STUDIES OF 1-CYANOSTEROIDS

ALASDAIR T. GLEN

Glen

SUMMARY

STUDIES OF 1-CYANOSTEROIDS

Cyanation of 5α-cholest-1-en-3-one with potassium cyanide and ammonium chloride in aqueous dimethylformamide gives 1α-cyano-5α-cholestan-3-one which reacts with bromine in acetic acid to give mainly 2α-bromo-1α-cyano-5α-cholestan-3-one. Dehydrobromination of the bromoketone with lithium chloride in dimethylformamide gives 1-cyano-5α-cholest-1-en-3-one and dehydrobromination of the mother liquors by the same method gives the same unsaturated ketone together with 1-cyano-cholesta-1,4-dien-3-one and 1α-cyano-cholest-4-en-3-one.

Hydrogenation of 1-cyano-5α-cholest-1-en-3-one in the presence of palladised charcoal gives 1β-cyano-5α-cholestan-3-one. Treatment of the 1β-cyano-ketone with bromine in acetic acid gives 2α-bromo-1β-cyano-5α-cholestan-3-one which, when dehydrobrominated by lithium chloride in dimethylformamide also yields 1-cyano-5α-cholest-1-en-3-one.

Both the 1α- and 1β-cyano-ketones are unaffected by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in benzene and in

dioxan containing a trace of hydrogen chloride.

Basic hydrolysis of 1α-cyano-5α-cholestan-3-one and 1β-cyano-5α-cholestan-3-one gives 3α-amino-1α-carboxy-3β-hydroxy-5α-cholestane lactam which forms an O-methyl derivative on treatment with methanolic hydrogen chloride. The epimerisation of the 1β-cyano-ketone to the 1α-cyano-ketone by the action of alkali has been demonstrated.

Treatment of 1-cyano-5α-cholest-1-en-3-one with alkali gives 5α-cholestan-1,3-dione.

Treatment of 1α-cyano-5α-cholestan-3-one with ethylene glycol and boron trifluoride etherate gives 1α-cyano-3-ethylenedioxy-5α-cholestane from which the ketone may be readily regenerated.

Reduction of 1α-cyano-5α-cholestan-3-one with sodium borohydride or aluminium iso-propoxide in iso-propyl alcohol gives 1α-cyano-5α-cholestan-3α-ol, from which the 3α-acetate was prepared, while reduction with sodium borohydride in methanol or lithium aluminium tri-tert-butoxyhydride in tetrahydrofuran gives an epimeric mixture of 1α-cyano-5α-cholestan-3-ols.

Reduction of 1α-cyano-5α-cholestan-3-one or 1α-cyano-5α-cholestan-3α-ol with lithium aluminium hydride in ether yields 1α-aminomethyl-5α-cholestan-3α-ol which forms the 1α,3α-diacetate. The diacetate is hydrolysed by aqueous methanolic sodium carbonate to the 1α-acetate. Both the amine and the monoacetate show evidence of intramolecular hydrogen bonding.

Rearrangement of 1α-cyano-5α-cholestan-3α-ol with hydrogen chloride in ether gives the 1α,3α-imino-ether, isolated as its hydrochloride which shows evidence of lactone formation on treatment with dilute hydrochloric acid.

The 3α-tosylate of 1α-cyano-5α-cholestan-3α-ol on hydrolysis with potassium acetate gives 1α-cyano-5α-cholest-2-ene.

Reduction of 1β-cyano-5α-cholestan-3-one with sodium borohydride in iso-propyl alcohol gives 1β-cyano-5α-cholestan-3ξ-ol which forms an acetate, while reduction with sodium borohydride in methanol gives a mixture of the epimeric 1β-cyano-5α-cholestan-3-ols.

Reduction of 1-cyano-5α-cholest-1-en-3-one with sodium borohydride in methanol gives 1-cyano-5α-cholest-1-ene-3ξ-ol.

Neither methyl magnesium iodide, methyl magnesium bromide, nor methyl lithium reacts with the 1-cyano group of 1α-cyano-3-ethylenedioxy-5α-cholestane, 1α-cyano-5α-cholestan-3α-ol or 1-cyano-5α-cholest-1-en-3 -ol, nor does the cyano group in these compounds undergo hydrolysis on treatment with alkali.

Reduction of 1α-cyano-5α-cholestan-3α-ol and 1α-cyano-3-ethylenedioxy-5α-cholestane with one molar equivalent of lithium aluminium hydride affords products which contain an imino group, while reduction of the latter compound with an excess of the same reagent gives a small yield of 3-ethylenedioxy-5α-cholestan-1α-al.

Attempted Stephen reduction of 1α-cyano-5α-cholestan-3α-ol gives 5α-cholestan-3α-ol 1α-amide as the only isolable product. The amide is oxidised by sodium dichromate to 3α-amino-1α-carboxy-3β-hydroxy-5α-cholestane lactam.

Formation of the insoluble acetic acid salt of the 1α-aminomethyl-5α-cholestan-3α-ol partially inhibits its deamination with nitrous acid in acetic acid but 1-methylene-5α-cholestan-3α-ol is detectable among the products formed. 3α-Amino-1α-carboxy-3β-hydroxy-5α-cholestane lactam is unaffected by treatment with nitrous acid.

The tosylhydrazone of 1α-cyano-5α-cholestan-3-one is reduced by sodium borohydride in dioxan to 1α-cyano-5α-cholestane and by sodium borohydride in methanol to give (in poor yield) a mixture of 1α-cyano-5α-cholestane and 1α-cyano-5α-cholest-2-ene.

1α-Cyano-5α-cholestane is unaffected by treatment with alkali or methyl magnesium iodide. Treatment of 1α-cyano-5α-cholest-2-ene with alkali results in partial isomerisation to 1-cyano-5α-cholest-1-ene.

The optical rotatory dispersion curves of 1α-cyano-5α-cholestan-3-one and 1β-cyano-5α-cholestan-3-one and their 2α-bromo derivatives show no anomalies.

The effect of a 1-cyano group on the chemical shift of the $C_{(18)}$ and $C_{(19)}$ methyl groups of a number of 5α-cholestane derivatives has been calculated.

STUDIES OF 1-CYANOSTEROIDS

A THESIS

submitted to

THE UNIVERSITY OF GLASGOW

in fulfilment of the

requirements for the

DEGREE OF DOCTOR OF PHILOSOPHY

by

ALASDAIR THOMAS GLEN

Department of Pure and Applied Chemistry,

The University of Strathclyde,

formerly,

The Royal College of Science and Technology, Glasgow.

October, 1964.

ACKNOWLEDGMENTS

The author wishes to express his sincere gratitude to Dr. J. McLean for his guidance and encouragement throughout this work. He also wishes to thank Dr. P. Bladon for nuclear magnetic resonance spectra and assistance in the interpretation of spectroscopic data, Dr. D. Savage for helpful discussion, Professor P.L. Pauson for providing laboratory facilities, and members of the technical staff of the Department of Pure and Applied Chemistry. Finally, he expresses his thanks to Drs. K. Kuriyama and K. Tori, Shionogi Research Laboratory, Osaka, Japan for measurement of optical rotatory dispersion curves.

SUMMARY

STUDIES OF 1-CYANOSTEROIDS

Cyanation of 5α-cholest-1-en-3-one with potassium cyanide and ammonium chloride in aqueous dimethylformamide gives 1α-cyano-5α-cholestan-3-one which reacts with bromine in acetic acid to give mainly 2α-bromo-1α-cyano-5α-cholestan-3-one. Dehydrobromination of the bromoketone with lithium chloride in dimethylformamide gives 1-cyano-5α-cholest-1-en-3-one and dehydrobromination of the mother liquors by the same method gives the same unsaturated ketone together with 1-cyano-cholesta-1,4-dien-3-one and 1α-cyano-cholest-4-en-3-one.

Hydrogenation of 1-cyano-5α-cholest-1-en-3-one in the presence of palladised charcoal gives 1β-cyano-5α-cholestan-3-one. Treatment of the 1β-cyano-ketone with bromine in acetic acid gives 2α-bromo-1β-cyano-5α-cholestan-3-one which, when dehydro-brominated by lithium chloride in dimethylformamide also yields 1-cyano-5α-cholest-1-en-3-one.

Both the 1α- and 1β-cyano-ketones are unaffected by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in benzene and in dioxan containing a trace of hydrogen chloride.

Basic hydrolysis of 1α-cyano-5α-cholestan-3-one and 1β-cyano-5α-cholestan-3-one gives 3α-amino-1α-carboxy-3β-hydroxy-5α-

cholestano lactam which forms an O-methyl derivative on treatment with methanolic hydrogen chloride. The epimerisation of the 1β-cyano-ketone to the 1α-cyano-ketone by the action of alkali has been demonstrated.

Treatment of 1-cyano-5α-cholest-1-en-3-one with alkali gives 5α-cholestan-1,3-dione.

Treatment of 1α-cyano-5α-cholestano-3-one with ethylene glycol and boron trifluoride etherate gives 1α-cyano-3-ethylenedioxy-5α-cholestane from which the ketone may be readily regenerated.

Reduction of 1α-cyano-5α-cholestan-3-one with sodium boro-hydride or aluminium iso-propoxide in iso-propyl alcohol gives 1α-cyano-5α-cholestan-3α-ol, from which the 3α-acetate was prepared, while reduction with sodium borohydride in methanol or lithium aluminium tri-tert-butoxyhydride in tetrahydrofuran gives an epimeric mixture of 1α-cyano-5α-cholestan-3-ols.

Reduction of 1α-cyano-5α-cholestan-3-one or 1α-cyano-5α-cholestan-3α-ol with lithium aluminium hydride in ether yields 1α-aminomethyl-5α-cholestan-3α-ol which forms the 1α,3α-diacetate. The diacetate is hydrolysed by aqueous methanolic sodium carbonate to the 1α-acetate. Both the amino and the monoacetate show evidence of intramolecular hydrogen bonding.

Rearrangement of 1α-cyano-5α-cholestan-3α-ol with hydrogen chloride in ether gives the 1α,3α-imino-ether, isolated as its hydrochloride which shows evidence of lactone formation on treatment with dilute hydrochloric acid.

The 3α-tosylate of 1α-cyano-5α-cholestan-3α-ol on hydrolysis with potassium acetate gives 1α-cyano-5α-cholest-2-ene.

Reduction of 1β-cyano-5α-cholestan-3-one with sodium boro-hydride in iso-propyl alcohol gives 1β-cyano-5α-cholestan-3ξ-ol which forms an acetate, while reduction with sodium borohydride in methanol gives a mixture of the epimeric 1β-cyano-5α-cholestan-3-ols.

Reduction of 1-cyano-5α-cholest-1-en-3-one with sodium borohydride in methanol gives 1-cyano-5α-cholest-1-en-3ξ-ol.

Neither methyl magnesium iodide, methyl magnesium bromide, nor methyl lithium reacts with the 1-cyano group of 1α-cyano-3-ethylenedioxy-5α-cholestane, 1α-cyano-5α-cholestane-3α-ol or 1-cyano-5α-cholest-1-en-3ξ-ol, nor does the cyano group in these compounds undergo hydrolysis on treatment with alkali.

Reduction of 1α-cyano-5α-cholestan-3α-ol and 1α-cyano-3-ethylenedioxy-5α-cholestane with one molar equivalent of lithium aluminium hydride affords products which contain an imino group, while reduction of the latter compound with an excess of the same reagent gives a small yield of 3-ethylene-dioxy-5α-cholestan-1α-al.

Attempted Stephen reduction of 1α-cyano-5α-cholestan-3α-ol gives 5α-cholestan-3α-ol 1α-amide as the only isolable product. The amide is oxidised by sodium dichromate to 3α-amino-1α-carboxy-3β-hydroxy-5α-cholestane lactam.

Formation of the insoluble acetic acid salt of 1α-aminomethyl-5α-cholestan-3α-ol partially inhibits its deamination with nitrous acid in acetic acid but 1-methylene-5α-cholestan-3α-ol is detectable among the products formed. 3α-Amino-1α-carboxy-3β-hydroxy-5α-cholestane lactam is unaffected by treatment with nitrous acid.

The tosylhydrazone of 1α-cyano-5α-cholestan-3-one is reduced by sodium borohydride in dioxan to 1α-cyano-5α-cholestane and by sodium borohydride in methanol to give (in poor yield) a mixture of 1α-cyano-5α-cholestane and 1α-cyano-5α-cholest-2-ene.

1α-Cyano-5α-cholestane is unaffected by treatment with alkali or methyl magnesium iodide. Treatment of 1α-cyano-5α-cholest-2-ene with alkali results in partial isomerisation to 1-cyano-5α-cholest-1-ene.

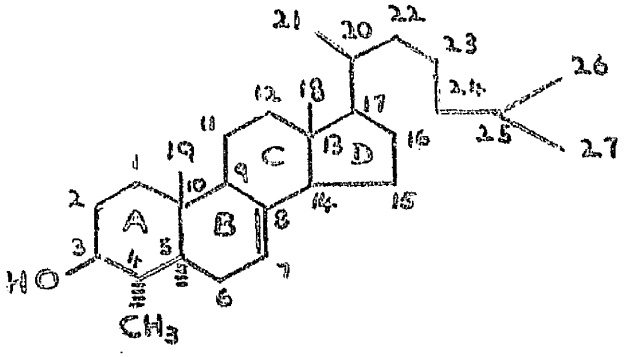
The optical rotatory dispersion curves of 1α-cyano-5α-cholestan-3-one and 1β-cyano-5α-cholestan-3-one and their 2α-bromo derivatives show no anomalies.

The effect of a 1-cyano group on the chemical shift of the $C_{(18)}$ and $C_{(19)}$ methyl groups of a number of 5α-cholestane derivatives has been calculated.

CONTENTS

INTRODUCTION

The only functional group present in ring A of most naturally occurring steroids is an oxygen function at $C_{(3)}$, but a few steroids have been isolated which are also methylated in ring A. An example is lophenol[1] (I) which was isolated from the giant cactus Lophocereus schotti, and was shown to be 4α-methyl-5α-cholest-7-en-3β-ol.



