ROUTES TO 11-OXYGENATED STEROIDS FROM ERGOSTEROL IN A PROJECTED PARTIAL SYNTHESIS OF CORTISONE

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by

ROBERT C. ANDERSON

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C O N T E N T S

HISTORICAL

INTRODUCTION

The discovery by Hench and his collaborators in 1949 (1) at the Mayo Clinic in Rochester, U.S.A., that 3:11:20-triketopregn-4-en-17α:21-diol (VII), known also as 17-hydroxy-11-dehydrocorticosterone, Kendall's compound E, or cortisone, possesses remarkable therapeutic activity in cases of chronic rheumatoid arthritis, has resulted in a revival of interest in the chemistry of adrenal cortical hormones and in a world-wide effort to devise a method of producing this compound in quantities sufficient to render practicable its use as a palliative for this dreadful condition.

About 1930 it was established that extracts of the cortex of the adrenal gland will prolong the life of an adrenalectomised animal (2). The preparation of these extracts varies; a typical method being extraction of minced adrenal glands with cold acetone, followed by partition of the extracted material between a number of immiscible solvent pairs to give a concentrate which, on extraction with ethyl acetate, and washing the solution with weak acid and alkali, yields a neutral concentrate. The yield of such a concentrate from beef adrenals is of the order of one per cent.

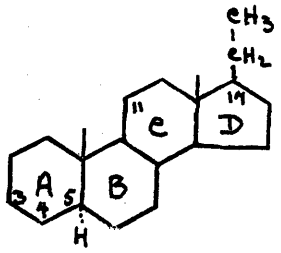
The isolation of individual compounds from the concentrate and their chemical study was undertaken about

1934 by Reichstein, Kendall, Pfiffner and others using a variety of analytical procedures; among these may be mentioned the use of the Girard reagent to effect separation into carbonyl and non-carbonyl fractions, partition techniques, and chromatography of acetylated extracts followed by hydrolysis of the separated components.

In the years which followed, twenty-eight crystalline substances were isolated from adrenal extracts and, with one exception, their structures were determined by degradation and interconversion and in some cases by methods of partial synthesis from compounds of established structure by unambiguous methods. In addition to the crystalline components isolated, there remains an amorphous fraction which possesses to a markedly high degree the capacity to prolong the life of an adrenal-ectomised animal. Considerable variation in the hormone content is observed in extracts from different animal species.

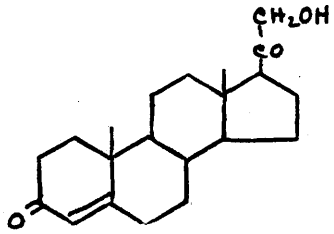
Most of the crystalline components are known to be pregnane derivatives and these either contain a 3-keto-Δ^4-unsaturated system or they are saturated, in which case they have the allo-configuration (I). Furthermore, adrenal cortical steroids hydroxylated at C_3 have, with one exception, the 3β-configuration. Although a number of the adrenal cortical steroids have not been available in sufficient quantity for physiological test, it is known

that six of them (II-VII) are capable of prolonging the life of an adrenalectomised animal. These six hormones



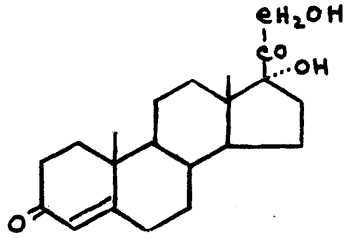
alloPregnane

I



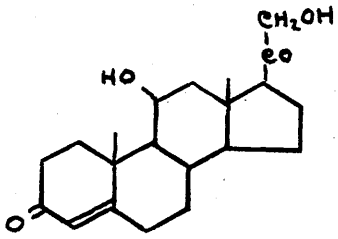
11-Deoxycorticosterone

II



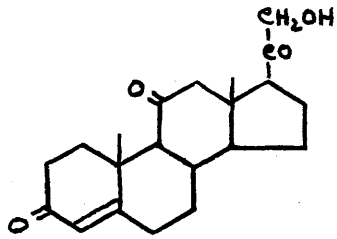
14-Hydroxy-11-deoxycorticosterone.

III



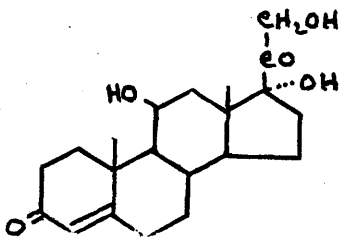
Corticosterone

IV



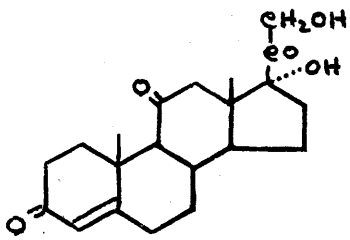
11-Dehydrocorticosterone

V



14-Hydroxycorticosterone

VI



14-Hydroxy-11-dehydrocorticosterone

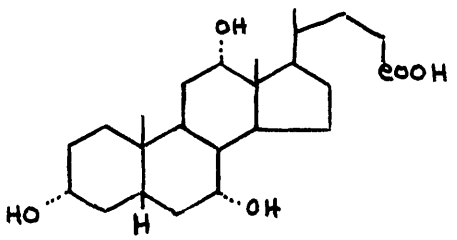
(Cortisone) VII

possess the 3-keto-Δ^4-unsaturated system and also an α-ketol side chain, both of which are necessary for their hormone activity.

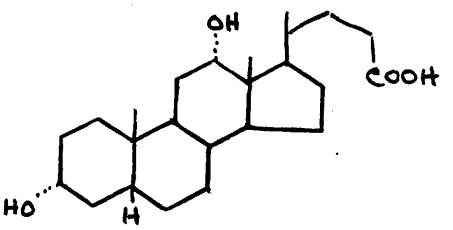
Cortisone (VII), one of these six physiologically active components, has a profound effect in rheumatoid arthritis, and to a lesser extent in rheumatic fever, in which respect it appears to be highly specific. It was isolated about the same time by Reichstein, Kendall and Pfiffner, and the constitution proposed by Reichstein (3) was verified shortly afterwards by its partial synthesis starting from 17-hydroxycorticosterone (VI)(4).

PARTIAL SYNTHESIS OF CORTISONE FROM DEOXYCHOLIC ACID.

Deoxycholic acid (IX), obtained from ox-bile or the more abundant bile acid cholic acid (VIII)(5), has been the centre of attraction as starting material in view of its relative abundance, its possession of a hydroxyl group in ring C, and of a side chain terminated by a



VIII



IX

carboxyl group, a factor expected to facilitate side chain degradation.

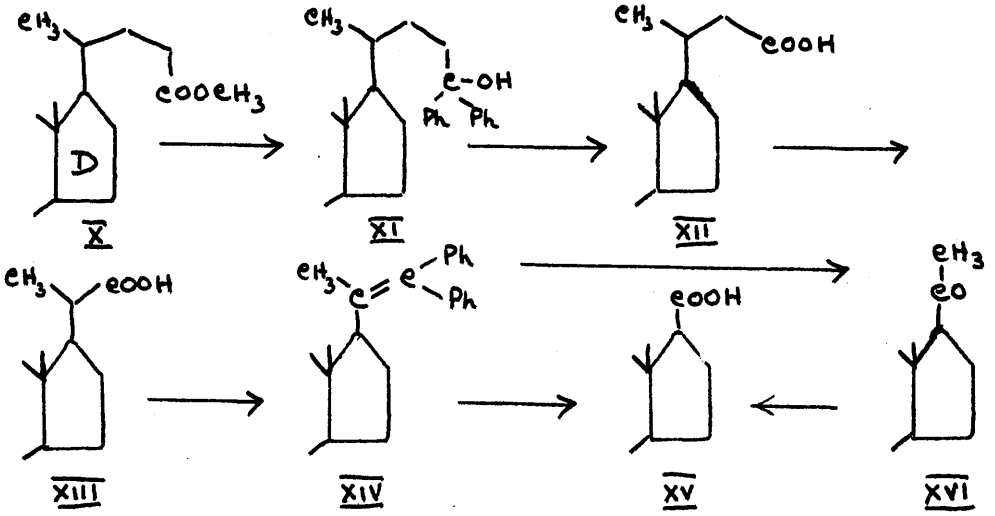
The synthesis of cortisone (VII) from deoxycholic acid (IX) involves the following steps.

1. Degradation of the bile acid side chain.
2. Elaboration of the dihydroxy-acetone side chain with configuration 17β.
3. Introduction of oxygen at C_{11} and removal of oxygen at C_{12}.
4. Formation of the 3-keto-Δ^4-unsaturated system.

An outline of the progress made is given below, under these four headings.

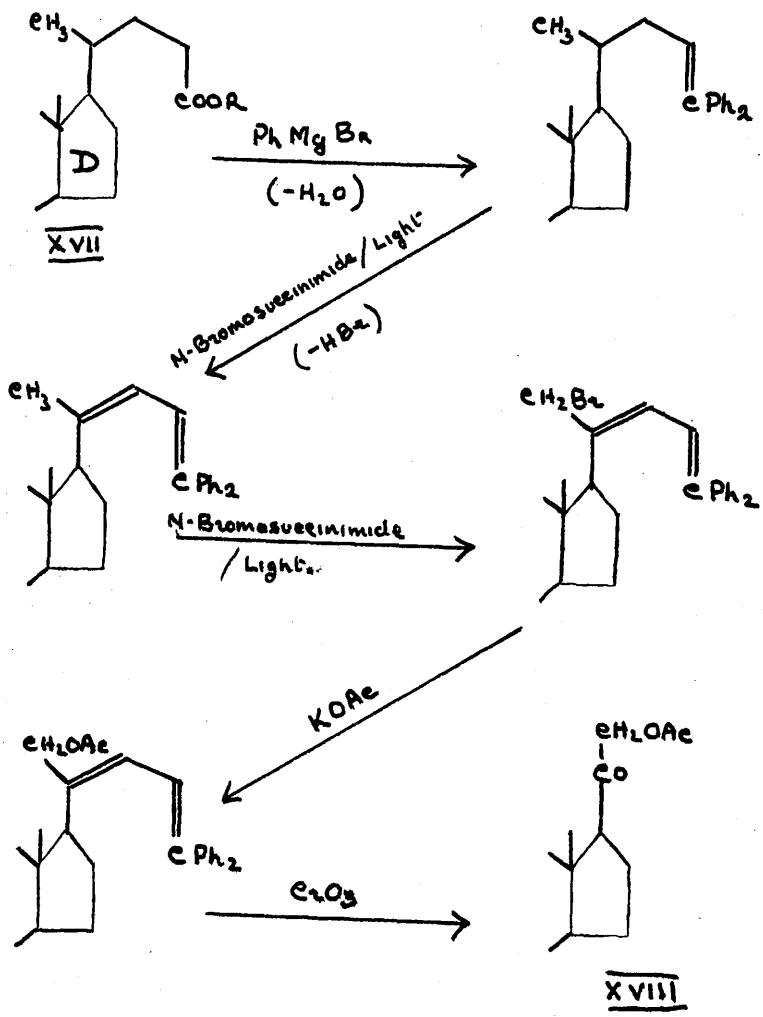
1. Degradation of the Bile Acid Side Chain.

The degradation of the side chain was initially accomplished by Wieland (6) using the method of Barbier (7). By the action of a Grignard reagent on the methyl



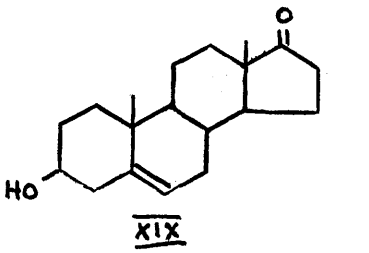
ester of deoxycholic acid (X) the carbinol (XI) is obtained. Dehydration with acetic anhydride, which simultaneously acetylates the two nuclear hydroxyl groups, followed by oxidation with chromium trioxide, gives the diacetate of nordeoxycholic acid (XII), which in turn is esterified and degraded in the same manner to give bisnordeoxycholic acid as the diacetate (XIII). Finally, this acid (XIII), after esterification, is converted into the diphenyl-ethylene (XIV) as before and oxidised with chromium trioxide to give the diacetate of etiodeoxycholic acid (XV). The latter compound can also be prepared from 3α:12α-diacetoxypregnan-20-one (XVI) obtained from the diphenylethylene (XIV) by ozonolysis.

Later, Miescher, Meystre and Wettstein (8) described a more efficient method for the degradation of the bile acid side chain to the stage of an α-ketol. An illustration of this method, shown below, depicts the conversion of a cholanic ester derivative (XVII) into a 21-acetoxy--20-ketopregnane derivative (XVIII).

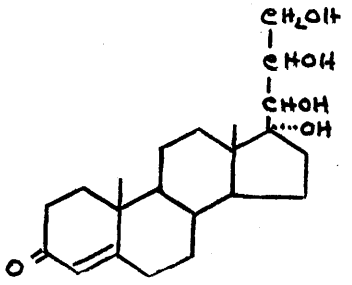


2. Elaboration of the Dihydroxy-acetone Side Chain with Configuration 17β.

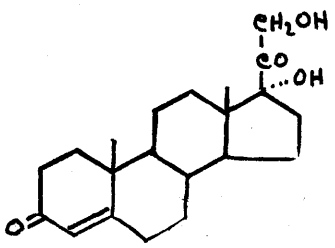
The first synthesis of this side chain was achieved by von Euw and Reichstein (9). Starting from 17α:20β: 21:22-tetrahydroxy-ω-homopregn-4-en-3-one (XX) obtained by Butenandt (10) from dehydroepiandrosterone (XIX), these workers synthesised 17-hydroxy-11-deoxycorticosterone (III).



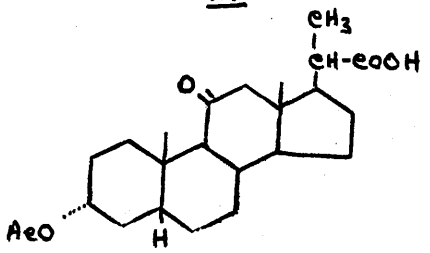
XIX



XX



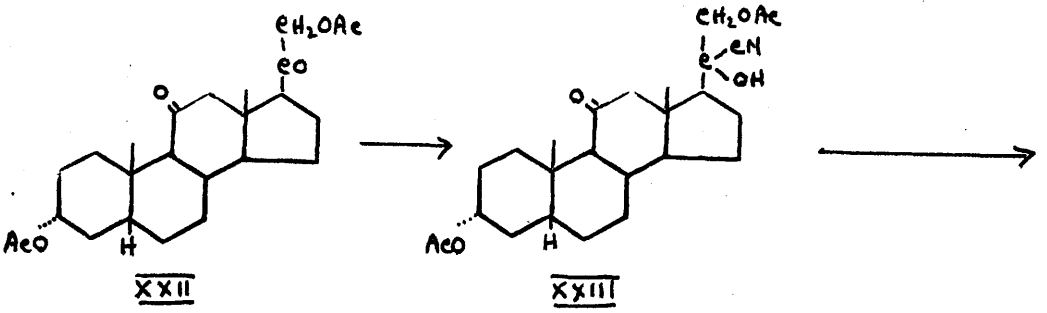
III



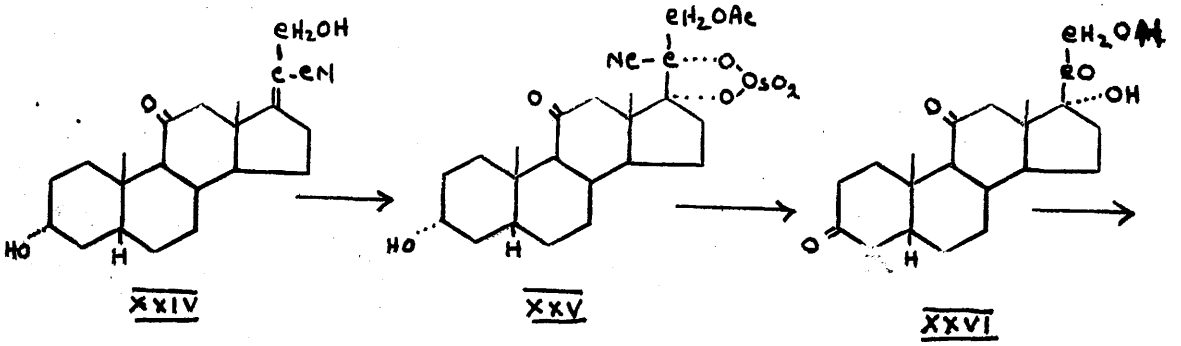
XXI

In 1946, Sarett (11) described a method which enabled the first synthesis of cortisone to be accomplished. The starting material is 3α-acetoxy-11-keto--bisnorcholanic acid (XXI) from which cortisone is obtained in a yield of a quarter of one per cent. The process involves the use of osmium tetroxide to introduce the 17α-hydroxyl group. A second method, also

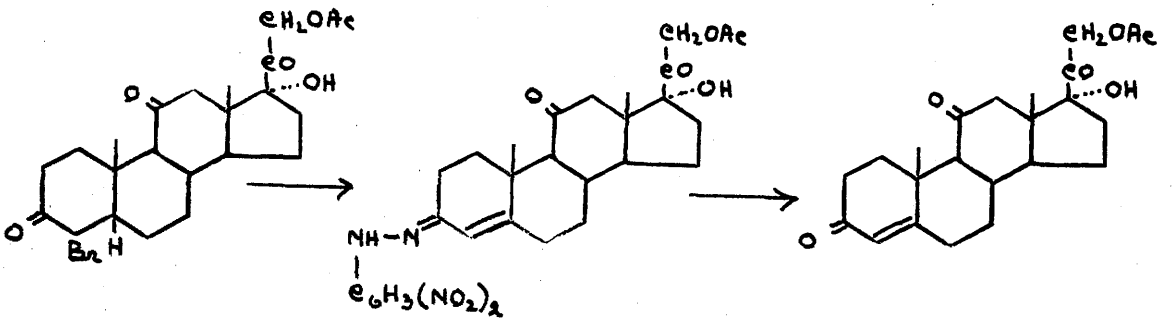
leading to the synthesis of cortisone, was described two years later by Sarett (12) in which the yield is greatly improved. The starting material is 3α:21--diacetoxypregnan-11:20-dione (XXII), the cyanhydrin (XXIII) of which on dehydration with phosphorous oxy-



XXII XXIII



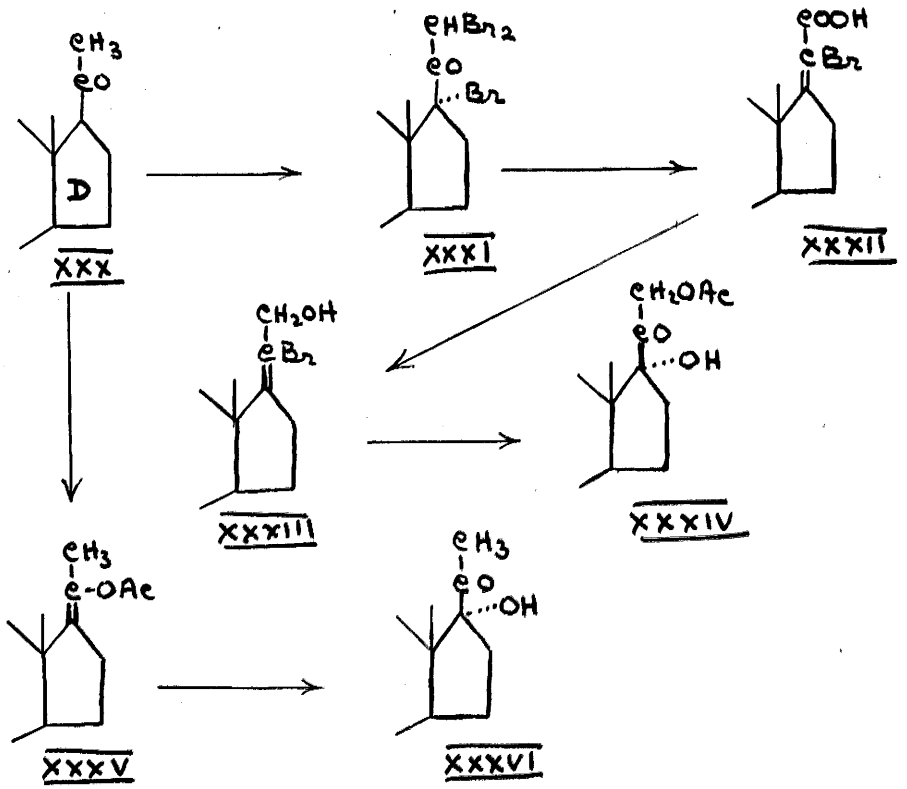
XXIV XXV XXVI



XXVII XXVIII XXIX

chloride in the presence of pyridine followed by saponification gives (XXIV). After acetylation of the 21-hydroxyl group, treatment with osmium tetroxide forms a cyclic ester (XXV) which is oxidised at the 3-position with chromium trioxide. The osmic ester is split with sodium bisulphite to give (XXVI) and, after acetylation of the 21-hydroxyl group and bromination at the 4-position the resulting compound (XXVII) is treated with 2:4-dinitrophenyl hydrazine. The product has the property of easily eliminating hydrogenbromide (13) with the formation of the 4:5-unsaturated compound (XXVIII). Regeneration of the ketone is achieved by the action of hydrogen bromide in the presence of pyruvic acid which acts as an acceptor, and cortisone in the form of its acetate (XXIX) is obtained in an overall yield of eighteen per cent starting from (XXII). A modification of this method has recently been described by Heer and Miescher (14) in which potassium permanganate is used in place of osmium tetroxide which is a very expensive reagent.

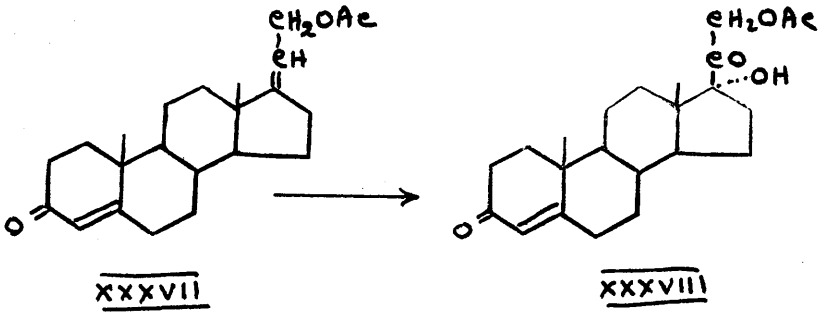
A method has been described by Wagner and Moore (15) in which a compound with the acetone side chain (XXX) is brominated to form a tribromide (XXXI) which is converted to (XXXII) by the action of alcoholic potash. The methyl ester of this acid is then reduced with lithium aluminium hydride to the 20-bromo-21-ol unsaturated compound (XXXIII).



This is treated, after acetylation, with osmium tetroxide to give the required dihydroxy-acetone side chain as its acetate (XXXIV). Gallagher and KritcheVsky (16) avoid the use of osmium tetroxide in a method which is fundamentally similar to that described above. This involves treatment of the enol-acetate (XXXV) of a 20-ketone (XXX) with perbenzoic acid followed by hydrolysis to give the 17α-hydroxylated compound (XXXVI). A 21-hydroxyl group can be introduced into (XXXVI) by bromination to give the required dihydroxy-acetone side chain (17).

Miescher and Smidlin (18) have reported a method using hydrogen peroxide with osmium tetroxide as a catalyst.

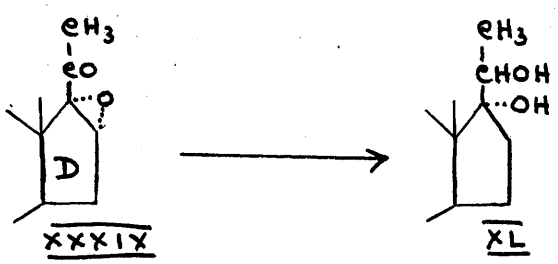
By this means 17-hydroxy-11-deoxycorticosterone acetate (XXXVIII) is obtained in fifty per cent yield from (XXXVII).



XXXVII XXXVIII

The elaboration of this type of side chain (XXXVII) from 17-keto steroids using ethoxyacetylene or by means of a Reformatsky reaction has been described by Heusser et al. (19).

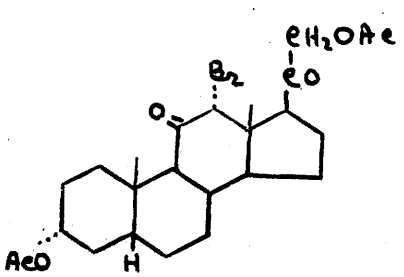
A different approach has been described by Plattner, Heusser, and Fuerer (20), who have prepared 17α:20--dihydroxypregnane derivatives (XL) by the reduction of 16α:17α-epoxy-20-ketones (XXXIX) with lithium aluminium hydride. Julian, Meyer, Karpel and Ryden (21) have used



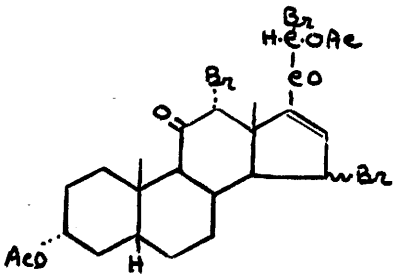
XXXIX XL

a similar method to form 17α-hydroxy-20-keto-compounds. The 20-keto group can be protected as a cyclic acetal during the reduction (22) or the oxide ring can be opened to form a bromohydrin and the bromine reduced out with

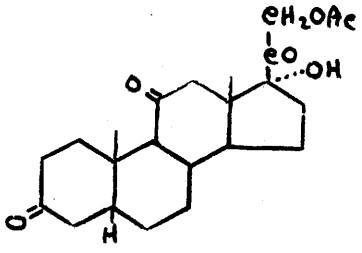
nickel without affecting the 20-keto group. A similar method has also been reported by Kendall (23) by means of which 4:5-dihydrocortisone acetate (XLIII) is obtained



XLI



XLII

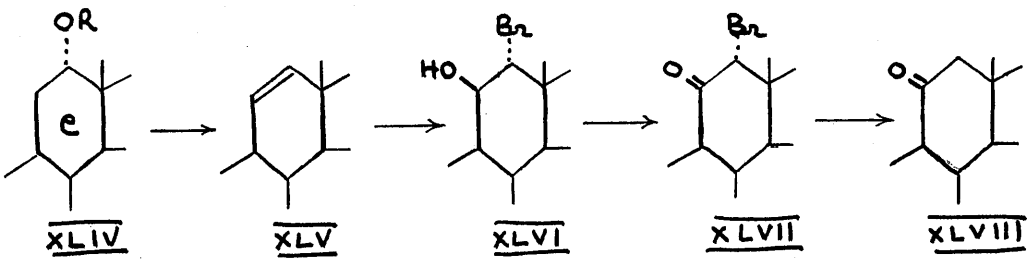


XLIII

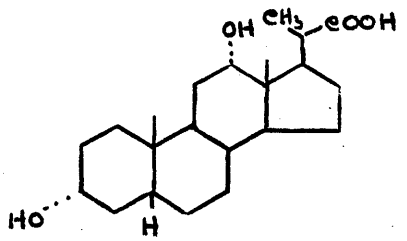
from (XLI) in about forty-five per cent yield. The essential intermediate compound is a brominated pregn--16-ene (XLII) which allows the formation of a 16α:17α--epoxide.

3. Introduction of Oxygen at C_{11} and Removal of Oxygen at C_{12}.

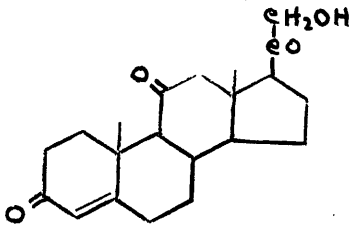
Route via an 11:12-unsaturated Derivative. This method was studied by Reichstein (24) with different derivatives of the bile acids; it consists in obtaining an 11-ethylene (XLV) by pyrolysis of a compound containing a free 12--hydroxyl group or one esterified with acetic, benzoic or anthraquinone β-carboxylic acid (XLIV). It is also possible to obtain the 11-ethylene by treatment of the 12-tosylate with pyridine under pressure (25), with an



XLIV XLV XLVI XLVII XLVIII



XLIX

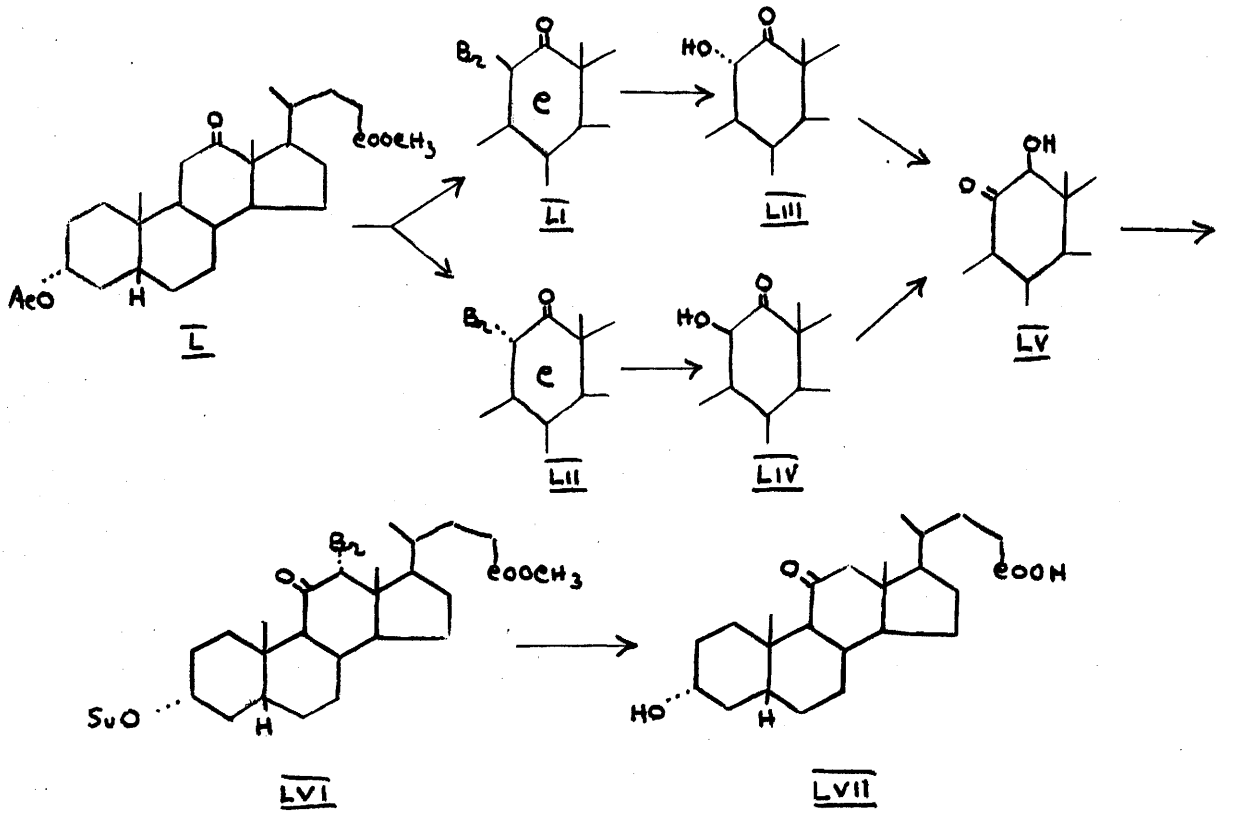


V

overall yield of about forty per cent in some cases. The 11-ethylene (XLV), on treatment with N-bromosuccinimide in aqueous acetone gives (XLVI) in about twenty-five per cent yield, mixed with compounds brominated in the 9-position and in the 11- and 12-positions. The bromohydrin, (XLVI) is oxidised to (XLVII) the 12-bromine atom of which is eliminated by reduction with zinc to give the 12-keto-compound (XLVIII). It was by this method that the synthesis of 11-dehydrocorticosterone (V) was effected by Reichstein (26) in 1943 starting from etiodeoxycholic acid (XLIX); the side chain was elaborated by a method described by Reichstein (27) using diazomethane.

