethylenedithioketal-derivative.

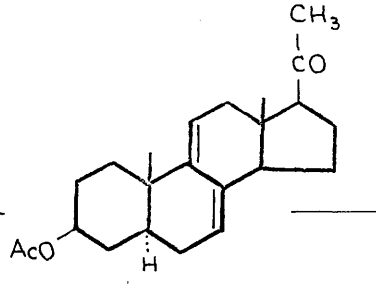
Aliphatic Peracid Route.

This method seems to be applicable only to compounds of the allo series; it does not apply to the 5β-series.

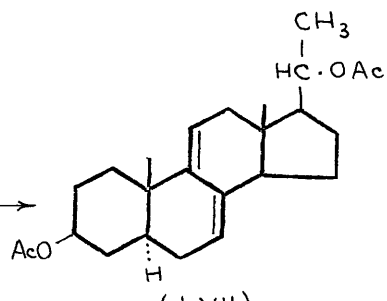
Diosgenin acetate (XXXI) is converted into 22a-allo-spirost-7-en-3β-yl acetate (XXXIII) as described above (42, 43), and the latter into 20-ketoallopregn-7-en-3β-yl acetate (LV), oxidation of which with mercuric acetate yields the 7:9(11)-diene (LVI) (51). In a sequence of reactions leading from diosgenin to cortisone, the



(LV)



(LVI)

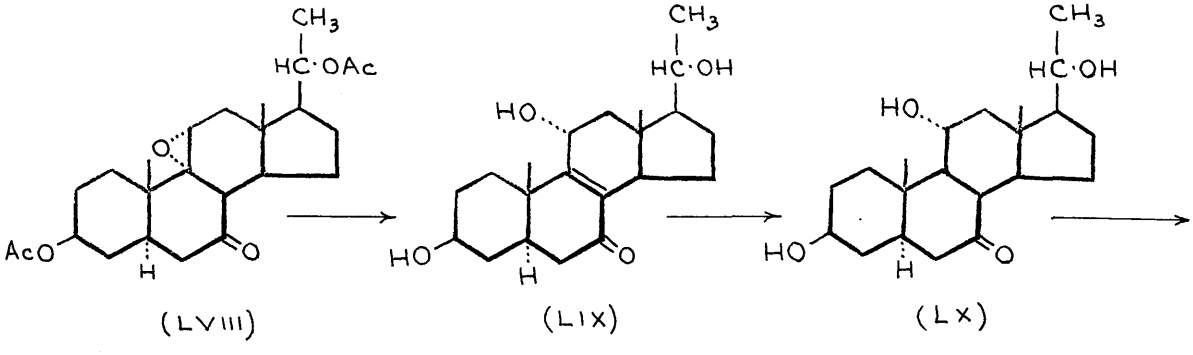


(LVII)

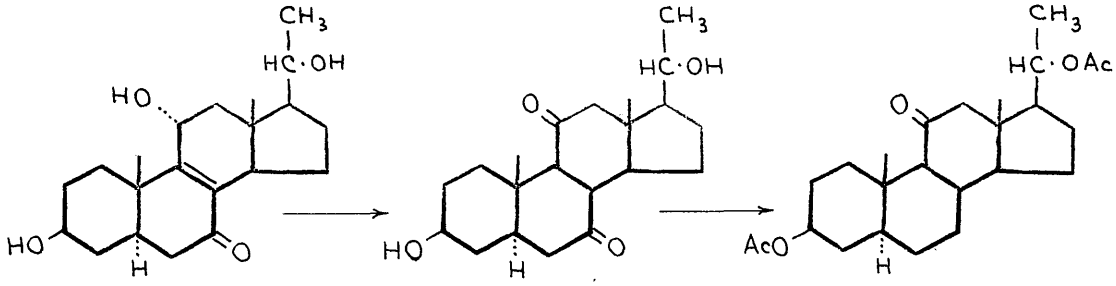
compound (LV) was reduced with lithium aluminium hydride followed by dehydrogenation of the diacetate with mercuric acetate to give 3β:20β-diacetoxyallopregna-7:9(11)-diene

(LVII) (52).

Oxidation (53) of the 7:9(11)-diene (LVII) with performic acid gives 3β:20β-diacetoxy-9α:11α-epoxy<u>allo</u>-pregnan-7-one (LVIII) which is isomerised by alkaline hydrolysis to give 3β:11α:20β-trihydroxy<u>allo</u>pregn-8-en--7-one (LIX). Catalytic hydrogenation of the double bond to (LX) followed by Wolff-Kishner reduction gives 3β:11α:20β-trihydroxy<u>allo</u>pregnane (LXI). Chromic acid oxidation of (LXI) yields the known (55) triketone (LXII), reduction of which with Raney nickel gives 11:20-diketo-<u>allo</u>pregnan-3β-ol (LXIII).

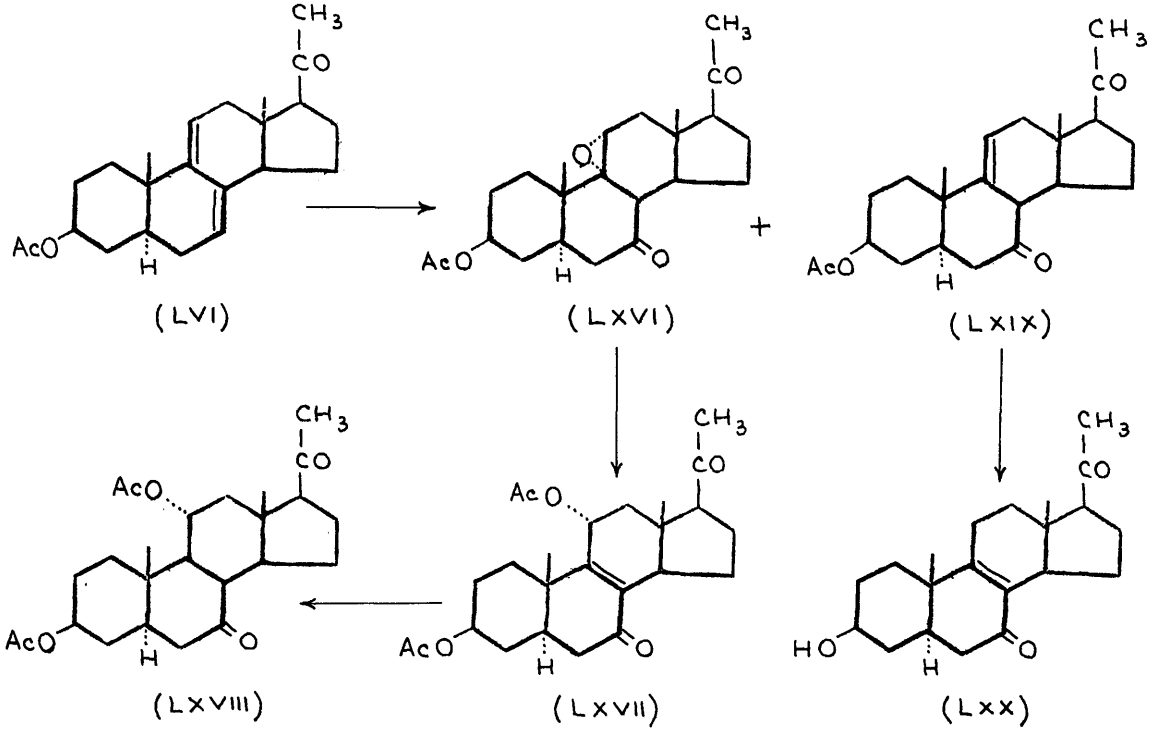


A variation of the method has been recently described by Djerassi and co-workers (54). Refluxing of 3β:11α:20β--trihydroxyallopregn-8-en-7-one (LIX) with potassium t-butoxide in t-butanol gives the isomeric 3β:20β--dihydroxyallopregnan-7:11-dione (LXIV). This is converted into 3β:20β-diacetoxyallopregnan-11-one (LXV) by formation of a 7-ethylenedithioketal and desulphurisation with Raney nickel.



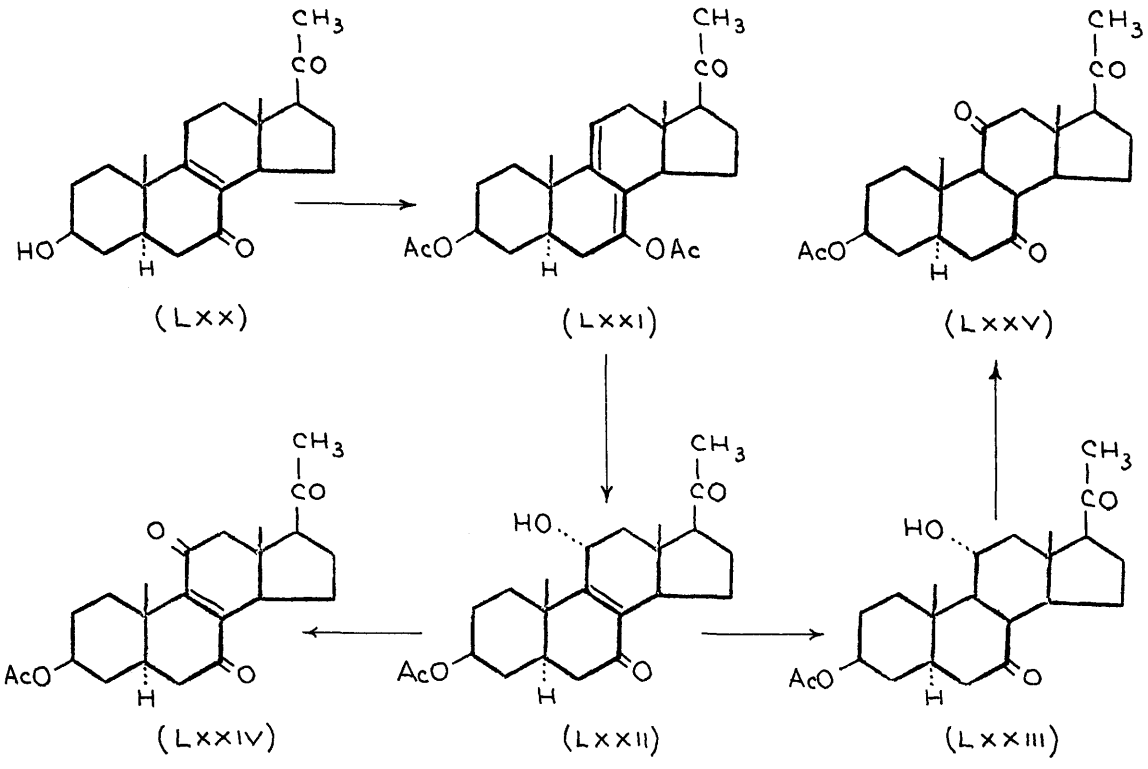
Performic acid oxidation (34) of 20-ketoallopregna--7:9(11)-dien-3β-yl acetate (LVI) yields 9α:11α-epoxy--7:20-diketoallopregnan-3β-yl acetate (LXVI). Alkaline hydrolysis followed by acetylation gives 3β:11α-diacetoxy--7:20-diketoallopregn-8-ene (LXVII), converted by catalytic hydrogenation into 3β:11α-diacetoxy-7:20-diketoallopregnane (LXVIII). Alkaline hydrolysis of the performic acid liquors from which (LXVI) had been removed, yielded the αβ-unsaturated ketone 7:20-diketoallopregn-8-en-3β-ol (LXX), presumably by rearrangement of the by-product $\Delta^{9(11)}$-7-ketone

(LXIX). Treatment of (LXX) with isopropenyl acetate in presence of p-toluenesulphonic acid gives the enol-acetate (LXXI), which is oxidised with monoperphthalic



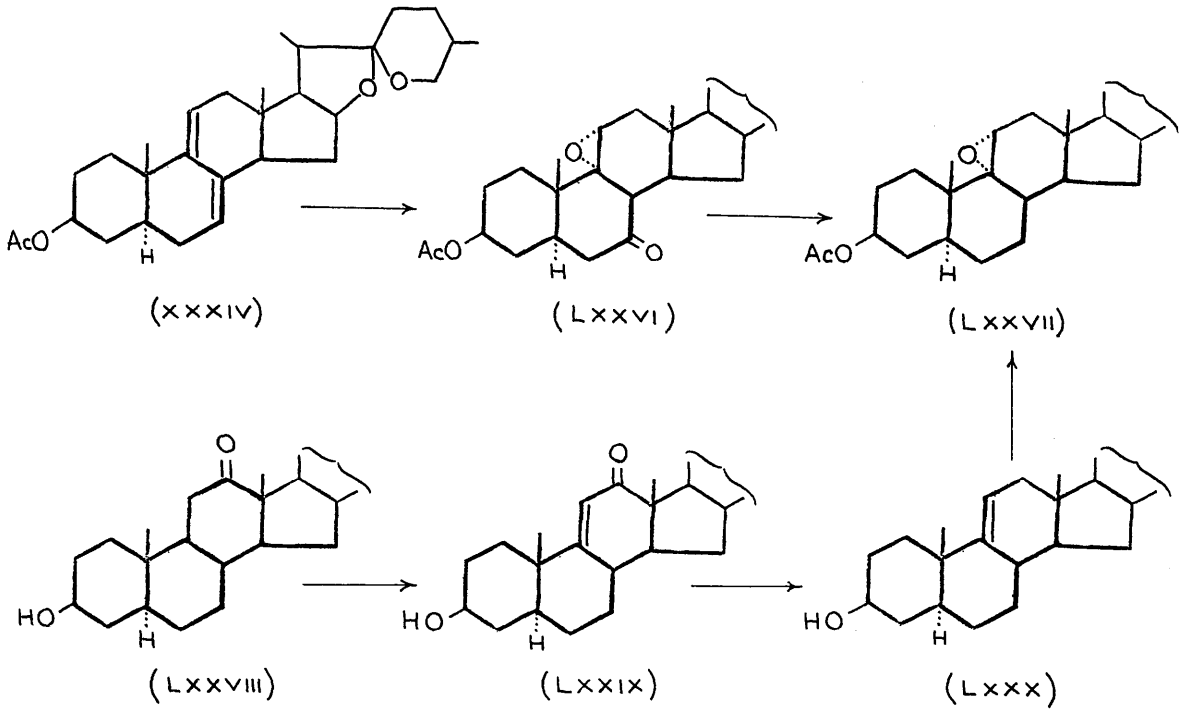
acid to 11α-hydroxy-7:20-diketoallopregn-8-en-3β-yl acetate (LXXII). Hydrogenation of the last compound gives 11α-hydroxy-7:20-diketoallopregnan-3β-yl acetate (LXXIII). A useful feature of this procedure (56) is that it leads to products containing a 3β-acetoxy- and 11α-hydroxy-groups thus allowing a differential treatment of the 3- and 11-substituents. Thus, chromic acid oxidation of (LXXII) gives 7:11:20-triketoallopregn-8-en-3β-yl acetate (LXXIV) and similar oxidation of (LXXIII) yields

7:11:20-triketo<u>allo</u>pregnan-3β-yl acetate (LXXV).



Performic acid oxidation (57, 53) of 22a-<u>allo</u>-spirosta-7:9(11)-dien-3β-yl acetate (XXXIV) obtained from diosgenin as described earlier, gives 9α:11α-epoxy--7-keto-22a-<u>allo</u>spirostan-3β-yl acetate (LXXVI). This was converted into 9α:11α-epoxy-22a-<u>allo</u>spirostan-3β-yl acetate (LXXVII) by Raney nickel desulphurisation of the 7-ethylenedithioketal. The last compound (LXXVII) was also obtained from hecogenin (LXXVIII) (56, 58), which was converted into 12-keto-22a-<u>allo</u>spirost-9(11)-en-3β-ol (LXXIX) (58) by oxidation with selenium dioxide. Wolff--Kishner reduction (31) of this compound gives (LXXX),

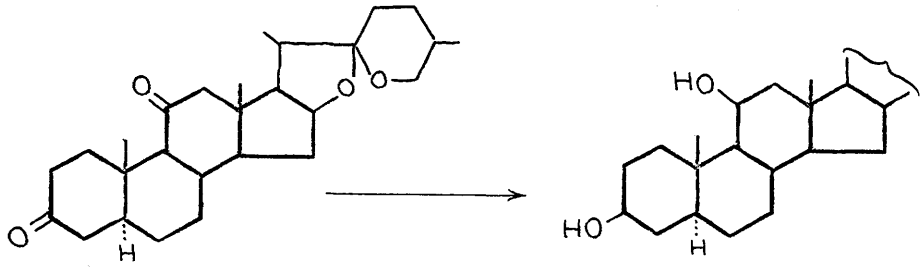
the acetate of which on treatment with perbenzoic acid gives the 9α:11α-epoxide (LXXVII).



The behaviour of the ketoxide (LXXVI) exactly parallels that of the related pregnane derivatives described above. Thus the compounds corresponding to (LIX)-(LXV) have been prepared using the same sequence of reactions (54). One additional 11-oxygenated sterol has been obtained by reduction of the corresponding diketone (LXXXI) with lithium aluminium hydride to 3β:11β--dihydroxy-22a-allospirostane (LXXXII) (cf. 59).

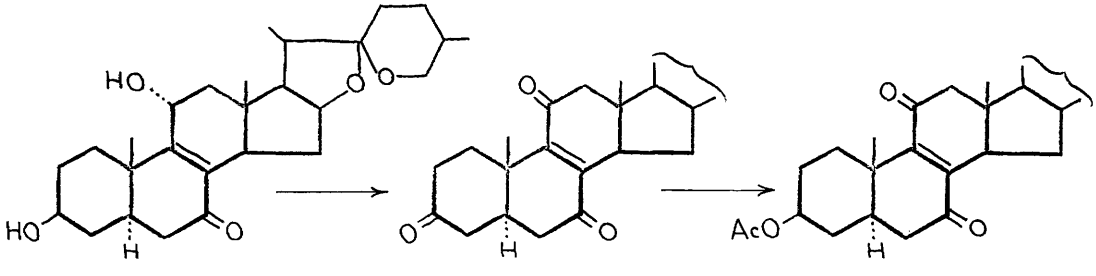
Furthermore, oxidation of 3β:11α-dihydroxy-22a-allo-

spirost-8-en-7-one (LXXXIII) with chromic acid yields the trione (LXXXIV), partial reduction of which, using



(LXXXI) (LXXXII)

a Raney nickel catalyst, followed by acetylation, gives 7:11-diketo-22a-allospirost-8-en-3β-yl acetate (LXXXV),



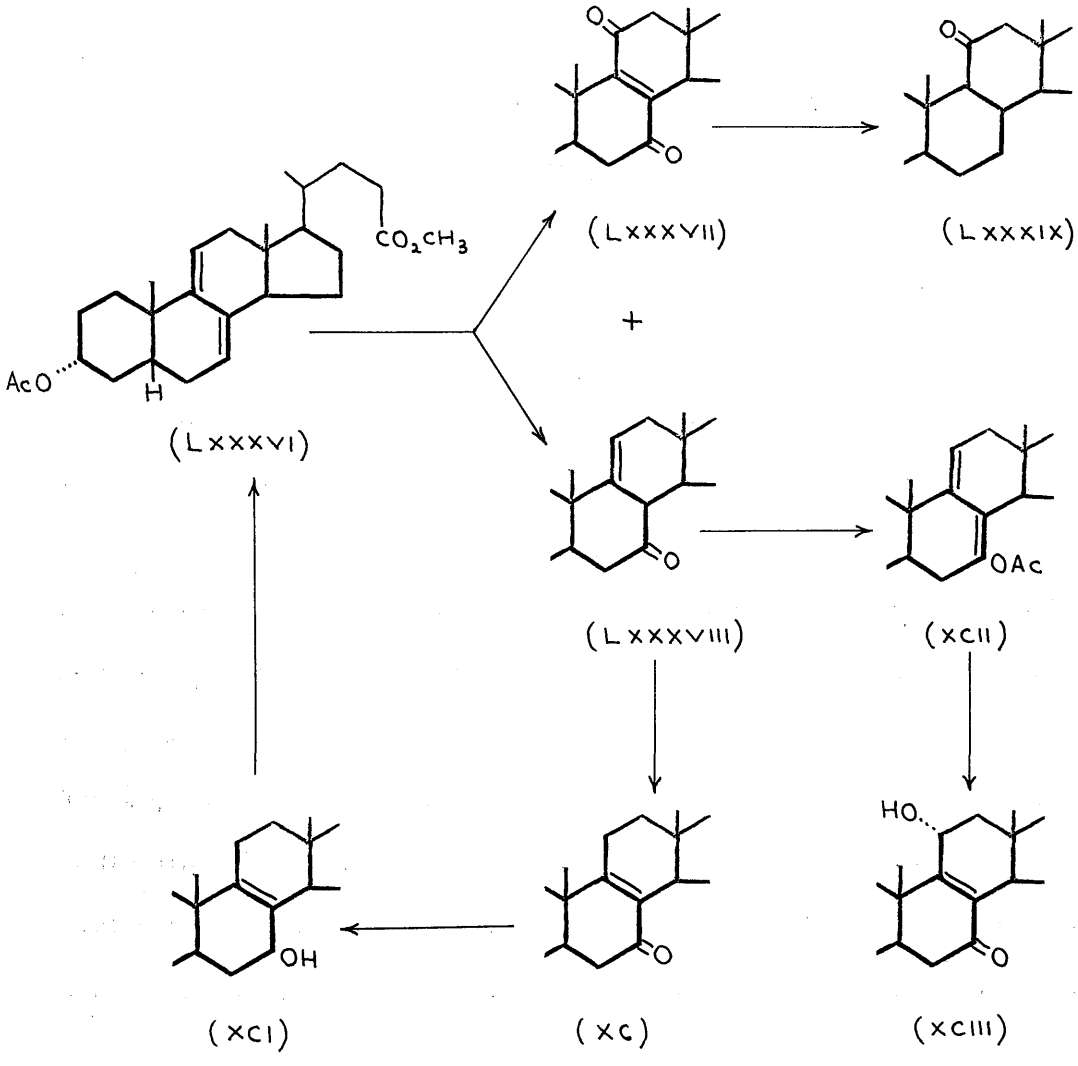
(LXXXIII) (LXXXIV) (LXXXV)

an intermediate in the synthesis of 11:20-diketoallo-pregnan-3β-yl acetate (XXXVI) from diosgenin using the perbenzoic acid route.

Other Routes.

In addition to other oxidizing agents for the conversion of 7:9(11)-dienes into 11-oxygenated steroids, Fieser and co-workers (60, 61, 62) investigated sodium

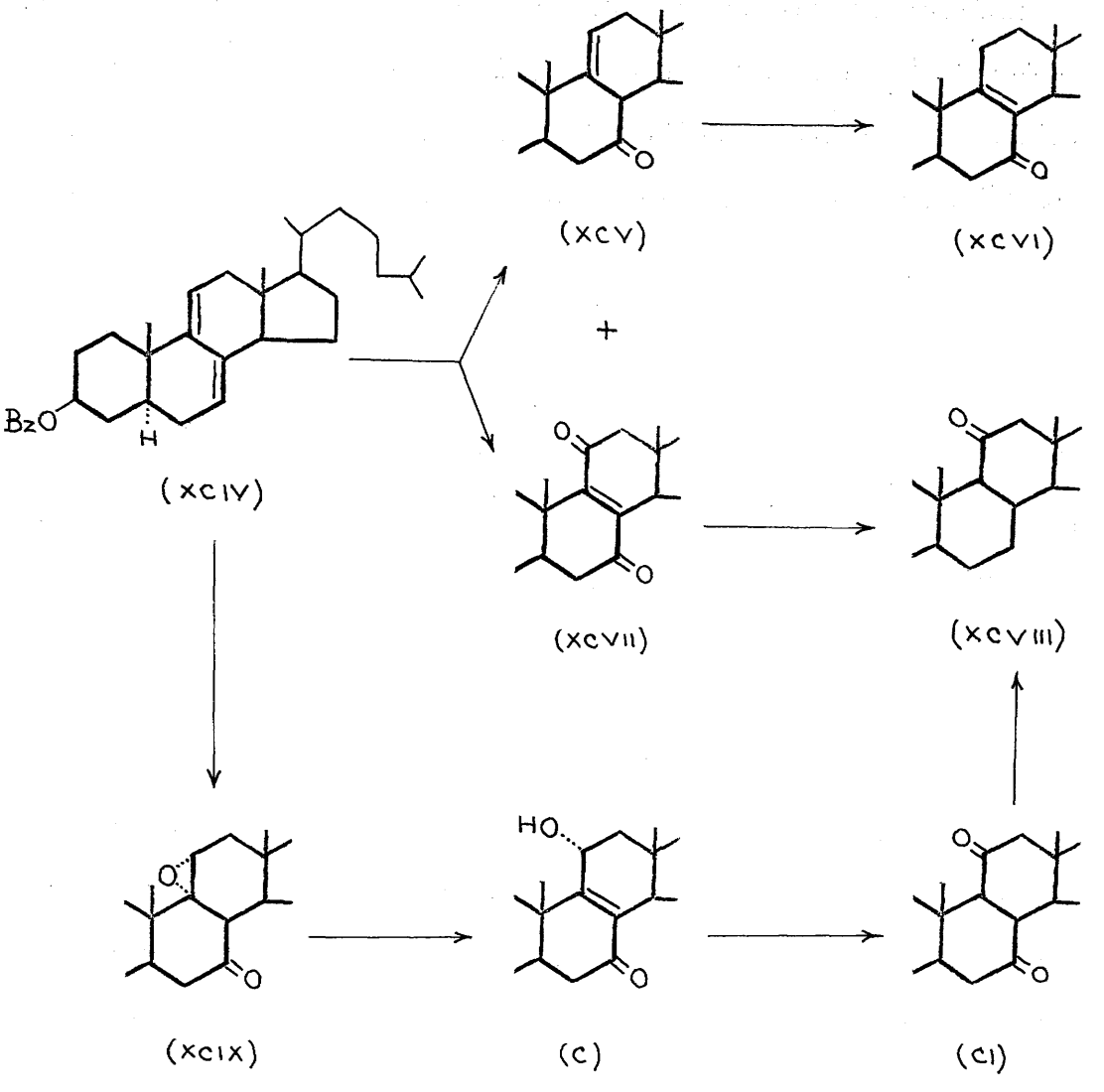
dichromate and N-bromosuccinimide. Oxidation of methyl 3α-acetoxychola-7:9(11)-dienate (LXXXVI) with sodium dichromate in acetic acid (32, 60) gave the $\Delta^{9(11)}$-7--ketone (LXXXVIII) and the Δ^{8}-7:11-diketone (LXXXVII). The latter product was transformed by reduction of the



double-bond with zinc and removal of the less hindered 7-keto group by Wolff-Kishner method into the methyl ester acetate of 11-ketolithocholic acid (LXXXIX). The $\beta\gamma$-unsaturated ketone (LXXXVIII) is easily isomerised to the conjugated ketone (XC), which can be converted back to the diene (LXXXVI) by reduction of the carbonyl group with sodium and amyl alcohol to (XCI) and dehydration with mineral acid, and oxidised through the enol acetate (XCII) to (XCIII) with perphthalic acid.

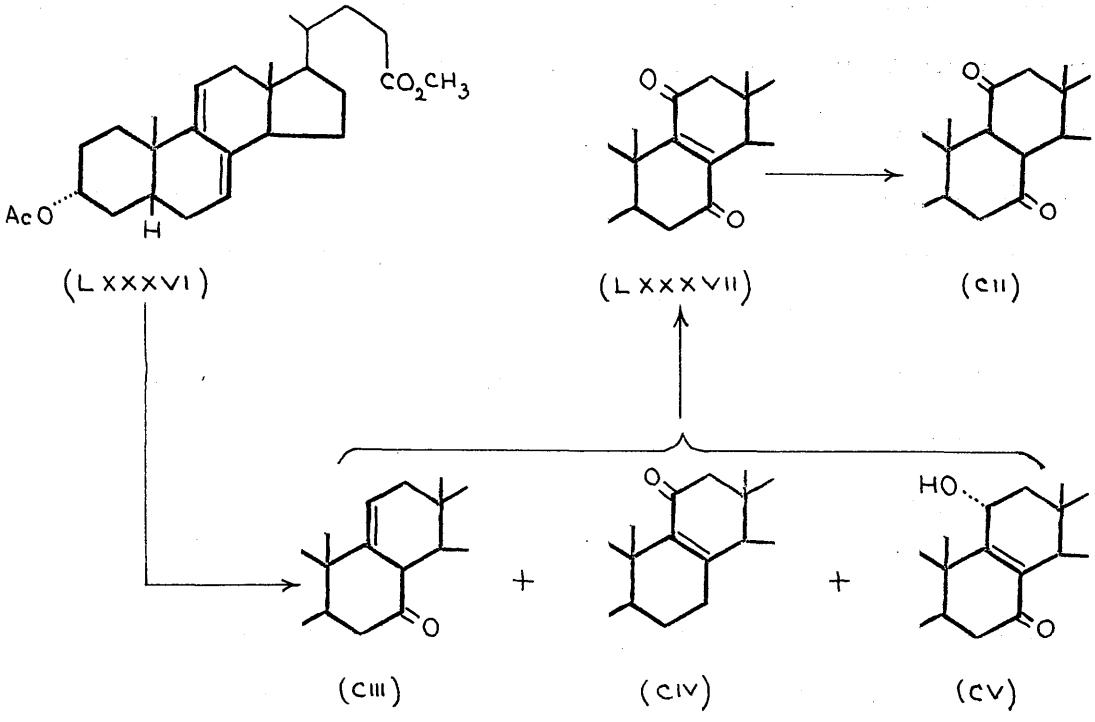
This method is also applicable to 7:9(11)-dienes of the 5α-series (61). Dichromate oxidation of 7:9(11)--cholestadienyl benzoate (XCIV) gave the Δ^{8}-7:11-diketone (XCVII) and the $\Delta^{9(11)}$-7-ketone (XCV) which was isomerised to the $\alpha\beta$-ketone (XCVI) by zinc and acetic acid. Reduction of (XCVII) with zinc and acetic acid followed by Wolff-Kishner reduction afforded 11-ketocholestanol (XCVIII). Oxidation of (XCIV) with hydrogen peroxide and ferrous sulphate (or peracetic acid) gave the 7-keto--9α:11α-epoxide (XCIX), convertible through (C) into (CI) by the methods reported by Djerassi *et al*. (33, 34).

Another method for use with either a *cis*- or a *trans*--A/B junction has been described (35, 62). 7:9(11)--Dienes of the bile acid (LXXXVI), cholesterol and ergosterol series have been converted into the saturated



7:11-diketones (CII) by reaction with N-bromosuccinimide in t-butanol-dilute sulphuric acid, followed by further oxidation with silver chromate and reduction with zinc and acetic acid. In the bile acid series initial products of reaction with the bromoimide have been characterised (62)

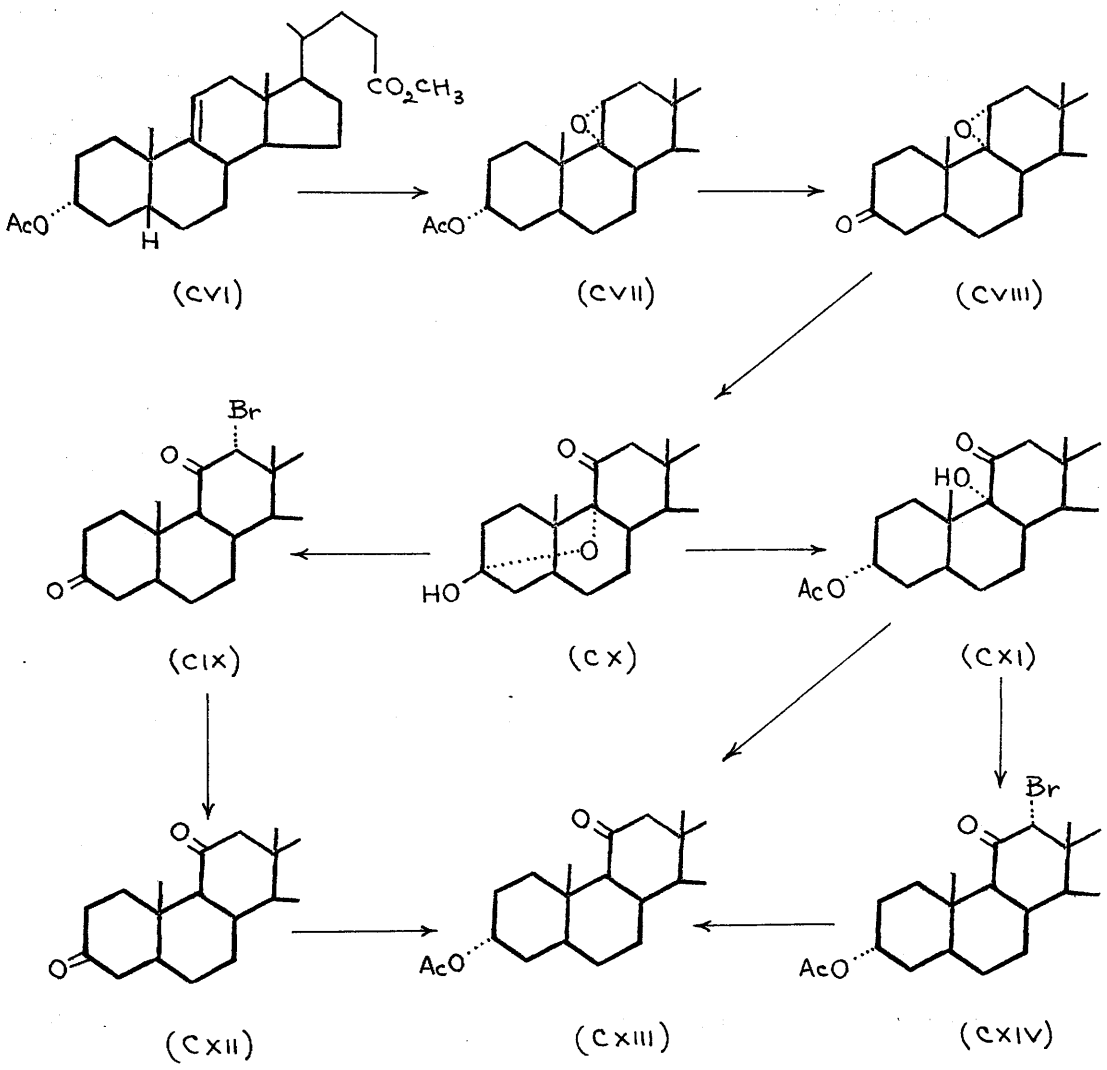
as the 9(11)-ene-7-one (CIII), 8-ene-11-one (CIV) and 8-ene-11α-ol-7-one (CV).



(LXXXVI) (LXXXVII) (CII) (CIII) (CIV) (CV)

A new route to 11-ketosteroids starting from 9(11)--unsaturated compounds (CVI) was described (63). By successive oxidation with perbenzoic acid and sodium dichromate the epoxides (CVII) and (CVIII) are prepared. The latter is oxidised with chromic acid to give the 3α:9α-epoxide (CX) which reacts with hydrogen bromide to form the bromodiketone (CIX). Reductive elimination of the bromo-atom leads to the diketone (CXII), and partial reduction with sodium borohydride, followed by acetylation,

to (CXIII). Reduction of (CX) with sodium borohydride gives rise to the formation of a mixture of triols which contains 3α:9α:11β-triol in largest amount. The 3-monoacetate of this triol can be oxidised to (CXI). The conversion of (CXI) to (CXIII) is accomplished either



(CVI) (CVII) (CVIII)

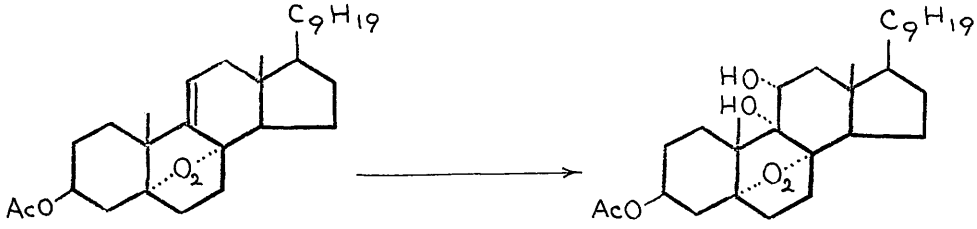
(CIX) (CX) (CXI)

(CXII) (CXIII) (CXIV)

by direct Clemmensen reduction or via the bromoketone (CXIV).

This type of method is applicable only to compounds with a cis-A/B ring junction, since the oxide ring in (CX) cannot be produced in the trans-A/B series.

In ergosterol series, Jones and co-workers (68) have recently reported that oxidation of 3β-acetoxy-5α:8α--epidioxyergost-9(11)-ene (CXV) with potassium permanganate in acetic acid gives the corresponding 9:11-diol (CXVI).

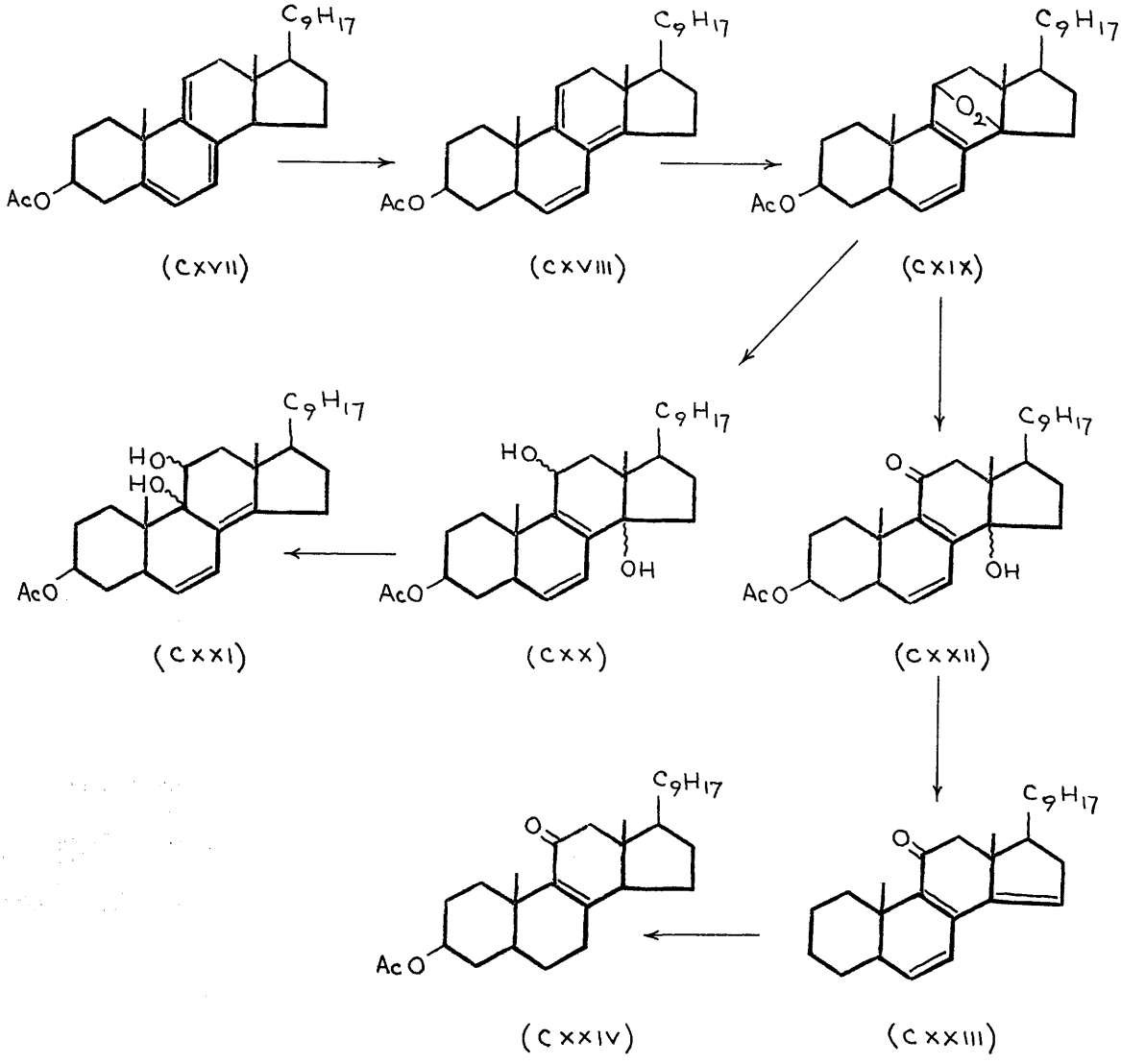


(CXV) (CXVI)

A new synthetic route has been recently devised by Laubach et al. (102) for the conversion of C-ring unsubstituted steroids to cortisone. Dehydroergosteryl acetate (CXVII), prepared by mercuric acetate dehydrogenation of ergosteryl acetate, was catalytically isomerised with liquid sulphur dioxide in over 80% yield to the 6:8(14):9(11):22-tetraene(CXVIII), which on photochemical peroxidation (103) afforded the 11:14-peroxide (CXIX). Selective hydrogenation of the peroxide over a lead-

-palladium catalyst gave a glycol (CXX), which underwent acid-catalyzed anionotropic rearrangement to a readily acylated isomer, the 9:11-diol (CXXI).

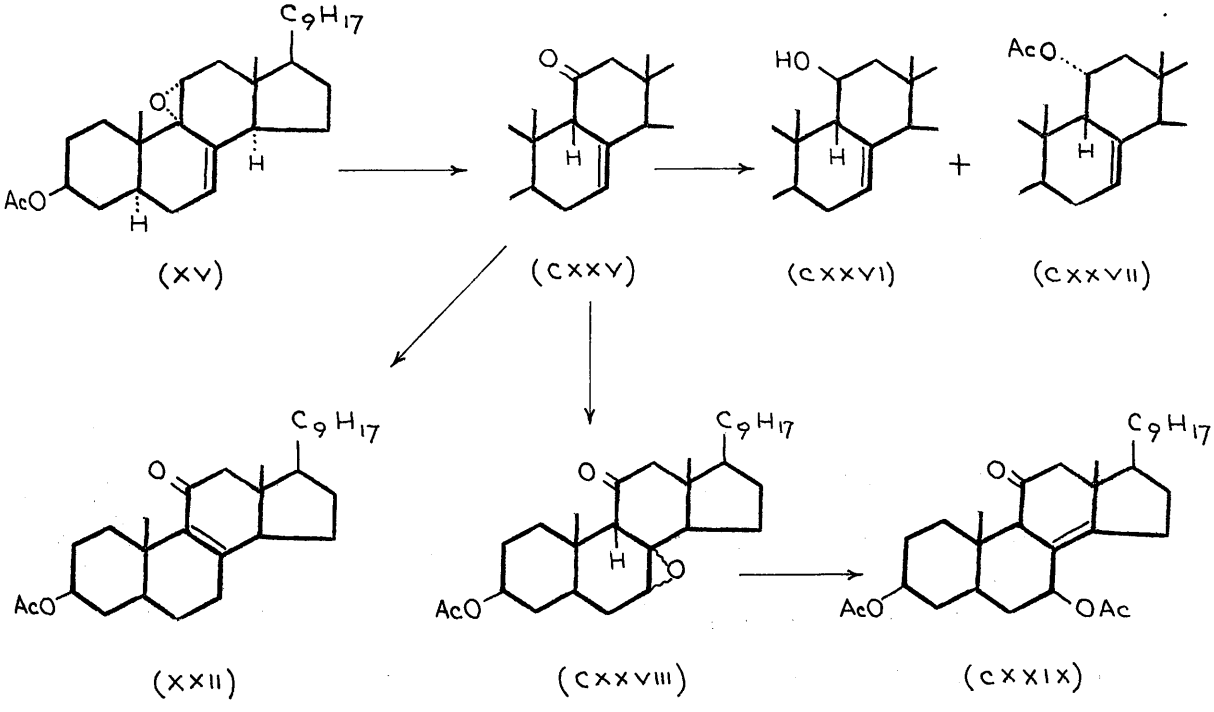
Mild base-catalyzed rearrangement of the peroxide (CXIX) yielded the 6:8:22-trien-14-ol-11-one (CXXII).



(CXVII) (CXVIII) (CXIX) (CXXI) (CXX) (CXXII) (CXXIV) (CXXIII)

Acid-catalyzed dehydration, followed by reacetylation led to the 6:8:14:22-tetraen-11-one (CXXIII), which on selective hydrogenation over W-7 Raney nickel gave the known intermediate, 3β-acetoxyergosta-8:22-dien-11-one (CXXIV).

