# S U M M A R Y

The first part of this thesis describes the preparation of 3β-hydroxy-11,20-dioxo-5α-pregnan-16α-ylmalonic acid, by the Michael addition of diethyl sodio malonate to 3β-acetoxy-5α-pregn-16-ene-11,20-dione, in the presence of an excess of diethyl malonate, and brief alkaline hydrolysis of the product. When the reaction was carried out with approximately equal molecular quantities of sodium and diethyl malonate the product was 3β-hydroxy-16α,24-cyclo-21-norcholan-11,20,23-trione

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It has not been found possible to prepare a 17α-hydroxy-16-dicarbomethoxymethyl compound or to carry out a Michael reaction between 3β-acetoxypregna-5,16-dien-20-one oxime and diethyl sodiomalonate.



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The second part of the work is mainly concerned with the photochemical oxygenation of three steroidal enol acetates and the identification of the products.

Photo-oxygenation of 3-acetoxyergosta-3,5,7,22-tetraene gave 3-acetoxyergosta-3,5,7,9(11),22-pentaene, 3-acetoxy-5α,8α-epidioxyergosta-3,6,22-triene and 3-acetoxy-5$\xi$,14$\xi$-dihydroxyergosta-3,8(9),22-trien-7-one. On treatment with alkali, the epidioxide underwent saponification accompanied by a novel re-arrangement to give 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one. Treatment of the epidioxide with sodium borohydride gave, depending on the solvent used, various compounds which were not identified, and catalytic hydrogenation gave a mixture of diols or triol which could not be separated to give any pure compound. Chromium trioxide-pyridine oxidation of ergosterol peroxide gave 5α,8α-epidioxyergosta-6,22-dien-3-one, which on alkali treatment gave 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one. Alkali treatment of 3-acetoxy-5$\xi$,14$\xi$-dihydroxyergosta-3,8(9),22-trien-7-one gave 7,14$\xi$-dihydroxyergosta-4,6,8(9),22-tetraen-3-one.

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T H E S I S

submitted to

THE UNIVERSITY OF GLASGOW

in fulfilment of the

requirements for the

DEGREE OF DOCTOR OF PHILOSOPHY

by

Thomas Sleigh

August, 1962.

# 16α-DICARBOMETHOXYMETHYL STEROIDS AND THE PHOTO-OXYGENATION OF STEROIDAL ENOL ACETATES.

# A C K N O W L E D G E M E N T S

. The author wishes to express his indebtedness to Dr. P. Bladon for the advice and encouragement given during these investigations, and to Professor P. L. Pauson for the opportunity to carry out this work. He is grateful to the staff of the micro-laboratory for microanalyses and ultraviolet and infrared spectra, and to Miss L. Semple for technical assistance. He would also like to express his appreciation to Dr. J. C. D. Brand, Glasgow University, for the nuclear magnetic resonance spectrum, to Dr. C. L. Hewett of Organon Laboratories Ltd. and Dr. R. M. Evans of Glaxo Laboratories Ltd. for gifts of chemicals and to the Cross Trust for a maintenance grant.

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# S U M M A R Y

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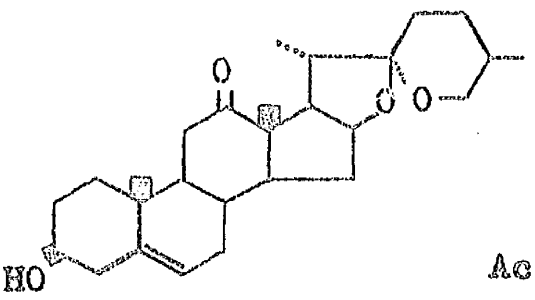
# Part 1.

## 16α-Dicarbomethoxymethyl Steroids.

## Introduction.

The ability of a wide variety of nucleophilic reagents to add to the double bond of $\Delta^{16}$-20-ketosteroids is well known. In investigating the use of the steroid sapogenin botogenin (I) as a starting material in the synthesis of cortisone intermediates, Marker[1] found that treatment of the oxidation product (II) of the diacetate of pseudobotogenin with methanolic potassium hydroxide gave a product which he thought to be 3β,17-dihydroxypregn-5-ene-12,20-dione (III).

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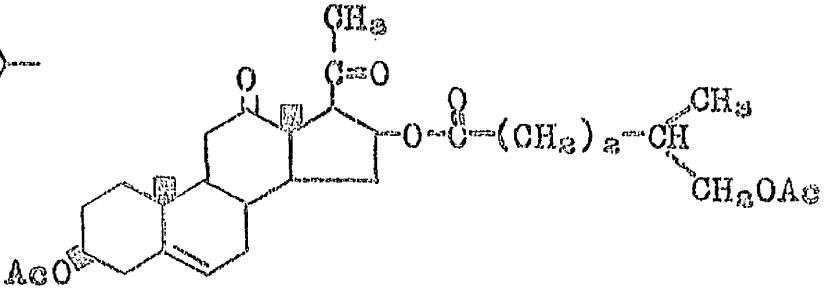


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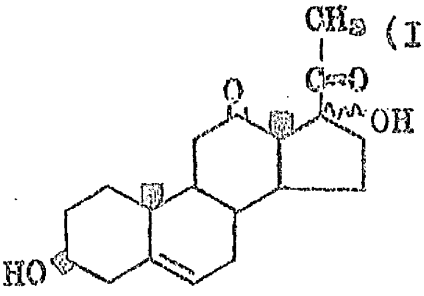
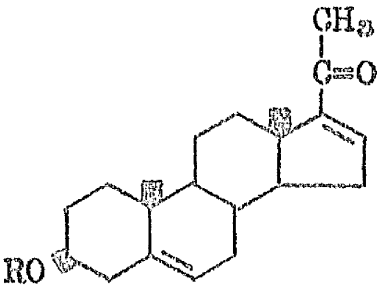



(III)

This compound was, however, shown to be the product of a base-catalysed addition of methanol to the αβ-unsaturated-20-ketosteroid, by Fukushima and Gallagher,[2] who obtained 3β-hydroxy-16α-methoxypregn-5-en-20-one (V), by refluxing 3β-acetoxypregna-5,16-dien-20-one (IV, R = Ac) with

methanolic potassium hydroxide.

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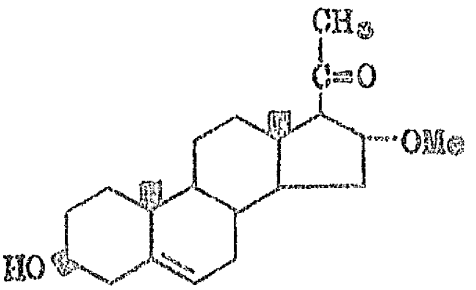
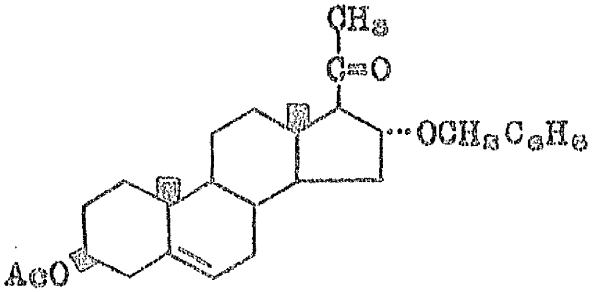



(V)

The 16-methoxy group was assumed to have the α-configuration as the reagent probably attacks the less hindered α-face of the molecule and this was confirmed by rotation data.[2]

The reaction was then seen as a convenient method of synthesising 16α-hydroxysteroids. Hirschmann et al[3] prepared 3β-acetoxy-16α-benzyloxypregn-5-en-20-one (VI) by reaction of 3β-hydroxypregna-5,16-dien-20-one (IV, R = H) and benzyl alcohol, then by successive reductions with lithium aluminium hydride and with sodium and alcohol obtained a mixture of 20-epimeric 3β,16α,20-trihydroxy-steroids (VII).

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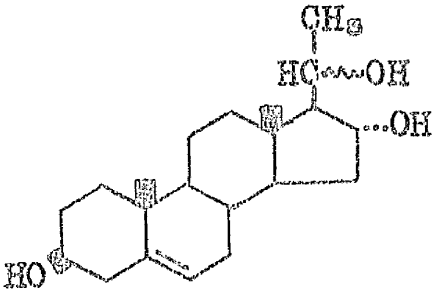
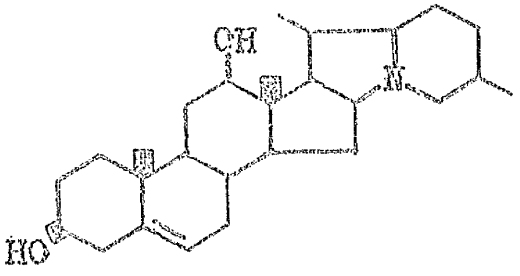



(VII)

It was also found that 3β-hydroxy-16α-methoxypregn-5-en-20-one (V) could be obtained by treating pregnadienolone (IV, R = H) in methanol, with acetyl chloride.[3]

Amines as well as alkoxides will take part in this reaction and, in synthetic approaches to the structure of steroidal alkaloids such as rubijervine (VIII), the Schering group prepared various 16-aminopregnenolones.[4] Thus, 3β-hydroxypregna-5,16-dien-20-one (IV, R = H) was treated with excess piperidine and aqueous potassium hydroxide to give 3β-hydroxy-16α-piperidinopregn-5-en-20-one (IX) and similarly with cyclohexylamine to give 3β-hydroxy-16α-cyclohexylaminopregn-5-en-20-one (X).

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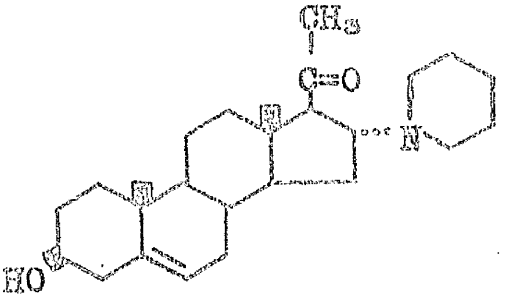


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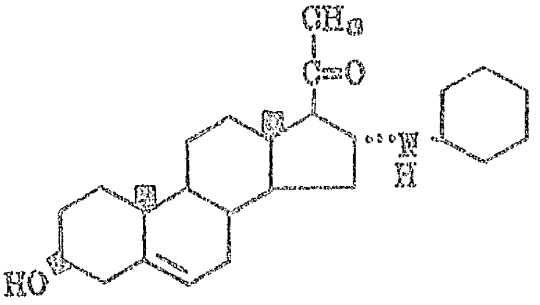
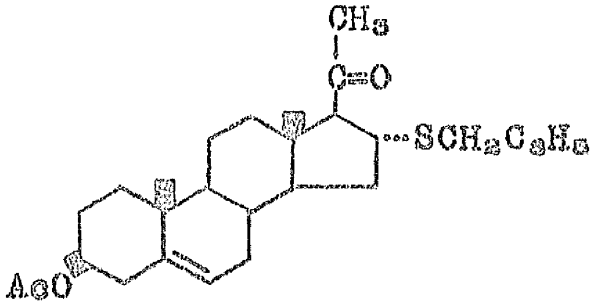



(X)

In a similar fashion, $\Delta^{16}$-20-ketosteroids will react with thiols to give 16-alkylthio derivatives. For instance, 3β-acetoxypregna-5,16-dien-20-one (IV, R = Ac) when refluxed with benzyl mercaptan and piperidine in benzene gives 3β-acetoxy-16α-benzylthiopregn-5-en-20-one (XI).[6]

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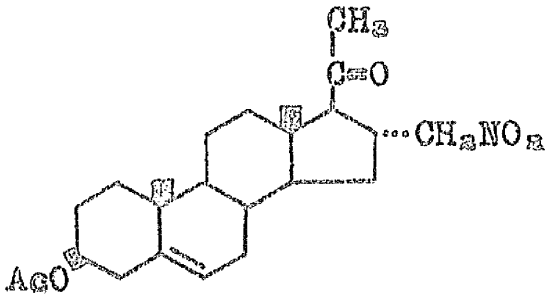
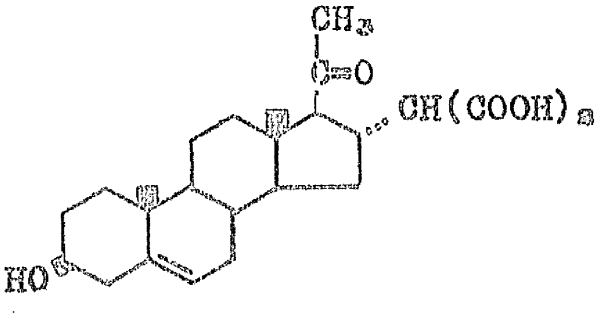



(XII)

Reaction will also take place with nitroparaffins under appropriate conditions,[7] so that in this case, 3β-acetoxypregna-5,16-dien-20-one (IV, R = Ac) dissolved in nitromethane and piperidine and kept at room temperature for five days gives 3β-acetoxy-16α-nitromethylpregn-5-en-20-one (XII).

The possibility that derivatives of progesterone containing carboxyl or methoxycarbonyl functions might have useful biological properties prompted Bladon[8] to investigate an extension of this type of reaction. He found that Michael reaction of 3β-acetoxypregna-5,16-dien-20-one (IV, R = Ac) and sodiomalonic ester gave, after brief

hydrolysis with aqueous alkali and acid treatment, the expected 3β-hydroxy-20-oxopregn-5-en-16α-ylmalonic acid (XIII). Prolonged hydrolysis, however, resulted in poor yields of the acid, due to (a) the splitting of the acid into hydrolysed starting materials and (b) further changes to an amorphous product, which was formulated as (XIV).

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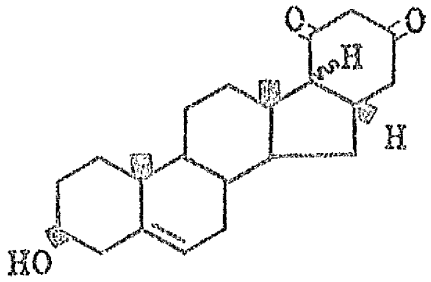
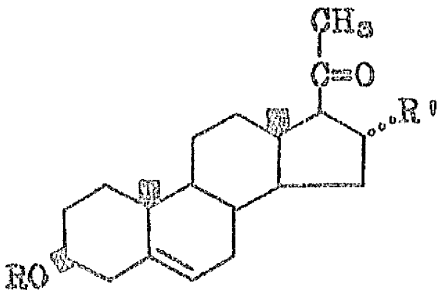



(XIV)

It has also been reported[9] that on heating a methanolic solution of the ketone (IV, R = H) under reflux with sodium cyanide, there was smooth addition of hydrocyanic acid to give a good yield of the 16α-cyano derivative (XV, R = H; R' = CN) which on vigorous alkaline hydrolysis gave 3β-hydroxy-16α-carboxypregn-5-en-20-one (XVI).

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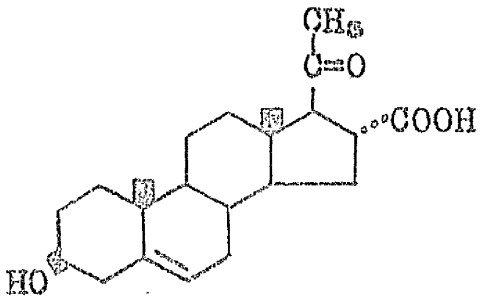



(XVI)

Mazur and Cella[10] later suggested, on the basis of molecular rotation measurements, that inversion of configuration takes place under the strong alkaline hydrolysis conditions and that the acid obtained is 3β-hydroxy-16β-carboxy-17α-pregn-5-en-20-one. This theory has recently received support from Crabbé and Romo,[11] who claim to have prepared other 16β-substituted 17α-progester ones, but the position is still not clear.

Mazur and Cella also carried out Michael reactions between 3β-acetoxypregna-5,16-dien-20-one (IV, R = Ac) and ethyl cyanoacetate, malononitrile, ethyl malonate, ethyl acetoacetate and acetylacetone.[10] Using ethanol as solvent and sodium ethoxide as base, the adducts XV, R = H; R' = $CH(COOC_2H_5)CHCN$ and XV, R = H; R' = $CH(CN)_2$ were obtained with ethyl cyanoacetate and malononitrile respectively. The second method used excess addend as solvent and preformed sodium enolate of the addend as base. The latter procedure yielded addends XV, R = Ac; R' = $CH(COOC_2H_5)_2$, XV, R = Ac; R' = $CH(COOC_2H_5)CHCOCH_3$ and XV, R = Ac; R' = $CH(COCH_3)_2$ with ethyl malonate, ethyl acetoacetate, and acetyl acetone respectively.

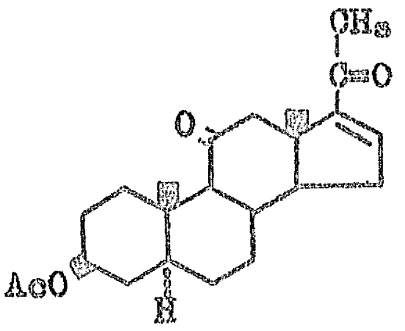
## The Michael reaction of 3β-Acetoxy-5α-pregn-16-ene-11,20-dione and malonic ester derivatives.

As an extension to the work carried out by Bladon[8] on the Michael reaction of 3β-acetoxypregna-5,16-dien-20-one (IV, R = Ac) and sodiomalonic ester, the analogous reaction between 3β-acetoxy-5α-pregn-16-ene-11,20-dione (XVII) and malonic ester derivatives has been investigated.

Using approximately the same experimental conditions as employed by Bladon in the preparation of the 16α-dicarboxymethyl derivative of 3β-acetoxypregna-5,16-dien-20-one (IV, R = Ac), it was found that the course of the Michael reaction between 3β-acetoxy-5α-pregn-16-ene-11,20-dione (XVII) and sodiomalonic ester was dependent to some extent on the relative concentrations of sodium and ethyl malonate present in the reaction mixture. Reaction between the two in the presence of excess of ethyl malonate gave, after brief alkaline hydrolysis and acidification, the desired 3β-hydroxy-11,20-dioxo-5α-pregnan-16α-ylmalonic acid (XVIII, R = R' = H). If, however, the reaction was carried out with approximately equal molecular quantities of sodium and diethyl malonate, then the product after hydrolysis and acidification was a poorly crystalline, colourless solid of indefinite melting point. This substance has been formulated as (XIX), because the ultraviolet spectra

in neutral and alkaline solution resemble the corresponding spectra of 5,5-dimethylcyclohexane-1,3-dione (dimedone). Further, as in the case of the corresponding compound (XIV) obtained from 3β-acetoxypregna-5,16-dien-20-one[8], the infrared spectrum had a strong band at 1590 cm.$^{-1}$, characteristic of the enol form of a β-diketone. The ill-defined melting point and poor recovery on crystallisation are probably due to the presence of ($C_{1,}$) isomeric substances and two enol forms. If the hydrolysis step was omitted, the product was the ethyl ester (XVIII, R = H; R' = Et), which was, however, not obtained crystalline.

The methyl ester (XVIII, R = H; R' = Me) was obtained by esterification of the acid (XVIII, R = R' = H) with diazomethane and chromatography of the product on deactivated alumina. If the Michael reaction was carried out using the sodio derivative of dimethyl malonate, thus avoiding the hydrolysis and methylation steps, the product was pure methyl ester (XVIII, R = H; R' = Me). Oxidation of the methyl ester with excess of chromic acid-sulphuric acid in acetone[12] gave dimethyl 3,11,20-trioxo-5α-pregnan-16α-ylmalonate (XX).



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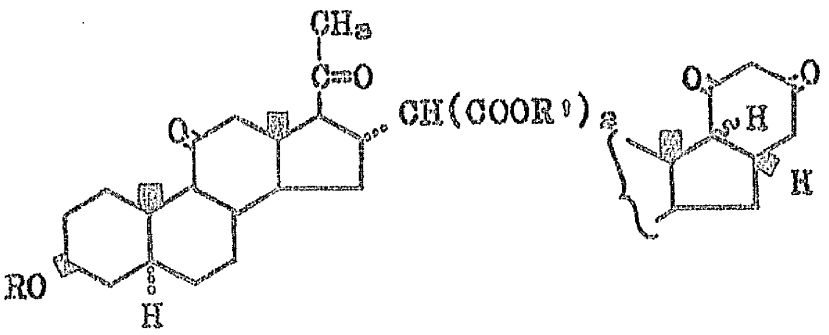

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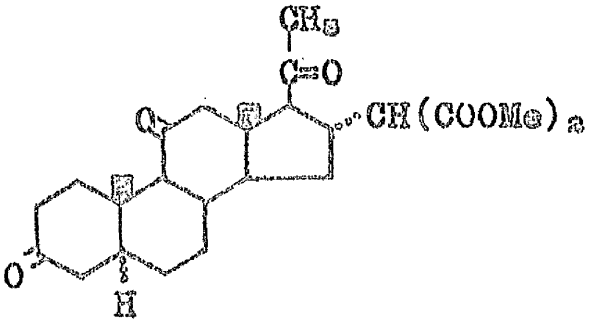

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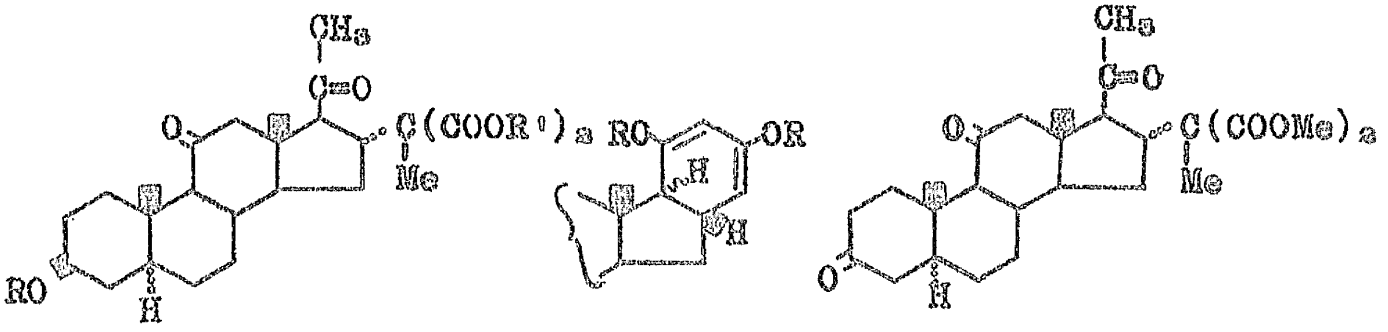


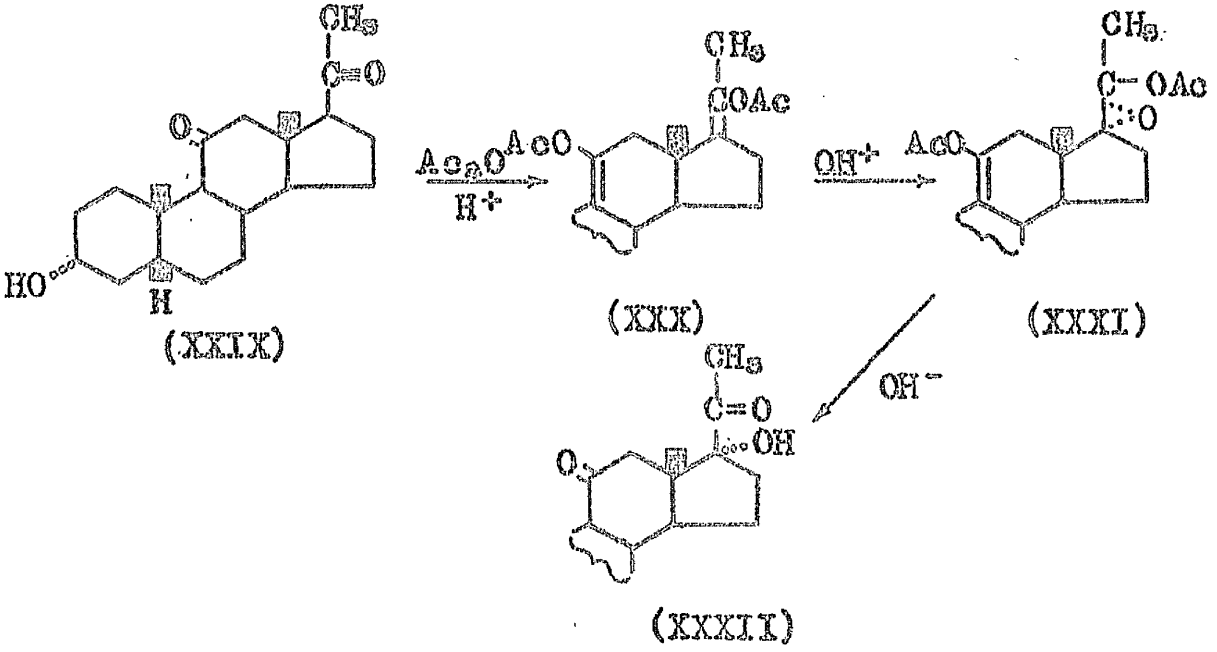
(XXI) (XXII) (XXIII)

The reaction between 3β-acetoxy-5α-pregn-16-ene-11,20-dione (XVII) and the sodio derivative of diethyl methylmalonate gave an impure acidic product after hydrolysis, even when an excess of the malonic ester was present. The ultraviolet spectra in neutral and alkaline solution showed

that this was probably a mixture of the desired acid (XXI, R = R' = H) and a cyclonorcholantrione (XXII, R = H). Methylation of this material, followed by chromatography of the product gave only a small quantity of a crystalline compound, melting over a wide range, which has been assigned the structure (XXII, R = Me). The ultraviolet spectrum in neutral solution again resembles that of dimedone, but in this case there is very little bathochromic shift on the addition of alkali. Cyclisation to the cyclonorcholantrione presumably takes place during the hydrolysis of the intermediate ester (XXI, R = H; R' = Et) since, if the hydrolysis step was omitted, the product was the oily ethyl ester (XXI, R = H; R' = Et). Furthermore, carrying out the reaction with the sodio derivative of dimethyl methylmalonate and chromatography of the product gave crystalline dimethyl (3β-hydroxy-11,20-dioxo-5α-pregnan-16α-yl)-methylmalonate (XXI, R = H; R' = Me). Oxidation of this with chromic acid-sulphuric acid in acetone gave dimethyl (3,11,20-trioxo-5α-pregnan-16α-yl)-methylmalonate (XXIII).

With the idea in mind that these compounds might eventually be converted into true cortisone analogues, it was decided to try to introduce a 17α-hydroxyl group into dimethyl 3β-hydroxy-11,20-dioxo-5α-pregnan-16α-ylmalonate

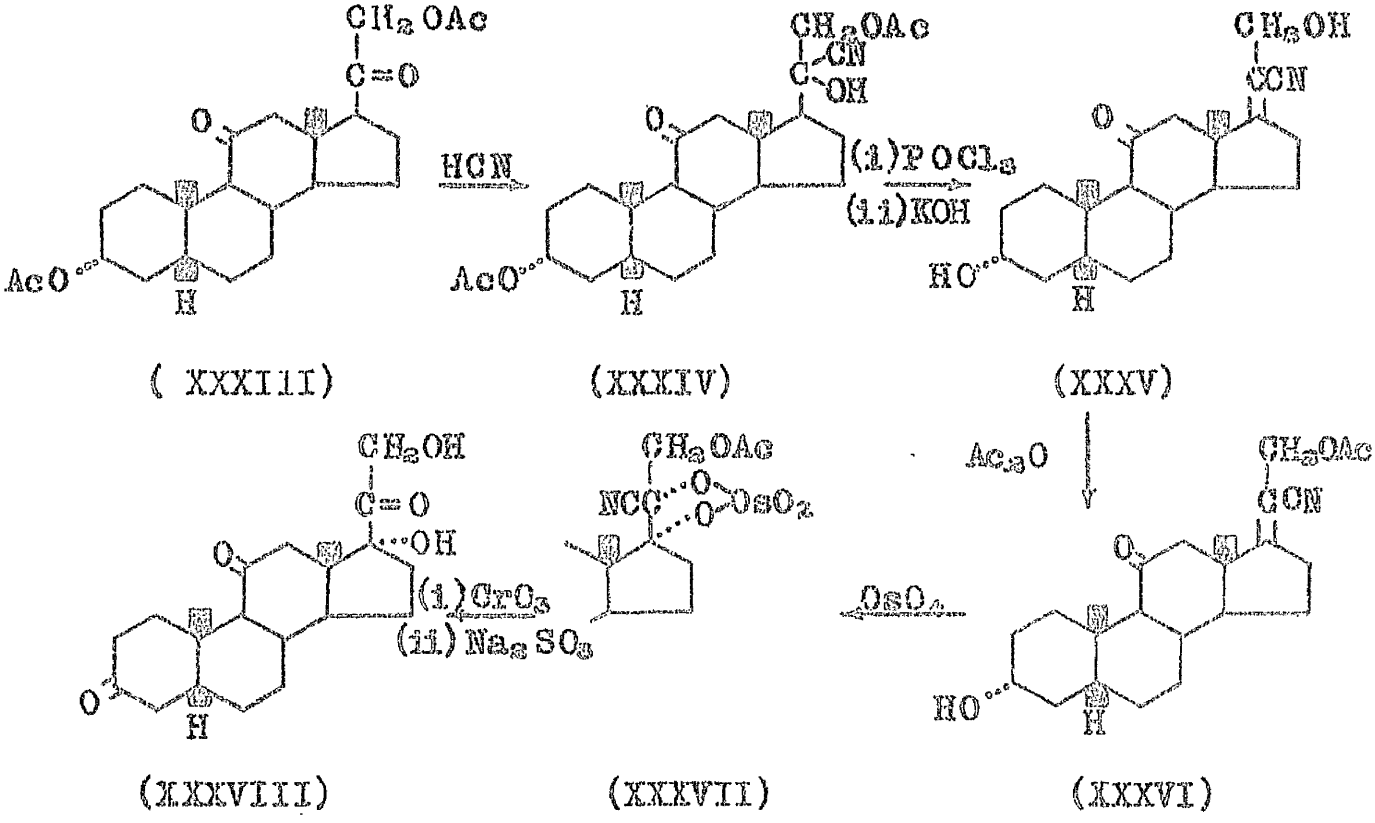
(XVIII, R = H; R' = Me). Among the more common methods of converting a 20-ketone to the 17α-hydroxy-20-ketone are the enol acetate method[13,14] and Sarett's method.[15]




(XXIX) (XXX) (XXXI) (XXXII)

The enol acetate method was developed by Kritchevsky, Gallagher and co-workers and enabled them to synthesize various 17α-hydroxycorticoids,[13] including cortisone.[14] Part of the cortisone synthesis involved reaction of 3α-hydroxypregnane-11,20-dione (XXIX) with acetic anhydride in the presence of toluene-p-sulphonic acid to form the dienol triacetate (XXX), which on oxidation with perbenzoic acid gave the 17,20-oxide (XXXI). Mild alkaline hydrolysis removed all three acetyl groups and opened the oxide ring to give 3α,17α-dihydroxypregnane-11,20-dione (XXXII).

Sarett, in a synthesis of cortisone[19] developed a general method for the introduction of a 17α-hydroxyl group. Treating one of his intermediates, 3α,21-diacetoxypregnane-11,20-dione (XXXIII) with hydrogen cyanide, he obtained the cyanhydrin (XXXIV), which was dehydrated with phosphorus oxychloride to the $\Delta^{17}$-unsaturated nitrile. The product was saponified (XXXV), and selective acetylation gave the 21-acetate (XXXVI). Treatment with osmium tetroxide gave the osmate ester (XXXVII), which was suitable for oxidation. Cleavage of the osmate ester grouping of the oxidation product and hydrolysis gave the 3-keto-17α-hydroxy compound (XXXVIII).




