"AN INVESTIGATION OF SOME ROUTES TO 11-OXYGENATED STEROIDS"

A Thesis in Two Parts

submitted by

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in fulfilment of the requirements for the Degree of Doctor of Philosophy of The University of Glasgow.

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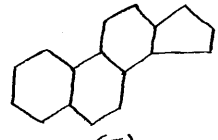
CONTENTS

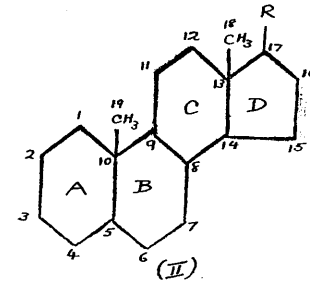
INTRODUCTION

INTRODUCTION

The steroids are naturally occurring crystalline compounds of high molecular weight derived from perhydro--1:2-cyclopentenophenanthrene (I) and with some exceptions, have the basic structure (II).



(I)



(II)

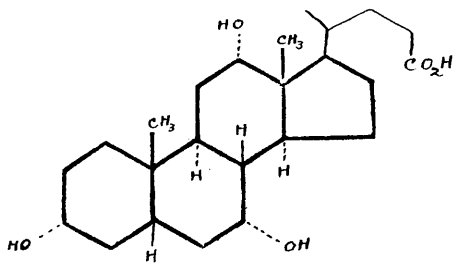
The carbon atoms at the ring junctions are asymmetric and could give rise to sixty-four stereoisomeric forms of (II) but only ~~three~~ four of these forms have been proved to exist in naturally occurring steroids. The introduction of a substituent to any of the methylene groups in the steroid nucleus creates a further asymmetric centre and the tertiary carbon atom, C_{17}, is also optically active.

The steroid molecule has been shown, by X-ray diffraction methods, to be an approximately planar structure (1) and stereochemical configurations at the centres of asymmetry are denoted by a full line for a group projecting above the plane of the molecule and a dotted line for a group projecting below the plane. The former are named "β" substituents and the latter "α".

In (II) the methyl groups at C_{10} and C_{13} and the side chain (R) at C_{17} are all of the β-(natural) configuration.

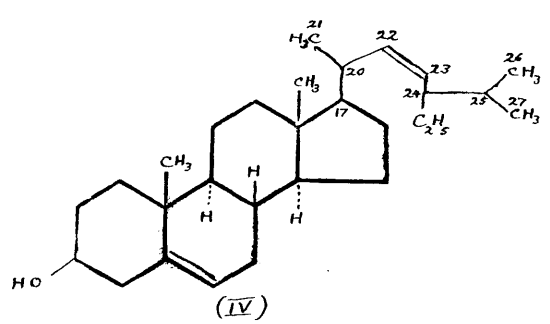
The steroids are classified into seven groups, namely bile acids, sterols, adrenal cortical hormones, sex hormones, cardiac-active steroids, steroid saponins and steroid alkaloids. The first three groups are of importance in the work to be discussed in this thesis and a brief review of the salient points in the structure of members of these groups is given below.

Bile Acids.— The bile acids are obtained by the saponification of animal bile in which they occur as water-soluble sodium salts in conjugation with the amino-acids glycine or taurine. The majority of naturally occurring bile acids are saturated compounds containing twenty-four carbon atoms, a carboxyl group and one, two or three hydroxyl groups. The hydroxyl groups are almost invariably of the α-configuration, one being at C_3 and the others, in most cases, either at C_7 or C_{12} or at both of these positions. The A/B ring junction is cis, resulting in C_5 having a hydrogen atom of the β-configuration attached to it, whereas the B/C and C/D ring fusions are trans. The stereochemical configuration of the bile acids is illustrated below in cholic acid (III), a 3:7:12-trihydroxy acid of wide occurrence.



(III)

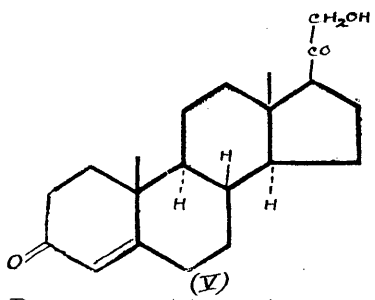
Sterols.— The sterols are crystalline alcohols occurring in the nonsaponifiable fractions of animal and plant fats. They contain one secondary hydroxyl group of β-configuration at C_3 and may be saturated or contain one, two or three double bonds. Their stereochemical configuration differs from that of the bile acids as the A/B ring fusion is trans. The sterol side chain consists of carbon and hydrogen only, contains eight to ten carbon atoms, frequently possesses an ethylenic linkage and can exhibit optical isomerism at C_{24} (see (IV) for side chain numbering) when a methyl or ethyl group is attached at this point. The normal configuration at C_{24}, as found in ergosterol, stigmasterol and most other phytosterols, is designated "*b*" and represented by a full line. The structure of a typical sterol, stigmasterol, is given in (IV).



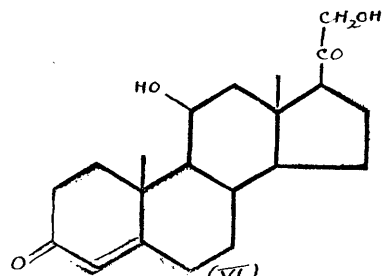
(IV)

Adrenal Cortical Hormones.— It was found that extracts of the adrenal cortex prolonged the life of adrenalectomized animals (2) and ~~an~~ investigation of the constituents of these extracts by Kendall, by Wintersteiner and Pfiffner, and by Reichstein, led to the isolation of twenty-eight crystalline substances, the adrenal cortical hormones, six of which are active in prolonging life. The latter are shown in chart I.

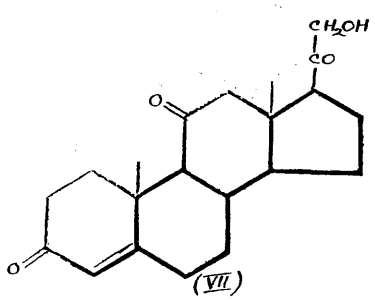
Chart I.



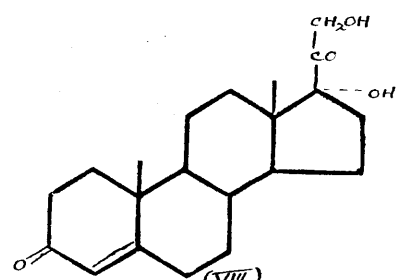
(V)
Deoxycorticosterone



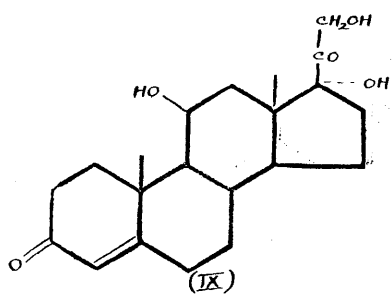
(VI)
Corticosterone



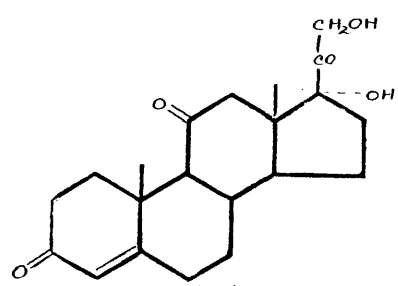
(VII)
Dehydrocorticosterone



(VIII)
17-Hydroxy-11-deoxycorticosterone



(IX)
17-Hydroxycorticosterone



(X)
17-Hydroxy-11-dehydrocorticosterone

The cortical hormones, like the sterols and bile acids, contain trans-fused B/C and C/D ring junctions and a β-orientated, carbon-containing side chain. The active hormones all possess an αβ-unsaturated ketone grouping in ring A and a ketol side chain. The inactive hormones are either saturated in ring A with a trans A/B ring junction, or contain a reduced side chain.

Of the six active hormones, 17-hydroxy-11-dehydrocorticosterone (X), also known as Kendall's Compound E, or cortisone, has proved of great efficiency in alleviating the effects of rheumatoid arthritis (3) and may have much wider therapeutic application. The quantity of cortisone available from adrenal glands is very small and the synthesis of this compound has been extensively investigated.

The Synthesis of Cortisone.— Readily available bile acids with an oxygen function in ring C, such as cholic acid and deoxycholic acid (3α:12α-dihydroxycholanic acid), were obvious starting materials for the synthesis of cortisone.

There were four main problems in such a synthesis: (i) introduction of an oxygen function at C_{11}, (ii) degradation of the bile acid side chain, (iii) synthesis of the dihydroxy acetone side chain and (iv) introduction of the

αβ-unsaturated ketone grouping in ring A. Sarett (4,5) was the first ~~person~~ to prepare cortisone from intermediates derived from 12-oxygenated bile acids.

Introduction of an Oxygen Atom at C_{11}.— The aim of the work described in this thesis is the introduction of an 11-oxygen atom into the steroid nucleus, part I being concerned with investigations in the bile acids and part II in the sterols. Consequently part I will deal in detail with the various methods for the preparation of 11-oxygenated bile acids and only brief mention of these methods will be given at this point. There are four methods (for references see part I of this thesis):

(a) the bromohydrin synthesis which involves addition of hypobromous acid to chol-11-enic acids prepared by dehydration of 12α-hydroxy bile acids;

(b) the bromination of 12-keto bile acids, hydrolysis of the resulting 11-bromo-12-ketones and rearrangement and reduction of the hydroxyketones thus formed;

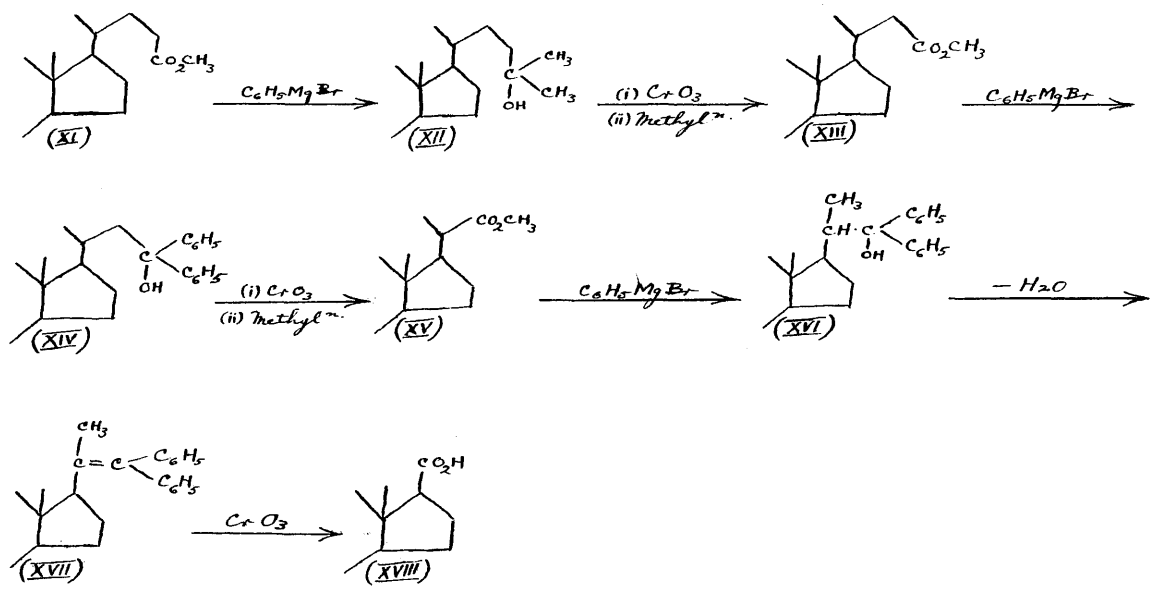
(c) through the formation of 3:9-oxidochol-11-enic acids obtained from chol-9(11)-enic acids;

(d) the oxidation of 7:9(11)-dienic bile acids.

Side Chain Degradation.— The degradation of the bile acid side chain was first accomplished by Wieland and his co-workers (6) using the Barbier-Wieland method

described by Barbier (7) in 1913. Chart II shows the method, involving carbinol formation and chromic acid oxidation of the latter compounds. By carrying out these reactions three times the cholanic acid side chain (XI) was degraded to the etianic acid side chain (XVIII).

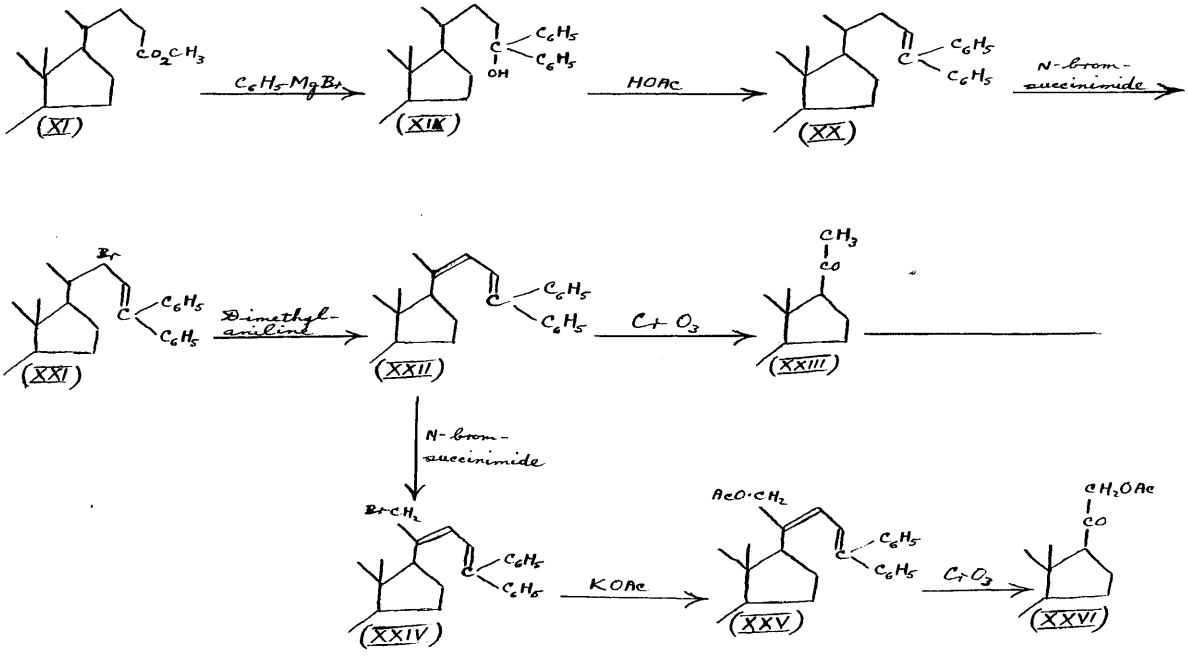
Chart II



This method gives low overall yields and Meystre and Miescher (8) have evolved a more efficient method of side chain degradation, formulated in chart III, which decreases the number of chromic acid oxidations required. The final product (XXIII) contains the progesterone side chain ($-CO.CH_3$) but Meystre and Wettstein (9) have devised a modification to produce the corticosterone side

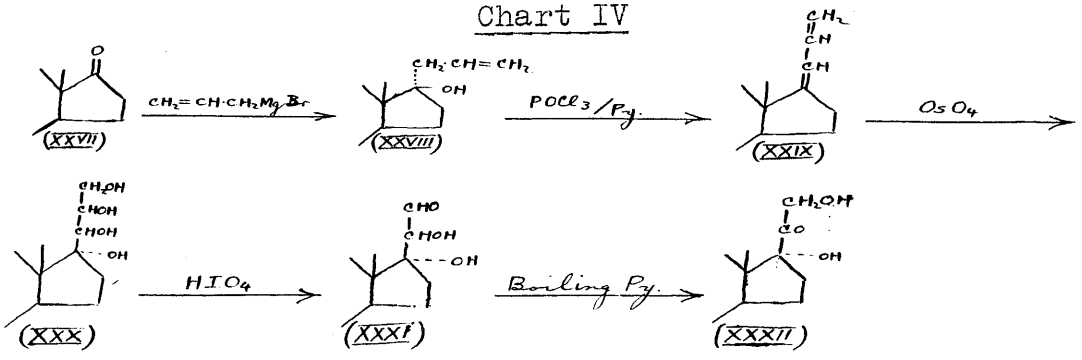
chain (XXVI).

Chart III



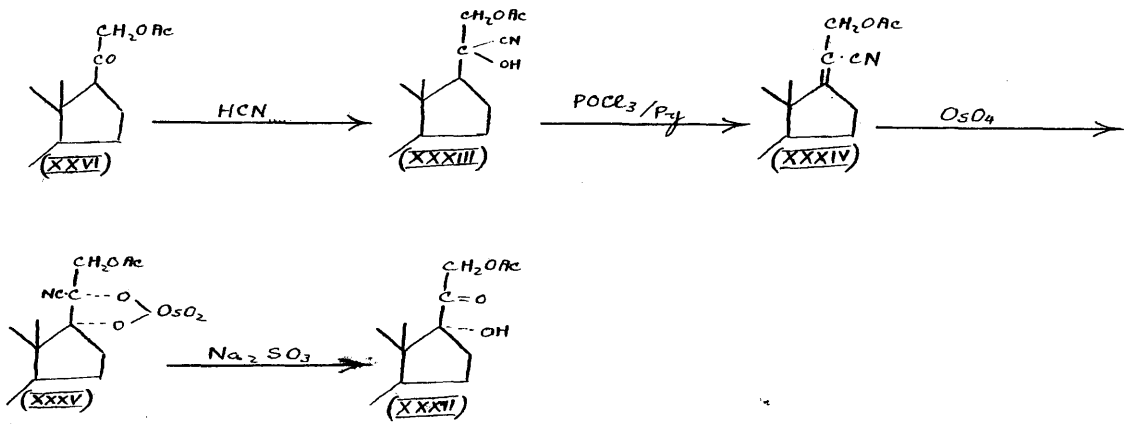
Synthesis of the Dihydroxyacetone Side Chain.— von Euw and Reichstein (10) first synthesised the dihydroxy-acetone side chain, using the tetrol (XXX) as starting material. Butenandt (11) prepared the latter from a 17-keto steroid (XXVII) as given below.

Chart IV



Sarett (5) in completing the synthesis of cortisone from deoxycholic acid, introduced the 17α-hydroxyl group into the corticosterone side chain (XXVI) by the following cyanohydrin synthesis (Chart V).

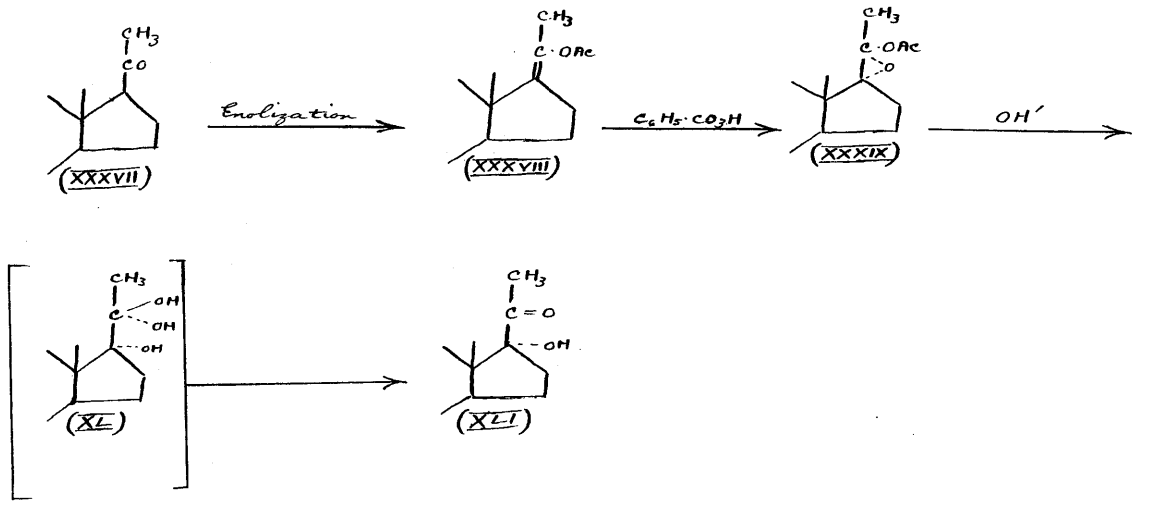
Chart V



The dehydration of the cyanohydrin (XXXIII) was found to proceed more satisfactorily with the 3-acetoxy than with the 3-keto derivative.

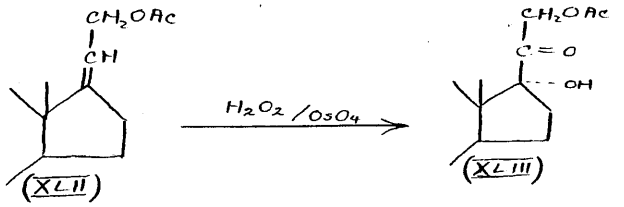
These two syntheses involve the use of osmium tetroxide for the introduction of the 17α-hydroxy group but Gallagher and his co-workers (12) have avoided the use of this expensive reagent in the preparation of the 17-hydroxyprogesterone side chain (XLI).

Chart VI



Julian et al (13) and Plattner, Heusser and Fuerer (14) have introduced 17α-hydroxyl groups into the corticosterone and progesterone side chains respectively through intermediate 16α:17α-oxido steroids, and Miescher and Schmidlin (15) have prepared the dihydroxy acetone side chain by direct oxidation, with hydrogen peroxide catalysed by osmium tetroxide, of (XLII) (Chart VII).

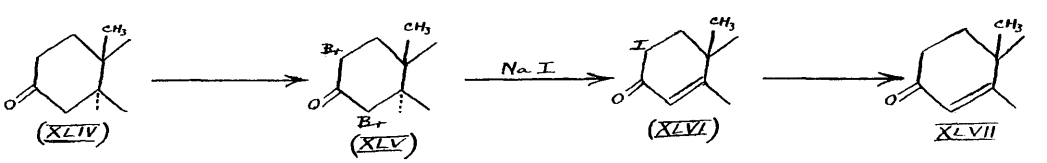
Chart VII



αβ-Unsaturated Ketone in Ring A.— Bromination of a 3-keto steroid with a cis A/B ring junction (e.g. a 3--keto bile acid) gives, as the main product, the 4-bromo-derivative which is dehydrobrominated, sometimes with difficulty, to the required αβ-unsaturated ketone. Dehydrobromination was also found to occur when the 2:4--dinitrophenylhydrazone of the 4-bromo-3-ketone was formed (16), the αβ-unsaturated ketone being generated by the action of pyruvic acid on the unsaturated phenylhydrazone.

Bromination of a 3-keto steroid with trans-fused rings A and B gives the 2-bromo compound normally but the 2:4-dibromide (XLV) can be obtained under certain conditions. The latter compound, on prolonged treatment with sodium iodide, yields the 2-iodo-αβ-unsaturated ketone (XLVI) which is converted to (XLVII) on reduction or collidine treatment (17).

Chart VIII



The synthesis of cortisone described above, is long and laborious and the overall yields are poor. However,

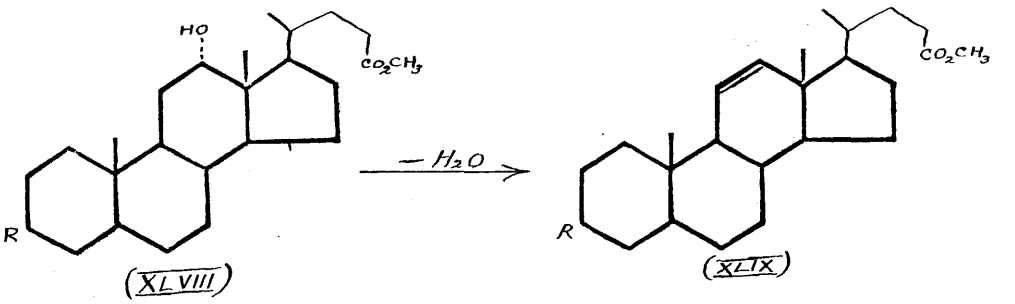
recent work on the oxidation of 7:9(11)-dienic steroids (see historical section of part II of this thesis for fuller details) has improved the synthesis of 11-oxygenated steroids and these compounds can now be obtained in good yield. Moreover the methods used are applicable to sterols with an ethylenic linkage in the side chain, facilitating side chain degradation and the synthesis of the dihydroxyacetone side chain.

PART I

11-OXYGENATED BILE ACIDS

HISTORICAL

Bile Acids Unsaturated at C_{11}.— The first successful approach to the synthesis of 11-oxygenated steroids was through Δ^{11}-derivatives (XLIX) prepared from 12α--hydroxy bile acids (XLVIII) (18).



Purely chemical methods of dehydration such as heating with mineral acids, phosphoryl chloride and pyridine ~~or tosyl chloride and pyridine~~, when applied to 12α-hydroxy steroids, gave mixtures containing only small quantities of Δ^{11}-compounds (19,20). von Euw and Reichstein (20) explained this by pointing out that the 12α-hydroxyl group is adjacent to a quaternary carbon atom at the 13-position which can give rise to various retropinacol rearrangements.