Route via an 11-Bromo-12-ketone. In this method, devised by Gallagher (28), the first step involves bromination of methyl 3α-acetoxy-12-keto-cholanate (L), a reaction first described by Marker and Lawson (29). The

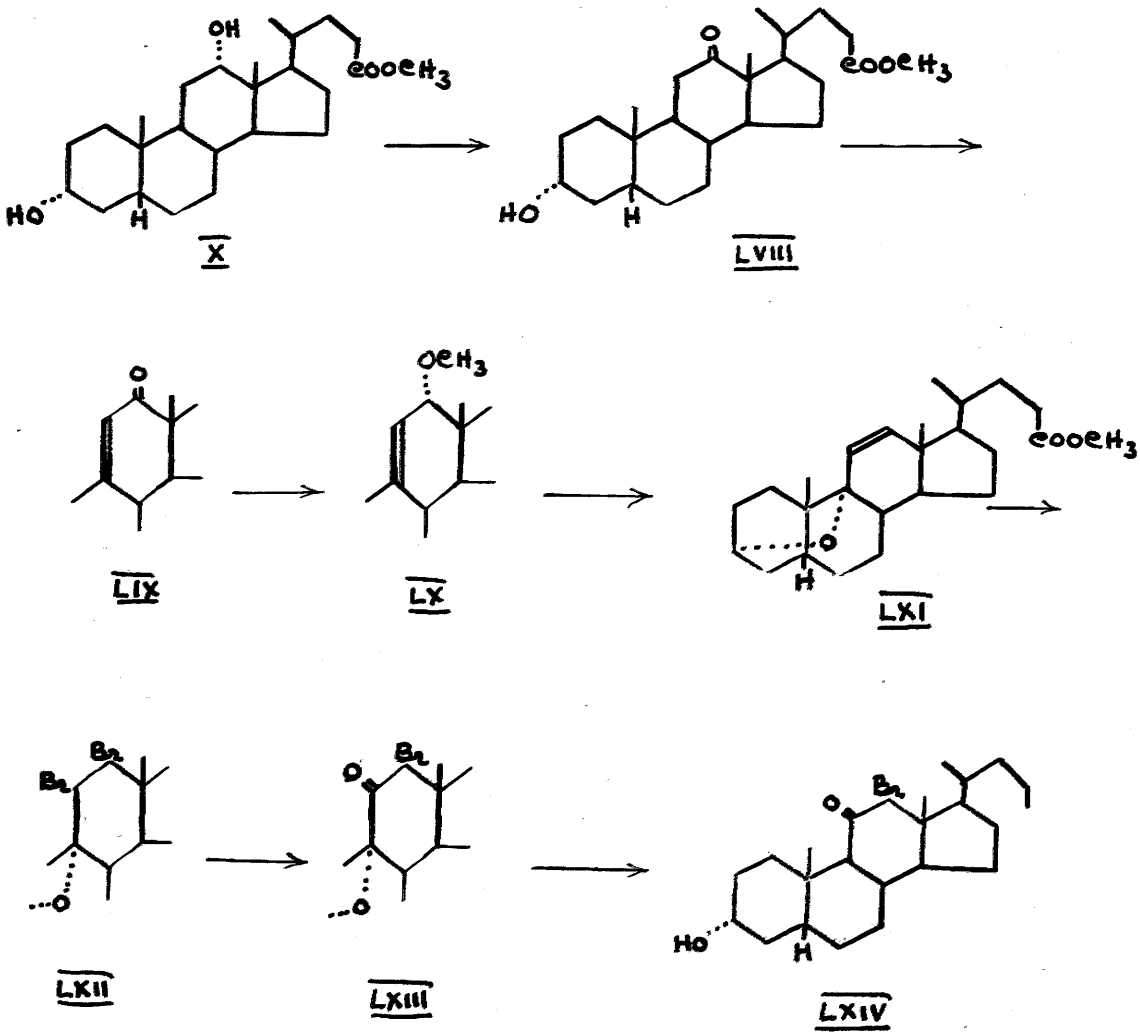
epimers (LI) and (LII) are obtained which are hydrolysed at room temperature to give the 3α:11β- and 3α:11α--dihydroxy-12-ketocholanic acids (LIII) and (LIV) respectively. Under very vigorous hydrolytic conditions both these acids are transformed into the Marker-Lawson acid (LV). Also the epimers (LI) and (LII) on vigorous



hydrolysis give the acid (LV) directly (30). After succinoylation at C_3, replacement of the 12-hydroxyl group by bromine (LVI) and removal of the bromine by reduction followed by hydrolysis at C_3, 3α-hydroxy-11-

ketocholanic acid (LVII) is obtained (31).

Route via a 3:9-Oxide. This method, elaborated by Kendall (32), is very efficient. In three steps



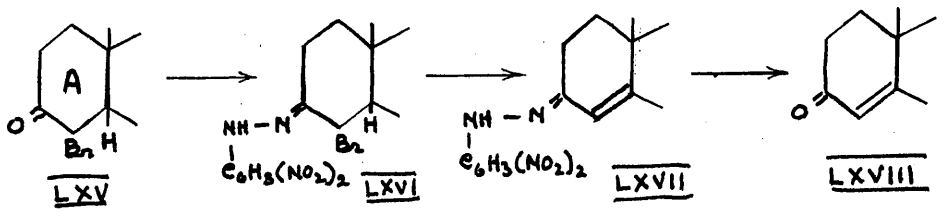
the methyl ester (X) of deoxycholic acid is transformed into methyl 3α-hydroxy-12-keto-cholanate (LVIII). The double bond in the 9(11)-position is introduced by oxidation with selenium dioxide to give (LIX) and the

12-keto-group is reduced to a hydroxyl group which is etherified with methanol (LX). The methoxyl group, rendered labile by the neighbouring double bond, can be replaced by an atom of chlorine with hydrogen chloride. The chlorine is then hydrolysed with sodium hydrogen carbonate with displacement of the double bond to the 11:12-position and the formation of an oxide link between the 3- and 9-positions (LXI). Two atoms of bromine are added to the double bond (LXII), and the bromine at C_{11}, which is labile, is replaced by a hydroxyl group; this is then oxidised to a ketone (LXIII). The bromine atom at C_{12} is eliminated using zinc, and the oxide link opened with hydrogen bromide with the fixation of a bromine atom in the 12-position (LXIV) which is in turn eliminated by the action of zinc. The 3:9-oxide bridge is stable in the presence of Grignard reagents and it is possible to degrade the side chain in its presence. The yields by this method are in general excellent; about sixty per cent from (X) to (LXIV).

4. Formation of the 3-Keto-Δ^4-unsaturated System.

The initial method consists of bromination at C_4 followed by elimination of hydrogen bromide between positions 4 and 5 by boiling with pyridine (33) with yields rarely exceeding fifty per cent. A notable

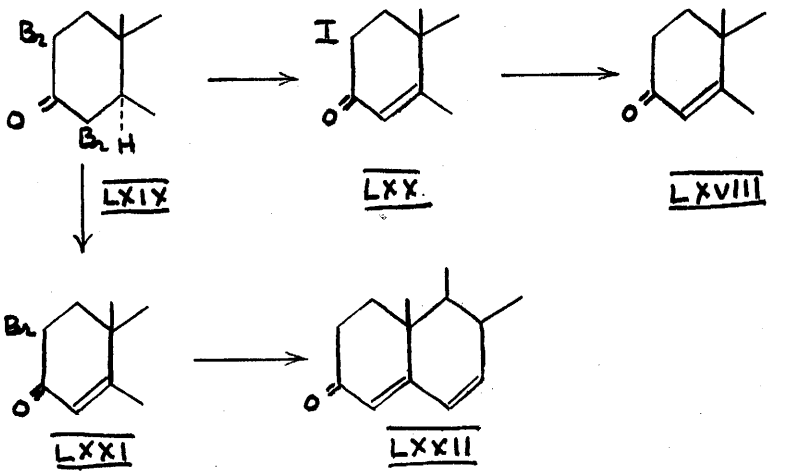
improvement is due to Mattox and Kendall (13); by condensation of a 4-bromoketone (LXV) with 2:4-dinitrophenylhydrazine, hydrogen bromide is simultaneously eliminated between the 4- and 5-positions with the formation of (LXVII) which is treated with hydrogen bromide



in the presence of pyruvic acid to give the required 3-keto-Δ^4-unsaturated compound (LXVIII) in ninety per cent yield. The reaction is known to proceed via the intermediate (LXVI) which has been isolated (34). Also, semicarbazide acetate has been used in place of 2:4--dinitrophenylhydrazine (35).

Formation of the 3-Keto-Δ^4-unsaturated System from allo-Steroids.

The introduction of a 4-double bond into 3-keto-compounds with rings A-B cis-fused (normal) is described above. When rings A and B are trans-fused (allo) a different procedure, described by Djerassi (36,37) has to be adopted. Bromination occurs first in the 2-position, but the 2:4-dibromo-derivatives (LXIX) can be made under the correct conditions. These can be converted into the

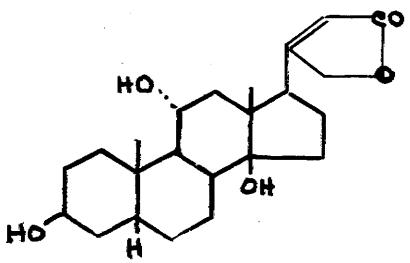


4-unsaturated 2-bromo-ketones (LXXI), which rather surprisingly give rise with 2:4-dinitrophenylhydrazine to the corresponding derivative of (LXXII)(36). The compounds (LXIX) by the brief action of sodium iodide form the 2-iodo-analogues, but by longer heating give (LXX) which are converted into the required unsaturated ketones (LXVIII) by the action of reducing agents, or simply by refluxing them with collidine (37). This is the only method at present available for the preparation of this system in ring A starting from allo-steroids.

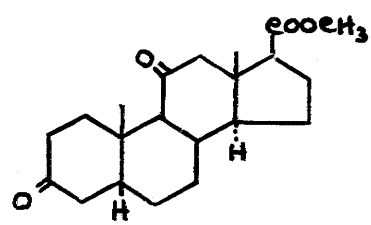
NEW STARTING MATERIALS

The partial synthetic method of preparing cortisone from deoxycholic acid is long and costly and the supply of ox-bile is limited, hence a search for new starting materials has been made. The goal is the finding of a steroid in the vegetable kingdom which can be obtained in large quantities and which possesses a structure capable of ready conversion into those of the adrenal cortical hormones.

Sarmentogenin. This compound (LXXIII) is structurally a most attractive starting material since it contains an 11α-hydroxy group; it has a side chain which is readily convertible into that of the adrenal-cortical hormones, it possesses a 3β-hydroxyl group, and rings A and B are cis-linked rendering the production of the 3-keto-Δ^4--unsaturated system a simple matter. It was characterised



LXXIII



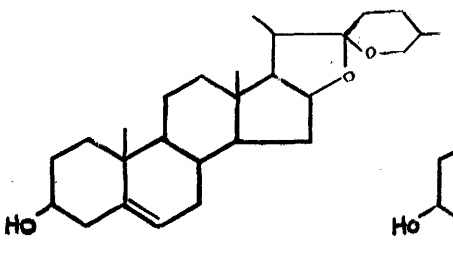
LXXIV

as the aglycone of the cardiac active glycoside sarment-

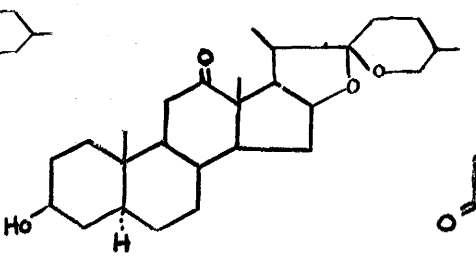
ocymarin by Jacobs and Heidelberger (38); its structure was definitely established by Katz (39) by degradation to the known methyl ester of 3:11-diketoetiocholanic acid (LXXIV). It occurs in the seeds of a Strophanthus species; a kilo of seeds yields a little over three grams of sarmentogenin and the yield from (LXXIII) to (LXXIV) is about fifty per cent, therefore, should sarmentogenin become reasonably available, a relatively simple route to cortisone is at hand.

Unfortunately the exact identification of the Strophanthus species from which sarmentocymarin is isolated is often difficult and a systematic study of the fruits and seeds of the numerous varieties of Strophanthus known has been undertaken (40).

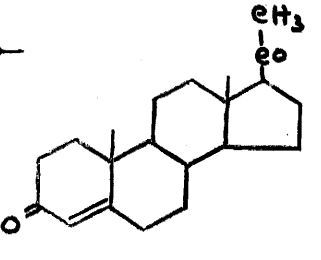
Steroidal Sapogenins. A series of compounds of this class have been isolated by Marker (14) from the rhizomes of Mexican dicsecrea. Among those of interest as starting materials may be mentioned diosgenin (LXXV) and hecogenin (LXXVI). It has been shown by Marker to be a relatively simple matter to convert a sapogenin into a 20-ketopregnane derivative, an example of which is the conversion of diosgenin into progesterone (LXXVII); also it has been converted into the corresponding 7:9(11)--diene (LXXVIII)(42) from which the acetate of the 11-keto-



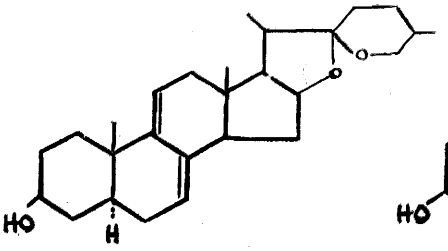
LXXV



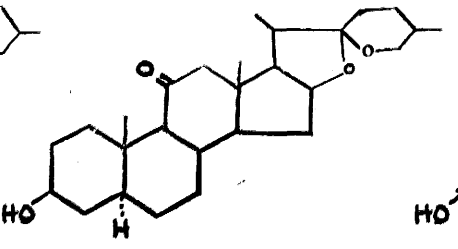
LXXVI



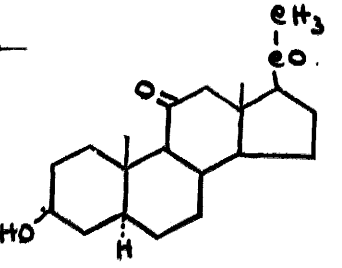
LXXVII



LXXVIII



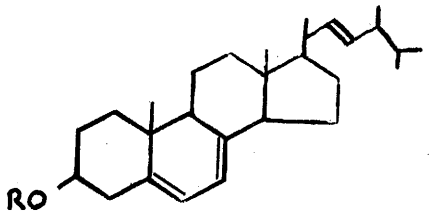
LXXIX



LXXX

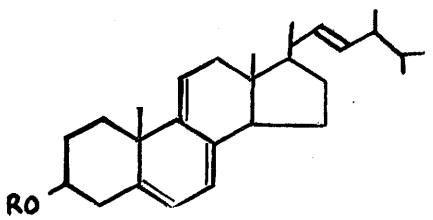
-sapogenin (LXXIX) has been prepared (43,44). Hecogenin (LXXVI) has also been transformed into the 11-keto--sapogenin (LXXIX) by Djerassi (45) using Gallagher's method of converting 12-ketones into 11-ketones by bromination (28,46). Since (LXXIX) has been converted into 11:20-diketo-allo-pregnan-3β-ol (LXXX)(47) which in turn has been transformed into cortisone (48) routes to cortisone from these two sapogenins are available.

Ergosterol. The only sterol which is potentially available in considerable quantity (since it is obtained as a by-product of yeast fermentation) and which possesses a promising structure from the point of view of the partial synthesis of cortisone is the mycosterol ergosterol (LXXXI, R=H).



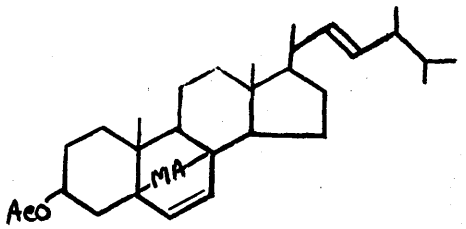
LXXXI

Bergmann and Stevens (49) were the first to suggest its use as a starting material. They point out that it could easily be converted into derivatives like 9(11)-dehydroergosterol (LXXXII, R=H) which possess unsaturation at C_{11}, thereby possibly lending themselves to the introduction of an oxygen atom at this point.

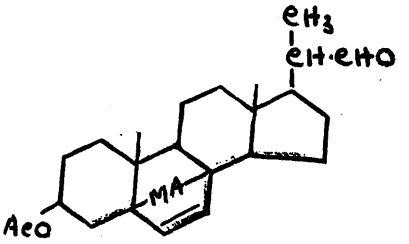


LXXXII

In addition they note the advantage which the ergosterol side chain has in possessing a 22-double bond. This, they observe, would facilitate removal of the side chain to permit its replacement by one of the typical side chains of the adrenal cortical hormones. In the latter endeavour these workers made considerable progress. The showed that protection of the conjugated nuclear double

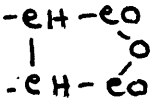


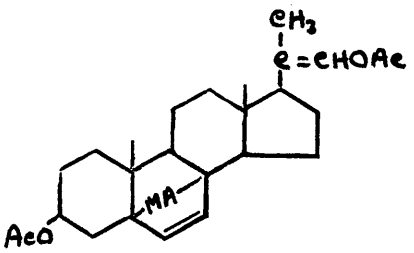
LXXXIII



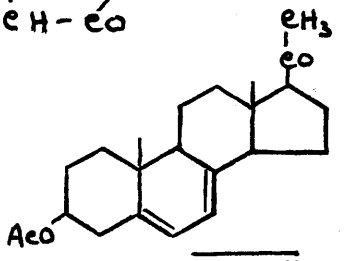
LXXXIV

MA =

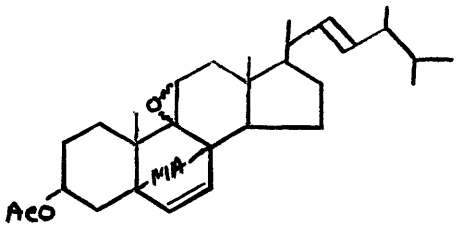




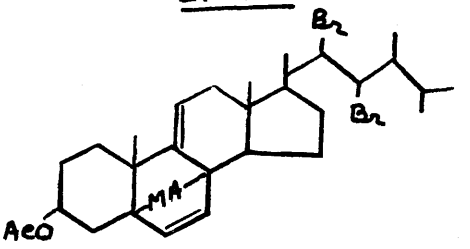
LXXXV



LXXXVI



LXXXVIII

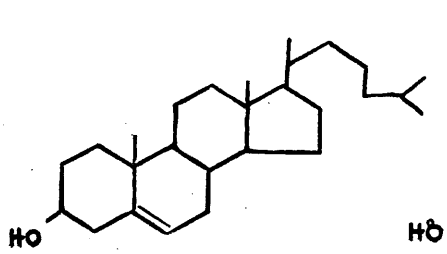


LXXXVII

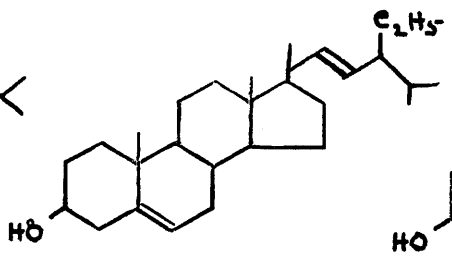
bonds in ergosteryl acetate (LXXXI; R=Ac) by means of maleic anhydride allowed the 22:23-double bond to be preferentially oxidised. Thus treatment of the maleic anhydride adduct (LXXXIII) with ozone gave an aldehyde (LXXXIV) which was converted into the enol-acetate (LXXXV) ozonolysis of which, followed by pyrolysis of the product gave 3β-acetoxypregna-5:7-dien-20-one (LXXXVI) (cf. 50). Less successful, however, were attempts to introduce either a hydroxyl or a ketone group at the 11-position

starting from 9(11)-dehydroergosteryl acetate maleic anhydride adduct-22:23-dibromide (LXXXVII). Although the epoxide (LXXXVIII) was obtained, pyrolysis of this to remove the maleic anhydride and regenerate the conjugated double bond system was accompanied by aromatisation of ring B.

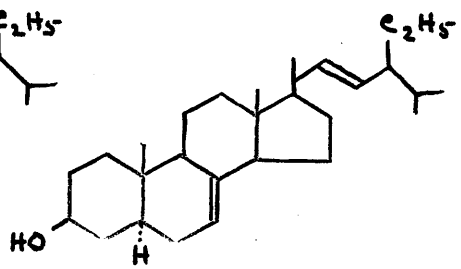
Other Sterols. Those of interest include cholesterol (LXXXIX) an abundant sterol of animal origin occurring in gall stones, brain tissue and in the spinal chord,



LXXXIX



XC



XCI

stigmasterol (XC) obtained from soya bean oil and α--spinasterol (XCI) isolated from spinach, senega root and from alfalfa. The two latter compounds possess a 22:23-double bond thereby providing a point of attack for side chain degradation. The saturated side chain of cholesterol on the other hand makes this compound less attractive from this point of view.

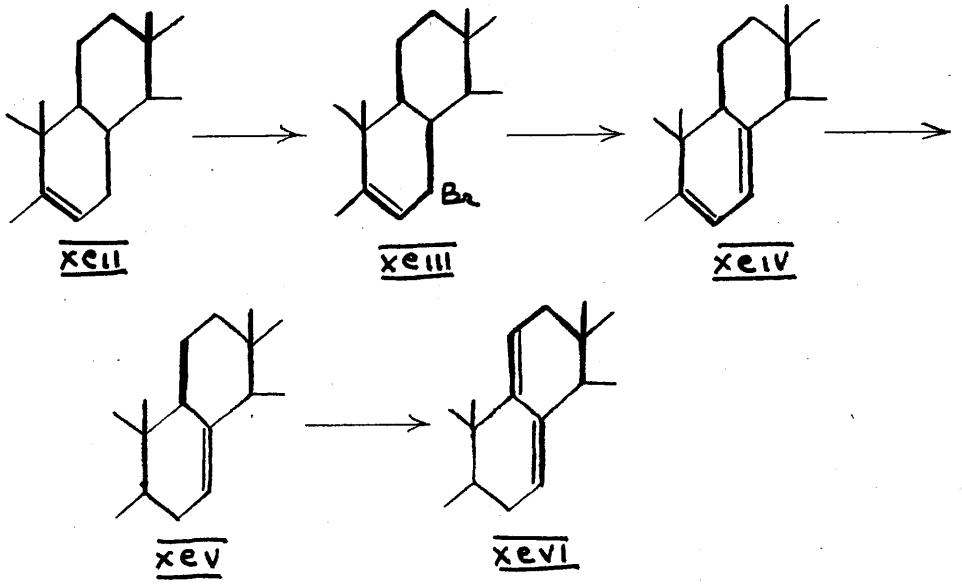
INTRODUCTION OF AN OXYGEN ATOM AT POSITION 11.

The introduction of an oxygen atom as a hydroxyl or ketone group into the 11-position of steroids in which ring C is saturated was not accomplished by purely chemical methods until 1951, when five groups of workers with Tishler, Fieser, Djerassi, Jeger and Spring respectively, published the results of investigations commenced about 1949 with this aim in view. This thesis represents a portion of the work of the latter group. The considerable data published since 1951 on this topic is outlined below apart from that having a direct bearing on the work described in this thesis, this latter being mentioned in the Theoretical section. All methods depend on the preliminary formation of either a 7:9(11)-diene or of a compound possessing an isolated 9(11)-double bond.

Routes via a 7:9(11)-Diene.

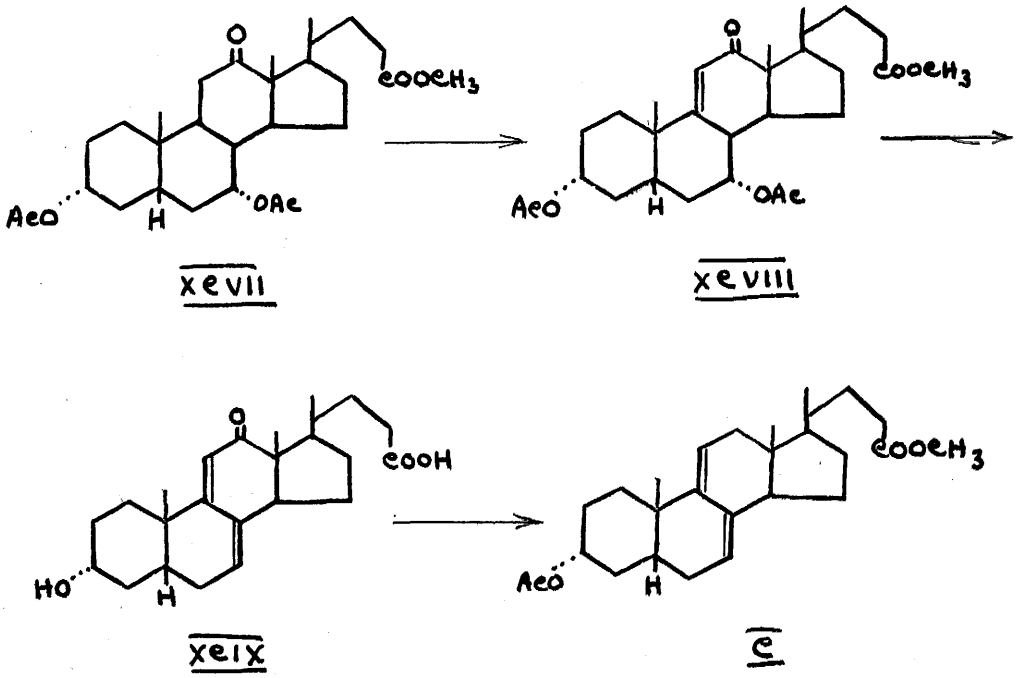
A general procedure (42,52,53) for the preparation of such dienes of the allo-series involves treatment of a 5-unsaturated steroid (XCII) with N-bromosuccinimide followed by dehydrobromination of the resulting allylic bromide (XCIII) with the formation of a 5:7-diene (XCIV), catalytic hydrogenation of which gives a 7-unsaturated steroid (XCV). Dehydrogenation of the latter compound gives the required 7:9(11)-diene (XCVI). The most common

reagent for this last step is mercuric acetate. This conversion has been effected with cholesterol (LXXXIX)



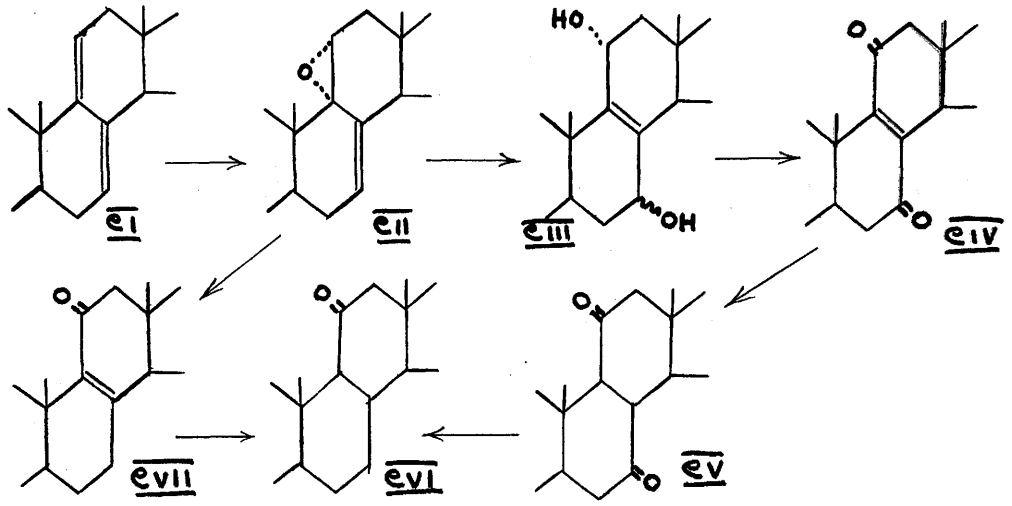
and stigmasterol (XC) derivatives (47) and with diosgenin (LXXVIII)(42) (see p. 21) all of which are represented by structure (XCII). In some cases the 7-double bond is already present as in ergosterol (LXXXI; R=H), represented by structure (XCIV) and α--spinasterol (XCI), represented by structure (XCV) both of which have been converted into the corresponding 7:9(11)-dienes (54,55). A convenient method of obtaining 7:9(11)-dienes of the normal-series is exemplified by the preparation of methyl 3α-acetoxychola-7:9(11)--dienate (C) (56,58,59) described below. The starting material is methyl 3α:7α-diacetoxy-12-ketocholanate (XCVII), which is dehydrogenated to (XCVIII) with selenium

dioxide. This compound (XCVIII) on treatment with alkali loses a molecule of acetic acid to give (XCIX) which, on Wolff-Kishner reduction followed by methylation and acetylation of the product gives the requires 7:9(11)--diene (C).



There are six different methods available for the conversion of 7:9(11)-dienic steroids into 11-oxygenated derivatives.

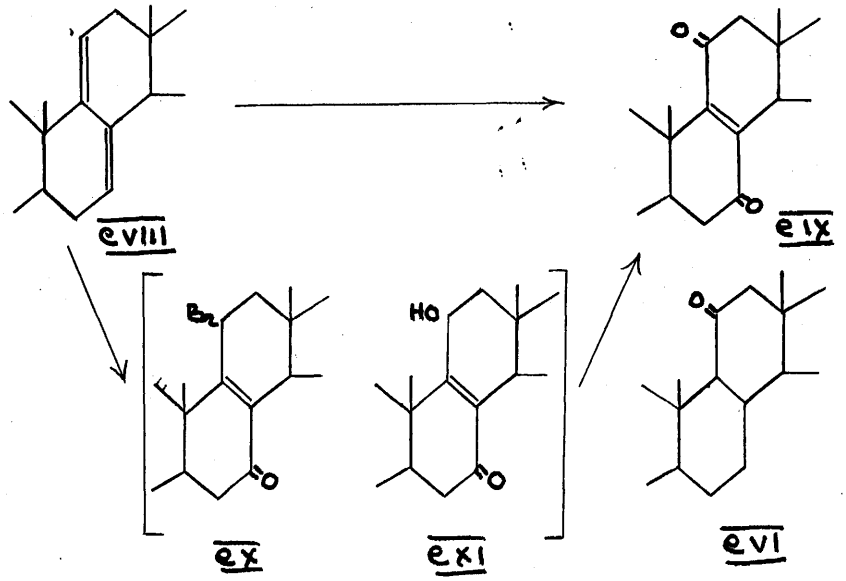
Two of these methods involve the initial formation of a monoxide (CII) by oxidation of a 7:9(11)-diene (CI) with perbenzoic acid (Tishler, 47) or monoperphthalic acid (Jeger, 46). This is rearranged with acid in the first method to give an 8-unsaturated-7:11-diol (CIII),



oxidised to the corresponding diketone (CIV), the 8--double bond of which is reduced with zinc and acetic acid to give (CV) and finally converted to the saturated 11-ketone (CVI) either by Wolff-Kischner reduction or by treatment with ethylene dithiol followed by desulphurisation with Raney nickel. This method is applicable to both the allo- and normal-series. The second method which has only been applied to the allo-series involves rearrangement of the monoxide (CII) with borontrifluoride (56) to give the 8-unsaturated-11-ketone (CVII) which on treatment with lithium in liquid ammonia gives the saturated 11-ketone (CVI)(57).