(XXXIII) (XXXIV) (XXXV)

(XXXVIII) (XXXVII) (XXXVI)

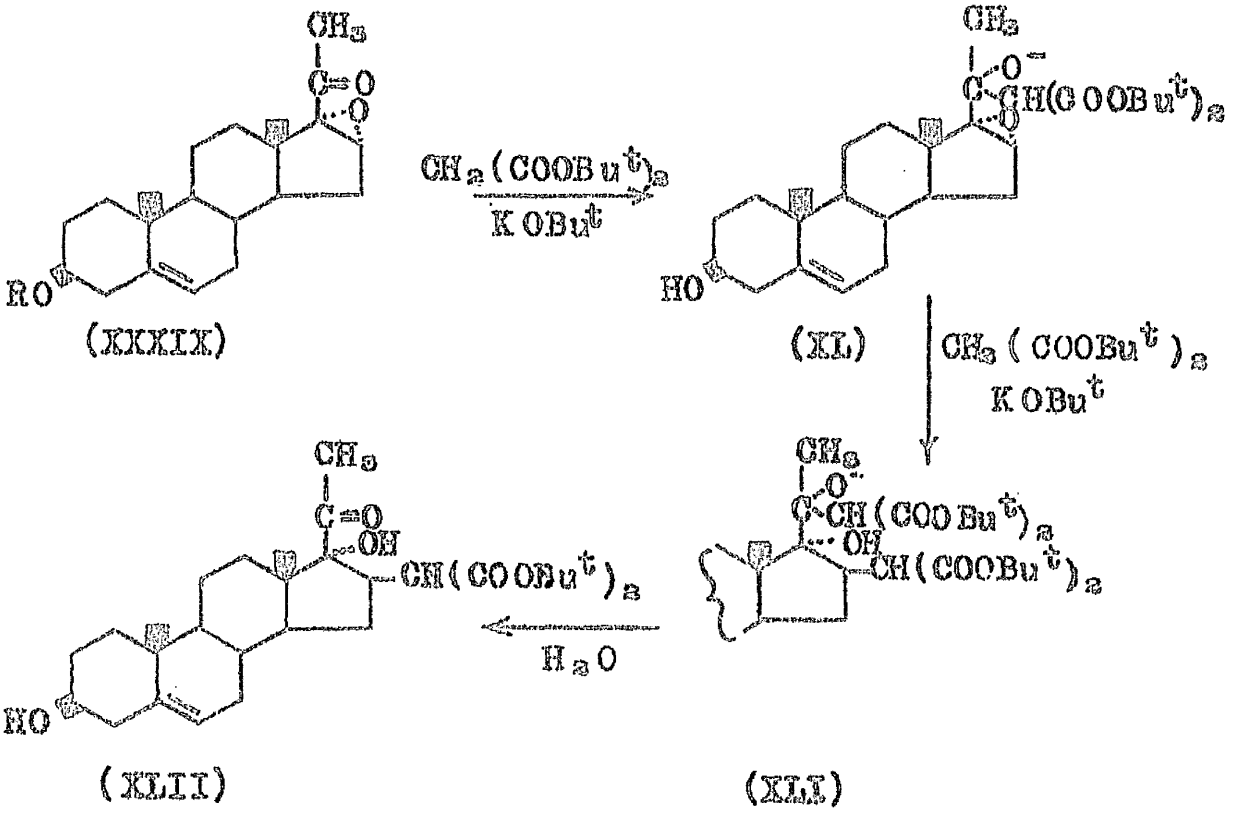
On applying the enol acetate method to dimethyl 3β-hydroxy-11,20-dioxo-5α-pregnan-16α-ylmalonate (XVIII, R = H; R' = Me), the initial stages of enol acetylation and epoxidation gave oils, which were used directly. The final mild hydrolysis gave a crystalline solid, which on re-esterification with diazomethane yielded unchanged starting material.

An attempt was then made to introduce the 17-OH group by means of Sarett's method. Again, the intermediate stages were obtained as oils and the final product itself as an uncrystallisable, black gum.

The reason for the failure of these methods is probably the steric hindrance caused by the presence of the large 16α-dicarbomethoxymethyl group.

A different approach to the problem was then tried. It was thought that the 17α-hydroxyl group and the 16-substituent might be introduced into a pregnane nucleus simultaneously, by treating a 16,17-epoxide with a potassio-malonic ester. The reaction was attempted on 3β-acetoxy-16,17-epoxypregn-5-en-20-one (XXXIX, R = Ac),[16] as this was immediately available and the potassio derivative of di-tert.-butyl malonate was chosen, as potassium tert.-butoxide is probably the alkoxide most likely to be sufficiently

basic to split the 16,17-epoxide linkage.



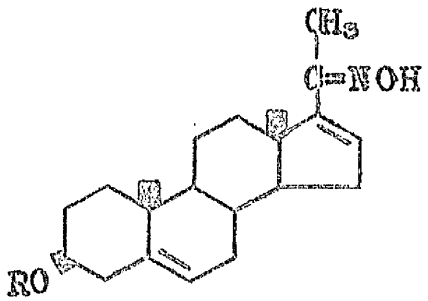

It was envisaged that the reaction might proceed by the route indicated, the 20-ketone reacting first to give the stable anion (XL), which would react further with di-tert.-butyl potassiomalonate with cleavage of the epoxide (XLI). On addition of water the anion should then revert to the 20-ketone, giving di-tert.-butyl 3β,17α-dihydroxy-20-oxopregn-5-en-16β-ylmalonate, the different method of insertion giving a 16β-derivative in this case.

In effect, when the reaction was carried out at the temperature of refluxing tert.-butanol, only hydrolysed

starting material (XXXIX, R = H) was obtained. Carrying it out at 150° in an autoclave was no more successful, giving an impure, high melting solid, which could not be purified by recrystallisation. The infrared spectrum of this compound indicated that it was perhaps an acid, so it was treated with diazomethane and the product chromatographed on deactivated alumina. This, however, gave only an uncrystallisable gum.

The reason for the failure of this reaction may be that once the stable anion (XL) is formed, steric hindrance, due to the bulky dibutoxymethyl group at C-20, protects the 16,17-epoxide group from the attack of a further quantity of di-tert.-butyl potassiomalonate. Addition of water then simply causes the reverse reaction, giving back the 16,17-epoxy-20-ketone (XXXIX, R = H).*

Possession of a quantity of 3β-acetoxypregna-5,16-dien-20-one oxime (XLIII, R = Ac) prompted investigation of whether a $\Delta^{16}$-20-oxime would take part in a Michael reaction in the same way as an αβ-unsaturated-20-ketone.




(XLIII)

* See additional note, p.33.

After refluxing the oxime (XLIII, R = Ac) and sodio-malonic ester in ethanol for three hours, however, all that was isolated was saponified starting material (XLIII, R = H) It would seem therefore, that the 16,17-double bond is not sufficiently activated by the 20-oxime group to enable it to take part in a Michael addition of malonic ester.

# E X P E R I M E N T A L

Melting points were determined on a Kofler hot stage. Unless otherwise stated, optical rotations were determined for chloroform solutions at room temperature, ultraviolet spectra for ethanol solutions and infrared spectra for potassium chloride discs. Petroleum ether had boiling point 60-80°. Alumina (Spence Grande 'H') was deactivated by the method of Farrar, Hamlet, Henbest and Jones.[17]

3β-hydroxy-11,20-dioxo-5α-pregnan-16α-ylmalonic acid (XVIII, R = R' = H).- Sodium (200 mg., 8 m moles) was dissolved in ethanol (25 ml.) and diethyl malonate (3.2 g., 20 m moles) added, followed by 3β-acetoxy-5α-pregn-16-en-11,20-dione (744 mg., 2 m moles). The mixture was refluxed for 3 hours under anhydrous conditions; then a solution of potassium hydroxide (4 g.) in 50% v/v aqueous ethanol (20 ml.) was added and refluxing continued for 5 min. Water was added and the solution extracted with ether to give a small neutral fraction (20mg.). Acidification of the aqueous layer gave a white solid (660 mg.). Two recrystallisations of a sample (100 mg.) of the dried material from ethanol-water gave 3β-hydroxy-11, 20-dioxo-5α-pregnan-16α-ylmalonic acid (XVIII, R = R' = H) as plates, m.p. 245-255° (decomp. begins at 220°), $[\alpha]_D$ + 94.6° (c 0.24 in methanol) (Found: C,66.0; H,8.2. $C_{24}H_{34}O_7$ requires C,66.3; H,7.9%), $\nu_{max.}$ 1740 (malonic acid),

1690 (11,20-dioxo) and a broad peak in the region of 2900 (enol) cm.$^{-1}$

If the reaction mixture was extracted with ether after the initial refluxing, there was isolated the corresponding ethyl ester (XVIII, R = H; R' = Et) as an oil (838 mg.), which could not be crystallised, even after chromatography on deactivated alumina.

In another experiment on the same scale, but using only 1.6 g. (10 m moles) of diethyl malonate, 640 mg. of crude acidic product was obtained. Two recrystallisations from ethanol-water gave a small, poorly crystalline sample of 3β-hydroxy-16α,24-cyclo-21-norcholan-11,20,23-trione (XIX), m.p. 210-320° (decomp.), $[\alpha]_D$ + 76.1° (c 0.57 in pyridine) (Found: C,74.2; H,8.5. $C_{23}H_{32}O_4$ requires C,74.2; H,8.7%), $\lambda_{max.}$ 259 mμ. (ε 15,000), $\lambda_{max.}$ (in NaOH) 284 mμ. (ε 24,900), $\nu_{max.}$ 1695 (11-oxo), 1590 (enol), 1030 and a broad peak in the region of 2900 (enol) cm.$^{-1}$ Dimedone under similar conditions had $\lambda_{max.}$ 257 mμ. (ε 16,600) and $\lambda_{max.}$ (in NaOH), 282 mμ. (ε 25,700).

Dimethyl 3β-hydroxy-11,20-dioxo-5α-pregnan-16α-ylmalonate (XVIII, R = H; R' = Me)(a). -- 3β-Hydroxy-11,20-dioxo-5α-pregnan-16α-ylmalonic acid (2.66 g.) was suspended in a little methanol and treated with excess of ethereal

diazomethane for 30 mins. The excess of diazomethane was destroyed by the addition of a few drops of acetic acid. Removal of the organic solvents under reduced pressure gave a yellow oil (2.56 g.), which was chromatographed on deactivated alumina (100 g.). Benzene-ether (1:1) eluted a yellow oil, which crystallised from ether to give impure dimethyl 3β-hydroxy-11,20-dioxo-5α-pregnan-16α-ylmalonate (XVIII, R = H; R' = Me) (1.39 g.) m.p. 150-160°. Two recrystallisations from methanol-isopropyl ether gave rosettes (624 mg.), m.p. 161-164°, $[\alpha]_D$ + 90.2° (c 0.88) (Found: C,67.6; H,8.3. $C_{26}H_{38}O_7$ requires C,67.5; H,8.3%). $\nu_{max.}$ 3445, (-OH), 1749 (malonate), and 1695 (11,20-dioxo) $cm.^{-1}$

(b) Sodium (100 mg., 4 m moles) was dissolved in methanol (15 ml.) and dimethyl malonate (1.32 g., 10 m moles) added, followed by 3β-acetoxy-11,20-dioxo-5α-pregn-16-ene (372 mg. 1 m mole). The solution was refluxed for 3 hrs. under anhydrous conditions, then cooled, water added and the product extracted twice with chloroform. The chloroform extracts were washed with water, dried over sodium sulphate and evaporated to dryness to give colourless, crystalline material (409 mg.), which was recrystallised from methanol-isopropyl ether to give dimethyl 3β-hydroxy-11,20-dioxo-5α-pregnan-16α-ylmalonate as rosettes (265 mg.),

m.p. 157-162°, $[\alpha]_D$ + 87.4° (c 0.78), $\nu_{max.}$ 3520 (-OH), 1750 (malonate) and 1698 (11,20-dioxo) cm.$^{-1}$ identical with material prepared by method (a).

Acetylation of this ester with acetic anhydride and pyridine at room temperature gave dimethyl 3β-acetoxy-11,20-dioxo-5α-pregnan-16α-ylmalonate (XVIII, R = Ac; R' = Me), crystals from methanol, m.p. 133-136.5°, $[\alpha]_D$ + 70.9° (c 0.80) (Found: C,67.0; H,8.1. $C_{28}H_{40}O_6$ requires C,66.6; H,8.0%), $\nu_{max.}$ 1762 (malonate), 1740 (-OAc) and 1710 (11,20-dioxo) cm.$^{-1}$ Dimethyl 3β-benzoyloxy-11,20-dioxo-5α-pregnan-16α-ylmalonate (XVIII, R = Bz; R' = Me), prepared with benzoyl chloride and pyridine formed feathery needles, m.p. 187-189°, $[\alpha]_D$ + 71.8° (c 0.65) from methanol (Found: C,69.9; H,7.45. $C_{35}H_{42}O_8$ requires C,69.9; H,7.5%), $\nu_{max.}$ 1755 (malonate), 1740 (-OBz), 1710 (11,20-dioxo) and 709 (-OBz) cm.$^{-1}$

Dimethyl 3,11,20-trioxo-5α-pregnan-16α-ylmalonate (XX).- Dimethyl 3β-hydroxy-11,20-dioxo-5α-pregnan-16α-ylmalonate (207 mg.) in acetone (10 ml.) was stirred vigorously at room temperature and chromic acid solution added (1 ml. of a solution of 26.7 g. of chromium trioxide and 23 ml. of concentrated sulphuric acid made up to 100 ml. with water). The excess of oxidant was destroyed after 5 min. by the

addition of dilute hydrochloric acid and sodium sulphite. The product was then extracted twice with ether, the combined ether extracts washed with sodium bicarbonate solution and water, dried over sodium sulphate and evaporated to dryness to give dimethyl 3,11,20-trioxo-5α-pregnan-16α-ylmalonate (XX) as platelets or needles (180 mg.) m.p. 190-193°, $[\alpha]_D$ + 103° (c 0.78) (Found: C,68.1; H,8.0. $C_{26}H_{38}O_7$ requires C,67.8; H,7.9%), $\nu_{max.}$ 1723 (malonate and 3-oxo) and 1702 (11,20-dioxo) cm.$^{-1}$

Reaction of diethyl methylmalonate and 3β-acetoxy-5α-pregn-16-ene-11,20-dione. - Sodium (100 mg., 4 m moles) was dissolved in ethanol (15 ml.) and diethyl methylmalonate (1.74 g., 10 m moles) added, followed by 3β-acetoxy-5α-pregn-16-ene-11,20-dione (372 mg., 1 m mole). The mixture was refluxed for 4 hours, with the exclusion of moisture; then a solution of potassium hydroxide (2 g.) in 50% v/v aqueous ethanol (10 ml.) was added and refluxing continued for 5 minutes, water was added and the solution extracted with ether to give a small neutral fraction (18 mg.). Acidification of the aqueous layer gave a slightly yellow solid (318 mg.) m.p. 175-240° (decomp.), $\lambda_{max.}$ 258 mμ. (ε 8,800), $\lambda_{max.}$ (in NaOH) 288 mμ. (ε 13,800), $\nu_{max.}$ 1710 (11,20-dioxo), 1651 and 1592 (enol), and a broad peak in the

region of 2900 (enol) cm.$^{-1}$ Recrystallisation from methanol-isopropyl ether gave crystalline material (152 mg.), m.p. 182-264° (decomp.).

All the material (recrystallised and mother liquor material) was esterified with diazomethane and the product passed through a column of deactivated alumina (10 g.) to give a gum (191 mg.) which, on crystallisation from ethyl acetate gave 3β-hydroxy-20,23-dimethoxy-24-methyl-16α,24-cyclo-21-norcholan-20,23-dien-11-one (XXII, R = Me) as tiny needles (19 mg.), m.p. 265-293° (decomp.), $\lambda_{max.}$ 248 mμ. (ε 13,400), $\lambda_{max.}$ (in NaOH) 254 mμ. (ε 14,600), $\nu_{max.}$ 1720 (11-oxo), 1678 and 1605 cms.$^{-1}$ Recrystallisation from chloroform-ethyl acetate gave needles (4 mg.), m.p. 278-294° (decomp.), $[\alpha]_D$ + 53.4° (c 0.15) (Found: C,73.4; H,9.4. $C_{28}H_{38}O_4$ requires C,73.5; H,9.2%). The mother liquors yielded a second crop of crystalline material (6 mg.).

Ether extraction of the reaction mixture after the initial refluxing gave the ethyl ester (XXI, R = H; R' = Et) as an oil (218 mg.) which could not be crystallised, even after chromatography on deactivated alumina (8 g.).

Dimethyl (3β-hydroxy-11,20-dioxo-5α-pregnan-16α-yl)-methylmalonate (XXI, R = H; R'=Me). - The reaction was carried out as above using dimethyl methylmalonate (1.46 g.,

10 m moles) instead of diethyl methylmalonate. Ether extraction after the initial refluxing gave yellow crystalline material (267 mg.), which was recrystallised twice from methanol-isopropyl ether to give impure product as rosettes (82 mg.), m.p. 173-175°, recrystallising and melting 186-204°, $[\alpha]_D$ + 88.7° (c 0.76).

Impure methyl ester (500 mg.) was dissolved in benzene and chromatographed on deactivated alumina (20 g.). Elution with 4:1 benzene-ether gave colourless, crystalline material, which after two recrystallisations from methanol-isopropyl ether gave pure dimethyl (3β-hydroxy-11,20-dioxo-5α-pregnan-16α-yl)-methylmalonate (XXI, R = H; R' = Me) as prisms (110 mg.), m.p. 224-227° (decomp.), $[\alpha]_D$ + 82.3° (c 0.8) (Found: C,67.8; H,8.3. $C_{27}H_{40}O_7$ requires C,68.0; H,8.5%), $\nu_{max.}$ 3581 (-OH), 1736 (malonate) and 1700 (11,20-dioxo) $cm.^{-1}$

Acetylation of the dimethyl ester with acetic anhydride and pyridine at room temperature gave dimethyl (3β-acetoxy-11,20-dioxo-5α-pregnan-16α-yl)-methylmalonate (XXI, R = Ac; R' = Me), crystals from hexane, m.p. 180-188° (decomp.). Recrystallisation from dichloromethane-hexane gave platelets, m.p. 187-189° (decomp.), $[\alpha]_D$ + 69.0° (c 1.1) (Found: C,67.2; H,8.2. $C_{29}H_{42}O_8$

requires C,67.2; H,8.2%), $\nu_{max.}$ 1735 (malonate and -OAc), 1710 (11,20-dioxo) and 1241 (-OAc, broad) cm.$^{-1}$

Dimethyl (3β-benzoyloxy-11,20-dioxo-5α-pregnan-16α-yl)-methylmalonate (XXI, R = Bz; R' = Me) prepared with benzoyl chloride and pyridine gave, after recrystallisation from dichloromethane-methanol, fine needles, m.p. 236-238° (decomp.), $[\alpha]_D$ + 64.8° (c 0.8) (Found: C,70.3; H,7.5. $C_{34}H_{44}O_9$ requires C,70.3; H,7.6%), $\nu_{max.}$ 1725 (-OBz), 1269 (-OBz, broad) and 710 (-OBz) cm.$^{-1}$

Dimethyl (3,11,20-trioxo-5α-pregnan-16α-yl)-methylmalonate (XXIII). - Dimethyl (3β-hydroxy-11,20-dioxo-5α-pregan-16α-yl)-methylmalonate (100 mg.) was oxidised with chromic acid-sulphuric acid in acetone, exactly as described above. Working up as before gave colourless, crystalline material (85 mg.), m.p. 229-240°. Two recrystallisations from ether-methanol gave dimethyl (3,11,20-trioxo-5α-pregnan-16α-yl)-methylmalonate (XXIII) as platelets (30 mg.), m.p. 238-241° (decomp.), $[\alpha]_D$ + 100.5° (c 0.9) (Found: C,67.3; H,8.25. $C_{27}H_{38}O_7, 0.5CH_3OH$ requires C,67.3; H,8.2%), $\nu_{max.}$ 1729 (malonate and 3-oxo) and 1695 (11,20-dioxo) cm.$^{-1}$

Enol acetylation of dimethyl 3β-hydroxy-11,20-dioxo-5α-pregnan-16α-ylmalonate with acetic anhydride-perchloric acid in carbon tetrachloride. - Dimethyl 3β-hydroxy-11,20-dioxo-5α-pregnan-16α-ylmalonate (100 mg.), suspended in carbon tetrachloride (2 ml.) was added to a mixture of acetic anhydride-perchloric acid [0.5 ml. of a solution of 4 drops of perchloric acid (72%) in 5 ml. of A.R. acetic anhydride] and the solution allowed to stand for 3 hours. The mixture was then diluted with ether and washed twice with ice-cold 5% sodium hydroxide solution and water, dried over sodium sulphate and evaporated to dryness to give a yellowish gum (116 mg.) which was used directly for the next step.

Attempted epoxidation of crude enolacetate of dimethyl 3β-hydroxy-11,20-dioxo-5α-pregnan-16α-ylmalonate. - The crude enolacetate from above was dissolved in chloroform (1 ml.), 1N monoperphthalic acid solution (3 ml.) added and the mixture allowed to stand for 3 days. The solution was diluted with ether, washed twice with ice-cold 5% sodium hydroxide solution and once with water, dried over sodium sulphate and evaporated to dryness to give a gum, which was treated immediately with boiling 0.8N methanolic potassium hydroxide solution (2 ml.). The mixture was warmed until

all the gum had dissolved, diluted with water and cooled. After acidification, a white solid was slowly precipitated. This was filtered off and dried over phosphorus pentoxide to give white, crystalline material (23 mg.), m.p. 175-184° (decomp.). Re-esterification with diazomethane gave dimethyl 3β-hydroxy-11,20-dioxo-5α-pregnan-16α-ylmalonate (XVIII, R = H; R' = Me), m.p. 154-162°. This material had an infrared spectrum identical with that of the starting material.

Treatment of dimethyl 3β-hydroxy-11,20-dioxo-5α-pregnan-16α-ylmalonate with hydrogen cyanide, followed by osmium tetroxide. - A mixture of dimethyl 3β-hydroxy-11,20-dioxo-5α-pregnan-16α-ylmalonate (110 mg.) in pyridine (1 ml.), liquid hydrogen cyanide (5 ml.) and triethylamine (4 drops) was kept overnight at room temperature. Dilute hydrochloric acid was added and the product (61 mg.) isolated with ether. This material (dried in vacuo) in pyridine (1 ml.) was treated with redistilled phosphorus oxychloride (0.1 ml.) and kept overnight at 20°C. Extraction with chloroform gave material (50 mg.), which showed $\lambda_{max.}$ 210 and 256 mμ. ( ε 58,900 and 3,500). This was dissolved in pyridine (2 ml.), treated with osmium tetroxide (48 mg.) and kept overnight at 20°C. Mannitol (500 mg.) and potassium hydroxide (100 mg.) were added and

the mixture warmed at 40° for 10 mins. After addition of dilute hydrochloric acid, the solution was extracted with chloroform (x 6), but no product was obtained. Ether extraction gave a blackish gum (112 mg.).

Malonyl chloride. - Malonic acid (52 g.) and phosphorus pentachloride (200 g.) were shaken together, with exclusion of moisture. When brisk evolution of hydrogen chloride ceased, the mixture was warmed at 55° for 30 minutes. The phosphorus oxychloride formed during the reaction was removed under reduced pressure and the residue distilled to give malonyl chloride (25 g.), b.p. 55° (water pump). The product was slightly brown and fluorescent.

Di-tert-butyl malonate. - A 1-litre three-necked flask was fitted with a thermometer, a mechanical stirrer, a reflux condenser protected by a calcium chloride tube and a dropping funnel, also protected by a calcium chloride tube. A mixture of tert-butyl alcohol (100 ml., about 1 mole), dried by distillation from sodium, and dimethylaniline (80 ml., 0.63 moles) was placed in the flask, and a solution of malonyl chloride (28.0 g., 0.2 moles) in dry alcohol-free chloroform (about 60 ml.)was added slowly from the dropping-funnel with stirring, while the reaction flask was cooled in an ice-bath. The reaction was strongly exothermic, so

the rate of dropping was regulated so that the temperature of the mixture did not exceed 30°. After the addition was complete (about 30 minutes), the reddish-brown mixture was heated under reflux for 4 hours. The mixture was then cooled, ice-cold 6N sulphuric acid (150 ml.) was added with stirring and the product extracted with three 250 ml. portions of ether. The combined ether extracts were washed once with 6N sulphuric acid, twice with water, twice with 10% potassium carbonate solution and once with saturated sodium chloride solution, and were finally dried over anhydrous sodium sulphate to which a small amount of potassium carbonate was added. The ether was removed by distillation at reduced pressure and the residue, to which a pinch of magnesium oxide was added, was distilled at reduced pressure. The yield of colourless di-tert.-butyl malonate, distilling at 96-98°/ 18 mm., was 18.0 g. (42%), $n_D^{23}$ 1.4190.

Treatment of 3β-acetoxy-16,17-epoxypregn-5-en-20-one with di-tert-butyl malonate at 80°. - Potassium (160 mg., 4 m moles) was dissolved in dry tert-butanol (15 ml.) and di-tert.-butyl malonate (2.16 g., 10 m moles) added, followed by 3β-acetoxy-16,17-epoxypregn-5-en-20-one (372 mg., 1 m mole). The mixture, which contained precipitated potassium tert.-butoxide, was refluxed for 12 hours under

anhydrous conditions, then the solution was cooled, water was added and the product extracted with ether. A reddish oil (990 mg.) was thus obtained, the weight showing that there was excess di-tert.-butyl malonate (approx. 450 mg.) present. This malonate proved very difficult to get rid of, so the product was hydrolysed by refluxing for 1 hour with potassium hydroxide (2 g.) in 50% v/v aqueous methanol (10 ml.). After about 5 minutes reflux, a yellow solid came down. At the end of the hour, the mixture was cooled, water was added and the mixture was extracted twice with chloroform. The combined chloroform extracts were dried over sodium sulphate and evaporated to dryness to give a yellow, crystalline solid (318 mg.), m.p. 185-194°. Recrystallisation from methanol gave 3β-hydroxy-16,17-epoxypregn-5-en-20-one (XXXIX, R = H) as slightly yellow plates (15 mg.), m.p. 190-194°, $[\alpha]_D$ - 7.1° (c 0.6), $\lambda_{max.}$ 208 mμ. ( ε 4,700), $\nu_{max.}$ 1705 (20-oxo), 1440, 1380 and 1055 cm.$^{-1}$ A second crop (95 mg.), m.p. 192-194.5° was obtained from the mother liquor.

Treatment of 3β-acetoxy-16,17-epoxypregn-5-en-20-one with di-tert-butyl malonate at 150°. - The reaction was done on exactly the same scale as above, but instead of carrying out the reaction at the temperature of refluxing tert.-butanol, the mixture was heated in an autoclave at 150° for

24 hours. After cooling the reaction mixture, water was added and the mixture extracted with chloroform to give a yellow-red gum (80 mg.). Acidification of the aqueous layer with concentrated hydrochloric acid gave a light brown solid (240 mg.), which melted only slightly up to 350°, $\lambda_{max.}$ 210, 230 and 294 mμ. ($E^{1\%}_{1\ cm.}$ 366.0, 340.0 and 205.8), $\nu_{max.}$ 3420, 2950 and 1630 (very broad) $cm.^{-1}$ Attempted recrystallisation was completely unsuccessful, so the compound was treated with diazomethane to give a dirty, brown froth (200 mg.), which was chromatographed on deactivated alumina (8 g.). Elution with 1:1 chloroform-benzene gave a gum which could not be crystallised.

Attempted Michael reaction of diethyl malonate and 3β-acetoxypregna-5,16-dien-20-one oxime. - Sodium (200 mg., 8 m moles) was dissolved in ethanol (25 ml.) and diethyl malonate (3.2 g., 20 m moles) added, followed by 3β-acetoxy-pregna-5,16-dien-20-one oxime (658 mg., 2 m moles). The mixture was refluxed for 3 hours with exclusion of moisture, then a solution of potassium hydroxide (5 g.) in 50% v/v aqueous ethanol (20 ml.) was added and the refluxing continued for 5 minutes. After cooling, water was added and the solution extracted with ether twice to give 3β-hydroxypregna-5,16-dien-20-one oxime (XLIII, R = H) as a white, crystalline, neutral fraction (534 mg.),

m.p. 200-225° (decomp.), $\lambda_{max.}$ 204 and 237 mμ. ( $\varepsilon$ 6,900 and 15,200), $\nu_{max.}$ 1444, 1370, 1245, 1050, 1038 and a broad peak in the region of 3000 cm.$^{-1}$

## References.


1. Marker, J. Amer. Chem. Soc., 1949, 71, 4149.
2. Fukushima and Gallagher, J. Amer. Chem. Soc., 1950, 72, 2306; ibid, 1951, 73, 196.
3. Hirschmann, Hirschmann and Daus, J. Amer. Chem. Soc., 1952, 74, 539.
4. Gould, Gruen and Hershberg, J. Amer. Chem. Soc., 1953, 75, 2510.
5. Gould, Shapiro and Hershberg, J. Amer. Chem. Soc., 1954, 76, 5567.
6. Rosenkranz, Djerassi and Romo, U.S.P. 2,697,108 (Chem. Abs., 1955, 49, 14043).
7. Dodson, U.S.P. 2,697,109 (Chem. Abs., 1955, 49, 14042).
8. Bladon, J., 1958, 3723.
9. Romo, Tetrahedron, 1958, 3, 37; Ellis, Petrow and Wedlake, J., 1958, 3748.
10. Mazur and Cella, Tetrahedron, 1959, 7, 130.
11. Crabbé and Romo, Chem. and Ind., 1962, 408.
12. Bladon, Fabian, Henbest, Koch and Wood, J., 1951, 2402.
13. Kritchevsky and Gallagher, J. Amer. Chem. Soc., 1951, 73, 184; Koechlin, Kritchevsky and Gallagher, ibid, 1951, 73, 189.

14. Kritchevsky, Garmaise and Gallagher, J. Amer. Chem. Soc., 1952, 74, 483.

15. Sarett, J. Amer. Chem. Soc., 1948, 70, 1454; ibid, 1949, 71, 2443.

16. Julian, Meyer, Carpel and Waller, J. Amer. Chem. Soc., 1950, 72, 5145.

17. Farrer, Hamlet, Henbest and Jones, J., 1952, 2657.

* Additional note for p.15.

Recent work (Sciaky, Gazzetta, 1961, 91, 562; Suvurev, Sokolova and Makarov, Izvest. Akad. Nauk S.S.S.R., Otdel. Khim. Nauk, 1961, 934) has shown that 16β-alkyl-17α-hydroxy-20-oxo steroids result from alkyl-metal compounds and 16α,17α-epoxy-20-ketones, in which the 20-ketone group has been protected by ethylenedioxy-ketal formation.

## Part 2.

## Photo-oxygenation of steroidal enol acetates.

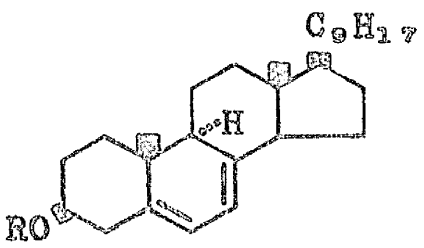
# INTRODUCTION

The ability of steroidal, conjugated dienes to take up oxygen with the formation of epidioxides, when irradiated with visible light in the presence of eosin is well known.