Pyrolytic methods gave better results although pyrolysis of the free or acetylated 12-hydroxy steroids (21,22) was not so successful as pyrolysis of the corresponding 12-benzoates (19,23,24). Reichstein et al (19) have obtained methyl 3-ketochol-11-enate (XLIX, R = O) in 40% yield by the thermal fission of amorphous methyl

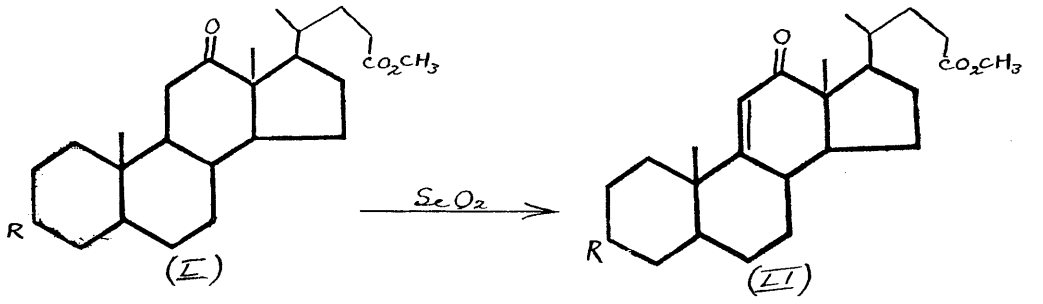
3-keto-12α-benzoyloxycholanate, formed by partial hydrolysis and oxidation of methyl 3-acetyl-12-benzoyl-deoxycholate. McKenzie, McGuckin and Kendall (24) have pyrolysed amorphous methyl 12-benzoyldeoxycholate to the corresponding Δ^{11}-compound (XLIX, R = OH) in 45% yield. Methyl 12-benzoyldeoxycholate was obtained by partial hydrolysis of methyl 3:12-dibenzoyldeoxycholate. Pyrolysis of methyl 3-acetyl-12-benzoyldeoxycholate was unsatisfactory as the 3-acetoxy group was in part eliminated with formation of a mixture of choladienic acids (19).

Thermal fission of the anthraquinone-β-carboxylates of 12α-hydroxy bile acids (25) introduced a Δ^{11}-double bond. As in the case of the free alcohol, acetate or benzoate, pyrolysis proceeded in better yield when the original bile acid side chain was intact than when it had been degraded.

In addition, treatment of the tosylates of 12α--hydroxy bile acids with pyridine under pressure or collidine gave the corresponding Δ^{11}-compound in reasonable yield. von Euw and Reichstein (20) have investigated this method and found that the yield of Δ^{11}-compound increased with decreasing length of side chain; methyl 3-keto-12α-tosyloxyetianate was converted to methyl 3-ketoeti-11-enate in 40% yield whereas methyl 3-keto-chol-11-enate was only obtained in 31% yield from the

corresponding tosylate. As in the pyrolysis of benzoates, the best results were obtained when the 3-position was occupied by a keto or unacylated hydroxyl group.

The above methods involved wasteful pyrolytic reactions but these were eliminated in the preparation of 12-ketochol-9(11)-enic acids (LI), required in the 3:9--oxide route to 11-oxygenated steroids (see below). Selenium dioxide dehydrogenation of 12-keto bile acids (L) introduced the necessary 9(11)-ethylenic linkage.

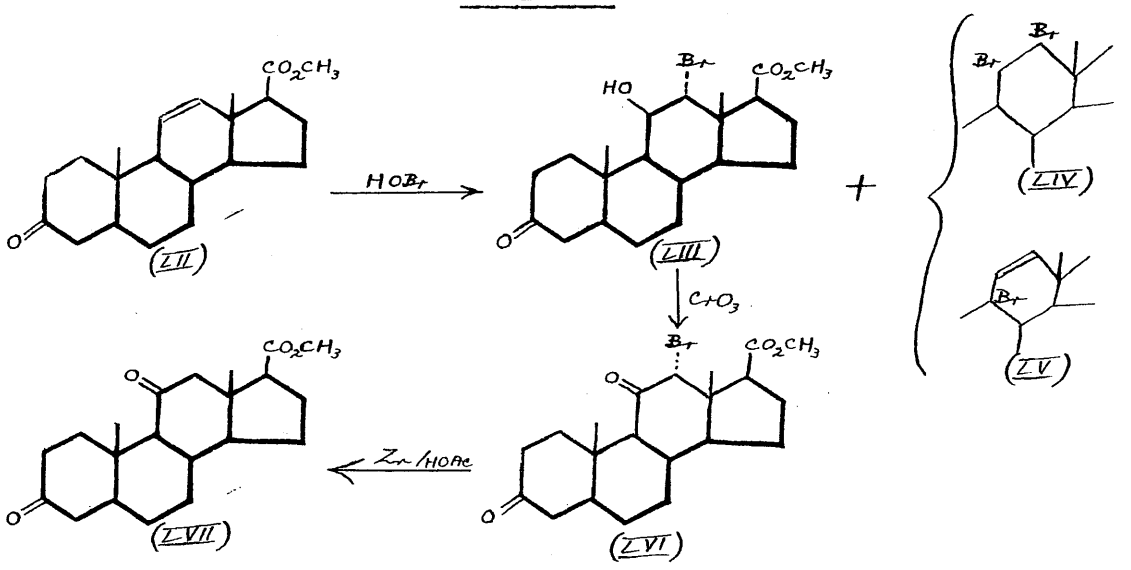


Schwenk and Stahl (26) applied the method to methyl 3α-acetoxy-12-ketocholanate (L, R = OAc) and Kendall et al (27) to methyl 3α-benzoyloxy-12-ketocholanate (L, R = OBz), the latter workers obtaining a 60% yield of 3α--hydroxy-12-ketochol-9(11)-enic acid.

Introduction of 11-Oxygen by the Bromohydrin Synthesis.— To introduce an oxygen atom at C_{11} in the steroid nucleus, from a bile acid containing an ethylenic linkage embracing C_{11}, Lardon and Reichstein (18) employed the bromohydrin

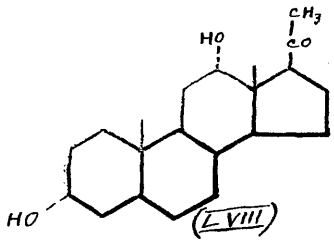
synthesis, starting from 3-ketoeti-11-enic acid methyl ester (LII) (chart IX).

Chart IX



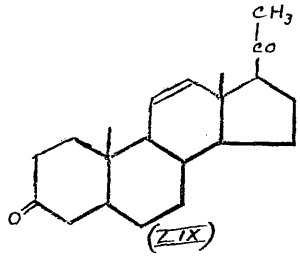
The formation of the bromohydrin (LIII) by hypobromous acid, generated by the action of aqueous acetone on N-bromacetamide, was accompanied by formation of the 11:12-dibromide (LIV) and the 9-bromo-11-ene (LV) but Sarett (4) improved the yield of (LIII) by using a trace of sulphuric acid as a catalyst and reconverting (LIV) to starting material by mild treatment of the reaction mixture with zinc and acetic acid. The bromohydrin synthesis has also been applied to (LIX) and (LXI), obtained from 20-keto-pregnan-3α:12α-diol (LVIII) and bisnordeoxycholic acid (LX) respectively (25,28), the latter being degradation products of deoxycholic acid.

Chart X

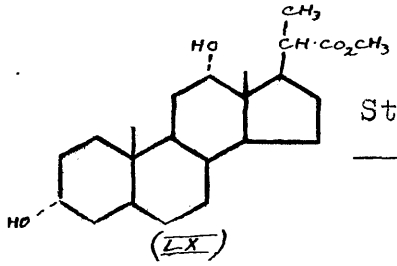


(LVIII)

3:12-Diacetate → 3-Hydroxy-12-acetate → 3-Keto-12-acetate → 3-Keto-12-anthraquinone-β-carboxylate

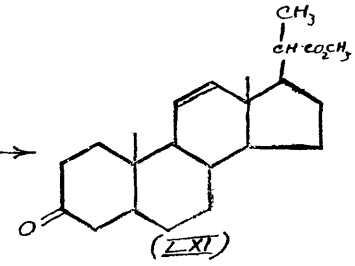


(LIX)



(LX)

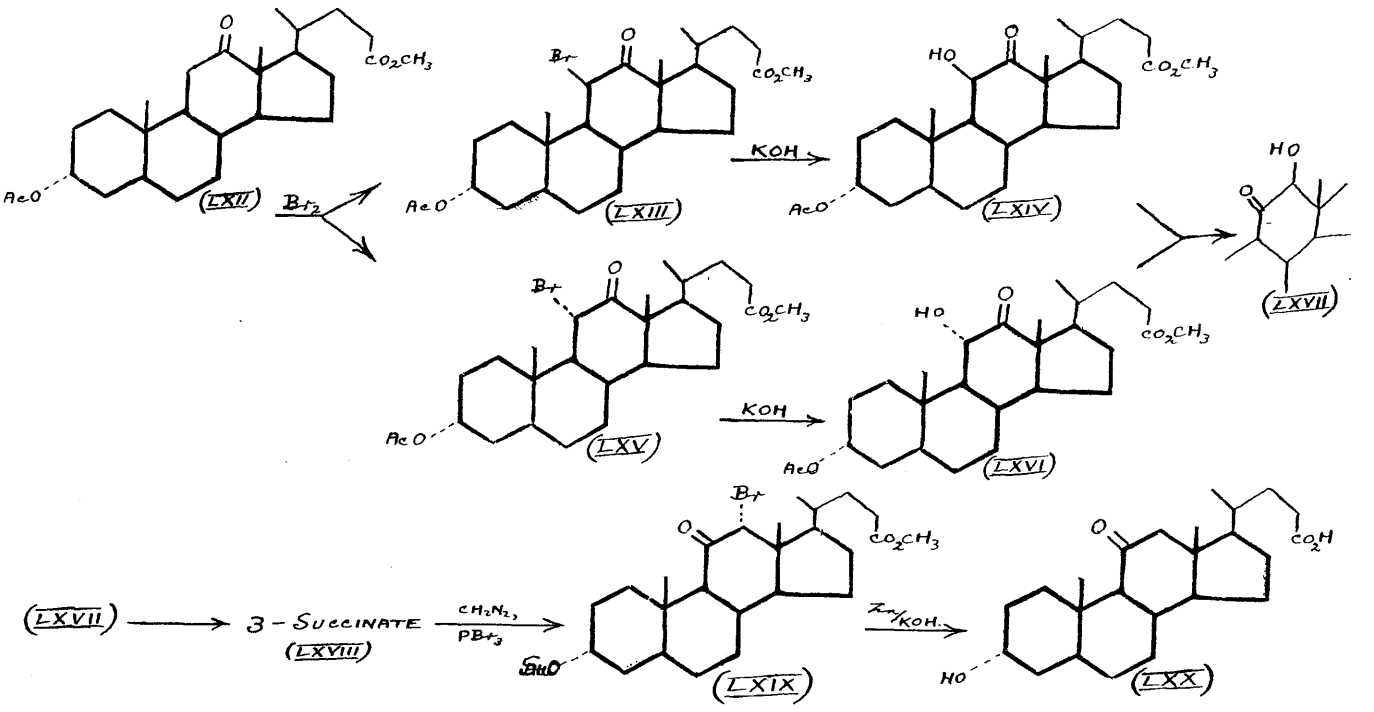
Steps as above → 3-Keto-12-benzoate →



(LXI)

Introduction of 11-Oxygen through 11-Bromo-12-ketones.— This method, introduced by Gallagher (29,30,31), does not involve the use of bile acids unsaturated at C_{11} but depends on the bromination of a 12-keto bile acid to a mixture of epimeric 11-bromo-12-ketones. The reaction sequence as applied to 3α-acetoxy-12-keto-cholanic acid methyl ester (LXII) is shown in chart XI.

Chart XI

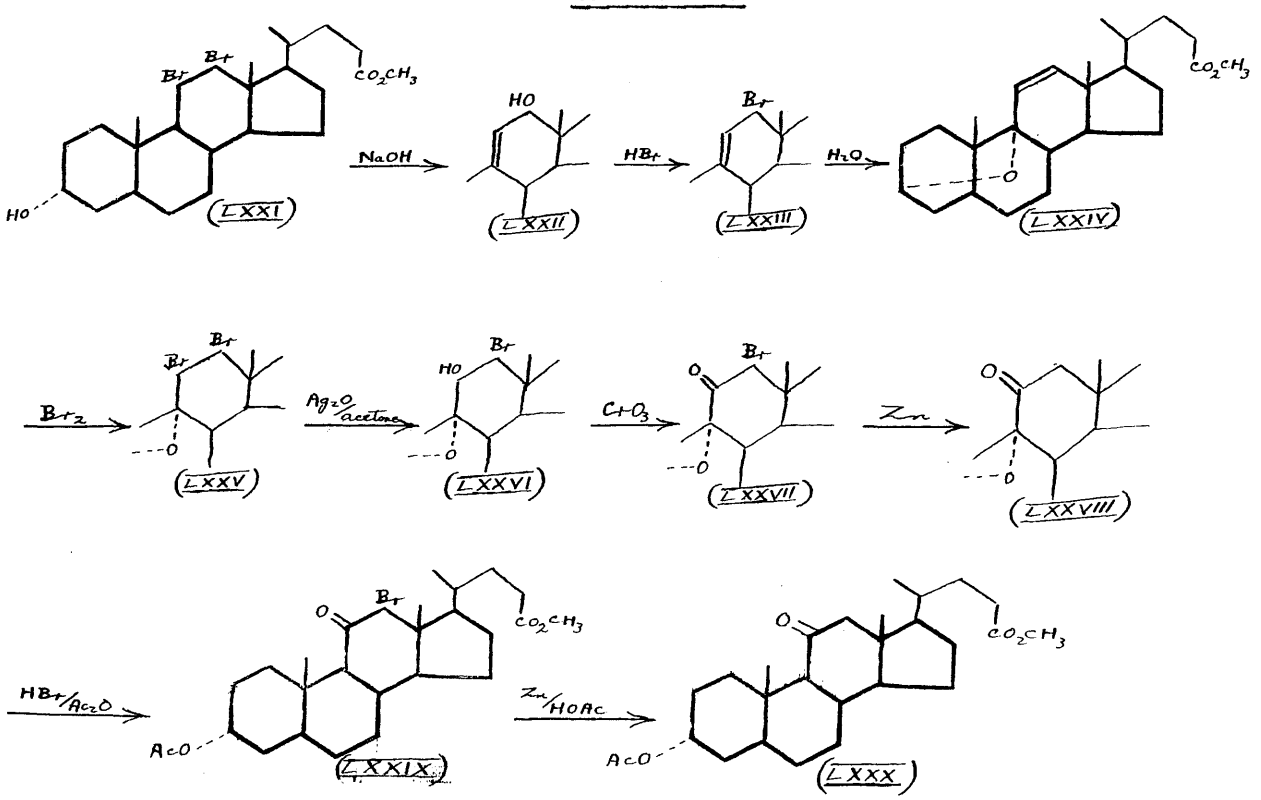


The bromoketones (LXIII) and (LXV) had previously been obtained as an amorphous mixture (32.33), but Gallagher and Lang (29) separated the methyl ester acetates by chromatography. The β-bromo-isomer (LXIII) has also been prepared from 3α-hydroxychol-11-enic acid α-oxide by the action of hydrogen bromide (34). Marker and Lawson (32) first carried out the hydrolysis of the bromoketones to give one hydroxyketone which has been proved to have the structure (LXVII) as Gallagher and his co-workers have been able to isolate (LXIV), and (LXVI), using milder hydrolysis conditions (35). The 12β-hydroxy group in (LXVII) has been proved by preparation of its diacetate

by oxidation of 3α:12β-diacetoxy-11β-hydroxycholanic acid (30).

Introduction of 11-Oxygen through 3:9-Oxide Formation.— Kendall et al (36) found that 11:12-dibromo-3α-hydroxycholanic acid (LXXI) gave the 12-hydroxy-chol-9(11)-enic acid (LXXII) on treatment with dilute alkali. The allylic alcohol (LXXII) was readily converted to the 12-bromo-derivative (LXXIII) which, when in chloroform solution, underwent allylic rearrangement on washing with water and formed the 3:9-oxide (LXXIV).

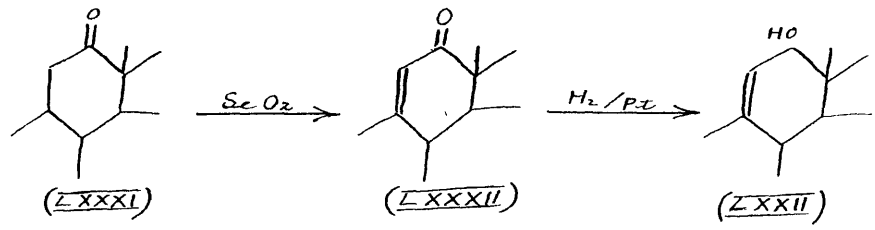
Chart XII



The structure of the 3:9-oxide (LXXIV) was confirmed by its chemical properties and the α-configuration is ~~favoured~~ compelled by the steric properties of C_3 and C_9 in the bile acid nucleus. Bromination of the 3:9-oxide gave a mixture of dibromides (37), one of which (LXXV) reacted as shown in chart XII, the 3:9-oxido-11-keto derivative (LXXVIII) being eventually formed. The oxide ring in (LXXVIII), although untouched by Raney nickel hydrogenation or Grignard reagents, was ruptured by hydrogen bromide in chloroform-acetic anhydride and the product (LXXIX) readily converted to the desired 11-ketone (LXXX).

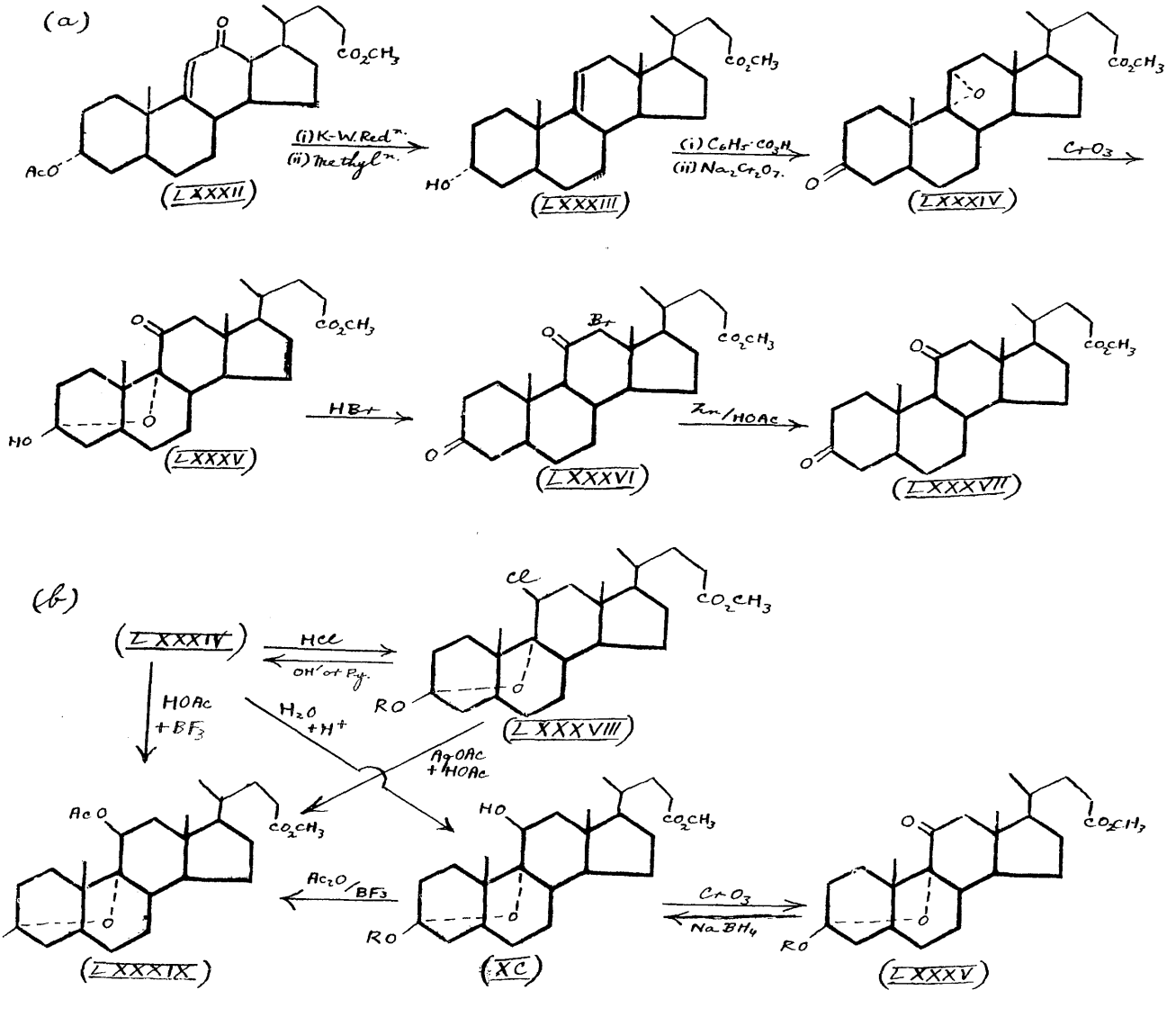
Kendall and his co-workers (27) have modified this synthesis to start from a 12-ketochol-9(11)-enic acid (LXXXII) prepared as described above, which, on hydrogenation in ethanol-acetic acid, gave a mixture of the allylic alcohols (LXXII) consisting mainly of the β-epimer. The procedure was then as in chart XII, with some modifications including degradation of the side chain while the oxide bridge was intact.

Chart XIII



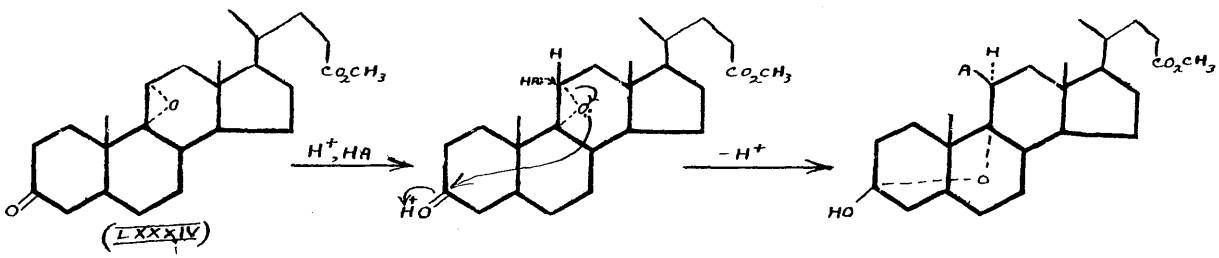
Since the work described in the theoretical and experimental sections of part I of this thesis was undertaken, Fieser et al (38,39,40) have synthesised 3:9-oxido bile acids with a function at C_{11} by routes differing from that described above. The routes are shown in chart XIV.

Chart XIV



A disadvantage of the above processes is the poor yield of lithochol-9(11)-enic acid (LXXXIII) obtained by Kishner-Wolff reduction of (LXXXII). The formation of the 3:9-oxide in (a) can proceed either as shown or by direct chromic acid oxidation of (LXXXIV). Route (a) has also been applied in the etianic acid series (39). Fieser (40) suggests that the conversion of 3-keto-9α:11α--oxidocholanic acid (LXXXIV) to a 3:9-oxide structure with a function at C_{11}, which only takes place under acid conditions, has the mechanism shown in chart XV.

Chart XV

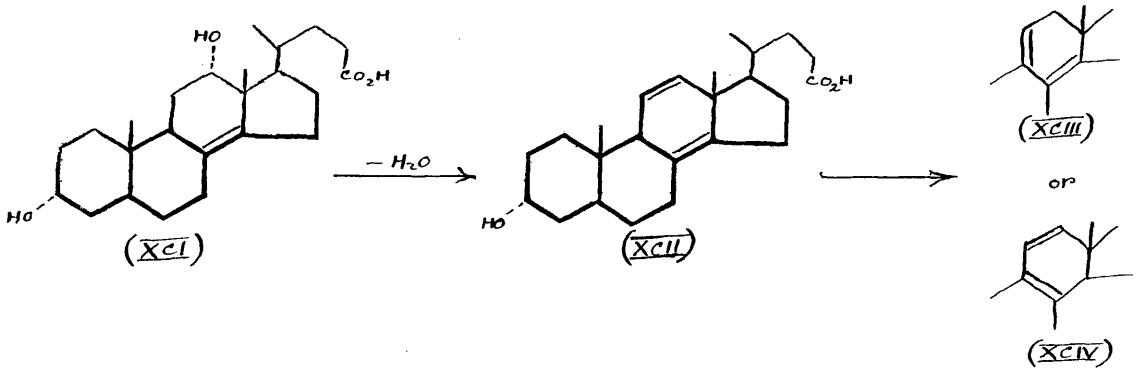


The presence of the 3α:9α-oxide group has been proved by treatment of the thioethyl derivative of (LXXXV) with Raney nickel to give the known 3α:9α-oxido--11-ketocholanic acid (LXXVIII) (40).