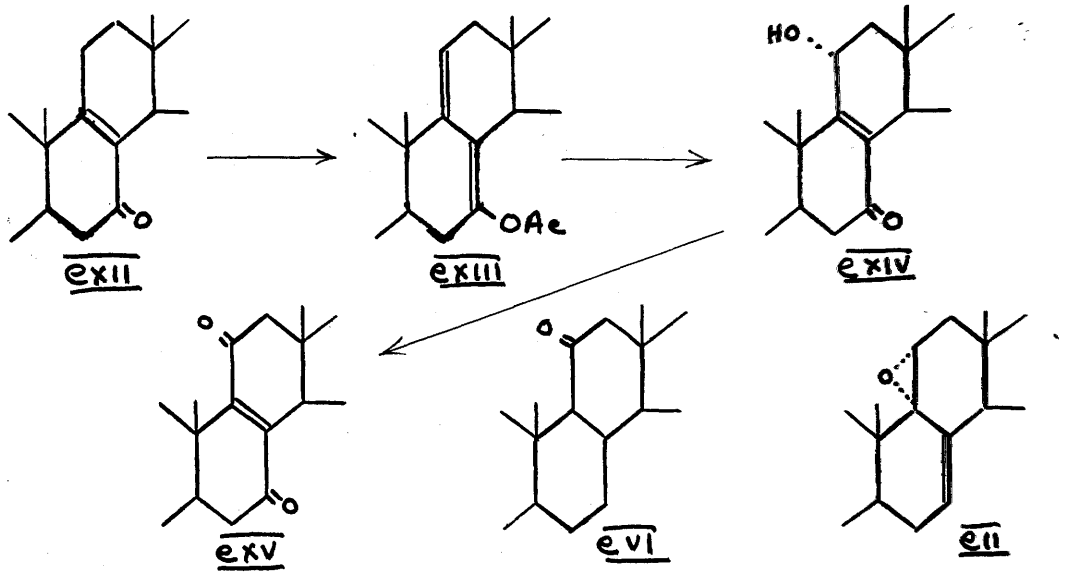
A further two methods which are applicable to both the allo- and the normal-series are described by Fieser (58,59). The first involves direct oxidation of a 7:9(11)-diene (CVIII) to an 8-unsaturated-7:11-diketone (CIX) with sodium dichromate (58). The second method (59) also provides a route to this type of compound (CIX)

by reacting a 7:9(11)-diene (CVIII) with excess N-bromo--succinimide in aqueous tertiary-butanol with the addition of silver nitrate followed by oxidation with



chromium trioxide. The authors suggest that the reaction proceeds via the intermediates (CX) and (CXI). The 8-unsaturated 7:11-diketone (CIX) is converted as before to a saturated 11-ketone (CVI).

A fifth method which is applicable to both the normal- and the allo-series is that described by Djerassi (60,61). The starting material is an 8-unsaturated-7--ketone (CXII) which gives a 7:9(11)-dienic steroid (CXIII) on enol-acetate formation. Treatment of (CXIII) with perbenzoic or monoperphthalic acid followed by hydrolysis gives an 8-unsaturated-7-keto-11α-ol (CXIV) which is converted by oxidation to an 8-unsaturated-7:11-

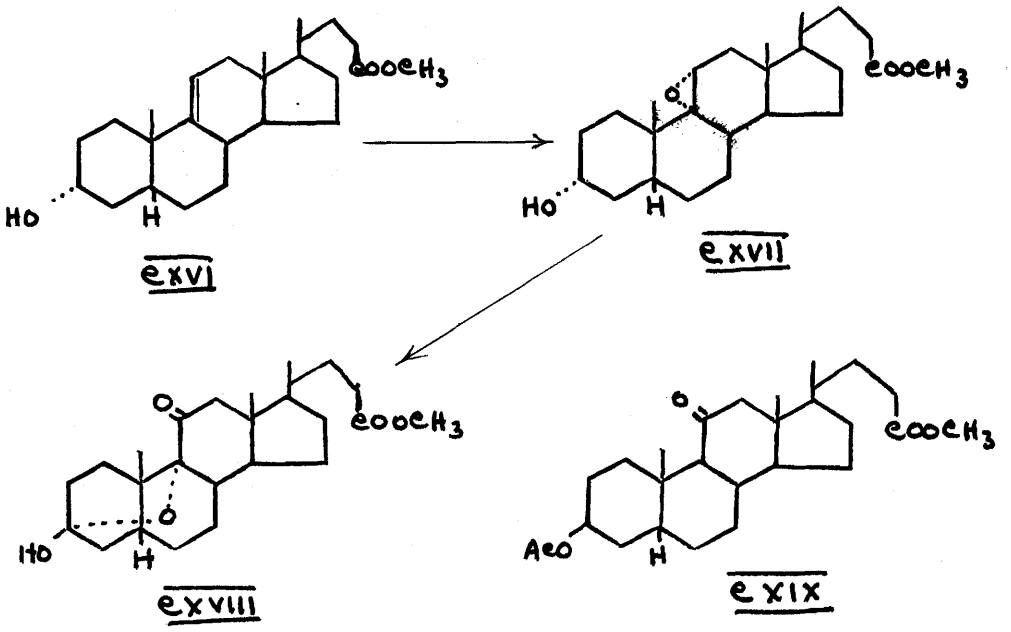


-diketone (CXV) and hence as before to a saturated-11--ketone (CVI). A method of obtaining a compound of the type (CXIV) is also described by Spring (62,63) starting from the monoxide (CII) in the ergosterol series.

Lastly there is the method described by Djerassi (43,60,64) and Spring (54,62,63) which is only applicable to steroids of the allo-series. This method is described in the Theoretical section of this thesis.

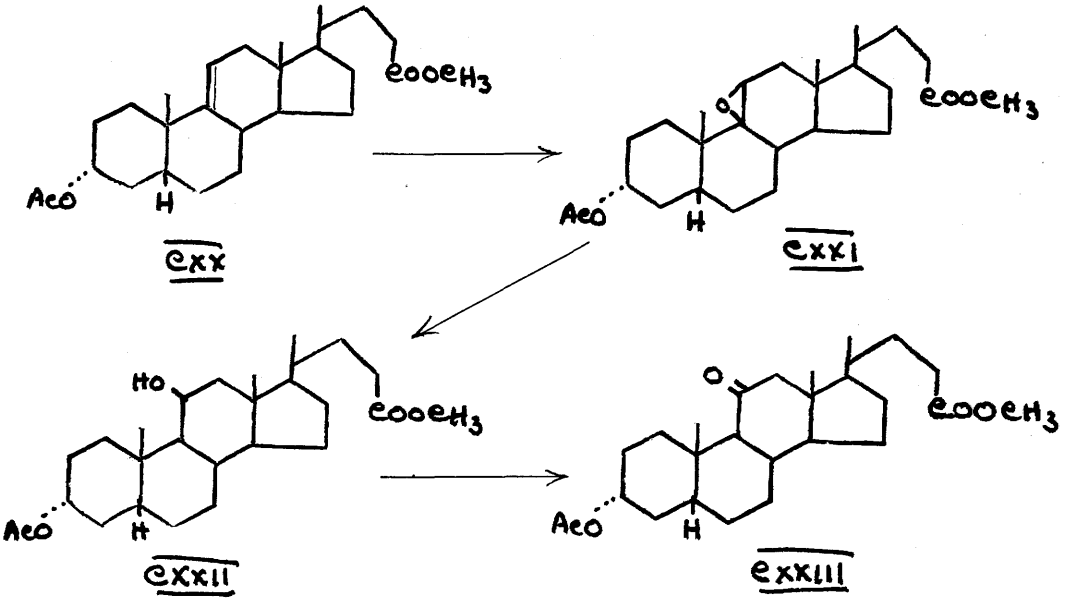
Routes via a Compound Containing Isolated 9(11)-Double Bond.

Fieser (65,60,67) has described a method which is only applicable to the normal-series in which methyl lithochol-9(11)-enate (CXVI) is treated with monoperphthalic acid to give the oxide (CXVII) which is oxidised to (CXVIII) with chromium trioxide. This compound (CXVIII) is converted into methyl 3β-acetoxy-11-keto-



-cholanate (CXIX) by two different methods.

Another method which has so far only been applied to the normal-series has been described recently by Constantin and Sarett (68). This involves oxidation of methyl 3α-acetoxychol-9(11)-enate (CXX) with potassium permanganate to give a monoxide (CXXI). The latter

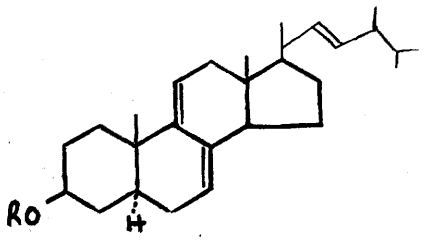


compound is converted by hydrogenolysis to methyl 3α--acetoxy-11β-hydroxycholanate (CXXII) and thence by oxidation to methyl 3α-acetoxy-11-ketocholanate (CXXIII).

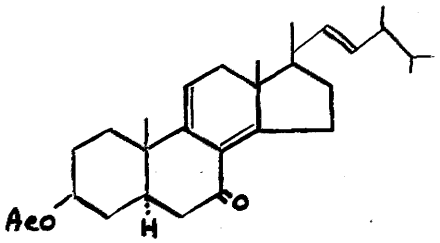
THEORETICAL

INTRODUCTION

The work described in this thesis has as its objective the development of routes to cortisone (VII) starting from ergosterol (LXXXI; R=H). In the Historical section (see p.23) an outline is given of the progress made in this direction by Bergmann and Stevens (49) starting from 9:11-dehydroergosterol (LXXXII; R=H) which they employ as the maleic anhydride adduct. In view of the difficulties encountered by these workers in liberating 5:7-unsaturated ergosterol derivatives from their maleic anhydride adducts in good yield, a different approach was considered desirable. Consequently other derivatives of ergosterol



CXXIV



CXXV

possessing a 9:11-double bond were sought as starting materials for this work. In this respect ergosterol-D (CXXIV; R=H) attracted attention since it was reported by Bergmann and Klacksmann (69) and by Barton (70) that it possesses a 7:9(11)-conjugated diene system. The

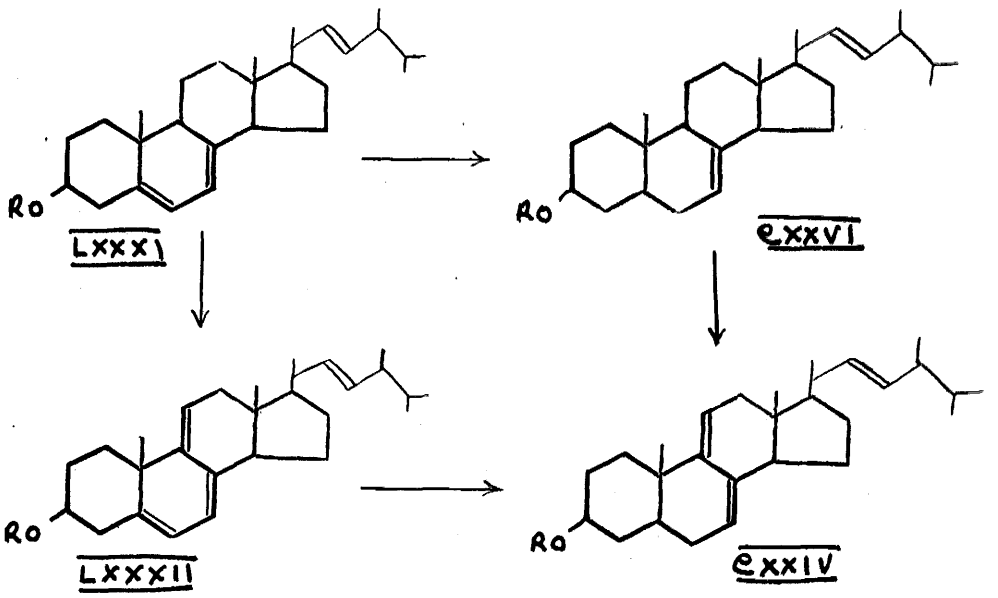
possibility of using this isomer of ergosterol as a starting material for the partial synthesis of adrenal-cortical steroids was first mentioned by the former authors (69).

Another potential starting material considered promising from a structural point of view was the triply unsaturated ketone obtained by Stavely and Ballenbock (71), since these authors identify this compound as 3β-acetoxy-ergosta-8(14):9(11):22-trien-7-one (CXXV).

The conversion of ergosterol into cortisone via derivatives of ergosterol-D is shown to be possible by experiments discussed in succeeding parts of this Theoretical section; an unsuccessful attempt to introduce an oxygen function at the 11-position of 3β-acetoxyergosta--8(14):9(11):22-trien-7-one (CXXV) is also described and, finally, experiments carried out with a view to establishing the structure of an intermediate in this work are discussed.

ERGOSTERYL-D ACETATE

Methods previously described for the preparation of ergosteryl-D acetate (CXXIV; R=Ac) involve either oxidation of 5-dihydroergosteryl acetate (CXXVI; R=Ac) with



mercuric acetate (72,73), perbenzoic acid (74), or selenium dioxide (75), or reduction of 9:11-dehydroergosterol (LXXXII; R=H) with sodium in ethanol (75). The most practicable method is that starting from 5-dihydroergosteryl acetate (CXXVI; R=Ac) and using mercuric acetate (72,73).

In the case of 5-dihydroergosteryl acetate (CXXVI; R=Ac) previously described methods of preparation are first, reduction of ergosterol (LXXXI; R=H) with sodium in ethanol (77,78,79) and secondly, the partial catalytic

hydrogenation of ergosteryl esters (80,81,82). Using a platinum catalyst and chloroform as solvent Barton and Cox (82) obtained a 30-35% yield of 5-dihydroergosteryl acetate starting from ergosteryl acetate (LXXXI; R=Ac) which they claim to be the best available method for the preparation of this compound.

A considerable improvement in yields in the conversion of ergosteryl acetate into 5-dihydroergosteryl acetate and of the latter into ergosteryl-D acetate was obviously necessary before the last named compound could be considered as a starting material for this work. With this aim in view ergosteryl acetate was catalytically hydrogenated under a variety of conditions as a result of which it was found that partial hydrogenation of it with Raney nickel as catalyst and cyclohexane as solvent gives 5-dihydroergosteryl acetate in 76% yield. A disadvantage of this method lies in the fact that ergosteryl acetate is not very soluble in cyclohexane. A further improvement of this method, however, was devised in which benzene is used as solvent. Since benzene is slowly hydrogenated in the presence of Raney nickel allowance had to be made for this fact. A blank experiment was carried out to measure the uptake of hydrogen by the benzene and, after experimentation, optimum conditions were found in which 5-dihydroergosteryl acetate is obtained in 92-95% yield.

Apart from the improved yield by the latter method it also has the advantage of allowing larger quantities of 5-dihydroergosteryl acetate to be prepared at the one time since ergosteryl acetate is easily soluble in benzene. Recently it has been reported (56; footnote) that Pannizon and Kägi have obtained a similar yield of 5--dihydroergosteryl acetate by hydrogenation of ergosteryl acetate in ether solution with Rupe nickel catalyst; also by Laubach and Brunnings (83) that 5-dihydroergosterol can be obtained in 80% yield from ergosterol by hydrogenation in dioxan solution with Raney nickel.

Now that 5-dihydroergosteryl acetate had been made easily available attention was focussed on devising a more efficient method of converting it into ergosteryl-D acetate. In this connection a modification of the existing method of preparation using mercuric acetate, employed by Bergmann and Stevens (49) for the oxidation of ergosteryl acetate (LXXXI; R=Ac) to 9:11-dehydroergosteryl acetate (LXXXII; R=Ac), was applied to 5-dihydroergosteryl acetate as a result of which the yield of ergosteryl-D acetate was slightly improved. The main advantage of this method, however, is that it permits the oxidation to be carried out on a much larger scale than by the previous method since chloroform, in which 5-dihydroergosteryl acetate is very soluble, is used

instead of ethanol, in which it is sparingly soluble. The use of this method has also been reported in a recent publication by Heusser et al (56).

This present method of preparation, although an improvement on all previous methods and affording, as it was thought, a reasonable yield (40%) of ergosteryl-D acetate, was still considered unsatisfactory when it was discovered that the product hitherto considered to be pure ergosteryl-D acetate was not quite homogeneous. This crude product yields almost pure ergosteryl-D acetate on purification by crystallisation to constant specific rotation value. The true yield of ergosteryl-D acetate by this method was thereby shown to be only 25%. Homogeneity of the product was verified by hydrolysis to the free alcohol followed by reacetylation. The specific rotation values, and the principal band of the absorption spectra of the products obtained in various methods of preparing ergosteryl-D acetate are listed in the table below. A comparison of these constants indicates that the impurity in crude ergosteryl-D acetate is a material which does not absorb above 2200Å and which has a negative specific rotation value. This suggests that the impurity is unreacted 5-dihydroergosteryl acetate ($[\alpha]_D$ -20.5). It would appear, therefore, that the removal of small amounts of 5-dihydroergosteryl acetate present in crude

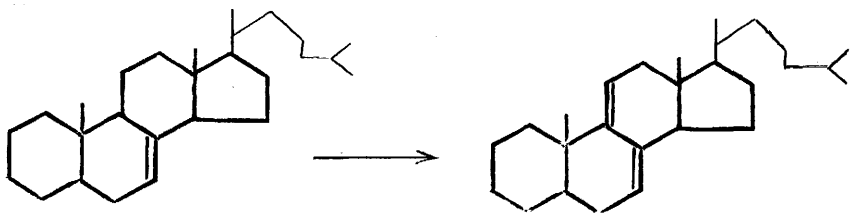
Ergosteryl-D Acetate

Method of Preparation	M.p.	$[\alpha]_D$	ε at 2420Å in Ethanol
Bromine (described later)	178-180°	+32,+33°	19,000
Chlorine (described later)	178-180	+33	18,000
Mercuric Acetate (described above)	175-177	+30	17,000
Perbenzoic Acid (74)	171	+25.7	—
Mercuric Acetate (56) (crude product)	169-170	+21	16,000

ergosteryl-D acetate is an extremely difficult task if purification by crystallisation is employed.

22:23-DIBROMOERGOSTA-7:9(11)-DIEN-3β-YL ACETATE (ERGOSTERYL-D ACETATE 22:23-DIBROMIDE)

In view of the unsatisfactory yield in the best available method of converting 5-dihydroergosteryl acetate into ergosteryl-D acetate a better method was sought. Hence, a study of the action of bromine on 5-dihydroergosteryl acetate was commenced since, according to Eck and Hollingsworth (84), oxidation of cholest-7-ene (CXXVII) in chloroform with bromine at -75° gives cholesta-7:9(11)--diene (CXXVIII). Treatment of 5-dihydroergosteryl



CXXVII CXXVIII

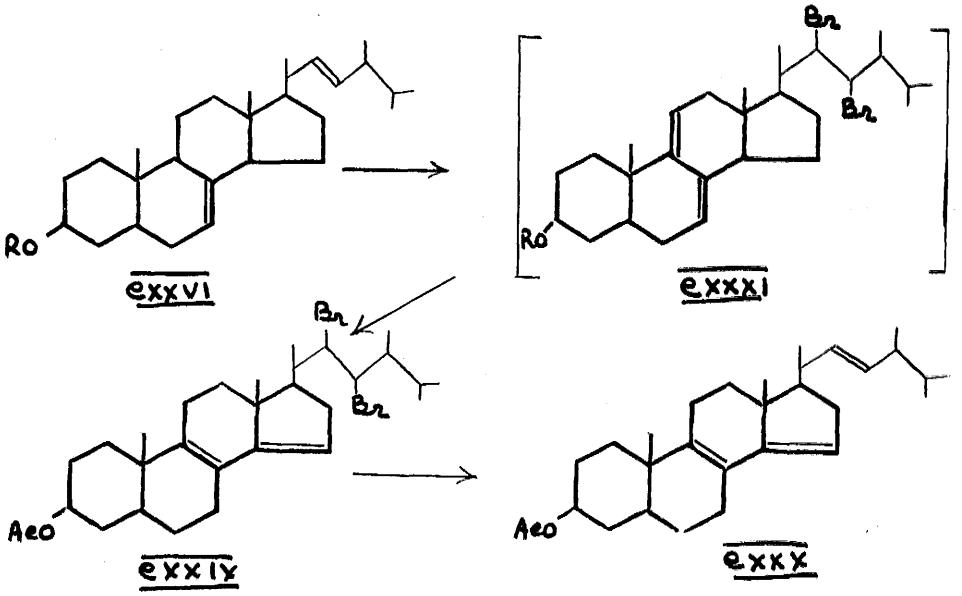
acetate (CXXVI; R=Ac) under similar conditions with one mol of bromine was found to give a dibromide $C_{30}H_{46}O_2Br_2$ in very poor yield. A similar product was obtained in better yield (11%) using two mols of bromine. This material is shown to be essentially 22:23-dibromoergosta--8:14-dien-3β-yl acetate (ergosteryl-B_1 acetate 22:23--dibromide) (CXXIX) by its ultra-violet absorption spectrum, and by its conversion into a compound having physical constants similar to those of ergosteryl-B_1 acetate (CXXX)

by debromination with zinc dust (see table below). The

	λ max.Å	ε max.	M.p.	$[\alpha]_D$
Dibromide (1 Mol of bromine)	2480	14,500	227-228° (decomp.)	-15°
Dibromide (2 Mols of bromine)	2480	14,000	227-228 (decomp.)	-20.5
Debrominated Product	2480	15,000	141-142	-52.2
Ergosteryl B_1 Acetate Windaus (76)	2480 (Ether)	16,000	142	-54
22:23-Dihydro-ergosteryl B_1 Acetate Barton (85)	2480	19,800		

only record of the ultra-violet absorption spectra of ergosteryl B_1 acetate (CXXX) is that described by Windaus (76) in which ether is used as solvent. Since the spectra of the products described above are recorded in ethanol a true comparison of the wave-lengths of maximum absorption cannot be made. However, the spectrum of the 8:14-conjugated diene system in the form of 22:23-dihydro-ergosteryl-B_1 acetate (CXXIX; Br=H) has recently been recorded in ethanol by Barton (85) and is identical in location with that found for the products reported above. It is noted, however, that though the intensity of absorption at 2480Å found for these products is in good

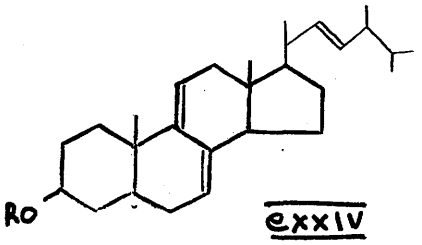
agreement with the figure quoted by Windaus (16,000), it is lower than the value given by Barton (19,800).



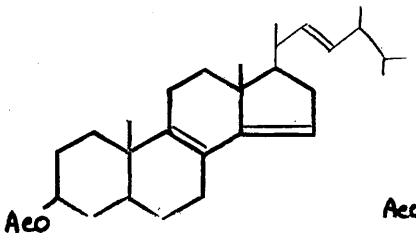
CXXVI CXXXI CXXIX CXXX

The production of the 8:14-conjugated diene system (CXXIX) probably involves the initial formation of the 7:9(11)-conjugated diene system (CXXI; R=Ac) which is then isomerised to the former by the hydrogen bromide produced during the reaction. In support of this view the analagous case can be quoted of the isomerism of ergosteryl-D acetate (CXXIV; R=Ac) with dry hydrogen chloride to give a mixture of ergosteryl-B_1 acetate (CXXX), ergosteryl-B_2 acetate (CXXXII) and ergosteryl-B_3 acetate (CXXXIII) (69,76).

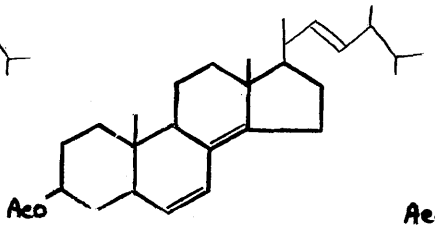
It was apparent, therefore, that this method in its present form was of no avail for the preparation of the 7:9(11)-diene system and, since isomerism of steroid



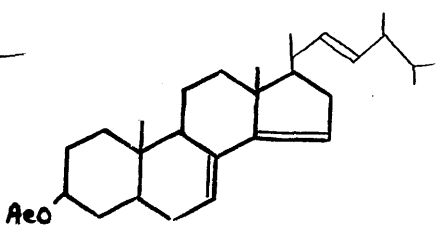
CXXIV



CXXX



CXXXII



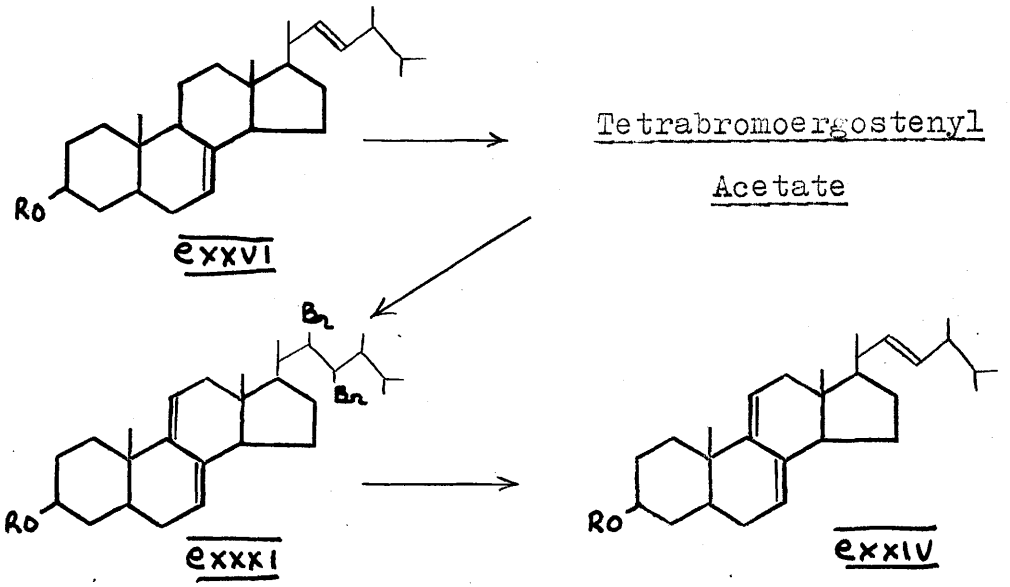
CXXXIII

nuclear diene systems with dry hydrogen chloride was known to occur readily in chloroform solution [cf. isomerism of ergosteryl acetate and of ergosteryl-D acetate (69,76)] it was decided that the use of this solvent should be avoided. Consequently it was decided to modify this bromination procedure by using a different solvent.

In this connection it was found that treatment of 5-dihydroergosteryl acetate (CXXVI; R=Ac) in ether solution at -60° with excess dry bromine in glacial acetic acid solution gives in good yield (48-53%) a tetrabromide $C_{30}H_{44}O_2Br_4$ which separates from the reaction mixture. This compound is identified as a tetrabromoergostenyl acetate by its conversion into a diene by the action of sodium iodide. This conversion is described below and

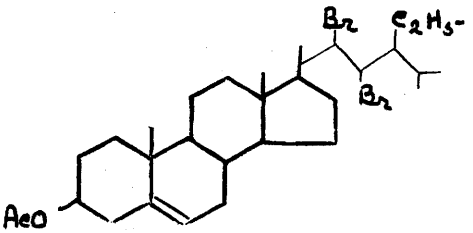
the structure of the tetrabromoergostenyl acetate is discussed in a later section.

The tetrabromoergostenyl acetate on treatment with sodium iodide in the cold yields a compound $C_{30}H_{46}O_2Br_2$



in excellent yield (90-95%) identified as 22:23-dibromo-ergosta-7:9(11)-dien-3β-yl acetate (ergosteryl-D acetate 22:23-dibromide) (CXXXI; R=Ac) first, by its conversion into ergosteryl-D acetate (CXXIV; R=Ac) by treatment of a solution of the material in a mixture of ethanol and ether with activated zinc dust and, secondly, by its ultra-violet absorption spectrum which is identical in location with that of ergosteryl-D acetate. The presence of two double bonds in the molecule was also indicated by the uptake of two oxygen atoms on treatment with excess perbenzoic acid. The first attempt to remove the side

chain bromine atoms involved treating a solution of ergosteryl-D acetate 22:23-dibromide in glacial acetic acid with zinc at 105-110° for $3^1/_2$ hours, which are similar to the conditions employed by Bergmann and Stevens (49) to remove side chain bromine atoms to give in their case the maleic anhydride adduct of 9:11-epoxyergosta--5:7:22-trien-3β-yl acetate (LXXXVIII) (see p. 25). This gives a product isomeric with ergosteryl acetate but which has physical properties indicating that isomerism of the nuclear double bond system has taken place. The milder debrominating conditions, using activated zinc in a mixture of ethanol and ether, which were successful in removing the side chain bromine atoms to give ergosteryl-D acetate are a slight modification of those employed by Fernholz and Stavely (86) for the debromination of stigmasterol 22:23-dibromide acetate (CXXXIV). These authors use zinc in moist ether at room temperature or zinc in boiling ethanol to remove the side chain bromine atoms.



CXXXIV

Hydrolysis of ergosteryl-D acetate 22:23-dibromide with aqueous methanolic potassium hydroxide solution gives 22:23-dibromoergosta-7:9(11)-dien-3β-ol (ergosteryl-D

22:23-dibromide) (CXXXI; R=H).

The conversion of ergosteryl-D acetate 22:23--dibromide into ergosteryl-D acetate is quantitative, hence pure ergosteryl-D acetate is now made available in 50% yield from 5-dihydroergosteryl acetate. In addition, ergosteryl-D acetate 22:23-dibromide is a useful intermediate for experiments directed towards the introduction of an oxygen atom at C_{11} since the 22:23--double bond is protected.

22:23-DICHLOROERGOSTA-7:9(11)-DIEN-3β-YL ACETATE.

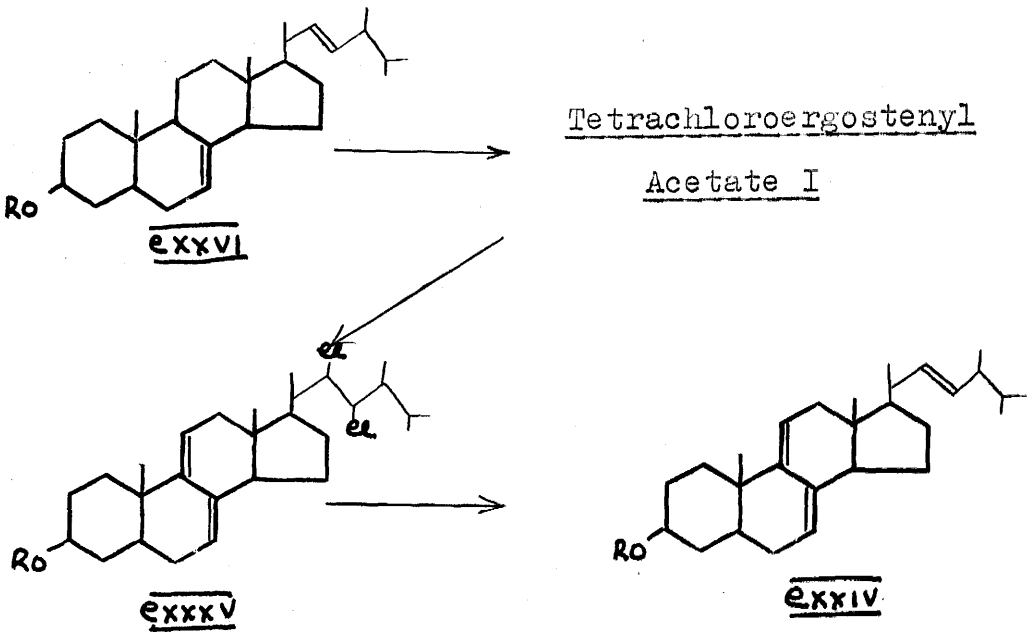
(ERGOSTERYL-D ACETATE 22:23-DICHLORIDE)

The promising results obtained in the bromination of 5-dihydroergosteryl acetate merited the investigation of similar reactions using chlorine in the expectation of obtaining analagous products. These, if obtained in as good yield as in the bromo-series, would be more attractive as intermediates from the point of view of expense.