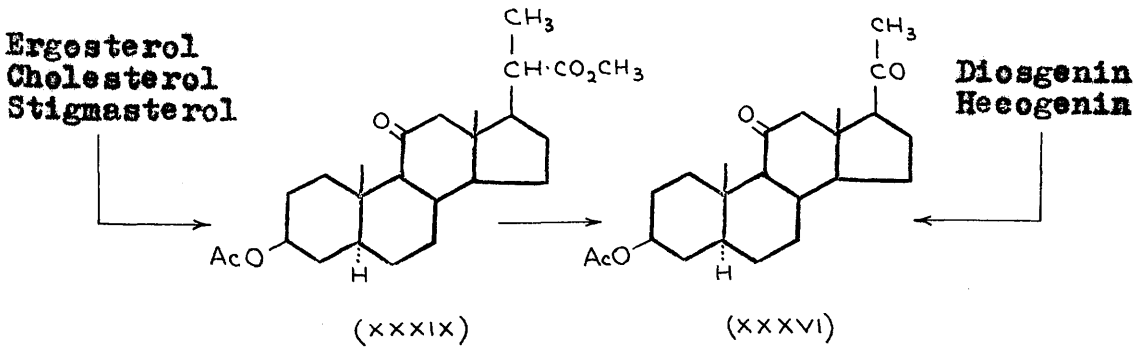
Recently Heusler and Wettstein (81) have reported the following series of transformations: Treatment of 9α:11α-epoxyergost-7-en-3β-yl acetate (XV) with boron trifluoride etherate in absolute ether gives the Δ^7-11-ketone (CXXV) which belongs to the 9β-series. This intermediate (CXXV) is rearranged with mineral acid or



boron trifluoride in benzene to the known conjugated Δ^{8}-11-ketone (XXII). Treatment of the unconjugated ketone (CXXV) with lithium aluminium hydride followed by acetylation gives rise to the compounds (CXXVI) and (CXXVII) with the unnatural 9β-configuration. Oxidation with monoperphthalic acid yields the ketoxide (CXXVIII) which is converted into (CXXIX) on treatment with boron trifluoride in dioxan, followed by acetylation. Similarly the Δ^{8}-11-ketones of the androstane and cholestane series have been prepared.

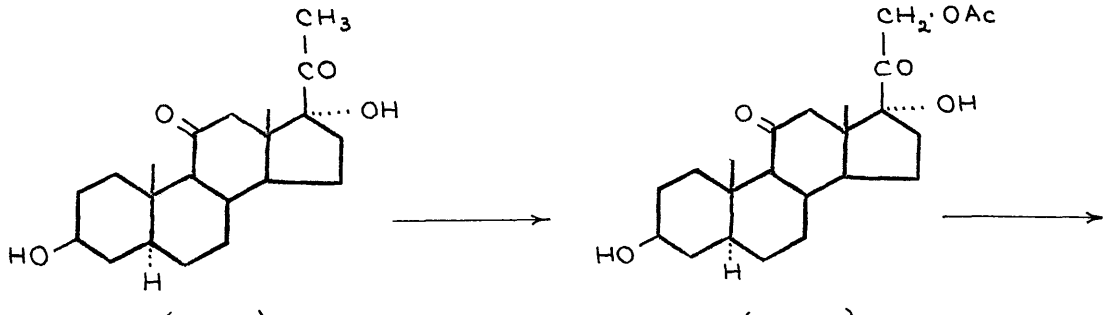
Conversion of 11-Oxygenated Steroids into Cortisone.

Ergosterol has been converted into 11-ketoergost-22--en-3β-yl acetate (XIX) as described above (29, 30). Ozonolysis of (XIX) followed by esterification gives methyl 3β-acetoxy-11-ketobisnorallocholanate (XXXIX), which is also obtained from methyl 3β-acetoxybisnorchol-5-enate (XXXVII), itself obtained from either cholesterol or

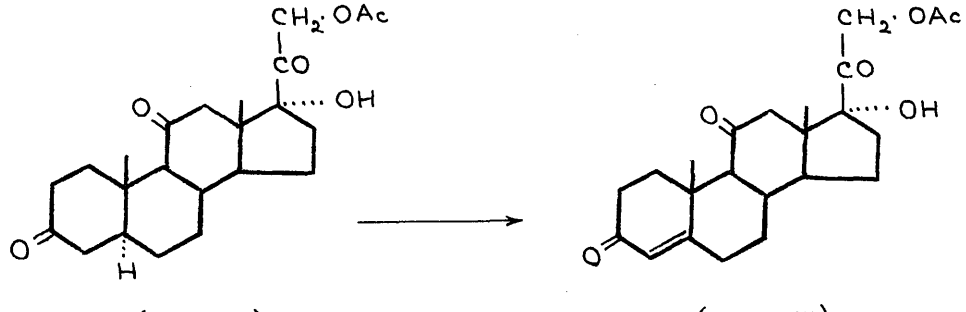


stigmasterol (29). Barbier-Wieland degradation of (XXXIX) gives 11:20-diketoallopregnan-3β-yl acetate (XXXVI) also obtained from diosgenin and hecogenin as described above.

The transformation of (XXXVI) to cortisone acetate (CXXXIII) includes introduction of two hydroxyl groups in positions 17 and 21 and of the αβ-unsaturated ketone in ring A (64, 65). Using the Gallagher method (66), 11:20-diketone (XXXVI) is converted into its 11:20-dienol--acetate, oxidation of which with perbenzoic acid followed by alkaline hydrolysis gives 3β:17α-dihydroxy-11:20--diketoallopregnane (CXXX). Bromination of the last



(CXXX) (CXXXI)

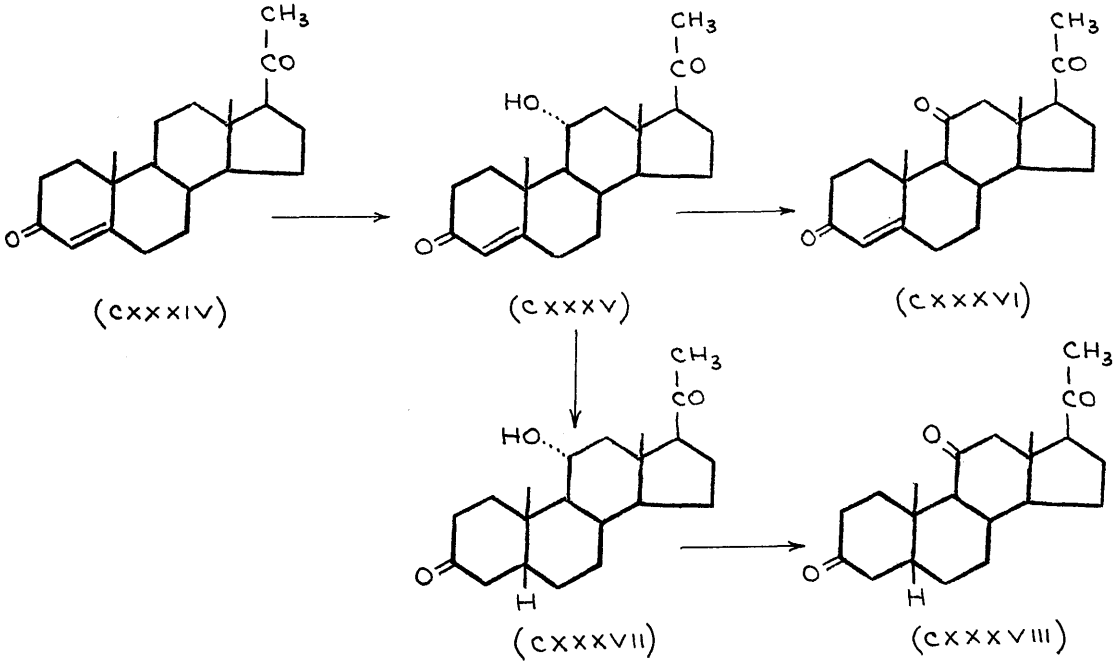


(CXXXII) (CXXXIII)

compound followed by treatment of the 21-bromo-derivative with sodium acetate (64) or with sodium iodide followed by potassium acetate (65) gives 3β:17α-dihydroxy-11:20--diketo<u>allo</u>pregnan-21-yl acetate (CXXXI). Oxidation of (CXXXI) with N-bromoacetamide yields 17α-hydroxy-3:11:20--triketo<u>allo</u>pregnan-21-yl acetate (CXXXII) (64, 65). Bromination and dehydrobromination of the last compound (64), or using a method previously employed with other 3--keto-<u>allo</u>-steroids (67), gives cortisone acetate (CXXXIII).

<u>Microbiological Oxidation.</u>

The ability of several micro-organisms to oxidise steroids at C_{11} in one simple step has been reported recently (70, 71, 73). Bio-oxygenation of progesterone (CXXXIV) yields 11α-hydroxyprogesterone (CXXXV), oxidation



(CXXXIV) (CXXXV) (CXXXVI)

(CXXXVII) (CXXXVIII)

of which gives the known 11-ketoprogesterone (CXXXVI). Catalytic reduction of (CXXXV) over palladised charcoal in presence of alkali (71) gives 3:20-diketopregnan-11α--ol (CXXXVII), which allows a simple part-synthesis of cortisone from progesterone in ten stages (72, 104).

Further studies revealed (74) that fungus Rhizopus nigricans Ehrb. in particular produces excellent yields (85-95%) of 11α-hydroxyprogesterone (CXXXV) from progesterone (CXXXIV).

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THEORETICAL

Introduction.

The work described in this thesis had as its object the development of routes to 11-oxygenated steroids from ergosterol in a projected partial synthesis of cortisone. The investigation was commenced in June 1951, at a stage when a general scheme for the synthesis of 11-keto--steroids from steroids containing a 5-ethylenic linkage such as ergosterol, diosgenin and stigmasterol had been reported in a preliminary note by Tishler and co-workers (29), and when Spring and co-workers (106, 107) had perfected the method for the preparation of 5-dihydro-ergosteryl acetate from ergosteryl acetate and considerably improved the yield of pure ergosteryl-D acetate, the starting material used in these investigations.

Since that time, many publications have appeared, describing various oxidation procedures directed towards the formation of 11-oxygenated steroids starting from 7:9(11)-dienic steroids. Many of these communications are in outline form only without experimental details. The experiments described in this thesis, performed during the same period, have in part been reported in a series of publications (107-114).

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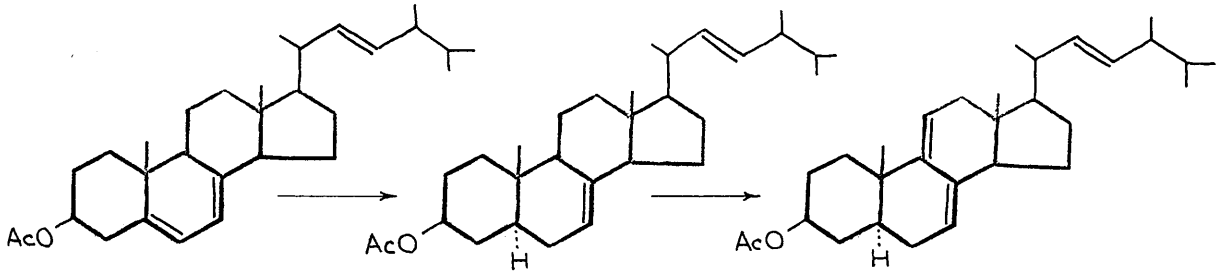
Ergosteryl-D Acetate.

The approach to the syntheses of 11-oxygenated steroids started from ergosteryl-D acetate [ergosta--7:9(11):22-trien-3β-yl acetate] (III) the simplest available derivative of ergosteryl acetate (I) containing unsaturation involving C_{11} and a side-chain ethylenic linkage. The most efficient method of preparation of ergosteryl-D acetate (III) consisted of the preparation of 5-dihydroergosteryl acetate (II), followed by oxidation with mercuric acetate to give (III).

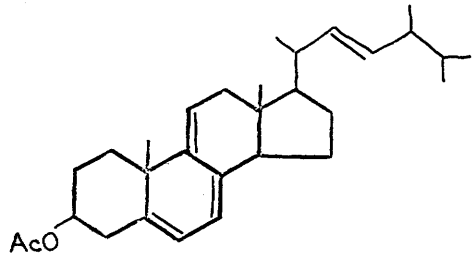


(I) (II) (III)

The preparation of 5-dihydroergosteryl acetate (II) has been reported by Heilbron and Sexton (14), Wieland and Benend (15) and Barton and Cox (16) by the partial hydrogenation of ergosteryl esters in a neutral solvent; the last-named authors obtained 30-35% yield after $1\frac{1}{2}$ - 2 hours reaction time, using a platinum catalyst and chloroform as solvent. Anderson, Stevenson and Spring (106) have isolated 5-dihydroergosteryl acetate of high

purity in practically quantitative yield (95%) by performing the hydrogenation in benzene solution with Raney nickel catalyst. The hydrogenation could be completed within 15 minutes. Since that time, other improved procedures have also been reported by various workers: Panizzon and Kägi (36; p.2123, footnote) obtained a 90-95% yield with Rupe nickel in ether solution; more recently Laubach and Brunings (75) reported a quantitative yield with Raney nickel in dioxan and, finally, Ruyle et al. (76) have effected the same reaction in similar yield using a Raney nickel catalyst and benzene as solvent.

Ergosteryl-D acetate was prepared by oxidation of 5-dihydroergosteryl acetate by means of mercuric acetate (19, 20), using a modification of the existing method, first employed by Bergmann and Stevens (22) for the



(IV)

preparation of dehydroergosteryl acetate (IV) from ergosteryl acetate in chloroform solution. Although the yield obtained in the oxidation of 5-dihydroergosteryl

acetate to ergosteryl-D acetate by mercuric acetate has been improved, the method is still far from satisfactory. A crude reaction product is isolable in 85% yield, which on purification by crystallisation, gives ergosteryl-D acetate, $[\alpha]_D$ +23°, in 48-50% yield. This product, however, is not quite homogeneous. Purification of this material to constant optical rotation by crystallisation proved extremely wasteful, lowering the yield of product with $[\alpha]_D$ $\not<$ +28° to 30%. Pure ergosteryl-D acetate, $[\alpha]_D$ +30°, exhibits the characteristic ultraviolet light absorption, viz, well-defined maxima at 2350 and 2420 Å (principal, ε = 18,000) with an inflection at 2510 Å, which appears to be characteristic of 7:9(11)-dienic steroids (77). A comparison of specific rotation values and the principal bands of the absorption spectra of the various samples prepared, suggests that the impurity is unreacted 5-dihydroergosteryl acetate ($[\alpha]_D$ -21°, showing no light absorption above 2200 Å). 5-Dihydroergosteryl acetate and ergosteryl-D acetate cannot be separated by chromatography.

Ergosteryl-D acetate was subsequently prepared in reasonable yield, according to the method developed by Anderson, Stevenson and Spring (106), using a bromination procedure (to be discussed) whereby a high purity was

readily obtained, as shown by comparison of its melting point, specific rotation and ultraviolet light absorption intensity with previously reported values.

Ergosteryl-D Acetate

Method of preparation	Source	M.p.	$[\alpha]_D$	ε at 2420 Å in EtOH
Bromine	This work	178-180°	+33°	19,000
Mercuric Acetate	This work	176°	+30°	18,000
Mercuric Acetate	(36)	169-170°	+21°	16,000
Mercuric Acetate	(16)	169°	+18°	13,200
Perbenzoic Acid	(78)	171°	+26°	—

Ergosteryl-D acetate, $[\alpha]_D$ +28°, was employed as starting material in the experiments described below. Homogeneity of the product was verified by alkaline hydrolysis to the free alcohol followed by reacetylation. Ergosteryl-D benzoate was also prepared from ergosterol-D by the usual method.

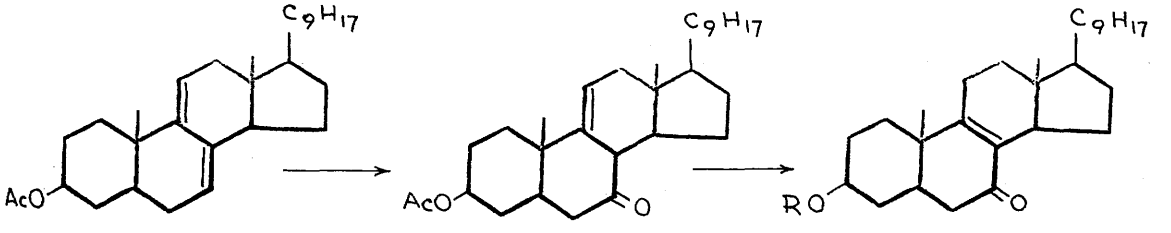
11-OXYGENATED STEROIDS FROM ERGOSTERYL-D ACETATE.

Oxidation with Chromic Acid.

The oxidation of ergosteryl-D acetate (III) with chromium trioxide in acetic acid was first examined under a variety of conditions. In all cases, 3β-acetoxyergosta--8:22-dien-7-one (VI) was obtained in poor yield. This αβ-unsaturated ketone was first obtained by Stavely and Bollenback (39), as a minor product of the oxidation of 5-dihydroergosteryl acetate (II) with chromium trioxide.

When ergosteryl-D acetate was oxidised with chromium trioxide in acetic acid at 50°, a crystalline compound was isolated, $C_{30}H_{46}O_3$, m.p.174-176°, $[\alpha]_D$ -54°, which does not exhibit selective absorption of high intensity above 2200 Å and gives a yellow colour with tetranitromethane in chloroform. This compound is isomeric with 3β-acetoxy-ergosta-8:22-dien-7-one (VI). It is converted into the αβ-unsaturated ketone (VI) by filtration of its solution in light petroleum-benzene through a column of alumina. Alkaline hydrolysis gives 3β-hydroxyergosta-8:22-dien-7-one (VI') showing a maximum at 2520 Å (ε = 11,000), which is also obtained on hydrolysis of 3β-acetoxyergosta-8:22--dien-7-one. The initial reaction product is an uncon-jugated ketone, 3β-acetoxyergosta-9(11):22-dien-7-one (V). Fieser and co-workers (35, 60, cf. 32) have shown that

oxidation of methyl 3α-acetoxychola-7:9(11)-dienate with sodium dichromate dihydrate gives methyl 3α-acetoxy-7--ketochol-9(11)-enate which, like the oxidation product from ergosteryl-D acetate, is readily isomerised by alkali to the corresponding Δ^{8}-7-ketone. Reaction with N-bromosuccinimide (62) gave the same unconjugated ketone. Dichromate oxidation of 7:9(11)-cholestadienyl benzoate (61) gave likewise the corresponding $\Delta^{8(11)}$-7-ketone (see "Historical"). Heusser and co-workers (37) described a compound, m.p.176-177°, $[\alpha]_D$ -58°, obtained by treatment of 7ξ:11α-dihydroxyergosta-8:22-dien-3β-yl acetate (XXIV) with hydrogen peroxide in acetic acid, as 3β-acetoxy-ergosta-9(11):22-dien-7-one (V). This is probably the

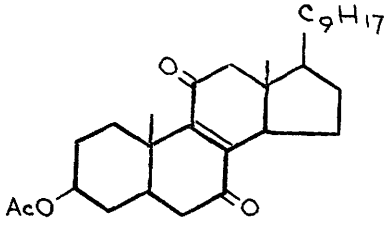


(III) (V) (VI, R=Ac ; VI', R=H)

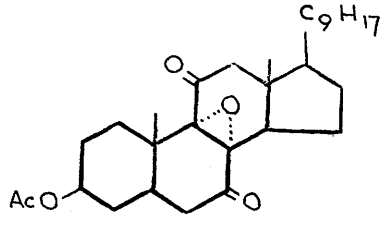
same as a compound (m.p.176-177°, $[\alpha]_D$ -43.5°) obtained by Tishler and co-workers (30) by controlled acid--isomerisation of 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate (XVIII). Further proof of structure employed for this ketone, and a discussion of the stereochemical

aspect is included later.

In addition to 3β-acetoxyergosta-8:22-dien-7-one (VI), oxidation of ergosteryl-D acetate with chromic anhydride gives, in very small yield, a second compound, m.p.127-128°, $[\alpha]_D$ -30°, showing a maximum at 2680 Å (ε = 3,300-4,700), which was not obtained in sufficient amount to allow a detailed investigation. The constants and the analysis ($C_{30}H_{44}O_{4-5}$), however, suggest a mixed crystal of 7:11-diketoergosta-8:22-dien-3β-yl acetate (VII) ($C_{30}H_{44}O_4$, m.p.133-135°, $[\alpha]_D$ +22°, ε_{2700} = 8,600) and 8α:9α-epoxy-7:11-diketoergost-22-en-3β-yl acetate (VIII) ($C_{30}H_{44}O_5$, m.p.130-132°, $[\alpha]_D$ -60°, no absorption of high intensity above 2200 Å). The same mixed crystal



(VII)



(VIII)

was later obtained on treatment of 7ξ:11α-dihydroxy-ergosta-8:22-dien-3β-yl acetate (XXIV) with chromium trioxide in acetic acid (cf. 36). Excess of oxidising agent promotes the formation of the ditertiary-epoxide (VIII). Separation of these two compounds by crystallis-

ation or chromatography is not easy. Fieser and co-workers (60, 61) obtained the corresponding Δ^{8}-7:11-diketones on dichromate oxidation of methyl 3α-acetoxychola-7:9(11)-dienate and 7:9(11)-cholestadienyl benzoate.

Oxidation of ergosteryl-D acetate with chromium trioxide in acetic acid containing sulphuric acid gives a product, which is almost certainly partially rearranged 3β-acetoxyergosta-9(11):22-dien-7-one (V), showing a maximum at 2520 Å (ε = 2,200). Chromatography of this product on alumina gives 3β-acetoxyergosta-8:22-dien-7-one. Again, a very small yield of the product, m.p.123-125°, was isolated by chromatography, which is probably a mixture of 8α:9α-epoxy-7:11-diketoergost-22-en-3β-yl acetate (VIII) and 7:11-diketoergosta-8:22-dien-3β-yl acetate (VII).

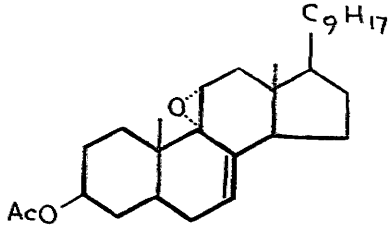
Oxidation with Performic Acid.

In view of the unpromising yields of the oxidation products described above, the oxidation of ergosteryl-D acetate with hydrogen peroxide in formic acid was next investigated.

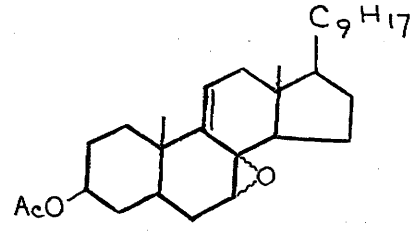
With one mol. of performic acid, a compound, $C_{30}H_{46}O_5$, m.p.194-197°, $[\alpha]_D$ +18° was obtained in good yield. This compound gives a pale yellow colour with tetranitromethane from which it follows that it is formed

by addition of oxygen to the conjugated system of ergosteryl-D acetate (in preference to the side-chain double bond), since the diene system of the last compound, in common with many conjugated dienes, gives a dark-brown colour with tetranitromethane. In support of this decision it was found that the compound $C_{30}H_{46}O_3$ does not exhibit selective ultra-violet absorption of high intensity above 2200 Å. The initial reaction product is unstable, simple crystallisation being accompanied by the appearance of selective light absorption with a maximum at 2540 Å. Hydrolysis of the initial reaction product with either dilute alkali or mineral acid is accompanied by rearrangement to give 3β-hydroxyergosta--8:22-dien-7-one (VI'). If the formation of a 7:11--oxide be excluded as sterically improbable, oxidation has occurred at either the 9:11- or at the 7:8-ethylenic bonds of ergosteryl-D acetate to give either an epoxide [(IX) or (X)] or a ketone [(XI) or (XII)]. The 9α:11α--configuration is ascribed to (IX) because of the well--established preferential rear attack by reagents of the 9- and the 11-position (40). The last compound (IX), obtained by the action of one mol. of perbenzoic acid on ergosteryl-D acetate (29, 108) is described later. The ease with which the compound, $C_{30}H_{46}O_3$, is rearranged to

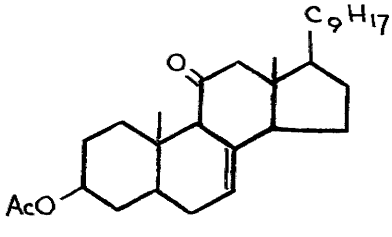
an αβ-unsaturated ketone (VI) by alkali and by simple crystallisation, is in marked contrast to the stability



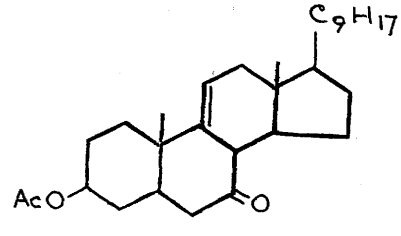
(IX)



(X)



(XI)



(XII)

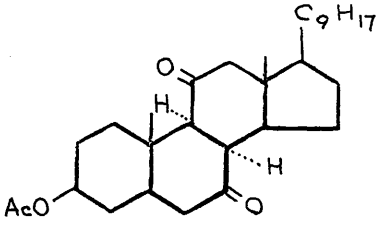
of the known ergosteryl-D epoxide (IX). The difference in reactivity between the epoxide and the compound, $C_{30}H_{46}O_3$, shows that the latter is not an epoxide, but rather a ketone [(XI) or (XII)]. That hydrolysis and rearrangement with alkali give 3β-hydroxyergosta-8:22--dien-7-one excludes (XI), and it is concluded that the compound $C_{30}H_{46}O_3$ is 3β-acetoxyergosta-9(11):22-dien-7-one (XII). These views are supported by the observation of Djerassi and co-workers (34) that treatment of the performic

acid mother-liquors, obtained from the conversion of 20-ketoallopregna-7:9(11)-dien-3β-yl acetate into 9α:11α--epoxy-7:20-diketoallopregnan-3β-yl acetate, with alkali gave 7:20-diketoallopregn-8-en-3β-ol presumably by rearrangement of the $\Delta^{8(11)}$-7:8-oxide and/or the $\Delta^{8(11)}$--7-ketone. So far, however, the value of the evidence is doubtful since the isomeric 7:8-oxides of ergosta--7:9(11):22-trien-3β-yl acetates are not known, and it is possible that one or both of these may readily rearrange to 3β-acetoxyergosta-8:22-dien-7-one (VI). It is unlikely that the oxidation product, $C_{30}H_{46}O_3$, is a 7:8--epoxide since it has been converted into 3β-acetoxy--9α:11α-epoxyergost-22-en-7-one (XVIII), by protection of the 22:23-ethylenic linkage by the addition of one mol. of bromine, followed by oxidation with perbenzoic acid and debromination of the product with zinc (108), identical with a compound obtained by the oxidation of ergosteryl-D acetate with two mols. of performic acid (to be discussed later).

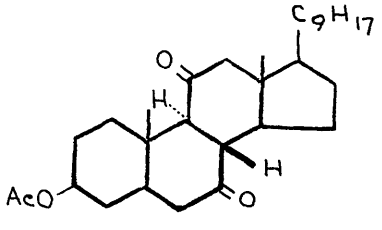
The structure of the oxidation product has been finally confirmed as 3β-acetoxyergosta-9(11):22-dien-7-one (XII) by an examination of its infra-red absorption spectrum which shows two well-resolved bands in the carbonyl region: one at 1740 cm.$^{-1}$ is ascribed to the

3β-acetate group and the other at 1715 cm.$^{-1}$ is ascribed to the 7-carbonyl group.

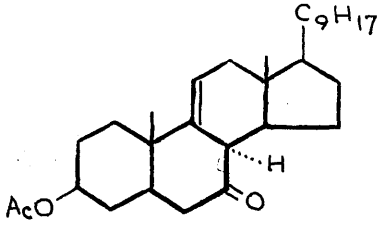
In view of the fact that the same structure, viz. 3β-acetoxyergosta-9(11):22-dien-7-one (XII), has been already ascribed to the compound (m.p.174-176°, $[\alpha]_D$ -54°) obtained by the oxidation of ergosteryl-D acetate with chromium trioxide and that the proof of structure employed by Heusser et al. (37) for this ketone is the same as that employed for the compound, m.p.194-197°, $[\alpha]_D$ +18° it is concluded that the two βγ-unsaturated ketones probably differ in orientation around C_8.



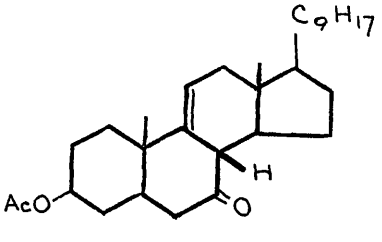
(XIII)



(XIV)



(XV)



(XVI)

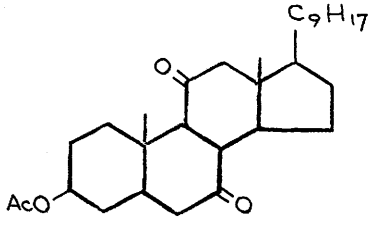
Later, a C_8 epimer (XIII) of 7:11-diketoergost-22--en-3β-yl acetate (XIV) has been discovered [(114); to be discussed] and a comparison of the molecular rotation relationships of the two diketones with those of the two unsaturated ketones confirms the original view and strongly suggests that the isomer of $[\alpha]_D$ +18° is 3β--acetoxy-8α-ergosta-9(11):22-dien-7-one (XV) and that the isomer of $[\alpha]_D$ -54° has the normal 8β-configuration (XVI).

Molecular Rotation Differences.

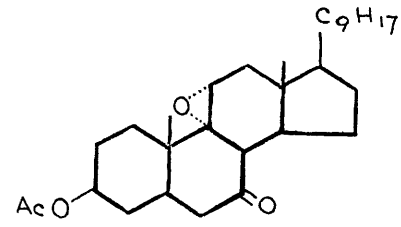
	C	$[\alpha]_D$	M.W.	$[M]_D$	$\Delta 8\beta \rightarrow 8\alpha$
7:11-diketone	8α	+30°	470	+141	+282
7:11-diketone	8β	-30°		-141	
$\Delta^{8(11)}$-7-ketone	8α	+18°	454	+82	+327
$\Delta^{8(11)}$-7-ketone	8β	-54°		-245	

Oxidation of ergosteryl-D acetate with two mols. of performic acid gave a compound, $C_{30}H_{46}O_4$, which does not show selective absorption of high intensity above 2200 Å. Two oxygen atoms have been introduced into the ergosteryl-D acetate molecule and, since the primary oxidation product of the performic acid oxidation of this compound has been shown to be 3β-acetoxyergosta-9(11):22-dien-7-one (XII), one oxygen atom must be present as a ketone at the 7-position. Since the remaining centres of attack are

the 9:11- and 22:23-double bonds, the second oxygen atom must either be in the nucleus or in the side chain. The latter possibility is excluded since (a) the remaining nuclear bond is attacked preferentially to the side-chain double bond, as is later proven by strong alkaline hydrolysis of the compound $C_{30}H_{46}O_4$, and (b) the compound $C_{30}H_{46}O_4$ is also obtained by performic acid oxidation of ergosteryl-D acetate 22:23-dibromide, in which the side-chain double bond is protected by bromine atoms, followed by zinc dust debromination, a step which introduces the 22:23-ethylenic linkage. It follows that the second



(XVII)



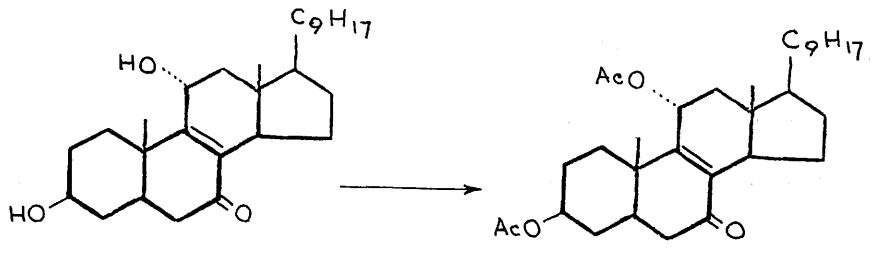
(XVIII)

oxygen atom is present either as a ketone at position 11, or as a 9:11-oxide. The possible structures for the compound $C_{30}H_{46}O_4$ are therefore 7:11-diketoergost-22-en--3β-yl acetate (XVII) and 9α:11α-epoxy-7-ketoergost-22--en-3β-yl acetate (XVIII) [the 9β:11β-configuration being excluded in view of the preferential rear attack of reagents at the 9- and the 11-positions (40)]. Since the

oxidation product differs, however, from the known 7:11--diketone (XVII) (29, 36, 109) it is therefore 9α:11α--epoxy-7-ketoergost-22-en-3β-yl acetate (XVIII). This compound can also be prepared from 3β-acetoxyergosta--9(11):22-dien-7-one (XII) by protection of the 22:23--ethylenic linkage by the addition of one mol. of bromine, followed by oxidation with perbenzoic acid and debromination of the product with zinc (108).

Relatively mild alkaline hydrolysis of 9α:11α--epoxy-7-ketoergost-22-en-3β-yl acetate (XVIII) gives a compound $C_{28}H_{44}O_3$ shown to be 3β:11α-dihydroxyergosta--8:22-dien-7-one (XIX) by the reactions now to be discussed. The structure ascribed to the compound $C_{28}H_{44}O_3$ is supported by its formation from the ketoxide (XVIII), by the ultra-violet absorption spectrum normally associated with an αβ-unsaturated ketone [maximum at 2520 Å (ε = 9000), and the infra-red spectrum which shows the existence of both the αβ-unsaturated ketone and hydroxyl groups. The presence of two hydroxyl groups in this compound is indicated by the fact that it forms a diacetate, $C_{32}H_{48}O_5$ [maximum at 2520 Å (ε = 10,400)] (XX). Since the precursor (XII) contains a ketone group at the 7--position it follows that the compound $C_{28}H_{44}O_3$ is either 3β:11α-dihydroxyergosta-8:22-dien-7-one (XIX) or the

corresponding 3β:11β-diol. Concerning the orientation of the 11-hydroxyl group in 3β:11-dihydroxyergosta-8:22--dien-7-one, the ease of acetylation to a diacetate at



(XIX) (XX)

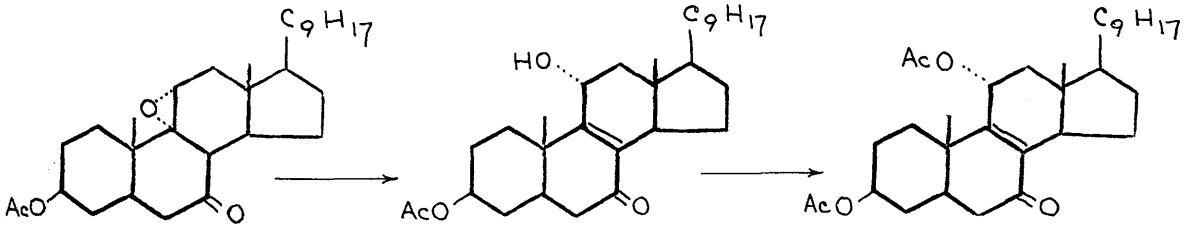
first sight precludes the possibility of the 11β-configuration, since it is a well established fact that an 11α-hydroxyl group can readily be acetylated whereas an 11β-hydroxyl group cannot (24). This deduction, however, may be invalidated by the effect of the 8:9-ethylenic linkage upon the accessibility of an 11β-hydroxyl group, which might exert an anomalous neighbouring-group effect (cf. 63). An argument based on analogy can be presented in favour of the 11α-configuration. Djerassi and co--workers (57, 33, 34) have employed a method for the introduction of an oxygen at C_{11} analogous to that described herein, which is applied to diosgenin and allopregnane derivatives. Oxidation of 3β:20β-diacetoxyallopregna--7:9(11)-diene with performic acid gives 3β:20β-diacetoxy--9α:11α-epoxyallopregnan-7-one, alkaline hydrolysis of

which yields 3β:11α:20β-trihydroxypregn-8-en-7-one which forms a triacetate. Catalytic hydrogenation of 3β:11α:20β--trihydroxypregn-8-en-7-one gives 3β:11α:20β-trihydroxypregnan-7-one which forms a triacetate (33, cf.34). Whilst it can be reasoned that the conversion of a 3:11:20--trihydroxypregn-8-en-7-one into a triacetate does not prove that the 11-hydroxyl group has the α-configuration, since the effect of a neighbouring group upon an 11β--hydroxyl group may be shown by an 8:9-unsaturated centre (63), triacetylation of the saturated 3:11:20-trihydroxypregnan-7-one proves the α-configuration for the 11--hydroxyl group in these compounds. Consequently, the 11-hydroxyl group in 3β:11-dihydroxyergosta-8:22-dien-7-one, obtained in an analogous manner from ergosteryl-D acetate, is assigned the α-configuration, from which it follows that the diacetate is 3β:11α-diacetoxyergosta-8:22-dien--7-one (XX).

It was considered necessary, nevertheless, to effect hydrolysis and acetylation on a related compound lacking the 8:9-ethylenic linkage. Correspondingly, it was conclusively shown that the ascribed 11α-configuration is correct, since catalytic hydrogenation (using platinum oxide) and alkaline hydrolysis of 3β:11α-diacetoxy-22:23--dibromoergost-8-en-7-one followed by acetylation gave

3β:11α-diacetoxyergost-22-en-7-one (to be discussed later).

Further investigation of 9α:11α-epoxy-7-ketoergost--22-en-3β-yl acetate (XVIII) revealed that on filtration of its benzene solution through a column of alumina, no material was eluted from the column until methanol was introduced to the eluting solvent. This behaviour suggested that the product contained one or more hydroxyl groups, and that the basicity of the alumina was sufficient to cause alkaline rearrangement of the 9α:11α-epoxy-7--ketone system to the 11α-hydroxy-8-en-7-one to give 3β-acetoxy-11α-hydroxyergost-8-en-7-one (XXI). The product exhibited light absorption at 2540 Å indicating an αβ-unsaturated ketone, and the infra-red spectrum showed



(XVIII) (XXI) (XX)

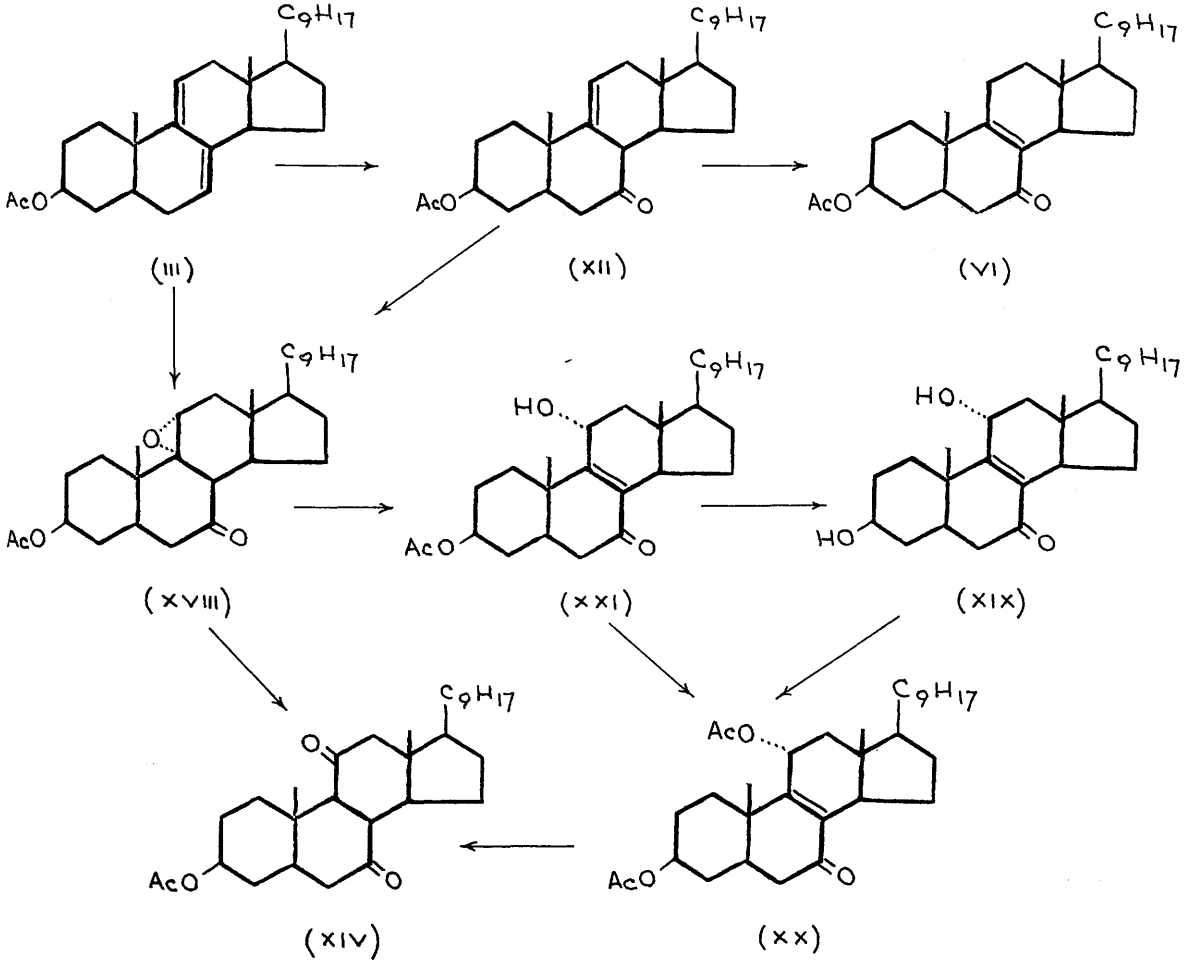
absorption bands in the ketone, acetate and hydroxyl regions. The structure (XXI) ascribed to this compound was confirmed by acetylation to the known 3β:11α-diacetoxy-ergosta-8:22-dien-7-one (XX). The important feature of

the above procedure is that the chromatographic rearrangement, unlike mild alkali rearrangement, affords selective protection at C_3.

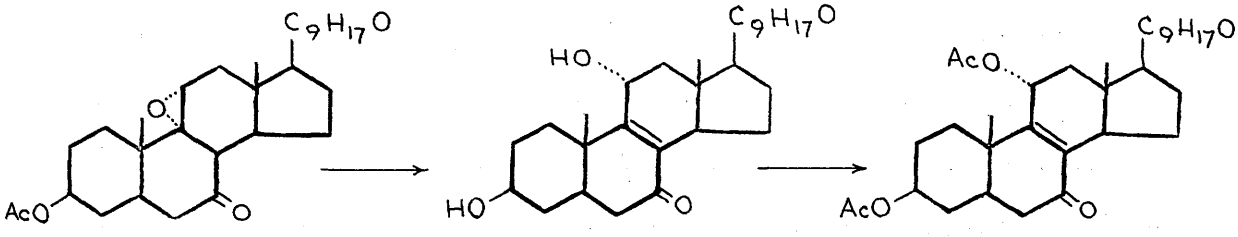
In contrast to treatment with mild alkali, hydrolysis of 9α:11α-epoxy-7-ketoergost-22-en-3β-yl acetate (XVIII) or 3β:11α-diacetoxyergosta-8:22-dien-7-one (XX) with strong aqueous-ethanolic potassium hydroxide followed by acetylation yielded a reaction mixture, from which 7:11--diketoergost-22-en-3β-yl acetate (XIV) was readily isolated by chromatography on alumina. The identity of the diketone (XIV) was established by direct comparison with a specimen prepared as described by Heusser et al. (36). A similar isomerisation of 6-ketocholest-4-en--3β-yl acetate into cholestane-3:6-dione was observed by Heilbron, Jones, and Spring (79) and the conversion of 6β:21-diacetoxypregn-4-en-3:20-dione into 21-hydroxyallopregnan-3:6:20-trione by treatment with alkali has been reported by Herzig and Ehrenstein (80). The isolation of 7:11-diketoergost-22-en-3β-yl acetate (XIV) proves without doubt the presence of an oxygen atom at C_{11} in the compound $C_{28}H_{44}O_3$ (hydrolysis of the ketoxide), and shows in addition that it can be either 3β:11α-dihydroxy-ergosta-8:22-dien-7-one (XIX) or 3β:7ξ-dihydroxyergosta--8:22-dien-11-one. If the identification of the performic

oxidation product $C_{30}H_{46}O_4$ as 9α:11α-epoxy-7-ketoergost--22-en-3β-yl acetate (XVIII) is correct, the latter possibility is excluded, and the compound $C_{28}H_{46}O_5$, therefore, must be 3β:11α-dihydroxyergosta-8:22-dien-7-one (XIX).

