T H E S I S

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THE UNIVERSITY OF GLASGOW

in fulfilment of the

requirements for the

DEGREE OF DOCTOR OF PHILOSOPHY

by

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

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ROUTES TO METHYL STEROIDS

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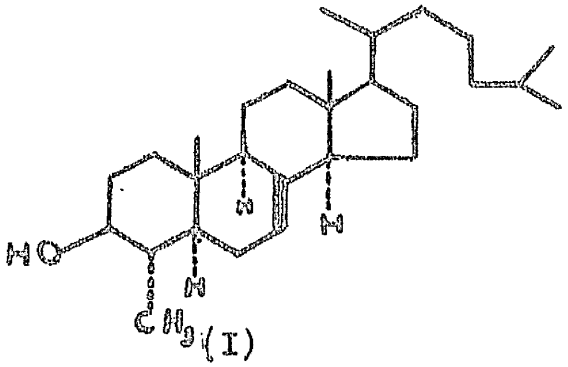
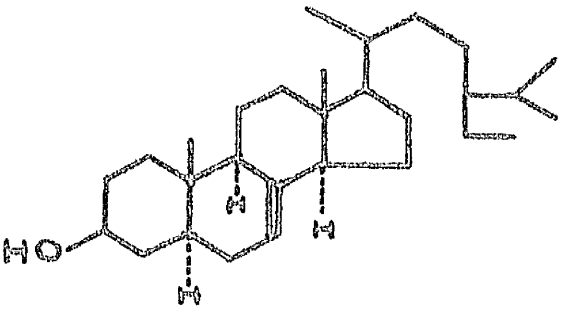
C O N T E N T S

INTRODUCTORY REVIEW

While carrying out extensive investigations into the constituents of giant cacti, Djerassi et al.[1,2,3,4] found that the non-saponifiable fraction of Lophocereus schotti contained n-octyl alcohol, lupeol, and two new sterols which were named lophenol and schottenol. A study of the chemistry of these two sterols[4] demonstrated that lophenol was 4α-methylcholest-7-en-3β-ol (I) and schottenol was stigmast-7-en-3β-ol (II).



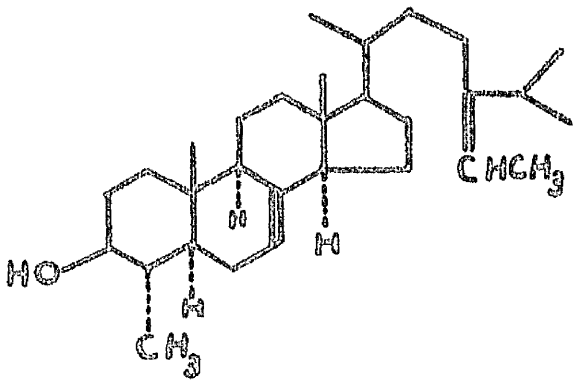
(I)



(II)

About the same time, Mazur, Weizmann, and Sondheimer[5] established the structure of another naturally occurring 4-methyl steroid named citrostadienol, showing it to be 4α-methylstigmasta-7,24(28)-dien-3β-ol (4α-methyl-24-ethylidenecholest-7-en-3β-ol) (III).

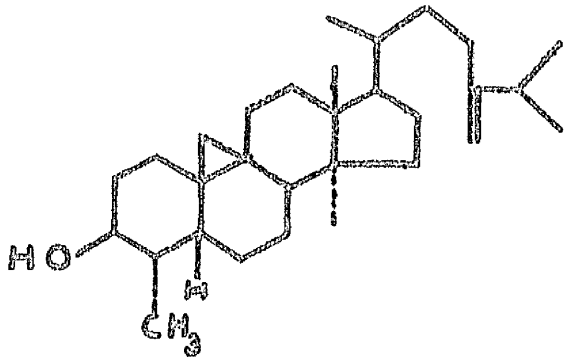
Our interest in 4-methyl steroids arose from the isolation of a compound which was obtained from rowan bark and



(III)

which was called sorbustadienol.[6] This steroid which was isolated in very small amount had m.p. 158-160°, $[\alpha]_D$ + 14° and the molecular rotation differences for sorbustadienol and its acetate were in close agreement with the corresponding figures for lophenol and citrostadienol.

Prior to the structural elucidation of lophenol and citrostadienol no naturally occurring monomethyl steroids were known, although cycloeucalenol (IV), a dimethyl steroid, had been isolated by King[7] in 1956 from tallow wood (Eucalyptus microcorys). Extensive characterisation of this compound indicated a close resemblance to cycloartenol (V) and Cox, King, and King[8b] eventually showed it to be 4β-demethyl-24-methylenecycloartanol (IV). Until recently[9] cholesterol had not been encountered in the plant kingdom and the isolation from plant sources of lophenol (I) is therefore unusual. More important however, is the fact that it is a 4-monomethyl steroid and it appears highly probable that compounds of this nature are intermediates



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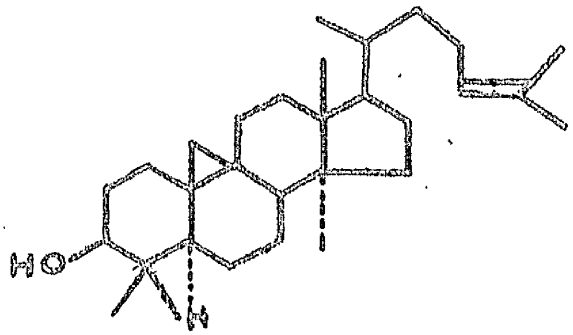
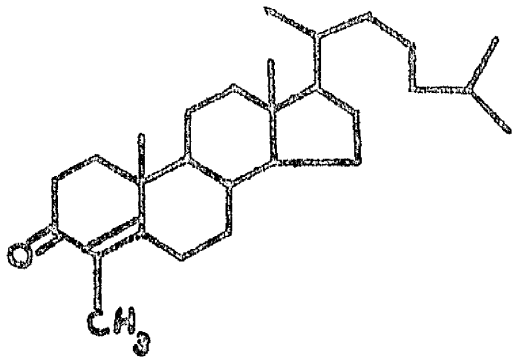


(V)

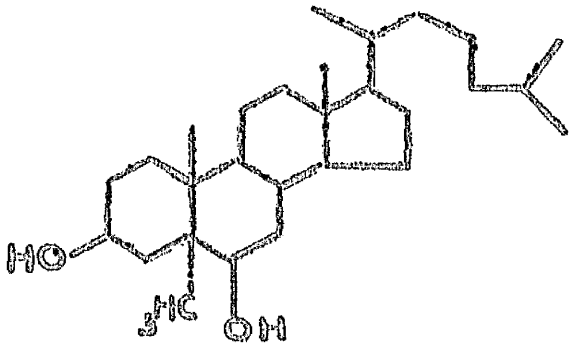
in the biogenesis of cholesterol from acetic acid.

The announcement[9] that certain steroidal Δ^4-3-ketones with a methyl substituent at the $C_{(2)}$-position possessed higher biological activity than the unmethylated hormones, aroused considerable interest in steroids containing a 4-methyl-3-oxo-Δ^4-system and in 1956 two groups of workers[10,11] described the first synthesis of a 4-monomethyl steroid, namely 4-methylcholest-4-en-3-one (VI).

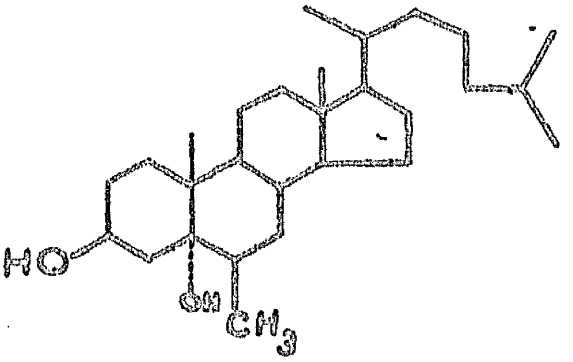


(VI)

Cholestane derivatives having methyl substituents at positions $C_{(5)}$ and $C_{(6)}$ have been known for some time. Urushibara and Chuman[12,13] prepared 5α-methylcholestan-3β,6β-diol (VII) by treatment of 5β,6β-epoxycoprostan-3β-ol with methylmagnesium iodide while Ushakov and Madaeva[14] synthesised the isomeric 6β-methylcholestan-3β,5α-diol (VIII) from 5α,6α-epoxycholestan-3β-ol and the same reagent.

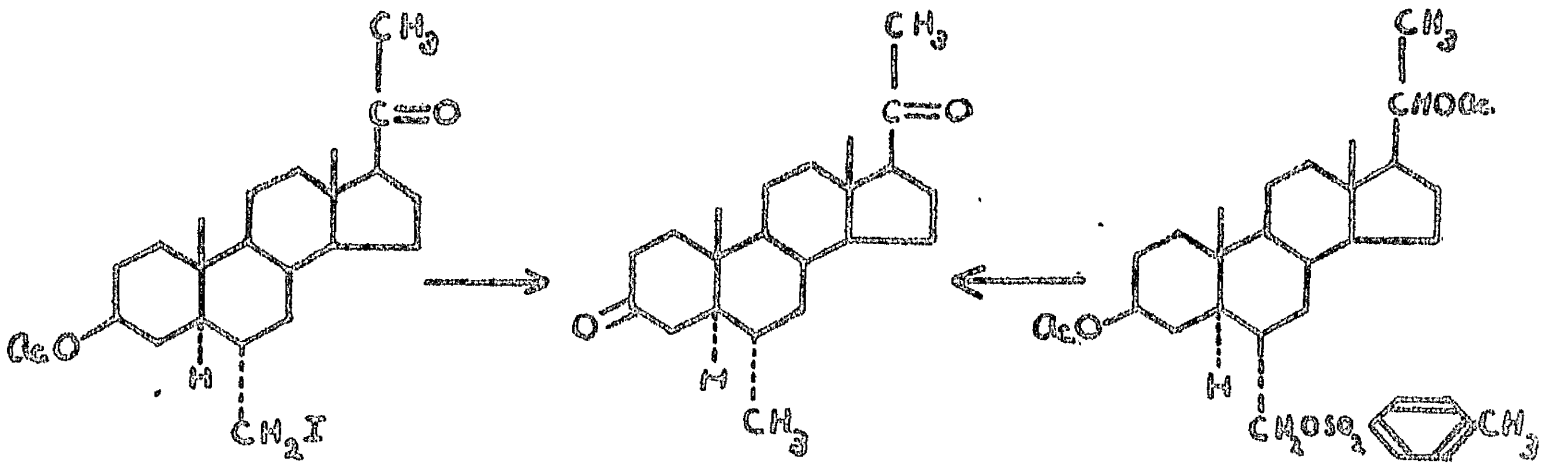


(VII)



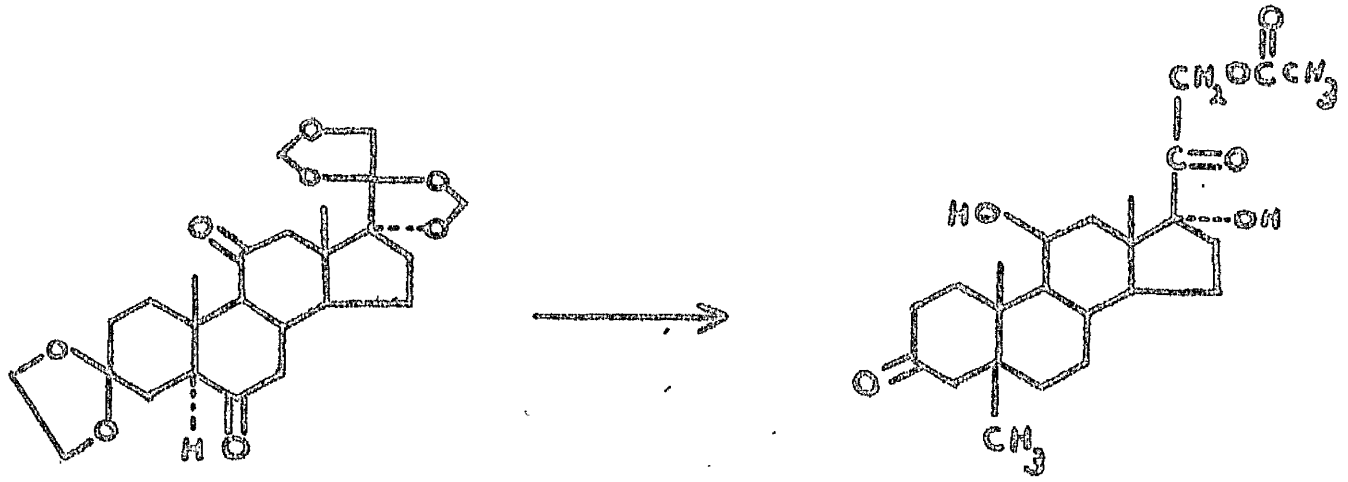
(VIII)

Alkyl substitution of the steroid nucleus at positions $C_{(5)}$ and $C_{(6)}$ has not been confined to the cholestane series. Thus 6α-methyl-5α-pregnan-3,20-dione (XI) has been synthesised by reduction of 3β-acetoxy-6α-iodomethyl-5α-pregnan-20-one (IX)[16] and 6α-tosylmethyl-5α-pregnan-3β,20β-diol diacetate (X)[16] with lithium aluminium hydride followed by oxidation of the reduced products.



(IX) (XI) (X)

The first synthesis of a 5-methyl pregnane was reported recently[17] by Fried, Arth, and Sarett who introduced the alkyl group at $C_{(5)}$ by treating a solution of 17α,20,20,21-bismethylene-dioxy-3-ethylenedioxy-alloprегnane-6,11-dione (XII) in xylene with sodium hydride and methyl iodide. Stepwise degradation of the product afforded 5-methylpregnan-11β,17α,21-triol-3,20-dione-17-acetate (XIII).

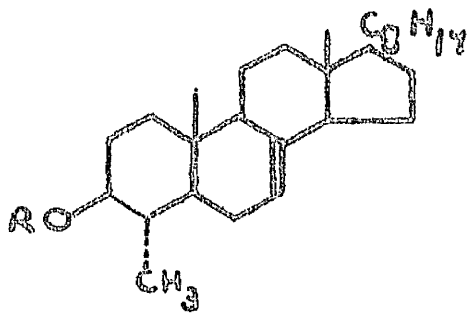


(XII) (XIII)

HISTORICAL

The Structure of Lophenol.

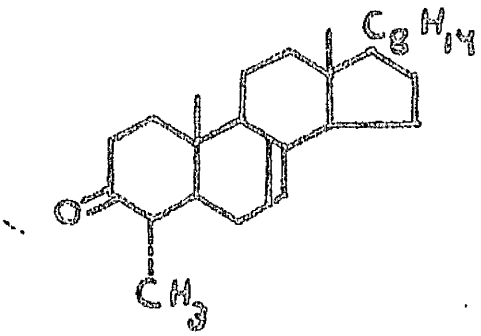
The analytical data obtained by Djerassi *et al.*[4] for the new sterol (XIV) from *Lophocereus schotti* suggested formulations ranging from $C_{27}H_{46}O$ to $C_{30}H_{52}O$. That the oxygen was contained in a secondary hydroxyl group was shown by the formation of an acetate (XV), a benzoate (XVI), and a ketone (XVII), lophenone.



(XIV) (R = H)

(XV) (R = CH_3CO)

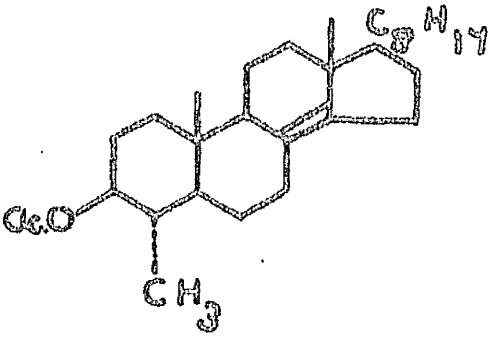
(XVI) (R = C_6H_5CO)



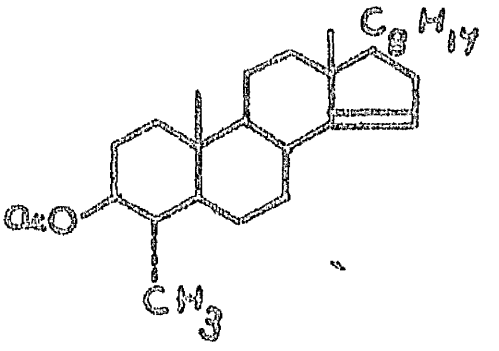
(XVII)

Lophenol (XIV) gave a yellow colour with tetranitromethane and showed ethylenic absorption in the ultraviolet[18,19] indicative of a trisubstituted double bond. The nature and position of the double bond was clarified by carrying out a series of interesting isomerisations which suggested that the sterol was closely related to the steroidal 5α-Δ^7- or Δ^8- alcohols. Reduction of lophenyl acetate (XV) by hydrogen and platinum in glacial acetic acid did

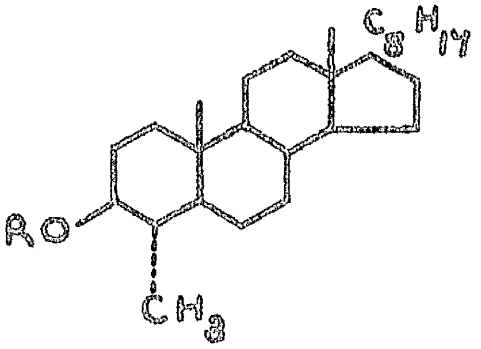
not yield the expected dihydro product, but gave the $\Delta^{8(14)}$-isomer (XVIII) which could be further isomerised with hydrogen chloride in chloroform to the Δ^{14}-acetate (XIX). While the double bond of the $\Delta^{8(14)}$-acetate (XVIII) was resistant to hydrogenation, that of the Δ^{14}-isomer was smoothly reduced to lophanyl acetate (XXI) using a platinum catalyst. The possibility of the double bond in lophenol being in the 8,9-position rather than the 7,8-position was rendered unlikely by the ultraviolet spectral data mentioned above,[10,19] coupled with the fact that oxidation of lophenyl acetate (XV) with mercuric acetate[31] afforded the corresponding $\Delta^{7,9(11)}$-diene (XXII); this reaction is characteristic of Δ^{7}-stenols.



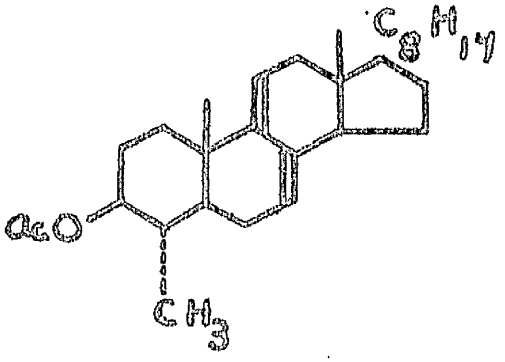
(XVIII)



(XIX)

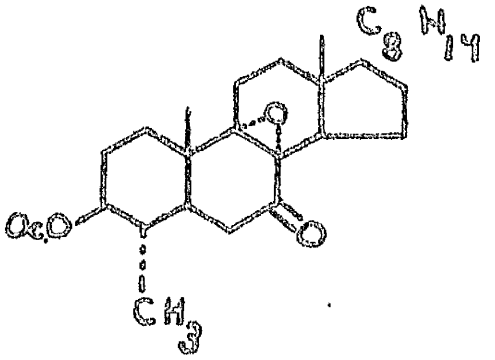


(XX) (R = H)
(XXI) (R = CH_3CO)

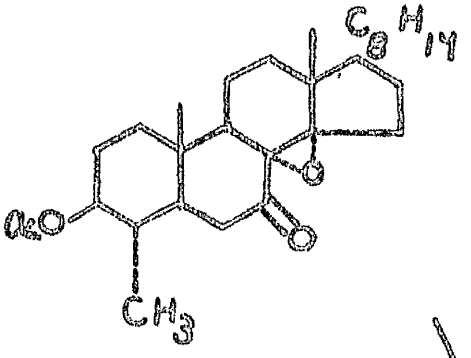


(XXII)

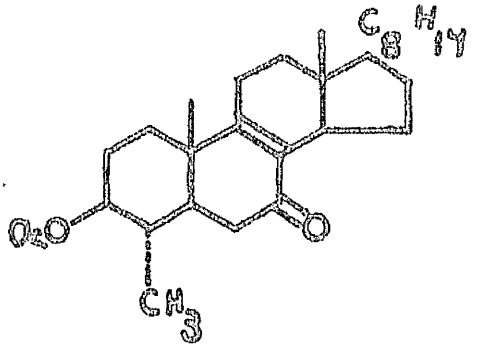
Treatment of lophenyl acetate (XV) with chromium trioxide in acetic acid afforded two isomeric acetoxy keto epoxides. By analogy with similar oxidation products in the Δ^7-ergostene[22,23,24] and Δ^7-cholestene[25,26] series the more dextrorotatory isomer was assigned the 7-oxo-8,9-oxido structure (XXIII), while the more laevorotatory was represented as the 7-oxo-8,14-oxide (XXIV). An improved method[25,26] for preparing these compounds consisted of stepwise oxidation, first with perbenzoic acid followed by chromium trioxide. By treatment with zinc,[22,23,24] these two compounds yielded the Δ^8-7-ketone (XXV) and the $\Delta^{8(14)}$-7-ketone (XXVI) respectively, characterised by their ultraviolet absorption spectra, while the same $\Delta^{8,14}$-dien-7-one (XXVII) was obtained by subjecting the two keto epoxides to hydrochloric acid treatment.



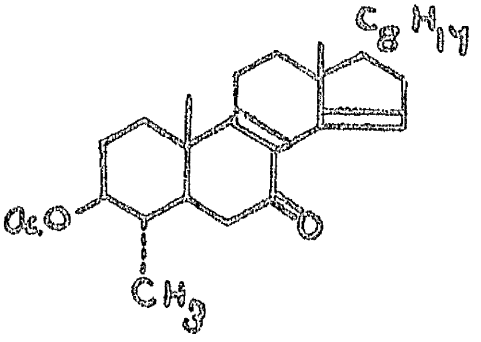
(XXIII)



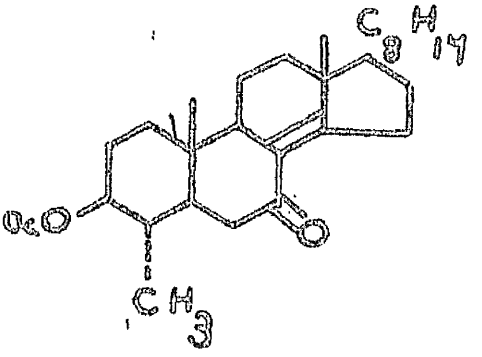
(XXIV)



(XXV)



(XXVII)



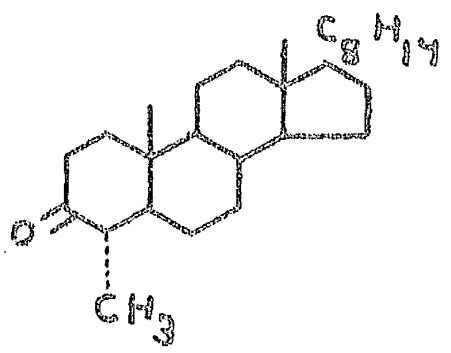
(XXVI)

One of the most interesting physical features of lophenol was that it displayed molecular rotation values which were characteristic of a tetracyclic triterpenoid rather than a steroid. Also, some samples of lophenol showed traces of another compound having a heteroannular diene chromophore[27] and originally these factors led Djerassi and his colleagues to believe that lophenol was a tetracyclic triterpenoid, since many naturally occurring compounds of this group are found admixed with the corresponding $\Delta^{7,9(11)}$-diene. With the exception of cycloeucalenol (IV) the tetracyclic triterpenoids are characterised

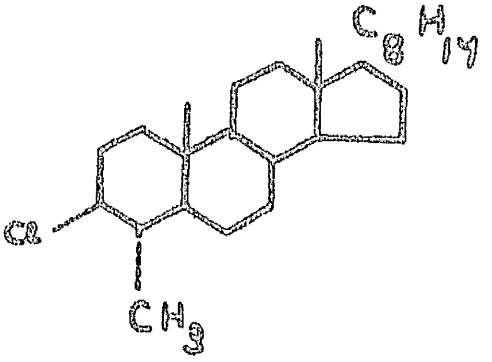
by having a 3-hydroxy-4,4-dimethyl grouping (or derived ketone) in ring A and the presence of this system is shown by the fact that compounds of this type readily undergo a retropinacolinic rearrangement[27] on treatment with phosphorus pentachloride. Under these conditions however, lophenol gave the 3α-chloro derivative (XXIX).

Nevertheless it was thought that the abnormal molecular rotation values were due to an extranuclear methyl substituent which was in the close vicinity of the hydroxyl group. Attempts to prove this by bromination were unsuccessful due to spontaneous dehydrobromination and consequent excessive bromine uptake.[28] The point was eventually proved by studying the effect of hemiketal formation on the rotatory dispersion curve of the saturated ketone lophanone (XXVIII). It is known[29] that hemiketal formation is inhibited where a ketone function is flanked by even one methyl group. The fact that cholestan-3-one suffered a reduction of about 65% in rotatory dispersion amplitude while lophanone (XXVIII) was only affected to the extent of 17% pointed to the methyl group being at $C_{(2)}$ or $C_{(4)}$. That the methyl group was at $C_{(4)}$ and had the stable α-(equatorial)-configuration was proved beyond doubt by comparison of the physical data for synthetic 4α-methylcholestan-3-one,

4α-methylcholestan-3β-ol, and 4α-methylcholestan-3β-yl acetate with those for lophanone (XXVIII), lophanol (XX), and lophanyl acetate (XXI) respectively. In view of this evidence lophenol was given the systematic name 4α-methylcholest-7-en-3β-ol.



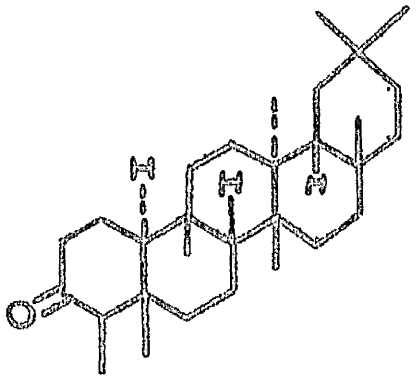
(XXVIII)



(XXIX)

The Structure of Citrostadienol.

By chromatography of the non-saponifiable fraction of grapefruit peel oil, Weizmann and Mazur[30] isolated friedelin (XXX), β-sitosterol (XXXI), and a new sterol which was named citrostadienol (XXXII). The problems involved in the structural elucidation of citrostadienol were not unlike those encountered with lophenol. Analyses of citrostadienol and its derivatives indicated the molecular formula $C_{30}H_{50}O$ for the parent alcohol and Weizmann and Mazur[30] suggested tentatively that this alcohol was a doubly unsaturated 3β-hydroxy-5α-steroid, one of the bonds being located at $C_{(7)}$ or $C_{(8)}$, the above assumptions



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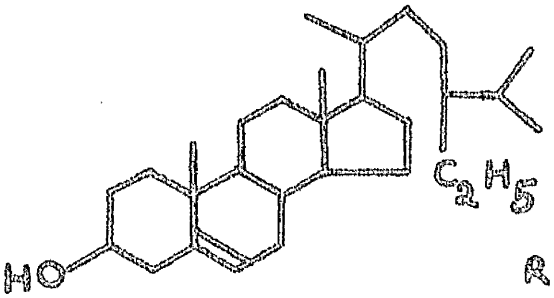
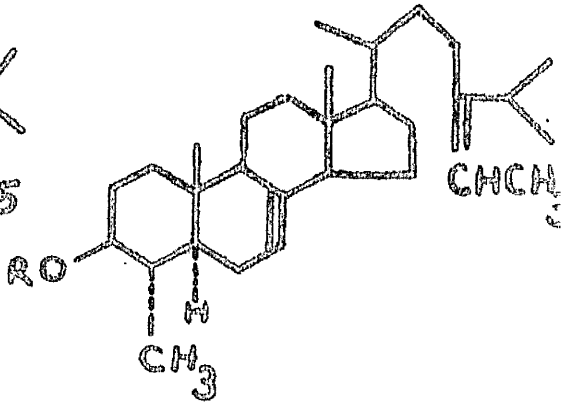


(XXXI)



(XXXII) (R = H)
(XXXIII)(R = CH_3CO)

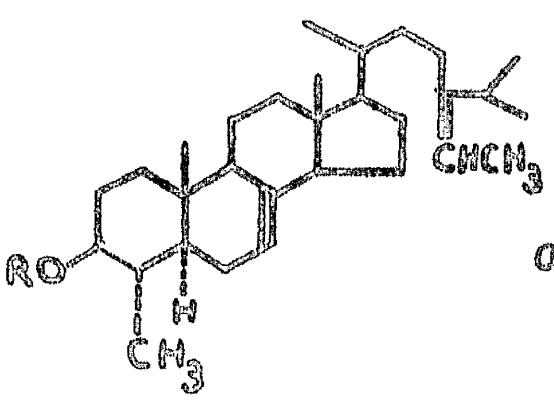
being based on the following evidence. Citrostadienol (XXXII) gave an insoluble digitonide, indicative of a 3β-hydroxyl group and the observation that citrostadienyl acetate (XXXIII) displayed a single unsplit band at 1252 $cm.^{-1}$ in the infrared[31] pointed to a 5α-configuration.

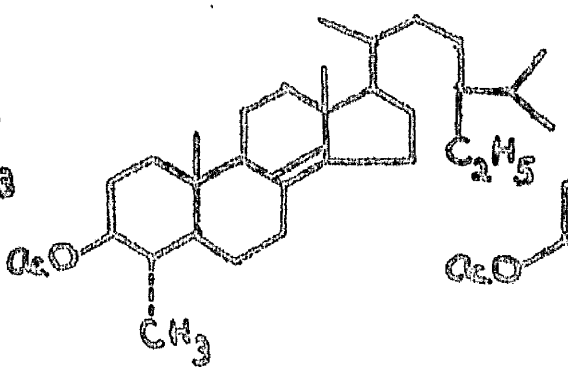
An extensive investigation into the complete structure of citrostadienol was carried out by Mazur, Weizmann, and Sondheimer.[32] Ozonolysis of citrostadienol (XXXII) yielded acetaldehyde and thus suggested the presence of a stigmast-24(28)-ene side chain. This type of side chain is not uncommon in plant sterols[33-36] and it was of considerable interest that β-sitosterol, a companion of

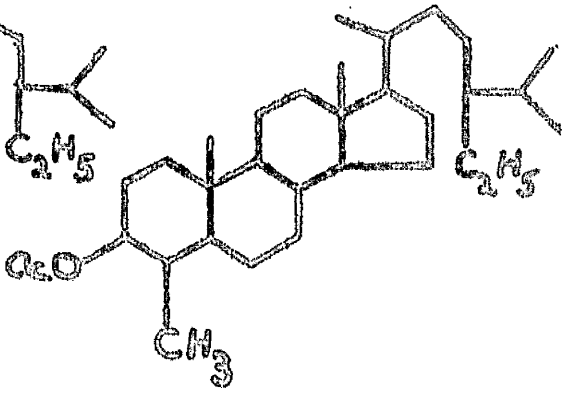
citrostadienol in grapefruit and orange peel oil[30] has the corresponding saturated side chain.

As in the case of lophenol, the molecular rotation values for citrostadienol were not consistent with those for sterols but rather, resembled those of the tetrasubstituted triterpenoids, viz. the shift in molecular rotation in passing from alcohol to acetate is positive.[37] The absence of a gem dimethyl group at C(4) was shown by the failure of citrostadienol (XXXII) to undergo the retropinacolinic rearrangement[27] on treatment with phosphorus pentachloride in benzene.

Hydrogenation of citrostadienyl acetate (XXXIII) in the presence of a platinum catalyst resulted in the uptake of one molar equivalent of hydrogen with the formation of isocitrostenyl acetate (XXXIV). The failure of this compound to furnish acetaldehyde on ozonolysis indicated that the side chain double bond had been saturated, while the ultraviolet absorption spectrum (λ max. 210 mu, ε 10,500) showed that the nuclear double bond had migrated to the $\Delta^{8(14)}$-position[18]. That the new position of the double bond is $\Delta^{8(14)}$- was confirmed when it was found that isocitrostenyl acetate (XXXIV) did not give a positive selenium dioxide test[38] and could be saturated by hydrogenation over platinum in acetic acid and hydrochloric acid to give citrostanyl acetate (XXXV).