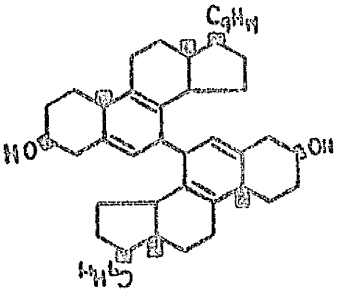
An epidioxide of this type was first prepared by Windaus and his co-workers at Göttingen in 1928. In investigating the structure of ergosterol and its ultraviolet irradiation products, their progress was constantly impeded by the difficulty of obtaining well-defined, crystalline intermediates. Windaus and his team therefore resorted to the circuitous method of preparing other derivatives of ergosterol, which were easily obtained crystalline.[1]

They found that in attemtping to use visible light in the presence of photosensitising dyes to bring about an isomerisation similar to that caused by ultraviolet light, the nature of the product obtained was dependent on whether or not oxygen was passed into the solution. In the presence of eosin alone, in alcoholic solution, ergosterol (I, R = H) was found to be converted by visible light into an extremely insoluble dehydrogenation product,[2] the so-called ergopinacol, the structure of which was at once recognised as being comprised of two steroid nuclei and is now known to be (II).[3,4] When oxygen was also present, the other

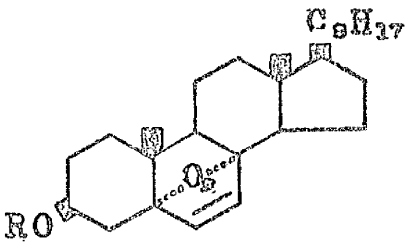
conditions being the same, Windaus and Brunken in 1928[5] obtained the peroxide addition compound, which was later shown to be (III, R = H).



(I)



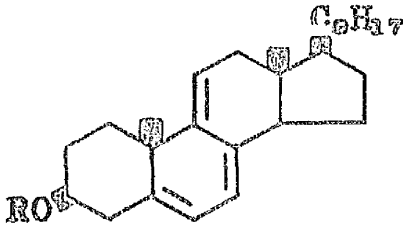
(II)



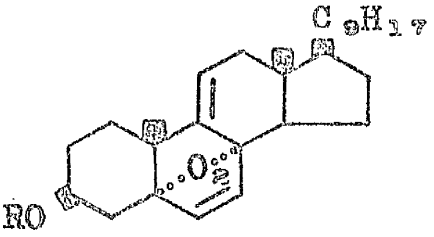
(III)

Ergosterol peroxide (III, R = H) is sensitive to acids but it differs from known 1,2-peroxides[6] in being stable to alkaline reagents. It has been isolated from the mycelium of the mould Aspergillus fumigatus.[7]

Dehydroergosterol (IV, R = H) was found to form an analagous epidioxide by Windaus and Linsert.[1] The structure of the epidioxide was then unknown, but after the formula of ergosterol had been established, Müller[8] proposed that the epidioxide has the, now accepted, structure V, R = H).



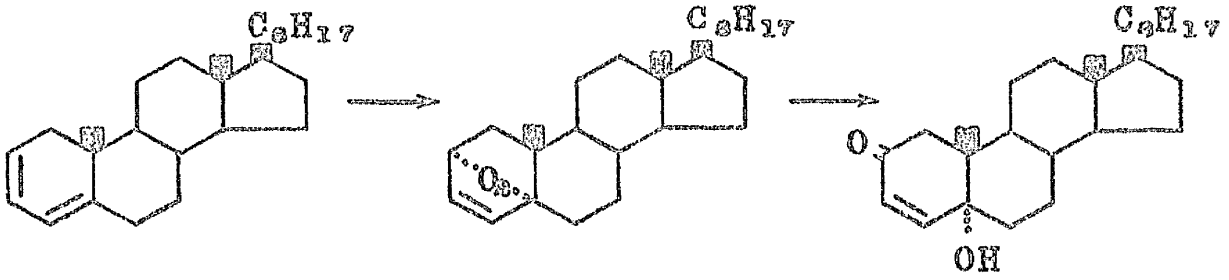
(IV)



(V)

Little work was then published on these epidioxides until the end of 1950, when a team of workers at Manchester embarked upon an intensive programme of investigation into the possibility of synthesising cortisone from dehydro-ergosterolperoxide, but most of this work was on hydrogenation.[9,10]

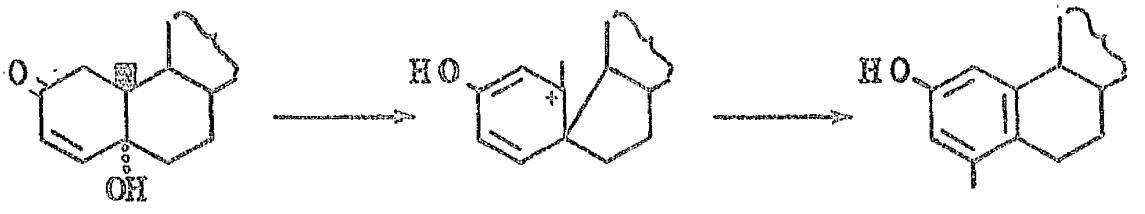
Skau and Bergmann[11] oxygenated an alcoholic solution of cholesta-2,4-diene (VI), while illuminating it with the light from a 200 watt bulb and obtained the epidioxide (VII), which on heating, isomerised to an αβ-unsaturated ketone. This was later shown to be 5-hydroxycholest-3-en-2-one (VIII),




(VI) (VII) (VIII)

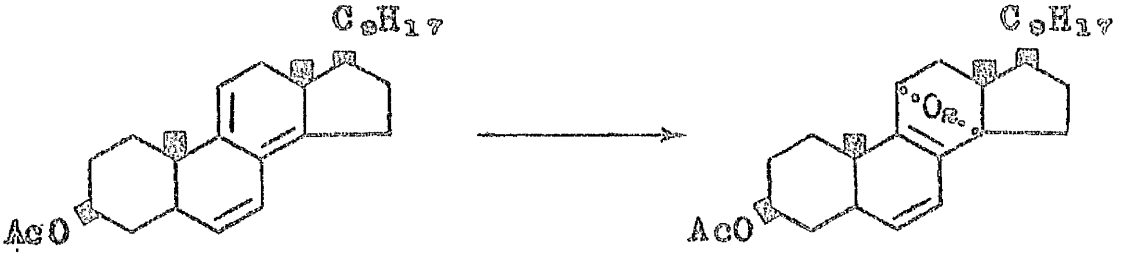
by Conca and Bergmann,[12] who also reported the acid-catalysed rearrangement of this compound to a phenol, but did not suggest a structure for their product. Davis and Halsall[13] have recently shown however, that the phenol has structure (X). They postulate that the first step in the

rearrangement is the formation of a carbonium ion at $C_9$. If then $C_9$, the most highly substituted carbon attached to $C_{10}$ migrates to form a spiran (IX) and if this spiran subsequently collapses by a second migration of the more highly substituted group, the phenol will have structure (X).



(VIII) (IX) (X)

Ring C epidioxides have been prepared by Laubach, Schreiber, Agnello and Brunings,[14] who obtained disappointing results using the method described earlier. Carrying out the reaction in 1:1 benzene-alcohol at 0-10°, with intense lighting however, they found that the reaction was complete in 30 minutes with a minimum of by-products. In this way, 3β-acetoxyergosta-6,8(14),9(11),22-tetraene (XI) was converted to 3β-acetoxy-11α,14α-epidioxyergosta-6,8(9),22-triene (XII).



(XI) (XII)

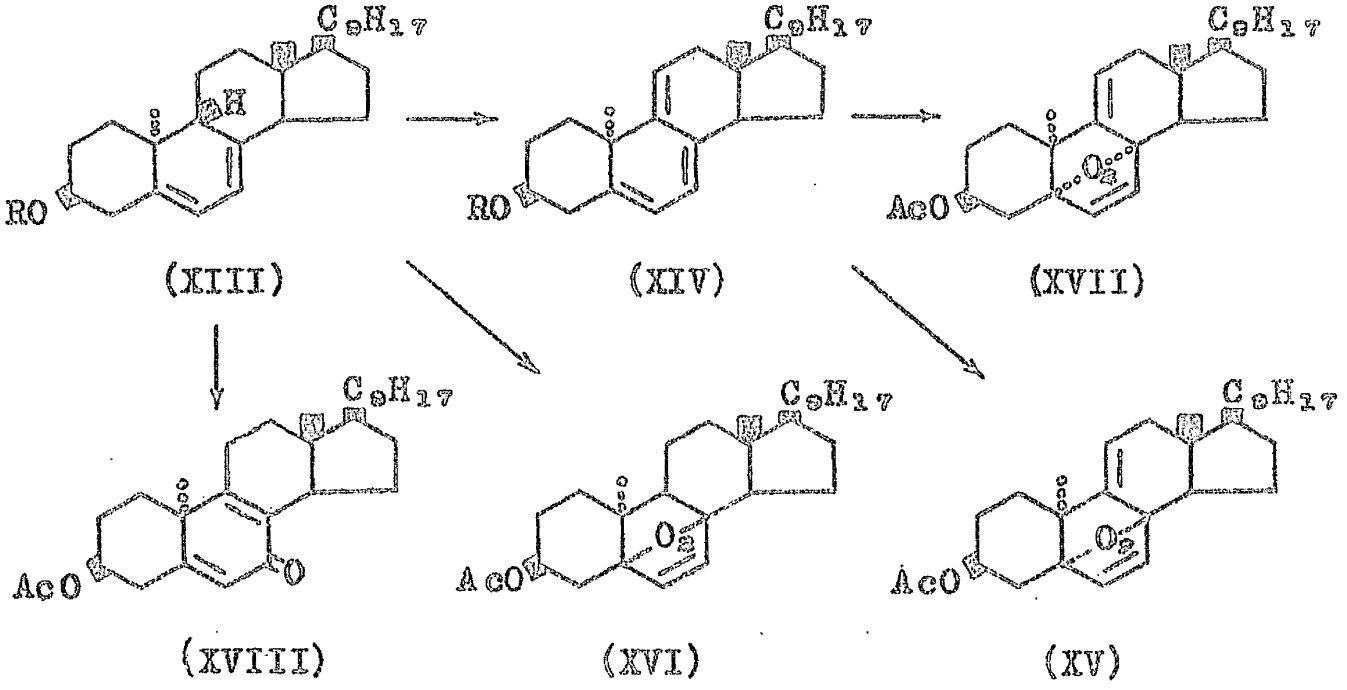
Epidioxides corresponding to those formed from ergosterol and dehydroergosterol have been prepared from lumisterol.[15] The method employed by Bladon, Clayton et al[9] and Clayton, Henbest and Jones[10] for the preparation of ergosteryl acetate peroxide and dehydroergosteryl acetate peroxide was used with lumisteryl acetate. A small quantity of pyridine was added to neutralise any acid produced by oxidative splitting of the side-chain double bond, alkali being avoided as it might have caused hydrolysis of the 3-acetate group. In the case of ergosterol and dehydroergosterol, the photo-oxygenation was carried out at the temperature of refluxing ethanol, mainly because of the low solubility of these compounds in ethanol. Lumisterol derivatives however, are much more soluble in ethanol, so the reaction could also be carried out at room temperature and it was thus shown that high temperature is not essential.

At high temperature, lumisteryl acetate (XIII, R = Ac) gave dehydrolumisteryl acetate (XIV, R = Ac), dehydro-lumisteryl acetate β-epidioxide (XV), lumisteryl acetate β-epidioxide (XVI), impure dehydrolumisteryl acetate α-epidioxide (XVII) and 3β-acetoxylumista-5,8(9),22-trien-7-one (XVIII).

At low temperature, lumisteryl acetate gave three

products, dehydrolumisteryl acetate (XIV, R = Ac), lumisteryl acetate β-epidioxide (XVI) and 3β-acetoxylumista-5,8(9),22-trien-7-one (XVIII).

At both high and low temperature, dehydrolumisteryl acetate gave dehydrolumisteryl acetate β-epidioxide and dehydrolumisteryl acetate α-epidioxide (XVII).



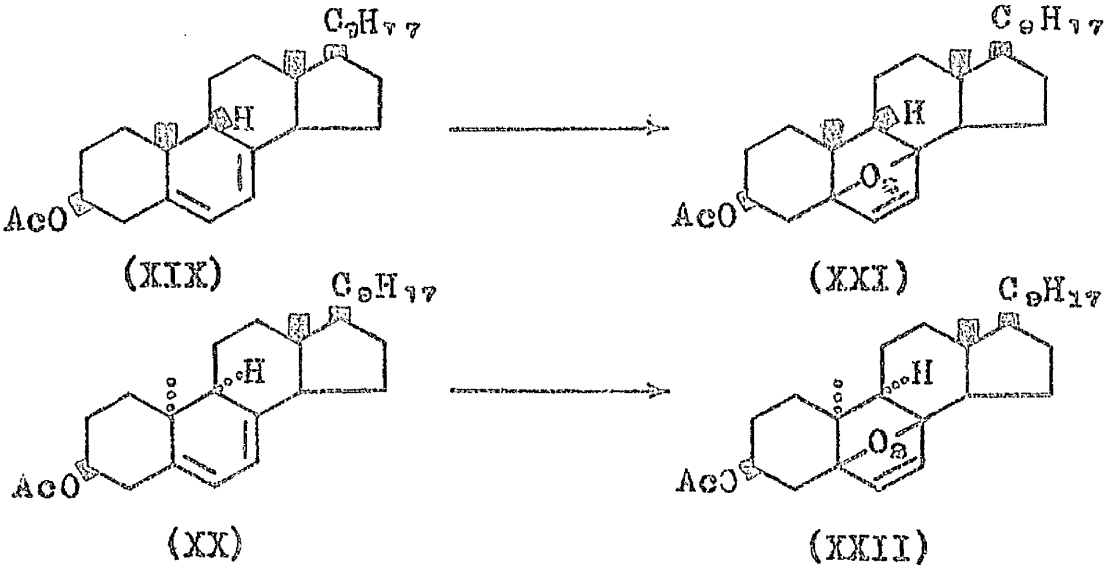

(XIII) (XIV) (XVII)

(XVIII) (XVI) (XV)

The fact that an α-epidioxide was not obtained from lumisteryl acetate, while two epidioxides were obtained from dehydrolumisteryl acetate probably means that this epidioxide is unstable. Also, since lumisteryl acetate gives the dehydrolumisteryl acetate epidioxides in the hot only, it may be assumed that lumisteryl acetate α-epidioxide

is initially formed but breaks down to dehydrolumisteryl acetate (XIV, R = Ac) and possibly the ketone (XVIII) at high temperature or in working up.

Photo-oxygenation of the other two 5,7-dienes, isomeric at $C_9$ and $C_{10}$, 9β-ergosterol[16] (isopyrocalciferol) and 9α-lumisterol[16] (pyrocalciferol) has also been carried out.[17] At the temperature of refluxing ethanol, both 9β-ergosteryl acetate (XIX) and 9α-lumisteryl acetate (XX) yield a 5,8-epidioxide (XXI and XXII) as the sole product.




(XIX) (XXI)

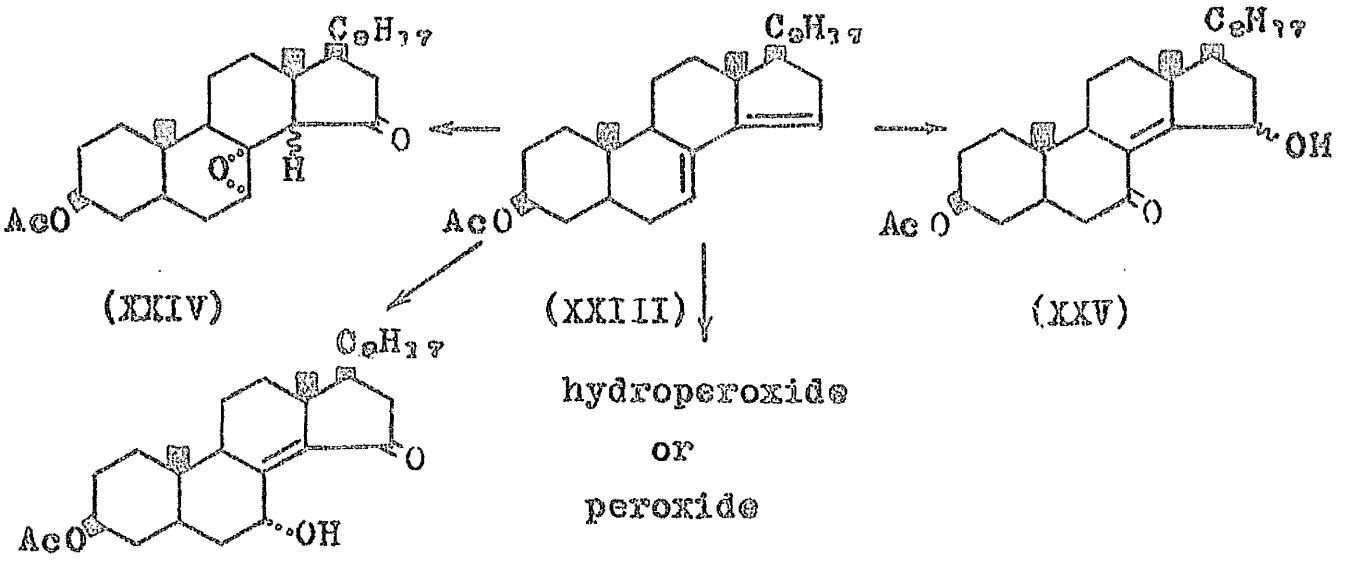
(XX) (XXII)

It is pointed out[17] that whilst the formation of 5,8-epidioxides is possible in both the 9,10-anti series (ergosterol and lumisterol) and the 9,10-syn series (9β-ergosterol and 9α-lumisterol), the reaction in the 9,10-anti series may be accompanied by dehydrogenation to a 5,7,9(11),22-tetraene,[15] while in the syn series no such

side reaction occurs.[x] Furthermore, the rate of reaction in the ergosterol series is slower than in the 9β-ergosterol series. These reactions indicate a greater reactivity of the dienic system in the syn series.

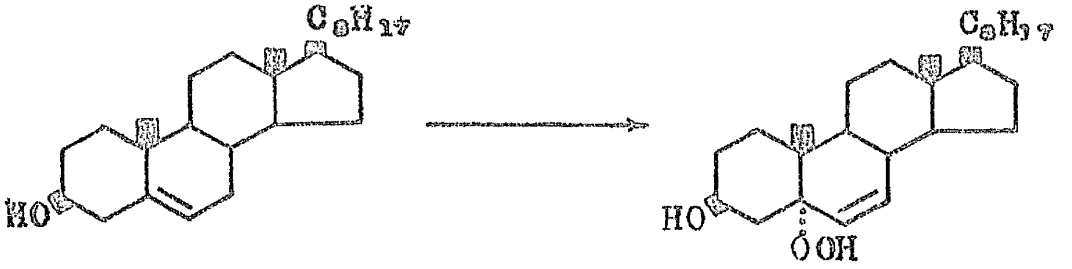
Work involving photo-oxidation of ergosterol-$B_3$ acetate, 3β-acetoxyergosta-7,14,22-triene (XXIII) has been carried out by Barton and Laws,[19] who also investigated the efficiency of various dyestuffs as sensitizers in the photo-oxygenation reaction. They found that erythrosin B, rose-bengal, eosin and phloxine were the most efficient and chose erythrosin B as their dyestuff, as it is slightly more efficient than the other three mentioned. Ergosterol $B_3$-acetate (XXIII) on photo-oxidation gave 3β-acetoxy-7α,8α-epoxy-14ξ -ergost-22-en-15-one (XXIV), 3β-acetoxy-15ξ -hydroxyergosta-8(14),22-dien-7-one (XXV), a hydroperoxide or peroxide, the structure of which was not elucidated and 3β-acetoxy-7α-hydroxyergosta-8(14),22-dien-15-one (XXVI).

[x] The present work shows that dehydroergosteryl acetate is not in fact formed as a by-product during the photo-oxygenation of ergosteryl acetate.



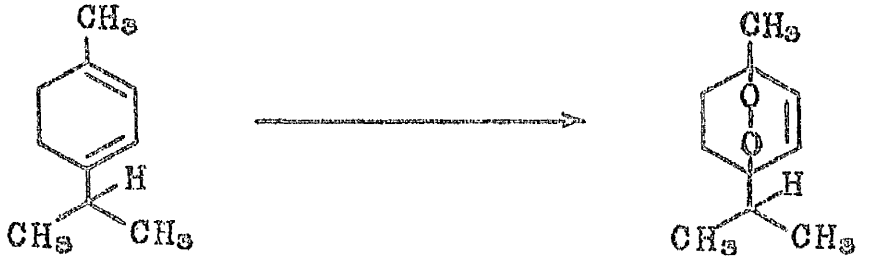

Barton and Laws pointed out that the cisoid nature of steroid heteroannular dienes is a strict necessity in the formation of peroxides, as it is in their reaction with maleic anhydride.

It can be seen from this work that photo-oxygenation can be used to produce compounds other than peroxides and another example of this is found in the cholesterol series. Schenk, Gollnick and Neumüller[19] found that cholesterol (XXVII), on irradiation in the presence of hematoporphyrin as sensitizer, combines with oxygen to give a substance regarded as the $\Delta^6$-5α-hydroperoxide (XXVIII).



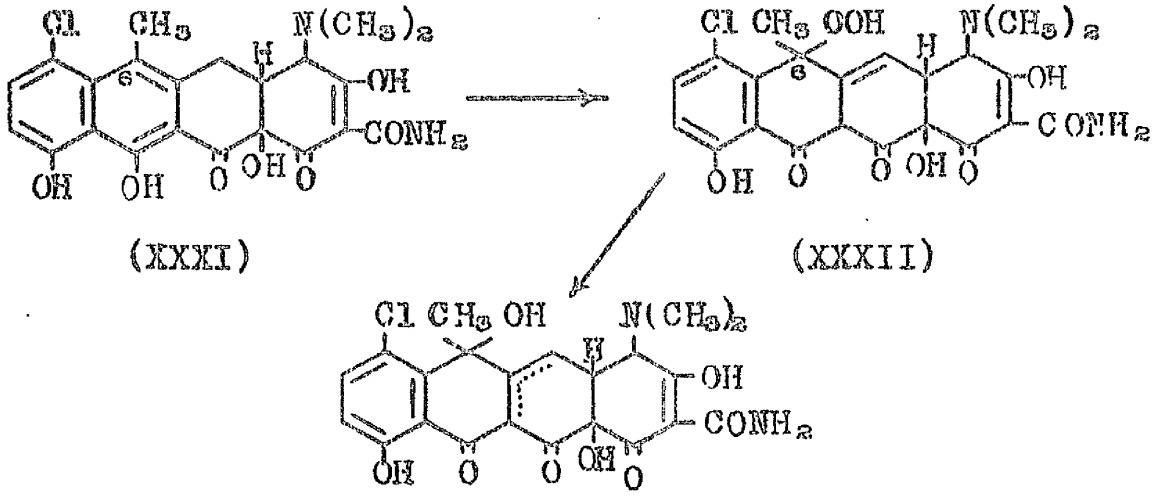
(XXVII) (XXVIII)

The use of photo-oxygenation is not confined to the steroid field and the technique has been used in most branches of organic chemistry in the synthesis of a variety of compounds. One of the best known examples is the synthesis of the monoterpene ascaridole (XXX), by photo-oxygenation of α-terpinene (XXIX),[20] but many such reactions are described in an interesting book by Schönberg.[21]



(XXIX) (XXX)

More recently, photo-oxygenation has been used to carry out one of the more difficult steps in the chemical synthesis of all tetracycline derivatives.[22] Photo-oxygenation of 7-chloroanhydrotetracycline (XXXI) gives the 6-hydroperoxy compound (XXXII), which is then catalytically reduced to 7-chlorodehydrotetracycline (XXXIII).



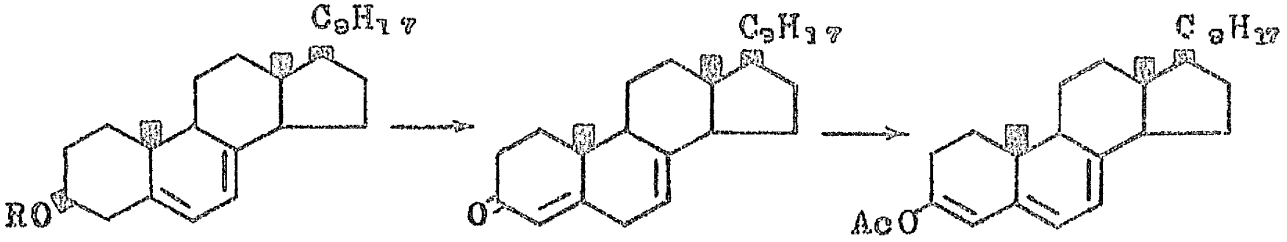
(XXXI) (XXXII)

(XXXIII)

The reaction is thought to simulate the biosynthesis of tetracycline.

Photo-oxygenation of 3-acetoxyergosta-3,5,7,22-tetraene.

In spite of the large amount of work which has been done on the photo-oxygenation of steroids, there is no record in the literature of the photo-oxygenation of a steroidal enol acetate, such as 3-acetoxyergosta-3,5,7,22-tetraene (XXXV), so it was decided to try and obtain an epidioxide from this compound and find out how its properties compared with those of ergosteryl acetate peroxide.



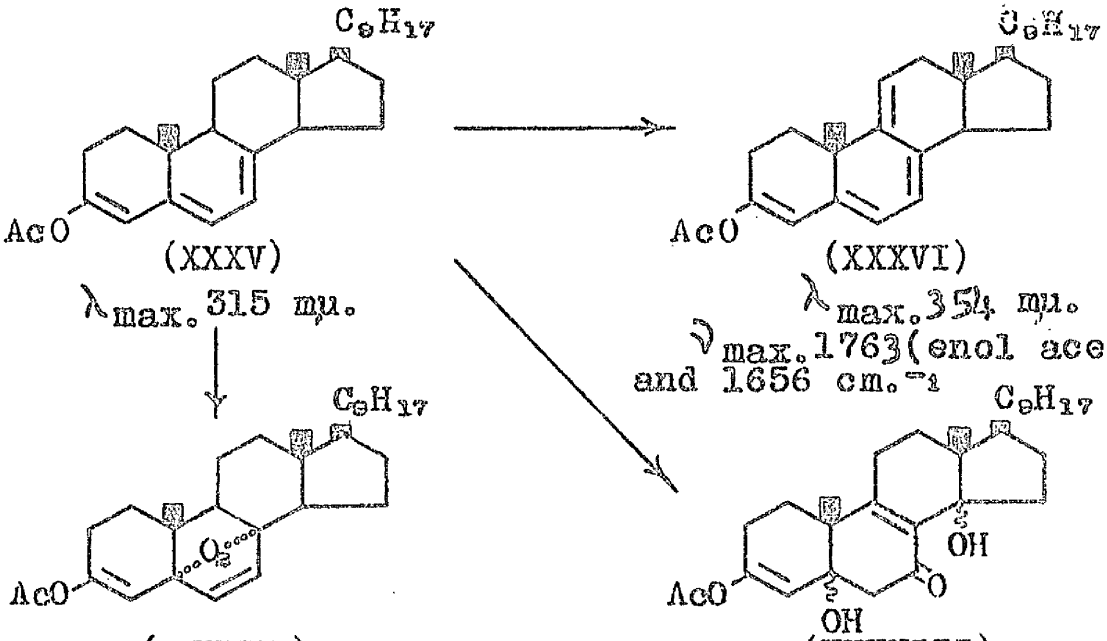

(I) (XXXIV) (XXXV)

3-Acetoxyergosta-3,5,7,22-tetraene (XXXV) was obtained from ergosterol (I, R = H), by way of the intermediate 3-ketone (XXXIV). Oppenauer oxidation[23] of ergosterol, using aluminium isopropoxide and acetone in benzene gave ergosta-4,7,22-trien-3-one (XXXIV),[23,24,25,26] which was converted to the enol acetate, on being refluxed with acetic anhydride and pyridine.[24]

As there is no reference in the literature to the photo-oxygenation of 3-acetoxyergosta-3,5,7,22-tetraene, the reaction was carried out by the method used in the preparation of lumisteryl acetate β-epidioxide at room temperature.[15] The enol acetate (XXXV) is not very soluble in ethanol, but rather than carry out the reaction at high temperature, which greatly increases the tendency to form by-products,[15] the starting material was dissolved in 1:1 benzene-ethanol, the procedure adopted by Laubach, Schreiber, Agnello and Brunings in the preparation of Ring C epidioxides.[14] Eosin was used as the sensitising dye and a small quantity of pyridine was again added to minimise the effect of any acid produced by oxidative splitting of the side-chain double-bond. Oxygenation was carried out, while the solution was irradiated with white light and under these conditions the reaction was about 85% complete in 12 hours.

The product obtained on evaporating the solution and removing the eosin was an impure, crystalline solid, which was chromatographed on deactivated alumina. The alkaline conditions of an alumina column however, seemed to cause decomposition or rearrangement of one or more of the photo-oxygenation products. In subsequent

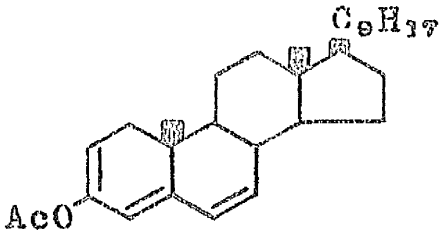
experiments therefore, silica gel was used, with considerably more success, allowing the crude product to be separated into three components. These were, in case of elution, 3-acetoxyergosta-3,5,7,9(11),22-pentaene (XXXVI), 3-acetoxy-5α,8α-epidioxyergosta-3,6,22-triene (XXXVII) and 3-acetoxy-5ξ,14ξ-dihydroxyergosta-3,8(9),22-trien-7-one (XXXVIII).




* $\lambda^{calc.}_{max.}$ refers to the value for the ultraviolet absorption, calculated by the Woodward-Fieser rules.[27] These rules are useful in calculating the approximate ultraviolet absorption maxima of conjugated enones, but they suffer from the disadvantage that they tend to break down at high degrees of unsaturation and substitution.

3-Acetoxyergosta-3,5,7,9(11),22-pentaene (XXXVI) is easily identified by its ultraviolet and infrared spectra and by comparison with an authentic sample, prepared by the method of Heilbron, Kennedy, Spring and Swain.[24] It is also interesting to note that while there is no record of dehydroergosteryl acetate being produced by photo-oxygenation of ergosteryl acetate, dehydrolumisteryl acetate is one of the photo-oxygenation products of lumisteryl acetate.[18]

3-Acetoxy-5α,8α-epidioxyergosta-3,6,22-triene (XXXVII) shows no ultraviolet absorption above 210 mμ. The infrared spectrum shows the presence of the enol acetate group. Inability to obtain a sample melting over less than 7-8°, led to a suspicion that the material was impure, either due to the fact that the supposedly pure starting material contained some of the isomeric 3-acetoxyergosta-2,4,6,22-tetraene (XXXIX)[24] or that the epidioxide was a mixture of two stereoisomers.



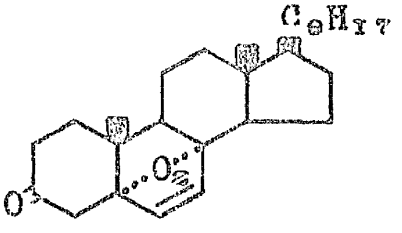

(XXXIX)

The reaction between ergosta-4,7,22-trien-3-one and acetyl chloride-acetic anhydride[24] and between ergosta-4,7,22-trien-3-one and isopropenyl acetate-concentrated sulphuric acid[28] gave enol acetates, identical with that prepared using pyridine-acetic anhydride. It would seem therefore, that the original sample was in fact homogeneous, thus ruling out the first of these possibilities. The second is largely ruled out by careful chromatography of the epidioxide, which fails to reveal the presence of any isomeric material. It was concluded that the somewhat wide melting-point of the epidioxide was due to decomposition of the compound as it neared the melting point temperature. This phenomenon was later found to be common to many of the compounds in the series, but in most cases, the melting-point can be made sharp by carrying out the determination in vacuo.

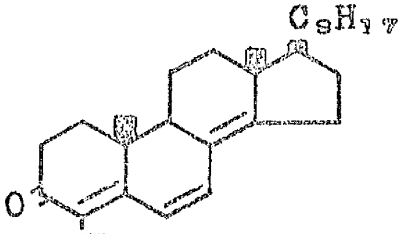
The epidioxide bridge most likely has the α-configuration, by analogy with ergosterol peroxide. The configuration of the epidioxide bridge in the latter compound was originally deduced by consideration of the "rule of rear attack", that certain reagents usually approach the steroid molecule from the less hindered (α)

side.[29,30] Dalton and Meakins point out[31] that there are many exceptions to this rule, especially when addition to ring A or B is involved,[32] and they provide chemical proof of rear approach in the oxidation of ergosterol. As the only difference between 3-acetoxy-ergosta-3,5,7,22-tetraene and ergosteryl acetate is the presence of the additional 3,4-double bond, it seems likely that the enol acetate will also form an α-epidioxide.