An analogous series of reactions was indeed found to take place though the yields unfortunately proved to be much lower than in the bromo-series. Treatment of 5-dihydroergosteryl acetate (CXXVI; R=Ac) in ether solution at -50° with excess chlorine in glacial acetic acid solution gives two isomeric tetrachlorides $C_{30}H_{44}O_2Cl_4$ which separate consecutively on concentration of the reaction mixture. These are shown to posses one double bond by their conversion into dienic compounds by treatment with sodium iodide in one case and with activated zinc dust in the other. These conversions are described below. The compounds are therefore tetrachloroergosteryl acetates.

Tetrachloroergostenyl acetate I, the first to separate from the reaction mixture (yield, 4%) appears to

to be directly analogous to the tetrabromoergostenyl acetate. It has a large positive specific rotation



value and is readily partially dechlorinated in the cold with sodium iodide yielding a compound $C_{30}H_{46}O_2Cl_2$ identified as 22:23-dichloroergosta-7:9(11)-dien-3β-yl acetate (ergosteryl-D acetate 22:23-dichloride) (CXXXV; R=Ac), first, by its conversion into ergosteryl-D acetate (CXXIV; R=Ac) by treatment of its solution in glacial acetic acid at 95° with zinc dust for 1 hour and, secondly, by its ultra-violet absorption spectrum which is identical in location with that of ergosteryl-D acetate.

Hydrolysis of ergosteryl-D acetate 22:23-dichloride (XCV; R=Ac) with aqueous methanolic potassium hydroxide solution furnishes 22:23-dichloroergosta-7:9(11)-dien-

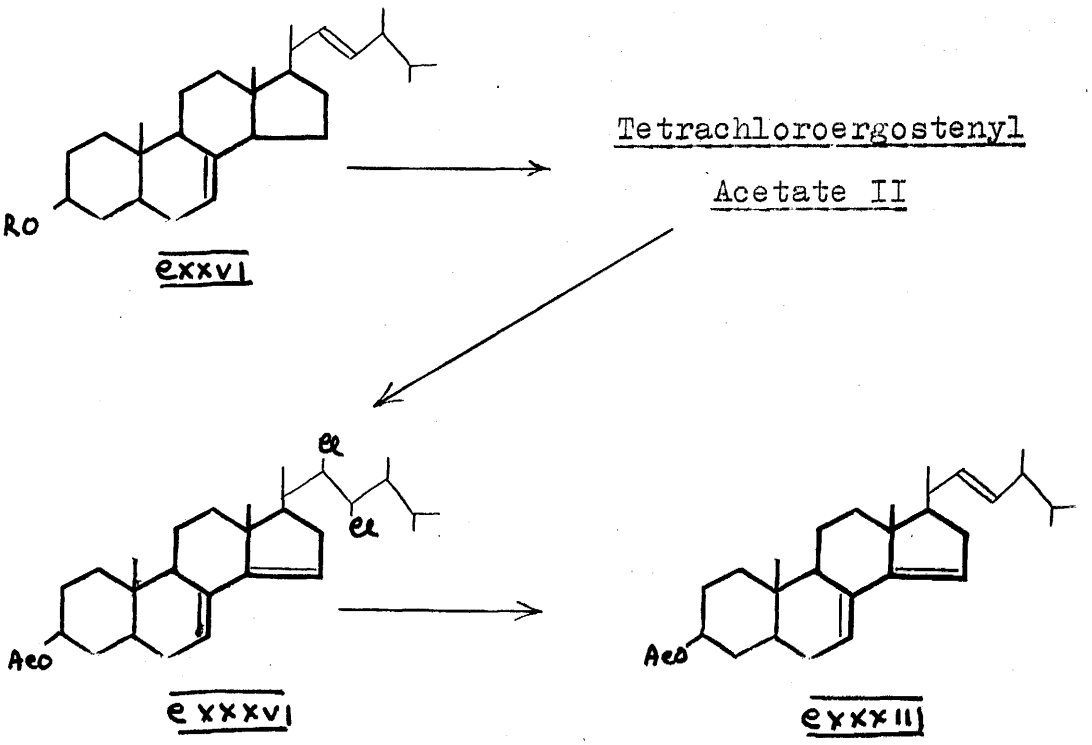
-3β-ol (CXXXV; R=H).

The chlorine atoms in the side chain appear to be more resistant to dehalogenation with zinc dust than the corresponding bromine atoms; thus ergosteryl-D acetate 22:23-dichloride is recovered unchanged when a solution of it in a mixture of ethanol and ether is treated with activated zinc dust. As has been shown previously, treatment of ergosteryl-D acetate 22:23--dibromide under such conditions gives a quantitative yield of ergosteryl-D acetate. The stability of the side chain chlorine atoms in this respect appeared at this point to jeopardise the chance of proving the structure of ergosteryl-D acetate 22:23-dichloride by direct conversion into ergosteryl-D acetate, since the more vigorous dehalogenating procedure using glacial acetic acid and zinc dust at 105-110° had been shown to isomerise the nuclear double bond system in the case of ergosteryl-D acetate 22:23-dibromide. Less vigorous modifications of this method were employed in the hope of success and, though treatment of ergosteryl-D acetate 22:23-dichloride in glacial acetic acid solution with zinc dust at room temperature for four hours yielded unchanged starting material, treatment of the glacial acetic acid solution with zinc dust at 95° for one hour was found to effect the desired conversion into

ergosteryl-D acetate.

The side chain chlorine atoms can also be removed by boiling a solution of ergosteryl-D acetate 22:23--dichloride in a mixture of benzene and ethanol with Raney nickel; hydrogenation of the 9:11-double bond simultaneously occurs, however, with the formation of 5-dihydroergosteryl acetate.

Tetrachloroergostenyl acetate II, obtained in extremely poor yield, no analogue of which has been found so far in the bromo-series, possesses a very large negative specific rotation value (-248°). Since the side chain chlorine atoms had been shown to be stable to mild dehalogenating conditions this material was treated in solution in a mixture of ethanol and ether with activated zinc dust in the expectation of removing only the nuclear chlorine atoms. This gives a compound $C_{30}H_{46}O_2Cl_2$ in low yield which is identified as 22:23--dichloroergosta-7:14-dien-3β-yl acetate (ergostenyl-B_3 acetate 22:23-dichloride) (CXXXVI), first, by its conversion into slightly impure ergosteryl-B_3 acetate (CXXXIII) by the action of zinc dust on its solution in glacial acetic acid and, secondly, by its ultra-violet absorption spectrum which is identical in location and intensity of absorption with that of ergosteryl-B_3 acetate (see table below). It possesses a large negative specific rotation



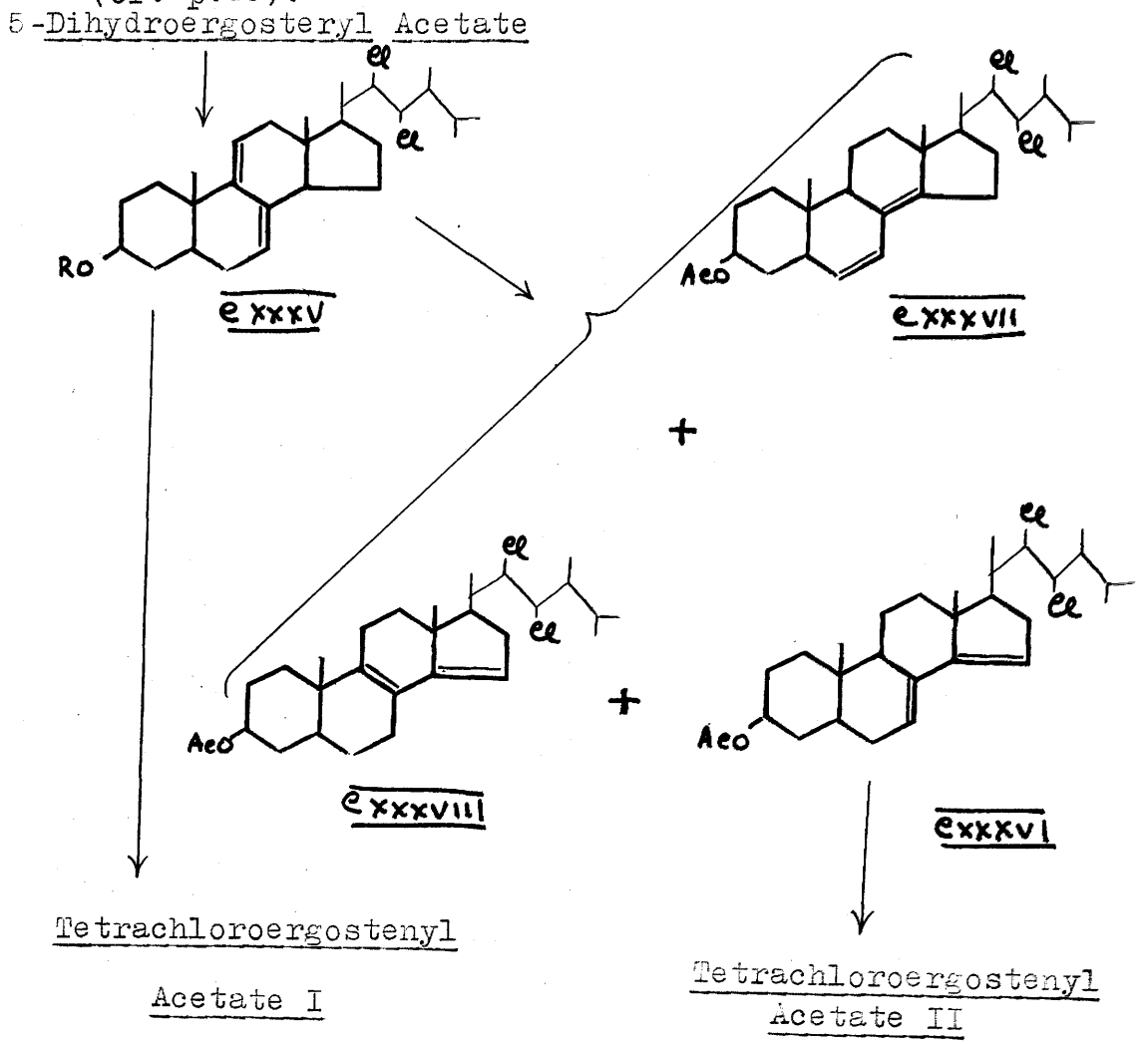
value as does ergosteryl-B_3 acetate.

	λ max. Å	ε max.	$[\alpha]_D$	M.p.	Mixed M.p.
Ergosteryl-B_3 Acetate 22:23-Dichloride	2420	10,000	-173		
Dechlorination Product	2420	8,800	-175	132-134°	132-136°
Ergosteryl-B_3 Acetate prepared as described by Barton (85)	2420	9,900	-218	138-140	

The following mechanism is put forward for the occurrence of the two isomeric tetrachloroergostenyl

acetates. This involves:

1. Trans addition of chlorine to the 22:23-double bond.
2. Introduction of a 9:11-olefinic bond due to attack by the chlorine followed by the elimination of hydrogen chloride (cf. 84).
3. Isomerism, in part, of the 7:9(11)-conjugated dien system (CXXXV; R=Ac) in the presence of hydrogen chloride to give a mixture of 8:14- (CXXXVIII), 6:8(14)- (CXXXVII) and 7:14-dien systems (CXXXVI) (cf. p.43).

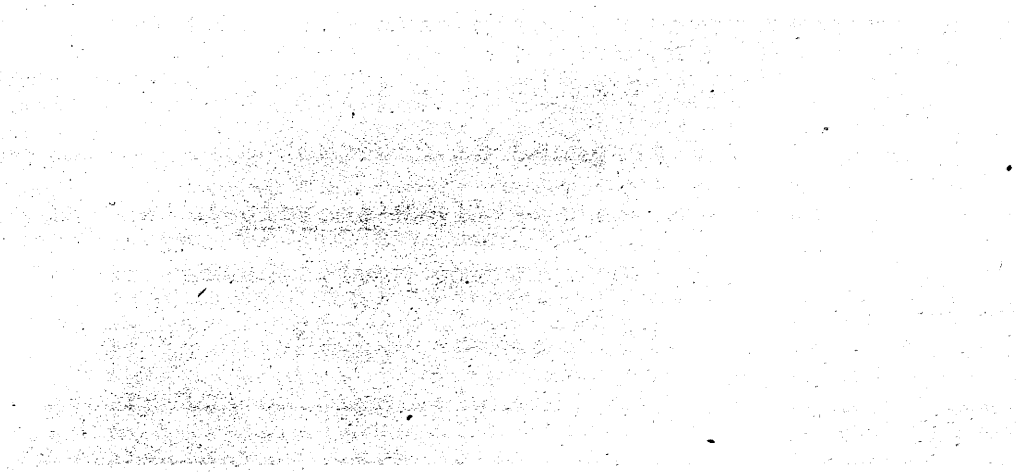


4. Addition of one molecule of chlorine to the 7:9(11)-dienic system (CXXXV; R=Ac) to give tetrachloro-ergostenyl acetate I and to the 7:14-conjugated dien system (CXXXVI) to give tetrachloroergostenyl acetate II.

It is of interest to note that ergosteryl-B_3 acetate (CXXXIII), i.e. the isomer of ergosteryl acetate possessing a 7:14-conjugated dien system, is obtained by isomerism of ergosteryl acetate in chloroform solution with dry hydrogen chloride in an equilibrium mixture with the other two isomers, ergosteryl-B_1 acetate (CXXX) and ergosteryl-B_2 acetate (CXXXII) (42) but, if the isomerism is carried out at a low temperature (-20°) the equilibrium lies in the direction of ergosteryl-B_3 acetate (CXXXIII) (85). As far as temperature is concerned, therefore, favourable conditions for the production of the 7:14--conjugated dien system exist in the chlorination experiment which is carried out at -50°.

The overall yield of pure ergosteryl-D acetate 22:23--dichloride from 5-dihydroergosteryl acetate is of the order of 2% by this method which was obviously far from satisfactory. In an attempt to improve the yield chlorination was carried out in chloroform solution containing pyridine which, it was hoped, would react with the hydrogen chloride as it was formed and thereby prevent

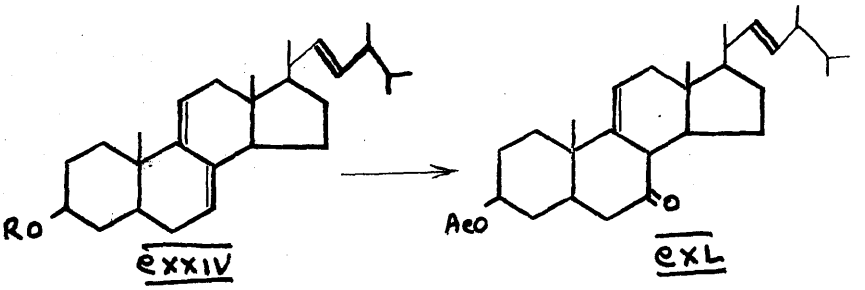
the postulated side reactions due to its isomerising effect. No attempt was made to isolate tetrachloro-ergostenyl acetate I, the reaction mixture being treated immediately with sodium iodide. By this method ergosteryl-D acetate 22:23-dichloride of reasonable purity can be obtained in 9% yield. Until this yield is greatly improved, however, it cannot be considered a practicable starting material for a synthesis of cortisone.



INTRODUCTION OF AN OXYGEN ATOM AT POSITION 11.

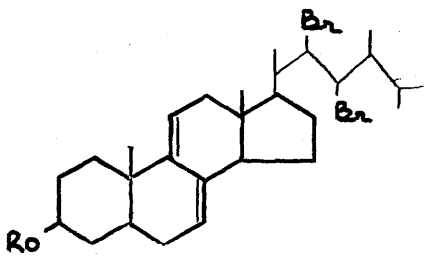
The reasonable yield in which ergosteryl-D acetate 22:23-dibromide (CXXXI; R=Ac) is obtained from 5-dihydro-ergosteryl acetate and the protection afforded by the bromine atoms in it to the side chain double bond makes this compound more attractive than ergosteryl-D acetate (CXXIV; R=Ac) as a stepping stone to 11-oxygenated steroids.

As a result of investigations using hydrogen peroxide in glacial acetic acid as oxidising agent it was found that oxidation of ergosteryl-D acetate 22:23-dibromide (CXXXI; R=Ac) with a large excess of peracetic acid gives a compound $C_{30}H_{46}O_4Br_2$ in reasonable yield (30%), which does not show selective absorption of high intensity above 2200Å and gives no colour with tetranitromethane in chloroform. This compound is assigned the structure 3β-acetoxy-22:23-dibromo-9α:11α-epoxyergostan-7-one (CXXXIX) for reasons given below. Debromination with zinc dust gives a compound $C_{30}H_{46}O_4$ which shows no selective absorption of high intensity above 2200Å and gives a faint yellow colour with tetranitromethane in chloroform. This compound was simultaneously obtained by a different route (63) and from a consideration of evidence from both methods of preparation a proof of its structure is



exxiv

exl



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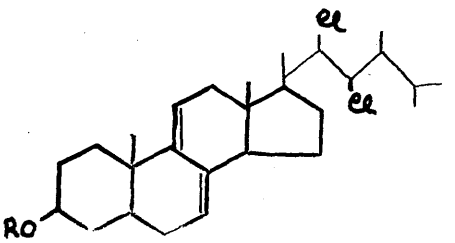

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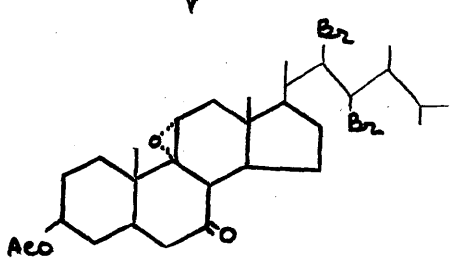

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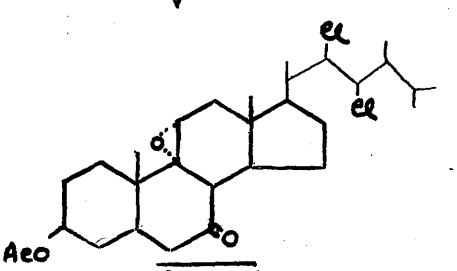
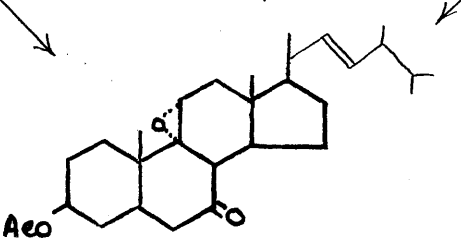


exlii



exlii

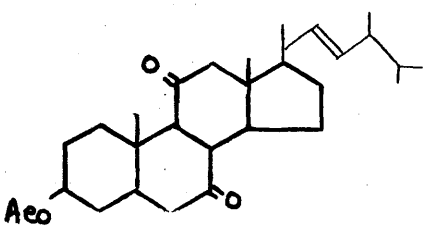
obtained as described below.

Investigations carried out by Spring (63) on the oxidation of ergosteryl-D acetate with hydrogen peroxide in formic acid showed that treatment of ergosteryl-D acetate (CXXIV; R=Ac) with one mol of performic acid resulted in oxidation of the 7-ethylenic bond with the formation of 3β-acetoxyergosta-9(11):22-dien-7-one (CXL), and that oxidation of ergosteryl-D acetate with two mols of performic acid gives a compound $C_{30}H_{46}O_4$ identical with that obtained by oxidation of ergosteryl-D acetate 22:23-dibromide with excess peracetic acid followed by debromination with zinc dust.

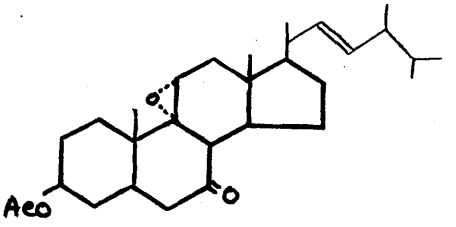
Two oxygen atoms have been introduced into the ergosteryl-D acetate molecule and, since the primary oxidation product of the performic acid oxidation of this compound has been shown to be (CXL), one of them must be present as a ketone group at the 7-position. Since the remaining centres of attack are 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 the compound $C_{30}H_{46}O_4$ is also obtained by peracetic 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. The latter step introduces a 22:23-double bond,

a fact which is supported by the light yellow colour given by the compound with tetranitromethane in chloroform.

It follows that the second oxygen atom is present either as a ketone at position 11 or as a 9:11-oxide. In the latter case there are theoretically two possibilities, viz., an oxide with the 9α:11α-configuration or with the 9β:11β-configuration. The latter possibility is excluded in view of the well established preferential rear attack of reagents at the 9- and the 11-positions (87). The possible structures for the compound $C_{30}H_{46}O_4$ are therefore 7:11-diketoergost-22-en-3β-yl acetate (CXLI)



CXLI



CXLII

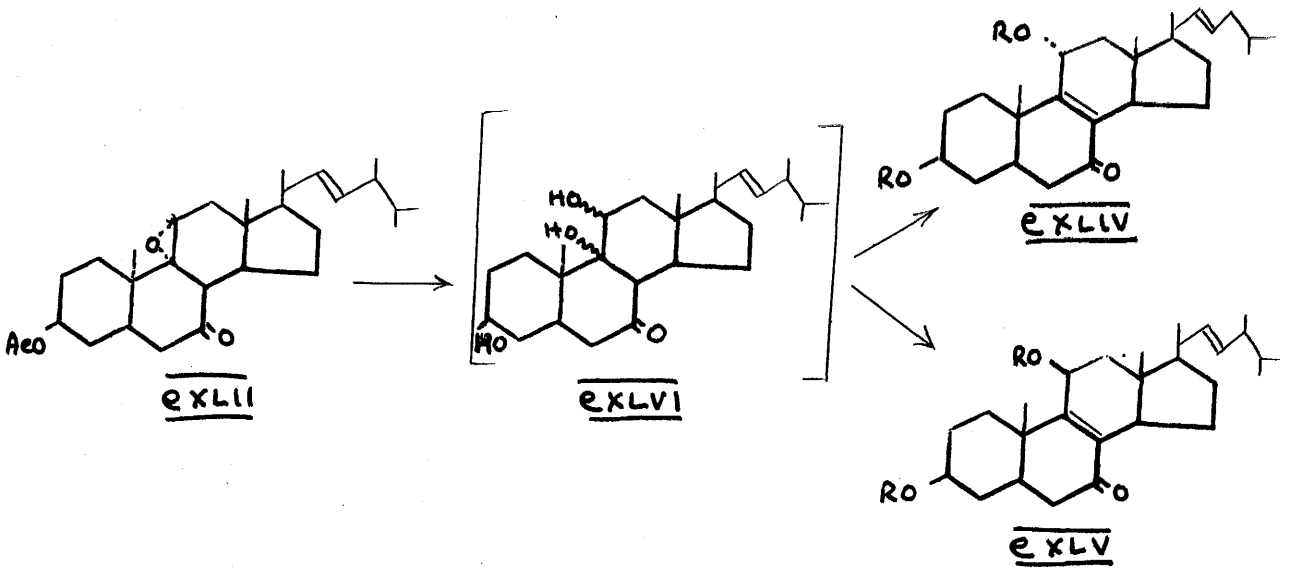
and 9α:11α-epoxy-7-ketoergost-22-en-3β-yl acetate (CXLII). It is however different from the 7:11-diketone (CXLI) which has recently been prepared by Chamberlin et al (47) and by Heusser et al (56) and therefore must be 9α:11α--epoxy-7-ketoergost-22-en-3β-yl acetate (CXLII). It also follows that the compound $C_{30}H_{46}O_4Br_2$, which yields (CXLII) on debromination with zinc dust, is 3β-acetoxy-

-22:23-dibromo-9α:11α-epoxyergostan-7-one (CXXXIX). The preparation of (CXLII) has also been described by Spring (63) starting from 3β-acetoxyergosta-9(11):22-dien-7-one (CXL). This involves protection of the 22-double bond in (CXL) by the addition of one mol of bromine, followed by oxidation with perbenzoic acid and debromination of the product with zinc.

An analogous series of reactions to these reported above have been carried out starting from ergosteryl-D acetate 22:23-dichloride (CXXXV; R=Ac) which, when treated with an excess of peracetic acid, gives a compound $C_{30}H_{46}O_2Cl_2$ showing no absorption of high intensity above 2200Å and giving no colour with tetranitromethane in chloroform. This compound is identified as 3β-acetoxy--22:23-dichloro-9α:11α-epoxyergostan-7-one (CXLIII) by its conversion into 9α:11α-epoxy-7-ketoergost-22-en-3β-yl acetate (CXLII) with zinc dust. The identity of (CXLII) was in turn established by direct comparison with a specimen obtained by the route via ergosteryl-D acetate 22:23-dibromide (CXXXI; R=Ac).

Saponification of 9α:11α-epoxy-7-ketoergost-22-en--3β-yl acetate (CXLII) gives a compound $C_{28}H_{44}O_3$ identified as 3β:11α-dihydroxyergosta-8:22-dien-7-one (CXLIV; R=H) for reasons given below. The presence of two hydroxyl groups in this compound is indicated by the fact

that it forms a diacetate $C_{32}H_{48}O_5$; also, its ultra-violet absorption spectrum [maximum at 2540Å (ε = 9100)] and that of the diacetate [maximum at 2520Å (ε = 10,200)], combined with the observation that both compounds give a faint yellow colour with tetranitromethane in chloroform,

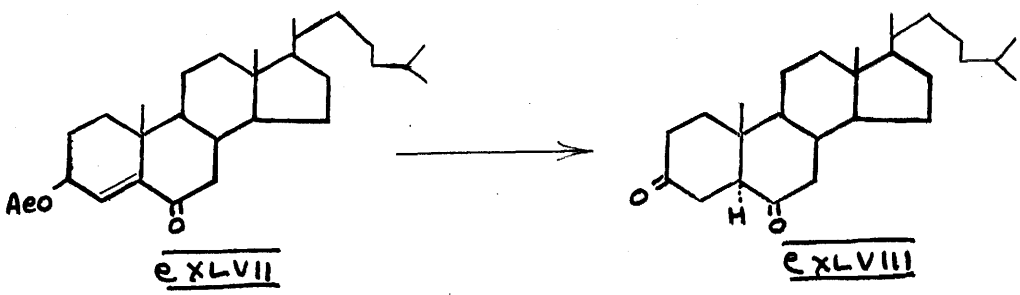


suggest that it is an $\alpha\beta$-unsaturated ketone. Furthermore, since the precursor (CXLII) contains a ketone group at the 7-position it follows that the compound $C_{28}H_{44}O_3$ is either 3β:11α-dihydroxyergosta-8:22-dien-7-one (CXLIV; R=H) or 3β:11β-dihydroxyergosta-8:22-dien-7-one (CXLV; R=H). The formation of such structures (CXLIV; R=H; CXLV; R=H) from (CXLII) could be readily explained by a mechanism involving hydrolysis of the 9:11-oxide with the transitory formation of a 9:11-diol (CXLVI), followed by elimination of a molecule of water involving the

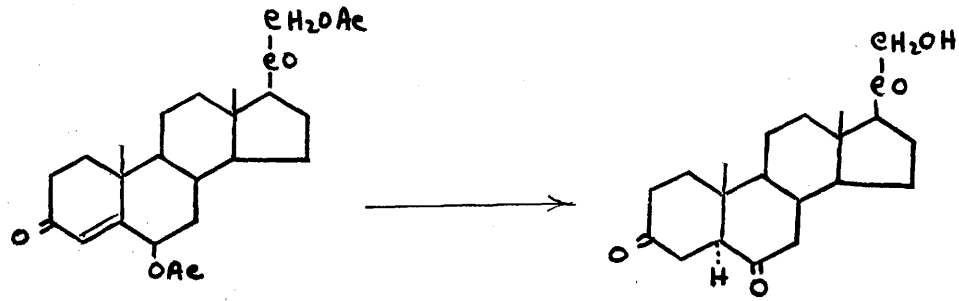
hydroxyl group at C_9 and the hydrogen atom at C_8. Such an intermediate (CXLVI) has in fact been isolated in the formation of (CXLIV) by another method (62,63).

In an endeavour to establish without doubt the presence of an oxygen atom at C_{11} in the compound $C_{28}H_{44}O_3$ a method was sought of converting it into a known 11--oxygenated steroid. In this connection it was noted that Heilbron, Jones and Spring (88) converted 6-keto-cholest-4-en-3β-yl acetate (CXLVII) into cholestan-3:6--dione (CXLVIII) by treatment with alkali; also that Herzig and Ehrenstein (89) obtained 21-hydroxyallopregnan--3:6:20-trione (CL) on similar treatment of 6β:21--diacetoxypregn-4-en-3:20-dione (CXLIX). In both cases an acetylated αβ-unsaturated 1:4-ketol system is first hydrolysed to the free ketol and then isomerised to a 1:4-dione system. Such a conversion of the diacetate of compound $C_{28}H_{44}O_3$ assuming either of the possible structures (CXLIV; R=Ac; CXLV; R=Ac) to be correct should give, after acetylation of the product, the corresponding 1:4-dione (CXLI) which is a known compound, and this in fact was found to be the case.

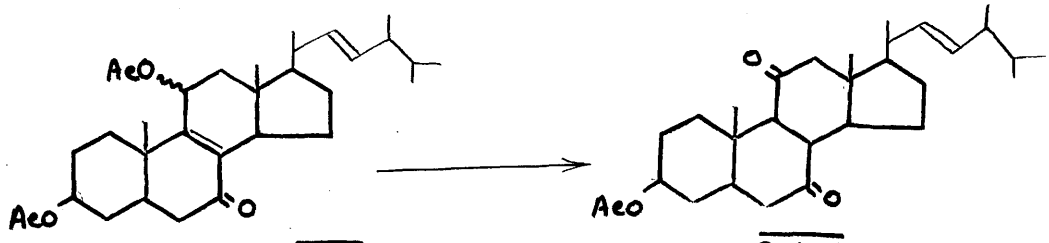
Treatment of the diacetate of the compound $C_{28}H_{44}O_3$ with strong aqueous-ethanolic potassium hydroxide followed by acetylation yielded a reaction mixture, one component of which was readily isolated in a pure state



CXLVII CXLVIII



CXLIX CL



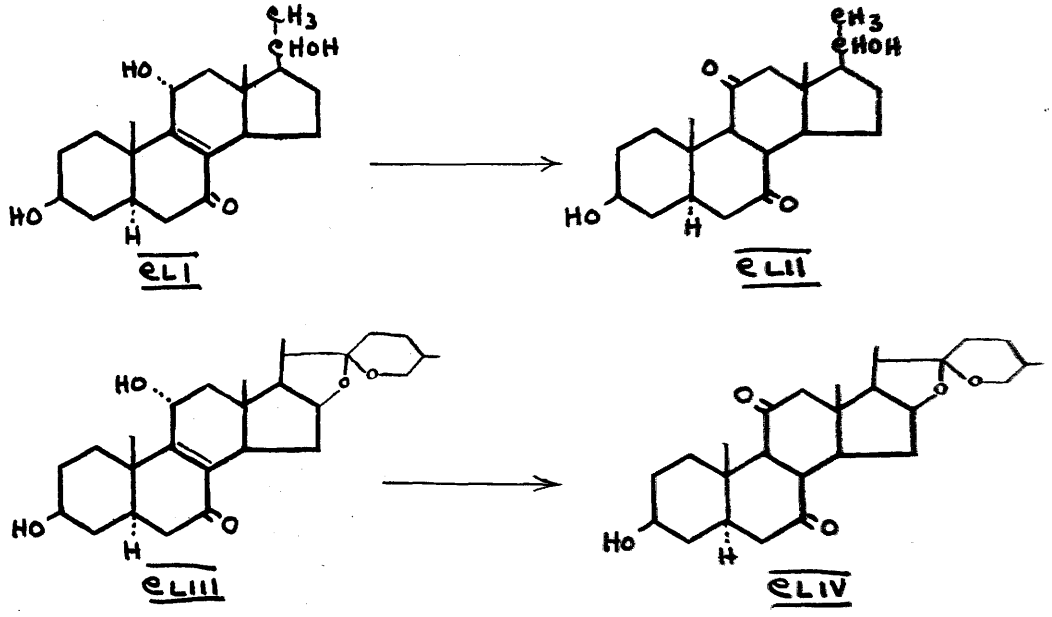
CXLIV (R=Ac); CXLV (R=Ac). CXLI

by chromatography on alumina. This material proved to be identical with 7:11-diketoergost-22-en-3β-yl acetate (CXL), the identity being established by direct comparison with a specimen prepared as described by Heusser et al (56).