THEORETICAL

It was decided to investigate the dehydration of apocholic acid (3α:12α-dihydroxychol-8(14)-enic acid) (XCI) as it was considered that the double bond already present in this acid would favour the introduction of a second ethylenic linkage in ring C of the steroid nucleus with possible formation of a conjugated diene system (XCIII or XCIV). The latter substances may be suitable starting materials for novel syntheses of 11-oxygenated steroids.

Chart XVI

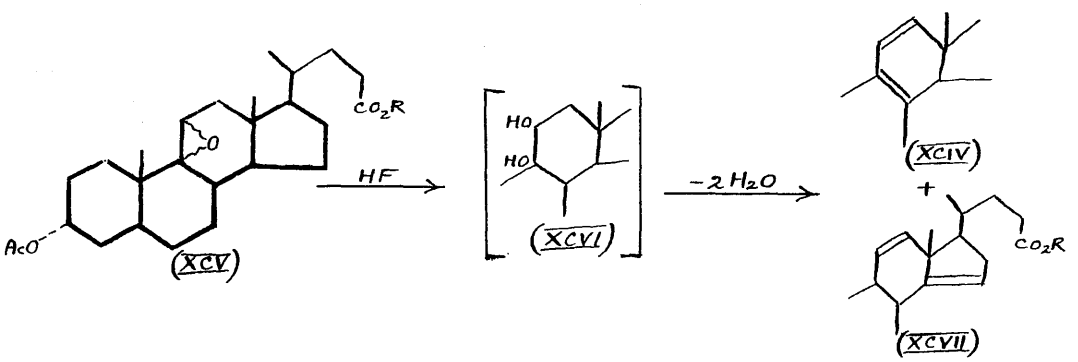


To achieve the dehydration it was intended to pyrolyse 12-acylated derivatives of apocholic acid. To obtain the 12-acylated derivatives partial hydrolysis of 3:12-diacyl derivatives of apocholic acid was attempted but, in contrast to the behaviour of corresponding derivatives of the related deoxycholic acid (19,24), both acyl groups of the apocholic acid diesters appeared equally

susceptible to hydrolysis and 12-monoacyl derivatives could not be isolated.

Hicks, Berg and Wallis (41) have claimed that the acid (XCIV) is formed on treatment of methyl 3α-acetoxy--9:11-oxidocholanate (XCV) with hydrogen fluoride. The product shows a maximum at 2440Å (ε = 420) in the ultraviolet absorption spectrum and would appear to be a mixture, the low intensity of absorption being due to migration of the 8:9-double bond to the 14:15-position (XCVII).

Chart XVII

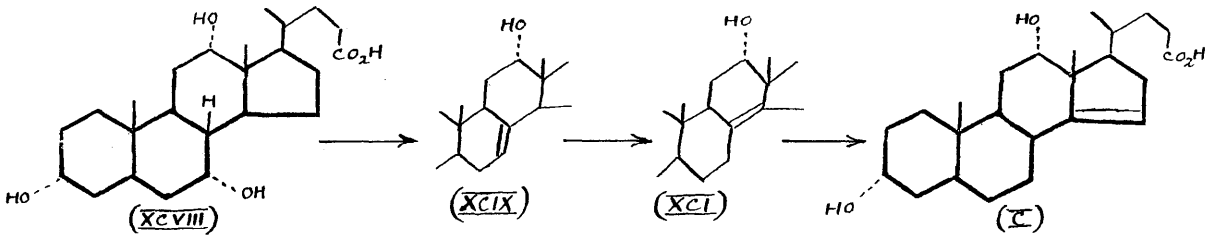


(XCV) (XCVI) (XCIV) (XCVII)

apoCholic Acid.— apoCholic acid can be obtained in 75% yield from cholic acid (XCVIII) by dehydration with zinc chloride in acetone (42) although a much lower yield is obtained when the reaction is performed in acetic acid (43,44). Callow suggested that the double bond of apocholic acid is in the 8:14-position (45) and Barton confirms this view (46). The dehydration probably

proceeds by normal trans elimination of the elements of water across the 7:8-position, giving 3α:12α-dihydroxychol-7-enic acid (XCIX) which isomerises to the chol--8(14)-enic acid.

Chart XVIII



The Δ^7-acid (XCIX) can be prepared by dehydration of methyl 3-acetylcholate (47, also this thesis) and is isomerised to apocholic acid by treatment with hydrogen and platinum catalyst (47). apoCholic acid itself can be further isomerised to the Δ^{14}-acid (C) by the action of hydrogen chloride (44). The acid ($\overline{C}$) is formed in very low yield during the preparation of apocholic acid (43,44).

Acylation of Methyl apoCholate. — Methyl apocholate is readily obtained by direct methylation of apocholic acid but the author has found that methyl cholate is recovered unchanged after treatment with zinc chloride in acetone. Partial benzoylation of methyl apocholate gives the 3--monobenzoyl derivative, further benzoylation of which

yields methyl 3:12-dibenzoyl-apocholate also obtained by benzoylation of methyl apocholate with excess benzoyl chloride. Attempts to hydrolyse the dibenzoate partially to 12-benzoylapocholic acid were unsuccessful, the only identifiable product being apocholic acid.

Pyrolytic treatment of methyl 3:12-dibenzoylapocholate gave benzoic acid and a resinous product which exhibited no intense absorption in the ultraviolet spectrum; the dibenzoate was recovered unchanged after refluxing with dimethylaniline.

In an attempt to prepare methyl 3-acetyl-12-benzoyl-apocholate, which it was hoped would be more amenable to partial hydrolysis than the dibenzoate, cholic acid was partially acetylated to give 3-acetylcholic acid characterised by esterification to the known methyl 3-acetylcholate. Attempted dehydration of 3-acetylcholic acid to 3-acetyl-apocholic acid, using zinc chloride in acetone, gave a resinous product and methylation gave an amorphous ester. Partial acetylation of methyl apocholate was also found to yield a resin and Berner, Lardon and Reichstein (47) have described methyl 3-acetylapocholate as amorphous. Benzoylation of the amorphous acetate did not give a crystalline product.

Methyl apocholate readily forms a crystalline 3:12--diacetate (44) but this ester, like the dibenzoate, could

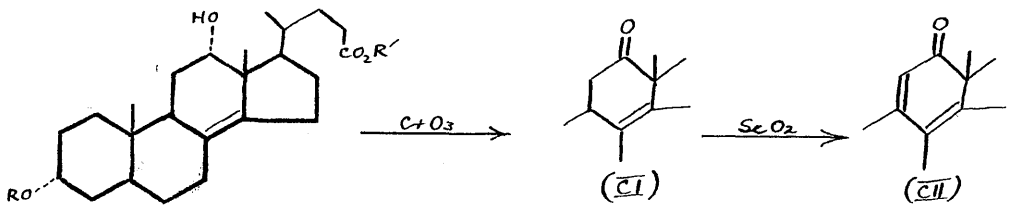
not be partially hydrolysed, the product being a gum.

Treatment of methyl 3-benzoylapocholate with p--toluene sulphonyl chloride converts it to methyl 3--benzoyl-12-tosylapocholate in good yield. Attempts to introduce a second ethylenic bond by treatment of this ester with collidine were unsuccessful, no crystalline reaction product being isolated. Tosylation of methyl apocholate gives methyl 3:12-ditosylapocholate but, as in the case of the dibenzoate and the diacetate, it was not possible to differentiate between the two acyl groups by partial hydrolysis. The ditosylate is rather resistant to alkali, being recovered unchanged after treatment with two or three moles of sodium hydroxide. Hydrolysis with a large excess of alkali gives apocholic acid. Treatment of the ditosylate by refluxing in collidine yielded an amorphous product showing selective absorption in the ultraviolet spectrum at a wavelength of 2520Å (ε = 7000) which may indicate the presence of a homoannular conjugated diene system.

3α-Hydroxy-12-ketochol-8(14)-enic Acid. — Attempts to introduce a diene system in ring C of apocholic acid, by removal of the 12-oxygen function in conjunction with one of the 11-hydrogen atoms, having proved unsuccessful, it was decided to investigate the selenium dioxide dehydrogenation of a 12-keto derivative of apocholic (CI) as a

means of introducing a $\Delta^{9(11)}$-ethylenic linkage (CII) (chart XIX).

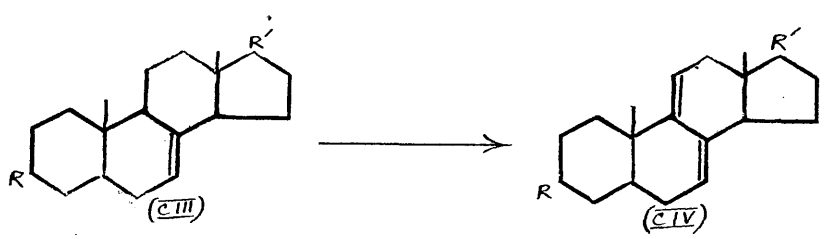
Chart XIX



Methyl 3-benzoylapocholate was unattacked by Oppenauer oxidation but chromic acid oxidation yielded amorphous methyl 3α-benzoyloxy-12-ketochol-8(14)-enate (CI, R= $C_6H_5.CO$, R'=CH_3), characterised as its 2:4-dinitrophenylhydrazone and by hydrolysis to the crystalline 3α-hydroxy--12-ketochol-8(14)-enic acid (CI, R=R'=H). Oxidation of methyl 3α-benzoyloxy-12-ketochol-8(14)-enate with selenium dioxide gave amorphous material showing no selective absorption in the ultraviolet spectrum.

3α:12α-Dihydroxychol-7-enic Acid.— A 7:9(11)-dienic system can be introduced into the sterol nucleus by dehydrogenation of Δ^7-sterols (chart XX).

Chart XX

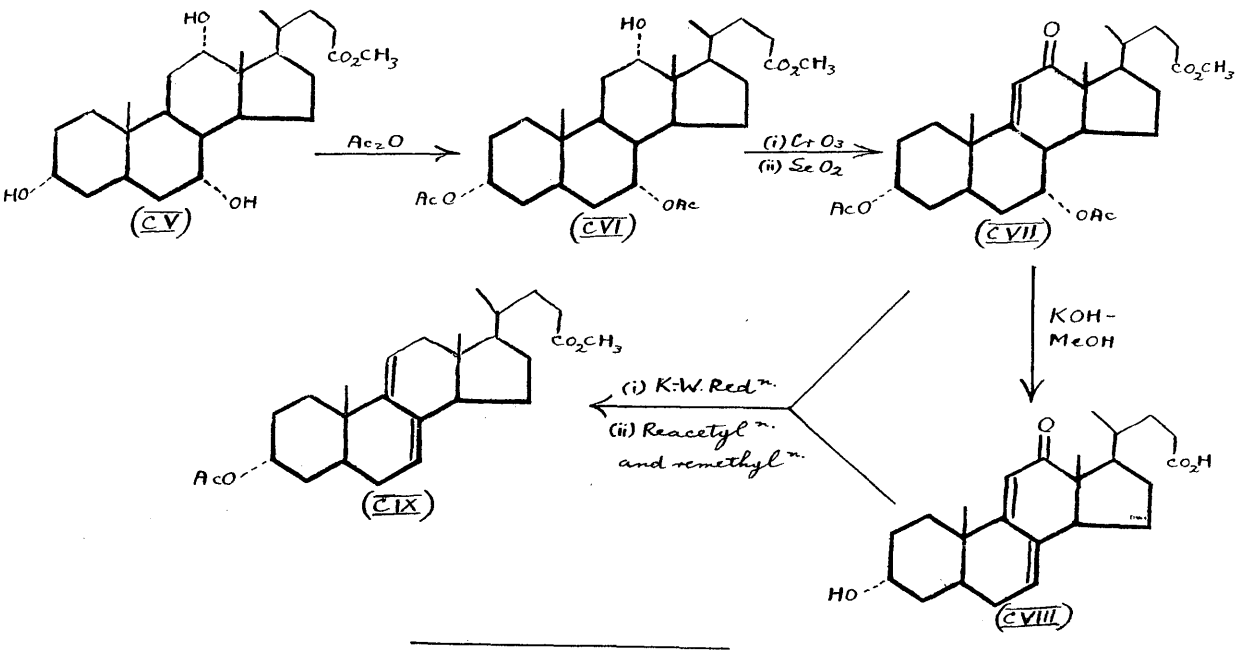


The oxidation has been performed on 5-dihydroergosterol (CIII, R=OH, R'=C_8H_{17}) using mercuric acetate (48,49), perbenzoic acid (50) or selenium dioxide (51) and on cholest-7-ene (CIII, R=H, R'=C_8H_{17}) using mercuric acetate or bromine (52). With a view to carrying out this reaction in the bile acid series, the preparation of chol-7-enic acids was investigated. Reichstein (47,53) has dehydrated methyl 3-acetylcholate to methyl 3α-acetoxy--12α-hydroxychol-7-enate by phosphoryl chloride or tosyl chloride in pyridine but the yield is poor. The author has carried out a similar dehydration of methyl 3-carbethoxycholate to methyl 3α-carbethoxyloxy-12α-hydroxychol-7-enate, moreover treatment of methyl 3-acetylcholate and methyl 3-carbethoxycholate with anhydrous copper sulphate (cf. Eck and Hollingsworth (54)) gave the corresponding Δ^7--esters but in all cases yields were low. Use of a number of other dehydrating agents, viz. magnesium perchlorate, phosphorus pentoxide, thionyl chloride in pyridine and zinc chloride in pyridine, on the saturated esters gave unchanged starting material only. Consequently this line of investigation was abandoned as reasonable supplies of chol-7-enic acids could not be obtained.

Since this work was carried out, Fieser (55) and Jeger and his co-workers (56) have prepared 7:9(11)-dienic bile acids and oxidised them to 11-oxygenated derivatives.

The latter reactions will be described in detail in part II of this thesis. The method of preparation of the 7:9(11)-dienes is shown in chart XXI.

Chart XXI



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EXPERIMENTAL

M.p.'s are uncorrected. Specific rotations were measured in chloroform, unless otherwise stated, using a 1 dm. tube at approximately 15°. The light petroleum used was of boiling range 60-80°.

apoCholic Acid.— (Devor and Marlow, loc.cit.). A mixture of cholic acid (5 g.) and fused zinc chloride (5 g.) in dry acetone (30 c.c.) was refluxed until solution was complete. The solution was then concentrated, on the steam-bath, to a brown syrup to which water (95 c.c.) acidified with acetic acid was added with stirring. The resulting precipitate was allowed to stand until it became cyrstalline, collected, washed with water and dissolved in ethanol (10 c.c.). The precipitation was repeated twice and the impure apocholic acid thus obtained dried in a desiccator in an atmosphere of nitrogen. The acid was then dissolved in ethanol (30 c.c.) and the solution filtered and concentrated to a syrup. The process was repeated and xylene (10 c.c.) added to the syrup. apo-Cholic acid (2.8 g.) separated as prisms and was collected, washed with xylene and dried at 135-140° in an atmosphere of nitrogen or in high vacuum; m.p.160-164°, $[\alpha]_D$ +51° (c, 1 in ethanol).

Methyl apoCholate.— apoCholic acid (25 g.) was dissolved, by gentle warming, in methanol (250 c.c.) containing con-

centrated hydrochloric acid (1 c.c.). The solution was kept overnight at room temperature, diluted with water and extracted with ether. After washing with aqueous sodium carbonate and water and drying (Na_2SO_4) the resulting ethereal solution of the neutral fraction was evaporated and the residue crystallised from methanol to yield methyl apocholate (17.5 g.) as prismatic needles, m.p.86-90°, $[\alpha]_D$ +45° (c, 1).

Methyl Cholate.— (a) Methanol (500 c.c.) was cooled to 0° and oleum (20 c.c.) carefully added. The mixture was shaken with cholic acid (50 g.) until solution was complete, and then stored for 18 hours at 0°. The solution was poured, slowly and with stirring, into iced water (4 l.) containing sodium hydroxide (40 g.) and on which methyl cholate had been sprinkled. Ethyl acetate (100 c.c.) was stirred into the aqueous suspension to neutralise excess alkali and the mixture kept at 0° for 48 hours. The precipitate was collected, washed with water, dried and dissolved in aqueous ethanol (750 c.c., 60% H_2O) from which methyl cholate (43 g.) separated as prisms, m.p.152-155°. (b) Cholic acid (10 g.) was dissolved, by warming, in methanol (50 c.c.) containing concentrated hydrochloric acid (0.5 c.c.). The solution was kept at 0° for 18 hours and methyl cholate (8.5 g.) separated as prisms. These were collected and washed with methanol at 0°, m.p.153-155°,

$[\alpha]_D$ +24° (c, 1).

Treatment of the mother liquors with sodium hydrogen carbonate, followed by filtration and concentration, yielded a further crop of less pure material.

Attempted Dehydration of Methyl Cholate.— (a) A mixture of methyl cholate (5 g.) and fused zinc chloride (5 g.) in dry acetone (30 c.c.) was heated under reflux until no more of the zinc chloride would dissolve. The reaction mixture was then worked up, as described for apocholic acid, to give unchanged methyl cholate (4.5 g.), m.p.152-155°, undepressed on mixing with an authentic specimen. (b) A mixture of methyl cholate (5 g.) and fused zinc chloride (5 g.) in dry acetone (30 c.c.) was allowed to stand at room temperature for one week. The solution was filtered from undissolved zinc chloride, concentrated and poured into water. The resulting precipitate was collected, washed with water, dried and crystallised from methanol as prisms (4.0 g.), m.p.153-156°, undepressed on mixing with methyl cholate.

Methyl 3-Benzoylapocholate.— A solution of methyl apocholate (1 g.) in dry benzene (10 c.c.) was treated with pyridine (1 c.c.) and benzoyl chloride (0.3 c.c.) and the mixture kept overnight at room temperature. The mixture was washed successively with dilute hydrochloric acid,

dilute sodium hydroxide solution and water and dried over sodium sulphate. The product was crystallised from methanol to yield methyl 3-benzoylapocholate (0.5 g.) as prisms, m.p.116-117°, $[\alpha]_D$ +56° (c, 1)

Analysis: Found: C,75.0; H,9.0

$C_{32}H_{44}O_5$ requires: C,75.0; H,8.9%.

Methyl 3:12-Dibenzoylapocholate.— (a) Methyl 3-benzoylapocholate (1.5 g.) in dry benzene (5 c.c.) was treated with pyridine (1 c.c.) and benzoyl chloride (1 c.c.) and the mixture kept at room temperature for 72 hours. The mixture was concentrated under reduced pressure, the residue dissolved in benzene and washed with dilute hydrochloric acid, dilute sodium hydrogen carbonate solution and water. After drying (Na_2SO_4) and decolourising with charcoal, the solution was evaporated and the residue crystallised from methanol, yielding methyl 3:12-dibenzoylapocholate (1.2 g.) as needles, m.p.110-112°, $[\alpha]_D$ +47° (c, 1)

Analysis: Found: C,76.4; H,8.2

$C_{39}H_{48}O_6$ requires: C,76.0; H,8.0%.

(b) A solution of methyl apocholate (5.0 g.) in pyridine (10 c.c.) was treated with benzoyl chloride (5 c.c.) and kept overnight at room temperature. The resinous reaction product was isolated in the usual way but could not be obtained crystalline. A solution in methanol was seeded

with a crystal of the dibenzoate prepared as described under (a). Crystallisation was rapid, the dibenzoate (5.6 g.) separating as small needles which, after recrystallisation from the same solvent, had m.p.110-112°, undepressed when mixed with the specimen described in (a).

Attempted Partial Hydrolysis of Methyl 3:12-Dibenzoylapocholate. — (a) Methyl 3:12-dibenzoylapocholate (7.5 g.) in ethanol (45 c.c.) was treated with a solution of sodium hydroxide (1.2 g., 2.5 moles) in water (6 c.c.). The mixture was refluxed for 30 minutes, cooled, acidified with concentrated hydrochloric acid (3 c.c.) and poured into water. The amorphous precipitate was collected and digested with hot water for 1 hour to remove benzoic acid. The product was dissolved in ether and extracted with dilute sodium hydrogen carbonate solution. The acid fraction thus obtained yielded a small quantity of crystals from ether-light petroleum, m.p.168-170°, undepressed when mixed with apocholic acid.

(b) Methyl 3:12-dibenzoylapocholate (5 g.) was dissolved in ethanol (50 c.c.) and 5N sodium hydroxide solution (3.5 c.c., 2 moles) added. The mixture was refluxed for 30 minutes, cooled, acidified with hydrochloric acid and poured into water. The amorphous precipitate was collected, steam-distilled to remove benzoic acid and dissolved in ethanol. Attempts to crystallise the

hydrolysis product by careful addition of water to the ethanol solution were unsuccessful.

The hydrolysis product was methylated in ethereal diazomethane solution and the neutral fraction from the esterification chromatographed but again no crystalline product was obtained.

Pyrolysis of Methyl 3:12-Dibenzoylapocholate.— Methyl 3:12-dibenzoylapocholate (5 g.) was heated in a distillation flask on a metal bath at 300°/3 mm. for $1\frac{1}{2}$ hours. Benzoic acid and a slightly yellow gum distilled over. The distillate was dissolved in ether and washed with dilute sodium hydrogen carbonate solution and water and dried (Na_2SO_4). The ether solution was evaporated to give a resinous product (3.0 g.) which was chromatographed but yielded no crystalline product and showed no intense selective absorption in the ultraviolet.

Treatment of Methyl 3:12-Dibenzoylapocholate with Dimethylaniline.— Methyl 3:12-dibenzoylapocholate (5 g.) was dissolved in dimethylaniline (125 c.c.) and the mixture refluxed for 8 hours, cooled and poured into dilute hydrochloric acid with stirring. The resulting suspension was extracted with ether, washed neutral and dried (Na_2SO_4).

The residue, obtained on evaporation of the ethereal solution, was crystallised from methanol as needles (3 g.),

m.p.110-112°, undepressed when mixed with starting material.

3-Acetylcholic Acid.— (a) A solution of cholic acid (5 g.) in glacial acetic acid (50 c.c.) was treated with concentrated hydrochloric acid (0.3 c.c.), heated on a water bath for 1 hour and kept overnight at room temperature. The reaction mixture was diluted with water, the precipitated solid collected, washed with water and dried. Crystallisation from ethyl acetate gave 3-acetylcholic acid as small prisms, m.p.207-208° (sinters at 165°), $[\alpha]_D$ +46° (c, 1 in ethanol).

(b) A solution of cholic acid (5 g.) in warm glacial acetic acid (50 c.c.) was treated with concentrated hydrochloric acid (0.5 c.c.). The solution was kept at room temperature for 48 hours and the reaction product isolated as described above to give 3-acetylcholic acid (2.9 g.) which, on recrystallisation from ethyl acetate, had m.p. 208-210° (sinters at 165°), $[\alpha]_D$ +47° (c, 1 in ethanol), m.p. undepressed when mixed with the specimen described above.

Analysis: Found: C,68.8; H,9.8

$C_{26}H_{42}O_6$ requires: C,69.3; H,9.3%.

The 3-monoacetate of cholic acid was characterised by methylation using diazomethane in ether-methanol solution. Methyl 3-acetylcholate separated from ether as needles, m.p.149-151°, $[\alpha]_D$ +42° (c, 1).

Analysis: Found: C,70.1; H,9.8

Calc. for $C_{27}H_{44}O_6$: C,69.8; H,9.5%.

Plattner and Heusser (Helv.Chim.Acta,1944,27,754) give m.p.149-150° (corr.) and $[\alpha]_D$ +48° for methyl 3--acetylcholate. A specimen prepared, as described by these authors, by partial acetylation of methyl cholate had m.p.148-150° and $[\alpha]_D$ +41° and gave no depression in m.p. when mixed with the specimen described above.

Attempted Dehydration of 3-Acetylcholic Acid.— 3-Acetylcholic acid (12 g.) and fused zinc chloride (12 g.) were refluxed together in dry acetone (75 c.c.) until all solid material had dissolved. The solution was then concentrated to a thin syrup to which was added water (250 c.c.) acidified with acetic acid. The resulting precipitate was collected, digested with water and dissolved in ethanol (25 c.c.). The precipitation was repeated twice to give an amorphous product (10 g.) which was unsaturated.

The product, which could not be crystallised, was dissolved in methanol (100 c.c.), concentrated hydrochloric acid (0.5 c.c.) added and the solution allowed to stand overnight at room temperature. After pouring into water, extracting with ether and washing with dilute sodium hydrogen carbonate solution, the amorphous neutral fraction (6.7 g.) thus obtained was dissolved in benzene-light

petroleum (1:9; 200 c.c.) and chromatographed on alumina (grade II, 150 g.) to give the following fractions:-

Fraction	Solvent		Eluate
(1)	Benzene-light petroleum (1:9)	1200 c.c.	4.5 g.
(2)	Benzene-methanol (95:5)	500 c.c.	1.0 g.

Fraction (1) would not crystallise and was unsaturated.

Fraction (2), which was saturated, crystallised from methanol, m.p.approx. 80°, but could not be obtained in pure form.