It was expected that alkaline hydrolysis of the epidioxide (XXXVII) would give the 3-ketone (XL), although there is no record in the literature of the preparation of this ketone from ergosterol peroxide. On treatment with alkali however, the epidioxide, in addition to being saponified, underwent an interesting rearrangement to yield 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one (XLI, R = H).



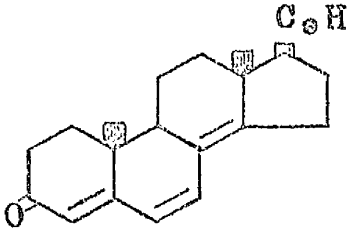

(XL)




(XLI, R=H)

$\lambda_{max.}$ 370 mμ. $\lambda^{calc.}_{max.}$ 391 mμ.
$\nu_{max.}$ 3425 (hydrogen bonded OH), 1656 (hydrogen bonded unsaturated ketone), and 1603 $cm.^{-1}$
1656 (hydrogen bonded unsaturated ketone), and 1603 $cm.^{-1}$

This compound is identified mainly by its ultraviolet and infrared spectra, which taken together, indicate a conjugated trienone, with a hydroxyl group adjacent to the carbonyl group. On this basis, (XLI, R = H) is the most likely structure for the rearrangement product. The $\Delta^{4,6,8(14)}$-trien-3-one system is preferred to the isomeric $\Delta^{4,6,8(9)}$-trien-3-one system, because of the predictions of the Woodward-Fieser rules.[27] The value of 391 mμ. predicted for the former system is much closer to the actual value of 370 mμ. than is the value of 420 mμ., predicted for the latter system, which contains a homoannular diene component.



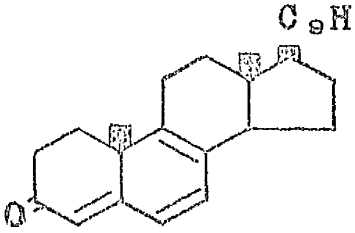

(XLII)

$\lambda_{max.}$ 350 mμ.

$\lambda^{calc}_{max.}$ 356 mμ.

$\nu_{max.}$ 1666, 1644 and 1588 cm.$^{-1}$

$[\alpha]_D$ + 588°




(XLIII)

$\lambda_{max.}$ 388 mμ.

$\lambda^{calc.}_{max.}$ 385 mμ.

$\nu_{max.}$ 1661, 1634 and 1594 cm.$^{-1}$

$[\alpha]_D$ + 749°

The ultraviolet and infrared absorption properties of the hydroxy-ketone (XLI, R = H) are similar to those of ergosta-4,6,8(14),22-tetraen-3-one (XLII)[33,34] and ergosta-4,6,8(9),22-tetraen-3-one (XLIII),[34] which absorb as shown. It can also be seen that the specific rotation of the hydroxy-ketone (+ 733°) is comparable with those of the compounds mentioned.

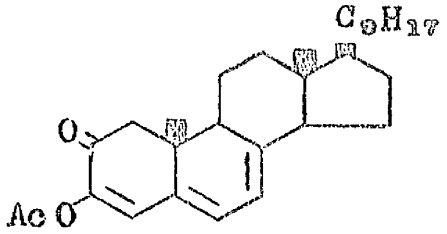
Acetylation of the hydroxy-ketone (XLI, R = H) with acetic anhydride-pyridine at room temperature gave 4-acetoxyergosta-4,6,8(14),22-tetraen-3-one (XLI, R = Ac) and treatment of it with benzoyl chloride-pyridine at room temperature gave the 4-benzoate (XLI, R = Bz). Both of these show an enol ester carbonyl peak in the infrared, together with disappearance of the hydrogen bonded hydroxyl peak and a slight shift of the 3-ketone peak to a higher frequency. Also, the main peak (370 mμ.) in the ultraviolet absorption spectrum of the hydroxy-ketone shows a bathochromic shift of about 20 mμ., when it is measured in alkaline solution as compared to neutral solution, and a similar shift is shown by the ultraviolet spectrum of the 4-acetate, presumably because hydrolysis of the acetate group takes place in alkaline

solution. The spectrum of the 4-benzoate however, shows no shift of this nature (see Table 1).

Table 1.

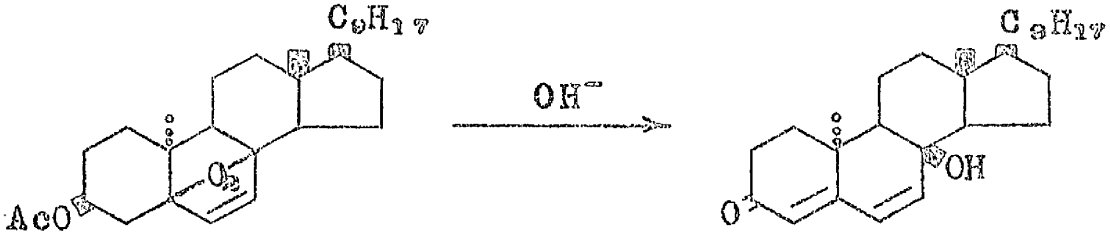
| Compound | 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one | | |
|---|---|---|---|
| | 4-hydroxy-ketone (XLI, R = H) | 4-acetate (XLI, R = Ac) | 4-benzoate (XLI, R = Bz) |
| $\lambda$max. (in EtOH) | 205, 262 and 370 mμ. ($\varepsilon$ 9,200, 6,600 and 21,000) | 250 and 362 mμ. ($\varepsilon$ 4,700 and 17,900) | 205, 232, 278 and 355 mμ. ($\varepsilon$ 15,500, 18,000, 6,800 and 25,200) |
| $\lambda$max. (in NaOH) | 225, 277 and 390 mμ. ($\varepsilon$ 36,100, 6,600 and 14,500) | 218 and 398 mμ. ($\varepsilon$ 13,200 and 11,200) | 218 and 355 mμ ($\varepsilon$ 42,500 and 22,600) |

The nuclear magnetic resonance spectrum of the 4-acetate (XLI, R = Ac) contained a symmetrical quartet of peaks due to the AB system of the $C_6$ ($\tau$ 3.82) and $C_7$ ($\tau$ 3.39) protons ($J$ 11c./sec.), together with a broad peak due to the side-chain olefinic protons ($\tau$ 4.72). The absence of a sharp singlet peak ($C_4$) in the olefinic region, together with the high positive rotation and the ultraviolet spectrum excluded the alternative structure (XLIV) for the acetate.



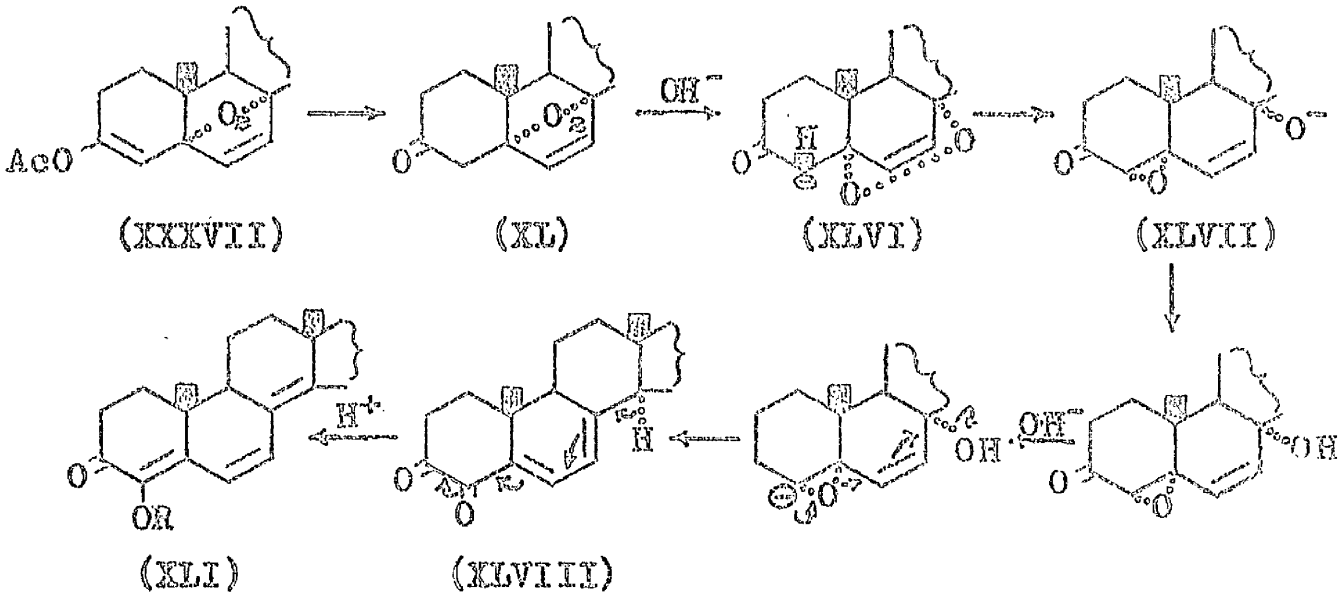

(XLIV)

Both ergosterol and dehydroergosterol peroxides are stable in alkaline solution,[10] as expected with compounds in which the epidioxide bridge joins two tertiary carbon atoms, so it is interesting that hydrolysis of 3-acetoxy-5α,8α-epidioxyergosta-3,6,22-triene (XXXVII) yields this hydroxy-ketone (XLI, R = H). This is not the first instance in which a ditertiary epidioxide system has been found to be unstable in alkaline solution: hydrolysis of lumisteryl acetate β-epidioxide (XVI) does not give the 3-hydroxy compound, but 8β-hydroxylumista-4,6,22-trien-3-one (XLV).[15] The structure of this compound was assigned on the basis of its infrared and ultraviolet spectra and optical rotation data.




(XVI) (XLV)

The following mechanism is advanced for the rearrangement of 3-acetoxy-5α,8α-epidioxyergosta-3,6,22-triene (XXXVII), on alkaline hydrolysis.

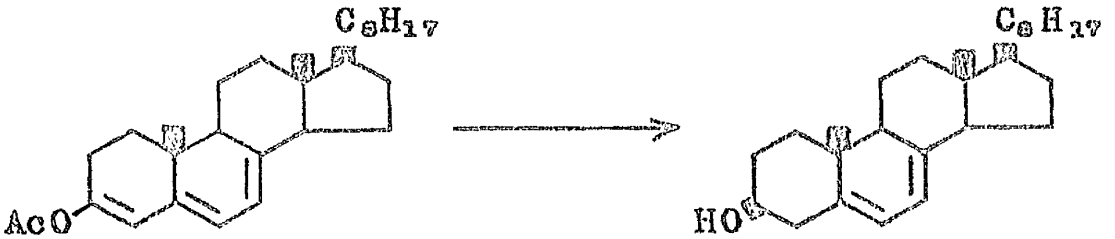



(XXXVII) (XL) (XLVI) (XLVII)

(XLI) (XLVIII)

The initial step is in fact, hydrolysis to the ketone (XL), which in alkaline solution forms the carbanion (XLVI). The negatively charged 4-position is sufficiently close to the 5α,8α-epidioxide bridge to attack it, with the formation of the intermediate epoxide (XLVII), which rearranges to form the diketone (XLVIII). Acidification then gives 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one (XLI, R = H).

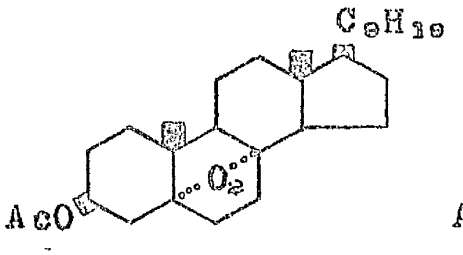
It was decided at this stage to try to obtain a direct relationship between the enol acetate epidioxide (XXXVII) and ergosterol peroxide (III, R = H) and the first method attempted was sodium borohydride reduction of the epidioxide to ergosterol peroxide. 3-Acetoxy-cholesta-3,5,7-triene(XLIX) has been directly reduced to 7-dehydrocholesterol (L) by this reagent,[35] so the same experimental conditions were used in this case.



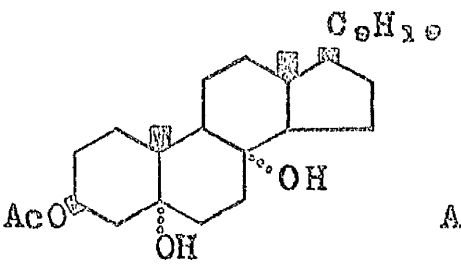

(XLIX) (L)

The reaction did not go as expected however, and varying the conditions gave several unexpected products, trienes or trienones, which will be described later.

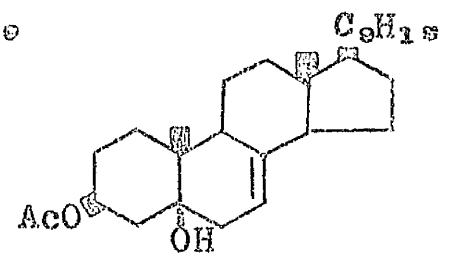
It was then decided to try and obtain correlation between the epidioxide (XXXVII) and ergosterol peroxide (III, R = H) by hydrogenating the epidioxide to a known ergosterol peroxide derivative (LI), or to an ergostanol (LII), or ergostenol (LIII), all of which have been obtained by catalytic hydrogenation of ergosteryl acetate peroxide.[10]



(LI)



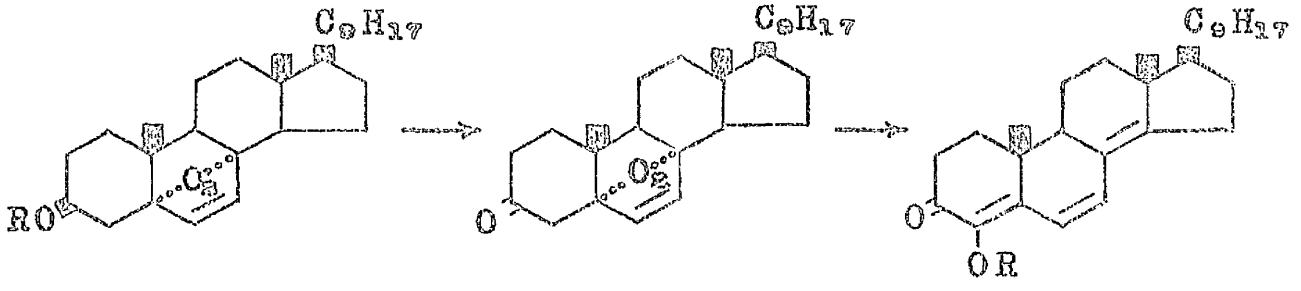
(LII)



(LIII)

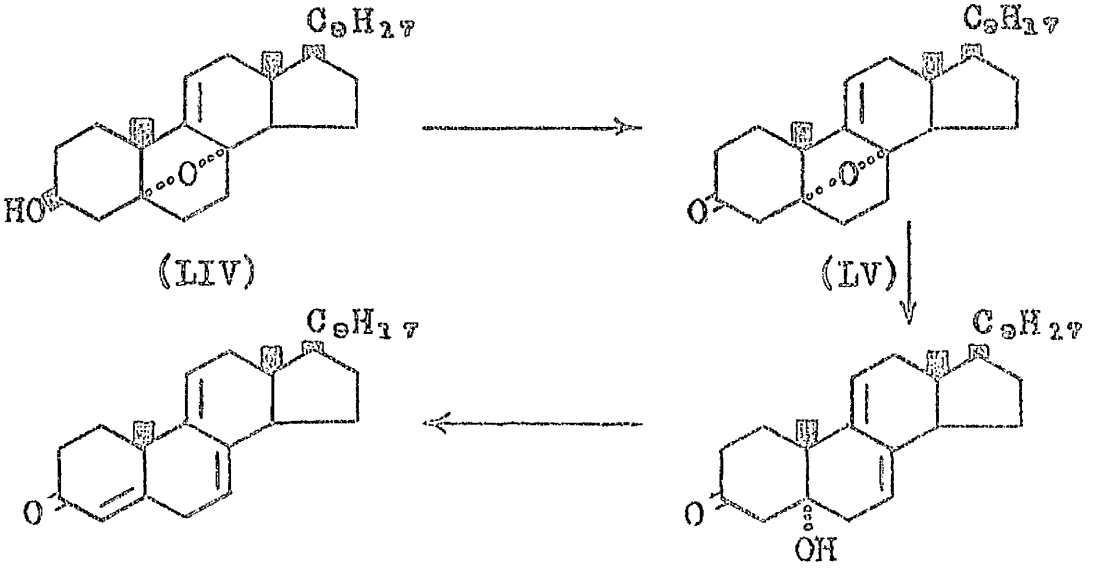
Unfortunately, catalytic hydrogenation of 3-acetoxy-5α,8α-epidioxyergosta-3,6,22-triene (XXXVII) with platinum oxide gave a mixture of diols or triols, in which hydrogenolysis of the 3-acetate group seemed to have taken place, and which could not be separated to give any recognisable compound.

Finally, to establish a relationship between the epidioxide (XXXVII) and ergosterol peroxide, an attempt was made to oxidize ergosterol peroxide to 5α,8α-epidioxy-ergosta-6,22-dien-3-one (XL), in the hope that alkali treatment of this compound would give 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one (XLI, R = H).



(III) (XL) (XLI)

As was mentioned earlier, there is no record in the literature of the preparation of the 3-ketone of ergosterol peroxide, but Oppenauer oxidation of 3β-hydroxy-5α,8α-epoxyergosta-9,22-diene (LIV) has been shown to give the 3-ketone (LV).[36] Isomerisation of this compound with a trace of mineral acid gave the 7,9-diene (LVI) which was dehydrated with the weak alkaline reagent, aluminium tert.-butoxide to give ergosta-4,7,9(11),22-tetraen-3-one (LVII).




Oppenauer oxidation of ergosterol peroxide[*] was carried out under the same conditions as used in the

[*] Ergosterol peroxide was prepared by a slight modification[16] of the original method of Windaus and Brunken.[6]

preparation of 5α,8α-epoxyergosta-9(11),22-dien-3-one (LV), but this did not give the desired product. Instead, there was isolated a small amount of 4-hydroxy-ergosta-4,6,8(14),22-tetraen-3-one (XLI, R = H), together with starting material (III, R = H), indicating that oxidation had probably taken place to some extent, but that any ketone formed had immediately rearranged under the conditions used in the oxidation.

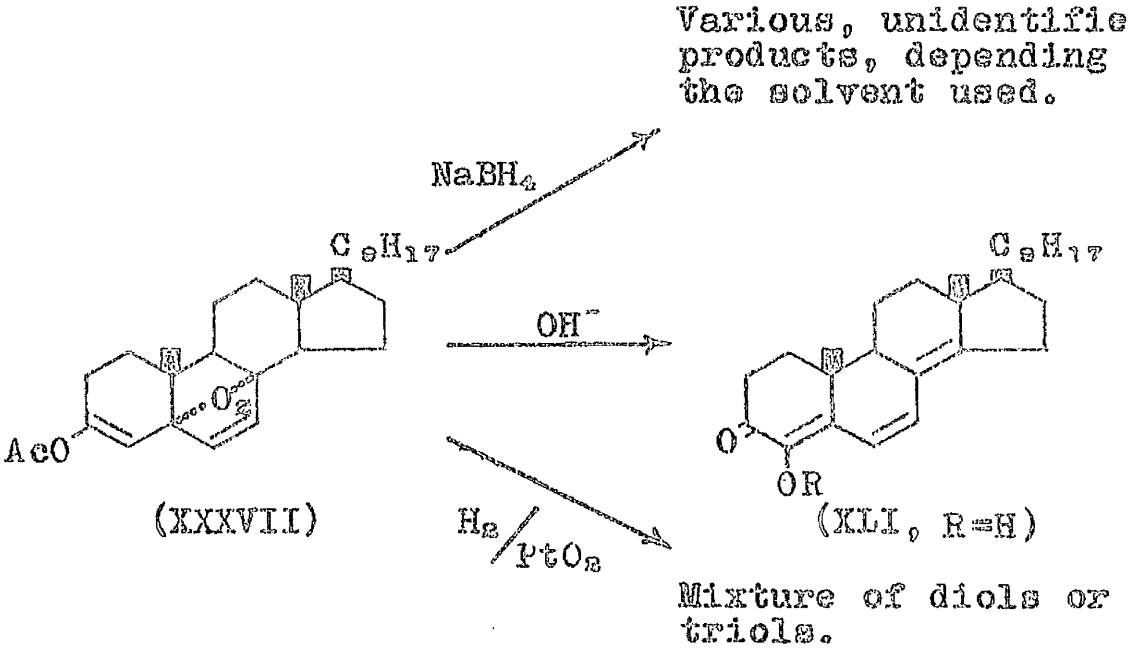
It was then decided to try chromium trioxide-pyridine oxidation, in the hope that this would allow isolation of the 3-ketone. It was found that the success of this oxidation was dependent on the method of working up. If the excess of oxidant was destroyed by adding dilute hydrochloric acid and sodium sulphite before carrying out the ether extraction then the initial, colourless oxidation product quickly deteriorated to a red gum. Chromatography of this gum gave a trace of 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one (XLI, R = H). Refluxing the gum with ethanolic potassium hydroxide solution, and chromatography of the product gave a little of the hydroxy ketone together with a colourless, crystalline compound which has been named Ketol A and which

showed ultraviolet absorption at 236 mμ. in ethanol and at 248 mμ. in alkaline solution. It showed peaks in the infrared at 3413 and 1681 cm.$^{-1}$, but no structure has been proposed for this compound. If the chromium trioxide-pyridine oxidation product was extracted with ether, without destroying the excess of oxidant, the extraction proved to be rather laborious, but it did give a colourless gum, which after fractional crystallisation, yielded two products. The first of these was the required 5α,8α-epidioxyergosta-6,22-dien-3-one (XL), the mother liquor of which gave an amorphous solid on trituration with petroleum ether. Crystallisation from ether-methanol gave a poorly crystalline solid, which absorbed in the ultraviolet at 240 mμ. and in the infrared at 3448 and 1656 cm.$^{-1}$ When the mother liquor of the initial trituration of this material was taken to dryness and the residue refluxed with ethanolic potassium hydroxide. 4-hydroxyergosta-4,6,8(14)22-tetraen-3-one (XLI, R = H) could be isolated from the product. In the same way, the crystallisation mother liquor of the poorly crystalline material was taken to dryness and the residue treated with ethanolic potassium hydroxide solution. Chromatography of

the product yielded Ketol A, which was identical with the material isolated during the attempted preparation of 5α,8α-epidioxyergosta-6,22-dien-3-one.

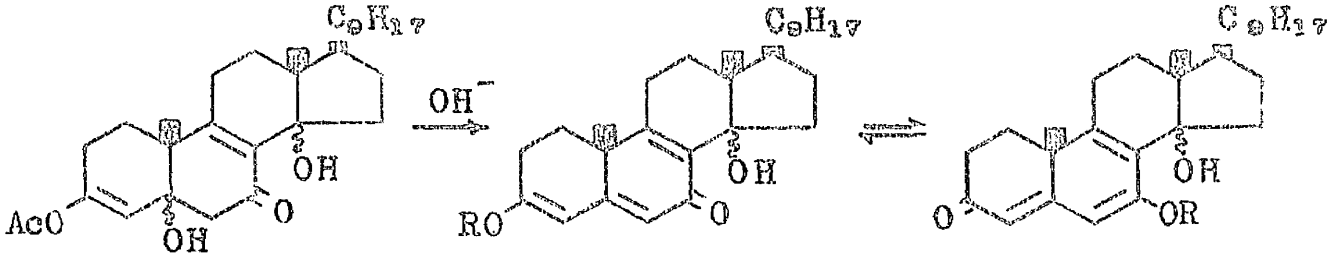
It would seem therefore that 5α,8α-epidioxyergosta-6,22-dien-3-one is extremely sensitive to traces of acid or alkali, giving poorly crystalline, decomposition products, which have not been identified. On being refluxed with ethanolic potassium hydroxide solution, it gave 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one, thus establishing the link between 3-acetoxy-5α,8α-epidioxy-ergosta-3,6,22-trien and ergosterol peroxide. Although this confirms that the structure of the enol acetate epidioxide is analogous to that of ergosterol peroxide, it does not prove the configuration of the epidioxide bridge.

The structure of the product of alkaline hydrolysis having been established, the reactions of 3-acetoxy-5α,8α-epidioxyergosta-3,6,22-triene (XXXVII) may be summarized by the following chart.




Due to the sensitivity of the molecule, all reactions have to be carried out under mild conditions and care has to be taken in working up, as most of the products are sensitive to traces of acids or alkali and to light and tend to deteriorate easily to intractable oils.

The structure of the third product of photo-oxygenation, 3-acetoxy-5$\xi$,14$\xi$-dihydroxyergosta-3,8(9),22-trien-7-one (XXXVIII) was assigned on the following evidence. The ultraviolet absorption suggested an $\alpha\beta$-unsaturated ketone and this was confirmed by the infrared




(XXXVIII) (LVIIIa, R=H) (LVIIIb, R=H)

$\lambda_{max.}$ 240 mμ. $\lambda^{calc.}_{max.}$ 298 mμ. $\lambda^{calc.}_{max.}$ 403 mμ.

$\lambda^{calc.}_{max.}$ 249mμ. $\lambda_{max.}$ 375 mμ.

$\nu_{max.}$ 3610, 3509 and 3360 (hydroxyl), 1768 (enol acetate) and 1695 $cm.^{-1}$ (αβ-unsaturated ketone).

$\nu_{max.}$ 3380 and 3236 (hydroxyl), and 1645 $cm.^{-1}$ (hydrogen bonded, unsaturated ketone).

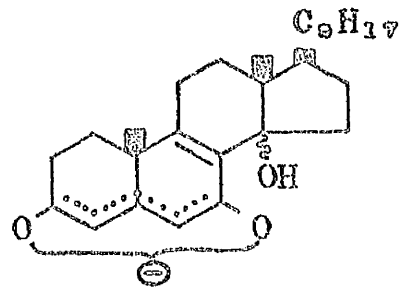
absorption, which also showed the presence of both hydrogen bonded and free hydroxyl groups and showed that the enol acetate grouping was still present. Analyses of the compound favour a molecular formula of $C_{30}H_{44}O_5$, showing that in addition to the enol acetate group and the ketone, there are two hydroxyl groups. Neither of these could be acetylated with acetic anhydride and pyridine at room temperature, so they must both be tertiary. The αβ-unsaturated system cannot be $\Delta^4$-6-one, as this would be in conjugation with the double bond of the enol acetate group, therefore it must be $\Delta^3$-7-one or

$\Delta^{7}$-6-one. This means that the hydroxyl groups must be at $C_5$ and $C_{14}$ and the $\Delta^{8(9)}$-7-one system is favoured, because of the compound's behaviour on treatment with ethanolic potassium hydroxide solution. The fact that one of the hydroxyl groups shows intramolecular hydrogen bonding in the infrared spectrum rules out a 9-hydroxy-$\Delta^{8(14)}$-7-one system.

Brief alkaline hydrolysis of 3-acetoxy-5$\xi$,14$\xi$-dihydroxyergosta-3,8(9),22-trien-7-one (XXXVIII) gave an unsaturated ketone, which was found to decompose on attempted recrystallisation. The pure material was obtained, simply by acidifying the reaction mixture and filtering off the product. This compound has been assigned the tautomeric structure (LVIIIa/LVIIIb, R = H), for the following reasons. The ultraviolet and infrared spectra indicate a conjugated trienone and possibly two hydroxyl groups.* Measurement of the ultraviolet spectrum in alkaline solution shows two very broad peaks at 263 and 444 mμ, probably due to the formation of the

* The hydroxyl region of the infrared spectrum cannot be studied in solution, because of the poor solubility of the compound and its tendency to decompose.

anion[*] (LIX) in this medium and micro-analysis suggests a molecular formula of $C_{28}H_{40}O_3$. Acetylation with acetic




(LIX)

anhydride and pyridine at room temperature gave a mono-acetate, which may have either structure (LVIIIa, R = Ac) or (LVIIIb, R = Ac). The second of these possibilities is favoured because of the nature of the compound's infra-red absorption. Examination of the 2-4 μ. region in dilute carbon tetrachloride solution reveals a hydroxyl peak at 3597 $cm.^{-1}$ indicating that the molecule is unlikely to contain a 7-keto group, as this would be hydrogen bonded to the adjacent 14-hydroxyl group.

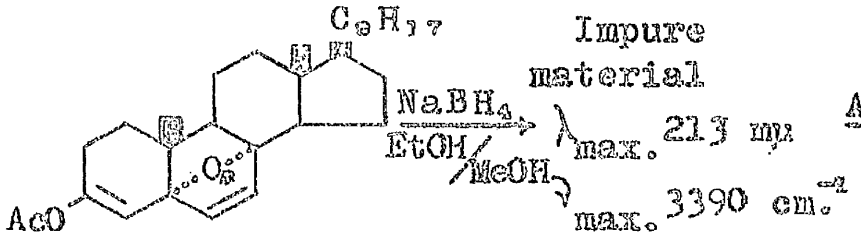
An attempt was made to carry out a dienone-phenol

[*] The ultraviolet absorption maximum of compounds of this nature can be calculated by the Electron Gas Theory.[17] This gives $\lambda_{max.} = 127 \frac{(j+1)^2}{j + \frac{3}{2}}$ mμ, where j = number of double bonds. In this case j = 3, as the 8(9)-double bond is not part of the resonance system and substituting j = 3 in the equation gives $\lambda_{max}$ 452 mμ.

rearrangement[16,30] of 3,14β -dihydroxyergosta-3,5,8(9),22-tetraen-7-one (LVIIIa, R = H), but this gave only water-soluble products, probably on account of the additional double bond at $C_3$.

Treatment of 3-acetoxy-5α,8α-epidioxyergosta-3,6,22-triene with sodium borohydride.

As was mentioned earlier, sodium borohydride reduction of 3-acetoxy-5α,8α-epidioxyergosta-3,6,22-triene was attempted, using the same conditions as were employed in the reduction of 3-acetoxycholesta-3,5,7-triene to 7-dehydrocholesterol.[35] This involved treatment of the enol acetate epidioxide in ether-methanol at 0°C, with sodium borohydride in ethanol, the borohydride being dissolved in ethanol, as it is more stable in that solvent. The initial product of the reaction was an impure, colourless crystalline solid, which had ultraviolet and infrared absorption as shown. This material could not be



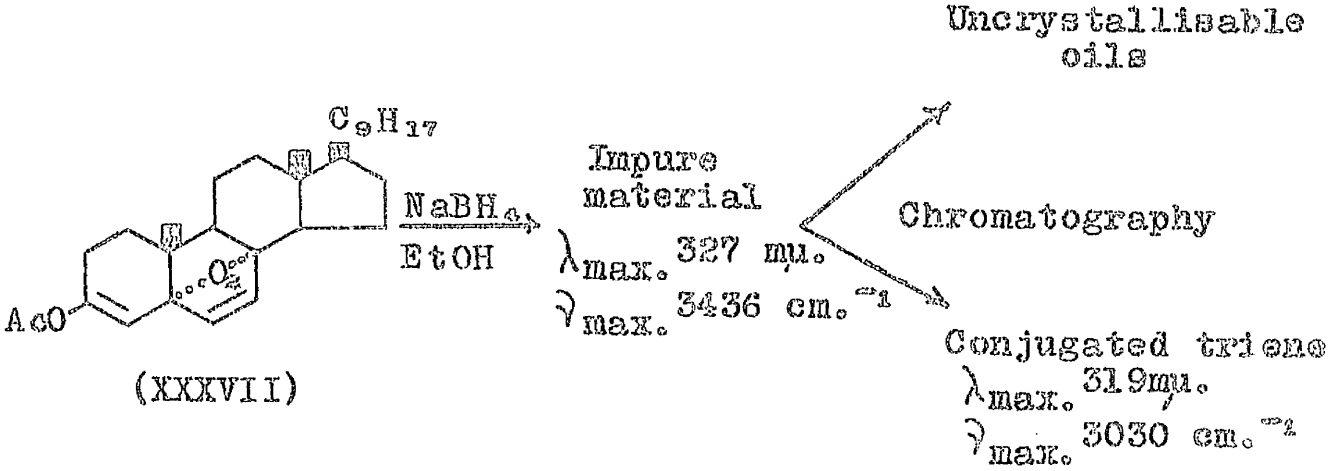

(XXXVII)

purified by recrystallisation and when it was chromatographed on deactivated alumina, rearrangement took place to give a sharp-melting, crystalline compound, which was

named Ketone III. This had ultraviolet and infrared absorption as shown, and these suggested that the structure of this compound is similar to that of the hydroxy-ketone (XLI, R = H). There is however, no hydroxyl absorption in the infrared, but the presence of a weak peak at 3021 $cm.^{-1}$ gave rise to the idea that the compound contains an ether linkage and that it might even be the methyl or ethyl ether of 4-hydroxyergosta-4,6,8(14), 22-tetraen-3-one. With this in mind, an attempt was made to form the methyl ether of the hydroxy-ketone, but this was unsuccessful. Ketone III was also found to have a specific rotation of only +2°, whereas the hydroxy-ketone has a rotation of +733° and it seems unlikely that ether formation would bring about such a change. The weak peak at 3021 $cm.^{-1}$ in the infrared spectrum is probably due to double bond absorption and Ketone III is most likely a conjugated trienone, but it has been found impossible to assign an exact structure.