(I)

Synthetic substituted steroids have been prepared for several reasons. The relatively rigid cyclohexane rings of the steroid nucleus have proved very suitable for the confirmation and extension of the ideas on the relationship of conformation to chemical activity put forward by Barton[2]. Two other main reasons concern the biological activity of steroid hormones. First is the problem of how a steroid reacts at a receptor site, whether it is the α- or β- face that is active. This

led to the synthesis and biological testing of steroids with bulky substituents which would interfere with absorption on a particular face, and the conclusion reached was that, in general, enzymic action occurred at the α- face of the steroid molecule[3]. Secondly, as steroid hormones became extensively used in medicine, investigations were conducted into the effects of modification of the molecule by the introduction of other functional groups. Until 1953 there was a widely held view that modification of the adrenal hormones, cortisone and hydrocortisone, always led to a decrease of activity, but in that year Fried and Salbo[4] reported that 9α-chloro and 9α-fluoro groups enhanced anti-inflammatory activity, and this finding stimulated considerable interest in modified steroid hormones. Since then, such substituents as carbonyl, hydroxyl, methyl, ethynyl, nitro, and cyano groups have been introduced with variable biological effect[3,5], and attempts are still being made to improve existing syntheses, and to prepare substituted steroids with increased activity but lacking unpleasant side effects.

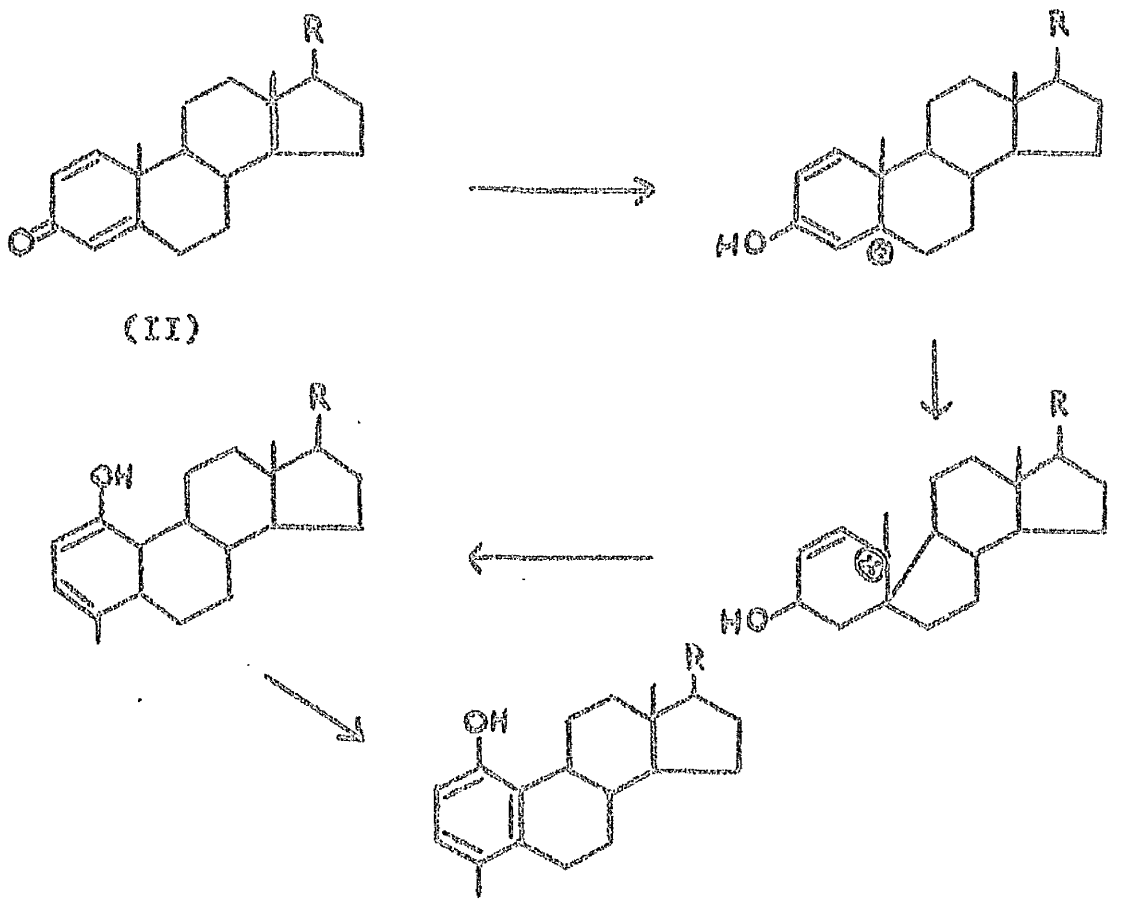
Some of the methods used to introduce carbon containing substituents into ring A are outlined in succeeding pages.

Synthetic Ring A Substituted Steroids

Substitution at $C_{(1)}$

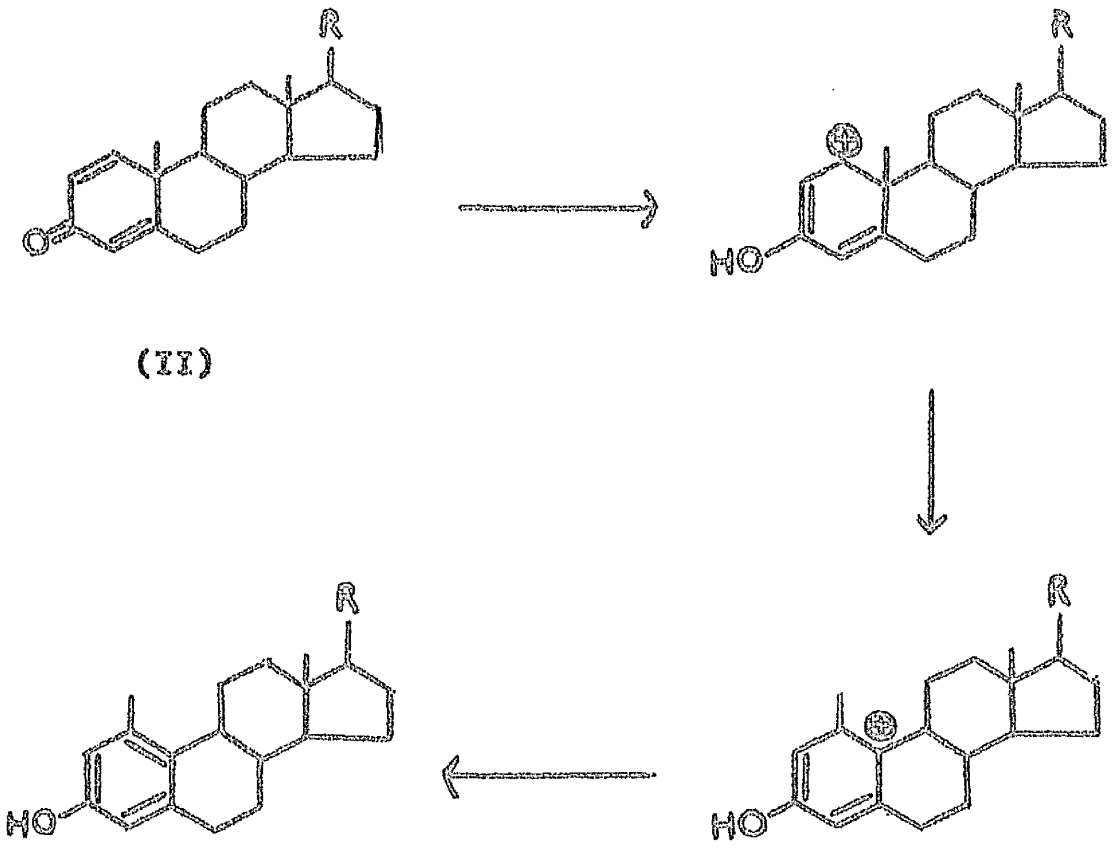
The first 1-methyl steroid was prepared at the expense of the methyl group at $C_{(10)}$ by the dienone - phenol rearrangement in which 1,4-dien-3-ones, such as (II) on treatment with acid, rearrange to 4-methyl-19-norsteroids[6,7] (III) (scheme A) or 1-methyl-19-norsteroids[8] (IV) (scheme B), both with ring A aromatic, according to the reaction conditions.

A



(II)

(III)

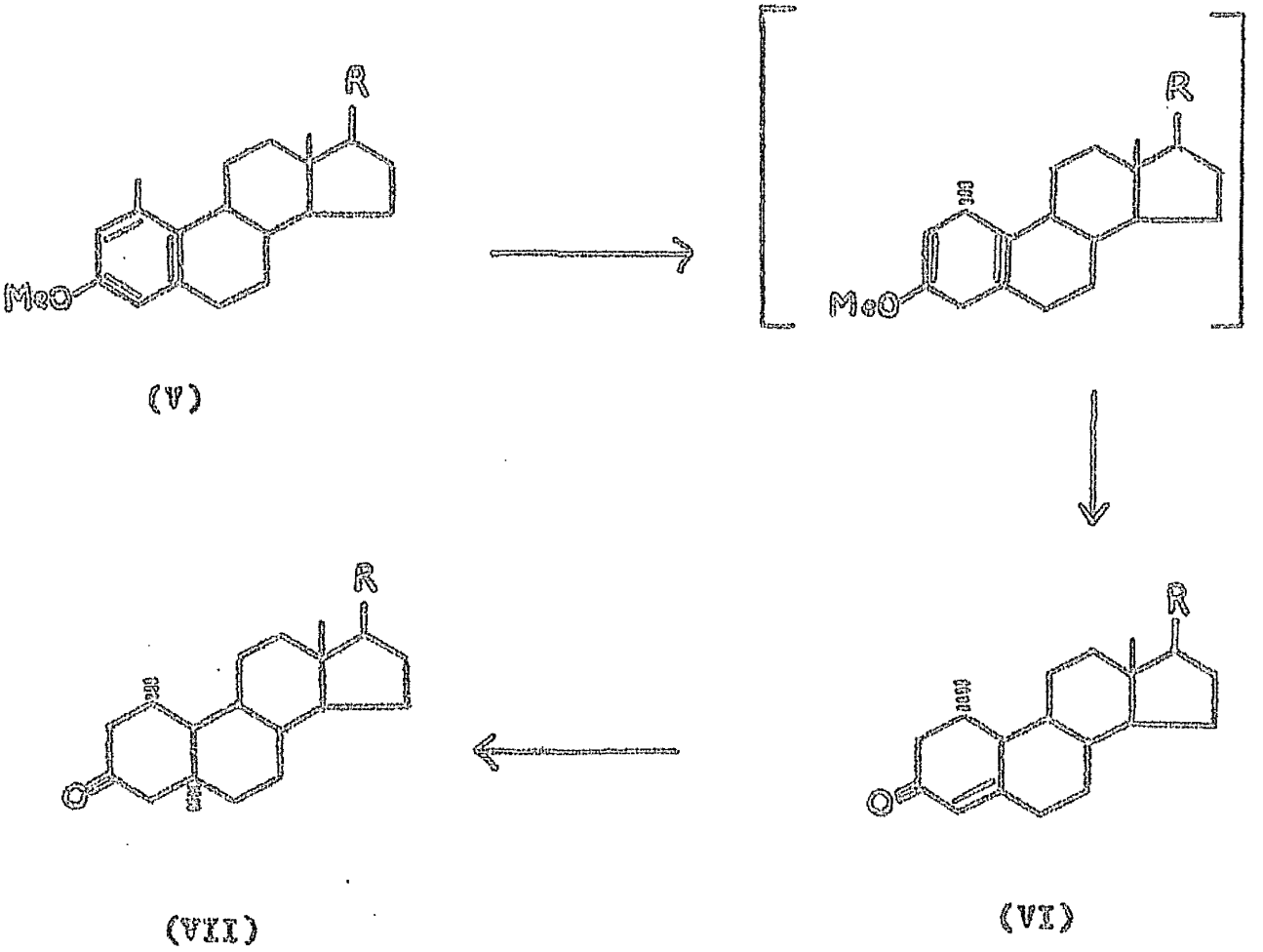


(II)

(IV)

A 1-methyl-oestrone derivative (IV) prepared by this method can readily be converted[9,10] into a 1-methyl-Δ^4-3-ketone (VI) by methylation of the phenol (IV) to give (V), followed by Birch reduction, and hydrolysis. Further reduction of the hydrolysis product (VI) with lithium and liquid ammonia gives

the 5α-steroid (VII) showing a trans junction between rings A and B[11]. Djerassi and his co-workers[12] have assigned the α-configuration to the $C_{(1)}$ methyl group on the basis of optical rotatory dispersion data and the unfavourable interaction of a 1β-methyl group with the 11α-hydrogen.



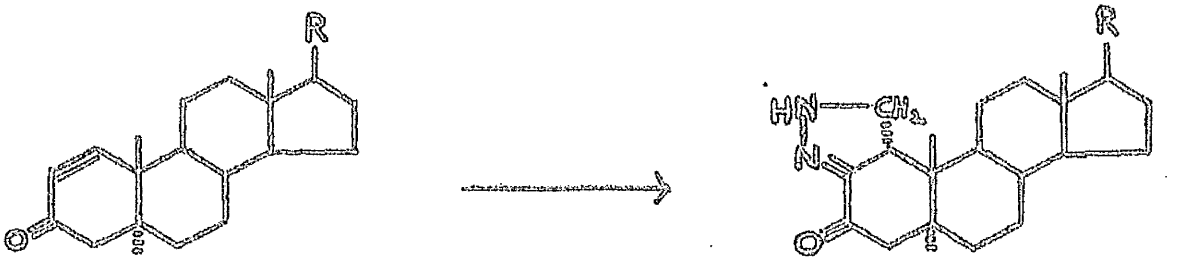
(V)

(VII)

(VI)

German workers[13,14,15] have treated steroidal Δ^1-3-ketones with diazomethane to form pyrazolino-steroids which may be cleaved

by several methods. Popper[13] found that 5α-androst-1-en-17β-ol-3-one (VIIIa) and 5α-cholest-1-en-3-one (VIIIb) formed $\Delta^{2'}$ pyrazoline derivatives (IXa) and (IXb) respectively, each of which on pyrolysis gave a mixture of the 1,2-methylene steroid and the Δ^{1}-1-methyl steroid (Xa) and (Xla), and (Xb) and (Xlb) respectively. When the pyrazoline derivative (IXb) is heated in quinoline to just below its melting point[14] it gives exclusively the 1-methyl derivative (Xlb) .