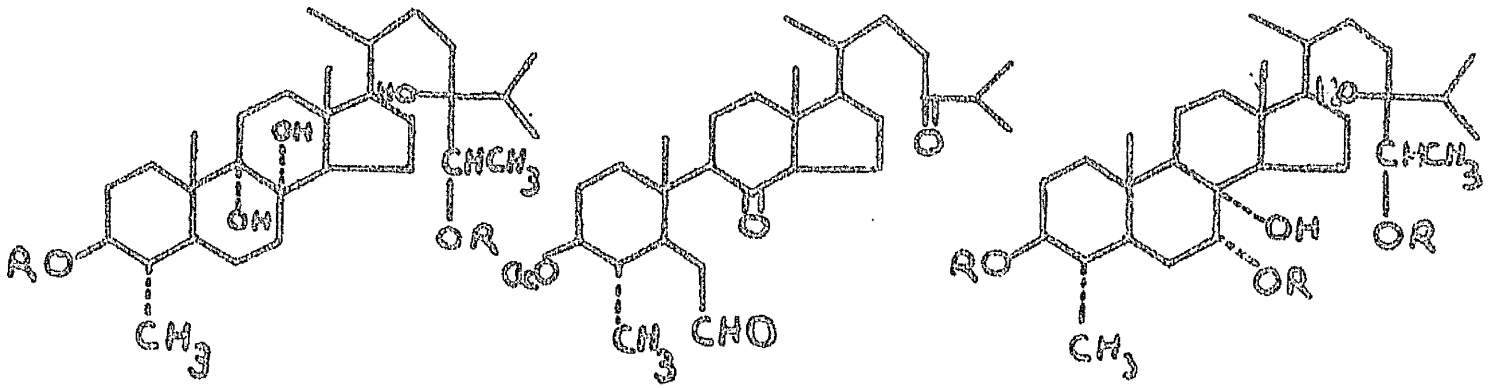




(XXXII) (R = H) (XXXIV) (XXXV)
(XXXIII)(R = CH_3CO)

The ultraviolet absorption spectrum (λ max. 209 mu, ε 5,500) of citrostadienol (XXXII) ruled out the 8,14-position for the nuclear double bond and only the Δ^{7}- or $\Delta^{8(9)}$-positions could account for the double bond shifting to $\Delta^{8(14)}$- on hydrogenation in acetic acid.[30] Masur, Weizmann, and Sondheimer[38] showed that the double bond was in fact at the 7,8-position as follows:- Citrostadienol (XXXII) was treated with osmium tetroxide and the resulting pentol was acetylated at room temperature. If the nuclear double bond is in the 7,8-position then the acetylated product should be the pentol triacetate (XXXIX), whereas if the double bond is in the 8,9-position the product should be the pentol diacetate (XXXVII). Analysis of the product showed it was a triacetate, and in addition cleavage

of the pentol with lead tetraacetate followed by acetylation furnished the diketo aldehyde (XL), which could only be derived from the pentol (XXXVIII).



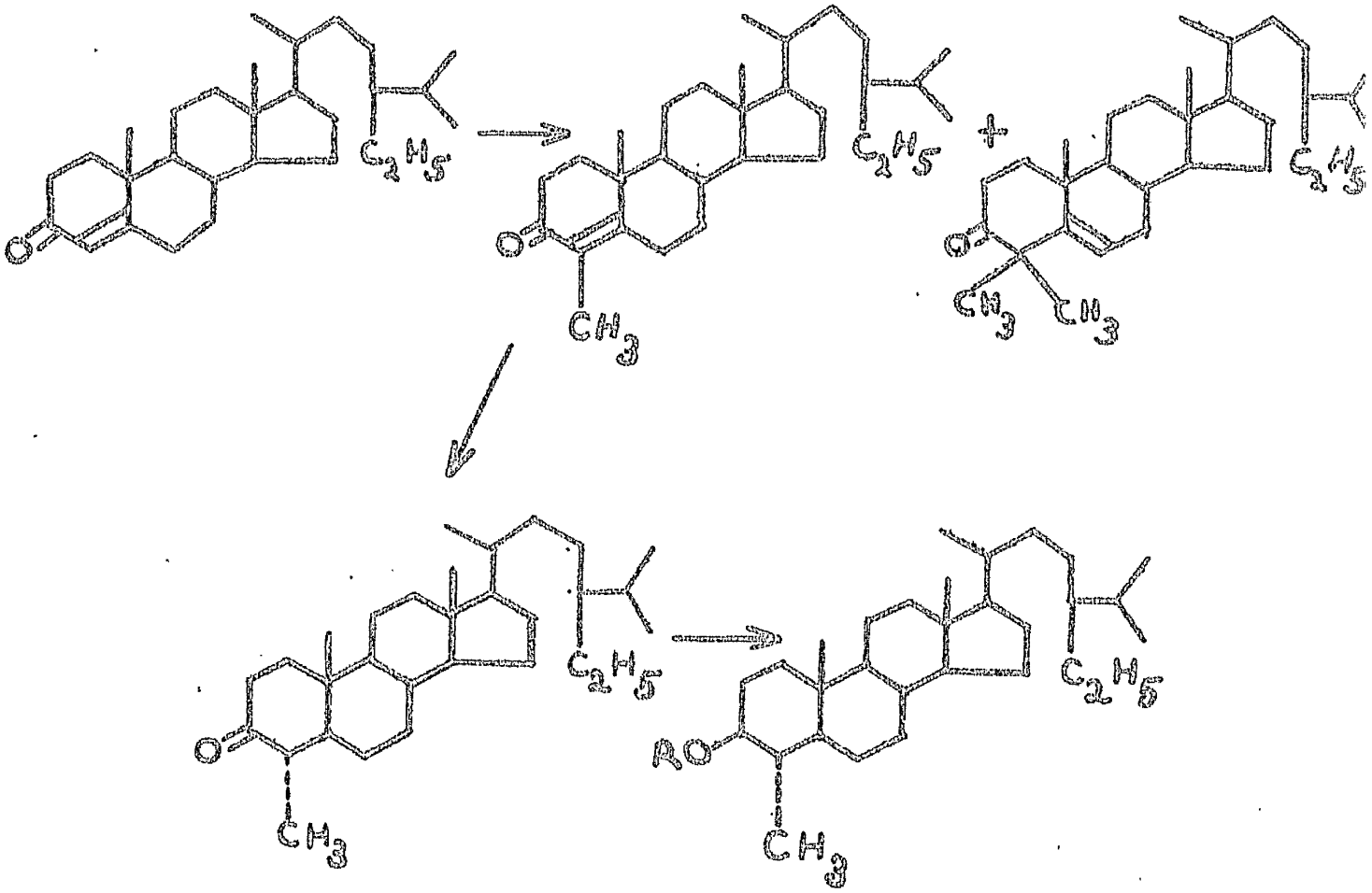
(XXXVI) (R = H)
(XXXVII) (R = CH_3CO)

(XL)

(XXXVIII) (R = H)
(XXXIX) (R = CH_3CO)

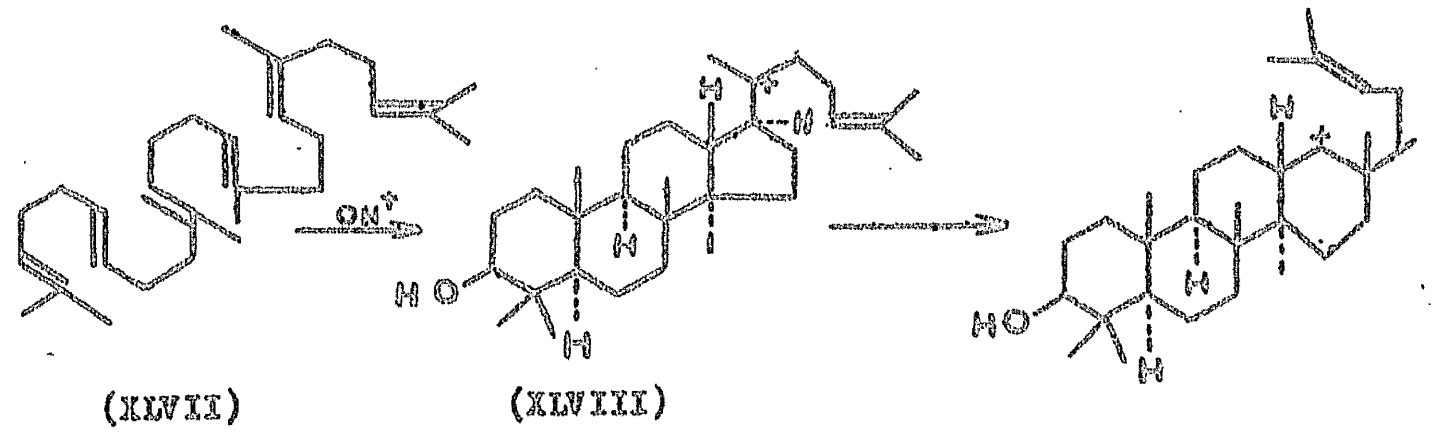
The final proof for the structure of citrostadienol came from synthetic studies. Treatment of stigmast-4-en-3-one (XLI) with methyl iodide and potassium tert-butoxide in tert-butyl alcohol afforded 4-methylstigmast-4-en-3-one (XLII) and 4,4-dimethylstigmast-5-en-3-one (XLIII). Reduction of the monomethyl derivative with lithium in liquid ammonia gave as the major product 4α-methylstigmastan-3-one (XLIV) which was identical with citrostanone prepared from the natural product. This saturated ketone was assigned the 4α-methyl-5α-configuration

by analogy with similar compounds in the cholestane series.[39] Reduction of this compound with lithium aluminium hydride gave 4α-methylstigmastan-3β-ol (XLV) which was identical with citrostanol, while the acetate of 4α-methylstigmastan-3β-ol (XLVI) was identical with citrostanyl acetate. Therefore citrostadienol may be represented as 4α-methylstigmasta-7,24(28)-dien-3β-ol (XXXII).

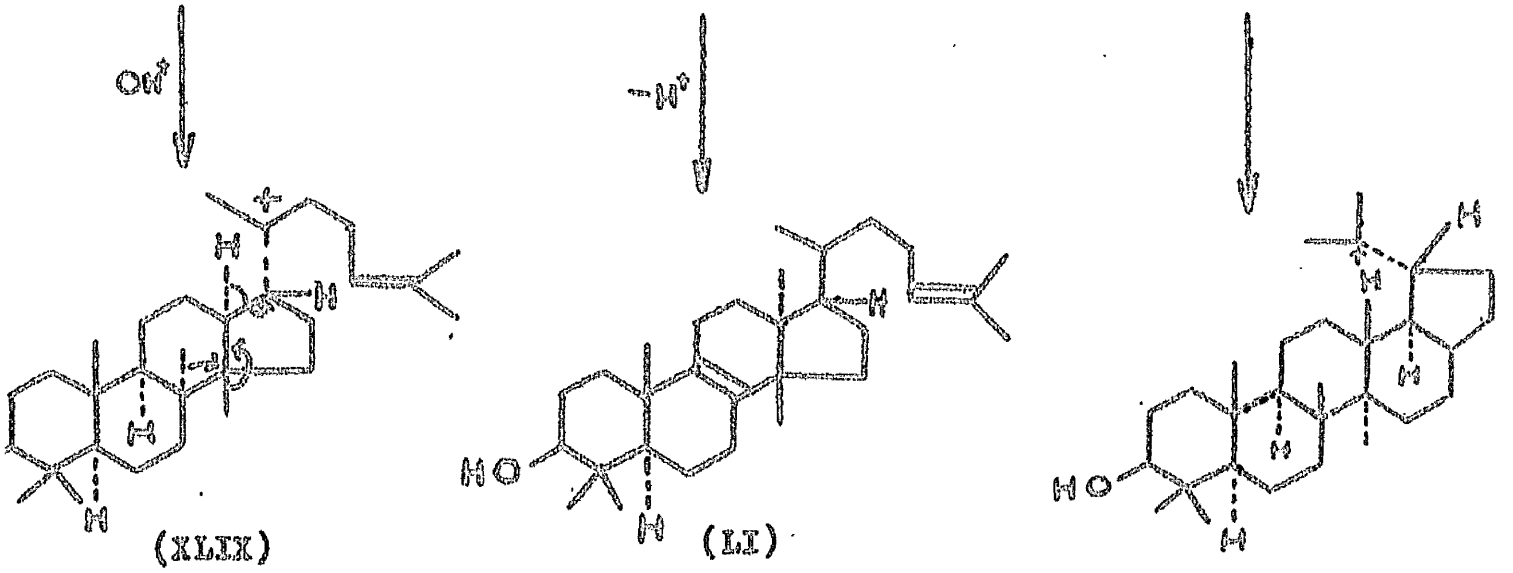


Biogenetic Implications.

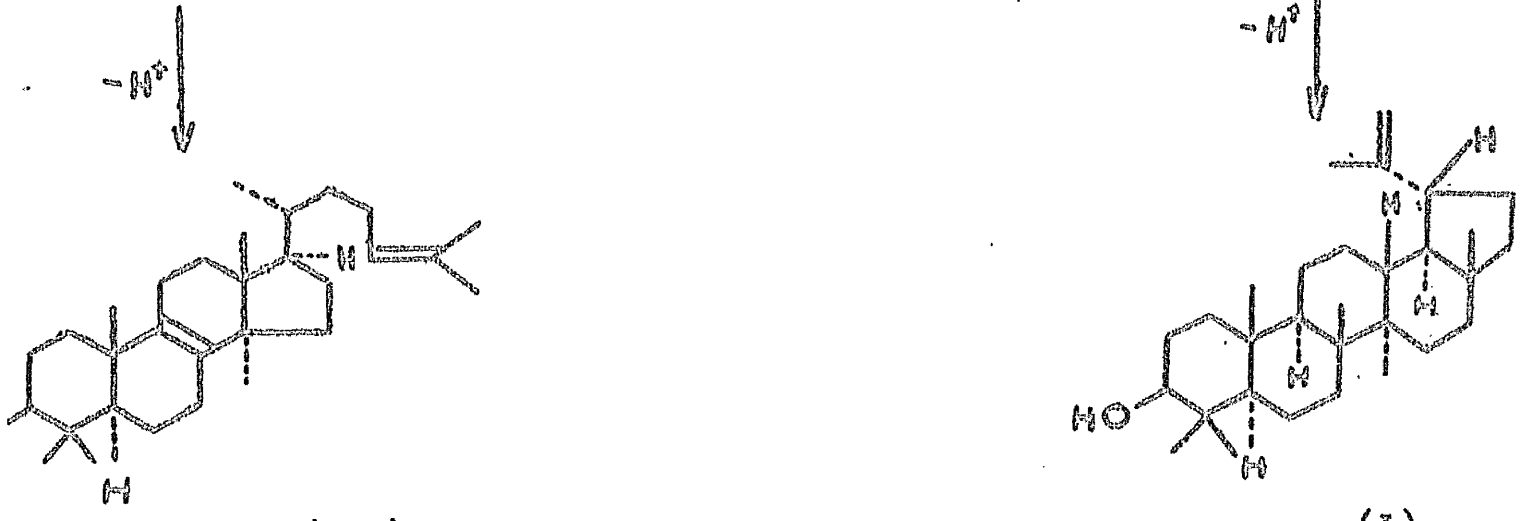
Bloch has shown experimentally[40-44] that squalene (XLVII) is an intermediate in the biosynthesis of lanosterol from acetic acid and that this tetracyclic triterpenoid can be converted into cholesterol both *in vivo* and in homogenised rat liver tissue. The molecule of natural squalene is now known to have a fully transoid arrangement throughout.[46] This is of considerable importance since, if the cyclisation is fully concerted, only this conformation can give rise to the stereochemistry found in the triterpenoids and steroids.[46-49] In the case of the biosynthesis of the tetracyclic triterpenoids such as lanosterol, the cyclisation is considered to be motivated by the attack of a cation at the double bond at one end of the squalene chain.[46-52] The cyclisation can proceed synchronously by two different paths. In the first the concerted closure of four rings leads to the formation of the carbonium ion (XLVIII) which is considered to be the precursor of the pentacyclic triterpenoids and of the euphol-tirucallol group of tetracyclic triterpenoids. The second path leads to the formation of the isomeric ion (XLIV), the precursor of the lanosterol group and thence the steroids. The formation of lupeol (L) and tirucallol (LI) from the carbonium ion (XLVIII) and of lanosterol (LII) from the ion (XLIV) is illustrated below.



(XLVII) (XLVIII)



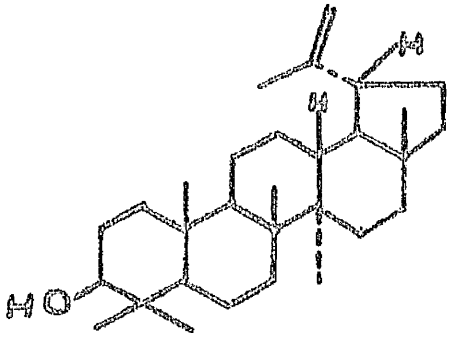
(XLIX) (LI)



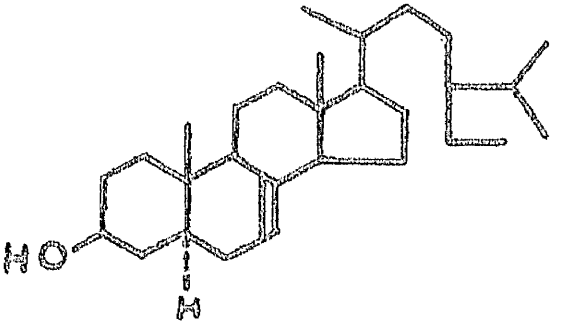
(LII) (L)

The experimental evidence[53] for the biogenesis of sterols in animals is impressive, being based mainly on the conversion of labelled squalene and other precursors into cholesterol. However, similar evidence for the biogenesis of plant sterols is not yet available and indeed the present highly unified picture[49,54] of the biogenesis of plant triterpenoids is mainly due to the isolation and establishment of the structures of products of incomplete or alternate cyclisation and/or methyl migration schemes which can all be derived from squalene.[56]

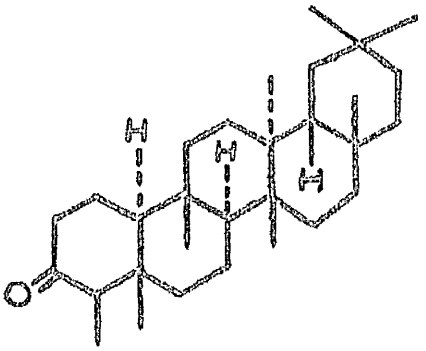
It is noteworthy that both lophenol and citrostadienol are found in nature accompanied by a triterpenoid and a steroid, namely lupeol (I) and stigmast-7-en-3β-ol (II), and by friedelin (XXX) and β-sitosterol (XXXI) respectively. This represents the kind of circumstantial evidence which permits the inclusion of plant sterols in the unified picture[53,54,56] of biogenesis by assuming demethylation of squalenoid cyclisation products as already demonstrated[53] in animal tissues.



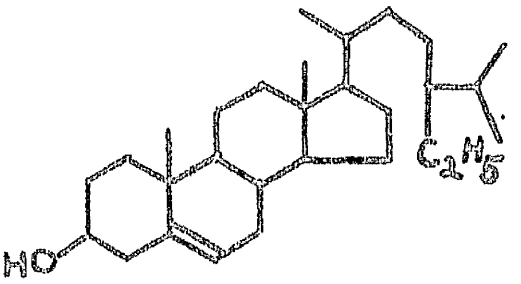
(I)



(II)



(XXX)



(XXXI)

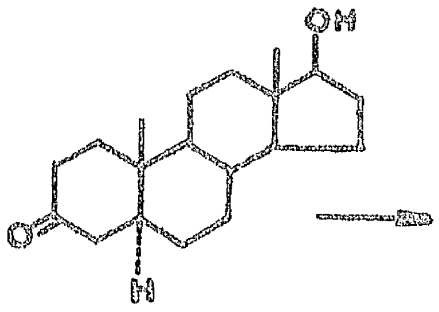
Synthetic Methyl Steroids

In the course of recent researches into the biological effects of modified steroids, the insertion of a methyl group at various positions has been of particular interest. Toromanoff[57] has published an excellent review of this work but since our own investigations deal with the introduction of methyl substituents into ring A, a resumé of methods already used for this purpose is presented below.

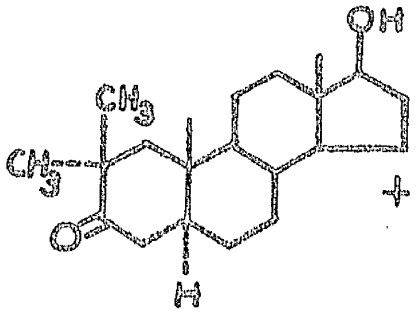
(1) Direct Methylation

The mode of attack of a methylating reagent such as methyl iodide and potassium tert-butoxide on a 3-oxo-steroid varies, depending on whether rings A and B are cis- or trans-fused and also on the presence of a 4,5-double bond. Thus in the 5α-series, methylation by this reagent is predominantly at C(2). Thus androstan-17β-ol-3-one (XXXII) gives a mixture of

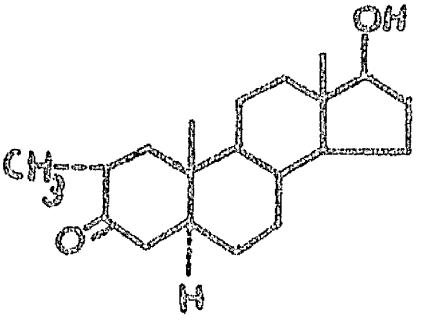
the 2,2-dimethyl (XXXIII) and 2-monomethyl (XXXIV) derivatives.[58]



(XXXII)

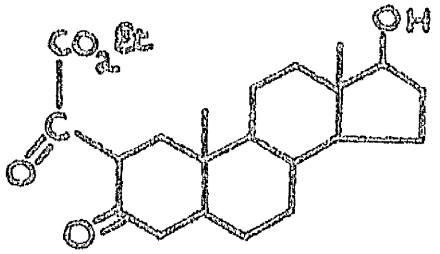


(XXXIII)

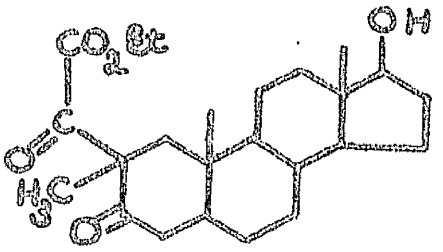


(XXXIV)

The above method results in a predominance of the dimethyl compound and a more convenient route to the monomethyl derivative involves the use of a 2-ethoxalyl or a 2-formyl intermediate. Thus the 2-ethoxalyl derivative (XXXV) yields the 2-methyl-2-ethoxalyl steroid (XXXVI), which on treatment with ethanolic sodium ethoxide furnishes 2α-methylandrostan-17β-ol-3-one (XXXIV).[58]

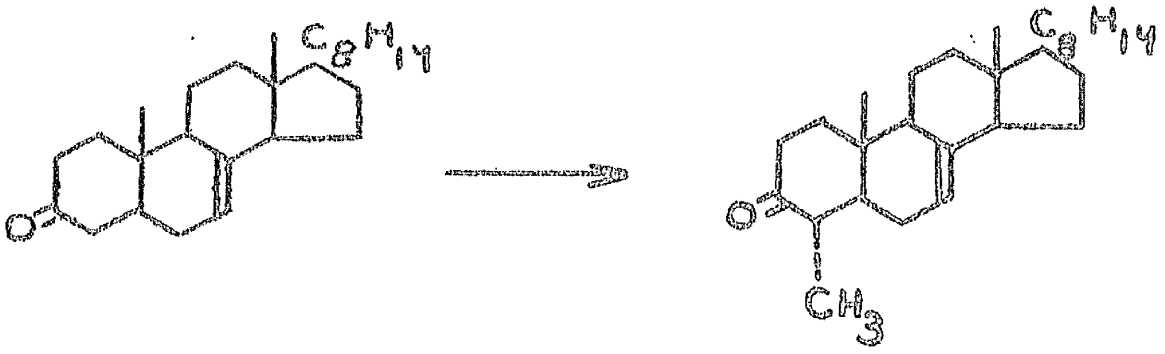


(XXXV)



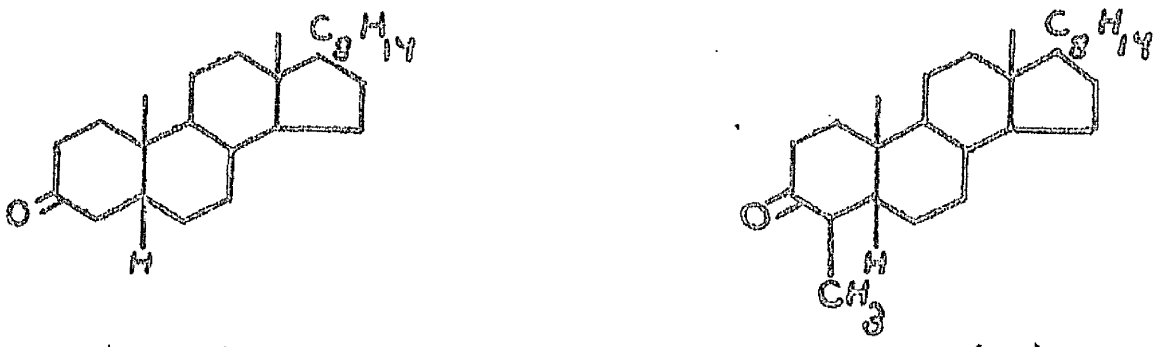
(XXXVI)

The direction of methylation can however be influenced by other steric factors as demonstrated by Mazur and Sondheimer[59] who found that cholest-7-en-3-one (XXXVII) is not methylated at the usual $C_{(2)}$ position but exclusively at $C_{(4)}$ to give 4α-methylcholest-7-en-3-one (XXXVIII).



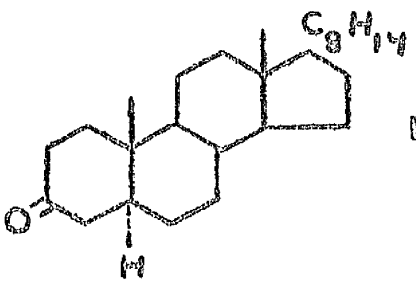
(XXXVII) (XXXVIII)

The cis-fusion of rings A and B directs the methylation towards position $C_{(4)}$. Mazur and Sondheimer[60] have shown that coprostan-3-one (XXXIX) reacts with methyl iodide in the presence of potassium tert-butoxide to form 4β-methylcoprostan-3-one (XL).

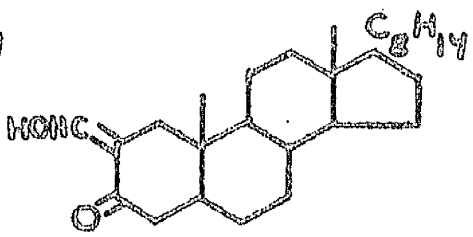


(XXXIX) (XL)

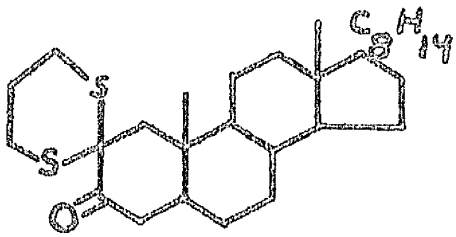
A most convenient method for the introduction of a 4-methyl group in the trans series involves blocking the more reactive position $C_{(2)}$, as has been achieved by Beton et al[61]. with respect to cholestan-3-one (XLI). Woodward[62] has shown that treatment of a solution of 2-hydroxymethylenecholestan-3-one (XLII) with trimethylene ditoluene-p-thiosulphonate and potassium acetate leads to the dithioketal (XLIII) and Beton and his co-workers, by varying the amounts of methyl iodide and potassium tert-butoxide, converted the dithioketal (XLIII) into a mixture, which after chromatography gave three compounds, one of which was shown to be the 4,4-dimethyl derivative of the dithioketal (XLIV). Desulphurisation of the other two materials with Raney nickel and subsequent oxidation gave 4α- (XLV) and 4β-methylcholestan-3-one (XLVI).



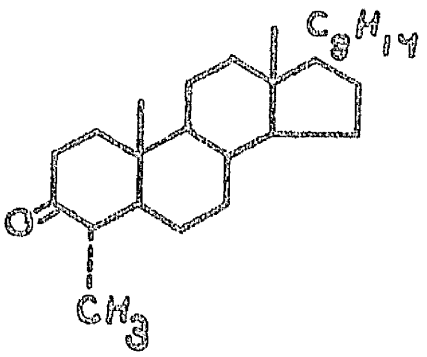
(XLI)



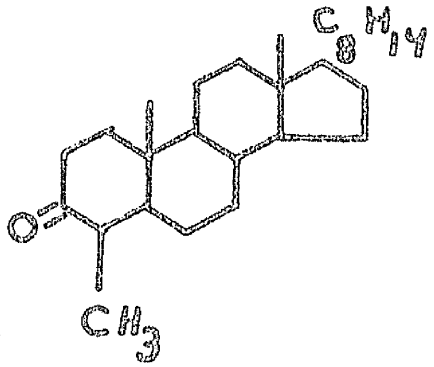
(XLII)



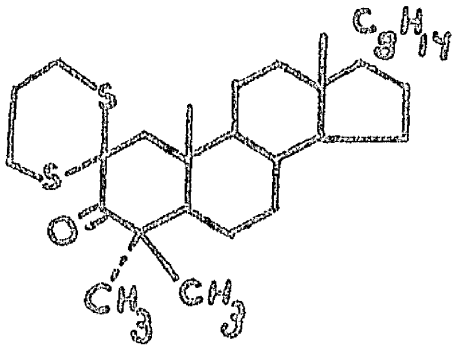
(XLIII)



(XLV)

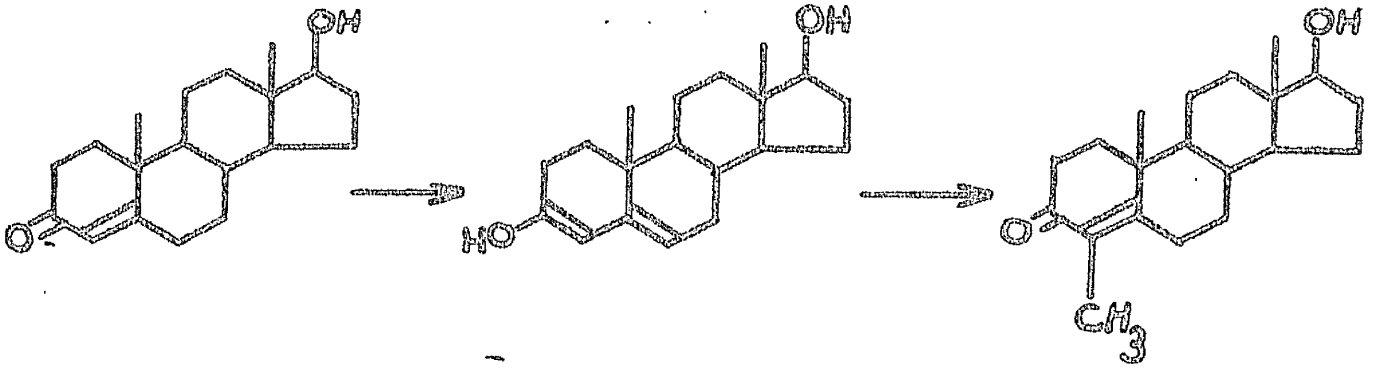


(XLVI)



(XLIV)

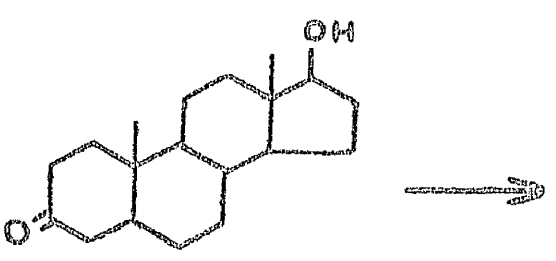
With respect to the 3-keto-4,5-ethylenic derivatives, alkylation of, for example, testosterone (XLVII) with methyl iodide and potassium tert-butoxide gives 4-methyltestosterone (XLVIII) in good yield,[69] attack at $C_{(4)}$ being favoured, since in the intermediate enol (XLIX) $C_{(4)}$ has high electron density.



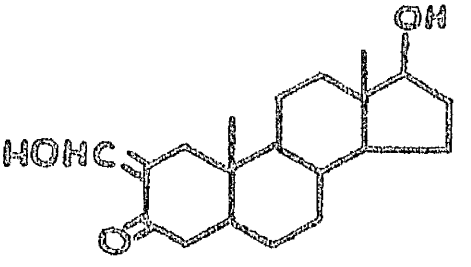
(XLVII) (XLIX) (XLVIII)

(2) Indirect Methylation

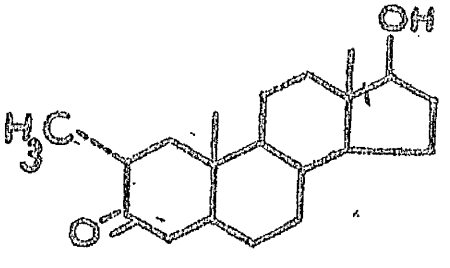
While direct methylation procedures afford good yields with 3-oxo-Δ^4-steroids, the reaction proceeds poorly with the corresponding dihydro-compounds and a more convenient preparation of methyl substituted derivatives of this type has been devised by Ringold et al.[66] The vicinal positions of steroidal ketones lend themselves very well to formylation by formyl esters under alkaline conditions and the above workers have prepared 2-hydroxymethylenedihydrotestosterone (LII) by condensing androstan-17β-ol-3-one (XXXII) with ethyl formate. Hydrogenation of this compound over a palladium-carbon catalyst yielded the thermodynamically unstable 2β-methyl derivative (LIII), which after contact with alkaline alumina gave 2α-methyldihydrotestosterone (XXXIV).



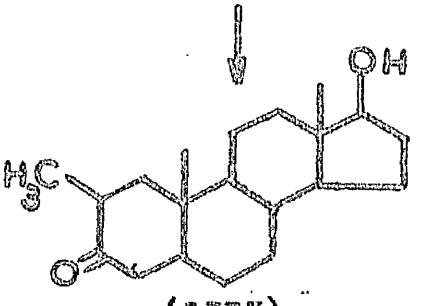
(XXXII)



(LII)

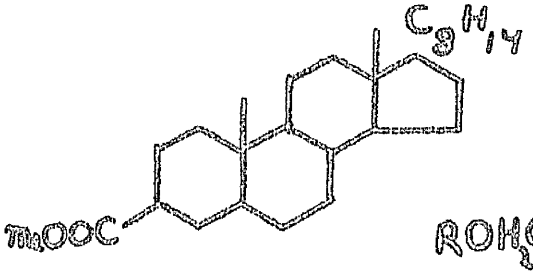


(XXXIV)

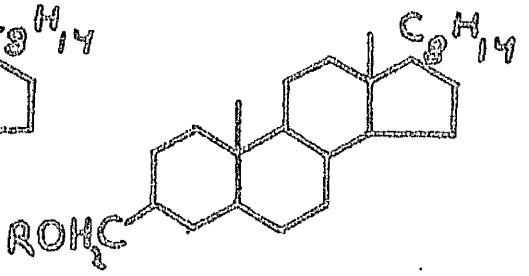


(LIII)

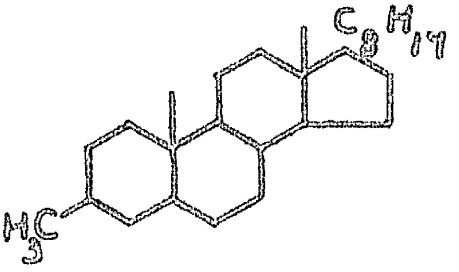
Barton[67] has prepared 3β-methylcholestane (LVII) by reduction of methylcholestane-3β-carboxylate (LIV) with lithium aluminium hydride to the primary alcohol (LV) followed by reduction of the derived toluene-p-sulphonate (LVI) with the same reagent.



