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SUMMARY

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Ergosterol has been converted to some 11-oxygenated steroids and to progesterone. The chemistry of some of the intermediates has been investigated.

Oxidation of 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate with one mol. of perbenzoic acid gave 22:23--dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate characterised as the alcohol. Rearrangement of this epoxide with mineral acid gave 22:23-dibromo-7-oxoergost-8-en-3β-yl acetate after reacetylation, which was debrominated with zinc dust to 7-oxoergosta-8:22-dien-3β-yl acetate. Treatment of 22:23-dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate with boron trifluoride afforded 22:23-dibromo-11--oxoergost-8-en-3β-yl acetate which on debromination gave 7-oxoergosta-8:22-dien-3β-yl acetate. Catalytic hydrogenation of 22:23-dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate and of 22:23-dibromo-11-oxoergost-8-en-3β-yl acetate in acetic acid yielded 22:23-dibromoergost-8(14)--en-3β-yl acetate. Reduction of 11-oxoergost-8:22-dien--3β-yl acetate with lithium in liquid ammonia gave 11-oxo--ergost-22-en-3β-ol acetylated to 11-oxoergost-22-en-3β-yl acetate. Reduction of 22:23-dibromo-11-oxoergost-8-en--3β-yl acetate under the same conditions, followed by reacetylation, afforded 3β:11α-diacetoxyergost-22-ene

characterised, by basic hydrolysis, as the diol.

Simple hydrolysis of 22:23-dibromo-11-oxoergost-8--en-3β-yl acetate gave the alcohol whereas stronger alkaline conditions gave, after reacetylation 22:23--dibromo-11-oxo-14β-ergost-8-en-3β-yl acetate or 22:23--dibromo-11-oxoergost-8(14)-en-3β-yl acetate, depending on the conditions employed. Treatment of 22:23-dibromo--11-oxoergost-8-en-3β-yl acetate or of 22:23-dibromo-11--oxoergost-8(14)-en-3β-yl acetate with hydrogen chloride or treatment of the latter with strong alkali also yielded 22:23-dibromo-11-oxo-14β-ergost-8-en-3β-yl acetate after reacetylation. Debromination of 22:23--dibromo-11-oxoergost-8(14)-en-3β-yl acetate and of 22:23--dibromo-11-oxo-14β-ergost-8-en-3β-yl acetate afforded 11-oxoergosta-8(14):22-dien-3β-yl acetate and 11-oxo-14β--ergosta-8:22-dien-3β-yl acetate respectively, which were characterised as their alcohols by basic hydrolysis. Enol acetylation of 22:23-dibromo-11-oxoergost-8-en-3β-yl acetate gave 3β:11-diacetoxy-22:23-dibromoergosta-7:9(11)--diene, whilst 22:23-dibromo-11-oxo-14β-ergost-8-en-3β-yl acetate, under the same conditions, afforded the corresponding 3β:11-diacetoxy-22:23-dibromo-14β-ergosta-7:9(11)--diene. Debromination of these enol acetates with zinc dust gave 3β:11-diacetoxyergosta-7:9(11):22-triene and

3β:11-diacetoxy-14β-ergosta-7:9(11):22-triene respectively. The course of the action of alkali on 22:23-dibromo-11--oxoergost-8-en-3β-yl acetate has been examined spectroscopically.

Hydrogenation of 22:23-dibromoergosta-7:9(11)-dien--3β-yl acetate gave 22:23-dibromoergost-8(14)-en-3β-yl acetate, or 22:23-dibromoergost-7-en-3β-yl acetate, depending on the solvent employed; they were characterised as the alcohols and as the derived benzoates. Debromination of 22:23-dibromoergost-8(14)-en-3β-yl acetate and of 22:23-dibromoergost-7-en-3β-yl acetate afforded ergosta--8(14):22-dien-3β-yl acetate and ergosta-7:22-dien-3β-yl acetate respectively. Treatment of 22:23-dibromoergost--8(14)-en-3β-yl acetate or of 22:23-dibromoergosta-7-en--3β-yl acetate with hydrogen chloride in chloroform gave mixed crystals (22:23-dibromo-''β''-dihydroergosteryl acetate). Likewise treatment of 22:23-dibromoergost-8(14)--en-3β-yl benzoate gave mixed crystals. Debromination of 22:23-dibromo-''β''-dihydroergosteryl acetate gave ''β''--dihydroergosteryl acetate characterised as the alcohol and derived benzoate. Hydrogenation of 22:23-dibromo-''β''--dihydroergosteryl acetate and removal of the residual unsaturated material yielded 22:23-dibromoergostan-3β-yl

acetate. Debromination of the latter gave ergost-22-en--3β-yl acetate converted by basic hydrolysis to the alcohol. Some anomalous molecular rotations are discussed.

Oxidation of ergosterol gave ergosterone which on rearrangement yielded isoergosterone. Partial reduction of the latter by either catalytic hydrogenation or by chemical methods afforded ergost-4:22-dien-3-one. Attempted 11-oxygenation of ergosta-4:22-dien-3-one by a microbiological method was unsuccessful. Selective catalytic hydrogenation of isoergosterone and of ergosta--4:22-dien-3-one gave 5β-ergost-22-en-3-one which when reduced with lithium aluminium hydride yielded 5β-ergost--22-en-3α-ol. Ozonolysis of the acetate of this compound gave 3α-acetoxybisnorcholan-22-al, characterised as its 2:4-dinitrophenylhydrazone. Oxidation of this aldehyde yielded 3α-acetoxybisnorcholanic acid, treatment of which with diazomethane, gave the methyl ester. Enol acetylation of 3α-acetoxybisnorcholan-22-al afforded an oily compound, which when ozonised yielded 20-oxopregnan-3α-yl acetate with 3α-acetoxypregnan-20α-ol as a by-product, the latter isolated as the diacetate. Basic hydrolysis of 20-oxopregnan-3α-yl acetate gave 20-oxopregnane-3α-ol, which when oxidised afforded pregnane-3:20-dione. Methods

exist for the conversion of the latter to progesterone.

CONTENTS

CONTRIBUTIONS TO PARTIAL SYNTHESES OF CORTISONE.

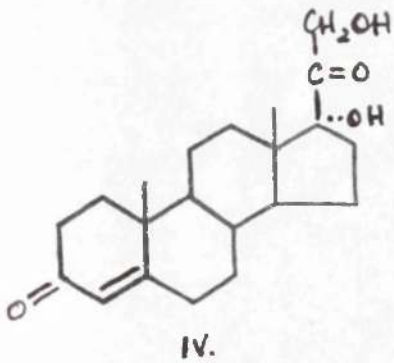
INTRODUCTORY REVIEW

INTRODUCTORY REVIEW

Since 1948 much chemical research has been devoted to the examination of possible partial, or total, synthetic routes to 17-hydroxy-11-dehydrocorticosterone, commonly know as cortisone (Kendall's compound E). The historical background of this and other adrenal cortical hormones originated, almost a quarter of a century ago, with clinical investigations on the physiological effects of an adrenal cortex extract. Rogoff and Stewart (1) in 1929, and Swingle and Pfiffner (2) in 1930, reported that extracts of the adrenal cortex could maintain the life of an adrenalectomised animal which would otherwise have died within a few days. As a result, an extensive series of chemical investigations (3,4,5), aimed at the isolation of the active principles, were initiated in 1935. This led within three years to the isolation of twenty-one crystalline compounds from the crude extract or cortin and, later, to the isolation of seven more. The residual amorphous fractions still possessed physiological activity and very recently there has been isolated (135) from them, what is probably the most physiologically active hormone, electrocortin.

Several methods, and combinations of methods, were

employed to isolate the individual substances. Extraction of the minced adrenal glands with acetone or alcohol gave a protein free concentrate and partition between various solvents gave a fat free extract (6,7,8,9). Further fractionation was accomplished in the formation or hydrolysis of the Girard derivatives, a method introduced by Reichstein (10) for the separation of ketonic from non-ketonic or inert ketonic material. Chromatography of the more stable acetates, however, proved the most efficient method of separation (11,12).



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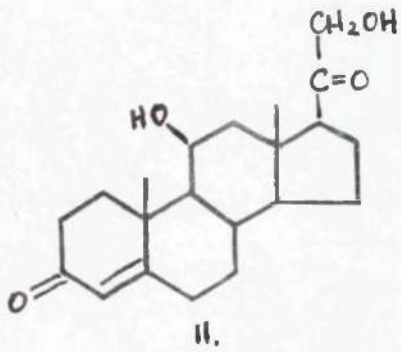

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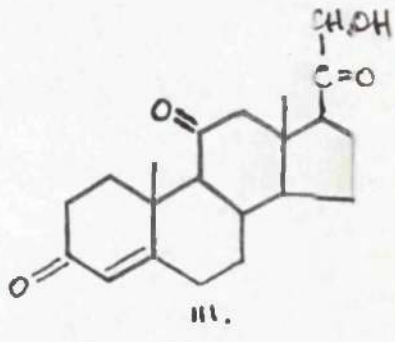

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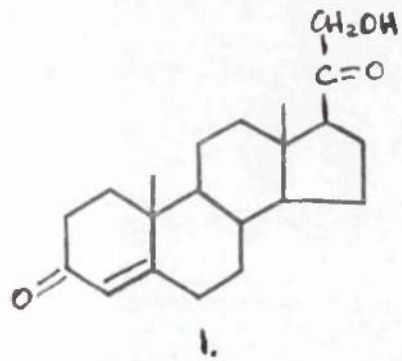

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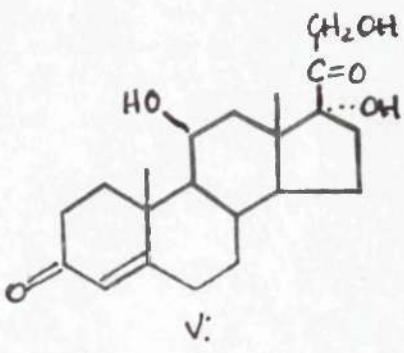

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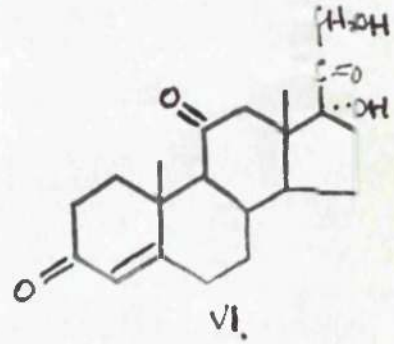

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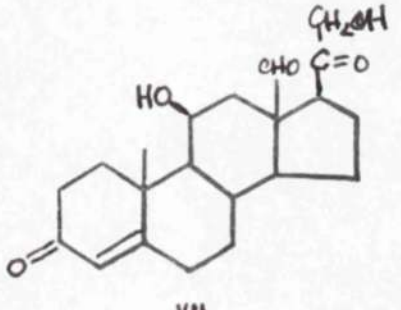


VII.

Seven of the isolated compounds showed activity in maintaining life in an adrenalectomised animal. Their structures are shown above (I-VII). They all posses an $\alpha\beta$-unsaturated ketonic grouping in ring-A, characteristic of progesterone and testosterone, and have a ketol side chain which is highly sensitive to alkali and acid. The $C_{(11)}$-ketone and $C_{(11)}$-β-hydroxyl groups, in agreement with the conformation theory (136,138) are hindered by the axial $C_{(10)}$ and $C_{(13)}$ methyl groups. A $C_{(11)}$ carbonyl group does not react with carbonyl reagents such as phenylhydrazine, hydroxylamine, or semicarbazide, and undergoes the Wolff-Kishner reaction with difficulty. It can, however, be reduced using lithium aluminium hydride, and can be catalytically hydrogenated with a platinum catalyst in acetic acid, both methods giving almost exclusively the 11β-hydroxy derivative. Using normal reaction conditions, this cannot be acylated, but acylation can be achieved using special conditions (13).

A number of companion cortical steroids have been isolated, all of which are reduction products of the hormones (I-VI) above, lacking either the unsaturated grouping in ring-A, or the side chain ketol function. They are inactive in the life maintenance test. Comparison of the seven active hormones indicates that oxygen

functions at $C_{(11)}$ and $C_{(17)}$ are not essential for life maintenance activity, but contribute significantly to other important physiological actions. The adrenal cortex through the medium of the steroidal material it secretes into the bloodstream, controls the electrolyte balance and the carbohydrate and protein metabolism in the liver and muscles. Of the isolated hormones electrocortin (VII) and deoxycorticosterone (I) are the most potent in the first type of activity, while the two 17--hydroxy-11-oxygenated steroids are slightly more active in the second type. In addition the adrenal cortical hormones are also concerned with resistance to shock and normal growth maintenance. Cortisone was recently reported to possess added therapeutic value (14) in that it alleviated the symptoms of rheumatoid arthritis. Since the work described in this thesis was completed, however, the initial promise shown by cortisone in this connection has diminished (141).

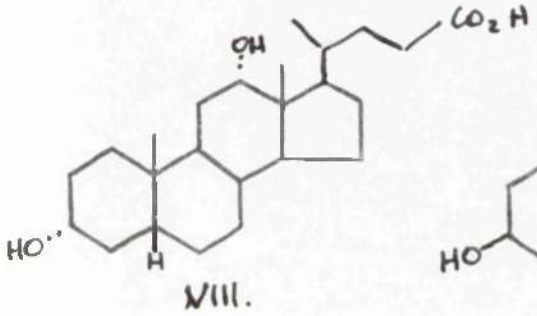
The elucidation of the structures of the various substances isolated from the adrenal cortex proceeded rapidly during 1937-1939, due largely to the researches of Reichstein and Kendall and their associates. Several methods were employed: (a) degradation to known compounds,

(b) correlation with hormones whose structures had already been deduced, and (c) partial syntheses from other steroids. The nature and position in the steroid nucleus of the inert oxygen atom, characteristic of many of these hormones, was finally deduced, by a partial synthesis (15,16) of 11-dehydrocorticosterone (III).

Investigation of the physiological properties of the 11-oxygenated cortical hormones in the biochemical, biological, and medical fields was severely limited by the minute quantities of material available for clinical use. The partial synthesis of cortisone had been achieved previously by Sarett (16,17) in 1946, starting from deoxycholic acid (VIII), a laborious procedure, which had provided, however, sufficient material for clinical testing. The therapeutic effects of cortisone in the treatment of rheumatoid arthritis (14) appeared to be highly specific, for no other compound, apart from the derived dihydrocortisone (V), had comparable action.

It thus became obvious that new synthetic routes were urgently required to produce cortisone economically and in quantity. The bile acid route has been thoroughly examined and despite many subsequent improvements in the procedures involved, it was felt that its complexity

rendered it impracticable, and that an examination of alternative starting materials was desirable. [The bile acid route is, however, the principal route by which cortisone has been made in quantity.]



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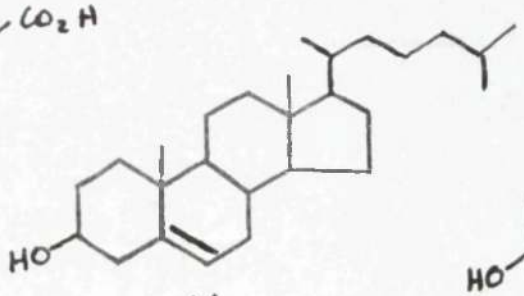

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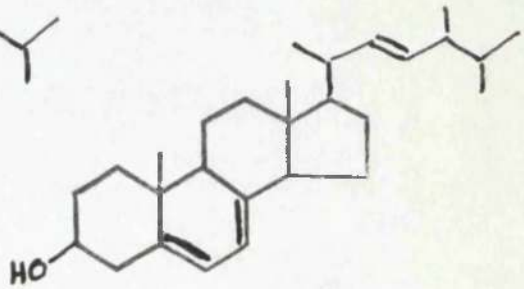

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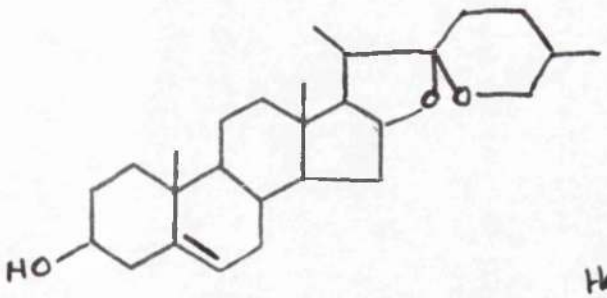

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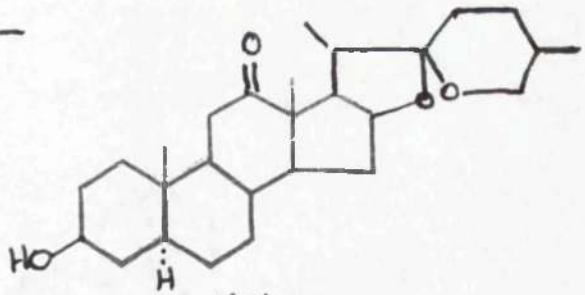

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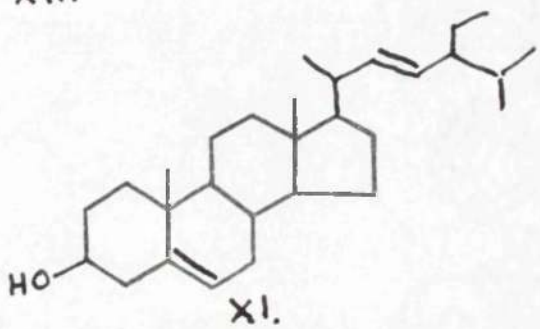
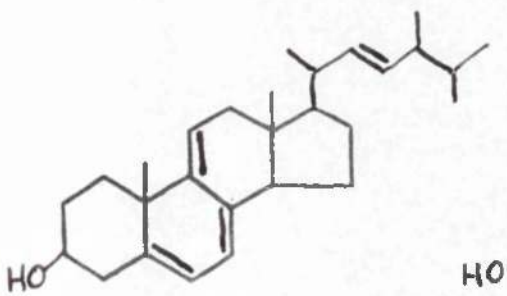


XI.

In the above connection cholesterol (IX), ergosterol (X), stigmasterol (XI), diosgenin (XII), and hecogenin (XIII), were considered. Although there are now methods for converting these steroids into cortisone, each method is

limited by the nature of the steroid employed. Apart from hecogenin, the above steroids are all unsubstituted in ring-C and consequently the problem of the introduction of an 11-oxygen function is more difficult. Cholesterol, in addition, has a saturated side chain and its conversion to a pregnane derivative is accompanied by drastic losses. Stigmasterol is not readily available and it seems unlikely that this position will improve. Ergosterol, the major sterol in the non-saponifiable fraction of yeast, seemed the most promising, since it is easily convertible to two derivatives, namely ergosta--5:7:9(11):22-tetraen-3β-ol (XIV) and ergosta-7:9(11):22--trien-3β-ol (XV) which, because of their $\Delta^{9(11)}$-bond, might form useful intermediates for the introduction of the necessary $C_{(11)}$-oxygen function. A further feature of ergosterol is that the side chain ethylenic linkage provides a point of attack for degradation to a $C_{(17)}$-acetyl group.



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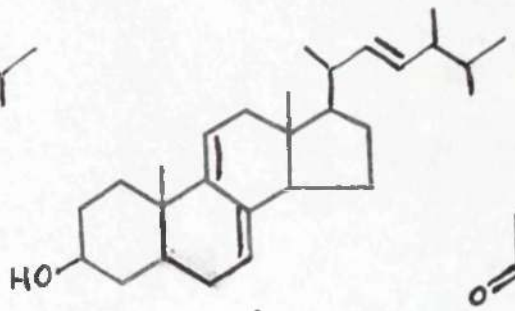

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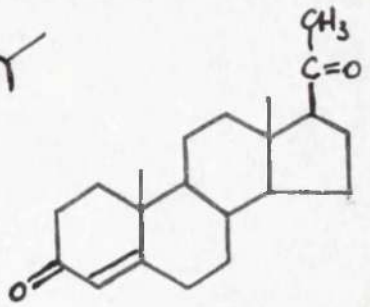


XVI.

In the case of cholesterol, stigmasterol, and diosgenin, the introduction of the $\Delta^{9(11)}$-bond is a lengthier and more wasteful procedure (139,122). However, the side chain of diosgenin is easily degraded (19) to pregnane derivatives and thus this sapogenin is a valuable starting material for ring-C unsubstituted hormones. Progesterone (XVI) itself is easily prepared from diosgenin (19), and in view of the recent microbiological hydroxylation (20) of progesterone at $C_{(11)}$, diosgenin may well assume greater importance. Hecogenin contains a $C_{(12)}$-keto group and seemed a promising starting material, but present methods of harvesting are poor, and purification of the crude material is attended by large losses (123). Improvements in the isolation of this steroidal sapogenin would undoubtedly give it greater prominence as a starting point (140) for a synthesis of cortisone.

The total synthesis of cortisone has been achieved by three different schools (21,124,125). The route due to Woodward and his associates (21) is the most direct and, although in its initial stages it did not appear a sound economic proposition, recent improvements (127) have brought it to a position where it must be regarded as highly competitive with any of the available partial syntheses.

HISTORICAL

HISTORICAL

THE PARTIAL SYNTHESIS OF 11-OXYGENATED STEROIDS, PARTICULARLY FROM ERGOSTEROL.

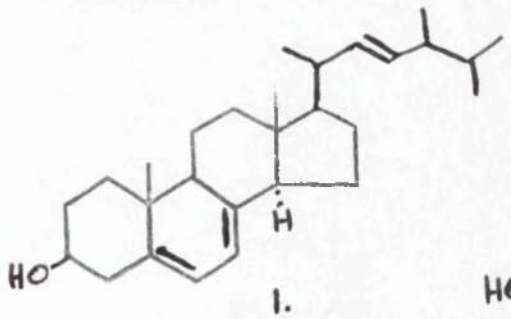
Since 1948, there have appeared many publications concerned with the partial synthesis of 11-oxygenated steroids, materials which might be regarded as possible intermediates for a synthesis of cortisone. The work described in this section is a precis of the methods and experiments employed by chemical research groups, other than that of this department, designed to convert ergosterol to $C_{(11)}$ oxygenated derivatives. The research work carried out in this field by the departmental team has been reported in a series of publications (109-114) and such papers that are pertinent will be discussed in the theoretical section of this thesis.

Ergosterol.

Ergosterol (I), first isolated from ergot, is the major sterol in the non-saponifiable fraction from yeast, which at present constitutes the main source. Widespread interest in ergosterol was first aroused in connection with its relationship to vitamin-D and consequently its chemical behaviour, particularly its photochemistry, has

been extensively investigated. With the establishment of its empirical formula $C_{28}H_{44}O$ by Windaus (22) in 1932, only a short period elapsed before its complete structure was elucidated.

Ergosterol can be converted by sodium-alcohol reduction (23,24) to ergosta-7:22-dien-3β-ol (II). Selective catalytic hydrogenation also gives either (II) or ergost-7-en-3β-ol (III)(25), depending on the conditions employed. The latter compound is isomerised (27) by a platinum catalyst, saturated with hydrogen to ergost--8(14)-en-3β-ol (IV) which can be further rearranged (27) with dry hydrogen chloride to ergost-14-en-3β-ol (β-ergostenol) (V). Barton (28) has confirmed the positions of the double bond in these stenols by molecular rotation methods.



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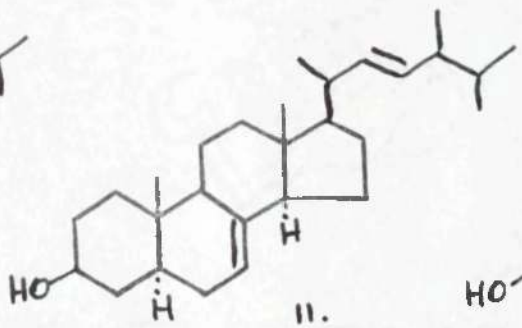

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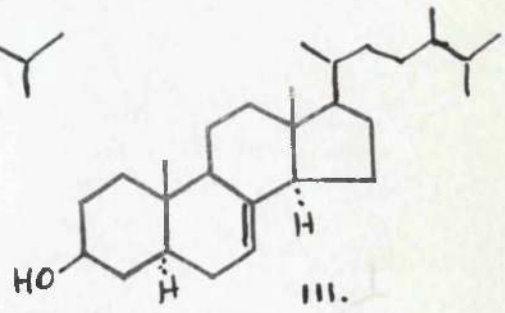

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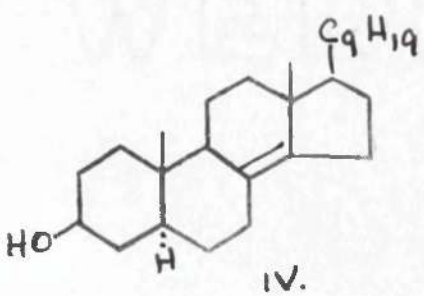

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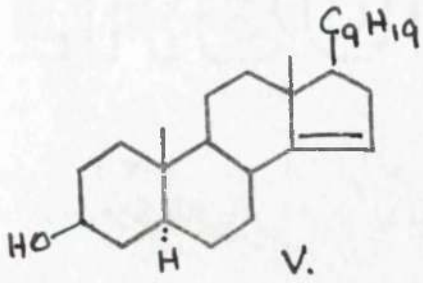
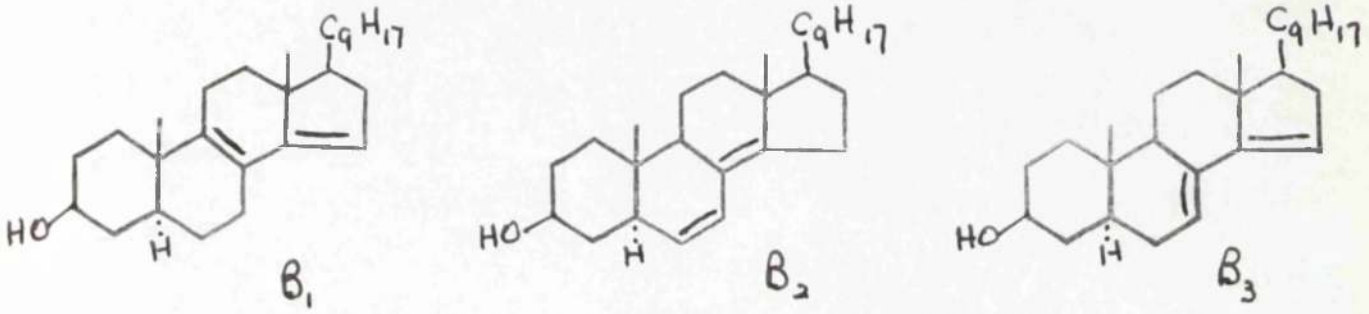


V.

Acid isomerisation of ergosterol (as acetate) leads to a separable mixture of ergosterol-B_1, ergosterol-B_2, and ergosterol-B_3. Each is converted to this mixture by acid treatment.



Windaus et al., (32) and Heilbron, Johnstone, and Spring (31) obtained (VI) by mercuric acetate oxidation of ergosta-7:22-dien-3β-ol and its structure, ergosta-

-7:9(11):22-trien-3β-ol, was finally confirmed by Barton (28) in 1946. Ergosterol itself undergoes a similar oxidation when treated with mercuric acetate (33) giving ergosta-5:7:9(11):22-tetraen-3β-ol (dehydroergosterol)(VII).

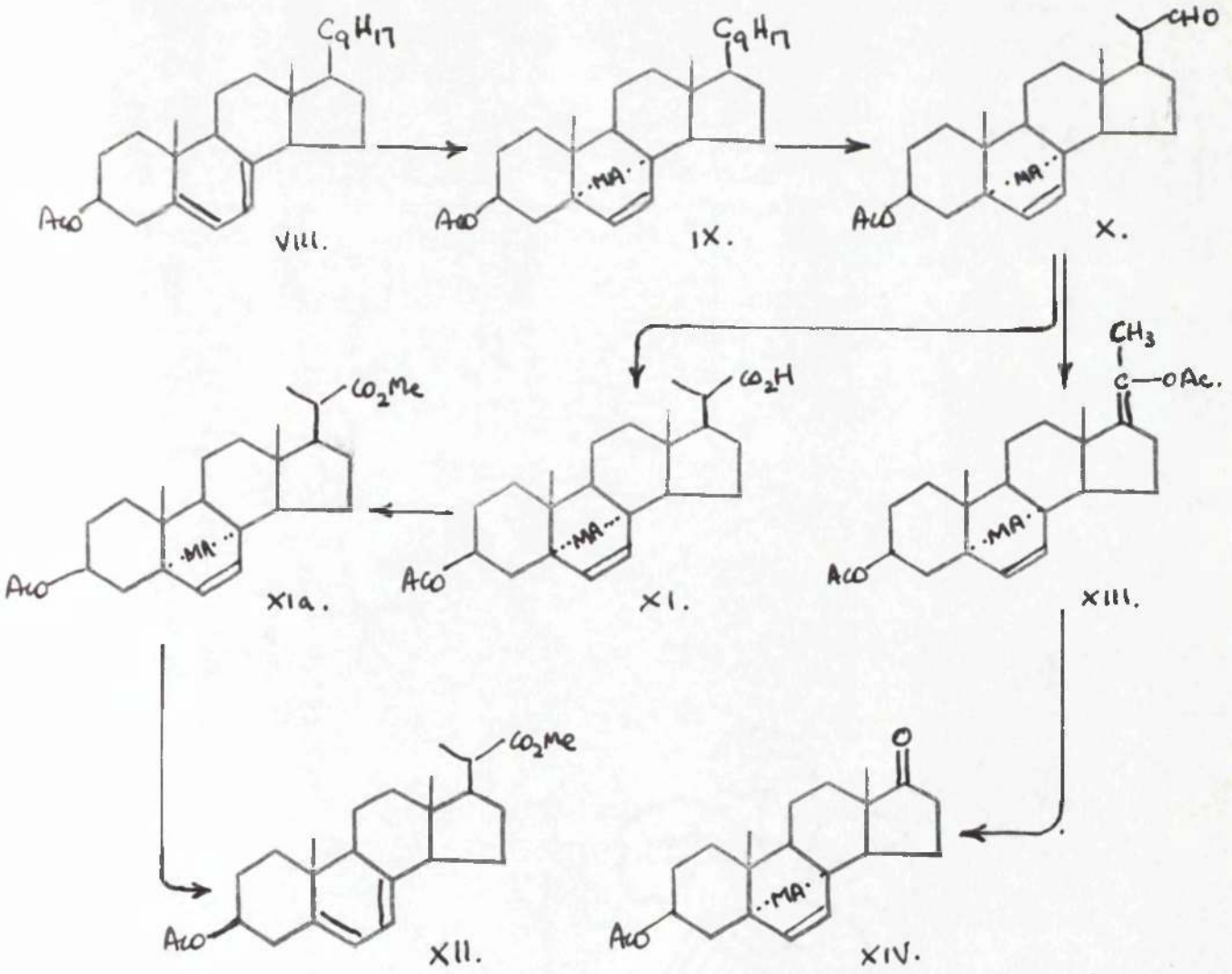
These compounds, (VI) and (VII), are the two ergosterol derivatives which primarily have been used in experiments designed to give $C_{(11)}$-oxygenated steroids and these methods will be described below.

Dehydroergosterol as an Intermediate.

(a) In 1948 Bergmann and Stevens (33) suggested the possible utilisation of dehydroergosterol as an intermediate in the synthesis of adrenal cortical hormones. They investigated two principal problems: firstly, the degradation of the side chain of ergosterol under conditions which would lead to the retention of the conjugated diene system in ring-B, and secondly, the introduction of an oxygen atom at $C_{(11)}$, followed by degradation of the side chain.

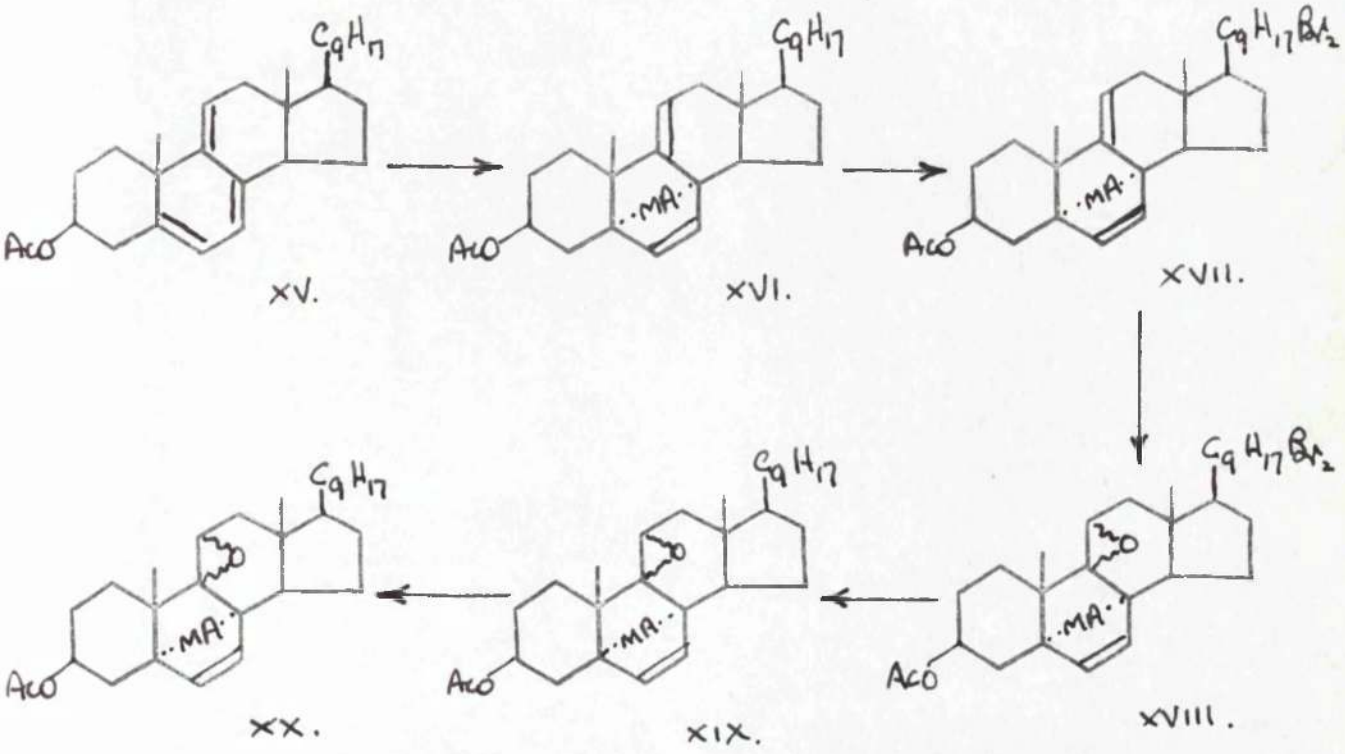
The initial stages of both problems met with reasonable success. Addition of maleic anhydride to ergosteryl acetate (VIII) gave the adduct (IX) which was preferentially oxidised at the 22:23-double bond with ozone, affording the aldehyde (X). Further oxidation of (X) gave the acid (XI), whose methyl ester (XIa) on pyrolysis yielded 3β--acetoxymethylbisnorchola-5:7-dienate (XII) with simultaneous elimination of maleic anhydride. Treatment of the aldehyde (X) with acetic anhydride in a sealed tube gave

an enol acetate, formulated as (XIII), which supposedly gave the maleic anhydride adduct of 17-oxoandrosta-5:7--dien-3β-yl acetate (XIV) on ozonolysis. Pyrolysis of the last compound, however, did not give any identifiable products.



In an attempt to introduce an oxygen atom at the 11-position, the maleic anhydride adduct (XVI) of dehydroergosteryl

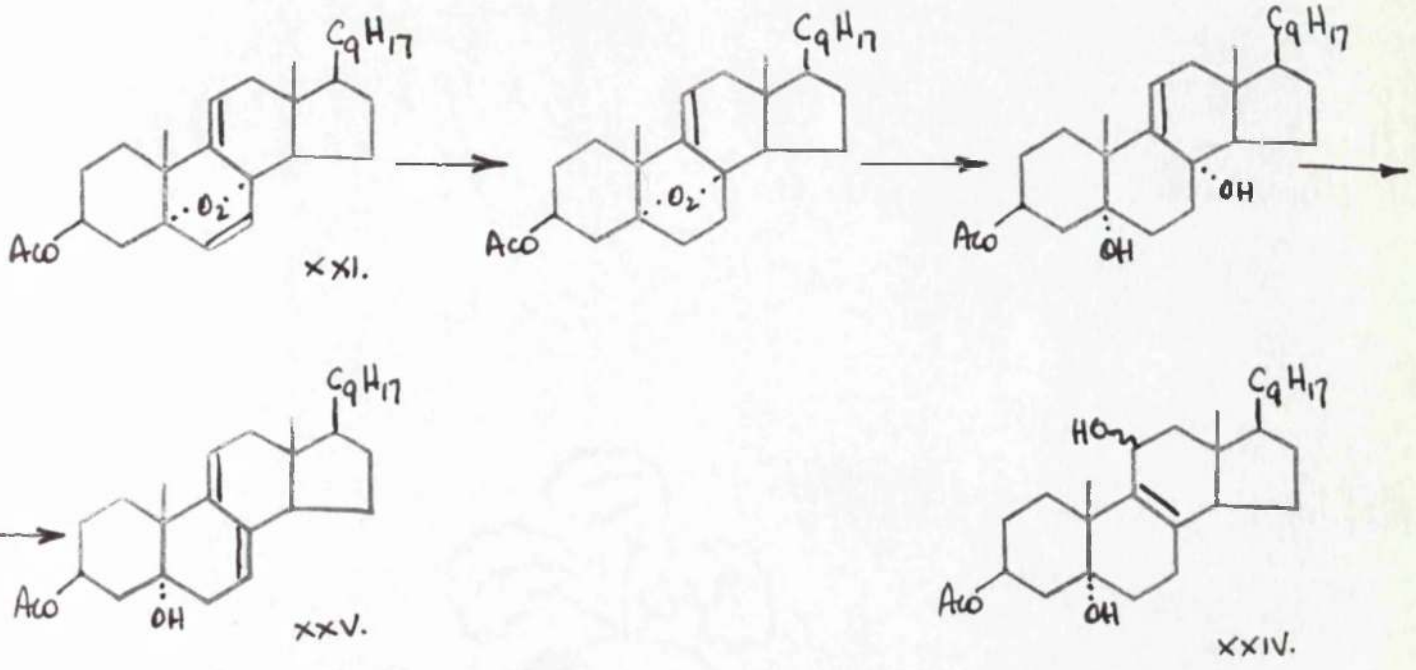
acetate (XV) was prepared and the side chain protected by conversion to the dibromide (XVII). Oxidation of this material with perbenzoic acid readily gave in high yield a 9:11-epoxide (XVIII) which on debromination with zinc in acetic acid yielded the desired adduct (XIX). Ozonolysis of the latter, followed by oxidation of the resulting aldehyde gave the bisnoracid (XX). Unfortunately pyrolysis of (XIX) brought about aromatisation of ring-B, and in view of the low yields involved, and the difficulties encountered in eliminating maleic anhydride from the adducts, the approach was abandoned at this stage.