(VIII)

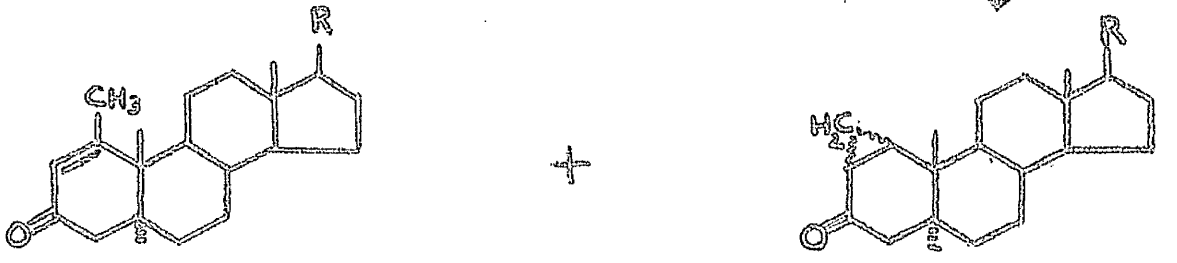
a, R = -OH

b, R = $-C_8H_{17}$

(IX)

a, R = -OH

b, R = $-C_8H_{17}$



(XI)

a, R = -OH

b, R = $-C_8H_{17}$

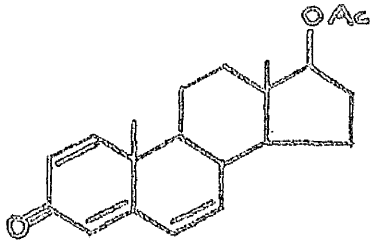
(X)

a, R = -OH

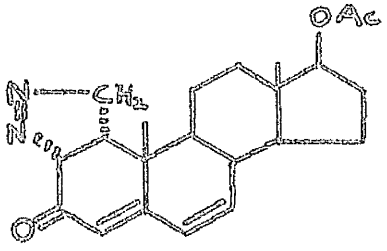
b, R = $-C_8H_{17}$

Wiechert and Kaspar[15] treated androsta-1,4,6-trion-17β-ol-3-one 17β-

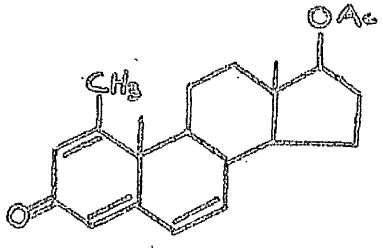
acetate (XII) with diazomethane and obtained the $\Delta^{1'}$-pyrazoline derivative (XIII). Examination of the infrared spectrum showed the presence of a $\Delta^{1'}$-pyrazoline as opposed to the $\Delta^{2'}$ compounds which Popper[13] had obtained. Pyrolysis under high vacuum, treatment with perchloric acid in acetone, or treatment with silica gel in carbon tetrachloride yielded mixtures of the 1α, 2α-methylene steroid (XIV) and the 1-methyl substituted derivative (XV)



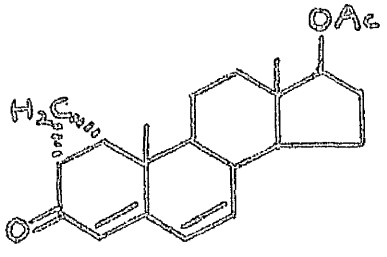
(XII)



(XIII)



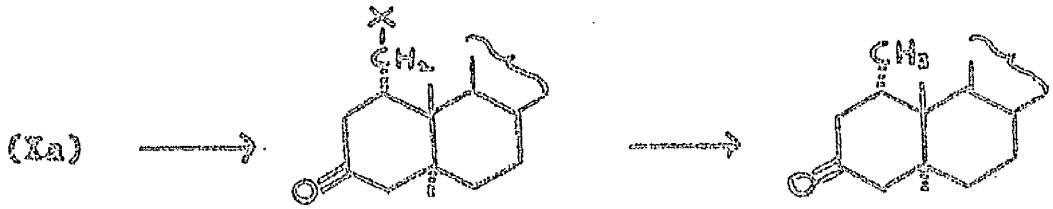
(XV)



(XIV)

1-Halogenomethyl steroids (XVI) were obtained by Wiechert[16] by treatment of the 1α,2α-methylene steroid (Xa) with potassium iodide in formic acid, or hydrogen chloride in methylene chloride,

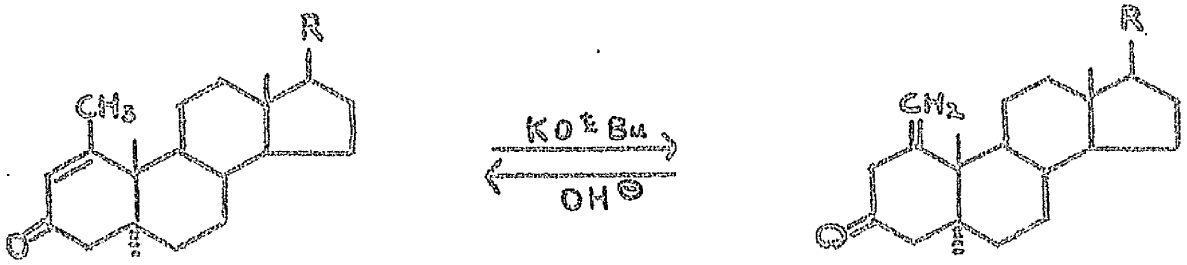
and were subsequently reduced by Raney nickel to the 1α-methyl-3-oxo-steroid (XVII).



(XVI) (XVII)

X = I or Cl

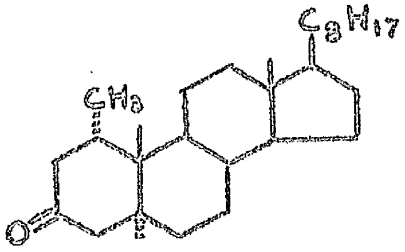
1-Methylene-3-oxo-steroids (XVIII) may be prepared by deconjugation of the corresponding 1-methyl-Δ^1-3-oxo-steroids with potassium tert-butoxide and ammonium chloride[17]. Reconjugation is effected with aqueous alkali.



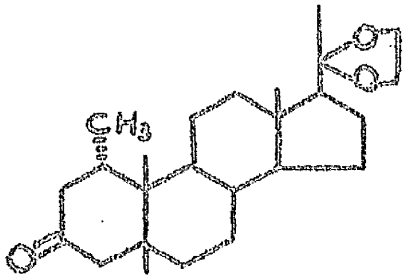
(XI) (XVIII)

Grignard reagents, in the presence of cuprous halides[18], add to the double bond of αβ-unsaturated ketones. Mori[19] and

Wachter[20] have used this reaction to prepare 1α-methyl-5α-cholestan-3-one (XIX) and the 5β-pregnane derivative (XX) respectively.

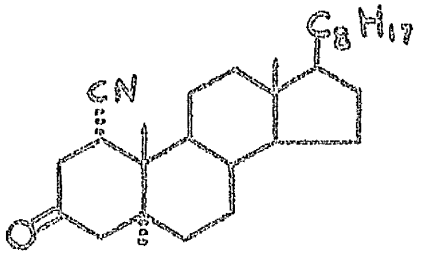


(XIX)



(XX)

Recently a cyano group has been introduced at $C_{(1)}$ by Bowers and Ringold[21], Julia, Lenares, and Simon[22], and ourselves[23] by means of a Michael type[24] reaction on the Δ^1-3-oxo-system and 1α-cyano-5α-cholestan-3-one (XXI) has been prepared in this way from 5α-cholest-1-en-3-one (VIIIb).[22,23]

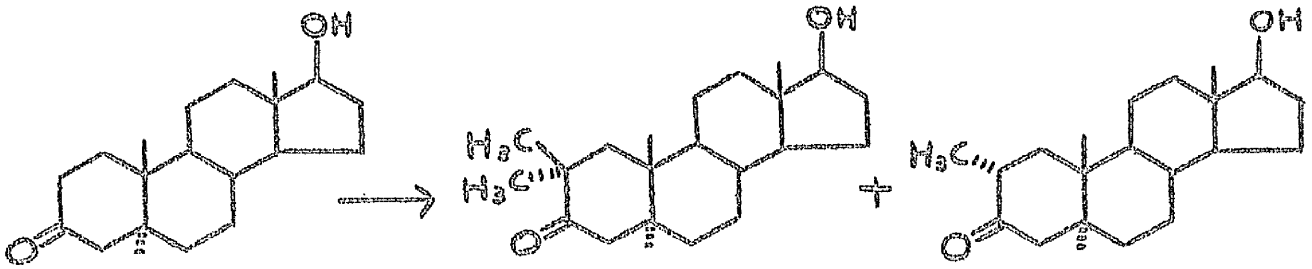


(XXI)

The reactions of 1-cyano-steroids will be discussed in a later section of this thesis.

Substitution at $C_{(2)}$

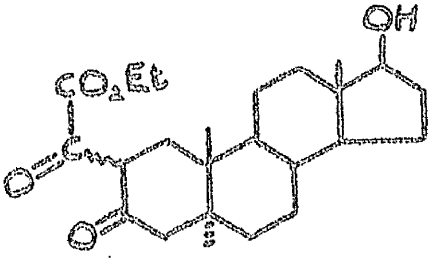
The presence of a carbonyl group at $C_{(3)}$ has in many cases facilitated the introduction of a carbon atom at $C_{(2)}$. 3-Oxo-steroids of the 5α-series enolise to give Δ^2-enols, thus reaction with methyl iodide under basic conditions yields the 2-methyl derivative. The reaction, however, does not stop at the monomethylated stage and the 2,2-dimethyl steroid is usually the major product. Methylation of 5α-androstan-17β-ol-3-one (XXII) gives 50% of the 2,2-dimethyl derivative (XXIII) and 10% of the 2α-methyl derivative (XXIV)[25].



(XXII) (XXIII) (XXIV)

In order to prepare 2-monomethyl steroids in good yield a procedure involving preliminary substitution with an easily

removable group was adopted. Ringold and Rosenkranz[25] formed a 2-ethoxalyl derivative (XXV) by treatment with ethyl oxalate in the presence of sodium hydride. After methylation with methyl iodide in the usual way, the ethoxalyl group was removed with sodium ethoxide to furnish 2α-methyl-5α-androstan-17β-ol-3-one (XXIV).

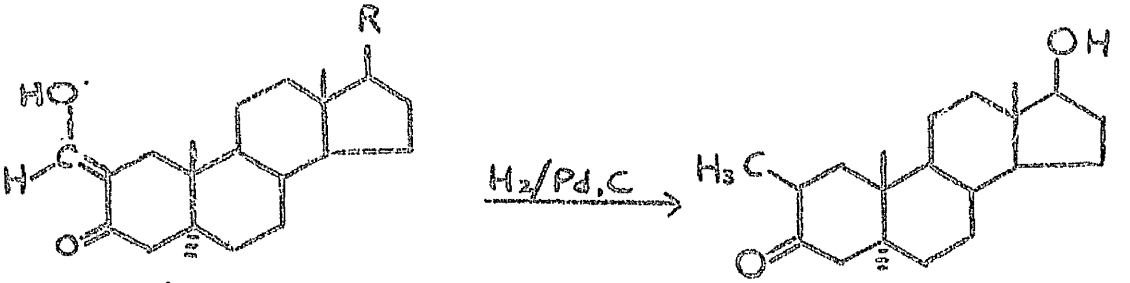


(XXV)

In 1938, 2-hydroxymethylene-5α-cholestan-3-one (XXVIa) was first prepared by Stiller and Rosenheim[26] who condensed 5α-cholestan-3-one with amyl formate in the presence of sodium. This type of derivative was later used as an intermediate in two routes to 2-methyl steroids. The hydroxymethylene group may be used in the same manner as an ethoxalyl group to prevent dimethylation[25], or it may itself be hydrogenated to a methyl group. 5α-Androstan-17β-ol-3-one (XXII) forms a 2-hydroxymethylene derivative (XXVIb) with ethyl formate and sodium hydride, hydrogenation of which, in methanol[27], in the presence of palladised charcoal gives

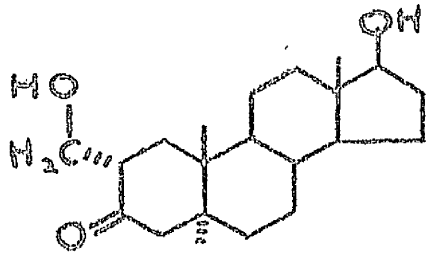
2β-methyl-5α-androstan-17β-ol-3-one (XXVII). This is the unstable axial (2β) epimer and contact with alkaline alumina converts it into the stable equatorial (2α) epimer (XXIV). Knox and Velarde[28] found that variation of the hydrogenation conditions led to only partial reduction of the hydroxymethylene group. Hydrogenation of (XXVIb), also in the presence of palladised charcoal, but in aqueous methanol or in tetrahydrofuran, yields the 2-hydroxymethyl derivative (XXVIII) and only a small quantity of the 2-methyl derivative (XXVII).

Further unsaturation may be introduced into the 2-hydroxymethylene-3-oxo system (XXVIa) and (XXVIb), thus causing it to exist in the keto form, by dehydrogenation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone to give the 1,2-dehydro derivative (XXIX)[29].