Monoacetylation of Methyl apoCholate.-- Methyl apocholate (5 g.) was dissolved in pyridine (10 c.c.) and acetic anhydride (1.2 c.c.) added. The mixture was kept overnight at room temperature, poured into iced water and allowed to stand for 2 hours. The aqueous suspension was extracted with ether, the ether extract washed successively with dilute hydrochloric acid, dilute sodium hydrogen carbonate solution and water, dried (Na_2SO_4) and the solvent removed under r.p. leaving a resinous residue (4.0 g.). The product was dissolved in benzene-light petroleum (1:4; 100 c.c.) and chromatographed on alumina (grade II, 140 g.) to give the following fractions:-

Fraction	Solvent		Eluate
(1)	Benzene-light petroleum (3:7)	700 c.c.	1.21 g.
(2)	Benzene-methanol (9:1)	400 c.c.	1.67 g.

Further fractions were eluted from the column but these were intractable oils.

Fraction (1) was amorphous.

Fraction (2) crystallised from methanol as needles, m.p.84-89°, undepressed on mixing with methyl apocholate.

Methyl 3:12-Diacetylapocholate.— (Ruzicka et al, loc.cit.). A mixture of methyl apocholate (7.5 g.) and dry pyridine (3 c.c.) dissolved in redistilled acetic anhydride (25 c.c.) was heated under reflux for $2^1/_2$ hours, cooled and poured into iced water. The aqueous suspension was extracted with ether-benzene and the extract washed with dilute hydrochloric acid, dilute sodium hydrogen carbonate solution and water. After drying over sodium sulphate the solution was evaporated to dryness under reduced pressure and the residue crystallised from ether to yield methyl 3:12--diacetylapocholate (6.5 g.), m.p.137-139°, $[\alpha]_D$ +89° (c, 2).

Partial Hydrolysis of Methyl 3:12-Diacetylapocholate.— Methyl 3:12-diacetylapocholate (5 g.) was dissolved in methanol (30 c.c.) and a solution of caustic potash (1.15 g., 2 moles) in water (5 c.c.) added. The mixture was kept overnight at room temperature, diluted with water and a small amount of neutral product removed by ether extraction. The aqueous solution was acidified with hydrochloric acid, extracted with ether and the ether extract washed with water, dried (Na_2SO_4) and the solvent removed under reduced

pressure, leaving a resinous product (3.5 g.). The product was dissolved in methanol (20 c.c.) containing hydrochloric acid (0.5 c.c.) and the solution kept overnight at room temperature. The esterified product was worked up in the usual way to give an amorphous neutral fraction (3.3 g.).

Methyl 3-Benzoyl-12-tosylapocholate.— A solution of methyl 3-benzoylapocholate (7 g.) and p-toluenesulphonyl chloride (7 g.) in dry pyridine (45 c.c.) was kept at 35-45° for 4 days. The mixture was poured into ice-water, the reaction product isolated by means of ether and crystallised from methanol to yield methyl 3-benzoyl-12-tosylapocholate (7.5 g.) as fine needles, m.p.106-111°, $[\alpha]_D$ +48° (c, 1).

Analysis:

Found: C,70.4; H,7.2

$C_{39}H_{50}O_7S$ requires: C,70.7; H,7.5%.

Treatment of Methyl 3-Benzoyl-12-tosylapocholate with Collidine.— Methyl 3-benzoyl-12-tosylapocholate (1.5 g.) was dissolved in redistilled collidine (b.p.173-174°, 15 c.c.) and the solution refluxed for 3 hours. The collidine was evaporated under reduced pressure and the residue dissolved in benzene. After the removal of some tar, the benzene solution was washed successively with dilute hydrochloric acid, dilute sodium hydrogen carbonate

solution and water, dried (Na_2SO_4) and the solvent removed under reduced pressure, leaving a resin (0.9 g.). The product could not be crystallised and showed no selective absorption in the ultraviolet spectrum above 2400Å.

Methyl 3:12-Ditosylapocholate.— A solution of methyl apocholate (5 g.) and p-toluenesulphonyl chloride (7.5 g.) in pyridine (10 c.c.) was maintained at 45-55° for 6 days. Water (10 c.c.) was added and, after 2 hours, the mixture was poured into ice-water. The crystalline precipitate (6.7 g.) was collected, washed with water, dried and recrystallised from chloroform-methanol from which methyl 3:12-ditosylapocholate separated as plates, m.p.151-152°, $[\alpha]_D$ +38° (c, 1.0).

Analysis: Found: C,65.6; H,7.5

$C_{39}H_{52}O_8S_2$ requires: C,65.7; H,7.3%.

Attempted Partial Hydrolysis of Methyl 3:12-Ditosylapocholate.— (a) A suspension of methyl 3:12-ditosylapocholate (3 g.) in ethanol (30 c.c.) to which 5N sodium hydroxide solution (2.5 c.c., 3 moles) was added, was refluxed for $1^1/_2$ hours when solution was complete. The mixture was cooled, poured into water and the slightly turbid solution acidified with hydrochloric acid. The precipitate was collected, washed with water, dried and crystallised from chloroform-methanol as plates, m.p.148-152°, $[\alpha]_D$ +38°

(c, 1.0), m.p. undepressed on mixing with methyl 3:12--ditosylapocholate.

(b) A suspension of methyl 3:12-ditosylapocholate (0.8 g.) in ethanol (10 c.c.), to which 5N sodium hydroxide solution (2.5 c.c., ca. 12 moles) was added, was heated under reflux for $1^{1}/_{2}$ hours when solution was complete. The solution was poured into water and acidified with hydrochloric acid. The precipitated solid was collected, washed with water, dried and crystallised from xylene to yield apocholic acid, m.p.168-170°, undepressed when mixed with an authentic specimen.

Treatment of Methyl 3:12-Ditosylapocholate with Collidine.— Methyl 3:12-ditosylapocholate (3 g.) was dissolved in sodium-dried, redistilled collidine (30 c.c.) and the mixture refluxed for 3 hours. The collidine was removed under reduced pressure and the residue dissolved in benzene. The benzene solution was washed successively with dilute hydrochloric acid, dilute sodium hydrogen carbonate solution and water, dried (Na_2SO_4) and the solvent removed under reduced pressure, leaving a resin (1.7 g.) which could not be crystallised. Light absorption: maximum at 2520Å (ε = 7700). Chromatography and hydrolysis of the product failed to yield crystalline material.

Attempted Oppenauer Oxidation of Methyl 3-Benzoylapocholate.— Methyl 3-benzoylapocholate (5 g., dried in high vacuum) was dissolved in a mixture of dry acetone (40 c.c., previously treated with potassium permanganate) and dry benzene (50 c.c.). The solution was refluxed on an oil-bath maintained at 75-85° and a solution of aluminium t-butoxide (4 g.) in dry benzene (25 c.c.) added in one portion to the boiling solution with the separation of a gelatinous precipitate. The mixture was refluxed at 75-85° for 8 hours, cooled, washed with dilute sulphuric acid (10%) and water, dried (Na_2SO_4) and the solvent removed under reduced pressure. The residue thus obtained crystallised from methanol as prisms (3.9 g.), m.p.113-115°, undepressed on mixing with starting material.

Oxidation of Methyl 3-Benzoylapocholate with Chromic Anhydride.— A solution of methyl 3-benzoylapocholate (4 g.) in stabilised glacial acetic acid (20 c.c.) was treated, dropwise with stirring, over 10 minutes with a solution of chromic anhydride (0.8 g.) in 85% acetic acid (6 c.c.). After 17 hours the solution was warmed with methanol and concentrated under reduced pressure. The residue was diluted with water, extracted with ether and the ether extract washed successively with dilute sulphuric acid, dilute sodium hydrogen carbonate solution and water and dried (Na_2SO_4). The amorphous residue, obtained on

evaporation of the ether, did not crystallise. The 2:4--dinitrophenylhydrazone of methyl 3α-benzoyloxy-12-keto-chol-8(14)-enate separated from chloroform-methanol as fine needles, m.p.228-230°.

Analysis: Found: C,66.0; H,6.6; N,8.4

$C_{38}H_{46}O_8N_4$ requires: C,66.5; H,6.7; N,8.2%.

3α-Hydroxy-12-ketochol-8(14)-enic Acid.— Hydrolysis of the amorphous ester (2 g.) described above was effected by treating its solution in alcohol (50 c.c.) with 5N sodium hydroxide solution (2 c.c.) and heating under reflux for 30 minutes. The acid fraction, isolated in the usual way, crystallised from acetone-light petroleum to give 3α-hydroxy-12-ketochol-8(14)-enic acid, m.p.135-140°.

Analysis: Found: C,74.1; H,9.6

$C_{24}H_{36}O_4$ requires: C,74.2; H,9.3%.

Treatment of Methyl 3α-Benzoyloxy-12-ketochol-8(14)-enate with Selenium Dioxide.— Amorphous methyl 3α-benzoyloxy--12-ketochol-8(14)-enate (5 g.) was dissolved in chloro-benzene-glacial acetic acid (4:1, 35 c.c.) and selenium dioxide (1.3 g.) and several drops of glacial acetic acid, containing hydrogen chloride, added to the solution. The mixture was refluxed for 72 hours, cooled, selenium removed by filtration and the filtrate washed with water and dried (Na_2SO_4). The solvent was removed under reduced

pressure, leaving a resin (3.7 g.) which could not be freed from selenium and could not be crystallised. The product showed no selective absorption above 2400Å in the ultraviolet spectrum.

Methyl 3-Acetylcholate.— (Reichstein et al, loc.cit.). Methyl cholate (solvent-free, 10 g.) was dissolved in dry benzene (25 c.c.) by heating under reflux and a solution of acetic anhydride (3.3 moles, 7.5 c.c.) in dry benzene (12 c.c.) added, over 20 minutes, to the refluxing solution. Refluxing was continued for a further 2 hours, the solution evaporated to dryness under reduced pressure and the residue dissolved in ether, washed with dilute sodium hydrogen carbonate solution and water and dried (Na_2SO_4). The ether solution was concentrated, light petroleum added until the solution became cloudy and the solution allowed to stand for several days. Methyl 3-acetylcholate (4.6 g.) separated as needles, m.p.148-150°, $[\alpha]_D$ +41° (c, 1.0).

Methyl 3-Carbethoxycholate.— (Fieser, J.Amer.Chem.Soc., 1949,71,3835). Methyl cholate (solvent free, 160 g.) was dissolved in dry pyridine (400 c.c.) and redistilled ethyl chloroformate (200 g.) added to the solution with stirring and cooling. The mixture was kept overnight at room temperature, then diluted with a large volume of water. A precipitate separated and became semi-crystalline after

standing for 2 days. The supernatant liquid was removed by decantation, the residue washed with water and crystallised from methanol to yield methyl 3-carbethoxycholate (80 g.), m.p.176-178°, $[\alpha]_D$ +42° (c, 1.0).

Methyl 3α-Acetoxy-12α-hydroxychol-7-enate.— (a) A solution of methyl 3-acetylcholate (5 g.) in dry pyridine (10 c.c.) was treated with redistilled phosphoryl chloride (0.5 c.c.) and the solution kept at room temperature for 16 hours. It was evaporated under reduced pressure and the residue dissolved in chloroform. The solution was washed successively with dilute hydrochloric acid, dilute sodium hydrogen carbonate solution and water and dried (Na_2SO_4) to give a resin (4.6 g.). A solution of the resin in benzene (100 c.c.) was chromatographed on acid-washed, activated alumina (grade II, 100 g.) to give the following fractions:-

Fraction	Solvent	Eluate
	Benzene (100 c.c.)	-
(1)	Benzene-methanol (99:1) (200 c.c.) Benzene-methanol (98:2) (100 c.c.)	0.51 g.
(2)	Benzene-methanol (98:2) (200 c.c.)	1.6 g.

Fraction (1) readily crystallised from aqueous methanol and gave a yellow coloration with tetranitromethane. Recrystallisation from ether-light petroleum gave methyl

3α-acetoxy-12α-hydroxychol-7-enate as prisms, m.p.114-117°, $[\alpha]_D$ +102° (c, 1.4).

Analysis: Found: C,72.4; H,9.3

Calc. for $C_{27}H_{42}O_5$: C,72.6; H,9.5%.

Grand and Reichstein, and Berner, Lardon and Reichstein (loc.cit.) give m.p.172-175° for the chol-7-enic acid ester although the specific rotation and constants of the corresponding acid are in good agreement with those found by the author.

Fraction (2) gave no coloration with tetranitromethane and crystallised from acetone-light petroleum to yield methyl 3-acetylcholate as prisms, m.p.149-151°, $[\alpha]_D$ +41° (c, 1.0), undepressed in m.p. when mixed with starting material.

(b) A solution of methyl 3-acetylcholate (5 g.) in dry xylene (25 c.c.) was heated with anhydrous copper sulphate (5 g.) in the presence of propionic acid (0.15 c.c.), using the conditions described by Eck and Hollingworth (loc.cit.). The filtrate, obtained after removal of the copper sulphate, was diluted with a mixture of dry xylene (25 c.c.) and light petroleum (50 c.c.) and chromatographed on acid-washed, activated alumina (grade II, 50 g.) to give the following fractions:-

Fraction	Solvent			Eluate
(1)	Benzene-light petroleum	(1:1)	200 c.c.	4.43 g.
	Benzene-light petroleum	(3:1)	100 c.c.	
	Benzene		200 c.c.	
(2)	Benzene-methanol	(99:1)	100 c.c.	0.38 g.
	Benzene-methanol	(95:5)	200 c.c.	

Fraction 1 crystallised from aqueous methanol to give prisms (0.9 g.), m.p.98-105°, which gave a yellow coloration with tetranitromethane. Recrystallisation from ether-light petroleum gave methyl 3α-acetoxy-12α-hydroxychol--7-enate as prisms, m.p.114-117°, $[\alpha]_D$ +102° (c,1.0); it does not depress the m.p. of the specimen prepared by method (a).

Fraction 2 from the chromatogram could not be obtained crystalline.

Methyl 3α-Carbethoxyloxy-12α-hydroxychol-7-enate.— (a) A solution of methyl 3-carbethoxycholate (5 g.) in dry pyridine (10 c.c.) and phosphoryl chloride (0.5 c.c.) was kept at room temperature for 18 hours. The reaction mixture was treated in the usual manner to give a resinous product (4.5 g.), a solution of which in benzene-light petroleum (1:1, 100 c.c.) was chromatographed on acid--washed, activated alumina (grade II, 100 g.) to give the following fractions:-

Fraction	Solvent	Eluate
(1)	Benzene-methanol (99:1) 100 c.c.)	0.64 g.
	Benzene-methanol (98:2) 300 c.c.)	
(2)	Benzene-methanol (98:2) 100 c.c.)	0.6 g.
	Benzene-methanol (9:1) 100 c.c.)	
(3)	Benzene-methanol (9:1) 200 c.c.	0.14 g.

Fractions 1 and 3 were not obtained crystalline. Fraction 2 crystallised from aqueous methanol to give methyl 3α-carbethoxyloxy-12α-hydroxychol-7-enate as needles from ether-light petroleum, m.p.112-115°, $[\alpha]_D$ +95° (c,1.2).

Analysis: Found: C,70.7; H,9.35

$C_{28}H_{44}O_6$ requires: C,70.6; H,9.3%.

(b) A solution of methyl 3-carbethoxycholate (4 g.) in dry xylene (20 c.c.) was refluxed for 6 hours in the presence of anhydrous copper sulphate (4 g.) and propionic acid (1 c.c.), the copper sulphate collected and the filtrate diluted with benzene (25 c.c.) and light petroleum (50 c.c.). The resulting solution was chromatographed on acid-washed, activated alumina (grade II, 40 g.) to give the following fractions:-

Fraction	Solvent	Eluate
(1)	Benzene-light petroleum (1:1) 100 c.c.)	
	Benzene-light petroleum (3:2) 200 c.c.)	2.43 g.
	Benzene-light petroleum (4:1) 100 c.c.)	

Fraction	Solvent		Eluate
(2)	Benzene	100 c.c.	
	Benzene-methanol (99:1)	100 c.c.	0.88 g.
	Benzene-methanol (98:2)	100 c.c.	

Fraction(1)crystallised as needles (400 mg.) from aqueous methanol, m.p.111-114°, $[\alpha]_D$ +93° (c, 0.8), m.p. undepressed on mixing with methyl 3α-carbethoxyloxy-12α--hydroxychol-7-enate.

Fraction(2)crystallised as prisms (250 mg.) from methanol, m.p.45-50°, mixed m.p. with fraction (1) = 106-110°.

Fraction (2) (200 mg.) was refluxed for 45 minutes with methanolic potassium hydroxide solution,(12 c.c., 8% of KOH), the mixture diluted with water, acidified with dilute hydrochloric acid and the granular precipitate collected and crystallised from acetone-light petroleum as prisms, m.p.207-209°, $[\alpha]_D$ +88° (c, 0.5 in dioxan), m.p. undepressed on mixing with 3α:12α-dihydroxychol-7-enic acid.

3α:12α-Dihydroxychol-7-enic Acid.— (a) Methyl 3α-acetoxy--12α-hydroxychol-7-enate (0.4 g.) was refluxed for 45 minutes with methanolic potassium hydroxide solution (15 c.c., 8% of KOH), the mixture diluted with water and the methanol evaporated.

The acid, separating on acidification of the solution, was collected, washed with water, dried and crystallised from ether-light petroleum to give 3α:12α-dihydroxychol--7-enic acid as small prisms, m.p.208-210°, $[\alpha]_D$ +93° (c, 1.1 in dioxan). Berner, Lardon and Reichstein (loc. cit.) give m.p.210-212°, $[\alpha]_D$ +92.6° (in dioxan).

(b) Methyl 3α-carbethoxyloxy-12α-hydroxychol-7-enate (110 mg.) was heated under reflux for 1 hour with a solution of methanolic potassium hydroxide solution (5 c.c., 10% of KOH). The acidic product was isolated as described in (a) and crystallised from acetone-light petroleum as prisms, m.p.204-208°, undepressed on mixing with 3α:12α--dihydroxychol-7-enic acid prepared as described above.

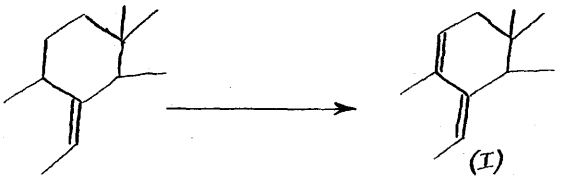
PART II

11-OXYGENATED STEROLS.

HISTORICAL

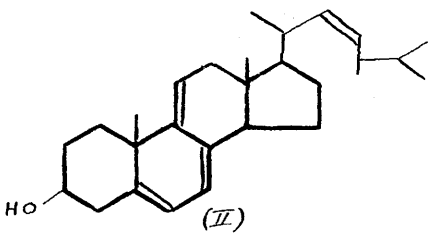
Sterols, as starting materials for 11-oxygenated steroids, have the disadvantage of containing no functional group in ring C but this is readily overcome in those sterols possessing a C_7-C_8 ethylenic linkage as they can be oxidised to products containing a 7:9(11)-dienic system (I) (chart I).

Chart I



(I)

Oxidation of Dehydroergosterol.— The first Δ^{11}-unsaturated sterol to be investigated with a view to introducing an oxygen atom at C_{11} was dehydroergosterol (II), obtained by mercuric acetate dehydrogenation of ergosterol (1,2).



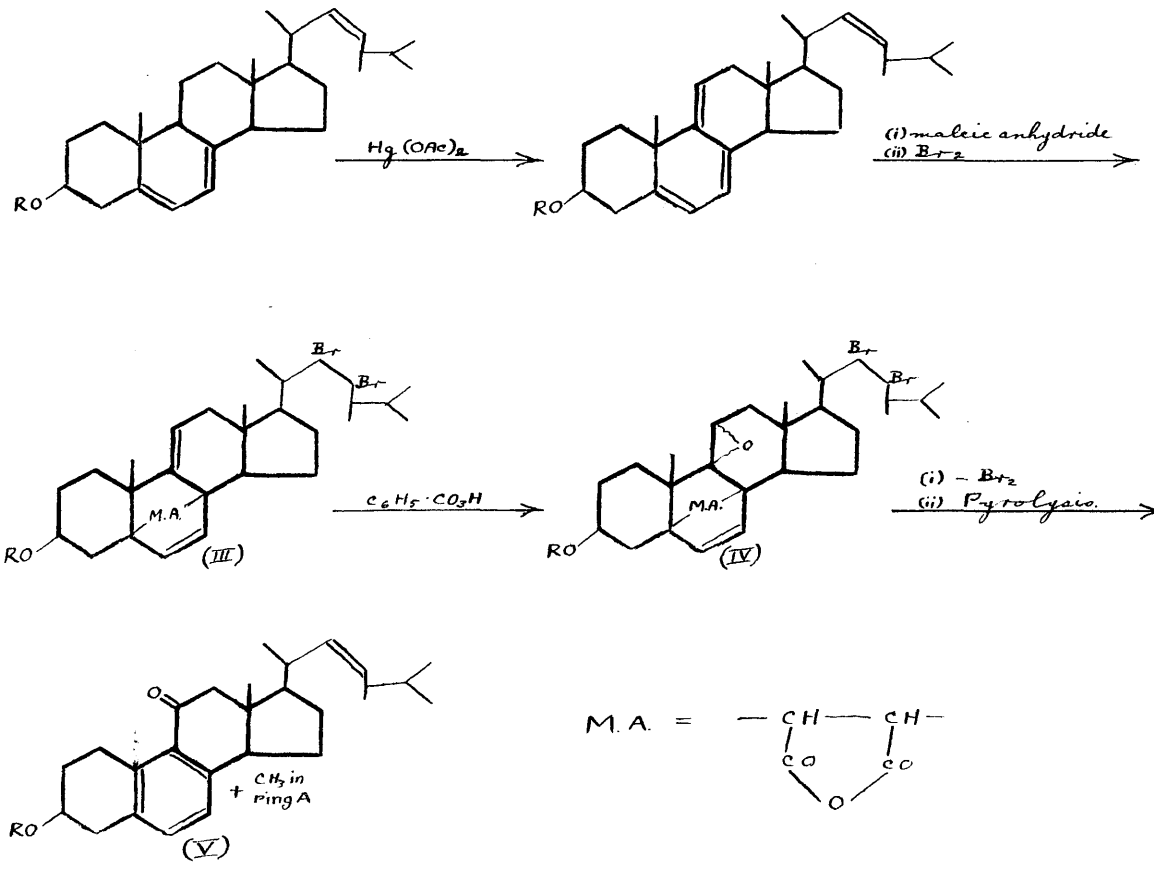
(II)

The structure of dehydroergosterol (II) has been deduced from its ultraviolet absorption spectrum which indicates the presence of a conjugated triene system and Muller (3) suggested that dehydroergosterol differs only from ergosterol in that it contains an additional double

bond in ring C. This was supported by Honigmann's observation that the maleic anhydride adducts of ergosterol and dehydroergosterol yield the same compound on absorption of two and three moles of hydrogen respectively (4), thus dehydroergosterol retains the system of conjugated double bonds present in ergosterol.

Bergmann and Stevens (2) protected the 5:6, 7:8 and 22:23-double bonds in dehydroergosterol by forming the 22:23-dibromo maleic anhydride adduct (III). The latter compound, on treatment with perbenzoic acid, gave the 9:11-monoxide (IV) as the 6:7-double bond in the adduct is inert.

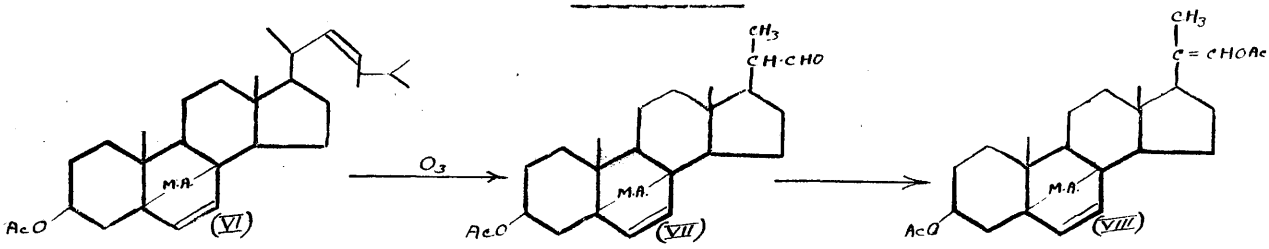
Chart II



(IV) was readily debrominated but the bromine-free derivative, on pyrolysis, rearranged to the 11-ketone with loss of maleic anhydride and aromatisation of ring B (V), shown by the ultraviolet absorption spectrum of the product.

Although this attempt to prepare an 11-oxygenated sterol failed, Bergmann and Stevens in the same paper (2) described the successful degradation of the sterol side chain by ozonolysis of the maleic anhydride adduct of ergosteryl acetate (VI) to the aldehyde (VII), the side chain double bond being oxidised preferentially.