The reactions discussed above can be summarised as follows:



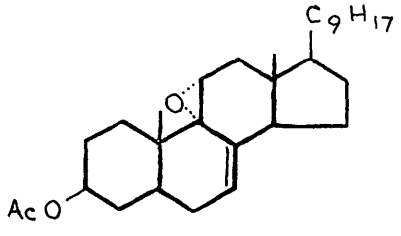
Oxidation of ergosteryl-D acetate with perbenzoic--formic acid gives a product which on alkaline hydrolysis and acetylation yields a crystalline compound, $C_{32}H_{48}O_6$, showing an absorption maximum at 2520 Å (ε = 9,700) and giving no colour with tetranitromethane. The yields were poor, and the insufficient amount of this compound did not allow a detailed investigation. The analysis and the absence of colour with tetranitromethane, however, suggest that the side-chain double bond was oxidised. It is possible that the following steps have taken place on oxidation, hydrolysis and acetylation:



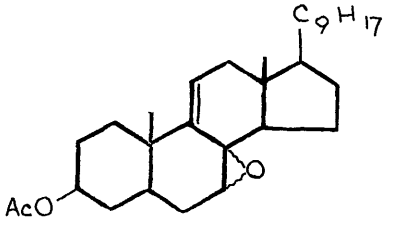
Oxidation with Perbenzoic Acid.

Parallel with the chromium trioxide and performic acid oxidation experiments described above, a study was made of the oxidation of ergosteryl-D acetate with perbenzoic acid. Controlled treatment of the latter compound with one equivalent of perbenzoic acid gives readily a

mono-epoxide in excellent yield. The preparation of this compound was reported almost simultaneously by Tishler and co-workers (29), and by Heusser and co-workers (36); the Swiss authors employed monoperphthalic acid for the oxidation of ergosteryl-D acetate. Since this epoxide, $C_{30}H_{46}O_3$, does not exhibit light absorption above 2200 Å, gives a yellow colour with tetranitromethane, and differs from the previously described unconjugated ketones (absence of a carbonyl group is shown by infra-red spectrum) it must be either 9α:11α-epoxyergosta-7:22--dien-3β-yl acetate (IX) or 7ξ:8ξ-epoxyergosta-9(11):22--dien-3β-yl acetate (X). Heusser et al. (36) favour



(IX)



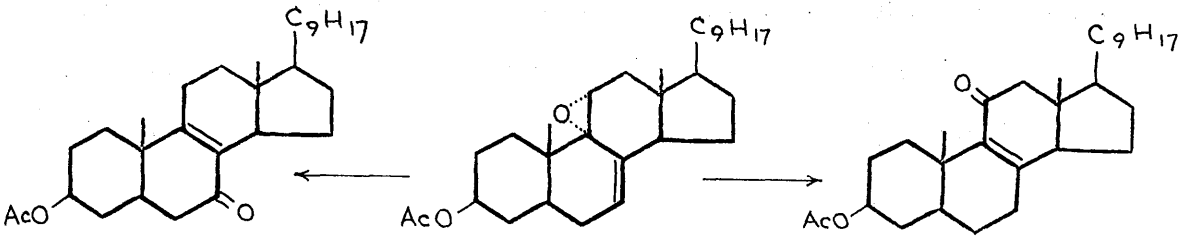
(X)

structure (IX), and Tishler et al. (30) have also since stated their preference for the Δ^7-9α:11α-epoxide structure. Since both chromic acid and performic acid had attacked the 7:8-ethylenic linkage preferentially to the 9:11-double bond of ergosteryl-D acetate, it was anticipated that perbenzoic acid would react in an

analogous manner. This, indeed, seemed to be the case when dilute mineral acid hydrolysis yielded the known 3β-hydroxyergosta-8:22-dien-7-one (VI). Consequently, in the preliminary communication (107) the oxide was given the alternative structure 7ξ:8ξ-epoxyergosta--9(11):22-dien-3β-yl acetate (X). This structure soon appeared unsatisfactory when an observation was made that catalytic hydrogenation of ergosteryl-D acetate 22:23--dibromide leads to initial saturation of the 9:11--ethylenic linkage with the formation of 22:23-dibromo-ergost-7-en-3β-yl acetate (111), which leads to the view that the monoepoxide of ergosteryl-D acetate 22:23--dibromide is likewise formed by saturation of the 9:11--ethylenic linkage and is therefore 9α:11α-epoxide.

The epoxide of ergosteryl-D acetate is ascribed the structure 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate (IX) since, according to the experimental conditions, it can be isomerised to two different αβ-unsaturated ketones. Firstly, with aqueous mineral acid, it is converted into 3β-acetoxyergosta-8:22-dien-7-one (VI), previously obtained by chromic acid oxidation of 5-dihydroergosteryl acetate (II) (39) and ergosteryl-D acetate (III)(108). In the second, place, the important observation was made by Heusser and co-workers (36) that treatment of the

epoxide with anhydrous Lewis acids (boron trifluoride in absolute benzene or ferric chloride) gives in high yield,



(VI) (IX) (XXII)

an isomeric αβ-unsaturated ketone to which was ascribed the structure 3β-acetoxyergosta-8:22-dien-11-one (XXII). Like 3β-acetoxyergosta-8:22-dien-7-one (VI) this isomer exhibits an ultraviolet absorption maximum at 2530 Å; neither of the two isomers is converted into the other on treatment with mineral acid. These considerations and the fact that there are no known transformations which cannot be explained by assumption of the 9α:11α-epoxy structure (the α-configuration is ascribed to the epoxy--group since attack at the 9:11-positions will be at the rear of the molecule), make the structure 9α:11α-epoxy-ergosta-7:22-dien-3β-yl acetate (IX) very probable.

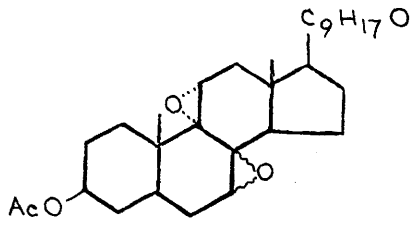
9α:11α-Epoxyergosta-7:22-dien-3β-yl acetate is smoothly hydrolysed with alkali to the corresponding 9α:11α-epoxyergosta-7:22-dien-3β-ol, which on reacetylation gives the parent acetate. The stability of this compound

to alkali is noteworthy in view of the extreme reactivity of 9α:11α-epoxy-7-ketoergost-22-en-3β-yl acetate (XVIII) under the same conditions.

Oxidation of ergosteryl-D acetate with two molecular proportions of perbenzoic acid did not give a homogeneous reaction product, apart from a small amount of 9α:11α--epoxyergosta-7:22-dien-3β-yl acetate. It is obvious that the second mol. of the oxidising agent attacked both the 7:8- and the 22:23-ethylenic linkages under the experimental conditions used. That this is the case, was proved by the fact that a beautifully crystalline compound was prepared in excellent yield, believed to be the 7:8,9:11--diepoxide, on treatment of ergosteryl-D acetate 22:23-dibromide with an excess of perbenzoic acid, followed by debromination of the product with zinc. Description of this compound is included in the section dealing with the bromo-compounds.

Treatment of ergosteryl-D acetate with excess of perbenzoic acid gives a crystalline compound, $C_{28}H_{46}O_3$, in good yield. It does not show selective light absorption above 2000 Å and does not give a colour with tetranitromethane. The analysis, supported by the last observation, suggest that the three oxygen atoms are distributed between the three ethylenic linkages. The

stability of this compound to alkali is demonstrated by alkaline hydrolysis to the free alcohol and reacetylation, a feature characteristic of the ergosteryl-D acetate



(XXIII)

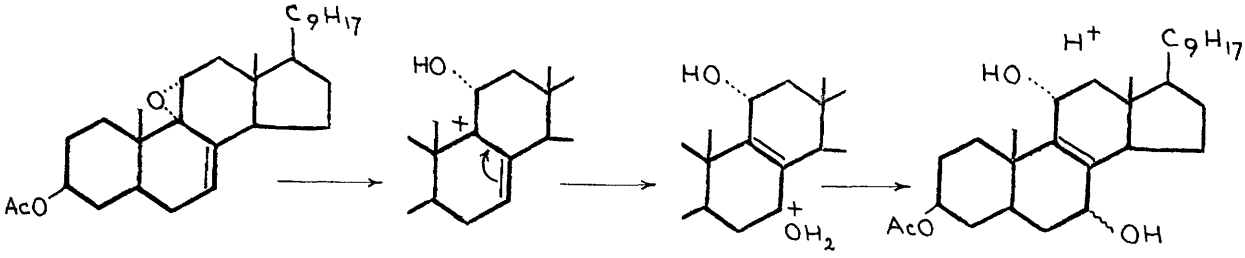
monoepoxide and diepoxide. The fact that the compound $C_{30}H_{46}O_5$ can be obtained from both the monoepoxide and diepoxide on treatment with excess perbenzoic acid, allows to fix two oxygens in the nucleus with reasonable certainty. The compound is probably a triepoxide (XXIII), although no more work has been done to prove the character of the oxygen atom in the side-chain.

7:11-Diketoergost-22-en-3β-yl Acetate.

In order to study the action of zinc on 7:11-diketo-ergosta-8:22-dien-3β-yl acetate (VII) and 8α:9α-epoxy--7:11-diketoergost-22-en-3β-yl acetate (VIII), the two compounds were prepared by the method employed by Heusser and co-workers (36).

Hydrolytic rearrangement of 9α:11α-epoxyergosta--7:22-dien-3β-yl acetate (IX) using dilute sulphuric acid

yields 7ξ:11α-dihydroxyergosta-8:22-dien-3β-yl acetate (XXIV). The following reaction scheme is proposed by Heusser et al. (loc.cit.):

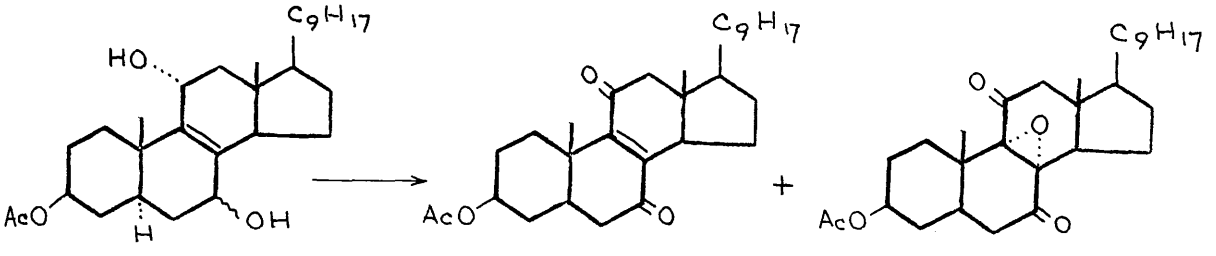


(IX) (XXIV)

The presence of two hydroxyl groups in (XXIV) is shown by acetylation to 3β:7ξ:11α-triacetoxyergosta-8:22-diene. The triol monoacetate (XXIV) was given the 11α-configuration in accordance with the reaction scheme depicted above.

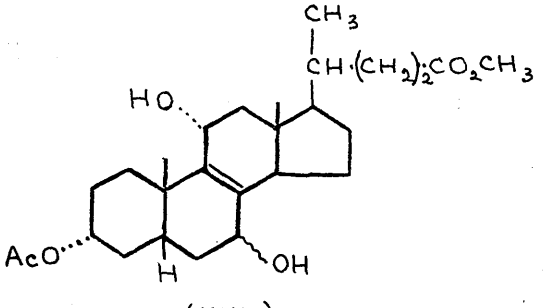
Oxidation of 7ξ:11α-dihydroxyergosta-8:22-dien-3β-yl acetate (XXIV) with chromic acid gives a mixture of 7:11--diketoergosta-8:22-dien-3β-yl acetate (VII) and 8α:9α--epoxy-7:11-diketoergost-22-en-3β-yl acetate (VIII). The two compounds were separated by crystallisation and chromatography. The unsaturated 7:11-diketone (VII) exhibits a light absorption with a maximum at 2700 Å (ε = 8,400), whereas the corresponding 8:9-epoxide does not show selective light absorption of high intensity above 2200 Å. The last compound (VIII) is obtained as major product when an

excess of oxidising agent is employed. The 8α:9α--configuration is given to the epoxide-group in (VIII) since a similar oxidation of the related methyl 3α-acetoxy--7ξ:11α-dihydroxychol-8-enate (XXV), even with an excess



(XXIV) (VII) (VIII)

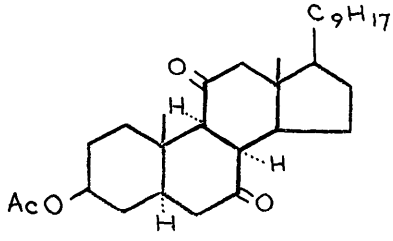
of chromic acid, gives only the corresponding unsaturated diketone and not a diketo-epoxide. It is reasoned that a ready explanation for this marked difference is to be found if the epoxide group in (VIII) is α-orientated, since addition of an α-epoxide group to the chol-8-ene derivative is considerably hindered.



(XXV)

Partial reduction of 7:11-diketoergosta-8:22-dien--3β-yl acetate (VII) and 8α:9α-epoxy-7:11-diketoergost-

-22-en-3β-yl acetate (VIII) with zinc and acetic acid at 100° gives the well-known 7:11-diketoergost-22-en-3β-yl acetate (XIV). An attempt was made to perform the same reaction in a neutral solvent, by treatment of 7:11--diketoergosta-8:22-dien-3β-yl acetate (VII) with zinc dust in ether-methanol. Surprisingly, the reaction gave a compound, $C_{30}H_{46}O_4$, $[\alpha]_D$ +30°, which does not show intense absorption above 2200 Å, and which on warming of



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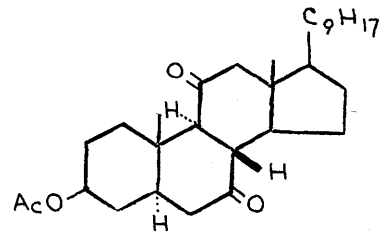
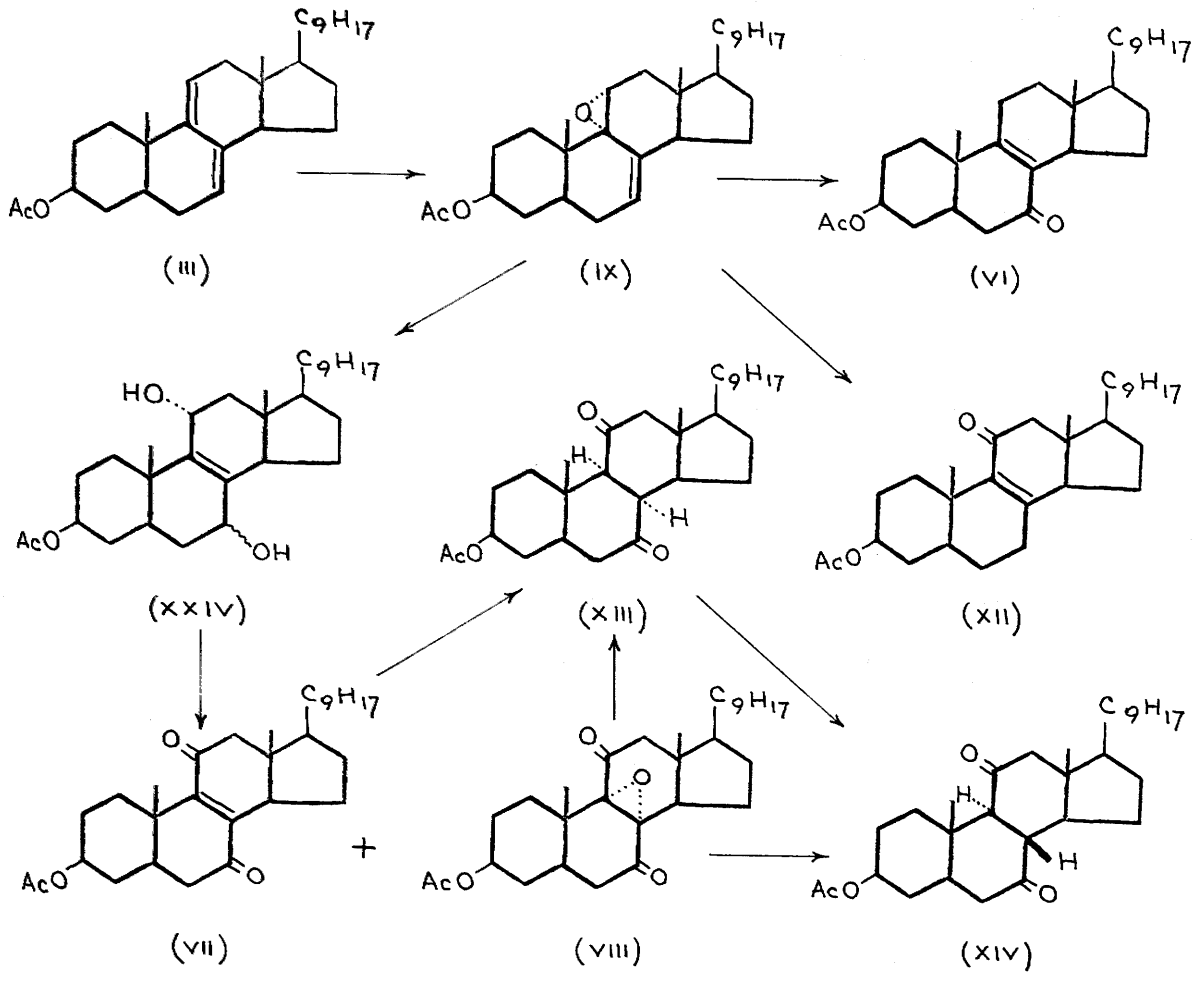


(XIV)

an acetic acid solution gives 7:11-diketoergost-22-en-3β-yl acetate (XIV), $[\alpha]_D$ -30°. It is also obtained by treatment of 8α:9α-epoxy-7:11-diketoergost-22-en-3β-yl acetate (VIII) in ether-methanol with zinc dust. The new compound is an isomer of 7:11-diketoergost-22-en-3β-yl acetate and it differs from the normal isomer in the configuration at C_8 (XIII). This compound was also prepared by different routes to be described and its structure is fully discussed on page 112.

The reactions of 9α:11α-epoxyergosta-7:22-dien-3β-yl

acetate (IX) described above can be summarised in the following way:



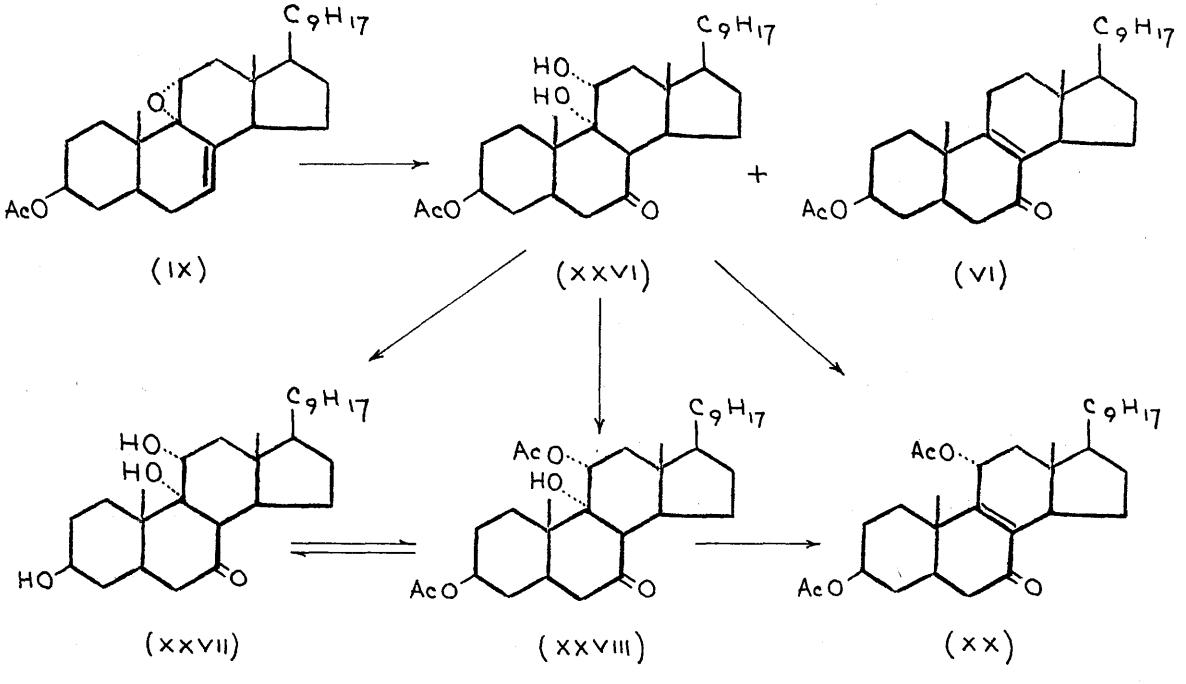
3β-Acetoxy-9α:11α-dihydroxyergost-22-en-7-one.

In an attempt to correlate ergosteryl-D monoxide (9α:11α-epoxyergosta-7:22-dien-3β-yl acetate, IX) with 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one (XVIII), a solution of the former was treated with one mol. of

bromine, to protect the side-chain ethylenic linkage during the subsequent oxidation stage, and then with an excess of perbenzoic acid. Debromination of the reaction mixture with zinc dust in acetic acid at 100° gave 3β--acetoxyergosta-8:22-dien-7-one (VI) and, in low yield, a crystalline compound $C_{30}H_{48}O_5$, identified as 3β-acetoxy--9α:11α-dihydroxyergost-22-en-7-one (XXVI) by the reactions described below.

The compound $C_{30}H_{48}O_5$ was isolated by several methods viz. by crystallisation, Girard's reagent and chromatography. The last method is by far the most efficient one: a solution of the reaction product in benzene is filtered through a column of alumina and the column washed with the same solvent until no more material is eluted. Subsequent washing with methanol gives the pure compound. On this behaviour, one or more hydroxyl groups were anticipated. The compound does not exhibit selective high-intensity ultra-violet absorption above 2200 Å. It is acetylated under normal conditions to a diacetoxy-derivative, $C_{34}H_{52}O_7$. Of the two oxygens to be accounted for, one is a ketone, which was indicated by its reaction with Girard's reagent. Treatment of the compound $C_{30}H_{48}O_5$, or its acetyl derivative $C_{34}H_{52}O_7$, with potassium hydroxide solution followed by acetylation gives the known 3β:11α-diacetoxyergosta-8:22-

-dien-7-one (XX) identical with the compound obtained on alkaline hydrolysis and acetylation of 3β-acetoxy-9α:11α--epoxyergost-22-en-7-one (XVIII) as described above. The formation of an αβ-unsaturated ketone (XX) indicates a



dehydration across C_8-C_9 bond, and thus allows the fixing of the hydroxyl group at C_9, which, being tertiary in character, cannot be acetylated. The acetylated product is, therefore, 3β:11α-diacetoxy-9α-hydroxyergost-22-en-7--one (XXVIII).

Mild alkaline hydrolysis of 3β-acetoxy-9α:11α-dihydroxy-ergost-22-en-7-one (XXVI) or 3β:11α-diacetoxy-9α-hydroxy-ergost-22-en-7-one (XXVIII) gives 3β:9α:11α-trihydroxy-

ergost-22-en-7-one (XXVII) which was reacetylated to the acetyl derivative (XXVIII). The infra-red absorption spectrum of 3β-acetoxy-9α:11α-dihydroxyergost-22-en-7-one (XXVI) shows bands at 1732 (3β-acetate group), 1710 (7--ketone group) and 3400 cm.$^{-1}$ (hydroxyl group).

The α-configuration of the 11-hydroxyl group follows from the formation of the known 3β:11α-diacetoxyergosta--8:22-dien-7-one (XX). The cis-orientation of the 9:11--hydroxyl groups with respect to each other, which was proved later, is discussed in the section describing the corresponding brominated derivatives (page 118).

---oOo---

Ergosteryl-D Acetate 22:23-Dibromide.

As a consequence of the examination of a possible preparation of ergosteryl-D acetate by bromination of 5--dihydroergosteryl acetate, a study commenced by Anderson and Spring (106), ergosteryl-D acetate 22:23-dibromide became available.

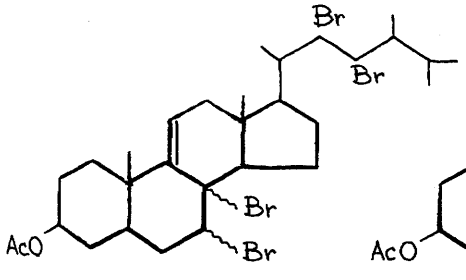
The introduction of an 11-oxygen function into the steroid nucleus starting from ergosterol and proceeding by way of the dibromide has certain attractive features, not the least of which is that the route avoids the preparation of ergosteryl-D acetate from 5-dihydroergosteryl acetate, an inefficient process using the best of the various methods available. More important, the use of ergosteryl-D acetate 22:23-dibromide as a starting material in the oxidation investigations of the 7:9(11)-ergostadiene derivatives, affords a ready means for the protection of the side-chain ethylenic linkage during the subsequent oxidation stage. It is particularly useful during oxidations involving an excess of oxidising agent, in which case the oxidation products are usually isolated in excellent yields. The protected side-chain ethylenic linkage permits selective nuclear hydrogenations in high yields under specified experimental conditions. The dibromo-derivatives usually have a much higher melting point, which makes them

easier to handle. They crystallise in well-defined, and in most cases, prismatic form. They are more insoluble than the corresponding 22-unsaturated compounds, and in some instances the product of high purity separates from the reaction mixture. The side-chain ethylenic linkage can be easily re-established prior to subsequent side-chain degradation in quantitative yield by debromination with zinc. A comparison of the molecular rotation of the various ergosterol derivatives described in the experimental section and in the publications (107-114) with their corresponding 22:23-dibromides shows that the dibromides have a higher positive rotation (mean value of 60 units). This can be utilized with advantage although in some cases the individual groups exert vicinal action causing divergences from the mean value (particularly in 11-substituted compounds).

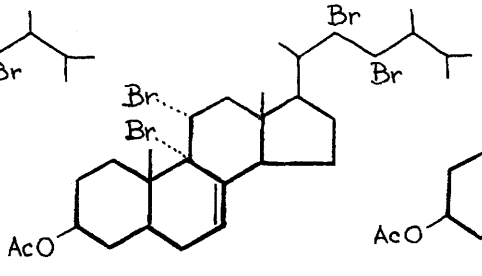
A study of the action of bromine on 5-dihydroergosteryl acetate was commenced since, according to Eck and Hollingsworth (83) oxidation of cholest-7-ene in chloroform with bromine at -75° gives cholesta-7:9(11)-diene. Treatment of 5-dihydroergosteryl acetate in ether solution at -60° with bromine gives in about 50% yield a tetrabromoergostenyl acetate which separates directly from the reaction mixture. This tetrabromide, which can also be

obtained directly from ergosteryl-D acetate, is moderately stable in the solid state, as are solutions of the compound in dioxan and benzene. However, its solutions in alcohol and particularly in chloroform, suffer profound decomposition after a short time at room temperature. Sodium iodide effects partial debromination to 22:23--dibromoergosta-7:9(11)-dien-3β-yl acetate (ergosteryl-D acetate 22:23-dibromide) (XXXII), the structure of which was established, first, by its conversion into ergosteryl-D acetate by debromination with zinc dust and, secondly, by its characteristic 7:9(11)-dienic ultra-violet absorption spectrum, which is identical in location with that of ergosteryl-D acetate.

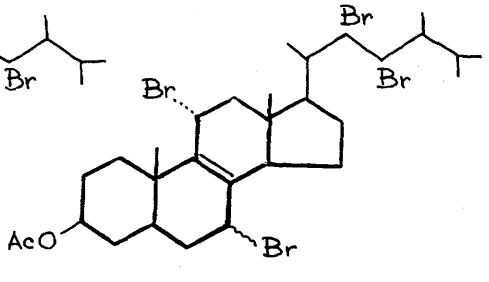
The structure of the intermediate tetrabromide has not been established with certainty (106, 107); the possibilities (XXIX), (XXX) and (XXXI), which could arise either by 1:4-addition of bromine or by allylic rearrangement of (XXIX) or (XXX), have been considered. The discrimination between these structures has not been possible



(XXIX)



(XXX)



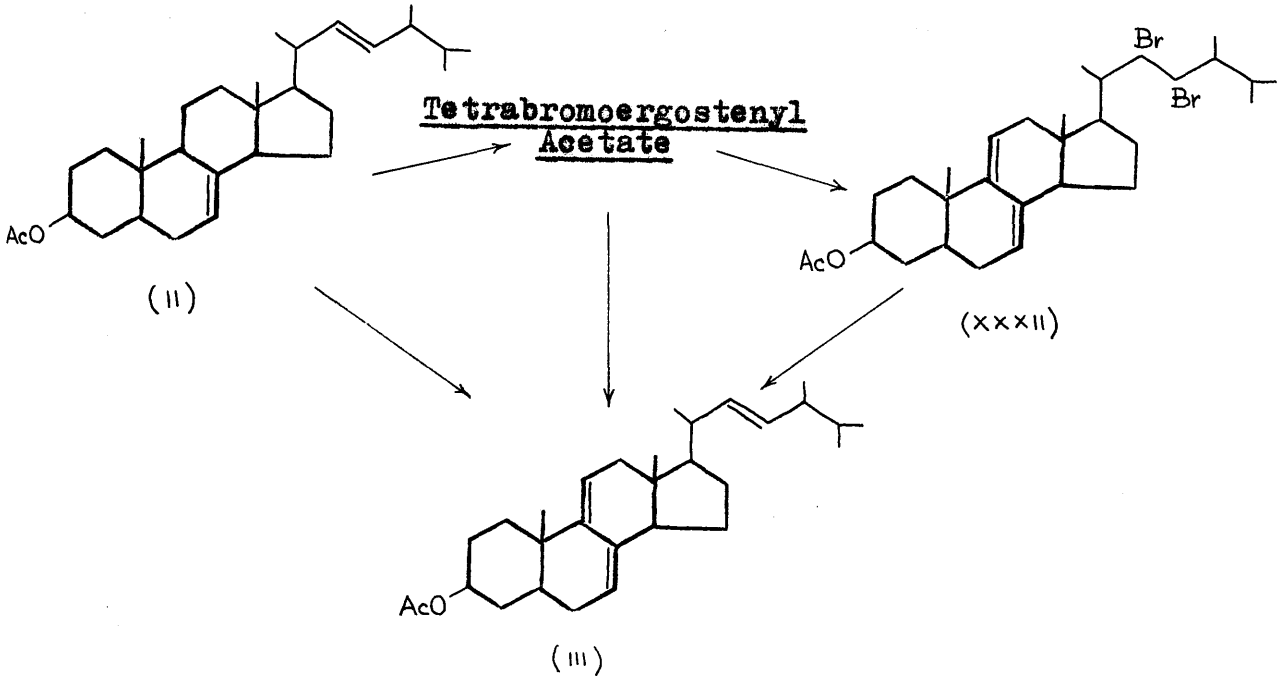
(XXXI)

because of the extremely labile nature of the compound. The last structure, however, seems to be more probable than the first two.

For the most efficient method of preparation of tetrabromoergostenyl acetate, the ether solvent should be dry, and the temperature should be allowed to rise from -60° to -10° over a period of 2 hours, whereafter the precipitated tetrabromide is removed by filtration from the rapidly decomposing reaction liquor. Attempts to obtain crystalline material from the tetrabromide filtrate proved unsuccessful due to the susceptibility to rearrangement and decomposition in common solvents at room temperature.

Treatment of a solution of tetrabromoergostenyl acetate at room temperature with ethanolic sodium iodide causes immediate liberation of iodine, to give 22:23--dibromoergosta-7:9(11)-dien-3β-yl acetate (XXXII) in almost quantitative yield (95%). This latter compound (XXXII) was debrominated in quantitative yield by heating an ether-ethanol solution with zinc dust to give ergosteryl-D acetate (III) of high purity. Ergosteryl-D acetate was also prepared in less pure form in 70% yield without isolation of the intermediate tetrabromide or dibromide by direct treatment of the reaction mixture

obtained by bromination of 5-dihydroergosteryl acetate (II) with zinc dust.



(II) (XXXII) (III)

In contrast to tetrabromoergostenyl acetate, 22:23--dibromoergosta-7:9(11)-dien-3β-yl acetate (XXXII) is extremely stable, and was hydrolysed by means of alkali to 22:23-dibromoergosta-7:9(11)-dien-3β-ol, characterised by reacetylation to (XXXII). Benzoylation of the alcohol in the usual manner gave 22:23-dibromoergosta-7:9(11)--dien-3β-yl benzoate, which was debrominated to ergosteryl-D benzoate on treatment with zinc dust in ether-methanol.

The Reduction of Ergosteryl-D Acetate 22:23-Dibromide.

Ergosteryl-D acetate 22:23-dibromide offers some advantage over ergosteryl-D acetate (XXXIII, R=C_9H_{17}) as a starting point for the synthesis of 11-keto steroids since nuclear oxidation is more efficiently effected in the first compound than in the case of ergosteryl-D acetate presumably because of partial attack at the side--chain ethylenic linkage of the latter. Parallel with the oxidation experiments described, a study was made of the catalytic reduction of ergosteryl-D acetate 22:23--dibromide. The side-chain halogens proved to be a satisfactory protection of the Δ^{22}-linkage of ergosteryl-D acetate under the hydrogenation conditions described in the experimental section, and this, together with the easy regeneration of the ethylenic linkage by zinc dust treatment has led to efficient methods for the preparation of the hitherto relatively inaccessible ergost-8(14):22--dien-3β-ol and ergost-22-en-3β-ol. Of these two compounds, the former has been described by Laubach and Brunings (75) since this work was completed; they prepared it by hydrogenation of ergosteryl-B acetate (ergosta--6:8(14):22-trien-3β-yl acetate) in a neutral solvent over Raney nickel. Ergost-22-en-3β-ol has been obtained by Barton, Cox and Holness (84) by partial hydrogenation of

isoergosterone in neutral solution to ergost-22-en-3-one followed by reduction of the latter with sodium and propanol.

Hydrogenation of ergosteryl-D acetate 22:23-dibromide in ethyl acetate over platinum leads to saturation of the 9:11-ethylenic bond and formation of 22:23-dibromoergost--7-en-3β-yl acetate (XXXIV, R=$C_9H_{17}Br_2$; R'=Ac) which was characterised by hydrolysis to 22:23-dibromoergost-7-en--3β-ol (XXXIV, R=$C_9H_{17}Br_2$; R'=H) and by the preparation of the benzoate of the latter.

The molecular rotation data (Table I) support the structure allocated to 22:23-dibromoergost-7-en-3β-ol, the △-values on acetylation and benzoylation being in good agreement with representative values observed for trans A/B 7-sten-3β-ols (cf. 85). Furthermore, the observed changes in molecular rotation accompanying saturation of the double bond (comparison with 22:23--dibromoergostan-3β-ol, its acetate and benzoate) are in good agreement with values for the saturation of comparable 7-stenols [cf. Barton (17); Barton and Cox (16)]. The structure allocated to 22:23-dibromoergost-7-en-3β-yl acetate was confirmed by its conversion into 5-dihydroergosteryl acetate (XXXIV, R=C_9H_{17}; R'=Ac) in high yield by treatment with zinc dust in ether-ethanol.

TABLE I

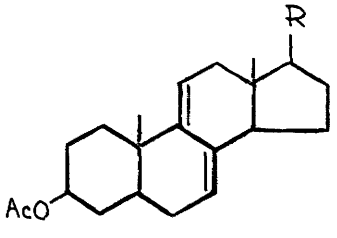
	$[M]_D$				
	Alcohol	**Acetate**	**Benzoate**	Δ_1	Δ_2
22:23-Dibromo-ergostan-3β-ol	+42	+12	+40	-30(-34)	-2(+2)
22:23-Dibromo-ergost-7-en-3β-ol	-47	-42	-26	+5(-6)	+21(+30)
Δ(saturation of F)	+89(+77±17)	+54(+57)	+66(+64)		
22:23-Dibromoergost--8(14)-en-3β-ol	+73	+27	+20	-46(-40)	-53(-42)
Δ(saturation of F)	-31(+9±8)	-15(+14±9)	+20(53±6)		

Standard values from Barton, J.,1945,813; 1946,512, are given in parentheses.

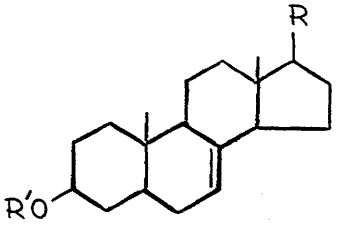
Hydrogenation of ergosteryl-D acetate 22:23-dibromide in acetic acid over platinum gives 22:23-dibromoergost--8(14)-en-3β-yl acetate (XXXV, R=$C_9H_{17}Br_2$; R'=Ac) characterised by hydrolysis to 22:23-dibromoergost-8(14)-en-3β-ol (XXXV; R=$C_9H_{17}Br_2$; R'=H) and by preparation of the corresponding benzoate. Furthermore, isomerisation of 22:23--dibromoergost-7-en-3β-yl acetate (XXXIV; R=$C_9H_{17}Br_2$; R'=Ac) in acetic acid solution by shaking with a platinum catalyst saturated with hydrogen gave 22:23-dibromoergost--8(14)-en-3β-yl acetate. The location of the double bond in these compounds follows from well-established considerations (see Fieser and Fieser, Natural Products Related to Phenanthrene, Reinhold Publ.Corp.,1949). Thus the molecular rotation changes on acetylation and benzoylation of the alcohol are in good agreement with representative values for other trans A/B 8(14)-sten-3β-ols (Table I), and the ultraviolet absorption spectrum of the compound agrees with that expected for an 8(14)-sterol [Bladen, Henbest and Woods; Halsall (87)]. However, although the molecular rotation changes on acetylation and benzoylation of 22:23-dibromoergost-8(14)-en-3β-ol are normal the △-values for saturation of the double bond are only in fair agreement with standard values for 8(14)-sterols. This indicates some degree of vicinal effect of the side

chain [cf. Mancera, Barton, Rosenkranz and Djerassi (85)].

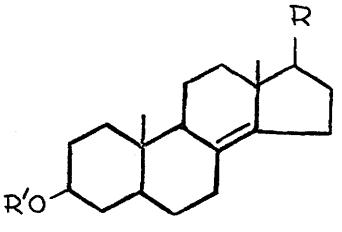
Debromination of 22:23-dibromoergost-8(14)-en-3β-yl acetate with zinc gives ergost-8(14):22-dien-3β-yl acetate (XXXV, R=C_9H_{17}; R'=Ac) hydrolysed by alkali to ergost--8(14):22-dien-3β-ol (XXXV, R=C_9H_{17}; R'=H). Hydrogenation of ergost-8(14):22-dien-3β-yl acetate (XXXV, R=C_9H_{17}; R'= Ac) in either ethyl acetate or acetic acid over platinum gives ergost-8(14)-en-3β-yl acetate (XXXV, R=C_9H_{19}; R=Ac) (α-ergostenyl acetate).



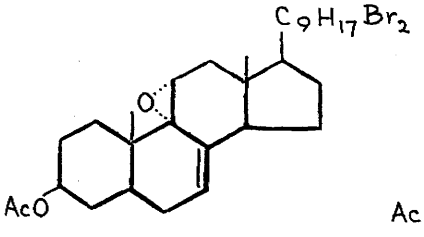
(XXXIII)



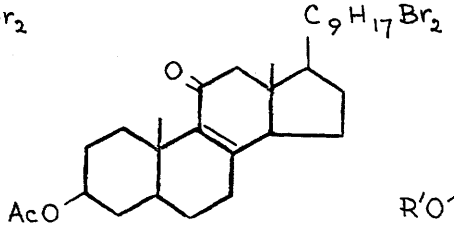
(XXXIV)



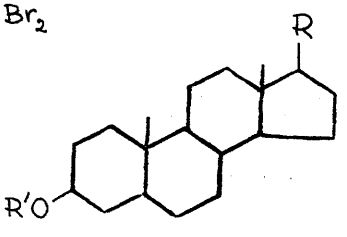
(XXXV)



(XXXVI)



(XXXVII)



(XXXVIII)

A study of the catalytic reduction of two oxidation products of ergosteryl-D acetate 22:23-dibromide, namely 22:23-dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate

(XXXVI) and 3β-acetoxy-22:23-dibromoergost-8-en-11-one (XXXVII) (described later) was included in this investigation. In acetic acid solution over a platinum catalyst both compounds suffered hydrogenolysis with the formation of 22:23-dibromoergost-8(14)-en-3β-yl acetate (XXXV, R=$C_9H_{17}Br_2$; R'=Ac). 3β-Acetoxy-22:23-dibromoergost-8-en-11-one was recovered unchanged after shaking with hydrogen either in ethyl acetate solution over platinum catalyst or in acetic acid solution over palladium black.

Treatment of 22:23-dibromoergost-8(14)-en-3β-yl acetate with dry hydrogen chloride (111) gave a mixed crystal which could not be resolved by crystallisation and a similar mixed crystal was obtained by treatment of 22:23-dibromoergost-8(14)-en-3β-yl benzoate with hydrogen chloride. Debromination of the acetate dibromide mixed crystal gave "β"-dihydroergosteryl acetate, further characterised by preparation of the alcohol and benzoate. The nature of "β"-dihydroergosteryl acetate was fully elucidated by Barton, Cox and Holness (84) as an inseparable equimolar mixture of ergost-8(14):22-dien-3β-yl acetate and ergost-14:22-dien-3β-yl acetate. The product obtained by isomerisation of 22:23-dibromoergost-8(14)--en-3β-yl acetate is therefore a mixture of 22:23-dibromo-

ergost-8(14)-en-3β-yl acetate and 22:23-dibromoergost--14-en-3β-yl acetate, and since it gives, on debromination, "β"-dihydroergosteryl acetate in nearly quantitative yield, it is inferred that it likewise is an equimolar mixture. The molecular rotations of 22:23--dibromoergost-14-en-3β-ol and its derivatives, calculated from the values for 22:23-dibromoergost-8(14)-en-3β-ol and those of the mixed crystal (22:23-dibromoergost-8(14)--sterol and 22:23-dibromoergost-14-sterol) are shown in Table II. Although the Δ_1 and Δ_2 values for acetylation and benzoylation of 22:23-dibromoergost-14-en-3β-ol are in reasonable agreement with standard values for Δ^{14}-sterols the changes in molecular rotation accompanying saturation of the double bond of this compound and its derivatives (comparison with corresponding derivatives of 22:23-dibromoergostan-3β-ol) are anomalous in this respect resembling 22-<u>isoallo</u>spirost-8(14)-en-3β-ol in which a strong vicinal effect of the sapogenin side chain was also observed (Mancera, Barton, Rosenkranz, and Djerassi, <u>loc.cit.</u>). Summarising, the 22:23-dibromo side chain exerts a profound vicinal effect upon the 14(15)--ethylenic linkage, a less pronounced effect upon the 8(14)-double bond and no effect upon a 7(8)-unsaturated centre.

TABLE II

	$[M]_D$				
	Alcohol	Acetate	Benzoate	Δ_1	Δ_2
22:23-Dibromoergost--8(14)-en-3β-ol	+73	+27	+20		
22:23-Dibromoergost--8(14)-en-3β-ol: 22:23-Dibromo-14-en--3β-ol (1:1)	+165	+126	+139		
22:23-Dibromoergost--14-en-3β-ol	+257	+225	+258	-32(-35±6)	+1(+30±2)
22:23-Dibromo-ergostan-3β-ol	+42	+12	+40		
Δ (saturation of F)	$-215\left(\begin{matrix}-24\\ +12\text{ or}\\ -7\end{matrix}\right)$	-213(-20±15)	-218(-59)		

Standard values are given in parentheses (Barton, 86).

A preparation of 22:23-dibromoergostan-3β-yl acetate (XXXVIII, R=$C_9H_{17}Br_2$; R'=Ac) was achieved (111) by isomerisation of 22:23-dibromoergost-8(14)-en-3β-yl acetate with dry hydrogen chloride, hydrogenation of the product followed by removal of unsaturated material using the method of Anderson and Nabenhauer (88). Attempts to improve the method of preparation by reduction of 22:23--dibromoergost-8(14)-5β-yl acetate in the presence of hydrochloric acid were not successful. Debromination of 22:23-dibromoergostanyl acetate gave ergost-22-en-3β-yl acetate (XXXVIII, R=C_9H_{17}; R'=Ac) characterised by hydrolysis to ergost-22-en-3β-ol (XXXVIII, R=C_9H_{17}; R'=H).

---oOo---

11-OXYGENATED STEROIDS FROM ERGOSTERYL-D ACETATE 22:23--DIBROMIDE.

Oxidation with Performic Acid.