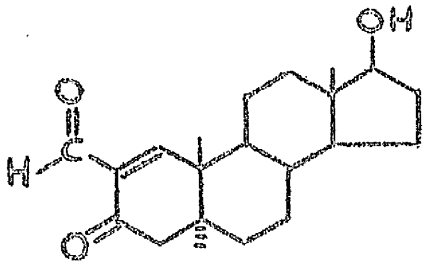


(XXVI)
a, R = $-C_8H_{17}$
b, R = -OH

(XXVII)

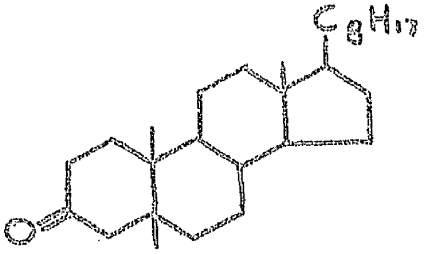


(XXVIII)

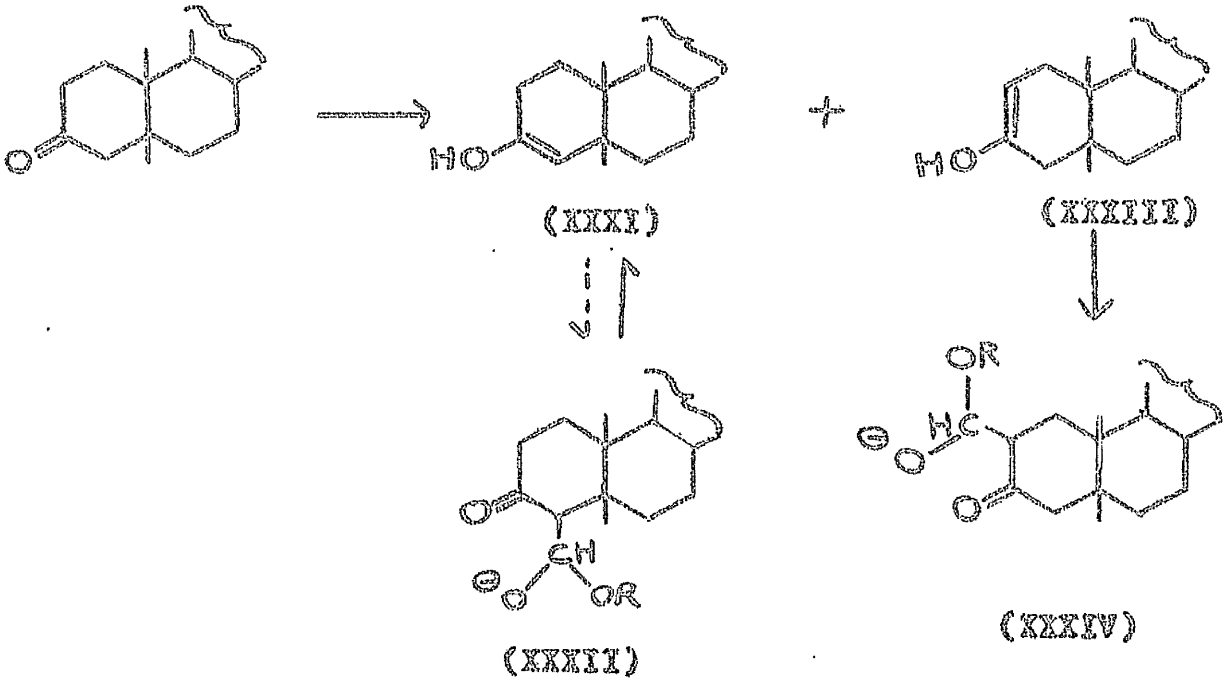


(XXIX)

It was found that 5β-stigmast-22-en-3-one, in which the A/B ring junction is cis, gives the 4-substituted derivative on formylation[30]. The reaction was reinvestigated[31] and it was found that alkylation at $C_{(4)}$ was not a general rule in the case of steroids having A/B cis-fused rings, for example, 5β-cholestan-3-one (XXX) gives the 2-formyl derivative, while 5β-stigmast-22-en-3-one in fact gives a mixture of 2- and 4-formyl derivatives in the ratio 1:1. Formylation at $C_{(2)}$ was not expected since A/B cis 3-ketones are known to enolise to give mainly the Δ^3-enols. The intermediate anion (XXXII) is considered to be sterically hindered by the 6α, 7α, and 9α protons so that equilibrium favours the Δ^3-enol (XXXI) and the small amount of Δ^2-enol (XXXIII) present is then attacked. The 2-substituted intermediate anion (XXXIV) is more stable and the final product is the 2-formyl derivative.

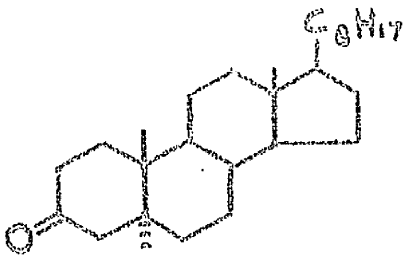


(XXX)

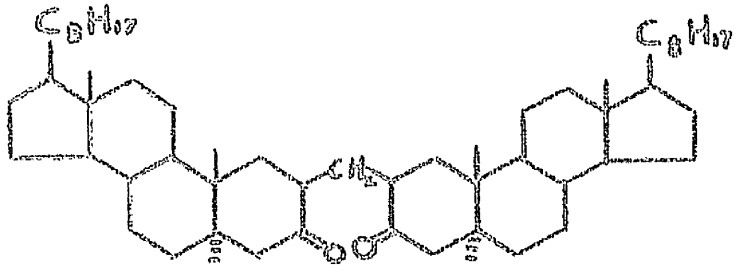


(XXXI) (XXXIII) (XXXII) (XXXIV)

Wald and Taurins[32] obtained an interesting result when they attempted to formylate 5α-cholestan-3-one (XXXV) with para-formaldehyde in the presence of morpholine or piperidine hydrochlorides; a dimer which was shown to have structure (XXXVI) was obtained.

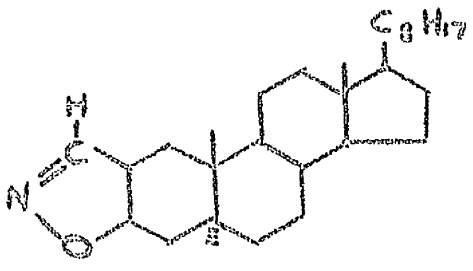


(XXXV)

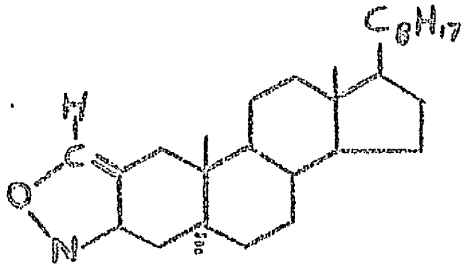


(XXXVI)

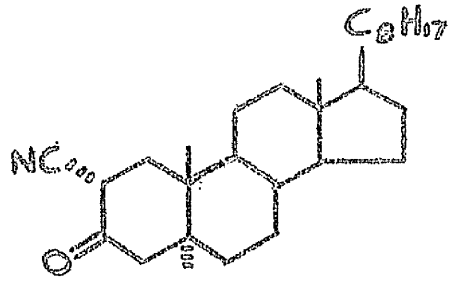
Treatment of 2-hydroxymethylene-5α-cholestan-3-one (XXVIa) with hydroxylamine hydrochloride gives a mixture of the two oxazoles (XXXVII) and (XXXVIII)[33]. The heterocyclic ring of (XXXVII) is cleaved with sodium ethoxide to give 2α-cyano-5α-cholestan-3-one (XXXIX).



(XXXVII)

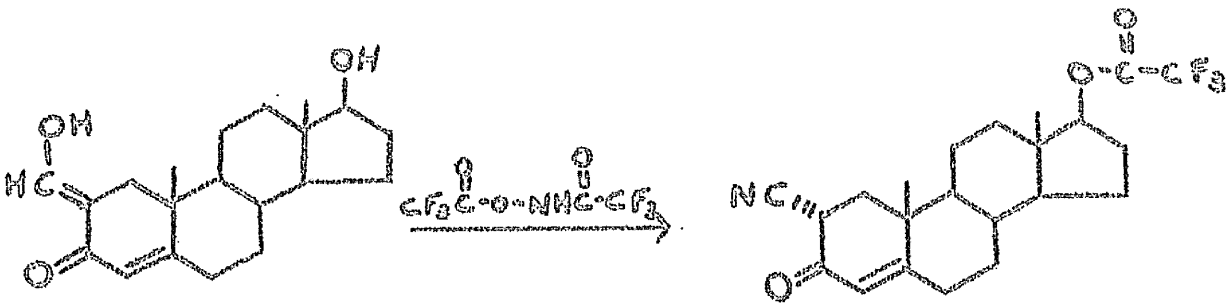


(XXXVIII)

(XXXVII) ⟶ 

(XXXIX)

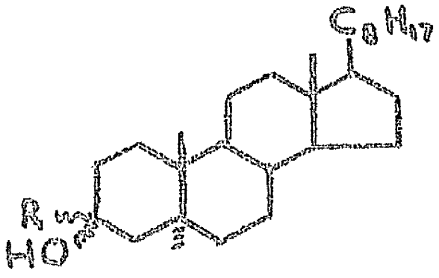
An alternative route to 2-cyanosteroids has been described by Kissman, Hoffman, and Weiss[34] who treated 2-hydroxymethylene-androst-4-en-17β-ol-3-one (XL) with O,N,-bis (trifluoroacetyl) hydroxylamine in benzene in the presence of pyridine and obtained the 2-cyano steroid (XLI) directly. Trifluoroacetylation of the 17β-hydroxyl group also occurs in the reaction.



(XL) (XLI)

Substitution at $C_{(3)}$

In ring A, the position which has been substituted with the greatest diversity of carbon containing functional groups is $C_{(3)}$. In 1937, Bolt and Backer[35] prepared a range of 3-alkyl-5α-cholestan-3-ols (XLIIa-g) in 55 - 75% yield by Grignard alkylation of 5α-cholestan-3-one(XXXV). Although these workers must have obtained epimeric products, they did not report the fact.



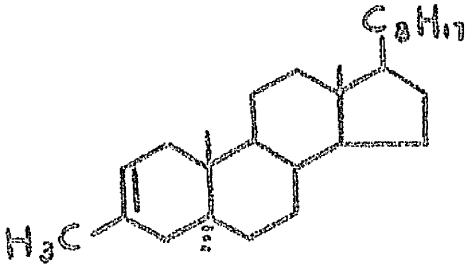
(XLII)

a, R = Me
b, R = i - Pr
c, R = t - Bu
d, R = cyclohexyl
e, R = phenyl
f, R = α-naphthyl
g, R = β-naphthyl

In the same year Farmer and Kon[36] prepared a mixture of 3-methyl-5α-cholestan-3-ols (XLIIa) by the same method and were able to obtain one pure epimer by fractional crystallisation.

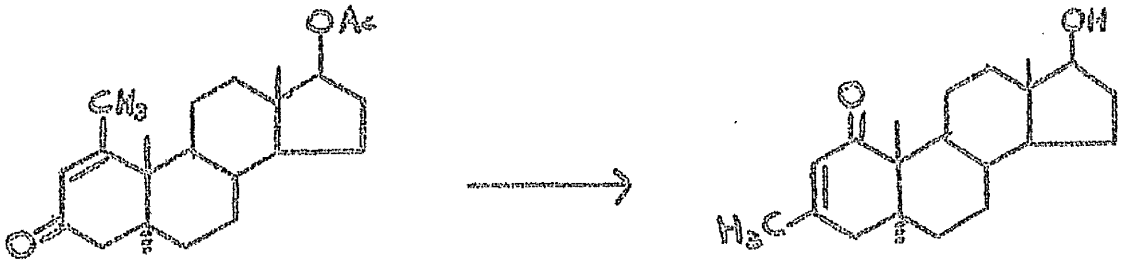
The preparation of a pure sample of each $C_{(3)}$ epimer was achieved by Kuwada and Miyasaka[37], by treatment of 5α-cholestan-3-one cyanohydrin with methyl Grignard reagent.

All the groups who had prepared 3-methyl-5α-cholestan-3-ols were able to dehydrate them to an olefin, but were uncertain of the position of the double bond. Barton[38] repeated the preparation of the epimeric 3-methyl derivatives, separated them and deduced the stereochemistry at $C_{(3)}$ by conformational analysis and showed the dehydration product to be 3-methyl-5α-cholest-2-ene (XLIII) by conversion to known compounds.



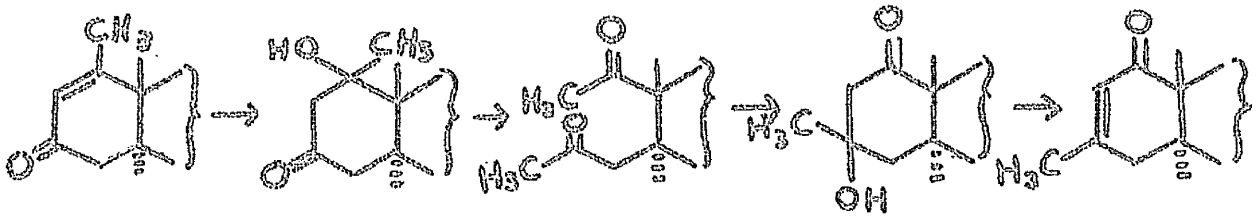
(XLIII)

The rearrangement of 1-methyl-Δ^1-3-oxo-steroids to the isomeric 3-methyl-Δ^2-1-oxo-steroid was observed by Bohlman and Rufer[39], who found that 1-methyl-5α-androst-1-en-17β-ol-3-one 17β-acetate (XLIV) on treatment with base yields 3-methyl-5α-androst-2-en-17β-ol-1-one (XLV).



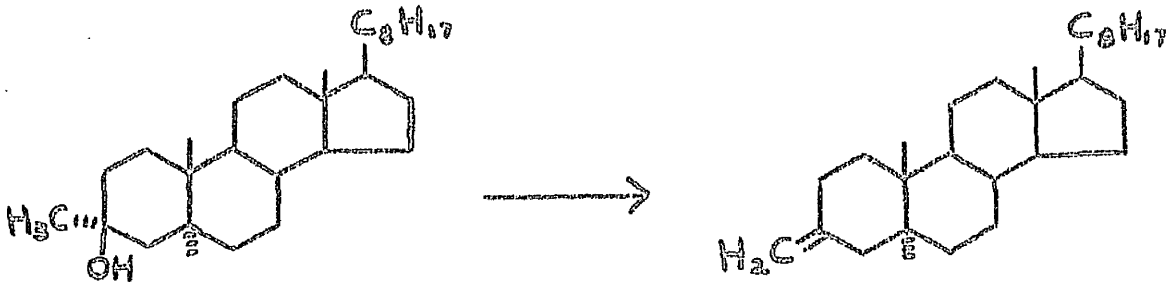
(XLIV) (XLV)

A retroaldol type of mechanism was proposed for the rearrangement, involving hydration of the double bond, opening of ring A, reformation of the ring by aldol condensation, and finally dehydration.