CHART III

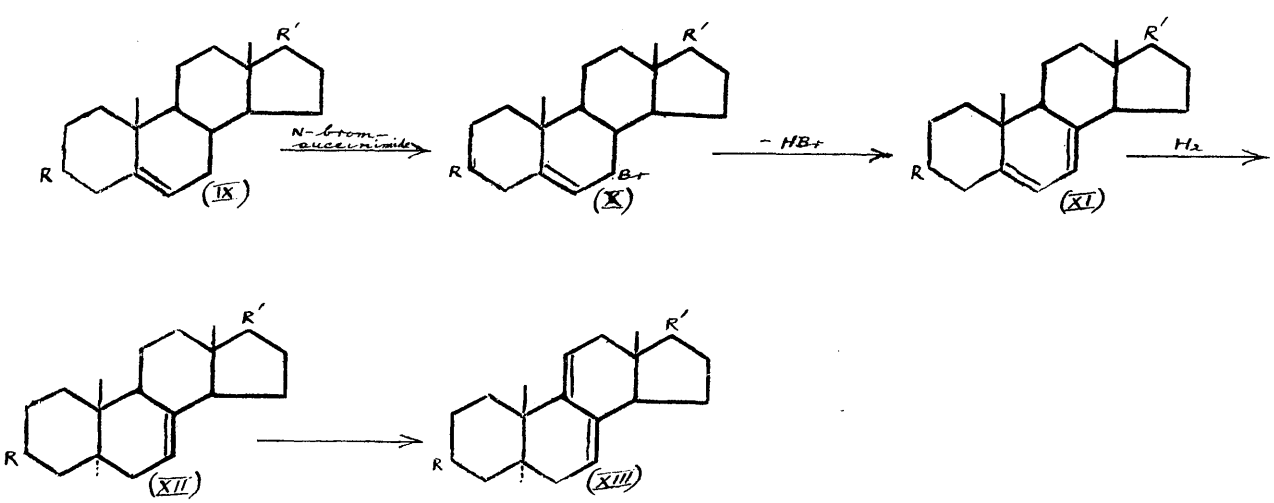


Comparison of this reaction with the much more laborious degradation of the bile acid side chain illustrates the benefit to be gained by using suitable sterols, unsaturated in the side chain, as precursors of 11-oxygenated steroids in the synthesis of cortisone.

Formation of 7:9(11)-Dienic Sterols. — There has already been described in this thesis (part I, p.29) the dehydrogenation of Δ^7-sterols to 7:9(11)-dienic systems. The

reaction did not proceed in a very satisfactory manner but a much improved method of transforming 5-dihydro-ergosterol into the corresponding 22:23-dibromo-7:9(11)--diene, by low temperature bromination followed by partial debromination, has recently been published (5). To obtain Δ^7-sterols (XII), which do not occur naturally to any great extent, it is usually necessary to start from a 5:7--dienic system (XI), such as is present in ergosterol, and hydrogenate it to the corresponding 5-dihydro derivative (5,6) which always has a trans A/B ring junction. Those sterols which only possess a C_5-C_6 ethylenic linkage (IX) (e.g. cholesterol, stigmasterol) can be converted to 5:7--dienic sterols by a number of methods, the most satisfactory of which is allylic bromination followed by dehydro bromination (e.g. cholesterol ⟶ dehydrocholesterol (7)) (chart IV).

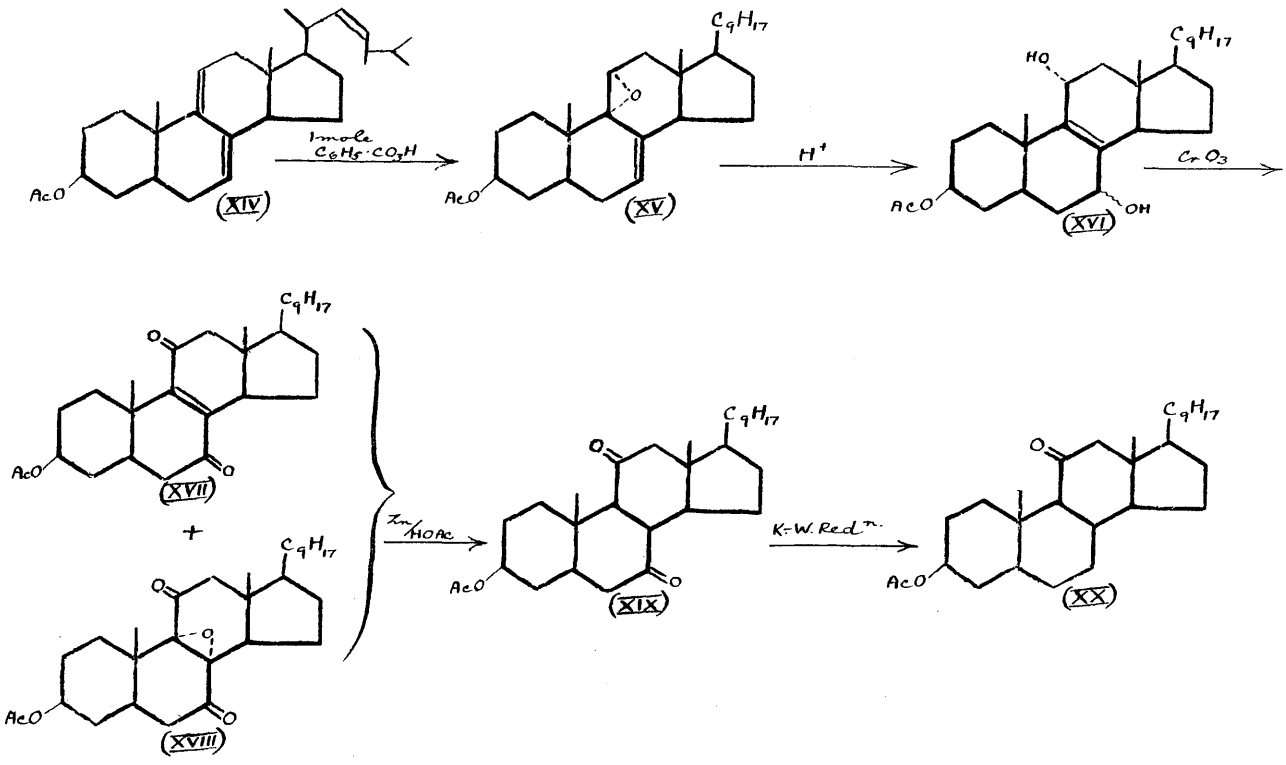
Chart IV



Oxidation of the 7:9(11)-Dienic System. — Since June, 1951, several methods have been described for the conversion of 7:9(11)-dienic steroids to 11-oxygenated steroids. These methods have greatly simplified the preparation of the latter compounds and should be advantageous in the synthesis of cortisone.

The first method to be described was that of Tishler and his co-workers (8), starting from ergosteryl-D acetate (ergosta-7:9(11):22-trien-3β-yl acetate) (XIV) and proceeding by the monoxide (XV) which Jeger (9) suggests is the 9α:11α-oxide. The α-configuration is supported by the fact that acid hydrolysis, under controlled conditions, of the oxide gives the 7ξ:11α-dihydroxy-8(9)-ene (XVI) which can be acetylated to the 7:11-diacetate (10) (11β--hydroxyl groups are sterically hindered and resist acetylation). The 7:11-dihydroxy-8(9)-ene can be oxidised to the 7:11-dion-8(9)-ene (XVII) and the corresponding 8α:9α--oxido derivative (XVIII) both of which, on reduction with zinc and acetic acid, give the 7:11-dione (XIX). The removal of the 7-keto group by the Wolff-Kishner method is not very satisfactory as the 11-keto group is also attacked to some extent, and this operation is performed more efficiently by formation of the 7-cycloethylenemercaptal derivative and treatment with Raney nickel although the last stage also reduces any ethylenic linkage in the sterol side chain (9).

Chart V

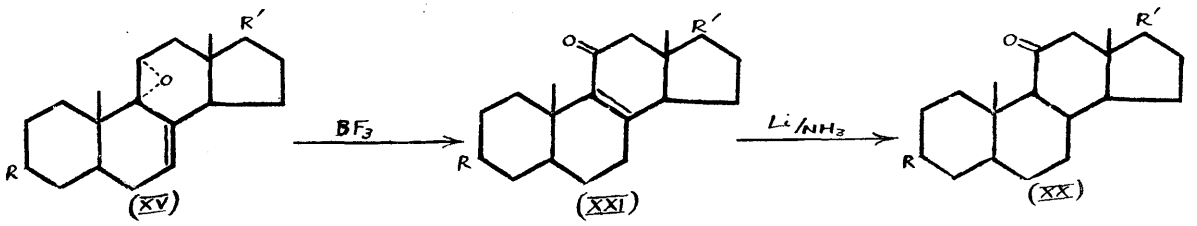


The reaction sequence has also been applied to diosgenin (a Δ^5-steroid sapogenin) and methyl 3β-acetoxy-bisnorchol-5-enate (after conversion to the 7:9(11)-diene) obtained by degradation of stigmasterol or cholesterol (8). Jeger has given a detailed account of the method as applied in the ergosterol (9), cholanic acid (9), cholestane (11) and androstane series (11). The synthesis is thus seen to be effective in steroids with either cis or trans A/B ring junctions.

Jeger (9) has also rearranged the oxide (XV) by the action of boron trifluoride etherate, to the αβ-unsaturated

ketone (XXI). The latter compound, on reduction with lithium in liquid ammonia, was converted to the 11-ketone (XX) in excellent yield (12).

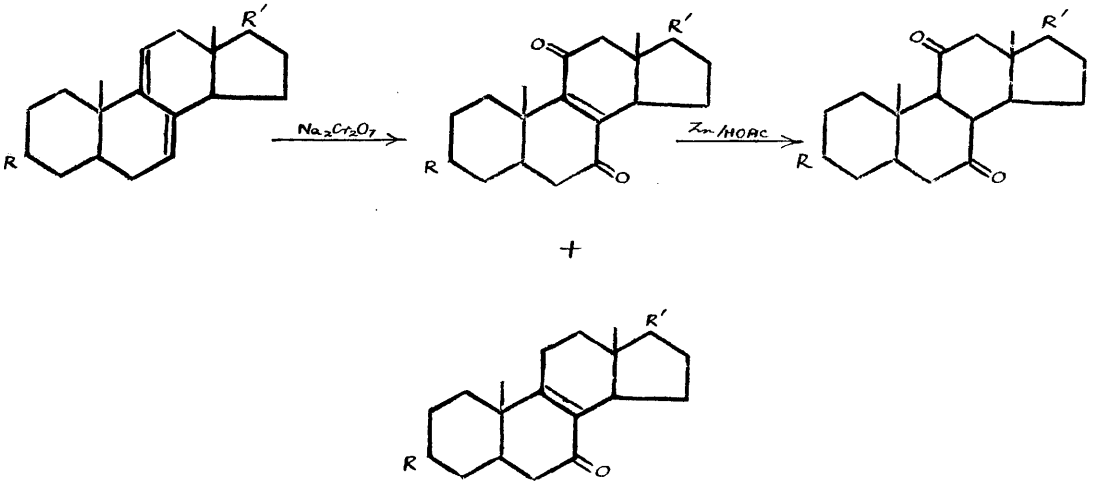
Chart VI



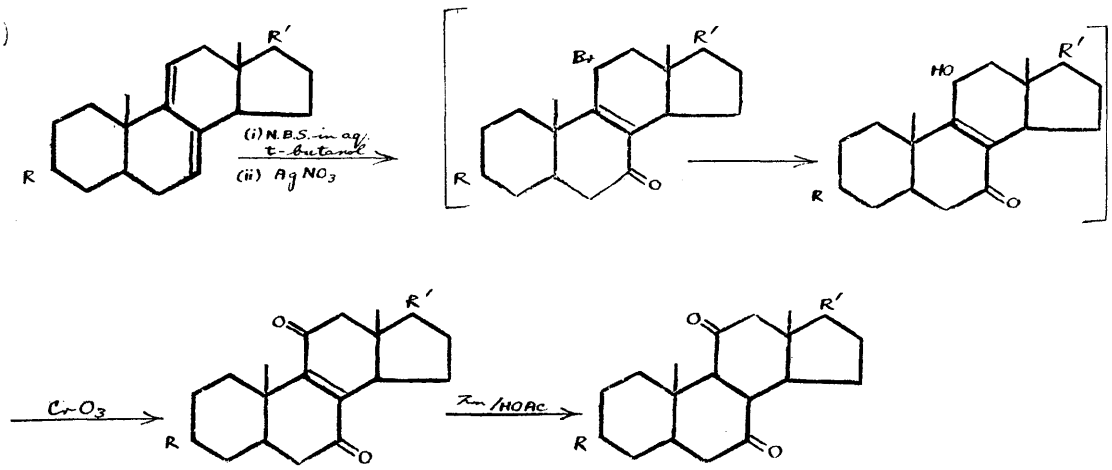
Fieser et al (13,14) have described two further methods for the formation of 7:11-diketo steroids, applicable in the cis or trans A/B series.

Chart VII

(a)

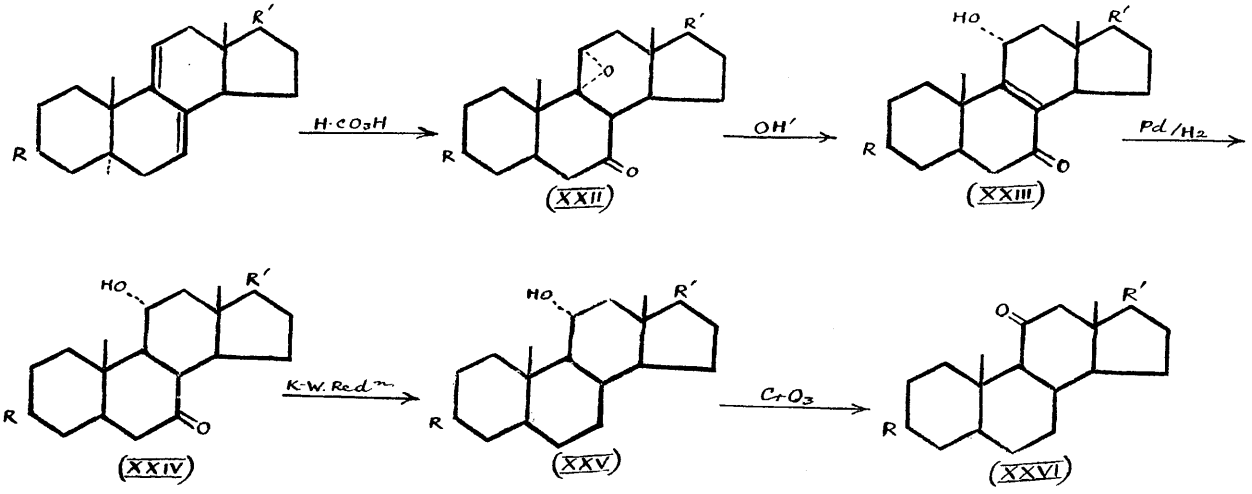


(b)



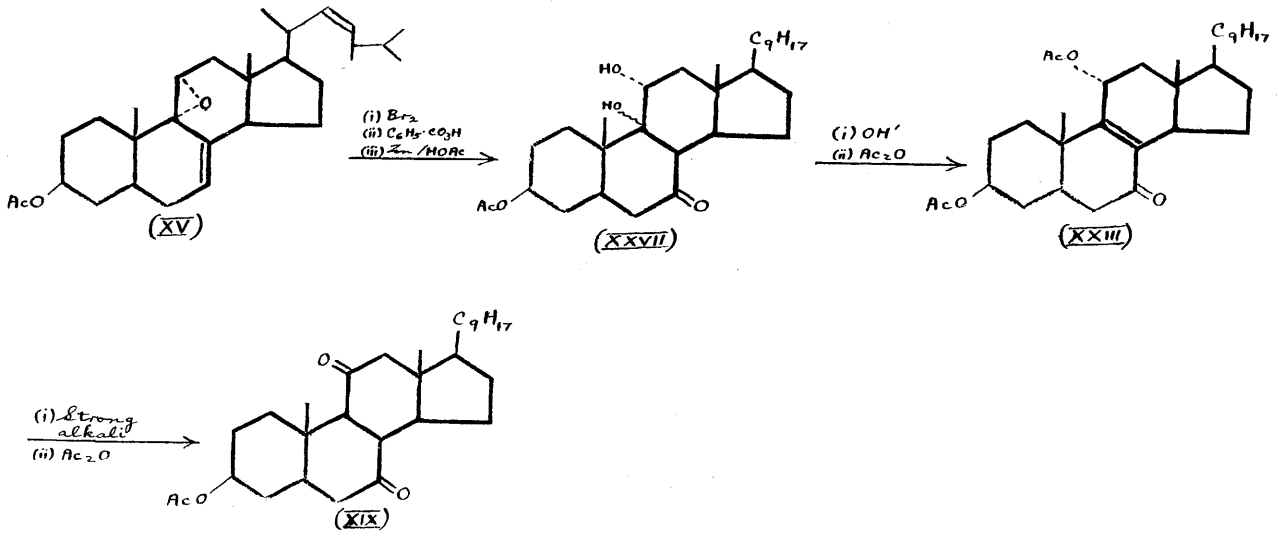
Djerassi and his co-workers (15,16) have accomplished the oxidation of the 7:9(11)-dienic system, in the allo-pregnane and sapogenin (unsubstituted in ring C) series, with performic acid to form a ketoxide (XXII) which, after hydrolysis, gives the 7-keto-11α-hydroxy-8(9)-ene (XXIII). This compound, in contrast to the diketo-8(9)-ene (XVII) and the dihydroxy-8(9)-ene (XVI), can be catalytically hydrogenated to the saturated derivative (XXIV) which is readily transformed to the 11-ketone (XXVI). The method is unsuccessful in the bile acid series as performic acid treatment of a 7:9(11)-dienic bile acid results only in oxidation at C_7 (14). The yield of ketoxide (XXII) obtained from ergosteryl-D acetate was low but became much better when the oxidation was performed on 22-dibromo-ergosteryl-D acetate (5).

Chart VIII



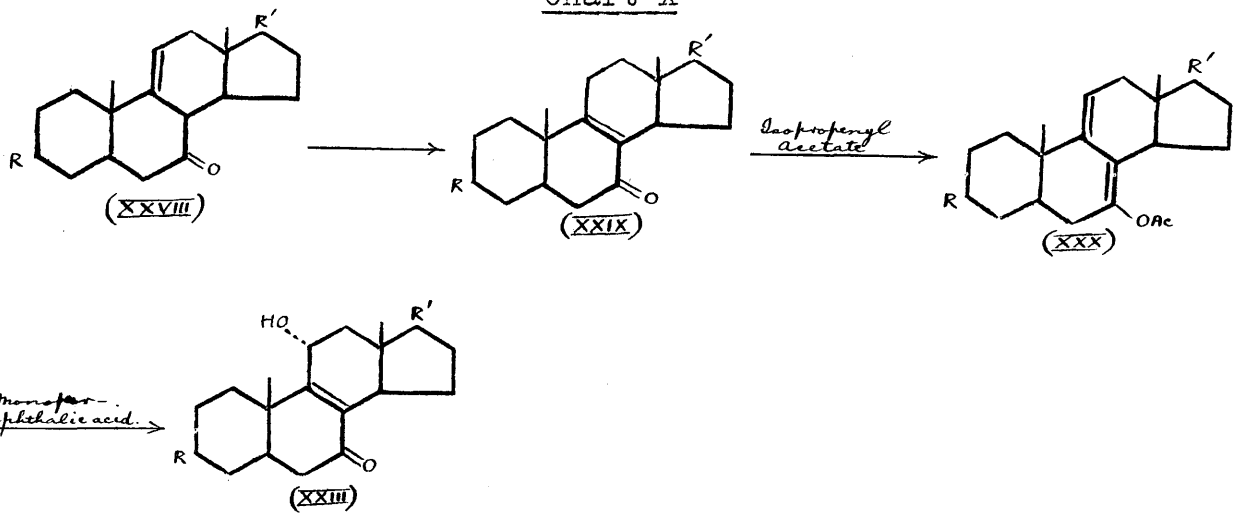
The hydroxy-enone (XXIII) has also been prepared from the oxide (XV) in the ergostane series and rearranged to the dione (XIX) by the action of concentrated alkali (5) (chart IX).

Chart IX



A further route to the hydroxy-enone (XXIII) starts from αβ-unsaturated ketones of the type (XXIX) (17) which are formed by rearrangement of the unconjugated unsaturated ketones (XXVIII) obtained as by-products of performic acid and chromate oxidations of 7:9(11)-dienes.

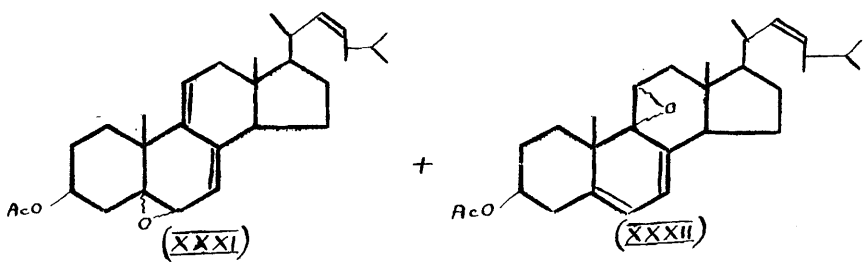
Chart X



THEORETICAL

Oxidation of Dehydroergosterol. — The unsuccessful attempt of Bergmann and Stevens (2) to obtain an 11-oxygenated steroid from the maleic anhydride adduct of dehydroergosterol has already been described. As the failure was caused by difficulties experienced in removing the maleic anhydride, it was decided to investigate the direct oxidation of dehydroergosterol, with all the double bonds in the molecule unprotected.

When dehydroergosteryl acetate was treated with one mole of perbenzoic acid it formed, in 10% yield, a well-defined, crystalline monoxide. The ultraviolet absorption spectrum of the product [maxima at 2400Å (ε = 7000) and 2800Å (ε = 5500)], however, indicated that it was not homogeneous but probably a mixed crystal of the 5:6- (XXXI) and 9:11-oxides (XXXII).



The 5:6-oxide, containing a 7:9(11)-dienic system, would show selective absorption at approximately 2400Å whereas the 9:11-oxide, containing a 5:7-dienic system, would have a maximum at approximately 2800Å in the ultra-

violet absorption spectrum. Fractional crystallisation and chromatography did not separate the mixed oxide into its components.

The reaction of the mixed oxide with maleic anhydride to give neutral and acid fractions can be regarded as further indication of the presence of (XXXI) and (XXXII) since the former would not form a maleic anhydride adduct, thus accounting for the neutral fraction whereas (XXXII) would form an adduct which could be hydrolysed to give the acid fraction.

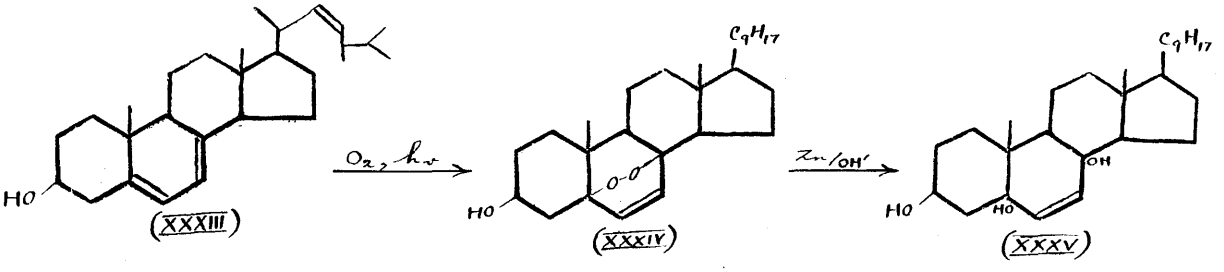
Acid hydrolysis of the mixed oxide did not attack the oxide groups, starting material being recovered after hydrolysis either at room temperature or 100° (reacetylation required in this case).

Oxidation of dehydroergosteryl acetate with molar proportions of performic acid, potassium permanganate, potassium dichromate and sodium bismuthate all resulted in formation of uncrystallisable resins sometimes accompanied by unchanged starting material.

These experiments indicate that the nuclear ethylenic linkages in dehydroergosterol cannot be differentiated in their susceptibility to oxidising agents unlike the 7:9(11)-dienic system which can be oxidised preferentially at one double bond (8,9,11).

Dehydroergosterol Peroxide and its Reduction.— When an alcoholic solution of ergosterol (XXXIII), containing a sensitizor such as eosin, is aerated in presence of light ergosterol peroxide (XXXIV) is formed (18) by 1:4-addition of oxygen across the conjugated diene system. The peroxide, which shows no intense selective absorption in the ultraviolet spectrum, is stable to alkali unlike 1:2-peroxides (19); moreover, on zinc-alkali reduction, it forms ergostadientriol I (XXXV) (20,21) which contains only one acylable hydroxyl group (22) and accords with the reduction product of a transannular peroxide. Bergmann has confirmed the transannular structure assigned to the peroxide by his investigations of the peroxidation of cholestadienes (23).

Chart XI

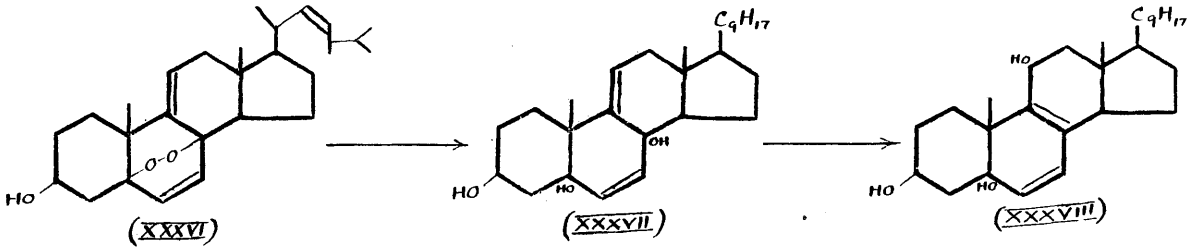


Dehydroergosterol forms a peroxide (XXXVI) under conditions similar to those for the peroxidation of ergosterol (20). The peroxide has no ultraviolet absorption spectrum and Muller has postulated that it also is of the

transannular type (3).