XV. XVI. XVII.

XVIII. XIX. XX.

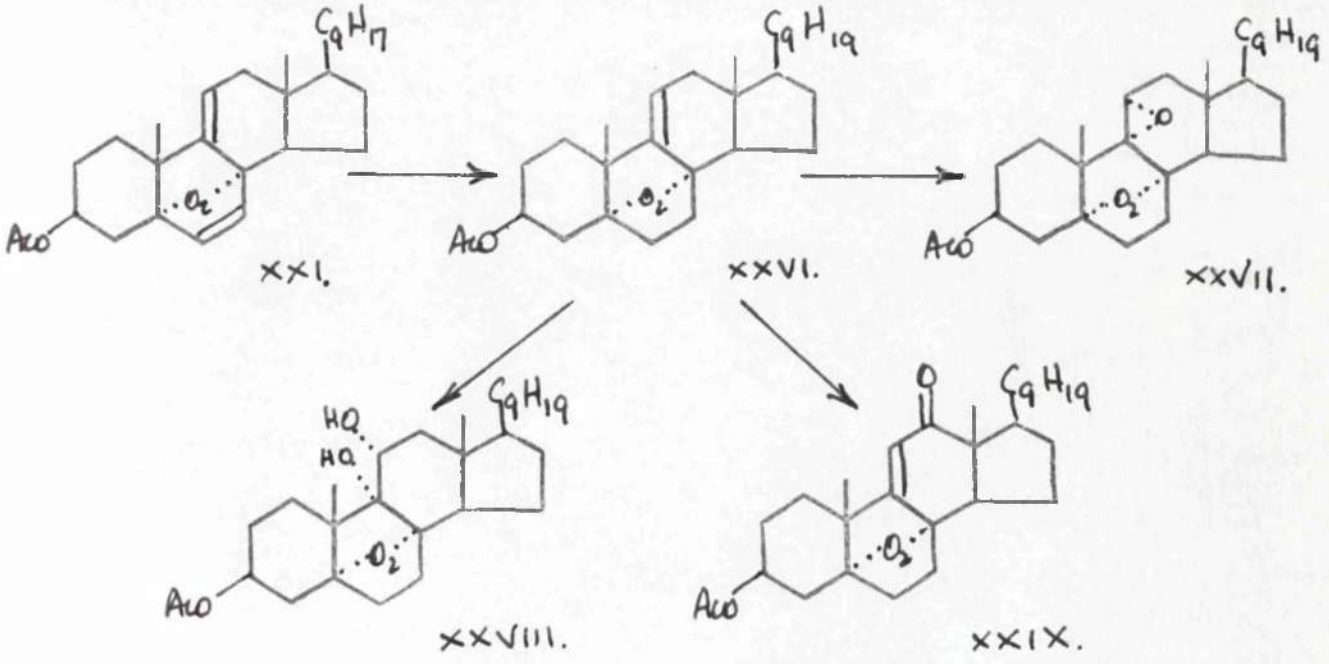
(b) No further successful attempts on the conversion of dehydroergosterol to 11-oxygenated steroids were reported until late 1952, when Jones and his co-workers published their experiments (34-38) in this field. Using as their starting material, 5α:8α-epidioxydehydroergosteryl acetate (XXI), obtained from dehydroergosterol by photoperoxidation and acetylation, they showed that it could be preferentially reduced with a specially prepared platinum catalyst to the dihydroderivative 5α:8α-epidioxyergosta-9(11):22--dien-3β-yl acetate (XXII) in about 50% yield. This in turn was hydrogenated giving 3β-acetoxyergosta-9(11):22--dien-5α:8α-diol (XXIII). It had been hoped that on treatment of this material with mineral acid, an anionotropic rearrangement would have occurred to give 3β--acetoxyergosta-8:22-dien-5α:11-diol (XXIV).



XXI. XXV. XXIV.

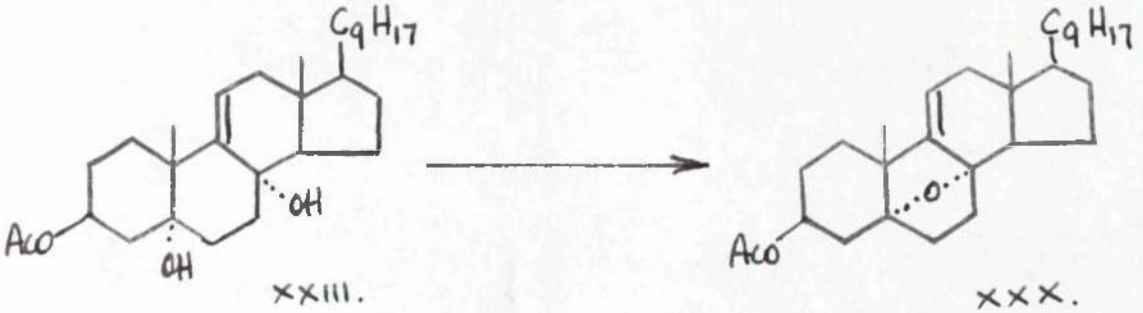
Only a simple dehydration occurred, however, the product being 3β-acetoxyergosta-7:9(11):22-trien-5α-ol (XXV).

Unfortunately it was not found possible to oxidise the $\Delta^{9(11)}$-bond of (XXII) selectively, as the side chain double bond proved to be very much more reactive. The lack of reactivity of the $\Delta^{9(11)}$-linkage was more forcibly demonstrated by the fact that 5α:8α-epidioxy-ergost-9(11)-en-3β-yl acetate (XXVI), produced by catalytic reduction of 5α:8α-epidioxydehydroergosteryl acetate (XXI), gave only small yields of 11-oxygenated compounds on oxidation.



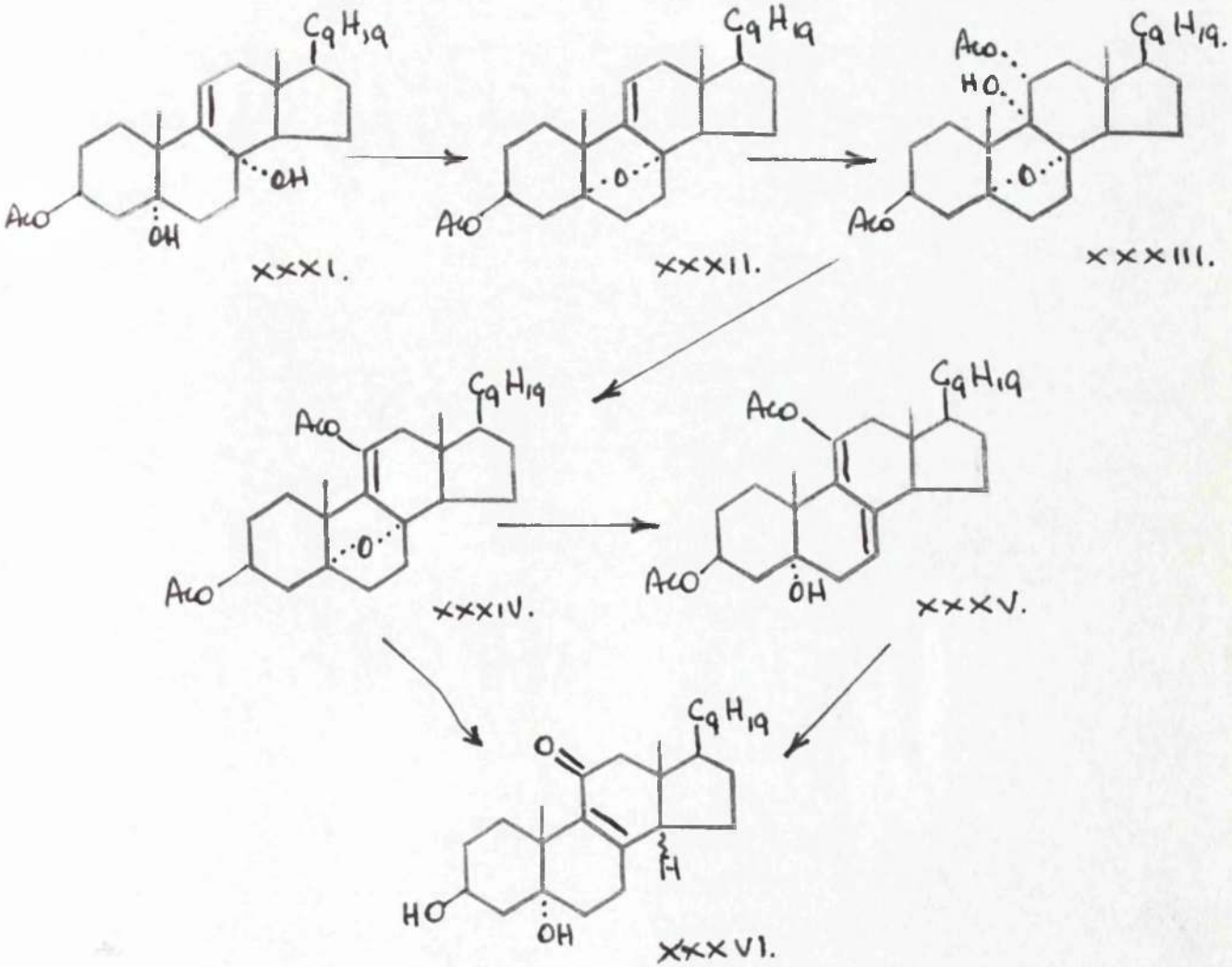
Thus perbenzoic acid gave 5α:8α-epidioxy-9α:11α-epoxy-ergostan-3β-yl acetate (XXVII) in 12% yield, potassium permanganate in acetic acid led to a 14% yield of 3β--acetoxy-5α:8α-epidioxyergostan-9α:11α-diol (XXVIII) together with 35% of the allylic oxidation product 12-oxo--5α:8α-epidioxyergost-9(11)-en-3β-yl acetate (XXIX), while chromium trioxide yielded only the latter material (XXIX). The unreactivity of the $\Delta^{9(11)}$-bond has been attributed to the steric hindrance caused by the 5α:8α-epidioxy bridge which shields it from attack on the rear(α) face. The use of such materials in a cortisone synthesis is inconceivable, due to the saturated side chain.

It was found, however, that, whereas treatment of 3β-acetoxyergosta-9(11):22-dien-5α:8α-diol (XXIII) with mineral acid gave (XXV), the use of an organic acid such as acetic acid led to the transannular epoxide 5α:8α--epoxyergosta-9(11):22-dien-3β-yl acetate (XXX), in which the $\Delta^{9(11)}$-bond was more reactive than in the 5α:8α--epidioxy analogue.



XXIII. XXX.

In this compound also the Δ^{22}-bond was found to be more reactive than the nuclear double bond and attempts at preferential oxidation gave only poor yields of 11-oxo-steroids, or were complicated by simultaneous degradation of the side chain. In the saturated side chain series, derived from the transannular dehydration of 3β-acetoxy-ergost-9(11)-en-5α:8α-diol (XXXI), oxidations were found to proceed in much better yield. A method of obtaining an 11-oxygenated steroid was described as outlined.



XXXI. XXXII. XXXIII.

XXXIV. XXXV.

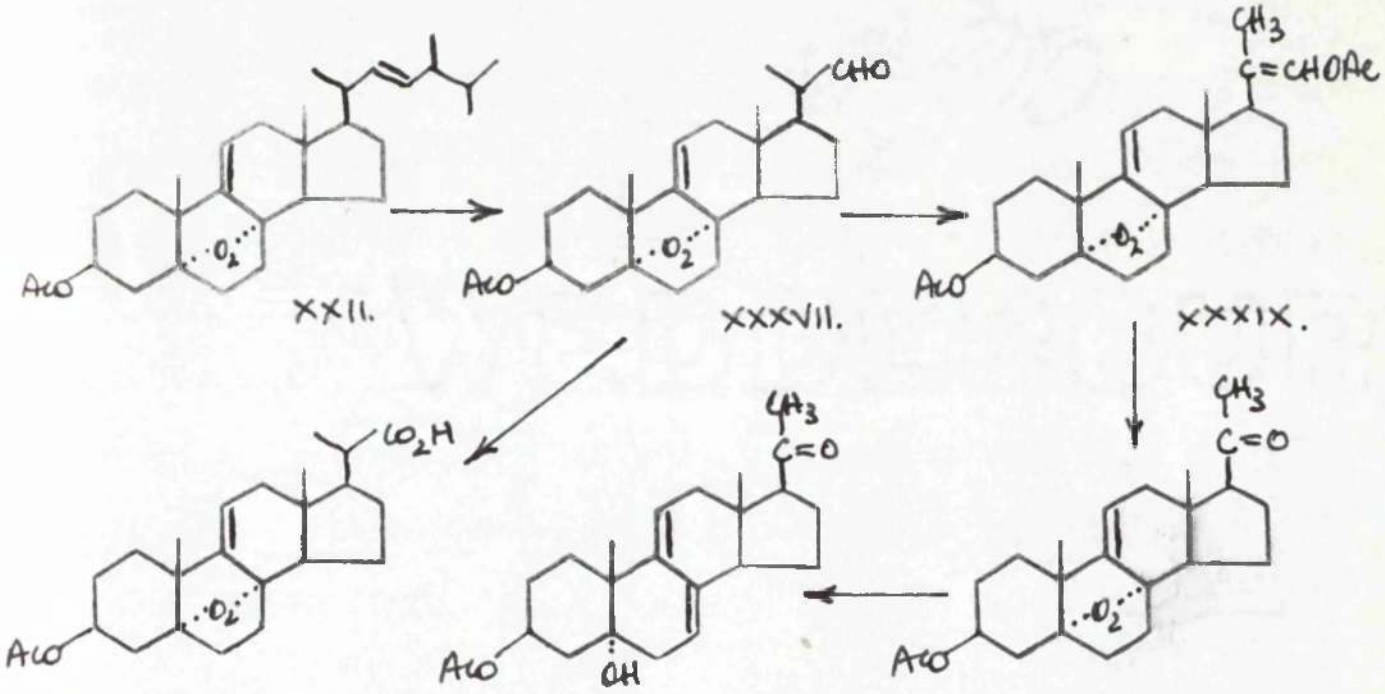
XXXVI.

Oxidation of (XXXII) with osmium tetroxide followed by acetylation afforded 3β:11α-diacetoxy-5α:8α-epoxyergostan--9α-ol (XXXIII), in 66% yield, readily dehydrated in 50% yield to the enol acetate (XXXIV), which was converted on acid treatment to the non-crystalline 3β:11-diacetoxyergosta-7:9(11)-dien-5α-ol (XXXV), identified by its ultraviolet absorption spectrum. Subsequent rearrangement of (XXXV) or of (XXXIV) with 5% alkali gave another non-crystalline substance, regarded as a 5α-hydroxy-11-oxo--8-ene (XXXVI) from a consideration of ultraviolet absorption data. In view of recent work (this thesis), it seems unlikely that (XXXVI) is a natural steroid, and the method of its production would seem more likely to give a 14β-steroid. The experimental detail, however, is scanty, making a decision difficult.

This study also included an investigation into methods of degrading the ergosterol side chain to a $C_{(17)}$-acetyl group (35). Using 5α:8α-epidioxyergosta-9(11):22-dien--3β-yl acetate (XXII) as a model, Jones et al., effected the degradation in the following way.

Ozonolysis of the epidioxide (XXII) and decomposition of the ozonide gave the aldehyde (XXXVII) in 35-50% yield together with 10% of the corresponding acid (XXXVIII). The

enol acetate (XXXIX), obtained in 60% yield from the aldehyde by treatment with acetic anhydride and potassium acetate, was ozonised to give the pregnane derivative (XL), which on reduction with zinc and acetic acid afforded 3β-acetoxy-20-oxopregna-7:9(11)-dien-5α-ol (XLI).



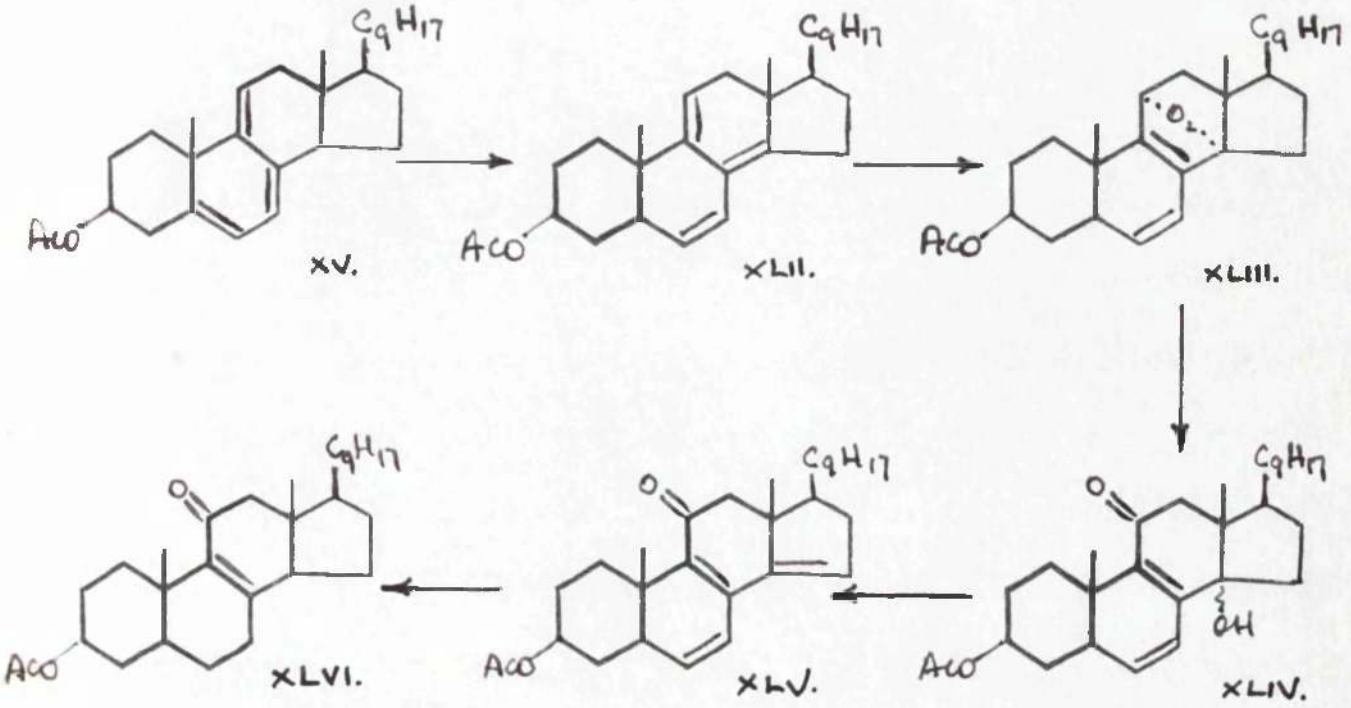
The low overall yield (7%) obtained in this series of reactions has been improved (40,114) on application of this method to less labile derivatives of ergosterol. This method has become the standard method of degrading the ergosterol side chain.

In view of the limited success which attended the attempts to obtain 11-oxygenated steroids by these routes the use of the derived 3β-acetoxyergosta-7:9(11):22-trien-

-5α-ol (XXV) was investigated. Since these experiments are closely related to the approach from ergosteryl-D acetate, they will be summarised in a later sub-section.

(c) A third and completely novel approach to the conversion of ring-C unsubstituted steroids to 11-oxygenated steroids, has been described by Laubach, Schreiber, Agnello, Lightfoot and Brunings (41). In contrast to the above methods, this elegant synthesis involves photochemical peroxidation of a homoannular ring-C diene, which introduces an 11-oxygen as an 11:14-epidioxy bridge.

Dehydroergosteryl acetate (XV) was catalytically isomerised with liquid sulphur dioxide in over 80% yield to ergosta-6:8(14):9(11):22-tetraen-3β-yl acetate (XLII),



which when photoperoxidised afforded 11:14-epidioxy-ergosta-6:8:22-trien-3β-yl acetate (XLIII). Base catalysed rearrangement of (XLIII) led to 3β-acetoxy-11--oxoergosta-6:8:22-trien-14ξ-ol (XLIV), which when treated with acid, underwent dehydration to 11-oxoergosta--6:8:14:22-tetraen-3β-yl acetate (XLV), selective hydrogenation of which gave the well-known 11-oxoergosta-8:22--dien-3β-yl acetate (XLVI). It is difficult to assess the value of this route as no experimental details have as yet been given.

Ergosterol-D as an Intermediate.

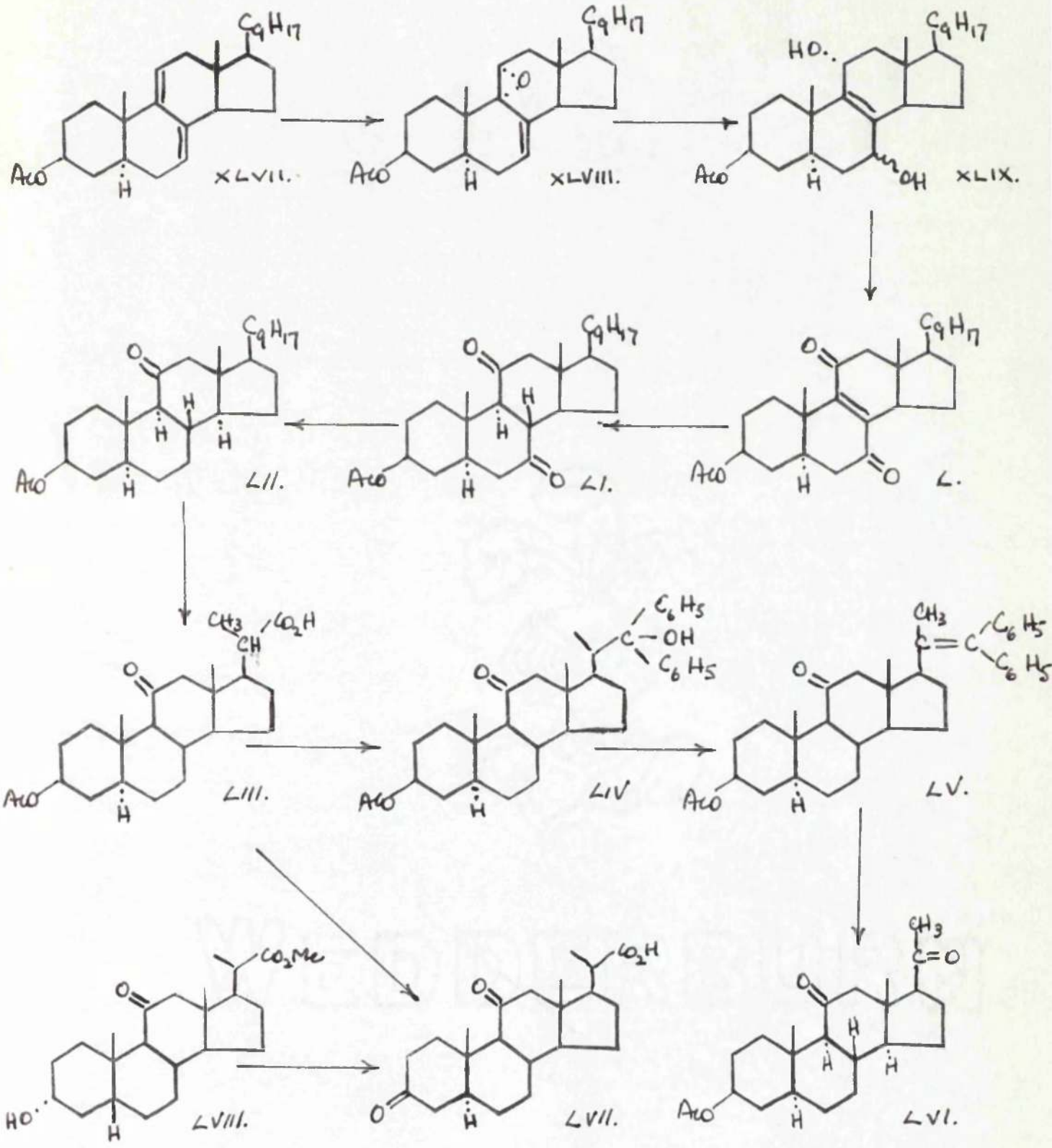
The use of this compound as a possible starting material for the partial syntheses of adrenal cortical hormones was first suggested by Bergmann and Klacsmann in 1948 in a paper (42) supplementary to their investigations on dehydroergosterol.

(a) Success in the preparation of an 11-oxygenated steroid from ergosteryl-D acetate was first achieved in the Merck Laboratories, the results of the investigation being reported in a preliminary note (43) by Tishler et al., in May, 1951. This was immediately followed by an independent publication (45) by Heusser et al., whose partial synthesis followed essentially the same pattern. The

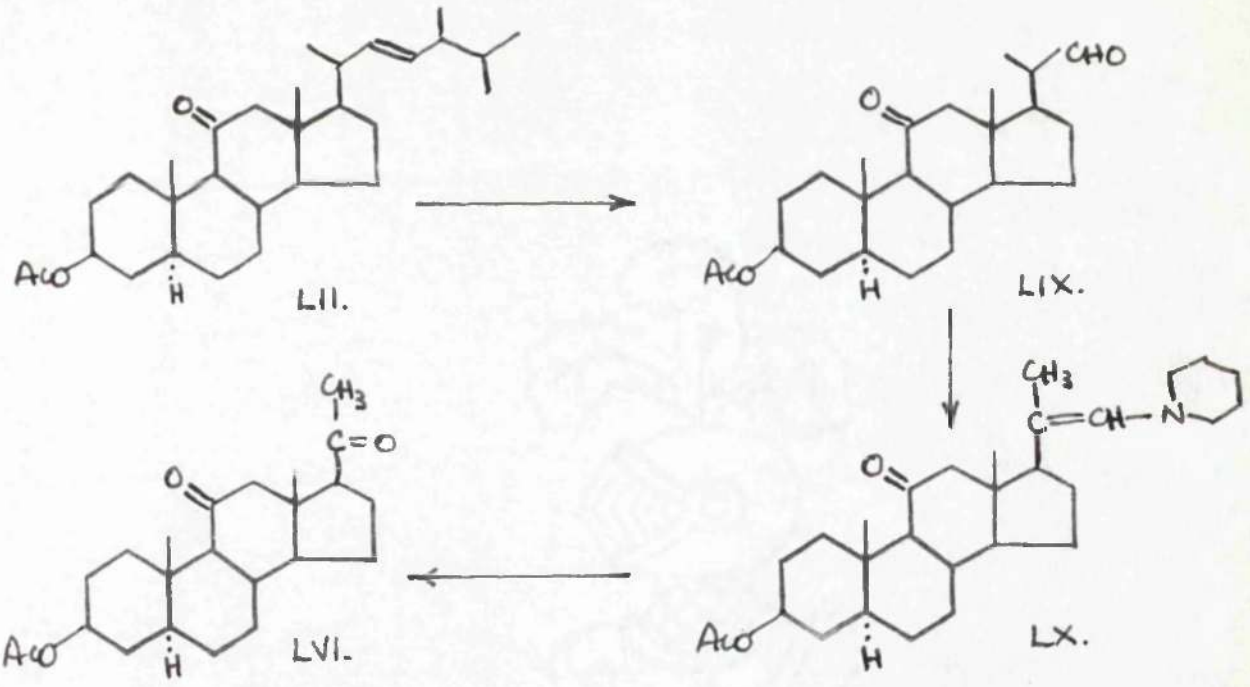
former workers have since issued a more complete communication (44), the details of which are described below.

Ergosteryl-D acetate (XLVII) was selectively oxidised at the $\Delta^{9(11)}$-bond with perbenzoic or monoperphthalic acid giving a monoepoxide (XLVIII) which when treated with a trace of mineral acid afforded 3β-acetoxyergosta-8:22--dien-7ξ:11α-diol (XLIX). Oxidation of this compound with chromium trioxide led to 7:11-dioxoergosta-8:22-dien-3β-yl acetate (L) which when reduced with zinc dust and acetic acid gave 7:11-dioxoergost-22-en-3β-yl acetate (LI), further reduced by the Huang-Minlon procedure (46) to 11-oxoergost-22-en-3β-yl acetate (LII). Identification of (LII), obtained in 50% overall yield, was carried out by ozonolysis to the acid (LIII), which was then hydrolysed and oxidised to 3:11-dioxo<u>bisnorallo</u>cholanic acid (LVII). This was then compared with an authentic specimen prepared, unmabiguously, from methyl-3α-hydroxy-11-oxo<u>bisnor</u>cholanate (LVIII).

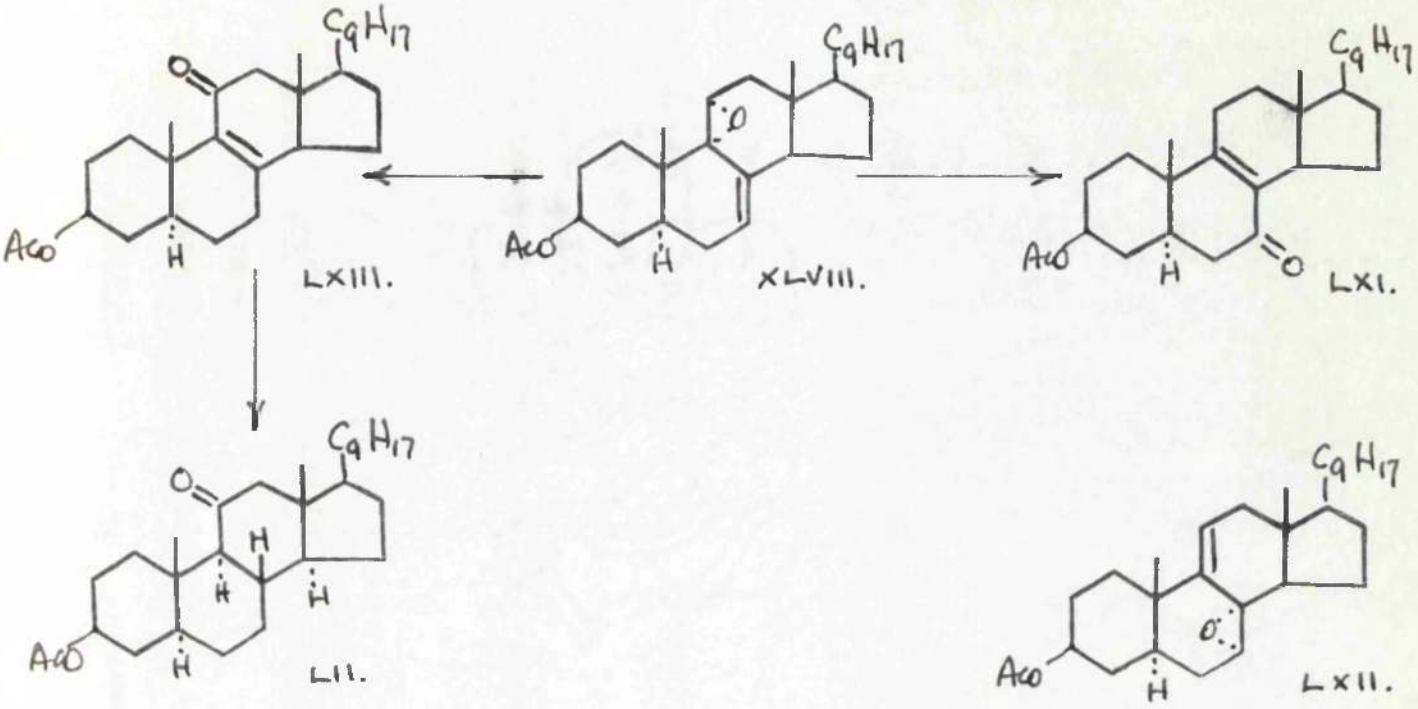
Treatment of the methyl ester of 3β-acetoxy-11-oxo-<u>bisnorallo</u>cholanic acid (LIII) with phenylmagnesium bromide and dehydration of the resulting diphenyl-carbinol (LII) yielded the diphenylethylene derivative (LV) which on ozonolysis gave 11:20-dioxo<u>allo</u>pregnan-3β-yl acetate (LVI).


C9H17
AcO
H
XLVII.
XLVIII.
HO
OH
XLIX.
L.
LI.
LII.
CH3
CO2H
CH
LIII.
C6H5
C
OH
LIV
LV.
CO2Me
HO
LVIII.
LVII.
LVI.

The side chain of 11-oxoergost-22-en-3β-yl acetate (LII) has also been degraded (133) by the standard method (35) giving (LVI) in high yield. An alternative method (133) based on the formation of an enamine (LX) [cf. Herr and Heyl (134)] of the intermediate aldehyde (LIX), followed by ozonolysis, gave only poor overall yields of 11:20--dioxoallopregnan-3β-yl acetate (LVI).



The Swiss workers (45) also found that the epoxide (XLVIII), on prolonged treatment with acid in methanol gave 7-oxoergosta-8:22-dien-3β-yl acetate (LXI). This reaction left some doubt as to the position of the epoxy-bridge in (XLVIII), as the intermediate diol (XLIX) might equally well have come from the isomeric epoxide (LXII).

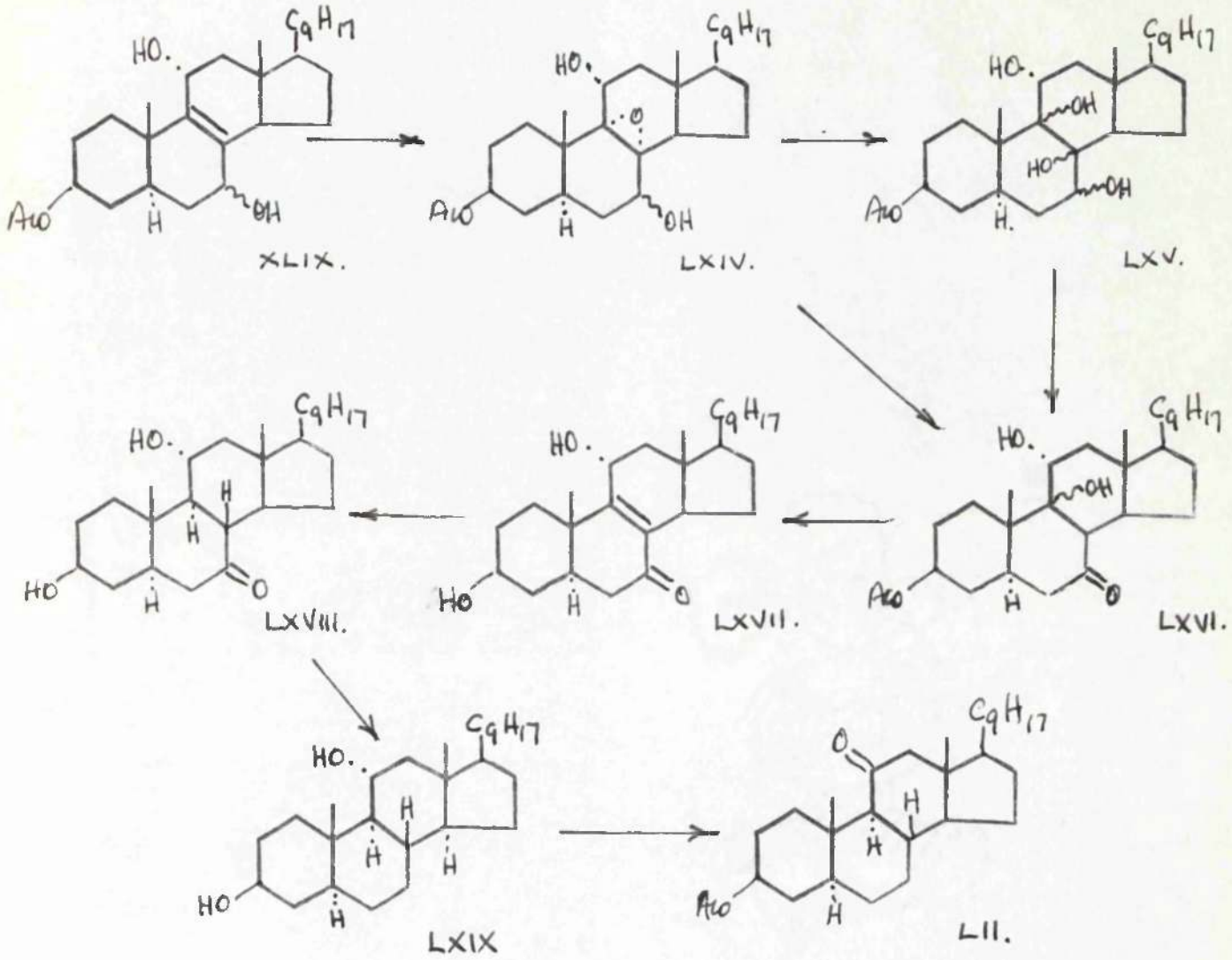


However, these investigators made the important discovery that the epoxide could be isomerised, under aprotic conditions, with a boron trifluoride (45), or ferric chloride (49), catalyst to a different αβ-unsaturated ketone which they postulated as being 11-oxoergosta-8:22--dien-3β-yl acetate (LXIII). This added considerable weight to the formulation of the epoxide as 9α:11α-epoxy-ergosta-7:22-dien-3β-yl acetate (XLVIII).

In a subsequent publication, Tishler et al., (48) reported a modification of their first route to 11-oxo-ergost-22-en-3β-yl acetate. They found that preferential reduction of the Δ^9-bond of 11-oxoergosta-8:22-dien-3β-yl acetate (LXIII) could be effected with lithium metal in

liquid ammonia giving (LII) after reacetylation, in 80% yield. This thus completed a route from ergosteryl acetate to a $C_{(21)}$ oxygenated steroid, involving only five stages, and having a reasonable overall yield. The reduction in addition confirmed the structures of (XLVIII) and of (LXIII).

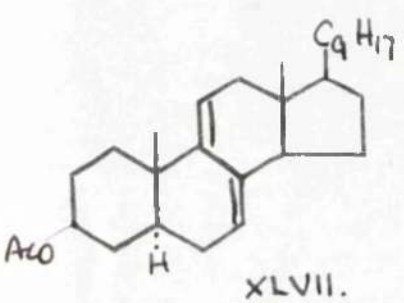
(b) Heusser, Anliker, Eichenberger and Jeger in a later communication (49) discussed alternative routes for the introduction of an 11-oxygen atom. Oxidation of the triol-monoacetate (XLIX) with monoperphthalic acid yielded 3β-acetoxy-8α:9α-epoxyergost-22-en-7ξ:11α-diol (LXIV), which when treated with a trace of sulphuric acid gave 3β-acetoxyergost-22-en-7ξ:8ξ:9ξ:11α-tetrol (LXV). This pentol-monoacetate, or its precursor (LXIV), was converted by hydrobromic acid to 3β-acetoxy-7-oxoergost-22-en-9ξ:11α-diol (LXVI) which, by alkaline dehydration, gave 7-oxoergosta-8:22-dien-3β:11α-diol (LXVII). Selective reduction of the Δ^8-bond of this compound was achieved by catalytic hydrogenation in an alkaline medium, and Wolff-Kishner reduction of the product (LXVIII) afforded ergost-22-en-3β:11α-diol (LXIX). The latter compound (LXIX) had been obtained previously by reduction of 11-oxoergost-22-en-3β-yl acetate (LII) with sodium in



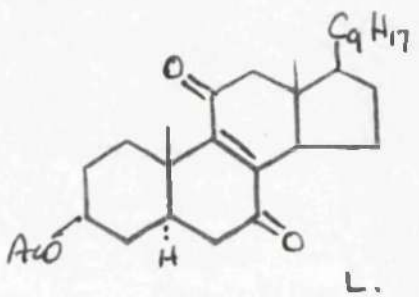
propanol (50), or by lithium in liquid ammonia (51), a method first described, by Djerassie et al., (52) in the diosgenin series.

(c) A less successful method of converting ergosteryl-D acetate to an 11-oxygenated steroid has been described by Fieser, Schneider, and Huang (53). Their procedure, a general one, consisted of oxidising 7:9(11)-dienes with

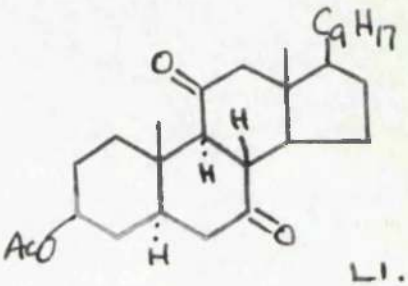
N-bromosuccinimide and treating the crude product with silver nitrate followed by further oxidation with silver chromate. In the case of ergosteryl-D acetate (XLVII), 7:11-dioxoergost-22-en-3β-yl acetate (L) was obtained which, in the crude state, was reduced with zinc dust and



XLVII.



L.



LI.