The other component is present as a mixture, probably with the 7:11-diketone (CXLI), and this mixture shows no selective absorption above 2200Å. It follows, therefore,

that this other component shows no absorption in this region. The 7:11-diketone has also been prepared by Spring (63) directly from 9α:11α-epoxy-7-ketoergost-22--en-3β-yl acetate (CXLII) using strong alkali. A second component is also isolated from this reaction which does not show selective absorption above 2200Å. It is extremely probable that this material is the same as that isolated in an impure state described above. The structure of this compound has not yet been established.

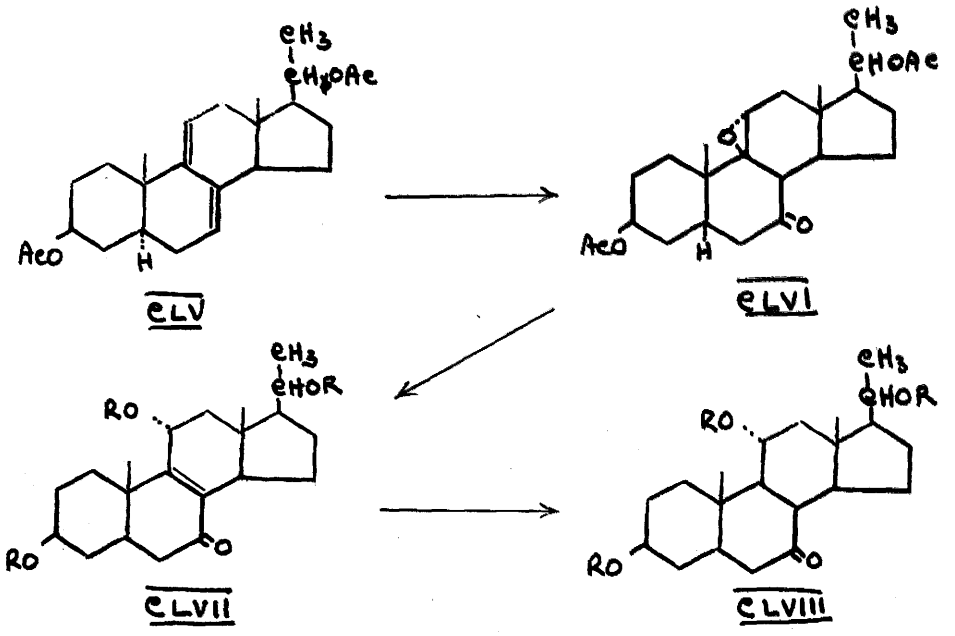
Rearrangements similar in nature to the conversion of 3β:11α-diacetoxyergosta-8:22-dien-7-one (CXLIV; R=Ac)



into 7:11-diketoergost-22-en-3β-yl acetate (CXLI) have recently been described by Djerassi (9C) in the allo-pregnane and diosgenin (22-isoallospirostane) series using potassium t-butoxide as is illustrated by the conversion

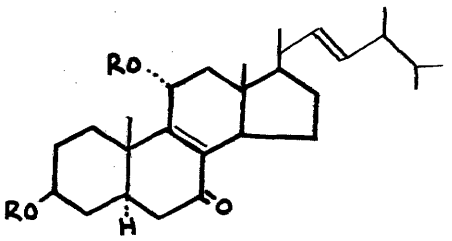
of (CLI) into (CLII) and (CLIII) into (CLIV) respectively.

The structure of the compound $C_{25}H_{44}O_5$ is therefore established apart from the configuration of the hydroxyl group at C_{11}. It is a well established fact that an 11α-hydroxyl group can readily be acetylated whereas an 11β-hydroxyl group cannot (91) and, since the compound $C_{28}H_{44}O_5$ readily forms a diacetate, it would appear that the 11β-configuration (CXLV; R=H) is excluded. This argument, however, may be fallacious since an 8-double bond may affect the accessibility of an 11β-hydroxyl group (cf. 67). However, an argument based on analogy can be presented in favour of the 11α-configuration (CXLIV; R=H). Djerassi and co-workers have employed a method for the introduction of an oxygen atom at C_{11} analogous to that described herein which is applied to diosgenin (isoallospirostane)(LXXV) and allopregnane (I) derivatives (43,60,64). These workers (64) obtain 3β:20β-diacetoxy-9α:11α-epoxyallopregnan-7-one (CLVI) by performic acid oxidation of 3β:20β-diacetoxyallopregna--7:9(11)-diene (CLV). The former, on saponification gives 3β:11α:20β-trihydroxyallopregn-8-en-7-one (CLVII; R=H) which forms a triacetate (CLVII; R=Ac). Catalytic hydrogenation of 3β:11α:20β-trihydroxyallopregn-8-en-7-one (CLVII; R=H) gives 3β:11α:20β-trihydroxyallopregnan-7-one (CLVIII; R=H) which forms a triacetate (CLVIII; R=Ac), a

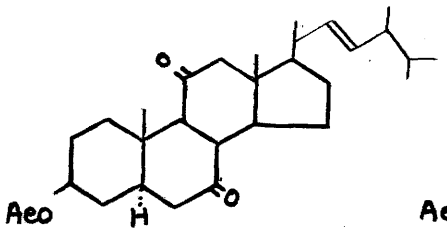


fact which proves the presence of an 11α-hydroxyl group in (CLVIII; R=H) and therefore also in its precursor (CLVII; R=H). Since saponification of 9α:11α-epoxy-7--keto-ergost-22-en-3β-yl acetate (CXLII), which possesses the same ketoxide structure as is present in (CLVI), results in the formation of the compound $C_{28}H_{44}O_5$, the latter is considered to possess an 11α-hydroxy group and is therefore 3β:11α-dihydroxyergosta-8:22-dien-7-one (CXLIV; R=H) from which it follows also that the diacetate is 3β:11α-diacetoxyergosta-8:22-dien-7-one (CXLIV; R=Ac).

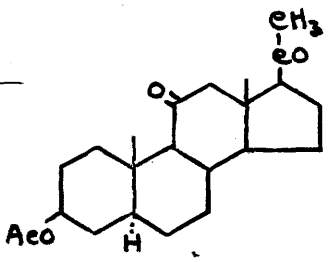
The preparation of 7:11-diketoergost-22-en-3β-yl acetate (CXLI) via ergosteryl-D acetate 22:23-dibromide (CXXXI; R=Ac) and ergosteryl-D acetate 22:23-dichloride (CXXXV; R=Ac), outlined herein, offers two alternative routes to cortisone (VII) starting from ergosterol since



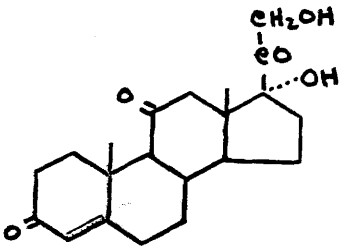
CXLIV



CXLI



CLIX

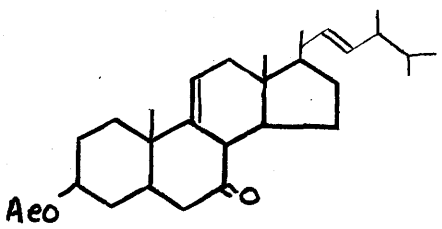


VII

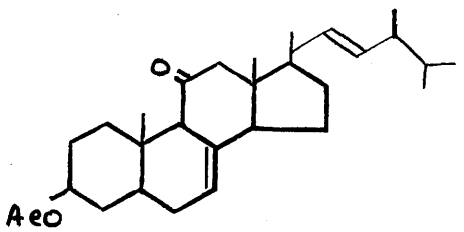
the diketone (CXLI) has been converted into 11:20--diketoallopregnan-3β-yl acetate (CLIX) (47), which has in turn been converted into cortisone (48,92).

THE CONFIGURATION AT C_8 IN 9α:11α-EPOXY-7-KETO-ERGOST-22-EN-3β-YL ACETATE AND IN ITS 22:23-DIBROMIDE AND 22:23-DICHLORIDE.

At an early stage in this work preliminary investigations were carried out on the oxidation of ergosteryl-D acetate with chromium trioxide, when it was found that treatment of ergosteryl-D acetate with three oxygen atoms followed by resolution of the product into ketonic and non-ketonic fractions using Girard's reagent furnished a compound $C_{30}H_{46}O_3$ present in the ketonic fraction which



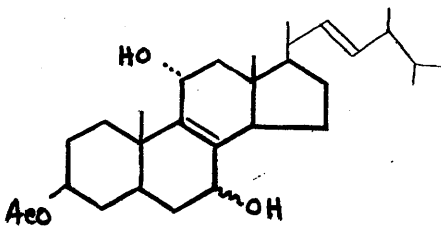
CXL



CLX

did not show light absorption of high intensity above 2200Å. It follows, therefore, that this compound is either 3β-acetoxyergosta-9(11):22-dien-7-one (CXL) or 3β-acetoxyergosta-7:22-dien-11-one (CLX). In view of the well established fact that 11-ketonic groups do not react with carbonyl reagents (91) however, the latter possibility is excluded and the compound must therefore

be 3β-acetoxyergosta-9(11):22-dien-7-one (CXL). This compound has recently been prepared by Jeger (93) by a different route. This involves treatment of 7ξ:11α--dihydroxyergosta-8:22-dien--3β-yl acetate (CLXI) with peracetic acid. No oxidation was found to take place; instead a dehydration occurred with the formation of 3β--acetoxyergosta-9(11):22-dien-7-one (CXL). The presence of a ketonic group in a six-membered ring is indicated by the infra-red absorption spectrum. Their product is identical in melting point and specific rotation value with those of the product obtained from oxidation of ergosteryl-D acetate with chromium trioxide (see table below).

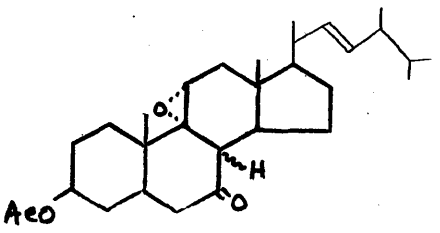


CLXI

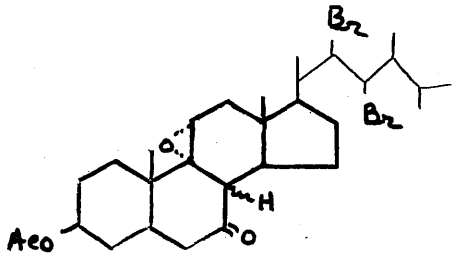
3β-Acetoxyergosta-9(11):22-dien-7-one

Preparation	M.p.	$[\alpha]_D$
This Thesis	176-178°	-55.5°
According to Jeger (93)	176-177	-58
According to Spring (63)	194-197	+20

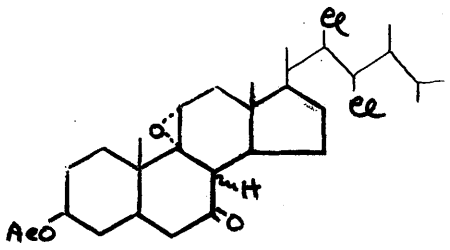
The preparation of 7-ketoergosta-9(11):22-dien-3β-yl acetate (CLX), however, has also been reported by Spring (63), as mentioned in the previous section (see p.58), and their product differs in physical properties from those of the compound described above. Since there is little doubt that both compounds contain a 7-ketone group and a 9:11-double bond, it follows that they must differ in the configuration of the hydrogen atom at C_8. No evidence is so far available to prove which one of the two isomeric ketones possesses the normal, i.e. α-configuration of the hydrogen atom at C_8 and at the moment it must suffice to call the ketone prepared by Spring (63) 3β-acetoxyergosta-9(11):22-dien-7-one I and that prepared as described in this thesis and by Jeger (93) as 3β--acetoxyergosta-9(11):22-dien-7-one II.



CLXII



CLXIII

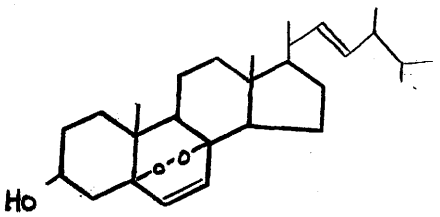


CLXIV

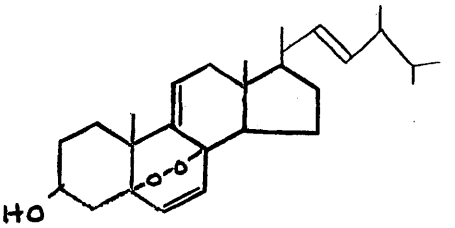
It follows, therefore, since the precursor of 9α:11α-epoxy-7-ketoergosta-22-en-3β-yl acetate (CXLII; CLXII) is 3β-acetoxyergosta-9(11):22-dien-7-one I that the configuration of the hydrogen atom at C_8 in this compound, and therefore in 3β-acetoxy-22:23-dibromo--9α:11α-epoxyergostan-7-one (CXXXIX; CLXIII) and 3β-acetoxy-22:23-dichloro-9α:11α-epoxyergostan-7-one (CXLIII; CLXIV) is at present unknown but is similar in configuration to that of the hydrogen atom at C_8 in 3β-acetoxyergosta-9(11):22-dien-7-one I.

3β-ACETOXYERGOSTA-8(14):9(11):22-TRIEN-7-ONE.

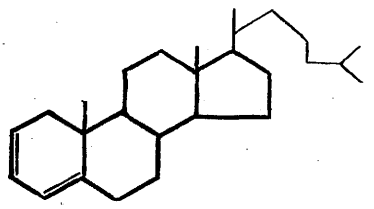
A property apparently characteristic of homoannular dienes, which react readily with maleic anhydride to form adducts, is the ability to add oxygen in the presence of artificial light and eosin to form transannular peroxides. For example, ergosterol (LXXXI; R=H) 9:11-dehydroergosterol (LXXXII; R=H) and cholesta-2:4-diene (CLXV), which react



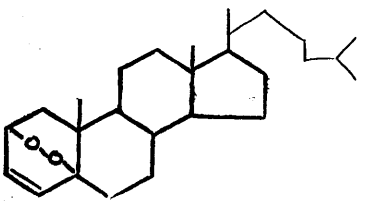
CLXVI



CLXVII



CLXV



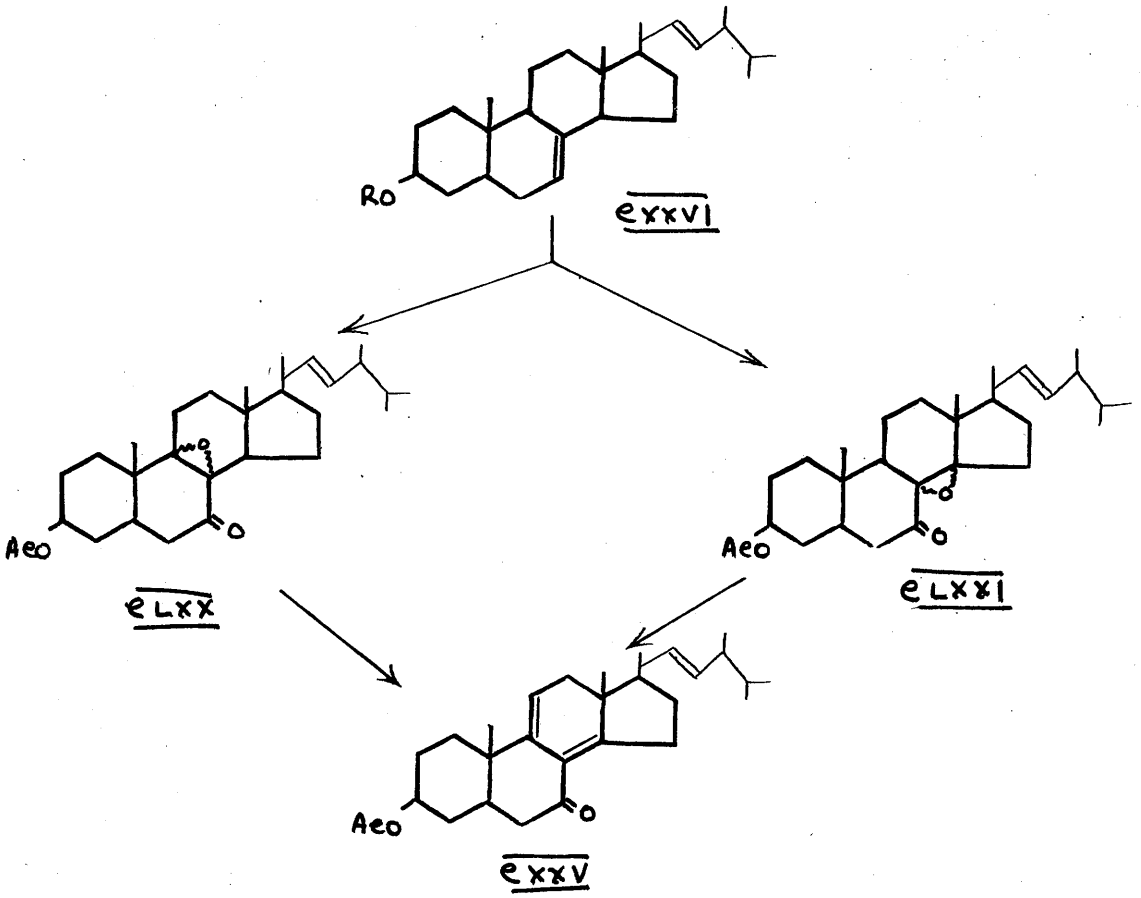
CLXVIII

with maleic anhydride readily, if subjected to the peroxidising conditions described above, give respectively 5:8-peroxidoergosta-6:22-dien-3β-ol (CLXVI)(77), 5:8--peroxidoergosta-6:9(11):22-trien-3β-ol (CLXVII)(94) and 2:5-peroxidocholest-3-ene (CLXVIII)(95,96). Heteroannular dienes, on the other hand, generally do not react with maleic anhydride, although there are some exceptions,

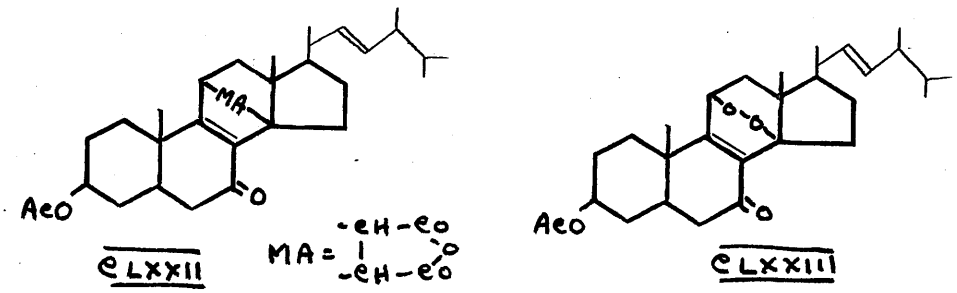
nor do they form peroxides, as is exemplified by the case of ergosteryl-D acetate (CXXIV; R=Ac) which does not form a maleic anhydride adduct (97) and an attempt to form a peroxide of this material, in an effort to introduce an oxygen atom at C_{11}, proved unsuccessful.

It was with a view to employing this peroxidation of homoannular dienic systems to introduce an oxygen atom at C_{11} that the triply unsaturated ketone prepared by Stavely and Ballenbock (71) attracted attention, since they suggest that this material is 3β-acetoxyergosta--8(14):9(11):22-trien-7-one (CXXV), i.e. possessing a homoannular dienic system in ring C. These authors oxidised 5-dihydroergosteryl acetate (CXXVI; R=Ac) with chromium trioxide and isolated the two ketoxides (CLXX) and (CLXXI), among other materials, from the reaction mixture. They found that both ketoxides, on treatment with hydrochloric acid, yielded the same triply unsaturated ketone (CXXV). Some of this material was prepared directly from 5-dihydroergosteryl acetate in 15% yield using Stavely and Ballenbock's method but without isolating the intermediate ketoxides.

First, an attempt was made to form the maleic anhydride adduct (CLXXII) of this material, the formation of which was expected if the peroxidation of the dienic system were possible.



Unfortunately, in this reaction and in an experiment attempting to form the transannular peroxide (CLXXIII)

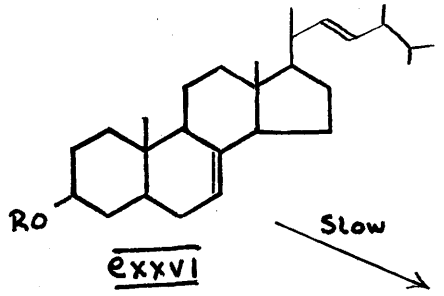


of the triply unsaturated ketone the sole product was unchanged starting material.

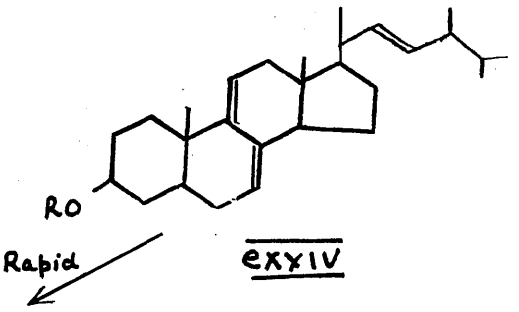
DISCUSSION ON THE STRUCTURE OF THE TETRABROMOERGOSTENYL ACETATE.

As mentioned previously, the conversion of the tetrabromide obtained by bromination of 5-dihydroergosteryl acetate (CXXVI; R=Ac) into ergosteryl-D acetate 22:23--dibromide (CXXXI; R=Ac), which possesses two nuclear double bonds, by the action of sodium iodide must mean that this compound contains one double bond which is in the nucleus, i.e. it is a tetrabromoergostenyl acetate. It follows also that two of the four bromine atoms are in the 22- and 23-positions in the side chain.

It has been found that the tetrabromide is also obtained by bromination of either 5-dihydroergosteryl acetate 22:23-dibromide (CLXXIV) prepared as described by Spring (98), ergosteryl-D acetate (CXXIV; R=Ac) or ergosteryl-D acetate 22:23-dibromide (CXXXI; R=Ac). In all methods of preparation the product separates from the reaction mixture due to its insolubility in ether. However, by the first two methods there is a time lag of 15 to 30 minutes before the product separates whereas by the last two methods, in which the starting material already possesses a 9(11)-olefinic bond, the product separates immediately. Those facts show that addition of bromine to the side chain double bond and to the

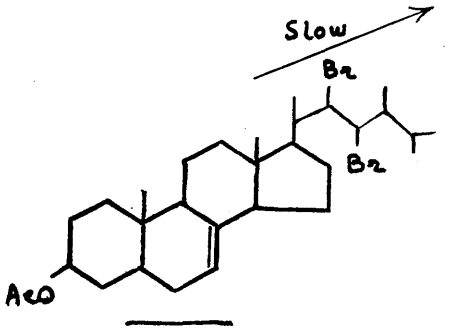


CXXVI

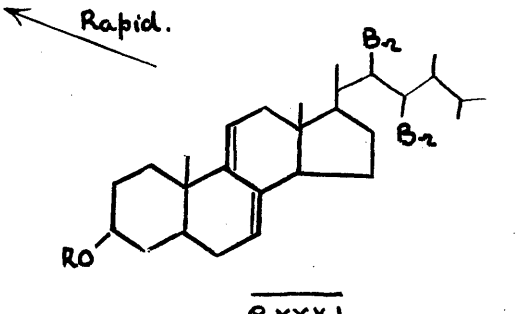


CXXIV

Tetrabromoergostenyl Acetate



CLXXIV



CXXXI

nuclear diene system are both rapid reactions whereas the oxidising action of the bromine introducing the 9(11)-double bond is a slow reaction. The mechanism of the reaction would therefore appear to be as follows:

1. Rapid addition of bromine to the side chain double bond and, since it is a well established fact that halogens add to a double bond in a trans fashion, this will be trans addition.

2. Slow dehydrogenation of the molecule due to the oxidising action of the bromine with the production of the 7:9(11)-conjugated-diene system and simultaneously

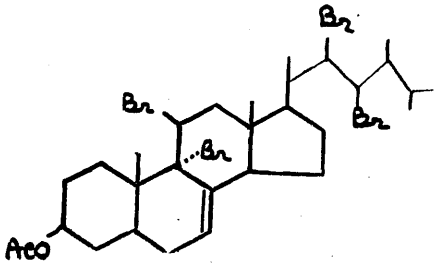
of hydrogen bromide.

3. Rapid addition of bromine to the 7:9(11)-conjugated diene system with the formation of the insoluble tetrabromide which therefore separates from the reaction mixture.

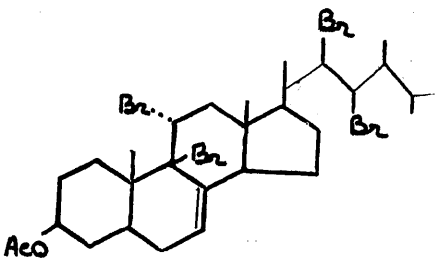
The problems remaining to be solved in elucidating the structure of the tetrabromoergostenyl acetate are therefore, first, the determination of the location and configuration of the two nuclear bromine atoms and, secondly, the determination of the configuration of substituents around C_{22} and C_{23}. The latter problem arises from the fact that trans addition of bromine to the side chain double bond, which produces two new asymmetric carbon atoms, can theoretically take place in two ways with the production of two optical isomers. Racemisation may have occurred; on the other hand only one of the two possible optical isomers may be stable due to steric conditions. No work has been carried out in investigating the occurrence of such isomers. From the point of view of this work it has not been found necessary to do so since removal of bromine from the side chain results in the formation of compounds retaining the natural configuration of the side chain double bond. The same problem arises with compounds containing chlorine in the side chain.

The first point to be settled in the former problem

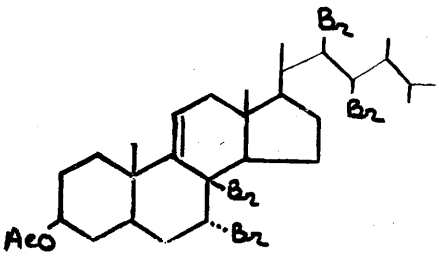
is whether 1:2- or 1:4-addition of bromine to the 7:9(11)-conjugated diene system has taken place. In the former case there are four possible structures for the tetrabromoergostenyl acetate, two resulting from trans addition to the 9(11)-double bond (CLXXV and CLXXVI) and two resulting from trans addition to the 7:8--double bond (CLXXVII and CLXXVIII). In the latter case four possible structures also exist (CLXXIX) depending on the configuration of the bromine atoms at positions 7 and 11.



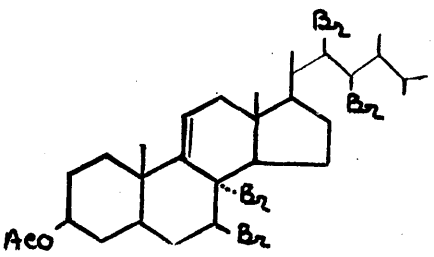
CLXXV



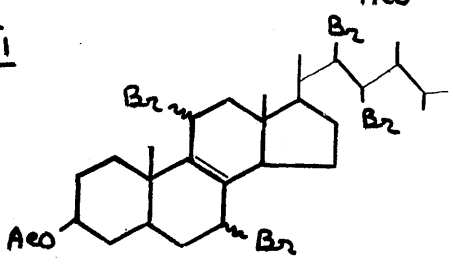
CLXXVI



CLXXVII



CLXXVIII



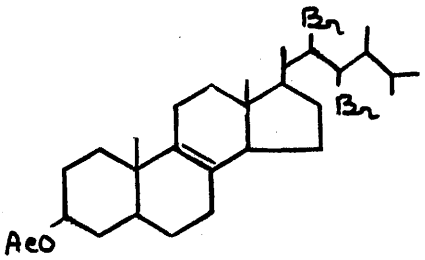
CLXXIX

The possibility of 1:4-addition is at first sight excluded by the fact that the two nuclear bromines are removed by the action of sodium iodide. This dehalogenation procedure is a modification of the Finkelstein reaction (99) which is a method of preparing alkyl iodides from reactive alkyl chlorides or bromides and depends on the fact that di-iodo-compounds containing the halogens united to adjacent carbon atoms are unstable and readily lose iodine to form unsaturated compounds. This evidence, on closer inspection, is not sufficient to exclude structures (CLXXIX) since no analogous reaction between sodium iodide and an allylic dibromide such as (CLXXIX) has been found reported in the literature. Furthermore there exists the possibility that a 7:11-di--iodide is first formed which then undergoes an allylic rearrangement to a 9:11- or 7:8-di-iodide with subsequent liberation of iodine. This, however, would involve the conversion of a secondary halide into a tertiary halide.

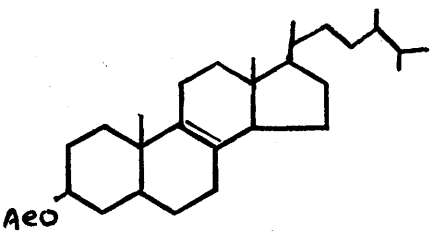
Since all possible structures for the tetrabromoergostenyl acetate are allylic bromides an endeavour was made to find out if it exists in an unstable allylic form. Conditions known to cause allylic rearrangement of butenyl bromides described by Young and Lane (100) using 48% hydrobromic acid were therefore applied to the tetrabromoergostenyl acetate, whereupon no rearrangement

was found to take place; unchanged starting material being recovered in 67% yield. It was therefore concluded that the tetrabromide exists in the stable allylic form.

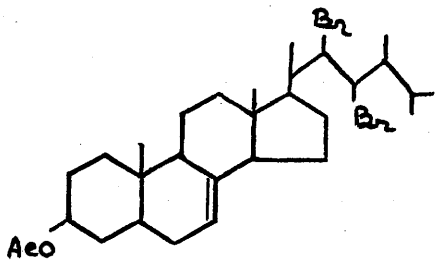
A catalytic hydrogenation of the tetrabromide in solution in dioxan, in which it is quite stable, using a platinum catalyst was carried out with a view to saturating the nuclear bromine atoms. Spring and co-workers (98) have shown that the side chain bromine atoms show considerable resistance to hydrogenation with platinum. In the event of any of the 1:4-bromo-structures (CLXXIX) being correct, since hydrogen bromide is soluble in dioxan, a two mol.absorption of hydrogen was expected with the formation of 22:23-dibromoergost-8-en-3β-yl acetate (CLXXX), an unknown compound, whose structure could be established by hydrogenation of the side chain bromine atoms to form the known ergost-8-en-3β-yl acetate (CLXXXI)(101). Also if structure (CLXXV) were correct, assuming no Walden inversion to take place on catalytic hydrogenation, the formation of the known 5-dihydroergosteryl acetate 22:23-dibromide (CLXXIV)(98) was to be expected and, in the event of the other three possible structures being correct the formation of the corresponding 22:23-dibromoergostan-3β-yl acetates, all of which are unknown compounds, was to be expected. In actual fact a one mol.absorption of hydrogen was observed and the



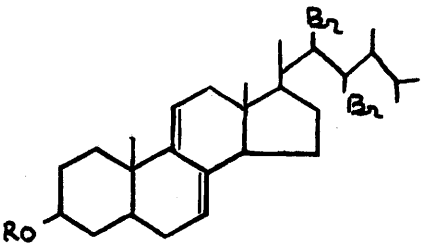
CLXXX



CLXXXI



CLXXIV



CXXXI

product was found to be ergosteryl-D acetate 22:23--dibromide (CXXXI; R=Ac), a result from which, unfortunately, no conclusive evidence can be obtained on the structure of the tetrabromide. It does, however, allow an argument to be put forward in favour of the 1:2-dibromo-structures (CLXXV-CLXXVIII) and against the 1:4-dibromo-structures (CLXXIX) based on the fact that a simpler and more probable mechanism can be postulated for the conversion of the former structures into ergosteryl-D acetate 22:23-dibromide than for a similar conversion of the latter structures.