Dehydration of 3α-methyl-5α-cholestan-3β-ol (XLVI) with phosphorus oxychloride and pyridine[38] yields 3-methylene-5α-cholestane (XLVII) together with 3-methyl-5α-cholest-2-ene (XLII), the former of which was also synthesised by a Wittig olefin synthesis[40] on 5α-cholestan-3-one (XXXV). The Wittig reagent (XLVIII) is prepared from triphenyl methyl-

phosphonium bromide and phenyl lithium.



(XLVI) (XLVII)

$Ph_3CH_3P^+Br^- \xrightarrow{PhLi} Ph_3P = CH_2 + PhH + LiBr$

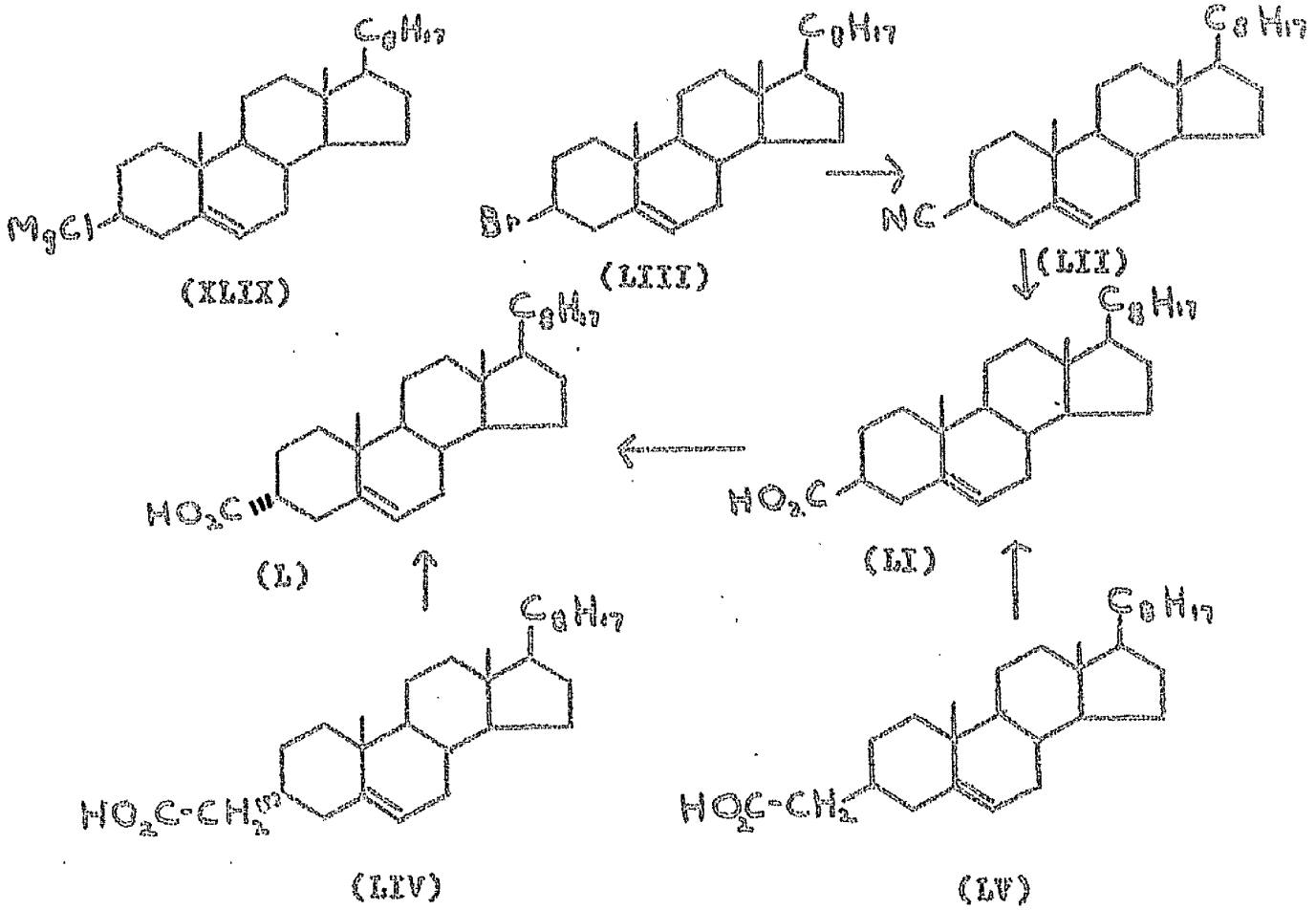
(XLVIII)

Dimethylsulphoxide metallated by sodium hydride has also been used to effect the same conversion[41].

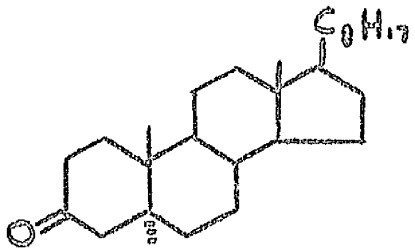
Marker and his co-workers[42], by treatment of cholesteryl 3β-magnesium chloride (XLIX) with carbon dioxide obtained what was thought to be a mixture of carboxylic acids epimeric at $C_{(3)}$, (L) and (LI). Baker and Squire[43], on repeating the reaction found that the product was a pure substance which was formulated as cholesteryl 3α-carboxylic acid (L), but Roberts, Sheppee and Stephenson[44] showed it to be cholesteryl 3β-carboxylic acid (LI). They synthesised it by the previous method and also by hydrolysis of 3β-cyano-cholest-5-one (LII) which was prepared from the corresponding 3β-bromo-steroid

(LIII). Epimerisation of the acid was effected by treatment with sodium in ethylene glycol, and the configuration at $C_{(3)}$ of each acid was decided by comparison with the products of Barbier-Wieland degradation of the known cholesteryl 3α-acetic acid (LIV) and cholesteryl 3β-acetic acid (LV) which had been unambiguously prepared by treatment of cholesteryl tosylates with diethyl malonate followed by partial decarboxylation.[45,46,47]

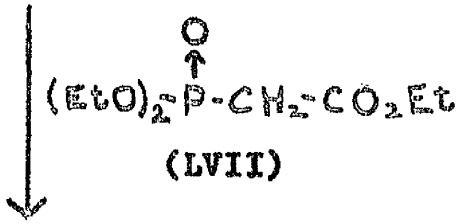
Shoppee's assignment of the 3β-configuration[44] to Marker's acid[42] was confirmed by Corey and Snoon.[48]



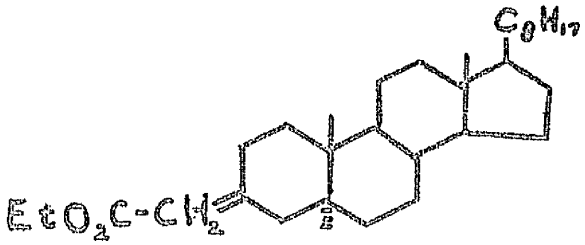
The unsaturated cholestanylidine acetic acid and the corresponding aldehyde (LVI) are also known. Bose[49] prepared the acid as its ethyl ester (LVIII) by a modified Wittig reaction. Triethylphosphonoacetate (LVII) and 5α-cholestan-3-one (XXXV) in the presence of sodium ethoxide afforded ethyl-5α-cholestanylidene-3-acetate (LVIII)



(XXXV)

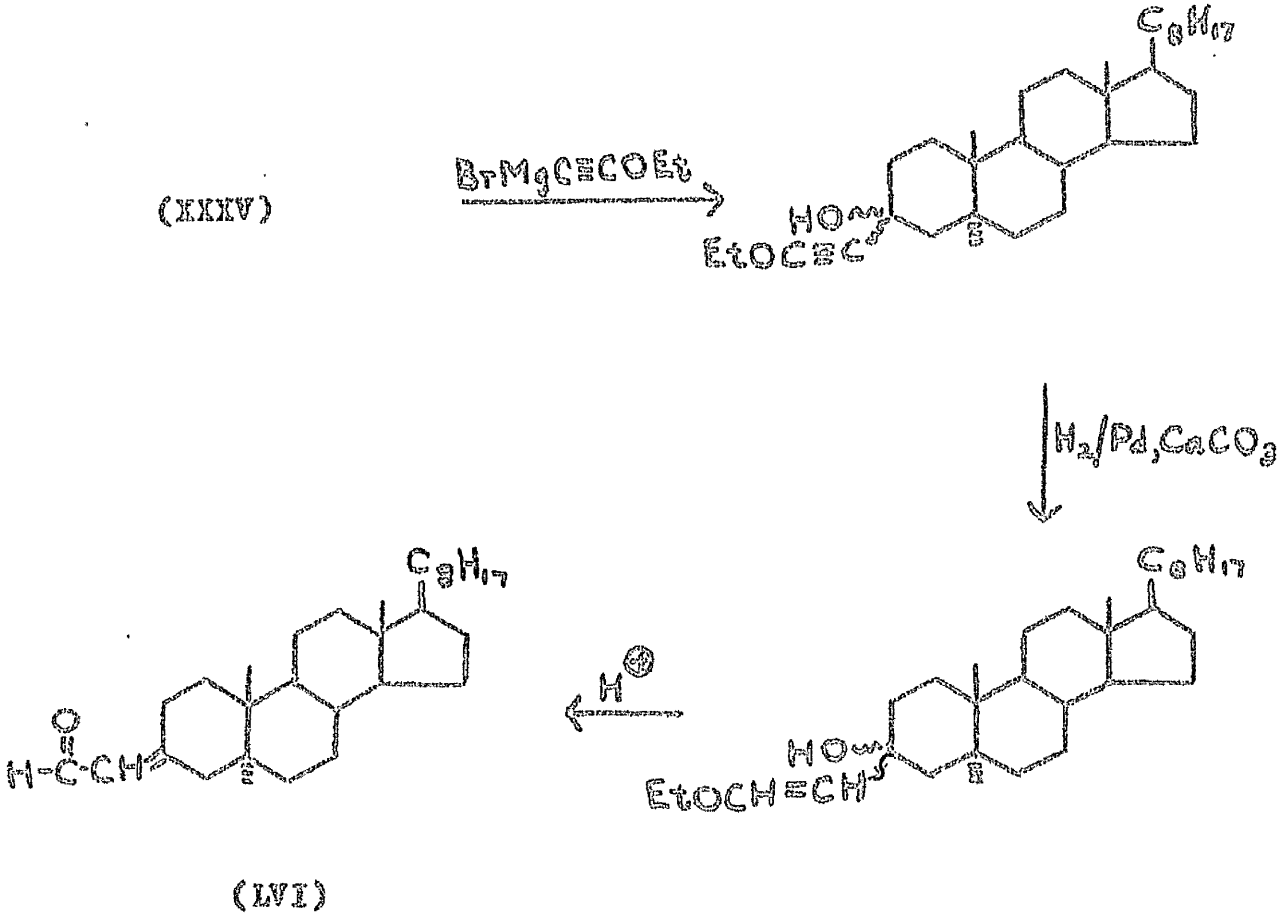


(LVII)



(LVIII)

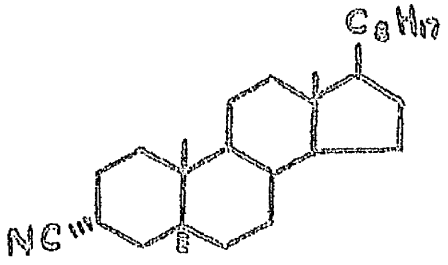
Milas and Priesing[50] obtained the aldehyde (LVI) from 5α-cholestan-3-one (XXXV) by an acetylenic synthesis.



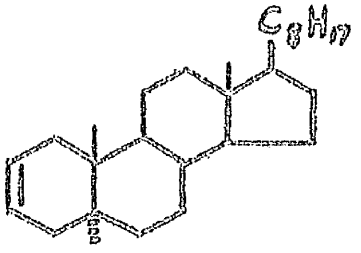
(LVI)

Shoppee[44] attempted to prepare 3-cyano-steroids from cholesteryl halides and potassium cyanide but the yields were of the order of 5%; however, by using an aprotic, dipolar, solvent = N-methy-pyrrolidine containing 5% of tert-butanol, Henbest and Jackson[51] obtained 3α-cyano-5α-cholestane (LIX) in 80% yield by treatment

of 5α-cholestanyl-3β-tosylate with calcium cyanide. As minor product (8%) 5α-cholest-2-one (LX) was formed, whereas this olefin was the main product obtained by Shoppee[44]. The epimeric 3α-tosylate with calcium cyanide gave 53% of olefin and 40% of the 3β-nitrile.