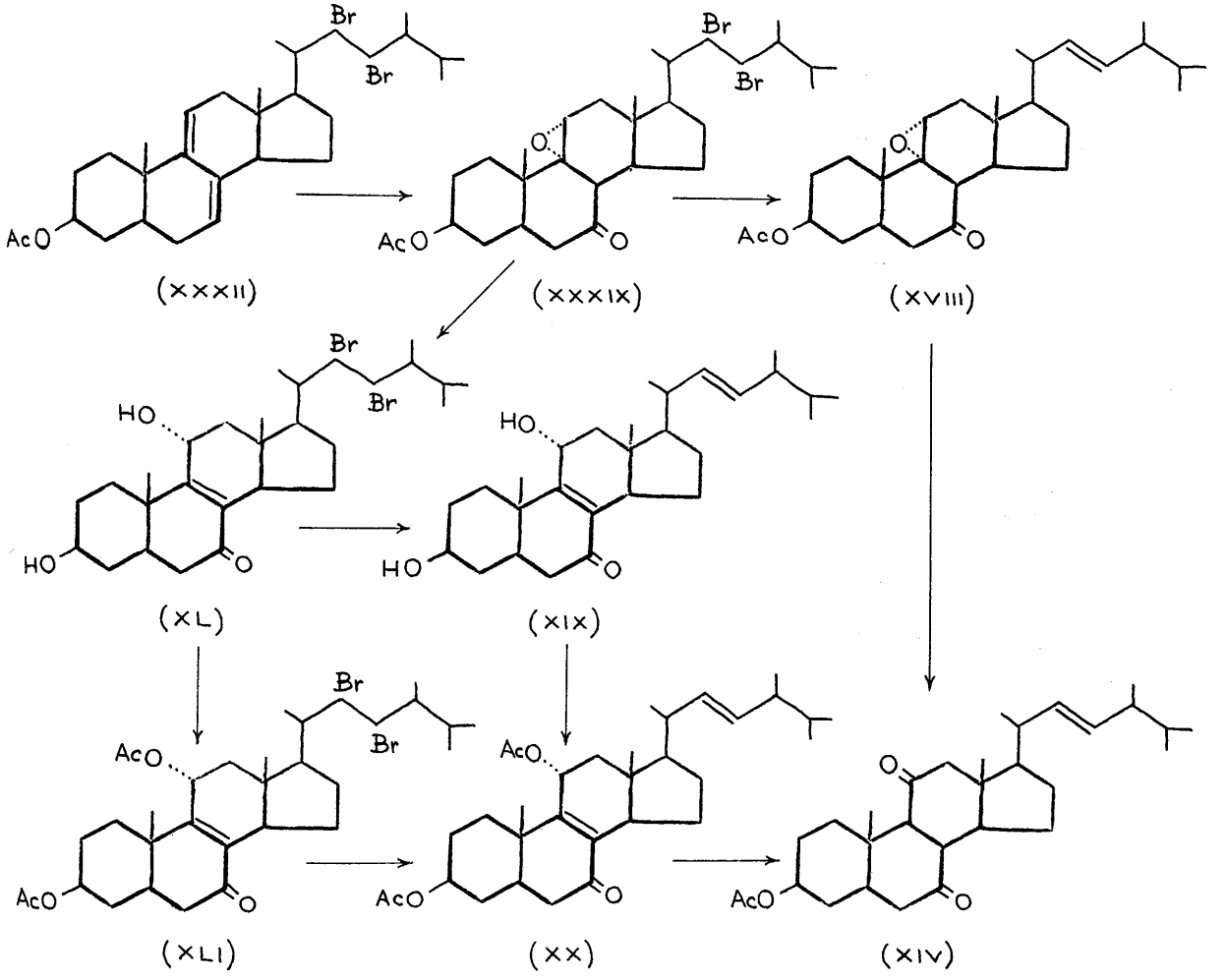
3β-Acetoxy-9α:11α-epoxyergost-22-en-7-one (XVIII) has been prepared directly from ergosteryl-D acetate by oxidation with performic acid (107, 108) as described before, and also by oxidation of ergosteryl-D acetate 22:23-dibromide (XXXII) followed by debromination of the intermediate 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan--7-one (XXXIX) (106). The last compound was later prepared by treatment of ergosteryl-D acetate 22:23--dibromide with hydrogen peroxide in acetic acid (110).

In an attempt to improve the yield of 3β-acetoxy--22:23-dibromo-9α:11α-epoxyergostan-7-one (XXXIX) attention was next turned to the oxidation of 22:23-dibromoergosta--7:9(11)-dien-3β-yl acetate (XXXII) with performic acid in a one phase solution made possible by the addition of ethyl acetate. The compound, $C_{30}H_{46}O_4Br_2$, obtained from the reaction mixture is dimorphous, separating from chloroform-methanol as plates, m.p.235-237° and from acetone as needles, m.p.220-221° (both forms posses the same specific rotation value), each form being convertible into the other by change of solvent. The compound was characterised as 3β-acetoxy-22:23-dibromo-9α:11α-epoxy-

ergostan-7-one (XXXIX) by the formation of a semi--carbazone and a 2:4-dinitrophenylhydrazone (good agreement for a non-conjugated ketone, cf. 89) and by zinc dust debromination, either in acetic acid or ether--methanol solution, to the known 3β-acetoxy-9α:11α-epoxy-ergost-22-en-7-one (XVIII), identical with the specimen obtained by performic acid oxidation of ergosteryl-D acetate, thus confirming the structure ascribed to (XVIII) on the evidence previously discussed.

Treatment of 3β-acetoxy-22:23-dibromo-9α:11α-epoxy-ergostan-7-one (XXXIX) with alkali gave 22:23-dibromo--3β:11α-dihydroxyergost-8-en-7-one (XL) in an exactly analogous manner to the formation of 3β:11α-dihydroxyergost--8-en-7-one (XIX) from 3β-acetoxy-9α:11α-epoxyergost-22--en-7-one (XVIII). The presence of the αβ-unsaturated ketone is shown in both the ultra-violet and infra-red spectra, the latter also confirming the presence of hydroxyl groups and absence of acetate groups. The dibromdiol (XL) was further characterised by debromination with zinc to give 3β:11α-dihydroxyergosta-8:22-dien-7-one (XIX), and by acetylation to 3β:11α-diacetoxy-22:23--dibromoergost-8-en-7-one (XLI). Debromination of the last compound gave the known, previously prepared 3β:11α--diacetoxyergosta-8:22-dien-7-one (XX). Both the ketoxide

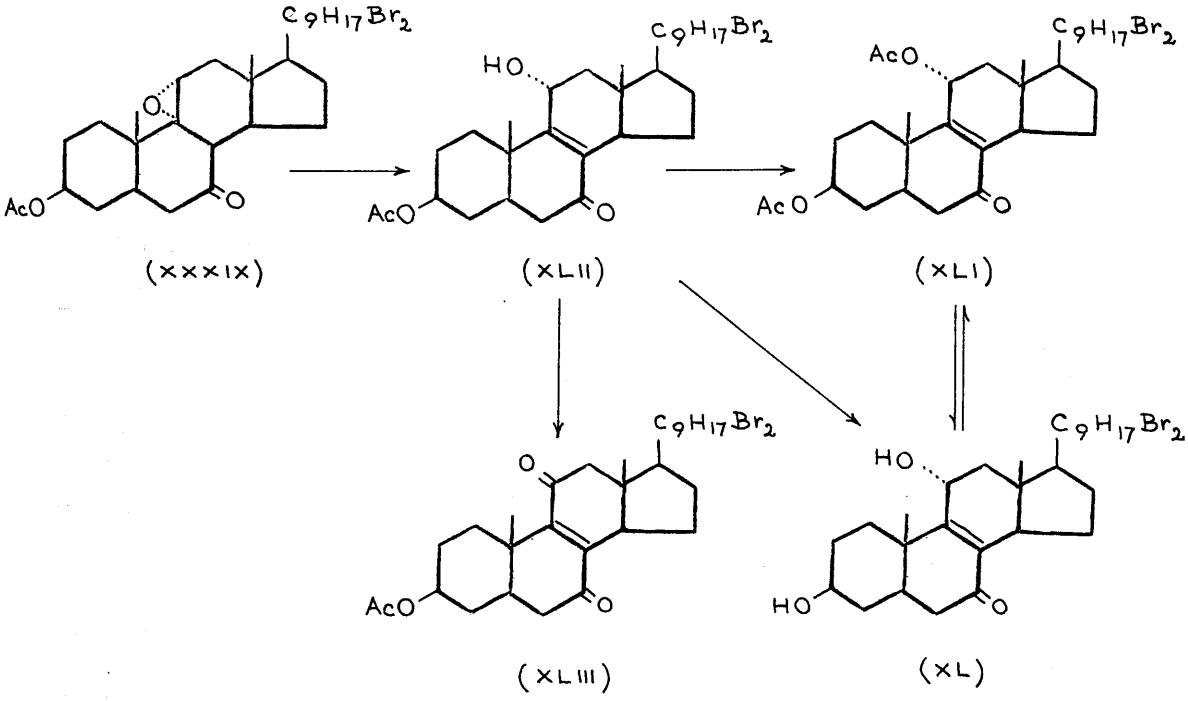
(XVIII) and diacetate (XX) were converted to 7:11--diketoergost-22-en-3β-yl acetate (XIV) by heating under reflux with strong alkali as described previously.



Numerous oxidations of ergosteryl-D acetate 22:23--dibromide with performic acid were carried out under a variety of conditions and at temperatures ranging from room temperature to 80°. The most efficient preparative method for 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one

(XLI) from ergosteryl-D acetate 22:23-dibromide is the oxidation of the last compound with performic acid in ethyl acetate at 40-45°, followed by alkaline hydrolysis and acetylation of the reaction product.

Filtration of a benzene solution of 3β-acetoxy-22:23--dibromo-9α:11α-epoxyergostan-7-one (XXXIX) through a column of alumina gives 3β-acetoxy-22:23-dibromo-11α--hydroxyergost-8-en-7-one (XLII) which is strongly

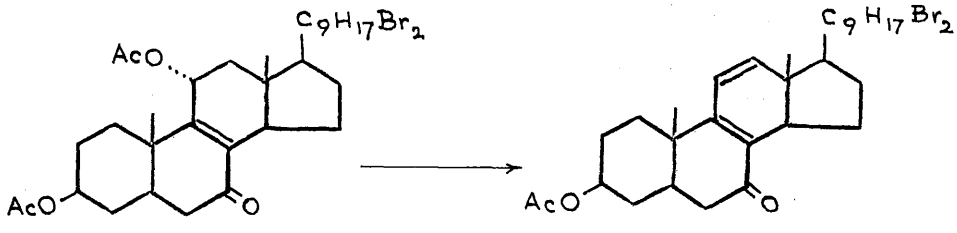


adsorbed by alumina. The compound was characterised by its ultra-violet absorption spectrum which exhibits a maximum characteristic of an αβ-unsaturated ketone, and the infra-red spectrum which shows bands in the ketone,

acetate and hydroxyl regions. Acetylation of this chromatography product gave 3β:11α-diacetoxy-22:23--dibromoergost-8-en-7-one (XLI) and alkaline hydrolysis yielded 22:23-dibromo-3β:11α-dihydroxyergost-8-en-7-one (XL). Oxidation of 3β-acetoxy-22:23-dibromo-11α-hydroxy-ergost-8-en-7-one (XLII) with chromic acid (1.1 atoms of oxygen) gives 22:23-dibromo-7:11-diketoergost-8-en-3β-yl acetate (XLIII) identified by its ultra-violet light absorption at 2700 Å and by direct comparison with an authentic specimen prepared as described by Budziarek, Johnson and Spring (109). This oxidation proceeds smoothly and in good yield in contrast to the oxidation of 22:23-dibromo-7:11α-dihydroxyergost-8-en-3β-yl acetate (XXIV) which gives a mixture of products including 22:23--dibromo-8α:9α-epoxy-7:11-diketoergostan-3β-yl acetate (LVIII) and 22:23-dibromo-7:11-diketoergost-8-en-3β-yl acetate (XLIII), which is described later (cf. 109).

In an attempt to convert 3β:11α-diacetoxy-22:23--dibromoergost-8-en-7-one (XLI) into 22:23-dibromo-7:11--diketoergostan-3β-ol (cf. the similar conversion of 6β-hydroxycholest-4-en-3-one into cholestane-3:6-dione by Ellis and Petrow, 90) the former compound was treated with methanolic hydrogen chloride. The product obtained after acetylation, however, had the molecular formula

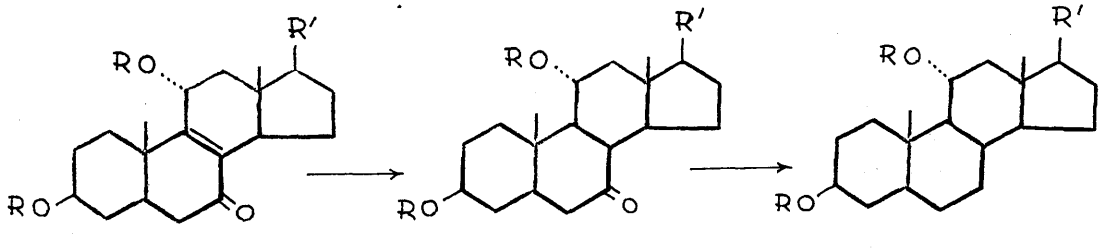
$C_{30}H_{44}O_3Br_2$ indicating that dehydration had occurred, and showed two absorption maxima at 2240 and 2960 Å in



(XLI) (XLIV)

the ultra-violet. This compound has been formulated as 3β-acetoxy-22:23-dibromoergosta-8:11-dien-7-one (XLIV). Similar dehydration of an 11α-hydroxy-7-keto--Δ^8-unsaturated steroid has been observed also in the sapogenin series by Romo, Stork, Rosenkranz and Djerassi (54).

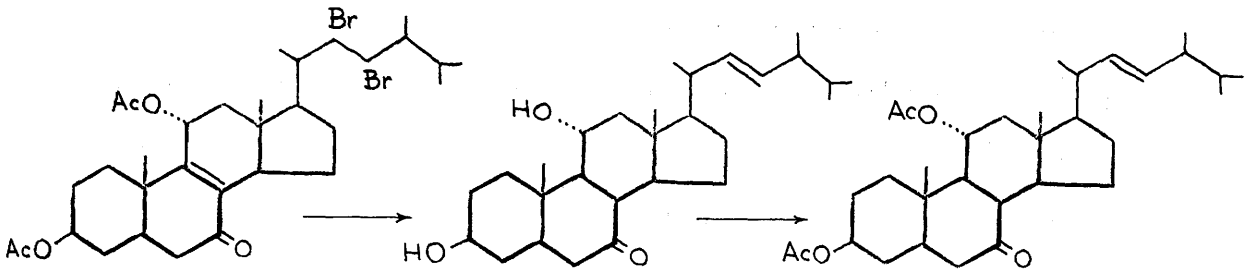
The next problem investigated in this series was the reduction of the 8(9)-ethylenic linkage in the 11--hydroxy-8-en-7-one system in order to permit the ready removal of the 7-keto group by Wolff-Kishner reduction, thus obviating the difficulty of performing this reaction on an αβ-unsaturated ketone.



The compounds chosen for this examination were 22:23--dibromo-3β:11α-dihydroxyergost-8-en-7-one (XL) and 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one (XLI), since due to the known difficulty of hydrogenating an 8(9)-double bond (91), it was considered that an unprotected 22(23)-double bond would be preferentially reduced.

A number of hydrogenation experiments performed on the two compounds in a variety of solvents and using various catalyst (platinum oxide, palladium black and palladised charcoal) resulted either in recovery of starting material or isolation of a non-homogeneous material. The required selective hydrogenation was finally successfully accomplished by performing the reaction in ethanolic potassium hydroxide. Hydrogenation of 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one (XLI) in ethanolic potassium hydroxide solution over platinum, using the conditions described in the Experimental section led to reduction of the 8(9)-ethylenic linkage which was accompanied by hydrolysis and side-chain debromination, with formation of 3β:11α-dihydroxyergost--22-en-7-one (XLV). This compound, which does not exhibit high intensity light absorption above 2200 Å and gives a pale yellow colour with tetranitromethane, was further characterised by acetylation to 3β:11α-diacetoxy-

ergost-22-en-7-one (XLVI).

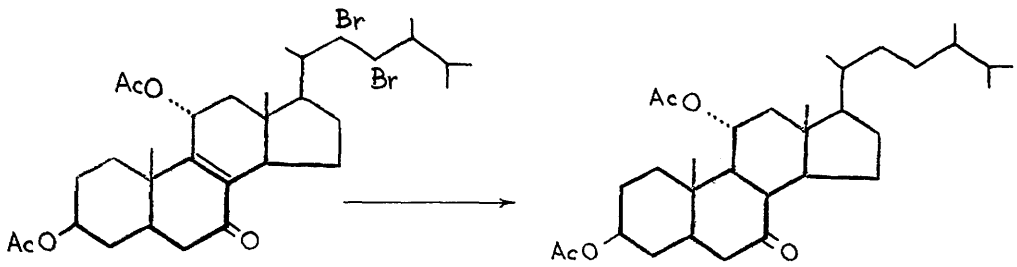


(XLI) (XLV) (XLVI)

This procedure, for reducing the ethylenic linkage of an αβ-unsaturated ketone, has been previously utilised by Chemerda, Chamberlin, Wilson and Tishler (64) (cf. also 92), and by this route the same compound (XLV) was also prepared by Heusser and co-workers (37), by selective reduction of 3β:11α-diacetoxyergosta-8:22-dien--7-one (XX). The latter workers also effected the Wolff-Kishner reduction of (XLVI) to 3β:11α-diacetoxy-ergost-22-ene.

Hydrogenation of 3β:11α-diacetoxy-22:23-dibromoergost--8-en-7-one (XLI) in ethanol over platinum and isolation by chromatography gave a crystalline compound, $C_{38}H_{52}O_8$, which does not exhibit light absorption of high intensity above 2000 Å, does not give a colour with tetranitromethane and shows a depression of melting point when mixed with 3β:11α-diacetoxyergost-22-en-7-one (XLVI). It is concluded that the catalytic hydrogenation led to reduction

of the 8(9)-ethylenic linkage accompanied by debromination



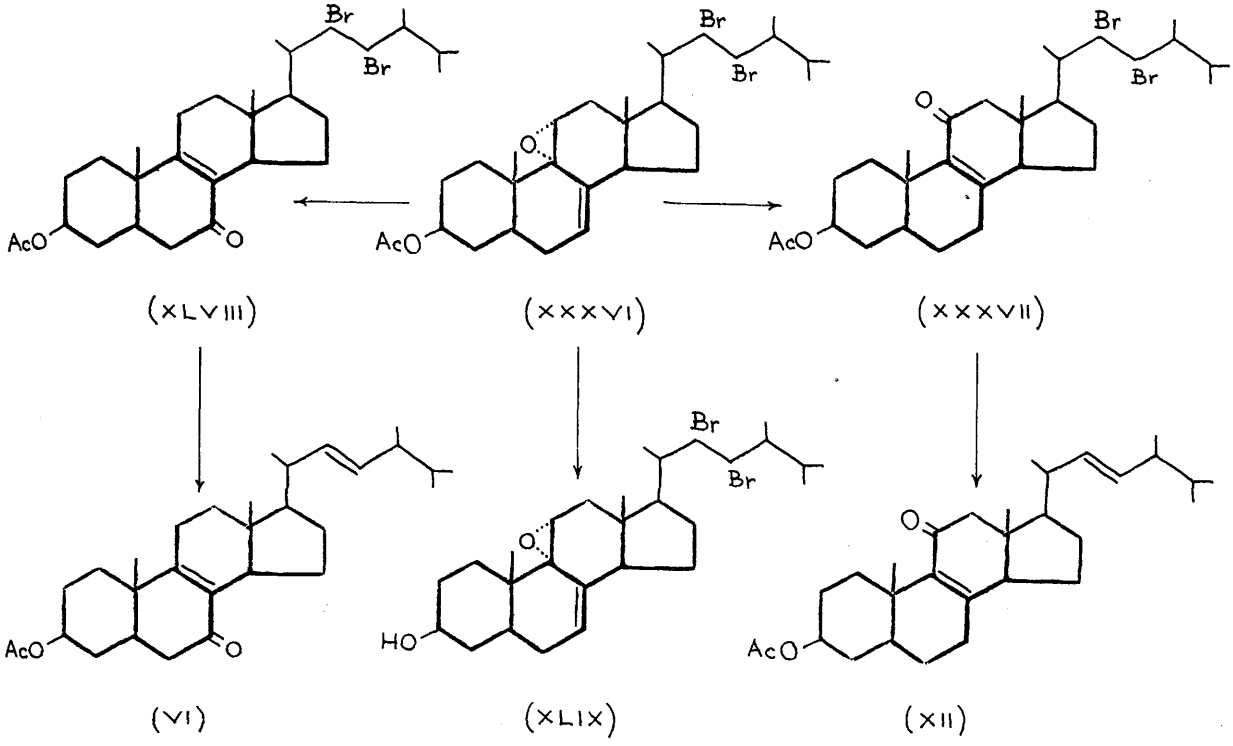
(XLI) (XLVII)

and saturation of the side-chain, with the formation of 3β:11α-diacetoxyergostan-7-one (XLVII).

Oxidation with Perbenzoic Acid.

Oxidation of ergosteryl-D acetate 22:23-dibromide (XXXII) with one molecular proportion of perbenzoic acid gives a monoepoxide in 70% yield. The reactions of this compound, which are described below, show that it is the 22:23-dibromide of ergosteryl-D acetate epoxide, itself obtained by similar oxidation of ergosteryl-D acetate (29) to which the structure 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate (IX) was ascribed by Heusser and co-workers (36). This is supported by the observation that catalytic hydrogenation of ergosteryl-D acetate 22:23-dibromide leads to initial saturation of the 9:11-ethylenic linkage, as described above, a fact which leads to the view that the monoepoxide of ergosteryl-D acetate 22:23-dibromide is likewise formed by saturation of the 9:11-ethylenic

linkage, and that it is therefore 22:23-dibromo-9α:11α--epoxyergost-7-en-3β-yl acetate (XXXVI).



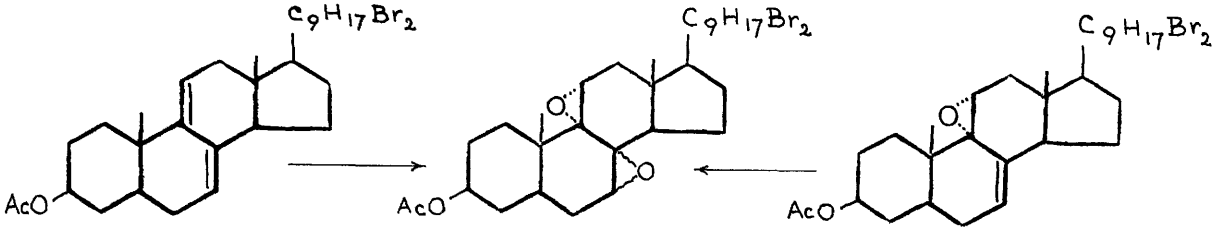
Alkaline hydrolysis of 22:23-dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate (XXXVI) yields 22:23-dibromo--9α:11α-epoxyergost-7-en-3β-ol (XLIX) which is reacetylated to the parent acetate. Treatment of 22:23-dibromo--9α:11α-epoxyergost-7-en-3β-yl acetate with aqueous hydrochloric acid, followed by acetylation of the product gives 3β-acetoxy-22:23-dibromoergost-8-en-7-one (XLVIII), debromination of which with zinc gives the known 3β--acetoxyergosta-8:22-dien-7-one (VI) (39, 36, 108). 3β--Acetoxy-22:23-dibromoergost-8-en-11-one (XXXVII) was

obtained in good yield by treatment of 22:23-dibromo--9α:11α-epoxyergost-7-en-3β-yl acetate (XXXVI) with boron trifluoride etherate in absolute benzene, using the method described by Heusser and co-workers (36) for the preparation of 3β-acetoxyergosta-8:22-dien-11-one (XII). The latter compound (XII) was obtained in excellent yield by debromination of (XXXVII) with zinc.

An attempt was made to debrominate 22:23-dibromo--9α:11α-epoxyergost-7-en-3β-yl acetate (XXXVI) with zinc dust in ether, but, surprisingly, the reaction gave quantitatively ergosteryl-D acetate of high purity.

Oxidation of either ergosteryl-D acetate 22:23--dibromide (XXXII) or 22:23-dibromo-9α:11α-epoxyergost--7-en-3β-yl acetate (XXXVI) with an excess of perbenzoic acid gives a compound, $C_{30}H_{46}O_4Br_2$, isolated in good yields. It does not give a colour with tetranitromethane and does not exhibit selective light absorption of high intensity above 2000 Å. Since the primary product of the perbenzoic acid oxidation is the 9α:11α-epoxide, and it differs from the known 3β-acetoxy-22:23-dibromo-9α:11α--epoxyergostan-7-one (XXXIX), it is presumably 22:23--dibromo-7ξ:8ξ,9α:11α-diepoxyergostan-3β-yl acetate (L). This structure is further supported by the infra-red absorption spectrum, which shows only one band in the

carbonyl region at 1736 cm.$^{-1}$ ascribable to the acetate group.

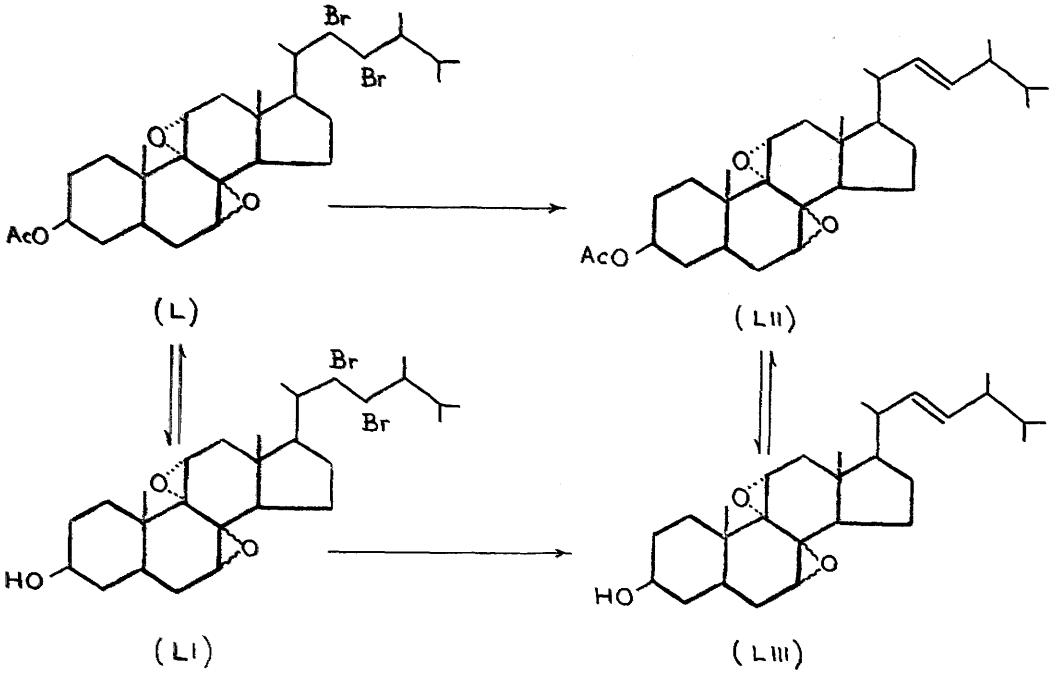


(XXXII) (L) (XXXVI)

22:23-Dibromo-7ξ:8ξ,9α:11α-diepoxyergostan-3β-yl acetate (L), is extremely stable to alkali, and it is hydrolysed with methanolic potassium hydroxide to 22:23--dibromo-7ξ:8ξ,9α:11α-diepoxyergostan-3β-ol (LI), characterised by reacetylation to the parent acetate. Further evidence of the absence of the ketone system is forthcoming from an examination of the infra-red absorption spectrum of the alcohol (LI). This shows the absence of any bands in the carbonyl region.

Debromination of 22:23-dibromo-7ξ:8ξ,9α:11α-diepoxy-ergostan-3β-yl acetate (L) with zinc dust in ether-methanol gives the beautifully crystalline 7ξ:8ξ,9α:11α-diepoxy-ergost-22-en-3β-yl acetate (LII), which is different from the known compounds. This is an important example in which the use of ergosteryl-D acetate 22:23-dibromide affords a ready means for the protection of the side-chain

ethylenic linkage during the oxidation stage. Oxidation of ergosteryl-D acetate with two mols. of perbenzoic



acid does not give a homogeneous reaction product (apart from a small amount of the monoxide) due to the partial attack of the side-chain double bond.

7ξ:8ξ,9α:11α-Diepoxyergost-22-en-3β-yl acetate (LII) is smoothly hydrolysed with alkali to the corresponding 7ξ:8ξ,9α:11α-diepoxyergost-22-en-3β-ol (LIII), which was also obtained by debromination of 22:23-dibromo-7ξ:8ξ,9α:11α--diepoxyergostan-3β-ol (LI), with zinc dust in ether--methanol. The infra-red light absorption spectrum of the alcohol (LIII) does not show any bands in the carbonyl region and thus confirms the structure. It was further

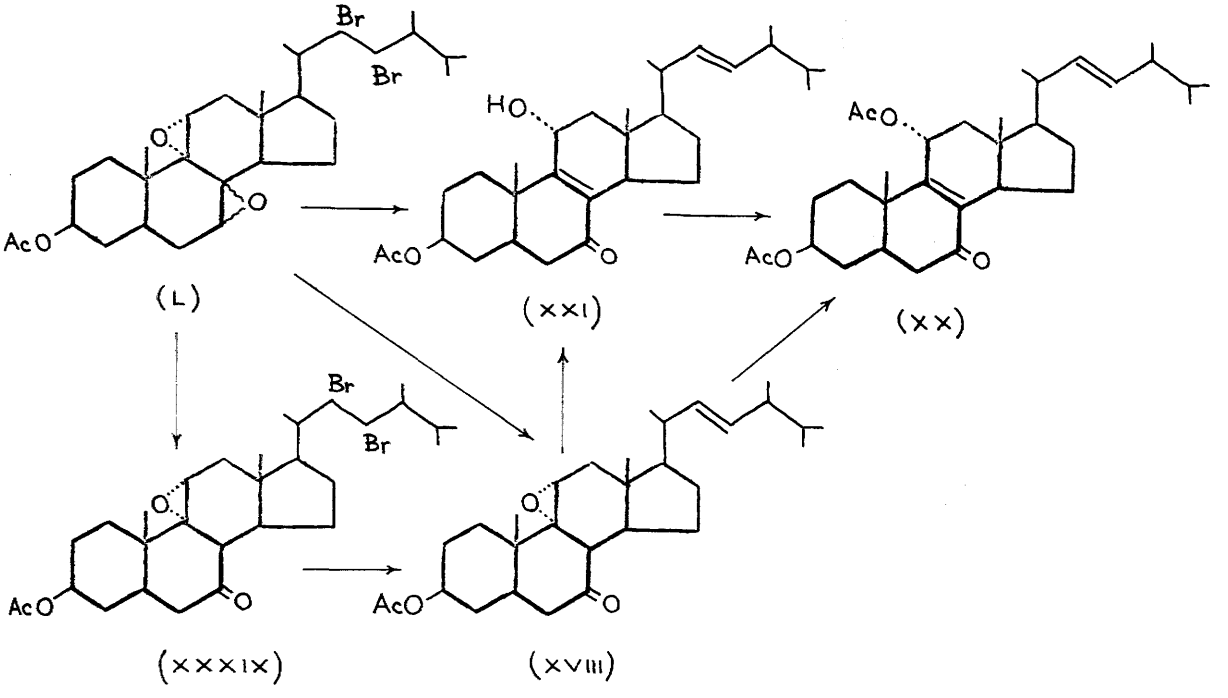
characterised by acetylation to the parent acetate (LII).

Oxidation of ergosteryl-D acetate 22:23-dibromide (XXXII) with excess perbenzoic acid followed by debromination of the product with zinc dust in acetic acid at 100° (6 hours) gives a mixture from which ergosteryl-D acetate and 3β-acetoxyergosta-8:22-dien-7-one (VI) were isolated in poor yield together with 3β-acetoxy-11α--hydroxyergosta-8:22-dien-7-one (XXI) by chromatography on alumina. Acetylation of the last compound (which proved difficult to purify) gave 3β:11α-diacetoxyergosta--8:22-dien-7-one (XX).

Treatment of 22:23-dibromo-7ξ:8ξ,9α:11α-diepoxyergostan-3β-yl acetate (L) with zinc dust in acetic acid at 100° (2 hours) gives 3β-acetoxy-9α:11α-epoxyergost-22--en-7-one (XVIII) in good yield. [Attempted chromatography of this ketoxide on alumina causes rearrangement to 3β-acetoxy-11α-hydroxyergosta-8:22-dien-7-one (XXI) as described earlier]. The corresponding 3β-acetoxy-22:23--dibromo-9α:11α-epoxyergostan-7-one (XXXIX) was obtained as a by-product when a solution of 22:23-dibromo--7ξ:8ξ,9α:11α-diepoxyergostan-3β-yl acetate in acetic acid was treated with aqueous hydrogen bromide at room temperature.

Although 22:23-dibromo-7ξ:8ξ,9α:11α-diepoxyergostan--3β-yl acetate (L) is very stable to alkali, treatment

with acid causes rearrangement. Thus hydrolysis with methanolic hydrogen chloride followed by acetylation,



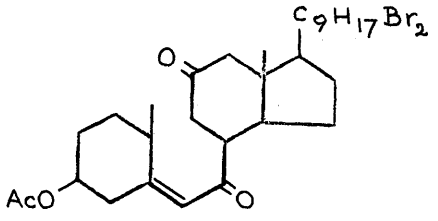
treatment with boron trifluoride etherate in benzene, aqueous hydrogen bromide in acetic acid, or heating in acetic acid at 100°, gives a compound, $C_{30}H_{48}O_4Br_2$, which does not give a colour with tetranitromethane and shows ultra-violet absorption maximum at 2400 Å (ε = 6000-7000; the intensity was practically unchanged after chromatography or further treatment with acid). The infra-red absorption spectrum shows bands at 1726 and 1240 (acetate group) and 1668 cm.$^{-1}$ ($\alpha\beta$-ketone group). It was recovered unchanged after treatment with chromic acid. The compound forms a 2:4-dinitrophenylhydrazone

(a mono-derivative) with Brady's reagent. The ultra-violet absorption spectrum (principal maximum at 3600 Å, ε = 24,000) is in good agreement with that expected for the 2:4-dinitrophenylhydrazone of a saturated ketone (89) (secondary maximum at 2360-80 Å, ε = 20,000). The rearranged compound is hydrolysed by methanolic potassium hydroxide to the corresponding alcohol; the infra-red absorption spectrum shows bands at 3460 (hydroxyl group) and 1676 cm.$^{-1}$ ($\alpha\beta$-ketone).

Debromination of the acetate with zinc dust in ether-methanol gives a compound, $C_{30}H_{46}O_4$, which is difficult to crystallise (low melting point; it forms readily a 2:4-dinitrophenylhydrazone). The same compound was obtained on treatment of 7ξ:8ξ,9α:11α-diepoxyergost-22-en-3β-yl acetate with aqueous hydrogen bromide in acetic acid. Reduction with lithium aluminium hydride followed by acetylation yields a diacetate, $C_{32}H_{50}O_5$, which was also obtained on similar treatment of the corresponding dibromide; it does not exhibit selective light absorption of high intensity above 2200 Å.

It is possible that one ring of the rearranged product is open and a structure such as (LIV) has been considered. The data available, however, does not permit putting forward any such structure with certainty. It is

interesting that although 3β-acetoxy-22:23-dibromo-9α:11α--epoxyergostan-7-one (XXXIX) is obtained as a minor



(LIV)

product (over 10%) on treatment of 22:23-dibromo--7ξ:8ξ,9α:11α-diepoxyergostan-3β-yl acetate (L) with hydrogen bromide in acetic acid, it is certainly not an intermediate compound in the formation of the final rearranged product, but a side-reaction, since the ketoxide is stable to acid and is recovered unchanged after treatment with hydrogen bromide in acetic acid or boron trifluoride in absolute benzene.

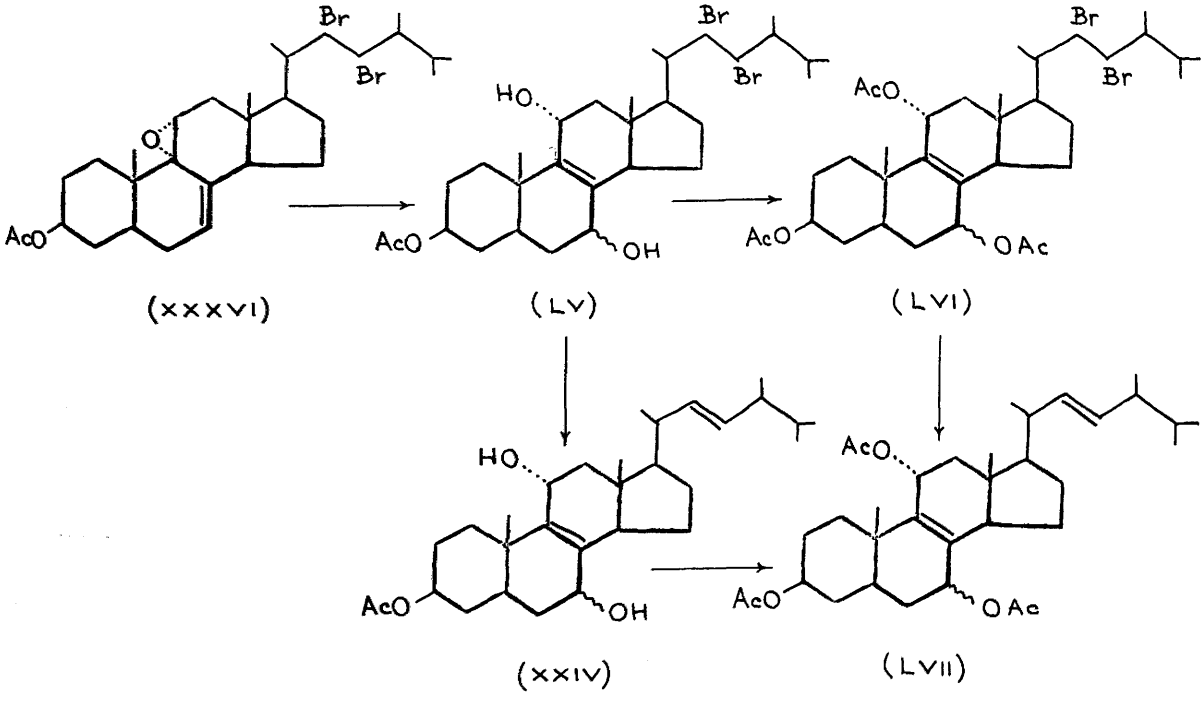
A digression into medicinal chemistry can conveniently be introduced at this stage. According to Feiser (96) evidence of low-order carcinogenicity of a variety of cholesterol-rich lipid fractions (97) and of high carcinogenic potency (98) of crude progesterone preparation derived from cholesterol by bromination, oxidation and debromination (99) suggests the existance of a possibly endogenous non-aromatic steroid carcinogen

related to or derived from cholesterol, and the consideration that the carcinogen is a product of oxidation of cholesterol cannot be excluded. It can be inferred that the Δ^7-cholestenol, which occurs naturally (96) with cholesterol, was converted into $\Delta^{7:9(11)}$-cholestadienol and hence that the carcinogen may be an oxidation product of this diene. In view of the demonstrated carcinogenicity of the diepoxide of vinylcyclohexane (100) it seems possible that the substance may be 7:8,9:11--diepoxycholestanol. This conceivably could be formed in lard-injected cholesterol (97) by the action of peroxides of lard on Δ^7-cholestenol; cholesterol administered in sesame oil, which contains a natural antioxidant preventing peroxidation, has given no tumors (98).

The possibility that the diepoxide of ergosterol-D may also have carcinogenic potency and the easy method of preparation has made sufficient quantities available for this investigation. Consequently, samples of the 7:8,9:11-diepoxide have been sent to The Royal Cancer Hospital, The Chester Beatty Research Institute, London, for testing. So far, however, the results are not yet available.

Reactions of 22:23-Dibromo-7ξ:11α-dihydroxyergost-8-en-3β-yl Acetate.

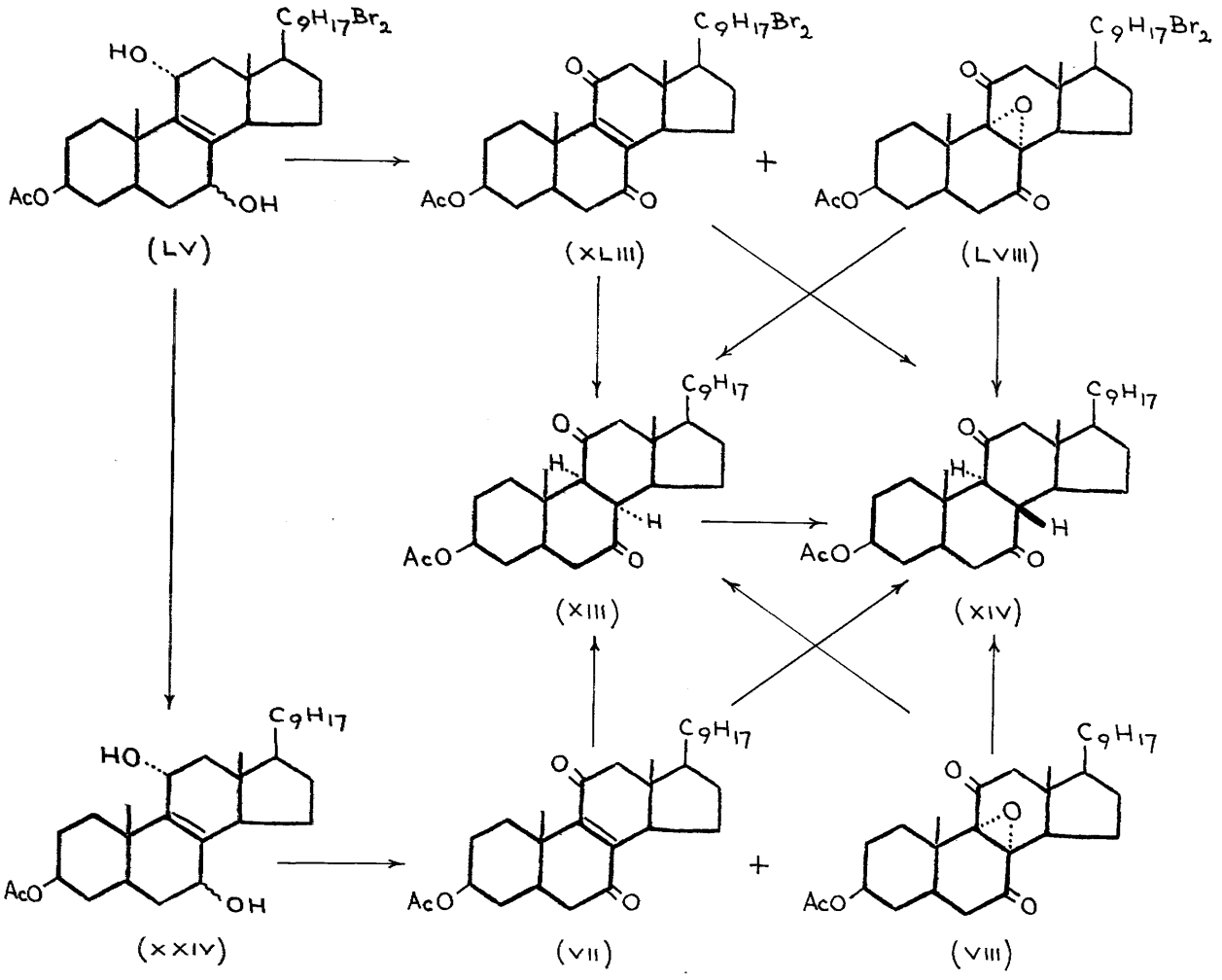
Controlled treatment of 22:23-dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate (XXXVI) with sulphuric acid in dioxan or tetrahydrofuran gives 22:23-dibromo-7ξ:11α-dihydroxyergost-8-en-3β-yl acetate (LV) in excellent yield. The latter compound is also obtained by treatment of 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate (IX) in chloroform with bromine. It acetylates readily to give 3β:7ξ:11α-triacetoxy-22:23-dibromoergost-8-ene (LVI), debromination of which with zinc dust yields 3β:7ξ:11α-triacetoxyergosta-8:22-dien (LVII) identical with a specimen prepared by acetylation of 7ξ:11α-dihydroxyergost-



-8:22-dien-3β-yl acetate (XXIV) obtained from 9α:11α--epoxyergosta-7:22-dien-3β-yl acetate (IX) previously described by Chamberlin et al. (29). Debromination of 22:23-dibromo-7ξ:11α-dihydroxyergost-8-en-3β-yl acetate (LV) with zinc gives 7ξ:11α-dihydroxyergosta-8:22-dien--3β-yl acetate (XXIV) in excellent yield.