The former conversion could be readily explained by hydrogenation of one of the nuclear bromine atoms followed by elimination of hydrogen bromide whereas the latter conversion would require first, hydrogenation of only one of the allylic, and therefore activated, bromine atoms in spite of the fact that there are two, secondly, an allylic rearrangement of the remaining bromine atom from a secondary position to a tertiary position and, finally, elimination of a molecule of hydrogen bromide.

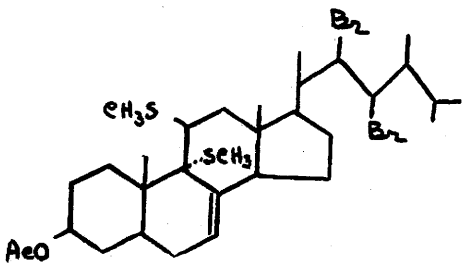
An attempt was made to replace the nuclear bromine atoms by methoxyl groups using sodium methoxide in the cold but no such replacement took place; instead the nuclear bromine atoms were removed as hydrogen bromide due to the alkaline conditions existing in the reaction mixture to give a product which is discussed later.

The idea of replacing the nuclear bromine atoms by methoxyl groups brought to mind the analogous reaction of replacing them by thiomethoxyl groups which in turn could be replaced by hydrogen using Raney nickel, thus offering another method of possibly saturating the nuclear bromine atoms. With this view in mind, the tetrabromide was treated in the cold with sodium methyl sulphide as a result of which a compound $C_{32}H_{52}O_2S_2Br_2$ was obtained which, since its precursor is a compound containing one nuclear double bond and two reactive nuclear bromine

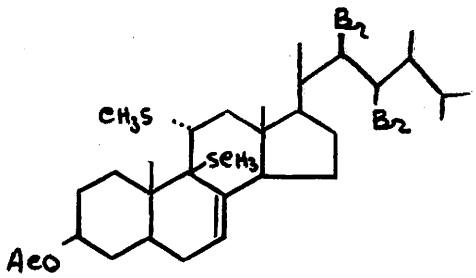
atoms, can be reasonably assumed to possess two nuclear methylthio groups and also one nuclear double bond. The presence of the latter is supported by the observation that the compound gives a deep yellow colour with tetranitromethane in chloroform.

The dimethylthio-compound was treated with Raney nickel in the expectation of replacing the methylthio groups by hydrogen but unfortunately this resulted, as in the case of hydrogenation of the tetrabromide, in the production of a 7:9(11)-conjugated diene system, i.e. ergosteryl-D acetate was formed. Raney nickel has previously been shown to remove side chain halogen atoms with simultaneous regeneration of the side chain double bond (see p.51). This conversion must involve removal of one methylthio group due to the desulphurising action of the Raney nickel followed by elimination of the other group as methyl mercaptan with the production of a double bond. The similarity in reaction of the tetrabromide and the methylthio-compound towards catalysed hydrogen suggests that they possess a similar structure, i.e. that the methylthio groups in the dimethylthio-compound are in the same positions as the nuclear bromine atoms in the tetrabromide. If this is the case then it can be reasoned, as follows, that the nuclear bromine atoms of the tetrabromide are either at C_7 and C_8 or at C_9 and C_{11},

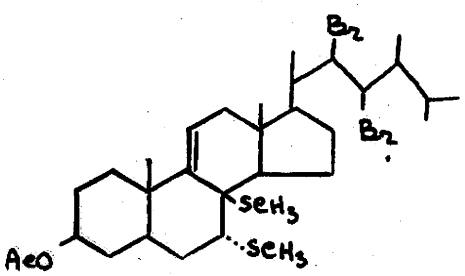
i.e. that 1:2-addition to the 7:9(11)-conjugated diene system has taken place in the formation of the tetrabromide. The possible structures for the dimethylthio-compound are, therefore, eight in number and are exactly analogous to those put forward for the tetrabromide, i.e. they either possess methylthio groups at C_8 and C_{11} (CLXXXII and CLXXXIII), at C_7 and C_8 (CLXXXIV and CLXXXV) or at C_7 and C_{11} (CLXXXVI).



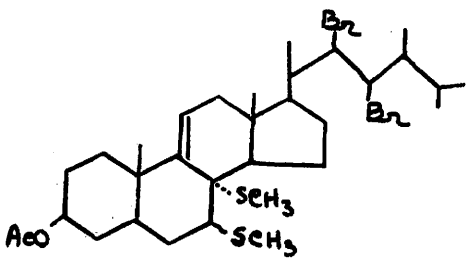
CLXXXII



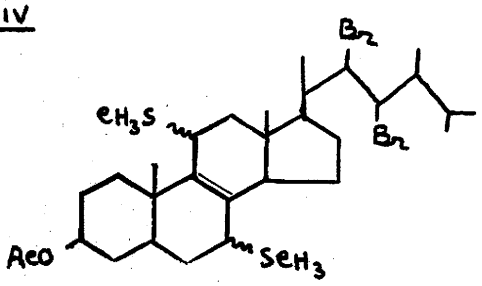
CLXXXIII



CLXXXIV



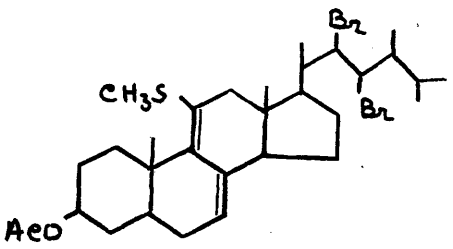
CLXXXV



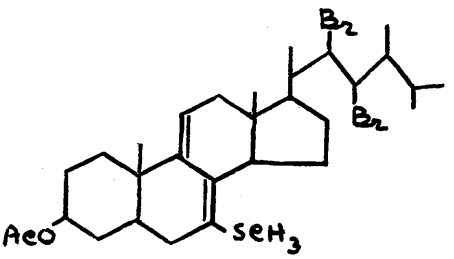
CLXXXVI

The conversion of the tetrabromide into ergosteryl-D acetate 22:23-dibromide by catalytic hydrogenation has been shown not to be sufficient evidence to eliminate the 1:4-dibromo structures (CLXXIX) as possibilities for the tetrabromide since the conversion of these structures into ergosteryl-D acetate 22:23-dibromide could be explained if an allylic rearrangement took place. However, the conversion of the dimethylthio-compound into ergosteryl-D acetate does eliminate the 1:4-dimethylthio-compound since no corresponding allylic rearrangement can occur in this case. It follows, therefore, that the methylthio-groups are either in at C_7 and C_8 or at C_9 and C_{11} and, assuming no rearrangement to have taken place during the replacement of the bromine atoms by methylthio-groups, the two nuclear bromine atoms in the tetrabromide must occupy similar positions.

A second product, a monomethylthio-compound, $C_{31}H_{48}O_2SBr_2$, was also isolated from the reaction mixture which is identified as either 22:23-dibromo-11-methylthioergosta-7:9(11)-dien-3β-yl acetate (CLXXXVII) or



CLXXXVII



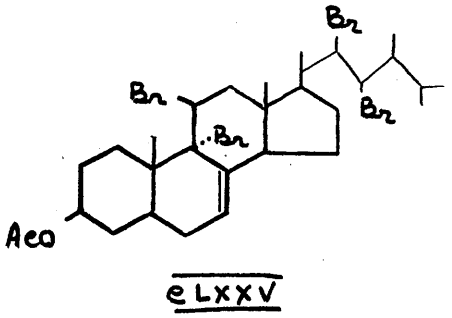
CLXXXVIII

22:23-dibromo-7-methylthioergosta-7:9(11)-dien-3β-yl acetate (CLXXXVIII) first, by its ultra-violet absorption spectrum which is identical in location and intensity of absorption with that of ergosteryl-D acetate and, secondly, by its conversion into ergosteryl-D acetate by treatment with Raney nickel.

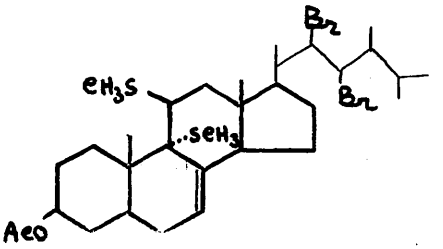
The evidence so far, therefore, though not of a conclusive nature, suggests that the nuclear bromine atoms of the tetrabromide are on adjacent carbon atoms either at C_7 and C_8 or at C_9 and C_{11}. Further evidence, also unfortunately of a non-conclusive nature, which is cited later suggests that the bromine atoms are in the 9- and 11-positions which means that the tetrabromide has either structure (CLXXV) or (CLXXVI). The latter structure (CLXXVI), however, is sterically unlikely to exist since the bromine atom at C_9 and the angular methyl group at C_{10} both have the β-configuration. Using Stuart models it has been found impossible to construct this molecule since the 9β-bromine atom and the 10β--angular methyl group cannot be accommodated simultaneously without causing rupture of a carbon to carbon bond.

It is tentatively suggested, therefore, that the tetrabromide is 9α:11β:22:23-tetrabromoergost-7-en-3β-yl acetate (CLXXV), that the dimethylthio compound is

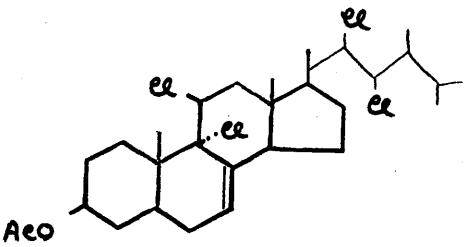
22:23-dibromo-9α:11β-dimethylthioergost-7-en-3β-yl acetate (CLXXXII), and that the monomethylthio-compound is 22:23-dibromo-11-methylthioergosta-7:9(11)-dien-3β-yl acetate (CLXXXVII). Assuming that the tetrachloroergostenyl acetate I is directly analogous to the tetrabromide, it follows also that this compound is 9α:11β:22:23-tetrachloroergost-7-en-3β-yl acetate (CLXXXIX).



CLXXV



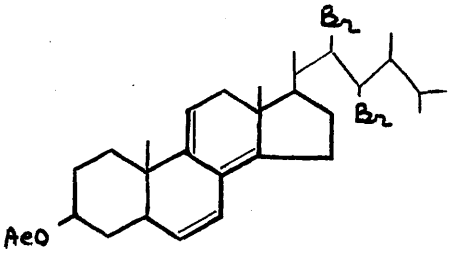
CLXXXII



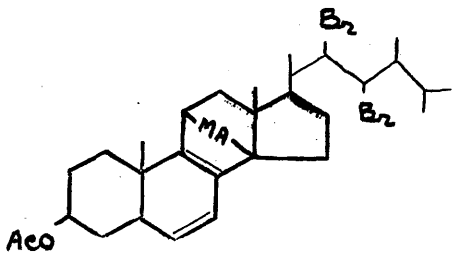
CLXXXIX

The final part of this section deals with partial dehydrobromination of tetrabromoergostenyl acetate. Treatment of tetrabromoergostenyl acetate with collidine, pyridine, acetone, or sodium methoxide, results in a partial dehydrobromination with the formation of a product, the analysis figures of which conform to the empirical formula $C_{30}H_{44}O_2Br_2$ except in the latter case, using

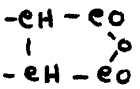
sodium methoxide, when hydrolysis of the 3β-acetoxyl group occurs simultaneously. This material has been shown to be a mixed crystal, 90% of which will react with maleic anhydride to form a mono-adduct $C_{30}H_{46}O_5Br_2$. The remaining 10% is shown, by experiments described later, to consist mainly of ergosteryl-D acetate 22:23--dibromide (CXXXI; R=Ac). In view of the relatively small amount (10%) of the non-adduct-forming material present, it is assumed that the ultra-violet absorption spectrum of the mixed crystal [maxima at 2280 (ε = 12,700; $E^{1\%}_{1cm.}$ = 212), 2340 (ε = 11,700; $E^{1\%}_{1cm.}$ = 195) and 2670Å (ε = 11,900; $E^{1\%}_{1cm.}$ = 199)] is essentially that of the adduct forming component. If this component possesses the steroid ring system, it must be a triene since its formation from tetrabromoergostenyl acetate involves the elimination of two bromine atoms as hydrogen bromide. However, no steroidal triene has been described which possesses an ultra-violet absorption spectrum similar to that of the mixed crystal. Furthermore, the only triene which could be visualised to give an adduct (CCI) whose ultra-violet absorption spectrum would be similar to that found [maxima at 2070 (ε = 3700; $E^{1\%}_{1cm.}$ = 53) and 2740Å (ε = 4500; $E^{1\%}_{1cm.}$ = 65)] is (CC) and, although the latter is not a known compound, an analogous triene in the chosterol series viz., cholesta-6:8(14):9(11)-trien-

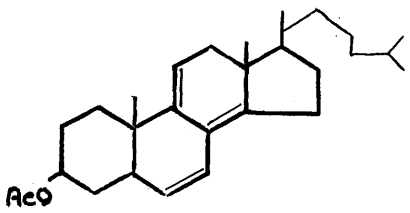


CCI

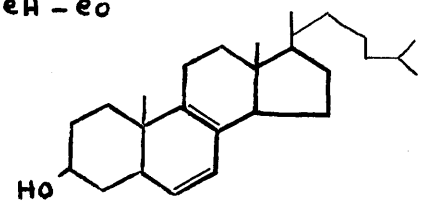


CCI

MA = 



CCII

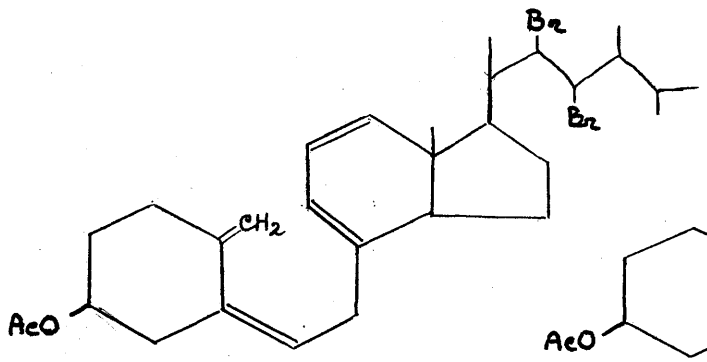


CCIII

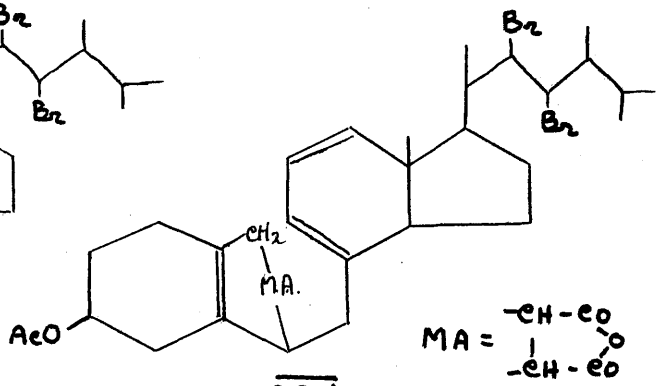
-3β-yl acetate (CCII) has been prepared, the ultra-violet absorption spectrum of which [maximum at 2840Å (ε = 9500) in ether] (102) is entirely different from that of the mixed crystal. The ultra-violet absorption spectrum of the adduct is very similar to that of iso-dehydrocholesterol (CCIII) [maximum at 2750Å (ε = 5000) in ether] (102), hence structure (CCI) could explain the band at 2740Å in the spectrum of the adduct although it does not explain the presence of the band at 2070Å. A triene structure is therefore not favoured for the adduct-forming component of the mixed crystal.

The ultra-violet absorption spectrum of the mixed

crystal could be interpreted as suggesting the presence of two different conjugated diene systems in the adduct-forming component. If this is the case then the compound is a tetraene. The formation of such a structure must involve ring opening. Also, the ultra-violet absorption spectrum of the adduct indicates that adduct formation has taken place across a conjugated diene system absorbing at 2280 and 2340Å since no absorption is observed at these wavelengths in its spectrum. Structures (CCIV) and (CCV) are tentatively suggested for the adduct-forming component



CCIV



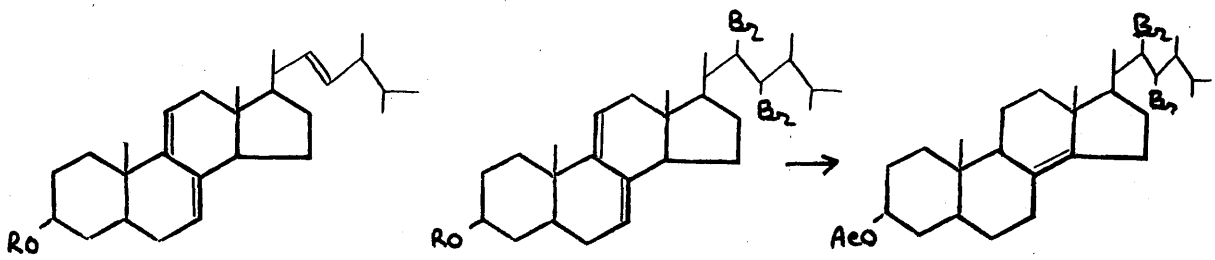
CCV

of the mixed crystal and its maleic anhydride adduct respectively in which it is suggested that the bands at 2280 and 2340Å in the spectrum of the mixed crystal represent a 5:10(19)-conjugated diene system and that the band 2670Å represents a 8:11-conjugated diene system. Using this formulation the band at 2070Å in the spectrum of the adduct can be attributed to a 5:10-double bond.

It is noted, however, that the intensity of absorption at 2670Å (ε = 11,900) in the spectrum of the mixed crystal is approximately double that at 2740Å (ε = 4500) in the spectrum of the adduct, and since, in this formulation, both these bands represent the same conjugated diene system, it is expected that both bands should absorb at the same wavelength and have similar intensity. An explanation for these discrepencies has not been found so far.

The minor component (10%) of the mixed crystal which does not react with maelic anhydride has been shown to consist mainly of ergosteryl-D acetate 22:23-dibromide (CXXXI; R=Ac) by the isolation of the last named compound from the mixed crystal. This is done by subjecting the mixed crystal to peroxidising conditions using oxygen in the presence of artificial light and eosin [cf. the preparation of ergosterol peroxide (77)] which results in resinification of the adduct-forming component. The non-adduct forming component is easily separated from the reaction mixture by chromatography. Presence of ergosteryl-D acetate 22:23-dibromide in the mixed crystal is also indicated, first, by the fact that the non-adduct forming component has an ultra-violet absorption spectrum [maxima at 2420Å (ε = 13,500) with an inflection at 2500Å (ε = 12,600)] which resembles that of ergosteryl-D

acetate 22:23-dibromide[maxima at 2350 (ε = 19,000), 2420Å (ε = 21,000) with an inflection at 2500Å (ε = 13,500)] and, secondly, by the isolation of impure ergosteryl-D acetate (CXXIV; R=Ac) by debromination of the mixed crystal followed by chromatography of the product.

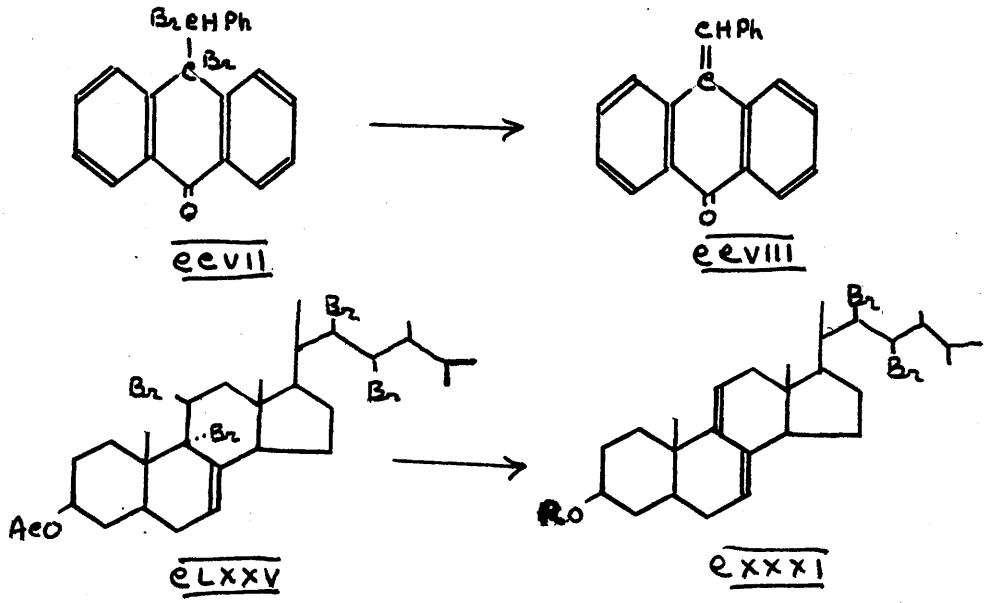


CXXIV CXXXI CCVI

A catalytic hydrogenation of the mixed crystal results in the formation of the known 22:23-dibromo-ergost-8(14)-en-3β-yl acetate (CCVI)(98) in 42% yield. Catalytic hydrogenation of ergosteryl-D acetate 22:23--dibromide (CXXXI; R=Ac) under the same conditions is known to give this compound (98) but, since ergosteryl-D acetate 22:23-dibromide is present in the mixed crystal in less than 10%, it follows that the majority of the hydrogenation product has originated from the adduct-forming component, which suggests that this component retains the steroid ring system. However, since a

tetraene structure, in which one of the rings of the steroid nuclear has been opened, is favoured for this compound it must be assumed that ring closure takes place on catalytic hydrogenation.

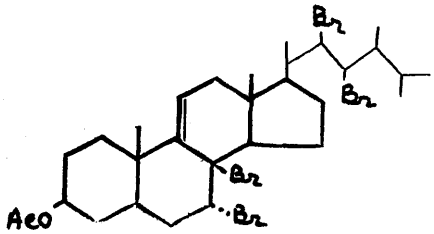
A reaction which appears to be analogous to the formation of ergosteryl-D acetate 22:23-dibromide (CXXXI; R=Ac) from the tetrabromide under dehydrohalogenating conditions has been reported by Cook (103) in which an attempt to partially dehydrobrominate benzilideneanthrone dibromide (CCVII) with pyridine, diethylamine, aqueous acetone, alcohol or dry silver oxide resulted in the loss



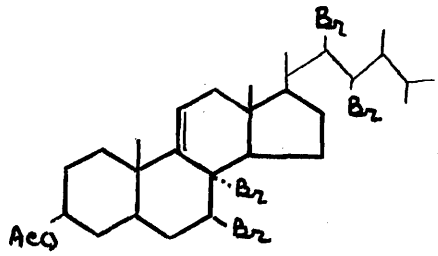
of bromine and the formation of benzilideneanthrone (CCVIII) as the main product of the reaction. It is of interest to note that benzilideneanthrone dibromide (CCVII)

resembles the suggested structure for the tetrabromide (CLXXV) in that there is only one hydrogen atom available for dehydrobromination purposes.

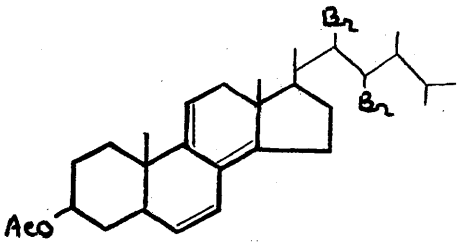
The following argument is put forward, therefore, against structures for the tetrabromide containing nuclear bromine atoms in the 7- and 8-positions (CLXXVII; CLXXVIII). Assuming such structures to be correct it is to be expected that dehydrobromination should occur



CLXXVII



CLXXVIII



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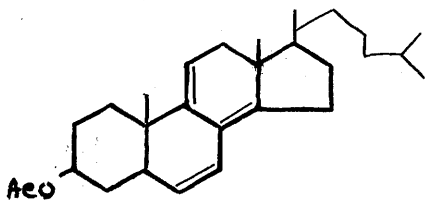

ee II

readily with the formation of compound (CC) since there are hydrogen atoms available at the 6- and 14-positions. However, as has been mentioned previously (see p. 89) an analogous compound to (CC) is known in the cholesterol series, viz. cholesta-6:8(14):9(11)-trien-3β-yl acetate

(CCII) the ultra-violet absorption spectrum of which is entirely different from that of the product obtained on dehydrobromination of the tetrabromide.

CONCLUSION

The conversion of ergosterol (LXXXI; R=H) into cortisone (VII) via ergosteryl-D acetate 22:23-dibromide (CXXXI; R=Ac) and via ergosteryl-D acetate 22:23--dichloride (CXXXV; R=Ac) has been shown to be possible. Furthermore, since it is considered that tetrabromo-ergostenyl acetate has a structure possessing a bromine atom at C_{11} (CLXXXII), this compound may prove to be an extremely attractive intermediate in the preparation of 11-oxygenated steroids if a method of replacing this bromine atom by a hydroxyl group is found.

EXPERIMENTAL

EXPERIMENTAL

All melting points are uncorrected. Specific rotation values were determined in chloroform solution (except where otherwise stated) in a 1 dm. tube at approximately 15°. Ultra-violet absorption spectra were measured in ethanol solution (except where otherwise stated) using a Unicam SP.500 spectrophotometer.

Ergosteryl Acetate.

Ergosterol [300 g.; m.p.156-162°; $[\alpha]_D$ -134° (c,2.0)] was dissolved in pure dry pyridine (1800 c.c.) in a five litre flask. Redistilled acetic anhydride (300 c.c.) was added and if any precipitation occurred, the mixture was slightly heated to obtain a homogeneous solution once more. The air in the reaction vessel was replaced by nitrogen, the flask stoppered and allowed to stand at room temperature for 18 hours. After a further lot of ergosterol (200 g.) had been similarly treated in a three litre flask with pure dry pyridine (1200 c.c.) and redistilled acetic anhydride (200 c.c.), the crystalline material which had separated from both experiments was filtered, washed with a little pure ice cold pyridine, hot water (6 litres in the first case and 4 litres in the second) and finally dried in an air oven about 50°.

The combined product was crystallised from chloroform--methanol furnishing pure ergosteryl acetate (310 g.) as glistening plates, m.p.173-175°.

$[\alpha]_D$ -93° (c,2.0).

On working up the mother liquors from the previous cyrstallisation a further 120 g. of reasonably pure ergosteryl acetate was obtained, m.p.167-170°.

$[\alpha]_D$ -93° (c,2.15).

Total yield 430 g. (86%).

5-Dihydroergosteryl Acetate.

1. Using Cyclohexane as Solvent.

Ergosteryl acetate (10 g.) was dissolved in cyclohexane (400 c.c.). Raney nickel sludge (W6; 8 c.c.) (104) previously washed with cyclohexane was added to the reaction mixture which was hydrogenated until 1.25 mols of hydrogen had been absorbed ($1^3/_4$ hours). After filtering to remove the catalyst the filtrate was evaporated to dryness and the residue crystallised once from a mixture of chloroform (50 c.c.) and methanol (65 c.c.) to give 5-dihydroergosteryl acetate as plates, m.p.178-180°. Yield 7.6 g. (76%).

$[\alpha]_D$ -20.5° (c,2.0).

The product gives a yellow colour with tetranitromethane in chloroform.

2. Using Benzene as Solvent.

A solution of ergosteryl acetate (35 g.) in benzene (300 c.c.; A.R.) was treated with a suspension of Raney nickel sludge (W6; 15-20 c.c.)(104) in benzene (50 c.c.; A.R.) and the mixture shaken at 17° with hydrogen under a slight positive pressure until the total absorption was 2140 c.c. (calc. 1900), of which, according to a blank experiment, approximately 150 c.c. was absorbed by the solvent; the time required for the absorption was approximately 15 minutes. The filtered reaction solutions from five such experiments were combined and the solvent 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°, $[\alpha]_D$ -19.5 (c,2.0), showing no high intensity absorption above 2200Å. A further quantity (69 g.) (total yield, 92%), m.p.177-179°, $[\alpha]_D$ -18 (c,1.8) was obtained from the mother liquors. In other experiments yields varying between 92% and 95% of material of similar melting point and specific rotation were obtained. Recrystallisation of the product from chloroform-methanol gave lustrous plates, m.p.180-182°.

$[\alpha]_D$ -20.5° (c,2.1)

Ergosteryl-D Acetate.

A solution of 5-dihydroergosteryl acetate (40 g.) in pure dry chloroform (560 c.c.) in a Winchester bottle was treated with a hot solution of mercuric acetate (65 g.) in glacial acetic acid (1040 c.c.). After shaking the reaction mixture for 18 hours the mercurous acetate, which had separated out, was filtered and washed with a little dry ether. The combined filtrate and washings were concentrated in vacuo to a volume of about 400 c.c., the solid, which had precipitated out, filtered, washed consecutively with a little glacial acetic acid and methanol and treated in the cold with excess dry chloroform. The resulting solution was filtered, thereby removing the last traces of mercury salts, the filtrate concentrated to a volume of about 150 c.c., heated to boiling and the product crystallised from it by the addition of methanol. Two crystallisations of this material from chloroform-methanol furnished impure ergosteryl-D acetate (16 g.; 40% yield), m.p.168-172°, $[\alpha]_D$ +22 (c,1.5). After two further crystallisations from the same solvent mixture almost pure ergosteryl-D acetate (10 g.; 25% yield) was obtained as large plates, m.p.175-177°.

$[\alpha]_D$ +30,+30° (c,1.9, 1.4)

Found: C,82.2; H,10.4

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

Light absorption: Maxima at 2350 (ε = 15,500) and 2420Å (ε = 17,000), with an inflection at 2510Å (ε = 12,500).

Ergosteryl-D acetate gives a brown colour with tetranitromethane in chloroform.

Ergosterol-D.

A solution of ergosteryl-D acetate (1.5 g.; $[\alpha]_D$ +30°) in ethanol (200 c.c.) was treated with a 20% aqueous solution of potassium hydroxide (10 c.c.) and the solution refluxed for one hour. The product was worked up via ether and crystallised from chloroform-methanol to give ergosterol-D (930 mg.) as felted needles, m.p.165-167°.

$[\alpha]_D$ +30, +30° (c,1.5, 1.4)

Found: C,82.9; H,11.34

Calc. for $C_{28}H_{44}O.{}^1/_2CH_3OH$: C,83.0; H,11.16%

Light absorption: Maxima at 2350 (ε = 15,500) and 2420Å (ε = 16,700) with an inflection at 2510Å (ε = 12,000).

The alcohol (1.6 g.) was reacetylated in warm, pure, dry pyridine (10 c.c.) and acetic anhydride (2 c.c.) by allowing it to stand in a nitrogen atmosphere for 16 hours at room temperature. The product, isolated via ether, was crystallised once from chloroform-methanol to give ergosteryl-D acetate as large plates, m.p.174-176°.

$[\alpha]_D$ +31° (c, 1.4)

Light absorption: Maxima at 2350 (ε = 15,300) and 2420Å (ε = 17,000) with an inflection at 2510Å (ε = 12,000).

Bromination of 5-Dihydroergosteryl Acetate in Chloroform Solution with 1 Mole of Bromine.