(LIX)

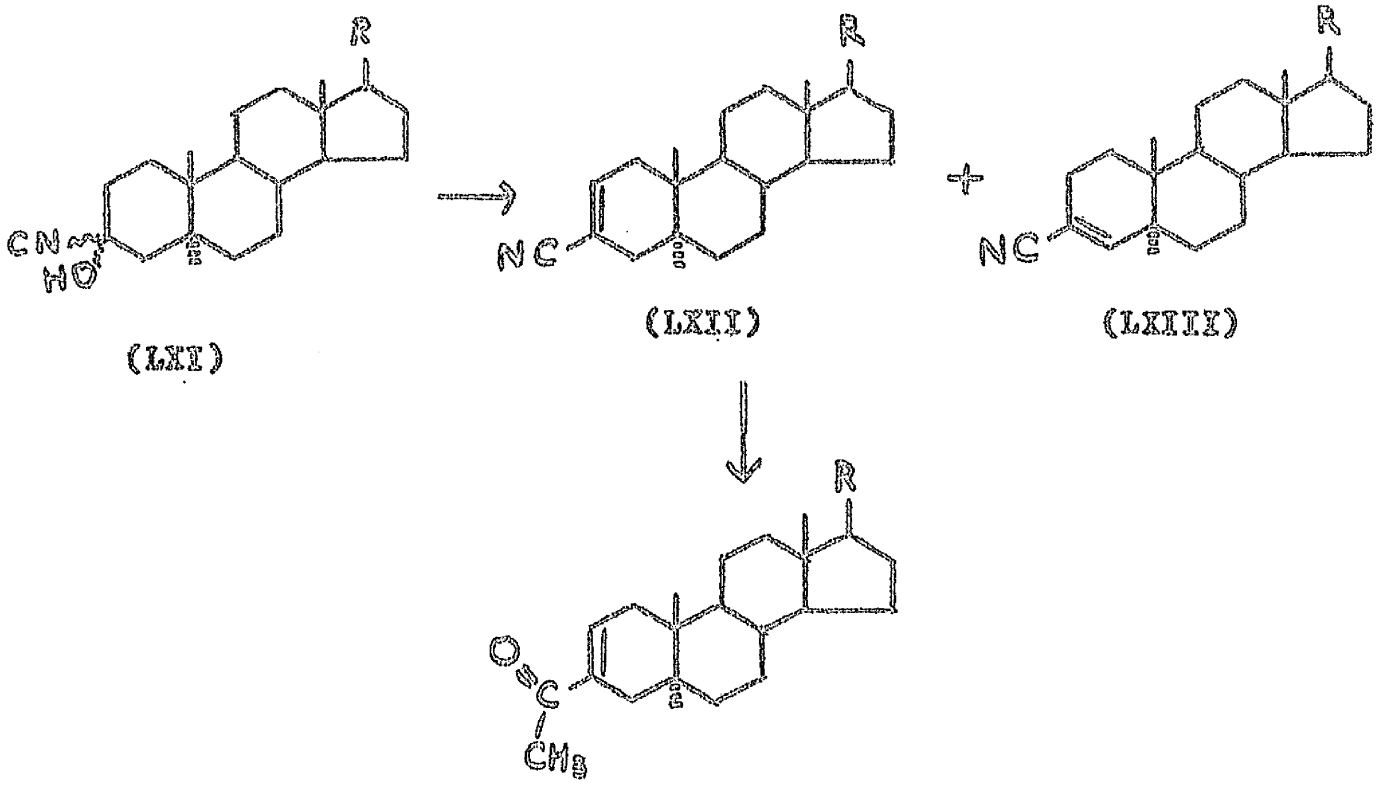


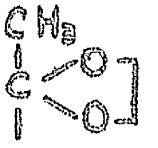
(LX)

Pohoryles, Gat and Sarel[52] using sodium cyanide in dimethyl-sulphoxide, obtained from 5α-cholestanyl-3β-chloride, a mixture of the 3α-cyanide (LIX) (60%), the 3β-cyanide (5%), and 5α-cholest-2-one (LX) (15%) after two hours heating at 180 - 190°. With longer reaction times equilibration took place and the 3α:3β ratio became 1:3.

Nathanson et al[53] have synthesised 3-methylketones (LXIV) from a mixture of epimeric cyanohydrins (LXI) formed by reaction of the corresponding 3-ketones with acetone cyanohydrin. The cyanohydrins (LXI) are dehydrated by phosphorus oxychloride to

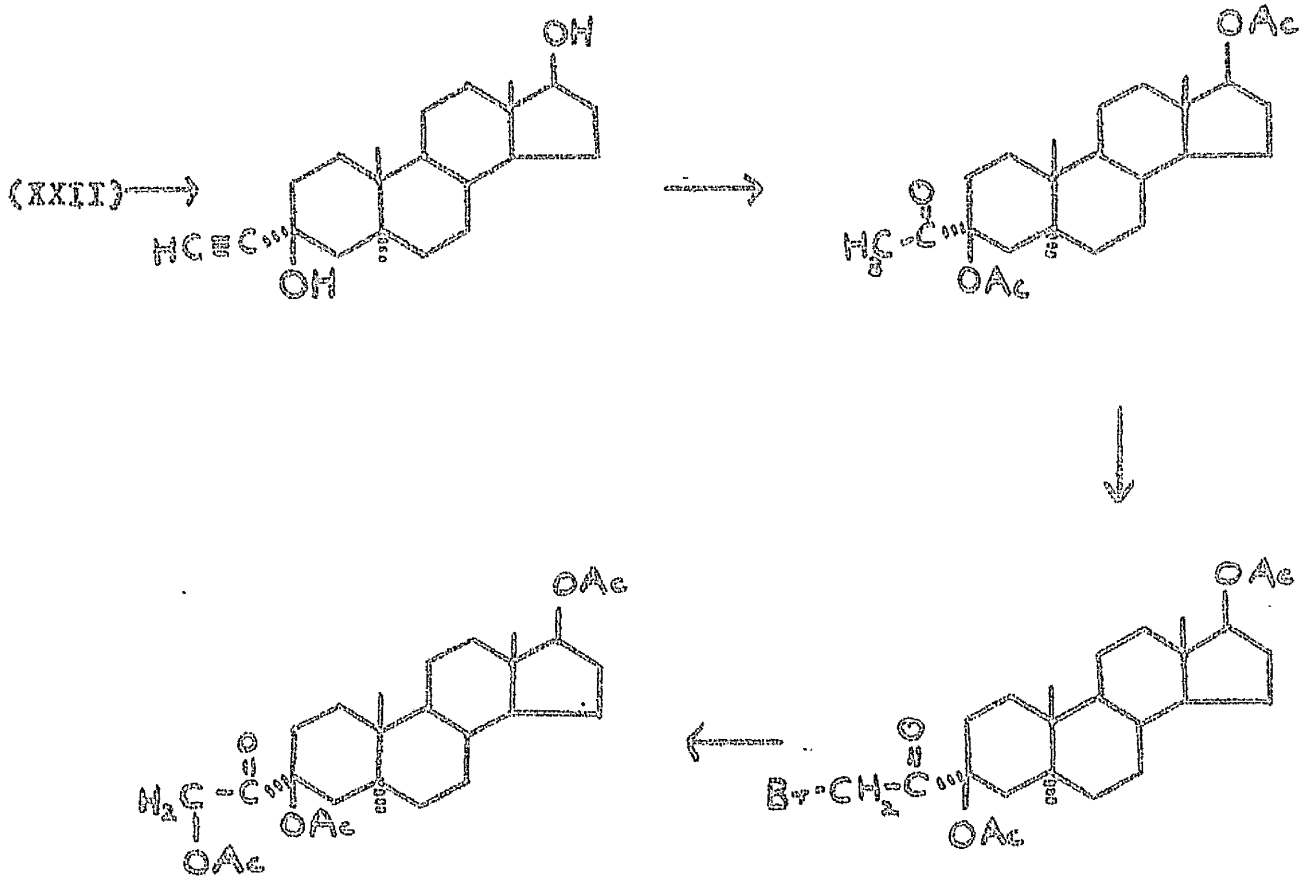
the isomeric 3-cyano-Δ^2- and 3-cyano-Δ^3-steroids (LXII) and (LXIII). Treatment of the Δ^2-olefin with methyl magnesium halide gives the corresponding 3-methylketones (LXIV).



R = C_8H_{17} or 

Kagan et al.[54], in a study of inverted cortical steroids, prepared a number of $C_{(3)}$ substituted compounds by ethynylation of 5α-androstan-17β-ol-3-one (LXII). The cortical side chain normally present at $C_{(17)}$ was introduced at $C_{(3)}$ by standard procedures involving hydration of the ethynyl carbinol to the methyl ketone and subsequent bromination, and acetylation of the latter

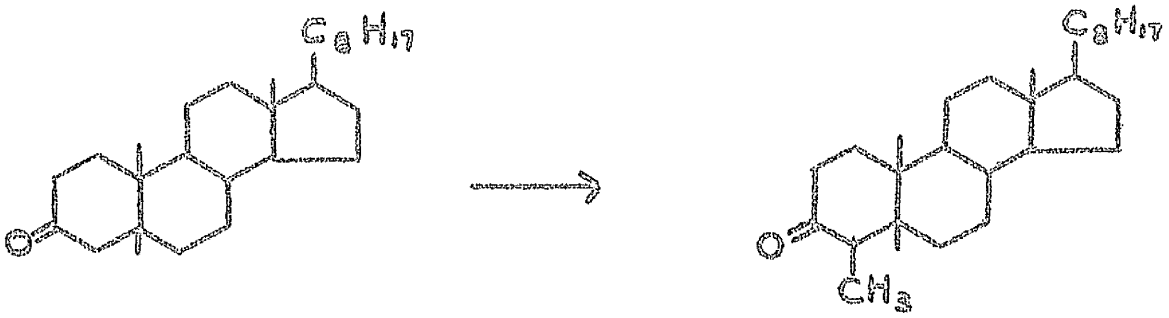
to yield 3α-(acetoxy-acetyl)-3β,17β-diacetoxy-5α-androstane (LXV).



(LXV)

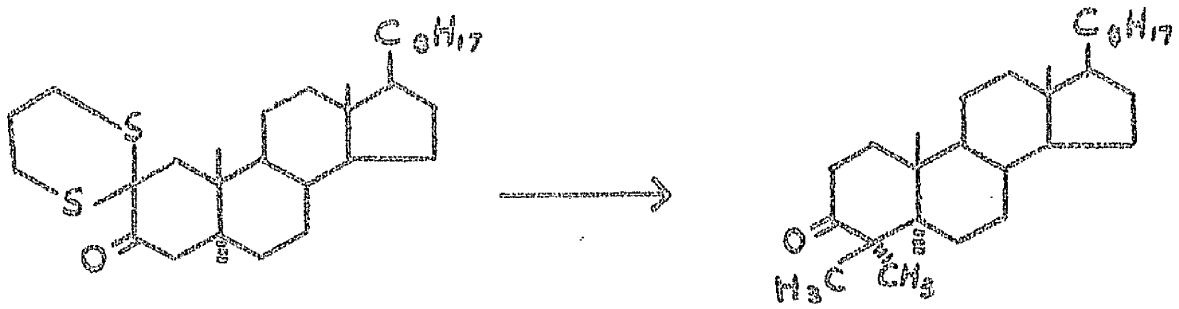
Substitution at $C_{(4)}$

Some of the procedures for introduction of carbon atoms at $C_{(2)}$ in the A/B trans series lead to substitution at $C_{(4)}$ when the A/B ring junction is cis. Thus 5β-cholestan-3-one (XXII), on treatment with methyl iodide[55] in the presence of potassium tert-butoxide affords 4β-methyl-5β-cholestan-3-one (LXVI).



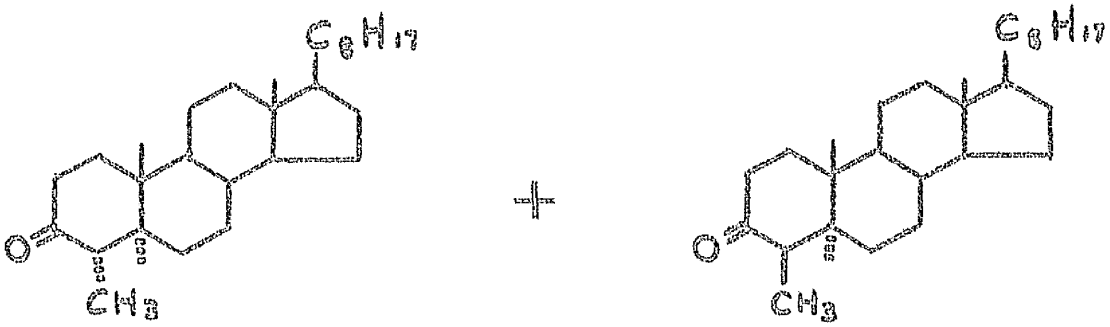
(XXX) (LXVI)

In the A/B trans series, successful introduction of a 4-methyl group requires blocking of the more reactive $C_{(2)}$ position, and this has been achieved by preparation of the dithioketal (LXVII) of 2-hydroxymethylene-5α-cholestan-3-one (XXVIa) by treatment with trimethylene ditoluene-p-thiosulphonate and potassium acetate[56]. Methylation of the dithioketal (LXVII) with methyl iodide followed by desulphurisation with Raney nickel and subsequent oxidation gives a mixture of 4,4-dimethyl-5α-cholestan-3-one (LXVIII), 4α-methyl-5α-cholestan-3-one (LXIX) and 4β-methyl-5α-cholestan-3-one (LXX)[57].