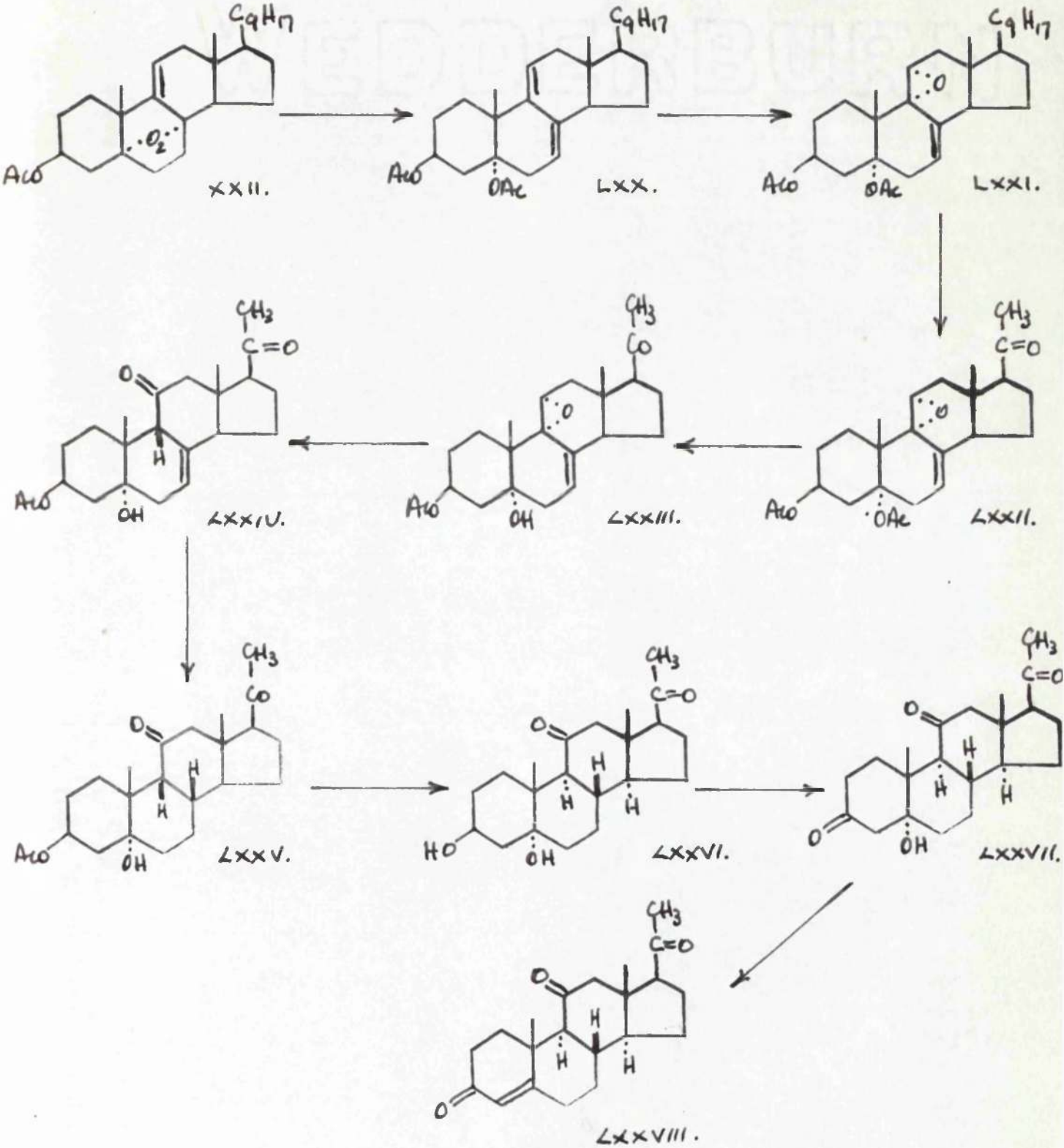
acetic acid to 7:11-dioxoergost-22-en-3β-yl acetate (LI), the conversion of which to 11:20-dioxo<u>allo</u>pregnan-3β-yl acetate (LVI) has already been described.

5α-<u>Hydroxyergosteryl-D Acetate as an Intermediate.</u>

The failure of ring-B epidioxy-, and epoxy-dehydro-ergosterol derivatives to undergo reactions leading to good yields of 11-oxygenated steroids, led Jones and his co-workers to approach the problem from a different aspect. They investigated the action of oxidising agents on derivatives of 3β-acetoxyergosta-7:9(11):22-trien-5α-ol (5α--hydroxyergosteryl-D acetate)(XXV) a compound obtained by

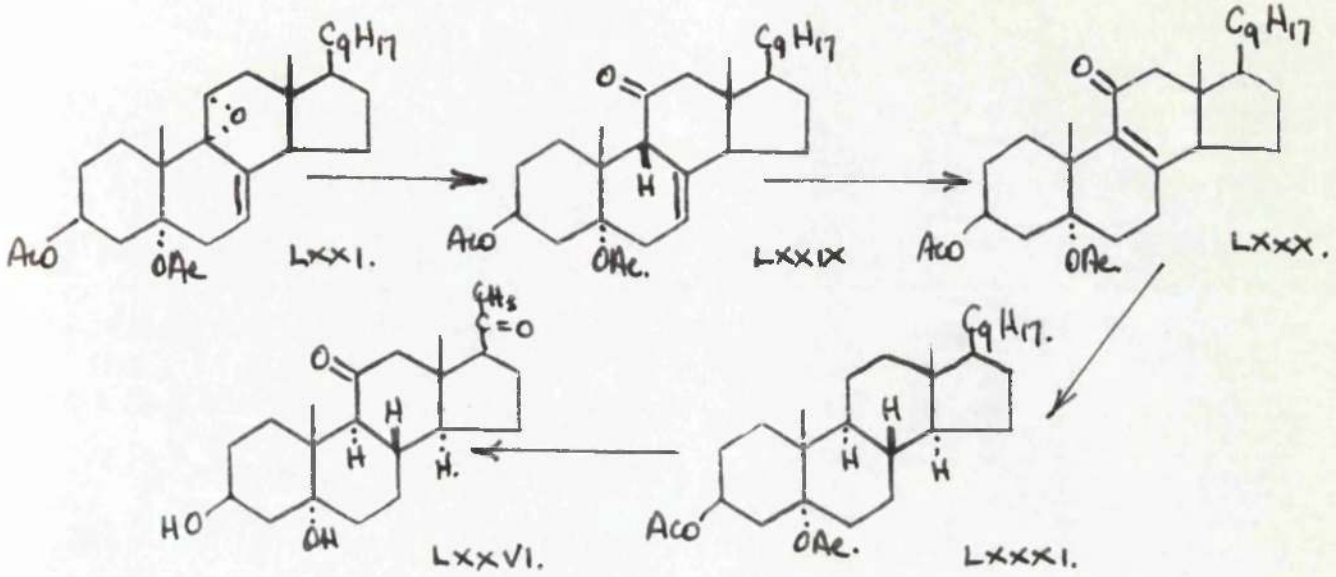
reductive fission of 5α:8α-epidioxyergosta-9(11):22-dien--3β-yl acetate (XXII). The outcome of this work, part of which was carried out in collaboration with a second group of workers, Evans, Hathway, Elks, Oughton, and Thomas, was the fabrication of a completely novel method of introducing an 11-oxygen atom (54,55). The method was applied to the synthesis of several compounds and the outline below demonstrates this route by a partial synthesis of 11:20-dioxo*allo*pregnan-3β:5α-diol (LXVI).

3β:5α-Diacetoxyergosta-7:9(11):22-triene (LXX) obtained by acetylation of (XXV) was epoxidised to yield 3β:5α-diacetoxy-9α:11α-epoxyergosta-7:22-diene (LXXI). Side chain degradation of this compound by the *standard method* (35) gave 3β:5α-diacetoxy-9α:11α-epoxy*allo*pregn--7-en-20-one (LXXII) which was converted to the 3β-acetoxy--5α-hydroxy derivative (LXXIII). At this juncture the synthesis departed from the conventional lines described above, for it was found that if the rearrangement of ''epoxyenes'' such as (LXXII) or (LXXIII) were carried out in ether instead of benzene using a boron trifluoride catalyst the product isolated in high yield was a non--conjugated, unsaturated, ring-C ketone [a reaction independently discovered by Heusler *et al.* (56)].



XXII. LXX. LXXI. LXXII. LXXIII. LXXIV. LXXV. LXXVI. LXXVII. LXXVIII.

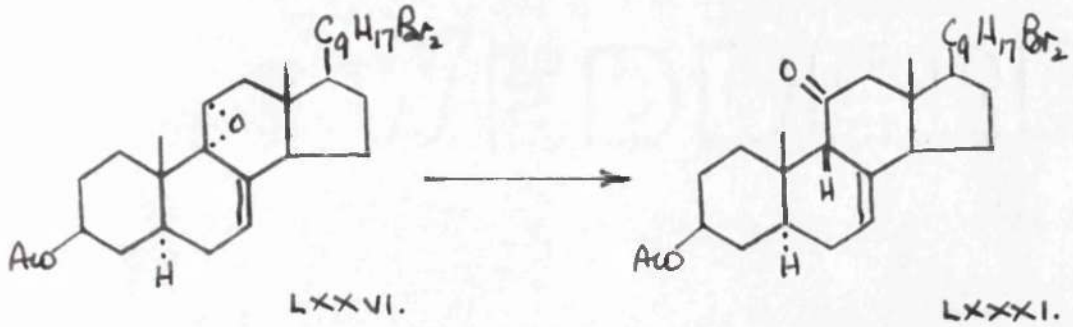
Rearrangement of (LXXIII) by this method gave 3β-acetoxy--11:20-dioxo-9β-allopregn-7-en-5α-ol (LXXIV). Catalytic hydrogenation of (LXXIV) effected reduction of the Δ^7-bond and 20-keto-groups and oxidation of the crude reaction product afforded 3β-acetoxy-11:20- dioxo-9β-allopregn-5α-ol (LXXV). Treatment of this material with 20% potassium hydroxide solution effected epimerisation at $C_{(9)}$ giving the required 11:20-dioxoallopregnan-3β:5α-diol (LXXVI). The latter compound was converted to cortisone (see later), and to 11-oxoprogesterone (LXXVIII) by oxidation at $C_{(3)}$ followed by base catalysed dehydration of the product (LXXVII) to give (LXXVIII). In a later paper (130), Jones et al., described an alternative method of preparing (LXXVI). Treatment of (LXXI) with boron trifluoride etherate gave (LXXIX) which was rearranged with alkali and reacetylated giving 3β:5α-diacetoxy-11-oxoergosta-8:22--diene (LXXX). Reduction of (LXXX) with lithium in liquid ammonia afforded 3β:5α-diacetoxy-11-oxoergost-22-ene(LXXXI), which was then degraded by the standard method (35) and hydrolysed to 11:20-dioxoallopregnan-3β:5α-diol (LXXVI). These elegant procedures carried out with materials having groups potentially capable of generating an αβ-unsaturated ketone in ring-A avoids the difficult problem of the



introduction of a Δ^{6}-bond into steroids possessing a ring A/B trans fusion. The latter problem is a serious stumbling block in the conversion to cortisone of all the previously described 11-oxygenated steroids derived from ergosterol.

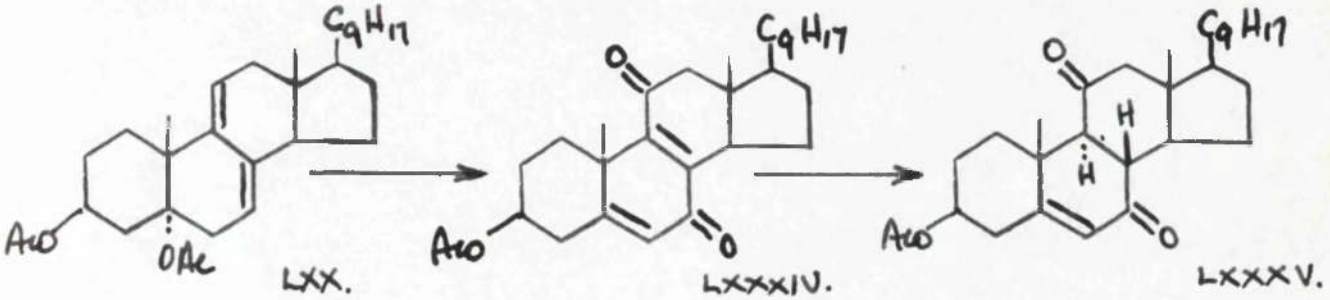
Other Methods.

(a) The method described above has also been applied to 22:23-dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate (LXXXII), and to the dichloro analogue (51). Rearrangement of either of these materials with boron trifluoride in ether gave the corresponding Δ^{7}-11-oxo-9β-compounds.

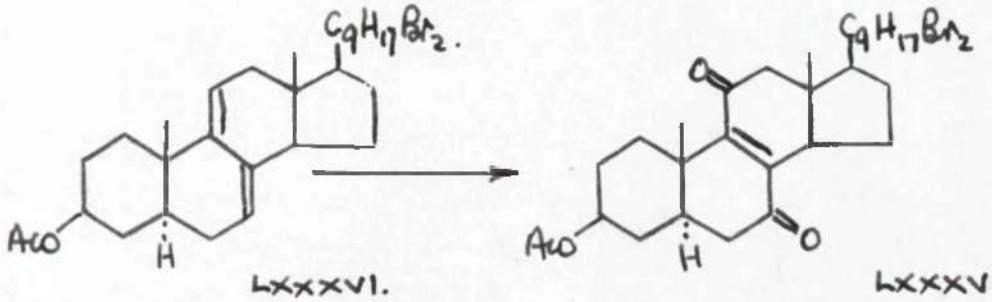


Hydrogenation of either of these compounds, however, gave poor yields of the saturated 11-keto compounds, as the conditions required to reduce the Δ^7-bond also caused some reductive dehalogenation of the side chain.

(b) A recent publication by Elks, Evans, Long and Thomas (53) describes the preparation of some 11-oxygenated steroids by sodium dichromate oxidation of several 7:9(11)-dienes. The most important of these was the oxidation of 3β:5α-diacetoxyergosta-7:9(11):22-triene (LXX) which afforded 7:11-dioxoergosta-5:8:22-trien-3β-yl acetate (LXXXIV) (chromatography caused dehydration of the 5α-acetoxy group). This was reduced with zinc dust and acetic acid to 7:11-dioxoergosta-5:22-dien-3β-yl acetate (LXXXV). Also described is the oxidation, with sodium dichromate, of 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate (LXXXVI) which gave, as the major product,



LXX. LXXXIV. LXXXV.



LXXXVI. LXXXVII.

22:23-dibromo-7:11-dioxoergost-5-en-3β-yl acetate (LXXXVII) previously obtained by Budziarek et al. (111). These oxidations proceeded in poor yield and have little, if any, preparative value.

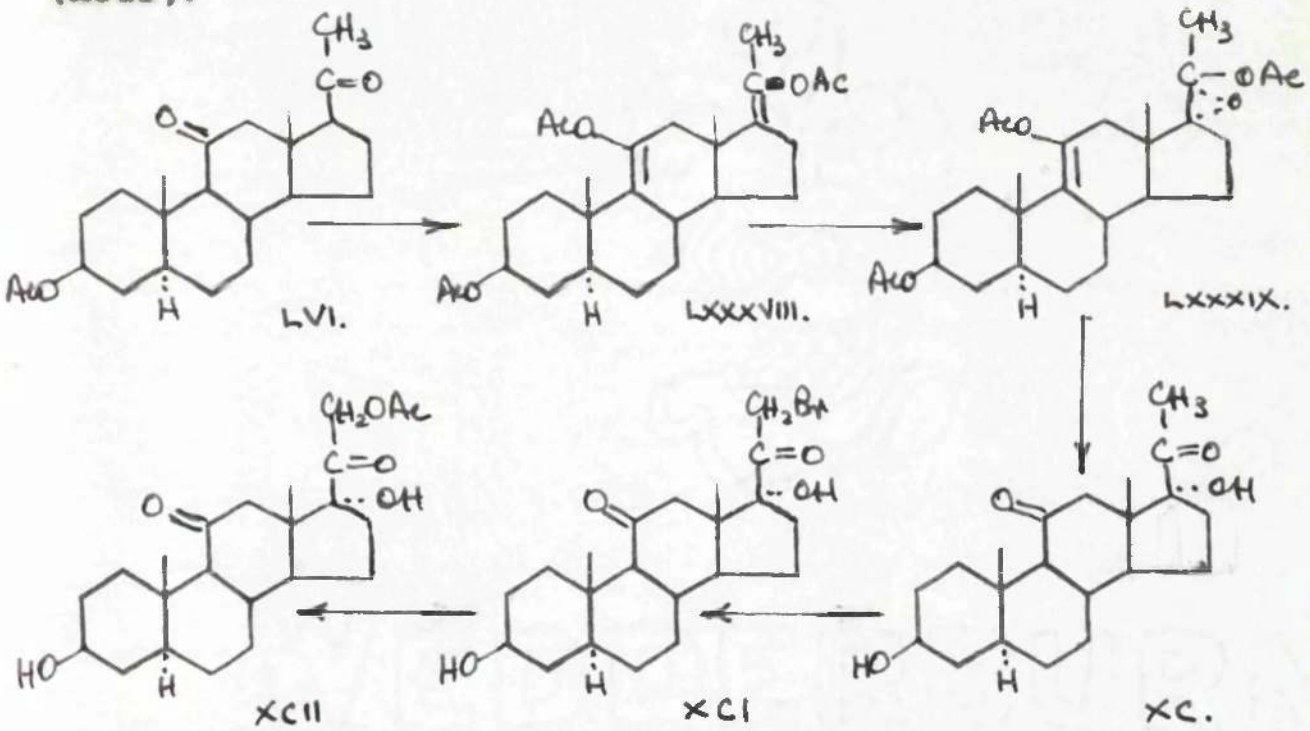
Cortisone from 11-Oxygenated Steroids.

Several routes have been outlined above for the syntheses of 11-oxo-17-acetyl steroids from ergosterol, and methods for the conversion of these to cortisone are described below.

(a) Cortisone from 7:11-dioxoallopregnan-3β-yl acetate:- The conversion of 7:11-dioxoallopregnan-3β-yl acetate [also obtained from cholesterol (44), stigmasterol (44), diosgenin (44,59) and hecogenin (60)] to cortisone requires the introduction of hydroxyl groups at $C_{(17)}$ and $C_{(21)}$, and the transformation of the 3β-acetoxyl group to a Δ^4-3-oxo-system.

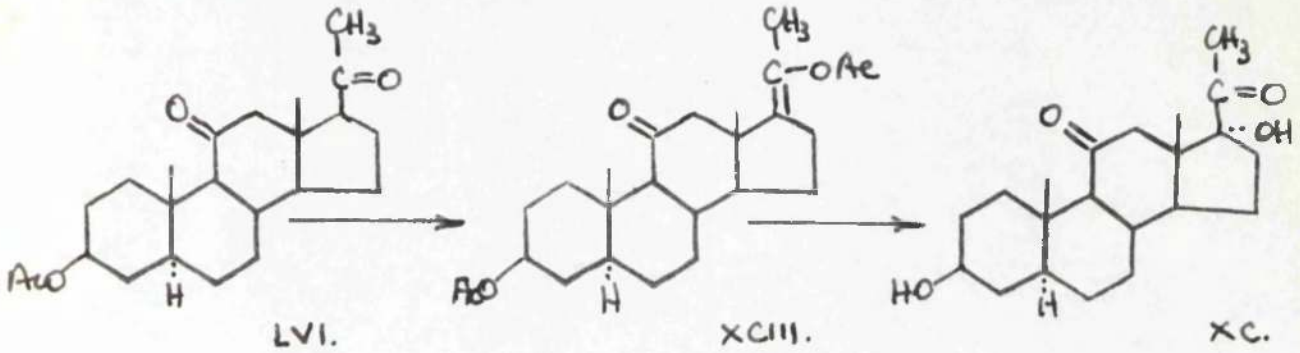
The elaboration of the side chain was achieved (47, 61) by the application of a method due to Gallagher (62, 63) for the conversion of an acetyl group at $C_{(17)}$ to a dihydroxyacetone group. Enol acetylation of 11:20-dioxo-allopregnan-3β-yl acetate (LVI) gave (LXXXVIII) which was preferentially oxidised, with perbenzoic acid, at the Δ^{17}-bond, yielding the 17α:20α-epoxide (LXXXIX). This

compound on mild alkaline hydrolysis afforded 11:20--dioxoallopregnane-3β:17α-diol (XC). Bromination of the latter in chloroform gave the monobromo intermediate (XCI), which when treated with sodium acetate solution afforded 21-acetoxy-11:20-dioxoallopregnane-3β:17α-diol (XCII).



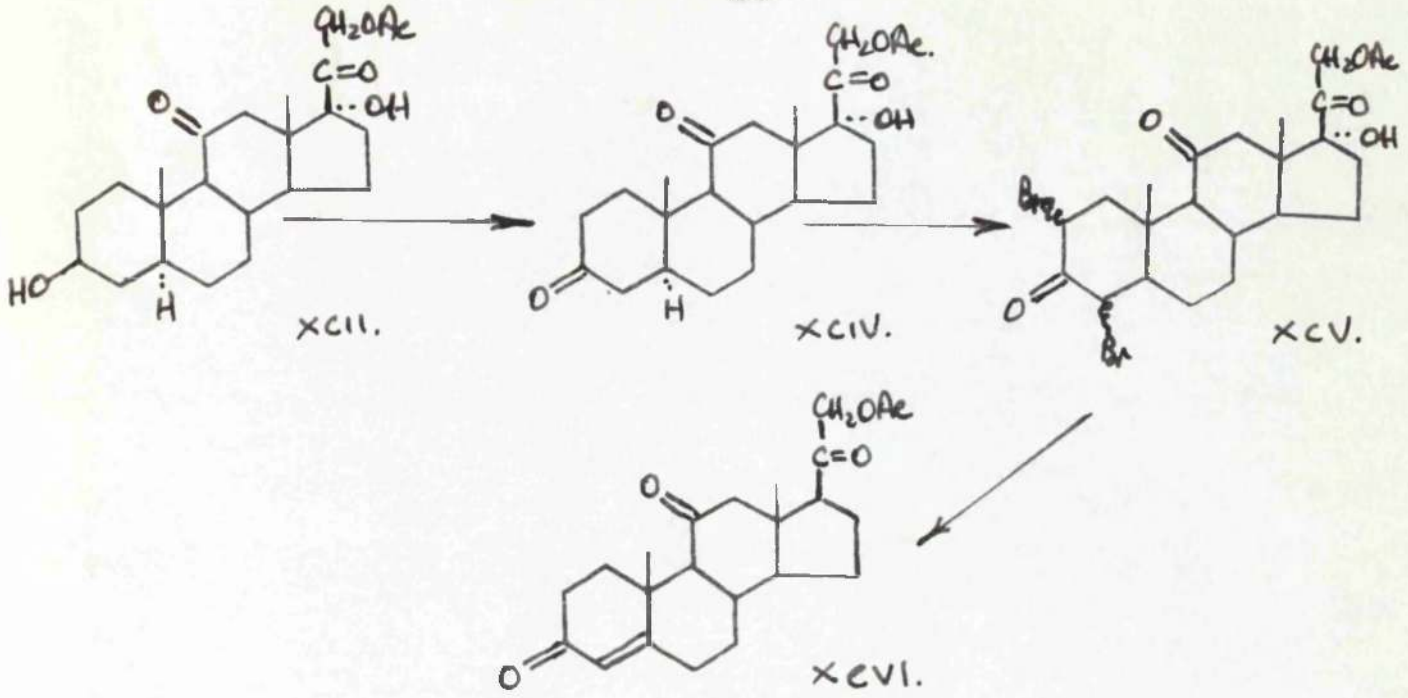
Barton et al., (132) found that this process gave poor yields due to simultaneous epoxidation of the $\Delta^{9(11)}$--bond. They developed a much superior method involving mono-enol acetylation of the $C_{(20)}$ ketone. This treatment of (LVI) with acetic anhydride, in carbon tetrachloride, in the presence of a catalytic trace of perchloric acid, gave the enol acetate (XCIII) which on

epoxydation and basic hydrolysis afforded 11:20-dioxo-allopregnane-3β:17α-diol (XC) in an overall yield of 92%.



The conversion of (XCII) to cortisone to cortisone acetate has been claimed by Tishler et al., (47) by a method involving bromination and dehydrobromination. Experimental details have not yet been disclosed.

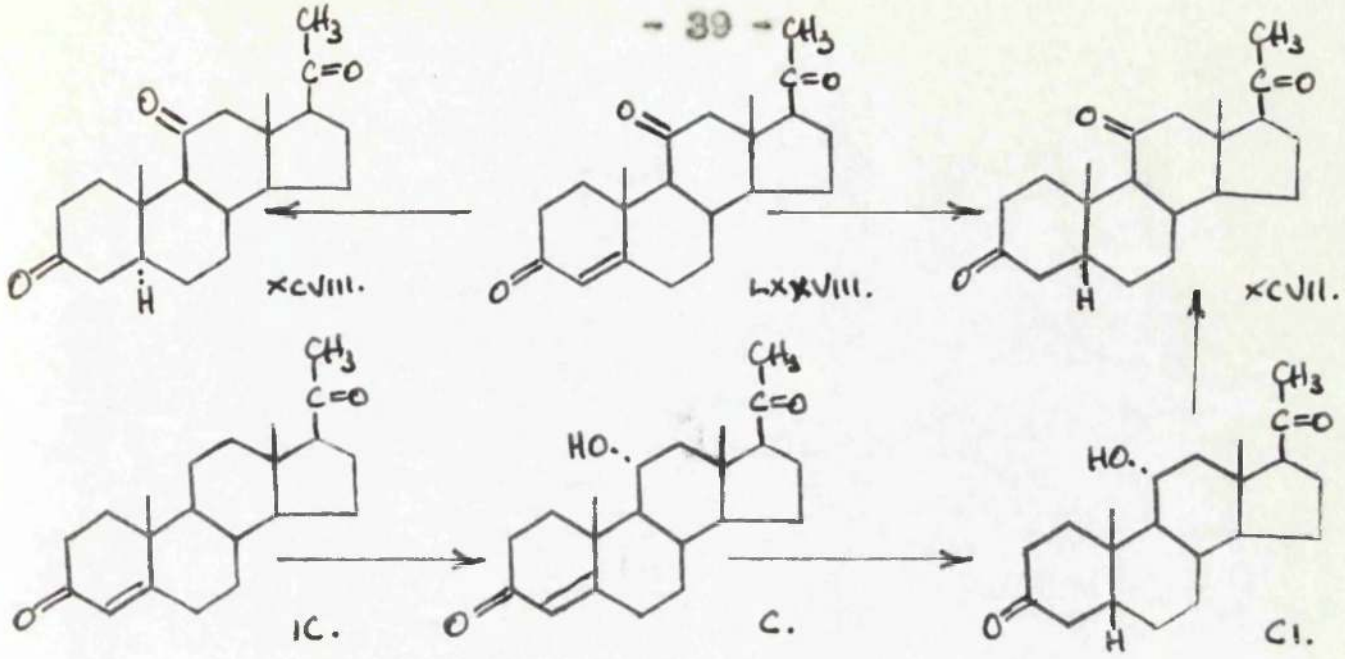
Djerassi and his co-workers have described a method for converting allosteroids into 3-oxo-Δ^4-derivatives and have completed the conversion of (XCII) to cortisone (65). Oxidation of 21-acetoxy-11:20-dioxoallopregnane-3β:17α-diol (XCII) with N-bromosuccinimide in tert.-butanol gave the triketone (XCIV), which on dibromination in anhydrous acetic acid, using a catalytic trace of hydrogen bromide, afforded the 2:4-dibromo derivative (XCV). When refluxed for 24 hours with sodium iodide in acetone, (XCV) yielded cortisone acetate (XCVI).



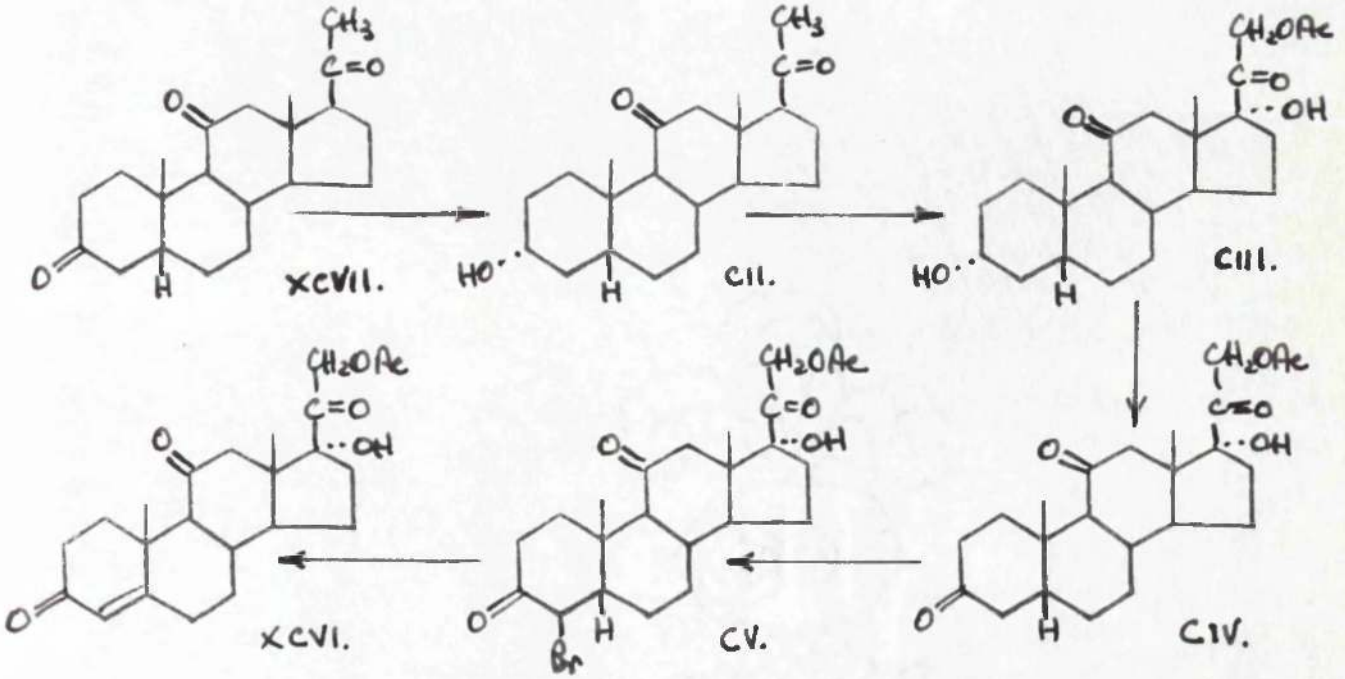
(b) Cortisone from pregnane-3:11:20-trione (67,136).

Pregnane-3:11:20-trione (XCVII) has been prepared by catalytic hydrogenation (66) of 11-oxoprgoesterone (LXXVIII), a procedure which also gave the allo isomer (XCVIII). A much superior method involves microbiological oxidation (20,137) of progesterone (IC) now available from ergosterol (105,106,114) which gave 11β-hydroxyprogesterone (C). Selective catalytic hydrogenation of this compound led to (CI), which on oxidation furnished pregnan-3:11:20-trione (XCVII) in high overall yield from progesterone.

Selective reduction (67) of pregnane-3:11:20-trione with sodium borohydride gave 11:20-dioxopregnan-3α-ol (CII)



the side chain of which was elaborated (136) by the Gallagher method (62,63). The resultant 21-acetoxy-11:20--dioxopregnane-3α:17α-diol (CIII) was then oxidised with N-bromosuccinimide in tert.-butanol to 21-acetoxy-3:11:20--trioxopregnan-17α-ol (CIV). Monobromination of this compound yielded the 4-bromo derivative (CV), which



afforded cortisone acetate on pyruvic acid cleavage of the derived semicarbazone.

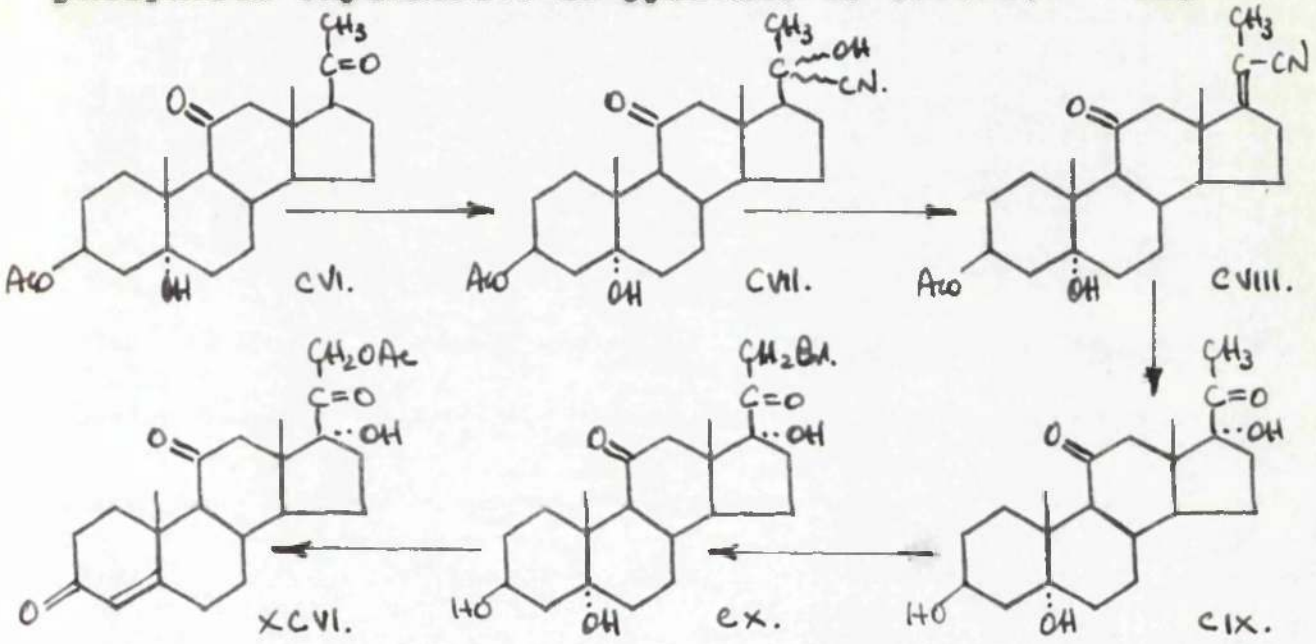
The introduction of Δ^4-3-oxo system by the above method is based on a procedure first discovered by Kendall (63), and is a general reaction for normal (A/B ring cis-fusion) steroids. An alternative method if dehydrohalogenation has recently been described by Holysz (69) involving the use of lithium chloride or other metallic chlorides, in dimethylformamide solution. The yields by this method are equally good and its simplicity compared with Kendall's method would seem to make it the reaction of choice.

(c) Cortisone from 11:20-dioxoallopregnane 3β:5α-diol.

Jones et al., (129,130) found that in order to elaborate the cortical side of 11:20-dioxoallopregnane--3β:5α-diol (LXXVI) and at the same time retain the 5α--hydroxyl group (for introducing the Δ^4-3-oxo grouping at a later stage) the procedure of Barett (131) was best employed. The widely used method of Gallagher et al., (62,63) proved to be too drastic, as simpler 5α-hydroxy compounds were readily dehydrated to Δ^5-steroids, under these conditions.

Addition of hydrogen cyanide to the monoacetate(CVI)

of (LXXVI) gave a cyanohydrin (CVII) in which the $C_{(20)}$--hydroxyl group was then selectively dehydrated with phosphorus oxychloride in pyridine to (CVIII). The



resulting nitrile (CVIII) still retaining the 5α-hydroxyl group was oxidised with osmium tetroxide and the product hydrolysed to the 17α-hydroxy compound (CIX). This on bromination gave (CX) which, when treated with potassium acetate, followed by oxidation of the $C_{(3)}$-hydroxy group, and dehydration of the 5α-hydroxyl, yielded cortisone acetate (XCVI).

THEORETICAL

THEORETICAL

Introduction.

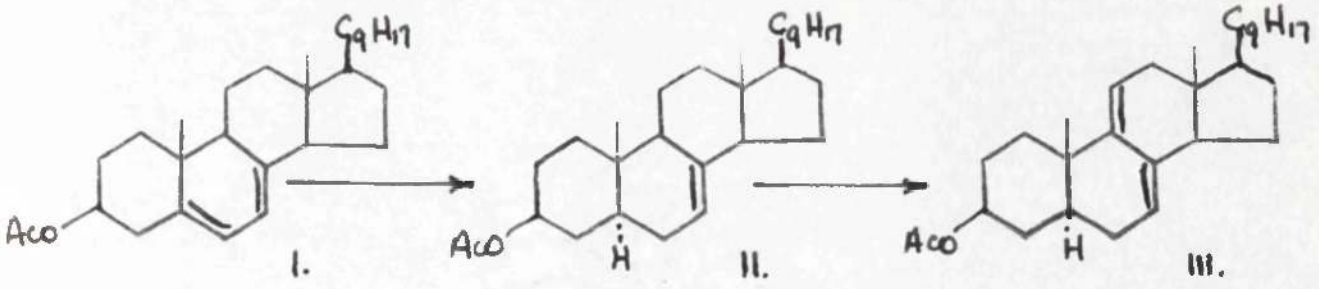
The work described in this thesis is part of a programme designed to effect a partial synthesis of cortisone from ergosterol, and had as its object the partial synthesis of 11-oxygenated steroids, and of materials suitable for 11-oxygenation by biological means. These investigations began in 1951 shortly after Tishler and his co-workers had outlined (44) a general scheme for the synthesis of 11-oxosteroids from steroids containing a Δ^5-bond such as ergosterol, diosgenin and stigmasterol. At this stage also, methods of obtaining ergosta-7:22-dien-3β-yl acetate, and its oxidation product ergosteryl- D acetate, were being considerably improved and a successful approach for the conversion of ergosterol to a $C_{(11)}$-oxygenated steroid (110), alternative to the one above, was nearing completion.

Since that time the latter work and the experiments described in this thesis, performed during the past three years, have been reported in a series of publications (109-114).

Ergosteryl-D Acetate and 22:23-Dibromoergosteryl-D Acetate.

The approach to the partial synthesis of 11-oxygenated steroids, possessing a two-carbon atom side chain, demanded two qualifications of the starting material, (a) that it should possess in ring-C, a function permitting ready oxygenation at $C_{(11)}$, and (b) that the side chain should be amenable to degradation to an acetyl group. Although ergosteryl-D acetate (III) fulfilled these conditions, difficulties had been encountered in its preparation. This consisted of hydrogenation of ergosteryl acetate (I) to ergosta-7:22-dien-3β-yl acetate (5:6--dihydroergosteryl acetate) (II) followed by oxidation with mercuric acetate to ergosteryl-D acetate (III).

Ergosta-7:22-dien-3β-yl acetate (II) had been prepared previously by Heilbron and Sexton (26), Wieland and Benend (25), and Barton and Cox (70), all by partial



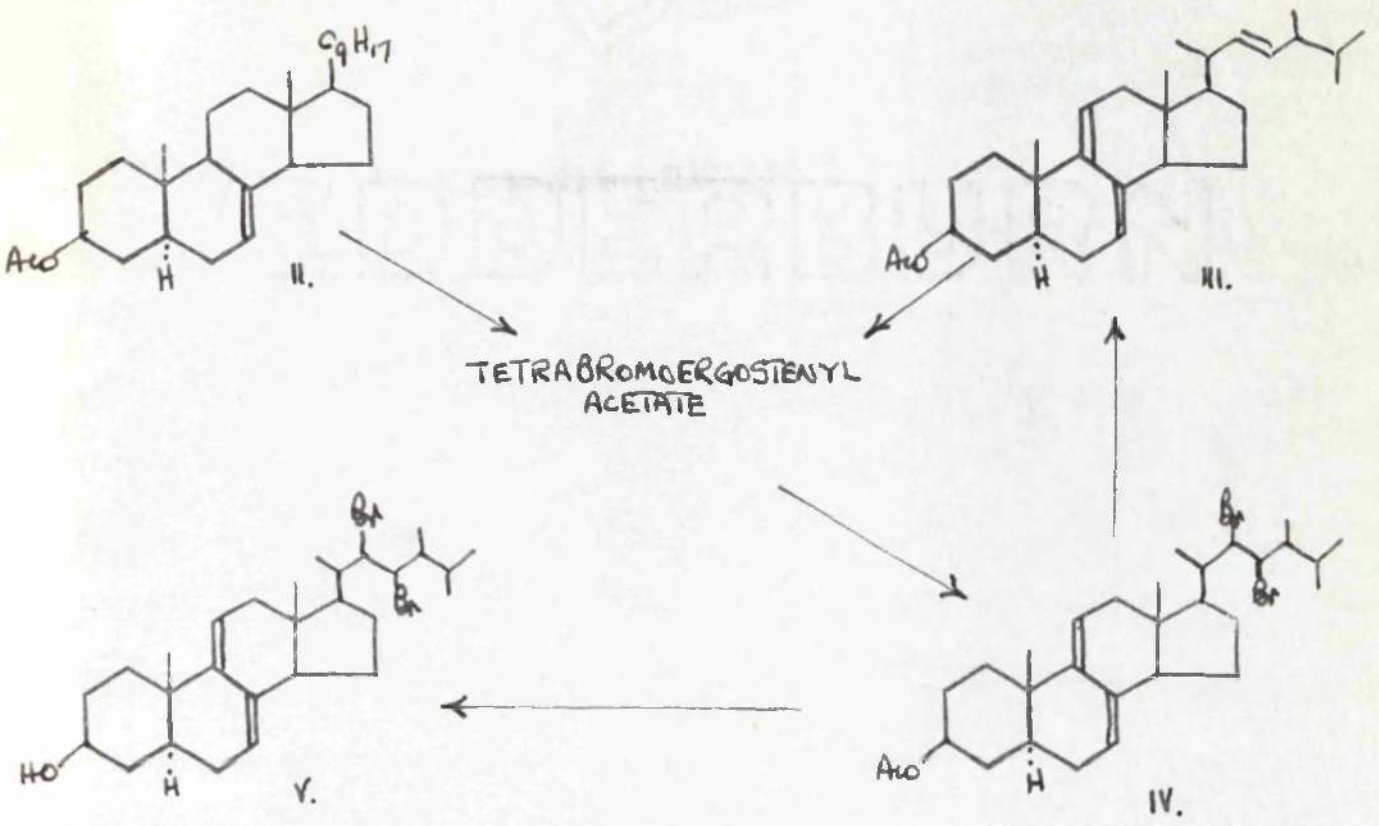
hydrogenation in a neutral solvent. None of the preparations gave good yields, the best (70) realising a 30-35% yield using a platinum catalyst and chloroform as solvent. Anderson, Stevenson, and Spring (110) investigating methods of improving the yield, carried out the hydrogenation in pure benzene with a Raney nickel catalyst and obtained ergosta-7:22-dien-3β-yl acetate in 90% yield.