(LIV)

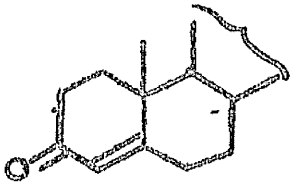


(LV) (R = OH)
(LVI) (R = $C_7H_7SO_3$)

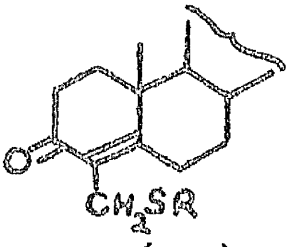


(LVII)

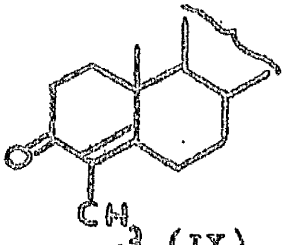
Recently, Kirk and Petrow[68] discovered a convenient and efficient route to 4-methyl-3-oxo-Δ^4-steroids (LX) by condensing the 3-oxo-Δ^4-steroid (LVIII) with formaldehyde and an organic thiol such as benzene-p-thiol in the presence of a tertiary base, to give the corresponding 4-organothiomethyl-3-oxo-Δ^4-steroid (LIX) which on treatment with Raney nickel in acetone gave the 4-methyl-3-oxo-Δ^4-steroid in overall yields of the order of 80%.



(LVIII)

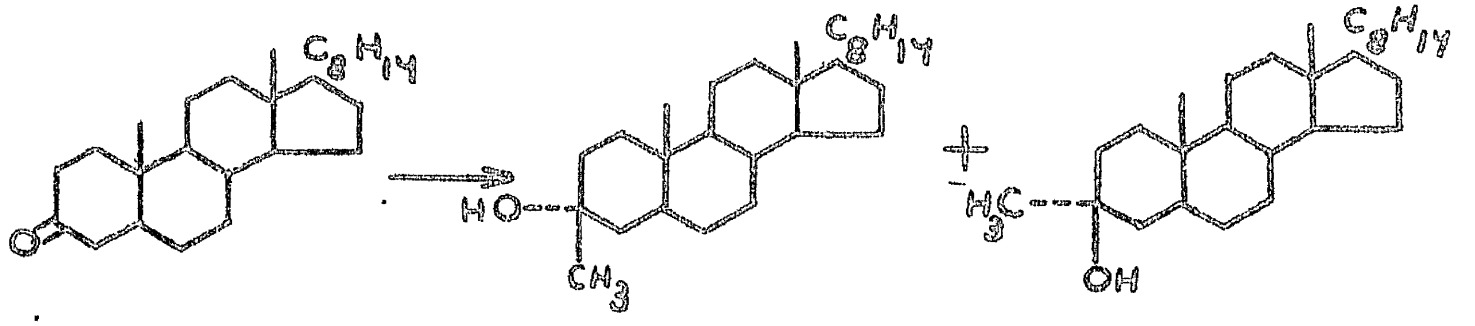


(LIX)

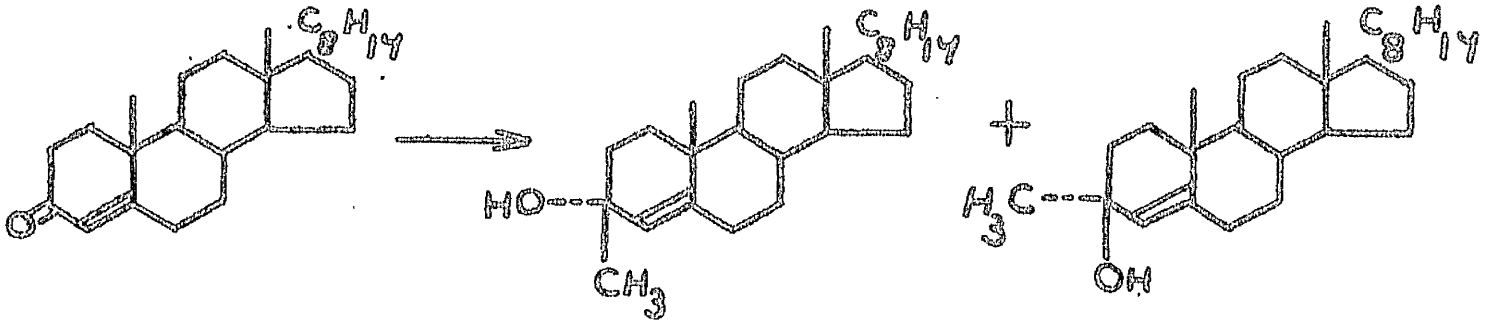


(LX)

The preparation of ring A methylated steroids *via* an oxo-derivative and a Grignard reagent has been confined to addition at $C_{(3)}$. Barton,[67] by treating cholestan-3-one (XLI) with methylmagnesium iodide, isolated 3-methylcholestan-3α- (LXI) and -3β-ol (LXII) while Musgrave[69] found that cholest-4-en-3-one (LXIII) with methylmagnesium iodide gave a molecular compound comprising the stereoisomers (LXIV) and (LXV).



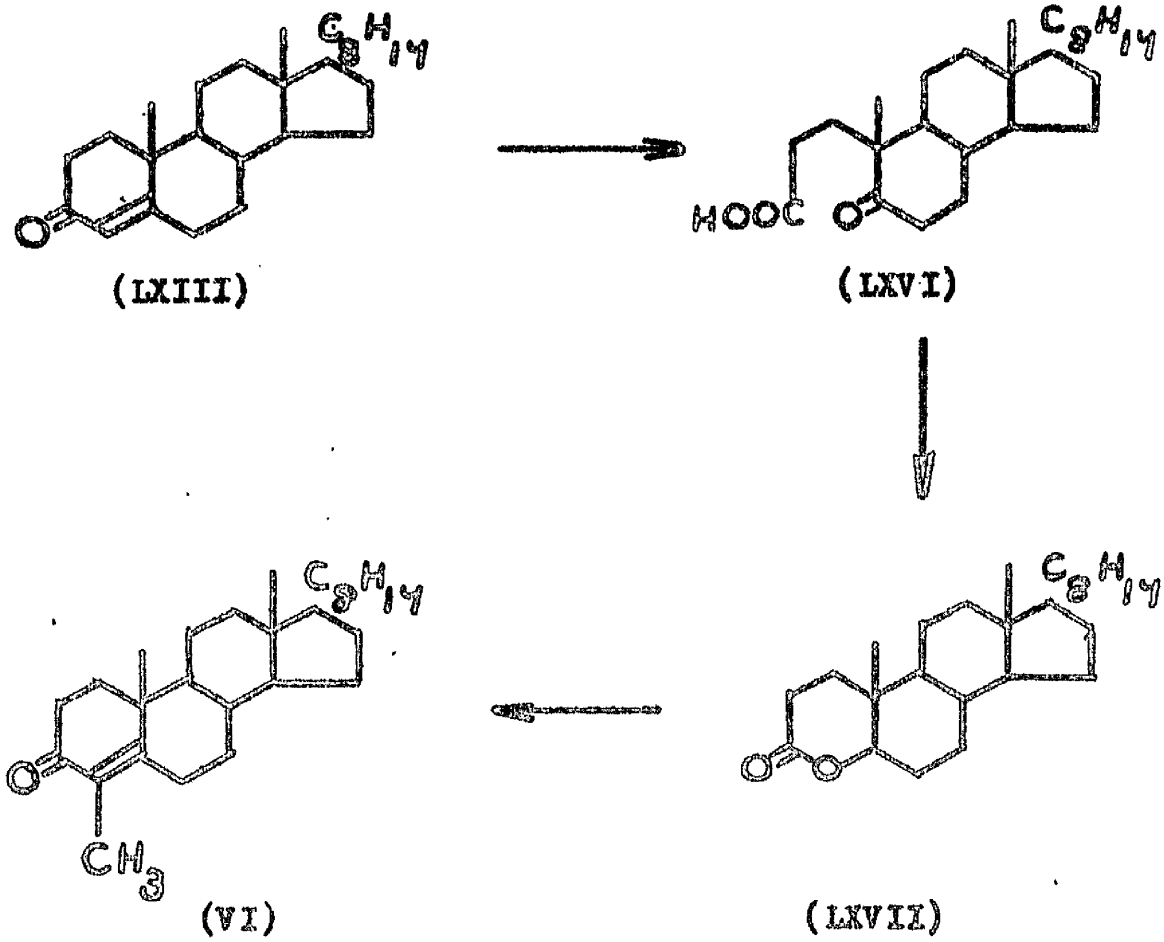
(XLI) (LXI) (LXII)



(LXIII) (LXIV) (LXV)

(3) Cleavage and Recyclisation.

The first synthesis of a 4-methyl steroid[10,11] involved the cleavage of cholest-4-en-3-one (LXIII) by ozonolysis[70,71] to the keto-acid (LXVI) which recyclised to the enol lactone (LXVII) on treatment with sodium acetate and acetic anhydride. Fujimoto,[72] and Heard and Ziegler[73] have shown that cholest-4-en-3-one (LXIII) can be obtained in high yield when the enol lactone (LXVII) is treated with methylmagnesium iodide and the product cyclised with base. Analogous treatment of (LXVII)



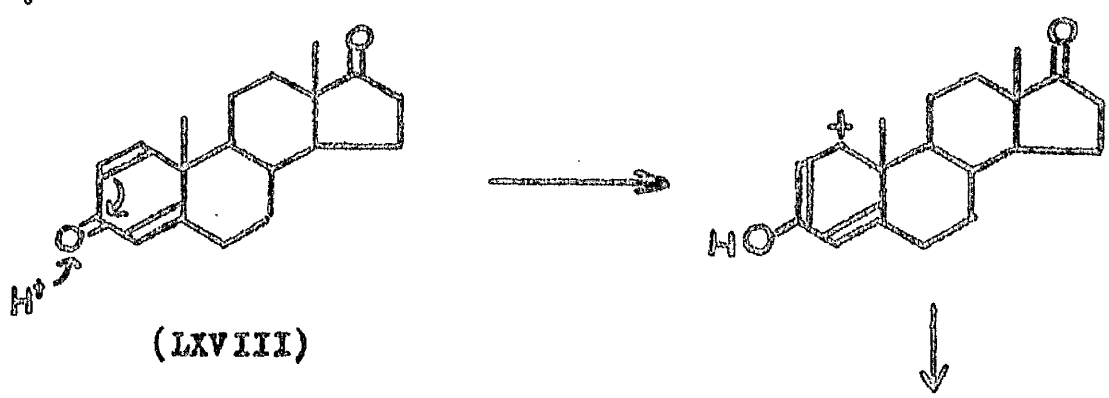
(LXIII) (LXVI) (VI) (LXVII)

with one equivalent of ethylmagnesium bromide was unsuccessful, but when an excess of the Grignard reagent was used, the required 4-methylcholest-4-en-3-one (VI) was obtained, after cyclisation with base.[10,11]

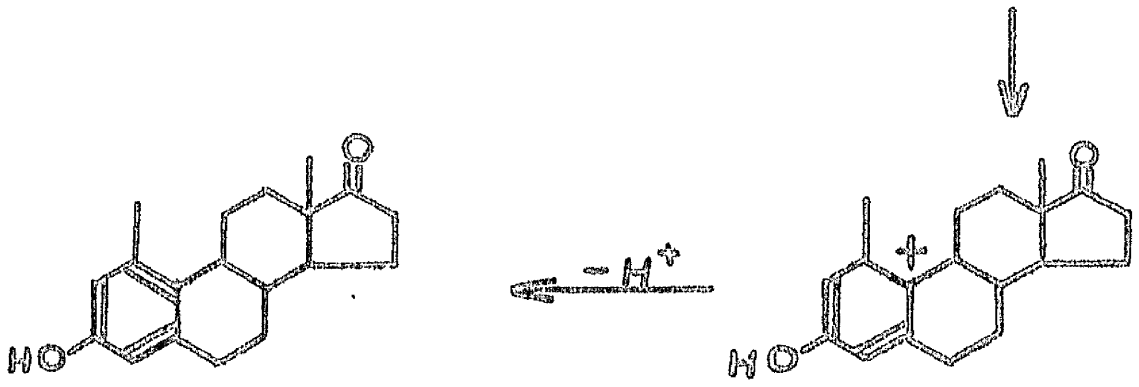
(4) Transposition

The steroid nucleus is susceptible to diverse structural rearrangements such as the dienone-phenol rearrangement,[74] where migration of a methyl group is effected without notable alteration in the steroid skeleton. Steroid dienones of the type (LXVIII) can aromatise to phenolic derivatives under the influence of acids in two different ways. The first corresponds to a simple migration of the methyl group from $C_{(10)}$ to $C_{(1)}$ (LXIX) as shown below in scheme (I); the second results in the reversal of ring A by a series of rearrangements of the type depicted in shheme (II) with the appearance of the hydroxyl group at $C_{(1)}$ and the methyl group at $C_{(4)}$ (LXX).

(I)

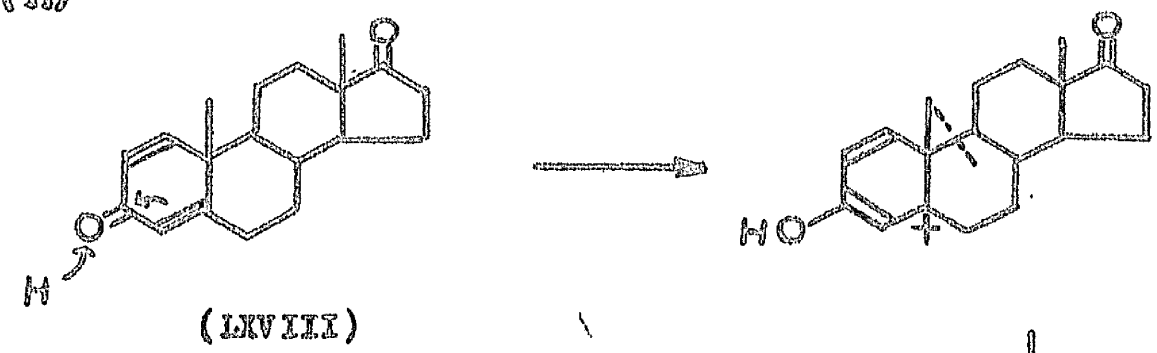


(LXVIII)

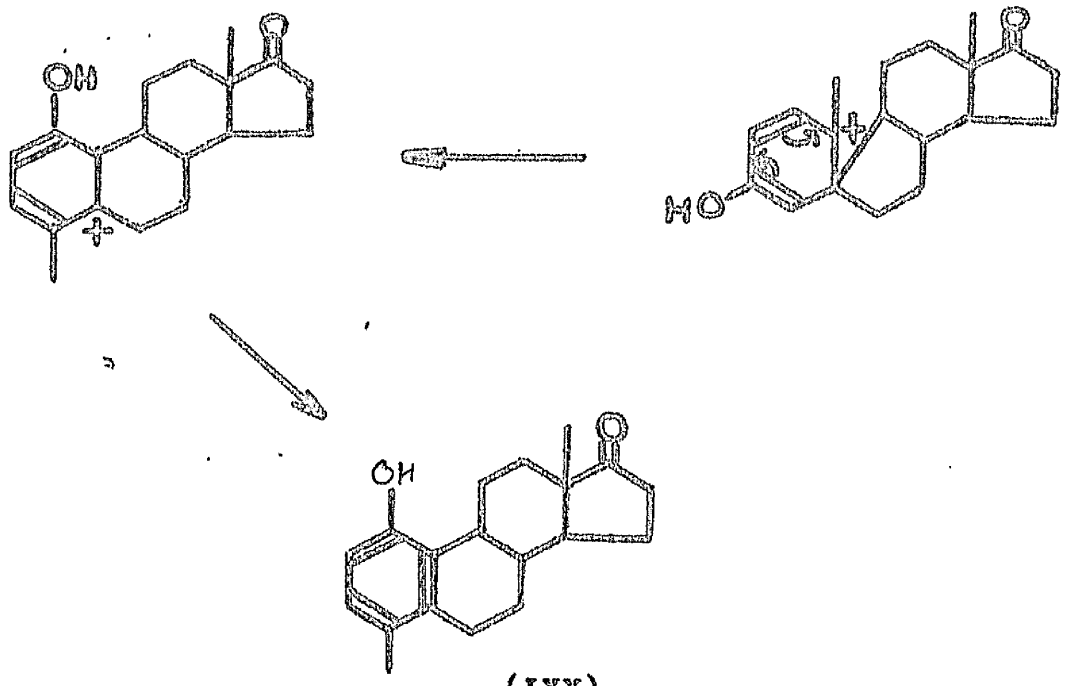


(LXIX)

(II)



(LXVIII)



(LXX)

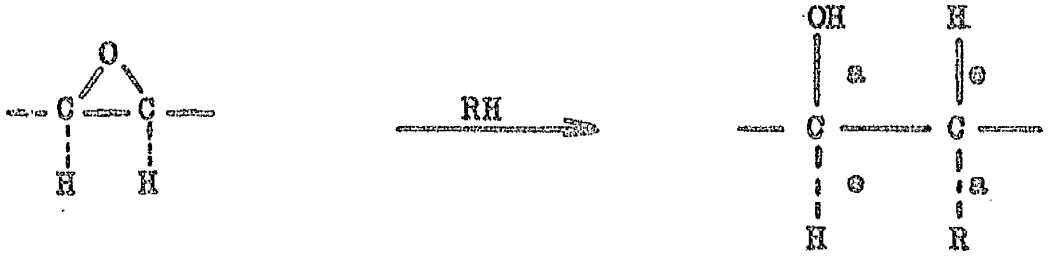
THEORETICAL

S E C T I O N I

The Action of Methylmagnesium Iodide on 4α,5α-Epoxycholestan-3β-yl Acetate.

Methylmagnesium iodide reacts with 4α,5α-epoxycholestan-3β-yl acetate to give exclusively 4β-methylcholestan-3β,5α-diol. Some aspects of the chemistry of this diol are described. Its structure has been established by conversion into 4-methylcholest-4-en-3-one, a compound of known constitution.

Treatment of 4α,5α-epoxycholestan-3β-yl acetate (LXXI) with an excess of methylmagnesium iodide gave a product, m.p. 187-189°, $[\alpha]_D$ + 23.5° in 90% yield. This material showed no colour with tetranitromethane and its infrared spectrum contained a strong band at 3333 cm^{-1} (hydroxyl). These observations together with the analytical data indicated that the substance was probably a methyl substituted cholestane diol. Steroidal epoxides are normally cleaved to give a diaxial product in which the resulting hydroxyl group retains the configuration of the original oxide.[75]

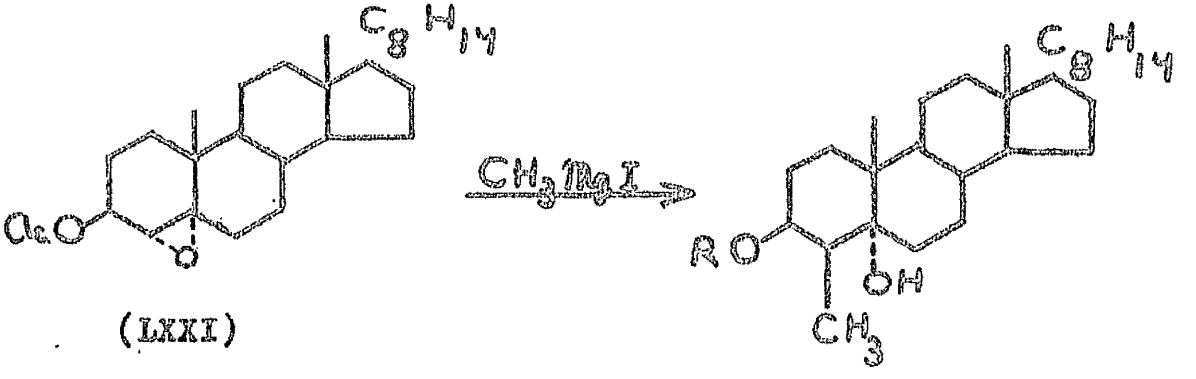


If such opening of the oxide ring occurs in the present case, the product would be 4β-methylcholestan-3β,5α-diol (LXXII) and the evidence presented below confirms this.

Oxidation of the diol (LXXII) with chromium trioxide in pyridine did not give 4β-methylcholestan-5α-ol-3-one (LXXIV) (although this saturated ketone must certainly be an intermediate) but afforded an αβ-unsaturated ketone, identified as the known 4-methylcholest-4-en-3-one (VI).[11] This conversion to the known ketone fixed the position of the methyl substituent at $C_{(4)}$

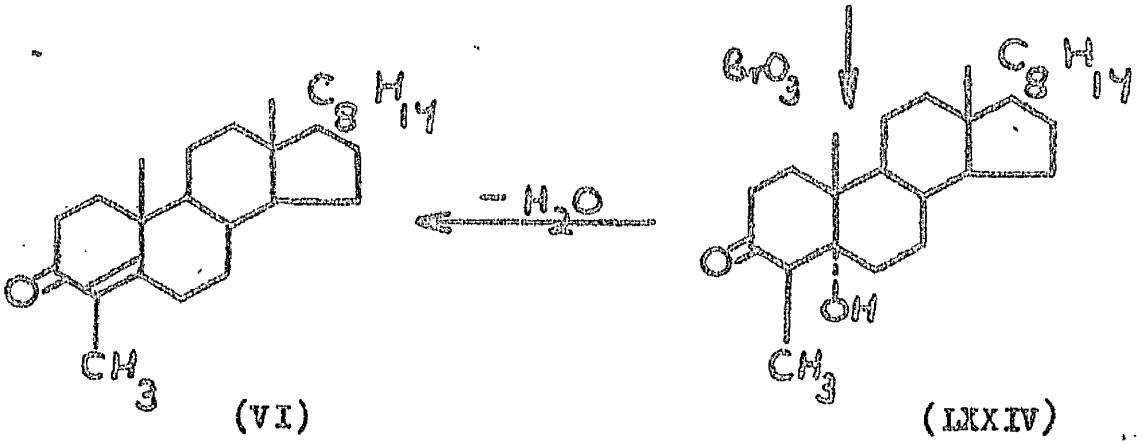
and eliminated the possibility of the new diol being 5α-methylcholestan-3β,4β-diol (LXXV) (still diaxial opening).

Further evidence in favour of the structure (LXXII) came from an examination of the infrared spectrum (in nujol) of 4β-methylcholestan-3β,5α-diol-3-acetate (LXXIII) which showed twin absorption bands in the acetate regions (1740, 1710, 1280 and 1245 cm.$^{-1}$). This behaviour is characteristic of steroids having a β-orientated 3-acetate group and an α-orientated 5-hydroxyl group[76] and it has been suggested[77] that the presence of the twin peaks is due to intermolecular bonding.



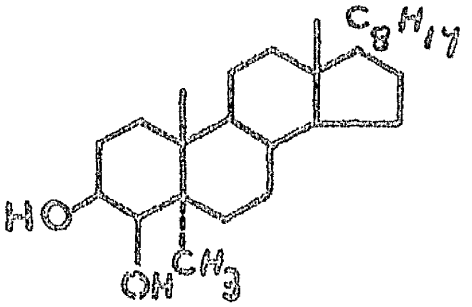
(LXXI)

(LXXII)(R = H)
(LXXIII)(R = CH_3CO)



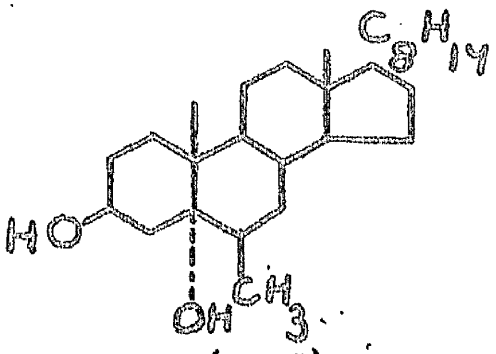
(VI)

(LXXIV)

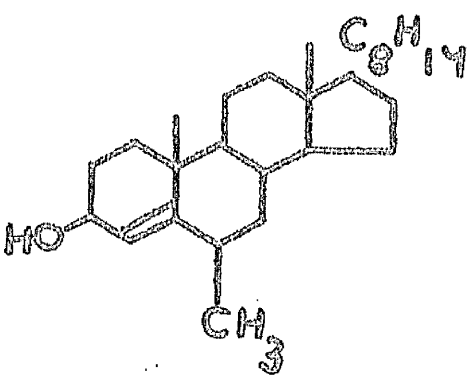


(LXXV)

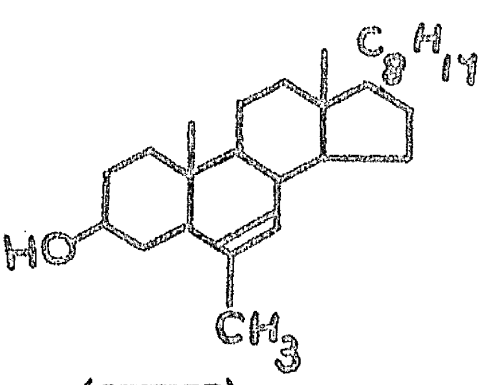
It has been reported[14,76] that acid catalysed dehydration of 6β-methylcholestan-3β,5α-diol (VIII) involves cis-elimination, the product being 6-methylcholest-5-en-3β-ol (LXXVII). Fieser and Rigaudy[78] have shown that the reaction does not proceed through 6β-methylcholest-4-en-3β-ol (LXXVI) (the expected product of dehydration), with subsequent isomerisation of the double bond to the tetrasubstituted 5(6)-position; these findings have been further substantiated by Turner.[79] In view of these unusual results it was decided to investigate the dehydration of 4β-methylcholestan-3β,5α-diol (LXXII) to see if this compound also behaved abnormally.



(VIII)



(LXXVI)

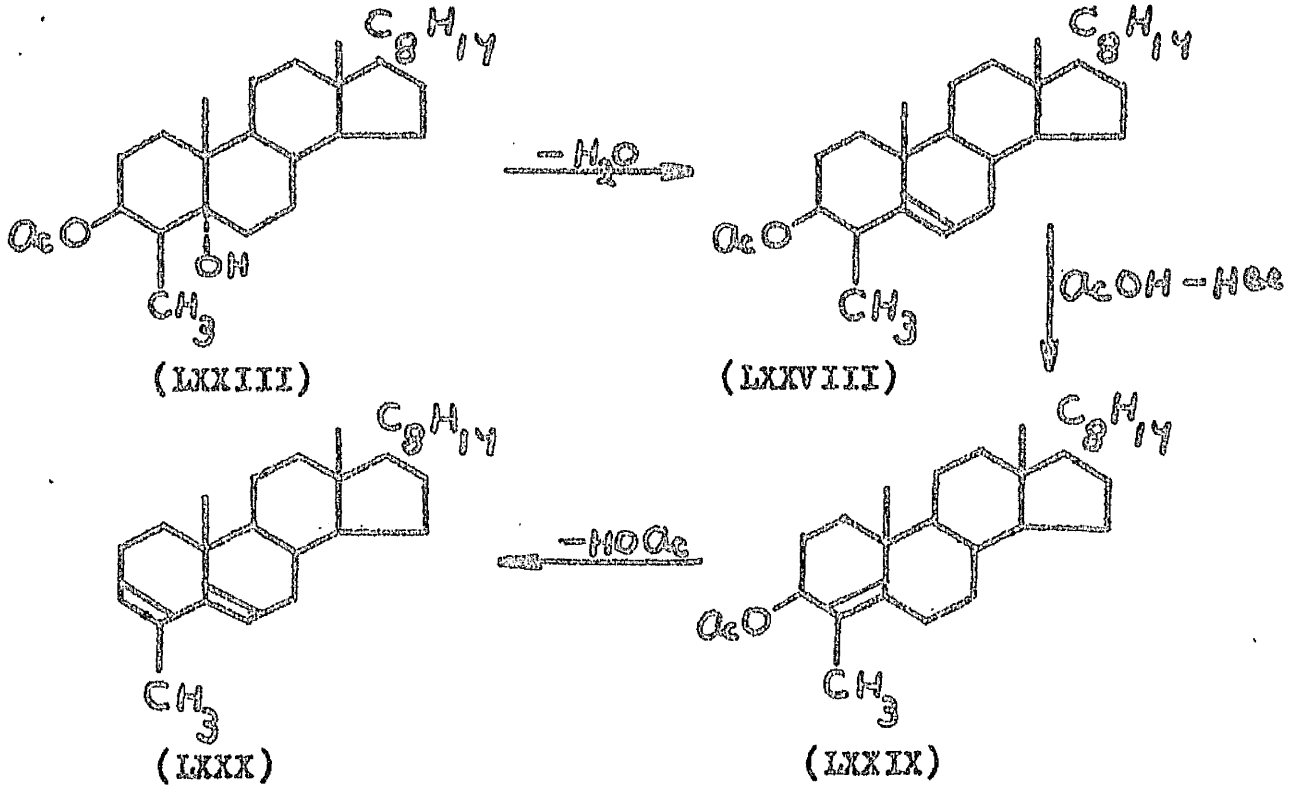


(LXXVII)

Dehydration of the diol monoacetate (LXXIII) with phosphorus oxychloride in pyridine yielded a compound which displayed the strong laevorotatory character of steroids containing a 5,6-double bond and whose ultraviolet absorption spectrum (λ max. 204 mu, ε 3,000) also suggested a trisubstituted ethylenic linkage. On the above evidence the product was formulated as 4β-methylcholest-5-en-3β-yl acetate (LXXVIII) and therefore dehydration has proceeded normally with trans-elimination of water. When dehydration was effected under the strongly acidic conditions of acetic anhydride - sulphuric acid the same product was obtained.

In an attempt to prepare the isomer (LXXIX) containing the tetrasubstituted double bond, 4β-methylcholest-5-en-3β-yl acetate (LXXVIII) was treated at 100° with hydrochloric acid in acetic acid. This reaction led to the isolation of a hydrocarbon whose ultraviolet absorption spectrum displayed triple peaks at 2320, 2380, and 2470 Å., typical of a heteroannular diene. This hydrocarbon must be 4-methylcholesta-3,5-diene (LXXX) and its formation from the acetate (LXXVIII) probably proceeds via the intermediate 4-methylcholest-4-en-3β-yl acetate (LXXIX) since in this compound the 3-acetate group is adjacent to a tetrasubstituted double bond and such a system has been shown[24] to facilitate the elimination of acetic acid with

the formation of a conjugated diene.



(LXXIII) (LXXVIII)

(LXXX) (LXXIX)

After the completion of this work, Julia and Lavaux[61] reported the preparation of some of the compounds described above. A comparison of the melting points and specific rotations of the derivatives prepared by these workers and by the author is shown in Table A.

It can be seen from Table A that with the exception of 4β-methylcholestan-3β,5α-diol-3-acetate (LXXIII) the specific rotation values are of the same order, but in all cases the melting points of the compounds prepared by Julia and

T A B L E A

		Author	Julia and Lavaux	
	m.p. (Kofler)	$[\alpha]_D$	m.p.	$[\alpha]_D$
4β-methylcholestan-3β,5α-diol (LXXII)	187-189°	+23.5°	181.5-182.5°	+17°
4β-methylcholestan-3β,5α-diol-3-acetate (LXXIII)	174-175	+ 4	167-168	+16
4β-methylcholest-5-en-3β-yl acetate (LXXVIII)	163-165	-71.5	157-159	-68
4-methylcholesta-3,5-diene (LXXX)	78-79	-91	71-72	-97

Lavaux are 6-7° lower than those reported in this thesis.

One other point of interest concerns the oxidation of 4β-methylcholestan-3β,5α-diol (LXXII). Julia and Lavaux found that their product (of 'chromic oxidation') was 4β-methylcholestan 3-one-5α-ol (LXXIV), a compound not isolated by us but postulated as an intermediate.

Appendix.