Repetition of the sodium borohydride treatment using ether and methanol alone as the solvents also gave an impure product. When this was chromatographed on deactivated alumina, it gave uncrystallisable mixtures and

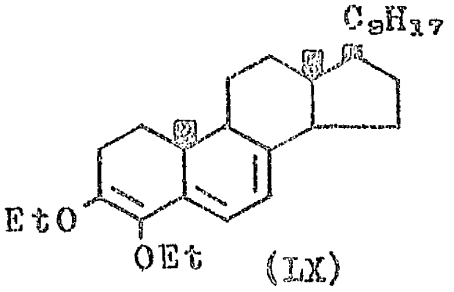
when it was chromatographed on silica gel, it gave only a trace of 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one, indicating that reduction was not taking place and that the conditions were merely causing rearrangement of the epidioxide. This was probably due to the instability of sodium borohydride in methanol.




(XXXVII)

In a third series of experiments, using ether and ethanol as the solvents, a completely different product was obtained. This was an impure, colourless, crystalline solid, the ultraviolet and infrared absorption of which suggested that it was a conjugated trienol. It could not be purified by recrystallisation and when it was chromatographed it gave uncrystallisable oils, apart from

one occasion, when chromatography on deactivated alumina gave a crystalline compound. The ultraviolet absorption of this compound indicated that it was a conjugated triene and a weak peak in the infrared at 3030 cm.$^{-1}$ again indicated the possible presence of an ether linkage. Micro-analysis shows that there are in fact two ethoxyl groups, so the structure (LX) was proposed for this

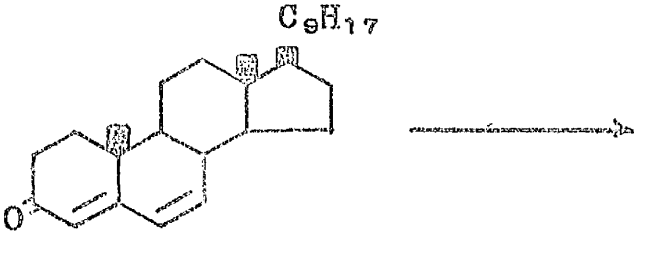



(LX)

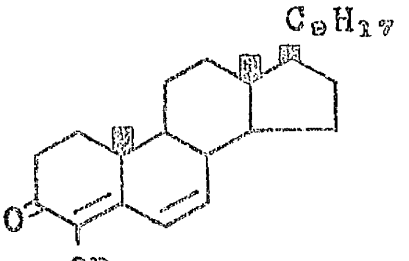
compound, but this has not been confirmed, because other attempts to obtain the compound gave unidentifiable oils.

Aerial oxidation of ergosta-4,6,22-trien-3-one and ergosta-4,6,8(14),22-tetraen-3-one.

An attempt was then made to obtain 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one by aerial oxidation of ergosta-4,6,22-trien-3-one (LXI). The trienone was dissolved in tert.-butanol, containing potassium tert.-butoxide and the solution stirred at room temperature for 24 hours.[※] The product obtained after working up the reaction mixture and chromatographing the crude product however, was 4-hydroxyergosta-4,6,22-trien-3-one (LXII, R = H), together with a little recovered starting material.



(LXI)



OR (LXII, R=H)

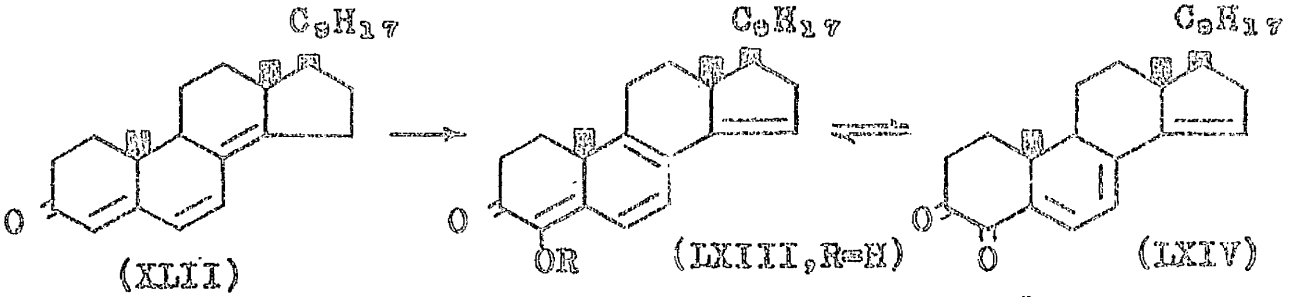
$\lambda_{max.}$ 288 mμ; $\lambda^{calc}_{max.}$ 315 mμ.

$\nu_{max.}$ 3401 (hydrogen bonded OH) and 1656 cm.$^{-1}$ (hydrogen bonded, unsaturated ketone)

※ Italian workers have recently reported the aerial oxidation of several 3-keto-$\Delta^4$-steroids using this method.[39] They obtained the corresponding 4-hydroxy-3-keto-$\Delta^{4,6}$-derivatives, along with 3,6-diketo-$\Delta^4$-derivatives.

The product was identified mainly by its infrared absorption. The ultraviolet absorption is considerably lower than that predicted for this type of compound, and on acetylation with acetic anhydride and pyridine at room temperature, the 4-hydroxy-3-ketone gives the 4-acetate (LXII, R = Ac), the ultraviolet absorption of which (307 mμ.) is rather higher than expected. This anomolous behaviour may be due to the compound's partial existence in a diketonic form.

As it appeared that the extra 8(14)-double bond could not be introduced by aerial oxidation, another attempt was made, using ergosta-4,6,8(14),22-tetraen-3-one (XLII). Under the same conditions, this gave a green, crystalline compound, which appears to contain one hydroxyl group and has been assigned the structure (LXIII, R = H). The deviation of the ultraviolet




$\lambda_{max.}^{calc.}$ 420 mμ. $\lambda_{max.}^{calc.}$ 393 mμ.

$\lambda_{max.}$ 270 and 371 mμ.

$\nu_{max.}$ 3378 (hydrogen bonded OH), 1642 and 1637 cm.$^{-1}$ (hydrogen bonded, unsaturated ketone)

absorption from the calculated value, the presence of twin peaks in the infrared spectrum and the green colour of the compound are probably due to its partial existence in the diketonic form (LXIV). This is borne out by examination of the visible absorption, which reveals a peak at 623 mμ. (ε 5.1), the value of the extinction coefficient suggesting that the compound exists as the diketone to the extent of approximately 25%.* It was thought possible that the initial step in the oxidation was the introduction of the 4-hydroxyl group, the extra double bond being introduced subsequently. However, allowing the reaction to proceed for only three hours, instead of twenty four, merely resulted in a lower yield of the compound (LXIII, R = H). Acetylation with acetic anhydride and pyridine at room temperature gave the 4-acetate (LXIII, R = Ac), which had infrared absorption at 1773 (enol acetate), 1669, 1647 and 1590 $cm.^{-1}$ (unsaturated ketone). It would seem therefore, that introduction of a hydroxyl group into an ergostenone nucleus by this method to give the desired

* Simple 1,2-diketones, such as diacetyl show a series of absorption bands in the region between 420 and 450 mμ. (ε ca. 20), and this absorption confers a yellow colour on these compounds.[40]

4-hydroxy-3-ketone (XLI, R = H) is rather difficult. The compounds obtained are interesting however, as the hydrogen bonded carbonyl groups absorb at a very low frequency in the infrared region. The main peaks in the infrared spectra of 4-hydroxyergosta-4,6,22-trien-3-one, 4-hydroxyergosta-4,6,8(9),14,22-pentaen-3-one and their acetates are given in Table 2.

Table 2.

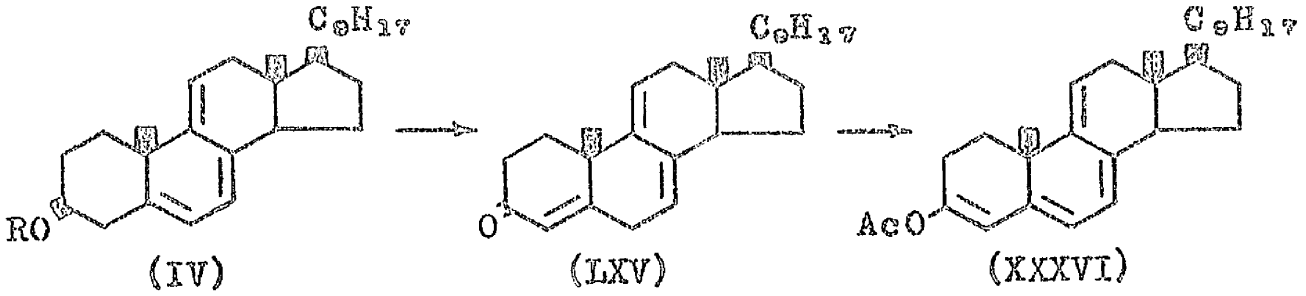
| Compound | 4-hydroxyergosta-4,6,22-trien-3-one | |
|---|---|---|
| | 4-hydroxy-ketone (LXII, R = H) | 4-acetate (LXII, R = Ac) |
| $\nu$ max. (in $CCl_4$) | 3448(OH), 1647, 1623 and 1575 cm.$^{-1}$ (3-ketone) | 1779(enol acetate), 1678, 1623 and 1585 cm.$^{-1}$ (3-ketone) |
| Compound | 4-hydroxyergosta-4,6,8(9),14,22-pentaen-3-one | |
| | 4-hydroxy-ketone (LXIII, R = H) | 4-acetate (LXIII, R = Ac) |
| $\nu$ max. (in $CCl_4$) | 3425(OH), 1642, 1637 and 1590 cm.$^{-1}$ (3-ketone) | 1773(enol acetate), 1669, 1653 and 1590 cm.$^{-1}$ (3-ketone). |

It can also be seen that the absorption due to the 3-ketone group moves to a higher frequency, when the effect of hydrogen bonding is removed, i.e. on acetylation.

## Photo-oxygenation of 3-acetoxyergosta-3,5,7,9(11),22-pentaene.

Having prepared an epidioxide from 3-acetoxyergosta-3,5,7,22-tetraene, it was decided to find out whether 3-acetoxyergosta-3,5,7,9(11),22-pentaene formed a similar epidioxide, and whether this epidioxide also underwent rearrangement on alkaline hydrolysis.

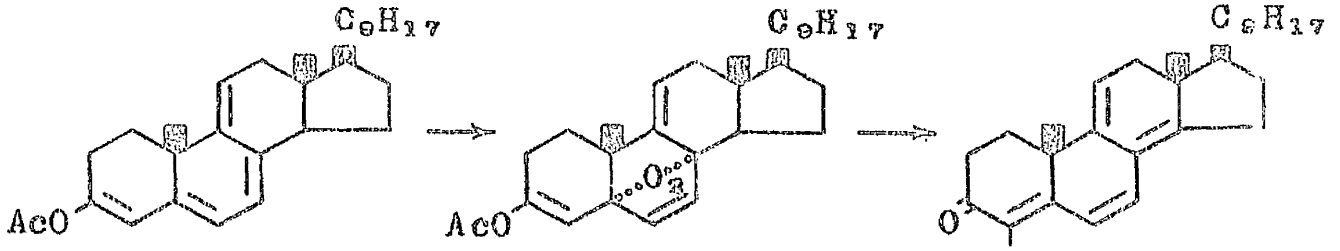
Dehydroergosterol (IV, R = H) was obtained from ergosterol by dehydrogenation with mercuric acetate[1] and was converted to the 3-ketone (LXV)[24] by Oppenauer oxidation.[23] In this case, aluminium tert.-butoxide was found to be preferable to isopropoxide and it was found to be unwise to chromatograph the crude product, as this led to decomposition and produced red gums. On one occasion, a batch of aluminium tert.-butoxide, which was pink in colour, produced an uncrystallisable product, but this was thought to be due to the presence of free base in the tert.-butoxide. In most cases the crude product was recrystallised once from acetone and used directly for the next step. Enol acetylation of the ketone (LXV), by refluxing it with acetic anhydride and pyridine gave 3-acetoxyergosta-3,5,7,9(11),22-pentaene (XXXVI).[24]




(IV) (LXV) (XXXVI)

The oxygenation and irradiation were carried out as described previously and it was found that the reaction went to completion in about 5.5 hours, compared with the 12 hours required for the complete photo-oxygenation of 3-acetoxyergosta-3,5,7,22-tetraene. The product obtained on evaporating the solution to dryness was a red gum, which was immediately chromatographed on silica gel, but no crystalline material could be isolated. It would seem therefore that the reaction product is even more sensitive to traces of acid or alkali than 3-acetoxy-5α,8α-epidioxy-ergosta-3,6,22-triene, which is not entirely unexpected with the additional 9(11)-double bond present in the molecule. Repetition of the photo-oxygenation gave the same red gum, but in this case, the gum was dissolved in chloroform and the solution passed through a short column of deactivated alumina as quickly as possible. This removed a great deal of the colour, but evaporation of the eluate still gave a red gum. Two recrystallisations from methanol however, gave 3-acetoxy-5α,8α-epidioxyergosta-

3,6,9(11),22-tetraene (LXVI) as pale orange needles.[*]




(XXXVI) (LXVI) (LXVII, R=H)

$\nu_{max.}$ 1761, 1681 and 1212 cm.$^{-1}$ (enol acetate)

$\lambda_{max.}$ 400 mμ.
$\lambda_{max.}^{calc.}$ 391 mμ.
$\nu_{max.}$ 3378 (OH), 1642, 1640 and 1527 cm.$^{-1}$ (unsaturated ketone)

The epidioxide shows no selective absorption in the ultraviolet, and the infrared spectrum shows the presence of the enol acetate group. On hydrolysis with dilute alkali, the epidioxide underwent rearrangement, to give 4-hydroxyergosta-4,6,8(14),9(11),22-pentaen-3-one (LXVII, R = H), which had ultraviolet and infrared absorption as shown and was thus identified in the same way as 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one (XLI, R = H). Acetylation with acetic anhydride-pyridine

[*] The pure product is probably completely colourless, but the presence of traces of eosin decomposition products, which are difficult to remove, make it slightly orange.

at room temperature gave the 4-acetate (LXVII, R = Ac) and treatment with benzoyl chloride-pyridine gave the 4-benzoate (LXVII, R = Bz).

It was recently pointed out[18] that the molecular rotation increments for the addition of oxygen to steroidal ring B dienes are of the same order in all cases. It is interesting therefore to compare the molecular rotation increments for the addition of oxygen to the two enol acetates with those of other ring B dienes (Table 3).

Table 3.

| Compound | $\Delta[M]_D^a$ |
|---|---|
| Ergosterol | +379[10] |
| Ergosteryl acetate | +300[18] |
| 22,23-Dihydroergosterol | +458[41] |
| 22,23-Dihydroergosteryl acetate | +377[42] |
| 3,17-Diacetoxyandrosta-5,7-diene | +488[42] |
| Lumisteryl acetate | -230[18] |
| 3-Acetoxyergosta-3,5,7,22-tetraene | +668[b] |
| 3-Acetoxyergosta-3,5,7,9(11),22-pentaene | +1579[b] |

(a) For formation of epidioxide
(b) Present work

It is also interesting at this stage to compare the infrared spectra of 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one, 4-hydroxyergosta-4,6,8(14),9(11),22-pentaen-3-one and their esters. The significant peaks are given in Table 4, from which it can be seen that both hydroxy-ketones show hydrogen bonded hydroxyl and hydrogen bonded carbonyl absorption. On esterification, there is as expected, complete disappearance of the hydroxyl peak and an associated shift of the 3-carbonyl peak to a higher frequency. In all cases, the carbonyl peaks of the $\Delta^{4,6,8(14),9(11)}$-compounds are at a lower frequency than those of the corresponding $\Delta^{4,6,9(14)}$-compounds, and the enol benzoate carbonyl frequency is lower than that of the enol acetate group.

The total combined mother liquors from the recrystallisation of the epidioxide (LXVI) were taken to dryness and the residual gum chromatographed on silica gel, in the hope that after having removed the bulk of the epidioxide, no decomposition would take place on the column. Only a further small amount of the epidioxide could be isolated however, so it would appear that if any by-product is formed during the photooxygenation, then it is very

Table 4.

| Compound | 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one | | |
|---|---|---|---|
| | 4-hydroxyketone (XLI, R = H) | 4-acetate (XLI, R = Ac) | 4-benzoate (XLI, R = Bz) |
| $\nu$ max. (in KCl) | 3400(OH), 1656(3-ketone) and 1600 cm.$^{-1}$ | 1773(enol acetate), 1681(3-ketone) and 1597 cm.$^{-1}$ | 1751 (enol benzoate), 1681(3-ketone), 1642 and 1600 cm.$^{-1}$ |
| Compound | 4-hydroxyergosta-4,6,8(14),9(11),22-pentaen-3-one | | |
| | 4-hydroxyketone (LXVII, R = H) | 4-acetate (LXVII, R = Ac) | 4-benzoate (LXVII, R = Bz) |
| $\nu$ max. (in KCl) | 3378(OH), 1642(3-ketone) and 1610 cm.$^{-1}$ | 1764(enol acetate), 1653(3-ketone), 1623 and 1585 cm.$^{-1}$ | 1732(enol benzoate), 1667(3-ketone) and 1626 cm.$^{-1}$ |

unstable and decomposes during the working-up process or on attempted separation.

An attempt was then made to oxidize dehydroergosterol peroxide (V, R = H) to the 3-ketone, to find out whether treatment of this ketone with ethanolic potassium hydroxide solution would give 4-hydroxyergosta-4,6,8(14),9(11),22-pentaen-3-one (LXVII, R = H). Treatment of dehydro-

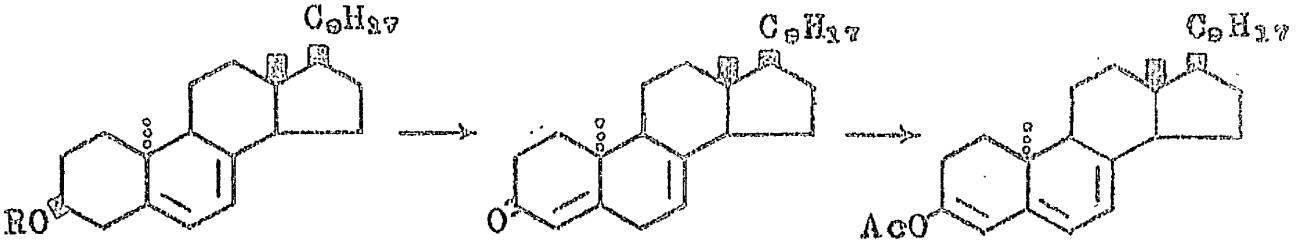
ergosterol peroxide with chromium trioxide-pyridine, which successfully oxidized ergosterol peroxide to 5α,8α-epidioxyergosta-6,22-dien-3-one was completely unsuccessful however, and left the peroxide unchanged.

Photo-oxygenation of 3-acetoxylumista-3,5,7,22-tetraene.

The lumisterol used as the starting material in this series was obtained as its 3,5-dinitrobenzoate, from Vitamin D residues.[*] Lumisteryl 3,5-dinitrobenzoate was distinguished from calciferyl 3,5-dinitrobenzoate, by the fact that calciferol compounds show a peak at 685 cm.$^{-1}$ in the infrared, probably due to the $C_{10}$-$C_{19}$ methylene group. This peak is completely absent in the spectra of lumisterol compounds. Hydrolysis of lumisteryl 3,5-dinitrobenzoate gave the sterol (XIII, R = H), which was oxidized to the 3-ketone (LXVIII),[84] by Oppenauer oxidation.[83] Aluminium tert.-butoxide was again found to be more suitable than isopropoxide, although on one occasion, the bad batch of aluminium tert.-butoxide mentioned earlier brought about no oxidation of the starting material. On all occasions, the crude oxidation product had to be chromatographed to separate the pure product from unchanged starting material, and the final yield was always poor. Refluxing lumista-4,7,22-trien-3-one (LXVIII) with acetic anhydride and pyridine gave

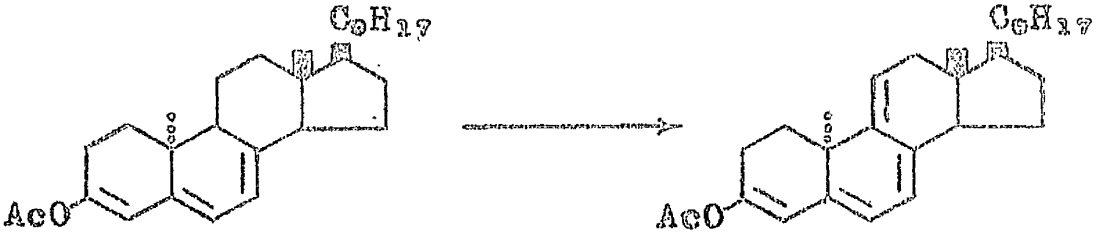
[*] These residues, obtained from Glaxo Laboratories Ltd. contain a mixture of calciferyl and lumisteryl 3,5-dinitrobenzoates, which can be separated by fractional crystallisation.

3-acetoxylumista,3,5,7,22-tetraene (LXIX).




(XIII) (LXVIII) (LXIX)

Photo-oxygenation of the enol acetate gave a red gum, which could not be crystallised, but chromatography on silica gel gave 3-acetoxylumista-3,5,7,9(11),22-pentaene (LXX), a series of oily fractions which were thought to be partially decomposed epidioxide, a trace of unidentifiable solid and an αβ-unsaturated ketone.




(LXIX)

$\lambda_{max.}$ 305, 317 and 330 mμ.

$\lambda_{max.}^{calc.}$ 318 mμ.

$\nu_{max.}$ 1754 (enol acetate), 1650 and 1221 cm.$^{-1}$

(LXX)

$\lambda_{max.}$ 320, 334 and 353 mμ.

$\lambda_{max.}^{calc.}$ 358 mμ.

$\nu_{max.}$ 1757 (enol acetate), 1661 and 1224 cm.$^{-1}$

There is no reference in the literature to the preparation of 3-acetoxylumista-3,5,7,9(11),22-pentaene (LXX), but it is identified by its ultraviolet and

infrared spectra. An attempt was made to synthesize this enol acetate, by oxidizing dehydrolumisterol (XIV, R = H) to the 3-ketone and enol acetylating the ketone, but Oppenauer oxidation of dehydrolumisterol with aluminium tert.-butoxide and acetone in benzene gave an impure product, which after chromatography on deactivated alumina gave unchanged dehydrolumisterol and an uncrystallisable red oil. The ultraviolet absorption of the oil however, showed that it contained a high proportion of αβ-unsaturated ketone, so it was refluxed directly with acetic anhydride and pyridine, but again a red oil was obtained, the ultraviolet spectrum of which showed that there was no enol acetate present.

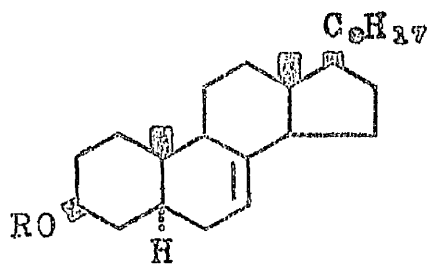
It was thought that the oily fractions obtained by chromatography of the crude photo-oxygenation product might contain some partially decomposed epidioxide, so they were refluxed briefly with ethanolic potassium hydroxide solution, to find out whether a rearrangement product could be obtained. The product of this reaction was a brown gum, even after chromatography on silica gel, so it would appear that the epidioxide of 3-acetoxylumista-3,5,7,22-tetraen is extremely unstable and quickly decomposes to give intractable oils.

The αβ-unsaturated ketone obtained, is thought to have come from an impurity in the lumisterol used, because a second photo-oxygenation experiment gave 3-acetoxylumista-3,5,7,9(11),22-pentaene as the sole crystalline product, although the two runs were carried out at completely different dilutions. The fact that the αβ-unseturated ketone shows no enol acetate peak in the infrared makes it very unlikely to have been a true photo-oxygenation product, so it was not investigated any further.

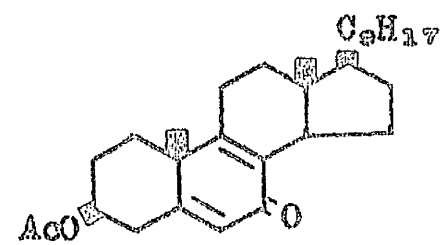
By-products from the photo-oxygenation of ergosteryl acetate.

By-products from the photo-oxygenation of ergosteryl acetate

The photo-oxygenation of ergosteryl acetate was carried out at one stage to obtain ergosterol peroxide for a synthesis which was described earlier, so it was decided to examine the mother liquors from the crystallisation of the peroxide, for the presence of any other products. Chromatography of the evaporated mother liquors on deactivated alumina gave 5,6-dihydroergosteryl acetate (LXXI; R = Ac), a further small quantity of ergosteryl acetate peroxide and 3β-acetoxyergosta-5,8(9),22-trien-7-one (LXXII).




(LXXI)

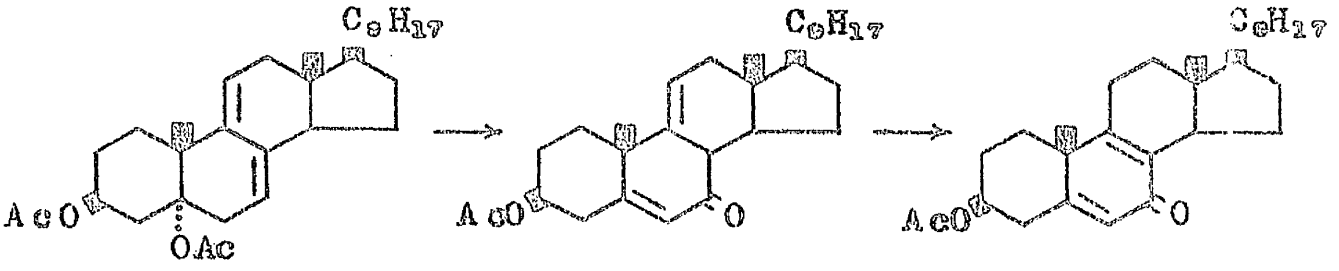



(LXXII)

5,6-Dihydroergosterol (LXXI, R = H) is usually present as an impurity in ergosterol and this is undoubtedly the source of the material obtained here.

The physical constants of the 3β-acetoxyergosta-

5,8(9),22-trien-7-one (LXXII) obtained, correspond quite closely with those of the same compound obtained by Elks et al., by oxidation of 3β,5α-diacetoxyergosta-7,9(11),22-triene (LXXIII) and rearrangement of the intermediate 3β-acetoxyergosta-5,9(11),22-trien-7-one (LXXIV).[43,44]




(LXXIII) (LXXIV) (LXXII)

The production of this cross-conjugated ketone (LXXII) by photo-oxygenation of ergosteryl acetate 5,8(9),22-trien-7-one (XVIII) from lumisteryl acetate.[15,41]

No trace of the expected 9(11)-dehydroergosteryl[15] acetate was detected.

yl
acetate was detected.

# E X P E R I M E N T A L

## General experimental procedures.

Melting points. These were determined on a Kofler hot stage, and also in certain specified cases, in a sealed, evacuated tube,

Optical rotations. Unless otherwise stated, these were determined for chloroform solutions at room temperature.

Ultraviolet spectra. Unless otherwise stated, these refer to ethanol solutions. Alterations in spectra in alkaline solution were observed by diluting ethanol solutions of the compounds with 0.1N NaOH. Blank solutions were prepared by a similar dilution of pure ethanol.

Infrared spectra. Unless otherwise stated, these are for potassium chloride discs. In certain specified cases, spectra were run in carbon tetrachloride solution at two concentrations: approx. 0.03M for the region 2-15 $\mu$. and approx. 0.0025M for the region 2-4 $\mu$. using 1 cm. Infrasil Quartz cells in the latter case.

Reagents. Alumina (Spence Grade 'H') was deactivated by the method of Farrar, Hamlet, Hembest and Jones.[46]

The silica gel used for chromatography was Hopkin and Williams, M.F.C. The eosin used was found to vary in purity, some samples giving impurities, which contaminated the photo-oxygenation products. Petroleum ether had boiling point 60-80°.

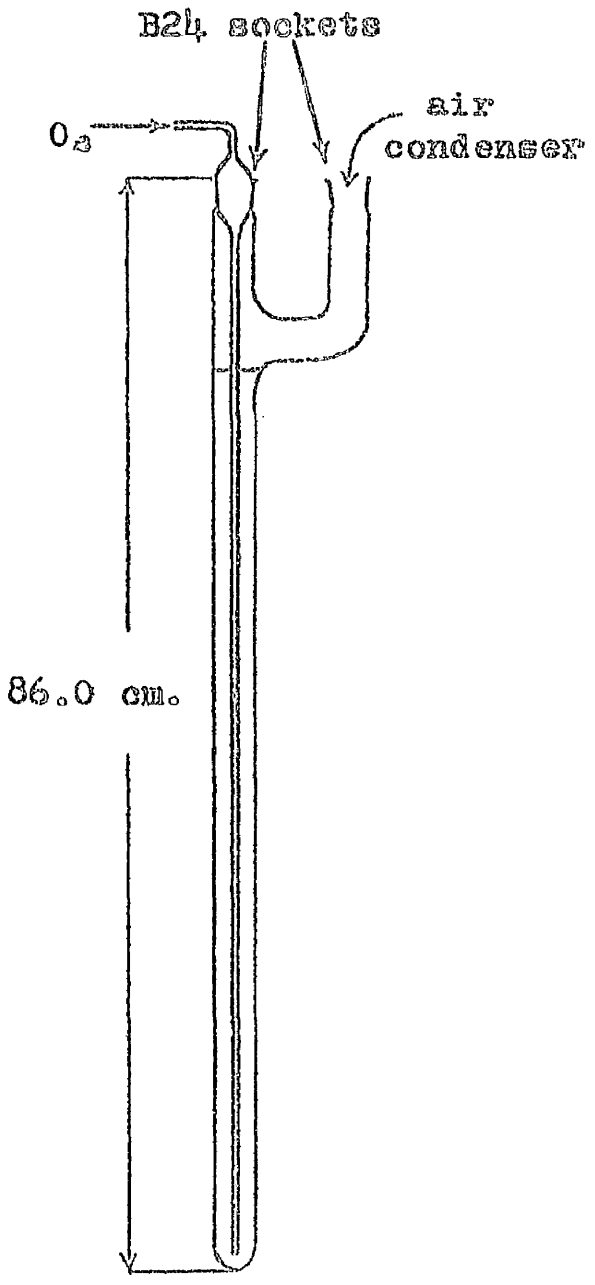
Photo-oxygenation techniques. Oxygenation and irradiation were carried out by two methods.