Oxidation of 22:23-dibromo-7ξ:11α-dihydroxyergost--8-en-3β-yl acetate (LV) with chromic acid gives a mixture of 22:23-dibromo-7:11-diketoergost-8-en-3β-yl acetate (XLIII and 22:23-dibromo-8α:9α-epoxy-7:11--diketoergostan-3β-yl acetate (LVIII). The oxidation of 22:23-dibromo-7ξ:11α-dihydroxyergost-8-en-3β-yl acetate with chromic acid parallels the similar oxidation of 7ξ:11α-dihydroxyergosta-8:22-dien-3β-yl acetate (XXIV) described by Heusser et al. (36) which gives a mixture of 7:11-diketoergosta-8:22-dien-3β-yl acetate (VII) and 8α:9α-epoxy-7:11-diketoergost-22-en-3β-yl acetate (VIII). Treatment of either (XLIII) or (LVIII, with zinc dust in acetic acid gives 7:11-diketoergost-22-en-3β-yl acetate (XIV) in quantitative yield. An attempt was made to limit this last reaction to simple debromination by treatment of 22:23-dibromo-7:11-diketoergost-8-en-3β-yl acetate (XLIII) with zinc dust in ether-methanol. The reaction gave a compound, $C_{30}H_{46}O_4$, $[\alpha]_D$ +30° which does not show

intense absorption above 2200 Å. Simple warming of an acetic acid solution of the compound, $C_{30}H_{46}O_4$, gives

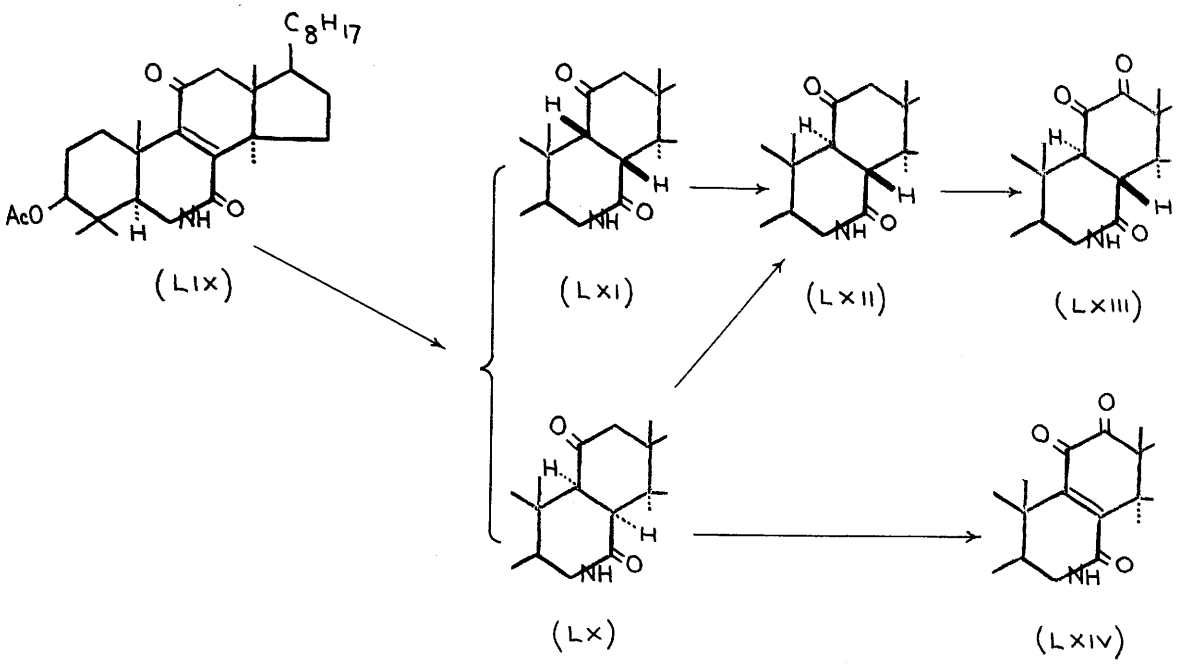


7:11-diketoergost-22-en-3β-yl acetate (XIV), $[\alpha]_D$ -28°. The compound, $C_{30}H_{46}O_4$, $[\alpha]_D$ +30°, is also obtained by treatment of 22:23-dibromo-8α:9α-epoxy-7:11-diketoergostan--3β-yl acetate (LVIII), 7:11-diketoergost-8:22-dien-3β-yl acetate (VII), and 8α:9α-epoxy-7:11-diketoergost-22-en-3β-yl acetate (VIII) in ether-methanol with zinc dust (see p.73);

the conversion of each of these compounds into 7:11--diketoergost-22-en-3β-yl acetate by treatment with zinc dust and acetic acid has previously been reported (29, 36, 109). The compound, $C_{30}H_{46}O_4$, $[\alpha]_D$ +30°, is therefore an isomeric 7:11-diketoergost-22-en-3β-yl acetate and it must differ from the latter in configuration at C_8 and/orC_9. The new isomer is 7:11-diketo-8α--ergost-22-en-3β-yl acetate (XIII), a cis-addition of hydrogen to the 8:9-double bond having occurred. This view is based on the probability that addition of hydrogen at C_9 occurs from the rear of the molecule to give a 9α-hydrogen, from which it follows that the new isomer must differ from the normal isomer in the configuration at C_8. Such a stereochemical course for the reduction of ene-1:4-diones has been also exemplified by Barton and co-workers (82), who describe a similar cis-addition of hydrogen to a 1:4-diketo-2:3-ene by treatment with zinc and acetic acid.

This view was later confirmed by Barton (105) in an extremely elegant series of transformations of stereochemical interest. Reduction of 7:11-diketo-6a-aza-B--homolanost-8-en-3β-yl acetate (LIX) with zinc dust and acetic acid affords two isomeric keto-amides, 8α:9α (LX) and 8β:9β (LXI), involving cis-addition of hydrogen.

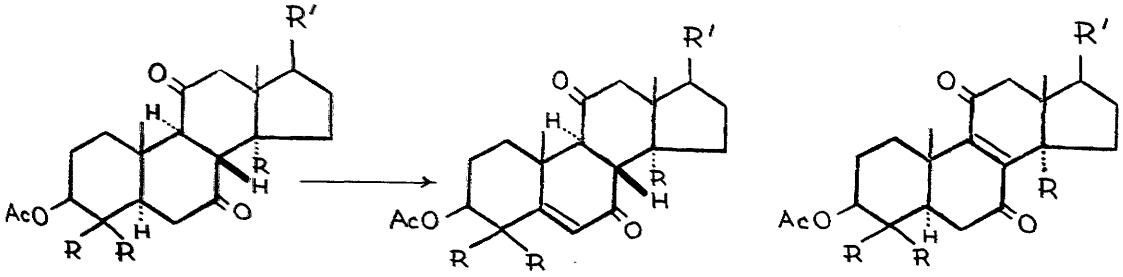
Both amides are isomerised by alkali to a further 8β:9α--stereoisomer (LXII), the more stable trans-form.



Oxidation of the amide (LXII) with selenium dioxide in acetic acid gives the saturated triketone (LXIII). This reaction is in marked contrast with the course of the selenium dioxide oxidation of the cis-form (LX), where the double bond is readily re-introduced between C_8 and C_9 to give the unsaturated triketone (LXIV). This led to the conclusion that the ready selenium dioxide oxidation of the system -CO-CH-CH-CO- to -CO-C=C-CO- is a stereospecific process in which the hydrogen atoms should be cis to each other.

Other cases have been described (105) where the

<u>trans</u>-stereochemistry of the hydrogen atoms of an ane--1:4-dione makes difficult selenium dioxide oxidation to an ene-1:4-dione. 7:11-Diketolanostanyl acetate (LXV; R=Me, R'=C_8H_{17}) gives 7:11-diketolanost-5-enyl acetate (LXVI; R=Me, R'=C_8H_{17}) and not 7:11-diketolanost--8-enyl acetate (LXVII; R=Me, R'=C_8H_{17}). Similarly



(LXV) (LXVI) (LXVII)

7:11-diketoergostanyl acetate (LXV; R=H, R'=C_9H_{19}) affords 7:11-diketoergost-5-enyl acetate (LXVI; R=H, R'=C_9H_{19}), and <u>not</u> 7:11-diketoergost-8-enyl acetate (LXVII; R=H, R'=C_9H_{19}).

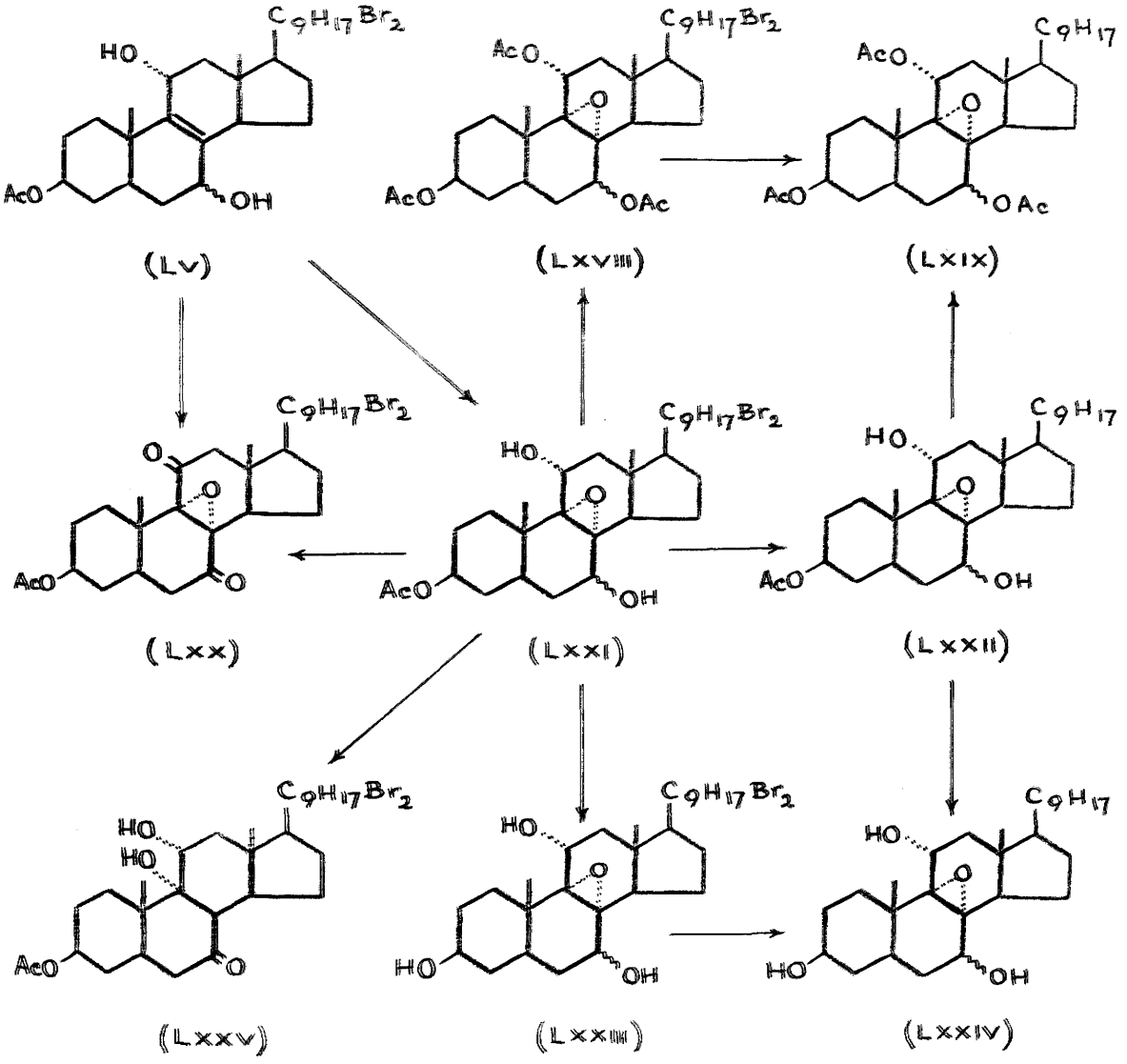
The discovery of 7:11-diketo-8α-ergost-22-en-3β-yl acetate (XIII) (114) enabled Barnes and Barton (105) to further support the generalisation that ready conversion of ane-1:4-diones to ene-1:4-diones requires a <u>cis</u>-relation for the eliminated hydrogen atoms and to confirm, at the same time, the structure of the new compound. They have shown that, as expected, selenium dioxide oxidation (in ethanol solution) of the compound gives in excellent yield

7:11-diketoergosta-8:22-dien-3β-yl acetate (LXVII; R=H, R'=C_9H_{17}) with preferential cis-elimination of hydrogen. Under the same conditions 7:11-diketoergostan-3β-yl acetate was not attacked.

22:23-Dibromo-9α:11α-dihydroxy-7-ketoergostan-3β-yl Acetate and Related Compounds.

Oxidation of 22:23-dibromo-7ξ:11α-dihydroxyergost--8-en-3β-yl acetate (LV) with perbenzoic acid gives, in excellent yield, 22:23-dibromo-8α:9α-epoxy-7ξ:11α--dihydroxyergostan-3β-yl acetate (LXXI) debromination of which with zinc dust in ether-methanol gives the known 8α:9α-epoxy-7ξ:11α-dihydroxyergost-22-en-3β-yl acetate LXXII) first prepared by Heusser and co-workers (37) by partial oxidation of 7ξ:11α-dihydroxyergosta-8:22-dien--3β-yl acetate (XXIV) with monoperphthalic acid. The dibromide (LXXI) was further characterised by acetylation to 3β:7ξ:11α-triacetoxy-22:23-dibromo-8α:9α-epoxyergostane (LXVIII), debromination of which gave the known 3β:7ξ:11α--triacetoxy-8α:9α-epoxyergost-22-ene (LXIX) (37, 109), and by alkaline hydrolysis to 22:23-dibromo-8α:9α-epoxy--3β:7ξ:11α-trihydroxyergostane (LXXIII) debromination of which gives 8α:9α-epoxy-3β:7ξ:11α-trihydroxyergost-22-ene (LXXIV). Oxidation of 22:23-dibromo-8α:9α-epoxy-7ξ:11α--dihydroxyergostan-3β-yl acetate (LXXI) with chromic acid

gives 22:23-dibromo-8α:9α-epoxy-7:11-diketoergostan-3β-yl acetate (LXX) previously obtained by the oxidation of 22:23-dibromo-7ξ:11α-dihydroxyergost-8-en-3β-yl acetate (LV) with chromic acid (109). The α-configuration of the 8:9-epoxide bridge in (LXXI) follows from the considerations used in the case of the corresponding diketone (LXX).

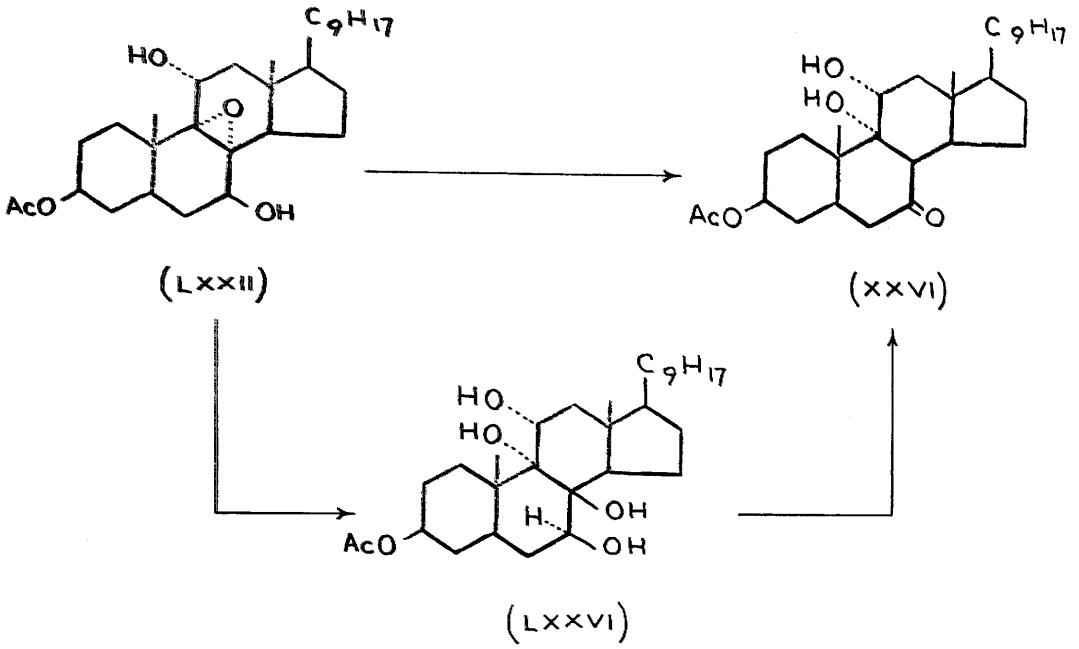


Treatment of 22:23-dibromo-8α:9α-epoxy-7ξ:11α--dihydroxyergostan-3β-yl acetate (LXXI) with aqueous hydrogen bromide in acetic acid (cf. 37) gives 3β-acetoxy--22:23-dibromo-9α:11α-dihydroxyergostan-7-one (LXXV) debromination of which gives 3β-acetoxy-9α:11α-dihydroxyergost-22-en-7-one (XXVI), obtained previously by treatment of 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate (IX) successively with one mol. of bromine, excess perbenzoic acid, and zinc and acetic acid. In a preliminary publication (107), the ξ-configuration was ascribed provisionally to the 9-hydroxyl group. Budziarek, Newbold, Stevenson and Spring (108) ascribed the β-orientation to the 9-hydroxyl group in (XXVI), the two hydroxyl groups being considered as trans-orientated with respect to each other "since they almost certainly originate by a hydrolytic cleavage of a 9α:11α-oxide intermediate." This argument does not now appear satisfactory since the instability of 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate and of 22:23-dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate to traces of mineral acid results in the addition of bromine being accompanied by hydrolytic rearrangement to give, in part, 22:23-dibromo-7ξ:11α-dihydroxyergost-8-en--3β-yl acetate (109) which may thus be the precursor of 3β-acetoxy-9α:11α-dihydroxyergost-22-en-7-one in the

reaction sequence described by Budziarek et al. (loc.cit.) (cf. 37). Support for this view was obtained from the observation that 3β-acetoxy-9α:11α-dihydroxyergost-22-en--7-one (XXVI) is obtained in high yield by treatment of 22:23-dibromo-8α:9α-epoxy-7ξ:11α-dihydroxyergostan-3β-yl acetate (LXXI) with zinc dust and acetic acid (112, 113). There is therefore no evidence for assuming the β-configuration for the 9-hydroxyl in (XXVI) and its derivatives.

The formation of 3β-acetoxy-22:23-dibromo-9α:11α--dihydroxyergostan-7-one (LXXV) from 22:23-dibromo-8α:9α--epoxy-7ξ:11α-dihydroxyergostan-3β-yl acetate (LXXI), the reactions of the former compound and inspection of the Stuart models seem to indicate the α-configuration of the 9-hydroxyl group in (LXXV). Since the 11-hydroxyl group is also α-orientated, it follows that (LXXV) is a cis-glycol. This view is further supported by Heusser and co-workers (37) who found that 8α:9α-epoxy-7ξ:11α--dihydroxyergost-22-en-3β-yl acetate (LXXII) is isomerised to 3β-acetoxy-9α:11α-dihydroxyergost-22-en-7-one (XXVI) either with hydrogen bromide in acetic acid, or with boron trifluoride etherate in absolute benzene. Treatment of the epoxide (LXXII) with aqueous sulphuric acid converts it into 7:8:9:11-tetrahydroxyergost-22-en-3β-yl acetate (LXXVI) which in its turn gives 3β-acetoxy-9α:11α-dihydroxy-

ergost-22-en-7-one (XXVI) on treatment with hydrogen bromide in acetic acid. A provisional allocation of



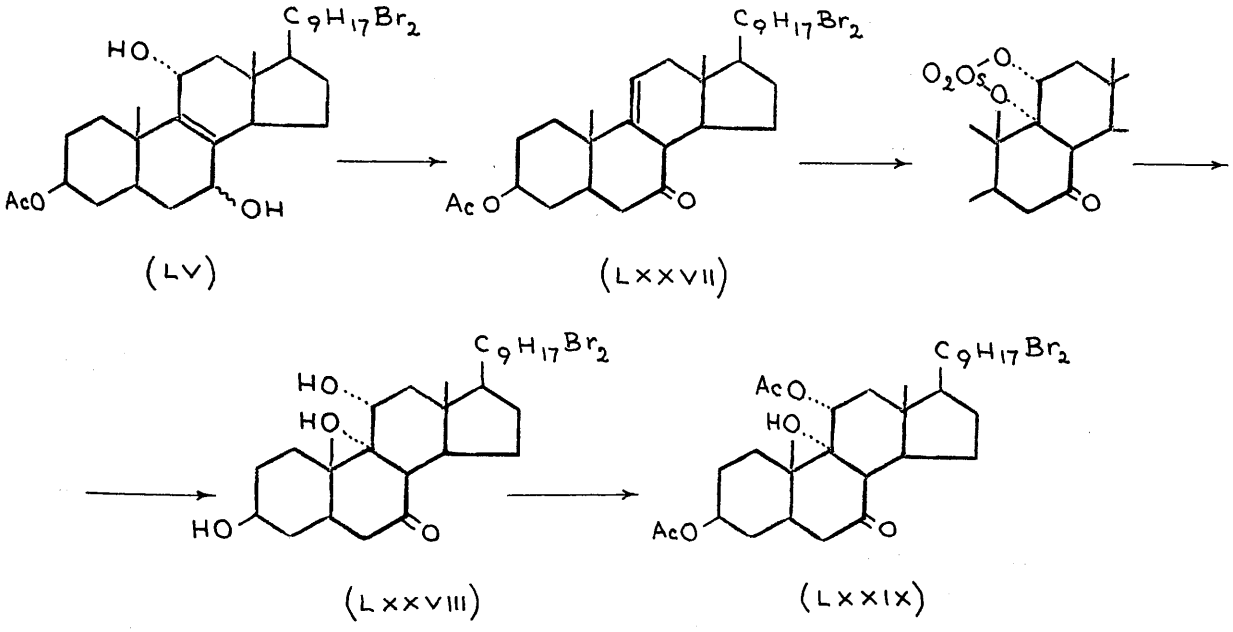
configurations to the hydroxyl groups in (XXVI) and (LXXVI) was made. Starting from the view that the 8:9--epoxide bridge and the 11-hydroxyl group in (LXXII) are both α-orientated, it is argued that the conversion of (LXXII) into (XXVI) by means of boron trifluoride etherate in absence of water requires that the 8α:9α--epoxide bridge is ruptured between C_8 and the oxygen with the consequence that the C_9-hydroxyl group is α--orientated. Since the tetrahydric alcohol (LXXVI) has been converted into (XXVI) the 9-hydroxyl group in the latter is also α-orientated. Since the 8- and 9-hydroxyl

groups in (LXXVI) result from an acid fission of the 8α:9α-epoxide bridge, they will be trans-orientated with respect to each other [in the cleavage of oxides a Walden inversion occurs at the carbon atom at which a carbon--oxygen bond is ruptured (cf. 24, p.221)], from which it follows that the 8-hydroxyl group in (LXXVI) is β-orientated. Concerning the orientation of the 7-hydroxyl group in (LXXVI) it is argued that the ready dehydration of (LXXVI) to the 7-ketone (XXVI) connotes a cis-glycol (trans-elimination of water) in which case the 7-hydroxyl group in (LXXVI) is β-orientated.

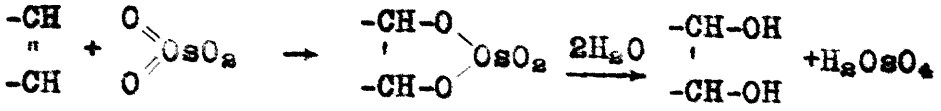
Support for this view is also forthcoming from the concept of equatorial and polar bonds (101). Inspection of the models (rings A, B and C in chair conformation) shows that the 8-hydroxyl group and the hydrogen at C_7 both form polar bonds, and, according to the concept, for ionic elimination reactions involving substituents on adjacent carbon atoms, the elimination proceeds most readily when the two substituents form polar bonds.

The α-configuration of the 9-hydroxyl group in (LXXV) has been finally proved beyond doubt [by Mr. D. Maclean in this laboratory (93)] by treatment of 3β-acetoxy-22:23--dibromoergost-9(11)-en-7-one (LXXVII) [itself obtained by treatment of 22:23-dibromo-7ξ:11α-dihydroxyergost-8-en-

-3β-yl acetate (LV) with boron trifluoride etherate in absolute benzene] with osmium tetroxide in absolute benzene to yield 3β:11α-diacetoxy-22:23-dibromo-9α--hydroxyergostan-7-one (LXXIX) [itself obtained by acetylation of 3β-acetoxy-22:23-dibromo-9α:11α-dihydroxyergostan--7-one (LXXV)] after hydrolysis (to decompose the addition--complex) followed by acetylation.



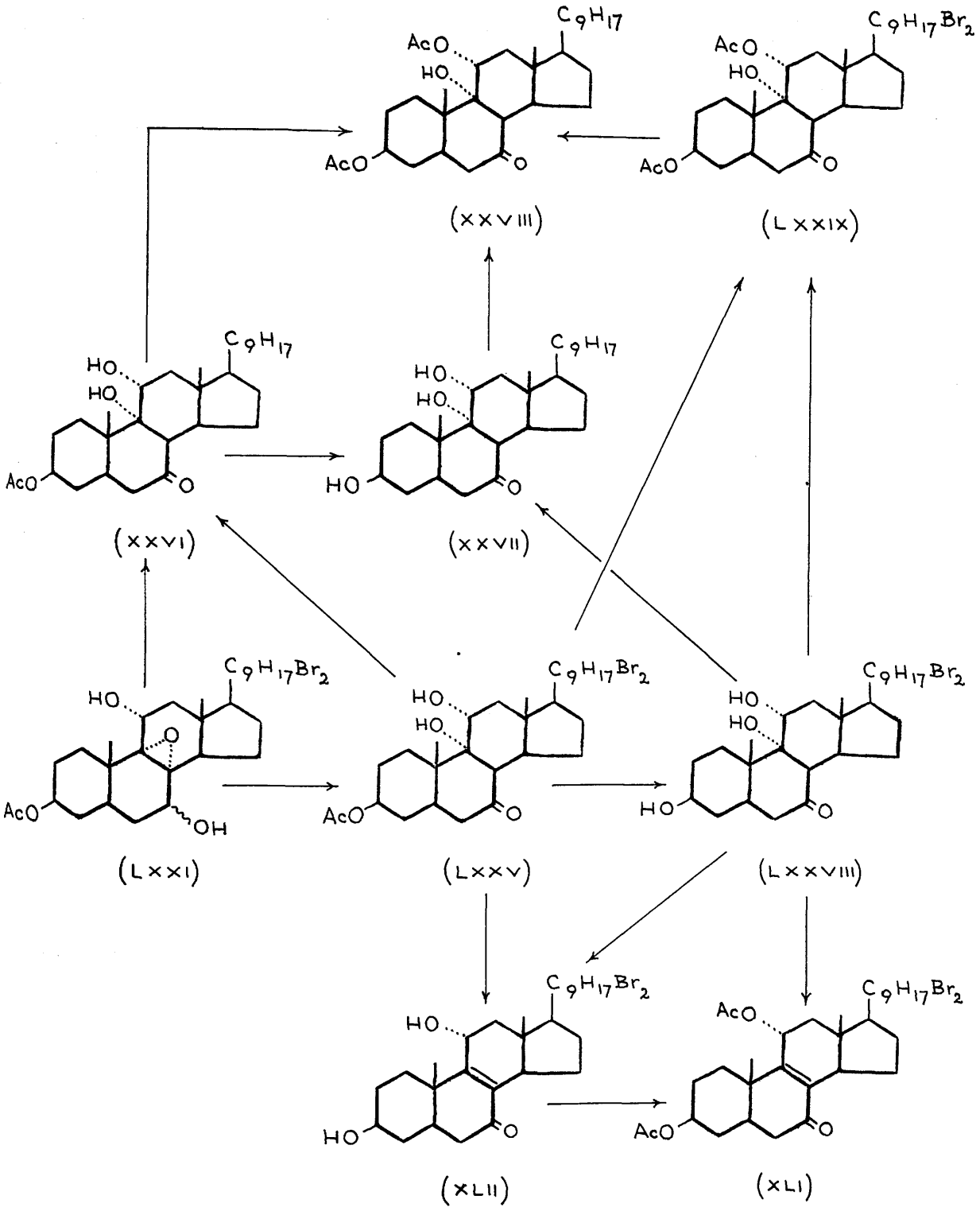
It is obvious that the addition-compound was formed by the attack of the molecule from the rear, the side which is considerably less hindered. Decomposition of the complex by hydrolysis yields a cis-glycol, which, according to Criegee (94), can be represented as follows:



Since the 11-hydroxyl group is α-orientated, it follows that the 9-hydroxyl group is also α-orientated.

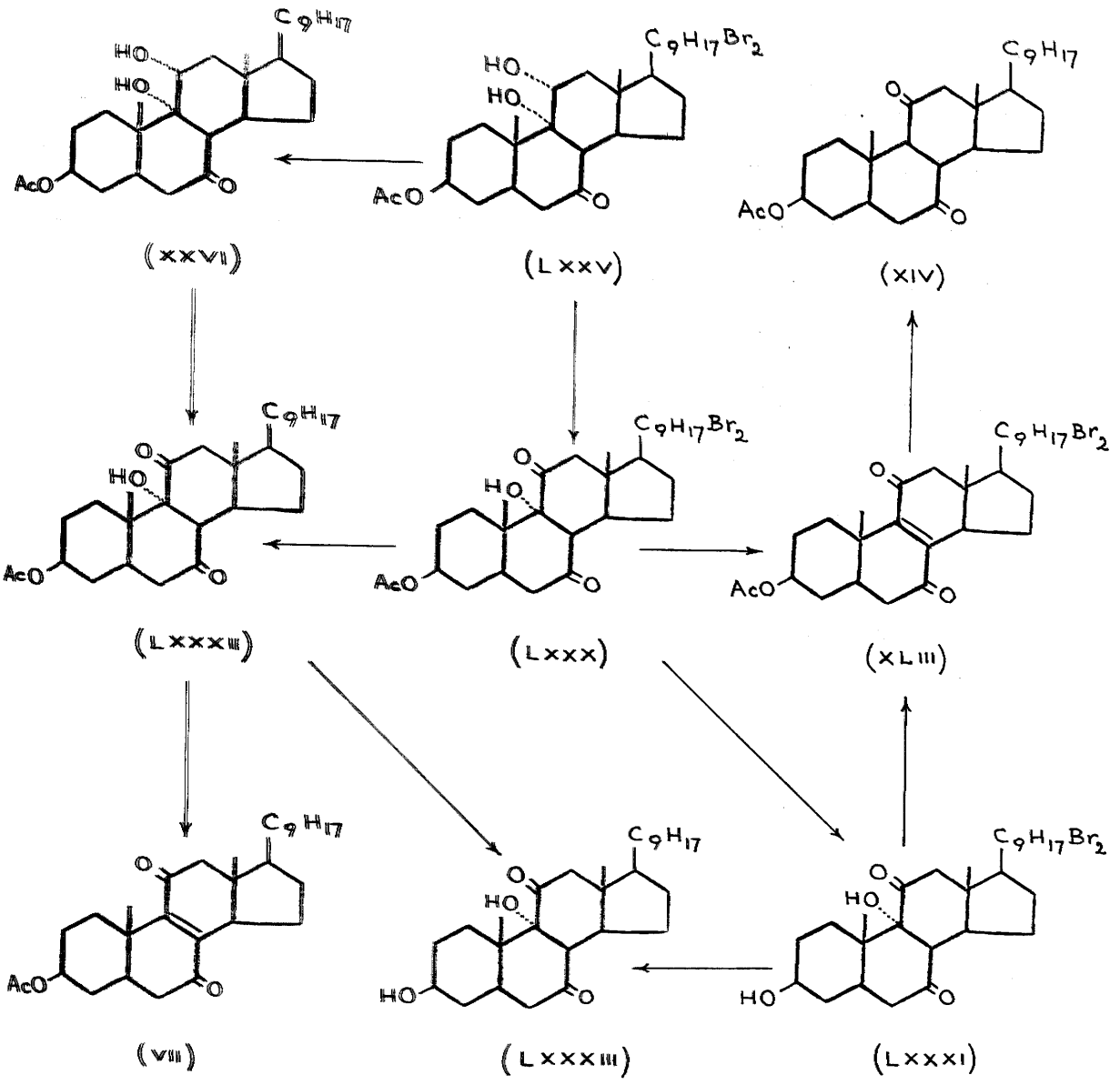
3β-Acetoxy-22:23-dibromo-9α:11α-dihydroxyergostan-7-one (LXXV) was further characterised by alkaline hydrolysis to 22:23-dibromo-3β:9α:11α-trihydroxyergostan-7-one (LXXVIII) and by acetylation to 3β:11α-diacetoxy-22:23-dibromo-9α-hydroxyergostan-7-one (LXXIX). Debromination of (LXXVIII) and (LXXIX) gave 3β:9α:11α-trihydroxyergost-22-en-7-one (XXVII) and 3β:11α-diacetoxy-9α-hydroxyergost-22-en-7-one (XXVIII) respectively.

Although treatment of 3β-acetoxy-22:23-dibromo-9α:11α-dihydroxyergostan-7-one (LXXV) with 1% alcoholic potassium hydroxide results in simple hydrolysis with the formation of 22:23-dibromo-3β:9α:11α-trihydroxyergostan-7-one (LXXVIII), using 10% methanolic potassium hydroxide, hydrolysis is accompanied by partial dehydration and formation of 22:23-dibromo-3β:11α-dihydroxyergost-8-en-7-one (XLII), which is also obtained by treatment of 22:23-dibromo-3β:9α:11α-trihydroxyergostan-7-one (LXXVIII) with 10% alkali. It was noted, however, that very drastic conditions had to be employed (18 hours) in order to induce dehydration across C_8-C_9 to (XLII); the dibromo-9:11-diol


C9H17
AcO
HO
AcO
O
(XXVIII)
C9H17Br2
AcO
HO
AcO
O
(LXXIX)
C9H17
HO
HO
AcO
O
(XXVI)
C9H17
HO
HO
HO
O
(XXVII)
C9H17Br2
HO
O
AcO
OH
(LXXI)
C9H17Br2
HO
HO
AcO
O
(LXXV)
C9H17Br2
HO
HO
HO
O
(LXXVIII)
C9H17Br2
HO
HO
O
(XLII)
C9H17Br2
AcO
AcO
O
(XLI)

is very stable to acid and alkali and should, therefore, have the normal β-configuration of hydrogen at C_8. This is further supported by the fact that 3β-acetoxy-22:23--dibromoergost-9(11)-en-7-one (LXXIX) [which was converted into 3β:11α-diacetoxy-22:23-dibromo-9α-hydroxy-ergostan-7-one (LXXIX)] was debrominated (93) to the more stable unconjugated ketone (XVI) previously described, which has probably the 8β-configuration.

Oxidation of 3β-acetoxy-22:23-dibromo-9α:11α--dihydroxyergostan-7-one (LXXV) with chromic acid gives 22:23-dibromo-9α-hydroxy-7:11-diketoergostan-3β-yl acetate (LXXX) which was recovered unchanged after heating with acetic anhydride, or with acetic anhydride to which concentrated hydrochloric acid had been added. Treatment of the last compound (LXXX) with 1% alcoholic potassium hydroxide effects simple hydrolysis with the formation of 22:23-dibromo-3β:9α-dihydroxyergostan-7:11-dione (LXXXI). Debromination of (LXXX) with zinc dust yields 3β-acetoxy--9α-hydroxy-7:11-diketoergost-22-ene (LXXXII), also obtained by chromic acid oxidation of 3β-acetoxy-9α:11α--dihydroxyergost-22-en-7-one (XXVI) (112). Controlled alkaline hydrolysis of (LXXXII) gives 3β:9α-dihydroxy--7:11-diketoergost-22-ene (LXXXIII) which is also obtained by zinc dust debromination of the corresponding dibromide (LXXXI).



Vigorous treatment of 22:23-dibromo-9α-hydroxy-7:11--diketoergostan-3β-yl acetate (LXXX) with alcoholic potassium hydroxide followed by acetylation of the reaction product, yields, as major product, 22:23-dibromo-7:11--diketoergost-8-en-3β-yl acetate (XLIII), identical with a specimen prepared by a different route by Budziarek,

Johnson, and Spring (109) who showed that treatment of (XLIII) with zinc dust and acetic acid gives the well--known 7:11-diketoergost-22-en-3β-yl acetate (XIV). Similar treatment of 3β-acetoxy-9α-hydroxy-7:11-diketo-ergost-22-ene (LXXXII) gives 7:11-diketoergost-8:22-dien--3β-yl acetate (VII) identical with a specimen prepared by Heusser and co-workers (36) by a different route.

Conclusion.

Ergosterol has been successfully converted by various procedures into 11α-hydroxy and 11-ketosteroids. The action of performic and perbenzoic acid on ergosteryl-D acetate and ergosteryl-D acetate 22:23-dibromide has been fully investigated as these two oxidising agents proved extremely promising. The introduction of an 11-oxygen function into the steroid nucleus proceeding by way of the dibromide has been shown to have many attractive features.

Novel methods for the preparation of 7:11-diketoergost--22-en-3β-yl acetate have been developed which offer an alternative route to cortisone from ergosterol since the diketone has been converted into 11:20-diketo-allopregnan--3β-yl acetate (64), which has been converted into cortisone (64, 65).

---oOo---

EXPERIMENTAL

Melting points are corrected.

Specific rotations were determined in chloroform solutions (unless otherwise stated) in a 1-dm. tube at room temperature.

Ultra-violet absorption spectra were measured in absolute ethanol solution (except where otherwise stated) with a Unicam SP. 500 spectrophotometer.

Micro-analyses were by Dr. A.C. Syme and Mr. Wm. McCorkindale, to whom grateful acknowladgements are due.

For chromatography, activated alumina (supplied by Savory and Moore), Grade II (except where stated) standardised according to Brockmann, was employed.

"Working up in the usual manner" means addition of water, extraction with ether, washing of the extract with sodium hydrogen carbonate solution, and water, drying ($MgSO_4$), and evaporation under reduced pressure.

Ergosteryl Acetate.

A solution of ergosterol (100 g.) in pyridine (600 c.c.) and acetic anhydride (100 c.c.) was kept in the dark for 18 hours at room temperature. The product, which had crystallised, was collected, washed with methanol and recrystallised from chloroform-methanol to give ergosteryl acetate as lustrous leaves, m.p.173-175°, $[\alpha]_D$ -93° (c, 2.0).

5-Dihydroergosteryl Acetate.

A solution of ergosteryl acetate (35 g.) in benzene (300 c.c.; Analar) was treated with a suspension of Raney nickel sludge (Org.Synth.,29,25) (W6; 15-20 c.c.) in benzene (50 c.c.), and the mixture shaken at 17° with hydrogen under slight positive pressure until the total absorption was 2140 c.c.(calc., 1900 c.c.) (time: 13-20 minutes), of which, according to a blank experiment, 150 c.c. were absorbed by the solvent. The filtered reaction solutions from five such experiments were combined, and the solvent was removed under reduced pressure to yield a crystalline residue, m.p.172-174°, which gave a yellow colour with tetranitromethane in chloroform. Crystallisation from chloroform-methanol gave 5-dihydroergosteryl acetate (93 g.) as lustrous plates, m.p.178--181°, $[\alpha]_D$ -20° (c, 2.0), showing no high-intensity

absorption above 2200 Å. [A further quantity (70 g.) (total yield, 93%), m.p.177-179°, $[\alpha]_D$ -18° (c, 1.8)]. Recrystallisation of the product from chloroform-methanol gave plates, m.p.180-182°, $[\alpha]_D$ -20.5° (c, 2.1) (Found: C,81.7; H,11.0. Calc. for $C_{30}H_{48}O_2$: C,81.8; H,11.0%).

Ergosteryl-D Acetate.

A solution of 5-dihydroergosteryl acetate (50 g.) in chloroform (700 c.c.) was treated with a solution of mercuric acetate (87 g., 1.2 mols) in stabilised glacial acetic acid (1.3 l.) added in one portion, and the mixture shaken for 6 hours at room temperature. After standing for 16 hours the precipitated mercurous acetate (63 g.) was removed by filtration and the filtrate concentrated under reduced pressure below 50° to a volume of 400 c.c., and cooled. The crystallised solid was collected, washed with cold methanol and dried (42 g.). Crystallisation from chloroform-methanol gave ergosteryl-D acetate as blades, m.p.169-172°, $[\alpha]_D$ +19° (c, 2.0). After four recrystallisations from chloroform-methanol the product (15 g.) was obtained as large blades, m.p.175-176°, $[\alpha]_D$ +30° (c, 1.8) (Found: C,82.1; H,10.6. Calc. for $C_{30}H_{46}O_2$: C,82.1; H,10.6%). Light absorption: Maxima at 2350 (ε = 17,000) and 2420 Å (ε = 18,300), and an inflection at 2510 Å (ε = 13,000). It gives a brown

colour with tetranitromethane in chloroform.

The crystalline solid collected from the mother-liquors (m.p.164-169°, $[\alpha]_D$ +7°) is suitable for similar re-treatment with mercuric acetate to yield ergosteryl-D acetate.

Ergosterol-D.

Hydrolysis of ergosteryl-D acetate with methanolic potassium hydroxide (3%) in the usual way gave ergosterol-D as felted needles from methanol-chloroform, m.p.165-167°, $[\alpha]_D$ +30° (c, 1.7). Light absorption: Maxima at 2350 (ε = 16,500) and 2420 Å (ε = 18,000), and an inflection at 2510 Å (ε = 12,500).

Acetylation of the alcohol using pyridine and acetic anhydride (at room temperature for 16 hours) gave ergosteryl-D acetate as large blades from methanol-chloroform, m.p.176°, $[\alpha]_D$ +31° (c, 1.5). Light absorption: Maxima at 2350 (ε = 17,000) and 2420 Å (ε = 18,300) with an inflection at 2510 Å (ε = 13,000).

Ergosteryl-D Benzoate.

A solution of ergosterol-D in pyridine and benzoyl chloride was heated at 100° for 2 hours. Crystallisation of the product from chloroform-methanol gave ergosteryl-D benzoate as blades, m.p.177-178°, $[\alpha]_D$ +31° (c, 1.9)

(Found: C,84.0; H,9.8. $C_{35}H_{46}O_2$ requires C,83.95; H,9.7%).

Oxidation of Ergosteryl-D Acetate with Chromic Acid.

(a) 3β-Acetoxyergosta-9(11):22-dien-7-one.

A solution of ergosteryl-D acetate (2.19 g.) in benzene (20 c.c.) and stabilised acetic acid (150 c.c.) was kept at 50° and treated with a solution of chromium trioxide in glacial acetic acid (31 c.c., 1.044N, equiv. to 3 atoms of O) added during 1 hour with stirring. After 1 hour's stirring at 50° the mixture was concentrated under reduced pressure to 20 c.c. and diluted with water. The precipitated solid was isolated by means of ether, in the usual way. Removal of the ether gave a pale yellow semicrystalline solid which crystallised from methanol as plates (0.63 g.). Two further crystallisations from methanol gave 3β-acetoxyergosta-9(11):22-dien-7-one as plates, m.p.174-175°, $[\alpha]_D$ -54° (c, 1.2) (Found: C,79.1; H,10.3. $C_{30}H_{46}O_3$ requires C,79.2; H,10.2%). It gives a yellow colour with tetranitromethane in chloroform and does not show selective absorption of high intensity above 2200 Å.

3β-Acetoxyergosta-8:22-dien-7-one.

A solution of this compound (0.37 g.) in light petroleum (b.p.60-80°)-benzene (5:1; 50 c.c.) was chromatographed on a column (12 x 2 cm.) of alumina (Grade II). Elution with light petroleum-benzene (1:2; 400 c.c.) and benzene (500 c.c.) gave a solid, m.p. ca.192-204° (320 mg.), which crystallised from methanol to give 3β--acetoxyergosta-8:22-dien-7-one as plates, m.p.208-211°, $[\alpha]_D$ -56° (c, 1.5), undepressed in m.p. when mixed with an authentic specimen, m.p.210-212° (Found: C,79.4; H,10.5. Calc. for $C_{30}H_{46}O_3$: C,79.2; H,10.2%). Light absorption: Maximum at 2520 Å (ε = 10,100). It gives a light yellow colour with tetranitromethane in chloroform.