During the preparation of 4α,5α-epoxycholestan-3β-yl acetate (LXXI) by the procedure of Henbest and Wilson,[82] a compound was isolated which had not been reported by previous workers[82,83] as a product of this reaction. Treatment of cholest-4-en-3β-yl acetate (LXXXI) with a solution of perbenzoic acid in benzene afforded a gum which on crystallisation from methanol gave the required 4α,5α-epoxycholestan-3β-yl acetate (LXXI). From the mother liquors another solid, m.p. 108-109°, $[\alpha]_D$ + 20° was obtained, the apparent composition, $C_{29}H_{48}O_3$, indicating that this material was isomeric with the

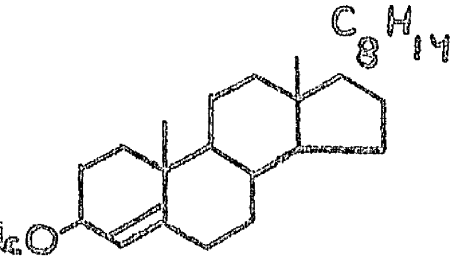
epoxide (LXXI). A comparison of the constants of (LXXI) and of 4β,5β-coprostan-3β-yl acetate (LXXXII) with those of the new compound (Table B) suggested that it was in fact a molecular complex of (LXXI) and (LXXXII). Mixed compounds of this type are not uncommon in the steroid field.[69,84,85]

TABLE B

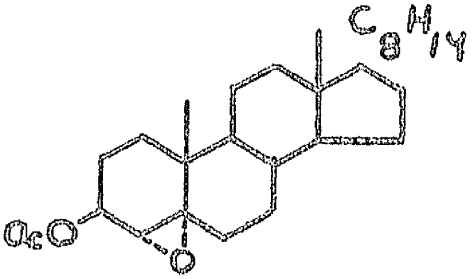
	m.p.	$[\alpha]_D$
4α,5α-Epoxycholestan-3β-yl Acetate (LXXI)	119°	+ 60°
4β,5β-Epoxycoprostan-3β-yl Acetate (LXXXII)	89	- 22
Molecular Complex	109	+ 20

Although the molecular complex could not be separated into its components by chromatography on alumina, the relationship was confirmed by the artificial preparation of the material by crystallisation of a mixture of equal parts of 4α,5α-epoxycholestan-3β-yl acetate (LXXI) and 4β,5β-epoxy-coprostan-3β-yl acetate (LXXXII). The resulting complex was indistinguishable from that prepared by the action of perbenzoic acid on the olefin (LXXXI).

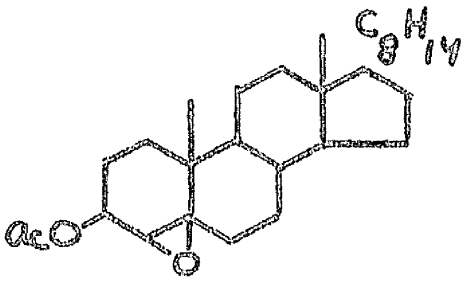
This molecular complex has also been obtained in this laboratory by Rowan[86] during the preparation of the 4,5-epoxides of cholestan-3β-yl acetate by the method of Plattner.[87]



(LXXXI)



(LXXI)



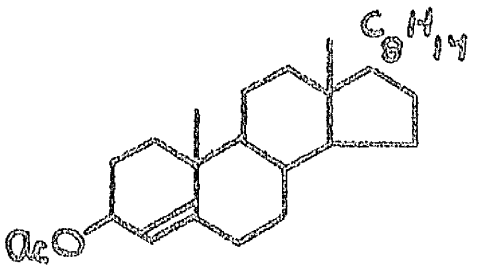
(LXXXII)

S E C T I O N II

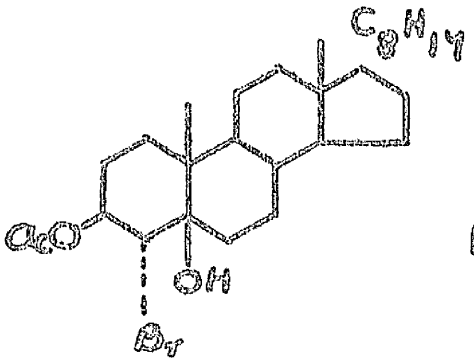
The Action of Methylmagnesium Iodide on 4β,5β-Epoxycoprostan-3β-ol

Methylmagnesium iodide reacts with 4β,5β-epoxycoprostan-3β-ol to give a variety of products, two of which have been identified. Experiments which show that one of these compounds is 4α-methylcholestan-3β,4β-diol are discussed and a synthesis of this diol by an unambiguous route is described. The second compound has been shown to be identical with the known cholest-5-en-3β,4β-diol.

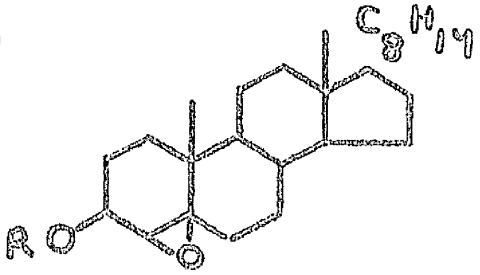
By a procedure essentially the same as that used by Collins,[83] 4β,5β-epoxycoprostan-3β-ol (LXXXIII) was prepared from cholest-4-en-3β-yl acetate (LXXXI) via the bromohydrin (LXXXIV).



(LXXXI)



(LXXXIV)



(LXXXIII)(R = H)
(LXXXII) (R = CH_3CO)

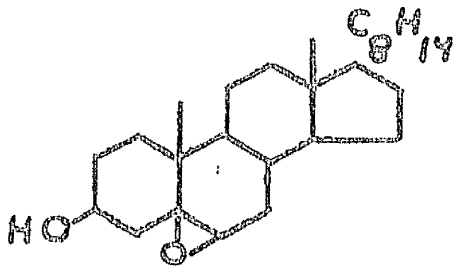
When a solution of this epoxide in ether-benzene was refluxed with an excess of methylmagnesium iodide, a complex mixture was obtained which on chromatography on alumina yielded several products, only two of these being obtained in quantities suitable for further investigation. The melting points and specific rotations of these materials are given in Table C.

TABLE C

Compound	Eluant	m.p.	$[\alpha]_D$	% yield
A	½% MeOH in ether	140-141°	+ 38.5°	1.6
B	1% MeOH in ether	202-203	+ 24.5	30
C	3% MeOH in ether	190-191	+ 17.5	1.8
D	3% MeOH in ether	203-205	+ 9.5	1.3
E	4% MeOH in ether	175-176	- 62	28

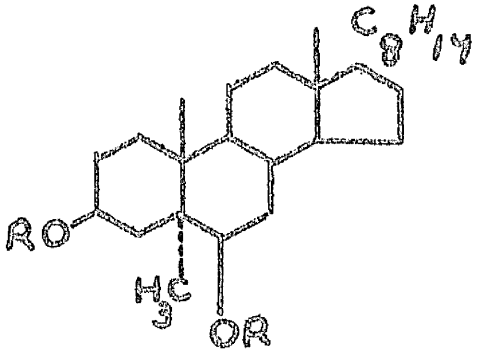
At this time Julia and Lavaux[81] reported the preparation of a diol, m.p. 188-190°, $[\alpha]_D$ + 27°, from the reaction between methylmagnesium iodide and 4β,5β-epoxycoprostan-3β-yl acetate (LXXXII) and these workers tentatively suggested that this compound was 5α-methylcholestan-3β,4β-diol (LXXV). In view of the discrepancies in constants reported by Julia and Lavaux with those obtained by the author (see Section I, Table A), it was difficult to determine whether their diol corresponded to B, C or D (Table C); a consideration of yield and rotation favoured B. The formation of the diol (LXXV) would follow the general rule of diaxial opening of steroidal epoxides and would be analogous to the product obtained by Urushibara and Chuman[12,13] from the similar reaction of methylmagnesium iodide with 5β,6β-epoxycoprostan-3β-ol (LXXXV). This product was shown by these workers to be 5α-methylcholestan-3β,6β-diol (VII) since it formed a diacetate (LXXXVI), a dibenzoate (LXXXVII), and a diketone (LXXXVIII) which could be converted to 5α-methylcholestane (LXXXIX) on Clemmensen reduction.

It was decided to carry out a fuller examination of the chemistry of the product B and the evidence obtained indicates that this compound is 4α-methylcholestan-3β,4β-diol (XC),



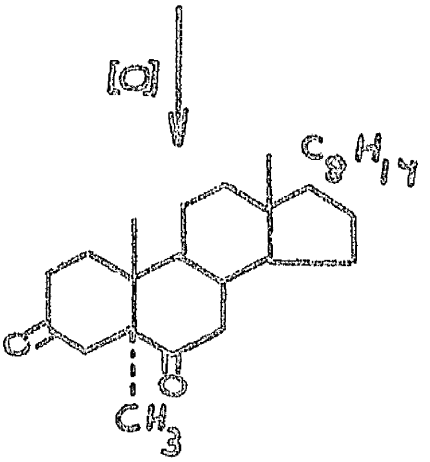
(LXXXV)

CH_3MgI



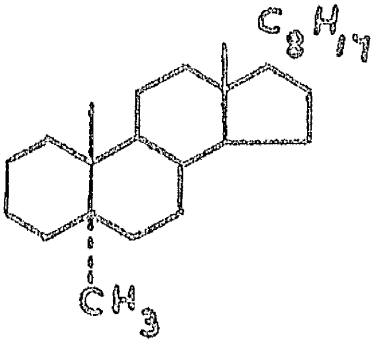
(VII) (R = H)
(LXXXVI) (R = CH_3CO)
(LXXXVII) (R = C_6H_5CO)

[O]



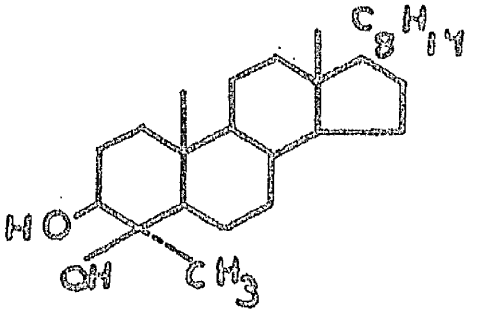
(LXXXVIII)

CLEMMENSEN REDUCTION

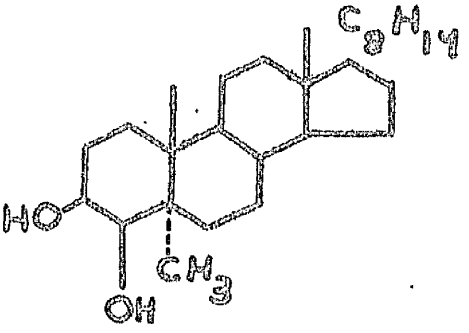


(LXXXIX)

although the investigation had been underway for some time before doubts arose concerning the correctness of structure (LXXV) proposed by Julia and Lavaux.



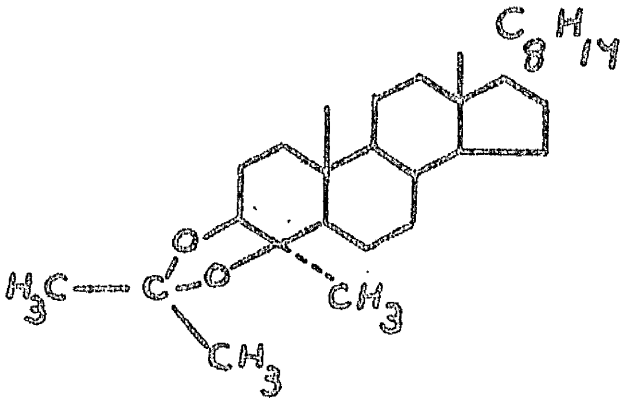
(XC)



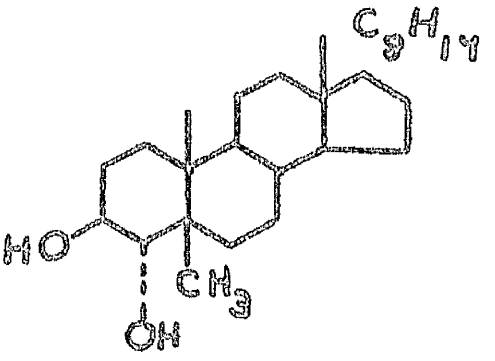
(LXXV)

The light absorption data (ultraviolet and infrared) and the analysis pointed to B being a saturated diol. In order to obtain information regarding the positions of the hydroxyl groups a quantitative examination of the reaction of B with sodium periodate was carried out. This experiment showed that a 1,2-glycol system was present, although the reaction rate was slow, the cleavage after 40 hr. and 90 hr. being 20% and 42% respectively. This is in keeping with the structure (LXXV) put forward by Julia and Lavaux and also with the alternative structure (XC) which we ascribe to the diol B. The slow rate of cleavage of the 1,2-glycol unit is presumably due to hindrance by the additional methyl substituent and by the $C_{(10)}$-methyl group, an effect which can be envisaged in both structures (LXXV) and (XC).

The possibility of the oxide ring having opened abnormally to give 5β-methylcoprostan-3β,4α-diol (XCII), (a diol which would be expected to react slowly with sodium periodate due to the diaxial orientation of the hydroxyl groups) could be eliminated on the following grounds. Compound B formed an acetonide (XCI) and on examination of a model of 5β-methylcoprostan-3β,4α-diol (XCII) shows that the diaxial nature of the glycol system makes acetonide formation sterically unlikely if not impossible.

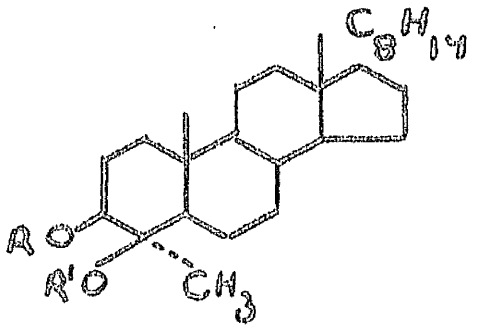


(XCI)

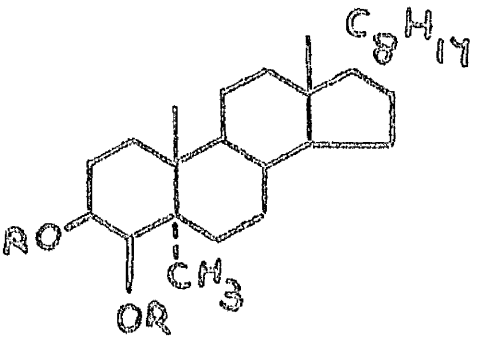


(XCII)

Treatment of the diol B with acetic anhydride in pyridine at room temperature gave crystals, m.p. 200-202° which depressed the melting point of the starting material and whose infrared spectrum showed the presence both of a hydroxyl group and an acetate group. It is therefore formulated as 4α-methylcholestan-3β,4β-diol-3-acetate (XCIII). At this stage in the investigation, when the structure (LXXV) was still favoured, the failure of B to form a diacetate was attributed to the highly hindered environment of the 4-hydroxyl group and it was considered that a diacetate might be obtained if more vigorous acetylating conditions were employed. When B was refluxed for 5 hr. with acetic anhydride, a mixture was obtained which furnished, after chromatography on alumina, the 3-monoacetate (XCIII) and a saturated diacetate now known to have the structure (XCIV) but which at the time was considered to be 5α-methylcholestan-3β,4β-diol diacetate (XCV).



(XCIII)(R = CH_3CO, R' = H)
(XCIV) (R = R' = CH_3CO)



(LXXV) (R = H)
(XCV) (R = CH_3CO)

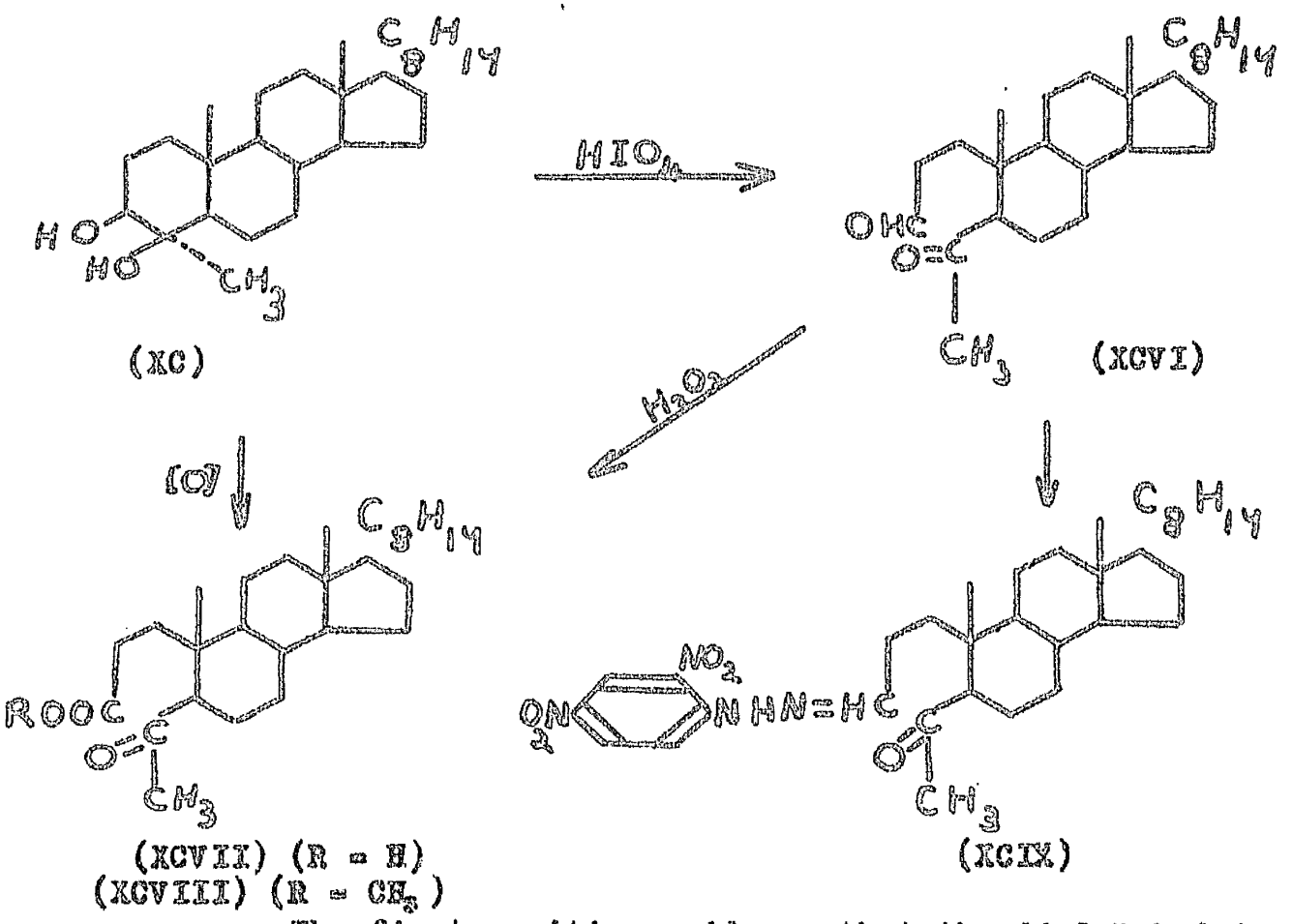
The infrared absorption of the diacetate showed no hydroxyl band and the acetate absorption showed two maxima in the carbonyl region at 1754 $cm.^{-1}$ and 1739 $cm.^{-1}$.

Oxidation of 4α-methylcholestan-3β,4β-diol (XC) with sodium dichromate in dilute sulphuric acid or with chromium trioxide in acetic acid gave a crystalline acid, m.p. 124-125°. When treated with diazomethane this acid gave a methyl ester which was smoothly reconverted to the original acid when saponified with methanolic potassium hydroxide at room temperature Therefore the acid did not contain a tertiary carboxyl group since methyl esters of tertiary acids are known to be resistant to dilute alkaline hydrolysis.[88] This information together with the fact that the infrared absorption spectrum of the compound showed a strong band at 1712 $cm.^{-1}$ (carbonyl) led to the conclusion that the acid was 4-methylcholestan-3,4-seco-4-oxo -3-carboxylic acid (XCVII). Support for this theory was obtained from a study of the cleavage of diol B with periodic

acid. When a solution of the diol in dioxan was treated with a solution of periodic acid in the same solvent, a non-crystalline carbonyl compound was obtained which is considered to be 4-methyl-3,4-seco-3,4-dioxocholestane (XCVI) on the basis of its facile oxidation with hydrogen peroxide in acetic acid to the previously obtained keto-acid (XCVII). The dicarbonyl compound (XCVI) formed a mono-2,4-dinitorphenyl-hydrazone whose infrared spectrum still showed strong carbonyl absorption at 1709 $cm.^{-1}$ and we consider this compound to have the structure (XCIX) although no real evidence is available for this formulation, apart from the fact that of the two carbonyl functions that at $C_{(3)}$ appears to be the less hindered.

A careful study of the above experiments shows that they do not provide sufficient evidence to allow the elimination of (LXXV) as a possible structure for B, since a diol of this formulation also lends itself to reactions similar to those cited above. Thus oxidation of the diol (LXXV) would give 5α-methylcholestan-3,4-seco-4-oxo-3-carboxylic acid (CI) if we accept the not unreasonable assumption that the tertiary aldehydic group is resistant to oxidation. From the cleavage of the diol (LXXV) with periodic acid the expected product would be the dialdehyde (C) and the formation of a mono-2,4-dinitrophenylhydrazone could once again be attributed to the

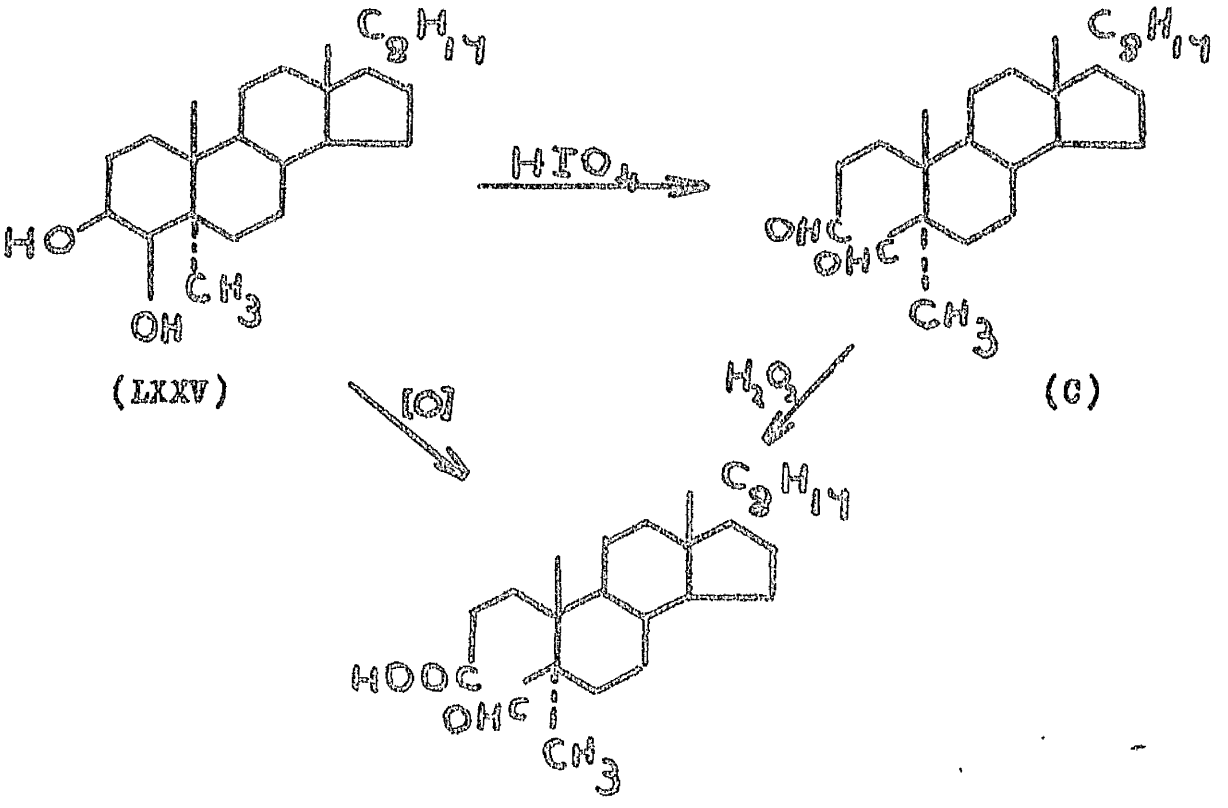
hindered tertiary aldehydic function.



(XC) (XCVI)

(XCVII) (R = H)
(XCVIII) (R = CH_3) (XCIX)

The first positive evidence that the diol B had the structure (XC) rather than (LXXV) came from the attempted oxidation of the monoacetate of B with chromium trioxide in pyridine. This resulted in a quantitative recovery of starting material and strongly suggested that a tertiary hydroxyl group was present. That the structure of B is (XC) was confirmed by the following series of reactions.

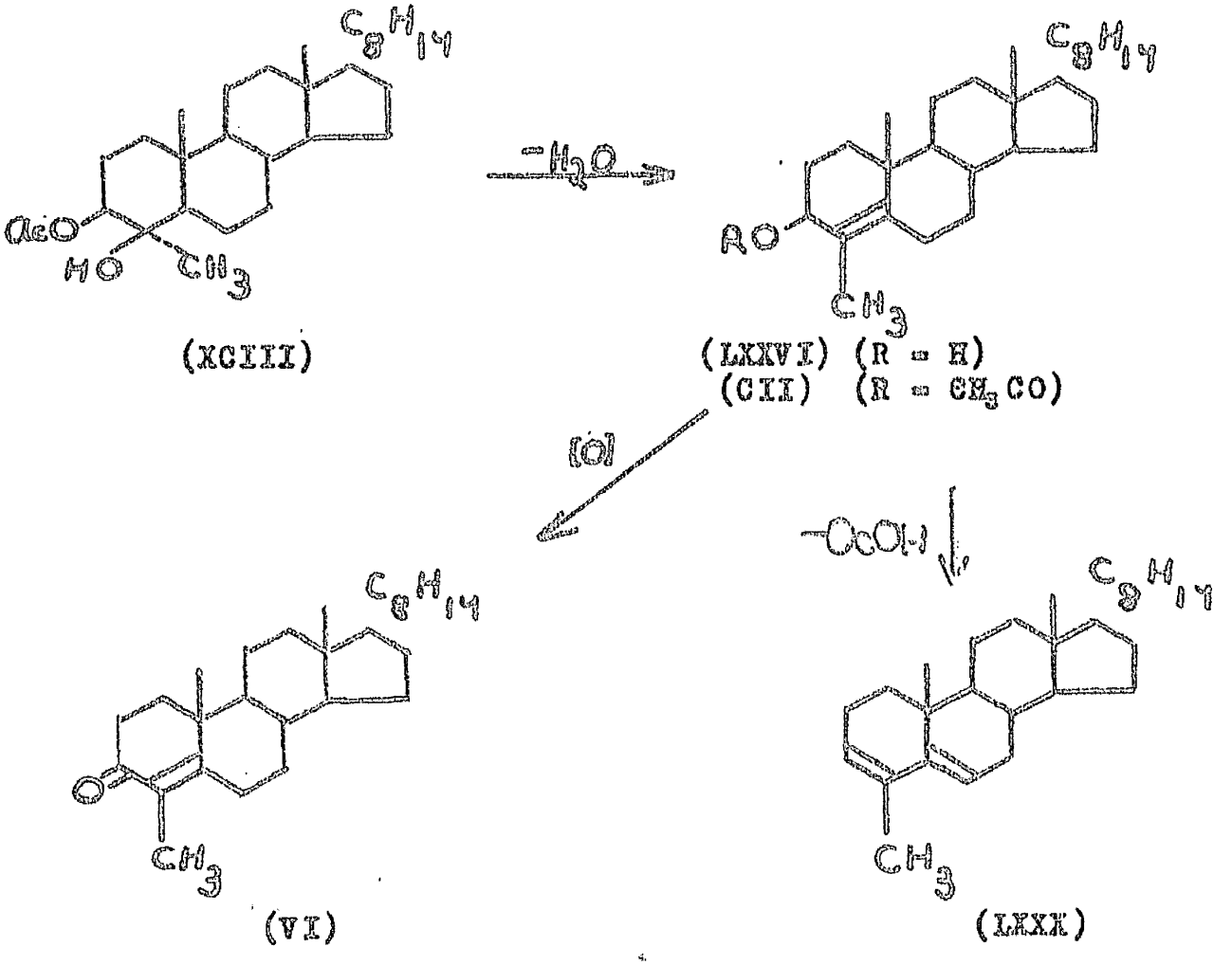


(CI)

Dehydration of 4α-methylcholestan-3β,4β-diol-3-acetate (XCIII) at 100° with phosphorus oxychloride in pyridine gave a mixture which on chromatography furnished a hydrocarbon and an acetate. The ultraviolet absorption of the hydrocarbon displayed triple peaks at 2320 Å. (ϵ 17,600), 2380 Å. (ϵ 18,600), and 2470 Å. (ϵ 11,600) which is typical of a steroid heteroannular $\Delta^{3,5}$-diene. This diene was readily shown to be 4-methylcholesta-3,5-diene (LXXX) since it was identical in all respects with the hydrocarbon obtained by treatment of 4β-methylcholest-5-en-3β-yl acetate with hydrochloric acid in acetic acid (Section I).

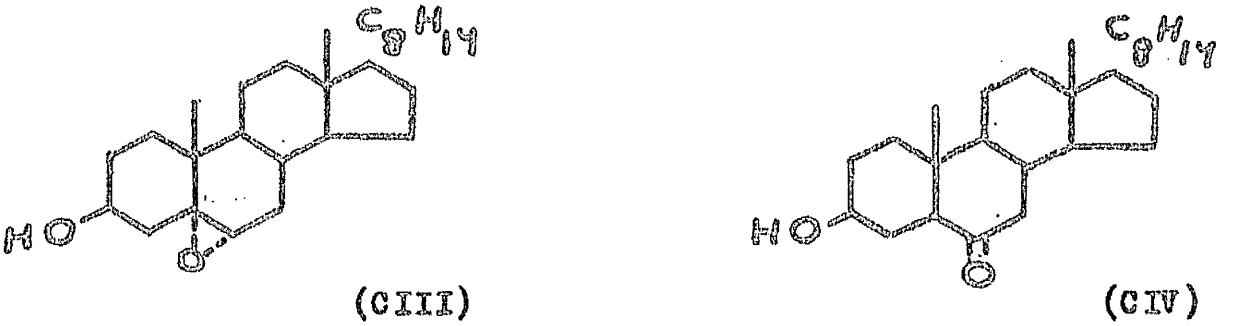
The ultraviolet absorption spectrum of the acetate obtained from the dehydration, exhibited a maximum at 2080 Å. (ε 11,000) and this relatively high intensity absorption is indicative of the presence of a tetrasubstituted double bond. The acetate is therefore considered to be 4-methylcholest-4-en-3β-yl acetate (CII) and since it contains an acetate group adjacent to a tetrasubstituted double bond the loss of acetic acid under the reaction conditions[24] can be envisaged with the formation of the hydrocarbon (LXXX). Structure (CII) for the acetate was confirmed when on treatment with lithium aluminium hydride it yielded 4-methylcholest-4-en-3β-ol (LXXVI) and this compound on oxidation with chromium trioxide in pyridine gave the known 4-methylcholest-4-en-3-one (VI). This conversion of the diol B into the 4-methyl ketone (VI) leads to the conclusion that B cannot be represented by structure (LXXV) since a methyl group migration from $C_{(5)}$ to $C_{(4)}$ would have to occur during the dehydration with phosphorus oxychloride in pyridine.

At this point it is relevant to note that 4β,5β-epoxy-coprostan-3β-ol (LXXXIII) undergoes rearrangement to the corresponding 4-ketone prior to reaction with methylmagnesium iodide. This is analogous to the reaction quoted by Gaylord



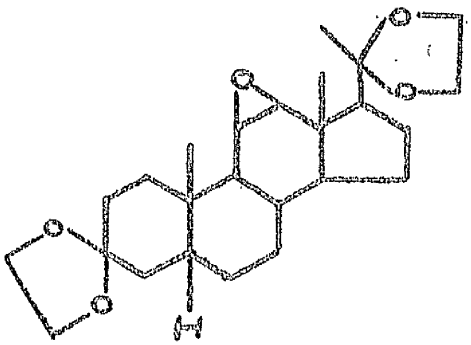
(XCIII) (LXXVI) (R = H)
(CII) (R = CH_3CO)
(VI) (LXXX)

and Becker[89] in which 5α,6α-epoxycholestan-3β-ol (CIII) reacts with phenylmagnesium bromide[90] to give 6-oxocholestan-3β-ol (CIV).

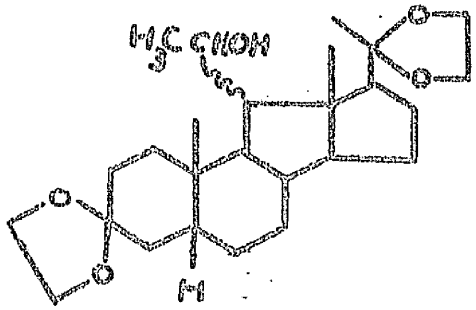


(CIII) (CIV)

Recently Christensen et al.[91] obtained a product from the reaction of methylmagnesium iodide and 11β,12β-epoxypregnan-3,20-dione-3,20-bis-(ethylene ketal) (CV) which they formulate as (CVI), thus assuming that a ring contraction has occurred.