Method (A).- The steroid was dissolved in benzene and ethanol. Eosin (a filtered solution of the dye in ethanol) and pyridine were added and the solution placed in a vertical, Pyrex glass tube, sealed at the lower end (see Fig.1). Oxygen was passed through, while the tube was illuminated by a 22", 20 watt, "warm white", fluorescent tube, placed vertically and parallel to the reaction tube. No refluxing of the solvents took place. Two solutions were often run simultaneously, using two tubes and the procedure repeated once or twice, before bulking the solutions and evaporating them all together. The reaction was followed spectrophotometrically by removing a sample (1 ml.) of the solution every hour or two hours, diluting this sample to 100 ml. with ethanol and determining the ultraviolet spectrum against a blank

solution, containing ethanol, benzene, pyridine and eosin in correct proportions. As the reaction proceeded, the intensity of the peak due to the 5,7-diene system slowly diminished.

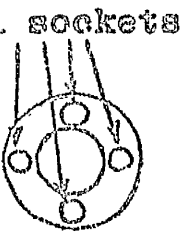
Method (B).- The solution was prepared as described in method (A), but it was placed in a large, annular Pyrex vessel (see Fig.2 and illustration). This allowed oxygen to be passed into the solution at three points and permitted more efficient use of the light, as the fluorescent tube passed down through the centre of the vessel. The reaction was followed by spectrographic analysis, as in method (A).

In both method (A) and method (B), the vessels were filled with solution to allow maximum use of the lighting. Changes in concentration did not appear to have any effect.

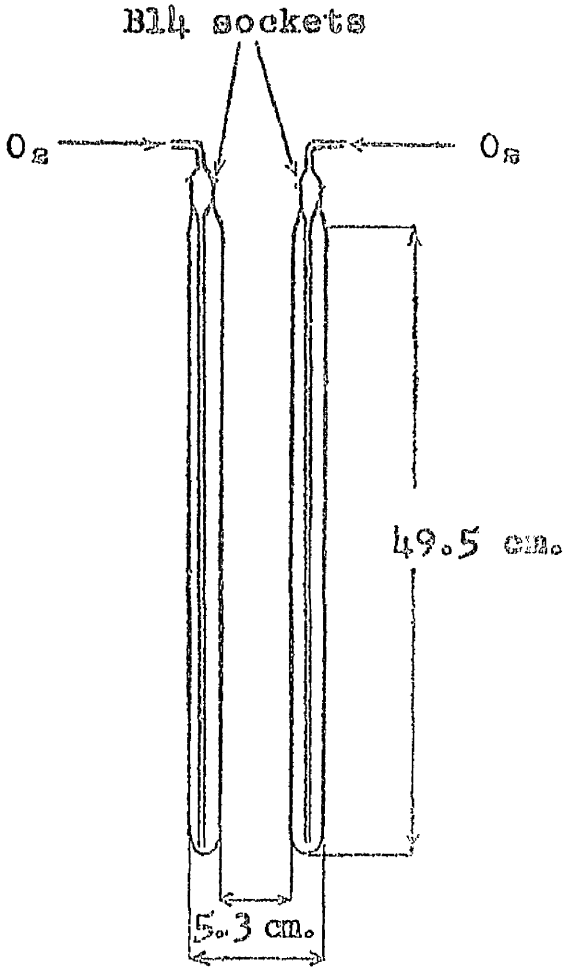



Inside diameter 2.7 cm.
Outside diameter 2.9 cm.
Capacity 430 ml.

Fig. 1




Plan of annular vessel




9.8 cm.
Capacity 1500 ml.

Section through annular vessel

Fig. 2

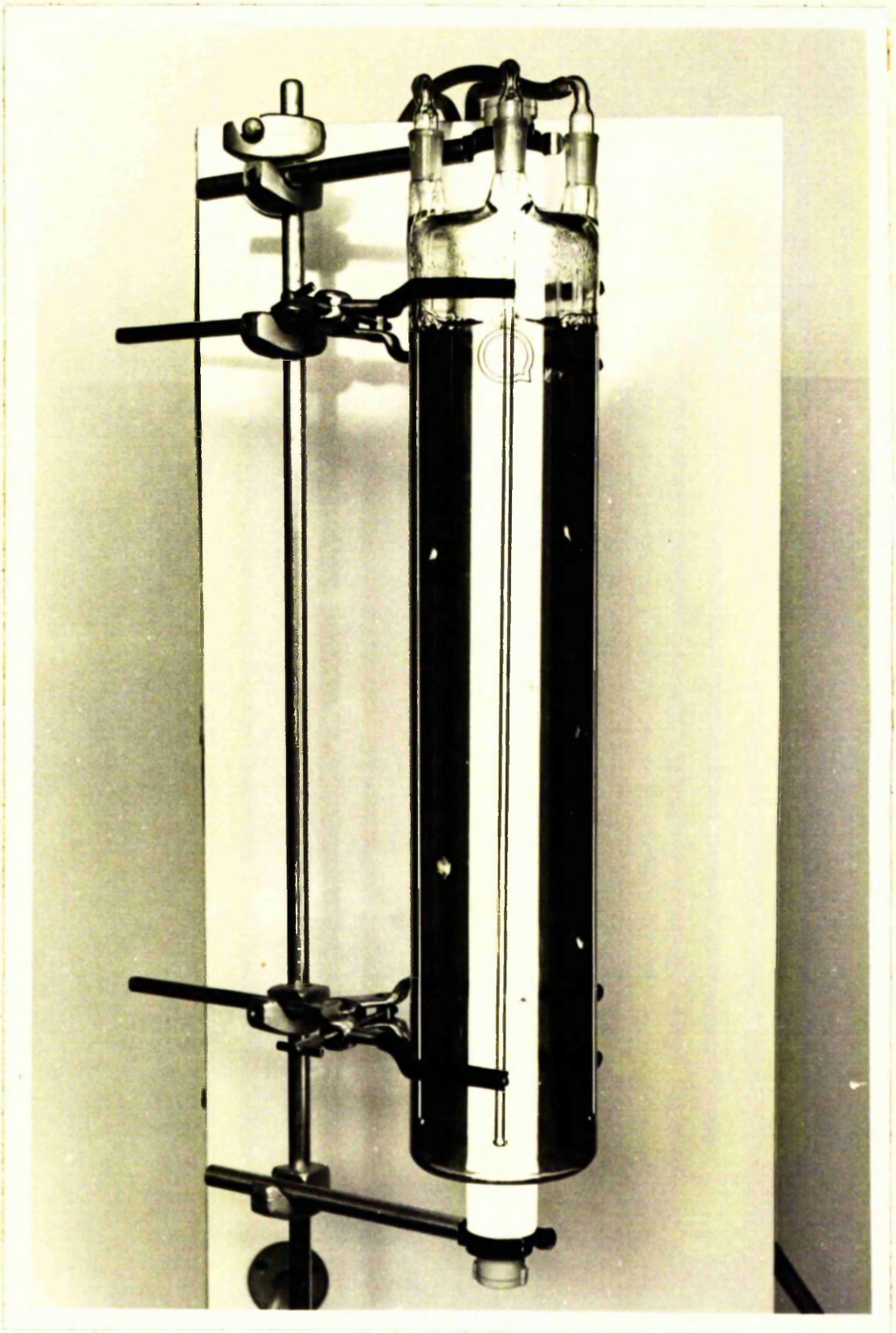


Photo-oxygenation of 3-acetoxyergosta-3,5,7,22-tetraene.

Ergosta-4,7,22-triene-3-one[24] (XXXIV).- Ergosterol (50 g.) was dissolved in benzene (1200 ml.) and the mixture distilled until no more water was entrained in the distillate (approx. 100 ml.). Aluminium isopropoxide (60 g.) and acetone (500 ml.) were then added, the mixture refluxed for 7 hours with stirring and allowed to stand overnight. The benzene solution was washed with water, dilute hydrochloric acid, potassium bicarbonate solution and finally with water. The combined washings were extracted twice with ether and the ether extracts washed with water. The benzene and ether solutions were combined, dried over sodium sulphate and evaporated to dryness under reduced pressure to give a yellow, crystalline solid (54.5 g.). Chromatography on deactivated alumina (500 g.) and recrystallisation of the material eluted by 1:1 petroleum ether-benzene from acetone-methanol gave the product as yellow needles (21.2 g.), m.p. 127-132° (partially recrystallises and melts up to 200°), $[\alpha]_D$ - 3.5° (c 1.02), $\lambda_{max.}$ 206 and 238 mμ. (ε 9,000 and 14,000), $\nu_{max.}$ 1682 (αβ-unsaturated ketone), 1629, 1460, 1370 and 966 cm.$^{-1}$ Heilbron et al[24] gave m.p. 132°,

$[\alpha]_D$ - 0.8°, $\lambda_{max.}$ 230 mμ.( ε 20,000). A second crop (4.75 g.), m.p. 123-131°, $[\alpha]_D$ - 5.5° (c 0.76) was obtained from the mother liquor.

3-Acetoxyergosta-3,5,7,22-tetraene[24] (XXXV).- A solution of ergosta-4,7,22-triene-3-one (24 g.) in pyridine (60 ml.) and acetic anhydride (60 ml.) was heated under reflux for 3 hours. Pale yellow plates (24.2 g.) came down on cooling and were recrystallised from ethyl acetate-methanol to give 3-acetoxyergosta-3,5,7,22-tetraene (XXXV) as almost colourless plates (20.5 g.), m.p. 141.5-151° (146-148° in vacuo), $[\alpha]_D$ - 153° (c 1.0), $\lambda_{max.}$ 206, 302, 315 and 330 mμ. ( ε 10,200, 18,000, 22,800 and 16,400), $\nu_{max.}$ 1767 (enol acetate), 1662 and 1640 cm.$^{-1}$ Heilbron et al[24] give m.p. 146°, $[\alpha]_D$ - 143°, $\lambda_{max.}$ 316.5 mμ. ( ε 21,400).

The foregoing preparations are typical of many which were carried out.

Enol acetylation of ergosta-4,7,22-trien-3-one, using acetic anhydride-acetyl chloride. - Ergosta-4,7,22-trien-3-one (1 g.) in acetic anhydride (8 ml.) and acetyl chloride (8 ml.) was heated under reflux for 6 hours. The excess acetyl chloride was removed under reduced pressure and the solution filtered to give a light brown

crystalline solid (683 mg.), m.p. 135-147.5°. Recrystallisation from ethyl acetate-methanol gave 3-acetoxyergosta-3,5,7,22-tetraene (XXXV) (556 mg.), m.p. 140-149°, $[\alpha]_D$ - 145°, (c 0.75), $\lambda_{max.}$ 210, 302, 315 and 330 mμ. ($\varepsilon$ 6,600, 17800 22,600 and 16,100), $\nu_{max.}$ 1765, 1661, 1462, 1368, 1216 and 1118 cm.$^{-1}$, identical with the infrared spectrum of material prepared above.

Enol acetylation of ergosta-4,7,22-triene-3-one.

Enol acetylation of ergosta-4,7,22-triene-3-one, using isopropenyl acetate-concentrated sulphuric acid.- A solution of ergosta-4,7,22-trien-3-one (1 g.) in isopropenyl acetate (3 ml.) and concentrated sulphuric acid (1 drop) was refluxed for two hours. The crystalline solid which was obtained on cooling was taken up in ether and the ether solution washed briefly with water and dried over sodium sulphate. Evaporation gave a brownish, crystalline solid (1.19 g.), which was recrystallised twice from ethyl acetate-methanol to give 3-acetoxyergosta-3,5,7,22-tetraene as pale yellow plates (XXXV) (157 mg.), m.p. 140-149°, $[\alpha]_D$ - 132° (c 0.75), $\lambda_{max.}$ 210, 302, 315, and 330 mμ. ($\varepsilon$ 8,700, 18,100, 22,400 and 16,300), $\nu_{max.}$ 1768, 1660, 1460, 1370, 1220 and 1118 cm.$^{-1}$

identical with the infrared spectrum of material prepared above. Evaporation of the combined mother liquors gave a dark red gum, which would yield no further crystalline material, even after chromatography on silica gel (30 g.).

Oxygenation and irradiation of 3-acetoxyergosta-3,5,7,22-tetraene (XXXV). - 3-Acetoxyergosta-3,5,7,22-tetraene (3 g.) was dissolved in benzene (125 ml.) and ethanol (125 ml.). Eosin (50 ml. of a filtered solution of 1 g. of the dye in 100 ml. of ethanol) and pyridine (2.5 ml.) were added and the solution oxygenated and irradiated by method (A). Spectroscopic analysis showed that the reaction was complete in about 12 hours, although it was not stopped until after 18 hours. The solution was then taken to dryness, the residue dissolved as far as possible in ether, the solution filtered and passed through a short column of deactivated alumina to remove any dissolved eosin. The resulting yellow solution was again taken to dryness and the gum (2.07 g.) crystallised from methanol to give a pale yellow solid (981 mg.), m.p. 104-152°, $\lambda_{max.}$ 207, and 258 mμ. ($E^{1\%}_{1cm.}$ 259 and 33.8), $\nu_{max.}$ 1772, 1689, 1460, 1450, 1380 and 1230 $cm.^{-1}$

The crystalline material and material from the mother liquor were recombined and chromatographed on deactivated alumina (80 g.). Elution with 4:1 petroleum ether-benzene gave a yellow gum, which was crystallised from methanol to give a pale yellow solid (341 mg.), m.p. 118-152°, $\lambda_{max.}$ 207 and 358 mμ. ($E^{1\%}_{1cm.}$ 262 and 59.7), $\nu_{max.}$ 1770, 1688, 1460, 1379 and 1229 cm.$^{-1}$ Elution with 1:4 petroleum ether-benzene gave a yellow solid (66 mg.), m.p. 150-193° (decomp.), $\lambda_{max.}$ 205 and 366 mμ. ($E^{1\%}_{1cm.}$ 230 and 252), $\nu_{max.}$ 1720, 1651, 1589, 1451 and 1371 cms.$^{-1}$

In another experiment, 3-acetoxyergosta-3,5,7,22-tetraene (10 g.) was dissolved in benzene (325 ml.) and ethanol (275 ml.). Eosin (a filtered solution of 1 g. of the dye in 100 ml. of ethanol) and pyridine (5 ml.) were added and the solution oxygenated and irradiated by method (A). The reaction was allowed to proceed for 16 hours, although spectroscopic analysis showed that it was complete after 12 hours. The reaction was then repeated with two further 10 g. portions of enol acetate, and again allowed to proceed for 16 hours in each case

The solutions from the three runs were combined and taken to dryness to give a deep red gum, which was dissolved as far as possible in 1:4 petroleum ether-benzene, filtered and chromatographed on silica gel (800 g.). Elution with 1:4 petroleum ether-benzene gave a yellow, crystalline solid, which after two recrystallisations from ethyl acetate-methanol gave 3-acetoxy-ergosta-3,5,7,9(11),22-pentaene (XXXVI) (55 mg.) as yellow plates, m.p. 143-160° (158-161° *in vacuo*), $[\alpha]_D$ - 234° (*c* 0.52)(Found: C,82.9; H,9.9. Calc. for $C_{30}H_{42}O_2$: C,82.9; H,9.7%), $\lambda_{max.}$ 206, 236, 338, 354 and 373 mμ. (ε 6,300, 12,300, 10,300, 13,700 and 10,500), $\nu_{max.}$ 1764 (enol acetate), 1656, 1458, 1368, 1211 and 1130 cm.$^{-1}$ Heilbron *et al*[24] give m.p. 161°, $[\alpha]_D$ - 212°, $\lambda_{max.}$ 356 mμ. (ε 17,400). Elution with 9:1 benzene-ether gave yellowish, crystalline material, which after two recrystallisations from methanol gave 3-acetoxy-5α,8α-epidioxyergosta-3,6,22-triene (XXXVII) as almost colourless needles (5.6 g.), m.p. 144-152° (148-149° *in vacuo*) $[\alpha]_D$ + 0.4° (*c* 0.94), $\lambda_{max.}$ 212 mμ. (ε 7,700), $\nu_{max.}$ 1764 (enol acetate), 1681, 1458, 1374 and 1227 cm.$^{-1}$ A sample (500 mg.) was given three further recrystallisations

from ethanol to give colourless needles (243 mg.), m.p. 144-152.5 (148-149° *in vacuo*), $[\alpha]_D$ + 1.1 (*c* 0.95). (Found: C, 77.3%; H, 9.3. $C_{30}H_{44}O_6$ requires C, 76.9%; H, 9.6%).

Elution with 7:3 benzene-ether gave orange crystalline material, which was recrystallised twice from methylene chloride-isopropyl ether to give 3-*acetoxy*-5ξ,14ξ-dihydroxyergosta-3,8(9),22-*trien*-7-*one* (XXXVIII), as almost colourless needles (1.01 g.) m.p. 182-200° (190-192° *in vacuo*), $[\alpha]_D$ - 111° (*c*, 0.55)(Found: C, 74.3%; H, 9.3 $C_{30}H_{44}O_6$ requires C, 74.3%; H, 9.15%), $\lambda_{max.}$ 240 mμ. (ε 10,500), $\nu_{max.}$ 3250 (hydrogen bonded hydroxyl), 1767 (enol acetate), 1692 (αβ-unsaturated ketone), 1634 and 1212 cm.$^{-1}$ $\nu_{max.}$ (in $CCl_4$) 3610 (free hydroxyl), 3509 (intermolecular hydrogen bonded hydroxyl) and 3360 cm.$^{-1}$ (intramolecular hydrogen bonded hydroxyl).

This photo-oxygenation reaction and the separation of the products is typical of several, which were carried out.

*Chromatography of 3-acetoxy-5α,8α-epidioxyergosta-3,6,22-triene.* - 3-Acetoxy-5α,8α-epidioxyergosta-3,6,22-triene (XXXVII) (520 mg.) was dissolved in 1:1 petroleum ether-benzene and chromatographed on silica gel (28 g.).

| Fraction | Volume | Solvent | Weight | Melting point | $[\alpha]_D$ | Description |
|---|---|---|---|---|---|---|
| 1 | 50 ml. | Petrol 50% Benzene 50% | 7 mg. | 144-152.5° | +8.3° | Fine, colourless needles. |
| 2 | " | " | 29 " | 143.5-152° | +1.6° | " |
| 3 | " | " | 20 " | 146-153° | +2.7° | Colourless, feathery needles. |
| 4 | " | " | 15 " | 141-153° | +6.3° | " |
| 5 | " | " | | | | " |
| 6 | " | " | 13 " | 145-152.5° | +2.7° | " |
| 7 | " | " | 6 " | 141-153° | +2.3° | " |
| 8 | " | " | | | | " |
| 9 | " | " | 12 " | 143-152° | +2.6° | " |
| 10 | " | " | 62 " | 140-152° | +7.8° | Pale, yellow crystall. solid. |
| 11 | 100 ml. | Petrol 30% Benzene 70% | | | | " |
| 12 | 50 ml. | " | | | | Colourless, feathery needles. |
| 13 | 100 ml. | " | 16 " | 138-152° | +5.0° | " |
| 14 | " | Benzene | 15 " | 126-152.5° | +2.6° | Slightly yellow needles |
| 15 | " | Benzene 90% Ether 10% | | | | " |

N.B. The weights, melting points and optical rotations all apply to the fractions after recrystallisation from methanol.

The ultraviolet and infrared absorption spectra of fractions 2, 9, 10 and 14 also showed that these fractions were identical.

4-Hydroxyergosta-4,6,8(14),22-tetraen-3-one (XLI, R = H). - 3-Acetoxy-5α,8α-epidioxyergosta-3,6,22-triene (1 g.) was dissolved in ethanol (40 ml.) and a solution of potassium hydroxide (2 g.) in water (5 ml.) added. The resulting orange-red solution was refluxed for 10 minutes during which time it became deep reddish-brown. The solution was cooled, acidified with concentrated hydrochloric acid and extracted twice with ether. The ether extracts were washed with water, dried over sodium sulphate and evaporated to dryness to give an orange, crystalline solid (875 mg.), which was dissolved in benzene and chromatographed on silica gel (40 g.).

Elution with benzene gave a yellow, crystalline solid, which was recrystallised from methylene chloride-isopropyl ether to give 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one (XLI, R = H) as fine, bright yellow needles (211 mg.), m.p. 187-198° (197-204 in vacuo), $[\alpha]_D$ + 733° (c 0.84) (Found: C,82.6; H,9.9. $C_{28}H_{40}O_2$ requires C,82.3; H,9.9%), $\lambda_{max.}$ 205, 262 and 370 mμ. (ε 9,200, 6,600 and 21,000), $\lambda_{max.}$ (in NaOH) 225, 277 and 390 mμ. (ε 36,100, 6,600 and 14,500), $\nu_{max.}$ 3400 (hydrogen bonded hydroxyl),

1656 (hydrogen bonded unsaturated ketone), 1600 and 1370 cm.$^{-1}$, $\nu_{max.}$ (in $CCl_4$) 3448 cm.$^{-1}$ (hydrogen bonded hydroxyl).

Acetylation with acetic anhydride and pyridine at room temperature gave 4-acetoxyergosta-4,6,8(14),22-tetraen-3-one (XLI, R = Ac) as yellow needles, m.p. 130-133° (from methylene chloride-methanol), $[\alpha]_D$ + 624° (c 0.60) (Found: C,80.1; H,9.5. $C_{30}H_{42}O_3$ requires C,79.95; H,9.3%), $\lambda_{max.}$ 250 and 362 mμ. (ε 4,700 and 17,900), $\lambda_{max.}$ (in NaOH) 218 and 398 mμ. (ε 13,200 and 11,200), $\nu_{max.}$ 1773 (enol acetate), 1681 (unsaturated ketone), 1597, 1319 and 1206 cm.$^{-1}$, $\tau$ values (in $CCl_4$) 3.82 doublet ($C_6$), 3.39 doublet ($C_7$) and 4.72 unresolved (side chain olefinic protons) (J 11 c/sec.).

The 4-benzoate (XLI, R = Bz), prepared from benzoyl chloride and pyridine, formed pale yellow crystals, m.p. 147-154°, $[\alpha]_D$ + 520° (c 0.59), from methylene chloride-methanol (Found: C,82.35 ; H,8.7 . $C_{35}H_{44}O_3$ requires C,82.0; H,8.65%), $\lambda_{max.}$ 205, 232, 278 and 355 mμ. (ε 15,500, 18,000, 6,800 and 25,200), $\lambda_{max.}$ (in NaOH) 218 and 355 mμ. (ε 42,500 and 22,600), $\nu_{max.}$ 1751 (enol benzoate) 1681 (3-ketone), 1642, 1600 and 701 cm.$^{-1}$

Catalytic hydrogenation of 3-acetoxy-5α,8α-epidioxy-ergosta-3,6,22-triene (XXXVII). - 3-Acetoxy-5α,8α-epidioxyergosta-3,6,22-triene (200 mg.) in ethyl acetate (25 ml.) was shaken with hydrogen and prereduced platinum oxide (150 mg.). Absorption ceased after 30 minutes, when 33-38 ml. of hydrogen had been consumed (30 ml. = 3 moles). The catalyst was removed by filtration and the solution evaporated to dryness *in vacuo* to give a white, crystalline solid (185 mg.), m.p. 160-203.5°, $[\alpha]_D$ - 55° (*c* 0.59), $\lambda_{max.}$ 208 and 214 mμ. ($E^{1\%}_{1cm.}$ 256 and 242), $\nu_{max.}$ 3367, 3268, 3226, 1462, 1379 and 971 cm.$^{-1}$ This material was dissolved in 7:3 petroleum ether-benzene and chromatographed on deactivated alumina (20 g.).

Elution with benzene gave a white, crystalline solid, which was recrystallised from methylene chloride-methanol to give long needles (67 mg.), m.p. 140-207°.

Elution with 7:3 benzene-ether gave a white, crystalline solid, which was again recrystallised from methylene chloride-methanol to give needles (15 mg.), m.p. 134-176°.

In another experiment, 3-acetoxy-5α,8α-epidioxy-ergosta-3,6,22-triene (500 mg.) in ethyl acetate (40 ml.)

was shaken with prereduced platinum catalyst (200 mg.) for 5 hours, by which time 122 ml. of hydrogen had been consumed and a white solid had been deposited. The white solid and the platinum oxide were filtered off and the filtrate evaporated to dryness to give a white, crystalline material (227 mg.), m.p. 140-206°, $[\alpha]_D$ - 43.3° (c 0.56), $\nu_{max.}$ 3280, 1742, 1471 and 1387 cm.$^{-1}$ The platinum oxide was then washed free of the white solid, using chloroform, and the chloroform solution evaporated to dryness to give a white, crystalline solid (214 mg.), m.p. 141-208°, $[\alpha]_D$ - 37.6° (c 0.46), $\nu_{max.}$ 3250, 1475 and 1389 cm.$^{-1}$

These two products were combined and allowed to stand overnight with pyridine and acetic anhydride. No acetylation took place however, and the material was recovered unchanged. This material was again dissolved in pyridine-acetic anhydride and the solution allowed to stand on the steam-bath for 1 hour, but this was equally unsuccessful.

The material was finally dissolved in 1:9 petroleum ether-benzene and chromatographed on deactivated alumina (25 g.).

Elution with 1:9 petroleum ether-benzene gave white, crystalline material (100 mg.), which after two

recrystallisations from isopropyl ether-methanol gave flat, colourless needles (18 mg.), m.p. 134-147°, $[\alpha]_D$ + 17.5° (c 0.49)(Found: C,83.2; H,12.0. Calc. for $C_{28}H_{48}O_2$: C,80.7; H,11.6%. Calc. for $C_{28}H_{48}O$: C,83.9; H,12.18%), $\nu_{max.}$ 3460, 1462 and 1377 cm.$^{-1}$

Elution with 9:1 benzene-ether gave white, crystalline material (45 mg.), which on recrystallisation from isopropyl ether gave fine, colourless needles (14 mg.), 142-199°, $[\alpha]_D$ - 44.6° (c 0.45) (Found: C,79.6; H,11.5. Calc. for $C_{28}H_{48}O_3$: C,80.7; H,11.6%), $\nu_{max.}$ 3195, 1460 and 1377 cm.$^{-1}$

Elution with 1:1 benzene-ether gave white, crystalline material (32 mg.), which on recrystallisation from isopropyl ether gave fine, colourless needles (14 mg.), m.p. 151-222°, $[\alpha]_D$ - 45.2° (c 0.49)(Found: C,73.5; H,10.8), $\nu_{max.}$ 3257, 1727, 1468 and 1377 cm.$^{-1}$

3β-Hydroxy-5α,8α-epidioxyergosta-6,22-diene (Ergosterol peroxide, III, R = H). $^{5,10}$ Ergosteryl acetate (20 g.) was dissolved in ethanol (3000 ml.) together with eosin (150 mg.) and the solution irradiated and heated under reflux by means of a 500 watt, tungsten filament lamp, while oxygen was passed into the solution

continuously. The reaction was stopped when the solution no longer showed an absorption maximum at 280 mμ. The solution was concentrated to 500 ml. and on cooling gave ergosteryl acetate peroxide (III, R = Ac) as plates (13.1 g.), m.p. 150-206°. Recrystallisation from chloroform-ethanol gave flat needles (9.57 g.), m.p. 199-211° (recrystallisation and much sweating from 146°). A second recrystallisation from chloroform-ethanol again gave flat needles (8.62 g.), m.p. 199-207° (recrystallisation 140-170° and much sweating 170-199°), $[\alpha]_D$ - 8.3° (c 0.99), $\nu_{max.}$ 1739 (acetate), 1458, 1370, 1225 and 1027 cm.$^{-1}$ Clayton, Henbest and Jones[10] give m.p. 199-206°, $[\alpha]_D$ - 19°.

Hydrolysis of the acetate with methanolic potassium hydroxide solution gave 3β-hydroxy-5α,8α-epidioxyergosta-6,22-diene (III, R = H) as a slightly yellow, crystalline solid, m.p. 176-181.5°. Recrystallisation from methanol gave almost colourless plates, m.p. 178-182° (recrystallises 85-90°), $[\alpha]_D$ - 24.6° (c 0.92), $\nu_{max.}$ 3448 (OH), 1458, 1372 and 1036 cm.$^{-1}$ Clayton, Henbest and Jones record m.p. 181-185°, $[\alpha]_D$ - 36°.

Oppenauer oxidation of ergosterol peroxide (III, R = H). - Ergosterol peroxide (750 mg.) in acetone (20 ml.) was refluxed for four hours with a 25% solution of aluminium tert.-butoxide in toluene (7.5 ml.). The solution was cooled and diluted with ether, the ether extract washed with water, dried over sodium sulphate and evaporated to dryness several times with xylene to remove mesityl oxide. The product was obtained as a yellow gum (634 mg.), which was dissolved in 1:1 petroleum ether-benzene and chromatographed on silica gel (30 g.).

Elution with 1:9 petroleum ether-benzene gave yellow, crystalline material (64 mg.), which was recrystallised twice from isopropyl ether to give 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one (XLI, R = H) as fine, pale yellow needles (9 mg.), m.p. 193-198° (195-196° in vacuo) $\lambda_{max.}$ 204, 214, 256 and 371 mμ. (ε 5,800, 4,400, 1,900 and 22,000), $\lambda_{max.}$ (in NaOH) 218, 225, 272 and 400 mμ. (ε 36,100, 31,000, 5,100 and 15,000), $\nu_{max.}$ 3378 (OH), 1645 (3-ketone) and 1590 cm.$^{-1}$, identical with the infrared spectrum of pure hydroxy-ketone. The combined mother liquors gave a second crop of yellow needles (5 mg.), m.p. 146-197°, $[\alpha]_D$ + 539° (c 0.45).

Elution with 4:1 benzene-ether gave almost colourless crystalline material (105 mg.), m.p. 174-180°, which on recrystallisation from methanol gave unchanged ergosterol peroxide as flat, colourless needles (80 mg.), m.p. 172-181°, $[\alpha]_D$ - 23.2° (c 0.52), $\nu_{max.}$ 3425 (OH), 1453 and 1370 cm.$^{-1}$ identical with the infrared spectrum of the starting material.

Chromium trioxide-pyridine oxidation of ergosterol peroxide. - Ergosterol peroxide (1.6 g.) in pyridine (15 ml.) was added to a slurry of chromium trioxide (1.6 g.) in pyridine (25 ml.) and the mixture allowed to stand overnight at room temperature. The excess of oxidant was destroyed by adding dilute hydrochloric acid and sodium sulphite, then the product was extracted twice with ether. The combined ether extracts were washed with dilute hydrochloric acid and water, dried over sodium sulphate and evaporated to dryness to give yellow, amorphous material (1.22 g.), $[\alpha]_D$ + 91° (c 1.12), $\nu_{max.}$ 3390, 1705, 1669, 1456, 1370 and 966 cm.$^{-1}$ This material quickly became yellow on standing, and attempts to crystallise it caused it to become darker and finally resulted in a brown gum, which could not be crystallised. This gum was dissolved

in benzene and chromatographed on silica gel (50 g.). Elution with benzene gave yellow, crystalline material (48 mg.), which was recrystallised twice from isopropyl ether to give 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one (XLI, R = H) as fine, yellow needles (6 mg.), m.p. 188-195° (decomp.) (192-194° *in vacuo*), $\nu_{max.}$ 3425 (OH), 1661 (3-ketone), 1600 and 1372 cm.$^{-1}$, identical with the infrared spectrum of pure hydroxy-ketone. The mother liquors gave a second crop (24 mg.), m.p. 173-194°, $[\alpha]_D$ + 542° (*c* 0.53).