A second crop (0.36 g.) combined with the residue (1.1 g.) from the methanol mother-liquors was dissolved in light petroleum-benzene (3:1; 50 c.c.) and chromatographed on a column of alumina (15 x 2.5 cm.).

Fr.	Solvent	Vol.	Wt.	Residue	m.p.
1.	l.petrol-benzene(3:1)	300 c.c.	45 mg.	yellow crystals	ca.140°
2.	" (1:1)	400	100	"	"
3.	" (1:2)	300	65	"	"
4.	benzene	600	143	"	ca.165-185
5.	benzene-ether(2:1)	200	42	"	160-178
6.	" (1:1)	200	110	"	150-170
7.	ether	200	30	yellow gum	
8.	ether-methanol(20:1)	200	500	"	
9.	methanol	200	80	"	

Fractions 1-3 crystallised from methanol to give a compound as prismatic needles, m.p.127-128.5°, $[\alpha]_D$ -30° (c, 0.9) (Found: C,75.15; H,9.3. $C_{30}H_{44}O_4$ requires C,76.9; H,9.5%; $C_{30}H_{44}O_5$ requires C,74.3; H,9.15%). Light absorption: Maximum at 2680 Å (ε = 4,700). It gives a yellow colour with tetranitromethane in chloroform.

Fractions 4 and 5 crystallised from methanol to give 3β-acetoxyergosta-8:22-dien-7-one as plates, m.p.201-206°, $[\alpha]_D$ -53° (c, 0.5) undepressed in m.p. on admixture with the specimen described above. Light absorption: Maximum at 2540 Å (ε = 9,100).

Later fractions did not give homogeneous material.

(b) Ergosteryl-D acetate (2.1 g.) suspended in stabilised glacial acetic acid (200 c.c.) was stirred at 15° and treated dropwise during 1 hour with a solution of chromium trioxide in acetic acid (30 c.c.; 1.044 N) containing sulphuric acid (2.5 c.c.; d,1.84). After a second hour's stirring the reaction mixture was treated as described under (a). Four crystallisations of the neutral fraction (0.7 g.) from methanol gave a compound, m.p.190-194°, undepressed on admixture with 3β-acetoxyergosta-8:22-dien-7-one, m.p.208-210°. Light absorption: Maximum at 2520 Å (ε = 2,200). Chromatography of this product on

alumina gave 3β-acetoxyergosta-8:22-dien-7-one as plates from methanol, m.p.208-210°, $[\alpha]_D$ -54° (c, 1.1). Light absorption: Maximum at 2520 Å (ε = 10,100).

The methanolic mother-liquors were combined, evaporated and the residue (1.0 g.) dissolved in light petroleum-benzene (1:1; 30 c.c.) was chromatographed on a column of alumina (12 x 2 cm.).

Fr.	Solvent	Vol.	Wt.	Residue	m.p.
1.	l.petrol-benzene(1:1)	400 c.c.	88 mg.	yellow crystals	90-100°
2.	" (1:2)	100	32	"	"
3.	benzene	400	150	"	150-180
4.	benzene-ether(5:1)	300	60	yellow gum	
5.	" (1:1)	100	10	"	
6.	ether	100	16	"	
7.	methanol	200	250	"	

Fractions 1 and 2 crystallised from methanol to give the compound $C_{30}H_{44}O_4$ as yellow prismatic needles, m.p.123-125°, $[\alpha]_D$ -32° (c, 0.4), undepressed in m.p. when mixed with a specimen described under (a). Light absorption: Maximum at 2680 Å (ε = 4,300).

Fraction 3 gave 3β-acetoxyergosta-8:22-dien-7-one as plates from methanol, m.p.203-205°, $[\alpha]_D$ -53° (c, 0.6) showing no depression of m.p. when mixed with the specimen described above. Light absorption: Maximum at 2520 Å (ε = 10,000).

Later fractions from the chromatogram did not crystallise.

3β-Hydroxyergosta-8:22-dien-7-one.

(a) Hydrolysis of 3β-acetoxyergosta-8:22-dien-7-one using methanolic potassium hydroxide (2%) in the usual way gave 3β-hydroxyergosta-8:22-dien-7-one which separated from methanol as plates, m.p.176-178°, $[\alpha]_D$ -44° (c, 0.9) (Found: C,78.0; H,11.1. $C_{28}H_{44}O_2.CH_3OH$ requires C,78.3; H,10.9%). Light absorption: Maximum at 2520 Å (ε = 11,000).

(b) Similar hydrolysis of 3β-acetoxyergosta-9(11):22-dien-7-one gave 3β-hydroxyergosta-8:22-dien-7-one as plates from methanol, m.p.175-177°, $[\alpha]_D$ -42° (c, 0.8), undepressed in m.p. when mixed with the specimen described above. Light absorption: Maximum at 2520 Å (ε = 11,000).

Oxidation of Ergosteryl-D Acetate with Performic Acid.

(a) One mol.

3β-Acetoxy-8α-ergosta-9(11):22-dien-7-one.

A mixture of ergosteryl-D acetate (2.2 g.) in benzene (20 c.c.), formic acid (20 c.c.; 90%), and hydrogen peroxide (0.65 c.c.; 30%) was stirred for 20 hours at room temperature. The reaction mixture was evaporated under reduced pressure below 50° (bath temp.) and the residue crystallised from methanol, to give 3β-acetoxy--8α-ergosta-9(11):22-dien-7-one (930 mg.) as small needles, m.p.196-198°, $[\alpha]_D$ +20°, +18° (c, 0.5, 1.0) (Found: C,78.8; H,10.2. $C_{30}H_{46}O_3$ requires C,79.2; H,10.2%). It gives a pale yellow colour with tetranitromethane in chloroform and does not show selective absorption of high intensity above 2200 Å. Repeated crystallisation from methanol did not appreciably alter the m.p. but caused the appearance of high intensity absorption at 2540 Å. Infra-red light absorption: Maxima at 1740 cm.$^{-1}$ (acetoxy group) and at 1715 cm.$^{-1}$ (nonconjugated ketone group).

3β-Hydroxyergosta-8:22-dien-7-one.

3β-Acetoxy-8α-ergosta-9(11):22-dien-7-one (250 mg.) was heated under reflux with aqueous methanolic potassium hydroxide (15 c.c.; 3%) for 2 hours. Isolation of the product by means of ether, followed by crystallisation

from methanol, gave 3β-hydroxyergosta-8:22-dien-7-one (150 mg.) as plates, m.p.178-180°, $[\alpha]_D$ -43° (c, 1.3) (Found: C,77.9; H,10.8. Calc. for $C_{28}H_{44}O_2.CH_3OH$: C,78.3; H,10.9%). Light absorption: Maximum at 2540Å (ε = 10,000).

3β-Acetoxyergosta-8:22-dien-7-one.

Acetylation of 3β-hydroxyergosta-8:22-dien-7-one using pyridine and acetic anhydride at 100° (1 hour), gave 3β-acetoxyergosta-8:22-dien-7-one as plates from methanol, m.p.209-211°, $[\alpha]_D$ -54° (c, 1.5) (Found: C,79.1; H,10.2. Calc. for $C_{30}H_{46}O_3$: C,79.2; H,10.2%). Light absorption: Maximum at 2540 Å (ε = 10,100).

(b) Two mols.

3β-Acetoxy-9α:11α-epoxyergost-22-en-7-one.

Ergosteryl-D acetate (2.2 g.) in benzene (20 c.c.) was stirred with a mixture of formic acid (20 c.c.; 90%) and hydrogen peroxide (1.2 c.c.; 30%) for 20 hours at 15°. The reaction mixture was evaporated under reduced pressure below 50°. Crystallisation of the residue from methanol gave 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one (360 mg.) as needles (which formed slowly from an initial gel), m.p.220-223°, $[\alpha]_D$ -85°, -87° (c, 0.5, 1.0) (Found: C,76.2; H,9.8. $C_{30}H_{46}O_4$ requires C,76.55; H,9.85%). The

compound gives a pale yellow colour with tetranitromethane in chloroform and does not exhibit high-intensity absorption above 2200 Å.

3β:11α-Dihydroxyergosta-8:22-dien-7-one.

3β-Acetoxy-9α:11α-epoxyergost-22-en-7-one (95 mg.) was heated under reflux for 1 hour in aqueous methanolic potassium hydroxide (6 c.c.; 3%). The product was isolated by means of ether and crystallised from acetone, to give 3β:11α-dihydroxyergosta-8:22-dien-7-one (60 mg.) as needles, m.p.214-215°, [α] -6° (c, 1.5) (Found: C,78.45; H,10.35. $C_{28}H_{44}O_3$ requires C,78.75; H,10.7%). Light absorption: Maximum at 2540 Å (ε = 8100). It gives a faint yellow colour with tetranitromethane.

3β:11α-Diacetoxyergosta-8:22-dien-7-one.

Acetylation of 3β:11α-dihydroxyergosta-8:22-dien-7-one using pyridine and acetic anhydride at 100° for 1 hour gave 3β:11α-diacetoxyergosta-8:22-dien-7-one which separated from methanol as needles, m.p.175-177°, $[\alpha]_D$ +13° (1.3) (Found: C,74.8; H,9.7. $C_{32}H_{48}O_5$ requires C,75.0; H,9.4%). Light absorption: Maximum at 2520 Å (ε = 10,400). It gives a light yellow colour with chloroformic tetranitromethane.

3β-Acetoxy-11α-hydroxyergosta-8:22-dien-7-one.

A solution of 3β-acetoxy-9α:11α-epoxyergost-22-en--7-one (200 mg.) in benzene (20 c.c.) was filtered through a column of alumina (12 x 1.5 cm.), and the column washed with the same solvent. The crystalline solid present in the benzene fraction (400 c.c.) and the benzene-ether fraction (1:1; 400 c.c.) was negligible. Evaporation of the ether-methanol fraction (10:1; 100 c.c.) gave a solid which after three crystallisations from aqueous methanol gave 3β-acetoxy-11α-hydroxyergosta--8:22-dien-7-one as felted needles, m.p.187-190° (sintering at 170°), $[\alpha]_D$ -29° (c, 0.8) (Found: C,76.3; H,10.1. $C_{30}H_{46}O_4$ requires C,76.55; H,9.85%). Light absorption: Maximum at 2540 Å (ε = 8000). It gives a light yellow colour with tetranitromethane in chloroform.

Acetylation of 3β-acetoxy-11α-hydroxyergosta-8:22--dien-7-one using pyridine and acetic anhydride gave 3β:11α-diacetoxyergosta-8:22-dien-7-one as needles from methanol, m.p.174-176°, $[\alpha]_D$ +12° (c, 1.1), undepressed in m.p. when mixed with the specimen described above. Light absorption: Maximum at 2520 Å (ε = 10,300).

7:11-Diketoergost-22-en-3β-yl Acetate.(with R.C.Anderson).

A solution of 3β:11α-diacetoxyergosta-8:22-dien-7-one

(140 mg.) in ethanol (66 c.c.) was treated with 50% aqueous potassium hydroxide (24 c.c.) and the mixture refluxed for 14 hours. The product isolated by means of ether and dried by evaporation of its solution in benzene, was acetylated in pyridine and acetic anhydride. The acetylated product was isolated by means of ether and its solution in benzene (25 c.c.) was chromatographed on Grade II-III alumina (8 x 1.25 cm.), and the column washed with benzene. Evaporation of the first fraction (270 c.c.) gave a crystalline solid (20 mg.), m.p.155-170°. The next fraction (200 c.c.) yielded a solid (15 mg.) which after two crystallisations from methanol gave 7:11--diketoergost-22-en-3β-yl acetate as small prismatic needles, m.p.196-198°, $[\alpha]_D$ -25° (c, 0.5) (Found: C,76.4; H,9.9. Calc. for $C_{30}H_{46}O_4$: C,76.55; H,9.85%). The compound did not show high-intensity absorption above 2200 Å. It was undepressed in m.p. when mixed with an authentic specimen prepared as described later (cf. 29,36).

Oxidation of Ergosteryl-D Acetate with Perbenzoic-Formic Acid.

A solution of ergosteryl-D acetate (1.9 g.) in dry chloroform (5 c.c.) and formic acid (5 c.c.; 98%) was treated dropwise with a solution of perbenzoic acid

(3 atoms of oxygen) in chloroform (10 c.c.) over 15 minutes with stirring while the flask was cooled in water. The homogeneous solution was kept at room temperature for 18 hours. The solvents were evaporated to dryness under reduced pressure below 50°.

Hydrolysis of the residue using methanolic potassium hydroxide (50 c.c.; 5%) and isolation by means of ether, gave froth (1.5 g.) which was dried in vacuum.

Acetylation of the product using pyridine (10 c.c.) and acetic anhydride (10 c.c.) gave a compound (200 mg.) which crystallised from methanol in prismatic needles, m.p.196-198°, $[\alpha]_D$ -4° (c, 2.3) (Found: C,73.0; H,9.1. $C_{33}H_{48}O_6$ requires C,72.7; H,9.2%). Light absorption: Maximum at 2520 Å (ϵ = 9700). It does not show a colour with tetranitromethane in chloroform.

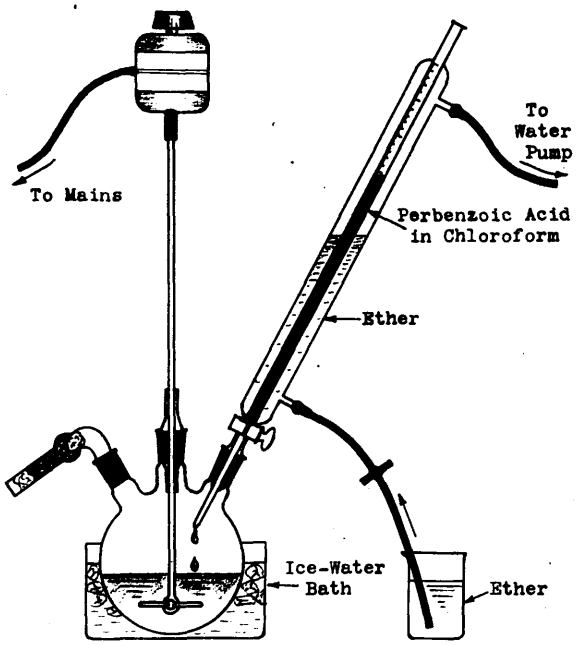
Oxidation of Ergosteryl-D Acetate with Perbenzoic Acid.

(a) One mol.

9α:11α-Epoxyergosta-7:22-dien-3β-yl Acetate.

A solution of ergosteryl-D acetate (1.0 g.) in chloroform (10 c.c.) was treated with perbenzoic acid (1.2 mols.) in chloroform (10 c.c.) added dropwise with stirring during 2 hours at -3°. The mixture was kept

[A convenient device for controlling the rate of oxidation and cooling the perbenzoic acid-solution is shown below:]



CONTROLLED OXIDATION WITH PERBENZOIC ACID.

at 0° for 20 hours. The solid residue, obtained by removal of the solvent at room temperature under reduced pressure, was dissolved in the minimum volume of boiling acetone. On cooling, the solution deposited 9α:11α--epoxyergosta-7:22-dien-3β-yl acetate (0.7 g.) as hexagonal plates, m.p.205-207°, which after two recrystallisations from the same solvent had m.p.211-213°, $[\alpha]_D$ -38° (c, 2.2) (Found: C,79.2; H,10.2. Calc. for $C_{30}H_{46}O_3$: C,79.2; H,10.2%). It gives a yellow colour with tetranitromethane in chloroform and does not show selective absorption of high intensity above 2200 Å.

Chamberlin *et al.* (29) report m.p.202-205°, $[\alpha]_D$ -35°, Heusser *et al.* (36) report m.p.205-207°, $[\alpha]_D$ -39.5°.

9α:11α-Epoxyergosta-7:22-dien-3β-ol.

A solution of 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate (500 mg.) in aqueous methanolic potassium hydroxide (60 c.c.; 2%) was heated under reflux for 2 hours. The solid (450 mg.) which separated on cooling was washed with water and twice crystallised from methanol to give 9α:11α--epoxyergosta-7:22-dien-3β-ol as flat needles, m.p.187--189°, $[\alpha]_D$ -41° (c, 1.3) (Found: C,78.5; H,10.8. $C_{28}H_{44}O_2.CH_3OH$ requires C,78.3; H,10.9%). It gives a yellow colour with tetranitromethane in chloroform and does not show high intensity light absorption above 2200 Å.

Acetylation of 9α:11α-epoxyergosta-7:22-dien-3β-ol was effected by heating on the steam bath for 1 hour with pyridine and acetic anhydride. The solid which separated on dilution with water was crystallised from acetone to give 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate as plates, m.p.210-212°, $[\alpha]_D$ -37° (c, 1.2), undepressed in m.p. when mixed with an authentic specimen (Found: C,79.3; H,10.3. Calc. for $C_{30}H_{46}O_3$: C,79.2; H,10.2%). It does not exhibit selective absorption of high intensity above 2200 Å.

(b) Two mols.

Ergosteryl-D acetate (1.0 g.) was treated with perbenzoic acid (2.2 mols.) exactly as described above. The residue crystallised from acetone in plates (270 mg.), m.p.190-200°. Recrystallisation from acetone gave 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate as plates, m.p.203-206°, undepressed on admixture with the specimen described above; $[\alpha]_D$ -38° (c, 1.1) (Found: C,79.5; H,10.3%).

The acetone mother-liquor did not give homogeneous material.

(c) Three mols.

(i) Ergosteryl-D acetate (2.19 g.) was treated with perbenzoic acid (3.5 mols.) as before. The reaction

mixture was shaken with sodium hydrogen carbonate solution and water, and dried. Evaporation of the solvent under reduced pressure gave a residue, which crystallised from methanol in plates (1.1 g.), m.p.190--195°. Three recrystallisations from the same solvent gave a compound as elongated plates, m.p.212-214°, $[\alpha]_D$-7° (c, 1.2) (Found: C,73.7; H,9.5. $C_{30}H_{46}O_5$ requires C,74.0; H,9.5%). The compound does not give a colour with tetranitromethane in chloroform and does not show selective absorption of high intensity above 2000 Å.

(ii) Similar treatment of 9α:11α-epoxyergosta-7:22--dien-3β-yl acetate (400 mg.) with perbenzoic acid (2.5 mols.) gave plates from methanol, m.p.209-211° (220 mg.), $[\alpha]_D$ -9° (c, 1.0), undepressed in m.p. when mixed with the specimen described above.

(iii) Treatment of 7ξ:8ξ,9α:11α-diepoxyergost-22--en-3β-yl acetate (200 mg.; described later) with perbenzoic acid (1.5 mols.) gave plates from methanol, m.p. 212-214° (160 mg.), $[\alpha]_D$ -6° (c, 1.2) (Found: C,73.9; H,9.5%), undepressed in m.p. when mixed with the specimen described above. It does not exhibit light absorption of high intensity above 2000 Å.

Hydrolysis of the acetate was effected by heating its solution in methanolic potassium hydroxide (4%) for

4 hours. Isolation by means of ether gave a product which crystallised from aqueous methanol in plates, m.p. 198-201°. Three recrystallisations from the same solvent gave plates, m.p.211-214°, $[\alpha]_D$ -10° (c, 1.5) (Found: C,73.1; H,10.4. $C_{28}H_{44}O_4.CH_3OH$ requires C,73.1; H,10.15%). It does not exhibit selective absorption of high intensity above 2000 Å.

Acetylation of the alcohol using pyridine and acetic anhydride gave the acetate as elongated plates from methanol, m.p.212-214°, $[\alpha]_D$ -8° (c, 1.0) undepressed in m.p. when mixed with the specimen described above (Found: C,73.9; H,9.5. $C_{30}H_{46}O_5$ requires C,74.0; H,9.5%). It does not give a colour with tetranitromethane in chloroform and does not show selective absorption of high intensity above 2000 Å.

3β-Hydroxyergosta-8:22-dien-7-one.

A solution of 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate (150 mg.) in aqueous methanolic hydrogen chloride (10 c.c.; 0.7%) was refluxed for 2 hours. The solution was concentrated and the solid (85 mg.) which separated on cooling crystallised thrice from methanol, to give 3β-hydroxyergosta-8:22-dien-7-one as plates, m.p.175-177°, undepressed with the specimen described above, $[\alpha]_D$ -45° (c, 0.5) (Found: C,78.5; H,11.1. Calc. for $C_{28}H_{44}O_2.CH_3OH$

C,78.3; H,10.9%). Light absorption: Maximum at 2540 Å (ε = 10,700). The alcohol gives a yellow colour with tetranitromethane in chloroform.

The same alcohol was obtained using aqueous methanolic sulphuric acid.

Acetylation of the alcohol with pyridine and acetic anhydride gave 3β-acetoxyergosta-8:22-dien-7-one which separated from methanol as plates, m.p.208-210°, $[\alpha]_D$ -55° (c, 1.1) (Found: C,79.0; H,10.3. Calc. for $C_{30}H_{46}O_3$: C,79.2; H,10.2%). Light absorption: Maximum at 2540 Å (ε = 10,000).

3β-Acetoxyergosta-8:22-dien-11-one.

Following the method described by Heusser et al.(36), a solution of 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate (1.0 g.) in dry benzene (30 c.c.) was treated with redistilled boron trifluoride-ether complex (10 drops) and the solution kept at room temperature for 3 days. The solution was diluted with ether, washed successively with water, sodium hydrogen carbonate solution, and water, and dried (Na_2SO_4). The solvents were removed under reduced pressure; the residue separated from methanol in flat needles (0.7 g.), m.p.120-123°. A solution of the solid in benzene (20 c.c.) was filtered through a short column

of activated alumina, and the column washed with the same solvent. Evaporation of the filtrate gave 3β-acetoxy-ergosta-8:22-dien-11-one which separated from methanol in blades, m.p.129-131°, $[\alpha]_D$ +105° (c, 1.7) (Found: C,79.2; H,10.2. Calc. for $C_{30}H_{46}O_3$: C,79.2; H,10.2%). Light absorption: Maximum at 2540 Å (ε = 9,600). It gives a pale yellow colour with tetranitromethane in chloroform.

Heusser et al. (loc.cit.) give m.p.122-123°, $[\alpha]_D$+92°.

7ξ:11α-Dihydroxyergosta-8:22-dien-3β-yl Acetate.

Following the method described by Heusser et al. (loc.cit.) a solution of 9α:11α-epoxyergosta-7:22-dien--3β-yl acetate (400 mg.) in dioxan (300 c.c.) was treated with sulphuric acid (55 c c.; 2N) added in one portion with shaking at room temperature. After 3 minutes the solution was poured into sodium hydrogen carbonate solution. The precipitate was extracted with ether and the ethereal solution washed with water till neutral. Removal of the ether gave a residue which separated from acetone in needles, m.p.230-232° (300 mg.). Recrystallisation from methanol gave 7ξ:11α-dihydroxyergosta-8:22--dien-3β-yl acetate as prismatic needles, m.p.250-252°, $[\alpha]_D$ +85° (c, 0.4) (Found: C,76.4; H,10.4. Calc. for

$C_{30}H_{48}O_4$: C,76.2; H,10.2%). It does not show selective absorption of high intensity above 2200 Å.

Chamberlin et al. (loc.cit.) give m.p.248-252°, $[\alpha]_D$ +85°, and Heusser et al. (loc.cit.) give m.p.270-272°, $[\alpha]_D$ +82°.

3β:7ξ:11α-Triacetoxyergosta-8:22-diene.

Acetylation of 7ξ:11α-dihydroxyergosta-8:22-dien-3β-yl acetate on the steam bath for 1 hour with acetic anhydride and pyridine gave 3β:7ξ:11α-triacetoxyergosta-8:22-diene which separated from methanol as prismatic needles, m.p.172-173°, $[\alpha]_D$ +90° (Found: C,73.0; H,9.4. Calc. for $C_{34}H_{52}O_6$: C,73.3; H,9.4%). It gives a pale yellow colour with tetranitromethane in chloroform and does not exhibit selective absorption of high intensity above 2200 Å.

Oxidation of 7ξ:11α-Dihydroxyergosta-8:22-dien-3β-yl Acetate with Chromic Acid.

(a) 8α:9α-Epoxy-7:11-diketoergost-22-en-3β-yl Acetate.

A suspension of 7ξ:11α-dihydroxyergosta-8:22-dien-3β-yl acetate (800 mg.) in stabilised glacial acetic acid (100 c.c.) was treated with a solution of chromium trioxide in acetic acid (3.1 atoms of O), and 2N sulphuric acid (1 c.c.) added in one portion. After shaking for

5 minutes at room temperature the solution was complete. It was kept overnight at room temperature. Water was added and the reaction mixture worked up using ether. The residue, after removal of the ether, crystallised from aqueous methanol in fine needles (300 mg.), m.p.117--120°. Two recrystallisations from aqueous methanol gave 8α:9α-epoxy-7:11-diketoergosta-22-en-3β-yl acetate as needles, m.p.130-132°, $[\alpha]_D$ -60° (c, 0.8) (Found: C,74.3; H,9.1. Calc. for $C_{30}H_{44}O_5$: C,74.3; H,9.15%). It gives a yellow colour with tetranitromethane in chloroform and does not show selective light absorption of high intensity above 2200 Å.

(b) 7:11-Diketoergosta-8:22-dien-3β-yl Acetate.

The mother-liquors from experiment (a) were evaporated to dryness, the residue dissolved in light petroleum--benzene (10 c.c., 4:1) and the solution filtered through a column of activated alumina (1.5 x 10 cm.). Evaporation of the solvents (150 c.c.) gave a solid, which crystallised from aqueous acetone to give 7:11-diketoergosta-8:22--dien-3β-yl acetate (240 mg.) as pale yellow, flat needles, m.p.133-135°, $[\alpha]_D$ +22° (c, 1.0) (Found: C,76.7; H,9.3. Calc. for $C_{30}H_{44}O_4$: C,76.9; H,9.5%). Light absorption: Maximum at 2700 Å (ε = 8,400).

7:11-Diketo-8α-ergost-22-en-3β-yl Acetate.

(a) A solution of 7:11-diketoergosta-8:22-dien-3β-yl acetate (200 mg.) in ether-methanol (1:1; 250 c.c.) was heated under reflux with zinc dust (2 g.) added portion-wise during 3 hours. The mixture was filtered and the solution slightly concentrated, when crystalline, small hexagonal plates separated. The solution was cooled, the solid collected, washed with methanol and dried (150 mg.; m.p.200-204°). Two recrystallisations from acetone gave 7:11-diketo-8α-ergost-22-en-3β-yl acetate as hexagonal plates, m.p.204-206°, $[\alpha]_D$ +30°, +27° (c, 0.6, 0.4; sparingly soluble in chloroform) (Found: C,76.6; H,9.9. $C_{30}H_{46}O_4$ requires C,76.55; H,9.85%). It does not show light absorption of high intensity above 2200 Å and gives a faint yellow colour with tetranitromethane in chloroform. A mixture with 7:11-diketoergost--22-en-3β-yl acetate (m.p.197-198°, $[\alpha]_D$ -28°) had m.p. 178-198°.

(b) Similar treatment of 8α:9α-epoxy-7:11-diketo-ergost-22-en-3β-yl acetate (300 mg.) in ether-methanol (1:1; 400 c.c.) with zinc dust (3 g.) gave 7:11-diketo--8α-ergost-22-en-3β-yl acetate (200 mg.) as hexagonal plates from acetone, m.p.203-205°, $[\alpha]_D$ +25° (c, 0.5) undepressed in m.p. when mixed with the specimen described under (a).

7:11-Diketoergost-22-en-3β-yl Acetate.

(a) 7:11-Diketo-8α-ergost-22-en-3β-yl acetate (80 mg.) in glacial acetic acid (3 c.c.) was heated on the steam bath for 45 minutes. The solution was diluted with water and extracted with ether. Removal of ether gave a solid, which was crystallised from methanol to yield 7:11-diketoergost-22-en-3β-yl acetate (70 mg.) as small, prismatic needles, m.p.196-198°, $[\alpha]_D$ -28°, -30° (c, 1.0, 1.2) (Found: C,76.7; H,10.0. Calc. for $C_{30}H_{46}O_4$: C,76.55; H,9.85%). The diketone does not show high intensity light absorption above 2200 Å. It is undepressed in m.p. when mixed with the specimens prepared as described under (b) and (c).

(b) A solution of 7:11-diketoergosta-8:22-dien-3β-yl acetate (300 mg.) in glacial acetic acid (25 c.c.) was heated on the steam bath for 3 hours with zinc dust (3 g.) added portionwise and then boiled under reflux for 15 minutes. Isolation of the product with ether gave 7:11-diketoergost-22-en-3β-yl acetate (250 mg.) as needles from methanol, m.p.197-198°, $[\alpha]_D$ -29° (c,0.8) (Found: C,76.5; H,9.9. Calc. for $C_{30}H_{46}O_4$: C,76.55; H,9.85%). It is undepressed in m.p. when mixed with the specimen described under (a). It gives a faint yellow colour with tetranitromethane in chloroform and does not

show high intensity light absorption above 2200 Å.

(c) Similar reduction of 8α:9α-epoxy-7:11-diketo-ergost-22-en-3β-yl acetate (400 mg.) with zinc dust and acetic acid gave a product isolated by means of ether. A solution of this product in benzene (20 c.c.) was filtered through a short column of alumina (5 x 1 c.c.) and the column washed with benzene (100 c.c.). Evaporation of the benzene filtrate gave 7:11-diketoergost-22--en-3β-yl acetate (300 mg.) as small prismatic needles from methanol, m.p.198-200°, $[\alpha]_D$ -30° (c, 1.3) undepressed in m.p. when mixed with the specimens described under (a) and (b).

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3β-Acetoxy-9α:11α-dihydroxyergost-22-en-7-one.

(a) A solution of 9α:11α-epoxyergosta-7:22-dien--3β-yl acetate (1.0 g.) in dry chloroform (10 c.c.) was treated dropwise during 15 minutes at 0° with a solution of bromine (1 mol.) in chloroform (5 c.c.) with stirring. A solution of perbenzoic acid (1.5 mols.) in chloroform (20 c.c.) was then added during 1 hour at -5° and the mixture kept for 2 days at 0°. The mixture was evaporated to dryness under reduced pressure at room temperature and the residue dissolved in glacial acetic acid (25 c.c.) and treated with zinc dust (10 g.) added in portions during 5 hours with stirring on the steam bath. The debrominated product was isolated by means of ether; it formed a crystalline solid which was recrystallised from acetone to give plates (250 mg.), m.p.198-205°, undepressed on admixture with 3β-acetoxyergosta-8:22-dien-7-one.

The acetone mother-liquors were evaporated and a solution of the residue (0.80 g.) in ethanol (20 c.c.) treated with glacial acetic acid (0.5 c.c.) and Girard's reagent T (0.5 g.). The mixture was heated under reflux for 90 minutes, cooled, and diluted with water (20 c.c.) containing crushed ice, and the pH was adjusted to 5.5-6 by sodium carbonate solution. The mixture was extracted with ether (2 x 20 c.c.) (Extract A). The pH of the

aqueous layer was adjusted to 2-3 by hydrochloric acid, the mixture extracted with ether (2 x 20 c.c.) and the extract washed with 5% sodium carbonate solution and then water, and dried (Na_2SO_4). Removal of the ether gave a solid (80 mg.) which after three crystallisations from methanol gave 3β-acetoxy-9α:11α-dihydroxyergost-22--en-7-one as rectangular plates, m.p.260-262°, $[\alpha]_D$ -66° (c, 1.3) (Found: C,74.0; H,10.0. $C_{30}H_{48}O_5$ requires C,73.7; H,9.9%). The compound gives a pale yellow colour with tetranitromethane in chloroform and does not show high-intensity absorption above 2200 Å.

Evaporation of extract A followed by crystallisation from methanol gave plates, m.p.200-210°, undepressed when mixed with 3β-acetoxyergosta-8:22-dien-7-one. (Light absorption: Maxima at 2440 Å, ε = 6,000 and at 2520 Å, ε = 6,300).

(b) Oxidation of 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate (1.0 g.) was effected as described above, with the difference that the reaction time with perbenzoic acid was 7 days. The debrominated product was crystallised from acetone to give plates, m.p.190-210°. This solid (160 mg.) in benzene (25 c.c.) was chromatographed on a column of Grade II alumina (12 x 1.5 cm.), and the column washed with benzene (500 c.c.) which gave 3β-acetoxy-

ergosta-8:22-dien-7-one (26 mg.) as plates, m.p.203-207°, from methanol. Light absorption: Maximum at 2540 Å (ϵ = 9,100).

Further elution of the column with benzene containing 1% of methanol (160 c.c.) gave a solid (115 mg.) which after two crystallisations from methanol gave 3β-acetoxy--9α:11α-dihydroxyergost-22-en-7-one as rectangular plates, m.p.261-263°, $[\alpha]_D$ -69° (c, 1.1), undepressed in m.p. when mixed with the specimen described above (Found: C,73.5; H,9.95%). The compound does not show high--intensity absorption above 2200 Å and gives a light yellow colour with tetranitromethane in chloroform.

The acetone mother-liquor was evaporated and chromatographed as above, to give 3β-acetoxyergosta-8:22-dien--7-one (60 mg.) together with 3β-acetoxy-9α:11α-dihydroxy-ergost-22-en-7-one which separated from methanol as plates, m.p.257-260°, undepressed when mixed with the specimen described above. It does not show selective absorption of high intensity in the ultra-violet region of the spectrum.

(c) 9α:11α-Epoxyergosta-7:22-dien-3β-yl acetate (1.0 g.) was oxidised with perbenzoic acid as described in (a) and the solution kept at 0° for 3 weeks. The reaction mixture was debrominated by zinc and acetic acid,

and the product directly crystallised from acetone to give 3β-acetoxy-9α:11α-dihydroxyergost-22-en-7-one (130 mg.) as plates, m.p.257-260°, undepressed on admixture with the specimen described above. Chromatography of the residue from the mother-liquors gave a further 45 mg. of this compound.

3β:11α-Diacetoxy-9α-hydroxyergost-22-en-7-one.

A solution of 3β-acetoxy-9α:11α-dihydroxyergost-22--en-7-one (130 mg.) in pyridine (5 c.c.) and acetic anhydride (5 c.c.) was kept at room temperature overnight. Isolation of the product by means of ether, followed by two crystallisations from light petroleum (b.p.60-80°) and two from methanol, gave 3β:11α-diacetoxy-9α-hydroxy-ergost-22-en-7-one as needles, m.p.197-198°, $[\alpha]_D$ -44° (c, 1.0) (Found: C,72.1; H,9.6. $C_{32}H_{50}O_6$ requires C,72.4; H,9.5%). It gives a pale yellow colour with tetranitromethane in chloroform, and does not show high--intensity light absorption above 2200 Å.

3β:9α:11α-Trihydroxyergost-22-en-7-one.

(a) Hydrolysis of 3β-acetoxy-9α:11α-dihydroxyergost--22-en-7-one using 2% aqueous methanolic potassium hydroxide, gave 3β:9α:11α-trihydroxyergost-22-en-7-one which separated from acetone (or methanol) in flat needles,

m.p.258-259°, $[\alpha]_D$ -71° (c, 1.1) (Found: C,75.2; H,10.4. $C_{28}H_{46}O_4$ requires C,75.3; H,10.4%). The compound did not show high-intensity absorption above 2200 Å.

(b) Similar hydrolysis of 3β:11α-diacetoxy-9α--hydroxyergost-22-en-7-one gave the triol, m.p.257-259°, $[\alpha]_D$ -70° (c, 0.7), showing no depression of mixed m.p. with the specimen described above.

Acetylation of 3β:9α:11α-trihydroxyergost-22-en-7-one using pyridine and acetic anhydride gave 3β:11α-diacetoxy--9α-hydroxyergost-22-en-7-one as needles from methanol, m.p.195-196°, $[\alpha]_D$ -43° (c, 0.8) undepressed in m.p. when mixed with the specimen described above.

3β:11α-Diacetoxyergosta-8:22-dien-7-one.

(a) 3β:11α-Diacetoxy-9α-hydroxyergost-22-en-7-one (100 mg.) was heated under reflux with aqueous methanolic potassium hydroxide (7 c.c.; 5%) for 8 hours. The solution was concentrated and the reaction product isolated by means of ether. A solution of this solid in pyridine (1 c.c.) and acetic anhydride (2 c.c.) was heated on the steam-bath for 2 hours. Isolation by means of ether gave a solid (60 mg.) which after crystallisation from methanol gave 3β:11α-diacetoxyergosta-8:22-dien-7-one as hard, flat needles, m.p.175-177°, $[\alpha]_D$ +14° (c, 0.6),

undepressed with the specimen described before (Found: C,75.0; H,9.55. Calc. for $C_{32}H_{48}O_5$: C,75.0; H,9.4%). Light absorption: Maximum at 2520 Å (ε = 10,400). The compound gives a faint yellow colour with tetranitromethane in chloroform.

(b) 3β-Acetoxy-9α:11α-dihydroxyergost-22-en-7-one was heated under reflux with aqueous methanolic potassium hydroxide (5%) for 24 hours. Working up as before followed by acetylation gave 3β:11α-diacetoxy-8:22-dien--7-one obtained as needles, m.p.174-176°, $[\alpha]_D$ +14° (c, 0.6), undepressed in m.p. when mixed with the specimen described above. Light absorption: Maximum at 2510 Å (ε = 10,300).

Tetrabromoergostenyl Acetate.

A solution of 5-dihydroergosteryl acetate (10 g.) in dry ether (1000 c.c.) was treated rapidly at 0° with a solution of dry bromine (5.1 c.c. = 4.4 mols.) in glacial acetic acid (50 c.c.). The mixture was cooled to -60° with shaking, and allowed to regain room temperature during 2 hours with frequent shaking. The solid (8.3-9.0 g.) was collected, washed with ether, and dried at room temperature under reduced pressure. Two crystallisations of a sample of the colourless amorphous solid from benzene-light petroleum (b.p.60-80°) gave tetrabromoergostenyl acetate as felted needles, m.p.128° (decomp.), $[\alpha]_D$ +240° (c, 1.2 in benzene) (Found: C,47.7; H,6.4; Br,42.5. Calc. for $C_{30}H_{46}O_2Br_4$: C,47.5; H,6.1; Br,42.2%). The compound decomposes on standing, and solutions in chloroform, acetone and acetic acid decompose with evolution of hydrogen bromide. Anderson, Stevenson and Spring (106) report $[\alpha]_D$ between +205° and +260° in various solvents.

22:23-Dibromoergosta-7:9(11)-dien-3β-yl Acetate. (Ergosteryl-D Acetate 22:23-Dibromide).

A solution of tetrabromoergostenyl acetate (9.0 g.) in warm benzene (500 c.c.) was treated with sodium iodide (25 g.) in ethanol (500 c.c.) added in one portion.

Iodine was immediately liberated. After standing for 20 hours at room temperature, the solution was diluted with water (500 c.c.), the benzene layer separated, and the aqueous phase extracted with benzene (300 c.c.). The combined extracts were washed with sodium hydroxide solution (2 x 200 c.c.; 1%), then with water, and dried (Na_2SO_4). Removal of the benzene gave an orange solid which was dissolved in a minimum volume of chloroform, precipitated by addition of methanol, and collected (7.9 g.). Crystallisation from chloroform-methanol gave 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate as prismatic needles, m.p.234-235°, $[\alpha]_D$ +32° (c, 1.4) (Found: C,60.0; H,7.8. Calc. for $C_{30}H_{46}O_2Br_2$: C,60.2; H,7.75%). Light absorption: Maxima at 2350 (ε = 19,000) and 2420 Å (ε = 21,000), and an inflection at 2500 Å (ε = 13,000). It gives a dark brown colour with tetranitromethane in chloroform.

22:23-Dibromoergosta-7:9(11)-dien-3β-ol.

A solution of the acetate (300 mg.) in benzene (5 c.c.) and aqueous methanolic potassium hydroxide (40 c.c.; 3%) was refluxed for 6 hours, and concentrated to 30 c.c. The crystals separating on cooling were recrystallised from methanol-chloroform, giving 22:23-dibromoergosta-7:9(11)-dien-3β-ol (270 mg.) as elongated

plates, m.p.230-231°, $[\alpha]_D$ +26° (c, 2.0) (Found: C,59.5; 59.0; H,8.4, 8.3. $C_{28}H_{44}OBr_2.CH_3OH$ requires: C,59.2; H,82.%). Light absorption: Maxima at 2350 (ε = 18,000) and 2420 Å (ε = 20,000), and an inflection at 2500 Å (ε = 13,500). The alcohol gives a brown colour with tetranitromethane in chloroform.

Acetylation of the alcohol using pyridine and acetic anhydride gave 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate as large, prismatic needles from methanol--chloroform, m.p.235-236°, $[\alpha]_D$ +33° (c, 1.2). Light absorption: Maxima at 2350 (ε = 20,000) and 2420 Å (ε = 21,000) and an inflection at 2500 Å (ε = 14,000).

22:23-Dibromoergosta-7:9(11)-dien-3β-yl Benzoate.

Treatment of 22:23-dibromoergosta-7:9(11)-dien-3β-ol with pyridine and benzoyl chloride at 100° for 3 hours gave 22:23-dibromoergosta-7:9(11)-dien-3β-yl benzoate as blades from methanol-chloroform, m.p.221-222°, $[\alpha]_D$ +28° (c, 1.9) (Found: C,63.8; H,7.4. $C_{35}H_{48}O_2Br_2$ requires: C,63.6; H,7.3%). Light absorption: Maxima at 2340 (ε = 30,200) and 2410 Å (ε = 27,400) and an inflection at 2500 Å (ε = 14,000). It gives a brown colour with tetranitromethane in chloroform.

Ergosteryl-D Benzoate.

A solution of 22:23-dibromoergosta-7:9(11)-dien-3β-yl benzoate (300 mg.) in ether-methanol (1:1; 100 c.c.) was treated with zinc dust (2 g.) (activated by washing with ammonium chloride solution) added portionwise and the mixture heated under reflux for 3 hours. The reaction mixture was filtered, concentrated and the residue treated with water and the precipitate extracted with ether. The ethereal solution was washed with water, dried (Na_2SO_4), and evaporated. Crystallisation of the residue from methanol-chloroform gave ergosteryl-D benzoate (200 mg.), m.p.176-178°, $[\alpha]_D$ +31° (c, 2.0) (Found: C,84.1; H,9.85. $C_{35}H_{48}O_2Br_2$ requires C,83.95; H,9.7%).

Ergosteryl-D Acetate.

A solution of 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate (1.0 g.) in ether-ethanol (1:1; 200 c.c.) was treated with zinc dust (5 g.) as before. Evaporation of ether gave plates, m.p.176-178° (0.7 g.). Crystallisation from methanol-chloroform gave ergosteryl-D acetate as elongated plates, m.p.178-180°, $[\alpha]_D$ +33° (c, 2.0) (Found: C,82.1; H,10.6. Calc. for $C_{30}H_{48}O_2$: C,82.1; H,10.6%). Light absorption: Maxima at 2350 (ε = 17,000) and 2420 Å (ε = 19,000), and an inflection at 2510 Å

(ε = 13,000). It gives a brown colour with tetranitromethane.

22:23-Dibromoergost-8(14)-en-3β-yl Acetate.

(a) A solution of ergosteryl-D acetate 22:23--dibromide (500 mg.) in stabilised glacial acetic acid (250 c.c.) was added to a suspension of freshly reduced platinum (from 200 mg. of platinum oxide) in acetic acid (15 c.c.), and the mixture shaken with hydrogen for 20 hours at room temperature. Removal of the catalyst by filtration, concentration of the filtrate under reduced pressure, addition of water, extraction with ether, washing of the extract with dilute sodium carbonate solution and water, drying (Na_2SO_4), and evaporation under reduced pressure gave 22:23-dibromoergost-8(14)-en--3β-yl acetate which separates from methanol-chloroform as elongated plates, m.p.192-193° (400 mg.), $[\alpha]_D$ +5°, +4.5° (c, 2.0, 7.0) (Found: C,60.1; H,8.3. $C_{30}H_{48}O_2Br_2$ requires C,60.0; H,8.0%). Light absorption: ε_{2100} 8,000, ε_{2150} 7500, ε_{2200} 5600, ε_{2250} 1400. It gives a deep yellow colour with tetranitromethane in chloroform.

After chromatography of a specimen on alumina the constants were unchanged.

The same product was obtained when chloroform-glacial

acetic acid (1:9 parts) was used as solvent for the hydrogenation (12 hours).