(CV)



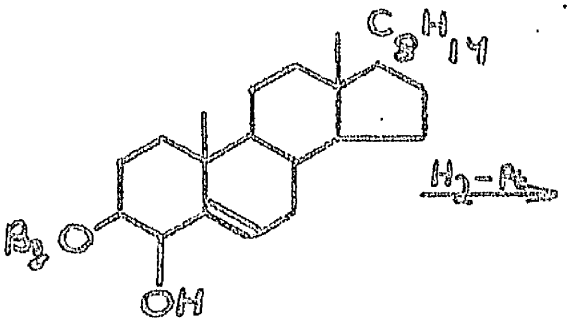
(CVI)

The above reactions appear to be the only cases of isomerisation of a steroid under Grignard conditions, although hypotheses of this nature have been suggested and finally rejected.[78,79]

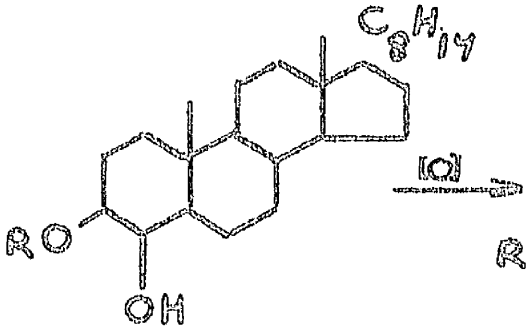
It was decided to attempt a synthesis of a derivative of (CX), namely 4-oxocholestan-3β-yl benzoate (CXI), with a view to studying the reaction of this compound with methylmagnesium iodide. A solution of cholest-5-en-3β,4β-diol-3-benzoate (CVII) was shaken with hydrogen in the presence of a platinum catalyst and after 12 hr. a volume of hydrogen equivalent to 4 moles had been absorbed. The ultraviolet

absorption spectrum of the product showed no maxima between 2000 Å. and 4000 Å. and in the infrared region the strong band at 715 $cm.^{-1}$ typical of a benzenoid system, was absent. This could only mean that under the reaction conditions hydrogenation had effected saturation of the aromatic ester as well as the Δ^{5}-double bond to give cholestan-3β,4β-diol-3-hexahydrobenzoate (CIX). This fact was confirmed by alkaline hydrolysis of the ester (CIX), when the known cholestan-3β,4β-diol (CVIII) was obtained. By oxidising the hydroxy-ester (CIX) in acetone solution with sodium dichromate in dilute sulphuric acid, the corresponding keto-ester, 4-oxocholestan-3β-yl hexahydrobenzoate (CXII) was isolated in 77% yield.

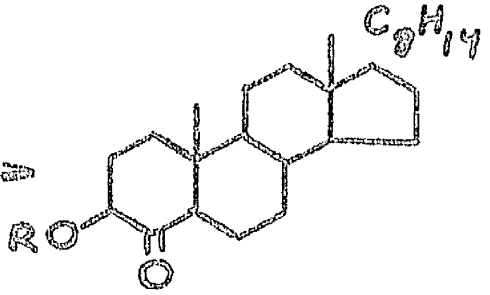
When a solution of the keto-ester (CXII) in ether-benzene was refluxed with a large excess of methylmagnesium iodide, a single compound was isolated in 70% yield. This proved to be identical to the diol B (XC), obtained by similar treatment of 4β,5β-epoxycoprostan-3β-ol (LXXXIII). The 4-methyl substituent in this compound is assigned the α-(equatorial)-configuration by analogy with the findings of Fieser and Rigaudy[78] who obtained 6α-methylcholestan-3β,6β-diol (CXIII) in high yield from the reaction of 6-oxocholestan-3β-ol (CIV) with methylmagnesium iodide.



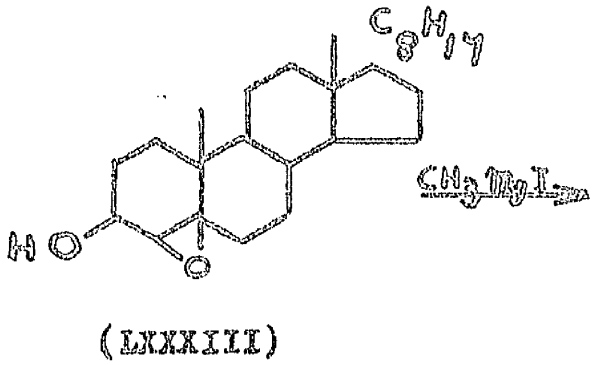
(CVII)



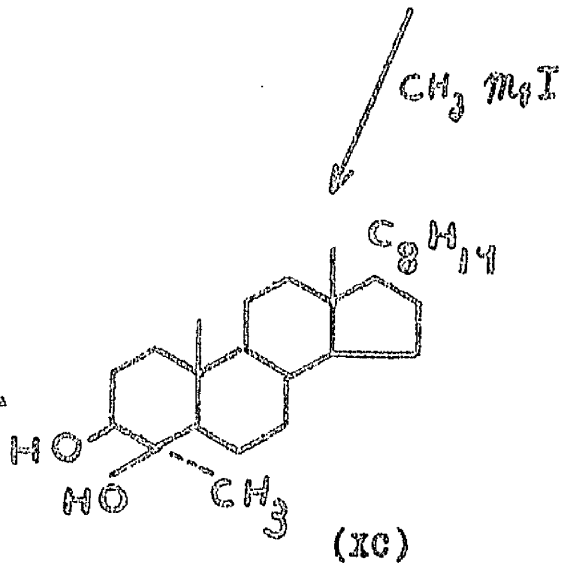
(CVIII) (R = H)
(CIX) (R = $C_6H_{11}CO$)



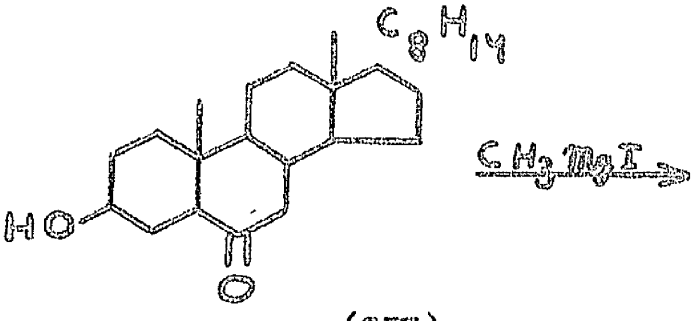
(CX)(R = H)
(CXI)(R = C_6H_5CO)
(CXII)(R = $C_6H_{11}CO$)



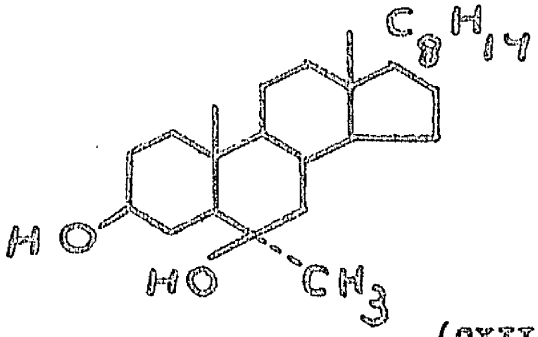
(LXXXIII)



(XC)



(CIV)

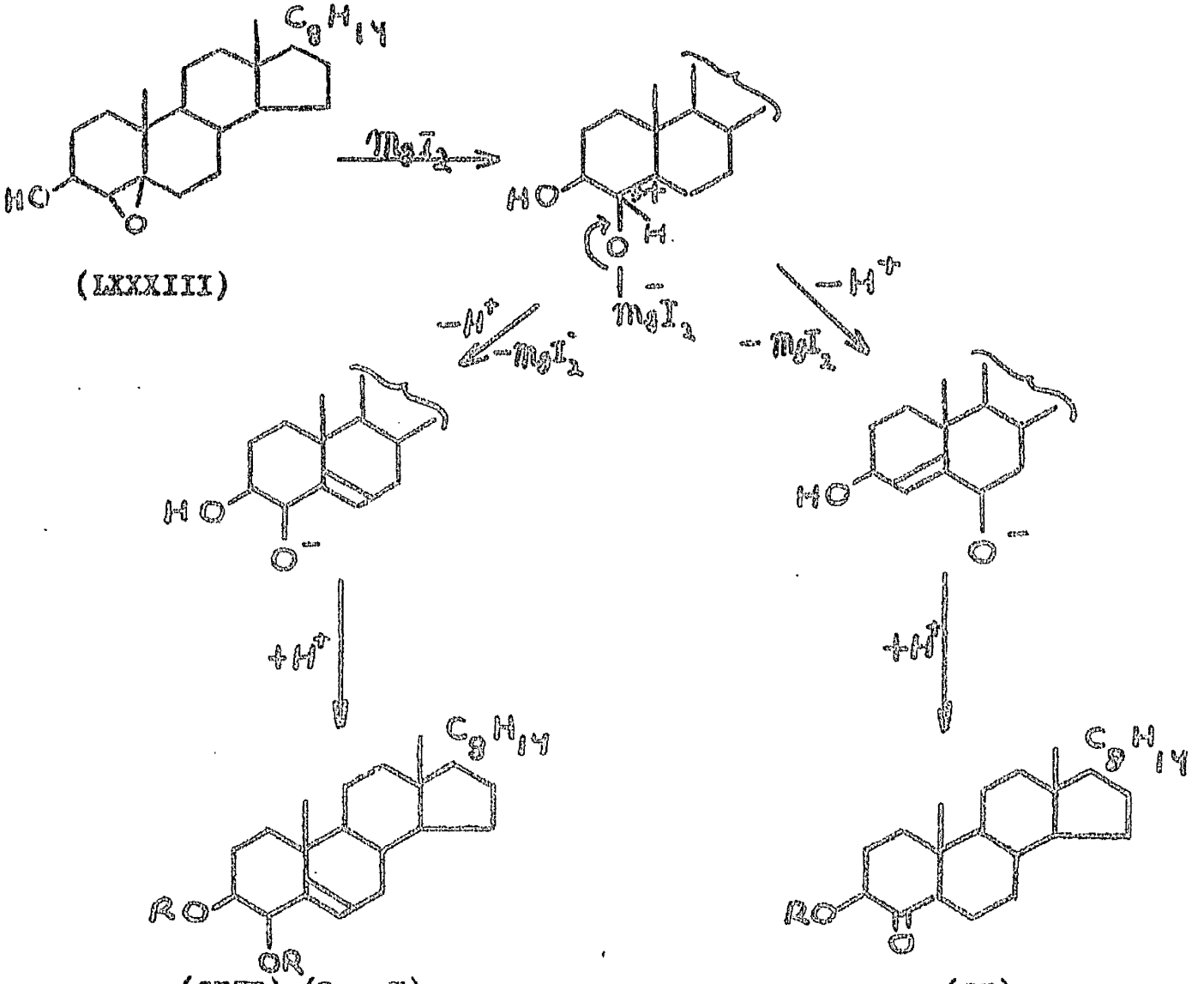


(CXIII)

Compound E

The other major product from the reaction of methylmagnesium iodide with 4β,5β-epoxycoprostan-3β-ol (LXXXIII) was shown to be cholest-5-en-3β,4β-diol (CXIV). The presence of a vicinal glycol system in this compound was indicated by the fact that it was readily cleaved with sodium periodate (after 4, 6, and 24 hr. the percentage oxidation was 65, 84, and 92 respectively). The ultraviolet absorption spectrum showed the presence of a double bond and the large negative specific rotation suggested that it was located between $C_{(5)}$ and $C_{(6)}$. Infrared comparison spectra of the diol E with an authentic sample of cholest-5-en-3β,4β-diol (CXIV) and of the corresponding diacetate with cholest-5-en-3β,4β-diol diacetate (CXV) revealed complete identity and the appropriate mixtures showed no depression in melting point. The diol E probably results from isomerisation of 4β,5β-epoxycoprostan-3β-ol (LXXXIII) under the influence of the magnesium iodide present in the Grignard reagent. The mechanism presented below besides explaining the formation of the diol (CXIV) also shows how the intermediate keto-alcohol (CX) could arise.

An analogous isomerisation of an epoxide to an unsaturated diol has been reported by Urushibara and Chuman[13] who found that 5β,6β-epoxycholestan-3β-ol (LXXXV) reacted with



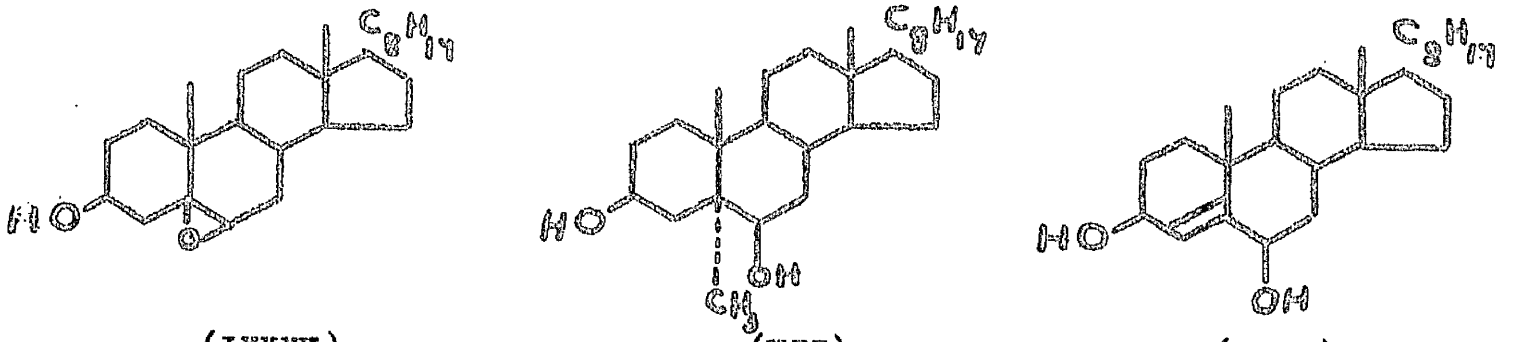
(LXXXIII)

(CXIV) (R = H)

(CXV) (R = CH_3CO)

(CX)

methylmagnesium iodide to give 5α-methylcholestan-3β,6β-diol (VII) and a small amount of cholest-4-en-3β,6β-diol (CXVI)



(LXXXV)

(VII)

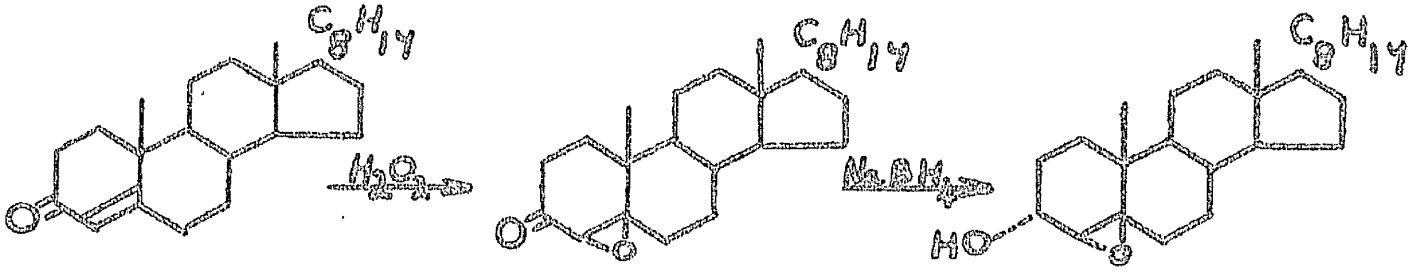
(CXVI)

SECTION III

The Action of Methylmagnesium Iodide on 4β,5β-Epoxycoprostan-3α-ol.

Methylmagnesium iodide reacts with 4β,5β-epoxycoprostan-3α-ol to give four isomeric diols A, B, C, and D. A is shown to be 4α-methylcholestan-3α,4β-diol by its conversion into 4-methylcholestan-3,4-seco-4-oxo-3-carboxylic acid, a compound already described in Section II. C is ascribed the formula 4α-methylcholestan-3α,5β-diol since it can be converted to the known 4-methylcholest-4-en-3-one. The diols B and D are shown to be epimers and evidence is presented which allows them to be formulated as 3α-methylcholestan-3β,4α-diol and 3β-methylcholestan-3α,4α-diol respectively.

The epoxide, 4β,5β-epoxycoprostan-3α-ol (CXVIII), was prepared by the action of alkaline hydrogen peroxide on cholest-4-en-3-one (LXIII)[93] followed by reduction of the intermediate 4β,5β-epoxycoprostan-3-one (CXVII) with sodium borohydride.[93]



(LXIII) (CXVII) (CXVIII)

Treatment of the epoxide (CXVIII) with an excess of methylmagnesium iodide gave a complex mixture, separable by careful chromatography on alumina into four components whose melting points and specific rotations are shown in Table D.

T A B L E D

Compound	Eluant	m.p.	$[\alpha]_D$	% Yield
A	ether	189-191°	+9°	16
B	¾% MeOH in ether	179-181	+7	15
C	1% MeOH in ether	154-155	+4	15
D	3% MeOH in ether	186-188	+10	7.5

The total yield of products was 53.5% and it is noteworthy that the specific rotations of the four compounds are similar.

The analytical data for all four substances indicated that they were isomeric diols of probable composition $C_{28}H_{50}O_2$. It was clear that more information was required regarding the positions of the hydroxyl groups and this was forthcoming from the quantitative treatment of the four diols with sodium periodate. The results of these experiments are tabulated below.

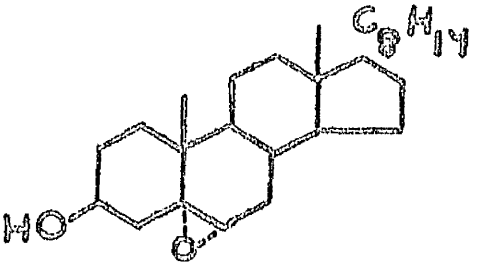
TABLE E

Compound	Percentage cleavage after 17 hr	42 hr.	92 hr.
A	0	0	0
B	22	32	35
C	0	0	0
D	59	85	95

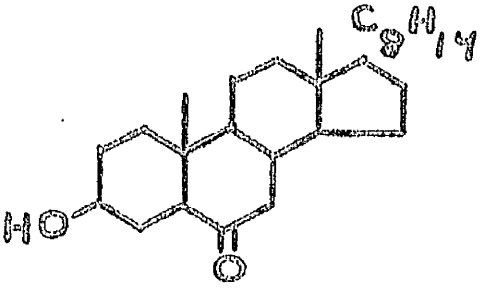
While the titration results for A failed to detect the occurrence of oxidation it was observed that after 92 hr. a slight crystalline precipitate (sodium iodate) had formed in the reaction solution, indicating that slow oxidation was taking place: the reaction mixtures containing B and D also deposited sodium iodate.

Compound A.

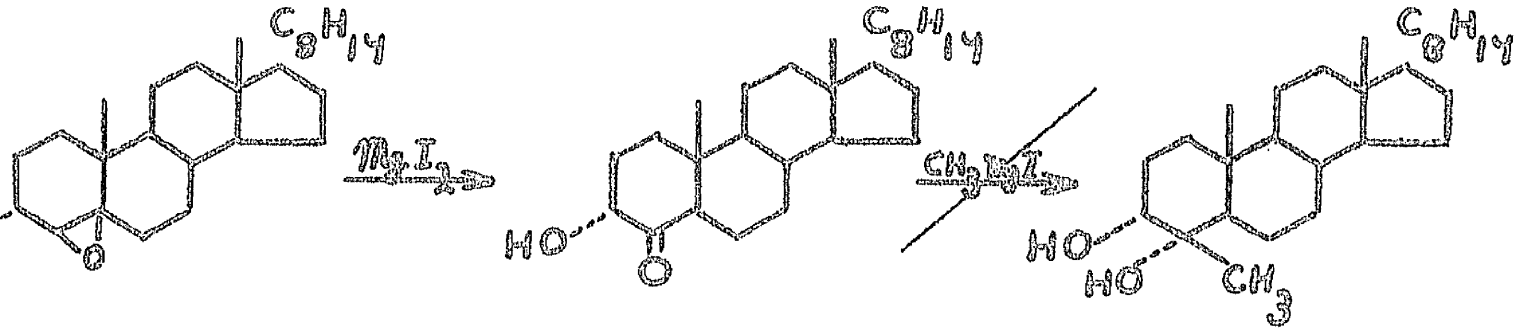
The diol A was transparent to ultraviolet light and and its infrared spectrum showed a strong hydroxyl peak at 3485 cm.$^{-1}$ Acetylation of this compound with acetic anhydride in pyridine at room temperature gave a diol monoacetate; therefore a tertiary hydroxyl group or a strongly hindered secondary hydroxyl group was present. Oxidation of A with chromic acid in acetone gave a carboxylic acid, m.p. 123-124°, $[\alpha]_D$ - 3° which proved to be identical in all respects to 4-methylcholestan-3,4-seco-4-oxo-3-carboxylic acid (XCVII), the keto-acid obtained by similar oxidation of 4α-methylcholestan-3β,4β-diol (XC) (Section II). This evidence indicates that A is epimeric with the diol (XC) and is either 4α-methylcholestan-3α,4β-diol (CXX) or 4β-methylcholestan-3α,4α-diol (CXXI). Such 4-methyl-4-hydroxy compounds could be formed by the action of methylmagnesium iodide on the epoxide (CXVIII), if the reaction in part, proceeds <u>via</u> the isomeric 4-oxocholestan-3α-ol (CXIX).



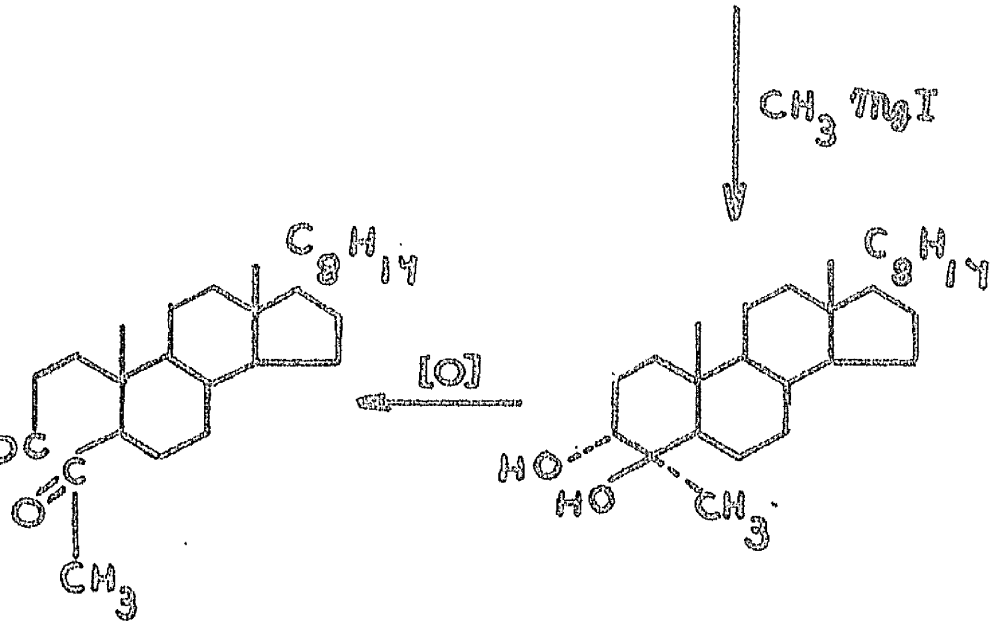
(CXIII)



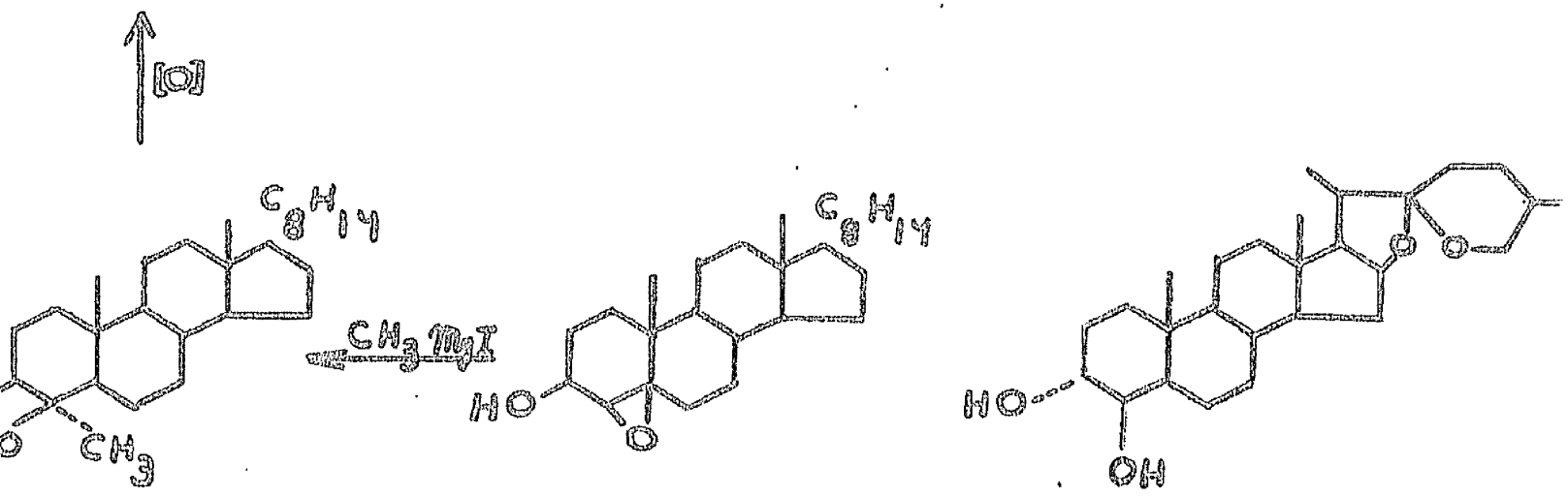
(CIV)



(CXVIII) (CXIX) (CXXI)



(XCVII) (CXX)



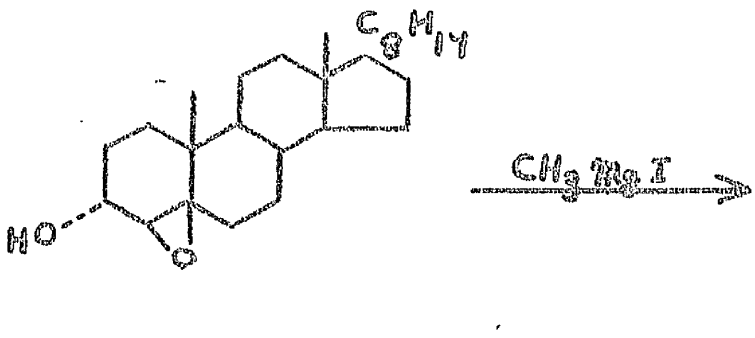
(XC) (LXXXIII) (CXXII)

Furthermore in Section II evidence is cited for an analogous isomerisation of the epimeric 4β,5β-epoxycoprostan-3β-ol (LXXXIII) under Grignard conditions. After the formation of the 4-ketone (CXIX), the reaction could proceed normally to give either (CXX) or (CXXI), but the former structure appears to be more likely for two reasons. Firstly, rear attack on the 4-ketone is preferred with formation of 4α-methylcholestan-3α,4β-diol (CXX), since the axial methyl group at $C_{(10)}$ hinders frontal attack. Secondly, the failure of sodium periodate to effect cleavage of the 1,2-glycol system in the diol A is characteristic of vicinal glycols having a trans-diaxial configuration as in structure (CXX). Djerassi and Ehrlich[93] have shown that the analogous oxidation of 22a,5α-spirostane-3α,4β-diol (CXXII) with lead tetra-acetate is an extremely slow process.

Compound C

Since the diol C did not react with sodium periodate and formed a monoacetate on acetylation at room temperature, the presence of a tertiary hydroxyl group at $C_{(5)}$ was suspected. Oxidation of C with chromic acid in acetone gave, in low yield, 4-methylcholest-4-en-3-one (VI)[10] which showed the presence of a methyl group at $C_{(4)}$. Assuming that the epoxide (CXVIII) opens in the normal diaxial manner when it reacts with the Grignard reagent, the two likely products would be

4α-methylcoprostan-3α,5β-diol (CXXIII) and 4β-methylcholestan-3α,5α-diol (CXXIV). We favour structure (CXXIII) for compound C since the 5-hydroxyl group has the same configuration as the original epoxide (CXVIII) and is axial to the ring wherein reaction has occurred. Hence structure (CXXIII) meets the requirements for the general rules concerning the opening of steroidal epoxides.



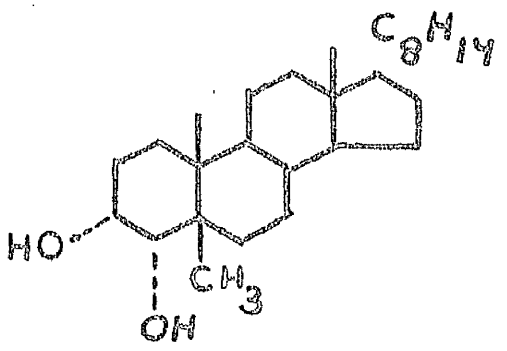
(CXVIII) (CXXIII)

(CXXIV) (VI)

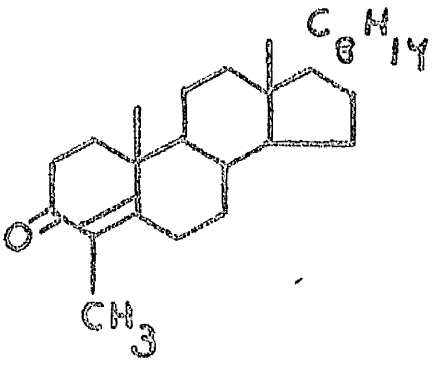
While the evidence obtained from oxidation of the diols A and C made the formulation of these compounds relatively straightforward, that from similar examination of products B and D proved to be more difficult to interpret. Both these diols could be oxidised with sodium periodate (Table E) and hence were vicinal glycols. Oxidation of B with sodium dichromate in dilute sulphuric acid or with chromium trioxide in pyridine yielded a saturated hydroxy-ketone whose infrared absorption spectrum displayed bands at 3559 $cm.^{-1}$ (hydroxyl) and 1712 $cm.^{-1}$ (carbonyl). Dehydration of this compound with phosphorus oxychloride in pyridine gave an αβ-unsaturated ketone, showing maximum absorption at 2380 Å. (ϵ 7,500) in the ultra-violet region. When the diol D was oxidised under the same conditions another keto-alcohol was obtained which was shown to be epimeric with that obtained by oxidation of B since it afforded the same αβ-unsaturated ketone on dehydration with phosphorus oxychloride in pyridine.

These reactions lead to the conclusion that the diols B and D are epimers and since they do not yield 4-methylcholest-4-en-3-one (VI) when subjected to oxidation followed by dehydration, the methyl substituent does not appear to be at position $C_{(4)}$. The possibility of the oxide ring having opened to give epimeric 5-methylcholestan-3,4-diols such as depicted by structures (CXXV) and (CXXVI) (both diaxial opening)

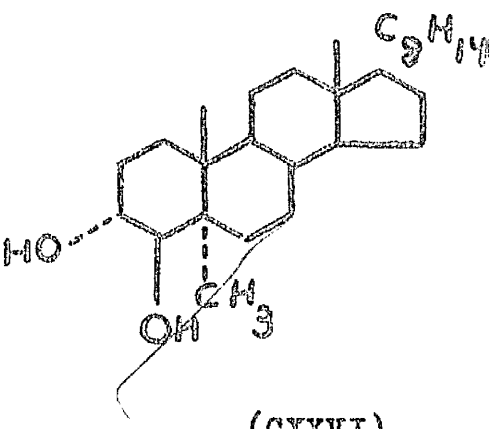
can be rejected since these formulations do not contain a tertiary hydroxyl group and besides they do not lend themselves to the series of reactions cited above.



(CXXV)



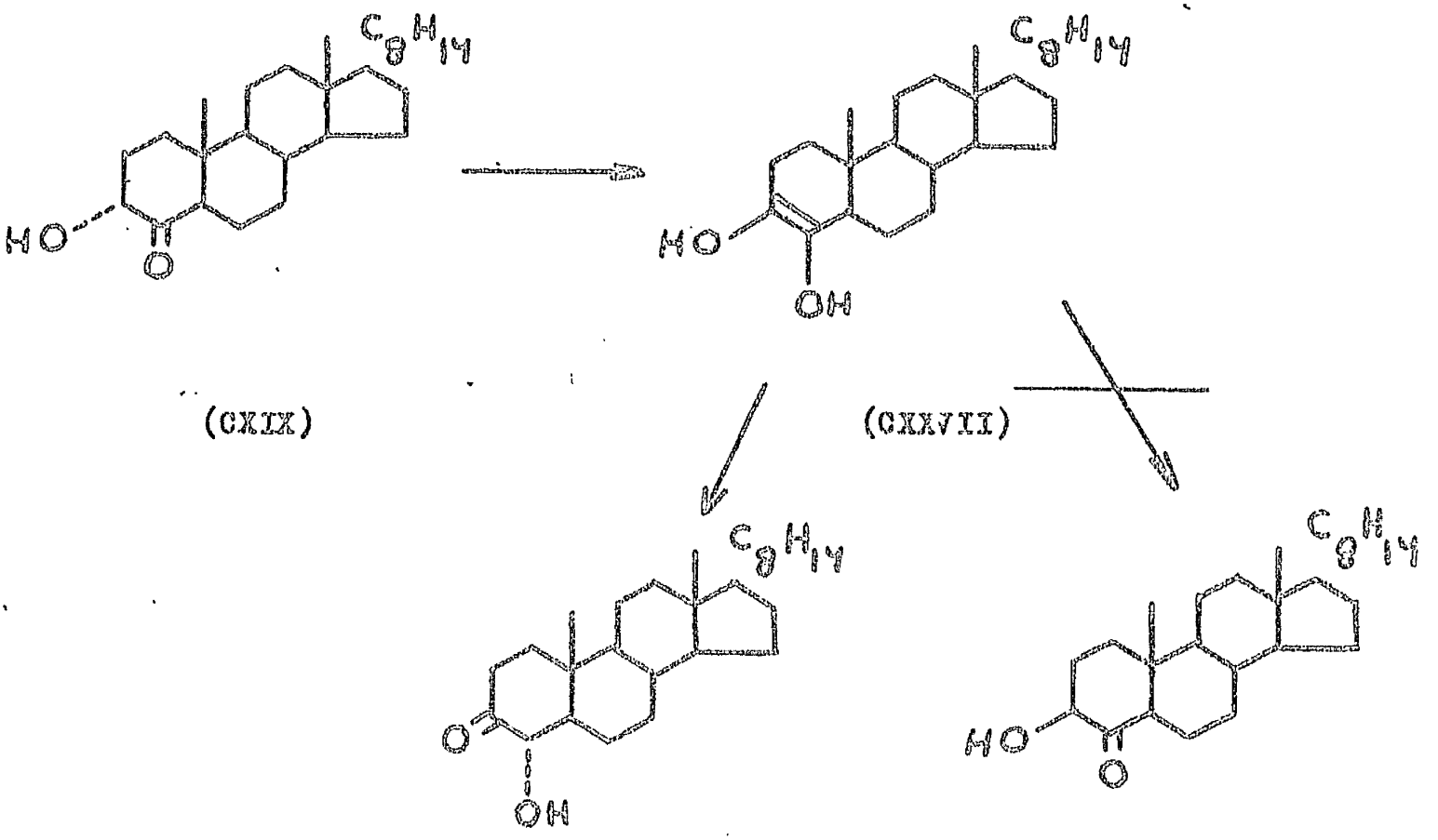
(VI)



(CXXVI)

In order to explain the isolation of the diols B and D we suggest that further partial rearrangement of 4-oxocholestan-3α-ol (CXIX) occurs with formation of the isomeric 3-oxocholestan-4α-ol (CXXVIII). Examination of structure (CXIX) reveals that the hydroxyl group has the less stable axial configuration and the conformational stability acquired in going to 3-oxocholestan-4α-ol (CXXVIII) (in which the 4-hydroxyl group has the more stable equatorial configuration) would be the driving force for this isomerisation. The transformation can be considered as proceeding via the enol (CXXVII), followed by ketonisation to give either 3-oxocholestan-4α-ol (CXXVIII) or 4-oxocholestan-3β-ol (CX) both of which have an equatorial hydroxyl group. The results obtained by the author suggest

that structure (CXXVIII) is preferred.