A solution of dry bromine (2 g.; 1.05 mole) in pure, dry chloroform (40 c.c.) at -70° was added all at once to a solution of 5-dihydroergosteryl acetate (5 g.) in pure, dry chloroform (250 c.c.) at -70°. The bromine colour disappeared immediately and the resulting mixture was allowed to stand for one hour, after which the temperature was -30°, when the solution began to take on a light green colour, which intensified to a dark green as room temperature was reached. The solvent was removed in vacuo below 30° and the resulting black-brown gummy residue dried by distillation with benzene, dissolved in benzene (120 c.c.) and perfused through an alumina column (Grade II — III; 15 x $2^1/_2$ cms.). Elution with 400 c.c. of benzene yielded a yellow gummy solid (5.6 g.) which was dissolved in light petroleum (b.p.60-80°) and perfused once again through an alumina column (Grade II — III; 15 x $2^1/_2$ cms.). Perfusion with 800 c.c. of the same solvent yielded a light yellow solid (3.0 g.) which, on crystallisation from chloroform-methanol afforded plates (1.0 g.), m.p.143-212°.

Repeated crystallisation of this material from chloroform--methanol furnished prismatic needles (40 mg.), m.p.227-228° (decomp.).

$[\alpha]_D$ -15° (c,1.2)

Found: C,60.0; H,7.95

$C_{30}H_{46}O_2Br_2$ requires: C,60.2; H,7.75%.

Light absorption: Maximum at 2480Å (ε = 14,500). The product gives a red-brown colour with tetranitromethane in chloroform.

Bromination of 5-Dihydroergosteryl Acetate in Chloroform Solution with 2 Moles of Bromine.

A solution of dry bromine (3.82 g.; 2 moles) in pure, dry chloroform (50 c.c.) at -70° was added all at once to a solution of 5-dihydroergosteryl acetate (5 g.) in pure, dry chloroform (250 c.c.) at -70°. The bromine colour disappeared almost immediately and the resulting mixture was allowed to stand for one hour, after which the temperature was -30°, when the solution began to take on a light green colour which intensified to a dark green as room temperature was reached. The solvent was removed in vacuo below 30° and the resulting black-brown gummy residue dissolved in 250 c.c. of a benzene/light petroleum (b.p.60-80°)(1:1) mixture and perfused through an alumina column (Grade II — III; 24 x 1 3/4 cms.) with

a further 400 c.c. of the same solvent mixture. The residue from evaporation of the total eluant, on crystallisation from chloroform-methanol, yielded prismatic needles (2.7 g.), m.p.214-218° (decomp.) which, after two similar crystallisations gave slightly impure 22:23-dibromoergosta-8:14-dien-3β-yl acetate (ergosteryl-B_1 acetate 22:23-dibromide) as prismatic needles (740 mg.; 11% yield), m.p.227-228° (decomp.).

$[\alpha]_D$ -20.5° (c,2.25)

Found: C,60.24; H,7.75; Br,26.9

$C_{30}H_{46}O_2Br_2$ requires: C,60.20; H,7.75; Br,26.65%.

Light absorption: Maximum at 2480Å (ε = 14,000). The product gives a red-brown colour with tetranitromethane in chloroform.

Ergosteryl-B_1 Acetate.

The ergosteryl-B_1 acetate 22:23-dibromide (250 mg.) described above was dissolved in ether (40 c.c.), ethanol (60 c.c.) and freshly activated zinc dust (1g; activated with aqueous ammonium chloride solution) added and the mixture stirred on a boiling water bath for 2 hours. After removal of the zinc dust, by filtration, ether was added to the filtrate which was washed with water, dried (Na_2SO_4) and evaporated to dryness to give a pure white solid (180 mg.), m.p.131-133°. Four crystallisations of

this material from chloroform-methanol furnished ergosteryl-B_1 acetate (20 mg.) as lustrous plates, m.p. 141-142°.

$[\alpha]_D$ -52.2 (c,1.15)

Found: C,81.84; H,10.7

Calc. for $C_{30}H_{48}O_2$: C,82.13; H,10.6%.

Light absorption: Maximum at 2480Å (ε = 15,000).

Tetrabromoergostenyl Acetate.

1. 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 (16 g.; 4.4 moles) in glacial acetic acid (50 c.c.). The bromine was dried by shaking with concentrated sulphuric acid. The mixture was immediately cooled to -60° using an acetone-solid carbon dioxide bath, with shaking and allowed to gain room temperature during two hours. After about 30 minutes a flocculant white solid began to separate out. The solid (8.3-9.0 g.; 48-53% yield) was collected, washed with ether and dried at room temperature under pressure. Two crystallisations of a sample of the colourless amorphous solid from benzene/light petroleum (b.p.60-80°) furnished tetrabromoergostenyl acetate as felted needles, m.p.126-127° (decomp.).

$[\alpha]_D$ +223, +225° (c,0.2, 0.2 in dioxan)

$[\alpha]_D$ +260° (c,1.8 in benzene)

Found: C,47.7; H,6.4; Br,42.5

$C_{30}H_{46}O_2Br_4$ requires: C,47.5; H,6.1; Br,42.16%

Tetrabromoergostenyl acetate decomposes on storage and solutions of the compound in chloroform undergo considerable discoloration with decomposition of the compound and liberation of hydrogen bromide. It is sparingly soluble in cold ether, reasonably soluble in dioxan and easily soluble in benzene and chloroform. A freshly prepared solution of tetrabromoergostenyl acetate in chloroform (c,1.1) had $[\alpha]_D$ +205°; after 90 minutes the value was +93°. It is quite stable in solution in dioxan and benzene.

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 15° the solution was diluted and shaken with water (500 c.c.), the benzene layer separated and the aqueous phase extracted with benzene (300 c.c.). The combined benzene solutions were washed with sodium

hydroxide solution (2 x 200 c.c.; 1%) then with water and dried (Na_2SO_4). Removal of the benzene by evaporation in vacuo and solution of the residual solid in the minimum volume of chloroform followed by addition of methanol and storage at 0° gave a solid. This solid was purified by percolation of its solution in benzene (approx. 100 c.c.) through a column of alumina (Grade II — III; 15 x 2.5 cm.) followed by washing with benzene (200 c.c.). Removal of solvent from the filtrate gave a solid (6.5 - 6.9 g.; 90-95% yield) which, on crystallisation from chloroform-methanol furnished 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate as prismatic needles, m.p.233-234° (decomp.).

$[\alpha]_D$ +32, +33° (c,1.4, 1.7)

Found: C,60.0; H,7.8; Br,27.0.

$C_{30}H_{46}O_2Br_2$ requires: C,60.2; H,7.75; Br,26.65%.

Light absorption: Maxima at 2350 (ε = 19,000), 2420Å (ε = 21,000) and an inflection at 2500Å (ε = 13,500). The dibromide gives a red-brown colour with tetranitromethane in chloroform.

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

A solution of ergosteryl-D acetate 22:23-dibromide (300 mg.) in benzene (5 c.c.) and aqueous methanolic

potassium hydroxide (40 c.c.; 3%) was refluxed for six hours and concentrated to a bulk of 30 c.c. The crystalline solid which separated on cooling was filtered and crystallised from chloroform-methanol to give 22:23--dibromoergosta-7:9(11)-dien-3β-ol (270 mg.) as elongated plates, m.p.230-231° (decomp.).

$[\alpha]_D$ +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,8.2%.

Light absorption: Maxima at 2350 (ε = 18,100) and 2420Å (ε = 20,000) and an inflection at 2500Å (ε = 13,500).

Debromination and Isomerism of Ergosteryl-D Acetate 22:23-Dibromide.

A stirred solution of ergosteryl-D acetate 22:23--dibromide (500 mg.) in glacial acetic acid (60 c.c.) heated to 105-110° was treated with zinc dust (11 g.) added portionwise over 15 minutes. Heating and stirring were continued for a further $3^1/_4$ hours, after which the reaction mixture was filtered, the filtrate diluted with water, the solid which separated allowed to settle, filtered, dried and crystallised from chloroform methanol to give plates (200 mg.), m.p.140-142°. A further crystallisation yielded plates (75 mg.) softening at 145°, m.p.147-149°.

$[\alpha]_D$ -45.6 (c,2.5)

Found: C,81.97; H,10.7

Calc. for $C_{30}H_{46}O_2$: C,82.20; H,10.5%.

Light absorption: Maximum at 2480Å (ε = 14,500). These constants resemble those of ergosteryl-B_1 acetate for which Windaus (76) quotes m.p.142°, $[\alpha]_D$ -54° with light absorption in ether with maximum at 2480Å (ε = 16,000).

Estimation of Uptake of Perbenzoic Acid by Ergosteryl-D Acetate 22:23-Dibromide.

Ergosteryl-D acetate 22:23-dibromide (1 g.) was dissolved in pure dry chloroform (25 c.c.) and a chloroform solution of perbenzoic acid (25 c.c.; 39.1 mg./c.c.; 4 moles) added. A blank solution was made up simultaneously. Both solutions were stored at 0° and the perbenzoic acid content in each was estimated from time to time. The table below records the uptake of oxidising agent. A 2 mole uptake requires 461 mg. of perbenzoic acid.

Time (hours)	1	18	44
Uptake of Perbenzoic acid (mgs.)	345	450	450

Debromination of Ergosteryl-D Acetate 22:23-Dibromide with the Formation of Ergosteryl-D Acetate.

1. Ergosteryl-D acetate 22:23-dibromide (2 g.) in a mixture of ether (200 c.c.) and ethanol (300 c.c.) was treated with zinc dust (10 g.) (freshly activated by shaking with a saturated aqueous solution of ammonium chloride for 15 minutes followed by washing with water till the washings were chloride free and finally shaken with ethanol, filtered and dried in an air oven at 80°) added in one portion. The stirred reaction mixture was heated under reflux for 2 hours, filtered, concentrated, and the residue treated with water and extracted with ether. The ethereal solution was washed with water, dried (Na_2SO_4) and evaporated. Crystallisation of the residue (m.p.178-180°; 1.4 g.; 95% yield) from chloroform-methanol furnished pure ergosteryl-D acetate as large plates, m.p.178-180°.

$[\alpha]_D$ +32, +33° (c,1.55, 1.63)

Found: C,82.1; H,10.7

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

Light absorption: Maxima at 2350 (ε = 17,000) and 2420Å (ε = 19,000) and an inflection at 2510Å (ε = 12,000). No depression in melting point was observed on admixture with a specimen of ergosteryl-D acetate prepared by the mercuric acetate method.

2. A solution of ergosteryl-D acetate 22:23--dibromide (500 mg.) in cyclohexane (100 c.c.) was treated with Raney nickel sludge (5 c.c.) previously washed with cyclohexane, and the mixture shaken with hydrogen for 18 hours. The mixture was filtered and the filtrate evaporated to dryness. The solid was crystallised from chloroform-methanol, to yield slightly impure ergosteryl-D acetate as plates (250 mg.), m.p.173-176°.

$$[\alpha]_D \ +27° \ (c,1.25).$$

The melting point was undepressed when the material was mixed with a specimen, m.p.175-177°, $[\alpha]_D$ +30°, obtained by oxidation of 5-dihydroergosteryl acetate with mercuric acetate.

Tetrachloroergostenyl Acetates.

A stirred solution of 5-dihydroergosteryl acetate (10 g.) in dry ether (1000 c.c.) at 0° was treated rapidly with dry chlorine (6.5 g.; 4 moles) in glacial acetic acid (100 c.c.) and immediately cooled to -50° and kept at this temperature for 75 minutes. The solution was allowed to attain room temperature, stored for 12 hours and then concentrated to a bulk of 150 c.c. under reduced pressure below 40°. The solid (1.7 g.), m.p. 126-128° (decomp.), which separated was collected (liquor A), washed with a little glacial acetic acid, and

thrice crystallised from benzene-light petroleum (b.p.60-80°) from which tetrachloroergostenyl acetate I separated as felted needles (540 mg.; 4% yield), m.p.167-168° (decomp.).

$[\alpha]_D$ +190° (c,1.15)

Found: C,62.13; H,8.0; Cl,24.8

$C_{30}H_{46}O_2Cl_4$ requires: C,62.10; H,8.0; Cl,24.4%.

Light absorption in chloroform: Maximum at 2460Å (ε = 4200).

Light absorption in cyclohexane: Maximum at 2320Å (ε = 3800).

The tetrachloride gives no colour with tetranitromethane in chloroform and solutions of the material are sufficiently stable to enable this solvent to be used for measuring the specific rotation value. However, on standing over long periods decomposition occurs with discoloration of the solution and liberation of hydrogen-chloride.

A second crop of crystalline solid (660 mg.), m.p.177° (decomp.) which separated from liquor A on standing, was crystallised from benzene-light petroleum (b.p.60-80°) to give tetrachloroergostenyl acetate II as needles, m.p.213-214° (decomp.).

$[\alpha]_D$ -247, -253° (c,1.1, 0.8)

Found: C,62.3; H,8.1; Cl,24.6

$C_{30}H_{46}O_2Cl_4$ requires: C,62.1; H,8.0; Cl,24.4%.

Light absorption: Maximum at 2280Å (ε = 9150, 10,400).

The tetrachloride gives no colour with tetranitromethane in chloroform.

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

1. Tetrachloroergostenyl acetate I (400 mg.) in dry benzene (30 c.c.) was treated with a solution of sodium iodide (1.5 g.) in ethanol (30 c.c.) when iodine was immediately liberated. After standing for 16 hours at 15° the reaction mixture was diluted with water, and the benzene solution washed with 5% sodium hydroxide solution, water, dried (Na_2SO_4) and evaporated to dryness. The residual white solid on dissolving in the minimum of chloroform and adding excess methanol yielded a solid (280 mg.; 60% yield) which on crystallisation from chloroform-methanol furnished 22:23-dichloroergosta--7:9(11)-dien-3β-yl acetate as long prismatic needles, m.p.235-237°.

$[\alpha]_D$ +43, +44° (c,0.5, 1.3).

Found: C,70.6; H,9.1; Cl,14.5

$C_{30}H_{46}O_2Cl_2$ requires: C,70.7; H,9.1; Cl,13.9%.

Light absorption: Maxima at 2360 (ε = 18,800) and 2420Å (ε = 21,000), with an inflection at 2500Å (ε = 14,000).

The dichloride gives a red-brown colour with tetranitromethane in chloroform.

2. A stirred solution of 5-dihydroergosteryl acetate (5 g.) in dry chloroform (100 c.c.) at -30°, to which pure dry pyridine (25 c.c.) had been added, was treated with a solution of dry chlorine (3.0 g.) in dry chloroform (50 c.c.), added dropwise over 30 minutes. A solution of sodium iodide (10 g.) in ethanol (150 c.c.) was immediately added, the reaction mixture stirred for 10 minutes and left at room temperature for 18 hours. After addition of chloroform (200 c.c.) the reaction mixture was washed with water, sodium thiosulphate solution to remove iodine, water, dried (Na_2SO_4), the chloroform solution concentrated in vacuo to small volume, treated with excess methanol and set aside at 0° for 30 minutes. The light yellow solid (1.4 g.) which had precipitated was filtered and crystallised from chloroform-methanol to give reasonably pure ergosteryl-D acetate 22:23-dichloride as long prismatic needles (500 mg.; 9% yield), m.p.217-219°.

$[\alpha]_D$ +38° (c,1.2).

A further crystallisation from chloroform-methanol furnished a purer product (300 mg.), m.p.223-225°.

$[\alpha]_D$ +42° (c,1.0).

Light absorption: Maxima at 2350 (ε = 16,000) and 2420Å (ε = 18,000) with an inflection at 2500Å (ε = 12,000).

22:23-Dichloroergosta-7:9(11)-dien-3β-ol (Ergosterol-D 22:23-Dichloride).

A solution of ergosteryl-D acetate 22:23-dichloride (430 mg.) in benzene (5 c.c.) and aqueous methanolic potassium hydroxide (85 c.c.; 3%) was refluxed for six hours, concentrated to a bulk of about 30 c.c. and diluted with water. The product was isolated via ether and the residual white solid on crystallisation from acetone furnished 22:23-dichloroergosta-7:9(11)-dien--3β-ol (80 mg.) as needles, m.p.215-216°.

$[\alpha]_D$ +33.5° (c,1.4)

Found: C,69.54; H,9.77; Cl,14.0

$C_{28}H_{44}OCl_2.H_2O$ requires: C,69.26; H,9.55; Cl,14.6%.

Light absorption: Maxima at 2370 (ε = 16,200) and 2440Å (ε = 17,700) with an inflection at 2520Å (ε = 12,500).

Attempt to Dechlorinate Ergosteryl-D Acetate 22:23-Dichloride.

1. Ergosteryl-D acetate 22:23-dichloride (50 mg.) in solution in a mixture of ether (40 c.c.) and ethanol (60 c.c.) was treated with activated zinc dust (500 mg.) added in one portion. The stirred reaction mixture was heated under reflux for 2 hours, filtered, concentrated, and the residue treated with water and extracted with ether. The ethereal solution was washed with water, dried (Na_2SO_4) and evaporated. Crystallisation of the residue from chloroform-methanol gave long prismatic

needles (35 mg.), m.p.235-237°, which, on admixture with starting material, showed no depression in melting point.

2. Ergosteryl-D acetate 22:23-dichloride (150 mg.) in solution in dry benzene (10 c.c.) was treated with glacial acetic acid (20 c.c.); the reaction mixture was stirred at room temperature while zinc dust (1 g.) was added portionwise over 15 minutes. Stirring at room temperature was continued for 4 hours after which the product was isolated via ether to give a white residual solid which crystallised from chloroform-methanol as long prismatic needles (80 mg.), m.p.233-235°, $[\alpha]_D$ +42° (c,1.1) which, on admixture with starting material, showed no depression in melting point.

Dechlorination of Ergosteryl-D Acetate 22:23-Dichloride.

1. With the Formation of 5-Dihydroergosteryl Acetate.

A solution of ergosteryl-D acetate 22:23-dichloride (70 mg.) in dry benzene (15 c.c.) was treated with Raney nickel sludge (4 c.c.) in ethanol (15 c.c.) and refluxed on a steam bath for 3 hours. After filtration to remove the catalyst, benzene (100 c.c.) was added to the filtrate which was washed with water, dried (Na_2SO_4) and evaporated, yielding a white solid residue; crystallisation of this from chloroform-methanol yielded plates (45 mg.), m.p.176-178°, $[\alpha]_D$ -12.5° (c,0.9) which gave a

yellow colour with tetranitromethane in chloroform. This material, after two crystallisations from chloroform--methanol furnished 5-dihydroergosteryl acetate as plates, m.p.180-182°.

$[\alpha]_D$ -17° (c,0.7).

Light absorption: Maximum at 2070 (ε = 4800) and 2420Å (= 520). The product showed no depression in melting point when mixed with an authentic specimen of 5-dihydro-ergosteryl acetate. The presence of a small amount of ergosteryl-D acetate in the product is indicated by the band at 2420Å.

2. With the Formation of Ergosteryl-D Acetate.

Ergosteryl-D acetate 22:23-dichloride (200 mg.) in solution in a mixture of benzene (10 c.c.) and glacial acetic acid (30 c.c.) was treated with zinc dust (1 g.), added portionwise over 10 minutes, while the stirred reaction mixture was heated on a steam bath. Heating and stirring were continued for a further 50 minutes, after which the mixture was filtered, the filtrate concentrated and the residue treated with water and extracted with ether. The ethereal solution was washed with water, dried (Na_2SO_4) and evaporated. Crystallisation of the residue from chloroform-methanol gave large plates (100mg.) m.p.171-174°, which, after one crystallisation from the same solvent mixture furnished pure ergosteryl-D acetate

as large plates (60 mg.), m.p.178-180°.

$[\alpha]_D$ +33° (c,1.1)

Found: C,82.4; H,10.5

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

Light absorption: Maxima at 2350 (ε = 16,500) and 2420Å (ε = 18,000) with an inflection at 2500Å (ε = 12,000). No depression in melting point was observed on admixture with an authentic speciman.

22:23-Dichloroergosta-7:14-dien-3β-yl Acetate (Ergosteryl-B_3 Acetate 22:23-Dichloride).

Tetrachloroergostenyl acetate II (385 mg.; $[\alpha]_D$ -257°) in solution in a mixture of ether (40 c.c.) and ethanol (60 c.c.) was treated with activated zinc dust (2 g.) added in one portion. The stirred reaction mixture was heated under reflux for two hours, filtered, the filtrate concentrated, and the residue treated with water and extracted with ether. The ethereal solution was washed with water, dried (Na_2SO_4) and evaporated. Three crystallisations of the residue from chloroform-methanol yielded 22:23-dichloroergosta-7:14-dien-3β-yl acetate (35 mg.) as prismatic plates, m.p.206-209°.

$[\alpha]_D$ -173, -169° (c,1.8, 1.4)

Found: C,70.54; H,9.22; Cl,14.20

$C_{30}H_{46}O_2Cl_2$ requires: C,70.70; H,9.10; Cl,13.92%.

Light absorption: Maximum at 2410, 2420Å (ε = 9000, 10,000). The dichloride gives a red-brown colour with tetranitromethane in chloroform.

Ergosta-7:14:22-trien-3β-yl Acetate (Ergosteryl-B_3 Acetate).

A stirred solution of ergosteryl-B_3 acetate 22:23--dichloride (35 mg.) in glacial acetic acid (15 c.c.) was heated with stirring on a steam bath and treated with zinc dust (200 mg.) added portionwise over 10 minutes. Heating and stirring were continued for one hour after which the product was isolated via ether in the form of a gummy solid, which yielded slightly impure ergosta--7:14:22-trien-3β-yl acetate as globules (4 mg.) from methanol, m.p.127-129°.

$[\alpha]_D$ -175° (c,0.33)

Light absorption: Maximum at 2420Å (ε = 8800).

A crystallisation of this material from aqueous methanol furnished fine needles, m.p.132-134° which, when mixed with a specimen of pure ergosteryl-B_3 acetate, m.p.138-140°, $[\alpha]_D$ -218°, light absorption: maximum at 2420Å (ε = 9900), prepared as described by Barton (85), melted at 132-136°.

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

A solution of ergosteryl-D acetate 22:23-dibromide (4.5 g.) in redistilled carbon tetrachloride (100 c.c.)

and glacial acetic acid (600 c.c.) (purified by refluxing over chromium trioxide) was treated with hydrogen peroxide solution (8 c.c.; 30%) added in one portion and heated to 95° with stirring over 25 minutes. The mixture was maintained at this temperature for 3 hours. The solution was then evaporated under reduced pressure, the partially crystalline residue treated with methanol (30 c.c.) and kept at 0° overnight and the solid (1.5 g.; m.p.218-221° (decomp.); 30% yield) collected. Recrystallisation from chloroform-methanol furnished 3β-acetoxy--22:23-dibromo-9α:11α-epoxyergostan-7-one as plates, m.p.234-235° (decomp.).

$[\alpha]_D$ -47° (c,1.7)

Found: C,56.9; H,7.4

$C_{30}H_{46}O_4Br_2$ requires: C,57.1; H,7.35%.

The compound shows no intensity of high absorption above 2200Å and gives no colour with tetranitromethane in chloroform.

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

A solution of ergosteryl-D acetate 22:23-dichloride (1 g.) in redistilled carbon tetrachloride (20 c.c.) and glacial acetic acid (150 c.c.) (purified by refluxing over chromium trioxide), was treated with hydrogen peroxide solution (2.2 c.c.; 30%) added in one portion and

heated to 90° with stirring over 30 minutes. The temperature of the reaction mixture was maintained at 90-95° for a further $2^1/_2$ hours during which time stirring was continued. The solution was then evaporated under reduced pressure, the partially crystalline residue treated with methanol (25 c.c.) and stored at 0° overnight and the solid (300 mg.; m.p.244-247° (decomp.; 28% yield) collected. Recrystallisation from chloroform-methanol furnished 3β-acetoxy-22:23-dichloro--9α:11α-epoxyergostan-7-one as plates, m.p.278-279° (decomp.).

$[\alpha]_D$ -59° (c,1.4)

Found: C,66.47; H,8.78; Cl,13.2

$C_{30}H_{46}O_4Cl_2$ requires: C,66.53; H,8.56; Cl,13.1%.

The compound shows no high intensity light absorption above 2200Å and gives no colour with tetranitromethane in chloroform.

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

1. A stirred solution of 3β-acetoxy-22:23-dibromo--9α:11α-epoxyergostan-7-one (1.3 g.; m.p.218-221°) in stabilised glacial acetic acid (150 c.c.) was treated at 95° over two hours with zinc dust (6 g.) added portionwise over 15 minutes. The zinc was removed by filtration and the filtrate concentrated under reduced pressure and

diluted with water. The mixture was ether extracted, the extract washed successively with sodium hydrogen carbonate solution and water and dried (Na_2SO_4). Removal of the ether gave a solid residue which on crystallisation from methanol furnished 3β-acetoxy-9α:11α-epoxyergost--22-en-7-one (600 mg.) as needles (which formed slowly from an initial gel), m.p.222-223°.

$[\alpha]_D$ -80.5° (c,1.0)

Found: C,76.38; H,10.10

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

The compound shows no high intensity absorption above 2200Å, gives a faint yellow colour with tetranitromethane in chloroform, and was undepressed in melting point when mixed with a specimen prepared from ergosteryl-D acetate as described by Spring et al. (63).

2. A stirred solution of 3β-acetoxy-22:23-dichloro--9α:11α-epoxyergostan-7-one (35 mg.) in glacial acetic acid (15 c.c.) was heated on a steam bath while zinc dust (200 mg.) was added portionwise over 10 minutes. The reaction mixture was heated with stirring for a further 50 minutes, filtered, the filtrate concentrated, treated with water and extracted with ether. The ether extract was washed with sodium hydrogen carbonate solution, water, dried (Na_2SO_4) and evaporated. The residual white solid, on crystallisation from methanol, yielded 3β-acetoxy-

-9α:11α-epoxyergost-22-en-7-one as needles (18 mg.)(which formed slowly from an initial gel), m.p.221-222°.

$[\alpha]_D$ -85° (c,1.2)

Found: C,76.33; H,9.98

Calc. for $C_{30}H_{46}O_4$: C,76.54; H,9.85%.

The material shows no high intensity light absorption above 2200Å, gives a faint yellow colour with tetranitromethane in chloroform and shows no depression in melting point when mixed with a specimen prepared as described in the previous experiment.

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

A solution of 3β-acetoxy-9α:11α-epoxyergost-22-en--7-one (100 mg.) in methanol (12 c.c.) was treated with 20% aqueous potassium hydroxide solution (2 c.c.) and the mixture refluxed for one hour. The cooled solution was diluted with water and ether extracted; the ether extract, after washing with water and drying (Na_2SO_4), was evaporated and the residue crystallised from acetone furnishing 3β:11α-dihydroxyergosta-8:22-dien-7-one (60 mg.) as needles, m.p.214-215°.

$[\alpha]_D$ -5.2 (c,2.0)

Found: C,78.54; H,10.5

$C_{28}H_{44}O_3$ requires: C,78.75; H,10.7%.

Light absorption: Maximum at 2540Å (ε = 9100).

The compound gives a faint yellow colour with tetranitromethane in chloroform.

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

1. 3β:11α-Dihydroxyergosta-8:22-dien-7-one (60 mg.) was heated in a steam bath with pure dry pyridine (2 c.c.) and redistilled acetic anhydride (2 c.c.) for one hour. After the addition of water to the reaction mixture the product was isolated via ether and the residual solid crystallised from methanol to give 3β:11α-diacetoxyergosta--8:22-dien-7-one (35 mg.) as needles, m.p.177-178°.

$[\alpha]_D$ +12° (c,1.75)

Found: C,75.2; H,9.8

$C_{32}H_{48}O_5$ requires: C,75.0; H,9.4%.

Light absorption: Maximum at 2520Å (ε = 10,200).

The product gives a light yellow colour with tetranitromethane in chloroform.

2. A solution of 3β-acetoxy-9α:11α-epoxyergost--22-en-7-one (150 mg.; m.p.217-219°) in benzene (2 c.c.) and methanol (25 c.c.) was treated with aqueous potassium hydroxide solution (20%; 4 c.c.) and the mixture refluxed for one hour. The cooled solution was diluted with water and extracted with ether. The extract was washed with water, dried (Na_2SO_4) and evaporated under reduced pressure. The residual solid was acetylated by heating

for one hour with pure dry pyridine (3 c.c.) and redistilled acetic anhydride (0.5 c.c.). The acetylated product, isolated by means of ether, separated as needles (50 mg.), m.p.172-173°, from aqueous methanol. Recrystallisation from the same solvent gave 3β:11α-diacetoxyergosta-8:22-dien-7-one, m.p.173-174°.

$[\alpha]_D$ +12.3 (c,1.55)

Found: C,75.2; H,9.6

Calc. for $C_{32}H_{48}O_5$: C,75.0; H,9.4%.

Light absorption: Maximum at 2520Å (ε = 9200).

The compound gives a light yellow colour with tetranitromethane in chloroform and the melting point was undepressed when mixed with the specimen described previously.

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

3β:11α-Diacetoxyergosta-8:22-dien-7-one (140 mg.) was dissolved in ethanol (66 c.c.) which had previously been distilled over solid potassium hydroxide. This solution was treated with a 50% aqueous solution of potassium hydroxide (4 c.c.) and the reaction mixture refluxed for 14 hours. It was then diluted with water and ether extracted; the ether extract washed with water, dried (Na_2SO_4) and evaporated, yielding a red-brown gum. This material was acetylated in pure dry pyridine (4 c.c.) and redistilled acetic anhydride (1 c.c.) by heating on

a steam bath for 1 hour. The product was isolated by means of ether as a red-brown gum which was dissolved in dry benzene (25 c.c.) and chromatographed on an alumina column (Grade II — III; 8 x 1.25 cm.). Elution with 270 c.c. of benzene furnished a white solid (20 mg.) which separated as felted needles from methanol, m.p.155-170°, $[\alpha]_D$ -18.2° (c,0.5), which showed no high intensity light absorption above 2200Å. Elution with a further 300 c.c. of benzene yielded a white solid (15 mg.) which, on crystallisation from methanol furnished 3β-acetoxy-ergost-22-en-7:11-dione as small prismatic needles (9 mg.), m.p.196-198°.

$[\alpha]_D$ -25° (c,0.5)

Found: C,76.36; H,9.94

Calc. for $C_{30}H_{46}O_4$: C,76.54; H,9.85%.

The compound showed no light absorption of high intensity above 2200Å and gave a light yellow colour with tetra-nitromethane in chloroform. No depression in melting point was observed when this material was mixed with a specimen prepared as described by Heusser at al. (47) who record m.p.195-196°, $[\alpha]_D$ -27° for this compound. Chamberlin et al. (54) give m.p.197-200°, $[\alpha]_D$ -30° for this material.

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

A stirred solution of ergosteryl-D acetate (2.7 g.) in carbon tetrachloride (50 c.c.) and glacial acetic acid (300 c.c.) (purified by refluxing over chromium trioxide) at 55° was treated with a solution of chromium trioxide (620 mg.; 3 oxygen atoms) in water (1 c.c.) diluted with glacial acetic acid (15 c.c.), added dropwise over one hour. The reaction mixture was stirred at the same temperature for a further 2 hours, allowed to cool, ethanol (30 c.c.) added, the solution stirred for 2 hours and finally concentrated to small volume (150 c.c.) in vacuo. Water (700 c.c.) was added and the mixture thoroughly extracted with ether. After acid oxidation products and acetic acid had been removed by washing with water and sodium hydrogen carbonate solution (5%) the ether extract was washed with water, dried (Na_2SO_4) and evaporated, yielding 2.66 g. of neutral oxidation product, m.p.82-120° which was treated with Girard's reagent 'T' as described below.