This experiment was repeated, but before evaporating the ether extracts to dryness, a little pyridine (0.5 ml.) was added to them. This gave a semi-crystalline, yellow solid (1.41 g.), which again deteriorated to a red gum on attempted recrystallisation. This gum (1.4 g.) in ethanol (40 ml.) was refluxed for 1 hour with a solution of potassium hydroxide (2.5 g.) in water (10 ml.). The deep, reddish-brown solution was cooled, acidified with concentrated hydrochloric acid and the product extracted twice with ether. The combined ether extracts were washed well with water, dried over sodium sulphate and evaporated to dryness to give a red gum (1.36 g.),

which was chromatographed on silica gel (50 g.). Elution with benzene gave yellow, crystalline material (63 mg.), which was recrystallised from isopropyl ether to give 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one as fine, bright yellow needles (14 mg.), m.p. 180-198° (192-194° in vacuo), $\nu_{max.}$ 3425 (OH), 1653 (3-ketone), 1595 and 1370 cm.$^{-1}$, identical with the infrared spectrum of pure hydroxy-ketone. Elution with 9:1 benzene-ether gave a yellowish, crystalline solid (248 mg.), which was recrystallised from methanol to give an almost colourless, crystalline solid (34 mg.), m.p. 205-247° (decomp.). A second recrystallisation from methylene chloride gave Ketol A as colourless, feathery needles (15 mg.), m.p. 205-243° (decomp.), $[\alpha]_D$ + 99° (c 0.57) (Found: C,79.9; H,9.95%), $\lambda_{max.}$ 213 and 236 mμ. ($E^{1\%}_{1cm.}$ 258 and 269), $\lambda_{max.}$ (in NaOH), 220 and 248 mμ. ($E^{1\%}_{1cm.}$ 507 and 276), $\nu_{max.}$ 3413, 1681, 1012 and 976 cm.$^{-1}$ Evaporation of the mother liquors and treatment of the residue with petroleum ether gave a second crop of almost colourless, crystalline material (56 mg.), m.p. 208-238° (decomp.).

The following procedure was then found to be the most satisfactory.

5α,8α-Epidioxyergosta-6,22-dien-3-one (XL). -

5α,8α-Epidioxyergosta-6,22-dien-3-one (XL). -

Ergosterol peroxide (1 g.) in pyridine (15 ml.) was added to a slurry of chromium trioxide (1 g.) in pyridine (10 ml.) and the mixture allowed to stand overnight at room temperature. A large volume of water (approx. 600 ml.) was added and the product extracted with ether. Before the ether and aqueous layers would separate however, the solution had to be centrifuged for 10 mins. The ether layer was pipetted off and the aqueous layer decanted into a separating funnel and re-extracted with ether. The combined ether layers were dried over sodium sulphate and evaporated at room temperature until only a small quantity of pyridine remained. Trituration with methanol gave almost colourless, crystalline material (280 mg.), m.p. 166-179°. The mother liquor (mother liquor A) was treated with petroleum ether (see below). Recrystallisation of the solid from ether-methanol (1 drop pyridine) gave 5α,8α-epidioxyergosta-6,22-dien-3-one (XL) as colourless plates (150 mg.), m.p. 162-180° (with a change of form to needles and decomposition)(161-165° in vacuo), $[\alpha]_D$ + 8.4° (c 0.55)(Found: C,78.6; H,10.0. $C_{28}H_{42}O_3$

requires C,78.8; H,9.9%), $\nu_{max.}$ 1709 (3-ketone), 1449, 1381, 1370 and 966 cm.$^{-1}$ The mother liquor gave a second crop of plates (15 mg.), m.p. 165-180°.

Mother liquor A was evaporated to dryness and the residue treated with petroleum ether to give buff, amorphous material (146 mg.). The mother liquor of this (mother liquor B) was treated with ethanolic potassium hydroxide (see below). The solid was crystallised from ether-methanol to give a poorly crystalline, buff solid (49 mg.), m.p. 178-188°, $[\alpha]_D$ + 29.8° (c 0.69) (Found: C,78.5; H,10.2%), $\lambda_{max.}$ 240 mμ. ($E^{1\%}_{1cm}$ 166.8), $\nu_{max.}$ 3448, 1656, 1458, 1368, 1041 and 966 cm.$^{-1}$ The mother liquor of this material (mother liquor C) was treated with ethanolic potassium hydroxide (see below).

Treatment of 5α,8α-epidioxyergosta-6,22-dien-3-one (XL) with ethanolic potassium hydroxide solution. - 5α,8α-Epidioxyergosta-6,22-dien-3-one (106 mg.) was dissolved in ethanol (2.5 ml.) and a solution of potassium hydroxide (0.2 g.) in distilled water (1 ml.) added. The reddish-brown solution was refluxed for 1 hour, then cooled, acidified with concentrated hydrochloric acid and extracted twice with ether. The combined

ether extracts were washed with water, dried over sodium sulphate and evaporated to dryness to give a reddish, crystalline solid (101 mg.), which was dissolved in benzene and chromatographed on silica gel (5 g.). Elution with benzene gave yellow, crystalline material (55 mg.), which was recrystallised twice from methylene chloride-isopropyl ether to give 4-hydroxyergosta-4,6,8(14),22-tetraene-3-one (XLI, R = H) as fine, bright yellow needles (16 mg.), m.p. 186-199° (decomp.), $[\alpha]_D$ + 774° (c 0.43) (Found: C,82.1; H,9.9. Calc. for $C_{28}H_{40}O_2$: C,82.3; H,9.9%), $\lambda_{max.}$ 206, 264 and 368 mμ. ( ε 6,600, 5,400 and 10,900), $\lambda_{max.}$ (in NaOH) 218, 282 and 404 mμ. ( ε 28,000, 5,400 and 9,100), $\nu_{max.}$ 3344 (OH), 1642 (3-ketone), 1587 and 1368 cm.$^{-1}$ Combined mother liquors gave a second crop of bright yellow needles (8 mg.), m.p. 144-187° (decomp.).

Treatment of mother liquor B material with ethanolic potassium hydroxide solution. - The mother liquor material (approx. 550 mg.) was treated with ethanolic potassium hydroxide solution, exactly as described above. Ether extraction gave a brown gum, which was dissolved in benzene

and chromatographed on silica gel (20 g.). Elution with benzene gave yellow, crystalline material (64 mg.), which was recrystallised from methylene chloride-isopropyl ether to give 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one (XLI, R = H) as yellow needles (25 mg.), m.p. 192-198° (decomp.), $\nu_{max.}$ 3436 (OH), 1656 (3-ketone), 1592 and 1370 cm.$^{-1}$, identical with the infrared spectrum of pure hydroxy-ketone.

Further elution gave only red gums, which could not be crystallised.

Treatment of mother liquor C material with ethanolic potassium hydroxide solution. - The mother liquor material (100 mg.) was treated with ethanolic potassium hydroxide solution, exactly as described above. Ether extraction gave a semi-crystalline, brown gum (91 mg.), which was dissolved in benzene and chromatographed on silica gel (5 g.). Elution with 1:1 benzene-ether gave crystalline-material (23 mg.), which was recrystallised from methylene chloride-petroleum ether to give Ketol A as almost colourless, crystalline material (10 mg.), m.p. 220-247° (decomp.), $\nu_{max.}$ 3436, 1681, 1014 and 976 cm.$^{-1}$,

identical with material obtained earlier. No other crystalline material was obtained.

Attempted acetylation of 3-acetoxy-5§ , 14§ -dihydroxyergosta-3,8(9),22-trien-7-one (XXXVIII). - 3-Acetoxy-5§ ,14§ -dihydroxyergosta-3,8(9),22-trien-7-one (106 mg.) was dissolved in pyridine (1.5 ml.) and acetic anhydride (1.5 ml.) and allowed to stand overnight at room temperature. Water was added and the product extracted twice with ether. The combined ether extracts were washed with dilute hydrochloric acid, potassium bicarbonate solution and water, dried over sodium sulphate and evaporated to dryness to give a slightly yellow, crystalline solid (104 mg.), m.p. 179-199°. Recrystallisation from methylene chloride-isopropyl ether gave unchanged starting material as almost colourless needles (70 mg.), m.p. 180-197°, $[\alpha]_D$ - 117° (c 0.51), $\lambda_{max.}$ 249 mμ. ( ε 11,600), $\nu_{max.}$ 3215 (OH), 1761 (enol acetate), 1689 (αβ-unsaturated ketone), 1626, 1361 and 1212 cm.$^{-1}$, identical with the infrared spectrum of the starting material.

7,14β -Dihydroxyergosta-4,6,8(9),22-tetraen-3-one (LVIIIb, R = H).- 3-Acetoxy-5β ,14β -dihydroxyergosta-3,8(9),22-trien-7-one (XXXVIII) (500 mg.) was suspended in ethanol (12.5 ml.) and potassium hydroxide (1 g.) in water (5 ml.) was added with stirring. The resulting red solution was stirred at room temperature for 1 hour, then acidified with concentrated hydrochloric acid to give a yellow solid, which was extracted with chloroform. The combined chloroform extracts were washed with water, dried over sodium sulphate and evaporated to dryness to give a yellow solid (425 mg.). Attempts were made to crystallise this material, but they resulted in decomposition to a red gum.

In another experiment, 3-acetoxy-5β ,14β dihydroxy-ergosta-3,8(9),22-trien-7-one (300 mg.) was treated in exactly the same way, but the product obtained on acidification was filtered off, washed well with water and dried *in vacuo*. This was found to be pure 7,14β-dihydroxyergosta-4,6,8(9),22-tetraen-3-one (LVIIIb, R = H) (222 mg.), m.p. 182-203° (decomp.), (202-206° with decomp. *in vacuo*), $[\alpha]_D$ + 172° (*c* 0.53 in pyridine)(Found: C,78.8;

H, 9.3. $C_{28}H_{40}O_3$ requires C, 79.2; H, 9.5%), $\lambda_{max.}$ 206, 240, 266 and 375 mμ. (ε 7,800, 8,200, 8,900 and 15,500), $\lambda_{max.}$ (in NaOH) 263 and 444 mμ. (ε 2,900 and 11,300), $\nu_{max.}$ 3410 (OH), 1642 (unsaturated ketone), 1597, 1582, 1456 and 1229 cm.$^{-1}$

Acetylation with acetic anhydride and pyridine at room temperature gave 7-acetoxy-14ξ-hydroxyergosta-4,6,8(9),22-tetraen-3-one (LVIIIb, R = Ac) as tiny, colourless needles, m.p. 157-159° (from n-hexane), $[\alpha]_D$ + 263° (c 0.52) (Found: C, 77.45; H, 9.1. $C_{30}H_{42}O_4$ requires C, 77.2; H, 9.1%), $\lambda_{max.}$ 345 mμ. (ε 26,200), $\lambda_{max.}$ (in NaOH) 265 and 440 mμ. (ε 2,900 and 3,200), $\nu_{max.}$ 3500 (OH), 1770 (enol acetate), 1658 (unsaturated ketone), 1603 and 1585 cm.$^{-1}$, $\nu_{max.}$ (in $CCl_4$) 3597 cm.$^{-1}$.

Attempted dienone-phenol rearrangement of 3,14ξ-dihydroxyergosta-3,5,8(9),22-tetraen-7-one (LVIIIa, R = H).- To a suspension of 3,14ξ-dihydroxyergosta-3,5,8(9)22-tetraen-7-one (64 mg.) in chloroform (1 ml.) and acetic anhydride (2 ml.) was added with shaking 0.5 ml. of a mixture of acetic anhydride (10 ml.) and concentrated sulphuric acid (1 ml.). The steroid dissolved immediately

to give a dark brown solution, which quickly turned dark blue and finally dark green. The solution was kept at room temperature for 6 hours, then ice-water was added with stirring and the solution neutralised by the dropwise addition of sodium carbonate solution, under chilling. This was allowed to stand overnight at 0°, then it was extracted twice with ether. The combined ether extracts were washed with water, dried over sodium sulphate and evaporated to dryness to give a trace of red oil. Re-extraction was no more successful, when the solution was alkaline or acidic.

Treatment of 3-acetoxy-5α,8α-epidioxyergosta-3,6,22-triene with sodium borohydride.

(a) Treatment of 3-acetoxy-5α,8α-epidioxyergosta-3,6,22-triene (XXXVII) in ether-methanol with sodium borohydride in ethanol. - To a stirred solution of 3-acetoxy-5α,8α-epidioxyergosta-3,6,22-triene (500 mg.) in dry ether (20 ml.) and dry methanol (60 ml.), cooled in an ice-bath was added over a period of half-an-hour, a solution of sodium borohydride (0.96 g.) in dry ethanol (30 ml.). The solution was stirred at ice-bath temperature for a further half-hour, then allowed to stand at room temperature for four hours. The solution was diluted with ether and washed four times with water. The dried ethereal solution was evaporated to dryness to give colourless, crystalline material (390 mg.), m.p. 103-137°, $[\alpha]_D$ + 73° (c 0.66), $\lambda_{max.}$ 204 and 213 mμ. ($E^{1\%}_{1cm.}$ 118 and 76), $\lambda_{max.}$ (in NaOH) 217, 226 and 246 mμ. ($E^{1\%}_{1cm.}$ 974, 703 and 275), $\nu_{max.}$ 3390, 1453 and 1374 cm.$^{-1}$

A sample of the colourless product (25 mg.), m.p. 103-137° after two recrystallisations from petroleum ether had m.p. 123-150°. The remainder of the material was dissolved in petroleum ether and

chromatographed on deactivated alumina (20 g.).

Elution with petroleum ether gave a yellow, crystalline solid (105 mg.), which after two recrystallisations from methylene chloride-methanol gave Ketone III as bright yellow plates (54 mg.), m.p. 152-155°, $[\alpha]_D$ + 2.1° (c 0.85)(Found: C,83.5; H,10.0), $\lambda_{max.}$ 205, 240 and 379 mμ. ($E^{1\%}_{1cm.}$ 207, 311 and 622), $\nu_{max.}$ 1672, 1637, 1542, 1459, 1368, 1352, 1143 and 966 cm.$^{-1}$, $\nu_{max.}$ (in $CCl_4$) 3021 cm.$^{-1}$ The combined mother liquors gave a second crop of bright yellow plates (32 mg.), m.p. 130-154°.

Further elution gave only small quantities of material, mostly non-crystalline.

Attempted acetylation of Ketone III. - Ketone III (32 mg.) was dissolved in pyridine (0.4 ml.) and acetic anhydride (0.2 ml.) and allowed to stand overnight at room temperature. Water was added and the solution extracted with ether. The ether extract was washed with dilute hydrochloric acid, potassium bicarbonate solution and water, dried over sodium sulphate and evaporated to dryness to give a yellow, crystalline solid (30 mg.), m.p. 100-152°. Two recrystallisations from methylene chloride-methanol gave unchanged starting material as

yellow plates (10 mg.), m.p. 143-154°, mixed m.p. 143-153.5°, $\lambda_{max.}$ 205, 240 and 380 mμ. ($E^{1\%}_{1cm.}$ 188, 282 and 596), $\nu_{max.}$ 1671, 1640 and 1545 cm.$^{-1}$

Attempted preparation of the methyl ether of 4-hydroxyergosta-4,6,8(14),22-tetraene-3-one (XLI, R = H).- 4-Hydroxyergosta-4,6,8(14),22-tetraen-3-one (80 mg.) was suspended in methanol (9 ml.) and treated with a saturated solution of hydrogen chloride in methanol (1 ml.). The mixture was allowed to stand at room temperature for two hours, with occasional swirling. Water was then added and the product extracted twice with ether. The combined ether extracts were washed with water, dried over sodium sulphate and evaporated to dryness to give unchanged starting material as a deep yellow, crystalline solid (74 mg.), m.p. 182-197°, $\nu_{max.}$ 3401 (-OH), 1650 (3-Ketone), 1592 and 1370 cm.$^{-1}$, identical with the infrared spectrum of pure hydroxy-ketone.

(b) Treatment of 3-acetoxy-5α,8α-epidioxyergosta-3,6,22-triene (XXXVII) in ether-methanol with sodium borohydride in methanol. - This was carried out exactly as described above, but the sodium borohydride was dissolved in

methanol, instead of ethanol. The crude product was obtained as a yellow, crystalline solid (458 mg.), which was chromatographed on silica gel (20 g.). Elution with benzene gave yellow, crystalline material, which on recrystallisation from methylene chloride-isopropyl ether gave 4-hydroxyergosta-4,6,8(14),22-tetraen-3-one (XLI, R = H) (37 mg.), m.p. 195-198.5°, $[\alpha]_D$ + 619° (c 0.62), $\lambda_{max.}$ 206, 256 and 369 mμ. (ε 9,400, 3,400 and 15,300), $\nu_{max.}$ 3425 (-OH), 1653 (3-ketone) and 1595 cm.$^{-1}$, identical with the infrared spectrum of pure hydroxy-ketone.

Repitition of this experiment gave the same crude product (430 mg.) but this was chromatographed on deactivated alumina instead of silica gel. Elution gave only mixtures however, none of which could be crystallised.

(c) Treatment of 3-acetoxy-5α,8α-epidioxyergosta-3,6,22-triene (XXXVII) in ether-ethanol with sodium borohydride in ethanol. - The epidioxide (500 mg.) in dry ether (20 ml.) and dry ethanol (60 ml.) was treated with sodium borohydride (0.96 g.) in dry ethanol (30 ml.) exactly as described

previously. The crude product was obtained as a white, crystalline solid (468 mg.), m.p. <143°, $\lambda_{max.}$ 204, and 327 mμ. ($E^{1\%}_{1cm.}$ 220 and 194), $\lambda_{max.}$ (in NaOH) 220, 248 and 376 mμ. ($E^{1\%}_{1cm.}$ 43, 33 and 43), $\nu_{max.}$ 3436, 1460 and 1387 cm.$^{-1}$ This was chromatographed on deactivated alumina (20 g.). Elution with 9:1 petroleum ether–benzene gave a slightly brown solid (127 mg.), which after three recrystallisations from methylene chloride–methanol gave slightly brownish needles (30 mg.), m.p. 90.5–103°, $[\alpha]_D$ + 184° (c 0.45). (Found: C,83.2; H,10.5; -OEt,24.4. $C_{32}H_{50}O_3$ requires C,82.3; H,10.8; -OEt,19.3%), $\lambda_{max.}$ 210 and 319 mμ. ($E^{1\%}_{1cm.}$ 321 and 401), $\lambda_{max.}$ (in NaOH) 219, 231 and 307 mμ. ($E^{1\%}_{1cm.}$ 243, 262 and 259), $\nu_{max.}$ 3030 (-OEt), 1460, 1377 and 1047 cm.$^{-1}$

Repetition of this experiment apparently gave the same crude product (441 mg.), but when this was chromatographed on deactivated alumina (20 g.), no material came off the column, until elution with 9:1 benzene–ether and this material could not be crystallised.

A second repetition of the experiment again gave the same crude product (443 mg.), which was chromatographed on silica gel (20 g.). Elution with 9:1 benzene–ether gave an oil which could not be crystallised.

Aerial oxidation of ergosta-4,6,22-trien-3-one and ergosta-4,6,8(14),22-tetraen-3-one.

Ergosta-4,6,22-trien-3-one[26] (LXI). - Ergosta-4,7,22-trien-3-one (XXXIV) (10.0 g.) in methanol (585 ml.) was heated under reflux with stirring until the steroid had completely dissolved. Heating was discontinued and when boiling had ceased, concentrated hydrochloric acid (approx. 3-4 drops) was added, giving a heavy, white precipitate of 3-methoxyergosta-3,5,7,22-tetraene. Heating was resumed and concentrated hydrochloric acid (25 ml.) added over a period of 25 minutes. Stirring and refluxing were then continued for a further 1.5 hours, by which time, the solid had been completely redissolved for about 30 minutes. Sodium bicarbonate (25 g.) was added to the stirred solution, then the sodium chloride was filtered off and the solution concentrated as far as possible, before bumping started. Water was added and the product extracted three times with petroleum ether. The combined extracts were washed with water, dried over sodium sulphate and evaporated to a small volume. The solution was allowed to cool, then stored overnight at 0°. Filtration gave ergosta-4,6,22-trien-3-one (LXI) as

pale yellow needles (3.45 g.), m.p. 95.5-107°. Recrystallisation from methanol gave pale yellow needles (2.85 g.), m.p. 100.5-108°, $[\alpha]_D$ - 10.7° (c 0.72), $\lambda_{max.}$ 215 and 283 mμ. (ε 12,600 and 24,300), $\nu_{max.}$ 1669, 1613 and 1582 cm.$^{-1}$ (unsaturated ketone). Lit.[26] gives m.p. 107-109°, $[\alpha]_D$ - 25°, $\lambda_{max.}$ 286mμ. (ε 26,900).

The initial mother liquor (petroleum ether) was taken to dryness and the resultant gum treated with methanol to give a second crop of yellow needles (2.51 g.). Recrystallisation from methanol again gave yellow needles (2.16 g.), m.p. 103-106°, $[\alpha]_D$ - 16.5° (c 1.02). The combined mother liquors gave a third crop of yellow needles (501 mg.), m.p. 97-108°.

Oxidation of ergosta-4,6,22-trien-3-one (LXI) in alkaline solution. - Potassium (468 mg.) was dissolved in tert.-butanol (15 ml.), then ergosta-4,6,22-trien-3-one (1 g.) was added and dissolved by warming the mixture slightly. The resulting solution quickly became yellow and after 1 hour solid came down, but the solution was allowed to stand at room temperature for 24 hours, in an open flask. Water was added, the solution acidified and the product extracted twice with ether. The ether

extracts were washed well with water and evaporated to dryness to give yellow, crystalline material (1.03 g.), which melted up to 148°. This material was dissolved in benzene and chromatographed on silica gel (40 g.).

Elution with benzene gave yellow, crystalline material (541 mg.), which after two recrystallisations from methylene chloride-methanol gave 4-hydroxyergosta-4,6,22-trien-3-one (LXII, R = H) as pale yellow needles (420 mg.), m.p. 156-159°, $[\alpha]_D$ - 151° (c 0.66) (Found: C, 81.4; H, 9.9. $C_{28}H_{38}O_2$ requires C, 81.9; H, 10.3%), $\lambda_{max.}$ 228 and 288 mμ. (ε 16,900 and 14,300), $\lambda_{max.}$ (in NaOH) 220, 257 and 300 mμ. (ε 7,500, 17,500 and 10,000), $\nu_{max.}$ 3378 (hydrogen bonded hydroxyl), 1661, 1639, 1616 and 1577 cm.$^{-1}$ (hydrogen bonded unsaturated ketone), $\nu_{max.}$ (in $CCl_4$) 3401 (hydrogen bonded hydroxyl), 1672, 1656, 1618 and 1575 cm.$^{-1}$ (hydrogen bonded unsaturated ketone). The mother liquor gave a second crop of pale yellow needles (74 mg.), m.p. 148-156°.

Further elution with benzene gave less pure material (174 mg.), which was recrystallised from methylene chloride-methanol to give 4-hydroxyergosta-4,6,22-trien-3-one as yellow needles (153 mg.), m.p. 155-158°.

Acetylation with acetic anhydride and pyridine at room temperature gave 4-acetoxyergosta-4,6,22-trien-3-one (LXII, R = Ac) as almost colourless needles, m.p. 161-163° (from methylene chloride-methanol), $[\alpha]_D$ - 72.0° (c 0.48) (Found: C,79.4; H,9.6. $C_{30}H_{44}O_3$ requires C,79.6; H,9.8%), $\lambda_{max.}$ 214, 267 and 307 mμ. (ε 14,300, 9,700 and 11,100), $\lambda_{max.}$ (in NaOH) 260 and 302 mμ. (ε 15,200 and 10,300), $\nu_{max.}$ 1761 (enol acetate), 1658, 1645, 1610 and 1575 (unsaturated ketone) cm.$^{-1}$, $\nu_{max.}$ (in $CCl_4$) 1779 (enol acetate), 1678, 1623 and 1585 cm.$^{-1}$ (unsaturated ketone).

Ergosta-4,6,8(14),22-tetraen-3-one[84] (XLII). - A solution of ergosterol (50 g.) and p-benzoquinone (100 g.) in toluene (1100 ml.) was distilled until no more moisture was entrained in the distillate, then aluminium tert.-butoxide (50 g.) was added and the solution refluxed for 1 hour. The solution was cooled and a stream of sulphur dioxide was passed through for 1 hour. The reaction mixture was filtered and the solid washed with ether and dilute sulphuric acid. The combined filtrate and washings were transferred to a separating funnel and

the organic layer washed six times with dilute sulphuric acid, twice with dilute sodium hydroxide solution and finally with water. The alkali washings were re-extracted with ether, the combined extracts dried over sodium sulphate and evaporated to dryness to give a gum, which was dissolved in benzene and chromatographed on deactivated alumina (500 g.).

Recrystallisation of the material eluted by 4:1 benzene-ether from methanol gave brown, crystalline material (8.06 g.), m.p. 113-116°. This material was dissolved in methylene chloride and the solution passed quickly through a short column of deactivated alumina (10 g.). The solution was evaporated to dryness and the residue recrystallised from methanol to give ergosta-4,6,8(14),22-tetraen-3-one (XLII) as orange platelets (6.26 g.), m.p. 114-116°, $[\alpha]_D$ + 590° (c 0.46) $\lambda_{max.}$ 235, 283 and 351 mμ. (ε 5,900, 9,300 and 33,400), $\nu_{max.}$ 1664 (3-ketone), 1637 and 1587 cm.$^{-1}$ Elks[94] gives m.p. 113-114°, $[\alpha]_D$ + 588°, $\lambda_{max.}$ 350 mμ. (ε 27,100), $\nu_{max.}$ 1666, 1644 and 1588 cm.$^{-1}$

The mother liquor gave a second crop of orange platelets (449 mg.), m.p. 112-116°.

Oxidation of ergosta-4,6,8(14),22-tetraen-3-one (XLII) in alkaline medium. - Potassium (440 mg.) was dissolved in tert.-butanol, then ergosta-4,6,8(14),22-tetraen-3-one (XLII) (1 g.) was added with stirring to give a deep red solution, which gradually became darker on standing. The mixture was allowed to stand at room temperature for 24 hours in an open flask, with occasional swirling. Water was added, the solution acidified and the product extracted with ether. The ether extract was washed well with water, dried over sodium sulphate and evaporated to dryness to give a dark red froth (1.07 g.), which was dissolved in petroleum ether and chromatographed on silica gel (40 g.).

Elution with benzene gave crystalline material (175 mg.), which after three recrystallisations from methylene chloride-methanol gave 4-hydroxyergosta-4,6,8(9),14,22-pentaen-3-one (LXIII, R = H) as green needles (51 mg.), m.p. 165-168°, $[\alpha]_D$ + 419° (c 0.27)(Found: C,82.9; H,9.55. $C_{28}H_{38}O_2$ requires C,82.7; H,9.4%), $\lambda_{max.}$ 208, 270, 371 and 623 mμ. (ε 13,200, 19,900, 9,300

and 5.1), $\lambda_{max.}$ (in NaOH) 229, 287 and 348 mμ. (ε 12,300 16,500 and 11,000), $\nu_{max.}$ 3378 (hydrogen bonded hydroxyl), 1664, 1629 and 1590 cm.$^{-1}$ (hydrogen bonded unsaturated ketone) $\nu_{max.}$ (in $CCl_4$) 3425 (hydrogen bonded hydroxyl), 1678, 1642, 1637 and 1590 cm.$^{-1}$ (hydrogen bonded, unsaturated ketone).

The combined mother liquors gave a second crop of green needles (27 mg.), m.p. 164-167° and a third crop (21 mg.), m.p. 163-167°

A second experiment was carried out on the same scale as above, but the reaction was stopped after three hours. The crude product (1.08 g.) was dissolved in benzene and chromatographed on silica gel (40 g.).

Elution with benzene gave crystalline material (132 mg.), which was recrystallised from methylene chloride-methanol to give 4-hydroxyergosta-4,6,8(9),14,22-pentaen-3-one (LXIII, R = H) as green needles (118 mg.), m.p. 163-169°. A second recrystallisation of a sample (45 mg.) again gave green needles (28 mg.), m.p. 164-168°, $[\alpha]_D$ + 405° (c 0.43).

Elution with 19:1 benzene-ether gave a red gum (354 mg.), which was crystallised from methylene chloride-methanol to give ergosta-4,6,8(14),22-tetraen-3-one (XLII). as brown crystals (200 mg.), m.p. 111-115°, $\nu_{max.}$ 1667, 1639 and 1587 cm.$^{-1}$, identical with the infrared spectrum of starting material.

The 4-acetate (XXIII, R = Ac), prepared with acetic anhydride-pyridine, was obtained as pale yellow needles (from methanol), m.p. 115-119°, $[\alpha]_D$ + 470° (c 0.45) (Found: C,80.1; H,9.0. $C_{30}H_{40}O_3$ requires C,80.3; H,9.0%), $\lambda_{max.}$ 222, 261 and 374 mμ. (ε 8,600, 22,400 and 12,800), $\lambda_{max.}$ (in NaOH) 221, 269 and 356 mμ. (ε 15,700, 20,800 and 14,000), $\nu_{max.}$ 1773 (enol acetate), 1669, 1647 and 1590 cm.$^{-1}$ (3-ketone), $\nu_{max.}$ (in $CCl_4$) 1773 (enol acetate), 1669, 1653 and 1590 cm.$^{-1}$ (3-ketone).

Photo-oxygenation of 3-acetoxyergosta-3,5,7,9(11),22-pentaen-3-one.

9(11)-Dehydroergosterol[1] (IV, R = H). - A boiling solution of mercuric acetate (116 g.) in ethanol (600 ml.) and glacial acetic acid (25 ml.) was added with stirring to a boiling solution of ergosterol (50 g.) in ethanol (2500 ml.) and the mixture refluxed for $2\frac{1}{2}$ hours. The mixture was cooled and left in the refrigerator overnight, then filtered and the residue washed with hot ethanol. The combined filtrate and washings were evaporated to about 200 ml. and on cooling gave a yellow, crystalline solid. Recrystallisation from chloroform-methanol gave 9(11)-dehydroergosterol (IV, R = H) as pale yellow needles (18.5 g.), m.p. 136-145°, $[\alpha]_D$ + 120° (c 0.67), $\lambda_{max.}$ 208, 314, 328 and 343 mμ. (ε 6,300, 6,100, 6,800 and 4,700), $\nu_{max.}$ 3448 (OH), 1456 and 1370 $cm.^{-1}$ Windaus and Linsert[1] give m.p. 146°, $[\alpha]_D$ + 149°.

The mother liquor gave a second crop (2.0 g.), m.p. 135-141°.

Ergosta-4,7,9(11),22-tetraen-3-one[24] (LXV). - 9(11)-Dehydroergosterol (IV, R = H) (46 g.) was dissolved in benzene (960 ml.) and the solution distilled until no more water was entrained in the distillate (approx. 50 ml.). Aluminium tert.-butoxide (50 g.) and acetone (400 ml.) were added and the mixture refluxed for 7 hours with stirring and allowed to stand overnight. The benzene solution was washed with dilute sulphuric acid and water, then with potassium bicarbonate solution and finally with water. The combined washings were extracted twice with ether and the extracts washed well with water. The benzene and ether solutions were combined, dried over sodium sulphate and evaporated to dryness under reduced pressure to give a red gum, which was redissolved in xylene and taken to dryness several times to remove mesityl oxide. The product was obtained as a red gum, which crystallised on cooling. Recrystallisation from acetone gave ergosta-4,7,9(11),22-tetraen-3-one (LXV) as yellow plates (13.8 g.), m.p. 124-149°, $[\alpha]_D$ + 214° (c 0.52), $\lambda_{max.}$ 244 mμ. (ε 25,100), $\nu_{max.}$ 1709, 1675 (αβ-unsaturated ketone) and 1626 cm.$^{-1}$ Heilbron et al[24] give m.p. 140-142°, $[\alpha]_D$ + 190°, $\lambda_{max.}$ 242 mμ. (ε 31,600).

This material was used for enol acetylation without further purification.