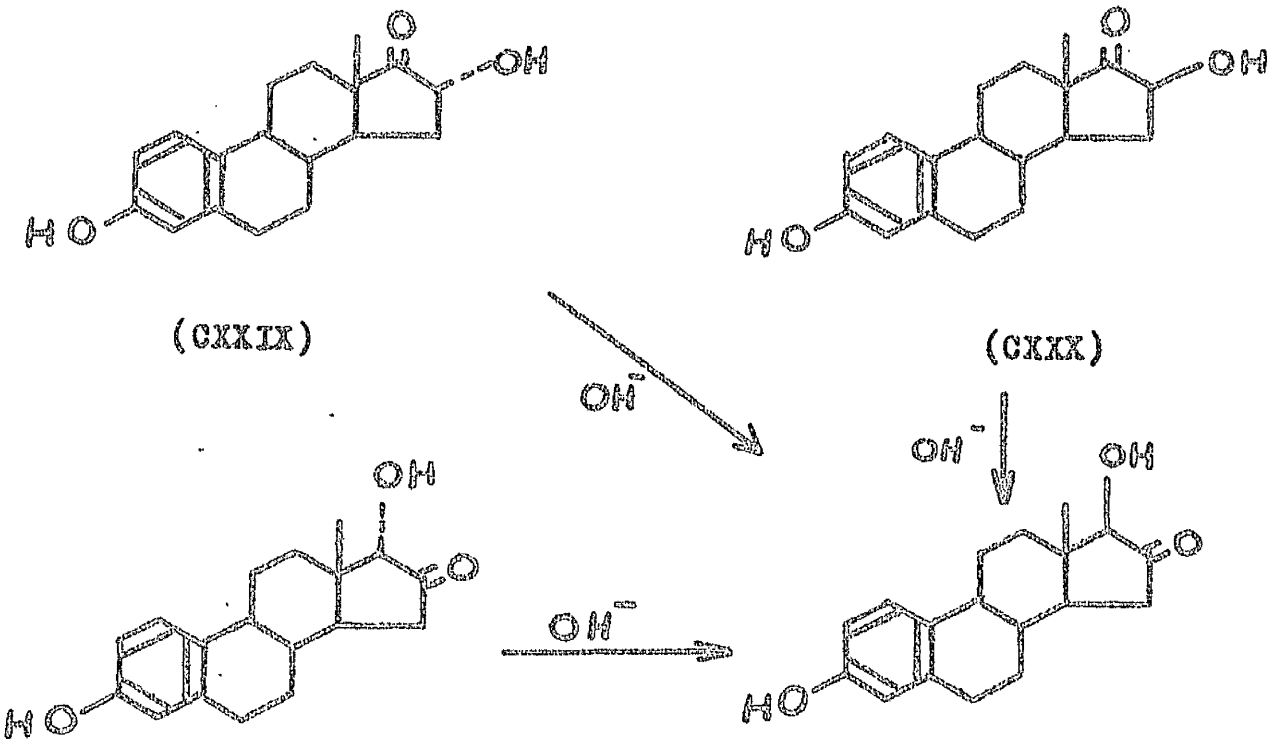


(CXIX) (CXXVII)

(CXXVIII) (CX)

Fishman[94] has shown that of the four possible C-16,17 ketols [(CXXIX), (CXXX), (CXXXI), (CXXXII)] derived from estrone, 16-oxoestradiol (CXXXII) has the more stable configuration since the other three forms rearrange to this compound in the presence of alkali.

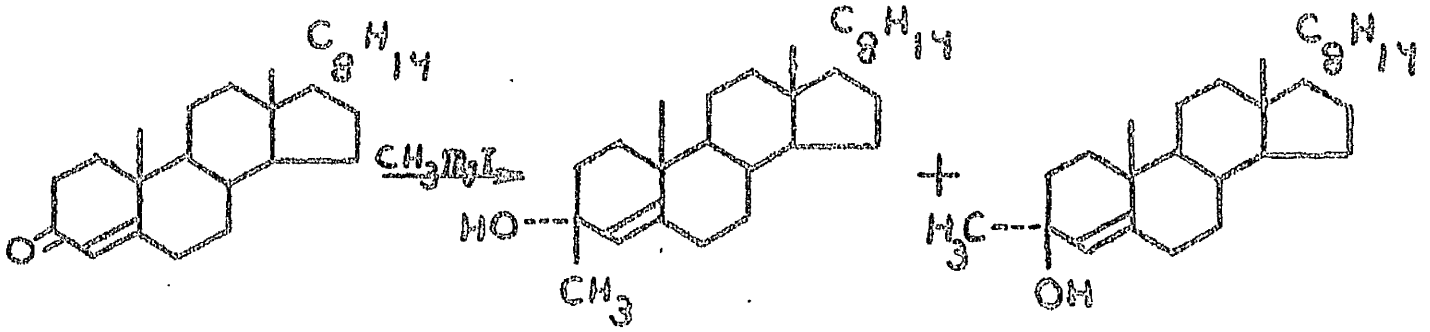
If we assume that the ketol (CXXVIII) is formed in the reaction mixture then a new factor is involved concerning the action of methylmagnesium iodide on the 3-oxo substituent.



(CXXIX) (CXXX)

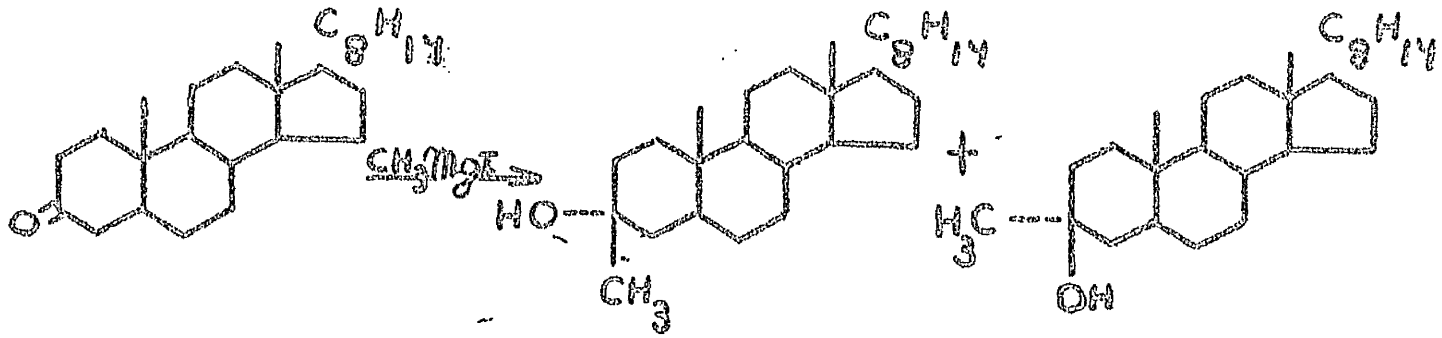
(CXXXI) (CXXXII)

Since the $C_{(3)}$ position is not so strongly influenced by the axial methyl group at $C_{(10)}$ as are positions $C_{(4)}$ and $C_{(6)}$, attack from the rear does not predominate and both epimers may be expected. Thus Musgrave[69] has treated cholest-4-en-3-one (LXIII) with methylmagnesium iodide and obtained a substance which he considered to be a molecular compound of 3α-methylcholest-4-en-3β-ol (LXV) and 3β-methylcholest-4-en-3α-ol (LXIV) since approximately 50% of this material was precipitated by digitonin.



(LXIII) (LXIV) (LXV)

The point is further emphasised by the findings of Barton et al.[67] who showed that cholestan-3-one (XLI) when treated with methylmagnesium iodide gave 3-methylcholestan-3α- (LXI) and -3β-ol (LXII) in almost equal amounts.

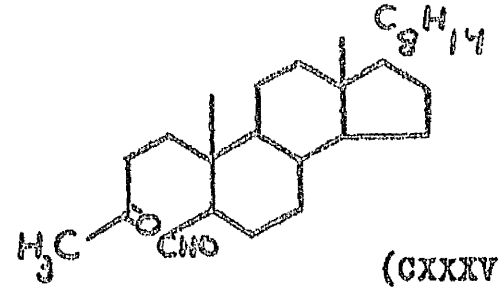


(XLI) (LXI) (LXII)

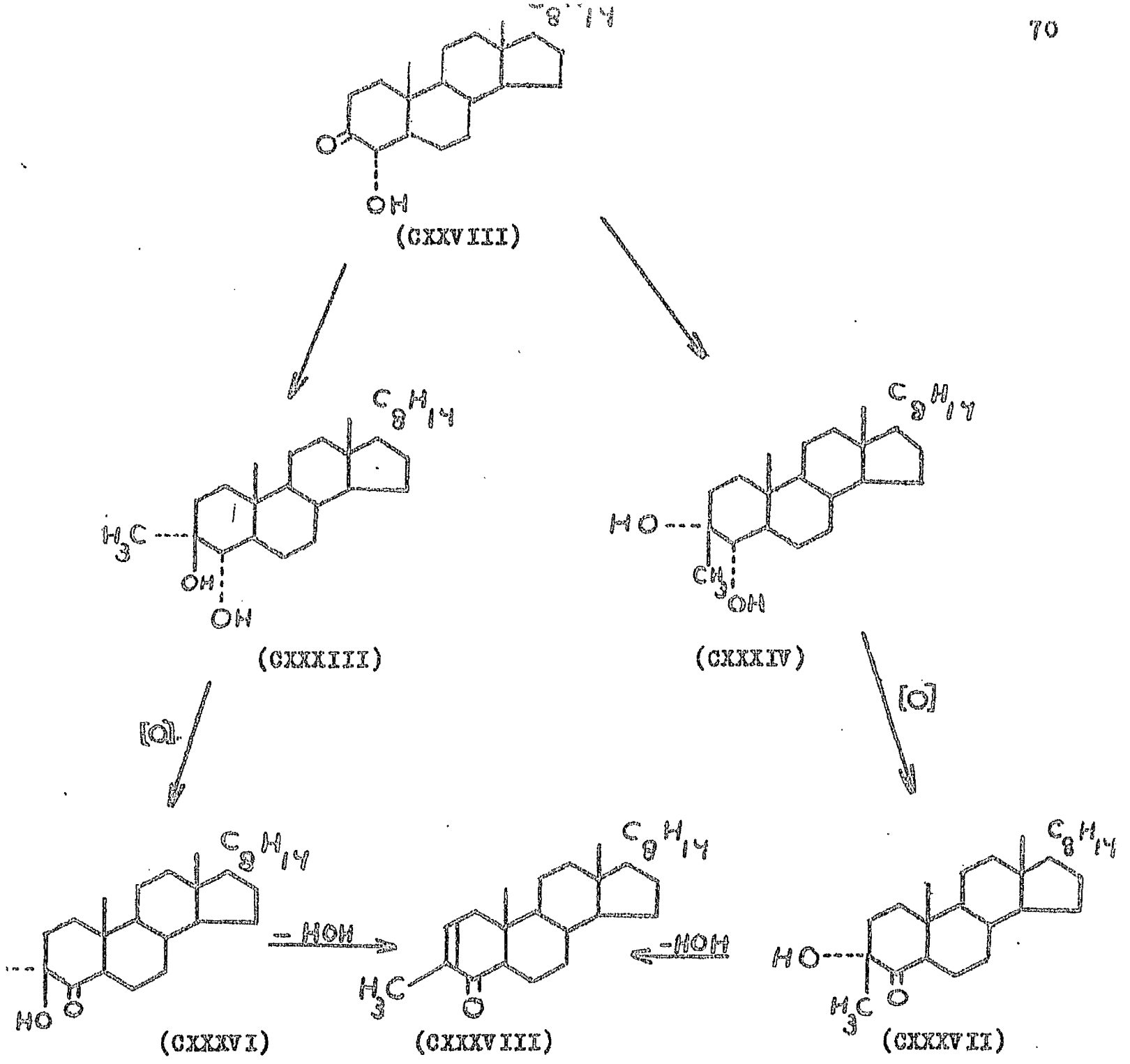
If we accept the above hypothesis of isomerisation, the action of the Grignard reagent on the intermediate keto-alcohol (CXXVIII) and the subsequent reactions of the products can be explained as follows.

Methylmagnesium iodide reacts with 3-oxocholestan-4α-ol (CXXVIII) to give two epimeric vicinal glycols 3α-methylcholestan-

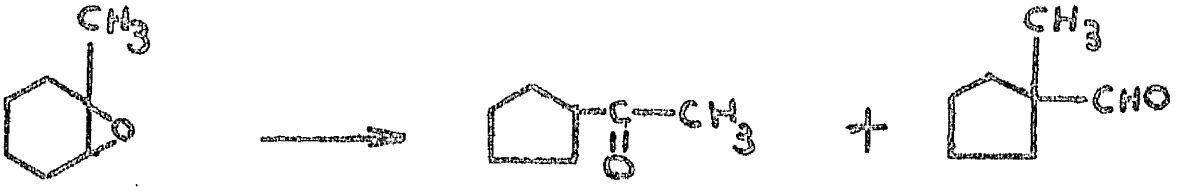
-3β,4α-diol (CXXXIII) and 3β-methylcholestan-3α,4α-diol (CXXXIV). From Table E it can be seen that the rate of cleavage of these diols with sodium periodate differs appreciably and since Djerassi and Ehrlich[93] have shown that a cis 3α,4α-diol is rapidly oxidised with lead tetra-acetate, compound D is assigned the structure (CXXXIV). Treatment of the epimeric diols with lead tetra-acetate gave non-crystalline products whose infrared spectra showed that they were identical and of probable constitution (CXXXV) since the infrared spectrum contained bands at 2740 and 1730 $cm.^{-1}$ (aldehyde) and 1712 $cm.^{-1}$ (ketone). Oxidation of the diols (CXXXIII) and (CXXXIV) with chromic acid in acetone furnished the corresponding epimeric keto-alcohols 3α-methyl-4-oxocholestan-3β-ol (CXXXVI) and 3β-methyl-4-oxocholestan-3α-ol (CXXXVII). The latter oxidations also gave intractable acidic products. Dehydration of the epimeric keto-alcohols (CXXXVI) and (CXXXVII) gave an αβ-unsaturated ketone formulated as 3-methylcholest-2-en-3-one (CXXXVIII) whose ultraviolet absorption spectrum (λ max. 237 mu, ε 7,500) is in keeping with the calculated wavelength of maximal absorption (237 mu) of an αβ-disubstituted αβ-unsaturated ketone.



(CXXXV)

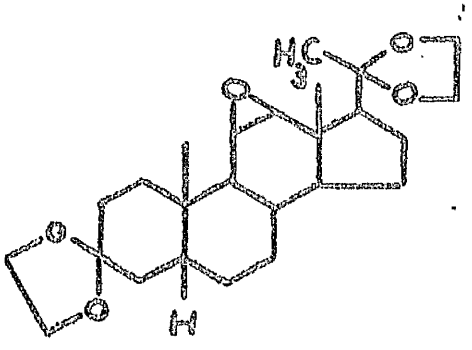


We also considered the possibility of ring contraction taking place during the Grignard reaction as many examples of this are known.[89] Thus 1-methylcyclohexene oxide (CXXXIX), in the presence of magnesium bromide rearranges to acetylcyclopentane (CXL) and methylcyclopentylaldehyde (CXLI).[93]

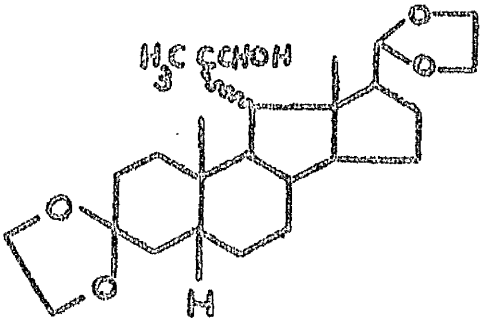


(CXXXIX) (CXL) (CXLI)

Furthermore Christensen et al.[91] have shown that 11β,12β-epoxypregnane-3,20-dione-3,20-bis-(ethylene ketal) (CV) reacts with methylmagnesium iodide to form a product which they formulate as (CVI).

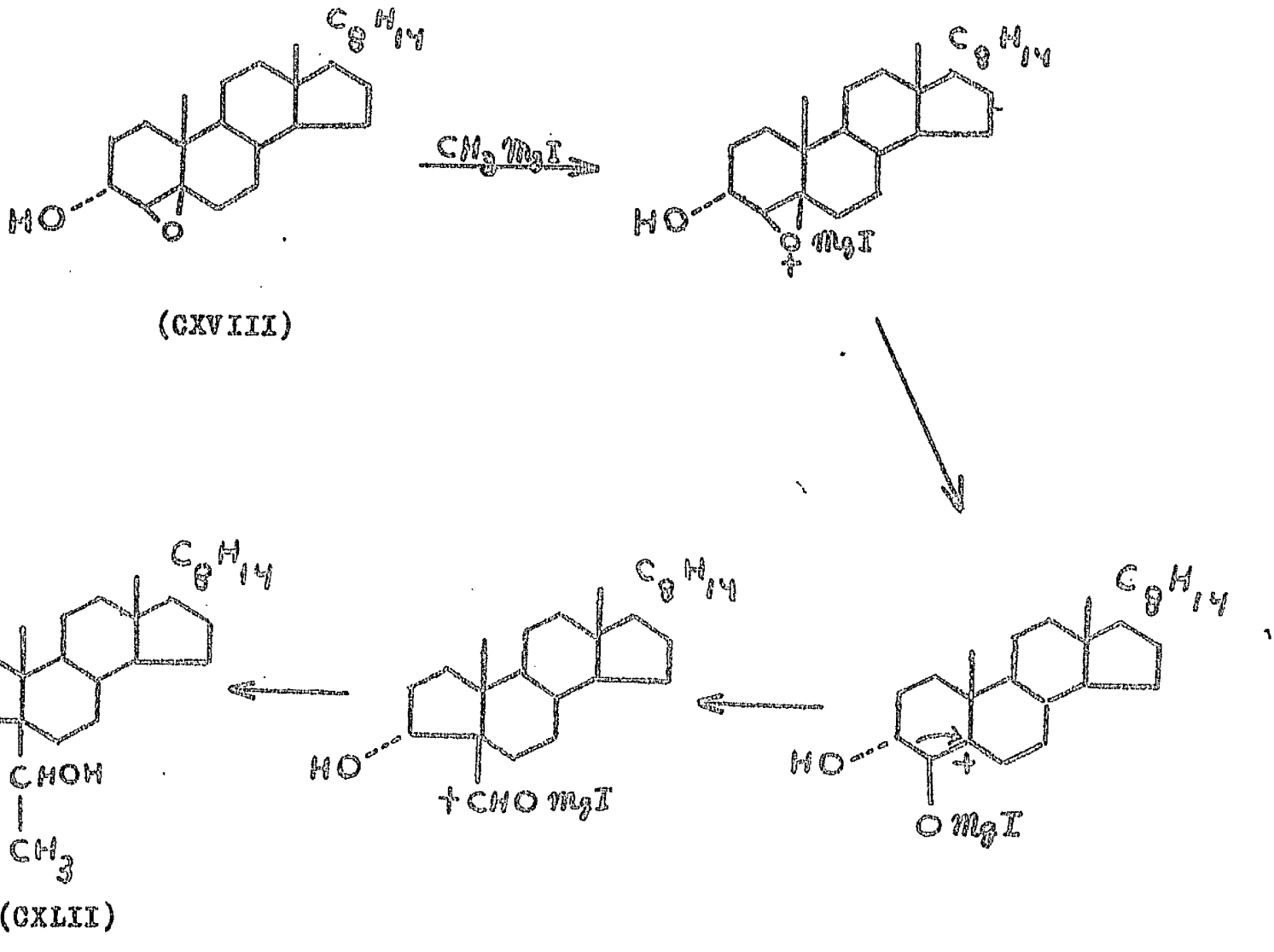


(CV)



(CVI)

If we assume a rearrangement similar to that described by Christensen et al.[91] we arrive at a ring contracted product of type (CXLII). This however, does not contain a vicinal glycol system and the possibility of such a contraction having occurred is excluded.



(CXVIII)

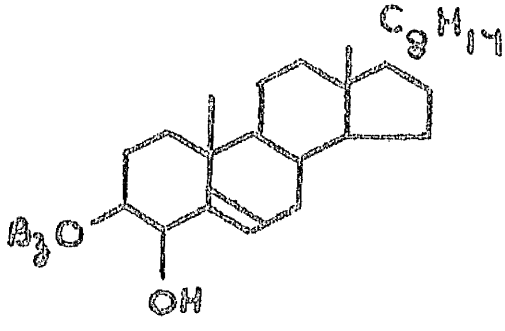
(CXLII)

SECTION IV

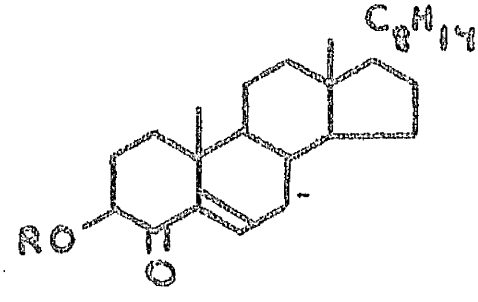
The Action of Methylmagnesium Iodide on 4-Oxocholest-5-en-3β-yl Benzoate.

Methylmagnesium iodide reacts with 4-oxocholest-5-en-3β-yl benzoate to give a mixture of 4α-methylcholest-5-en-3β,4β-diol and 4β-methylcholest-5-en-3β,4α-diol and also two epimeric saturated ketones tentatively formulated as 4α,6α-dimethylcholestan-3-one and 4β,6α-dimethylcholestan-3-one.

In an attempt to introduce a methyl substituent at $C_{(4)}$ in the cholestane nucleus the action of methylmagnesium iodide on 4-oxocholest-5-en-3β-yl benzoate (CXLIV) has been investigated. This benzoate has previously been prepared in this department by Ikan and McLean,[96] the mode of preparation being essentially the same as that used by Rosenheim and Starling[97] for the preparation of the corresponding acetate (CXLIII). Cholest-5-en-3β,4β-diol-3-benzoate (CVII) was brominated at room temperature and without isolation, the dibromide was oxidised with chromium trioxide in acetic acid. Debromination of the product with sodium iodide in ethanol afforded 4-oxocholest-5-en-3β-yl benzoate (CXLIV).



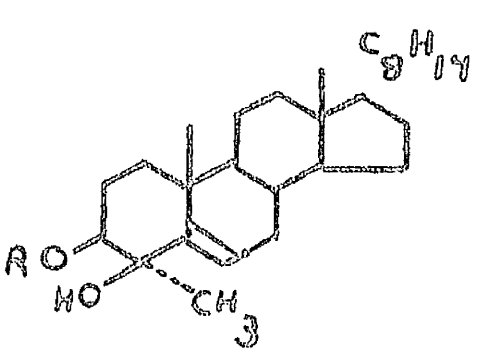
(CVII)

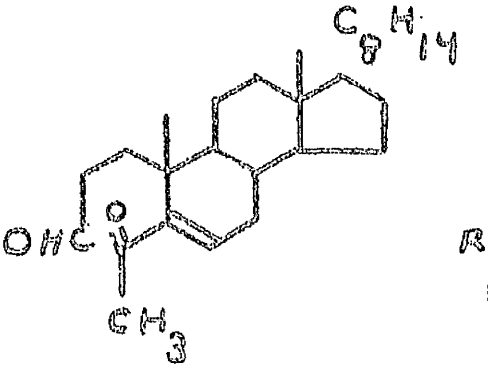


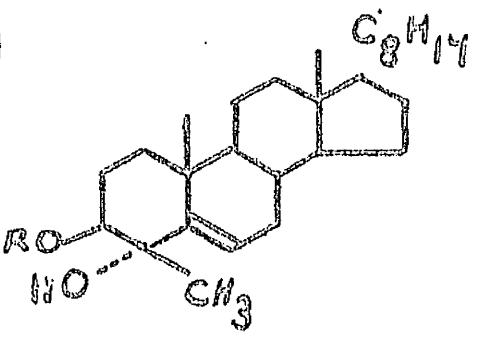
(CXLIII, R = CH_3CO)
(CXLIV, R = C_6H_5CO)

When a solution of the benzoate (CXLIV) in ether-benzene was treated with an excess of methylmagnesium iodide, a complex mixture was obtained which after chromatography on alumina yielded two saturated ketones, a fraction containing phenyldimethylcarbinol and an amorphous solid. A detailed examination of this amorphous material indicated that it was a mixture of epimeric diols, viz., 4α-methylcholest-5-en-3β,4β-diol (CXLV) and 4β-methylcholest-5-en-3β,4α-diol (CXLVII), formed by the normal 1,2-addition of methylmagnesium iodide to the αβ-unsaturated ketone system. Careful chromatography failed to resolve this mixture, which gave a yellow colour with tetranitromethane and showed strong hydroxyl absorption at 3360 $cm.^{-1}$ in the infrared. Treatment of the mixture with periodic acid gave a non-crystalline neutral product which exhibited maximum absorption at 2350 Å.(ϵ 9,600) in the ultraviolet. This characteristic is in keeping with that expected from 4-methyl-3,4-seco-3,4-dioxocholest-5-ene (CXLIX) the product which would be formed by cleavage of the glycol system in both diols (CXLV) and (CXLVII).

In an attempt to form the acetonide derivatives, the mixture of epimers was treated at room temperature with acetone containing a few drops of concentrated hydrochloric acid. Isolation of the product, however, gave the known 4-methyl-cholest-4-en-3-one (VI) in 72% yield, thus confirming that a



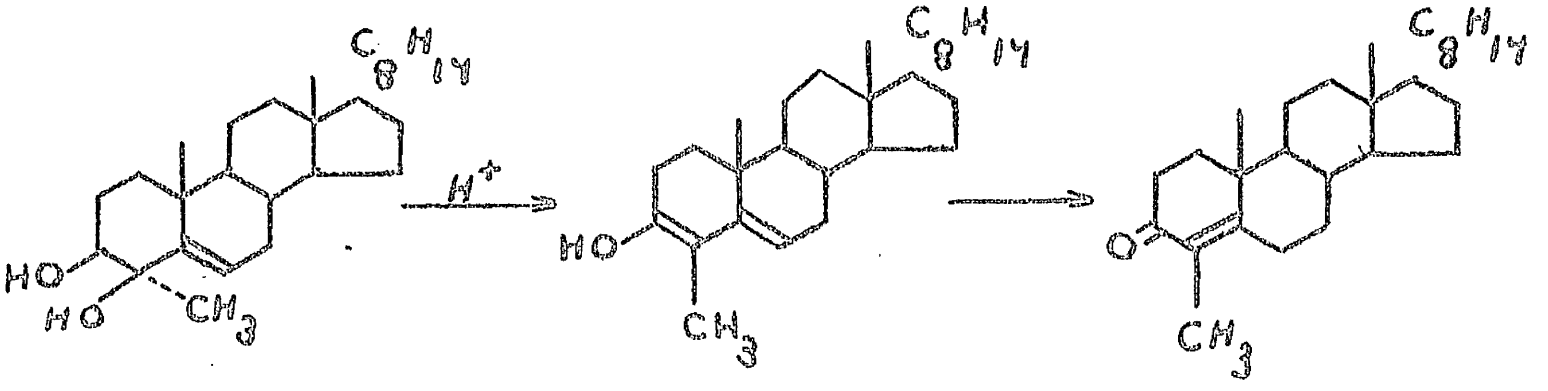




(CXLV, R = H) (CXLIX) (CXLVII, R = H)
(CXLVI, R = CH_3CO) (CXLVIII, R = CH_3CO)

methyl substituent was present in the diols at $C_{(4)}$. Also, the high yield of the αβ-unsaturated ketone (VI) suggests that the percentage of the 4α-methyl derivative (CXLV) in the epimeric mixture is fairly high since the 4-hydroxyl group in 4β-methylcholest-4-en-3β,4α-diol (CXLVII) is not suitably orientated for the normal trans - elimination of water with the hydrogen atom at $C_{(5)}$. Thus the αβ-unsaturated ketone (VI) must be almost entirely derived from 4α-methylcholest-4-en-3β,4β-diol (CXLV) by trans- elimination of water to give the

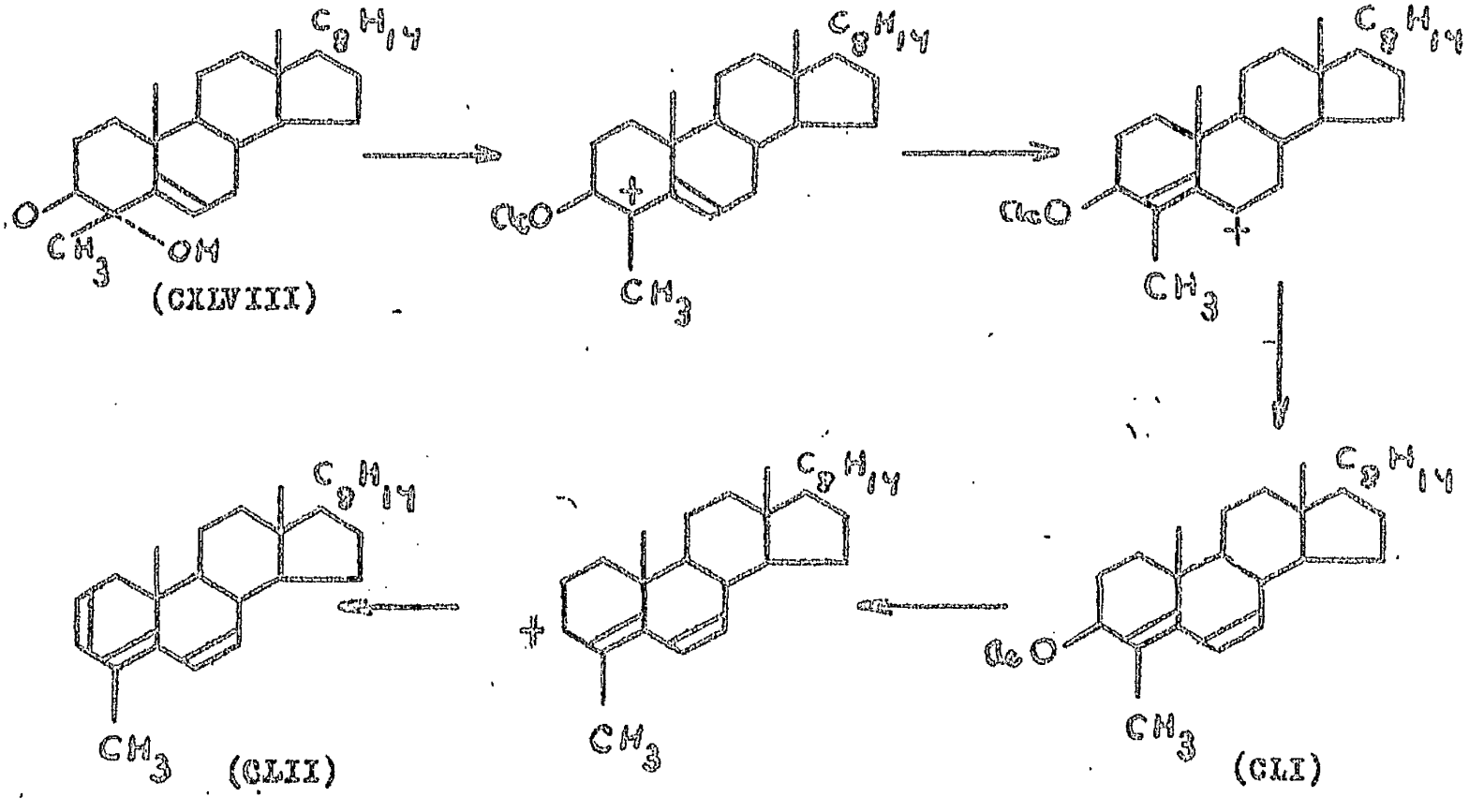
intermediate dienol (CL) which then undergoes rearrangement to 4-methylcholest-4-en-3-one (VI).