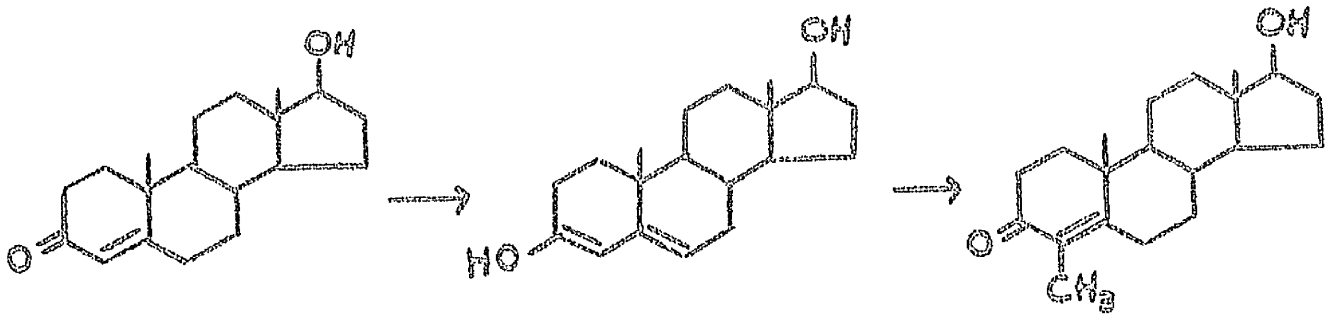
(LXVII) (LXVIII)

+



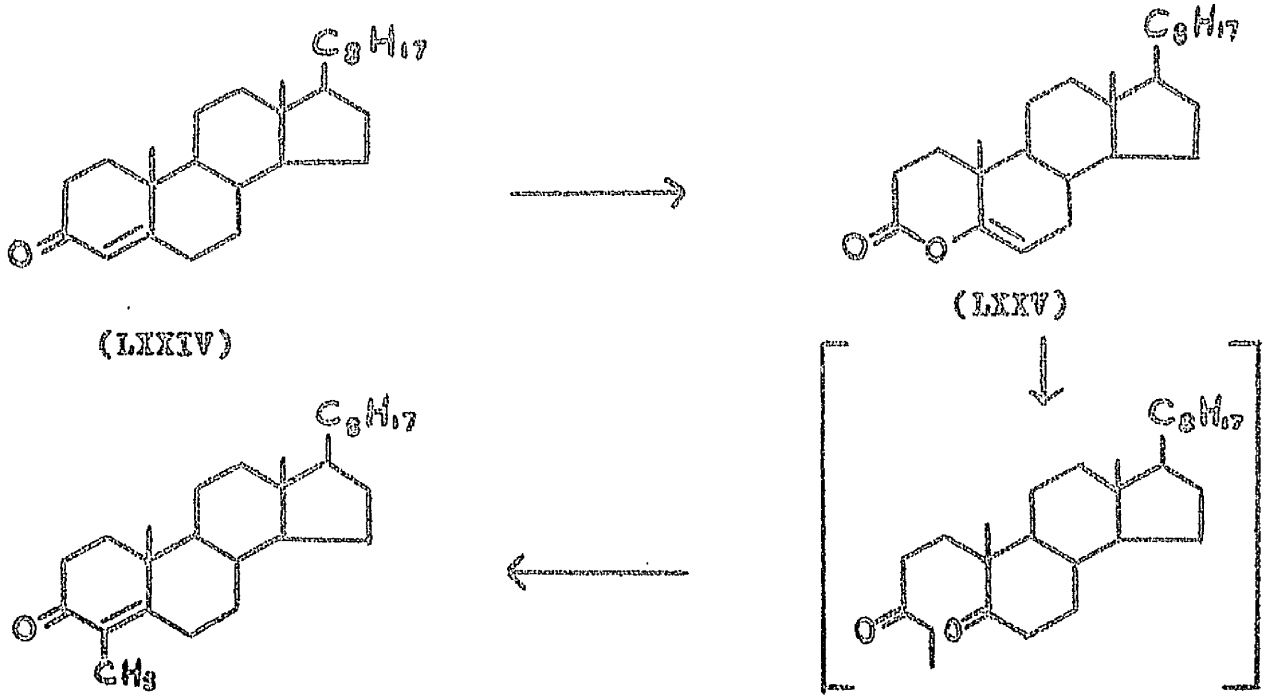
(LXIX) (LXX)

If, however, a 3,4-double bond is present, methylation takes place directly at $C_{(4)}$, since the intermediate enol (LXXI) has high electron density at this position, thus testosterone (LXXII) yields 4-methyl-androst-4-en-3-one (LXXIII)[58] on treatment with methyl iodide.



(LXXII) (LXXI) (LXXIII)

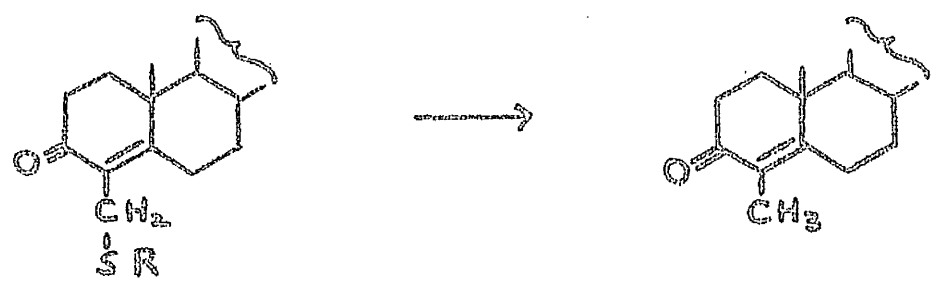
Cholest-4-en-3-one (LXXIV)on ozonolysis and recyclisation with sodium acetate and acetic anhydride affords the enol lactone (LXXV)[59] which on treatment with ethyl Grignard reagent followed by ring closure of the postulated intermediate gives 4-methyl-cholest-4-en-3-one[60,61,62].



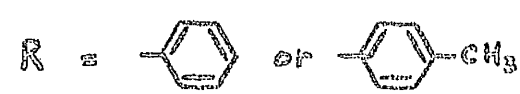
(LXXIV) (LXXV)

(LXXVI)

The yields of 4-methyl-Δ^4-steroids were greatly improved by a method developed by Kirk and Petrow[63] who first prepared a thiomethyl derivative (LXXVII) of the unsaturated ketone by treatment with benzene or toluene thiol and formaldehyde in the presence of triethylamine. Desulphurisation with deactivated Raney nickel affords the methyl derivative (LXXVIII) in 80% yield. These workers applied this reaction to many steroids including those with a similar unsaturated system elsewhere in the molecule and it appeared to be specific to the $C_{(4)}$ position.



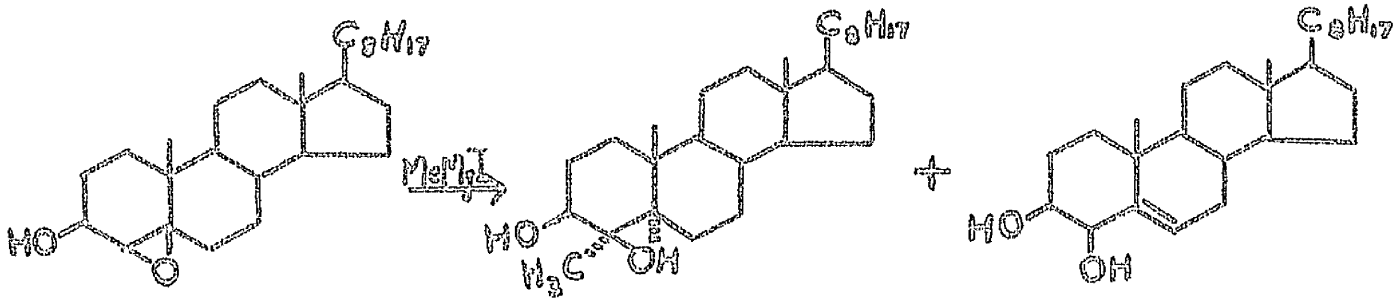
(LXXVII) (LXXVIII)



Cleavage of epoxides with Grignard reagents has proved to be a route to 4-methyl steroids; 4α,5α-epoxycholestan-3β-yl acetate (LXXIX) and methyl magnesium iodide yield exclusively 4β-methyl-cholestan-3β ,5α-diol[64,65] (LXXX).

(LXXIX) (LXXX)

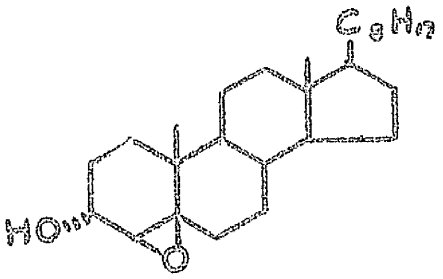
More than one product is obtained when a similar reaction is carried out in the 5β-cholestane series[64]. Two compounds identified from the reaction between 4β,5β-epoxycoprostan-3β-ol (LXXXI) and methyl magnesium iodide are 4α-methyl-5α-cholestan-3β,4β-diol (LXXXII) and cholest-5-en-3β,4β-diol (LXXXIII).



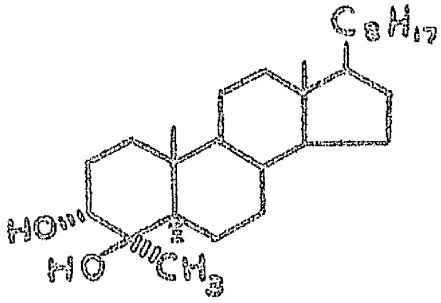
(LXXXI) (LXXXII) (LXXXIII)

Four products, none in more than 16% yield, are obtained when 4β,5β-epoxy-coprostan-3α-ol (LXXXIV) is treated with the same Grignard reagent[64]. They are 4α-methyl-5α-cholestan-3α,

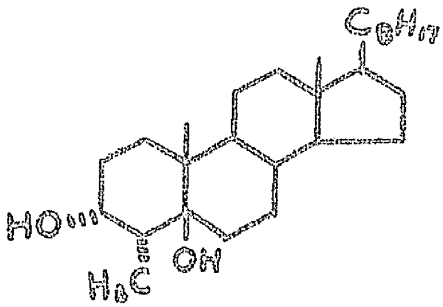
4β-diol (LXXXV), 4α-methyl-cholestan-3α,5β-diol (LXXXVI), 3α-methyl-5α-cholestan-3β,4α-diol (LXXXVII), and 3β-methyl-5α-cholestan-3α,4α-diol (LXXXVIII).



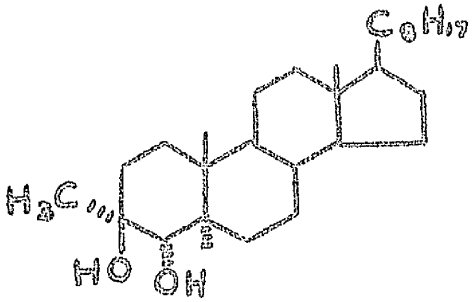
(LXXXIV)



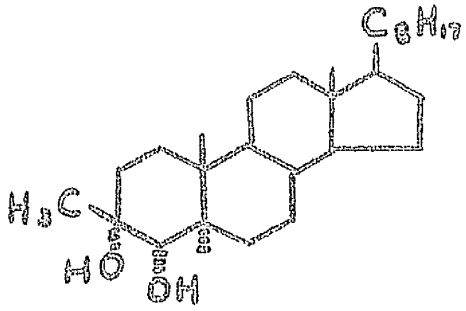
(LXXXV)



(LXXXVI)

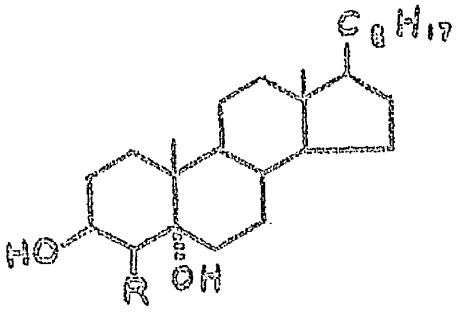


(LXXXVII)



(LXXXVIII)

Julia and Moutonnier[66] have recently prepared a number of 4β-alkyl derivatives (LXXXIX) by treatment of 4α,5α-epoxycholestan-3β-yl-acetate (LXXIX) with the appropriate Grignard reagent.

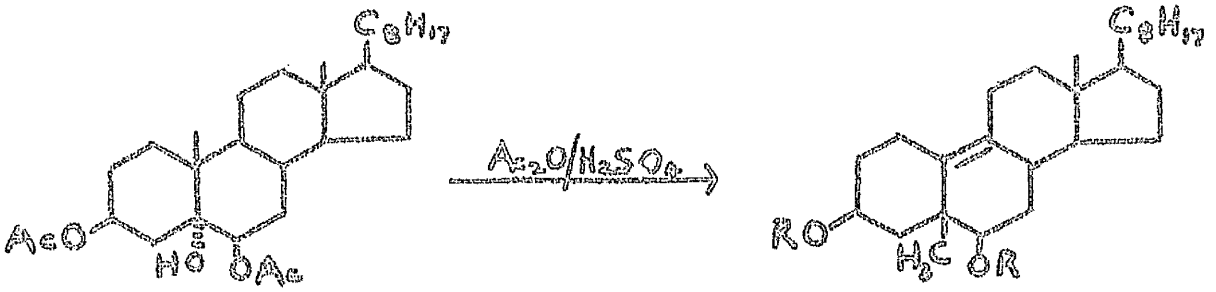


R = -Et, $-CH=CH_2$, $-C\equiv CH$, or 

(LXXXIX).

Substitution at $C_{(5)}$

The first steroid with a carbon containing substituent at the angular $C_{(5)}$ position was Westphalen's diol (XCIa), prepared in 1915[67]. It was a number of years, however, before the structure of the diol was fully elucidated. A suggestion by Lettré and Muller[68] that the diol (as the diacetate (XCIb) obtained by treatment of cholestan-3β,5α,6β-triol 3,6-diacetate (XC) with acetic anhydride and sulphuric acid was the 3,6-diacetate of 5β-methyl-19-norcholest-9(10)-en-3β,6β-diol (XCIa) was verified by Bladon, Henbest, and Wood[69] who confirmed the position of the double bond by ultraviolet spectroscopy, and by Ellis and Petrow[70] who studied the reactions of the diol (XCIa).

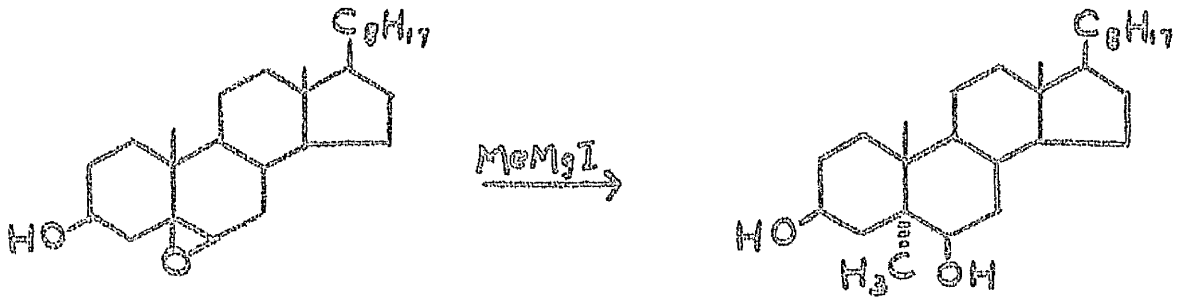


(XC) (XCI)

a, R = H

b, R = Ac

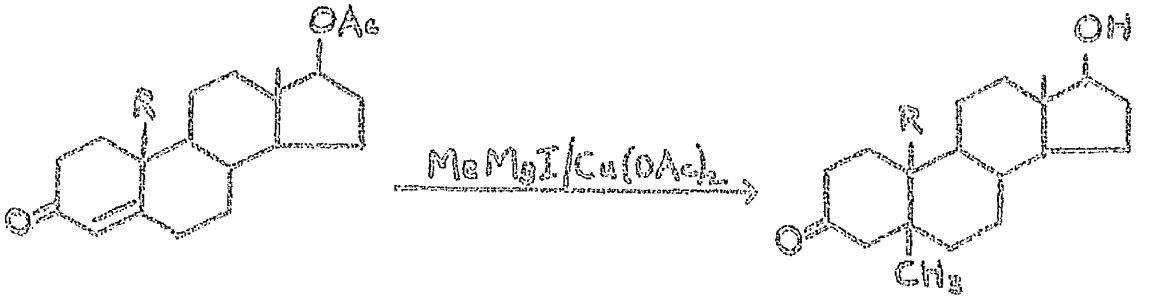
Another example of the utility of epoxides in the synthesis of carbon substituted steroids is the preparation of 5α-methyl-cholestan-3β,6β-diol[71] (XCIII) from the 5β,6β-epoxide (XCII) by treatment with methyl magnesium iodide.