(b) A solution of 3β-acetoxy-22:23-dibromoergost--8-en-11-one (500 mg.) in glacial acetic acid (100 c.c.) was shaken with hydrogen over pre-reduced platinum oxide (200 mg.) for 10 hours. Working up in the usual manner gave 22:23-dibromoergost-8(14)-en-3β-yl acetate as plates (from methanol-chloroform), m.p.189-191° (250 mg.), $[\alpha]_D$ +3° (c, 1.0), undepressed in m.p. when mixed with the specimen described under (a) (Found: C,60.2; H,8.4%).

The same product was obtained when glacial acetic acid (100 c.c.)-concentrated hydrochloric acid (3 drops) was used as solvent and palladium black as catalyst for the hydrogenation (68 hours).

(c) A solution of 22:23-dibromo-9α:11α-epoxyergost--7-en-3β-yl acetate (500 mg.) in glacial acetic acid (80 c.c.) was shaken with hydrogen for 4 hours over platinum (from 100 mg. of platinum oxide). Working up in the usual way gave 22:23-dibromoergost-8(14)-en-3β-yl acetate (450 mg.) as plates (from methanol-chloroform), m.p.191-192°, $[\alpha]_D$ +4° (c, 1.1), undepressed in m.p. when mixed with the specimen described under (a) and (b) (Found: C,60.0; H,8.1%).

22:23-Dibromoergost-8(14)-en-3β-ol.

A solution of 22:23-dibromoergost-8(14)-en-3β-yl acetate (500 mg.) in benzene (10 c.c.) and methanolic potassium hydroxide (80 c.c.; 1%) was refluxed for 1 hour. The solution was diluted with water and extracted with ether. Removal of the ether gave a solid, which crystallised from methanol-chloroform to give 22:23--dibromoergost-8(14)-en-3β-ol as plates, m.p.213-214°, (420 mg.), $[\alpha]_D$ +13° (c, 1.8) (Found: C,60.3; H,8.6. $C_{28}H_{46}OBr_2$ requires C,60.2; H,8.3%).

Acetylation of the alcohol using pyridine and acetic anhydride gave 22:23-dibromoergost-8(14)-en-3β-yl acetate as elongated plates from methanol-chloroform, m.p.192-193°, $[\alpha]_D$ +4° (c, 1.6).

22:23-Dibromoergost-8(14)-en-3β-yl Benzoate.

A solution of 22:23-dibromoergost-8(14)-en-3β-ol in pyridine and benzoyl chloride was heated at 100° for 1 hour and kept at room temperature for 2 hours. Crystallisation of the product from chloroform-methanol gave 22:23-dibromoergost-8(14)-en-3β-yl benzoate as plates, m.p.242-243°, $[\alpha]_D$ +3° (c, 5.0) (Found: C,63.4; H,7.8. $C_{35}H_{50}O_2Br_2$ requires C,63.4; H,7.6%).

22:23-Dibromoergost-7-en-3β-yl Acetate (with F.Johnson).

A solution of ergosteryl-D acetate 22:23-dibromide (500 mg.) in ethyl acetate (100 c.c.) was shaken with hydrogen over pre-reduced platinum oxide (100 mg.) for 14 hours. Working up in the usual way gave 22:23--dibromoergost-7-en-3β-yl acetate (5-dihydroergosteryl acetate 22:23-dibromide) as needles (from methanol-ethyl acetate), m.p.224° (400 mg.), $[\alpha]_D$ -7° (c, 2.0) (Found: C,60.3; H,8.2. $C_{30}H_{48}O_2Br_2$ requires C,60.0; H,8.0%). Light absorption: ε_{2100} 5000, ε_{2150} 3620, ε_{2200} 1700. It gives a yellow colour with tetranitromethane in chloroform.

It is isomerised by shaking its solution in acetic acid, with a platinum catalyst and hydrogen for 4 hours, to give 22:23-dibromoergost-8(14)-en-3β-yl acetate.

Hydrolysis of the acetate using methanolic potassium hydroxide (2%) gave 22:23-dibromoergost-7-en-3β-ol as plates from methanol, m.p.222-223°, $[\alpha]_D$ -8° (c, 1.3) (Found: C,59.1; H,8.6. $C_{28}H_{46}OBr_2.CH_3OH$ requires C,59.0; H,8.5%).

The benzoate, prepared in the usual way, separates from methanol-chloroform as needles, m.p.205°, $[\alpha]_D$ -4° (c, 4.0) (Found: C,63.4; H,7.8. $C_{35}H_{50}O_2Br_2$ requires C,63.4; H,7.6%).

Ergosta-8(14):22-dien-3β-yl Acetate.

A solution of 22:23-dibromoergost-8(14)-en-3β-yl acetate (m.p.191-193°; 200 mg.) in ether-methanol (50 c.c.; 1:2) was refluxed with zinc dust (2 g.) added portionwise during 3 hours. The solution was filtered, concentrated, and diluted with water. Isolation by means of ether gave ergosta-8(14):22-dien-3β-yl acetate, as plates (from methanol), m.p.122-123.5° (120 mg.), $[\alpha]_D$ -25° (c, 1.1) (Found: C,81.5; H,11.2. Calc. for $C_{30}H_{48}O_2$: C,81.8; H,11.0%). Light absorption: ε_{2150} 8000, ε_{2150} 7000, ε_{2200} 4900. It gives a deep yellow colour with tetranitromethane in chloroform.

Ergosta-8(14):22-dien-3β-yl Benzoate.

A solution of 22:23-dibromoergost-8(14)-en-3β-yl benzoate (500 mg.) in pyridine (30 c.c.) containing water (3 drops) was heated with zinc dust (4 g.) for 3 hours on the steam-bath. The mixture was filtered, and the filtrate concentrated under reduced pressure and diluted with water. Working up using ether gave ergosta-8(14):22-dien-3β-yl benzoate (380 mg.) as flat needles (from methanol-chloroform), m.p.126-127°, $[\alpha]_D$ -24° (c,4.1) (Found: C,83.8; H,10.2. $C_{35}H_{50}O_2$ requires C,83.6; H,10.0%). It gives a deep yellow colour with tetranitro-

methane in chloroform.

Ergosta-8(14):22-dien-3β-ol.

A solution of ergosta-8(14):22-dien-3β-yl acetate (60 mg.) in methanolic potassium hydroxide (20 c.c.; 3%) was refluxed for 2 hours, then concentrated and diluted with water. Isolation by means of ether gave ergosta--8(14):22-dien-3β-ol which crystallised from methanol or acetone as elongated plates, m.p.126-127° (40 mg.), $[\alpha]_D$ -19°, -20° (c, 1.5, 1.0) (Found: C,84.2; H,11.8. $C_{28}H_{46}O$ requires C,84.35; H,11.6%). It gives a deep yellow colour with tetranitromethane.

Acetylation of the alcohol using pyridine and acetic anhydride gave ergosta-8(14):22-dien-3β-yl acetate as plates (from methanol), m.p.123-124°, $[\alpha]_D$ -27° (c,1.3).

Ergost-8(14)-en-3β-yl Acetate.

A solution of ergosta-8(14):22-dien-3β-yl acetate (100 mg.) in ethyl acetate (60 c.c.) was shaken with hydrogen over freshly reduced platinum oxide catalyst for 5 hours. The filtered reaction mixture was concentrated, giving plates (80 mg.) which on recrystallisation from methanol gave ergost-8(14)-en-3β-yl acetate as plates, m.p.109-110°, $[\alpha]_D$ +4° (c, 2.0), showing no depression of m.p. when mixed with an authentic sample,

m.p.108-109°, $[\alpha]_D$ +3°, prepared by hydrogenation of ergosteryl-D acetate in glacial acetic acid (Found: C,81.2; H,11.4. Calc. for $C_{30}H_{50}O_2$: C,81.4; H,11.4%). Light absorption: ε_{2100} 7000, ε_{2150} 6400, ε_{2200} 4600. It gives a yellow colour with tetranitromethane in chloroform.

The same product in similar yield was obtained by using chloroform or glacial acetic acid as solvent for the hydrogenation.

Oxidation of Ergosteryl-D Acetate 22:23-Dibromide with Performic Acid.

3β-Acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one.

A solution of ergosteryl-D acetate 22:23-dibromide (5.0 g.) in ethyl acetate (250 c.c.) was treated with a mixture of formic acid (50 c.c.; 98-100%) and hydrogen peroxide (30%; 3.75 c.c. = 4 atoms of oxygen) added in one portion with shaking. The suspension was shaken at room temperature for 6 hours when solution was complete. The clear solution was kept at room temperature for 40 hours. It was washed with water, the ethyl acetate layer separated, and the aqueous layer extracted with ethyl acetate. The combined ethyl acetate extracts were washed with sodium hydrogen carbonate solution and water and dried (Na_2SO_4). Removal of the solvent under reduced pressure and crystallisation of the residue from acetone gave 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one (1.5 g.) as needles, m.p.220-221°, $[\alpha]_D$ -46° (c, 1.6), not altered by recrystallisation from the same solvent. Recrystallisation from methanol-chloroform gave a second modification as plates, m.p.235-237°, $[\alpha]_D$ -47° (c, 1.5). The two forms are unchanged after drying in a high vacuum at 100°) (Found: C,57.1; H,7.4. Calc. for $C_{30}H_{46}O_4Br_2$: C,57.1; H,7.35%). It does not give a colour with

tetranitromethane in chloroform, or exhibit high-intensity light absorption above 2000Å.

Alkaline hydrolysis and acetylation of the residue from the acetone mother-liquors gives 3β:11α-diacetoxy--22:23-dibromoergost-8-en-7-one, as described below.

3β-Acetoxy-9α:11α-epoxyergost-22-en-7-one.

A solution of 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one (600 mg.) in ether-methanol (1:1; 200 c.c.) was heated under reflux with zinc dust (5 g.) added portionwise during 3 hours. The product isolated by means of ether was crystallised from methanol to give 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one as felted needles (initial gel formation), m.p.227-229°, $[\alpha]_D$ -89°, -87° (c, 1.0; 1.5) (Found: C,76.7; H,9.9. Calc. for $C_{30}H_{46}O_4$: C,76.55; H,9.85%). Mixed m.p. with the specimen, m.p.223°, described before, was 224-228°. Light absorption: Maximum at 2050 Å (ε = 1,500). It gives a pale yellow colour with tetranitromethane in chloroform.

3β-Acetoxy-22:23-dibromo-11α-hydroxyergost-8-en-7-one.

A solution of 3β-acetoxy-22:23-dibromo-9α:11α--epoxyergostan-7-one (300 mg.) in benzene (30 c.c.) was filtered through a column of alumina (12 x 2 cm.). After

elution with benzene, benzene-ether (1:1) and ether (eluate: negligible), elution with ether-methanol (2:1; 100 c.c.) gave a solid (260 mg.) which, thrice crystallised from methanol, yielded 3β-acetoxy-22:23-dibromo-11α--hydroxyergost-8-en-7-one as needles, m.p.206-208°, $[\alpha]_D$ -15° (c, 1.7) (Found: C,57.0; H,7.5. Calc. for $C_{30}H_{46}O_4Br_2$: C,57.1; H,7.35%). Light absorption: Maximum at 2520 Å (ε = 8,000). Infra-red light absorption: Maxima at 1732 cm.$^{-1}$ (acetate), 1669 cm.$^{-1}$ (αβ-ketone) and 3700 cm.$^{-1}$ (hydroxyl). It gives no colour with tetranitromethane in chloroform.

22:23-Dibromo-3β:11α-dihydroxyergost-8-en-7-one.

(a) A solution of 3β-acetoxy-22:23-dibromo-9α:11α--epoxyergostan-7-one (650 mg.) in benzene (5 c.c.) and aqueous methanolic potassium hydroxide (50 c.c.; 3%) was heated under reflux for 3 hours. Isolation by means of ether gave a product, which crystallised from methanol in flat needles, m.p.230-231° (550 mg.). Recrystallisation from methanol gave 22:23-dibromo-3β:11α-dihydroxyergost-8-en-7-one as blades, m.p.231-232°, $[\alpha]_D$ +4° (c,1.7) (Found: C,57.3; H,7.7. $C_{28}H_{44}O_3Br_2$ requires C,57.1; H,7.5%). Light absorption: Maximum at 2520 Å (ε = 8,200). It gives no colour with tetranitromethane.

(b) A solution of 3β-acetoxy-22:23-dibromo-11α--hydroxyergost-8-en-7-one (100 mg.) in methanolic potassium hydroxide (20 c.c.; 2%) was heated under reflux for 2 hours. The product, isolated by ether, gave 22:23-dibromo-3β:11α-dihydroxyergost-8-en-7-one (80 mg.) as plates (from methanol), m.p.231-232°, $[\alpha]_D$ +3° (c, 1.4), undepressed in m.p. when mixed with the specimen described above. Light absorption: Maximum at 2520 Å (ε = 8,000).

(c) A solution of 3β:11α-diacetoxy-22:23-dibromo-ergost-8-en-7-one (600 mg.) in benzene (10 c.c.) and methanolic potassium hydroxide (3%; 40 c.c.) was refluxed for 3 hours. 22:23-Dibromo-3β:11α-dihydroxyergost-8--en-7-one (400 mg.), isolated by means of ether, crystallised from methanol as elongated plates, m.p.232°, $[\alpha]_D$ +4° (c, 1.3), undepressed in m.p. when mixed with the specimens described above. Light absorption: Maximum at 2510 Å (ε = 8000).

3β:11α-Diacetoxy-22:23-dibromoergost-8-en-7-one.

(a) Acetylation of 22:23-dibromo-3β:11α-dihydroxy-ergost-8-en-7-one using pyridine and acetic anhydride (at 100°) gave 3β:11α-diacetoxy-22:23-dibromoergost-8--en-7-one as prismatic needles from methanol-chloroform,

m.p.161-163° (air dried) or m.p.201-202° after prolonged drying at 100° in a high vacuum; $[\alpha]_D$ +18° (c, 1.5) (Found: C,57.1; H,7.3. $C_{28}H_{48}O_5Br_2$ requires C,57.1; H,7.2%). Light absorption: Maximum at 2520 Å (ε = 10,000). Infra-red light absorption: Maxima at 1738 and 1243 cm.$^{-1}$ (acetate) and 1690 cm.$^{-1}$ ($\alpha\beta$-ketone). It gives no colour with tetranitromethane.

(b) Acetylation of 3β-acetoxy-22:23-dibromo-11α-hydroxyergost-8-en-7-one (acetic anhydride-pyridine) gave 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one which separated from methanol-chloroform as prismatic needles, m.p.162-163° and 200-202° (after drying at 100° in vacuum), $[\alpha]_D$ +18° (c, 0.8) (Found: C,56.7; H,7.5%). Light absorption: Maximum at 2520 Å (ε = 10,000). The mixed m.p. was undepressed on admixture with a specimen described above.

Preparative Method for 3β:11α-Diacetoxy-22:23-dibromo-ergost-8-en-7-one.

A solution of ergosteryl-D acetate 22:23-dibromide (5.0 g.) in ethyl acetate (250 c.c.) was treated dropwise with a mixture of formic acid (98-100%; 50 c.c.) and hydrogen peroxide (30%; 3.75 c.c.) over a period of 4 hours at 40-45° with mechanical stirring. The clear solution was kept at room temperature for 2 days, washed

with water and sodium hydrogen carbonate solution, and the solvent removed under reduced pressure. The white crystalline residue was hydrolysed by refluxing its solution in benzene (15 c.c.) with methanolic potassium hydroxide (2%; 100 c.c.) for 3 hours. Isolation by means of ether gave a crystalline solid, which was acetylated in pyridine (50 c.c.) and acetic anhydride (50 c.c.) on the steam bath for 3 hours. Working up using ether gave a solid which crystallised from methanol-chloroform to yield 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one (2.5 g.) as prismatic needles, m.p.156-158°, $[\alpha]_D$ +19° (c, 1.5) (Found: C,57.3; H,7.5. Calc. for $C_{32}H_{48}O_5Br_2$: C,57.1; H,7.2%). Light absorption: Maximum at 2510 Å (ε = 11,000). It is undepressed in m.p. when mixed with the specimens described above.

3β:11α-Diacetoxyergosta-8:22-dien-7-one.

A solution of 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one (200 mg.) in ether-methanol (1:1; 60 c.c.) was heated under reflux with zinc dust (2 g.) added in portions for 3 hours. The product, isolated by means of ether, was crystallised from methanol to give 3β:11α-diacetoxyergosta-8:22-dien-7-one (130 mg.) as needles, m.p.175-177°, $[\alpha]_D$ +12° (c, 1.2) (Found: C,75.2; H,9.5. Calc. for $C_{32}H_{48}O_5$: C,75.0; H,9.4%). Light absorption:

Maximum at 2510 Å (ε = 10,300). The m.p. was undepressed when the product was mixed with the specimen described before.

3β:11α-Dihydroxyergosta-8:22-dien-7-one.

A solution of 22:23-dibromo-3β:11α-dihydroxyergost--8-en-7-one (160 mg.) in methanol (50 c.c.) was heated under reflux and treated with zinc dust (1 g.) added portionwise over a period of 3 hours. Working up using ether gave a product which on crystallisation from acetone gave 3β:11α-dihydroxyergosta-8:22-dien-7-one (100 mg.) as prismatic needles, m.p.214-215°, $[\alpha]_D$ -7° (c, 1.0) (Found: C,78.5; H,10.5. Calc. for $C_{28}H_{44}O_3$: C,78.45; H,10.35%). Light absorption: Maximum at 2540 Å (ε = 8,600). It is undepressed in m.p. when mixed with the specimen (m.p.215°, $[\alpha]_D$ -6°) obtained by alkaline hydrolysis of 3β-acetoxy--9α:11α-epoxyergost-22-en-7-one.

22:23-Dibromo-7:11-diketoergost-8-en-3β-yl Acetate (with R. Stevenson).

A solution of 3β-acetoxy-22:23-dibromo-11α-hydroxy-ergost-8-en-7-one (177 mg.) in stabilised glacial acetic acid (25 c.c.) was treated with a solution of chromic anhydride (21 mg.) in glacial acetic acid (0.63 c.c.) and then with sulphuric acid (2N; 2 drops). The mixture was

shaken for 5 minutes, kept at room temperature overnight and then filtered from a fine flocculent precipitate (20 mg.; m.p.245°). The solution was diluted with water and the neutral product (140 mg.) isolated by means of ether. Crystallisation of the neutral product from chloroform-methanol gave 22:23-dibromo-7:11-diketoergost--8-en-3β-yl acetate as plates, m.p.249-250°, $[\alpha]_D$ +29° (c, 1.0). Light absorption: Maximum at 2700 Å (ε = 8,100). The m.p. was undepressed when mixed with an authentic specimen, m.p.250-251°, $[\alpha]_D$ +27° described earlier.

3β-Acetoxy-22:23-dibromoergosta-8:11-dien-7-one.

A solution of 3β:11α-diacetoxy-22:23-dibromoergost--8-en-7-one (300 mg.) in methanolic hydrogen chloride (5%; 60 c.c.) was refluxed for 4 hours. The product was isolated by means of ether and acetylated using pyridine and acetic anhydride at 100°. Crystallisation of the acetylated product from methanol-chloroform gave plates (200 mg.), m.p.260-262°, which were purified by chromatography of a solution in benzene (20 c.c.) on alumina (10 x 2 cm.). The solid eluted with benzene (300 c.c.) was crystallised from methanol-chloroform to give 3β-acetoxy-22:23-dibromoergosta-8:11-dien-7-one as thick plates, m.p.263-264°, $[\alpha]_D$ -13° (c, 1.0) (Found:

C,59.0; H,7.5. $C_{30}H_{44}O_3Br_2$ requires C,58.7; H,7.2%). **Light absorption:** Maxima at 2240 (ε = 18,000) and 2960 Å (ε = 6,000).

3β:11α-Dihydroxyergost-22-en-7-one.

A solution of 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one (250 mg.) in ethanolic potassium hydroxide (0.1M; 100 c.c.) was shaken with hydrogen and pre-reduced platinum catalyst (from 50 mg. of PtO_2) at room temperature for 2 hours. The solution was filtered, concentrated to a volume of 30 c.c., diluted with water, and extracted with ether. Removal of the ether left a crystalline residue, which was crystallised from aqueous acetone (or aqueous methanol) to yield 3β:11α-dihydroxyergost-22-en-7-one (170 mg.) as long fine needles, m.p.206-207°, $[\alpha]_D$ -76° (c, 0.8) (Found: C,74.8; H,10.9. Calc. for $C_{28}H_{46}O_3.H_2O$: C,74.95; H,10.8%). It does not exhibit high intensity light absorption above 2200 Å, and gives a faint yellow colour with tetranitromethane in chloroform.

Starting material was recovered on attempted hydrogenation in ethanol (20 minutes), ethyl acetate (5 hours) over platinum, ethanol over palladium black (6 hours), and ethyl acetate (12 hours) over palladised charcoal.

3β:11α-Diacetoxyergost-22-en-7-one.

Acetylation of 3β:11α-dihydroxyergost-22-en-7-one using pyridine and acetic anhydride at 100° (2 hours) gave 3β:11α-diacetoxyergost-22-en-7-one, which separates from methanol as soft plates, m.p.141-142°, $[\alpha]_D$ -67°, -68° (c, 0.7, 0.6) (Found: C,74.8; H,10.0. Calc. for $C_{32}H_{50}O_6$: C,74.7; H,9.8%). It does not exhibit high intensity light absorption above 2200 Å, and gives a faint yellow colour with tetranitromethane in chloroform. Heusser et al.(37, p.949, footnote) give m.p.123-124°, $[\alpha]_D$ -60° for this compound.

3β:11α-Diacetoxyergostan-7-one.

A solution of 3β:11α-diacetoxy-22:23-dibromoergost-8-en-7-one (500 mg.) in ethanol (300 c.c.) was shaken with hydrogen over prereduced platinum oxide (from 200 mg. of PtO_2) at room temperature for 20 hours. The solution was filtered, concentrated to a volume of 50 c.c. under reduced pressure, and diluted with water. Isolation by means of ether gave a very soluble gum, which did not crystallise. A solution of this product in light petroleum (40-60°)-benzene (1:1) was filtered through a column of alumina (10 x 2 cm.). Elution with the same solvent (300 c.c.) gave a solid (50 mg.) which did not

separate in homogeneous crystals from methanol. Evaporation of the benzene fraction (200 c.c.) gave a product (250 mg.) which crystallised from methanol to give 3β:11α-diacetoxyergostan-7-one as hard thick plates, m.p.139-141°, $[\alpha]_D$ -50° (c, 0.6) (Found: C,74.0; H,10.4. $C_{32}H_{52}O_5$ requires C,74.4; H,10.1%). The compound does not exhibit light absorption of high intensity above 2000 Å, and does not give a colour with tetranitromethane in chloroform. Mixed m.p. with 3β:11α-diacetoxyergost-22-en-7-one (m.p.141-142°) was 126-132°.

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Oxidation of Ergosteryl-D Acetate 22:23-Dibromide with Perbenzoic Acid.

22:23-Dibromo-9α:11α-epoxyergost-7-en-3β-yl Acetate.

A solution of ergosteryl-D acetate 22:23-dibromide (4.39 g.) in chloroform (20 c.c.) was treated dropwise with perbenzoic acid (1.2 mols.) in chloroform (20 c.c.) added with stirring during 3 hours at 0°. The mixture was kept at 0° for a further 2 hours. The solution was concentrated under reduced pressure at room temperature, until solid separated, when the mixture was diluted with ether. The solution was shaken successively with 3% sodium carbonate and water and dried ($MgSO_4$). The solid obtained by removal of the solvents at room temperature, under reduced pressure, crystallised from pure acetone in needles, m.p.207-208° (3.4 g.). Three further crystallisations from acetone gave 22:23-dibromo-9α:11α--epoxyergost-7-en-3β-yl acetate as flat needles, m.p.218--219°, $[\alpha]_D$ -26° (c, 1.7) (Found: C,58.5; H,7.6. $C_{30}H_{46}O_3Br_2$ requires C,58.6; H,7.55%). It gives a yellow colour with tetranitromethane in chloroform and does not show selective absorption of high intensity above 2200 Å.

Attempted crystallisation of the epoxide from technical acetone or methanol caused rearrangement.

22:23-Dibromo-9α:11α-epoxyergost-7-en-3β-ol.

A solution of 22:23-dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate (400 mg.) in benzene (20 c.c.) and methanolic potassium hydroxide (80 c.c.; 2%) was heated under reflux for 2 hours. Precipitation with water and isolation by means of ether gave a product which after three crystallisations from methanol gave 22:23-dibromo-9α:11α-epoxyergost-7-en-3β-ol as plates, m.p. 200-202° (350 mg.), $[\alpha]_D$ -27° (c, 0.9) (Found: C,58.0; H,8.0. $C_{28}H_{44}O_2Br_2.CH_3OH$ requires C,57.6; H,8.0%). It gives a yellow colour with tetranitromethane in chloroform and does not show selective absorption of high intensity above 2200 Å.

Acetylation of the alcohol using pyridine and acetic anhydride at room temperature gave 22:23-dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate as needles from acetone, m.p.218°, $[\alpha]_D$ -25° (c, 1.5) (Found: C,58.5; H,7.55%). It does not exhibit selective absorption of high intensity above 2200 Å. Mixed m.p. with the authentic specimen described above was undepressed.

Ergosteryl-D Acetate.

(a) A solution of 22:23-dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate (1 g.) in ether was treated under reflux with zinc dust (10 g.) added portionwise during

3 hours. The solution was filtered, shaken with water and dried. Removal of ether gave a solid which crystallised from acetone to give quantitatively ergosteryl-D acetate as plates, m.p.177-179°, $[\alpha]_D$ +32°. Light absorption: Maxima at 2350 (ε = 16,400), 2420 (ε = 18,800) and 2510 Å (ε = 12,800). It gives a red-brown colour with tetranitromethane in chloroform.

9α:11α-Epoxyergosta-7:22-dien-3β-yl acetate was recovered unchanged on treatment with zinc dust under similar conditions.

(b) Treatment of an acetic acid solution of 22:23--dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate with zinc dust at room temperature for 4 hours gave also ergosteryl-D acetate, m.p.176-178°, undepressed when mixed with an authentic specimen,

3β-Acetoxy-22:23-dibromoergost-8-en-7-one.

A solution of 22:23-dibromo-9α:11α-epoxyergost-7-en--3β-yl acetate (700 mg.) in benzene (7 c.c.) was refluxed with aqueous methanolic hydrochloric acid (100 c.c.; 1%) for 1½ hours. A solution of the product, isolated in the usual manner, in pyridine (15 c.c.) was heated with acetic anhydride (10 c.c.) for 4 hours at 100°. The acetylated product (m.p.233°; 120 mg.) isolated by means of ether was purified by filtration of its solution in

benzene (10 c.c.) through a short column of activated alumina. Elution with the same solvent gave 3β-acetoxy-22:23-dibromoergost-8-en-7-one which crystallised from methanol in plates, m.p.241-242°, $[\alpha]_D$ -29° (c, 1.5) (Found: C,58.6; H,7.6. $C_{30}H_{46}O_3Br_2$ requires C,58.6; H,7.55%). Light absorption: Maximum at 2520 Å (ε = 10,000). It does not give a colour with tetranitromethane in chloroform.

3β-Acetoxyergosta-8:22-dien-7-one.

A solution of 3β-acetoxy-22:23-dibromoergost-8-en-7-one (50 mg.) in ether-ethanol (40 c.c.; 1:1) was refluxed for 2 hours with zinc dust (1 g.) added portionwise. After filtration, the solution was concentrated and poured into water. Isolation by means of ether gave a solid which crystallised from methanol to give 3β-acetoxyergosta-8:22-dien-7-one as plates, m.p.209-211°, $[\alpha]_D$ -56° (c, 0.5) (Found: C,79.3; H,10.3. Calc. for $C_{30}H_{46}O_3$: C,79.2; H,10.2%); it is undepressed in m.p. when mixed with an authentic specimen. Light absorption: Maximum at 2520 Å (ε = 10,000). It gives a yellow colour with tetranitromethane in chloroform.

3β-Acetoxy-22:23-dibromoergost-8-en-11-one.

A solution of 22:23-dibromo-9α:11α-epoxyergost-7--en-3β-yl acetate (1 g.) in dry benzene (50 c.c.) was treated with redistilled boron trifluoride etherate (15 drops) and the solution kept at room temperature for 3 days. The solution was diluted with ether (50 c.c.) and washed successively with water, sodium hydrogen carbonate solution, water and dried over sodium sulphate. The solvents were removed under reduced pressure and the residue crystallised from methanol-chloroform from which it separated in elongated plates, m.p.190° (0.73 g.). Light absorption: Maximum at 2530 Å (ε = 9100). A solution of the solid in benzene (25 c.c.) was filtered through a short column of activated alumina, and the column washed with the same solvent. Evaporation of the filtrate gave 3β-acetoxy-22:23-dibromoergost-8-en--11-one which crystallised from methanol-chloroform in blades, m.p.201-202°, $[\alpha]_D$ +98° (c, 1.7) (Found: C,58.7; H,7.7. $C_{30}H_{46}O_3Br_2$ requires C,58.6; H,7.55%). Light absorption: Maximum at 2530 Å (ε = 9800). It gives no coloration with tetranitromethane in chloroform.

3β-Acetoxyergosta-8:22-dien-11-one.

A solution of 3β-acetoxy-22:23-dibromoergost-8-en--11-one (300 mg.) in ether (60 c.c.) and methanol (60 c.c.)

was treated under reflux with zinc dust (3 g.) added portionwise for 2 hours. The mixture was filtered, the filtrate concentrated and the crystalline solid (m.p. 120-130°; 200 mg.) separating collected and thrice crystallised from methanol to give 3β-acetoxy-8:22-dien--11-one as elongated plates, m.p.129-131°, $[\alpha]_D$ +105° (c, 1.6) (Found: C,79.2; H,10.2. Calc. for $C_{30}H_{46}O_3$: C,79.2; H,10.2%); it is undepressed in melting point when mixed with an authentic specimen prepared according to Heusser et al. (36) who give m.p.122-123°, $[\alpha]_D$ +92° for this compound. Light absorption: Maximum at 2530 Å (ε = 9000).

22:23-Dibromo-7ξ:8ξ-9α:11α-diepoxyergostan-3β-yl Acetate.

(a) A solution of ergosteryl-D acetate 22:23-dibromide (2 g.) in dry chloroform (30 c.c.) was treated dropwise with perbenzoic acid (3 mols.) in chloroform (20 c.c.) added with stirring during 3 hours at 0°. The mixture was kept at -3° for 2 days, shaken successively with 3% sodium carbonate and water and dried ($MgSO_4$). The crystalline solid obtained by removal of the solvent at 30-40° under reduced pressure crystallised from acetone in needles, m.p.197-202° (1.4 g.). These further crystallisations from acetone gave 22:23-dibromo-7ξ:8ξ-9α:11α--diepoxyergostan-3β-yl acetate as needles, m.p.214-216°,

$[\alpha]_D$ +1.3° (c, 2.3) (Found: C,57.1; H,7.5. $C_{30}H_{46}O_4Br_2$ requires C,57.1; H,7.35%). It does not give a colour with tetranitromethane in chloroform and does not show selective absorption of high intensity above 2000 Å. Infra-red light absorption: Maxima at 1736 and 1248 cm.$^{-1}$ (acetate group).

Chromatography of a benzene solution of the compound on alumina gave a solid which crystallised from acetone in prismatic needles, m.p.218-220°, $[\alpha]_D$ +1.7° (c, 3.5) undepressed in m.p. when mixed with the specimen described above.

(b) A solution of 22:23-dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate (20 g.) in chloroform (180 c.c.) was treated with perbenzoic acid (1.5 mols.) in chloroform added in one portion at 0° with shaking. The mixture was kept at -4° for 24 hours and then for 12 hours at room temperature. It was shaken successively with water, sodium hydrogen carbonate solution, water and dried ($MgSO_4$). Removal of the solvent at -40° under reduced pressure gave a solid, which crystallised from acetone as needles, m.p.203-206° (15.9 g.). Recrystallisation from acetone gave 22:23-dibromo-7ξ:8ξ-9α:11α-diepoxyergostan-3β-yl acetate as needles, m.p.214-216° (10.1 g.), $[\alpha]_D$+1.8° (c, 5.5) (Found: C,57.4; H,7.6%). It is undepressed in

m.p. when mixed with the specimen described above.

Chromatography of this compound on alumina in benzene solution raised the m.p. to 219-220°, $[\alpha]_D$ +1.7° (c, 5.0).

22:23-Dibromo-7ξ:8ξ-9α:11α-diepoxyergostan-3β-ol.

A solution of 22:23-dibromo-7ξ:8ξ-9α:11α-diepoxyergostan-3β-yl acetate (1.0 g.) in aqueous methanolic potassium hydroxide (220 c.c.; 2%) was heated under reflux for 3 hours. The crystalline solid (m.p.215-217°; 650 mg.) which separated on cooling, was collected and crystallised from acetone to give 22:23-dibromo-7ξ:8ξ-9α:11α-diepoxyergostan-3β-ol as prisms, m.p.225-227°, $[\alpha]_D$ +2.7° (c, 4.4) (Found: C,55.7; H,7.5. $C_{28}H_{44}O_3.H_2O$ requires C,55.5; H,7.6%). It does not give a colour with tetranitromethane in chloroform and does not show selective light absorption of high intensity above 2200 Å. Infra-red light absorption spectrum shows a wide band at 3340 $cm.^{-1}$ (hydroxyl group) (no bands in the ketone-acetate region).

Acetylation of the alcohol using pyridine-acetic anhydride gave 22:23-dibromo-7ξ:8ξ-9α:11α-diepoxyergostan-3β-yl acetate as needles, m.p.220-221° (from acetone), $[\alpha]_D$ +1.8° (c, 3.2), undepressed in m.p. when mixed with the specimen described above.

7ξ:8ξ-9α:11α-Diepoxyergost-22-en-3β-yl Acetate.

A solution of 22:23-dibromo-7ξ:8ξ-9α:11α-diepoxyergostan-3β-yl acetate (5 g.) in ether (300 c.c.) and methanol (500 c.c.) was refluxed for 3 hours with zinc dust (15 g.) added portionwise. The mixture was filtered, the filtrate concentrated and the crystalline solid (m.p.220-222°; 3.1 g.) separating collected and crystallised from methanol to give 7ξ:8ξ-9α:11α-diepoxyergost-22-en-3β-yl acetate as soft, elongated plates, m.p.221-223°, $[\alpha]_D$ -4° (c, 3.2) (Found: C,76.4; H,9.8. $C_{30}H_{46}O_4$ requires C,76.55; H,9.85%). It gives a pale yellow colour with tetranitromethane in chloroform and does not exhibit selective light absorption of high intensity above 2200 Å (ε_{2200} = 1200). Infra-red light absorption: Maxima at 1740 and 1252 cm.$^{-1}$ (acetate group).

7ξ:8ξ-9α:11α-Diepoxyergost-22-en-3β-ol.

(a) A solution of 7ξ:8ξ-9α:11α-diepoxyergost-22-en-3β-yl acetate (500 mg.) in aqueous methanolic potassium hydroxide (120 c.c.; 2%) was heated under reflux for 2 hours. The solution was concentrated and the crystalline solid, which separated on cooling, collected (m.p.227-228°; 450 mg.), and crystallised from methanol to give 7ξ:8ξ-9α:11α-diepoxyergost-22-en-3β-ol as elongated plates, m.p.227-228°, $[\alpha]_D$ -1.5° (c, 2.2) (Found: C,75.5; H,10.4.

$C_{28}H_{44}O_3.CH_3OH$ requires C,75.6; H,10.5%). It gives a pale yellow colour with tetranitromethane and does not show selective absorption of high intensity above 2100 Å. Infra-red light absorption: Maximum at 3340 cm.$^{-1}$ (hydroxyl group) (absence of any bands in the ketone--acetate region).

(b) A solution of 22:23-dibromo-7ξ:8ξ-9α:11α--diepoxyergostan-3β-ol (300 mg.) in ether-methanol (1:1; 120 c.c.) was refluxed with zinc dust (3 g.) added portionwise during 3 hours. The mixture was filtered, the filtrate concentrated and the crystalline solid separating (200 mg.) collected and crystallised from methanol to give 7ξ:8ξ-9α:11α-diepoxyergost-22-en-3β-ol as plates, m.p.227-229°, $[\alpha]_D$ -2° (c, 2.6), undepressed in m.p. on admixture with the specimen described above.

Acetylation of the alcohol using pyridine-acetic anhydride gave 7ξ:8ξ-9α:11α-diepoxyergost-22-en-3β-yl acetate which crystallised from methanol in blades, m.p.222-223°, $[\alpha]_D$ -4° (c, 3.5), undepressed in m.p. when mixed with the specimen described above.

3β-Acetoxy-9α:11α-epoxyergost-22-en-7-one.

A solution of 22:23-dibromo-7ξ:8ξ-9α:11α-diepoxy-ergostan-3β-yl acetate (500 mg.) in acetic acid (15 c.c.) was heated with zinc dust (2 g.) on the steam bath for

2 hours. Isolation by means of ether gave a product which crystallised from methanol (initial gel formation) to give 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one as needles, m.p.222-225°, $[\alpha]_D$ -86° (c, 1.2), undepressed in m.p. when mixed with an authentic specimen, m.p.223-225°.

3β-Acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one.

A solution of 22:23-dibromo-7ξ:8ξ-9α:11α-diepoxyergostan-3β-yl acetate (8 g.) in acetic acid was treated with aqueous hydrogen bromide (80 c.c.; 48%) added portionwise with shaking at room temperature. The mixture was kept overnight at room temperature. The crystalline solid which separated from the dark-green solution was collected (m.p.238-240°; 1.4 g.), washed with methanol and crystallised from methanol-chloroform to give 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one as plates, m.p.237-239°, $[\alpha]_D$ -48° (c, 1.0) (Found: C,57.4; H,7.4. Calc. for $C_{30}H_{46}O_4Br_2$: C,57.1; H,7.35%). It is undepressed in m.p. when mixed with an authentic specimen, m.p.236-237°. Infra-red light absorption: Bands at 1720 (wide) and 1254 cm.$^{-1}$ (acetate group). It was recovered unchanged on further treatment with either (a) aqueous hydrogen bromide in acetic acid solution or (b) boron trifluoride etherate in absolute benzene.

Debromination in ether-methanol with zinc dust gave 3β-acetoxy-9α:11α-epoxyergost-22-en-7-one as needles (initial gel formation from methanol), m.p.227-229°, $[\alpha]_D$ -89° (c, 1.0) (Found: C,76.7; H,9.9. Calc. for $C_{30}H_{46}O_4$: C,76.55; H,9.85%). Mixed m.p. with the specimen, m.p.223-225°, was 224-228°.

3β:11α-Diacetoxyergosta-8:22-dien-7-one.

A solution of ergosteryl-D acetate 22:23-dibromide (2 g.) in chloroform (50 c.c.) was treated dropwise with a solution of perbenzoic acid (3 atoms of oxygen) in chloroform over a period of 3 hours at 0°. The solution was kept at -5° for 2 days and evaporated to dryness under reduced pressure at 30-35°. A solution of the partially crystalline solid in glacial acetic acid was treated with zinc dust (20 g.) added portionwise over a period of 6 hours with stirring at 100°. Working up in the usual manner gave a crystalline solid, m.p.190° (400 mg.) a solution of which in light petroleum (b.p.60-80°)-benzene (50 c.c.; 1:1) was chromatographed on activated alumina (2 x 12 cm.). A light petroleum-benzene (150 c.c.; 1:1) eluate gave ergosteryl-D acetate which crystallised from methanol in plates, m.p.173-176° (50 mg.), $[\alpha]_D$ +25° (c, 0.6). Light absorption: Maxima at 2350 and 2430 Å (ε = 16,500 and 17,500 respectively). It gives

a red-brown colour with tetranitromethane in chloroform. A benzene eluate (600 c.c.) gave 3β-acetoxyergosta-8:22--dien-7-one, which crystallised from methanol in plates, m.p.208-210° (100 mg.), $[\alpha]_D$ -54° (c, 0.7). Light absorption: Maximum at 2540 Å (ε = 9100). It gives a yellow colour with tetranitromethane in chloroform. Washing the column with ether-methanol (100 c.c.; 20:1) gave a product, m.p.170-180° (200 mg.). Light absorption: Maximum at 2520 Å (ε = 7200) undepressed when mixed with 3β-acetoxy-11α-hydroxyergosta-8:22-dien-7-one, m.p.186--189°. This proved difficult to purify; acetylation using pyridine (2 c.c.) and acetic anhydride (2 c.c.) at 100° for 3 hours gave 3β:11α-diacetoxyergosta-8:22-dien--7-one as flat needles from methanol, m.p.173-175°, $[\alpha]_D$ +12° (c, 0.9) undepressed in m.p. when mixed with an authentic specimen, m.p.174-176° (Found: C,74.8; H,9.7. Calc. for $C_{32}H_{48}O_5$: C,75.0; H,9.4%). Light absorption: Maximum at 2520 Å (ε = 10,000).

Treatment of 22:23-Dibromo-7ξ:8ξ-9α:11α-diepoxyergostan--3β-yl Acetate with:

(a) Methanolic Hydrogen Chloride.

A solution of 22:23-dibromo-7ξ:8ξ-9α:11α-diepoxy-ergostan-3β-yl acetate (200 mg.) in aqueous methanolic hydrogen chloride (15 c.c.; 2%) was heated under reflux

for 6 hours. Isolation by means of ether gave a product (light absorption: Maximum at 2400 Å, ε = 6600) which was acetylated at 100° (1 hour) using pyridine-acetic anhydride. Working up gave a product which crystallised from methanol to give a compound as needles, m.p.183-185° (100 mg.), $[\alpha]_D$ -45° (c, 1.7) (Found: C,57.3; H,7.8. $C_{30}H_{48}O_4Br_2$ requires C,57.1; H,7.35%). Light absorption: Maximum at 2400 Å (ε = 6600). It does not give a colour with tetranitromethane in chloroform. Infra-red light absorption: Maxima at 1726 and 1240 cm.$^{-1}$ (acetate) and 1668 cm.$^{-1}$ ($\alpha\beta$-ketone).

Chromatography of a benzene solution of the substance on alumina gave needles (from methanol), m.p.188-189°, $[\alpha]_D$ -46° (c, 1.6), undepressed in m.p. with the specimen described above. Light absorption: Maximum at 2400 Å (ε = 7400).

(b) Boron Trifluoride.

A solution of 22:23-dibromo-7ξ:8ξ-9α:11α-diepoxy-ergostan-3β-yl acetate (300 mg.) in dry benzene (20 c.c.) was treated with redistilled boron trifluoride etherate (6 drops) and the solution kept at room temperature for 3 days. The solution was diluted with ether (20 c.c.) and shaken successively with water, sodium hydrogen carbonate solution, water and dried (Na_2SO_4). The solid

(250 mg.), obtained on removal of the solvent under reduced pressure, was dissolved in light-petroleum (b.p. 60-80°)-benzene (1:1; 25 c.c.) and the solution filtered through a column of alumina (10 x 2 cm.). Washing with the same mixture of solvents gave no solid. Washing with benzene (300 c.c.) gave a product (50 mg.), which crystallised from methanol in needles, m.p.182-183°. Light absorption: Maximum at 2400 Å (ε = 6400). Evaporation of the benzene-ether fraction (200 c.c.; 5:1) gave a solid (180 mg.) which crystallised from methanol in needles, m.p.185-187°, $[\alpha]_D$ -47° (c, 1.4) (Found: C,57.0; H,7.8%). Light absorption: Maximum at 2400 Å (ε = 7000). It is undepressed in m.p. when mixed with the specimen described above.

(c) Hydrogen Bromide in Acetic Acid.

A solution of the dibromo-diepoxide (8 g.) in acetic acid (800 c.c.) was treated with aqueous hydrogen bromide (80 c.c.; 48%) added in portions with shaking. The solution was kept at room temperature for 48 hours. The crystalline by-product (3β-acetoxy-22:23-dibromo-9α:11α--epoxyergostan-7-one (m.p.238-240°, 1.4 g.), separating from the solution, was removed by filtration, the filtrate diluted with water and extracted with ether. Working up in the usual manner gave a product which crystallised

from methanol in needles, m.p.186-188° (5.5 g.), $[\alpha]_D$ -47° (c, 1.5) (Found: C,57.2; H,7.7%). Light absorption: Maximum at 2400 Å (ε = 6100). Infra-red light absorption: Maxima at 1726, 1668 and 1240 cm.$^{-1}$.

Chromatography on alumina (benzene solution) gave needles (from methanol), m.p.190-191°, $[\alpha]_D$ -48° (c,1.1), undepressed in m.p. when mixed with the specimens described above. Light absorption: Maximum at 2400 Å (ε = 6300) (The intensity varied from 5700 to 7000 in different experiments performed).