Subsequently other workers have reported various improved procedures: Pannizon and Kägi (72) obtained a similar yield with Rupe nickel in ether solution, Laubach and Brunings (71) reported a quantitative yield with Raney nickel in dioxan and, more recently, Ruyle et al., (73) have found that the reaction can be efficiently carried out at 3 atm. pressure in benzene solution with a Raney nickel catalyst.

Ergosteryl-D acetate was originally prepared by the oxidation of ergosta-7:22-dien-3β-yl acetate (II) with mercuric acetate using a modification of a method first used by Bergmann and Stevens (33) for the preparation of dehydroergosteryl acetate from ergosteryl acetate. The method was found to be unsatisfactory, for although an 85% yield of crude material, $[\alpha]_D$ ⩾ 18°, could be realised, its purification to constant optical rotation was extremely

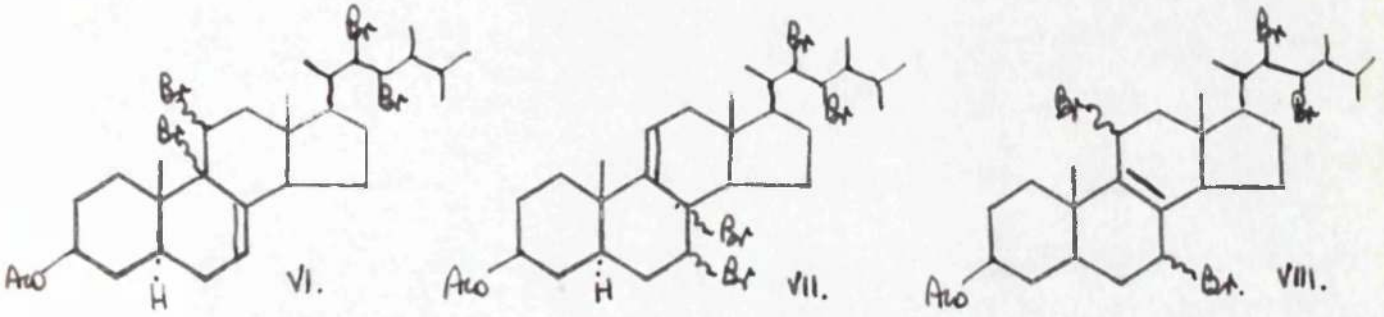
wasteful, the yield of product with $[\alpha]_D$ < 23° not exceeding 30%.

In a search for alternative methods of preparing ergosteryl-D acetate, Spring and his co-workers (110) studied the action of bromine on ergosta-7:22-dien-3β-yl acetate, since Eck and Hollingsworth (74) had found that oxidation of cholest-7-ene in chloroform with bromine at -75° gave cholesta-7:9(11)-diene. The former workers found that bromination of ergosta-7:22-dien-3β-yl acetate in ether at -60° led to the separation of a tetrabromo-ergostenyl acetate in 48-53% yield. This tetrabromide which can also be obtained directly from ergosteryl-D acetate is moderately stable in the solid state, as are its solutions in ether or dioxan. However, alcohol and particularly chloroform solutions, suffer profound decomposition, even after a short period at room temperature. Sodium iodide in ethanol-benzene effects partial debromination giving 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate [dibromoergosteryl-D acetate] (IV), the structure of which was first established by its conversion to ergosteryl-D acetate and secondly by its characteristic ultraviolet absorption, identical in location with that of ergosteryl-D acetate. In contrast with tetrabromo-



ergostenyl acetate, 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate is markedly stable and can be hydrolysed with ease to the corresponding alcohol (V).

The structure of the intermediate tetrabromide has been established by Merck and Co., (Private communication, R. Stevenson) by chemical methods. They have shown that of the three probable structures (VI), (VII) and (VIII), the tetrabromo-compound has the structure (VIII).



Confirmation of this could possibly be obtained by spectroscopic methods. The tetrabromide shows a high light intensity absorption at 2570 Å (ϵ = 8750) and comparison of this with the ultraviolet absorption spectra of 4:5-dibromo-3:4-dimethylhex-2-ene and of 2:5-dibromo--3:4-dimethylhex-3-ene, or materials containing these systems, might show some correlation, and enable a complete decision between the 1:2 and 1:4 structures to be made.

22:23-Dibromoergosta-7:9(11)-dien-3β-yl acetate, obtained in 90% yield by sodium iodide debromination of the tetrabromide, undergoes debromination quantitatively when heated with zinc dust in ether-ethanol giving ergosteryl-D acetate (III). The latter material can also be obtained in 75% yield by bromination of ergosta--7:22-dien-3β-yl acetate in ether, followed by treatment with zinc dust, without isolation of the intermediate tetrabromide.

These investigations (110) thus provided excellent methods of obtaining ergosta-7:22-dien-3β-yl acetate, ergosteryl-D acetate, and 22:23-dibromoergosta-7:9(11)--dien-3β-yl acetate.

11-Oxygenated Steroids from 22:23-Dibromoergosta-7:9(11)--dien-3β-yl Acetate.

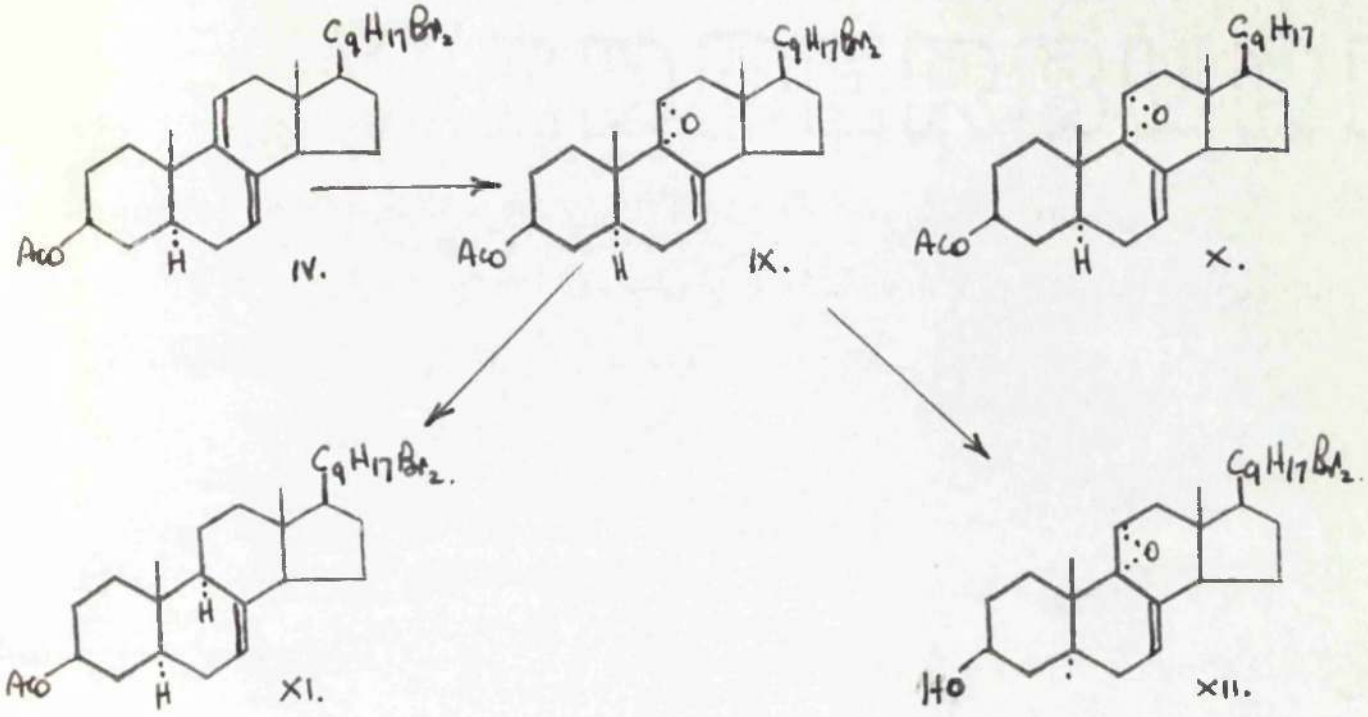
The introduction of an 11-oxygen function into the steroid nucleus starting from ergosterol and proceeding by way of 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate (IV) has many attractive features, not the least of which is that the bromine atoms afford an excellent method of protecting the side chain. 22:23-Dibromoergosta-7:9(11)--dien-3β-yl acetate is especially useful during oxidations, involving an excess of oxidising agent, where attack on an unsaturated side chain may take place. In particular the dibromo-side chain permits selective nuclear hydrogenation in high yield under specified experimental conditions.

The dibromo-derivatives usually crystallise in well defined forms and are considerably more insoluble in the common organic solvents, than their corresponding Δ^{22}--unsaturated analogues. In addition, the side chain ethylenic linkage can be regenerated, in quantitative yield, by debromination with zinc prior to side chain degradation. A comparison of the molecular rotations of the various ergosterol derivatives with those of the corresponding 22:23-dibromides shows that the 22:23-dibromo--steroids have a higher positive rotation (mean value of

60 units; see Experimental; 109-113). In some cases the individual groups in the nucleus exert some vicinal action causing divergence from the mean value.

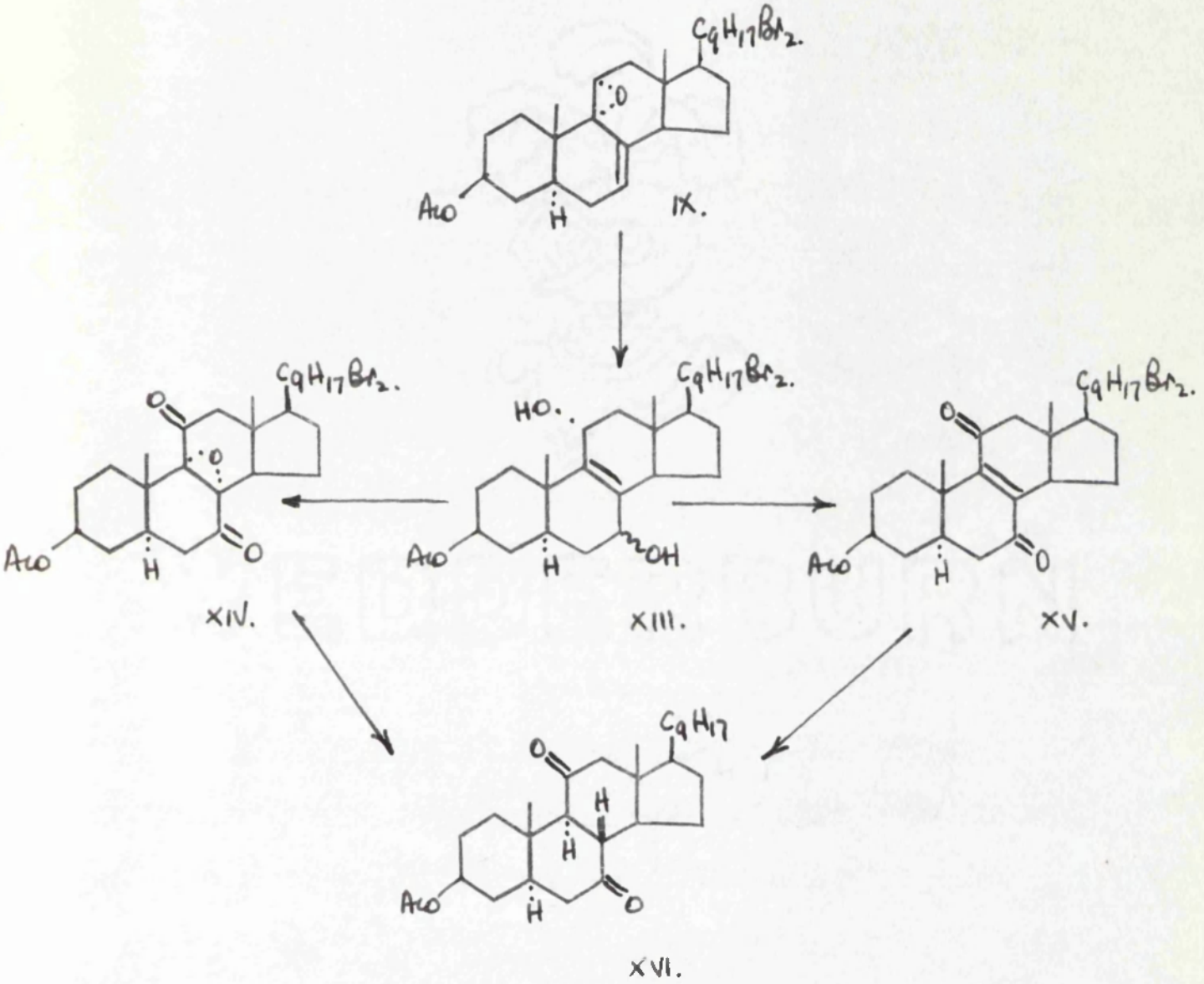
Since the initially devised route (110) from 22:23--dibromoergosta-7:9(11)-dien-3β-yl acetate to an 11--oxygenated steroid, involving peracetic acid oxidation, had not given high yields the problem was approached from an alternative direction. Oxidation of 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate (IV) with one mol. of perbenzoic acid in chloroform gave a monoepoxide in 70% yield. The reactions of this compound, which are described below, show that it is the 22:23-dibromide of ergosteryl-D acetate epoxide [itself obtained by a similar oxidation (44)] and to which the structure 9α:11α-epoxyergosta-7:22-dien-3β-yl acetate (X) was ascribed by Heusser et al., (45). This is supported by the observation that catalytic hydrogenation of 22:23-dibromoergosta--7:9(11)-dien-3β-yl acetate leads to initial saturation of the $\Delta^{9(11)}$-bond with the formation of 22:23-dibromoergost-7-en-3β-yl acetate (XI).

Alkaline hydrolysis of 22:23-dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate (IX) yielded 22:23-dibromo--9α:11α-epoxyergost-7-en-3β-ol (XII) reacetylation of



which gave the parent acetate The epoxy-bridge is stable to all alkaline conditions, but under the influence of acid catalysts, four distinct compounds can be obtained, all in high yield, depending on the experimental conditions chosen.

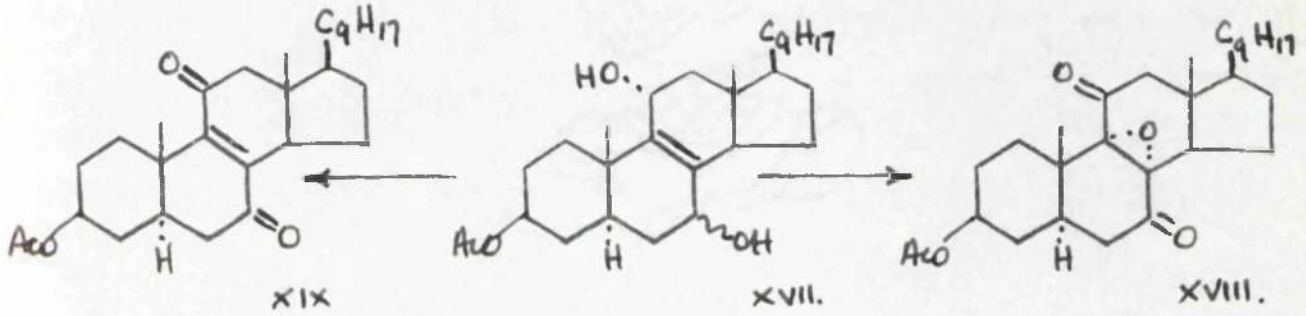
Controlled treatment (Budziarek, Ph.D. Thesis) of 22:23-dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate (IX) with sulphuric acid in aqueous dioxan gave 3β-acetoxy--22:23-dibromoergost-8-en-7ξ:11α-diol (XIII) in excellent yield. The latter compound was also formed when 9α:11α--epoxyergosta-7:22-dien-3β-yl acetate (X) was treated with bromine in chloroform solution or when 22:23-dibromo-



-9α:11α-epoxyergost-7-en-3β-yl acetate (IX) was crystallised from acetone containing a trace of acid.

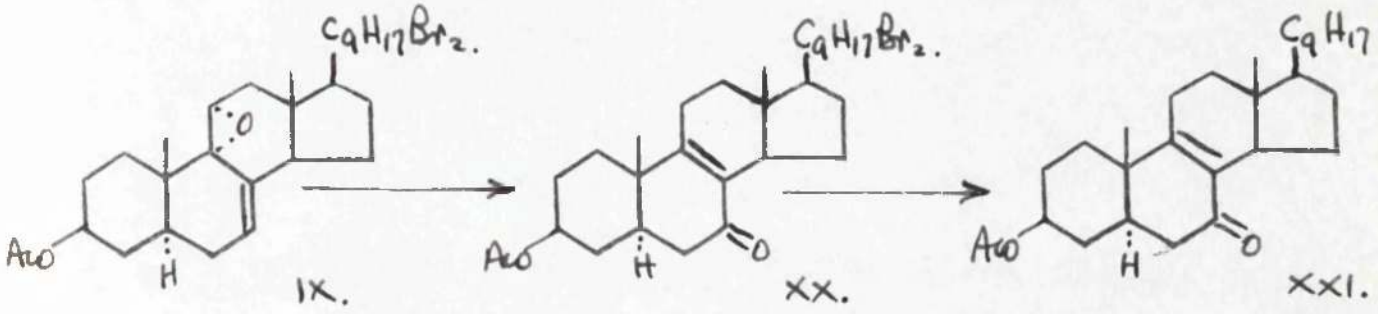
Oxidation of (XIII) with chromium trioxide in acetic acid gave a mixture of 22:23-dibromo-3α:9α-epoxy-7:11--dioxoergostan-3β-yl acetate (XIV) and 22:23-dibromo-7:11--dioxoergost-8-en-3β-yl acetate (XV). This parallels the similar oxidation (45) of 3β-acetoxyergosta-8:22-dien-

-7ξ:11α-diol (XVII) to 8α:9α-epoxy-7:11-dioxoergost-22--en-3β-yl acetate (XVIII) and 7:11-dioxoergosta-8:22--dien-3β-yl acetate (XIX), although only the latter compound was isolated when the oxidation was conducted in



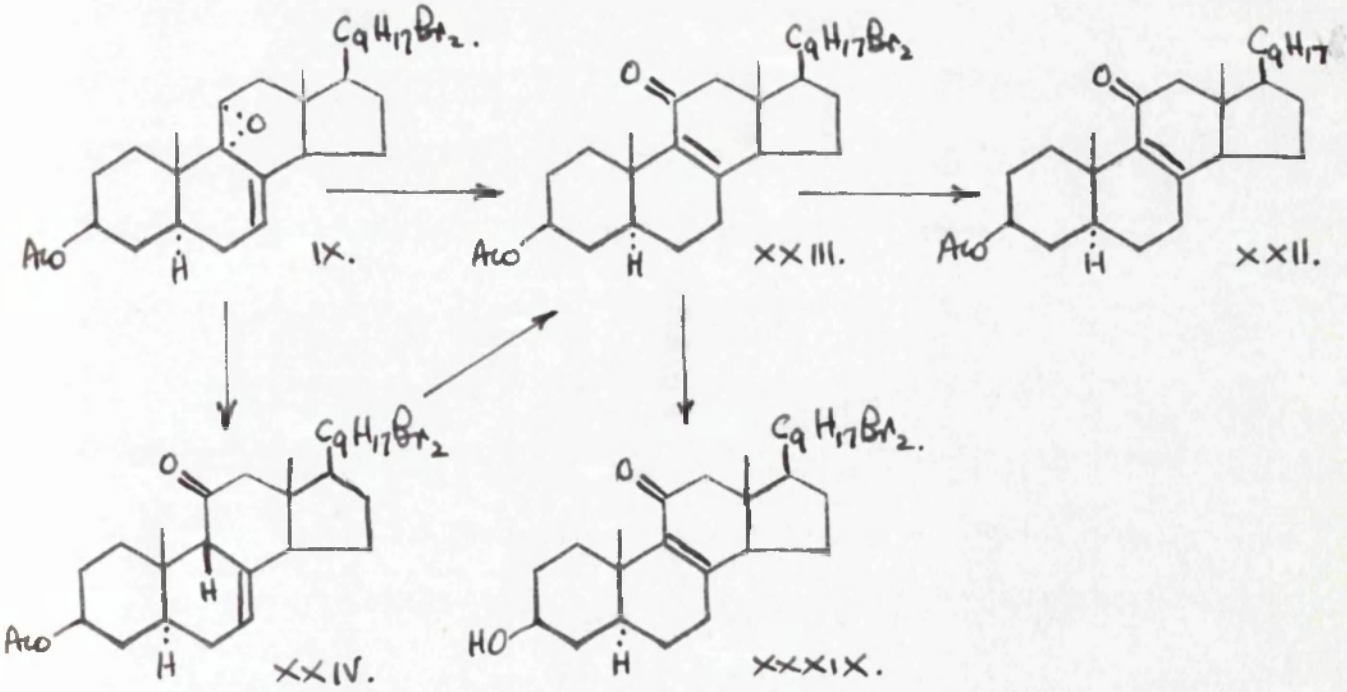
acetone solution (44). Reduction of (XIV) or (XV) with zinc dust in acetic acid afforded 7:11-dioxoergost-22--en-3β-yl acetate (XVI), debromination being simultaneously effected.

Prolonged treatment of 22:23-dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate (IX) with hydrochloric acid in aqueous methanol, followed by reacetylation of the product, yielded 22:23-dibromo-7-oxoergost-8-en-3β-yl acetate (XX),



debromination of which with zinc afforded the known 7-oxoergosta-8:22-dien-3β-yl acetate (XXI) identical with that obtained by previous workers (45, 75,109).

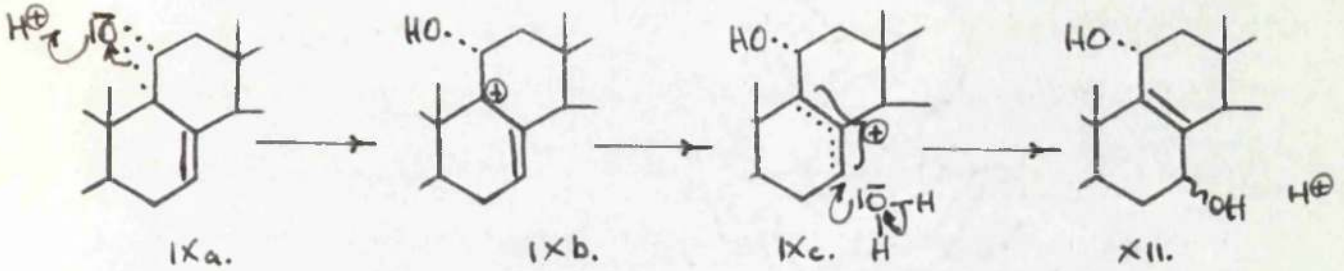
Treatment of 22:23-dibromo-9α:11α-epoxyergost-7-en--3β-yl acetate with boron trifluoride etherate in benzene, using the method described by Heusser et al., (45) for the preparation of 11-oxoergosta-8:22-dien-3β-yl acetate (XXII), gave 22:23-dibromo-11-oxoergost-8-en-3β-yl acetate (XXIII) in 80% yield. Debromination of (XXIII) with zinc yielded (XXII) quantitatively. The hydrolysis



of (XXIII) did not proceed in a simple manner and its reactions with alkali will be discussed in a later section.

Elks *et al.*, (57) have recently shown, in accordance with earlier work (54), that 22:23-dibromo-9α:11α-epoxy-ergost-7-en-3β-yl acetate when treated with boron trifluoride etherate in *ether solution* gives rise to 22:23--dibromo-11-oxo-9β-ergost-7-en-3β-yl acetate (XXIV). The same product could be isolated, when benzene was used as solvent, provided the reaction was quenched after about 15 secs. The marked susceptibility of this epoxide to rearrangement under the influence of acids, merits comment, but before discussing these reactions it is necessary to consider two important facts: (a) that one of the more important factors governing the course of a chemical reaction is the nature of the solvent employed (118), and (b) that the formation and subsequent rearrangement of a carbonium ion is dependent on the stability it may derive from its environment (119). Recognising these basic tenets, the reactions of the epoxide possibly proceed by the following mechanisms.

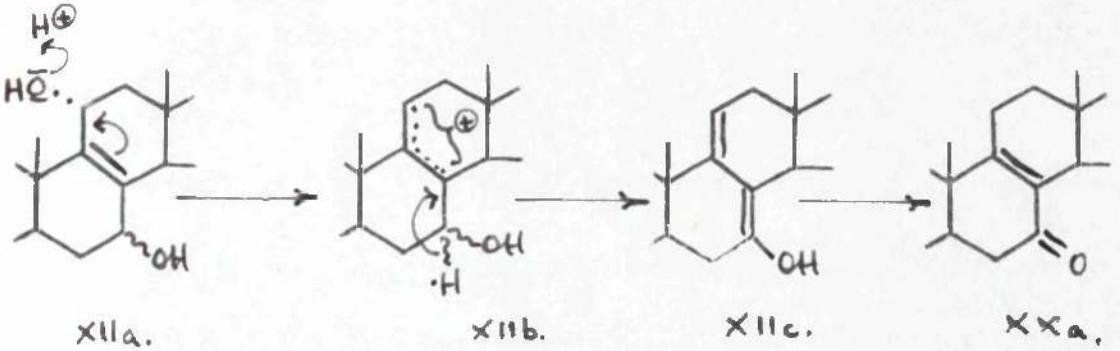
In highly polar solvents, using an acidic catalyst, the rearrangement takes the course outlined below (45) giving (XII) as the first isolated product. Proton fission of the epoxide (IXa) generates a carbonium ion at $C_{(9)}$ (IXb) which immediately passes into the form (IXc), a much



IXa. IXb. IXc. XII.

more stable state due to the distribution of the ⊕ve charge over three carbon atoms instead of one, and to the electron-donating character of the solvent which by solvating the ion causes even greater distribution of the charge; both effects lower the energy of the system. The distributed ion (IXc) then abstracts an anion from the solvent, in this case $OH^{\ominus}$ (aqueous medium) giving (XII). Henbest et al., (77) found that if acetic acid were used as the solvent, an acetate group appeared at $C_{(7)}$, as might have been expected, if the above formulation of the reaction is correct.

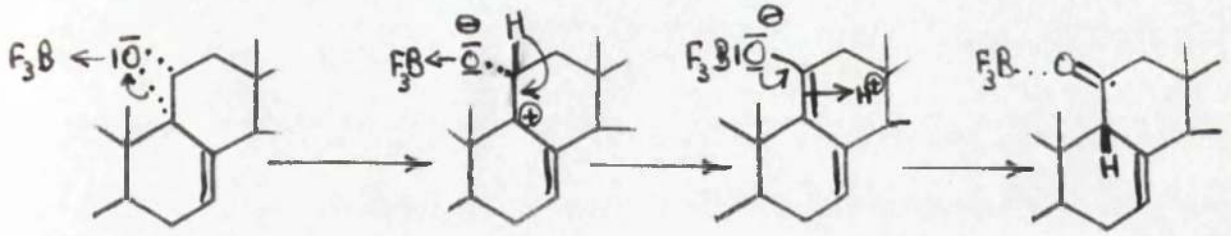
The diol (XII) in the same medium can then undergo a similar further rearrangement, as shown to the 7-oxo--8-ene (XXa).



XIIa. XIIb. XIIc. XXa.

Here again the polar solvent stabilises the distributed ion (XIIb). The system ultimately attains complete stability by rearrangement, and ejection of a proton from $C_{(7)}$, giving the enol form (XIIc), which becomes (XXa).

In weakly polar or aprotic solvents, carbonium ions are only stabilised to a small extent by the medium and thus may assume alternative charge distributed forms. Thus the ion (IXe) which would possibly be formed at $C_{(9)}$



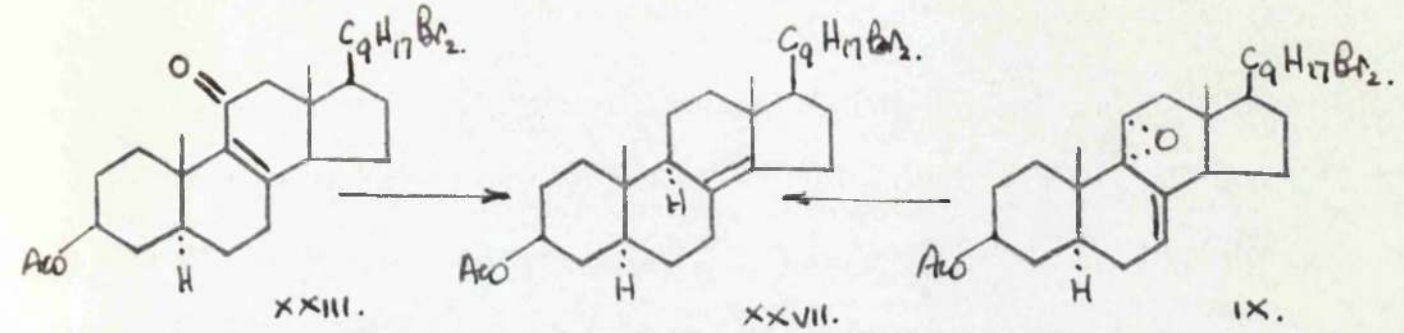
IXd. IXe IXf. XXIVa.

when the epoxide (IXd) is treated with boron trifluoride in ether, could not undergo complete solvation by ether, and takes the shortest alternative route to stability in the formation of the synartetic ion (IXf) (121,118). The latter's formation is, without doubt, simultaneous with the fission of the 9α:11α-epoxy-bridge, a full ⊕ve charge never appearing at $C_{(9)}$ at any one instant. This transition complex (IXf) then attains complete stability by the formation of a $C_{(11)}$-ketone and attachment of the

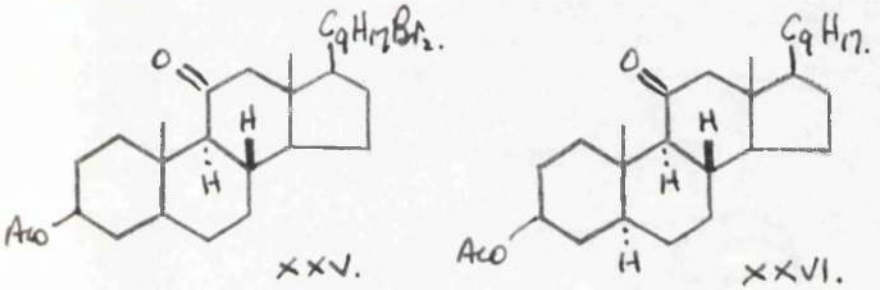
former $C_{(11)}$-β-hydrogen at $C_{(9)}$ giving (XXIVa).

In benzene where boron trifluoride etherate displays an even greater electron withdrawing capacity (without doubt due to the greater dissociation of the etherate in benzene, i.e. mass action effect, $RORBF_3$ + inert solvent $\rightleftharpoons$ ROR + BF_3), the formation of (XXIVa) is immediately followed by conjugation of the Δ^7-bond with the $C_{(11)}$--ketone, the $C_{(9)}$-β-hydrogen atom undergoing a 1:3 shift to $C_{(7)}$ giving (XXIII). This rearrangement also occurs very slowly in ether. Other strongly electron-withdrawing reagents (Lewis acids) such as magnesium bromide (76) and ferric chloride (49) will also cause these arrangements, in the same solvents, and by similar mechanisms.

Since 22:23-dibromo-11-oxoergost-8-en-3β-yl acetate had been obtained in good yield from the 9α:11α-epoxide, it was felt that a stereospecific reduction of the Δ^8--bond of the former compound might lead to 22:23-dibromo--11-oxoergostan-3β-yl acetate (XXV) and thus provide a much more convenient synthesis of 11-oxoergost-22-en-3β-yl acetate (XXVI). In view of the protection afforded the Δ^{22}-bond by the bromine atoms catalytic hydrogenation of the Δ^8-bond of (XXIII) was attempted by several methods.



XXIII. XXVII. IX.

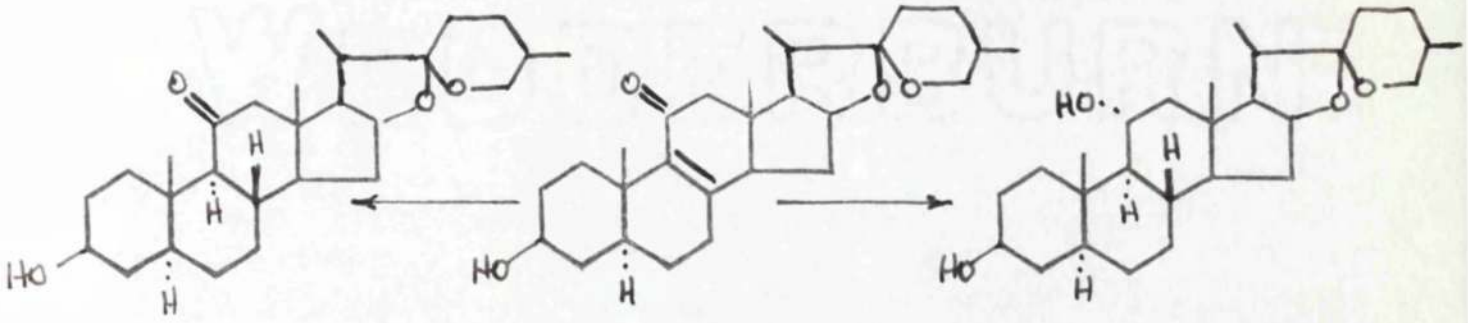


XXV. XXVI.

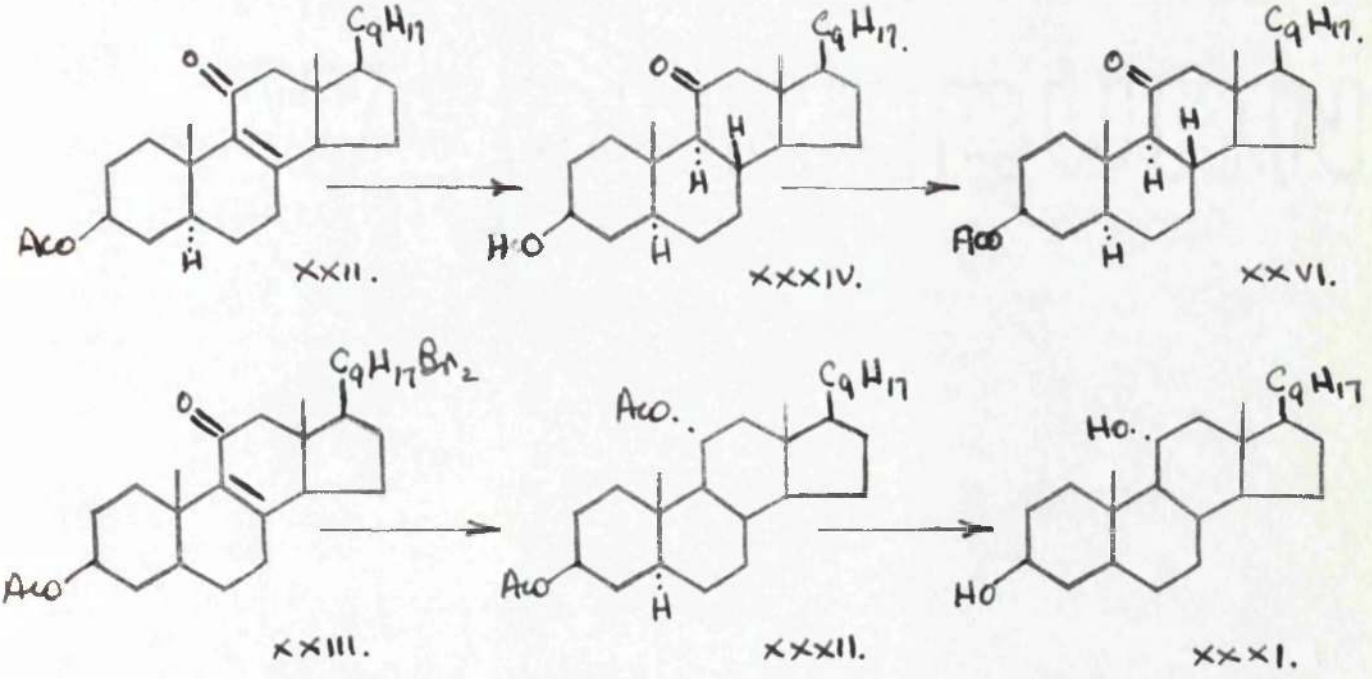
Hydrogenation of 22:23-dibromo-11-oxoergost-8-en-3β-yl acetate and likewise of 22:23-dibromo-9α:11α-epoxy-ergost-7-en-3β-yl acetate (IX) in glacial acetic acid, using a platinum catalyst, caused hydrogenolysis of the carbonyl or epoxide group respectively, and migration of the double bond in each compound giving, in high yield, 22:23-dibromoergost-8(14)-en-3β-yl acetate (XXVII). Attempted saturation of the 8(9)-ethylenic linkage using a palladium catalyst with glacial acetic acid as solvent, or a platinum oxide catalyst in ethyl acetate solution, resulted in a negligible uptake of hydrogen, and only starting material was recovered from the reactions.

Parallel with these attempts at catalytic hydrogenation, several methods of chemical reduction were also investigated. Treatment of (XXIII), in refluxing amyl alcohol, with sodium metal gave only a non-crystallisable gum, whilst aluminium amalgam in moist ether gave a mixture of reduction products, inseparable by crystallisation, and which resinified during chromatography.

Before an outlined programme of such reduction experiments could be completed, Tishler *et al.*, (48) in a brief communication reported that 11-oxoergosta-8:22--dien-3β-yl acetate (XXII) could be reduced to 11-oxoergost-22-en-3β-yl acetate (XXVI) by means of lithium metal in liquid ammonia. Simultaneously Djerassi and his co-workers (73) stated that with the analogous diosgenin derivative (XXVIII) limited quantities of lithium in liquid ammonia reduced the Δ^{9}-bond to the natural 8β:9α-configuration. In the presence of alcohol and an



excess of lithium, the 11-keto group also underwent reduction giving the 11α-hydroxy derivative (XXIX). When this reduction procedure was applied to 22:23-dibromo--11-oxoergost-8-en-3β-yl acetate, in the absence of alcohol, only one product, ergost-22-en-3β:11α-diol (XXXI), isolated as its diacetate (XXXII), was obtained, irrespective of whether limited or excessive amounts of lithium were used. Even when the reaction time was limited to two minutes, the sole product after reacetylation was the diacetate, the reduction, as before, being accompanied by debromination.



However, reduction of 11-oxoergosta-8:22-dien-3β-yl acetate (XXII) with limited quantities of lithium in

liquid ammonia afforded 11-oxoergost-22-en-3β-ol (XXXIV). Acetylation yielded (XXVI) identified by comparison with a specimen prepared according to Heusser et al (45) and Chamberlin et al., (44).