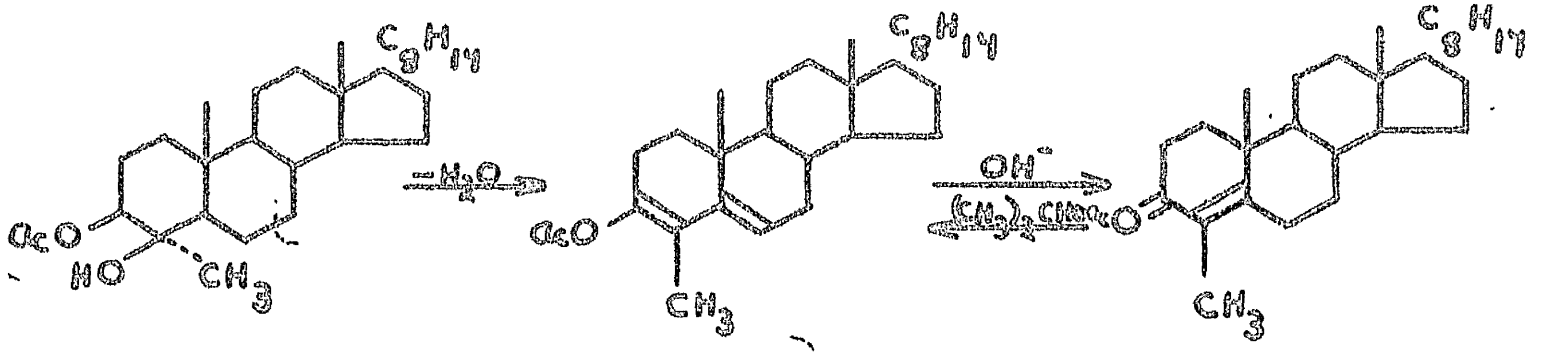
(CXLV) (CL) (VI)

Acetylation of the mixture of epimeric diols gave material which could not be resolved by chromatography on alumina. Bands at 3521 cm.$^{-1}$ (hydroxyl) and 1739 cm.$^{-1}$ and 1263 cm.$^{-1}$ (acetate) in the infrared spectrum indicated the presence of the diol monoacetates (CXLVI) and (CXLVIII). Dehydration of the mixed diol monoacetates with phosphorus oxychloride in pyridine and chromatography of the product on alumina gave a non-crystalline hydrocarbon and an acetate. The ultraviolet absorption spectrum of the hydrocarbon contained maxima at 2080, 3000, and 3100 Å. reminiscent of a cholesta-2,4,6-triene system. An authentic specimen of cholesta-2,4,6-triene obtained for comparison showed strong ultraviolet absorption at 2070, 2960, and 3060 Å. and it is concluded that the hydrocarbon described above is largely

4-methylcholesta-2,4,6-triene (CLII), the methyl substituent at $C_{(4)}$ being responsible for the bathochromic displacement of approximately 4 mu. This hydrocarbon is probably derived from 4β-methylcholest-5-en-3β,4α-diol-3-acetate (CXLVIII) and its formation depends on the inability of this diol monoacetate to undergo dehydration by the normal trans- elimination process. The reaction could be visualised as proceeding by the path shown below with the formation of 4-methylcholesta-4,5-dien-3-yl acetate (CLI) as an intermediate. It has been shown[24] that compounds of this type containing an acetate group allyl to a tetrasubstituted double bond readily loose acetic acid and in the case under discussion 4-methylcholesta-2,4,6-triene (CLII) would be the expected product.



The acetate obtained from the above dehydration is considered to be 4-methylcholesta-3,5-dien-3-yl acetate (CLIII), on the basis of the following evidence: the acetate bands in the infrared spectrum occurred at 1754 and 1217 cm.$^{-1}$, this shift to the lower wavelength being characteristic of enol acetates and many examples are cited in the literature.[98] The ultraviolet absorption spectrum was also in keeping with that expected of a compound having the structure (CLIII) since it did not exhibit the absorption maxima normally associated with steroid $\Delta^{3,5}$-dienes, but rather showed a single maximum at 2360 Å. (18,000), characteristic of a dienyl acetate.[99] The dienyl acetate was readily converted into 4-methylcholest-4-en-3-one (VI)



(CXLVI) (CLIII) (VI)

on treatment with dilute methanolic potassium hydroxide or even by contact with alumina for a few hours. That the compound was in fact 4-methylcholesta-3,5-dien-3-yl acetate (CLIII) was established beyond doubt when it was obtained by acetylation

of 4-methylcholest-4-en-3-one (VI) using isopropenyl acetate.

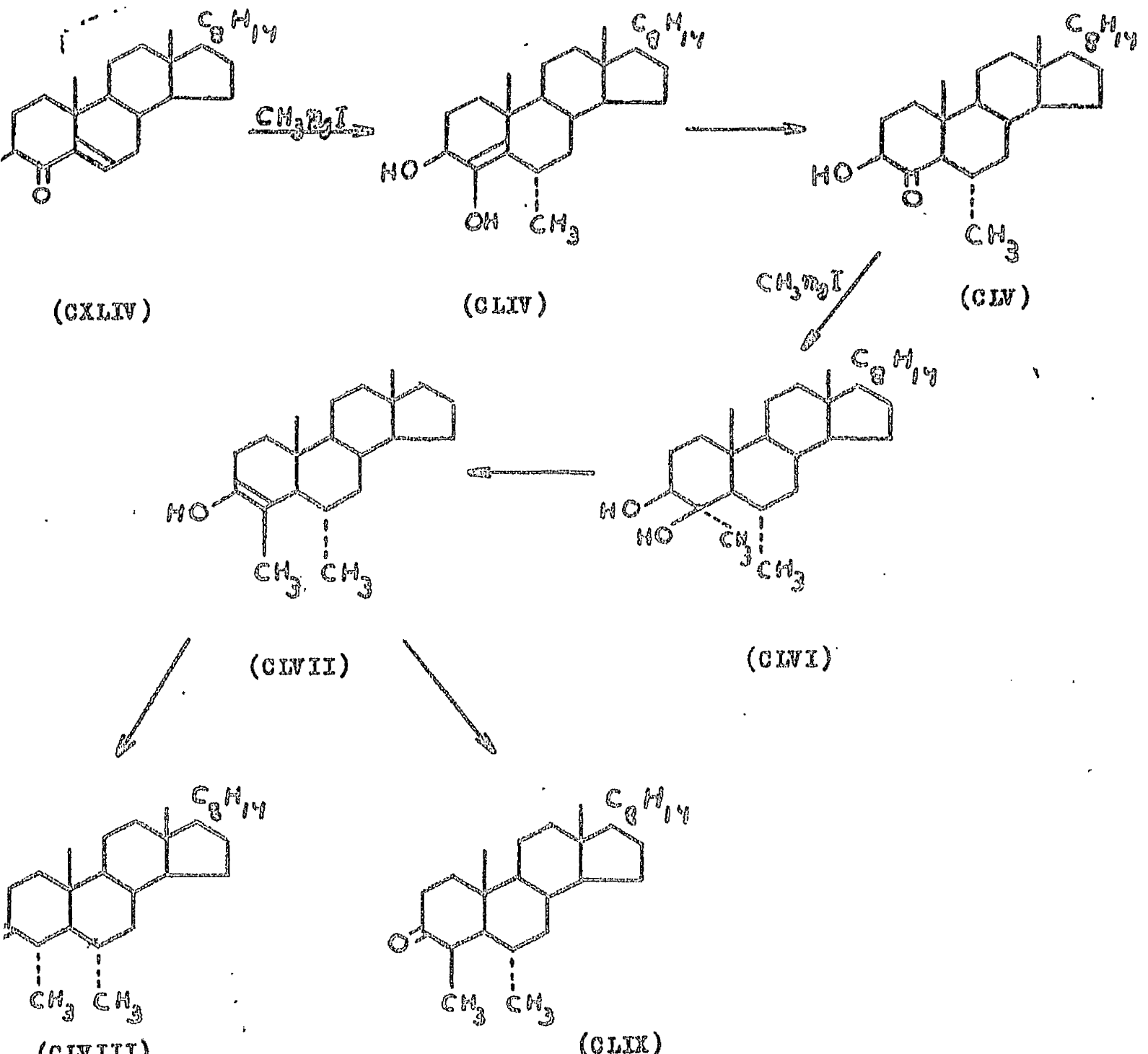
Atwater[100] has recently also prepared this compound (CLIII) by treatment of 4-methylcholest-4-en-3-one (VI) with a mixture of acetyl chloride and acetic anhydride and the constants quoted by him are in complete agreement with those obtained by the author.

Analyses of the two saturated ketonic products mentioned above (p.75) indicated that each contained only one atom of oxygen per molecule and suggested that they were isomers of probable molecular formula $C_{29}H_{50}O$. No definite explanation regarding the structures of these compounds has been obtained but on the evidence available the more stable ketone (m.p. 102-103°, $[\alpha]_D$ - 7.5°) is formulated as 4α,6α-dimethylcholestan-3-one (CLVIII). This compound is stable to treatment with acid or base but on refluxing a solution of the second ketone, (m.p. 109-110°, $[\alpha]_D$ - 35°), in methanol containing potassium hydroxide it was smoothly converted into the more stable ketone (CLVIII). This shows that the compounds are isomers, epimeric at the centre adjacent to the carbonyl group and the less stable compound is therefore formulated as 4β,6α-dimethylcholestan-3-one (CLIX), in which the 4-methyl group is axial. It is possible to explain the formation of these saturated ketones if the reaction between methylmagnesium iodide and 4-oxocholest-

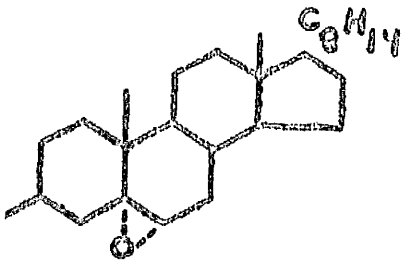
-5-en-3β-yl benzoate (CXLIV) proceeds in a stepwise manner, as follows:-

The initial attack involves the 1,4-addition of the Grignard reagent to the αβ-unsaturated ketone system with the formation of the enol (CLIV), the 6-methyl group being assigned the α-(equatorial)-configuration since the entering group should approach from the less hindered α-face of the molecule. We must now assume that the enol (CLIV) undergoes rearrangement to give the ketone (CLV) which will then react with a second mole of methylmagnesium iodide to form 4α,6α-dimethylcholestan-3β,4β-diol (CLVI). Dehydration of this diol would lead to the enol (CLVII) which is the parent of the two epimeric dimethyl ketones (CLVIII) and (CLIX).

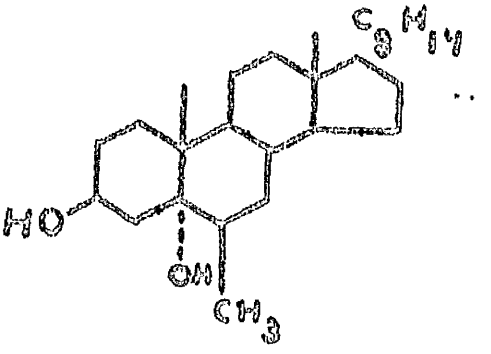
Initially the ketones (CLVIII) and (CLIX) would be present in equal amounts in the reaction mixture but under the conditions the conversion of a considerable proportion of the 4β-methyl compound (CLIX) to the 4α-methyl epimer (CLVIII) would be expected and this is confirmed by the isolation of larger amounts of the more stable isomer (CLVIII). The spontaneous dehydration step is not without precedent since Ushakov and Madeava[14] found that if the reaction between



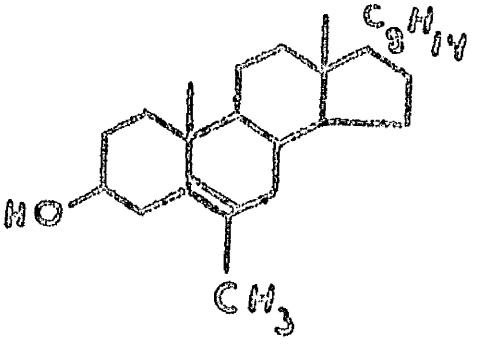
methylmagnesium iodide and 5α,6α-epoxycholestan-3β-ol (CIII) is prolonged for 7 hr., dehydration occurs with the formation of 6-methylcholesterol (LXXVII).



(CIII)



(VIII)



(LXXVII)

EXPERIMENTAL

All melting points are uncorrected. Specific rotations were determined in chloroform solution in a 1 dm. tube at approximately 15°C. Ultraviolet absorption spectra were measured in ethanol solution, using a Unicam SP.500 spectrophotometer. Infrared absorption spectra were measured in Nujol mulls, (unless otherwise stated) with a Grubb Parsons S.4 double beam spectrophotometer with sodium chloride optics. Grade II alumina and light petroleum (b.p. 60-80°) were used for chromatography.

The author wishes to thank Dr. A. C. Syme and Mr. W. McCorkindale for the microanalyses and ultraviolet absorption measurements, and Miss J. Goldie for the infrared absorption spectra.

SECTION I

Treatment of 4α,5α-Epoxycholestan-3β-yl Acetate with Methylmagnesium Iodide. - A solution of 4α,5α-epoxycholestan-3β-yl acetate (400 mg.) in dry benzene (30 ml.) and dry ether (30 ml.) was added to methylmagnesium iodide, [prepared from magnesium (0.11 g.), methyl iodide (0.64 g.), and ether (30 ml.)], and the solution was refluxed for 4 hr. The reaction mixture was cooled and poured on a mixture of crushed ice (10 g.) and ammonium chloride (1 g.). After 1 hr. the organic layer was

separated, washed several times with water, and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure gave a white

water to give 4β-methylcholestan-3β,5α-diol (340 mg.) as plates one-

water to give 4β-methylcholestan-3β,5α-diol (340 mg.) as plates, m.p.185-187°. Two recrystallisations from acetone-water gave an analysis sample, m.p. 187-189°, $[\alpha]_D$ + 23.5° (c, 1.2); infrared absorption at 3333 $cm.^{-1}$ (hydroxyl). The material gave no colour with tetranitromethane in chloroform and showed no selective light absorption in the ultraviolet region (Found: C,80.2; H,12.2. $C_{28}H_{50}O_2$ requires C,80.3; H,12.0%).

4β-Methylcholestan-3β,5α-diol-3-acetate.- 4β-Methyl-cholestan-3β,5α-diol (100 mg.) in pyridine (3 ml.) and acetic anhydride (3 ml.) was allowed to stand at room temperature for 18 hr. The mixture was poured into water, acidified with dilute hydrochloric acid, and extracted with ether. The ethereal extract was worked up in the normal manner and evaporation of the solvent gave a white solid (100 mg.). The crude product was recrystallised several times from methanol to give 4β-methyl-cholestan-3β,5α-diol-3-acetate (74 mg.) as blades, m.p. 174-175°, $[\alpha]_D$ + 4° (c, 1.7); infrared absorption at 3480 $cm.^{-1}$ (hydroxyl), 1740 $cm.^{-1}$, 1710 $cm.^{-1}$, 1280 $cm.^{-1}$, and 1245 $cm.^{-1}$ (acetate) (Found: C,77.9; H,11.2. $C_{30}H_{52}O_3$ requires C,78.2; H,11.4%).

Hydrogenolysis of this compound with lithium aluminium hydride regenerated the original diol, m.p. and mixed m.p. 185-187°.

Treatment of 4β-Methylcholestan-3β,5α-diol with Chromium Trioxide in Pyridine. - A solution of the diol (250 mg.) in pyridine (2.5 ml.) was mixed intimately with a slurry of chromium trioxide (250 mg.) in pyridine (2.5 ml.) and the reaction mixture was left at room temperature for 24 hr. The neutral product (200 mg.), isolated by means of ether, was dissolved in light petroleum (15 ml.) and filtered through a column of alumina (7.5 g.). Elution with light petroleum-benzene (4:1) afforded a crystalline solid (165 mg.) which after two recrystallisations from methanol gave 4-methylcholest-4-en-3-one as needles, m.p. 103-104°, $[\alpha]_D$ + 104° (c, 2.0); $\lambda_{max.}$ 2500 Å. (ε, 15,700); infrared absorption at 1665 cm.$^{-1}$ and 1613 cm.$^{-1}$ (α,β,β-trisubstituted α,β-unsaturated ketone). Sondheimer and Mazur[11] quote, m.p. 102-103°, $[\alpha]_D$ + 110°; $\lambda_{max.}$ 2510 Å. (ε, 14,300); infrared absorption at 1661 cm.$^{-1}$ and 1618 cm.$^{-1}$ for this compound.

Dehydration of 4β-Methylcholestan-3β,5α-diol-3-acetate with Phosphorus Oxychloride in Pyridine. - The diol monoacetate (125 mg.) in pyridine (10 ml.) was treated with phosphorus oxychloride (3 ml.) and the solution was allowed to stand at

room temperature for 24 hr. The product (120 mg.), isolated by means of ether, was recrystallised several times from chloroform-methanol to give 4β-methylcholest-5-en-3β-yl acetate (112 mg.) as fine white needles, m.p. 163-165°, $[\alpha]_D$ - 71.5° (c, 1.8). The compound gave a yellow colour with tetranitromethane in chloroform and showed light absorption maximum at 2040 Å. (ε, 3,800) (Found: C, 81.0; H, 11.1. $C_{30}H_{50}O_2$ requires C, 81.4; H, 11.4%).

Dehydration of 4β-methylcholestan-3β,5α-diol with Acetic Anhydride-Sulphuric Acid. - 4β-Methylcholestan-3β,5α-diol (200 mg.) in acetic anhydride (25 ml.) was refluxed for 2 hr. and the solution, (cooled to 100°), was treated with concentrated sulphuric acid (3 drops). The dark green reaction mixture was maintained at 100° for a further 10 min., cooled to room temperature, and the precipitate filtered and washed with water. The material was recrystallised several times from chloroform-methanol to give 4β-methylcholest-5-en-3β-yl acetate (180 mg.) as needles, m.p. 162-163°, $[\alpha]_D$ - 70° (c, 1.5), identical (m.p. and mixed m.p.) with the material obtained in the previous reaction.

Treatment of 4β-Methylcholest-5-en-3β-yl Acetate with Mineral Acid. - 4β-Methylcholest-5-en-3β-yl acetate (70 mg.) was dissolved in a mixture of acetic acid (19 ml.) and

concentrated hydrochloric acid (1 ml.) and the solution was heated at 100° for 3 hr. The mixture was cooled, poured into water, and extracted with ether. The ether solution was washed with sodium bicarbonate solution, dried over anhydrous sodium sulphate, and evaporated to give a pale yellow gum. A solution of the gum in light petroleum (5 ml.) was filtered through alumina (5 g.). Light petroleum (50 ml.) eluted a colourless gum (54 mg.) which crystallised from chloroform-methanol to give 4-methylcholesta-3,5-diene as prismatic needles, m.p. 78-79°, $[\alpha]_D$ - 91° (c,1.6), λmax.2320 Å. (ϵ, 17,600), 2380 Å. (ϵ, 18,600), 2470 Å. (ϵ, 11,600) (Found: C,88.2; H,12.2. $C_{28}H_{46}$ requires C,87.9; H,12.1%).

Treatment of Cholest-4-en-3β-yl Acetate with Perbenzoic Acid. - Cholest-4-en-3β-yl acetate (1 g.) in dry benzene (50 ml.) was treated with a 0.29 M. solution (10 ml.) of perbenzoic acid in benzene. After 3 days at room temperature the reaction mixture was poured into 5% potassium hydroxide solution and the organic layer was allowed to separate. The benzene extract was washed with water, dried (Na_2SO_4), and evaporated under reduced pressure to give a gum (1.09 g.) which was dissolved in hot methanol (30 ml.). The white crystalline solid which separated on cooling was collected and recrystallised from methanol to give 4α,5α-epoxycholestan-

-3β-yl acetate (550 mg.) as blades, m.p. 119-120°, $[\alpha]_D$ + 56° (c, 1.8), (lit.[83], m.p. 119-120°, $[\alpha]_D$ + 55°).

When the mother liquors from this crystallisation were reduced in bulk a molecular complex (210 mg.) of 4α,5α-epoxycholestan-3β-yl acetate and 4β,5β-epoxycoprostan-3β-yl acetate separated as needles, m.p. 104-107°. Two recrystallisations from methanol gave needles, m.p. 108-109°, $[\alpha]_D$ + 20° (c, 1.7); infrared absorption at 1739 $cm.^{-1}$ and 1235 $cm.^{-1}$ (acetate) (Found: C, 78.3; H, 10.7. $C_{29}H_{48}O_3$ requires C, 78.3; H, 10.9%).

Chromatography of Molecular Complex. - The molecular complex (100 mg.) in light petroleum (10 ml.) was chromatographed on alumina (3 g.). Elution with light petroleum (150 ml.) gave a gum (95 mg.) which crystallised from methanol as needles, m.p. 107-108°, identical (m.p. and mixed m.p.) with the starting material.

Preparation of the Molecular Complex from its Components. - Equal quantities (30 mg.) of 4α,5α-epoxycholestan-3β-yl acetate, m.p. 119-120°, $[\alpha]_D$ + 56° and 4β,5β-epoxycoprostan-3β-yl acetate, m.p. 88-89°, $[\alpha]_D$ - 23.5° were mixed and recrystallised twice from methanol to give needles, m.p. 108-109°, $[\alpha]_D$ + 18° (c, 2.0). A mixed m.p. determined with a sample of the molecular complex showed no depression.

SECTION II

Treatment of 4β,5β-Epoxycoprostan-3β-ol

with Methylmagnesium Iodide. - A solution of 4β,5β-epoxycoprostan-3β-ol (9.2 g.) in dry benzene (180 ml.) and dry ether (180 ml.) was added to methylmagnesium iodide, [prepared from magnesium (4.4 g.), methyl iodide (26.08 g.), and ether (110 ml.)], and the solution was refluxed for 4 hr., cooled, and poured on a mixture of crushed ice (500 g.) and ammonium chloride (50 g.). Ether (300 ml.) was added and after the solution had been shaken vigorously, the organic layer was separated, washed several times with water, dried (Na_2SO_4), and evaporated under reduced pressure to give a waxy solid (9.3 g.). The crude product was dissolved in a minimum of benzene and chromatographed on alumina (400 g.). The chromatogram was developed as indicated in the summary below.

Fraction	Eluant	Vol.(ml.)	Wt.(g.)	Description
1-5	Benzene	500	-	-
6-15	Ether	1000	-	-
16-31	½% Methanol in ether	1600	0.137	Gum
32-34	½% Methanol in ether	300	0.151	Gum
35-56	1% Methanol in ether	6000	3.503	White resin
57-63	2% Methanol in ether	2100	-	-

Fraction	Eluant	Vol.(ml.)	Wt.(g)	Description
64-85	3% Methanol in ether	3900	0.273	White solid
86-90	3% Methanol in ether	1500	0.105	White solid
91-99	3% Methanol in ether	2700	0.269	White resin
100-109	4% Methanol in ether	1000	0.086	White resin
110-121	5% Methanol in ether	1200	3.170	White solid

Fractions (16-31) were non-crystalline.

Fractions (32-34) were combined and crystallised several times from acetone-water to give an unidentified diol (75 mg.) as blades, m.p. 140-141°, $[\alpha]_D$ + 38.5° (c,0.8). The compound gave no colour with tetranitromethane in chloroform and was transparent to ultraviolet light; infrared absorption at 3290 and 3125 cm.$^{-1}$ (hydroxyl) (Found: C,80.2; H,11.6. $C_{28}H_{50}O_2$ requires C,80.3; H,12.0%).

Fractions (35-56) were bulked and crystallised many times from light petroleum to give 4α-methylcholestan-3β,4β-diol (2.86 g.) as lustrous plates, m.p. 202-203°, $[\alpha]_D$ + 24.5° (c,1.0). The compound gave no colour with tetranitromethane in chloroform and showed no selective light absorption in the ultraviolet region; infrared absorption at 3356 cm.$^{-1}$ (hydroxyl) (Found: C,80.2; H,11.9. $C_{28}H_{50}O_2$ requires C,80.3; H,12.0%).

Fractions (64-85) were combined and crystallised several times from light petroleum give a second unidentified diol (172 mg.) (c,2.0). The compound was transparent to ultraviolet light; infrared absorption at 3220 cm.$^{-1}$ (hydroxyl) (Found: C,80.6; H,12.1. $C_{28}H_{50}O_2$ requires C,80.3; H,12.0%).

Fractions (86-90) were combined and crystallised many times from acetone but a pure material was not obtained.

Fractions (91-99) were bulked and crystallised numerous times from acetone to give a third unidentified diol (124 mg.) as plates, m.p. 203-205°, $[\alpha]_D$ + 9.5° (c,2.0). The compound was transparent to ultraviolet light; infrared absorption at 3484 cm.$^{-1}$ (hydroxyl) (Found: C,80.1; H,12.2. $C_{28}H_{50}O_2$ requires C,80.3; H,12.0%).

Fractions (110-121) were combined and crystallised twice from acetone to give cholest-5-en-3β,4β-diol (2.58 g.) as needles, m.p. 175-176°, $[\alpha]_D$ - 62° (c,2.1). The compound gave a yellow colour with tetranitromethane in chloroform and showed light absorption at 2080 Å. (ε, 4,200). The m.p. of a mixture of this material with an authentic sample showed no depression.

The above diol (50 mg.) in acetic anhydride (1 ml.) was refluxed for 1 hr., and the product which separated from the cold reaction mixture was recrystallised from methanol to give cholest-5-en-3β,4β-diol diacetate, m.p. and mixed m.p. 169-170°, $[\alpha]_D$ - 94° (c,2.0).

Sodium Periodate Titration of 4α-Methylcholestan-3β,4β-diol.[101,102] - An aqueous solution of sodium periodate (2.5 ml.; 0.2017N) was added to a solution of the diol (59.3 mg.) in alcohol and the volume made up to 50 ml. by addition of a further quantity of alcohol. Portions (10 ml.) were removed after suitable time intervals and the excess of sodium periodate was estimated iodimetrically by the method of Barnaby.[103] A blank containing only the aqueous alcoholic solution of sodium periodate was prepared and treated in the same manner as the sample. The results of the titrations are tabulated below.

Reaction Time (Hrs.)	Blank Titration (ml. of 0.1N sodium arsenite)	Sample Titration (ml. of 0.1N sodium arsenite)	Titration Difference (ml. of 0.1 N sodium arsenite)
5	1.08	1.08	-
18	1.08	1.08	-
40	1.07	0.96	0.11
90	1.07	0.83	0.24

The above results indicate that after 40 hr. and 90 hr. the oxidation of the 1,2 glycol unit had occurred to the extent of 20% and 42% respectively.

Sodium Periodate Titration of 3β,4β-Dihydroxycholest-5-ene. - An alcoholic solution of the diol (51.2 mg.) was treated in the manner described above with the following results.

Reaction Time (Hrs.)	Blank Titration (ml. of 0.1N sodium arsenite)	Sample Titration (ml. of 0.1 N sodium arsenite)	Titration Diffnce. (ml. of 0.1 N sodium arsenite)
4	1.07	0.74	0.33
6	1.07	0.64	0.43
24	1.06	0.59	0.47

The above results show that the cleavage of the 1,2-glycol unit was 65, 85 and 92% after 4, 6 and 24 hrs. respectively.

The Acetone Derivative of 4α-Methylcholestan-3β,4β-diol. - 4α-Methylcholestan-3β,4β-diol (100 mg.) and dry acetone (10 ml.) was treated with concentrated hydrochloric acid (3 drops) and the mixture was shaken until a clear solution resulted. The shaking was continued for a further 2 hr., when sodium bicarbonate was added and, after 10 minutes, the solid was filtered off and the filtrate taken to dryness. The white solid which remained, was dissolved in light petroleum and chromatographed on alumina (5 g.). Elution with light petroleum gave a crystalline fraction (41 mg.) which after two recrystallisations from methanol gave 4α-methylcholestan-3β,4β-diol acetonide (35 mg.) as needles, m.p. 135-137°, $[\alpha]_D$ + 11° (c, 1.5) (Found: C, 81.2; H, 12.0. $C_{31}H_{54}O_2$ requires C, 81.2; H, 11.9%).

4α-Methylcholestan-3β,4β-diol-3-acetate. - 4α-Methyl cholestan-3β,4β-diol (105 mg.) in pyridine (3 ml.) and acetic anhydride (3 ml.) was kept at room temperature for 18 hr.

Isolation of the acetylated product with ether gave a white solid, m.p. 186-195°. Several recrystallisations from methanol gave 4α-methylcholestan-3β,4β-diol-3-acetate (81 mg.) as matted needles, m.p. 200-202°, $[\alpha]_D$ + 29.5° (c,1.0). The compound was transparent to ultraviolet light; infrared absorption at 3509 cm.$^{-1}$ (hydroxyl), 1724 cm.$^{-1}$ and 1266 cm.$^{-1}$ (acetate) (Found: C,78.5; H,11.2. $C_{30}H_{52}O_3$ requires C,78.2; H,11.4%).

4α-Methylcholestan-3β,4β-diol Diacetate. - A solution of 4α-methylcholestan-3β,4β-diol (200 mg.) in acetic anhydride (5 ml.) was refluxed for 5 hr. The cold reaction mixture was poured into water and the neutral product extracted with ether in the usual manner. Evaporation of the solvent gave a white solid which was dissolved in light petroleum and chromatographed on alumina (5 g.). Elution with light petroleum-benzene (4:1) gave a crystalline solid (110 mg.) which after three recrystallisations from methanol gave 4α-methylcholestan-3β,4β-diol diacetate (93 mg.) as plates, m.p. 154-155°, $[\alpha]_D$ + 3° (c,1.4). The compound was transparent to ultraviolet light; infrared absorption (in carbon tetrachloride) at 1754 cm.$^{-1}$ and 1739 cm.$^{-1}$ (acetate) (Found: C,76.2; H,11.1. $C_{32}H_{54}O_4$ requires C,76.4; H,10.8%).

Elution with benzene gave a white solid (73 mg.) which after several crystallisation from methanol gave 4α-methylcholestan-3β,4β-diol-3-acetate (52 mg.) as needles, m.p. 200-202°.

The compound was identical with that prepared in the previous reaction.

Oxidation of 4α-Methylcholestan-3β,4β-diol with Chromic Acid. - 4α-Methylcholestan-3β,4β-diol (450 mg.) in stabilised acetic acid (37.5 ml.) and water (2.5 ml.) was heated to 60° on a water bath. A solution of chromium trioxide (450 mg.) in stabilised acetic acid (4 ml.) and water (0.5 ml.) was added and the reaction mixture maintained at 60° for a further 2.5 hr. The excess of chromic acid was destroyed with methanol (2 ml.) and the solution diluted with water, extracted with ether (3 x 50 ml.), and the ethereal extract washed with 7% sodium hydoxide solution (3 x 50 ml.). The alkaline wash was acidified with dilute hydrochloric acid and the precipitate extracted with ether (300 ml.). Evaporation of the solvent under reduced pressure gave a gum (430 mg.) which after three crystallisations from methanol gave 4-methylcholestan-3,4-seco-4-oxo-3-carboxylic acid (230 mg.) as plates, m.p. 124-125°, $[\alpha]_D$ - 1.5° (c,1.4); infrared absorption at 2674 cm.$^{-1}$ (carboxylic acid), 1712 cm.$^{-1}$ (carbonyl) (Found: C,78.0; H,10.9. $C_{29}H_{48}O_3$ requires C,77.7; H,11.2%).

The acid (100 mg.) was treated with an excess of diazomethane in ether. Crystallisation of the product from methanol gave 4-methylcholestan-3,4-seco-4-oxo-3-carboxylic acid methyl ester as plates, m.p. 77-78°; infrared absorption

at 1739 cm.$^{-1}$ (ester), 1706 cm.$^{-1}$ (ketone) (Found: C,78.3; H,11.2. $C_{29}H_{50}O_3$ requires C,78.0; H,11.3%).

Hydrolysis of 4-methylcholestan-3,4-seco-4-oxo-3-carboxylic Acid Methyl Ester. - The ester (70 mg.) and sodium hydroxide in methanol (0.5%; 10 ml.) were shaken together until solution was complete and the reaction mixture was allowed to stand at room temperature for 24 hr. The solution was acidified with dilute hydrochloric acid, extracted with ether, and the ethereal solution was washed with 7% sodium hydroxide solution (100 ml.). The alkaline wash was acidified with dilute hydrochloric acid and the precipitate extracted with ether. Evaporation of the solvent gave a gum (55 mg.) which crystallised from methanol to give 4-methylcholestan-3,4-seco-4-oxo-3-carboxylic acid (40 mg.) as plates, m.p. and mixed m.p. 124-125°.

Treatment of 4α-Methylcholestan-3β,4β-diol with Periodic Acid. - The diol (590 mg.) was dissolved in dioxan (25 ml.) and a solution of periodic acid ($HIO_4.2H_2O$; 670 mg.) in water (4 ml.) was added. The white precipitate which formed was redissolved by shaking and a further quantity of dioxan (5 ml.) was added. The opalescent solution was allowed to stand at room temperature for 48 hr., when a crystalline precipitate (HIO_3) was formed. The dioxan was removed under reduced pressure, water was added, and the resulting gum was extracted with ether. The extract was

washed with water, dried over anhydrous sodium sulphate, and the solvent evaporated to give a yellow gum (570 mg.) which was dissolved in light petroleum and chromatographed on alumina (15 g.). Elution with light petroleum-benzene (1:1) gave 4-methyl-3,4-seco-3,4-dioxocholestane as a non-crystalline gum (250 mg.), infrared absorption at 1712 cm.$^{-1}$ (carbonyl). The gum (80 mg.) in methanol (2 ml.) was treated with a solution of 2,4-dinitrophenylhydrazine in methanol and the resulting yellow precipitate was filtered, dried, and recrystallised from chloroform-methanol to give the mono-2,4-dinitrophenylhydrazone of 4-methyl-3,4-seco-3,4-dioxocholestane (65 mg.) as blades, m.p. 197-199°, $[\alpha]_D$ - 17° (c, 1.4); infrared absorption showed a strong band at 1709 cm.$^{-1}$ (carbonyl) (Found: C, 68.5; H, 8.8; N, 9.6. $C_{34}H_{52}O_8N_4$ requires C, 68.4; H, 8.8; N, 9.4%).