It was hoped that reduction of dehydroergosterol peroxide, under suitable conditions, would result in formation of the triol (XXXVII) which might be capable of isomerization to an 11-oxygenated derivative of the structure (XXXVIII) (chart XII).

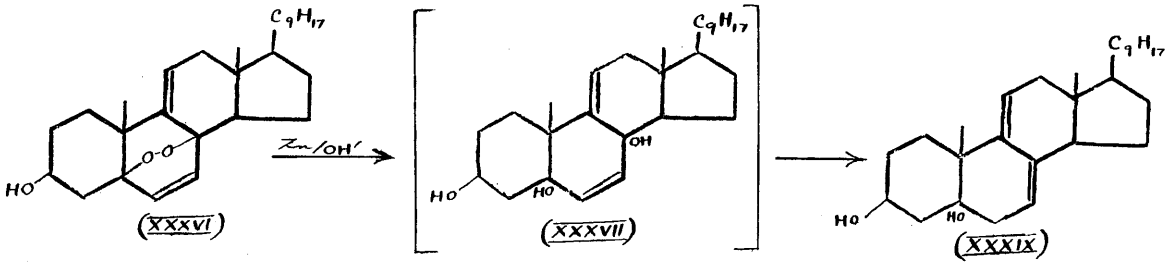
Chart XII



Zinc-Alkali Reduction of Dehydroergosterol Peroxide.— Windaus found that zinc-alkali reduction of dehydroergosterol peroxide did not proceed as in the case of ergosterol peroxide to a triol but gave a diol instead (24). The diol was shown, by acetylation, to contain a secondary and a tertiary hydroxyl group (24). It also contains three double bonds, two of which are conjugated to form a heteroannular diene, indicated by ultraviolet absorption spectrum measurements and the nonreactivity of the compound towards maleic anhydride (3). Experimental evidence has shown that the diol is ergosta-7:9(11):22-

-trien-3β:5§-diol (XXXIX) (3) and it has been suggested that it is formed as illustrated in chart XIII, the triol (XXVII) being an unstable intermediate with the tertiary hydroxyl group at C_8 being replaced by hydrogen. The 9:11-double bond may have an activating influence on the 8-hydroxyl group as ergostadientriol I (XXXV) is unaffected by zinc and alkali.

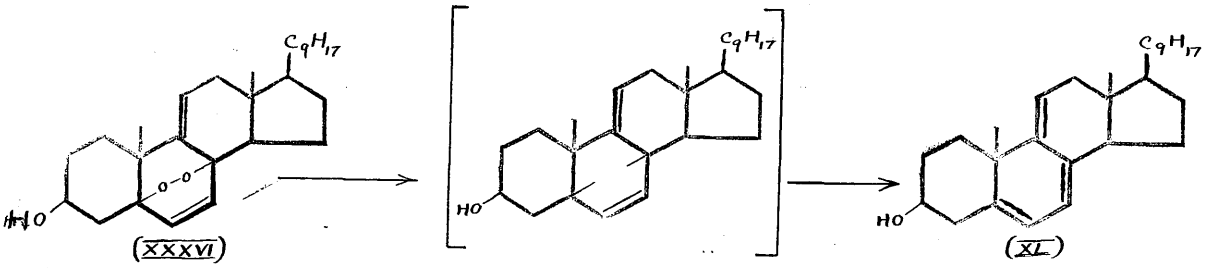
Chart XIII



The author, on repeating the zinc-alkali reduction of dehydroergosterol peroxide, isolated the diol described above and dehydroergosterol. It is unlikely that the latter compound, also isolated when other reducing agents were used, is formed by dehydration of (XXXIX) as the diol appears to be stable under dehydrating conditions, e.g. acetylation results only in the formation of a 3β-acetoxy group. It is possible that the dehydroergosterol results from the direct removal of the two peroxide oxygen atoms as water, leaving C_5 and C_8 each with a free valency and the 6:7-double bond moves into conjugation with these to

give the triene system.

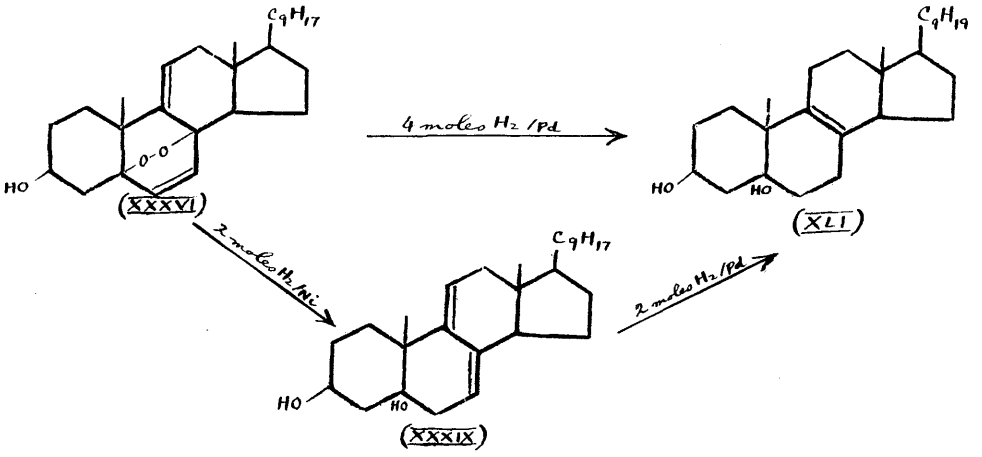
Chart XIV



Catalytic Hydrogenation of Dehydroergosterol Peroxide. — With the aim of obtaining ergosta-6:9(11):22-trien-3β:5ξ:8ξ-triol (XXXVII) or a rearrangement product of this compound (see chart XII), the catalytic hydrogenation of dehydroergosterol peroxide was studied.

Hydrogenation of dehydroergosterol peroxide, using palladium catalyst, results in the absorption of four moles of hydrogen with formation of ergost-8-en-3β:5ξ-diol (XLI) which can also be obtained by catalytic hydrogenation of ergosta-7:9(11):22-trien-3β:5ξ-diol in presence of palladium (3). The following experiments by the author suggest that, under the prevailing reaction conditions, dehydroergosterol peroxide is attacked by two moles of hydrogen simultaneously with direct formation of ergosta--7:9(11):22-trien-3β:5ξ-diol, i.e. not through the intermediate trientriol (XXXVII) (see chart XIII).

Chart XV



Dehydroergosterol peroxide was catalytically hydrogenated, using Raney nickel catalyst. The reaction mixture absorbed two moles of hydrogen and the triendiol (XXXIX) separated from solution in 70% yield. When the reaction was stopped after absorption of one mole of hydrogen the reaction product consisted of unchanged starting material (36%), ergosta-7:9(11):22-trien-3β:5ξ--diol (57%) and a small quantity of material (4%) which appeared to be a mixed crystal of dehydroergosterol and ergosterol-D, the latter being formed by hydrogenation of the dehydroergosterol.

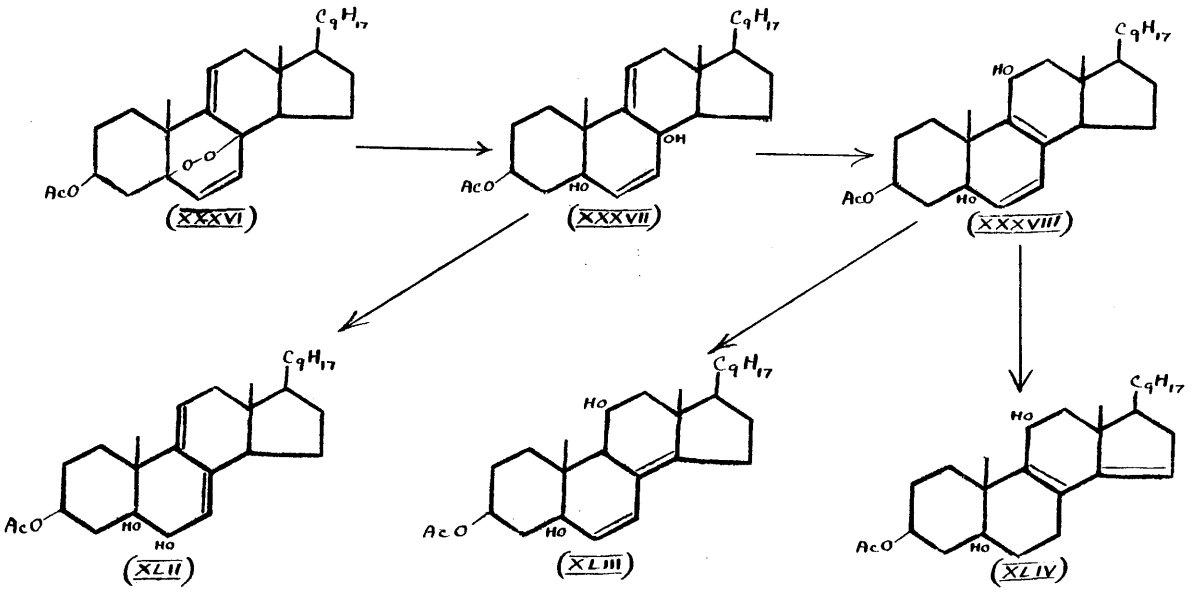
Aluminium Amalgam Reduction of Dehydroergosterol Peroxide.— Three reductions of the acetate of dehydroergosterol peroxide with aluminium amalgam were carried out, the quantity of reducing agent being varied in each experiment. When the ratio of reducing agent to peroxide acetate was 8:1 ($^{W}/_{w}$), dehydroergosteryl acetate, ergosta-7:9(11):22-

-trien-3β:5ξ-diol 3-acetate and a compound, $C_{30}H_{46}O_4$ (hereafter named compound A) were isolated. When the ratio of reducing agent to peroxide acetate was 5:1, the same compounds were obtained but when the ratio was 3:1, in addition to the three compounds listed above, unchanged starting material and a substance of doubtful homogeneity with the ultraviolet absorption spectrum of a conjugated triene, were isolated.

The analysis of compound A conforms to that of the monoacetate of an ergostatrientriol ($C_{30}H_{46}O_4$) but its ultraviolet absorption spectrum [maximum at 2470Å (ε = 20,000), heteroannular conjugated diene] shows that it is neither (XXXVII) which would exhibit no selective absorption in the ultraviolet spectrum or (XXXVIII) which is a homoannular conjugated diene (see chart XII). Compound A is recovered unchanged when further acetylation is attempted therefore the free hydroxyl groups present in it must be tertiary or sterically hindered secondary (e.g. 11β-hydroxyl). It is not attacked by periodic acid, therefore it is not a vic. glycol. On the basis of this evidence it is suggested that compound A may have the structure (XLIII) or (XLIV) (chart XVI).

The structure (XLII) can be discounted as it would be attacked by periodic acid and would be capable of further acylation at the secondary 6-hydroxyl group. The

Chart XVI



formation of (XLIII) or (XLIV) necessitates the isomerization of the 6:8(9)-diene system; isomerization of a 6:8(9)-diene to an 8(9):14-diene has been described in the coprostane series (25).

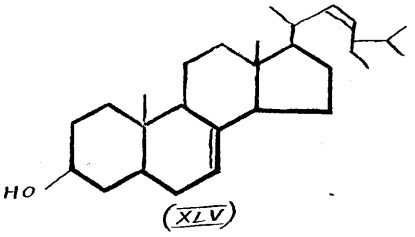
Compound A, on hydrolysis, gave the corresponding ergostatrientriol but the latter could not be obtained by aluminium amalgam reduction of dehydroergosterol peroxide, dehydroergosterol and ergosta-7:9(11):22-trien-3β:5ξ-diol only being isolated. It is probable that organo-metallic complexes are formed by interaction of the metallic reducing agent and dehydroergosterol peroxide as only approximately 50% of the starting material was recovered as reaction product, the remainder being liberated by treatment of the

insoluble residue, derived from the aluminium amalgam, with dilute mineral acid. No similar effects were experienced when the peroxide acetate was being reduced.

Lithium Aluminium Hydride Reduction of Dehydroergosterol Peroxide. — In an unsuccessful attempt to increase the yield of compound A, dehydroergosterol peroxide was reduced by lithium aluminium hydride. Two experiments were carried out, the first with a large excess of lithium aluminium hydride and a short reaction time, the second with approximately molar proportions of reducing agent and a long reaction time. In both instances the reaction product was very complex, containing unchanged starting material, ergosta-7:9(11):22-trien-3β:5ξ-diol, mixed crystals of ergosterol and dehydroergosterol, and small quantities of other, unidentified crystalline substances. It is interesting to note that ergosterol is formed during the reduction as it has probably been derived from the dehydroergosterol present by hydrogenation of the 9:11-ethylenic linkage of that compound; reduction of dehydroergosterol normally results in removal of the 5:6-double bond to give ergosterol-D (26).

α-Spinasterol and Stigmasta-7:9(11):22-trien-3β-ol. — α-Spinasterol (stigmasta-7:22-dien-3β-ol) (XLV) is one of the few naturally occurring sterols containing a 7:8-double bond and for this reason it is an especially suitable

starting material for the preparation of 11-oxygenated steroids by the methods, involving a 7:9(11)-dienic system, described earlier in this thesis.



(XLV)

α-Spinasterol has been isolated from spinach (27), senega root (28) and lucerne (29). The location of one double bond at the 22:23-position in the side chain was established by ozonolysis experiments (30). The position of the other double bond was variously postulated as being C_8-C_{14} (30,31) and C_8-C_9 (32) on the basis of hydrogenation and oxidation experiments but Barton and Cox (33) eventually proved it to be C_7-C_8. This structure was confirmed by the partial synthesis of α-spinasterol from stigmasterol (34).

The author isolated α-spinasterol, as its acetate, by chromatography of the non-saponifiable fraction of lucerne, acetylation of the portion eluted by benzene--methanol (199:1) and further chromatography and finally crystallisation of the acetylated material. Examination of the mother liquors from the acetylated material revealed the presence of more crystalline solid which could not be obtained in pure form and may contain the isomeric β- and γ-spinasteryl acetates differing from the α-isomer in the

position of the side chain ethylenic linkage (35,36).

α-Spinasteryl acetate was dehydrogenated to stigmasta--7:9(11):22-trien-3β-yl acetate (XLVI) in 25% yield by mercuric acetate. The product, which exhibited the characteristic ultraviolet absorption spectrum of a 7:9(11)-dienic steroid, was obtained in better yield (45%) and purer form by bromination of α-spinasteryl acetate followed by direct zinc dust debromination of the reaction product, both operations being carried out at low temperature. These reactions proceed through the formation of an intermediate tetrabromide (XLVII) which can be isolated from the bromination mixture in crystalline form but decomposes on standing. The tetrabromide can be partially debrominated by sodium iodide to 22:23-dibromostigmasta--7:9(11)-dien-3β-yl acetate (XLVIII), a stable compound which exhibits the 7:9(11)-diene absorption spectrum. This may indicate that the tetrabromide is 9:11:22:23--tetrabromostigmast-7-en-3β-yl acetate, formed by direct addition of bromine at C_{22} and C_{23} and by replacement of hydrogen by bromine at C_9 and C_{11}. The dibromide, on treatment with zinc dust, is transformed in good yield to stigmasta-7:9(11):22-trien-3β-yl acetate.

It was found that low temperature bromination of α-spinasteryl acetate followed by zinc dust debromination at a higher temperature did not result in satisfactory

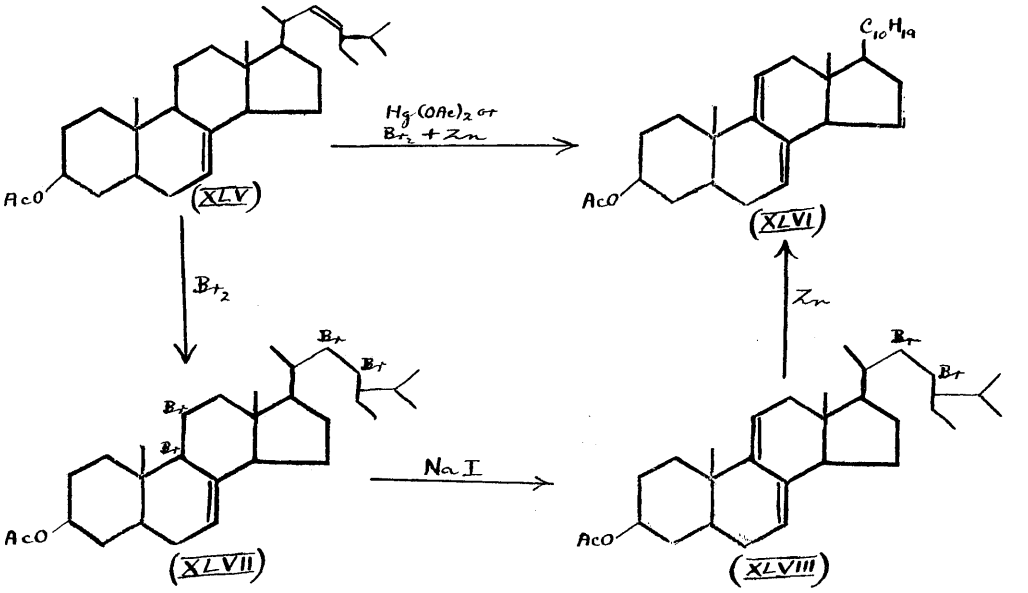
formation of stigmasta-7:9(11):22-trien-3β-yl acetate. The product, obtained by refluxing the bromination mixture with zinc dust, was formed in very low yield (5%), had a specific rotation of -17° as compared with a value of +45° for the 7:9(11)-dienic steroid and its ultraviolet absorption spectrum indicated the presence of approximately 50% of the latter compound. On the basis of this evidence the product would appear to be contaminated by a substance showing no selective absorption in the ultraviolet spectrum and having a specific rotation of approximately -80°. Debromination at 0° gave a 35% yield of 7:9(11)-diene of 75% purity (based on ultraviolet absorption spectrum data).

The bromination-debromination reactions of α-spinasteryl acetate follow a course similar to that for the bromination of the analogous 5-dihydroergosteryl acetate (5,37). The bromination of 5-dihydroergosteryl acetate was investigated in a search for improved methods of preparation of ergosteryl-D acetate as Eck and Hollingsworth (38) have described the preparation of cholesta-7:9(11)-diene by oxidation of cholest-7-ene at a low temperature with bromine. Tetrabromoergostenyl acetate is obtained in 50% yield by low temperature bromination of 5-dihydroergosteryl acetate. The tetrabromide, whose structure has not been elucidated, is unstable and closely resembles tetrabromostigmastenyl acetate in physical and chemical properties; it is highly probable that the two tetrabromides have the same nuclear

structure. Partial debromination of tetrabromoergostenyl acetate by sodium iodide gives 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate (ergosteryl-D acetate 22:23-dibromide). This compound, unlike 22:23-dibromostigmasta-7:9(11)-dien-3β-yl acetate, can be obtained in good overall yield from the parent Δ^7-sterol and, as it has no side chain double bond, can undergo oxidation of the 7:9(11)-dienic system in a more satisfactory manner than ergosteryl-D acetate (5,37). For purposes of side chain degradation the side chain ethylenic linkage is readily regenerated from the dibromide by the action of zinc dust.

Low temperature chlorination of 5-dihydroergosteryl acetate has yielded two isomeric tetrachlorides. Treatment of one of these isomers with sodium iodide gives ergosteryl-D acetate 22:23-dichloride (37).

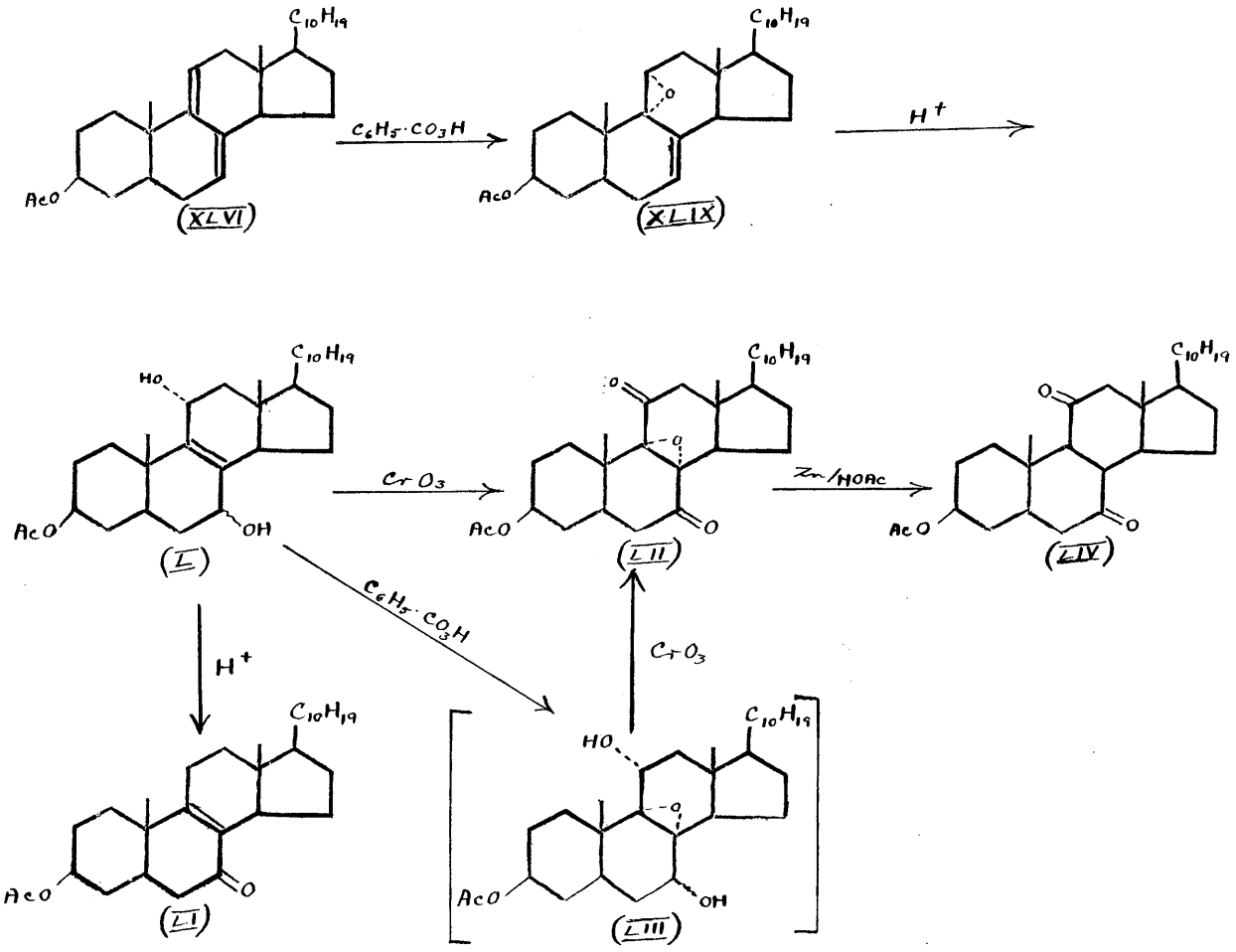
Chart XVII



Oxidation of Stigmasta-7:9(11):22-trien-3β-yl Acetate.— It was decided to convert stigmasta-7:9(11):22-trien-3β-yl acetate to an 11-oxygenated derivative by the method described by Tishler (8) and Jeger (9,11) for the oxidation of 7:9(11)-dienic steroids. The reaction sequence is outlined in chart (XVIII).

Treatment of the trienyl acetate (XLVI) with one mole of perbenzoic acid gave the crystalline monoxide (XLIX) which, on hydrolysis with sulphuric acid, was converted to stigmasta-8:22-dien-3β:7ξ:11α-triol 3-acetate (L). If the hydrolysis is allowed to proceed for too long a time, the dientriol monoacetate (L) is decomposed to a substance showing the ultraviolet absorption spectrum of an αβ-unsaturated ketone (LI). Jeger has described

Chart XVIII



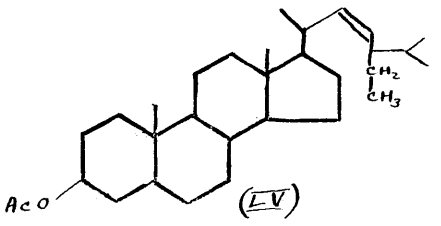
this reaction in the ergostane series (9). As the dien-triol monoacetate (L) has a high positive optical rotation and the oxide (XLIX) and the αβ-unsaturated ketone (LI) both have negative rotations, the optimum hydrolysis time was gauged by following the reaction in a polarimeter tube. This showed that a reaction time of 12 to 20 minutes was most satisfactory. The overall yield of dientriol mono-acetate (L) from stigmasta-7:9(11):22-trien-3β-yl acetate

(XLVI) was improved by carrying out the hydrolysis on the crude perbenzoic acid oxidation product rather than on the crystalline oxide. Chromic acid oxidation of stigmasta-8:22-dien-3β:7ξ:11α-triol 3-acetate gave 8α:9α--epoxy-7:11-diketostigmast-22-en-3β-yl acetate (LII) in 25% yield, accompanied by a trace of 7:11-diketostigmasta--8:22-dien-3β-yl acetate whose presence was indicated by the ultraviolet absorption spectrum of the mother liquors. The yield of dione-oxide (LII) is much improved by oxidising the dientriol monoacetate (L) with approximately one mole of perbenzoic acid and treating the amorphous oxidation product with chromic acid. The perbenzoic acid oxidation product is probably the oxido-diol (LIII) but it could not be obtained in crystalline form and characterized. 7:11-Diketostigmast-22-en-3β-yl acetate (LIV) was formed practically quantitatively by zinc and acetic acid reduction of the dione-oxide (LII).