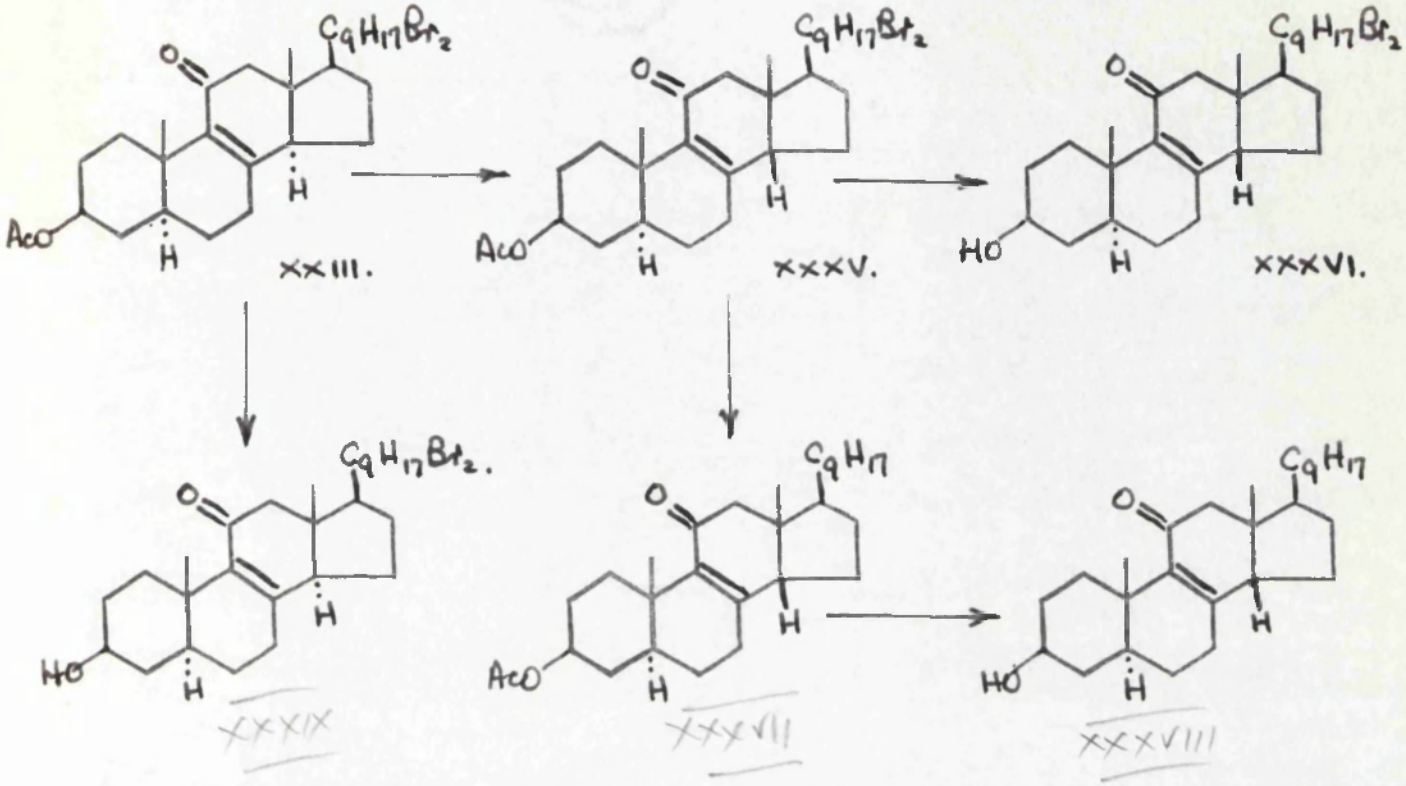
It is, therefore, considered likely that the lithium bromide produced by debromination of the side chain may exert a catalytic effect in furthering the reduction of the $C_{(11)}$-ketone, although no experiments were performed to confirm this point of view.

Hydrolysis of 3β:11α-diacetoxyergost-22-ene gave the diol reacetylation of which afforded the parent diacetate. The constants obtained for these two compounds are identical with these found by previous workers (50).

The Action of Alkali on 22:23-Dibromo-11-oxoergost-8-en-3β-yl Acetate.

Preliminary attempts to saponify 22:23-dibromo-11-oxoergost-8-en-3β-yl acetate (XXIII) were complicated by an epimerisation occurring at $C_{(14)}$, and even initial attempts to obtain the 14β-epimer, using conditions described by Djerassi (79) for epimerisation of the diosgenin analogue, were not very successful. In view of this, the effect of alkaline conditions on (XXIII) was investigated.

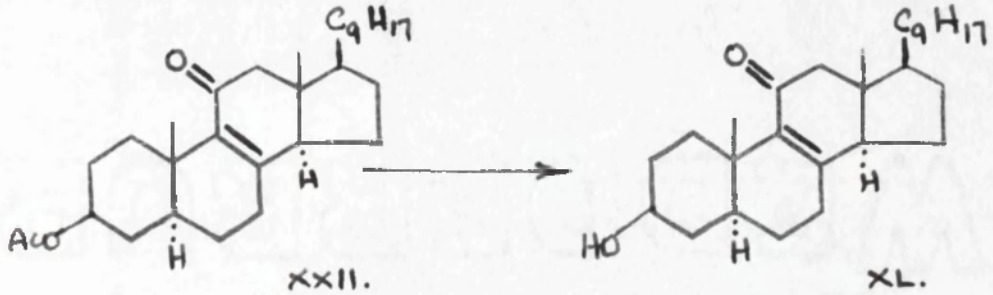
It was found that the desired 22:23-dibromo-11-oxo-14β-ergost-8-en-3β-yl acetate (XXXV) could be obtained, in good yield, by refluxing 22:23-dibromo-11-oxoergost-8-en-3β-yl acetate with 5% methanolic potassium hydroxide for 5-6 hours followed by reacetylation. Shorter reaction periods of 1-2 hours led to mixtures, difficultly separable by crystallisation and giving at best only small quantities of the required 14β-epimer. The best method of obtaining the 14β-epimer (XXXV), however, consisted of treating the 14α-epimer (XXIII) with dry hydrogen chloride in refluxing glacial acetic acid, followed by reacetylation. 22:23-Dibromo-11-oxo-14β-ergost-8-en-3β-yl acetate was characterised as the alcohol (XXXVI) by basic hydrolysis,



XXIII. XXXV. XXXVI.

XXXIX XXXVII XXXVIII

reacetylation affording the parent acetate. Debromination of the latter with zinc dust yielded 11-oxo-14β-ergosta--8:22-dien-3β-yl acetate (XXXVII) alkaline hydrolysis of which led to the alcohol (XXXVIII). Treatment of 22:23--dibromo-11-oxoergost-8-en-3β-yl acetate with methanolic potassium carbonate, under conditions which did not lead to any epimerisation at $C_{(14)}$ gave 22:23-dibromo-11-oxo-ergost-8-en-3β-ol (XXXIX). Likewise, 11-oxoergosta-8:22--dien-3β-yl acetate (XXII) gave the corresponding



XXII. XL.

alcohol (XL). Reacetylation of these alcohols regenerated the acetates.

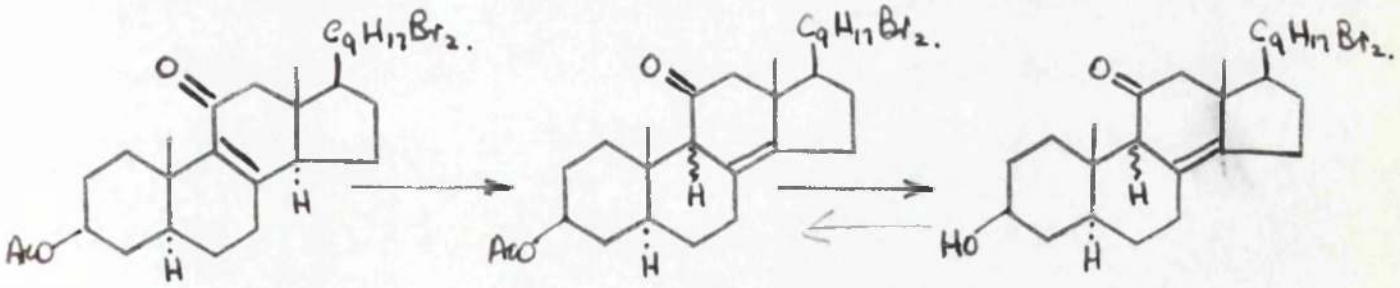
In all of the 14β-steroids of this type, that have so far been examined, the ultraviolet absorption peak of the ring-C-αβ-unsaturated ketone appears approximately 50 Å lower in wavelength than the peak for the 14α--analogues, as shown in the table below.

	Absorption Max.	
	x=α	x=β
22:23-Dibromo-11-oxo-14x-ergost--8-en--3β-yl acetate	2530Å	2480Å
22:23-Dibromo-11-oxo-14x-ergost-8-en-3β-ol	2540Å	2490Å
11-Oxo-14x-ergosta-8:22-dien-3β-yl acetate	2530Å	2480Å
11-Oxo-14x-ergosta-8:22-dien-3β-ol	2540Å	2500Å
3β:7β-Diacetoxy-22:23-dibromo-11-oxo--14x-ergost-8-ene	2490Å	2440Å
3β:7β-Diacetoxy-11-oxo-14x-ergosta--8:22-diene	2490Å	2450Å

The last two 14β-compounds, in the table, (for samples of which the author is indebted to J.Grigor of this Department) were postulated as having a 14β-hydrogen atom in a previous paper (115) and the positions of their ultraviolet maxima confirm the assignment of these configurations.

Djerassi *et al.*, (79) describe 11-oxo-22a-spirost-3-en--3β-yl acetate and its 14β-isomer, both as having maxima at 2520 Å. No explanation of this anomaly is apparent.

Treatment of 22:23-dibromo-11-oxoergost-3-en-3β-yl acetate (XXIII) with a 12% solution of potassium hydroxide in ethanolic benzene for a short period, followed by reacetylation, gave a product which, from a consideration of its physical and chemical properties, is considered to be 22:23-dibromo-11-oxoergost-3(14)-en-3β-yl acetate (XLI). Basic hydrolysis at 15° gave the alcohol (XLII), acetylation of which gave the parent acetate.



XXIII XLI XLII

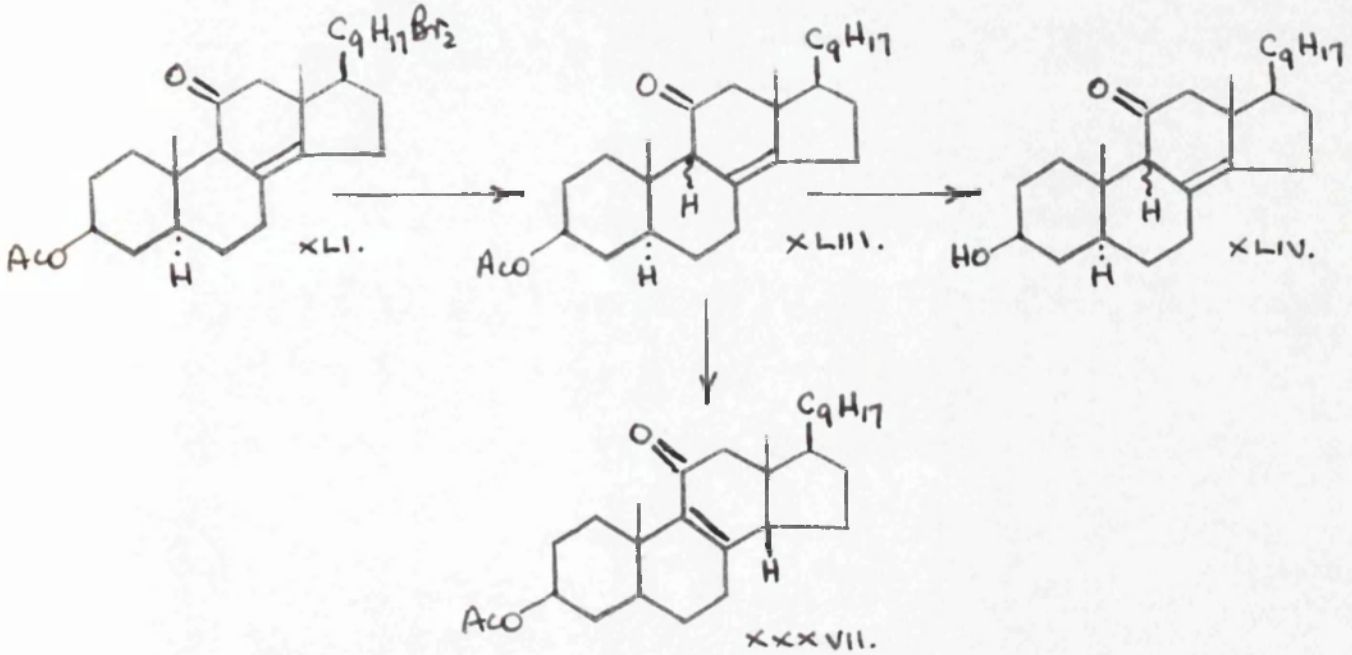
The structure of this steroid and its alcohol were determined by the following date:-

(a) Neither compound showed significant light absorption above 2200Å; below 2200Å the curve characteristics were indicative of a tetrasubstituted double bond (30,31).

(b) Examination of the infra-red spectra of the alcohol and its acetate indicated the presence of a non--conjugated ketone in the former and of ketone and acetate groups in the latter (see Experimental).

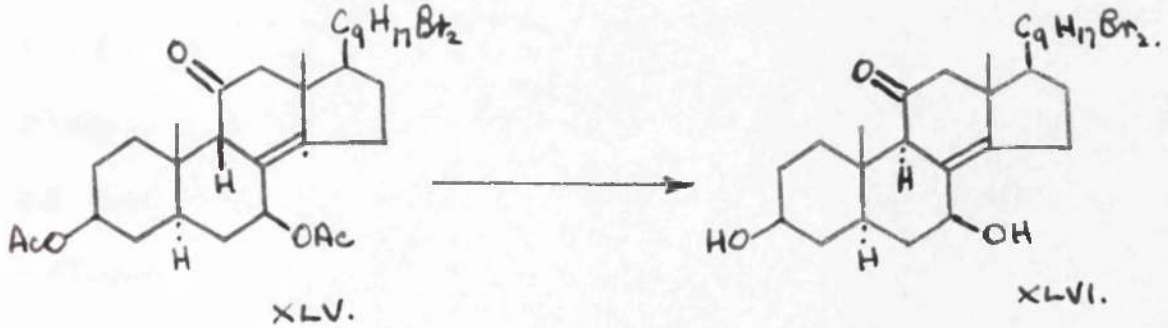
(c) Reconjugation of the $\Delta^{8(14)}$-bond with the $C_{(11)}$ ketone could be effected by prolonged refluxing with dilute alkali, or by treatment with dry hydrogen chloride in chloroform in boiling glacial acetic acid. Hydrogen chloride in chloroform effected reconjugation very slowly, only 10% of the crude reaction product being 22:23-dibromo--11-oxo-14β-ergost-8-en-3β-yl acetate after 3 hours, as shown by spectrometric data.

Debromination of 22:23-dibromo-11-oxoergost-8(14)-en--3β-yl acetate with zinc gave 11-oxoergosta-8(14):22-dien--3β-yl acetate (XLIII) characterised as the alcohol (XLIV)



by hydrolysis, at 15°, with dilute alkali. Treatment of 11-oxoergosta-8(14):22-dien-3β-yl acetate (XLIII) with dilute alkali, at reflux temperature, for 5 hours, afforded 11-oxo-14β-ergosta-8:22-dien-3β-yl acetate (XXXVII) after reacetylation.

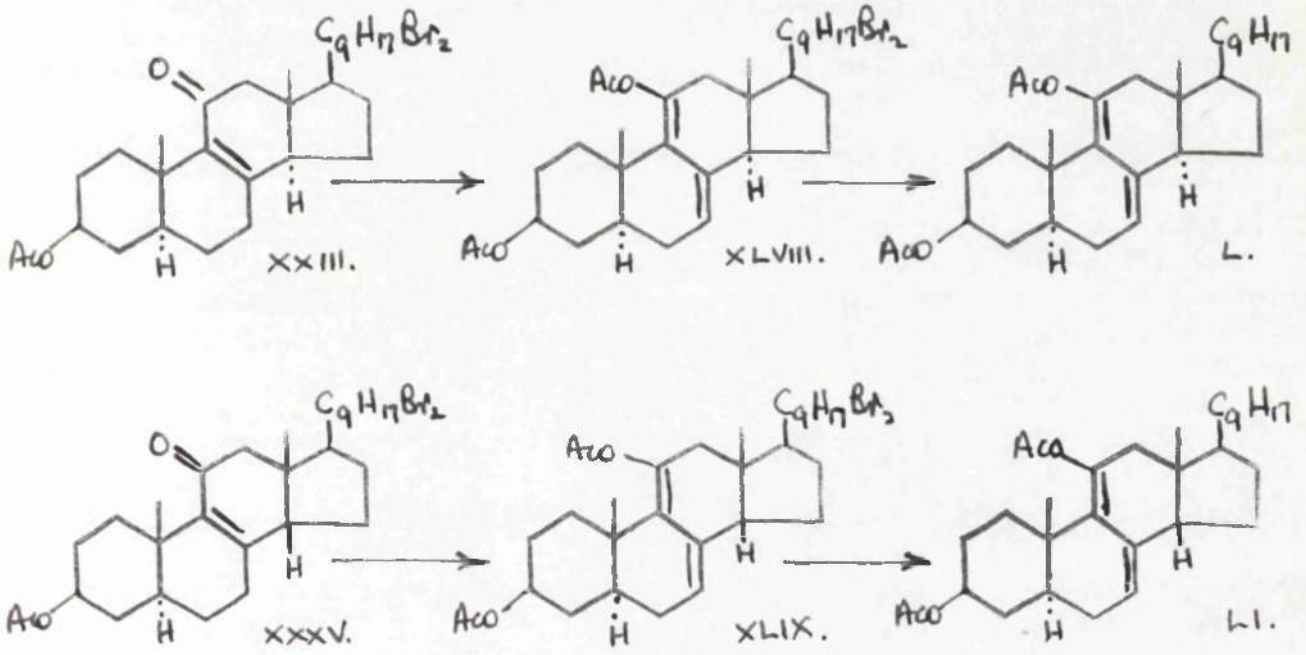
The configuration of the C(9)-hydrogen atom in the $\Delta^{8(14)}$-11-oxo-compounds is considered to be 9α in view of the fact that the initially formed 22:23-dibromo-11-oxo-ergost-8(14)-en-3β-ol (XLIV) was produced under equilibrating conditions. This is also supported by the action of alkali on 3β:7β-diacetoxy-22:23-dibromo-11-oxo-9β--ergost-8(14)-ene (XLV) which gives initially 22:23--dibromo-11-oxo-9α-ergost-8(14)-en-3β:7β-diol (XLVI)(115), indicating the 9α configuration to be the most stable in these $\Delta^{8(14)}$-compounds.



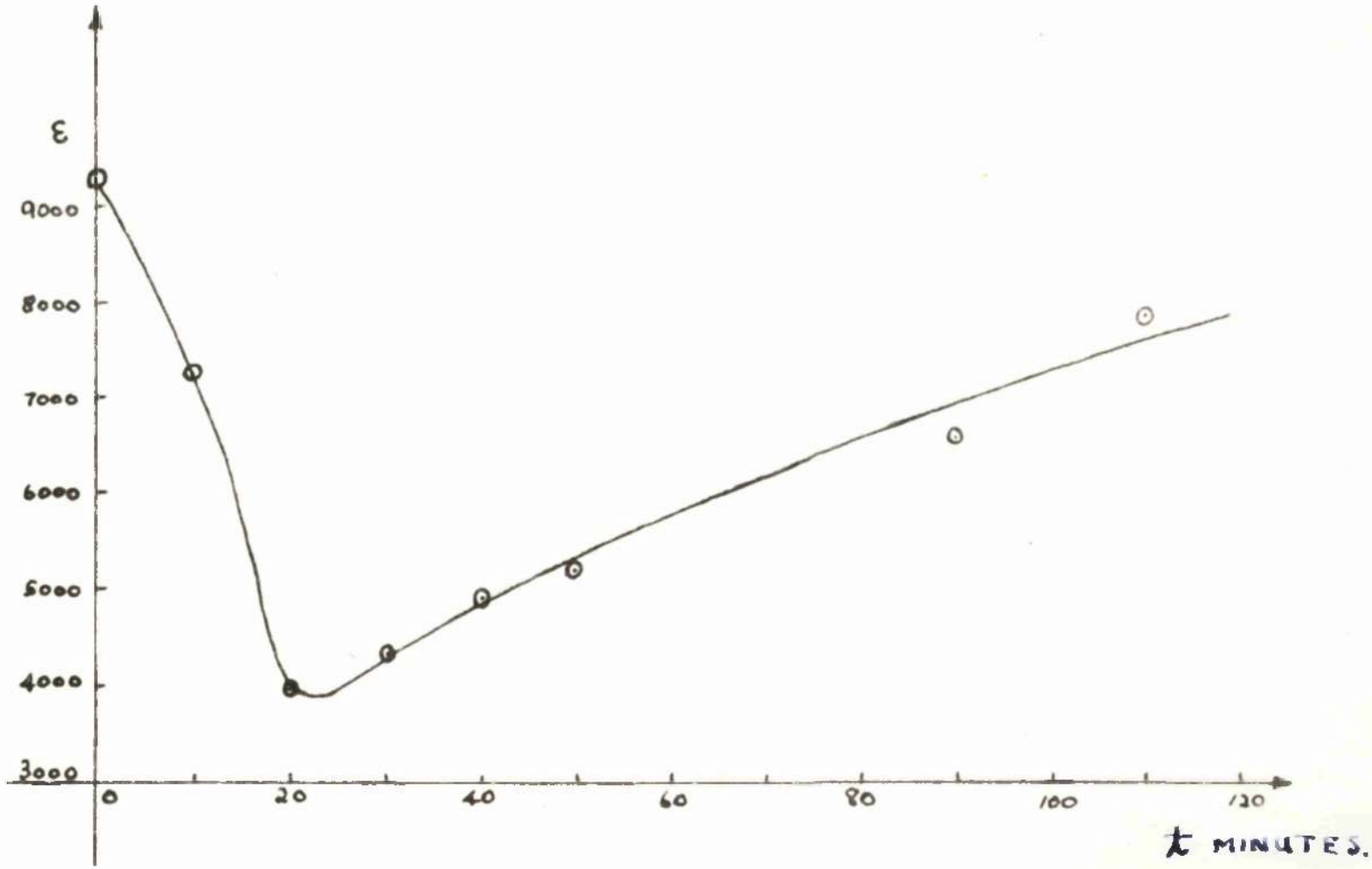
XLV. XLVI.

Whereas 22:23-dibromo-11-oxo-ergost-8-en-3β-yl acetate (XXIII) and its 14β-epimer (XXXV) undergo enol

acetylation, with isopropenyl acetate in the presence of an acid catalyst, to give 3β:11-diacetoxy-22:23--dibromoergosta-7:9(11)-dien (XLVIII) and 3β:11-diacetoxy--22:23-dibromo-14β-ergosta-7:9(11)-diene (XLIX) respectively, 22:23-dibromo-11-oxoergost-8(14)-en-3β-yl acetate



(XLI) was recovered unchanged under these conditions, no ring-C homoannular diene being formed. Debromination of the enol acetates (XLVIII) and (XLIX) gave 3β:11--diacetoxyergosta-7:9(11)-diene (L) and 3β:11-diacetoxy--14β-ergosta-7:9(11)-diene (LI). The heteroannular nature of these four dienes was established by an examination of their ultraviolet absorption spectra, all of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which showed an absorption peak with no fine structure in the region 2390-2450 Å [cf. Djerassi et al., (83)].

In view of the results obtained by the action of strong alkali on 22:23-dibromo-11-oxoergost-8-en-3β-yl acetate the reaction was followed spectrometrically in order to determine whether an equilibrium mixture of the $\Delta^{8(14)}$-isomer and the 14β-isomer was being produced, and to give a rough indication of the most suitable reaction time, to obtain a maximum yield of the former material. In the examination of this reaction, samples of the reaction mixture were withdrawn over a period of approximately 2 hours. Each specimen was worked up, acetylated and the crude acetate examined for absorption intensity at 2430-2530 Å. The results, shown in the graph opposite, indicate that under the conditions of the experiment, the Δ^{8}-bond moves out of conjugation with the $C_{(11)}$-ketone quite rapidly to give the $\Delta^{8(14)}$-11-oxo-steroid, while reconjugation occurs more slowly to yield the 14β-steroid. The latter compound, in the acetate form, was the only substance which was isolated from the final reaction mixture. From the minimum point on the curve, it can be inferred that the maximum yield of 22:23-dibromo-11-oxo-ergost-8(14)-en-3β-yl acetate might be obtained after

22 minutes. The solubility of the latter, however, is similar to that of the starting material and experimentally a reaction period of 30 minutes was found to be an optimum for a good yield and easy purification.

Treatment of pure 22:23-dibromo-11-oxo-14β-ergost--8-en-3β-yl acetate (XXXV) with a 12% solution of potassium hydroxide in ethanol-benzene for 2.5 hours, followed by reacetylation, gave a product whose ultraviolet absorption spectrum showed a peak at 2480 Å, only slightly lower in intensity than that of pure starting material. Extensive examination of this material failed to reveal the presence of any 22:23-dibromo-11-oxoergost--8(14)-en-3β-yl acetate (XLI) indicating that 22:23--dibromo-11-oxo-14β-ergost-8-en-3β-ol is stable to the action of alkali. As expected, treatment of 22:23--dibromo-11-oxoergost-8(14)-en-3β-yl acetate with strong alkali solution gave the 14β-isomer.

The facile migration of the Δ^{8}-bond out of conjugation with the $C_{(11)}$-ketone demonstrates the thermodynamically stable nature of the $\Delta^{8(14)}$-bond. In addition, the Δ^{8}-bond is in an unfavoured position with regard to the two trans linked systems of rings A and B and of rings C and D (89), although in view of the reconjugation which

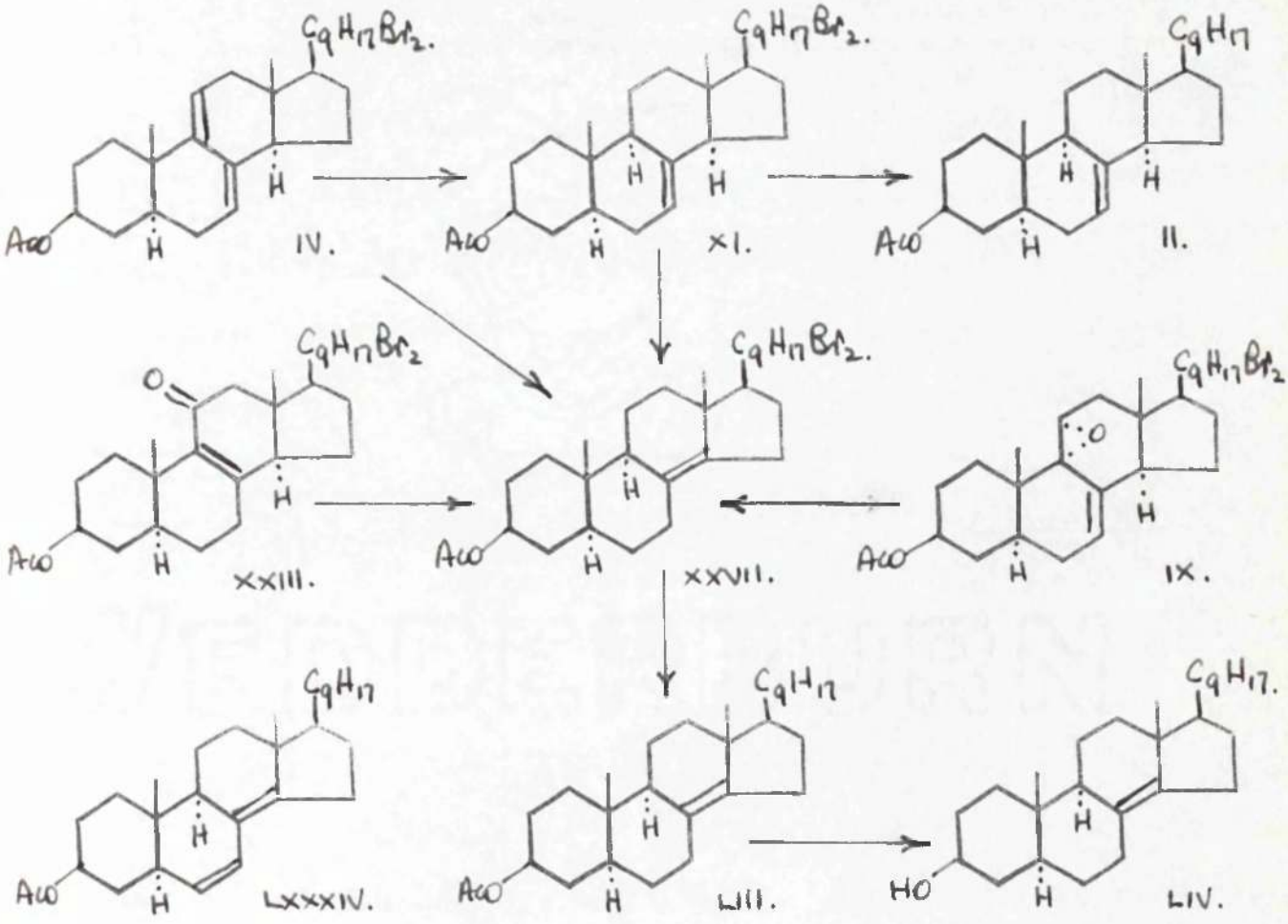
can take place, the most critical factor is the stable cis-fusion of the C/D indene system. In this connection it is interesting to note that Barton and Laws (32) have shown that indane systems of similar molecules are the most stable when the C/D ring fusion is trans.

The Reduction of 22:23-Dibromoergosta-7:9(11)-dien-3β-yl Acetate.

Concurrently with the oxidation experiments that were carried out on 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate (IV) a study was made of its catalytic reduction (113). 22:23-Dibromoergosta-7:9(11)-dien-3β-yl acetate offers some advantage over ergosteryl-D acetate as a starting point for the synthesis of 11-oxo-steroids, since nuclear oxidation is more efficiently effected in the former than in the latter, presumably because of partial attack at the side chain ethylenic linkage. The side chain halogen atoms also provided a satisfactory protection of the 22:23-double bond of ergosteryl-D acetate under the hydrogenation conditions used and, coupled with the easy regeneration of the Δ^{22}-bond by zinc dust, has led to efficient methods for the preparation of ergosta-8(14):22--dien-3β-ol (LIV) and ergost-22-en-3β-ol (LIX). Of these compounds the former has been described by Laubach and Brunings (71) since this work was completed. They prepared it by hydrogenation of ergosta-6:8(14):22-trien-3β-yl acetate (LXXXIV) in a neutral solvent over Raney nickel. Ergost-22-en-3β-ol has been obtained by Barton, Cox, and Holness (84) by partial hydrogenation of isoergosterone

(LXII) to 3-oxoergost-22-ene (LXXXIII) followed by reduction of the latter with sodium and propanol.

Hydrogenation of 22:23-dibromoergosta-7:9(11)-dien--3β-yl acetate (IV) in ethyl acetate over platinum gave 22:23-dibromoergost-7-en-3β-yl acetate (XI) which was characterised by hydrolysis to the alcohol, and as the derived benzoate. The molecular rotation data (Table I) support the structure allocated to 22:23-dibromoergost-7--en-3β-ol. Furthermore, the observed changes in molecular



rotation accompanying saturation of the double bond (comparison with 22:23-dibromoergostan-3β-ol, its acetate and benzoate) are in agreement with values for the saturation of comparable stenols (23,35). The structure allocated to 22:23-dibromoergost-7-en-3β-yl acetate was confirmed by its conversion, in high yield, to ergosta--7:22-dien-3β-yl acetate (11) by zinc dust treatment.

Hydrogenation of 22:23-dibromoergosta-7:9(11)-dien--3β-yl acetate in acetic acid over platinum gave 22:23--dibromoergost-8(14)-en-3β-yl acetate (XXVII) characterised as in the case of the Δ^7-isomer. Furthermore, shaking 22:23-dibromoergost-7-en-3β-yl acetate (XI) in acetic acid with a platinum catalyst saturated with hydrogen caused isomerisation to 22:23-dibromoergost--8(14)-en-3β-yl acetate (XXVII). The location of the double bond in these compounds follows from well established considerations (36). Thus the molecular rotation changes on acetylation Δ_1 and benzoylation Δ_2 of the alcohol, are in good agreement with representative values for other 5α-st-8(14)-en-3β-ols, and the ultraviolet absorption spectrum of the acetate agrees with that expected for a $\Delta^{8(14)}$-stenol (30,31) or its acetate. However, although the molecular rotation changes on

TABLE I

	M_D				
	Alcohol	Acetate	Benzoate	Δ_1	Δ_2
22:23-Dibromo-ergostan-3β-ol	+42°	+12°	+40°	-30°(-34°)	-2°(+2°)
22:23-Dibromo-ergost-7-en-3β-ol	-47°	-42°	-26°	+5°(-6°)	+21°(+30°)
Δ (saturation of F)	+39°(+77°)	+54°(+57°)	+66°(+64°)	—	—
22:23-Dibromoergost--8(14)-en-3β-ol	+73°	+27°	+20°	-46°(-40°)	-53°(-42°)
Δ (saturation of F)	-31°(+9°)	-15°(+14°)	+20°(+53°)	—	—

Standard values from Barton (28,38) are given in parentheses.

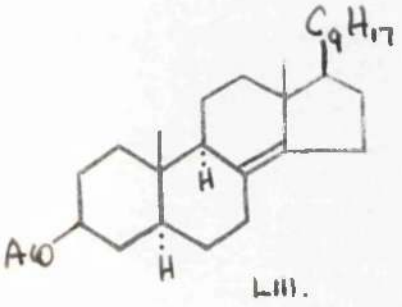
acetylation and benzoylation of 22:23-dibromoergost--8(14)-en-3β-ol are normal, the Δ-values for saturation of the double bond are only in fair agreement with standard values for 8(14) stenols. This indicates some degree of vicinal action of the side chain [cf. Mancera et al., (87)].

Debromination of 22:23-dibromoergost-8(14)-en-3β-yl acetate with zinc afforded ergosta-8(14):22-dien-3β-yl acetate (LIII) which was characterised by hydrolysis to the alcohol (LIV).

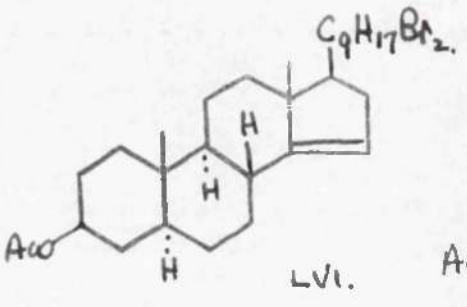
As has already been mentioned previously, (XXVII) was also obtained when, either 22:23-dibromo-11-oxoergost--8-en-3β-yl acetate, or 22:23-dibromo-9α:11α-epoxyergost--8-en-3β-yl acetate was hydrogenated in acetic acid using a platinum catalyst.

Treatment of 22:23-dibromoergost-8(14)-en-3β-yl acetate or benzoate with dry hydrogen chloride in chloroform gave mixed crystals which could not be resolved by crystallisation. Debromination of the acetate dibromide mixed crystal gave ''β''-dihydroergosteryl acetate characterised by the preparation of the corresponding alcohol and benzoate. ''β''-Dihydroergosteryl acetate was shown by Barton, Cox and Holness (84), to be an inseparable

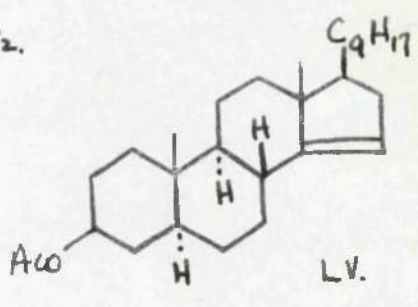
equimolar mixture of ergosta-8(14):22-dien-3β-yl acetate (LIII) and ergosta-14:22-dien-3β-yl acetate (LV).



LIII.



LVI.



LV.

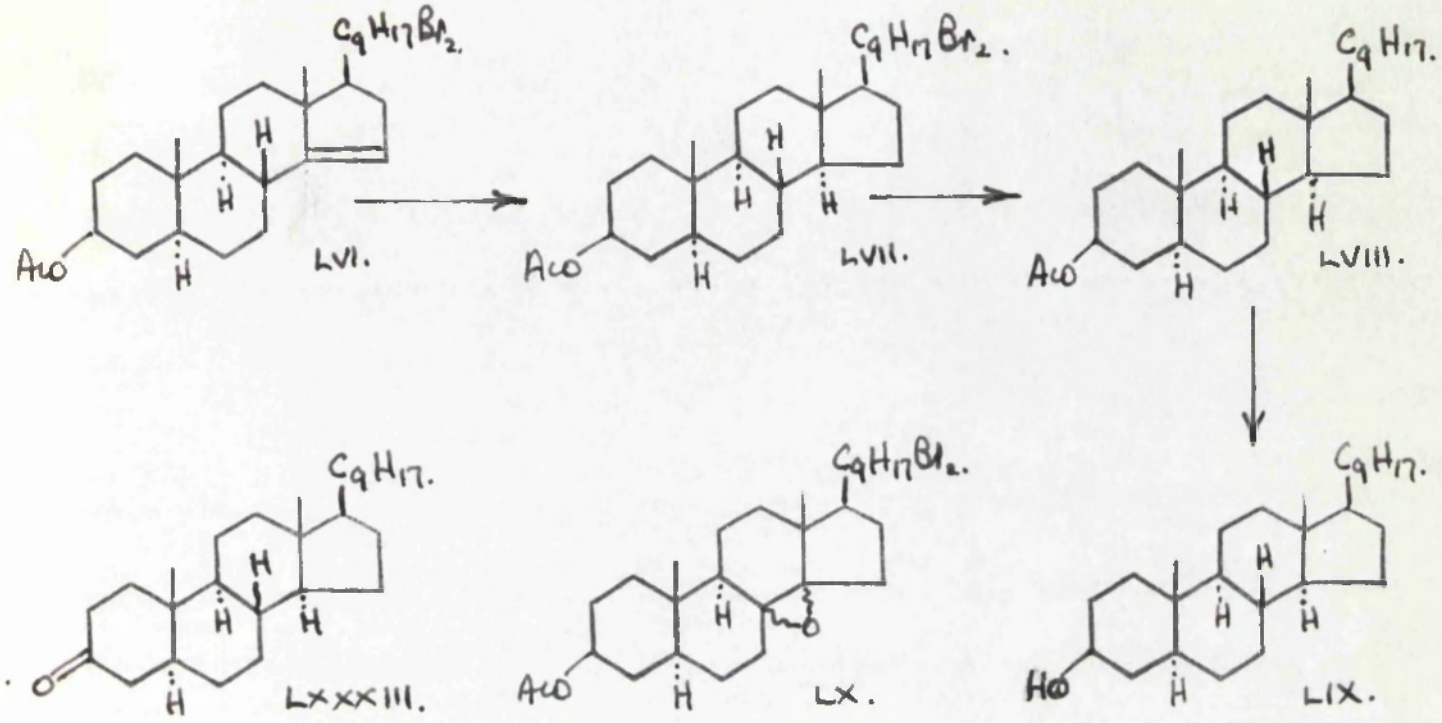
The product obtained by isomerisation of 22:23-dibromo--ergost-8(14)-en-3β-yl acetate is, therefore, a mixture of 22:23-dibromoergost-8(14)-en-3β-yl acetate and 22:23--dibromoergost-14-en-3β-yl acetate (LVI), and, since it affords ''β''-dihydroergosteryl acetate in nearly quantitative yield, it is inferred that it, likewise, is an equimolar mixture. The molecular rotations of 22:23--dibromoergost-14-en-3β-ol and its derivatives, calculated from the values for 22:23-dibromoergost-8(14)-en-3β-ol and of the mixed crystals (22:23-dibromoergost-8(14)-en-3β-ol and 22:23-dibromoergost-(14)-en-3β-ol) are shown in Table II.

TABLE II

	M_D				
	Alcohol	Acetate	Benzoate	Δ_1	Δ_2
22:23-Dibromoergost--8(14)-en-3β-ol	+73°	+27°	+20°	-46°(-40°)	-53°(-42°)
[22:23-Dibromoergost--8(14)-en-3β-ol + 22:23--dibromoergost-14-en-3β--ol (1:1)]	+165°	+126°	+139°	—	—
22:23-Dibromoergost--14-en-3β-ol	+257°	+225°	+253°	-32°(-35°)	+1°(+30°)
22:23-Dibromoergostan--3β-ol	+42°	+12°	+40°	-30°(-34°)	-2°(+2°)
Δ(Saturation of ⊨)	-215°(-24°)	-213°(-20°)	-218°(-59°)	—	—

Although the Δ values for acetylation and benzoylation of 22:23-dibromoergost-14-en-3β-ol are in reasonable agreement with standard values for Δ^{14}-stenols, the changes in molecular rotation accompanying saturation of the double bond of this compound and its derivatives (comparison with the corresponding derivatives of 22:23--dibromoergostan-3β-ol) are anomalous, in this respect resembling 22α-allospirost-8(14)-en-3β-ol in which a strong vicinal effect of the sapogenin side chain was also observed (87). Summarising, the dibrominated side chain exerts a profound vicinal effect upon the 14(15)-, a less pronounced effect upon the 8(14)-, and no effect upon a 7(8)-ethylenic linkage.