Treatment of 4-Methyl-3,4-seco-3,4-dioxocholestane with Hydrogen Peroxide. - The non-crystalline carbonyl compound (60 mg.) in acetic acid (2 ml.) was treated with aqueous hydrogen peroxide (30%, 1 ml.) and the solution was heated at 100° for 3 hr. Extraction of the acidic product in the manner described previously gave a gum (40 mg.) which crystallised from methanol to yield 4-methylcholestan-3,4-seco-4-oxo-3-carboxylic acid as plates, m.p. and mixed m.p. 124-125°.

Treatment of 4α-Methylcholestan-3β,4β-diol-3-acetate with Chromium Trioxide. - A solution of the diol monoacetate (100 mg.) in pyridine (3 ml.) was mixed with a slurry of chromium trioxide (100 mg.) in pyridine (1 ml.) and the reaction mixture was left at room temperature for 20 hr. Isolation of the product by means of ether gave a white solid which crystallised from methanol to give unchanged starting material.

The Action of Phosphorus Oxychloride in Pyridine on 4α-Methylcholestan-3β,4β-diol-3-acetate. - Phosphorus oxychloride (10 ml.) was added to a solution of the diol monoacetate (1 g.) in dry pyridine (40 ml.) and the mixture was kept at 100° for 3 hr. The excess phosphorus oxychloride was destroyed by the addition of crushed ice and the neutral product was isolated by means of ether. Evaporation of the solvent afforded a gum (850 mg.) which was dissolved in light petroleum and chromatographed on alumina (25 g.). Elution with light petroleum (100 ml.) gave a fraction (260 mg.) which crystallised from chloroform-methanol to give 4-methylcholesta-3,5-diene (210 mg.) as needles, m.p. 78-79°, $[\alpha]_D$ - 92° (c, 2.1). The identity of this compound with the 4-methylcholesta-3,5-diene prepared previously (page 88) was established by mixed m.p. determination.

Further elution with light petroleum (250 ml.) gave a crystalline fraction (390 mg.) which gave, after two crystallisations from methanol, 4-methylcholest-4-en-3β-yl acetate (260 mg.)

as needles, m.p. 113-114°, $[\alpha]_D$ + 37° (c, 1.1); $\lambda_{max.}$ 2080 Å. (ε, 11,000); infrared absorption at 1739 cm.$^{-1}$ and 1250 cm.$^{-1}$ (acetate) (Found: C, 81.0; H, 11.7. $C_{30}H_{50}O_2$ requires C, 81.4; H, 11.4%).

4-Methylcholest-4-en-3β-ol. - 4-Methylcholest-4-en-3β-yl acetate (145 mg.) in dry ether (20 ml.) was added to a suspension of lithium aluminium hydride (100 mg.) in dry ether (20 ml.) and the mixture was boiled gently under reflux for 2.5 hr. Ether (50 ml.) was added and the excess hydride was decomposed by the cautious addition of crushed ice. The suspension was washed with dilute sulphuric acid (5N), water, and dried (Na_2SO_4). The residue remaining after evaporation of the ether was recrystallised several times from methanol to give 4-methylcholest-4-en-3β-ol (110 mg.) as needles, m.p. 171-173°, $[\alpha]_D$ + 58° (c, 1.7); $\lambda_{max.}$ 2100 Å. (ε, 7,500) (Found: C, 84.2; H, 12.2. $C_{28}H_{48}O$ requires C, 83.9; H, 12.1%).

Oxidation of 4-Methylcholest-4-en-3β-ol with Chromium Trioxide. - The alcohol (75 mg.) in pyridine (3 ml.) was treated with a slurry of chromium trioxide (100 mg.) in pyridine (1 ml.) and the mixture was allowed to stand for 20 hr. at room temperature. The product was isolated by means of ether, dissolved in light petroleum, and chromatographed on alumina (3 g.). Elution with light petroleum-benzene (2:1) gave a crystalline fraction (50 mg.)

which crystallised from methanol as needles, m.p. 103-104°, identical (m.p., mixed m.p., and infrared absorption) with 4-methylcholest-4-en-3-one.

Hydrogenation of Cholest-5-en-3β,4β-diol-3-benzoate. - The benzoate (1 g.) in ether (100 ml.), ethyl acetate (50 ml.), and acetic acid (20 ml.) was shaken with hydrogen over a platinum catalyst (from 100 mg. of platinum oxide) for 12 hr. at room temperature. The volume of hydrogen absorbed at N.T.P. was 180 ml. (4 moles). The catalyst was removed by filtration and the filtrate reduced to 50 ml. by evaporation of the solvent *in vacuo*. The product which separated on cooling was collected and recrystallised once from chloroform-methanol to give cholestan-3β,4β-diol-3-hexahydrobenzoate (780 mg.) as needles, m.p. 213-215°, $[\alpha]_D$ + 4.5° (c, 1.1); infrared absorption at 1709 cm.$^{-1}$ (with a shoulder at 1730 cm.$^{-1}$) and 1244 cm.$^{-1}$ (ester). The compound gave no colour with tetranitromethane in chloroform and showed no selective light absorption in the ultraviolet region. A mixture with the starting material (m.p. 212-213°) had m.p. 200-202° (Found: C, 79.0; H, 11.2. $C_{34}H_{58}O_3$ requires C, 79.3; H, 11.4%).

Hydrolysis of Cholestan-3β,4β-diol-3-hexahydrobenzoate. - The ester (100 mg.) was refluxed with 3% methanolic potassium hydroxide solution (30 ml.) for 3 hr. The product (71 mg.),

isolated by means of ether was recrystallised several times from acetone to give cholestan-3β,4β-diol as plates, m.p. 199-201°, $[\alpha]_D$ + 16° (c,0.8). Rosenheim and Starling[104] give m.p. 202-203°, $[\alpha]_D$ + 18.8° for this compound.

Oxidation of Cholestan-3β,4β-diol-3-hexahydrobenzoate with Sodium Dichromate in Sulphuric Acid. - A solution of cholestan-3β,4β-diol-3-hexahydrobenzoate (960 mg.) in boiling acetone (150 ml.) was treated dropwise with Kiliani reagent (0.176 g. sodium dichromate/ml.) till a permanent yellow colour was observed. The solution was cooled, poured into water (400 ml.), and the white precipitate was filtered and dissolved in ether. The solution was washed with saturated sodium bicarbonate solution, water, and dried (Na_2SO_4). Evaporation of the solvent gave a white crystalline solid which was recrystallised several times from chloroform-methanol to give 4-oxocholestan-3β-yl hexahydrobenzoate (740 mg.) as lustrous plates, m.p. 183-185°, $[\alpha]_D$ - 7° (c,1.8); infrared absorption at 1745 cm.$^{-1}$ and 1242 cm.$^{-1}$ (ester), 1724 cm.$^{-1}$ (ketone). The compound gave no colour with tetranitromethane in chloroform and showed no selective light absorption in the ultraviolet region (Found: C,79.4; H,11.3. $C_{34}H_{56}O_3$ requires C,79.6; H,11.0%).

Treatment of 4-Oxocholestan-3β-yl Hexahydrobenzoate with Methylmagnesium Iodide. - A solution of 4-oxocholestan-3β-yl hexahydrobenzoate (3.18 g.) in dry ether (150 ml.) and dry benzene (150 ml.) was added to the Grignard reagent [prepared from magnesium (1.44 g.), methyl iodide (8.76 g.), and dry ether (100 ml.)]. The reaction mixture was refluxed for 4 hr., cooled, and poured on crushed ice (150 g.) and ammonium chloride (15 g.). After 1 hr., ether (400 ml.) was added, the solution was shaken vigorously, and the organic extract was washed thoroughly with water and dried (Na_2SO_4). Evaporation of the solvent gave a resinous mass (2.98 g.) which after several crystallisations from acetone gave 4α-methylcholestan-3β,4β-diol (1.75 g.) as prismatic needles, m.p. 202-203°, $[\alpha]_D$ + 24° (c,1.9). The compound was identical (m.p., mixed m.p., and infrared absorption) with the diol obtained from the reaction of methylmagnesium iodide with 4β,5β-epoxycholestan-3β-ol (page 91).

SECTION III

Treatment of 4β,5β-Epoxycoprostan-3α-ol with Methylmagnesium Iodide. - A solution of 4β,5β-epoxycoprostan-3α-ol (7 g.) in dry ether (175 ml.) and dry benzene (175 ml.) was added to methylmagnesium iodide, [prepared from magnesium (2.52 g.), methyl iodide (14.88 g.), and dry ether (200 ml.)],

and the reaction mixture was refluxed for 4 hr., cooled, and poured on a mixture of crushed ice (250 g.) and ammonium chloride (25 g.). The mixture was treated with ether (250 ml.) and the organic extract was washed several times with water and dried over anhydrous sodium sulphate. Evaporation of the solvent afforded a resinous mass (7.3 g.) which was dissolved in a minimum of ether and absorbed on a column of alumina (210 g.). The chromatogram was developed as indicated in the summary below.

Fraction	Eluant	Vol.(Ml.)	Wt.(g)	Description
1-5	Ether	500	0.122	Yellow gum
6-18	Ether	1300	1.218	Resinous gum
19-48	Ether	3000	1.361	Crystalline solid
49-77	½% Methanol in Ether	2900	1.140	White solid
78-109	1% Methanol in Ether	3200	1.147	White resin
110-119	5% Methanol in Ether	1000	0.599	White resin

Fractions (1-5) were non-crystalline.

Fractions (6-18) were combined and crystallised three times from methanol to give 4α-methylcholestan-3α,4β-diol (1.14 g.) as needles, m.p. 189-191°, $[\alpha]_D$ + 9° (c,0.8). The compound gave no colour with tetranitromethane in chloroform and showed no selective light absorption in the ultraviolet region;

infrared absorption at 3484 cm.$^{-1}$ (hydroxyl) [Found: C,78.6; H,11.8. $C_{28}H_{50}O_2 \cdot \frac{1}{2}CH_3OH$ requires C,78.7; H,12.1%].

Fractions (19-48) were bulked and crystallised twice from acetone to give a material (1.26 g.) as blades, m.p. 159-161°, $[\alpha]_D$ + 23° (c,2.0). The compound was shown to be identical with starting material (m.p., mixed m.p. and infrared absorption).

Fractions (49-77) were combined and crystallised from acetone to give 3α-methylcholestan-3β,4α-diol (1.10 g.) as prisms, m.p. 179-181°, $[\alpha]_D$ + 7° (c,1.6). The compound was transparent to ultraviolet light; infrared absorption at 3460 cm.$^{-1}$ (hydroxyl) (Found: C,80.4; H,12.1. $C_{28}H_{50}O_2$ requires C,80.3; H,12.0%).

Fractions (78-109) were combined and crystallised from light petroleum and ultimately from acetone to give 4α-methylcholestan-3α,5β-diol (1.08 g.) as plates, m.p. 154-155°, $[\alpha]_D$ + 4° (c,1.7). The compound gave no colour with tetranitromethane in chloroform and was transparent to ultraviolet light; infrared absorption at 3613 cm.$^{-1}$ and 3247 cm.$^{-1}$ (hydroxyl) (Found: C,80.2; H,11.9. $C_{28}H_{50}O_2$ requires C,80.3; H,12.0%).

Fractions (110-119) were combined and crystallised twice from acetone to give 3β-methylcholestan-3α,4α-diol (0.54 g.) as needles, m.p. 186-188°, $[\alpha]_D$ + 10° (c,1.7). The compound gave no colour with tetranitromethane in chloroform and showed no selective light absorption in the ultraviolet region; infrared

absorption at 3636 cm.$^{-1}$ and 3460 cm.$^{-1}$ (hydroxyl) (Found: C,79.9; H,11.7. $C_{28}H_{50}O_2$ requires C,80.3; H,12.0%)

Sodium Periodate Titration of 4α-Methylcholestan-3α,4β-diol. - An alcoholic solution of the diol (65.4 mg.) was treated in the manner described for 4α-methylcholestan-3β,4β-diol (page 93) After 92 hr. a slight precipitate ($NaIO_3$) was observed but the titration results indicated that no oxidation had occurred.

Sodium Periodate Titration of 3α-Methylcholestan-3β,4α-diol. - An alcoholic solution of the diol (49.9 mg.) was treated in the manner described above with the following results:

Reaction Time Hr.	Blank Titration (ml. of 0.1N sodium arsenite)	Sample Titration (ml. of 0.1N sodium arsenite)	Titration Diffnce. (ml. of 0.1 N sodium arsenite)
17	1.07	0.97	0.10
42	1.06	0.91	0.15
92	1.06	0.89	0.17

These results show that the cleavage of the 1,2-glycol unit was 21, 31, and 36% after 17, 42, and 92 hr. respectively.

Sodium Periodate Titration of 4α-Methylcholestan-3α,5β-diol. - An alcoholic solution of the diol (53.5 mg.) was treated in the manner described above. After 92 hr. the titration results indicated that no oxidation had taken place.

Sodium Periodate Titration of 3β-Methylcholestan-3α,4α-diol. - An alcoholic solution of the diol (50 mg.) was treated in the manner described above with the following results:

Reaction Time Hr.	Blank Titration (ml. of 0.1N sodium arsenite)	Sample Titration (ml. of 0.1N sodium arsenite)	Titration Diffnce (ml. of 0.1 N sodium arsenite)
17	1.07	0.79	0.28
42	1.07	0.66	0.41
92	1.07	0.61	0.46

The above results indicate that the cleavage of the 1,2-glycol unit was 60, 86, and 96% after 17, 42, and 92 hr. respectively.

4α-Methylcholestan-3α,4β-diol-3-acetate. - A solution of 4α-methylcholestan-3α,4β-diol (100 mg.) in pyridine (5 ml.) and acetic anhydride (5 ml.) was heated at 100° for 1 hr. and allowed to stand at room temperature overnight. The acetylated product was extracted with ether in the normal manner and evaporation of the solvent gave a white solid (112 mg.) which crystallised from methanol to give 4α-methylcholestan-3α,4β-diol-3-acetate as needles, m.p. 162-163°, $[\alpha]_D$ - 22° (c,1.0). The compound was transparent to ultraviolet light; infrared absorption at 3571 cm.$^{-1}$ (hydroxyl), 1727 cm.$^{-1}$ 1709 cm.$^{-1}$ and 1250 cm.$^{-1}$ (acetate) (Found: C,77.9; H,11.2. $C_{30}H_{52}O_3$ requires C,78.2; H,11.4%)

Oxidation of 4α-Methylcholestan-3α,4β-diol with Sodium Dichromate in Sulphuric Acid. - A solution of the diol (60 mg.) in acetone (10 ml.) was treated dropwise with the Kiliani reagent (0.176 g. sodium dichromate/ml.) till a permanent yellow colour

was attained. The reaction mixture was poured into water, extracted with ether (2 x 50 ml.), and the extract was washed with dilute sodium hydroxide solution (2 x 25 ml.). The alkaline wash was acidified with dilute hydrochloric acid and extracted with ether (3 x 50 ml.). Evaporation of the solvent gave a gum (45 mg.) which crystallised from methanol as plates, m.p. 120-123°. Two further recrystallisations from methanol yielded 4-methylcholestan-3,4-seco-4-oxo-3-carboxylic acid (35 mg.), m.p. 124-125°, $[\alpha]_D$ - 3° (c, 1.2). The compound was identical (infrared absorption) with the acid obtained by oxidation of 4α-methylcholestan-3β,4β-diol (page 96).

4α-Methylcholestan-3α,5β-diol-3-acetate. - A solution of 4α-methylcholestan-3α,5β-diol (100 mg.) in pyridine (3 ml.) and acetic anhydride (3 ml.) was allowed to stand at room temperature for 20 hr. The reaction mixture was poured into water and the product (110 mg.), isolated by means of ether, was crystallised from methanol to afford 4α-methylcholestan-3α,5β-diol-3-acetate (93 mg.) as blades, m.p. 101-109°. A sample was dried under vacuum for 4 hr. at 65° and had m.p. 111-112°, $[\alpha]_D$ + 8° (c, 1.0). The compound showed no selective light absorption in the ultraviolet region; infrared absorption at 3546 $cm.^{-1}$ (hydroxyl), 1727 $cm.^{-1}$ and 1272 $cm.^{-1}$ (acetate) (Found: C, 78.2; H, 11.3. $C_{30}H_{52}O_3$ requires C, 78.2; H, 11.4%).

Oxidation of 4α-Methylcholestan-3α,5β-diol with Sodium Dichromate in Sulphuric Acid. - A solution of the diol (53 mg.) in acetone (10 ml.) was treated dropwise with the Kiliani reagent (0.176 g. sodium dichromate/ml.) till the solution assumed a permanent yellow colour. The reaction mixture was poured into water and the product was extracted with ether in the usual manner. Evaporation of the solvent gave a yellow gum (49 mg.) which was dissolved in light petroleum and absorbed on a column of alumina (3 g.). Elution with light petroleum-benzene (2:1) gave a fraction (15 mg.) which after two crystallisations from methanol gave 4-methylcholest-4-en-3-one as needles, m.p. 102-104°; identical (m.p. mixed m.p. and infrared absorption) with an authentic specimen.

Oxidation of 3α-Methylcholestan-3β,4α-diol with Sodium Dichromate in Sulphuric Acid. - A solution of 3α-methylcholestan-3β,4α-diol (51 mg.) in acetone (20 ml.) was treated dropwise with the Kiliani reagent (0.176 g. sodium dichromate/ml.) till the solution remained pale yellow. The reaction mixture was poured into water, extracted with ether, and the extract was washed with dilute sodium hydroxide solution (2 x 25 ml.), water, and dried (Na_2SO_4). Evaporation of the solvent gave a crystalline solid (26 mg.) which was recrystallised several times from methanol to yield 3α-methylcholestan-4-one-3β-ol as blades,

m.p. 189-192°, $[\alpha]_D$ + 1° (c,1.1); infrared absorption at 3559 cm.$^{-1}$ (hydroxyl), 1712 cm.$^{-1}$ (ketone) (Found: C,80.95; H,11.5. $C_{28}H_{48}O_2$ requires C,80.7; H,11.6%).

The alkaline wash from the above reaction was acidified with dilute hydrochloric acid and extracted with ether. Evaporation of the solvent gave a gum (16 mg.) which could not be purified by crystallisation.

Oxidation of 3α-Methylcholestan-3β,4α-diol with Chromium Trioxide in Pyridine. - A solution of the diol (275 mg.) in pyridine (5 ml.) was mixed intimately with a slurry of chromium trioxide (300 mg.) in pyridine (3 ml.) and the reaction mixture was left at room temperature for 24 hr. Isolation of the neutral product by means of ether gave a white solid (250 mg.) which was recrystallised from methanol to give a compound, identical (m.p. mixed m.p. and infrared absorption) with that prepared in the above reaction.

Treatment of 3α-Methylcholestan-4-one-3β-ol with Phosphorus Oxychloride in Pyridine. - A solution of 3α-methylcholestan-4-one-3β-ol (100 mg.) in dry pyridine (10 ml.) was treated with phosphorus oxychloride (1 ml.) and the solution was allowed to stand at room temperature overnight. The product, isolated by means of ether was a gum (85 mg.) which crystallised on trituration with methanol. Two recrystallisations from

methanol gave 3-methylcholest-2-en-4-one as needles, m.p. 126-127°, $[\alpha]_D$ + 52° (c,1.1); $\lambda_{max.}$ 2380 Å. (ε, 7,500); infrared absorption at 1678 cm.$^{-1}$ and 1603 cm.$^{-1}$ (αβ-disubstituted αβ-unsaturated ketone) (Found: C,84.1; H,11.6. $C_{28}H_{46}O$ requires C,84.35; H,11.6%).

Oxidation of 3β-Methylcholestan-3α,4α-diol with Sodium Dichromate in Sulphuric Acid. - A solution of the diol (54 mg.) in acetone (10 ml.) was treated dropwise with the Kiliani reagent (0.176 g. sodium dichromate/ml.) until the solution remained pale yellow. The reaction mixture was poured into water, extracted with ether (3 x 50 ml.) and the extract was washed with dilute sodium hydroxide solution (2 x 25 ml.), water, and dried over anhydrous sodium sulphate. Evaporation of the solvent gave a crystalline solid (25 mg.) which was recrystallised twice from methanol to give 3β-methylcholestan-4-one-3α-ol as needles, m.p. 125-126°, $[\alpha]_D$ - 9° (c,1.1); infrared absorption at 3584 cm.$^{-1}$ and 3509 cm.$^{-1}$ (hydroxyl), 1712 cm.$^{-1}$ (ketone) (Found: C,80.3; H,11.3. $C_{28}H_{48}O_2$ requires C,80.7; H,11.6%).

The alkaline wash from the above reaction was acidified with dilute hydrochloric acid and extracted with ether. The extract was washed several times with water, dried (Na_2SO_4) and the solvent evaporated to give a gum (12 mg.) which could

not be purified by crystallisation.

Treatment of 3β-Methylcholestan-4-one-3α-ol with Phosphorus Oxychloride in Pyridine. - A solution of 3β-methylcholestan-4-one-3α-ol (130 mg.) in dry pyridine (10 ml.) was mixed with phosphorus oxychloride (3 ml.) and heated at 100° for 3 hr. The product (110 mg.) isolated by means of ether was dissolved in light petroleum and chromatographed on alumina (3 g.). Elution with light petroleum gave a fraction (74 mg.) which crystallised from methanol to give 3-methylcholest-2-en-4-one as monoclinic needles, m.p. 125-127°, $[\alpha]_D$ + 50° (c, 0.9). The compound was shown to be identical with that prepared by dehydration of 3α-methylcholestan-4-one-3β-ol (mixed m.p. 125-127°) and by the coincidence of their infrared absorption spectra. The m.p. of a mixture of the compound and starting material was depressed by 10°

Treatment of 3α-Methylcholestan-3β,4α-diol and of 3β-Methylcholestan-3α,4α-diol with Lead Tetraacetate. - (a) A solution of 3α-Methylcholestan-3β,4α-diol (120 mg.) in chloroform (15 ml.) and dry benzene (50 ml.) was treated with lead tetraacetate (300 mg.). The solution rapidly became yellow and after standing at room temperature for 12 hr. an orange precipitate had formed. Ethylene glycol (3 drops) was added and after

10 min. the reaction mixture was filtered through kieselguhr and the clear filtrate evaporated under reduced pressure to give a yellow gum (148 mg.). The gum was dissolved in ether and the solution washed with saturated sodium bicarbonate solution, water, and dried over anhydrous sodium sulphate. Evaporation of the solvent gave a gum, which from its absorption spectrum appeared to be 3-methyl-3,4-seco-3,4-dioxocholestane; infrared absorption at 2740 cm.$^{-1}$ and 1730 cm.$^{-1}$ (aldehyde), 1712 cm.$^{-1}$ (ketone).

(b) A solution of 3β-methylcholestan-3α,4α-diol (80 mg.) in chloroform (10 ml.) and dry benzene (40 ml.) was treated with lead tetraacetate (200 mg.) in the manner described above. The product (86 mg.) was a non-crystalline gum, identical (infrared absorption) with the material obtained in (a).

SECTION IV

Cholest-5-en-4-on-3β-yl Benzoate. - A solution of cholest-5-en-3β,4β-diol-3-benzoate[104] (10 g.) in chloroform (60 ml.) was treated with a solution of bromine (1.4 ml.) in glacial acetic acid (40 ml.) with intermittent cooling. After standing at room temperature for 2 hr. the reaction mixture was diluted with benzene (140 ml.) and a solution of chromium

trioxide (6.6 g.) in water (60 ml.) and glacial acetic acid (140 ml.) was added. The mixture was stirred vigorously for 5 hr. and the organic layer was separated, washed with water, and dried over anhydrous sodium sulphate. The dry extract was heated to 80°, treated with a 10% solution of sodium iodide in ethanol (100 ml.), and the mixture was refluxed for a further 10 min., cooled, and poured into dilute sodium hydroxide solution. The organic layer was separated, washed with water, and dried (Na_2SO_4). Evaporation of the solvent under vacuum gave a dark brown mass which was crystallised several times from chloroform methanol to give cholest-5-en-4-on-3β-yl-benzoate as stout white needles, m.p. 157-158°, $[\alpha]_D$ - 30° (c, 2.2); $\lambda_{max.}$ 2020 Å. (ε, 13,000), 2320 Å. (ε, 19,500); infrared absorption at 1724 cm.$^{-1}$ and 1271 cm.$^{-1}$ (benzoate), 1709 cm.$^{-1}$ and 1634 cm.$^{-1}$ (αβ-unsaturated ketone). (Found: C, 81.0; H, 9.8. $C_{34}H_{48}O_3$ requires C, 80.9; H, 9.6%).

Treatment of Cholest-5-en-4-on-3β-yl Benzoate with Methylmagnesium Iodide. - A solution of cholest-5-en-4-on-3β-yl benzoate (5.0 g.) in dry ether (125 ml.) and dry benzene (125 ml.) was added to methylmagnesium iodide, [prepared from magnesium (1.37 g.), methyl iodide (8.46 g.), and dry ether (35 ml.)]. The yellow solution was heated under reflux for 4 hr., cooled, and poured on a mixture of crushed ice (140 g.) and ammonium

chloride (15 g.). Ether (300 ml.) was added, the solution was shaken, and the organic layer which separated was washed several times with water and dried over anhydrous sodium sulphate. The solvent was evaporated to give a yellow gum (5.3 g.) which was dissolved in benzene and absorbed on a column of alumina (150 g.) The chromatogram was developed as indicated below.

Fraction	Eluant	Vol. (ml.)	Wt. (g.)	Description
1-5	Benzene	500	1.86	Yellow gum
6-10	Ether	500	0.47	Yellow gum
11-13	2% Methanol in ether	300	0.21	Colourless gum
14-18	5% Methanol in ether	500	0.65	White solid

Fractions (1-5) were combined dissolved in light petroleum and rechromatographed on a column of alumina (60 g.).

Elution with light petroleum (500 ml.) gave a fraction (710 mg.) which was crystallised several times from methanol to give 4α,6α-dimethylcholestan-3-one (520 mg.) as colourless needles, m.p. 102-103°, $[\alpha]_D$ - 7.5° (c, 1.6). The compound gave no colour with tetranitromethane in chloroform and showed no selective light absorption in the ultraviolet region; infrared absorption at 1712 $cm.^{-1}$ and 1700 $cm.^{-1}$ (ketone) [Found: C, 84.3; H, 12.0. $C_{29}H_{50}O$ requires C, 84.0; H, 12.15%].

Further elution with light petroleum (200 ml.) gave a

colourless gum (220 mg.) which was crystallised several times from methanol to give 4β,6α-dimethylcholestan-3-one (175 mg.) as feathery needles, m.p. 109-110°, $[\alpha]_D$ - 35° (c,1.7). The compound gave no colour with tetranitromethane in chloroform and was transparent to ultraviolet light; infrared absorption at 1712 $cm.^{-1}$ (ketone) (Found: C,84.4; H,12.2. $C_{29}H_{50}O$ requires C,84.0; H,12.15%).

Elution with light petroleum benzene (1:2) gave intractable material (0.91 g.).

Fractions (6-10) from the original column could not be crystallised. The infrared absorption spectrum of this material showed a band at 700 $cm.^{-1}$ (aromatic ring system).

Fractions (11-13) could not be crystallised.

Fractions (14-18) could not be purified by crystallisation. The material gave a yellow colour with tetranitromethane in chloroform; $\lambda_{max.}$ 2040 Å. (ε, 4,700); infrared absorption at 3360 $cm.^{-1}$ (hydroxyl). This material will be referred to as "the epimeric diols" in the experiments described below.

Treatment of the Epimeric Diols with Periodic Acid. - A solution of the epimeric diols (50 mg.) in dioxan (3 ml.) was treated with a solution of periodic acid ($HIO_4.2H_2O$; 65 mg.) in water (0.4 ml.). The white precipitate was redissolved by shaking and a further quantity of dioxan (1 ml.) was added.

The solution was allowed to stand at room temperature for 48 hr., the solvent was evaporated under reduced pressure, and the residue dissolved in ether. The ethereal solution was washed with water, dried (Na_2SO_4), and evaporated to give a gum, which from spectroscopic data, probably was 4-methyl-3,4-seco-3,4-dioxocholest-5-ene (47 mg.); $\lambda_{max.}$ 2350 Å. (ϵ, 9,600).

Treatment of the Epimeric Diols with Mineral Acid. - A suspension of the epimeric diols (267 mg.) in dry acetone (30 ml.) was treated with concentrated hydrochloric acid (5 drops) and the mixture was shaken for 2 hr., when a clear solution was obtained. Solid sodium bicarbonate was added and after 10 min., the solid was removed by filtration and the filtrate was evaporated to dryness to give a gum (255 mg.) which was dissolved in light petroleum and absorbed on a column of alumina (8 g.).

Elution with light petroleum gave a fraction (18 mg.) which was not further examined.

Elution with light petroleum-benzene (1:1) gave a gum (210 mg.) which was crystallised several times from methanol to give material (184 mg.), m.p. 103-104°, $[\alpha]_D$ + 107° (*c*, 1.8). The compound was identical (m.p., mixed m.p., and infrared absorption) with an authentic specimen of 4-methylcholest-4-en-3-one.

Acetylation of the Epimeric Diols. - The epimeric diols (590 mg.) in pyridine (10 ml.) and acetic anhydride (10 ml.) were heated at 100° for 2 hr. The acetylated product (612 mg.), isolated by means of ether, was dissolved in benzene and chromatographed on alumina (20 g.). Elution with benzene-ether (1:2) gave a white solid (480 mg.) which could not be purified by crystallisation, but from spectroscopic data appeared to be a monoacetate of a diol. The material gave a yellow colour with tetranitromethane in chloroform; $\lambda_{max.}$ 2040 Å. (ε, 6000); infrared absorption at 3521 cm.$^{-1}$ (hydroxyl); 1712 cm.$^{-1}$ and 1263 cm.$^{-1}$ (acetate).

Treatment of the Mixed Epimeric Diol Monoacetates with Phosphorus Oxychloride in Pyridine. - A solution of the monoacetates (350 mg.) in dry pyridine (20 ml.) was treated with phosphorus oxychloride (5 ml.) and the mixture was heated at 100° for 3 hr. Isolation of the product in ether in the usual way gave a yellow semi-solid mass (300 mg.) which was dissolved in light petroleum (10 ml.)and chromatographed on a column of alumina (10 g.).

Elution with light petroleum (100 ml.) gave a gum which from spectroscopic data appeared to contain 4-methylcholesta-2,4,6-triene (79 mg.); $\lambda_{max.}$ 2080 Å., 3000 Å., and 3100 Å. On standing for a few days the gum became yellow.

Further elution with light petroleum (300 ml.) gave a crystalline solid (116 mg.), m.p. 105-112°. The material was recrystallised several times from chloroform-methanol to give 4-methylcholesta-3,5-dien-3-yl acetate as plates, m.p. 114-115°, $[\alpha]_D$ - 102° (Ω,1.7). The compound gave a brown colour with tetranitromethane in chloroform; $\lambda_{max.}$ 2360 Å. (ε, 18,000), infrared absorption at 1754 $cm.^{-1}$ and 1217 $cm.^{-1}$ (enol acetate). Atwater[100] gives m.p. 114-116°, $[\alpha]_D$ - 102° for this compound.

In another experiment the crude dehydration product was absorbed on a column of alumina and left overnight. Elution with light petroleum-benzene (1:1) gave 4-methylcholest-4-en-3-one, m.p. and mixed m.p. 102-104°.

Saponification of 4-Methylcholesta-3,5-dien-3-yl Acetate. - A solution of 4-methylcholesta-3,5-dien-3-yl acetate (35 mg.) in 3% methanolic potassium hydroxide solution (20 ml.) was heated under reflux for 1 hr. The mixture was acidified with dilute hydrochloric acid and the product was extracted by means of ether. The extract was washed with saturated sodium bicarbonate solution, water, and the solvent evaporated to furnish a colourless gum (30 mg.) which crystallised from methanol to give 4-methylcholest-4-en-3-one identified by m.p. mixed m.p. and infrared absorption.

Treatment of 4-Methylcholest-4-en-3-one with Isopropenyl Acetate. - A solution of 4-methylcholest-4-en-3-one (200 mg.) in

isopropenyl acetate (25 ml.) was treated with concentrated sulphuric acid (1 drop) and the reaction mixture was heated at 100° for 5 hr. Solid sodium bicarbonate was added and the solution was evaporated to dryness under reduced pressure to give a semi-solid mass which was extracted with ether. The ethereal solution was washed with water, dried over anhydrous sodium sulphate, and evaporated to give a dark red gum (185 mg.) which yielded crystals on trituration with methanol. The product was crystallised several times from chloroform-methanol to give 4-methylcholesta-3,5-dien-3-yl acetate as lustrous plates, m.p. 113-115°, $[\alpha]_D$ - 101° (c, 2.1). The compound was identical (m.p., mixed m.p., and infrared absorption) with that obtained by dehydration of the epimeric diol monoacetates (page 119).

Conversion of 4β,6α-Dimethylcholest-3-one into 4α,6α-Dimethylcholest-3-one. - A solution of 4β,6α-dimethylcholest-3-one (50 mg.) in 1% methanolic potassium hydroxide (20 ml.) was heated under reflux for 5 hr. The product (45 mg.) isolated by means of ether in the usual way was dissolved in light petroleum and absorbed on a column of alumina (3 g.). Elution with the same solvent gave a fraction (40 mg.) which crystallised from methanol to yield needles, m.p. 103-104°, $[\alpha]_D$ - 8° (c, 1.3), identical (m.p., mixed m.p., and infrared absorption) with 4α,6α-dimethylcholest-3-one.

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