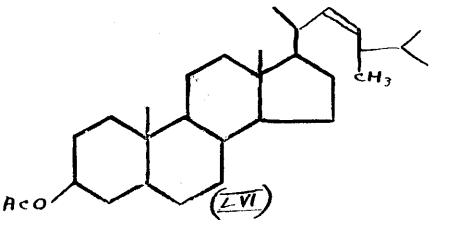
The configurations assigned to the oxide and hydroxyl groups in compounds (XLIX), (L) and (LII) are based on those of the analogous compounds in the ergostane series (9).

The table below lists the specific rotations ($[\alpha]_D$) and molecular rotations ($M_D = \frac{[\alpha]_D \times \text{mol.wt.}}{100}$) of a number of derivatives of stigmast-22-en-3β-yl acetate (LV) and the corresponding derivatives of ergost-22-en-3β-yl acetate

(LVI). The molecular rotation differences (ΔM_D) between these two series of compounds can be regarded as constant within the limits of experimental error and show that the introduction of an additional methylene group in the ergost-22-ene side chain causes a molecular rotation increment of approximately +60°.



(LV)



(LVI)

Derivative	Ergost-22-en-3β-yl Acetate		Stigmast-22-en-3β-yl Acetate		Δ M_D
	$[\alpha]_D$	M_D	$[\alpha]_D$	M_D	
Δ^7-ene	-20.5° (39)	-90°	-5°	-23°	+67°
$\Delta^{7:9(11)}$-diene	+33° (39)	+145°	+45°	+204°	+59°
Δ^7-ene-9α:11α-oxide	-35° (8) -39.5° (9)	-144° -180°	-20°	-94°	+50° +86°
Δ^8-ene-7ξ:11α-diol	+82° (9)	+388°	+93°	+453°	+65°
7:11-diketo-8α:9α-oxide	-63° (9)	-287°	-49°	-244°	+43°
7:11-dione	-27° (9) -31° (39)	-127° -149°	-16°	-78°	+49° +71°

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EXPERIMENTAL

Ergosteryl Acetate.— Ergosterol (100 g.) was dissolved in dry pyridine (400 c.c.) by warming in a stream of nitrogen. When no solid remained, redistilled acetic anhydride (100 c.c.) was added to the warm solution which was then kept at room temperature for 20 hours in the absence of light. The ergosteryl acetate which separated was collected, washed with pyridine and water, dried and recrystallised from chloroform-methanol as plates (82 g.), m.p.170-172°, $[\alpha]_D$ -93° (c, 1.5).

Dehydroergosterol.— (Windaus and Linsert, loc.cit.). Ergosterol (50 g.) was dissolved by refluxing in ethanol (2500 c.c.) and added to a solution of mercuric acetate (170 g.) in glacial acetic acid (200 c.c.) and ethanol (400 c.c.). The mixture was refluxed for 40 minutes, mercury salts removed by filtration and the filtrate concentrated to small bulk under reduced pressure. Crystals separated which were collected and recrystallised from chloroform-methanol to yield dehydroergosterol as plates (15 g.), m.p.146-148°, $[\alpha]_D$ +153° (c, 1.6).

Dehydroergosteryl Acetate.— (Bergmann and Stevens, loc. cit.). Ergosteryl acetate (50 g.) was dissolved in chloroform (700 c.c.) and added to a solution of mercuric acetate (95 g.) in glacial acetic acid (1200 c.c.). The mixture was shaken for 24 hours, mercury salts collected

and the filtrate diluted with water (3 litres). The chloroform layer was separated, the aqueous layer washed with chloroform and the combined chloroform solutions concentrated under reduced pressure at low temperature. Methanol was added to the concentrated solution, crystals separated, were collected and recrystallised from chloroform-methanol from which dehydroergosteryl acetate separated as plates (20 g.), m.p.144-146°, $[\alpha]_D$ +196° (c,1.2).

Perbenzoic Acid Oxidation of Dehydroergosteryl Acetate.— Dehydroergosteryl acetate (1.5 g.) was dissolved in chloroform (10 c.c.), the solution cooled to 0° and treated with a solution of perbenzoic acid in chloroform (12 c.c., 40 mg./c.c., 1 atom O), also at 0°. The reaction mixture was maintained at 0° for 4 hours, when it gave no reaction with potassium iodide reagent, then washed with dilute sodium hydrogen carbonate and water and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue digested with methanol, the methanolic mother liquors rapidly darkening. The resulting amorphous yellow solid (0.4 g.) crystallised from methanol as felted needles (150 mg.), m.p.191-193°, $[\alpha]_D$ -169° (c, 1.0).

Analysis: Found: C,79.5; H,9.7

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

Note: $C_{30}H_{44}O_3$ = dehydroergosteryl acetate monoxide.

Light absorption: maxima at 2400Å (ε = 7000) and 2800Å (ε = 5500).

Repetition of the experiment at -10° with dropwise addition of the perbenzoic acid over several hours (3-7 hours) did not increase the yield of oxidation product.

Attempted Hydrolysis of the Oxidation Product.— (a) The oxidation product (200 mg.) was dissolved in dioxan (55 c.c.), water (5 c.c.) and sulphuric acid, (10%, 0.2 c.c.) added to the solution and the mixture kept at room temperature for 90 hours. It was then diluted with water and ether extracted. The ether extract was washed with dilute sodium hydrogen carbonate and water, dried (Na_2SO_4) and the solvent removed. The amorphous residue crystallised from methanol-chloroform as needles (150 mg.), m.p.188-189°, undepressed on mixing with starting material.

(b) The oxidation product (120 mg.) was dissolved in dioxan (25 c.c.), and water (5 c.c.) and sulphuric acid (10%, 0.5 c.c.) added. Precipitation occurred but the mixture was heated under reflux and the precipitate redissolved. Refluxing was continued for $2^1/_2$ hours, the mixture cooled and diluted with water. The amorphous reaction product was isolated by ether and a portion

(70 mg.) acetylated in the usual way. The acetylated material crystallised from methanol-chloroform as fine, felted needles, m.p.187-189°, undepressed on mixing with starting material, $[\alpha]_D$ -165° (c, 1.3).

Treatment of the Oxidation Product with Maleic Anhydride.— The oxidation product (200 mg.) and maleic anhydride (150 mg.) were dissolved in dry xylene (5 c.c.) and the solution heated under reflux for 8 hours. The solvent and excess maleic anhydride were removed under reduced pressure and the amorphous, pale yellow residue dissolved in ether (50 c.c.). The ethereal solution was shaken with 4N sodium hydroxide (2 x 50 c.c. portions) and an acid fraction isolated from the aqueous layer in the usual way.

Evaporation of the ethereal layer yielded the neutral fraction. The acid and neutral fractions were both acetylated but in neither case could be induced to crystallise.

Dehydroergosterol Peroxide.— (Windaus and Linsert, loc. cit.). Dehydroergosterol (10 g.) was dissolved in ethanol (1200 c.c.) and eosin (10 mg.) added to the solution which was irradiated with a water-cooled white light whilst oxygen was slowly bubbled through it for 8 hours. The solution was evaporated to dryness under reduced pressure,

the residue dissolved in benzene (100 c.c.) and adsorbed on a column of alumina (grade II, 50 g.) to remove eosin. The alumina was washed with benzene-methanol (100 c.c., 3:1) and the washings evaporated to give dehydroergosterol peroxide which crystallised as needles (6 g.) from methanol, m.p.160-163°, $[\alpha]_D$ +79° (c, 1.7).

Analysis: Found: C,78.8; H,9.6.

Calc. for $C_{28}H_{42}O_3$: C,78.9; H,9.9%.

Dehydroergosterol Peroxide Acetate.— Dehydroergosteryl acetate (7.5 g.) was dissolved in ethanol (1200 c.c.) and eosin (8 mg.) added to the solution which was treated as described for the preparation of dehydroergosterol peroxide. Dehydroergosterol peroxide acetate separated from methanol as needles (5 g.), m.p.175-177°, $[\alpha]_D$ +91° (c, 1.0).

Analysis: Found: C,76.7; H,9.1

$C_{30}H_{44}O_4$ requires: C,76.9; H,9.4%.

Dehydroergosterol peroxide acetate (1 g.) was dissolved in methanolic potassium hydroxide solution (5%, 40 c.c.) and the mixture refluxed for 2 hours. The solution was cooled and dehydroergosterol peroxide (0.7 g.) separated as needles, m.p.162-165°, undepressed when mixed with a sample prepared as described above, $[\alpha]_D$ +80° (c, 1.5).

Reduction of Dehydroergosterol Peroxide by Zinc/Potassium Hydroxide.— (cf. Windaus et al, loc.cit.). Dehydroergo-

sterol peroxide (1.5 g.) was dissolved in ethanol (10 c.c.) by heating under reflux. Potassium hydroxide (0.5 g.) in aqueous ethanol (90%, 5 c.c.) and zinc dust (4 g.) were added to the solution and refluxing continued for 2 hours. The zinc was collected, washed with boiling ethanol and the combined filtrate and washings concentrated to give a crystalline solid, m.p.150-210°. Light absorption: Maxima at 2360Å (ε = 10,000), 2440Å (ε = 11,000), 3120Å (ε = 5500), 3260Å (ε = 6000) and 3400Å (ε = 3800). Recrystallisation of this product from ethyl acetate yielded ergosta-7:9(11):22-trien-3β:5ξ-diol as plates, m.p.223-226°, $[\alpha]_D$ +44° (c,1.0).

Analysis: Found: C,81.5; H,10.6.

Calc. for $C_{28}H_{44}O_2$: C,81.8; H,10.8%.

Acetylation of ergosta-7:9(11):22-trien-3β:5ξ-diol in the usual way gave ergosta-7:9(11):22-trien-3β:5ξ-diol 3-acetate as plates from methanol-chloroform, m.p.220-223°, $[\alpha]_D$ +47.5° (c, 1.1).

Analysis: Found: C,79.1; H,9.9.

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

A portion of the crude reduction product was acetylated in the usual way and the acetylated material (0.5 g.) dissolved in benzene-light petroleum (1:5, 60 c.c.) and chromatographed on acid-washed, activated alumina (grade II, 15 g.) to give the following fractions:

Fraction	Solvent		Eluate
-	Benzene-light petroleum (1:5)	60 c.c.	-
(1)	Benzene-light petroleum (1:4)	200 c.c.	0.29 g.
-	Benzene-light petroleum (1:4)	100 c.c.	-
-	Benzene	100 c.c.	-
(2)	Benzene-methanol (99:1)	100 c.c.	0.20 g.

Fraction 1 crystallised from methanol-chloroform as plates, m.p.141-144°, undepressed on mixing with dehydroergosteryl acetate, $[\alpha]_D$ +198° (c, 1·5).

Fraction 2 crystallised from methanol-chloroform as plates, m.p.222-224°, undepressed on mixing with ergosta--7:9(11):22-trien-3β:5ξ-diol 3-acetate, $[\alpha]_D$ +50° (c,1.2).

Catalytic Hydrogenation of Dehydroergosterol Peroxide.— (a) A solution of dehydroergosterol peroxide (1 g.) in ethyl acetate (70 c.c.) was catalytically hydrogenated in presence of Raney nickel. After 61 c.c.(1.1 moles) of hydrogen had been absorbed, the hydrogenation was stopped, the catalyst collected, washed with ethyl acetate and the combined filtrate and washings evaporated to dryness under reduced pressure to give an amorphous residue which was acetylated with acetic anhydride in pyridine at room temperature. The acetylated product (0.93 g.) was dissolved in light petroleum (100 c.c.) and chromatographed on acid-washed, activated alumina (grade II, 30 g.).

Fraction	Solvent	Eluate
(1)	Benzene-light petroleum (1:3) 300 c.c.	0.04 g.
(2)	Benzene-light petroleum (1:3) 100 c.c.)	
	Benzene-light petroleum (2:3) 300 c.c.)	
	Benzene-light petroleum (1:1) 100 c.c.)	0.36 g.
	Benzene-light petroleum (3:2) 100 c.c.)	
	Benzene-light petroleum (3:1) 100 c.c.)	
(3)	Benzene-light petroleum (9:1)1100 c.c.	0.57 g.

Fraction 1 crystallised from chloroform-methanol as plates, m.p.154-157°. Light absorption: maxima at 2430Å (ε = 6500), 3100Å (ε = 4700), 3250Å (ε = 5200) and 3400Å (ε = 3200) with an inflection at 2260Å (ε = 6000).

Fraction 2, on crystallisation from methanol, yielded dehydroergosterol peroxide acetate as needles, m.p. and mixed m.p.174-176°, $[\alpha]_D$ +92.5° (c,1.7).

Fraction 3 crystallised from chloroform-methanol as plates, m.p.218-221°, undepressed on mixing with ergosta--7:9(11):22-trien-3β:5ξ-diol 3-acetate, $[\alpha]_D$ +49° (c, 2.0).

(b) A solution of dehydroergosterol peroxide acetate (1 g.) in ethyl acetate (100 c.c.) was shaken with hydrogen in presence of Raney nickel catalyst. 115 c.c. (2.2 moles) of hydrogen were quickly absorbed and a crystalline solid separated from the reaction mixture, further uptake of hydrogen being very slow. The reaction mixture was warmed to dissolve the separated solid, the Raney nickel removed

by filtration and washed with ethyl acetate. The combined filtrate and washings were concentrated and glistening plates (0.6 g.) of ergosta-7:9(11):22-trien-3β:5ξ-diol 3-acetate separated, m.p. and mixed m.p.215-220°, $[\alpha]_D$ +46° (c, 1.6).

Further concentration of the mother-liquors yielded a second crop (100 mg.) of less pure triendiol acetate.

Aluminium Amalgam.— (Weygand, Org.Prep.,p.9). Aluminium turnings, activated by treatment with 10% sodium hydroxide solution, were washed with water and treated with 1% mercuric chloride solution. The resulting amalgam was washed successively with water, ethanol and ether to give a product which effervesced vigorously in moist ether. The amalgam must be used immediately after preparation.

Aluminium Amalgam Reduction of Dehydroergosterol Peroxide Acetate.— (a) Dehydroergosterol peroxide acetate (6 g.) was dissolved in moist ether (240 c.c.) and aluminium amalgam (50 g.) added to the solution which was stored at room temperature for 24 hours. The insoluble residue was removed by filtration and washed with ether, the combined filtrate and washings being evaporated to dryness under reduced pressure to give an amorphous residue. The residue was fractionally crystallised from chloroform-methanol, two fractions being obtained (fraction I, 1.5 g.; fraction II, 3.5 g.).

A portion of fraction I (0.70 g.) was dissolved in benzene-light petroleum (1:1; 50 c.c.) and chromatographed on acid-washed, activated alumina (grade II; 20 g.) to give the following fractions:

Fraction	Solvent	Eluate
(1)	Benzene-light petroleum (1:1) 100 c.c.)	0.20 g.
	Benzene-light petroleum (3:2) 100 c.c.)	
(2)	Benzene-methanol (99:1) 200 c.c.	0.50 g.

Fraction (1) crystallised from chloroform-methanol, yielding dehydroergosteryl acetate as plates, m.p.146-148°, undepressed on mixing with an authentic specimen, $[\alpha]_D$ +190° (c, 1.3).

Fraction (2) crystallised from chloroform-methanol as plates, m.p.216-218°, undepressed on mixing with ergosta-7:9(11):22-trien-3β:5ξ-diol 3-acetate.

A portion of fraction II (1.80 g.) was dissolved in benzene-light petroleum (1:1; 100 c.c.) and chromatographed on acid-washed, activated alumina (grade II, 50 g.).

Fraction	Solvent	Eluate
(1)	Benzene-light petroleum (1:1) 100 c.c.	0.26 g.
(2)	Benzene-light petroleum (3:2) 100 c.c.)	1.53 g.
	Benzene-light petroleum (4:1) 100 c.c.)	
	Benzene 100 c.c.)	
	Benzene-methanol (98:2) 100 c.c.)	

Fraction (1) crystallised from chloroform-methanol as plates, m.p.144-146°, undepressed on mixing with dehydroergosteryl acetate.

Fraction (2) crystallised from methanol as prisms, m.p.142-144°, $[\alpha]_D$ -66° (c,1.7). Light absorption: maximum at 2470Å (ε = 20,000).

Analysis: Found: C,76.8; H,10.1; active H,0.32.

$C_{30}H_{46}O_4$ requires: C,76.6; H,9.9; active H,0.42%.

Note: $C_{30}H_{46}O_4$ = ergostatrientriol monoacetate. This substance was named compound A.

(b) Dehydroergosterol peroxide acetate (5 g.) in moist ether (200 c.c.) was treated with aluminium amalgam (25 g.) for 18 hours at room temperature and worked up as described in (a) to give two fractions (fraction I, 0.90 g.; fraction II, 2.43 g.).

Fraction I was recrystallised from ethyl acetate to give ergosta-7:9(11):22-trien-3β:5ξ-diol 3-acetate as glistening plates, m.p.220-223°, undepressed on mixing with an authentic specimen.

A portion of fraction II (1.0 g.) was dissolved in benzene-light petroleum (1:1; 100 c.c.) and chromatographed on acid-washed, activated alumina (grade II, 30 g.)as follows:

Fraction	Solvent	Eluate
(1)	Benzene-light petroleum (1:1) 150 c.c.	0.11 g.
(2)	Benzene-light petroleum (3:2) 100 c.c.)	
	Benzene-light petroleum (3:1) 200 c.c.)	
	Benzene-light petroleum (9:1) 100 c.c.)	0.42 g.
	Benzene 100 c.c.)	
(3)	Benzene-methanol (99:1) 200 c.c.	0.12 g.

Fraction (1) yielded dehydroergosteryl acetate from chloroform-methanol as plates, m.p.144-147°, undepressed on mixing with an authentic specimen.

Fraction 2 crystallised from methanol as prisms, m.p.142-145°, undepressed on mixing with compound A, $[\alpha]_D$ -63° (c,0.5).

Fraction (3) would not crystallise.

(c) A solution of dehydroergosterol peroxide acetate (5 g.) in moist ether (200 c.c.) containing aluminium amalgam (15 g.) was kept at room temperature for 14 hours and worked up as described in (a) to give two fractions (fraction I, 1.2 g.; fraction II, 3.0 g.).

Fraction I, on recrystallisation from ethyl acetate, afforded ergosta-7:9(11):22-trien-3β:5ξ-diol 3-acetate as plates, m.p.217-219°, undepressed on mixing with an authentic specimen, $[\alpha]_D$ +47° (c, 1.0).

Fraction II (2 g.) was dissolved in benzene-light petroleum (1:2; 150 c.c.) and chromatographed on acid-

washed, activated alumina (grade II, 60 g.) to give the following fractions:

Fraction	Solvent		Eluate
(1)	Benzene-light petroleum	(1:2) 200 c.c.	0.11 g.
(2)	Benzene-light petroleum	(1:1) 100 c.c.	0.42 g.
(3)	Benzene-light petroleum	(1:1) 200 c.c.)	0.09 g.
	Benzene-light petroleum	(3:2) 100 c.c.)	
(4)	Benzene-light petroleum	(3:1) 100 c.c.)	
	Benzene-light petroleum	(9:1) 100 c.c.)	1.11 g.
	Benzene	500 c.c.)	
(5)	Benzene-methanol	(1:1) 100 c.c.	0.09 g.

Fraction (1) crystallised from chloroform-methanol as plates, m.p.144-146°, undepressed on mixing with dehydroergosteryl acetate, $[\alpha]_D$ +199° (c,0.5).

Fraction (2) crystallised from a large bulk of methanol as clumps of matted needles, m.p.140-146°, $[\alpha]_D$ -240° (c,0.45).

Analysis: Found: C,81.8; H,9.5%.

Light absorption: maxima at 2510Å (ε = 3300), 2600Å (ε = 3330), 3250Å (ε = 9800) and 3410Å (ε = 9700).

Fraction (3)crystallised from methanol as needles, m.p.169-172°, undepressed on mixing with starting material, $[\alpha]_D$ +95° (c,1.0).

Fraction (4) crystallised from methanol as prisms,

m.p.142-145°, undepressed on mixing with compound A, $[\alpha]_D$ -66° (c, 1.7).

Fraction 5 could not be crystallised.

Attempted Acetylation of Compound A.— (a) Compound A (150 mg.) was dissolved in a mixture of dry pyridine (1 c.c.) and redistilled acetic anhydride (0.15 c.c.), kept at room temperature for 18 hours and worked up in the usual way to give unchanged starting material.

(b) A solution of compound A (250 mg.) and fused sodium acetate (100 mg.) in redistilled acetic anhydride (2 c.c.) was refluxed for 1 hour. Prisms separated from the solution on cooling, were collected, washed with water and dried, m.p. and mixed m.p.140-142°, $[\alpha]_D$ -65° (c, 1.4).

Hydrolysis of Compound A.— A solution of compound A (340 mg.) in methanolic potassium hydroxide solution (7%, 15 c.c.) was heated under reflux for 2 hours, cooled and diluted with water. The precipitate which separated was isolated and crystallised from aqueous methanol as needles (220 mg.), m.p.133-136°, $[\alpha]_D$ -75° (c, 0.9).

Analysis: Found: C,79.0; H,10.7.

$C_{28}H_{44}O_3$ requires: C,78.5; H,10.4%.

Note: $C_{28}H_{44}O_3$ = ergostatrientriol.

Attempted Periodic Acid Oxidation of Compound A.— Compound A (100 mg.) was dissolved in methanol (15 c.c.) and treated

with a solution of periodic acid (160 mg.; 4 moles) in water (1.2 c.c.). The mixture was stored at room temperature for 22 hours, diluted with water and the granular precipitate which separated collected, washed with water and dried. The product was crystallised from methanol as prisms, m.p.141-143°, undepressed on mixing with starting material.

Aluminium Amalgam Reduction of Dehydroergosterol Peroxide.— A solution of dehydroergosterol peroxide (2 g.) in moist ether (100 c.c.) was shaken with aluminium amalgam (20 g.) for 24 hours. Insoluble material was removed from the solution by filtration and washed with ether, the combined filtrate and washings being evaporated to dryness under reduced pressure to give an amorphous residue (fraction I, 0.74 g.).

The insoluble material from the reaction after unchanged aluminium had been removed, was digested with dilute hydrochloric acid, and the resulting suspension extracted with ether. The ether extract was washed with water, dried (Na_2SO_4) and the ether removed under reduced pressure to give a slightly yellow, amorphous residue (fraction II, 0.93 g.).

A portion of fraction I was acetylated with acetic anhydride in pyridine at room temperature and worked up

in the usual way. The acetylated product (0.6 g.) was dissolved in benzene-light petroleum (1:2; 75 c.c.) and chromatographed on acid-washed, activated alumina (grade II, 18 g.).

Fraction	Solvent			Eluate
(1)	Benzene-light petroleum	(2:1)	50 c.c.	0.10 g.
	Benzene-light petroleum	(7:3)	50 c.c.	
(2)	Benzene-light petroleum	(9:1)	400c.c.	0.43 g.
	Benzene		100c.c.	
	Benzene-methanol	(99:1)	100c.c.	
(3)	Benzene-methanol	(99:1)	100c.c.	0.06 g.

Fraction (1) crystallised from chloroform-methanol as plates, m.p.144-146°, undepressed on mixing with dehydroergosteryl acetate.

Fraction(2)crystallised from chloroform-methanol as plates, m.p.218-221°, undepressed on mixing with ergosta-7:9(11):22-trien-3β:5ξ-diol 3-acetate, $[\alpha]_D$ +48° (c, 1.0).

Fraction (3) would not crystallise.

A portion of fraction II was acetylated and chromatographed but no crystalline products were obtained from it.