A preparation of 22:23-dibromoergostan-3β-yl acetate (LVII) was achieved by isomerisation of 22:23-dibromoergost-8(14)-en-3β-yl acetate with dry hydrogen chloride, hydrogenation of the mixed crystal with a platinum catalyst, and removal of unsaturated material by Anderson and Nabenhauer's method (90). Alternatively, the crude hydrogenation product was treated with an excess of perbenzoic acid and the oxidation mixture chromatographed. In addition to (LVII) a compound $C_{30}H_{48}O_3Br_2$ was isolated. This is regarded as 22:23-dibromo-8ξ:14ξ-epoxyergostan-



-3β-yl acetate (LX).

The increasing importance of progesterone, due to its easy biological oxygenation (20) to 11α-hydroxy-progesterone prompted an attempt to obtain better yields of 22:23-dibromoergostan-3β-yl acetate. This compound could possibly form a useful precursor for a synthesis of progesterone. Attempts to improve this hydrogenation, however, (a) by hydrogenation of 22:23-dibromoergost-8(14)--en-3β-yl acetate or of 22:23-dibromoergosta-7:9(11)-dien--3β-yl acetate in the presence of hydrochloric acid (122, 132) at 0° or 80°, (b) by repeated rearrangements and hydrogenations of 22:23-dibromoergost-7-en-3β-yl acetate,

and (c) hydrogenation of 22:23-dibromoergosta-7:9(11)--dien-3β-yl acetate in hydrogen chloride-saturated ether, were not successful. (Some, but not all, of these experiments are reported in the Experimental). The chief reason for this failure appears to be that the long periods of hydrogenation needed for saturation of the nucleus caused marked reductive debromination of the side chain.

Debromination of 22:23-dibromoergostan-3β-yl acetate gave ergost-22-en-3β-yl acetate (LVIII) characterised, by basic hydrolysis, as the alcohol (LIX).

The Conversion of Ergosterol to Progesterone.

It has recently been shown that microbiological oxidation of progesterone (LXXIX) gives a 90% yield of 11α-hydroxyprogesterone (LXXXII) which can be transformed into cortisone by a ten step route of high overall efficiency (87). The lack of any abundant indigenous steroid easily convertible to progesterone, coupled with the limited availability of progesterone itself, prompted the exploration of a possible partial synthesis from ergosterol (I). [For a summary of alternative available methods of preparing progesterone, vide (133)] The prime aim was to obtain a nuclear saturated Δ^{22}-steroid while retaining an oxygen function at $C_{(3)}$. Subsequent to this the side chain could be degraded to give a suitable pregnane derivative. Both of these objects were achieved by the methods described below (114).

Oxidation of ergosterol to ergosta-4:7:22-trien-3--one (ergosterone) (LXI) by the Oppenauer method (91) gave a highly coloured product which required extensive purification, with consequent poor yield. A considerable improvement was made by using aluminium tert.-butoxide in toluene with cyclohexanone as a hydrogen acceptor, although the removal of self condensation products of

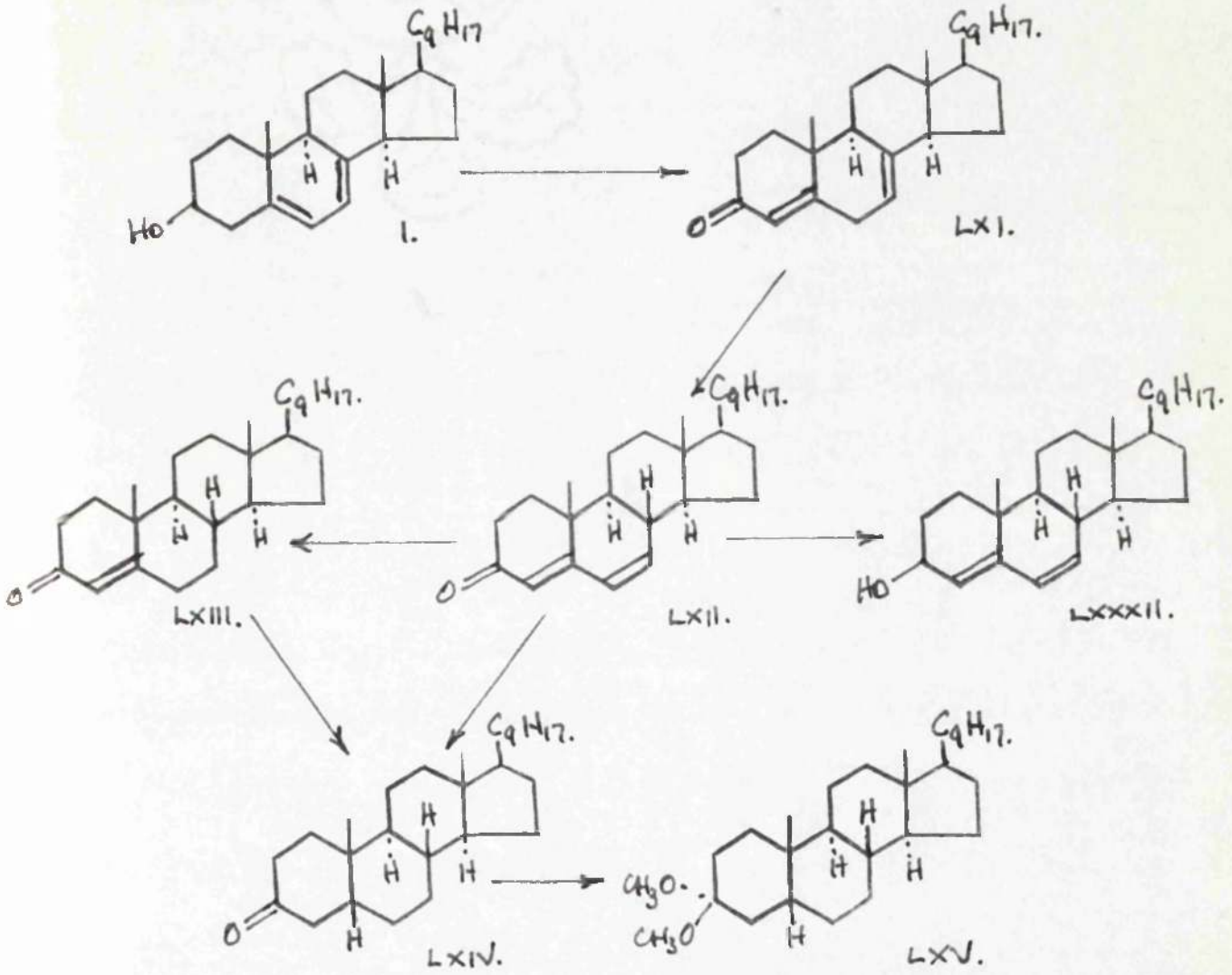
cyclohexanone was necessary, before good yields of ergosterone were obtained.

The method of Barton et al., (34) was used for the rearrangement of ergosterone to ergosta-4:6:22-trien-3--one (isoergosterone) (LXII). Boron trifluoride in ether failed to effect any rearrangement.

In connection with this project it was also decided to attempt the $C_{(11)}$ microbiological oxidation of ergosta--4:22-dien-3-one (LXIII). However, the only available method, at that stage, of obtaining ergosta-4:22-dien-3--one was that due to Barton, Cox, and Holness (34) who prepared it by a selective catalytic hydrogenation of isoergosterone. The yield, moreover, had been so poor that the method could not be deemed of any preparative value, and alternative methods were sought.

Fieser et al. (92) had found that a 12-oxo-7:9(11)--diene system could be reduced with zinc dust in acetic acid to the 12-oxo-8-ene steroid, which then rearranged under the influence of acid or alkali to the 12-oxo-9(11)--ene compound. Application of this reduction to isoergosterone gave, after chromatography, a minute quantity of the desired ergosta-4:22-dien-3-one (LXIII). Zinc dust in ethanol effected no reduction whatsoever, while

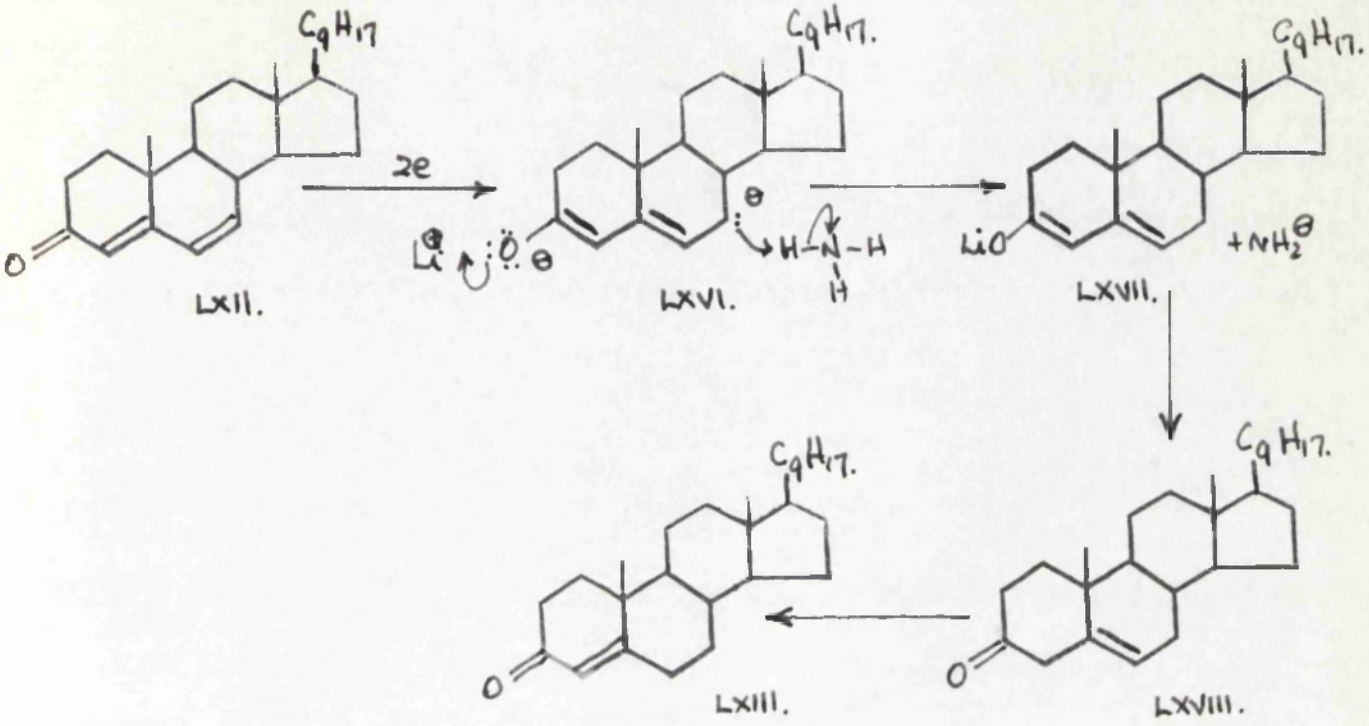
lithium aluminium hydride in ether gave only ergosta--4:6:22-trien-3β-ol (LXXXII).



With the failure of this attempt, attention was turned to the possibility of employing a hydrogenation procedure. Reduction of isoergosterone in cyclohexane with a Raney nickel catalyst proceeded very rapidly at first but was arrested after the absorption of approximately 2 mols. of

hydrogen. Chromatography of the complex product yielded only two pure materials of unknown constitution, which were not further investigated. When the hydrogenation was carried out using a palladium-strontium catalyst in pure benzene, and the reaction stopped after the absorption of 1 mol. of hydrogen, ergosta-4:22-dien-3--one was obtained in 40% yield. Attempts to improve this yield by employing different solvents, or by stopping the reaction after the absorption of 0.8 mol., or of 1.2 mol. of hydrogen, were not successful. A much more satisfactory synthesis of ergosta-4:22-dien-3-one was evolved by reducing isoergosterone with a blue solution of lithium metal in liquid ammonia, in the presence of methanol. In the light of this result it is interesting to recall that reduction of Δ^{8}-11-oxo-steroids with lithium in liquid ammonia gave the corresponding saturated 11-oxo-steroid (43,73), whereas with excess lithium in liquid ammonia, the saturated 11α-hydroxy-steroid was obtained. More recently Barton, Ives, and Thomas (93) have found that reduction of Δ^{4}-3-oxo-steroids with lithium in liquid ammonia gave the saturated 5α-ketones.

The reduction of the dienone system may possibly proceed by the following mechanism; 1:6 addition of two



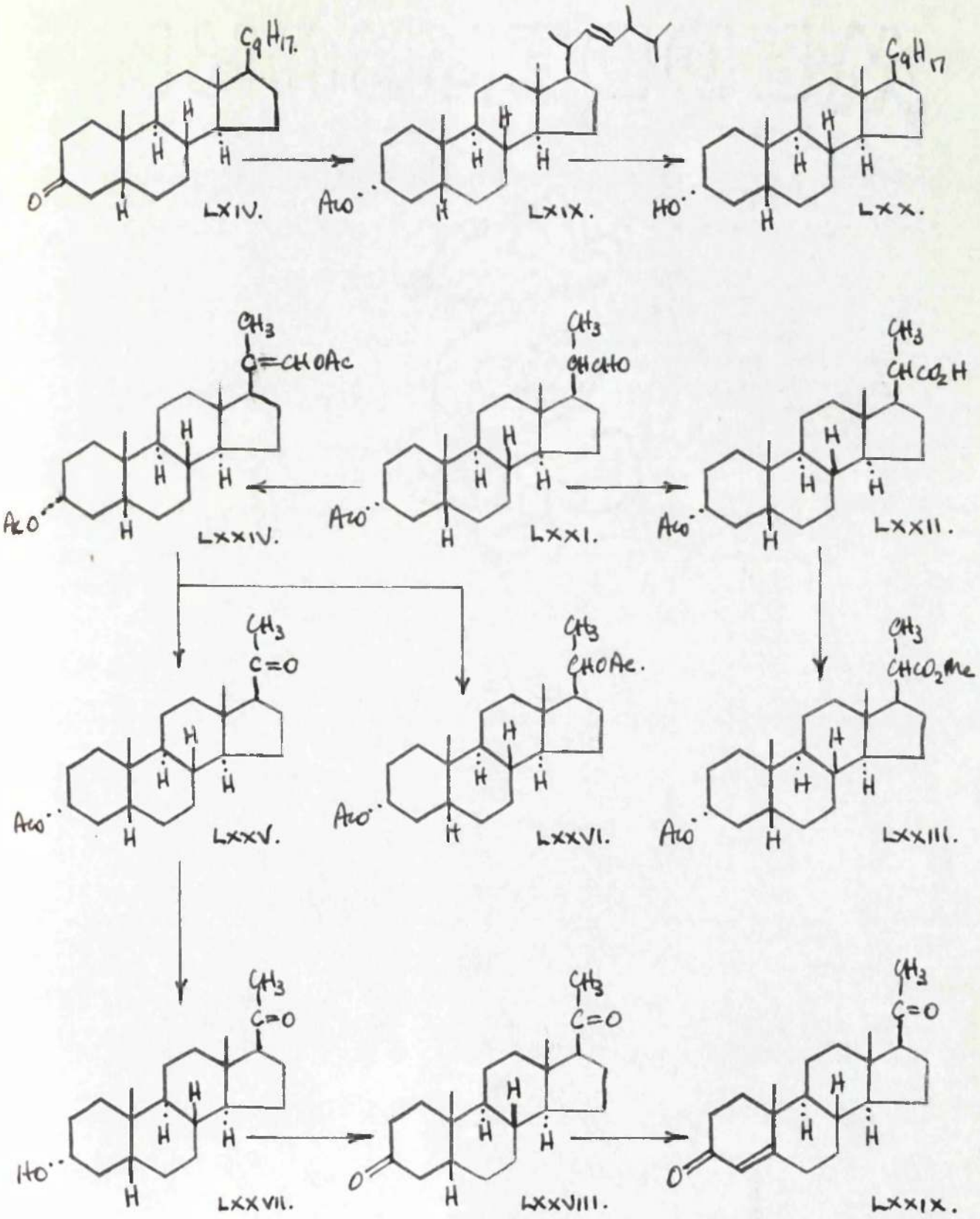
electrons would give the dianionoid system (LXVI) which by the attachment of a proton, from the methanol, at $C_{(7)}$ and a lithium cation at the $C_{(3)}$ oxygen atom would afford the metal enolate (LXVII). There is good reason to believe that this would be stable to further reduction (94). Subsequent working up would afford the Δ^5-3-oxo-steroid (LXVIII), which, under the experimental conditions, would isomerise to ergosta-4:22-dien-3-one [cf. Birch (95)].

Microbiological oxidation, according to Peterson et al., (20) of ergosta-4:22-dien-3-one was attempted using the mould Rhizopus Nigricans (see Experimental). Extensive examination of the recovered steroidal material failed

to reveal the presence of any transformation products, and no other work was done in this direction.

Selective hydrogenation of isoergosterone with a palladium-charcoal catalyst in the presence of alkali gave 5β-ergost-22-en-3-one (LXIV) in almost quantitative yield. The latter compound was also obtained, when ergosta-4:22-dien-3-one was reduced under the same conditions, although in much lower yield. When 5β-ergost--22-en-3-one was crystallised from methanol, containing a trace of mineral acid, it readily formed a ketal (LXV), from which the parent ketone was regenerated by hydrolysis with aqueous mineral acid. The facile formation of this ketal is in accord with the observations of Fieser (96) on the reductive methylation of 3-oxo-5β--steroids. Both the ketal and 5β-ergost-22-en-3-one gave the same 2:4-dinitrophenylhydrazone.

Reduction of 5β-ergost-22-en-3-one (LXIV) with lithium aluminium hydride followed by acetylation gave 5β-ergost-22-en-3α-yl acetate (LXIX), in good yield, hydrolysis of which then gave 5β-ergost-22-en-3α-ol (LXX). The last compound had been prepared by Barton, Cox, and Holness (34) by sodium-propanol reduction of 5β-ergost--22-en-3-one (LXIV), which they obtained by careful

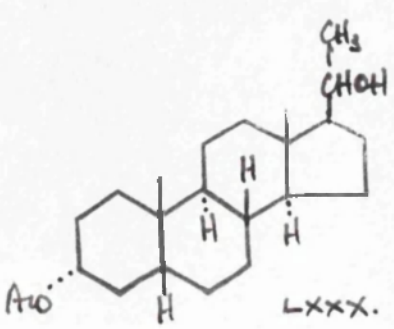

LXIV.
LXIX.
LXX.
LXXIV.
LXXI.
LXXII.
LXXV.
LXXVI.
LXXIII.
LXXVII.
LXXVIII.
LXXIX.

fractionation of the complex mixture derived from a partial hydrogenation of isoergosterone in the presence of a platinum catalyst in ethyl acetate solution. The side chain of 5β-ergost-22-en-3β-yl acetate was then degraded to an acetyl group according to the method shown in the scheme (preceding page).

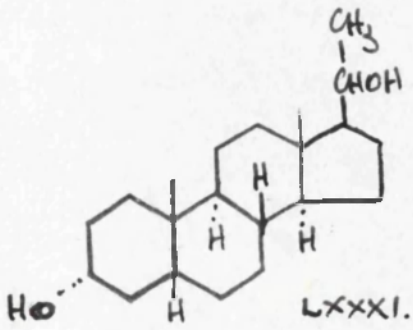
Ozonolysis of 5β-ergost-22-en-3β-yl acetate (LXIX) in chloroform at -45° followed by decomposition of the ozonide with zinc dust and acetic acid gave a mixture of 3α-acetoxybisnorcholan-22-al (LXXI) and 2:3-dimethylbutyraldehyde, each of which was characterised by preparation of its 2:4-dinitrophenylhydrazone. The aldehyde (LXXI) was further characterised, and identified, by oxidation, either with chromic acid or ozone, to 3α-acetoxybisnorcholanic acid (LXXII), which had previously been obtained by side chain degradation of lithocholic acid (97,98). Treatment of 3α-acetoxybisnorcholanic acid with diazomethane in ether gave the corresponding methyl ester (LXXIII).

Although attempted enol acetylation of 3α-acetoxybisnorcholan-22-al (LXXI) using acetic anhydride in carbon tetrachloride and a perchloric acid catalyst failed to give a pure product, treatment of the aldehyde with acetic

anhydride-potassium acetate gave an oily enol acetate (LXXIV) which defied crystallisation. Ozonolysis of this material followed by decomposition of the ozonide with zinc dust and acetic acid gave 20-oxopregnan-3α-yl acetate (LXXV) as the major product. Basic hydrolysis gave the alcohol (LXXVII). The latter compound had previously been prepared from lithocholic acid (99) and also from pregnane-3:20-dione (LXXVIII) by preferential reduction either catalytically (100,101) or with sodium borohydride (67). A second amorphous (minor) product from the ozonolysis of the enol acetate (LXXIV) was readily separated by chromatography since it was much more strongly held by alumina. On acetylation it yielded 3α:20α-diacetoxypregnane (LXXVI), after chromatographic purification. The isolation of this diacetate is attributed to the formation of a small quantity of 3α-acetoxypregnan-20α-ol (LXXX), during the zinc dust decomposition



LXXX.



LXXXI.

of the ozonolysis product. The diol (LXXXI) has been isolated from pregnancy urine by Hartmann and Locher (103) and has been obtained (101) by sodium and iso-propyl alcohol reduction of 3α-hydroxypregnane-20-one (LXXVII).

Oxidation of 3α-hydroxypregnane-20-one with chromium trioxide in acetic acid yielded pregnane-3:20-dione (LXXVIII) from which progesterone may be obtained by the method of Butenandt and Schmidt (104).

[Pregnane-3:20-dione (LXXVIII) was identified by comparison with a specimen prepared by a modification of a method due to Butenandt and Fleisher (102) involving catalytic reduction of progesterone. 3α-Hydroxy-pregnane-20-one (LXXVII) was identified by comparison with a specimen prepared by sodium borohydride reduction of pregnane-3:20-dione (67)].

Since this work was completed, similar partial syntheses of progesterone from ergosterol have been briefly described by the Upjohn group (105) and by Dalgleish, Green and Poole (106).

Methods for converting progesterone to cortisone have been described in the preceding section of this thesis.

EXPERIMENTAL

EXPERIMENTAL

Ergosta-7:22-dien-3β-yl Acetate (5:6-Dihydroergosteryl Acetate). - A solution of ergosteryl acetate (35 g.) in benzene (300 c.c.) was added to a suspension of Raney nickel sludge (W.6; 15-20 c.c.) (Org.Synth.,22, 25) in pure benzene (50 c.c.) and the mixture shaken with hydrogen. When the hydrogen uptake corresponded to 2140 c.c. (Theo = 1900 c.c.) the reaction was arrested and the catalyst removed by filtration. Evaporation of the filtrate in vacuo yielded a solid which crystallised from methanol-chloroform giving ergosta-7:22-dien-3β-yl acetate (25 g.) as lustrous plates, m.p.178-180°, $[\alpha]_D$ -20° (c, 1.4). Light absorption: it showed no high intensity absorption above 2200 Å. It gave a yellow colour with tetranitromethane in chloroform. Concentration of the mother liquors yielded a further quantity (7g.) of ergosta-7:22-dien-3β-yl acetate, which after a further crystallisation, had m.p.180-181°, $[\alpha]_D$ -21° (c, 2.0).

Ergosta-7:9(11):22-trien-3β-yl Acetate (Ergosteryl-D Acetate). - A solution of ergosta-7:22-dien-3β-yl acetate (100 g.) in chloroform (1400 c.c.) was added to a solution of mercuric acetate (174 g.) in stabilised

glacial acetic acid (2600 c.c.) and the mixture divided into two equal portions. Each portion was shaken in a Winchester bottle for 18 hours at 15°. After standing for a further six hours the precipitated mercurous acetate was filtered, and the filtrates combined and concentrated below 50°, under reduced pressure, to 800 c.c. The crystalline solid (78 g.), which separated on cooling was collected, washed with methanol, and dried in vacuo. Three recrystallisations from chloroform-methanol gave ergosta-7:9(11):22-trien-3β-yl acetate (38 g.) as elongated plates, m.p.175°, $[\alpha]_D$ +28° (c, 1.6). Light absorption: maxima at 2350 Å (ϵ = 16,500) and 2420 Å (ϵ = 17,800), with an inflexion at 2510 Å (ϵ = 12,700). It gives a red-brown colour with tetranitromethane in chloroform.

Tetrabromoergostenyl Acetate. - Ergosta-7:22-dien-3β-yl acetate (10 g.) in pure dry ether (1 l.) was cooled to -20° and a solution of dry bromine (5.1 c.c.) in glacial acetic acid (60 c.c.) added in one portion with swirling. The mixture was then quickly cooled in an external acetone-carbon dioxide bath to -45° and allowed to come to room temperature over 2 hours. The white flocculent precipitate (9.3 g.) which had separated

was filtered, washed with ether, and dried *in vacuo* for 15 minutes at 15°, m.p.114-118°. A specimen after two crystallisations from benzene-petrol had m.p.127-128° (decomp.). The crude material (m.p.114-118°) was used for further reaction.

22:23-Dibromoergosta-7:9(11)-dien-3β-yl Acetate. - A solution of tetrabromoergostenyl acetate (20 g.) in benzene (300 c.c.) was treated with a solution of sodium iodide (30 g.) in ethanol (300 c.c.) at 15°. After standing for 16 hours, the mixture was poured into a solution of sodium thiosulphate (3 l., 4%) to remove free iodine. The yellow benzene layer was separated, washed with water, and dried (Na_2SO_4). Removal of the benzene under reduced pressure below 60° gave a yellow crystalline solid, which crystallised from chloroform-methanol giving 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate as thick colourless prismatic rods (12.5 g.), m.p.228-230°, $[\alpha]_D$ +31° (c, 1.8). Light absorption: Maxima at 2350 (ε = 19,000) and 2420 Å (ε = 21,000) with an inflexion at 2500 Å (ε = 13,000). With tetranitromethane in chloroform it gives a red-brown colour.

Ergosta-7:9(11):22-trien-3β-yl Acetate (Ergosteryl-D Acetate). - 22:23-Dibromoergosta-7:9(11)-dien-3β-yl acetate (10 g.) in ether (500 c.c.), containing glacial acetic acid (1 c.c.), was stirred at room temperature for five hours with zinc dust (20 g.). The solution was filtered to remove excess zinc and the filtrate washed with water, dried (Na_2SO_4) and evaporated under reduced pressure. The crystalline residue (6.9 g.), m.p.175--176°, was recrystallised from chloroform-methanol, affording pure ergosta-7:9(11):22-trien-3β-yl acetate as large elongated plates, m.p.178°, $[\alpha]_D$ +32° (c, 1.4). Light absorption: Maxima at 2350 (ε = 17,000) and 2420 Å (ε = 19,000) with an inflexion at 2510 Å (ε = 12,000). It gives a red-brown colour with tetranitromethane in chloroform.

22:23-Dibromo-9α:11α-epoxyergost-7-en-3β-yl Acetate.- 22:23-Dibromoergosta-7:9(11)-dien-3β-yl acetate (10 g.) was dissolved in chloroform (240 c.c.) and treated dropwise with a solution of perbenzoic acid (1.2 mols) in chloroform (50 c.c.) over a period of 3 hours. During the addition the solution was stirred and maintained at -10° by an ice salt bath; the perbenzoic acid was kept at 0° by using a jacketed burette, the jacket containing

ether through which a stream of air was drawn. This device maintained the perbenzoic acid at 0°. The reaction mixture was kept at 0° for a further 12 hours, then washed with sodium carbonate solution (5%), water, and dried (Na_2SO_4). Removal of the chloroform under reduced pressure below 50° gave a white solid which was crystallised from pure acetone or petrol to give 22:23--dibromo-3α:11α-epoxyergost-7-en-3β-yl acetate as needles (6.8 g.), m.p.218°, $[\alpha]_D$ -24°, -26° (c, 1.5, 1.7). It gives a yellow colour with tetranitromethane in chloroform and does not show high intensity light absorption above 2200 Å.

Crystallisation from acetone led to the separation of a microcrystalline solid, m.p.200°, very insoluble in the usual solvents except pyridine, and undepressed in melting point when mixed with a specimen of 3β-acetoxy--22:23-dibromoergost-8-en-7ξ:11α-diol (m.p.204°) (Found: C,56.6; H,8.0. Calc. for $C_{30}H_{48}O_4Br_2$: C,57.0; H,7.65%).

Crystallisation from technical methanol gave a material of m.p.231-232° after three crystallisations, showing the characteristic absorption of an αβ-unsaturated ketone (ε = 6000). This compound is probably impure 22:23-dibromo-7-oxoergost-8-en-3β-yl acetate.

22:23-Dibromo-9α:11α-epoxy-7-en-3β-ol. - 22:23--Dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate (0.30 g.) was dissolved in benzene (40 c.c.) and added to a solution of potassium hydroxide (1 g.) in methanol (50 c.c.). The mixture was refluxed for 2 hours, then poured into water and extracted with ether. The ethereal layer was separated, washed with water, dried (Na_2SO_4), and taken to dryness under reduced pressure. Crystallisation of the residue from methanol gave 22:23-dibromo-9α:11α-epoxyergost-7-en-3β-ol as plates, m.p.200-202°, $[\alpha]_D$ -27° (c, 0.7) (Found: C,53.0; H,8.0. $C_{28}H_{44}O_2Br_2.CH_3OH$ requires C,57.6; H,8.0%). It gives a yellow colour with tetranitromethane in chloroform and does not show any selective light absorption above 2200 Å.

22:23-Dibromo-7-oxoergost-8-en-3β-yl Acetate. - 22:23-Dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate (0.70 g.) in methanol (100 c.c.), and benzene (7 c.c.) was treated with concentrated hydrochloric acid (2 c.c.) and the mixture refluxed for 1.5 hours. The solution was then diluted with water and the precipitate filtered, washed with water and dried. The solid was reacetylated using pyridine-acetic anhydride. Working up in the usual way and crystallisation of the product from methanol

gave yellow plates, m.p.233° further purified by percolation of a solution in benzene (10 c.c.) through a column of alumina (2.5 x 5.0 cm.) which was eluted with benzene (10 c.c.). Evaporation of the combined eluates gave a white residue recrystallised three times from methanol giving 22:23-dibromo-7-oxoergost-8-en-3β-yl acetate as plates, m.p.241-242°, $[\alpha]_D$ -29° (c, 1.5) (Found: C,58.6; H,7.56. $C_{30}H_{46}O_3Br_2$ requires C,58.6; H,7.55%). Light absorption: Max. at 2520 Å (ϵ = 10,000). It gives only a faint yellow colour with tetranitromethane in chloroform.

7-Oxoergosta-8:22-dien-3β-yl Acetate. - 22:23-Dibromo--7-oxoergost-8-en-3β-yl acetate (0.05 g.) was dissolved in ether (20 c.c.) and ethanol (20 c.c.). Zinc dust (1 g.) was added and the mixture refluxed for 2 hours. The excess zinc dust was removed and the filtrate concentrated to small bulk. Isolation of the product in the usual way, and crystallisation from methanol furnished 7-oxoergosta-8:22-dien-3β-yl acetate (0.032 g.) as plates, m.p.209-211°, $[\alpha]_D$ -56° (c, 0.5) (Found: C,79.1; H,10.3. Calc. for $C_{30}H_{46}O_3$: C,79.2; H,10.2%). It was undepressed in melting point when mixed with an authentic specimen.

Light absorption: Max. at 2520 Å (ϵ = 10,000). It gives a yellow colour with tetranitromethane in chloroform.

22:23-Dibromo-11-oxoergost-8-en-3β-yl Acetate. - 22:23-Dibromo-9α:11α-epoxyergost-7-en-3β-yl acetate (1g.) in dry benzene (50 c.c.) was treated with dry, redistilled, boron trifluoride etherate (15 drops) and the solution kept at 15° for 3 days. The mixture was then diluted with ether, (100 c.c.), washed with sodium hydrogen carbonate solution, water and dried (Na_2SO_4). The solvents were removed under reduced pressure and a solution of the residual yellow solid in benzene (25 c.c.) was percolated through a column of alumina (8 x 1.25 cm.). The column was further eluted with benzene (200 c.c.). The combined eluates were evaporated to dryness *in vacuo* and the residue (0.73 g.) recrystallised from chloroform-methanol to give 22:23-dibromo-11-oxoergost-8-en-3β-yl acetate as blades, m.p.200-202°, $[\alpha]_D$ +83° (c, 1.1) (Found: C,58.75; H,7.7. $C_{30}H_{46}O_3Br_2$ requires C,58.6; H,7.55%). Light absorption: Max. at 2530 Å (ϵ = 9300). It does not give a coloration with tetranitromethane.

22:23-Dibromo-11-oxoergost-8-en-3β-ol. - A solution of 22:23-dibromo-11-oxoergost-8-en-3β-yl acetate (0.4 g.) in benzene (30 c.c.) was added to a solution of potassium carbonate (1 g.) in water (5 c.c.) and methanol (30 c.c.) and the mixture refluxed for 1.5 hours. Isolation of the product by the usual procedure afforded 22:23-dibromo--11-oxoergost-8-en-3β-ol (0.22 g.) as plates from methanol, m.p.131-133°, $[\alpha]_D$ +96° (c, 1.3) (Found: C,57.3; H,7.94. $C_{28}H_{44}O_2Br_2.CH_3OH$ requires C,57.6; H,8.0%). Light absorption: Max. at 2540 Å (ϵ = 8500). It gives no colour with tetranitromethane in chloroform.

11-Oxoergosta-8:22-dien-3β-yl Acetate. - 22:23--Dibromo-11-oxoergost-8-en-3β-yl acetate (2.5 g.) was dissolved in ether (200 c.c.) and methanol (200 c.c.) and the mixture refluxed with zinc dust (6.0 g.). The excess zinc was removed by filtration and the filtrate concentrated to small bulk, diluted with water and extracted with ether. Working up in the usual way gave 11-oxoergosta-8:22-dien-3β-yl acetate (1.54 g.) as glistening plates from methanol, m.p.133-134°, $[\alpha]_D$ +104° (c, 1.0) (Found: C,79.6; H,10.4. Calc. for $C_{30}H_{46}O_3$: C,79.3; H,10.3%). Light absorption: Max. at 2530 Å (ϵ = 9000). It gives a faint yellow colour with tetra-

nitromethane in chloroform and shows no depression in melting point when mixed with an authentic specimen, m.p.129-130°, $[\alpha]_D$ +102° prepared according to Heusser et al., (45) who gave m.p.122-123°, $[\alpha]_D$ +92°.

Hydrogenation of 22:23-Dibromo-9α:11α-epoxyergost--7-en-3β-yl Acetate. - (a) A solution of 22:23-dibromo--9α:11α-epoxyergost-7-en-3β-yl acetate (0.44 g.) in glacial acetic acid (30 c.c.) was added to prereduced platinum catalyst (from 0.1 g. PtO_2) in glacial acetic acid (50 c.c.). The mixture was shaken with hydrogen until absorption ceased (32 c.c.; 1 mol = 32 c.c.). The catalyst was removed by filtration and the bulk of the acetic acid taken off under reduced pressure. The residual liquid was diluted with water and the granular precipitate filtered, washed with water and dried. Crystallisation from chloroform-methanol afforded glistening plates of 22:23-dibromoergost-8(14)-en-3β-yl acetate (0.22 g.), m.p.189-190°, $[\alpha]_D$ +3° (c, 1.0) (Found: C,60.0; H,8.1. $C_{30}H_{48}O_2Br_2$ requires C,60.0; H,8.0%). It gives a deep yellow colour with tetranitromethane in chloroform. Light absorption: no high intensity absorption above 2200 Å. Below 2200 Å the curve exhibits the characteristics of a tetrasubstituted double bond,

ϵ_{2100}3000, ϵ_{2150}7500, ϵ_{2200}5600, ϵ_{2230}1400.

(b) Using the same experimental conditions as above with dioxane as solvent, the starting material was recovered unchanged.

Hydrogenation of 22:23-Dibromo-11-oxoergost-8-en-3β-yl Acetate. - (a) 22:23-Dibromo-11-oxoergost-8-en-3β-yl acetate (0.5 g.) in pure acetic acid (100 c.c.) was added to prereduced platinum catalyst (from PtO_2, 0.2 g.) in pure acetic acid (50 c.c.). After shaking the mixture with hydrogen for 10 hours the catalyst was removed and the filtrate concentrated, diluted with water and the product isolated in the usual way. Crystallisation from chloroform-methanol gave 22:23-dibromoergost-8(14)-en-3β-yl acetate (0.25 g.) as lustrous plates, m.p.189-190°, $[\alpha]_D$ +3.5 (c, 1.0) (Found: C,60.2; H,8.4%). Mixed with a specimen prepared in the previous experiment, it showed no depression in melting point.

(b) Attempted hydrogenation using a palladium oxide catalyst with acetic acid as solvent or a platinum oxide catalyst with ethyl acetate as solvent effected no reduction, starting material being recovered unchanged.

Chemical Reductions of 22:23-Dibromo-11-oxoergost--3-en-3β-yl Acetate. - (a) Sodium-Amyl alcohol:- 22:23--Dibromo-11-oxoergost-3-en-3β-yl acetate (0.3 g.) in n-amyl alcohol (40 c.c.) was heated to reflux and sodium (0.6 g.) added as fast as reaction would permit, then allowed to cool. Isolation of the product as usual yielded a non-crystallisable gum. No crystalline material could be isolated on chromatography.

(b) Aluminium Amalgam:- A solution of 22:23-dibromo--11-oxoergost-3-en-3β-yl acetate (0.4 g.) in ether (100 c.c.) was added to amalgamated aluminium foil (5 g.) covered with ether, and a few drops of water added. The solution was allowed to stand at 15° for 24 hours with the addition of water (0.5 c.c.) after 6 hours. Processing in the usual way gave a white solid which was crystallised from methanol, giving a mixture of plates and prisms (0.16 g.), m.p.135-150°. After four recrystallisations the product had m.p.167-170° showing negligible light absorption and still obviously a mixture from the two crystalline forms present. Chromatography failed to effect any separation.

(c) Aluminium isopropoxide failed to effect any reduction.

3β:11α-Diacetoxyergost-22-ene. - 22:23-Dibromo-11-oxoergost-8-en-3β-yl acetate (0.8 g.) in dry ether (200 c.c.) was added over 15 minutes to a stirred deep blue solution of lithium metal (0.5 g.) in anhydrous liquid ammonia (250 c.c.). Stirring was continued for a further 2 hours (disappearance of blue colour) and the solution then allowed to stand overnight to permit evaporation of the excess ammonia. Water and ether were added and the product, isolated in the usual way, was acetylated using pyridine-acetic anhydride. Slow crystallisation of the acetylated product from aqueous methanol furnished 3β:11α-diacetoxyergost-22-ene, as hard needles (0.32 g.), m.p.128-129°, $[\alpha]_D$ -31° (c, 0.9). Two further recrystallisations gave the pure diacetate, m.p.132-133°, $[\alpha]_D$ -36° (c, 1.2) (Found: C,76.8; H,10.4. Calc. for $C_{32}H_{52}O_4$: C,76.75; H,10.5%). It gives a pale yellow colour with tetranitromethane in chloroform. [Heusser et al., (50) give m.p.128-129°, $[\alpha]_D$ -33° for this material.]

Ergost-22-en-3β:11α-diol. - 3β:11α-Diacetoxyergost-22-ene (0.1 g.) was dissolved in methanol (20 c.c.) and added to a solution of potassium hydroxide (1 g.) in water (3 c.c.) and methanol (30 c.c.). The mixture was

refluxed for 1 hour, diluted with water and the precipitate filtered, washed with water, and dried *in vacuo*. The product was recrystallised from methanol to give ergost-22-en-3β:11α-diol (0.07 g.), m.p.165°, $[\alpha]_D$ -24°(c,0.9) (Found: C,80.7; H,11.6. Calc. for $C_{28}H_{48}O_2$: C,80.7; H,11.6%).

Reacetylation of a sample of this diol by the usual method gave the diacetate, m.p.132-133°, $[\alpha]_D$ -36° (c,1.0), undepressed in melting point when mixed with the specimen prepared above.