A solution of the neutral oxidation product (2.66 g.) in methanol (100 c.c.) and glacial acetic acid (10 c.c.) was treated with Girard's reagent 'T' (2.3 g.) and the reaction mixture refluxed for one hour. After cooling, it was poured into 5% aqueous sodium hydrogen carbonate solution (180 c.c.) containing eight ice cubes (to give

a final pH 7.6 indicating that 90% of the acetic acid had been neutralised). Some water was added and the aqueous mixture extracted with ether (4 x 250 c.c.). The aqueous layer (920 c.c.) was treated with concentrated hydrochloric acid (60 c.c.; s.g. 1.16) and left for one hour. The mixture was then ether extracted (4 x 250 c.c.), the ether extract washed with sodium hydrogen carbonate solution (5%), water, dried (Na_2SO_4) and evaporated to give the ketonic fraction (710 mg.), m.p.127-156° which, after three crystallisations from pure dry methanol furnished 3β-acetoxyergosta-9(11):22-dien-7-one II (170mg.), m.p.176-178°.

$[\alpha]_D$ -55.5° (c,1.4)

Found: C,79.52; H,10.57

$C_{30}H_{46}O_3$ requires: C,79.24; H,10.2%.

The compound shows no high intensity light absorption above 2200Å. Jeger quotes m.p.176-177°, $[\alpha]_D$ -58 (93) for this compound.

3β-Acetoxyergosta-8(14):9(11):22-trien-7-one.

5-Dihydroergosteryl acetate (20 g.) was dissolved in benzene (600 c.c.) and glacial acetic acid (1200 c.c.) added. A solution of chromium trioxide (16 g.) in 90% acetic acid (200 c.c.) was added dropwise with stirring at room temperature. After standing for 24 hours, ethanol

(100 c.c.) was added, the mixture allowed to stand for 2 hours, and concentrated to small volume in vacuo. Water was added and the mixture was thoroughly extracted with ether. After acid oxidation products and acetic acid had been removed by washing with water and 2N sodium hydroxide solution, the ether extract was washed with water, dried (Na_2SO_4) and evaporated, yielding 18 g. of neutral oxidation product.

This material (18 g.) was dissolved in boiling ethanol (600 c.c.), concentrated hydrochloric acid (50 c.c.) added and the mixture refluxed for 2 hours. The reaction mixture was concentrated in vacuo to a volume of about 200 c.c., diluted with water, and ether extracted. The ether extract was washed with water, dried (Na_2SO_4) and evaporated yielding a dark red gum (17 g.) which was treated with Girard's reagent 'T' as described below.

The dark red gum (17 g.) was dissolved in boiling ethanol (180 c.c.) and glacial acetic acid (20 c.c.) added. Girard's reagent 'T' (17 g.) was then added, the mixture refluxed for one hour, allowed to cool, poured into a beaker containing eight ice cubes and ice cold sodium hydrogen carbonate solution (5%; 400 c.c.) gradually added. The volume was made up to 1500 c.c. by dilution with water and exhaustively extracted with ether (3 x 500 c.c.). The aqueous layer, after acidification with

concentrated hydrochloric acid (75 c.c.) and being allowed to stand for one hour, was extracted with ether (4 x 300c.c.), the ether extract dried (Na_2SO_4) and evaporated, yielding a solid ketonic fraction (6.3 g.), m.p.128-130°.

This ketonic material (6.3 g.) was acetylated in pure dry pyridine (40 c.c.) and redistilled acetic anhydride (7 c.c.) by storing at room temperature for 24 hours. The mixture was concentrated in vacuo, the residue extracted with ether, the ether extract washed with water, dried (Na_2SO_4) and evaporated. A solution of the residue in light petroleum (b.p.60-80°)-benzene (4:1; 500 c.c.) was perfused through a neutral alumina column (Grade II — III; 12 x 4 cm.). Elution with 750 c.c. of the same solvent mixture yielded a white solid (4.5 g.), m.p.178-182°, which, on one crystallisation from methanol (300 c.c.) furnished 3β-acetoxyergosta-8(14):9(11):22--trien-7-one (2.93 g.; 15% yield) as rosettes of fine white needles, m.p.187-189°.

$[\alpha]_D$ -50°(c,1.7)

Light absorption: Maximum at 2980Å (ε = 5000).

Attempt to Form the Maleic Anhydride Adduct of 3β-Acetoxy-ergosta-8(14):9(11):22-trien-7-one.

A solution of 3β-acetoxyergosta-8(14):9(11):22--trien-7-one (500 mg.) and maleic anhydride (500 mg.) in

dry benzene (5 c.c.) was refluxed for 4 hours on a steam bath. The benzene was removed by distillation in vacuo at 100° and unreacted maleic anhydride was sublimed off at the same temperature at 10^{-1} mm. pressure. The residue, on crystallisation from methanol, yielded rosettes of fine white needles (410 mg.), m.p.188-190°. Light absorption: Maximum at 2990Å (ε = 5300). The product, on admixture with starting material, showed no depression in melting point.

Attempts to Form the Transannular Peroxide of 3β-Acetoxy-ergosta-8(14):9(11):22-trien-7-one.

The following apparatus was used which is that employed by Windaus (77) for the preparation of ergosterol peroxide from ergosterol. A 200 watt Osram bulb was suspended in a litre beaker with a side arm through which a continual stream of water flowed to cool the bulb. This was suspended into the reaction vessel in the form of a two litre beaker into which a slow stream of oxygen was bubbled. The reaction vessel had an outer wrapping of aluminium foil which acted as a reflector.

3β-Acetoxyergosta-8(14):9(11):22-trien-7-one (1.0g.) dissolved in ethanol (95%; 1000 c.c.) containing eosin (5 mg.) was illuminated in the presence of oxygen for 7 hours in the apparatus described above. The ethanol solution was evaporated in vacuo, the solution of the

residue in dry benzene (40 c.c.) perfused through a neutral alumina column (Grade II — III; 8 x 2.5 cm.) and eluted with a benzene:chloroform (4:1) mixture (250 c.c.). The eluant, on evaporation, yielded a solid, m.p.187-189°, which, on crystallisation from methanol furnished rosettes of fine white needles (710 mg.), m.p. 188-190°, which showed no depression in melting point on admixture with starting material.

Attempted Peroxidation of Ergosteryl-D Acetate.

The apparatus used was that described previously for the attempted peroxidation of 7-ketoergosta-8(14):9(11):22-trien-3β-yl acetate.

Ergosteryl-D acetate (500 mg.) was dissolved in boiling 95% ethanol (1200 c.c.), eosin (6 mg.) was added and the mixture poured into the reaction vessel and illuminated for $6^{1}/_{2}$ hours while a slow stream of oxygen was bubbled through it. The mixture was then evaporated in vacuo, the residue dissolved in light petroleum (b.p. 60-80°) and perfused through an alumina column (Grade II — III; 12 x 4.5 cm.). Elution with one litre of the same solvent removed nothing from the column. When the eluant was changed to a mixture of benzene and ethanol (4:1) the first 125 c.c. of this eluant yielded a white solid (440 mg.), m.p.166-173° which on one

crystallisation from chloroform-methanol furnished ergosteryl-D acetate as large plates, m.p.173-175° which showed no depression in melting point on admixture with starting material.

Tetrabromoergostenyl Acetate. (see also p.105)

2. From 5-Dihydroergosteryl Acetate 22:23-Dibromide.

5-Dihydroergosteryl acetate 22:23-dibromide (450mg.) in solution in dry ether (50 c.c.) was treated with a solution of dry bromine (0.25 c.c.) in glacial acetic acid (5 c.c.) as described for 5-dihydroergosteryl acetate (see p.105). After about 15 minutes tetrabromoergostenyl acetate (350 mg.), m.p.127° (decomp.) began to separate out which crystallised as felted needles from benzene-light petroleum (b.p.60-80°), m.p.127-128° (decomp.).

$[\alpha]_D$ +255° (c,1.1 in benzene)

The product showed no depression in melting point when mixed with a specimen prepared from 5-dihydroergosteryl acetate.

3. From Ergosteryl-D Acetate.

Ergosteryl-D acetate (1.0 g.) in solution in dry ether (100 c.c.) was treated with a solution of dry bromine (0.51 c.c.) in glacial acetic acid as described for 5-dihydroergosteryl acetate. The product in this case separated out immediately to give tetrabromoergo-

stenyl acetate (1.05 g.) as felted needles from benzene-light petroleum (b.p.60-80°), m.p.123-124° (decomp.).

$[\alpha]_D$ +259° (c,1.3 in benzene)

The product showed no depression in melting point when mixed with a specimen prepared from 5-dihydroergosteryl acetate.

4. From Ergosteryl-D Acetate 22:23-Dibromide.

Ergosteryl-D acetate 22:23-dibromide (600 mg.) in solution in dry ether (50 c.c.) was treated with a solution of dry bromine (0.3 c.c.) in glacial acetic acid (5 c.c.) as described for 5-dihydroergosteryl acetate. The product, as in the previous experiment, separated out immediately to give tetrabromoergostenyl acetate (510 mg.), m.p.123-124° (decomp.) which crystallised as felted needles from benzene-light petroleum (b.p.60-80°), m.p.125-127° (decomp.).

$[\alpha]_D$ +260° (c,1.2 in benzene)

The product showed no depression in melting point when mixed with a specimen prepared from 5-dihydroergosteryl acetate.

Treatment of the specimens of tetrabromoergostenyl acetate prepared from 5-dihydroergosteryl acetate 22:23--dibromide, ergosteryl-D acetate and ergosteryl-D acetate 22:23-dibromide with sodium iodide in each case gave ergosteryl-D acetate 22:23-dibromide as prismatic needles

from chloroform-methanol which did not depress the melting point of a specimen prepared as described previously (see p.106).

$[\alpha]_D$ +30°, +31°, +31° (c,1.4, 2.0, 1.5) respectively.

Attempt to Allylic Rearrange Tetrabromoergostenyl Acetate.

Hydrobromic acid solution (3 c.c.; 46-48%) was added to a solution of tetrabromoergostenyl acetate (3g.) in benzene (150 c.c.; AR) and the biphase system shaken at 15° for 60 hours. The mixture was washed with water (7 x 100 c.c.), dried (Na_2SO_4) and concentrated to small volume at room temperature, light petroleum (b.p.60-80°) (50 c.c.) added and the solid (1.3 g.), m.p.122-123° (decomp.),

$[\alpha]_D$ +265° (c,0.8 in benzene)

which separated as felted needles, was filtered. By cooling, two further crops of felted needles, one (400mg.), m.p.122-123° (decomp.)

$[\alpha]_D$ +260° (c,1.2 in benzene)

and the other (300 mg.), m.p.122-123° (decomp.)

$[\alpha]_D$ +259° (c,1.0 in benzene)

were obtained. All three fractions showed no depression in melting point when mixed with an authentic specimen of tetrabromoergostenyl acetate. The total recovery of unchanged starting material is therefore 2.0 g. (67%).

Catalytic Hydrogenation of Tetrabromoergostenyl Acetate to give Ergosteryl-D Acetate 22:23-Dibromide.

Platinum oxide (150 mg.) was hydrogenated in suspension in pure dioxan (50 c.c.). A solution of tetrabromoergostenyl acetate (600 mg.) in pure dioxan (80 c.c.) was added and the mixture shaken with hydrogen. After $1\frac{1}{4}$ hours one mole of hydrogen had been absorbed, no appreciable absorption occurring after this time. The catalyst was filtered, the filtrate diluted with water and the solid (380 mg.) which separated filtered, dried and crystallised from chloroform-methanol to give prismatic needles (160 mg.), m.p.226-228° (decomp.).

Light absorption: Maxima at 2350 (ε = 16,000) and 2420Å (ε = 18,000) with an inflection at 2500Å (ε = 12,300).

One more crystallisation from the same solvent mixture furnished ergosteryl-D acetate 22:23-dibromide (90 mg.) as prismatic needles, m.p.227-229° (decomp.).

$[\alpha]_D$ +31° (c,1.4)

Light absorption: Maxima at 2350 (ε = 17,000) and 2420Å (ε = 19,500) with an inflection at 2500Å (ε = 12,500).

The product showed no depression in melting point on admixture with an authentic specimen.

Treatment of Tetrabromoergostenyl Acetate with Sodium Methyl Sulphide.

A solution of tetrabromoergostenyl acetate (13 g.) in dry benzene (300 c.c.) was treated with a solution of sodium methyl sulphide (8 moles) in absolute ethanol (100 c.c.)(105) and left at room temperature for one hour. Sodium bromide was immediately precipitated. The mixture was filtered, the filtrate washed with water till the washings were neutral to litmus, dried (Na_2SO_4) and evaporated in vacuo at about 50°. The light yellow solid residue was acetylated in pure dry pyridine (50 c.c.) and redistilled acetic anhydride (20 c.c.) by standing at room temperature for 14 hours during which time a solid had crystallised out. The mixture, after cooling at 0° for 5 hours, was filtered (liquor A) yielding a solid (3.35 g.) partly as clusters of fine needles and partly as prismatic plates. The component crystallising as needles was isolated by shaking the solid mixture with dry ether (200 c.c.) for 15 minutes, in which it is insoluble, filtered (950 mg.) and crystallised from benzene to give 22:23-dibromo-dimethylthio-ergostenyl acetate as fine felted needles, m.p.217-218° (decomp.).

$[\alpha]_D$ +39.5° (c,2.0)

Found: C,55.7; H,7.83; S,9.0; Br,23.1

$C_{32}H_{52}O_2S_2Br_2$ requires: C,55.48; H,7.57; S,9.26; Br,23.1%.

Light absorption: Maximum at 2240Å (ε = 8800).

In dioxan : Maximum at 2330Å (ε = 8350).

The product gives a deep yellow colour with tetranitromethane in chloroform.

Liquor A was treated with methanol to destroy excess acetic anhydride and left for 30 minutes. Water was then added to the mixture which was extracted with ether (2 x 200 c.c.). The ether extract was washed with dilute sulphuric acid, sodium hydrogen carbonate solution, water, dried (Na_2SO_4) and evaporated in vacuo yielding a yellow gummy solid. Solution of this material in benzene (20 c.c.) followed by the addition of excess methanol with storing at 0° for 30 minutes gave a solid (2.4 g.), m.p.157-170° (decomp.) which was filtered. Crystallisation of this material from ethyl acetate furnished 22:23-dibromomethylthioergosta-7:9(11)-dien--3β-yl acetate (400 mg.) as small prismatic needles, m.p.203° (decomp.).

$[\alpha]_D$ +32° (c,1.4)

Found: C,58.0; H,7.8; S,4.60; Br,25.1

$C_{31}H_{48}O_2SBr_2$ requires: C,57.75; H,7.5; S,4.97; Br,24.8%.

Light absorption: Maxima at 2360 (ε = 15,800) and 2420Å (ε = 17,000) with an inflection at 2510Å (ε = 12,400).

The compound gives a red-brown colour with tetranitromethane in chloroform.

Desulphurisation of 22:23-Dibromo-dimethylthioergostenyl Acetate with the Formation of Ergosteryl-D Acetate.

1. A solution of 22:23-dibromo-dithiomethoxyergostenyl acetate (550 mg.) in benzene (25 c.c.) was treated with Raney nickel sludge (5 c.c.) in benzene (25 c.c.) and the mixture refluxed for one hour. The catalyst was filtered, the filtrate evaporated and the residue crystallised from chloroform-methanol to give ergosteryl-D acetate (200 mg.) as large plates, m.p.178-180°.

$[\alpha]_D$ +32° (c,2.0)

Found : C,82.32; H,10.76

Calc.for $C_{30}H_{46}O_2$: C,82.10; H,10.60%.

Light absorption: Maxima at 2350 (ϵ = 16,300) and 2420Å (ϵ = 17,700) with an inflection at 2500Å (ϵ = 12,000). The product gives a red-brown colour with tetranitromethane in chloroform and shows no depression in melting point on admixture with an authentic specimen.

2. A solution of 22:23-dibromo-dimethylthioergostenyl acetate (100 mg.) in benzene (50 c.c.) was treated with Raney nickel sludge (6 c.c.) in ethanol (60 c.c.) and the mixture shaken at room temperature for 14 hours. The catalyst was filtered, washed with benzene (100 c.c.)

and the combined filtrate and washings washed with water, dried (Na_2SO_4) and evaporated in vacuo. The residual solid, on crystallisation from chloroform-methanol yielded slightly impure ergosteryl-D acetate (40 mg.) as plates, m.p.174-176°.

$[\alpha]_D$ +28° (c,1.3)

Light absorption: Maxima at 2050 (ε = 5600), 2350 (ε = 11,700) and 2420Å (ε = 13,200) with an inflection at 2500Å (ε = 8600). The product, on admixture with an authentic specimen of ergosteryl-D acetate, showed no depression in melting point.

Desulphurisation of 22:23-Dibromo-methylthioergosta-7:9(11)--dien-3β-yl Acetate with the Formation of Ergosteryl-D Acetate.

A solution of 22:23-dibromo-methylthioergosta-7:9(11)--dien-3β-yl acetate (170 mg.) in benzene (10 c.c.) was treated with Raney nickel sludge (2 c.c.) in benzene (10 c.c.) and the mixture refluxed for one hour. The catalyst was filtered, the filtrate evaporated and the residual white solid, on crystallisation from chloroform--methanol furnished ergosteryl-D acetate (50 mg.) as plates, m.p.178-180°.

$[\alpha]_D$ +32° (c,0.94)

Found: C,82.36; H,10.7

Calc. for $C_{30}H_{46}O_2$: C,82.10; H,10.6%.

Light absorption: Maxima at 2350 ($\mathcal{E}$ = 15,500) and 2420Å ($\mathcal{E}$ = 17,200) with an inflection at 2500Å ($\mathcal{E}$ = 11,500). The product, on admixture with an authentic specimen, showed no depression in melting point.

Dehydrobromination of Tetrabromoergostenyl Acetate.

1. Using Collidine.

A solution of tetrabromoergostenyl acetate (8.27 g.) in dry benzene (300 c.c.) was treated with collidine (50 c.c.) (purified by distillation over sodium) and the mixture heated with stirring on a steam bath for 20 minutes. A buff coloured precipitate of collidine hydrobromide (3.8 g.; theoretical for two moles, 4.4 g.) separated during the reaction which was filtered, washed with a little dry benzene, the yellow filtrate plus washings washed successively with dilute sulphuric acid solution, sodium hydrogen carbonate solution, water, dried (Na_2SO_4) and evaporated. The residual solid was dissolved in the minimum of chloroform in the cold and excess methanol gradually added to precipitate a light yellow solid (4.72 g.), m.p.194-195° (decomp.) which was filtered. One crystallisation of this material from chloroform-methanol yielded flat rectangular prisms (3.6 g.; 55% yield), m.p.200-201° (decomp.).

$[\alpha]_D$ -56° (c,2.0)

This material was crystallised three times from the same solvent mixture to give flat rectangular prisms (200 mg.), m.p.200-201° (decomp.).

$[\alpha]_D$ -59.3 (c,2.3)

Found: C,60.12; H,7.45; Br,27.5

$C_{30}H_{44}O_2Br_2$ requires: C,60.41; H,7.44; Br,26.7%.

Light absorption: Maxima at 2280 (ε = 12,700; $E^{1\%}_{1cm.}$ = 212), 2340 (ε = 11,700; $E^{1\%}_{1cm.}$ = 195) and 2670Å (ε = 11,900; $E^{1\%}_{1cm.}$ = 199). The product gives a red-brown colour with tetranitromethane in chloroform. An identical product is obtained if the washing with dilute sulphuric acid is omitted.

2. Using Pyridine.

Pure dry pyridine (80 c.c.) was added to a solution of tetrabromoergostenyl acetate (14 g.) in dry benzene (400 c.c.) and the mixture heated on the steam bath for 30 minutes. The buff-coloured precipitate of pyridine hydrobromide, which separated soon after heating was begun, was filtered and washed with a little dry benzene. The combined filtrate and washings were washed successively with water, dilute sulphuric acid solution, sodium hydrogen carbonate solution, again with water and dried (Na_2SO_4). The benzene solution was concentrated to small volume in vacuo, excess methanol added and stored at 0° for 30 minutes. The light yellow crystalline

solid (5 g.), m.p.192-194° (decomp.), which had separated, was filtered and crystallised twice from chloroform--methanol to give flat rectangular prisms (2.3 g.), m.p. 199-200° (decomp.).

$[\alpha]_D$ -54° (c,1.2)

Light absorption: Maxima at 2280 (ε = 11,400; $E^{1\%}_{1cm.}$ = 190), 2350 (ε = 10,300; $E^{1\%}_{1cm.}$ = 172), and 2660Å (ε = 10,400; $E^{1\%}_{1cm.}$ = 174). The product gives a red-brown colour with tetranitromethane in chloroform and showed no depression in melting point when mixed with the product prepared using collidine.

3. Using Acetone.

A suspension of tetrabromoergostenyl acetate (200mg.) in bench acetone (15 c.c.) was refluxed for 10 minutes during which the solid dissolved to give a yellow solution which was acid to litmus. Water (5 c.c.) was added, the mixture shaken for a moment and set aside to cool. The white solid (90 mg.), m.p.182-184° (decomp.) was filtered and crystallised from chloroform-methanol to give prismatic needles, m.p.193-194° (decomp.).

$[\alpha]_D$ -55° (c,1.35)

Found: C,60.1; H,7.88

$C_{30}H_{44}O_2Br_2$ requires: C,60.41; H,7.44%.

Light absorption: Maxima at 2260 (ε = 13,400; $E^{1\%}_{1cm.}$ = 224), 2330 (ε = 13,000; $E^{1\%}_{1cm.}$ = 218) and 2670Å (ε = 10,000; $E^{1\%}_{1cm.}$ = 168).

The product gives a red-brown colour with tetranitromethane in chloroform.

4. Using Sodium Methoxide.

A solution of tetrabromoergostenyl acetate (1.4 g.) in dry benzene (35 c.c.) was treated with a solution of clean dry sodium (350 mg.; 8 moles) in pure dry methanol (14 c.c.). The mixture was allowed to stand at room temperature for one hour, diluted with benzene, washed with water till the washings were neutral to litmus, dried (Na_2SO_4) and evaporated in vacuo. The residual gum was heated with methanol (30 c.c.), stored at -0° for one hour and the solid (280 mg.), which had separated, was filtered and crystallised from chloroform-methanol yielding colourless needles (50 mg.), m.p.188-189° (decomp.).

$[\alpha]_D$ -56° (c,1.5)

Found: C,59.00; H,8.11

$C_{28}H_{42}OBr_2.CH_3OH$ requires: C,59.38; H,7.9%.

Light absorption: Maxima at 2280 (ε= 12,000; $E^{1\%}_{1cm.}$ = 201), 2350 (ε = 11,200; $E^{1\%}_{1cm.}$ = 188) and 2680Å (ε = 10,000; $E^{1\%}_{1cm.}$ = 166). The product colours on standing in air for 24 hours, and gives a red-brown colour with tetranitromethane in chloroform.

Reactions of the Product obtained from Dehydrobromination of Tetrabromoergostenyl Acetate using either Collidine or Pyridine hereafter called 'the Dibromide'.

Debromination of the Dibromide with the Isolation of impure Ergosteryl-D Acetate.

A solution of the dibromide (2 g.) in ether (200 c.c.) and ethanol (300 c.c.) was treated with activated zinc dust (10 g.) added in one portion, by heating with stirring on a boiling water bath for 2 hours. The zinc dust was filtered and ether added to the filtrate which was washed with water, dried (Na_2SO_4) and evaporated to give a white solid (1.24 g.), m.p.134-151°. This material, on crystallisation from acetone yielded crystals (450mg.), m.p.145-156°, which were crystallised once more from acetone to give curved blades, m.p.150-164°.
Light absorption: Maxima at 2280 (ε = 10,300; $E^{1\%}_{1cm.}$ = 236), 2350 (ε = 12,000; $E^{1\%}_{1cm.}$ = 273), 2520 (ε = 8900; $E^{1\%}_{1cm.}$ = 203), 2660 (ε = 7100; $E^{1\%}_{1cm.}$ = 163) and 2720Å (ε = 7000; $E^{1\%}_{1cm.}$ =161) with an inflection at 2420Å (ε = 9700; $E^{1\%}_{1cm.}$ = 222).
This material was combined with that recovered from the crystallisation mother liquors (total: 1 g.), dissolved in a light petroleum (b.p.80-100°):benzene (1:2) mixture (180 c.c.) and chromatographed on an alumina column (Grade II — III; 14 x 2.5 cm.). The chromatogram is

shown below.

Eluting Solvent	Vol.(c.c.)	Wt.(mg.)	Fraction No.	M.P.
Light Petroleum: Benzene (2:1)	300	0		
"	100	10	1	
"	100	60	2	172-175°
"	100	40	3	
"	100	10	4	
"	100	5	5	
"	100	5	6	166-169°
"	100	5	7	
"	200	5	8	
" (1:1)	200	trace	9	
" (1:2)	200	trace	10	
Benzene	300	0		
Benzene: Ether (1:1)	200	trace	11	
Benzene: Methanol(99:1)	200	650	12	
"	200	170	13	
"	100	0		
	Total =	960		

Fractions 1 to 3 were combined and crystallised from chloroform-methanol to give impure ergosteryl-D acetate as plates, m.p.172-175°.

$[\alpha]_D$ +13° (c,1.5)

Found: C,82.0; H,10.7

Calc. for $C_{30}H_{46}O_2$: C,82.1; H,10.7%.

Light absorption: Maxima at 2360 ($\mathcal{E}$ = 12,200) and 2430Å ($\mathcal{E}$ = 13,700) with an inflection at 2510Å ($\mathcal{E}$ = 9300). The product gave a red-brown colour with tetranitromethane in chloroform.

Fractions 4 to 8 were combined and, on crystallisation from chloroform-methanol yielded plates, m.p.166-169°.

$[\alpha]_D$ -27° (c,1.0)

Light absorption: Maxima at 2360 ($\mathcal{E}$ = 9200), 2430 ($\mathcal{E}$ = 9200) and 2680Å ($\mathcal{E}$ = 3300) with inflections at 2280 ($\mathcal{E}$ = 7000) and 2510Å ($\mathcal{E}$ = 6900). The product gave a red-brown colour with tetranitromethane in chloroform.

Fractions 9 to 13 were gums which evaded all attempts to crystallise them.

The Formation of a Maleic Anhydride Adduct from the Dibromide.

A solution of the dibromide (500 mg.) in dry benzene (50 c.c.) was treated with excess maleic anhydride (500 mg.; 6.1 moles) by refluxing the solution for 10 hours on a boiling water bath. After standing overnight the solution was evaporated, excess maleic anhydride sublimed off by heating the residue under high vacuum at

95°, and the remaining solid, on crystallisation from acetone, yielded fine needles (170 mg.), m.p.226-229° (decomp.). After several crystallisations from the same solvent the pure adduct was obtained as very fine needles, m.p.230-231° (decomp.).

$[\alpha]_D$ +27° (c,1.0)

Found: C,58.57; H,6.88

$C_{34}H_{48}O_5Br_2$ requires: C,58.79; H,6.68%.

Light absorption: Maxima at 2070 (ε = 3700; $E^{1\%}_{1cm.}$ = 53) and 2740Å (ε = 4500; $E^{1\%}_{1cm.}$ = 65). The compound gives a red-brown colour with tetranitromethane in chloroform.

Peroxidation of the Dibromide with the Isolation of Ergosteryl-D Acetate 22:23-Dibromide.

The apparatus described previously for the attempted peroxidation of 7-ketoergosta-8(14):9(11):22-trien-3β-yl acetate was used in this experiment (see p.131).

A suspension of the dibromide (5 g.) in 95% ethanol (1200 c.c.) containing eosin (6 mg.) was heated to boiling. Complete solution was not obtained and the suspension was transferred to the reaction vessel and illuminated for 5 hours during which a slow stream of oxygen was bubbled through the reaction mixture. After this time a considerable amount of solid still remained in suspension. This solid (3.2 g.), m.p.197-199° (decomp.), $[\alpha]_D$ -50° (c,2.8), which was starting material, was filtered.

The filtrate was evaporated, the residual gummy solid dissolved in dry benzene (50 c.c.) and perfused through an alumina column (Grade II — III; 14 x 2 cm.) with benzene. The first 100 c.c. of benzene eluant yielded a solid which on crystallisation from ethanol yielded prisms (190 mg.), m.p.220-221° (decomp.)

$[\alpha]_D$ +8° (c,2.8)

Light absorption: Maxima at 2360 (ε = 13,500) and 2420Å (ε = 16,000) with an inflection at 2500 (ε = 11,000) and 2700Å (ε = 2300). This material was twice crystallised from ethyl acetate to give ergosteryl-D acetate 22:23--dibromide (20 mg.) as prismatic needles, m.p.226-228° (decomp.).

$[\alpha]_D$ +30° (c,0.8)

The product gave a red-brown colour with tetranitromethane in chloroform and on admixture with an authentic specimen of ergosteryl-D acetate 22:23-dibromide showed no depression in melting point.

The second 100 c.c. of benzene eluant yielded a small amount of solid which crystallised from ethanol as badly formed plates, m.p.213-216° (decomp.).

Light absorption: Maxima at 2360 (ε = 14,000) and 2420Å (ε = 15,200) with inflections at 2510 (ε = 11,000) and 2700Å (ε = 3500). Further elution with benzene and benzene-methanol (99:1) yielded uncrystallisable gums.

Catalytic Hydrogenation of the Dibromide with the Formation of 22:23-Dibromoergost-8(14)-en-3β-yl Acetate.

Platinum oxide (50 mg.) was hydrogenated in suspension in glacial acetic acid (20 c.c.) (purified by refluxing over chromium trioxide), a solution of the dibromide (130 mg.; m.p.200-201° (decomp.); $[\alpha]_D$ -59°) in glacial acetic acid (150 c.c.) added and the mixture hydrogenated for 20 hours. The catalyst was filtered, the filtrate evaporated, the solid residue crystallised from chloroform-methanol to give plates (55 mg.; 42% yield), m.p.188-190° (decomp.) which, on one more crystallisation from the same solvent mixture furnished 22:23-dibromoergost-8(14)--en-3β-yl acetate as plates, m.p.190-191° (decomp.).

$[\alpha]_D$ +3.6° (c,1.6)

Found: C,60.2; H,8.13; Br,26.3

Calc. for $C_{30}H_{48}O_2Br_2$: C,59.98;H,8.05; Br,26.6%.

Light absorption: Maximum at 2110Å (ε = 10,000).

The product gave a deep yellow colour with tetranitromethane and on admixture with an authentic specimen, m.p.190-191°, $[\alpha]_D$ +4.0° (98) it showed no depression in melting point.

Resolution of the Dibromide into Adduct-forming and Non-adduct-forming Components.

A solution of the dibromide (3.3 g.) in dry benzene

(60 c.c.) was treated with excess maleic anhydride (3.3g.; 6.1 moles) by refluxing for 3 hours on a boiling water bath. The solution was then evaporated and excess maleic anhydride sublimed off by heating the residue under high vacuum at 95°. The resulting solid was hydrolysed by dissolving in benzene (30 c.c.) adding ethanol (100 c.c.) (redistilled over solid potassium hydroxide) and aqueous potassium hydroxide solution (20 c.c.; 20%) and refluxing the mixture on a boiling water bath for one hour. The claret coloured mixture was then diluted with water and extracted with ether (3 x 150 c.c.). The ether extract was washed with sodium hydrogen carbonate solution, water, dried (Na_2SO_4) and evaporated to give a solid neutral fraction (345 mg.; 11% yield). This material was acetylated in pure dry pyridine (5 c.c.) and redistilled acetic anhydride (5 c.c.) by heating on a steam bath for one hour. On cooling a crystalline solid separated (90 mg.), m.p.221-222° (decomp.) which was filtered and crystallised twice from chloroform-methanol to give prismatic needles (30 mg.), m.p.226-227° (decomp.).

$[\alpha]_D$ -9.2° (c,1.0)

Light absorption: Maximum at 2420Å (ε = 13,500) with an inflection at 2500Å (ε = 12,600).

The alkaline, aqueous layer, after the neutral material had been extracted, was acidified with dilute

sulphuric acid solution, a solid being precipitated, and the mixture was extracted with ether (3 x 150 c.c.), the ether extract washed with water, dried (Na_2SO_4) and evaporated to give the acid fraction as a light yellow gummy solid (3.2 g.; 91% yield).

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