Attempts to chromatograph the product gave red gums, which gave only a small amount of crystalline material. The use of aluminium isopropoxide, instead of tert.-butoxide resulted in incomplete oxidation of the sterol.

3-Acetoxyergosta-3,5,7,9(11),22-pentaene[24] (XXXVI).- A solution of ergosta-4,7,9(11),22-tetraen-3-one (LXV) (13.6 g.) in pyridine (35 ml.) and acetic anhydride (35 ml.) was heated under reflux for 3 hours. Yellow plates (11.9 g.), m.p. 151-162° separated on cooling and were recrystallised from ethyl acetate-methanol to give 3-acetoxyergosta-3,5,7,9(11),22-pentaene (XXXVI), as flat yellow needles (10.8 g.), m.p. 150-162° (158-161° *in vacuo*), $[\alpha]_D$ - 242° (c 0.77), $\lambda_{max.}$ 200, 237, 340, 357 and 376 mμ. (ε 9,100, 16,000, 13,700, 17,900 and 13,100), $\nu_{max.}$ 1764 (enol acetate), 1658, 1229, 1208, 1199 and 1114 cm.$^{-1}$ Heilbron *et al*[24] give m.p. 161°, $[\alpha]_D$ - 232°, $\lambda_{max.}$ 356 mμ. (ε 17,400).

The foregoing preparations are typical of several which were carried out.

Oxygenation and irradiation of 3-acetoxyergosta-3,5,7,9(11),22-pentaene (XXXVI). - 3-acetoxyergosta-3,5,7,9(11),22-pentaene (XXXVI) (10.0 g.) was dissolved in benzene (645 ml.) and ethanol (650 ml.). Eosin (100 ml. of a filtered solution of 1 g. of the dye in 100 ml. of ethanol) and pyridine (5 ml.) were added and the solution oxygenated and irradiated by method (B). Spectroscopic analysis showed that the reaction was complete in 5.5 hours. The solution was taken to dryness to give a red gum (11.8 g.), which in the first experiment was dissolved in 1:1 petroleum ether-benzene and chromatographed on silica gel. This led to the formation of uncrystallisable oils, so in a second experiment, the crude red gum (11.8 g.) was dissolved in chloroform and passed quickly through a column of deactivated alumina (100 g.), This removed most of the eosin and evaporation of the eluate gave a red gum, which was crystallised from ether-methanol to give pink, crystalline material (6.32 g.), m.p. 129-155°. The mother liquor gave a second crop of yellow needles (575 mg.) m.p. 150-154° and a third crop of pink, crystalline material (326 mg.), m.p. 120-147°. The mother liquor

was then taken to dryness and chromatographed on silica gel (see below).

Two recrystallisations of the first crop (6.32 g.) from ether-methanol gave 3-acetoxy-5α,8α-epidioxyergosta-3,6,9(11),22-tetraene (LXVI) as pale orange needles (4.62 g.), m.p. 152-155°, $[\alpha]_D$ + 113° (c 0.52)(Found: C,77.3; H,9.0. $C_{30}H_{42}O_4$ requires C,77.2; H,9.1%), $\nu_{max.}$ 1761 (enol acetate), 1681 and 1212 cm.$^{-1}$

The recrystallisation mother liquors gave pink, crystalline material (1.35 g.), m.p. 110-150°.

The initial mother liquors on evaporation gave a red gum (2.38 g.), which was dissolved in benzene and chromatographed on silica gel (100 g.). The only crystalline material which was obtained however, was a further small quantity of epidioxide (LXVI). The other fractions were red oils, which would give no crystalline material.

4-Hydroxyergosta-4,6,8(14),9(11),22-pentaen-3-one (LXVII, R = H). - 3-Acetoxy-5α,8α-epidioxyergosta-3,6,9(11),22-tetraene (1 g.) was dissolved in ethanol (28 ml.) and a solution of potassium hydroxide (1.4 g.) in water (7 ml.) added. The solution was refluxed for

5 minutes, during which time it quickly became deep red The solution was cooled, acidified with concentrated hydrochloric acid and extracted twice with ether. The ether extracts were washed three times with water, dried over sodium sulphate and evaporated to dryness to give a deep yellow, crystalline solid (868 mg.), which was dissolved in benzene and chromatographed on silica gel (40 g.).

Elution with benzene gave a yellow, crystalline solid (552 mg.), which was recrystallised from methylene chloride-isopropyl ether to give 4-hydroxyergosta-4,6,8(14), 9(11),22-pentaen-3-one (LXVII, R = H) as bright yellow needles (391 mg.), m.p. 187-198° (decomp.). A sample (94 mg.) was given a second recrystallisation from methylene chloride-isopropyl ether to give bright yellow needles (79 mg.), m.p. 189-201° (decomp.), (193-200° with decomp., *in vacuo*), $[\alpha]_D$ + 230° (c 0.57)(Found: C,82.0, 81.8; H,9.5, 9.6. $C_{28}H_{38}O_2$,0.25$CH_3OH$ requires C,81.9; H,9.5), $\lambda_{max.}$ 202, 281 and 400 mμ. (ε 9,600, 8,400 and 11,900), $\lambda_{max.}$ (in NaOH) 221 and 310 mμ. (ε 17,600 and 11,700), $\nu_{max.}$ 3378 (hydrogen bonded hydroxyl), 1656, 1642, 1610 and 1582 (hydrogen bonded unsaturated ketone)

and 1527 cm.$^{-1}$, $\nu_{max.}$ (in $CCl_4$) 3442 cm.$^{-1}$ (hydrogen bonded hydroxyl).

The combined mother liquors gave a second crop of yellow needles (70 mg.), m.p. 185-197° (decomp.) and a third crop (36 mg.), m.p. 173-196°.

Acetylation with acetic anhydride and pyridine at room temperature gave the 4-acetate (LXVII, R = Ac) as yellow prisms (from methylene chloride-methanol), m.p. 199-209° (205-209° with decomp, in vacuo), $[\alpha]_D$ + 62° (c 0.57) (Found: C, 80.2; H, 9.1. $C_{30}H_{40}O_3$ requires C, 80.3; H, 9.0%), $\lambda_{max.}$ 207, 271 and 405 mμ. (ε 11,200, 8,600 and 12,100), $\lambda_{max.}$ (in NaOH) 221, 270 and 407 mμ. (ε 16,100, 10,800 and 10,500), $\nu_{max.}$ 1764 (enol acetate), 1653, 1623 and 1585 (unsaturated ketone) and 1534 cm.$^{-1}$

The 4-benzoate (LXVII, R = Bz), prepared from benzoyl chloride and pyridine, formed pale yellow, feathery needles, m.p. 197-213° (212-215° in vacuo), $[\alpha]_D$ + 123° (c 0.56) from methylene chloride-acetone (Found: C, 82.3; H, 8.2. $C_{35}H_{42}O_3$ requires C, 82.3; H, 8.3), $\lambda_{max.}$ 235, 271 and 404 mμ. (ε 18,800, 7,600 and 10,900), $\lambda_{max.}$ (in NaOH) 235, 280 and 400 mμ. (ε 16,400, 12,000 and 7,600), $\nu_{max.}$ 1732 (enol benzoate), 1667 and

1626 (unsaturated ketone) and 1543 cm.$^{-1}$

Chromium trioxide-pyridine oxidation of dehydroergosterol peroxide (V, R = H). - The dehydroergosterol peroxide[1,9] was obtained by alkaline hydrolysis of a sample (5 g.) of dehydroergosteryl acetate peroxide (V, R = Ac). The product was recrystallised from methylene chloride-methanol to give dehydroergosterol peroxide (V, R = H) as colourless plates (4.04 g.), m.p. 155-165.5°, $[\alpha]_D$ + 72.5 (c 0.52), $\nu_{max.}$ 1453, 1370, 1071, 1031 and 976 cm.$^{-1}$ Bladon et al[9] give m.p.161-165.5°, $[\alpha]_D$ + 80°.

Dehydroergosterol peroxide (V, R = H) (1 g.) in pyridine (15 ml.) was added to a slurry of chromium trioxide (1 g.) in pyridine (10 ml.) and the mixture allowed to stand overnight at room temperature. Water was added and the mixture extracted three times with ether, centrifuging each time to get the layers to separate. The combined ether extracts were washed well with water, dried over sodium sulphate and evaporated to dryness to give a reddish-brown gum (912 mg.), which was crystallised from methanol to give slightly brown plates (142 mg.), m.p. 147-165°. The mother liquor gave a

second crop of yellowish plates (197 mg.), m.p. 146-165°. The two crops were combined and recrystallised from methanol to give dehydroergosterol peroxide (V, R = H) as slightly brown needles (240 mg.), m.p. 148-166.5° (161-163° *in vacuo*), $[\alpha]_D$ + 78.1° (*c* 0.60), $\nu_{max.}$ 1453, 1370, 1070, 1031 and 976 cm.$^{-1}$, identical with the infrared spectrum of the starting material.

Photo-oxygenation of 3-acetoxylumista-3,5,7,22-tetraene.

Extraction of lumisteryl 3,5-dinitrobenzoate from crude Vitamin D residues.[x] - The crude residues (approx. 8 Kg.) were dissolved in hot benzene (approx. 9 l.) in a large drum, and methanol added to the hot solution until it just became turbid (approx. 10 l.). The solution was allowed to stand at about 0°C. for three days, by which time yellow crystals had formed on the sides of the drum and a semi-crystalline sludge had been deposited on the bottom. After the supernatant liquor had been syphoned off, the yellow crystals from the sides were scooped off and recrystallised from the minimum quantity of ethyl acetate to give calciferyl 3,5-dinitrobenzoate (Crop 1) as bright yellow needles (82.6 g.), m.p. 138-141° (recrystallises at about 90°), $[\alpha]_D$ + 12.4° (c 0.49), $\nu_{max.}$ 1724 (3,5-dinitrobenzoate), 1631, 1548, 1342, 1279 and 685 ($=CH_2$) cm.$^{-1}$ Lit.[46] gives m.p. 148-149°.

The sludge from the bottom of the drum was divided into two portions and filtered.

[x] The residues were a gift from Glaxo Laboratories Ltd. The procedure is based on a method outlined by Professor E. R. H. Jones in a personal communication to Dr. P. Bladon.

The first portion of sludge, on crystallisation from the minimum quantity of ethyl acetate gave calciferyl 3,5-D.N.B. (Crop 2) as bright yellow needles (250.9 g.), m.p. 138-141° (recrystallises at about 90°), $\nu_{max.}$ 1724 (3,5-D.N.B.), 1634, 1550, 1342, 1274 and 685 (=$CH_2$) cm.$^{-1}$ The mother liquor of this material was concentrated and ethanol added, and on standing overnight gave lumisteryl 3,5-dinitrobenzoate (Crop 2a) as brownish, crystalline material (8.5 g.), m.p.123-140°, $\nu_{max.}$ 1724 (3,5-D.N.B.), 1629, 1548, 1340 and 1274 cm.$^{-1}$ On reduction to very small volume, the mother liquor gave brown, semi-crystalline material, which was recrystallised from the minimum volume of ethyl acetate to give lumisteryl 3,5-D.N.B. (Crop 2b) as yellow prisms (7.2 g.), m.p. 128-141°, $\nu_{max.}$ 1727 (3,5-D.N.B.), 1626, 1534, 1340 and 1276 cm.$^{-1}$ Lit.[46] gives m.p. 139-141°.

The second portion of sludge was crystallised from the minimum volume of ethyl acetate to give calciferyl 3,5-D.N.B. (Crop 3) as stout, yellow needles (56.6 g.), m.p. 138-141° (recrystallises at about 90°), $\nu_{max.}$ 1724 (3,5-D.N.B.), 1637, 1548, 1342, 1273 and 685 (=$CH_2$) cm.$^{-1}$ Concentration of the mother liquor and

addition of ethanol gave lumisteryl 3,5-D.N.B. (Crop 3a) as a hard, brown, crystalline solid, which was ground up

1733 (3,5-D.N.B.), 1658, 1548, 1340 and 1277 cm.$^{-1}$) $\nu_{max.}$

1733 (3,5-D.N.B.), 1658, 1548, 1340 and 1277 cm.$^{-1}$

The mother liquor was then taken to dryness to give a red gum, which was dissolved in the minimum quantity of hot ethyl acetate and the solution was allowed to stand for two days. This gave lumisteryl 3,5-dinitrobenzoate (Crop 3b) as yellow prisms (16.0 g.), m.p. 122-135°, $\nu_{max.}$ 1727 (3,5-D.N.B.), 1658, 1548, 1340 and 1277 cm.$^{-1}$

The four crops of lumisteryl 3,5-D.N.B. (2a, 2b, 3a and 3b) were combined, dissolved in ethyl acetate and passed through a short column of charcoal and celite. The solution was taken to small volume and ethanol added. On cooling, lumisteryl 3,5-dinitrobenzoate separated as tiny, yellow needles (44.1 g.), m.p. 131-141°, $[\alpha]_D$ + 16.2° (c 0.54), $\nu_{max.}$ 1730 (3,5-D.N.B.), 1629, 1548, 1340 and 1279 cm.$^{-1}$ The mother liquor was concentrated and cooled to give a second crop of yellow needles (3.5 g.), m.p. 130-141°.

A second batch of residues (1200 g.), proved rather better and gave lumisteryl 3,5-dinitrobenzoate

(26.5 g.), m.p. 141-144°, together with a little calciferyl 3,5-dinitrobenzoate (5.5 g.), m.p. 142-144°.

Lumisterol[47] (XIII, R = H). - Alkaline hydrolysis of lumisteryl 3,5-dinitrobenzoate gave lumisterol (XIII, R = H) as a white, crystalline solid, which was recrystallised from acetone-methanol to give colourless needles, m.p. 103-118.5°, $[\alpha]_D$ - 182° (c 0.50), $\lambda_{max.}$ 207, 276 and 282 mμ. (ε 6,100, 9,900 and 9,600), $\nu_{max.}$ 3401 (OH), 1458, 1368 and 1022 cm.$^{-1}$ Lit.[47] gives m.p. 118°, $[\alpha]_D$ + 191°. The mother liquor gave a second crop of almost colourless needles (1.40 g.), m.p. 105-119°.

Lumista-4,7,22-trien-3-one[24] (LXVIII). - Lumisterol (14.7 g.) in benzene (350 ml.) was treated with aluminium tert.-butoxide (18 g.) and acetone (150 ml.), exactly as described in the preparation of ergosta-4,7,9(11),22-tetraen-3-one (see p.133). The crude product was obtained as a deep yellow gum (14.2 g.), which was dissolved in benzene and chromatographed on deactivated alumina (560 g.),

Elution with benzene gave yellow, crystalline material (3.34 g.), which was recrystallised from ethyl

acetate-methanol to give lumista-4,7,22-trien-3-one (LXVIII) as yellow needles (2.56 g.), m.p. 135-139°, $[\alpha]_D$ + 18.6° (c 0.59), $\lambda_{max.}$ 207 and 242 mμ. (ε 10,300 and 13,600), $\nu_{max.}$ 1678 and 1610 cm.$^{-1}$ (αβ-unsaturated ketone). Heilbron et al[84] give m.p. 139-140°, $[\alpha]_D$+48.7°, $\lambda_{max.}$ 229 mμ. (ε 17,000).

Elution with 9:1 benzene-ether gave lumisterol, which was combined with comparative material from another preparation. The total material (6.35 g.)was recrystallised twice from acetone-methanol to give lumisterol as yellow crystals (3.53 g.), m.p. 107-118°.

This is typical of several preparations, which were carried out.

3-Acetoxylumista-3,5,7,22-tetraene[84] (LXIX).- Lumista-4,7,22-trien-3-one (LXVIII)(3.1 g.) was dissolved in pyridine (7.5 ml.) and acetic anhydride (7.5 ml.) and the solution refluxed for 3 hours. The excess reagents were removed under reduced pressure and the residue taken up in ether. The ether solution was washed with dilute hydrochloric acid, potassium bicarbonate solution and water, dried over sodium sulphate and evaporated to dryness to give a red gum (3.48 g.). Recrystallisation

from ethyl acetate-methanol gave 3-acetoxylumista-3,5,7,22-tetraene (LXIX) as yellow needles (1.73 g.), m.p. 92-99° (94-98° *in vacuo*), $[\alpha]_D$ + 312° (*c* 0.57), $\lambda_{max.}$ 210, 305, 317 and 330 mμ. (ε 8,200, 16,400, 18,600 and 12,500), $\nu_{max.}$ 1754 (enol acetate), 1650 and 1221 cm.$^{-1}$ Heilbron *et al*[24] gave m.p. 98°, $[\alpha]_D$ + 294°, $\lambda_{max.}$ 315 mμ. (ε 19,100).

This preparation is typical of several which were carried out.

*Oxygenation and irradiation of 3-acetoxylumista-3,5,7,22-tetraene (LXIX).* - 3-Acetoxylumista-3,5,7,22-tetraene (3 g.) was dissolved in benzene (333 ml.) and ethanol (335 ml.). Eosin (30 ml. of a filtered solution of 1 g. of the dye in 100 ml. of ethanol) and pyridine (2 ml.) were added and the solution oxygenated and irradiated by method (B). Spectroscopic analysis showed that the reaction was 85% complete after 16 hours. The reaction was stopped and the solution taken to dryness to give a red gum, which could not be crystallised, so it was dissolved in petroleum ether and chromatographed on silica gel (100 g.).

Elution with 1:9 petroleum ether-benzene gave yellow, crystalline material (282 mg.), which was recrystallised twice from methylene chloride-methanol to give 3-acetoxylumista-3,5,7,9(11),22-pentaene (LXX) as yellow needles (154 mg.), m.p. 119.5-129° (126-129° in vacuo), $[\alpha]_D$ + 639° (c 0.55)(Found: C,82.8 ; H, 9.5 . $C_{30}H_{42}O_2$ requires C,82.9; H,9.7%), $\lambda_{max.}$ 234, 320, 334 and 353 mμ. (ε 10,500, 12,200, 12,400 and 9,800), $\nu_{max.}$ 1757 (enol acetate), 1661, 1562, 1224 and 1124 cm.$^{-1}$

Elution with 4:1 benzene-ether gave an orange, semi-crystalline solid (1.59 g.) which could not be recrystallised, but which was thought to consist of partially decomposed epidioxide. This material was treated with ethanolic potassium hydroxide solution (see below).

Elution with 1:1 benzene-ether gave slightly pink, crystalline material (445 mg.), which was recrystallised twice from methylene chloride-isopropyl ether to give an αβ-unsaturated ketone as almost colourless needles (77 mg.), m.p. 165-177°, $[\alpha]_D$ + 59° (c 0.58)(Found: C,76.7; H,9.25), $\lambda_{max.}$ 240 mμ. ($E^{1\%}_{1cm.}$ 382), $\nu_{max.}$ 3378

(hydrogen bonded hydroxyl) and 1669 (αβ-unsaturated ketone) cm.$^{-1}$, $\nu_{max.}$ (in $CCl_4$) 3590 (free hydroxyl), and 3478 cm.$^{-1}$ (intermolecular hydrogen bonded hydroxyl). This material was not examined further.

Other fractions from the chromatography gave traces of poorly crystalline material, which were not examined.

In a second experiment, 3-acetoxylumista-3,5,7,22-tetraene (2.26 g.) was dissolved in benzene (122.5 ml.) and ethanol (125 ml.). Eosin (50 ml. of a filtered solution of 1 g. of the dye in 100 ml. of ethanol) and pyridine (2.5 ml.) were added and the solution oxygenated and irradiated by method (A). Spectroscopic analysis showed that only 18% of the starting material was left after 24 hours. The solution was then evaporated and the residue chromatographed on silica gel (100 g.).

Elution with benzene gave yellow, crystalline material (221 mg.), which was recrystallised twice from methylene chloride-methanol to give 3-acetoxylumista-3,5,7,9(11),22-pentaene (LXX) as yellow needles (78 mg.), m.p. 112-127°, $[\alpha]_D$ + 577° (c 0.45), $\lambda_{max.}$ 229, 315, 331

and 349 mμ. (ε 12,200, 16,300, 15,500 and 10,800), $\nu_{max.}$ 1757 (enol acetate), 1653, 1225 and 1122 cm.$^{-1}$

No other significant fractions were obtained from the column.

Treatment of semi-crystalline product (4:1 benzene-ether) with ethanolic potassium hydroxide solution. - The crude material (1.59 g.) was dissolved in ethanol to give an orange solution. To this was added a solution of potassium hydroxide (3.2 g.) in water (8 ml.), giving a dark red solution, with green fluorescence. This was refluxed for 5 minutes, cooled, acidified with concentrated hydrochloric acid and the product extracted twice with ether. The combined ether extracts were washed with water, dried over sodium sulphate and evaporated to dryness to give a brown gum (1.22 g.), which was dissolved in benzene and chromatographed on silica gel (60 g.), but no crystalline material was obtained.

Dehydrolumisterol (XIV, R = H). - Alkaline hydrolysis of a sample (1.22 g.) of dehydrolumisteryl acetate (XIV, R = Ac) gave colourless, crystalline material

(1.06 g.), which was recrystallised from methylene chloride-methanol to give 9(11)-dehydrolumisterol (XIV, R = H) as colourless needles (907 mg.) m.p. 139-142°, $[\alpha]_D$ + 169° (c 0.51)(Found: C,83.4 ; H,10.5 . $C_{28}H_{44}O$, $0.5CH_3OH$ requires C,83.4; H,10.8%), $\lambda_{max.}$ 212, 313 and 325 mμ. (ε 8,800, 11,000 and 11,900), $\nu_{max.}$ 3425 (OH), 1451, 1368, 1009 and 971 cm.$^{-1}$ The mother liquor gave a second crop of slightly yellow needles (120 mg.), m.p. 134-141°.

Oppenauer oxidation of 9(11)-dehydrolumisterol (XIV, R = H). - 9(11)-Dehydrolumisterol (920 mg.) was dissolved in dry benzene (15 ml.), then aluminium tert.-butoxide (9 g.) and acetone (7.5 ml.) were added and the mixture refluxed with stirring for 6 hours. The mixture was cooled, diluted with benzene and washed with dilute sulphuric acid. The aqueous layer was re-extracted twice with ether and the combined ether and benzene layers washed with dilute sulphuric acid, potassium bicarbonate solution and water, dried over sodium sulphate and evaporated to dryness to give an orange gum (1.1 g.). Treatment with acetone-methanol gave an orange, crystalline

solid (62 mg.), m.p. <190°, $\nu_{max.}$ 1709, 1656 and 1587 cm.$^{-1}$ The crystalline material and the mother liquor were recombined and taken to dryness. The resulting gum was dissolved in benzene and chromatographed on deactivated alumina (50 g.).

Elution with benzene gave poor separation. The first fraction (295 mg.) on treatment with ethyl acetate-methanol gave a small amount of waxy solid. This was recombined with the mother liquor, which was taken to dryness, to give a red gum, $\lambda_{max.}$ 238, 308 and 320 mμ. ($E^{1\%}_{1cm.}$ 387, 180 and 184). The second fraction (157 mg.) was recrystallised from ethyl acetate-methanol to give dehydrolumisterol (XI, R = H) as slightly yellow needles (38 mg.), m.p. 138-142°, $\nu_{max.}$ 3367 (OH), 1451, 1370, 1010 and 971 cm.$^{-1}$, identical with the infrared spectrum of the starting material. None of the other fractions (4-10) could be crystallised.

The residue from fraction 1 was dissolved in petroleum ether and rechromatographed on deactivated alumina (20 g.). Elution with 4:1 petroleum ether-benzene gave yellow crystalline material (65 mg.) which

was recrystallised from ethyl acetate-methanol to give dehydrolumisterol as yellow needles (21 mg.), m.p. 133-142°, $\nu_{max.}$ 3356 (OH), 1453, 1368, 1010 and 971 cm.$^{-1}$, identical with the infrared spectrum of the starting material. The column then had to be stripped with 9:1 ether-methanol before the rest of the material could be recovered. This material was combined with fractions 4-10 from the first chromatography and the solution taken to dryness to give a red gum (340 mg.), $\lambda_{max.}$ 242 mμ. ($E^{1\%}_{1cm.}$ 327).

As the ultraviolet absorption of this gum seemed to indicate that it consisted mainly of αβ-unsaturated ketone, an attempt was made to carry out enol acetylation.

Attempted enol acetylation of non-crystalline Oppenauer oxidation product. - The crude gum (340 mg.) was dissolved in pyridine (2.5 ml.), and acetic anhydride (2.5 ml.) and the solution refluxed for three hours. The excess of reagents was removed under reduced pressure and the residue taken up in ether. The ethereal solution was washed with dilute hydrochloric acid, potassium bicarbonate solution and water, dried over sodium sulphate and evaporated to dryness to give a dark red gum (330 mg.),

$\lambda_{max.}$ 215 and 237 mμ. ($E^{1\%}_{1cm.}$ 220 and 242).

The ultraviolet absorption shows that the desired enol acetate was not obtained.

By-products from the photo-oxygenation of

By-products from the photo-oxygenation of

ergosteryl acetate.

Chromatography of ergosteryl acetate peroxide mother liquors. - The crude residues (11.6 g.) from the crystallisation of ergosteryl acetate peroxide were dissolved in 1:1 petroleum ether-benzene and chromatographed on deactivated alumina (350 g.).

Elution with 1:1 petroleum ether-benzene gave almost colourless, crystalline material (526 mg.), which was recrystallised twice from methylene chloride-methanol to give 5,6-dihydroergosteryl acetate (LXXI, R = Ac) as colourless plates (163 mg.), m.p. 175-187°, $[\alpha]_D$ - 27.6° (c 0.64), $\nu_{max.}$ 1736 (3-acetate), 1453, 1364, 1245 and 1030 cm.$^{-1}$ Windaus and Brunken[5] give m.p. 180-181°, $[\alpha]_D$ - 20°. The combined mother liquors gave a second crop of colourless plates (106 mg.), m.p. 169-184°.

Elution with benzene gave colourless crystalline material (2.57 g.), which was recrystallised from chloroform-ethanol to give ergosteryl acetate peroxide (III, R = Ac) as flat colourless needles (1.51 g.), m.p. 187-208° (recrystallises at 130°), $\nu_{max.}$ 1733 (3-acetate), 1453, 1361 and 1239 cm.$^{-1}$

Elution with 9:1 benzene-ether gave yellow, crystalline material (680 mg.), which after three recrystallisations from methylene chloride-isopropyl ether gave 3β-acetoxyergosta-5,8(9),22-trien-7-one (LXXII) as almost colourless needles (11 mg.), m.p. 195-210° (recrystallises 172-190°), $[\alpha]_D$ - 36.6° (c 0.56), $\lambda_{max.}$ 205 and 246 mμ. (ε 10,500 and 16,600), $\nu_{max.}$ 1733 (3-acetate), 1664, 1626 and 1587 cm.$^{-1}$ (cross-conjugated αβ-unsaturated ketone). Lit.[43] gives m.p. 199-204°, $[\alpha]_D$ - 32°, $\lambda_{max.}$ 245 mμ. (ε 11,800), $\nu_{max.}$ (in $CS_2$) 1738 (3-acetate), 1658 and 1628 (αβ-unsaturated ketone) and 967 cm.$^{-1}$ The combined mother liquors gave a second crop of pale yellow needles (104 mg.) m.p. <208°.

## REFERENCES


1. Windaus and Linsert, Annalen, 1928, 465, 148.
2. Windaus and Borgeaud, Annalen, 1928, 460, 235.
3. Inhoffen, Naturwiss., 1937, 25, 125.
4. Windaus and Roosen-Runge, Berichte, 1940, 73, 321.
5. Windaus and Brunken, Annalen, 1928, 460, 225.
6. Kohler, J. Amer. Chem. Soc., 1906, 36, 177.
7. Wieland and Prelog, Helv. Chim. Acta, 1947, 30, 1028.
   Fiore, Arch. Biochem., 1948, 16, 161.
8. Müller, Z. physiol. chem., 1935, 231, 75.
9. Bladon, Clayton, Greenhalgh, Henbest, Jones, Lovell, Silverstone, Wood and Woods, J., 1952, 4883.
10. Clayton, Henbest and Jones, J., 1953, 2015.
11. Skau and Bergmann, J. Org. Chem., 1938, 3, 166.
12. Conca and Bergmann, J. Org. Chem., 1953, 18, 1104.
13. Davis and Halsall, J., 1962, 1833.
14. Laubach, Schreiber, Agnello and Brunings, J. Amer. Chem. Soc., 1956, 78, 4746.
15. Bladon, J., 1955, 2176.
16. Castells, Jones, Meakins and Williams, J., 1959, 1159.
17. Dauben and Fonken, J. Amer. Chem. Soc., 1959, 81, 4060.
18. Barton and Laws, J., 1954, 52.

19. Schenck, Gollnick and Neumüller, Annalen, 1957, 603, 46.

20. Schenck and Zeigler, Naturwiss., 1944, 32, 157.

21. Schönberg, "Präparative Organische Photochemie", Springer-Verlag, Vienna, 1958.

22. Scott and Bedford, J. Amer. Chem. Soc., 1962, 84, 2271.

23. Oppenauer, Rec. Trav. Chim., 1937, 56, 137.

24. Heilbron, Kennedy, Spring and Swain, J., 1938, 869.

25. Wetter and Dimroth, Berichte, 1937, 70, 1665.
Johnson, Newbold and Spring, J., 1954, 1302.

26. Shepherd, Donia, Campbell, Johnson, Holysz, Slomp, Stafford, Pederson and Ott, J. Amer. Chem. Soc., 1955, 77, 1212.

27. Fieser and Fieser, "Steroids", Rheinhold Publishing Corporation, New York, 1959, p.19.

28. Dauben and Eastham, J. Amer. Chem. Soc., 1951, 73, 3260.

29. Fieser, Experientia, 1950, 6, 312.

30. Gallagher and Kritchevsky, J. Amer. Chem. Soc., 1950, 72, 882.

31. Dalton and Meakins, J., 1961, 1880.

32. See, inter al, Petrov, Rosenheim and Starling, J., 1943, 135; Henbest and Wilson, ibid, 1956, 3289; Johnson, Rogier, Szmuszkovicz, Hadler, Ackermann, Bhattacharyya, Bloom, Stalmann, Clement, Bannister and Wynberg, J. Amer. Chem. Soc., 1956, 78, 6289.

33. Barton and Bruun, J., 1951, 2728.

34. Elks, J., 1954, 468.

35. Dauben and Eastham, J. Amer. Chem. Soc., 1951, 73, 4463; Dauben, Eastham, Micheli, Takemura, Mandell and Chemerda, ibid, 1953, 75, 3255.

36. Clayton, Crawshaw, Henbest, Jones, Lovell and Wood, J., 1953, 2009.

37. Kuhn, Angew. Chem., 1959, 71, 93; see also, idem, Fortschr. Chem. org. Naturstoffe, 1958, 16, 169; 1959, 17, 404.

38. Barton and Thomas, J., 1953, 1842; Kyosuke Tsuda, KoArima and Ryoichi Hayatsu, J. Amer. Chem. Soc., 1954, 76, 2933.

39. Camerino, Patelli and Sciaky, Tetrahedron Letters, 1961, 554.

40. Gillam and Stern, "Electronic Absorption Spectra", E. Arnold Ltd., London, 1957, p.122.

41. Windaus and Langer, Annalen, 1934, 508, 105.

42. Value from Mathieu and Petit, "Constants Selectionées, Pouvoir Rotatoire Naturel, Vol.1, Steroids", Masson et Cie, Paris, 1956.

43. Elks, Evans, Long and Thomas, J., 1954, 451.

44. cf. Elks, Evans, Oughton and Thomas, J., 1954, 463; Burke, Turnbull and Wilson, J., 1953, 3237.

45. Farrar, Hamlet, Henbest and Jones, J., 1952, 2657.

46. Askew, Bourdillon, Bruce, Callow, Philpot and Webster, Proc. Roy. Soc., 1932, B109, 488.

47. Windaus, Dithmar and Fernholz, Annalen, 1932, 493, 259.

Products from 3-acetoxyergosta-3,5,7,22-tetraene.