Attempted Reduction of the Δ^8*-bond of 22:23-Dibromo--11-oxoergost-8-en-3β-yl Acetate by Lithium in Liquid Ammonia.* - A solution of 22:23-dibromo-11-oxoergost-8-en--3β-yl acetate (0.8 g.) in ether (150 c.c.) was added with stirring to a deep blue solution of lithium metal (0.056 g.; 7 atoms) in anhydrous liquid ammonia (200 c.c.) and ether (100 c.c.). Addition of the dibromo-steroid solution was discontinued after 65 c.c. had been added, the blue colour of the liquid ammonia solution having disappeared. The mixture was allowed to stand at 13° for 6 hours. Isolation of the product in the usual way afforded a white solid, [light absorption: max. at 2560 Å (ϵ = 400) indicating the almost complete conversion

of the αβ-unsaturated ketone grouping] which was reacetylated using pyridine-acetic anhydride. The crude acetate was refluxed with zinc dust (1 g.) in ether (30 c.c.) for 0.5 hours to debrominate any remaining dibromosteroid material. Working up in the usual way and crystallisation of the product from aqueous methanol gave 3β:11α--diacetoxyergost-22-ene (0.105 g.) as needles, m.p.132--133°, $[\alpha]_D$ -36° (c, 1.05), giving no melting point depression when mixed with an authentic specimen.

11-Oxoergost-22-en-3β-ol. - 11-Oxoergosta-3:22-dien--3β-yl acetate (0.7 g.) in dry ether (25 c.c.) was added with stirring to a blue solution of lithium metal (0.045g. 4 atoms) in liquid ammonia (120 c.c.). After 7 minutes when addition was complete the solution became colourless. Lithium (6 mg.) was added (reappearance of blue colour) and stirring continued for a further 2 minutes. Ammonium chloride (1 g.) was added to destroy excess lithium and the product worked up as described in previous reductions of this nature. The material obtained was completely saponified using a solution of potassium hydroxide (1 g.) in methanol (50 c.c.) and water (1 c.c.) and refluxing for 1 hour. Working up in the usual way and crystallising the product four times from methanol gave needles of

11-oxoergost-22-en-3β-ol, m.p.166-167°, $[\alpha]_D$ +26° (c,0.35). Yield (0.25 g.) = 35% (Found: C,80.7; H,11.4. Calc. for $C_{28}H_{46}O_2$: C,81.1; H,11.2%). It showed no selective light absorption above 2200 Å, and when mixed with an authentic specimen, m.p.167-169°, $[\alpha]_D$ +25°, prepared according to Heusser *et al.* (45) showed no depression in melting point. It gave a faint yellow colour with tetranitromethane in chloroform.

11-Oxoergost-22-en-3β-yl Acetate. - 11-Oxoergost-22-en-3β-ol (0.1 g.) was dissolved in pyridine (2 c.c.) and acetic anhydride added (1.5 c.c.). After standing at 15° for 12 hours isolation of the product by the usual procedure gave 11-oxoergost-22-en-3β-yl acetate as needles from methanol, m.p.124-125°, $[\alpha]_D$ +12.6° (c, 0.8) (Found: C,79.3; H,10.9. Calc. for $C_{30}H_{48}O_3$: C,78.9; H,10.6%). It gave no depression in melting point when mixed with an authentic specimen prepared according to Heusser *et al.* (45).

9α:11α-Epoxyergosta-7:22-dien-3β-yl Acetate. - A solution of ergosta-7:9(11):22-trien-3β-yl acetate (10 g.) in chloroform (90 c.c.) was cooled to -5° in an ice-salt bath. A solution of perbenzoic acid (1.3 mol) in

chloroform (73 c.c.) was added with stirring over 3 hours. After standing at 0° for a further two hours, the reaction mixture was shaken with sodium carbonate solution, the chloroform layer separated, washed with water and dried (Na_2SO_4). Removal of the chloroform under reduced pressure, below 35°, gave a white crystalline residue which was crystallised from acetone. 9α:11α-Epoxyergost--7:22-dien-3β-yl acetate (4.9 g.) separated as elongated plates, m.p.205°. This product was used for further reaction. A specimen purified from acetone had m.p.210°, $[\alpha]_D$ -40° (c, 1.6) (Found: C,79.2; H,10.3. Calc. for $C_{30}H_{46}O_3$: C,79.2; H,10.2%). Light absorption: no selective absorption above 2200 Å. Chamberlin et al., (44) give m.p.202-205°, $[\alpha]_D$ +35°. Heusser et al. (45) report m.p.205-207°, $[\alpha]_D$ -39.5°.

3β-Acetoxyergosta-8:22-diene-7ξ:11α-diol [after Heusser et al., (45)]. - 9α:11α-Epoxyergosta-7:22-dien--3β-yl acetate (0.2 g.) was dissolved in pure dioxane (150 c.c.). Sulphuric acid (27 c.c.; 2N) was added and the mixture shaken for exactly 3 minutes, poured into saturated sodium bicarbonate solution (150 c.c.) and immediately extracted with ether (200 c.c.). The ethereal layer was separated, washed with water, dried (Na_2SO_4)

and taken to dryness under reduced pressure. The total material from ten such runs was combined, triturated with acetone (15 c.c.), and filtered. The solid thus obtained was dried to give 3β-acetoxyergosta-8:22-dien--7ξ:11α-diol, (1.44 g.), m.p.230°, used without purification in further reactions.

7:11-Dioxoergosta-8:22-dien-3β-yl Acetate [after Heusser et al. (45)]. - 3β-Acetoxyergosta-8:22-dien-7ξ::11α-diol (1.4 g.) was cooled to 0° in an ice-salt bath, and a solution of chromic acid (1.35 g.) in glacial acetic acid (315 c.c.) added, together with sulphuric acid (5 c.c.; 2N), over a period of 1 min. The mixture was stirred vigorously until all the solid had gone into solution, and allowed to stand at 15° for 24 hours. Methanol (50 c.c.) was added to destroy the excess chromic acid, followed by water (1500 c.c.) and the mixture then extracted with ether (400 c.c.). The ether layer was separated, washed successively with sodium hydrogen carbonate solution, 5% ferrous sulphate solution, water, and dried (Na_2SO_4). Removal of the ether under reduced pressure gave a gummy residue (1.16 g.) which was taken up in glacial acetic acid (100 c.c.) and zinc dust (1 g.) added. The mixture was heated to reflux point and a

further quantity of zinc dust (2 g.) added during 0.5 hours. Refluxing was continued for 2 hours, the liquid reduced to small bulk in vacuo and then diluted with water and ether. The liquid was filtered to remove excess zinc dust, the ethereal layer separated, washed with water, sodium hydrogen carbonate solution and dried (Na_2SO_4). Removal of the ether under reduced pressure afforded a pale brown solid (1.09 g.) which was taken up in benzene (50 c.c.) and absorbed on to a column of alumina (10 x 1.2 cm.). Further elution with benzene (400 c.c.), combination of the eluates and evaporation to dryness yielded a white solid which crystallised from acetone-water, affording 7:11-dioxoergost-22-en-3β-yl acetate (0.25 g.) as flocculent white needles, m.p.197--198°, $[\alpha]_D$ -27° (c, 1.5).

11-Oxoergost-22-en-3β-ol [after Chamberlin et al., (44)]. - 7:11-Dioxoergost-22-en-3β-yl acetate (2.5 g.), powdered potassium hydroxide (1.15 g.) and diethylene glycol (12.5 c.c.) together with 90% hydrazine hydrate (1.25 c.c.) were warmed in an open flask. The temperature was raised to 130-140° for 1 hour, with stirring, and then to 180-190° where it was maintained for an

additional 2 hours. The reaction mixture was cooled, diluted with water and benzene, the organic layer separated, washed with water, and dried (Na_2SO_4). Removal of the benzene under reduced pressure gave a brown solid which crystallised from methanol as pale brown needles, m.p.167-169°. This was taken up in methanol treated with charcoal, and allowed to crystallise. 11-Oxoergost--22-en-3β-ol separated as long fine needles (1.25 g.), m.p.167-169°, $[\alpha]_D$ +25° (c, 1.3).

A specimen of the above alcohol was acetylated using acetic anhydride-pyridine. Working up afforded 11-oxoergost-22-en-3β-yl acetate which crystallised from methanol as long fine needles, m.p.124°, $[\alpha]_D$ +11° (c,1.5).

22:23-Dibromo-11-oxo-14β-ergost-8-en-3β-yl Acetate.- (a) A solution of 22:23-dibromo-11-oxoergost-8-en-3β-yl acetate (1 g.) in methanol (200 c.c.) was added to potassium hydroxide (13.5 g.) in methanol (100 c.c.) containing water (10 c.c.), and the mixture refluxed for 6 hours. Dilution of the reaction mixture with water and isolation of the steroidal material by means of ether gave a gummy solid which was acetylated using pyridine (15 c.c.)-acetic anhydride (10 c.c.). Working up in the usual way and crystallisation of the product from chloroform-methanol, or acetone-methanol, afforded rhombohedral crystals of 22:23-dibromo-11-oxo-14β-ergost-8-en-3β-yl acetate (0.625 g.), m.p.171-172°. Two further recrystallisations from chloroform-methanol gave the pure compound (0.5 g.), m.p.183-184° (S.169°), $[\alpha]_D$ +93°. +92° (c, 1.0, 1.5) (Found: C,58.5; H,7.6. $C_{30}H_{48}O_3Br_2$ requires C,58.6; H,7.55%). Light absorption: Max. at 2480 Å (ε = 9,300). It shows a faint yellow colour with tetranitromethane in chloroform.

(b) 22:23-Dibromo-11-oxoergost-8-en-3β-yl acetate (0.75g.) was dissolved in glacial acetic acid (50 c.c.) and a stream of hydrogen chloride passed through the mixture at the reflux temperature. The reaction mixture was

taken to dryness, under reduced pressure, and the residue acetylated using pyridine (3 c.c.)-acetic anhydride (5 c.c.) at 90° for 3 hours. Isolation of the acetate through ether and crystallisation from acetone-methanol yielded 22:23-dibromo-11-oxo-14β-ergost-8-en-3β-yl acetate (0.54 g.) as rhombohedra, m.p. and mixed m.p.132--133°, $[\alpha]_D$ +91° (c, 1.5). Light absorption: Max. at 2430 Å (ϵ = 9000).

22:23-Dibromo-11-oxo-14β-ergost-8-en-3β-ol. - 22:23--Dibromo-11-oxo-14β-ergost-8-en-3β-yl acetate (0.192 g.) in methanolic potassium hydroxide (60 c.c.; 2%) was refluxed for 0.5 hours. Isolation of the product by means of ether afforded 22:23-dibromo-11-oxo-14β-ergost--8-en-3β-ol which separated from methanol as plates or needles (0.1 g.), m.p.203-204°, $[\alpha]_D$ +106° (c, 0.8) (Found: C,53.7; H,8.0. $C_{28}H_{44}O_2Br_2$ requires C,53.8; H,7.8%). Light absorption: Max. at 2490 Å (ϵ = 8,600).

Reacetylation of a specimen of the alcohol (0.065 g.) by the usual method gave the acetate (0.04 g.), m.p.183--184°, $[\alpha]_D$ +91° (c, 1.0).

11-Oxo-14β-ergosta-8:22-dien-3β-yl Acetate. - 22:23--Dibromo-11-oxo-14β-ergost-8-en-3β-yl acetate (0.165 g.)

in ether-methanol (40 c.c.; 1:1) was refluxed for 2.5 hours with zinc dust (2 g.). Isolation of the product in the usual way gave 11-oxo-14β-ergosta-8:22-dien-3β-yl acetate (0.11 g.) which separated from methanol as glistening blades, m.p.113-114°, $[\alpha]_D$ +134° (c, 1.3) (Found: C,79.2; H,10.5. $C_{30}H_{46}O_3$ requires C,79.3; H,10.3%). Light absorption: Max. at 2430 Å (ϵ = 9000). It gives a faint yellow colour with tetranitromethane.

A specimen of the alcohol (0.12 g.) was hydrolysed by methanolic potassium hydroxide (60 c.c.; 2%) at 15° during 2 days. 11-Oxo-14β-ergosta-8:22-dien-3β-ol crystallised slowly from aqueous methanol as plates, m.p.43° (indistinct), $[\alpha]_D$ +152° (c, 1.5) (Found: C,78.4; H,10.6. $C_{28}H_{44}O_2.H_2O$ requires C,78.1; H,10.8%). Light absorption: Max. at 2500 Å (ϵ = 8600). This alcohol is very soluble in all the common organic solvents. Reacetylation of a specimen, using pyridine-acetic anhydride, regenerated the acetate, m.p. and mixed m.p.112-113°, $[\alpha]_D$ +134° (c,1.5).

22:23-Dibromo-11-oxoergost-8(14)-en-3β-yl Acetate. - A solution of 22:23-dibromo-11-oxoergost-8-en-3β-yl acetate (1 g.) in benzene (45 c.c.) was added to a refluxing solution of potassium hydroxide (10 g.) in dry ethanol (45 c.c.). The mixture was refluxed for 0.5 hours and

then poured into water (300 c.c.), extracted with ether (200 c.c.), and the organic layer separated, dried (Na_2SO_4) and taken to dryness under reduced pressure. Reacetylation of the residue, working up in the usual way, and recrystallisation of the product three times from chloroform-methanol furnished 22:23-dibromo-11-oxo-ergost-8(14)-en-3β-yl acetate (0.4 g.) as needles, m.p. 195-196°, $[\alpha]_D$ +50°, +51° (c, 1.25, 0.9) (Found: C,53.9; H,7.7. $C_{30}H_{46}O_3Br_2$ requires C,53.6; H,7.55%). Light absorption: (a) it shows no high intensity absorption above 2200 Å. ϵ_{2100}8200, ϵ_{2150}7100, ϵ_{2200}3750, ϵ_{2250}1730; (b) the infra-red absorption spectrum shows peaks at 1724 cm.$^{-1}$ and 1736 cm.$^{-1}$ indicative of ketone and acetoxyl groups respectively. It gives a deep yellow colour with tetranitromethane in chloroform.

22:23-Dibromo-11-oxoergost-8(14)-en-3β-ol. - A solution of 22:23-dibromo-11-oxoergost-8(14)-en-3β-yl acetate (0.143 g.) in methanol (30 c.c.) and benzene (10 c.c.) was added to methanolic potassium hydroxide (32 c.c., 4%) and the mixture allowed to stand at 15° for 2 days. Isolation of the product through ether and crystallisation from methanol afforded 22:23-dibromo--11-oxoergost-8(14)-en-3β-ol (0.09 g.) as plates,

m.p.135-136°, $[\alpha]_D$ +67° (c, 1.0) (Found: C,59.2; H,8.0. $C_{28}H_{44}O_2Br_2$ requires C,58.8; H,7.75%). Light absorption: (a) ϵ_{2100}3650; (b) peak at 1724 cm.$^{-1}$ in the infra-red spectrum, indicative of an isolated ketone. It gives a deep yellow colour with tetranitromethane in chloroform. Reacetylation of a specimen of this alcohol (0.035 g.) gave the acetate (0.032 g.) as needles from methanol, m.p. and mixed m.p.195-196°, $[\alpha]_D$ +50° (0.8).

Investigation of the Action of 12% Potassium Hydroxide Solution on 22:23-Dibromo-11-oxoergost-8-en-3β-yl Acetate. - A solution of 22:23-dibromo-11-oxo-ergost-8-en-3β-yl acetate (1 g.) in benzene (45 c.c.) was heated to reflux temperature and added to a boiling solution of potassium hydroxide (10 g.) in ethanol (45 c.c.). At appropriate intervals, samples (2 c.c. each) were withdrawn from the solution and each individual aliquot worked up in the usual way and the product reacetylated. Isolation of the acetates gave a gummy residue in each case, and this was triturated with methanol to induce solidification. The methanol was then removed in vacuo and the residual solids examined spectroscopically for maxima and their intensities in the region 2430-2530 Å. (For graph see theoretical).

Time (min.)	0	10	20	30	40	50	90	110
ϵ	9200	7350	4000	4350	4900	5200	6600	8400

Crystallisation of the end product gave 22:23-dibromo-11--oxo-14β-ergost-8-en-yl acetate, m.p. and mixed m.p.182°, $[\alpha]_D$ +91° (c, 1.3).

Treatment of 22:23-Dibromo-11-oxo-14β-ergost-8-en--3β-yl Acetate with 12% Potassium Hydroxide Solution. - A solution of 22:23-dibromo-11-oxo-14β-ergost-8-en-3β-yl acetate (0.5 g.) in benzene (22.5 c.c.) was added to a refluxing solution of potassium hydroxide (5 g.) in dry ethanol (22.5 c.c.). The mixture was refluxed for 2.5 hours and the product isolated by dilution with water and ether extraction. Reacetylation gave the acetate as a brown gummy material which was triturated with methanol. After removal of the methanol under reduced pressure a representative sample of the solid was examined for light absorption intensity at 2430 Å (ϵ = 8900). Crystallisation of the remainder gave successive crops of starting material (0.45 g.), m.p.180°, $[\alpha]_D$ +92° (c, 1.4), mixed melting point 182°. The residual gummy material from the mother liquor showed a light absorption of ϵ_{2430} 6000, indicating the presence of mainly starting material.

Treatment of 22:23-Dibromo-11-oxoergost-8(14)-en-3β-yl Acetate with 12% Potassium Hydroxide Solution. - 22:23-Dibromo-11-oxoergost-8(14)-en-3β-yl acetate (0.2g.) in benzene (9 c.c.) was added to a refluxing solution of potassium hydroxide (2 g.) in ethanol (9 c.c.), and refluxing continued for 2.5 hours. Working up in the usual way, reacetylation, and isolation of the acetate followed by recrystallisation from acetone-methanol yielded 22:23-dibromo-11-oxo-14β-ergost-8-en-3β-yl acetate (0.12 g.) as rhombohedra, m.p.183°, $[\alpha]_D$ +89° (c, 1.6), mixed melting point 183°.

11-Oxoergosta-8(14):22-dien-3β-yl Acetate. - 22:23-Dibromo-11-oxoergost-8(14)-en-3β-yl acetate (0.15 g.) in ether-methanol (30 c.c.; 1:1) was refluxed with zinc dust (2 g.) for 2.5 hours. The debrominated steroid isolated in the usual way, afforded plates from methanol of 11-oxoergosta-8(14):22-dien-3β-yl acetate (0.07 g.), m.p.112°, $[\alpha]_D$ +41.5 (c, 0.83) (Found: C,79.3; H,10.5. $C_{30}H_{46}O_3Br_2$ requires C,79.3; H,10.3%). Light absorption: it shows no high intensity absorption above 2200 Å; ϵ_{2130}6100, ϵ_{2150}5800, ϵ_{2200}3600. It gives a bright yellow colour with tetranitromethane in chloroform.

11-Oxoergosta-8(14):22-dien-3β-ol. - A solution of 11-oxoergosta-8(14):22-dien-3β-yl acetate (0.185 g.) in methanol (30 c.c.) was treated with a solution of potassium hydroxide (1 g.) in methanol (30 c.c.) and the mixture allowed to stand at room temperature for 2 days. Isolation through ether yielded 11-oxoergosta-8(14):22--dien-3β-ol (0.096 g.) which crystallised from methanol--water in fine needles, m.p.113-114°, $[\alpha]_D$ +63° (c,0.94) (Found: C,81.3; H,10.6. $C_{28}H_{44}O_2$ requires C,81.5; H,10.75%). Light absorption: no high intensity light absorption above 2200 Å.

Reacetylation of the alcohol (0.021 g.) regenerated the acetate (0.015 g.), m.p. and mixed m.p.113-114°, $[\alpha]_D$ +40° (c, 0.75).

Rearrangement of 22:23-Dibromo-11-oxoergost-8(14)--en-3β-yl Acetate. - (a) (Using hydrogen chloride). 22:23-Dibromo-11-oxoergost-8(14)-en-3β-yl acetate (0.12g.) in acetic acid (20 c.c.) was treated under reflux with a stream of dry hydrogen chloride for 3 hours. The product, worked up in the usual way, was reacetylated by pyridine-acetic anhydride at 15° for 18 hours. Isolation of the acetate followed the usual procedure and two recrystallisations from methanol gave 22:23-dibromo-11-

-oxo-14β-ergost-8-en-3β-yl acetate (0.07 g.) as rhombohedral plates, m.p.134°, mixed m.p.134°, $[\alpha]_D$ +94° (c, 1.1) (Found: C,68.9; H,7.8%).

(b) (Using dilute alkali). Refluxing a solution of 22:23-dibromo-11-oxoergost-8(14)-en-3β-yl acetate (0.112 g.) in methanol (30 c.c.) with potassium hydroxide (1.2 g.) in methanol (30 c.c.) for 3 hours and reacetylation of the isolated product gave 22:23-dibromo-11-oxo-14β-ergost-8-en-3β-yl acetate which after three crystallisations from methanol had m.p.132° (S.168°), mixed melting point 132-134°, $[\alpha]_D$ +94° (c, 1.25). Light absorption: Max. at 2470 Å (ε = 9000).

Rearrangement of 11-Oxoergosta-8(14):22-dien-3β-yl Acetate. - A solution of 11-oxoergosta-8(14):22-dien-3β-yl acetate (0.08 g.) in methanol (30 c.c.) was added to a solution of potassium hydroxide (1 g.) in methanol (30 c.c.) and water (1 c.c.). The mixture was refluxed for 5 hours and the product, isolated in the usual manner, was reacetylated. Crystallisation of the acetate twice from aqueous methanol afforded 11-oxo-14β-ergosta-8:22-dien-3β-yl acetate (0.02 g.), m.p. and mixed m.p.110°, $[\alpha]_D$ +130° (c, 0.9).

Attempted Enol Acetylation of 22:23-Dibromo-11--oxoergost-8(14)-en-3β-yl Acetate. - 22:23-Dibromo-11--oxoergost-8(14)-en-3β-yl acetate (0.07 g.) was dissolved, by warming, in isopropenyl acetate (20 c.c.) and a microdrop (≒ 0.01 c.c.) of concentrated sulphuric acid. The solution was heated to 90° for 3 hours and, after the addition of anhydrous sodium acetate (0.3 g.), taken to dryness under reduced pressure. The residual solid was taken up in ether-water and the ethereal layer separated, washed with sodium hydrogen carbonate solution, water and dried (Na_2SO_4). Removal of the ether under reduced pressure and crystallisation of the residue furnished starting material (0.05 g.) as blades from methanol (0.05 g.), m.p.196°, $[\alpha]_D$ +50° (c, 1.2). Light absorption: λ_{max}^{EtOH} 9300. It gave no depression in melting point when mixed with a specimen of starting material.

3β:11-Diacetoxy-22:23-dibromoergosta-7:9(11)-diene.- 22:23-Dibromo-11-oxoergost-8-en-3β-yl acetate (0.4 g.) was dissolved in isopropenyl acetate (10 c.c.) and a microdrop of concentrated sulphuric acid added. After heating the mixture at 90° for four hours, sodium acetate (0.2 g.) was added, and the excess isopropenyl acetate

removed under reduced pressure. The residue was taken up in ether and the ethereal layer separated, washed with sodium hydrogen carbonate, water and dried (Na_2SO_4). Removal of the ether and crystallisation of the residue from methanol furnished 3β:11-diacetoxy-22:23-dibromo-ergosta-7:9(11)-diene (0.31 g.) as plates, m.p.208-210°. Three further recrystallisations gave the pure diacetate (0.19 g.), m.p.213-214°, $[\alpha]_D$ +36°, +33° (c, 1.4, 1.8). Light absorption: Max. at 2390 Å (ε = 15,200) (Found: C,58.67; H,7.6. $C_{32}H_{48}O_4Br_2$ requires C,58.5; H,7.4%). It gives a red-brown colour with tetranitromethane in chloroform.

3β:11-Diacetoxy-22:23-dibromo-14β-ergosta-7:9(11)--diene. - 22:23-Dibromo-11-oxo-14β-ergost-8-en-3β-yl acetate (0.4 g.) in isopropenyl acetate (10 c.c.) was treated with a microdrop of concentrated sulphuric acid and the mixture heated at 90° for 3 hours. Anhydrous sodium acetate (0.2 g.) was added and the product isolated as in the previous experiment. Crystallisation from methanol afforded 3β:11-diacetoxy-22:23-dibromo-14β--ergosta-7:9(11)-diene (0.2 g.) as flocculent needles, m.p.151-153°, $[\alpha]_D$ -32°, -34° (c, 1.3, 1.75) (Found:

C,58.75; H,7.55. $C_{32}H_{48}O_4Br_2$ requires C,58.53; H,7.4%). It gives a red-brown coloration with tetranitromethane in chloroform

3β:11-Diacetoxyergosta-7:9(11):22-triene. - A solution of 3β:11-diacetoxy-22:23-dibromoergosta-7:9(11)-diene (0.5 g.) in ether-methanol (50 c.c.; 1:1) was refluxed with zinc dust (3 g.) for 3.5 hours. The excess zinc dust was removed and the filtrate diluted with water. 3β:11--Diacetoxyergosta-7:9(11):22-triene isolated through ether, crystallised as large blades from methanol (0.22 g.), m.p. 116-117°, $[\alpha]_D$ +33° (c, 2.0) (Found: C,77.7; H,10.0. $C_{32}H_{48}O_4$ requires C,77.4; H,9.7%). Light absorption: Max. at 2400 Å (ε = 15,000). It gives a red-brown colour with tetranitromethane in chloroform.

3β:11-Diacetoxy-14β-ergosta-7:9(11):22-triene. - Debromination of 3β:11-diacetoxy-22:23-dibromo-14β-ergosta--7:9(11)-diene (0.433 g.), as in the previous experiment, afforded 3β:11-diacetoxy-14β-ergosta-7:9(11):22-triene (0.23 g.) as long fine needles from aqueous methanol, m.p. 30-31°, $[\alpha]_D$ -14° (c, 1.2) (Found: C,77.2; H,9.7. $C_{32}H_{48}O_4$ requires C,77.4; H,9.7%). Light absorption: Max. at 2440Å (ε = 15,000). It gives a red-brown coloration with tetranitromethane in chloroform.

22:23-Dibromoergost-8(14)-en-3β-yl Acetate. - (a) A solution of 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate (0.5 g.) in glacial acetic acid (250 c.c.) was added to a suspension of platinum catalyst (from 0.2 g. PtO_2) in acetic acid (15 c.c.) and the mixture shaken with hydrogen at room temperature for 20 hours. Isolation of the product in the usual way gave 22:23-dibromoergost-8(14)--en-3β-yl acetate (0.4 g.) which separated as lustrous plates from chloroform-methanol, m.p.190-191°, $[\alpha]_D$ +5°, +4.5° (c, 2.0, 7.0) (Found: C,60.1; H,8.3. $C_{30}H_{48}O_2Br_2$ requires C,60.0; H,8.0%). It showed no depression in melting point when mixed with a specimen prepared by a previously described method.

(b) A solution of 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate (3 g.) in chloroform (60 c.c.) was added to glacial acetic acid (400 c.c.) in which there was suspended platinum oxide (0.4 g.). After shaking with hydrogen for 12 hours the catalyst was removed, the filtrate concentrated to 50 c.c. under reduced pressure, and the product precipitated by the gradual addition of water. The coarse crystalline solid, thus obtained, was filtered, washed, dried at 80° and crystallised from chloroform--methanol. 22:23-Dibromoergost-8(14)-en-3β-yl acetate

(2.3 g.) separated as large plates, m.p.189-191°, $[\alpha]_D$ +3.5° (c, 2.6). Light absorption: λ_{max} 3000. Mixed with a specimen from (a) it had m.p.190°.

22:23-Dibromoergost-8(14)-en-3β-ol. - A solution of 22:23-dibromoergost-8(14)-en-3β-yl acetate (0.25 g.) in benzene (10 c.c.) was added to methanolic potassium hydroxide (40 c.c.; 1%) and the mixture refluxed for 1 hour. Isolation of the product in the usual way and crystallisation from chloroform-methanol yielded 22:23--dibromoergost-8(14)-en-3β-ol (0.2 g.) as lustrous plates, m.p.213-214°, $[\alpha]_D$ +13° (c, 1.6) (Found: C,60.3; H,8.6. $C_{28}H_{46}OBr_2$ requires C,60.2; H,8.3%).

Reacetylation of the alcohol (0.05 g.) using pyridine--acetic anhydride at 90° for 1 hour gave the acetate (0.04 g.), m.p.138-139°, $[\alpha]_D$ +4.9° (c, 2.5).

22:23-Dibromoergost-8(14)-en-3β-yl Benzoate. - 22:23--Dibromoergost-8(14)-en-3β-ol (from 1.5 g. of acetate) was dissolved in pyridine (35 c.c.) and redistilled benzoyl chloride (3 c.c.) added. The mixture was kept at 90° for 1 hour and then allowed to stand overnight at 15°. Isolation of the benzoate through ether and crystallisation from chloroform-methanol yielded 22:23-dibromoergost-

-8(14)-en-3β-yl benzoate (1.25 g.) as plates, m.p.242-243°, $[\alpha]_D$ +3° (c, 5.0, 4.5) (Found: C,63.4; H,7.8. $C_{35}H_{50}O_2Br_2$ requires C,63.4; H,7.6%). Light absorption: ϵ_{230} 15,000. It gave a bright yellow colour with tetranitromethane in chloroform.

Ergosta-8(14):22-dien-3β-yl Acetate (with R.Budziarek).- A solution of 22:23-dibromoergost-8(14)-en-3β-yl acetate (0.2 g.) in ether-ethanol (60 c.c.; 1:1) was refluxed with zinc dust (1.5 g.) for 2 hours. The solution was filtered, concentrated, diluted with water and extracted with ether. Isolation of the product afforded ergosta-8(14):22-dien-3β-yl acetate (0.15 g.) as plates from chloroform-methanol, m.p.122-123.5°, $[\alpha]_D$ -25° (c, 1.1) (Found: C,81.5; H,11.2. $C_{30}H_{48}O_2$ requires C,81.8; H,11.0%). Light absorption: ϵ_{218} 8000, ϵ_{215} 7000, ϵ_{200} 4900. It gives a deep yellow colour with tetranitromethane in chloroform [Laubach and Brunings (71) give m.p.122.6-124°, $[\alpha]_D$ -26.5°].

Ergosta-8(14):22-dien-3β-ol (with R.Budziarek). - A solution of ergosta-8(14):22-dien-3β-yl acetate (0.1 g.) in methanolic potassium hydroxide (20 c.c.; 3%) was refluxed for 2 hours then concentrated, and diluted with

water. Isolation by means of ether gave ergosta-8(14):22--dien-3β-ol which crystallised from methanol as elongated plates (0.06 g.), m.p.126-127°, $[\alpha]_D$ -19° (c, 1.5) (Found: C,84.2; H,11.8. $C_{28}H_{46}O$ requires C,84.35; H,11.6%).

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

22:23-Dibromoergost-7-en-3β-yl Acetate. A solution of 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate (0.5g.) in ethyl acetate (100 c.c.) was added to platinum catalyst (from 0.115 g. PtO_2), in ethyl acetate (20 c.c.). The suspension was shaken for 5 hours with hydrogen. Removal of the catalyst and evaporation of the filtrate to dryness *in vacuo* gave a white solid which was recrystallised from chloroform-methanol or ethyl acetate-methanol. 22:23--Dibromoergost-7-en-3β-yl acetate (0.4 g.) separated as needles, m.p.224° (decomp.), $[\alpha]_D$ -7° (c, 2.0, 1.4) (Found: C,60.3; H,8.2. $C_{30}H_{48}O_2Br_2$ requires C,60.0; H,8.0%). Light absorption: $\epsilon_{2100}5000$, $\epsilon_{2150}3620$, $\epsilon_{2200}1700$. It gives a yellow colour with tetranitromethane in chloroform.

22:23-Dibromoergost-7-en-3β-ol. - A solution of 22:23-dibromoergost-7-en-3β-yl acetate (0.2 g.) in benzene (15 c.c.) was added to methanolic potassium hydroxide (25 c.c.; 2%) and refluxed for 1 hour. Processing the reaction mixture in the usual way yielded 22:23--dibromoergost-7-en-3β-ol (0.165 g.) as plates from methanol, m.p.222-223°, $[\alpha]_D$ -8° (c, 1.3) (Found: C,59.1; H,8.6. $C_{28}H_{46}OBr_2.CH_3OH$ requires C,59.0; H,8.5%).

Reacetylation gave the parent acetate as needles from methanol chloroform, m.p.225°, $[\alpha]_D$ -6° (c, 1.5).

22:23-Dibromoergost-7-en-3β-yl Benzoate. - 22:23--Dibromoergost-7-en-3β-ol (from 0.9 g. of acetate) in benzene (20 c.c.) was added to a solution of benzoyl chloride (3 c.c.) in pyridine (50 c.c.). After heating at 90° for 2 hours the product was isolated in the usual way. Crystallisation from chloroform-methanol furnished needles of 22:23-dibromoergost-7-en-3β-yl benzoate (0.6g.), m.p.205° (decomp.), $[\alpha]_D$ -4° (c, 4.0) (Found: C,63.4; H,7.8. $C_{35}H_{50}O_2Br_2$ requires C,63.4; H,7.6%). Light absorption: Max. at 2300 Å (ϵ = 16,000).

Ergosta-7:22-dien-3β-yl Acetate. - A solution of 22:23-dibromoergost-7-en-3β-yl acetate (0.25 g.) in ether-

-ethanol (60 c.c.; 1:1) was refluxed with zinc dust (1.5 g.) for 2 hours. The solution was filtered, concentrated, and diluted with water. Isolation by means of ether afforded ergosta-7:22-dien-3β-yl acetate (0.15 g.) as plates from chloroform-methanol, m.p.132-133°, $[\alpha]_D$ -20° (c, 1.2) undepressed in melting point when mixed with an authentic sample (Found: C,82.1; H,11.1. Calc. for $C_{30}H_{48}O_2$: C,81.8; H,11.0%). It gives a yellow colour with tetranitromethane in chloroform.

Isomerisation of 22:23-Dibromoergost-7-en-3β-yl Acetate. - 22:23-Dibromoergost-7-en-3β-yl acetate (0.5 g.), in acetic acid (300 c.c.) was shaken with a platinum catalyst in hydrogen for 4 hours. Working up through ether gave 22:23-dibromoergost-8(14)-en-3β-yl acetate as plates (0.4 g.) from chloroform-methanol, m.p. and mixed m.p.190°, $[\alpha]_D$ +4° (c, 3.0).

Treatment of 22:23-Dibromoergost-8(14)-en-3β-yl Acetate with Hydrogen Chloride. - Dry hydrogen chloride was passed into a solution of 22:23-dibromoergost-8(14)--en-3β-yl acetate (1 g.) in dry chloroform (20 c.c.) for 90 minutes at 0°. The solvent was removed under reduced pressure and the solid crystallised from chloroform-

-methanol giving plates, m.p.225-226° (decomp.) (0.6 g.), $[\alpha]_D$ +21° (c, 2.0) (Found: C,60.1; H,8.0. $C_{30}H_{48}O_2Br_2$ requires C,60.0; H,8.0%). Light absorption: ϵ_{2800}4000, ϵ_{2100}3000, ϵ_{2150}1250, ϵ_{2200}620. The ''compound'' gave a yellow colour with tetranitromethane in chloroform.

Hydrolysis of this ''<u>acetate</u>'' (0.3 g.) in benzene (20 c.c.) by refluxing with methanolic potassium hydroxide (45 c.c.; 2%) for 2 hours gave the corresponding "<u>alcohol</u>" (0.24 g.), m.p.216-219° which after three recrystallisations from methanol separated as plates, m.p.219-220°, $[\alpha]_D$ +29° (c, 1.9) (Found: C,58.7; H,8.7. $C_{28}H_{46}OBr_2$. CH_3OH requires C,59.0; H,8.5%).

Acetylation of the alcohol (pyridine-acetic anhydride) gave the acetate which separated from chloroform-methanol as plates, $[\alpha]_D$ +22° (c, 1.3), m.p.223-225° (decomp.) alone or mixed with the acetate described above.

Refluxing the acetate (0.3 g.) in ether-ethanol (40 c.c.; 1:1) with zinc dust (1 g.) gave "β"-dihydroergosteryl acetate which separated from methanol-chloroform as needles, m.p.106-107° (0.2 g.), $[\alpha]_D$ -18° (c,1.6) (Found: C,82.1; H,11.4. Calc. for $C_{30}H_{48}O_2$: C,81.8; H,11.0%). Light absorption: ϵ_{2100}2250, ϵ_{2150}730. It gives a deep yellow colour with tetranitromethane in

chloroform. Barton, Cox, and Holness (34) give m.p.104.5°, $[\alpha]_D$ -17°, for "β"-dihydroergosteryl acetate.

Hydrolysis of the acetate with methanolic potassium hydroxide (2%) followed by crystallisation of the product from chloroform-methanol gave "β"-dihydroergosterol, m.p. 115-116°, $[\alpha]_D$ -11° (c, 1.2). Barton, Cox and Holness (34) gave m.p.116°, $[\alpha]_D$ -9° for this compound. Benzoylation of the alcohol by the usual procedure gave the benzoate as plates from chloroform-methanol, m.p.118°, $[\alpha]_D$ -9.5° (c, 1.7) (lit., m.p.116°, $[\alpha]_D$ -3°).

Treatment of 22:23-Dibromoergost-8(14)-en-3β-yl Benzoate with Hydrogen Chloride. Dry hydrogen chloride was passed through a solution of 22:23-dibromoergost-8(14)--en-3β-yl benzoate (1 g.) in dry chloroform (30 c.c.) for 2 hours at 0°. Evaporation to dryness under reduced pressure gave a pale yellow solid which separated from chloroform-methanol as plates (0.86 g.), m.p.229-231°, $[\alpha]_D$ +21° (c, 2.0). Four further recrystallisations from the same solvent gave mixed crystals, m.p.232° (decomp.), $[\alpha]_D$ +21°, +20° (c, 2.4, 4.0) (Found: C,63.3; H,7.8. $C_{35}H_{50}O_2Br_2$ requires C,63.4; H,7.6%). A solution of the benzoate (0.075 g.) in benzene (25 c.c.) was refluxed with methanolic potassium hydroxide (25 c.c.; 3%) for four hours

gave the alcohol as plates (0.055 g.) from methanol, m.p. 219-220°, $[\alpha]_D$ +29° (c, 1.3). The alcohol was undepressed when mixed with the alcohol obtained by isomerisation of 22:23-dibromoergost-8(14)-en-3β-yl acetate followed by hydrolysis.

Attempts to saturate completely 22:23-Dibromoergosta--7:9(11)-dien-3β-yl Acetate and 22:23-Dibromoergost-7--en-3β-yl Acetate. - (a) A solution of 22:23-dibromoergosta-7:9(11)-dien-3β-yl acetate (5 g.) in chloroform (150 c.c.) was added to prereduced platinum catalyst (from 0.5 g. PtO_2) in chloroform (50 c.c.) with sodium sulphate (1 g.) in suspension to remove water. The mixture was shaken with hydrogen for six hours, the catalyst removed and the filtrate cooled to 0°. Dry hydrogen chloride was passed through the solution for 2 hours, and the solvent removed under reduced pressure. The solid residue was hydrogenated again as above. No absorption of hydrogen occurred, but after the addition of glacial acetic acid (100 c.c.), hydrogen (50 c.c.) was absorbed during 12 hours. This isomerisation and hydrogenation procedure was repeated twice with the product [total hydrogen absorbed = 150 c.c.]. Working up gave a crystalline substance which still exhibited a

deep yellow colour with tetranitromethane and which when crystallised from chloroform-methanol gave plates (2.5g.), m.p.109-112°, unchanged by further crystallisation and exhibiting a negative Beilstein test. Chromatographic purification of this material failed to yield an identifiable product.

(b) A solution of 22:23-dibromoergost-7-en-3β-yl acetate (1 g.) in glacial acetic acid (400 c.c.) was treated with dry hydrogen chloride at 15° for 5 minutes. The resulting solution was shaken with hydrogen in the presence of platinum oxide (0.1 g.) for 26 hours. Working up gave a product, m.p.214-215° showing a deep yellow colour with tetranitromethane in chloroform. (Using a temperature of 60° in this reaction did not improve the result). When the hydrogenation time was increased to 50 hours, no significant difference appeared in (a) the colour the product gave with tetranitromethane in chloroform or (b) the absorption spectrum of the product (ϵ_{309} 3000).