(d) Acetic Acid at 100°.

A solution of the dibromo-diepoxide (500 mg.) in acetic acid (30 c.c.) was heated on the steam bath for 45 minutes. Working up gave a product which crystallised from methanol in felted needles, m.p.187-189° (430 mg.), $[\alpha]_D$ -46° (c, 1.2), showing no depression of mixed m.p. with the specimens described above. Light absorption: Maximum at 2400 Å (ε = 6500).

The dibromo-diepoxide was recovered unchanged (a) after keeping its acetic acid solution at room temperature overnight, and (b) after treatment with 2N sulphuric acid in tetrahydrofuran for 4 hours.

The diepoxide was recovered unchanged after heating its acetic acid solution at 100° for 2 hours.

The 2:4-Dinitrophenylhydrazone of the acetate was obtained (in methanol with Brady's reagent) as yellow, felted needles (from methanol-methylene chloride), m.p. 212-214° (Found: C,53.3; H,6.1. $C_{36}H_{50}O_7N_4Br_2$ requires C,53.3; H,6.2%). Light absorption: Maxima at 2360 Å (ε = 19,600) and 3600 Å (ε = 24,000) (wide bands). Infra-red light absorption: Bands at 1732 and 1233 cm.$^{-1}$ (acetate), 1668 cm.$^{-1}$ ($\alpha\beta$-ketone), 1611, 1585 and 1510 cm.$^{-1}$ (substituted aromatic ring, etc.).

Hydrolysis of the rearranged compound (m.p.184-186°, ε_{3400} = 6100; 500 mg.) effected by heating in aqueous methanolic potassium hydroxide (3%) under reflux for 3½ hours, gave a product which crystallised from methanol in needles (470 mg.), m.p.147-149° and 242-245° (sec.). further crystallisations from methanol gave needles, m.p.148-150° and 244-247° (sec.), $[\alpha]_D$ -45° (c, 1.3) (Found: C,57.0 and 57.1; H,8.0 and 8.05. $C_{28}H_{44}O_5Br_2$ requires C,57.1; H,7.6%). Ultra-violet light absorption: Maximum at 2400 Å (ε = 6200). Infra-red light absorption: Maxima at 3460 (hydroxyl) and 1676 cm.$^{-1}$ ($\alpha\beta$-ketone).

The 2:4-Dinitrophenylhydrazone of the alcohol was obtained (in methanol with Brady's reagent) as yellow, felted needles, m.p.226-227° (from methanol-methylene chloride) (Found: C,53.3; H,6.0. $C_{34}H_{48}O_8N_4Br_2$

requires C,53.2; H,6.3%). Ultra-violet light absorption: Maxima at 2360 Å (ε = 20,000) and 3600 Å (ε = 24,000). Infra-red light absorption: Bands at 3240 (hydroxyl), 1668 (αβ-ketone) 1611, 1585, 1510, 1324 and 1269 cm.$^{-1}$.

Acetylation of the alcohol using pyridine-acetic anhydride gave the parent acetate which crystallised from methanol in needles, m.p.190-191°, $[\alpha]_D$ -47° (c, 1.1) (Found: C,57.3; H,7.7%). Light absorption: Maximum at 2400 Å (ε = 6800). It is undepressed in m.p. when mixed with the specimens described above.

Chromic Acid Oxidation of the acetate (m.p.186-188°, ε_{2400} = 6100) in acetic acid at room temperature (12-24 hours) resulted in recovery of the starting material of higher m.p. (202-204° from methanol), $[\alpha]_D$ -52° (c, 1.5), undepressed when mixed with the starting material (Found: C,57.1; H,7.8%). Light absorption: Maximum at 2400 Å (ε = 6300-7200 from several attempted experiments).

Treatment with Ethanolic Hydrogen Chloride (3%) under reflux for 4 hours followed by acetylation and chromatography gave starting material of higher melting point (204-206° from methanol), $[\alpha]_D$ -49° (c, 1.0) (Found: C,57.6; H,7.4%). Ultra-violet light absorption: Maximum at 2400 Å (ε = 6600-7400 from several experiments).

Debromination of the acetate (1 g.) in ether-methanol

with zinc dust (refluxing for 3 hours) gave a gum which resisted all attempts to induce crystallisation. Chromatography on alumina (benzene solution) gave fractions of gum which crystallised with difficulty, after prolonged standing in contact with methanol allowing slow evaporation for several days, to yield a compound as soft, felted needles, m.p.76-79° (pool cleared at 84°), $[\alpha]_D$ -60° (c, 1.1) (Found: C,76.55; H,10.1. $C_{30}H_{48}O_4$ requires C,76.55; H,9.85%). Ultra-violet light absorption: Maximum at 2400 Å (ε = 5600).

The 2:4-Dinitrophenylhydrazone of the debrominated compound (gum)(in methanol with Brady's reagent) crystallised beautifully in yellow, prismatic needles from methanol, m.p.132-135° (Found: C,66.1; H,7.6. $C_{36}H_{50}O_7N_4$ requires C,66.4; H,7.7%). Ultra-violet light absorption: Maxima at 2380 Å (ε = 19,000) and 3600 Å (ε = 23,000). Infra-red light absorption: Maxima at 1729 and 1233 cm.$^{-1}$ (acetate), 1668 cm.$^{-1}$ ($\alpha\beta$-ketone), 1611, 1585, 1503 and 1324 cm.$^{-1}$

Treatment of 7ξ:8ξ-9α:11α-Diepoxyergost-22-en-3β-yl Acetate with Hydrogen Bromide in acetic acid (1 g. in 25 c.c. + 2 c.c. 48% HBr) as described for the dibromide (red → blue → green solution) gave a gum, which after chromatography on alumina (in benzene solution) crystallised

slowly from methanol in felted needles, m.p.72-76°, undepressed when mixed with the specimen described above. Light absorption: Maximum at 2400 Å (ε = 5800).

The 2:4-dinitrophenylhydrazone crystallised from methanol in yellow needles, m.p.131-133°, undepressed when mixed with the specimen described above.

Lithium Aluminium Hydride Reduction of the above gum (0.5 g. from debromination experiment) effected by refluxing in ether for 3 hours, followed by acetylation using pyridine-acetic anhydride, gave a gum, which was chromatographed in benzene solution on alumina. The fractions collected crystallised slowly from methanol in clusters of soft prisms, m.p.84-87° (cleared at 94°), $[\alpha]_D$ +37° (c, 0.6) (Found: C,74.8; H,10.2. $C_{32}H_{50}O_5$ requires C,74.7; H,9.8%). It does not exhibit selective light absorption of high intensity above 2200 Å (ε_{2100} = 5200), and gives a yellow colour with tetranitromethane in chloroform.

Similar treatment of the dibromide (m.p.184-186°) gave a gum, which after chromatography on alumina crystallised with difficulty from methanol in soft prisms, m.p.80-83°, undepressed when mixed with the specimen described above. It does not show selective light absorption of high intensity above 2200 Å.

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22:23-Dibromo-7ξ:11α-dihydroxyergost-8-en-3β-yl Acetate.

(a) Powdered 22:23-dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate (600 mg.) was shaken vigorously in dioxan (50 c.c.) containing aqueous sulphuric acid (2N; 10 c.c.). After a few seconds solution was complete and almost immediately solid separated. After 15 minutes the crystalline solid was filtered off and washed successively with methanol, water, methanol, and ether (small prisms, m.p.204-205°; 420 mg.). Two crystallisations from pyridine gave 22:23-dibromo-7ξ:11α-dihydroxyergost-8-en-3β-yl acetate as needles, m.p.216-217°, $[\alpha]_D$ +93° (c, 0.3 in pyridine) (Found: C,56.8; H,7.8. $C_{30}H_{48}O_4Br_2$ requires C,57.0; H,7.65%). It does not show selective absorption of high intensity above 2200 Å; it is very sparingly soluble in most organic solvents including chloroform.

(b) Attempted crystallisation of the dibromo-epoxide from technical acetone led to the separation of a microcrystalline solid, m.p.200° which is very insoluble in most organic solvents, including chloroform, and is undepressed in m.p. when mixed with 22:23-dibromo-7ξ:11α-dihydroxyergost-8-en-3β-yl acetate, m.p.204° (Found: C,56.6; H,8.0%).

(c) 9α:11α-Epoxyergosta-7:22-dien-3β-yl acetate

(200 mg.) in chloroform (10 c.c.) was treated dropwise with a solution of bromine (1 mol.) in chloroform during 20 minutes at -4°. The colourless solution was kept overnight at -5°. Chloroform was removed under reduced pressure at room temperature. The residue separated from acetone as a microcrystalline powder (100 mg.), very insoluble in most organic solvents including chloroform, and has m.p. 200°, undepressed when mixed with 22:23-dibromo-7ξ:11α-dihydroxyergost-8--en-3β-yl acetate, m.p.204°.

(d) [Preparative method.] A solution of 22:23--dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate (20 g.) in tetrahydrofuran (100-105 c.c.) was treated with aqueous sulphuric acid (2N; 6 c.c.) added in one portion with shaking. The solution was kept at room temperature for 4 hours. The crystalline solid which separated, was collected and washed well with methanol (16.5 g.; m.p. 207-210°).

3β:7ξ:11α-Triacetoxy-22:23-dibromoergost-8-ene.

A suspension of 22:23-dibromo-7ξ:11α-dihydroxyergost--8-en-3β-yl acetate (400 mg.) in pyridine (15 c.c.) and acetic anhydride (15 c.c.) was heated on the steam bath for 30 minutes after which time solution was complete; the solution was kept overnight at room temperature.

Most of the solvents were removed under reduced pressure at 60-80°, the residue diluted with water, and the product isolated by means of ether. Crystallisation from methanol gave 3β:7ξ:11α-triacetoxy-22:23-dibromo-ergost-8-en as prisms, m.p.171-172° (350 mg.), $[\alpha]_D$ +77° (c, 1.9) (Found: C,56.8; H,7.5. $C_{34}H_{52}O_6Br_2$ requires C,57.0; H,7.3%). It gives a pale yellow colour with tetranitromethane in chloroform and does not show selective absorption of high intensity above 2200 Å.

3β:7ξ:11α-Triacetoxyergosta-8:22-diene.

A solution of 3β:7ξ:11α-triacetoxy-22:23-dibromo-ergost-8-ene (100 mg.) in ether-methanol (30 c.c.; 1:1) was refluxed for 3 hours with zinc dust (2 g.) added portionwise. After filtration, water was added and the product isolated by means of ether. Crystallisation from methanol gave 3β:7ξ:11α-triacetoxyergosta-8:22-diene as prismatic needles, m.p.172° (90 mg.), $[\alpha]_D$ +88° (c, 1.2) (Found: C,73.5; H,9.6. $C_{34}H_{52}O_6$ requires C,73.3; H,9.4%). It gives a pale yellow colour with tetranitromethane in chloroform and does not exhibit selective absorption of high intensity above 2200 Å. Mixed m.p. with a specimen m.p.172-173°, $[\alpha]_D$ +90°, obtained by acetylation of 7ξ:11α-dihydroxyergost-8-en-

-3β-yl acetate, was undepressed.

7ξ:11α-Dihydroxyergosta-8:22-dien-3β-yl Acetate.

A solution of 22:23-dibromo-7ξ:11α-dihydroxyergost--8-en-3β-yl acetate (m.p.204°; 200 mg.) in pyridine (30 c.c.) containing water (3 drops) was heated with zinc dust (2 g.; added in portions) for 3 hours on the steam bath. The mixture was filtered and the filtrate evaporated to dryness under reduced pressure. The residue separated from acetone in felted needles, m.p. 231-234° (150 mg.), which on recrystallisation from methanol separated as prismatic needles, m.p.251-253° (unchanged after drying for 12 hours at 100° and 0.01 mm.), $[\alpha]_D$ +85° (c, 0.4) identical with a specimen obtained by the treatment of ergosteryl-D acetate epoxide with sulphuric acid in dioxan (m.p.248-250°, $[\alpha]_D$ +83°). It gives a pale yellow colour with tetranitromethane in chloroform and does not exhibit selective absorption of high intensity above 2200 Å (Found: C,76.6; H,10.5. Calc. for $C_{30}H_{48}O_4$: C,76.2; H,10.2%). Chamberlin, Ruyle, Erickson, Chemerda, Aliminosa, Erickson, Sita, and Tishler (29) give m.p.248-252°, $[\alpha]_D$ +85°, and Heusser, Eichenberger, Kurath, Dällenbach and Jeger (36) give m.p.270-272°, $[\alpha]_D$ +82°.

22:23-Dibromo-8α:9α-epoxy-7:11-diketoergostan-3β-yl Acetate.

A suspension of 22:23-dibromo-7ξ:11α-dihydroxyergost--8-en-3β-yl acetate (m.p.204°; 3.6 g.) in glacial acetic acid (400 c.c.) was treated portionwise with a solution of chromic anhydride in acetic acid (1.05N; 2.5 O), the mixture stirred for 2 hours at room temperature, and then kept overnight. After adding a little methanol, the solution was concentrated under reduced pressure and diluted with water. The product, isolated by means of ether, separated from methanol-chloroform as plates (1.7 g.), m.p.240°. A solution of this solid in benzene (100 c.c.) was chromatographed on activated alumina (2 x 8 cm.). Evaporation of the first benzene fraction (300 c.c.) gave a solid (200 mg.) which after crystallisation from methanol gave 22:23-dibromo-8α:9α-epoxy-7:11--diketoergostan-3β-yl acetate as flat needles, m.p.210--212°, $[\alpha]_D$ -44° (c, 1.8) (Found: C,56.2; H,7.2. $C_{30}H_{44}O_5Br_2$ requires C,55.9; H,6.9%). It gives no coloration with tetranitromethane in chloroform and does not show selective absorption of high intensity above 2000 Å.

22:23-Dibromo-7:11-diketoergost-8-en-3β-yl Acetate.

Evaporation of the second benzene fraction (500 c.c.) from the above chromatogram gave 22:23-dibromo-7:11--diketoergost-8-en-3β-yl acetate (600 mg.) separating from methanol-chloroform as hexagonal plates, m.p.250--251°, $[\alpha]_D$ +27, +24° (c, 1.9, 0.9) (Found: C,57.2; H,7.25. $C_{30}H_{44}O_4Br_2$ requires C,57.3; H,7.05%). Light absorption: Maximum at 2690 Å (ε = 8200). It does not give a colour with tetranitromethane in chloroform.

7:11-Diketoergost-22-en-3β-yl Acetate.

(a) A solution of 22:23-dibromo-7:11-diketoergost--8-en-3β-yl acetate (50 mg.) in glacial acetic acid (25 c.c.) was treated with zinc dust (1 g.) and heated on the steam bath for 3 hours and then boiled under reflux for 15 minutes. Isolation of the product using ether gave 7:11-diketoergost-22-en-3β-yl acetate (30 mg.) as needles from methanol, m.p.195-196°, $[\alpha]_D$ -26° (c,0.4) (Found: C,76.4; H,10.0. Calc. for $C_{30}H_{46}O_4$: C,76.55; H,9.85%); it is undepressed in melting point when mixed with an authentic specimen prepared as described by Budziarek et al. (loc.cit.). It gives a faint yellow coloration with tetranitromethane in chloroform and does not show high intensity light absorption above 2200 Å.

(b) Similar reduction of 22:23-dibromo-8α:9α-epoxy--7:11-diketoergostan-3β-yl acetate (100 mg.) with zinc dust and acetic acid gave a product isolated by means of ether. A solution of this product in benzene (20 c.c.) was filtered through a column of alumina (5 x 1 cm.) and the column washed with benzene (250 c.c.). Evaporation of the benzene filtrate gave 7:11-diketoergost-22-en-3β-yl acetate (60 mg.), m.p.197-198°, $[\alpha]_D$ -28° (c, 1.2); it is undepressed in m.p. when mixed with the specimen described under (a).

7:11-Diketo-8α-ergost-22-en-3β-yl Acetate.

(a) A solution of 22:23-dibromo-7:11-diketoergost--8-en-3β-yl acetate (150 mg.) in ether-methanol (1:1; 200 c.c.) was heated under reflux with zinc dust (2 g.) added portionwise during 3 hours. The mixture was filtered and the solution slightly concentrated, when small hexagonal plates separated. The solution was cooled, the solid collected, washed with methanol and dried (100 mg.), m.p.200-204°. Two recrystallisations from acetone gave 7:11-diketo-8α-ergost-22-en-3β-yl acetate as hexagonal plates, m.p.204-206°, $[\alpha]_D$ +30°, +28° (c, 0.6, 0.5) (Found: C,76.7; H,10.1. $C_{30}H_{46}O_4$ requires C,76.5; H,9.85%). It does not show light

absorption of high intensity above 2200 Å. A mixture with 7:11-diketoergost-22-en-3β-yl acetate (m.p.197-198°, $[\alpha]_D$ -28°) had m.p.178-198°.

(b) Treatment of 22:23-dibromo-8α:9α-epoxy-7:11--diketoergostan-3β-yl acetate (60 mg.) in refluxing ether-methanol (1:1; 80 c.c.) with zinc dust (1 g.) for 3 hours gave 7:11-diketo-8α-ergost-22-en-3β-yl acetate (35 mg.) as hexagonal plates from acetone, m.p.203-206°, $[\alpha]_D$ +27° (c, 0.5), undepressed in m.p. when mixed with the specimen described under (a).

(c) Similar treatment of either 7:11-diketoergost--8:22-dien-3β-yl acetate or 8α:9α-epoxy-7:11-diketoergost--22-en-3β-yl acetate gave 7:11-diketo-8α-ergost-22-en--3β-yl acetate as described earlier.

7:11-Diketoergost-22-en-3β-yl Acetate.

7:11-Diketo-8α-ergost-22-en-3β-yl acetate (80 mg., $[\alpha]_D$ +30°) in glacial acetic acid (3 c.c.) was heated on the steam bath for 45 minutes. The solution was diluted with water and extracted with ether. Removal of ether gave a solid, which was crystallised from methanol to yield 7:11-diketoergost-22-en-3β-yl acetate (70 mg.) as small, prismatic needles, m.p.196-198°, $[\alpha]_D$ -28° (c, 1.2) (Found: C,76.7; H,10.0. Calc. for $C_{30}H_{46}O_4$: C,76.5;

H,9.85%). The diketone does not show high intensity light absorption above 2200 Å. It is undepressed in m.p. when mixed with a specimen prepared as described above (109).

22:23-Dibromo-8α:9α-epoxy-7ξ:11α-dihydroxyergostan-3β-yl Acetate.

A solution of perbenzoic acid in chloroform (5%; 1.1 mol.) was added slowly with stirring at 0° to a suspension of 22:23-dibromo-7ξ:11α-dihydroxyergost-8-en-3β-yl acetate (15 g.) in chloroform (150 c.c.) and the mixture kept at room temperature for 4 hours, when solution was complete. The mixture was washed with water, sodium hydrogen carbonate solution, water, and dried ($MgSO_4$). The solvent was removed under reduced pressure and the solid crystallised twice from acetone from which 22:23--dibromo-8α:9α-epoxy-7ξ:11α-dihydroxyergostan-3β-yl acetate (12 g.) separated as needles, m.p.245-246°, $[\alpha]_D$ +16°, +15° (c, 2.0, 1.6) (Found: C,55.3; H,7.7. $C_{30}H_{48}O_5Br_2$ requires C,55.6; H,7.5%).

3β:7ξ:11α-Triacetoxy-22:23-dibromo-8α:9α-epoxyergostane.

A solution of 22:23-dibromo-8α:9α-epoxy-7ξ:11α--dihydroxyergostan-3β-yl acetate (700 mg.) in pyridine (5 c.c.) and acetic anhydride (5 c.c.) was heated on the

steam bath for 3 hours. The product, isolated by means of ether, separated from methanol-chloroform as prismatic needles (740 mg.), m.p.214-215°. Two recrystallisations from the same solvent gave 3β:7ξ:11α-triacetoxy-22:23--dibromo-8α:9α-epoxyergostane, m.p.220-221°, $[\alpha]_D$ +4°, +4° (c, 1.0, 4.0) (Found: C,55.4; H,7.3. $C_{34}H_{52}O_7Br_2$ requires C,55.7; H,7.2%).

3β:7ξ:11α-Triacetoxy-8α:9α-epoxyergost-22-ene.

A solution of 3β:7ξ:11α-triacetoxy-22:23-dibromo--8α:9α-epoxyergostane (400 mg.) in ether-methanol (1:1; 100 c.c.) was refluxed with zinc dust (4 g.) added portionwise over 3 hours. Removal of zinc by filtration and concentration of the solution gave prisms (300 mg.), m.p.160-162°. Two recrystallisations from methanol gave 3β:7ξ:11α-triacetoxy-8α:9α-epoxyergost-22-ene as prisms, m.p.165-166°, $[\alpha]_D$ +3° (c, 3.0) (Found: C,71.6; H,9.3. Calc. for $C_{34}H_{52}O_7$: C,71.3; H,9.15%). Heusser, Anliker, Eichanberger and Jeger (37) give m.p.158-159°, $[\alpha]_D$ +6° for this compound.

8α:9α-Epoxy-7ξ:11α-dihydroxyergost-22-en-3β-yl Acetate.

A solution of 22:23-dibromo-8α:9α-epoxy-7ξ:11α--dihydroxyergostan-3β-yl acetate (500 mg.) in ether-methanol (1:1; 100 c.c.) was heated under reflux with zinc dust

(5 g.) added portionwise over 3 hours. Removal of zinc by filtration and concentration of the solution with an addition of a few drops of water, gave felted needles (340 mg.), m.p.125-130°. Four recrystallisations from aqueous methanol gave 8α:9α-epoxy-7ξ:11α-dihydroxyergost--22-en-3β-yl acetate as felted needles, m.p.130-131° (unchanged after prolonged drying at 100°, 10^{-3}mm. pressure, $[\alpha]_D$ +19°, +18° (c, 1.3, 1.2) (Found: C,73.5; H,10.0. Calc. for $C_{30}H_{48}O_5$: C,73.7; H,9.9%). Heusser et al. (37) give m.p.147-148°, $[\alpha]_D$ +16° for this compound.

Acetylation of 8α:9α-epoxy-7ξ:11α-dihydroxyergost--22-en-3β-yl acetate using pyridine and acetic anhydride gave 3β:7ξ:11α-triacetoxy-8α:9α-epoxyergost-22-ene as prisms from methanol, m.p.164.5-166°, $[\alpha]_D$ +2° (c, 2.9), undepressed in m.p. when mixed with the specimen described above.

22:23-Dibromo-8α:9α-epoxy-3β:7ξ:11α-trihydroxyergostane.

(a) A solution of 22:23-dibromo-8α:9α-epoxy-7ξ:11α--dihydroxyergostan-3β-yl acetate (700 mg.) in methanolic potassium hydroxide (2%; 50 c.c.) was heated under reflux for 2 hours. The solution was diluted with water, the solid collected (very sparingly soluble in ether), and washed with water until the filtrate was neutral to litmus.

After drying, the product was crystallised from acetone giving needles (550 mg.), m.p.235-237°. Two recrystallisations from acetone gave 22:23-dibromo-8α:9α-epoxy--3β:7ξ:11α-trihydroxyergostane as needles, m.p.241-242°, $[\alpha]_D$ +29° (c, 0.5) (Found: C,55.7; H,7.75. $C_{28}H_{46}O_4Br_2$ requires C,55.45; H,7.65%).

(b) A solution of 3β:7ξ:11α-triacetoxy-22:23-dibromo--8α:9α-epoxyergostane (100 mg.) in methanolic potassium hydroxide (2%; 50 c.c.) and benzene (2 c.c.) was heated under reflux for 2 hours. Crystallisation of the product from acetone gave 22:23-dibromo-3β:7ξ:11α-trihydroxy--8α:9α-epoxyergostane (60 mg.) as needles, m.p.239-240°, $[\alpha]_D$ +27° (c, 0.4) undepressed in m.p. when mixed with the specimen described above.

Acetylation of 22:23-dibromo-8α:9α-epoxy-3β:7ξ:11α--trihydroxyergostane using pyridine and acetic anhydride gave 3β:7ξ:11α-triacetoxy-22:23-dibromo-8α:9α-epoxy-ergostane which separated as prismatic needles from methanol-chloroform, m.p.220-221°, $[\alpha]_D$ +4° (c, 2.8) undepressed in m.p. when mixed with the specimen described above.

8α:9α-Epoxy-3β:7ξ:11α-trihydroxyergost-22-ene.

(a) A solution of 8α:9α-epoxy-7ξ:11α-dihydroxyergost--22-en-3β-yl acetate (300 mg.) in methanolic potassium

hydroxide (1%; 60 c.c.) was heated under reflux for 2 hours. The product, isolated by means of ether, separated from acetone in flat needles (250 mg.), m.p. 160-163°. Three recrystallisations from acetone gave 8α:9α-epoxy-3β:7ξ:11α-trihydroxyergost-22-ene as flat needles, m.p.166-167°, $[\alpha]_D$ +32° (c, 1.0) (Found: C,74.9; H,10.5. $C_{28}H_{46}O_4$ requires C,75.3; H,10.4%).

(b) A solution of 22:23-dibromo-8α:9α-epoxy-3β:7ξ:11α--trihydroxyergostane (250 mg.) in ether-methanol (1:1; 60 c.c.) was heated under reflux with zinc dust added portionwise over 3 hours. Removal of zinc by filtration and concentration of the solution gave flat needles (170 mg.), m.p.164-166°, which on recrystallisation from acetone gave 8α:9α-epoxy-3β:7ξ:11α-trihydroxyergost-22--ene, m.p.166-167°, $[\alpha]_D$ +31° (c, 0.9) showing no depression in m.p. when mixed with the specimen described above.

(c) A solution of 3β:7ξ:11α-triacetoxy-8α:9α-epoxy-ergost-22-ene (100 mg.) in methanolic potassium hydroxide (2%; 40 c.c.) was heated under reflux for 2 hours. The product, isolated by means of ether, was crystallised from acetone to give 8α:9α-epoxy-3β:7ξ:11α-trihydroxy-ergost-22-ene (60 mg.) as flat needles, m.p.165-166°, $[\alpha]_D$ +30° (c, 0.8) undepressed in m.p. when mixed with the specimens described above.

Acetylation of 8α:9α-epoxy-3β:7ξ:11α-trihydroxy-ergost-22-ene using pyridine and acetic anhydride gave 3β:7ξ:11α-triacetoxy-8α:9α-epoxyergost-22-ene which separated from methanol as prisms, m.p.164-166°, $[\alpha]_D$ +3° (c, 2.7) showing no depression in m.p. when mixed with the specimen described above.

22:23-Dibromo-8α:9α-epoxy-7:11-diketoergostan-3β-yl Acetate. (with Dr. G.T. Newbold).

A solution of 22:23-dibromo-8α:9α-epoxy-7 :11α--dihydroxyergostan-3β-yl acetate (216 mg.) in glacial acetic acid (15 c.c.) was treated with a solution of chromium trioxide in acetic acid (1.7 c.c.; 1N) diluted with glacial acetic acid (10 c.c.) added over $1\frac{1}{2}$ hours with stirring at room temperature. After standing overnight the solution was heated to 45-50° for 30 minutes, treated with methanol, evaporated to small bulk under reduced pressure and diluted with water. The product was isolated by means of ether and crystallised from methanol-chloroform to give 22:23-dibromo-8α:9α-epoxy-7:11--diketoergostan-3β-yl acetate as flat needles (160 mg.), m.p.210-212°, $[\alpha]_D$ -43° (c, 1.0) (Found: C,55.9; H,7.2. Calc. for $C_{30}H_{44}O_5Br_2$: C,55.9; H,6.9%). It was undepressed in m.p. when mixed with a specimen prepared by Budziarek, Johnson and Spring (loc.cit.).

3β-Acetoxy-22:23-dibromo-9α:11α-dihydroxyergostan-7-one.

A solution of 22:23-dibromo-8α:9α-epoxy-7ξ:11α--dihydroxyergostan-3β-yl acetate (5 g.) in glacial acetic acid (40 c.c.) was treated with aqueous hydrogen bromide (48%; 1.5 c.c.) at room temperature and the mixture kept for 1 hour. The crystalline solid which separated from the blue solution was collected, washed successively with a little acetic acid and methanol and dried (4.2 g.; m.p. 246-248°). Three recrystallisations from acetone gave 3β-acetoxy-22:23-dibromo-9α:11α-dihydroxyergostan-7-one as hexagonal prisms, m.p.250-251°, $[\alpha]_D$ -36°, -35° (c, 2.5, 2.1) (Found: C,55.6; H,7.6. $C_{30}H_{48}O_5Br_2$ requires C,55.6; H,7.5%).

3β-Acetoxy-9α:11α-dihydroxyergost-22-en-7-one.

(a) A solution of 3β-acetoxy-22:23-dibromo-9α:11α--dihydroxyergostan-7-one in ether-methanol (1:1) was heated under reflux with zinc dust for 4 hours. The product, isolated in the usual manner, was 3β-acetoxy--9α:11α-dihydroxyergost-22-en-7-one (yield, quantitative) which separated from methanol as rectangular plates, m.p. 267-269°, $[\alpha]_D$ -69°, -67.5° (c, 1.0, 0.8), undepressed in m.p. when mixed with the specimen described by Budziarek, Newbold, Stevenson and Spring (108) (Found: C,74.0; H,10.1. Calc. for $C_{30}H_{46}O$: C,73.7; H,9.9%).

(b) A solution of 22:23-dibromo-8α:9α-epoxy-7ξ:11α--dihydroxyergostan-3β-yl acetate (400 mg.) in acetic acid (30 c.c.) was heated on the steam bath with zinc dust (4 g.) added portionwise over 4 hours with stirring. Working up using ether, gave a product which crystallised from methanol in rectangular plates (210 mg.), m.p.262-263°. Recrystallisation from methanol gave 3β-acetoxy-9α:11α--dihydroxyergost-22-en-7-one, m.p.267-269°, $[\alpha]_D$ -67° (c, 0.9), showing no depression in m.p. when mixed with the specimen described above.

3β:9α:11α-Trihydroxyergost-22-en-7-one.

Hydrolysis of 3β-acetoxy-9α:11α-dihydroxyergost-22--en-7-one (100 mg.) using 2% methanolic potassium hydroxide (30 c.c.), gave 3β:9α:11α-trihydroxyergost-22-en-7-one (70 mg.) which separated from acetone (or methanol) in flat needles, m.p.258-259°, $[\alpha]_D$ -71° (c, 1.1) (Found: C,75.2; H,10.4. $C_{28}H_{46}O_4$ requires C,75.3; H,10.4%).

3β:11α-Diacetoxy-22:23-dibromo-9α-hydroxyergostan-7-one.

A solution of 3β-acetoxy-22:23-dibromo-9α:11α--dihydroxyergostan-7-one (400 mg.) in pyridine (20 c.c.) and acetic anhydride (10 c.c.) was heated on the steam bath for 3 hours. The solid separating from the mixture on standing was collected, washed with methanol and dried

(330 mg.; m.p.256-257°). After two recrystallisations from acetone (or methanol-chloroform) 3β:11α-diacetoxy--22:23-dibromo-9α-hydroxyergostan-7-one was obtained as felted needles, m.p.259-260°, $[\alpha]_D$ -29°, -27° (c, 1.6, 0.9) (Found: C,55.65; H,7.5. $C_{32}H_{50}O_6Br_2$ requires C,55.65; H,7.3%).

3β:11α-Diacetoxy-9α-hydroxyergost-22-en-7-one.

(a) 3β:11α-Diacetoxy-22:23-dibromo-9α-hydroxyergostan--7-one (150 mg.) was debrominated by heating its solution in ether-methanol (1:2; 100 c.c.) with zinc dust (2 g.) for 4 hours under reflux. The reaction product, isolated in the usual manner, crystallised from methanol to yield 3β:11α-diacetoxy-9α-hydroxyergost-22-en-7-one (100 mg.) as needles from methanol, m.p.194-196°, $[\alpha]_D$ -43° (c, 1.0), undepressed in m.p. when mixed with the specimen described by Budziarek et al. (108) (Found: C,72.6; H,9.6. Calc. for $C_{32}H_{50}O_6$: C,72.4; H,9.5%).

(b) Acetylation of 3β:9α:11α-trihydroxyergost-22-en--7-one using pyridine and acetic anhydride gave 3β:11α--diacetoxy-9α-hydroxyergost-22-en-7-one as needles from methanol, m.p.193-195°, $[\alpha]_D$ -42° (c, 0.8), undepressed in m.p. when mixed with the specimens described above.

22:23-Dibromo-3β:9α:11α-trihydroxyergostan-7-one.

(a) A solution of 3β-acetoxy-22:23-dibromo-9α:11α--dihydroxyergostan-7-one (300 mg.) in methanolic potassium hydroxide (1%; 200 c.c.) was heated under reflux for 2 hours. The product isolated in the usual manner (250 mg.; m.p.258-260°) was crystallised from acetone to give 22:23-dibromo-3β:9α:11α-trihydroxyergostan--7-one as felted needles, m.p.262-263°, $[\alpha]_D$ -45°, -42° (c, 0.25, 0.3) (Found, after drying in high vacuum over P_2O_5 at 100° for varying periods up to 7 days: C,54.0; 54.1; H,7.75, 7.9. $C_{28}H_{46}O_4Br_2.H_2O$ requires C,53.9; H,7.75%). It is sparingly soluble in chloroform and separates as thick, elongated plates from methanol-chloroform.

Acetylation of the triol, using pyridine and acetic anhydride gave 3β:11α-diacetoxy-22:23-dibromo-9α-hydroxy-ergostan-7-one in quantitative yield; the diacetate separated from acetone as felted needles, m.p.259-260°, $[\alpha]_D$ -28° (c, 0.8), undepressed in m.p. when mixed with the specimen described above (Found: C,55.6; H,7.6%).

Debromination of the triol by refluxing its solution in methanol with zinc dust for 4 hours, gave in quantitative yield, 3β:9α:11α-trihydroxyergost-22-en-7-one which separated from methanol as flat needles, m.p.257-259°, $[\alpha]_D$ -70° (c, 0.7), undepressed in m.p. when mixed with the specimen described above.

(b) A solution of 3β-acetoxy-22:23-dibromo-9α:11α--dihydroxyergostan-7-one (300 mg.) in dry acetone (200 c.c.) was treated with a stream of dry hydrogen chloride for 30 minutes, at 18° and the mixture kept at room temperature for two days. The solvent was removed under reduced pressure and the product isolated by means of ether. Crystallisation from acetone (or methanol) gave 3β:9α:11α-trihydroxy-22:23-dibromoergostan-7-one as small plates, m.p.258-259°, $[\alpha]_D$ -43° (c, 0.2). (Found: C,54.1; H,7.9). It does not exhibit selective absorption of high intensity above 2000 Å and the m.p. of a mixture with the specimen described under (a) was undepressed.

3β:11α-Diacetoxy-22:23-dibromoergost-8-en-7-one.

A solution of 22:23-dibromo-3β:9α:11α-trihydroxy-ergostan-7-one (150 mg.) in 10% methanolic potassium hydroxide (50 c.c.) was refluxed for 18 hours. The product, isolated by means of ether, was acetylated by warming for 1 hour with pyridine and acetic anhydride. The acetylated product, also isolated by means of ether, crystallised from methanol giving 3β:11α-diacetoxy-22:23--dibromoergost-8-en-7-one as needles (70 mg.), m.p.158--160°, $[\alpha]_D$ +18° (c, 0.8) (Found: C,57.3; H,7.5. Calc. for $C_{32}H_{48}O_5Br_2$: C,57.1; H,7.2%). Light absorption:

Maximum at 2520 Å (ε = 9000). It was undepressed in m.p. when mixed with the specimen described by Budziarek, Stevenson and Spring (110).

22:23-Dibromo-3β:11α-dihydroxyergost-8-en-7-one.

A solution of 3β-acetoxy-22:23-dibromo-9α:11α--dihydroxyergostan-7-one (200 mg.) in methanolic potassium hydroxide (10%; 100 c.c.) was heated under reflux for 18 hours. The product, isolated using ether, was crystallised four times from methanol to yield 22:23-dibromo--3β:11α-dihydroxyergost-8-en-7-one (120 mg.) as flat needles, m.p.228-230°, $[\alpha]_D$ +4° (c, 1.8) (Found: C,57.3; H,7.6. Calc. for $C_{28}H_{44}O_3Br_2$: C,57.1; H,7.5%). Light absorption: Maximum at 2520 Å (ε = 8000). The m.p. of a mixture with the specimen described by Budziarek, Stevenson and Spring (110) was undepressed.

22:23-Dibromo-9α-hydroxy-7:11-diketoergostan-3β-yl Acetate.

A solution of 3β-acetoxy-22:23-dibromo-9α:11α--dihydroxyergostan-7-one (4.0 g.) in glacial acetic acid (1000 c.c.) was treated with a solution of chromium trioxide in glacial acetic acid (1 N; 25 c.c.) added dropwise over 30 minutes at 35-40° with stirring. The solution was kept at room temperature overnight, treated with a little methanol and concentrated under reduced

pressure. After dilution with water, the product was isolated by means of ether, and crystallised from methanol-chloroform to give 22:23-dibromo-9α-hydroxy-7:11--diketoergostan-3β-yl acetate (1.6 g.) as blades, m.p.256--258°, $[\alpha]_D$ +1.5° (c, 4.5) (Found: C,55.7; H,7.3. $C_{30}H_{46}O_5Br_2$ requires C,55.7; H,7.2%). It does not exhibit light absorption of high intensity above 2000 Å.

22:23-Dibromo-9α-hydroxy-7:11-diketoergostan-3β-yl acetate was recovered unchanged (a) after heating its solution in acetic anhydride under reflux for 24 hours, and (b) after heating its solution in acetic anhydride containing concentrated hydrochloric acid (3 drops) under reflux for 12 hours.

22:23-Dibromo-3β:9α-dihydroxyergostan-7:11-dione.

A solution of 22:23-dibromo-9α-hydroxy-7:11-diketo-ergostan-3β-yl acetate (150 mg.) in methanolic potassium hydroxide (1%; 200 c.c.) was refluxed for 30 minutes. The product, isolated in the usual manner, crystallised from acetone or methanol to give 22:23-dibromo-3β:9α--dihydroxyergostan-7:11-dione (120 mg.) as needles, m.p. 234-235°, $[\alpha]_D$ +16° (c, 1.7) (Found: C,56.0; H,7.5. $C_{28}H_{44}O_4Br_2$ requires C,55.6; H,7.3%).

Reacetylation of the alcohol (50 mg.) using pyridine (0.5 c.c.) and acetic anhydride (1 c.c.) at 100° gave the

acetate (40 mg.) which separates from methanol-chloroform as blades, m.p.256-258°, $[\alpha]_D$ +2° (c, 1.6), undepressed in m.p. when mixed with the specimen described above.

3β-Acetoxy-9α-hydroxy-7:11-diketoergost-22-ene.

(a) A solution of 22:23-dibromo-9α-hydroxy-7:11--diketoergostan-3β-yl acetate (160 mg.) in ether-methanol (1:1; 120 c.c.) was heated under reflux with zinc dust (2 g.) added portionwise over $3\frac{1}{2}$ hours. The product was isolated by means of ether and crystallised from aqueous methanol to give 3β-acetoxy-9α-hydroxy-7:11--diketoergost-22-ene (110 mg.) as felted needles, m.p.183--185°, $[\alpha]_D$ -23° (c, 1.6) (Found: C,73.85; H,9.75. $C_{30}H_{48}O_5$ requires C,74.0; H,9.5%). It does not exhibit light absorption of high intensity over 2200 Å (ε_{2050} = 3000, ε_{2150} = 1100).

The same product was obtained using zinc and acetic acid at 100°.

(b) A solution of 3β-acetoxy-9α:11α-dihydroxyergost--22-en-7-one (70 mg.) in glacial acetic acid (30 c.c.) was treated with a solution of chromium trioxide in glacial acetic acid (1 N; 0.4 c.c.) added in one portion with shaking at room temperature. The solution was kept at room temperature overnight. A little methanol was added, the solution concentrated under reduced pressure,

diluted with water and the oily product isolated by means of ether. After standing for two days with methanol the solid was collected and crystallised from aqueous methanol to give 3β-acetoxy-9α-hydroxy-7:11-diketoergost--22-ene as felted needles, m.p.177-180°, $[\alpha]_D$ -25° (c,1.4), undepressed in m.p. when mixed with the specimen described above.

7:11-Diketoergost-8:22-dien-3β-yl Acetate.

A solution of 3β-acetoxy-9α-hydroxy-7:11-diketo-ergost-22-ene (70 mg.) in aqueous methanolic potassium hydroxide (5%; 30 c.c.) was refluxed for 3 hours. The yellow solution was concentrated and diluted with water. Isolation by means of ether gave a yellow gum, which was acetylated using acetic anhydride in pyridine. A solution of the acetylated product in light petroleum (b.p.60-80°)--benzene (3:1; 10 c.c.) was filtered through a column of alumina (Grade I/II; 10 x 1 cm.) and the column washed with the same solvent mixture. Evaporation of the first fraction (60 c.c.) gave a yellow gum (40 mg.) which proved difficult to crystallise. Evaporation of the second fraction (light petroleum-benzene, 1:1; 60 c.c.) gave a yellow semicrystalline solid (15 mg.) which crystallised from aqueous methanol to give 7:11-diketoergost-8:22--dien-3β-yl acetate as yellow, soft, flat needles,

m.p.132-135°, undepressed when mixed with a specimen prepared according to Heusser et al. (36). Light absorption: Maximum at 2700 Å (ε = 8600).

3β:9α-Dihydroxy-7:11-diketoergost-22-ene.

(a) 3β-Acetoxy-9α-hydroxy-7:11-diketoergost-22-ene (150 mg.) in methanolic potassium hydroxide (3%; 8 c.c.) was heated gently on the steam bath for 10 seconds when solution was complete whereafter a crystalline solid separated immediately. The solid was collected, washed with methanol and dried (120 mg., needles, m.p.244-246°). Three recrystallisations from methanol gave 3β:9α--dihydroxy-7:11-diketoergost-22-ene as needles, m.p.255--256°, $[\alpha]_D$ -8° (c, 0.4 in chloroform-methanol, 20:1) (Found: C,75.5; H,10.1. $C_{28}H_{44}O_4$ requires C,75.6; H,10.0%). It does not exhibit light absorption of high intensity above 2200 Å.

Reacetylation of the diol (30 mg.) using pyridine (0.5 c.c.) and acetic anhydride (1 c.c.) at 100° gave the acetate (25 mg.) which crystallised from methanol in felted needles, m.p.183-185°, $[\alpha]_D$ -23° (c, 1.5), undepressed in m.p. when mixed with the specimen described above.

(b) A solution of 22:23-dibromo-3β:9α-dihydroxy-ergostan-7:11-dione (50 mg.) in ether-methanol (1:1;30c.c.)

was heated under reflux with zinc dust (0.5 g.) added portionwise over 3 hours. The product, isolated by means of ether, was crystallised from methanol to yield 3β:9α-dihydroxy-7:11-diketoergost-22-ene as needles, m.p.254-256°, $[\alpha]_D$ -7° (c, 0.4 in chloroform-methanol, 20:1), undepressed in m.p. when mixed with the specimen described above.

22:23-Dibromo-7:11-diketoergost-8-en-3β-yl Acetate.

A solution of 22:23-dibromo-9α-hydroxy-7:11-diketoergostan-3β-yl acetate (400 mg.) in methanolic potassium hydroxide (3%; 120 c.c.) was refluxed for 3 hours. The product was isolated by means of ether and acetylated using acetic anhydride and pyridine. A solution of the acetylated product in benzene (40 c.c.) was filtered through a column of alumina (Grade I/II; 1.5 x 12 cm.). Evaporation of the first two benzene fractions (250 c.c.) gave a solid (250 mg.) which crystallised from methanol--chloroform to yield 22:23-dibromo-7:11-diketoergost-8--en-3β-yl acetate as hexagonal plates, m.p.257-259°, $[\alpha]_D$ +30° (c, 1.8) (Found: C,57.6; H,7.2. Calc. for $C_{30}H_{44}O_4Br_2$: C,57.3; H,7.05%); it is undepressed in m.p. when mixed with the specimen described by Budziarek, Johnson, and Spring (109). Light absorption: Maximum at 2700 Å (ε = 9600).

Evaporation of later benzene fractions gave a yellow solid (100 mg.) which crystallised from methanol--chloroform in yellow elongated plates, m.p.253-255°, $[\alpha]_D$ +66° (c, 1.2) (Found: C,57.8; H,7.2). Light absorption: Maximum at 2100 Å (ε = 8000), 2660 Å (ε = 6300) and 3300 Å (ε = 2600).

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