Lithium Aluminium Hydride Reduction of Dehydroergosterol Peroxide.— (a) Lithium aluminium hydride (2 g.) was

heated under reflux in dry ether (150 c.c.) until no more would dissolve. A solution of dehydroergosterol peroxide (2 g.) in dry ether (100 c.c.) was added dropwise to the refluxing solution over 45 minutes. The mixture was heated under reflux for a further $4^1/_2$ hours, poured carefully on to ice and acidified with dilute sulphuric acid. The ethereal layer was separated, the aqueous layer twice extracted with ether and the combined ethereal solutions washed with dilute sodium hydrogen carbonate solution and water and dried (Na_2SO_4). The ether was removed under reduced pressure, giving an amorphous residue which was acetylated with acetic anhydride in pyridine at room temperature in the usual way. The acetylated material (2.0 g.) was dissolved in benzene-light petroleum (1:1; 100 c.c.) and chromatographed on acid-washed, activated alumina (grade II, 50 g.) to give the following fractions:

Fraction	Solvent		Eluate
(1)	Benzene-light petroleum	(1:1) 100 c.c.	0.08 g.
(2)	Benzene-light petroleum	(1:1) 50 c.c.	0.12 g.
(3)	Benzene-light petroleum	(3:2) 100 c.c.	0.39 g.
(4)	Benzene-light petroleum	(3:2) 300 c.c.	
	Benzene-light petroleum	(3:1) 100 c.c.	
	Benzene-light petroleum	(9:1) 100 c.c.	0.67 g.
	Benzene	100 c.c.	
	Benzene-methanol	(99:1) 100 c.c.	

Fraction	Solvent	Eluate
(5)	Benzene-methanol (98:2) 200 c.c.	0.77 g.

Fraction (1) could not be crystallised.

Fraction (2) crystallised from chloroform-methanol as plates, m.p.158-161°, undepressed on mixing with a synthetic mixed crystal of ergosteryl acetate and dehydroergosteryl acetate of m.p.162-164° (see experiment (b) below). Light absorption: maxima at 2720Å (ε = 8200), 2820Å (ε = 8200), 2940Å (ε = 6000), 3100Å (ε = 2900) and 3250Å (ε = 2600) with an inflection at 3400Å (ε = 1900).

Fraction (3) crystallised as needles, m.p.170-172°, undepressed on mixing with dehydroergosterol peroxide acetate, $[\alpha]_D$ +95° (c, 0.6).

Fraction (4) crystallised from chloroform-methanol as plates, m.p.217-220°, undepressed on mixing with ergosta--7:9(11):22-trien-3β:5ξ-diol 3-acetate, $[\alpha]_D$ +45° (c, 1.1).

Fraction (5) would not crystallise.

(b) Lithium aluminium hydride (0.4 g., 70% soluble in ether) was added to dry ether (50 c.c.) and heated under reflux until no more solid dissolved. A solution of dehydroergosterol peroxide (2 g.) in dry ether (50 c.c.) was added dropwise, over 10 minutes, to the refluxing solution and refluxing continued for a further 21 hours. The reaction mixture was worked up and acetylated as described in (a) to give an amorphous product, part of which

(1.7 g.) was dissolved in benzene-light petroleum (1:4; 100 c.c.) and chromatographed on acid-washed, activated alumina (grade II, 50 g.) as follows:

Fraction	Solvent		Eluate
(1)	Benzene-light petroleum	(1:4) 150 c.c.	0.04 g.
(2)	" " "	(1:4) 100 c.c.	0.24 g.
(3)	" " "	(1:4) 400 c.c.	0.44 g.
(4)	" " "	(1:4) 300 c.c.	0.07 g.
(5)	" " "	(1:4) 200 c.c.	0.06 g.
(6)	" " "	(1:1) 100 c.c.	0.08 g.
(7)	" " "	(1:1) 600 c.c.	0.24 g.
(8)	" " "	(4:1) 500 c.c.	0.15 g.
(9)	Benzene	100 c.c.	0.03 g.
-	Benzene-methanol	(199:1) 200 c.c.	-
(10)	Benzene-methanol	(9:1) 50 c.c.	0.37 g.

Fraction (1) crystallised as plates from chloroform-methanol, m.p.149-153°, mixed m.p. with fraction 2 = 148-154°. This fraction is probably similar to fraction (2) in composition.

Fraction (2) crystallised from chloroform-methanol as plates, m.p.160-163°, undepressed on mixing with a synthetic mixed crystal of ergosteryl acetate and dehydroergosteryl acetate of m.p.162-164°, $[\alpha]_D$ +16° (c, 1.0).

Analysis: Found: C,82.1; H,10.7

Required: C,82.4; H,10.3%.

The "required" values are for a mixed crystal, ratio of ergosteryl acetate to dehydroergosteryl acetate ≒ 6:4, calculated from optical rotation and light absorption. Light absorption: maxima at 2720Å (ε = 7500), 2820Å (ε = 8000), 2940Å (ε = 6000), 3120Å (ε = 4100), 3260Å (ε = 4400) and 3420Å (ε = 2900).

Fraction (3) crystallised from methanol as needles, m.p.170-173°, undepressed when mixed with dehydroergosterol peroxide acetate, $[\alpha]_D$ +91° (c, 0.6).

Fraction 4 crystallised from chloroform-methanol as plates, m.p.248-250°, pale yellow colour with tetranitromethane. There was insufficient pure material available for analysis.

Fraction (6) crystallised from chloroform-methanol as plates, m.p.216-220°, undepressed on mixing with ergosta-7:9(11):22-trien-3β:5ξ-diol 3-acetate.

Fractions 5, 7,8,9 and 10 did not crystallise satisfactorily, were combined (0.47 g.), dissolved in light petroleum (75 c.c.) and rechromatographed on acid-washed, activated alumina (grade II, 15 g.).

Fraction	Solvent		Eluate
-	Light petroleum	125 c.c.	-
-	Benzene-light petroleum (1:4)	100 c.c.	-
(1)	" " " (1:1)	100 c.c.	0.03 g.
(2)	" " " (1:1)	200 c.c.	0.08 g.
(3)	" " " (1:1)	250 c.c.	0.10 g.
-	" " " (1:1)	150 c.c.	-
-	" " " (3:1)	200 c.c.	-
-	Benzene	100 c.c.	-
(4)	Benzene-methanol (99:1)	100 c.c.	0.22 g.

Fraction (1) would not crystallise.

Fraction (2) crystallised from chloroform-methanol as plates, m.p.216-219°, undepressed on mixing with ergosta-7:9(11):22-trien-3β:5ξ-diol 3-acetate.

Fraction (3), on crystallisation from methanol, yielded ergosta-7:9(11):22-trien-3β:5ξ-diol 3-acetate, m.p. and mixed m.p.214-220°. The mother liquors contained a second, lower melting component which was not isolated in pure form.

Fraction (4), on long standing in methanol, deposited a small quantity of needles, m.p.185-188°, no colour with tetranitromethane.

Analysis: Found: C,74.4; H,10.7%,

α-Spinasteryl Acetate.— An extract from lucerne was supplied by Dr. W. Mitchell of Stafford Allen and Sons, Ltd., to whom the author expresses his thanks. The non-saponifiable matter (105 g.), from lucerne, in benzene (2 l.) was chromatographed on alumina (grade II, 1000 g., 50 x 6 cm.). The fractions eluted with benzene (7.5 l.), benzene-ether (9:1, 1 l.), benzene-ether (7:3, 1 l.), benzene-ether (1:1, 3 l.) and benzene-methanol (199:1, 3 l.) were discarded. Continued elution with benzene-methanol (199:1, 8 l.) gave a brown wax (approx. 50 g.). A solution of this wax in pyridine (200 c.c.) and acetic anhydride (40 c.c.) was kept at room temperature. After 18 hours the separated crystalline solid (8 g.) was collected and recrystallised from methanol-chloroform to give α-spinasteryl acetate (2.7 g.) as plates, m.p.182-185°, $[\alpha]_D$ -5° (c, 1.9).

Analysis: Found: C,82.0; H,11.4

Calc. for $C_{31}H_{50}O_2$: C,81.9; H,11.1%.

A solution of the solid, obtained from the acetic-anhydride-pyridine mother liquors by precipitation with water, in benzene (1 l.) was chromatographed on alumina (grade II, 1000 g., 50 x 6 cm.) and the column washed with benzene (2 l.). The solid (12 g.) obtained by evaporation of the benzene filtrate was crystallised from chloroform-methanol to give α-spinasteryl acetate (2.8 g.), m.p.177-

180°, $[\alpha]_D$ -3° (c, 2.5).

α-Spinasterol.— A solution of α-spinasteryl acetate (100 mg.) in methanolic potassium hydroxide solution (2%; 20 c.c.) was heated under reflux for 2 hours, and cooled. The crystalline solid which separated was isolated and crystallised from chloroform-methanol to give α-spinasterol as plates, m.p.167-169°, $[\alpha]_D$ -1° (c, 2.0).

Analysis: Found: C,82.2; H,11.8

Calc. for $C_{29}H_{48}O.{}^1/_2H_2O$: C,82.6; H,11.7%.

Tetrabromostigmastenyl Acetate.— A solution of α-spinasteryl acetate (500 mg.) in dry ether (35 c.c.) was treated at 0° with a solution of dry bromine in glacial acetic acid (10%; 3.5 c.c.) with shaking. The mixture was immediately cooled to -50° and then allowed to attain 0° over 4 hours. The ether was removed under reduced pressure at room temperature and the solid (190 mg.; m.p.123-127°) separating from the acetic acid solution collected and crystallised from light petroleum from which tetrabromostigmastenyl acetate separated as clusters of small plates, m.p. 130-132° (decomp.), $[\alpha]_D$ +239° (c, 0.5 in benzene).

Analysis: Found: C,48.65; H,6.4; Br,40.8

$C_{31}H_{48}O_2Br_2$ requires: C,48.2; H,6.3; Br,41.4%.

The tetrabromide is unstable and becomes discoloured on standing.

22:23-Dibromostigmasta-7:9(11)-dien-3β-yl Acetate.— A solution of tetrabromostigmastenyl acetate (130 mg.) in benzene (10 c.c.) was mixed with one of sodium iodide (0.6 g.) in ethanol (10 c.c.). After 18 hours the mixture was diluted with water, the benzene layer separated and the aqueous layer extracted with benzene. The combined benzene solutions were washed successively with sodium thiosulphate solution and water and dried (Na_2SO_4). The solution was concentrated to a bulk of 50 c.c. under reduced pressure and percolated through a column of acid-washed, activated alumina (grade II, 1.5 g., 2 x 1 cm.). The filtrate was evaporated and the residual solid crystallised from methanol-chloroform to give 22:23-dibromostigmasta-7:9(11)-dien-3β-yl acetate as needles (70 mg.), m.p.203-205° (decomp.), $[\alpha]_D$ +35° (c, 0.6).

Analysis: Found: C,60.8; H,8.2

$C_{31}H_{48}O_2Br_2$ requires: C,60.8; H,7.9%.

Light absorption: maxima at 2350Å (ε = 16,000) and 2420Å (ε=18000) with an inflection at 2500Å (ε = 12,000). The dibromide gives an orange-red colouration with tetranitromethane in chloroform.

Stigmasta-7:9(11):22-trien-3β-yl Acetate.— (a) From 22:23--dibromostigmasta-7:9(11)-dien-3β-yl acetate. A solution of the dibromide (53 mg.) in a mixture of ether (10 c.c.) and ethanol (15 c.c.) was heated under reflux for 3 hours

with activated zinc dust (300 mg.). The zinc was removed by filtration, the filtrate washed with water, dried and the solvent removed under reduced pressure. The residue was crystallised from methanol-chloroform to give stigmasta-7:9(11):22-trien-3β-yl acetate as plates (28 mg.), m.p.164-167°, $[\alpha]_D$ +45° (c, 0.7).

Analysis: Found: C,82.4; H,10.8

$C_{31}H_{48}O_2$ requires: C,82.2; H,10.7%.

Light absorption: maxima at 2360Å (ε = 17,000) and 2420Å (ε = 19,000) with an inflection at 2500Å (ε = 12,500). The compound gives an orange-red coloration with tetranitromethane in chloroform.

(b) By mercuric acetate dehydrogenation of α-spinasteryl acetate. A solution of α-spinasteryl acetate (1.5 g.) in dry chloroform (25 c.c.) was mixed with a solution of mercuric acetate (3.5 g.) in glacial acetic acid; mercurous acetate quickly separated. The mixture was shaken for 22 hours, the mercurous acetate (2.5 g.) collected and washed with chloroform. The combined filtrate and washings were concentrated under reduced pressure below 50° and the solid (450 mg.), which separated on cooling, recrystallised from methanol-chloroform to give stigmasta-7:9(11):22-trien-3β-yl acetate as plates (390 mg.), m.p.159-163°, undepressed on mixing with a specimen prepared as described in (a), $[\alpha]_D$ +40° (c, 2.4).

Analysis: Found: C,82·1; H,11·0%.

Light absorption: maxima at 2360Å (ε = 16,000) and 2420Å (ε = 18,000) with an inflection at 2500Å (ε = 12,000).

(c) By bromination of α-spinasteryl acetate. A solution of α-spinasteryl acetate (400 mg.) in dry ether (30 c.c.) at 0° was treated with a solution of dry bromine in glacial acetic acid (8%; 2.5 c.c.) with shaking. The solution was cooled to approximately -40°, maintained at this temperature for 3 hours, treated with activated zinc dust (3 g.) and the mixture stirred for 3 hours at -40° and kept at 0° overnight. After filtration, the solution was washed with water, dried (Na_2SO_4) and the solvent removed under reduced pressure. A solution of the solid residue in benzene (100 c.c.) was filtered through a column of alumina (grade II, 10 g., 5 x 1.7 cm.) and the column washed with benzene (250 c.c.). The combined benzene filtrates were evaporated and the residue crystallised from methanol-chloroform to give stigmasta--7:9(11):22-trien-3β-yl acetate as plates (185 mg.) m.p.163-166°, $[\alpha]_D$ +46° (c,0.7).

Analysis: Found: C,82.2; H,10.8%.

Light absorption: maxima at 2360Å (ε = 16,000) and 2420Å (ε = 17,000) with an inflection at 2500Å (ε = 10,500).

Bromination-Debromination of α-Spinasteryl Acetate.—

(a) α-Spinasteryl acetate (500 mg.) in dry ether (50 c.c.) was treated at -10° with a solution of dry bromine in glacial acetic acid (10%; 2.5 c.c.). The mixture was cooled to -40°,

allowed to reach room temperature over 4 hours, then washed with water, diluted with ether (50 c.c.), activated zinc dust (6 g.) and ethanol (150 c.c.) added and the mixture heated under reflux for 3 hours. After filtering, the solution was washed with water, the ethereal layer separated, dried (Na_2SO_4) and evaporated to leave a pale yellow resin. The resin, on digesting with methanol, gave an amorphous solid (250 mg.) which exhibited an orange-red colour with chloroformic tetranitromethane. The solid, after many recrystallisations from chloroform-methanol, yielded plates (27 mg.), m.p.160-164°, $[\alpha]_D$ -17° (c,0.7). Light absorption: maxima at 2360Å (ε = 7600) and 2420Å (ε = 8500) with an inflection at 2500Å (ε = 6000).

(b) α-Spinasteryl acetate (500 mg.) in dry ether (50 c.c.) was treated at -5° with a solution of dry bromine in glacial acetic acid (10%; 2.5 c.c.). The mixture was cooled to approximately -40°, maintained at this temperature for 4 hours, and then placed in an ice bath and stirred with activated zinc dust (3.5 g.) for $^1/_2$ hour. The zinc was collected and ethanol (50 c.c.) and zinc dust (5 g.) added to the filtrate. The mixture was heated under reflux for $2^1/_2$ hours and working up as in (a) gave a product which crystallised from chloroform-methanol, as plates (175 mg.) m.p.155-159°, $[\alpha]_D$ +30° (c,1.0). Light absorption: maxima at 2360Å (ε = 12,400) and 2420Å (ε = 14,000) with an inflection at 2500Å (ε = 9000).

Stigmasta-7:9(11):22-trien-3β-ol.— Stigmasta-7:9(11):22--trien-3β-yl acetate (100 mg.) in methanolic potassium hydroxide solution (1%; 30 c.c.) was heated under reflux for 1½ hours. The solution was cooled (needles separated), diluted with water and the product isolated by ether. The product was crystallised from chloroform-methanol to give stigmasta-7:9(11):22-trien-3β-ol as needles (70 mg.), m.p. 164-165°, $[\alpha]_D$ +44° (c,0.5).

Analysis: Found: C,84.5; H,11.2

$C_{29}H_{46}O$ requires: C,84.8; H,11.3%.

Light absorption: maxima at 2360Å (ε = 14,000) and 2430Å (ε = 16,000) with an inflection at 2500Å (ε = 10,500).

Stigmasta-7:9(11):22-trien-3β-ol was acetylated with acetic anhydride in pyridine at room temperature to give stigmasta-7:9(11):22-trien-3β-yl acetate as plates, m.p. 161-163°, $[\alpha]_D$ +45° (c,0.8).

9α:11α-Epoxystigmasta-7:22-dien-3β-yl Acetate.— Stigmasta--7:9(11):22-trien-3β-yl acetate (200 mg.) was dissolved in dry chloroform (1 c.c.), the solution cooled to ca. -5° and treated with a solution of perbenzoic acid in chloroform (39.5 mg./c.c., 2.1 c.c., 1.35 atoms O) added dropwise with stirring during 3 hours. The reaction mixture was kept overnight at 0°, diluted with chloroform, washed with sodium hydrogen carbonate solution and water, and dried (Na_2SO_4). The residue, obtained on removal of the solvent under reduced pressure at about 40°, crystallised from acetone to give 9α:11α-epoxystigmasta-7:22-dien-3β-yl acetate

as plates (75 mg.), m.p.204-208°. Further recrystallisation from acetone raised the m.p. to 209-212°, $[\alpha]_D$ -20°, -17° (c,1·4, 0.6).

Analysis: Found: C,79.6; H,10.6

$C_{31}H_{48}O_3$ requires: C,79.4; H,10.3%.

The epoxide gave a pale yellow colour with tetranitromethane and showed no selective absorption in the ultraviolet spectrum above 2200 Å.

Sulphuric Acid Hydrolysis of 9α:11α-Epoxystigmasta-7:22--dien-3β-yl Acetate.— The epoxide (50 mg.) was dissolved in pure dioxan (37.5cc)and 2N sulphuric acid (6.75 c.c.) added. The mixture was shaken until cloudiness disappeared (approximately 20 seconds), then placed in a 4 dcm. polarimeter tube and readings taken as follows. Zero = 0.07°.

Time (minutes)	Reading	Time (minutes)	Reading
0	-	17	0.32°
1	-	18	0.32°
2	0.18°	19	0.32°
3	0.16°	20	0.31°
4	0.20°	21	0.32°
5	0.23°	22	0.32°
6	0.27°	23	0.31°
7	0.27°	24	0.31°
8	0.28°	25	0.31°
9	0.30°	26	0.31°
10	0.31°	27	0.30°
11	0.31°	28	0.30°
12	0.32°	29	0.30°
13	0.33°	30	0.30°
14	0.32°	35	0.29°
15	0.33°	40	0.27°
16	0.32°	45	0.26°
		After 12 hours	359.99°

The reaction mixture was kept at room temperature for a total of 84 hours and then poured into a mixture of ether and aqueous sodium hydrogen carbonate solution and thoroughly shaken. The ether layer was separated, washed with water, dried (Na_2SO_4) and the solvent evaporated. The residue was acetylated in the usual manner, the acetylated product isolated by ether and crystallised from methanol as plates. After several recrystallisations from the same solvent, the product had m.p.179-185°, $[\alpha]_D$ -33° (c,0.4). Light absorption: maximum at 2500 Å (ε = 8000). (No analysis available).

Stigmasta-8:22-dien-3β:7ξ:11α-triol 3-Acetate. — (a) 9α:11α-Epoxystigmasta-7:22-dien-3β-yl acetate (50 mg.) in pure dioxan (37.5 c.c.) was treated with 2N sulphuric acid (6.75 c.c.) and the mixture allowed to stand at room temperature for 15 minutes. It was poured into ether--aqueous sodium hydrogen carbonate solution and well shaken. The ether layer was separated, washed with water, dried (Na_2SO_4) and the solvent removed under reduced pressure. The residue crystallised from acetone to give stigmasta-8:22-dien-3β:7ξ:11α-triol 3-acetate (32 mg.) as needles (slowly formed from an initial gel), m.p.230-238°, (inserted at 215°), $[\alpha]_D$ +90°, +93° (c,0·6, 0.7).

Analysis: Found: C,76.2; H,10.6

$C_{31}H_{50}O_4$ requires: C,76.5; H,10.4%.

The compound showed no selective absorption above 2200 Å in the ultraviolet spectrum. The m.p. appears to be no criterion of purity; if heating is commenced at room temperature, the compound melts over a wide temperature range.

(b) Stigmasta-7:9(11):22-trien-3β-yl acetate (1.3 g.) was treated with perbenzoic acid as described on p. 115 The amorphous oxidation product (1.1 g.) which showed no ultraviolet absorption above 2200Å, was dissolved in pure dioxan (500 c.c.), 2N sulphuric acid (125 c.c.) added and the mixture allowed to stand at room temperature for 12 minutes. The reaction mixture was worked up as described in (a) to give stigmasta-8:22-dien-3β:7ξ:11α-triol 3--acetate as needles (330 mg.) from acetone (separated as gel), m.p.231-238° (inserted at 220°), alone or when mixed with a specimen prepared as described in (a), $[\alpha]_D$ +90°, +90° (c,0.75, 0.7).

Analysis: Found: C,76.75; H,10.3%.

8α:9α-Epoxy-7:11-diketostigmast-22-en-3β-yl Acetate.— (a) Stigmasta-8:22-dien-3β:7ξ:11α-triol 3-acetate (320 mg.) in suspension in dry chloroform (2 c.c.) at -3° was treated with chloroformic perbenzoic acid solution (40 mg./c.c., 3.5 c.c., 1.5 atoms O) added dropwise with stirring during

45 minutes. The reaction mixture, which still contained suspended solid, was kept at 0° for 3 hours, when all solid had dissolved, then at room temperature for $1^1/_2$ hours. The mixture was diluted with chloroform, washed with sodium hydrogen carbonate solution and water, dried and evaporated under reduced pressure. The resulting amorphous residue (330 mg.) was dissolved in stabilized glacial acetic acid (20 c.c.) and N/1 chromic acid-acetic acid solution (3.5 c.c., 2.6 atoms O), diluted with stabilized glacial acetic acid (25 c.c.), added dropwise with stirring at room temperature during $2^1/_2$ hours. The reaction mixture was kept at room temperature for 18 hours, treated with methanol (10 c.c.) and water (1 c.c.) and allowed to stand for a further 2 hours at room temperature. The mixture was concentrated under reduced pressure, diluted with water and the reaction product isolated by ether to give 8α:9α-epoxy-7:11-diketostigmast-22-en-3β-yl acetate as long needles (135 mg.) from aqueous methanol, m.p.137-140°. The product was recrystallised from aqueous methanol, m.p.149-151°, $[\alpha]_D$ -50°, -49° (c,0.9, 0.6).

Analysis: Found: C,74.4; H,9.0

$C_{31}H_{46}O_5$ requires: C,74.7; H,9.3%.

The compound showed no selective absorption in the ultraviolet above 2200Å.

(b) A suspension of stigmasta-8:22-dien-3β:7ξ:11α-triol

3-acetate (80 mg.) in a solution of chromic acid in glacial acetic acid (21.5 c.c., 1.03 mg. active O/c.c., 10 drops $2NH_2SO_4$/50 c.c.) was stirred at 0° for 5 minutes. The suspended solid quickly dissolved and the resulting solution was kept for 16 hours at room temperature. Methanol (10 c.c.) and water (2 c.c.) were added and the mixture allowed to stand for a further 2 hours. Isolation of the reaction product as in (a) gave 8α:9α-epoxy-7:11-diketostigmast-22-en-3β-yl acetate as needles (20 mg.), m.p.142-145°. Further recrystallisation raised the m.p. to 151-153°, undepressed on mixing with a specimen prepared as described in (a).

Analysis: Found: C,75.0; H,9.8%.

The amorphous residue (40 mg.) from the oxidation mother liquors was dissolved in petrol (25 c.c.) and adsorbed on acid-washed, activated alumina (grade II, 3.5 x 1 cm.). Petrol-benzene [(1:1), 100 c.c.] eluted an amorphous fraction (8 mg.) which gave needles from aqueous acetone, m.p.123-137°. Light absorption: maximum at 2650Å (ε = 1600).

7:11-Diketostigmast-22-en-3β-yl Acetate. — A solution of 7:11-diketo-8α:9α-epoxystigmast-22-en-3β-yl acetate (77 mg.) in glacial acetic acid (8 c.c.), containing zinc dust (80 mg.) in suspension was heated to boiling point over a period of 20 minutes. Heating under reflux was continued

for 40 minutes during which zinc dust (200 mg.) was added in four portions. The reaction mixture was cooled, filtered and diluted with ether. The ethereal solution was washed with water, dried (Na_2SO_4) and the solvent removed under reduced pressure, leaving 7:11-diketo-stigmast-22-en-3β-yl acetate as an amorphous residue (73 mg.), m.p.178-184°. On crystallisation from aqueous acetone, the dione was obtained as prismatic needles, m.p.187-189°, $[\alpha]_D$ -16°, -15° (c,1.0, 0.7).

Analysis: Found: C,77.1; H,9.8

$C_{31}H_{48}O_4$ requires: C,76.8; H,10.0%.

The product does not show high intensity light absorption above 2200Å.