(c) 22:23-Dibromoergosta-7:9(11)-dien-3β-yl acetate (1 g.) in ether (60 c.c.) was added to a saturated solution of hydrogen chloride in ether (25 c.c.). Reduced platinum catalyst (from 0.3 g. PtO_2) was washed twice with methanol and three times with dry ether and then

added as a suspension in dry ether (20 c.c.) to the reaction flask (vol. 250 c.c.). The flask was charged with hydrogen, stoppered, and shaken vigorously for 110 hours. Isolation of the product and crystallisation from chloroform-methanol gave 22:23-dibromoergost-7-en-3β-yl acetate (0.5 g.) as needles, m.p. 216-218°, $[\alpha]_D$ -5° (c, 2.8). The total crude material was treated according to the Nabenhaur-Anderson procedure (90). No saturated material was isolated.

22:23-Dibromoergostan-3β-yl Acetate. - (a) Dry hydrogen chloride was passed through a solution of 22:23-dibromoergost-8(14)-en-3β-yl acetate (2.0 g.) in chloroform (25 c.c.) for 2 hours at 0°. The solvent and hydrogen chloride were removed under reduced pressure and the residual solid (1.23 g.), m.p. 217-219°, recrystallised from chloroform-methanol. The product (0.8 g.) was dissolved in acetic acid-ether (100 c.c.; 1:1) and shaken with hydrogen over a platinum catalyst (from 0.2 g. PtO_2), for 40 hours. The catalyst was removed and the solution reduced to small bulk under reduced pressure. Working up in the usual way gave a solid which was dissolved in carbon tetrachloride (30 c.c.). Acetic anhydride (10 c.c.) containing concentrated

sulphuric acid (10 c.c.) was added slowly with shaking and cooling over five minutes, and shaking continued for a further five minutes. Water (10 c.c.) was added and the mixture extracted with carbon tetrachloride. The combined extracts were washed with water, sodium hydrogen carbonate solution, water, and dried (Na_2SO_4). Removal of the ether gave a pale yellow solid (0.37 g.) which crystallised from chloroform-methanol giving 22:23-dibromoergostan-3β-yl acetate (0.25 g.) as plates, m.p.242-243° (decomp.), $[\alpha]_D$ +3°, +2.7° (c, 2.2, 3.0) (Found: C,60.4; H,8.6. Calc. for $C_{30}H_{50}O_2Br_2$: C,59.9; H,8.4%). It does not give a colour with tetranitromethane in chloroform and does not show high intensity absorption above 2000 Å. Barton, Cox, and Holness (34) give decomp. ca. 226° for this compound.

(b) A crude specimen of the mixed crystals of 22:23-dibromoergost-3(14)-en-3β-yl acetate and 22:23-dibromoergost-14-en-3β-yl acetate (0.8 g., m.p.223-225°) was hydrogenated as in (a) above. Of the product (0.2 g.) was dissolved in chloroform (10c.c.) and perbenzoic acid (0.18 g.) in chloroform (3 c.c.) added. The solution was allowed to stand for 42 hours and then worked up in the usual way. The isolated solid was dissolved in

petrol (120 c.c.) and chromatographed on alumina (10 x 1.2 cm.). Elution of the column with benzene-petrol mixtures (2:3; 350 c.c.) and evaporation of the eluates to dryness gave a solid (0.048 g.) which separated from chloroform-methanol as plates (0.035 g.), m.p. 237-238°, $[\alpha]_D$ +3° (c, 2.2). It showed no depression in melting point when mixed with a specimen prepared as in (a) above.

Further elution of the column with benzene (250 c.c.) gave a second material which crystallised from chloroform--methanol as plates, m.p. 212-213° (0.02 g.), $[\alpha]_D$ +21.5° (c, 1.2) (Found: C,58.5; H,7.9. $C_{30}H_{48}O_3Br_2$ requires C,58.4; H,7.8%). This compound, probably 22:23-dibromo--3α:14α-epoxyergostan-3β-yl acetate shows no selective absorption above 2200 Å and gives no colour with tetranitromethane in chloroform.

22:23-Dibromoergostan-3β-ol. - A solution of 22:23--dibromoergostan-3β-yl acetate (0.115 g.) in benzene (20 c.c.) and methanolic potassium hydroxide (20 c.c.; 2%) was heated under reflux for 1 hour. Isolation by means of ether gave 22:23-dibromoergostan-3β-ol which crystallised from chloroform-methanol as plates, m.p. 239-240°, (0.035 g.), $[\alpha]_D$ +7° (c, 1.45) (Found: C,58.4; H,8.9. $C_{28}H_{48}OBr_2.CH_3OH$ requires C,58.8; H,8.9%).

22:23-Dibromoergostan-3β-yl Benzoate. - 22:23--Dibromoergostan-3β-ol (0.07 g.) in pyridine (10 c.c.) was heated with benzoyl chloride (1 c.c.) at 90° and allowed to stand at 15° for 24 hours. 22:23-Dibromoergostan-3β-yl benzoate (0.06 g.) was isolated in the usual way and crystallised from chloroform-methanol as plates, m.p.218°, $[\alpha]_D$ +6° (c, 2.5) (Found: C,63.6; H,8.2. $C_{35}H_{52}O_2Br_2$ requires C,63.3; H,7.9%).

Ergost-22-en-3β-yl Acetate. - A solution of 22:23--dibromoergostan-3β-yl acetate (0.15 g.) in ether-ethanol (75 c.c.; 1:1) was refluxed with zinc dust (1 g.), added over 2 hours. Working up in the usual way yielded ergost-22-en-3β-yl acetate which crystallised from chloroform-methanol as plates (0.06 g.), m.p.157°, $[\alpha]_D$ -20°, -18° (c, 1.3, 1.4) (Found: C,81.0; H,11.4. Calc. for $C_{30}H_{50}O_2$: C,81.4; H,11.4%). Barton, Cox and Holness (84) give m.p.155.5°, $[\alpha]_D$ -17° for this compound.

Ergost-22-en-3β-ol. - A solution of ergost-22-en-3β--yl acetate (0.04 g.) in benzene (2 c.c.) was heated under reflux for 1 hour with methanolic potassium hydroxide (2 c.c.; 2%). Isolation by means of ether gave ergost--22-en-3β-ol (0.025 g.) which crystallised from chloroform-

-methanol as plates, m.p.153-154°, $[\alpha]_D$ -10° (c, 1.3) (Found: C,81.9; H,12.2. Calc. for $C_{28}H_{48}O.\frac{1}{2}H_2O$: C,82.1; H,12.1%).

An Improved Preparation of Aluminium tert.-Butoxide (cf. Org.Synth.,21,8). - Aluminium turnings (30 g.) were covered with an aqueous solution of sodium hydroxide (200 c.c., 3%) and the solution allowed to stand until hydrogen was being freely generated (approx. 3 mins.). The liquid was then decanted, the aluminium washed four times with water and covered with a solution of mercuric chloride (200 c.c., 5%). After 60 secs. the liquid was again decanted and the amalgamated aluminium washed four times with water, three times with dry methanol, three times with dry ether, stored under dried ether and used within 15 mins. The ether was decanted off and anhydrous tert.-butanol (100 c.c., distilled twice over sodium) added. A crystal of iodine was added to promote the reaction which began almost immediately, signified by the gentle liberation of hydrogen. The mixture was refluxed for 40 hours, tert.-butanol (350 c.c.) being added after 4 hours; benzene was also added at intervals to keep the aluminium tert.-butoxide in solution. At the end of this period the solution was diluted with benzene (2 litres) and the liquid decanted from any undissolved aluminium. After centrifuging for 30 mins., at 2500 r.p.m., the clarified liquor was decanted from

the sludge and evaporated to dryness in an inert atmosphere giving a crystalline residue of pure aluminium tert.-butoxide (330 g.). Yield approx. 82% based on the tert.-butanol used.

Ergosterone (Ergosta-4:7:22-trien-3-one). -
(a) Oppenauer conditions. Ergosterol (20 g.) was dissolved in dry benzene (300 c.c.) and pure dry acetone (120 c.c.), and the mixture added to a solution of aluminium tert.-butoxide (32 g.) in dry benzene (200 c.c.). The mixture was then refluxed for four hours, poured into sulphuric acid (1 l., 10%) and the benzene layer separated, washed with water, sodium hydrogen carbonate solution, and finally dried (Na_2SO_4). The dark brown liquid was taken to dryness under reduced pressure and the residual gum recrystallised from acetone-methanol. Two further recrystallisations from acetone-petrol gave ergosta-4:7:22-trien-3-one (3.4 g.) as pale yellow needles, m.p.130-131°, $[\alpha]_D$ -11° (c, 1.3). Light absorption: ϵ_{2400}13,300.
(b) The following modified procedure proved more convenient. Recrystallised ergosterol (50 g.) was dissolved in a mixture of dry toluene (500 c.c.) and cyclohexanone (375 g.) by gentle heating. The solution was

brought to reflux temperature and 50 c.c. of the solvent distilled to remove traces of moisture. A solution of aluminium tert.-butoxide (50 g.) in dry toluene (400 c.c.) was quickly added to the hot solution and the mixture refluxed for 1.75 hours. After 0.5 hours the solution became gelatinous, becoming more mobile towards the end of the reaction. The cooled mixture was washed with sulphuric acid (4 x 1000 c.c., 4%), water, sodium hydrogen carbonate solution, water and then dried (Na_2SO_4). The solvents were removed under reduced pressure and the residue heated at 138-140° 10^{-4} mm., to remove self condensation products of cyclohexanone. Crystallisation of the residual viscous oil from acetone-petroleum (b.p.60--80°) gave ergosta-4:7:22-trien-3-one (36.5 g.), m.p.123--126° suitable for further reaction. The mother liquors were taken to dryness and the residue taken up in ether (ca. 40 c.c.). A further quantity of ketone (4 g.) separated on standing at 0°. [Total yield of crude material (40.5 g.) = 81%]. Recrystallisation of the total product yielded almost pure ergosterone (30 g.), m.p.131°, as pale yellow needles. A specimen recrystallised four times from acetone-light petroleum (b.p.60-80°) furnished the pure material as soft white needles, m.p.134°, $[\alpha]_D$-12°

(c, 4.5) (Found: C,84.3; H,10.5. Calc. for $C_{28}H_{42}O$: C,85.2; H,10.7%). Light absorption: Max. at 2400 Å (ϵ = 13,300). It gives a pale yellow colour with tetranitromethane in chloroform.

For ergosterone, Oppenauer (91) gives m.p.131.5-132°, $[\alpha]_D$ -15.7°; Heilbron, Kennedy, Spring, and Swain (107) give m.p.132°, $[\alpha]_D$ -0.8°; Wetter and Dimroth (108) give m.p.132°, $[\alpha]_D$ -0.5°, -0.8°.

Note: The use of freshly prepared aluminium tert.-butoxide is essential in the reproduction of the above yield.

isoErgosterone (Ergosta-4:6:22-trien-3-one). - A solution of ergosta-4:7:22-trien-3-one (18 g.) in dry chloroform (150 c.c.) was cooled to 0° by an ice bath and treated with a stream of dry hydrogen chloride for 1 hour. The reaction mixture which had become blood-red in colour, was poured into a solution of potassium carbonate (1 l., 10%) and the organic layer separated, washed with water, and dried (Na_2SO_4). Removal of the chloroform under reduced pressure gave a semisolid gum which was crystallised from aqueous acetone. Ergosta-4:6:22-trien-3-one (14.5g.) separated as thick yellow prismatic needles, m.p.100° of suitable purity for further reaction. Further purification proved wasteful. A specimen recrystallised three

times from ethyl acetate-methanol separated as pale yellow needles, m.p.105-107°. Sublimation at 150° (10^{-4} mm.) removed colour and crystallisation of the sublimate gave hard prismatic needles of pure isoergosterone, m.p.106-107°, $[\alpha]_D$ -24.5° (c, 0.9) (Found: C,84.9; H,10.9. Calc. for $C_{28}H_{42}O$: C,85.2; H,10.7%). Light absorption: Maxima at 2340 (ϵ = 23,000) and 2060 Å (ϵ = 11,000). It gives a pale yellow colour with tetranitromethane in chloroform.

Heilbron, Kennedy, Spring, and Swain (107) give m.p.108°, $[\alpha]_D$ -30°. Wetter and Dimroth (108) give m.p.110°. Barton, Cox, and Holness (84) give m.p.105° for isoergosterone.

On one occasion the crude reaction product (5.75g.) (from which the last traces of hydrogen chloride are difficult to remove) was crystallised directly from ethyl acetate-methanol. Yellow needles (2.5 g.), m.p.138-140° separated, which on further recrystallisation gave the pure material as yellow needles identified as 3-methoxy-ergosta-3:5:7:22-tetraene, m.p.137-138°, $[\alpha]_D$ -98° (c,1.7) (Found: C,85.0; H,10.8. Calc. for $C_{29}H_{48}O$: C,85.0; H,11.1%). Light absorption: Maxima at 3200 (ϵ = 23,000) and 3350 Å (ϵ = 16,400), a spectrum indicative of a

$\Delta^{3:5:7}$-triene system [cf. ergosta-3:5:7:22-tetraen-3β--yl acetate with a maximum at 3165 Å (ϵ = 21,400) and 3:6-dibenzoyloxycholesta-3:5:7-triene with a maximum at 3160 Å (ϵ = 20,000)]. It gives a dark brown colour with tetranitromethane in chloroform. Oppenauer (91) gives m.p.140-141° for this compound.

Attempted Rearrangement of Ergosta-4:7:22-trien--3-one with BF_3/Et_2O Complex. - A solution of ergosta--4:7:22-trien-3-one (0.4 g.) in pure dry ether (25 c.c.) was treated with boron trifluoride-ether complex and allowed to stand at room temperature for 48 hours. Working up in the usual way and crystallisation of the product from methanol yielded pale yellow needles, m.p. 129-130°, (0.325 g.), $[\alpha]_D$ -11.2° (c, 1.05), undepressed in melting point with starting material.

Hydrogenation of Ergosta-4:6:22-trien-3-one with a Raney Nickel Catalyst. - Ergosta-4:6:22-trien-3-one (0.5 g.) was dissolved in cyclohexane (30 c.c.) and added to Raney nickel sludge (2 c.c. W.6) in cyclohexane (70 c.c.). The mixture was shaken with hydrogen and the absorption, which at first occurred rapidly, slowed down after the absorption of approximately 2 mols. of hydrogen.

After 2.3 mols of hydrogen had been absorbed, the reaction was stopped, the catalyst removed by filtration, and the filtrate taken to dryness under reduced pressure. The solid residue, which showed no selective absorption of high intensity above 2200 Å, was dissolved in light petroleum (50 c.c., b.p.60-80°) and chromatographed on ethyl acetate washed alumina (14 x 1.5 cm.). 50 c.c. Fractions were collected.

Fraction No.	Eluant	Weight	Characteristics	
1-4	Petrol	-		
5-6	Petrol:Benzene (9:1)	-		
8-14	Petrol:Benzene (8:2)	99 mg.	Cryst.Solid	A
15-21	''	66	'' ''	B
22-26	''	41	'' ''	C
27-28	''	22		
29-32	''	33	'' ''	D
33-36	''	19		
37-40	''	20	'' ''	E
41-44	''	5		
45	Benzene	-		
46	Benzene:Methanol (19:1)	100	'' ''	F

No fraction yielded a weight of material greater than 20 mg. and the fractions were thus combined arbitrarily. Product A was crystallised three times from methanol--acetone from which it separated as fine needles, m.p. 193°, $[\alpha]_D$ -12° (c, 0.85) (Found: C,83.9; H,12.1. $C_{28}H_{48}O$ requires C,83.9; H,12.1%). It shows no selective absorption above 2200 Å. It gives a pale yellow colour with tetranitromethane in chloroform.
Product F was crystallised three times from methanol giving plates, m.p.145°, $[\alpha]_D$ -8° (c, 1.2) (Found: C,83.4; H,12.2. $C_{28}H_{48}O$ requires C,83.9; H,12.1%. $C_{28}H_{46}O$ requires C,84.5; H,11.6%). It shows no selective light absorption above 2200 Å and gives a pale yellow colour with tetranitromethane in chloroform.
Products B, C, D, and E failed to crystallise well and were not investigated further.

Ergosta-4:6:22-trien-3β-ol. - Ergosta-4:6:22-trien--3-one (0.5 g.) was added to a warm solution of lithium aluminium hydride (1.5 g.) in ether (50 c.c.), the mixture refluxed for 12 minutes, then allowed to stand overnight. The excess lithium aluminium hydride was decomposed with ethyl acetate (10 c.c.) and the resulting paste diluted with water. Hydrochloric acid (100 c.c.,3%)

was added and the mixture ether extracted. Isolation of the product in the usual way, and crystallisation from acetone-light petroleum (b.p.40-60°) furnished soft glistening plates of ergosta-4:6:22-trien-3β-ol (0.2 g.), m.p.129-130°, $[\alpha]_D$ -20° (c, 0.8) (Found: C,84.8; H,11.2. $C_{28}H_{44}O$ requires C,84.8; H,11.2%). Light absorption: Maxima at 2340 Å (ϵ = 23,000) and 2400 Å (ϵ = 25,000) with an inflexion at 2470 Å (ϵ = 16,000). It gives a red-brown colour with tetranitromethane in chloroform.

Attempted Saturation of the Δ^6-Bond of Ergosta--4:6:22-trien-3-one using Zinc Dust:Ethanol. - Ergosta--4:6:22-trien-3-one (0.33 g.) was taken up in ethanol (25 c.c.), activated zinc dust (2.0 g.) added, and the solution refluxed for 4.5 hours. Isolation of the product by the usual method and crystallisation from ethyl acetate-methanol gave pale yellow needles of starting material (0.25 g.), m.p. and mixed m.p. 104°, $[\alpha]_D$ +22° (c, 1.3).

Ergosta-4:22-dien-3-one. - By reduction of iso-ergosterone. (a) With a palladium catalyst. Ergosta--4:6:22-trien-3-one (0.5 g.) in thiophen-free benzene (20 c.c.) was added to a suspension of palladised strontium

carbonate catalyst (0.25 g., 2% Pd) in benzene (10 c.c.). The mixture was shaken with hydrogen for 45 minutes when absorption approximated to 1 mol. Filtration and evaporation to dryness under reduced pressure gave an oily product which was crystallised from aqueous acetone. Three further recrystallisations from acetone-methanol afforded long hard needles of ergosta-4:22-dien-3-one (0.2 g.), m.p.129-130°, $[\alpha]_D$ +42° (c, 1.4) (Found: C,84.9; H,11.3. Calc. for $C_{28}H_{44}O$: C,84.8; H,11.2%). Light absorption: Max. at 2400 Å (ϵ = 13,700). It gives a pale yellow colour with tetranitromethane in chloroform. Barton et al., (84) give m.p.127.5-128.5°, $[\alpha]_D$ +43°, +44°.

The use of ''Analar'' light petroleum or reagent grade benzene as the solvent in this reaction gave reduced yields. Moreover, when the reaction was stopped after the absorption of either 0.8 mols. or more than one mol. of hydrogen, yields were again less than 40%.

(b) With lithium: liquid ammonia. A solution of ergosta--4:6:22-trien-3-one (1.0 g.) in dry ether (50 c.c.), containing methanol (5 c.c.), was added during 3 minutes to a stirred blue solution of lithium metal (0.5 g.) in liquid ammonia (200 c.c.). Stirring was continued for 35 minutes and the ammonia allowed to evaporate overnight.

The residue was diluted with water, extracted with ether, and the product isolated in the usual way. Two recrystallisations from aqueous acetone yielded ergosta-4:22--dien-3-one (0.6 g.) as long colourless needles, m.p.129--130°, $[\alpha]_D$ +41° (c, 0.9) (Found: C,84.8; H,11.2%). Light absorption: Max. at 2420 Å (ε = 18,000). It shows no depression in melting point when mixed with a specimen prepared by method (a) above.

(c) With zinc dust-acetic acid. Ergosta-4:6:22-trien--3-one (0.97 g.) was dissolved in stabilised glacial acetic acid (40 c.c.) and zinc dust (5 g.) added. The mixture was refluxed for 60 hours. Working up through ether gave a brown non-crystallisable gum (0.813 g.) which was dissolved in light petroleum (30 c.c., b.p.60--80°) and chromatographed on alumina (16 x 1.2 cm.). Elution of the column with light petroleum (350 c.c., b.p.60-80°) gave a non-crystalline gum (0.035 g.). Elution with petroleum-benzene (100 c.c., 1:1) gave a material (0.035 g.) which, after two crystallisations from ethyl acetate-acetone, had m.p.123-125°, $[\alpha]_D$ +33° (c, 0.5). Light absorption: Max. at 2420 Å (ε =17,000). It showed no depression when mixed with an authentic specimen of ergosta-4:22-dien-3-one. Further elution of the column with more polar solvents yielded only non-

-crystallisable viscous oils.

Attempted Biological Oxidation of Ergosta-4:22-dien--3-one with Rhizopus Nigricans. - The medium used to support the growth of the fungus had the following composition: bacto-tryptone (20 g.), corn-steep (3.0 g.), and technical glucose (50 g.), diluted with tap-water to 1 litre of solution. A solution of the medium (1.5 l.) was prepared the the pH adjusted to 4.4, using concentrated hydrochloric acid. The liquid was then placed in a 5 l. aspirator bottle equipped at the base with a tube containing a cotton wool filter, for the introduction of a stream of sterile air, and a thistle funnel at the top for the introduction of spores and steroidal material. The whole unit was sterilised at 10 lbs. per sq.in. for 15 minutes, and after cooling, the medium was inoculated with the spores of Rhizopus Nigricans taken from an agar slope. The medium was aerated for 24 hours at the rate of 500 c.c. air per minute, the growth of the mould becoming prolific during this period. A solution of ergosta-4:22-dien-3-one (0.4 g.) in alcohol (22 c.c.) and water (7 c.c.) was then introduced and growth of the culture permitted for a further 40 hours, with continued aeration. The introduction of the air was sufficient to

give good turbulence and provide thorough mixing of the steroid solution. At the end of this period the mycelium was filtered and, after drying in air, extracted with acetone (150 c.c.) in a Soxhlet apparatus. The filtrate was extracted with chloroform (200 c.c.). Both extracts were taken to dryness and the residual solids combined, dissolved in benzene (50 c.c.), and chromatographed on neutral alumina (12.5 x 1.5 cm.). Elution of the column with benzene (200 c.c.) and evaporation under reduced pressure gave a solid residue (0.41 g.). Further elution of the column gave non-crystallisable oils (0.02 g.). The solid material from the column was crystallised from methanol-acetone giving ergosta-4:22--dien-3-one (0.21 g.), m.p.123-129°, $[\alpha]_D$ +42° showing no depression in melting point when mixed with starting material. Re-chromatography of the material (0.155 g.) obtained from the mother liquors of the above crystallisation yielded only a further quantity (0.075 g.) of ergosta-4:22-dien-3-one. No other crystalline product apart from the latter could be isolated.

5β-Ergost-22-en-3-one. - (a) Ergosta-4:6:22-trien--3-one (2.0 g.) was dissolved in dry ethanol (30 c.c.) and added to a solution of potassium hydroxide (1.5 g.)

in dry ethanol (30 c.c.). This solution was added to a suspension of 10% palladised charcoal (0.25 g.) in dry ethanol (10 c.c.) and shaken with hydrogen until absorption ceased (40 minutes). The catalyst was removed by filtration, the filtrate concentrated, and diluted with water. Following the usual isolation procedure, there was obtained a crystalline mass, m.p.104-108°, identified as almost pure 5β-ergost-22-en-3-one (2 g.), which was used without further purification in subsequent reactions. A specimen crystallised twice from ethanol separated as lustrous plates, m.p.110°, $[\alpha]_D$ -5.2° (c, 2.1) (Found: C,84.6; H,11.8. Calc. for $C_{28}H_{46}O$: C,84.35; H,11.6%). Light absorption: Max. at 2070 (ϵ = 1,000) and 2700 Å (ϵ = 107). Peak at 1723 cm.$^{-1}$. 5β-Ergost-22-en-3-one gives a faint yellow colour with tetranitromethane in chloroform. Barton et al., (84) give m.p.110.5°, $[\alpha]_D$ -2°, -1°, for this material. The presence of traces of water markedly reduce the yields from this reaction.

(b) A solution of ergosta-4:22-dien-3-one in dry ethanol (45 c.c.) containing potassium hydroxide (0.8 g.) was hydrogenated as in (a) using 10% palladised charcoal catalyst (0.125 g.). Isolation of the product and crystallisation from ethanol yielded soft plates of 5β-ergost-

-22-en-3-one (0.45 g.), m.p. and mixed m.p.109-110°, $[\alpha]_D$ -5° (c,2.0) (Found: C,84.2; H,11.7%).

5β-Ergost-22-en-3-one (0.1 g.) in ethanol (6 c.c.) was treated with Brady's reagent (5 c.c.) at 40°. After standing at 15° for 14 hours, the orange precipitate was removed by filtration and recrystallised three times from benzene-ethanol. 5β-Ergosta-22-en-3-one 2:4-dinitrophenylhydrazone separated as small yellow needles, m.p. 197-198° (Found: N,9.8. Calc. for $C_{34}H_{50}O_4N_4$: N,9.7%). Barton et al., (34) give m.p.199°.

3:3-Dimethoxy-5β-ergost-22-ene. - On two occasions crystallisation of 5β-ergost-22-en-3-one from dry technical methanol (i.e. containing a trace of acid) afforded 3:3-dimethoxy-5β-ergost-22-ene (72% yield), as blades, m.p.34-35.5°, $[\alpha]_D$ -5.5° (c, 2.5) (Found: C,81.4; H,11.9; -OMe,14.7. $C_{30}H_{52}O_2$ requires C,81.0; H,11.8; -OMe,14%). Light absorption: Max. at 2040 Å (ϵ = 1900). It shows no absorption peak in the infra-red carbonyl region and does not absorb in the 2600-3200 Å zone in the ultra-violet spectrum.

A specimen of this compound (0.07 g.) in methanol (10 c.c.) treated with Brady's reagent at 40° yielded the 2:4-dinitrophenylhydrazone of 5β-ergost-22-ene-3-one,

which was recrystallised from benzene-ethanol, m.p. and mixed m.p.198°.

Hydrolysis of 3:3-Dimethoxy-5β-ergost-22-ene. - The dimethyl ketal (0.1 g.) in dioxan (40 c.c.) was added to water (10 c.c.) containing concentrated sulphuric acid (0.5 c.c.) and the mixture refluxed for 1 hour. The product, isolated by means of ether, crystallised from ethanol affording plates (0.065 g.) of 5β-ergost-22-en--3-one, m.p. and mixed m.p.109-111°, $[\alpha]_D$ -5.4° (c,1.7).

5β-Ergost-22-en-3α-yl Acetate. - 5β-Ergost-22-en-3--one (5.92 g.) in dry ether (100 c.c.) was added during 15 minutes to a boiling solution of lithium aluminium hydride (6.0 g.) in dry ether (250 c.c.) and the mixture refluxed for 1 hour. Acetone (50 c.c.) was then added dropwise and the pasty mass diluted with water (200 c.c.). Isolation of the product was accomplished in the usual way and acetylation was carried out using a mixture of pyridine (40 c.c.) and acetic anhydride (20 c.c.) at 90° for 2 hours. Working up through ether and crystallisation from ethanol or ethyl acetate-methanol afforded 5β-ergost-22-en-3α-yl acetate (4.5 g.) as plates, m.p.111--113°, used as such for further reaction. A sample twice

recrystallised from ethyl acetate-methanol gave the pure acetate, m.p.114-115°, $[\alpha]_D$ +9.2° (c, 1.3) (Found: C,81.6; H,11.7. Calc. for $C_{30}H_{50}O_2$: C,81.4; H,11.4%). Light absorption: it shows no selective absorption of high intensity above 2200 Å. It gives a faint yellow colour with tetranitromethane in chloroform. Barton et al., (34) give m.p.114-115°, $[\alpha]_D$ +16°.

5β-Ergost-22-en-3α-ol. - A solution of 5β-ergost-22-en-3α-yl acetate (0.136 g.) was refluxed with methanolic potassium hydroxide (25 c.c.; 4%). Isolation through ether gave 5β-ergost-22-en-3α-ol in quantitative yield. It separated from methanol as needles, m.p.149-150°, $[\alpha]_D$ -6.2° (c, 1.6) (Found: C,83.6; H,12.3. Calc. for $C_{28}H_{48}O$: C,83.9; H,12.1%). Barton et al. (34) give m.p. 149-150°, $[\alpha]_D$ -4°. Reacetylation of the alcohol with acetic anhydride-pyridine at 90° gave 5β-ergost-22-en-3α-yl acetate, m.p. and mixed m.p.114-115°, $[\alpha]_D$ +10° (c,1.4).

3α-Acetoxybisnorcholan-22-al. - 5β-Ergost-22-en-3α-yl acetate (5 g.) was dissolved in pure dry chloroform and cooled to -45° in an acetone-carbon dioxide freezing mixture. A stream of dry ozonised oxygen (14.7 c.c.; O_3 min.) was passed through the solution (at -45 ±3°)

until a faint blue colour developed. The reaction mixture was allowed to attain room temperature, glacial acetic acid (50 c.c.) and zinc dust (10 g.) were added, and the mixture stirred for 2 hours. Excess zinc dust and zinc acetate were separated and the filtrate subjected to steam distillation, 1 l. of distillate being collected. The non-volatile product was isolated by means of ether and crystallised from ethanol, to give the aldehyde as plates, m.p.115-118°. Recrystallisation from ethanol or aqueous acetone afforded 3α-acetoxybisnorcholan-22-al (3 g.) as plates, m.p.121-123° sintering at 115°, $[\alpha]_D$ +36° (c, 1.25) (Found: C,77.1; H,10.4. $C_{24}H_{38}O_3$ requires C,77.0; H,10.2%). The aldehyde does not show high light intensity absorption above 2200 Å nor give a colour with tetranitromethane in chloroform.

The 2:4-dinitrophenylhydrazone of this aldehyde was prepared by treatment of the aldehyde (0.075 g.) in ethanol (3 c.c.) with Brady's reagent. The precipitate formed after 0.5 hours was filtered, dissolved in benzene (50 c.c.) and percolated through a column of alumina (5g; 4 x 1 cm.). Removal of the benzene from the eluate, and crystallisation of the residue from ethyl acetate, gave yellow needles, m.p.205° (Found: N,9.3. $C_{30}H_{42}O_6N_4$

requires N,10.1%).

Extraction of the steam distillate with chloroform gave an oil which on treatment with Brady's reagent yielded the 2:4-dinitrophenylhydrazone of 2:3-dimethylbutyraldehyde which separated from methanol as orange blades, m.p.125° (lit., m.p.124-125°).

3α-Acetoxybisnorcholanic Acid. - (a) 3α-Acetoxybisnorcholan-22-al (0.225 g.) in glacial acetic acid (20 c.c.) was treated at 15° with a solution of chromium trioxide (0.05 g.) in glacial acetic (10 c.c.) during 10 minutes. After 18 hours, methanol (5 c.c.) was added to destroy the excess chromium trioxide; the solution was then diluted with water, ether extracted, and the ether extract shaken with sodium hydroxide solution (5%). The insoluble precipitate was removed by filtration and suspended in aqueous hydrochloric acid. Ether extraction, followed by isolation of the ether soluble portion yielded, after reacetylation, 3α-acetoxybisnorcholanic acid (0.175 g.). It separated as needles from acetone-light petroleum (b.p.60-80°), m.p.221-222°, $[\alpha]_D$ +21° (c, 1.4) (Found: C,73.8; H,9.8. Calc. for $C_{24}H_{38}O_4$: C,73.8; H,9.8%). Sawlewicz and Reichstein (98) give m.p.213-219° for this acid.

(b) 3α-Acetoxybisnorcholan-22-al (3 g.) was dissolved in chloroform (120 c.c.) and ozonised (17.5 c.c.; O_3 min.) at -45° for 20 minutes. The chloroform was removed under reduced pressure below 45° and the residue boiled with water for 1 hour. The granular solid which formed was crystallised from acetone-petroleum to give 3α-acetoxybisnorcholanic acid (2 g.), m.p.221-222°, $[\alpha]_D$ +21° (c, 1.4) (Found: C,74.05; H,9.8%).

Methyl-3α-Acetoxybisnorcholanate. - A solution of 3α-acetoxybisnorcholanic acid (0.5 g.) in ether was added to an ethereal solution of diazomethane (100 c.c.) and the mixture allowed to stand overnight. Glacial acetic acid was then added dropwise until the yellow colour disappeared from the reaction mixture. The ether was removed under reduced pressure and the residue crystallised from aqueous acetone. Methyl-3α-acetoxybisnorcholanate (0.46 g.) separated as plates, m.p.108-109°, $[\alpha]_D$ +29° (c, 2.8) (Found: C,74.3; H,10.2. $C_{25}H_{40}O_4$ requires: C,74.2; H,10.0%). It gives no colour with tetranitromethane in chloroform.

Attempted Enol Acetylation of 3α-Acetoxybisnorcholan--22-al. - A solution of 3α-acetoxybisnorcholan-22-al

(0.51 g.) in dry carbon tetrachloride (4.5 c.c.) was treated with acetic anhydride (0.5 c.c.) containing a trace (0.05 c.c.) of perchloric acid (65%). The solution was allowed to stand at room temperature for 75 minutes. Isolation of the product in the usual way afforded a non-crystallisable gum which was dissolved in petrol (50 c.c., n.p.60-80°) and chromatographed on alumina (15 g.). No crystallisable material was eluted.

Enol Acetylation of 3α-Acetoxybisnorcholan-22-al. - 3α-Acetoxybisnorcholan-22-al (3.4 g.) was dissolved in acetic anhydride (35 c.c.) and anhydrous potassium acetate (1.75 g.) added. The mixture was refluxed for 6 hours and allowed to stand overnight. Isolation through ether afforded a non-crystallisable oil which was dissolved in benzene (20 c.c.). Light petroleum (180 c.c.; b.p.60-80°) was added, a small quantity of an insoluble precipitate removed, and the solution percolated through a column of alumina (50 g.; 20 x 1.5 cm.). The column was washed with light petroleum-benzene (400 c.c.; 9:1), the eluates combined, and evaporated to dryness under reduced pressure, giving a colourless oil (2.63 g.) which failed to crystallise from any of the usual organic solvents. In contrast to the parent

aldehyde, this oil gave a yellow colour with tetranitromethane in chloroform and assuming it to be crude 3α:22--diacetoxybisnorchol-20(22)-ene, it was used for further reaction.

20-Oxopregnan-3α-yl Acetate. - The crude enol acetate of 3α-acetoxybisnorcholan-22-al (2.63 g.) was dissolved in chloroform (120 c.c.) and ozonised air passed through the solution at -45° until the blue colour of excess ozone appeared (22 minutes). There was then added to the reaction mixture glacial acetic acid (40 c.c.) and zinc dust (10 g.). After stirring for 2 hours the excess zinc dust was filtered and the filtrate diluted with water. The chloroform layer was separated, washed with water, sodium hydrogen carbonate solution, and dried (Na_2SO_4). Removal of the chloroform under reduced pressure gave a colourless glass (2.34 g.), which was dissolved in light petroleum (80 c.c.; b.p.60-80°) and chromatographed on alumina (50 g.; 20 x 2.5 cm.). The chromatogram was developed as follows.

Fraction No.	Vol.	Eluant	Weight	Description
1-3	240 c.c.	Petrol	-	
4-5	160	Petrol:Benzene(9:1)	296 mg.	Colourless gum
6	30	''	130	Cryst.from petrol
7	30	''	160	''
8	30	''	35	''
9	30	''	78	''
10	110	''	30	''
11	30	''	49	''
12	30	''	46	''
13	30	''	36	''
14	30	''	30	''
15	250	''	95	''
16	200	''	43	''
17	400	''	72	''
18	100	''	10	''
19	100	Petrol:Benzene(1:1)	31	''
20	100	''	76	''
21	100	''	58	''
22	100	''	36	Partially cryst. from petrol
23	100	''	20	Non-cryst.gum
24	100	''	12	''
25	100	Benzene:Methanol (1:1)	279	Amorphous solid
26	100	''		from petrol

Fractions 6-21 were combined and crystallised from light petroleum (b.p.60-80°). 20-Oxopregnan-3α-yl acetate (1.2 g.) separated as thick rods, m.p.100-101°, $[\alpha]_D$ +123° (c, 0.7) (Found: C,76.8; H,10.1. Calc. for $C_{26}H_{36}O_3$: C,76.6; H,10.1%). The keto-acetate was undepressed in m.p. when mixed with a specimen (m.p.99-100°) prepared by reduction of pregnane-3:20-dione followed by acetylation [Mancera et al. (67)].

Fractions 1-5 and fractions 22-24 could not be crystallised and were not further examined.

3α:20α-Diacetoxypregnane. - Fractions 25 and 26 were combined and acetylated using pyridine-acetic anhydride at 80°. The product was chromatographed in light petroleum (30 c.c.; b.p.60-80°) on alumina (10 x 1 cm.). Light petroleum (250 c.c.; b.p.60-80°) eluted a solid (0.17 g.) which when crystallised from petrol (b.p.60-80°) afforded 3α:20α-diacetoxypregnane as needles, m.p.130°, $[\alpha]_D$ +35° (c, 1.1) (Found: C,74.0; H,10.0. Calc. for $C_{25}H_{40}O_4$: C,74.2; H,10.0%). Hartmann and Locher (103) give m.p. 132-133°, $[\alpha]_D$ +35.3° (in benzene).

3α-Hydroxypregnane-20-one. - 20-Oxopregnan-3α-yl acetate (0.237 g.) was dissolved in methanol (20 c.c.)

and refluxed 1 hour with a solution of potassium carbonate (0.4 g.) in methanol-water (15 c.c.; 2:1). Isolation by means of ether gave 3α-hydroxypregnane-20-one (0.214 g.) which crystallised from acetone-light petroleum (b.p.60--30°) as needles, m.p.147-148°, $[\alpha]_D$ +110° (c, 0.9) (Found: C,79.2; H,10.9. Calc. for $C_{21}H_{34}O_2$: C,79.2; H,10.8%). When mixed with a specimen (m.p.145-147°) prepared according to Mancera et al. (67) there was no depression in melting point. Meystre and Miescher (99) give m.p.154°, $[\alpha]_D$ +110°.

Reacetylation of a specimen (0.12 g.) of the alcohol using pyridine-acetic anhydride at 80° regenerated the acetate. Two recrystallisations from petrol (b.p.60-80°) gave 20-oxopregnan-3α-yl acetate, m.p. and mixed m.p.99--100°, $[\alpha]_D$ +21° (c, 0.5).

Pregnane-3:20-dione. - 3α-Hydroxypregnan-20-one (0.15 g.) was dissolved in stabilised glacial acetic acid (20 c.c.) and a solution of chromium trioxide (0.04 g.) in pure acetic acid added at 15° during 8 minutes with stirring. After standing overnight the reaction mixture was processed in the usual way, and afforded pregnane--3:20-dione as rosettes of thick rods, m.p.120-121° (lit., 123°), $[\alpha]_D$ +112° (c, 1.0) (Found: C,79.5; H,10.5.

Calc. for $C_{21}H_{34}O_2$: C,79.7; H,10.2%).

Pregnane-3:20-dione. - (From progesterone). Progesterone (2 g.) was dissolved in ethanol (50 c.c.) and added to a freshly reduced 10% palladium-charcoal catalyst (0.25 g.) in ethanol (80 c.c.) containing potassium hydroxide (1.5 g.). The mixture was shaken with hydrogen for 6 hours, the catalyst removed by filtration, and the product isolated by means of ether. Two recrystallisations from light petroleum (b.p.60-80°) furnished a slightly impure pregnane-3:20-dione, m.p.116--118°, (0.4 g.) $[\alpha]_D$ +103° (c, 1.2).

3α-Hydroxypregnane-20-one. -[According to Mancera et al. (67)]. Pregnane-3:20-dione (0.375 g.) was dissolved in ethanol (13.5 c.c.) and a solution of sodium borohydride (0.065 g.) in ethanol (13.5 c.c.) added. The solution was allowed to stand at room temperature for 1 hour, and then a few drops of glacial acetic acid added to destroy excess borohydride. The mixture was concentrated under reduced pressure and the product isolated in the usual way. Crystallisation from acetone-light petroleum (b.p.60-80°) yielded 3α-hydroxypregnane-20-one as needles, m.p.145-147°, $[\alpha]_D$ +103° (c, 1.3).

A specimen was acetylated and the acetate crystallised from petroleum (b.p.60-80°); 3α-acetoxypregnane-20-one separated as thick needles, m.p.99-100°, $[\alpha]_D$ +121° (c, 0.8).

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