(XCII) (XCIII)

Birch and Smith[72] found that a methyl Grignard reagent catalysed by cupric acetate adds to the double bond of Δ^4-3-oxo-steroids

in high yield in the case of 19-nortestosterone acetate (XCIVa) but in low yield with testosterone acetate (XCIVb) to give 5β-methyl-testosterone (XCVa) and 5β-methyl-testosterone (XCVb) respectively. The use of cupric acetate is of interest since normally cuprous halides are used to effect this type of Grignard reaction[18,19,20].



(XCIV) (XCV)

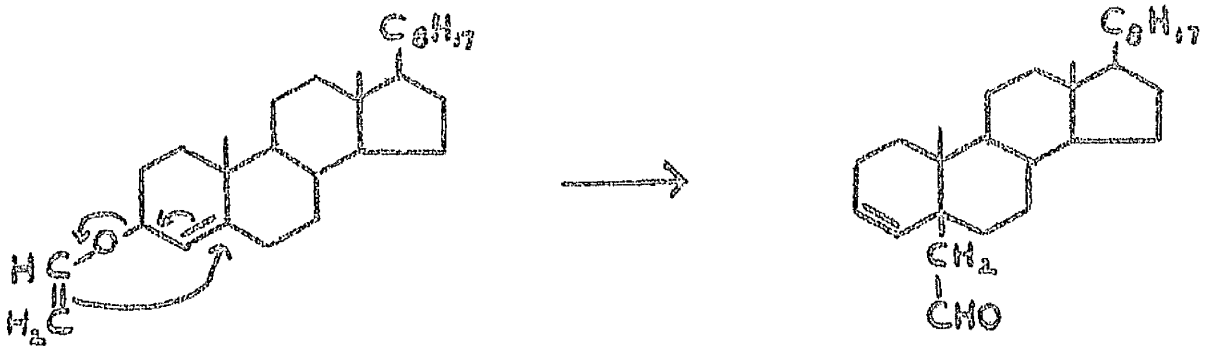
a, R = -H

b, R = -CH_3

a, R = -H

b, R = -CH_3

The first instance of introduction of a functional group other than methyl at $(C)_5$ was recorded by Burgstahler and Nordin[73] who found that Claisen rearrangement of the vinyl ether (XCVI) cholest-4-en-3β-ol affords the 5β-aldehyde (XCVII).



(XCVI) (XCVII)

In 1961, Nagata and his co-workers[74], and Bowers[75] introduced a nitrile group at $C_{(5)}$ by addition of the elements of hydrogen cyanide to the double bond of Δ^4-3-oxo-steroids and prepared 5α- and 5β-cyanocholestan-3-one (XCVIIIa) and (XCIXa), 5α- and 5β-cyano-androstan-17β-ol-3-one (XCVIIIb) and (XCIXb) and 5α- and 5β-cyano-pregnan-3,20-dione (XCVIIIc) and (XCIXc).

(XCVIII) (XCIX)

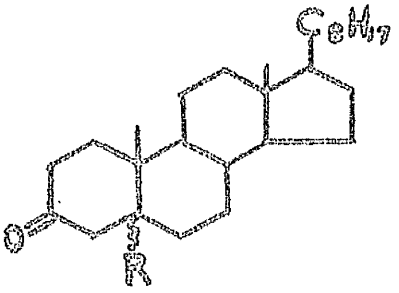
a, R = $-C_8H_{17}$ a, R = $-C_8H_{17}$

b, R = -OH b, R = -OH

c, R = $-COCH_3$ c, R = $-COCH_3$

Nagata's group later reported the transformation of each of the epimeric 5-cyano-cholestan-3-ones (XCVIIIa) and (XCIXa) to

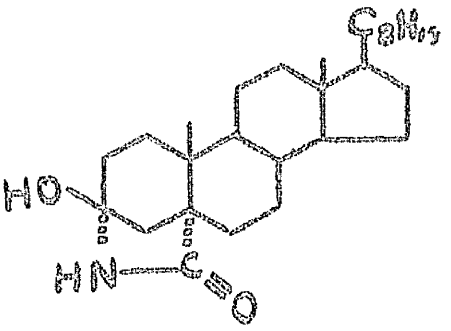
the 5α-and 5β-carboxylic acids (Ca) and (Cb) by hydrolysis to their respective lactams (CI) and (CII) followed by cleavage of their O-methyl-N-mesyl derivatives (CIII) and (CIV) with alkali.



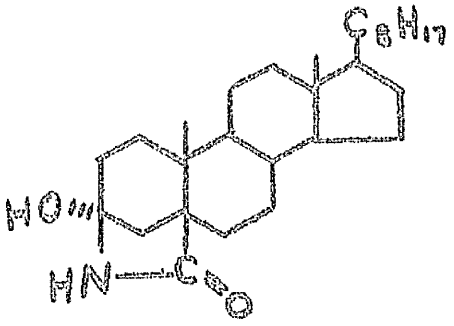
(C)

a, R = $-CO_2H$

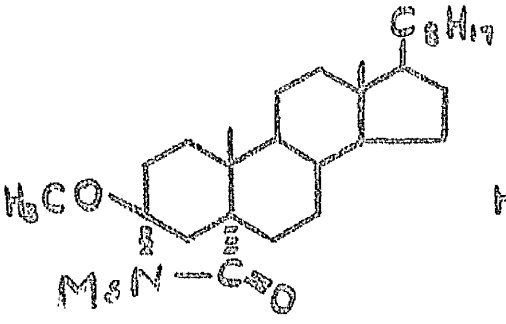
b, R = $-CO_2H$



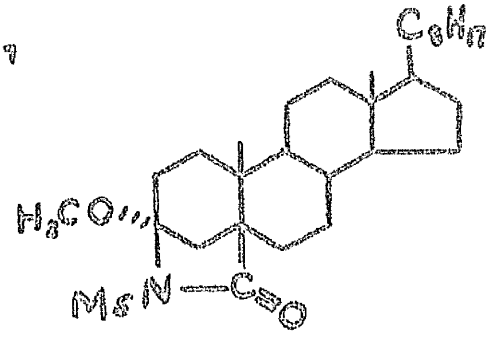
(CI)



(CII)



(CIII)

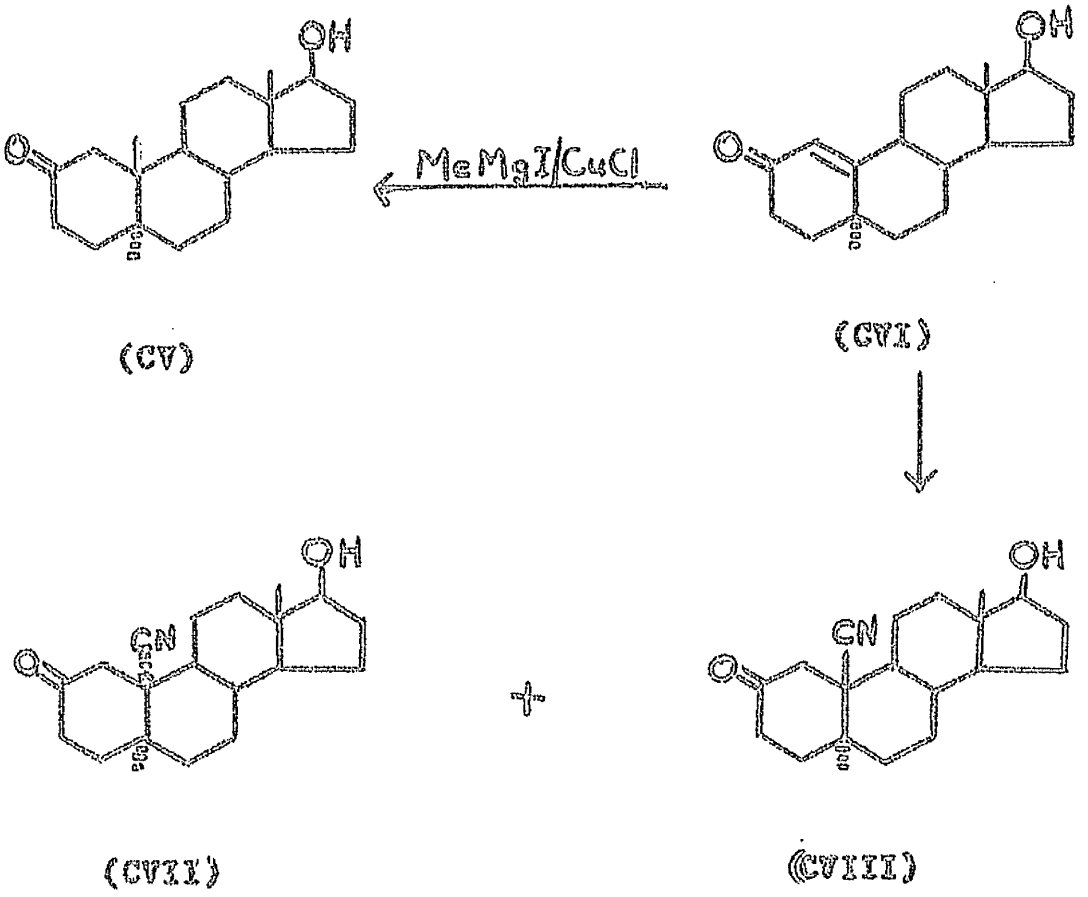


(CIV)

Substitution at $C_{(10)}$

The $C_{(10)}$ position is normally substituted by a methyl group and interest in this position has been more concerned with the reactions of the methyl group and with its removal rather than addition of a carbon atom to the relatively rare 19-nor-steroids. Torrigo® and Fishman[77], however, prepared 5α-androstan-17β-ol-2-one (CV) by treatment of 5α-androstan-1(10)-en-17β-ol-2-one (CVI) with methyl

Grignard reagent in the presence of cuprous chloride. These workers also found that the same unsaturated ketone (XVI) adds the elements of hydrogen cyanide to give the epimeric 10α- and 10β-cyanoketones (CVII) and (CVIII) respectively, in the ratio of 5:2.

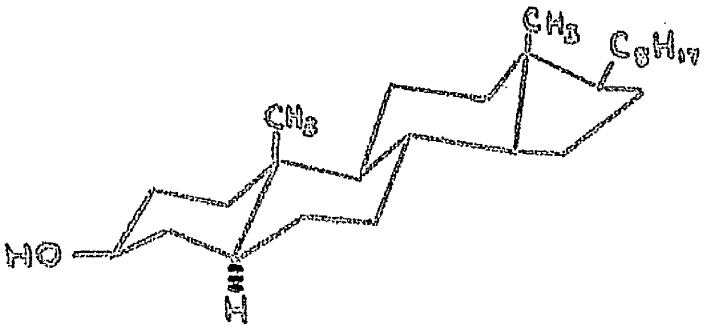


(CV) (CVI) (CVII) (CVIII)

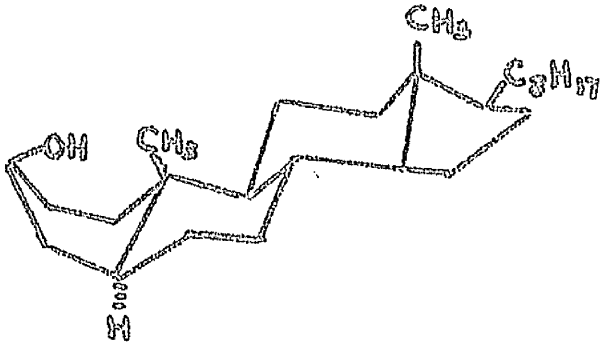
Conformations of ring A

In a simple steroid such as 5α-cholestan-3β-ol, rings B and C are held in the chair conformation by their trans fusion to rings A and D. Ring A is more flexible but is also considered to adopt the chair conformation (CIX) which has a minimum of 1,3 interactions.

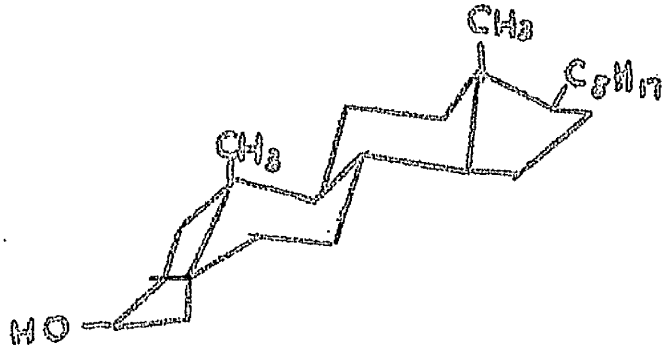
If ring A adopted the boat conformation (CX), there would be a strong interaction between the $C_{(10)}$ methyl group and the $C_{(3)}$ hydroxyl group. In the 5β-series where rings A and B are cis fused, the stable conformation of a molecule such as 5β-cholestan-3α-ol is also the all chair conformation (CXI).



(CIX)



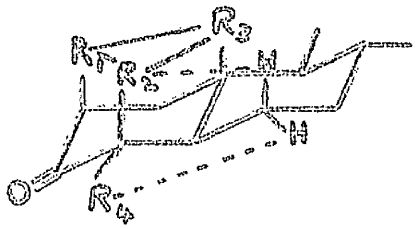
(CX)



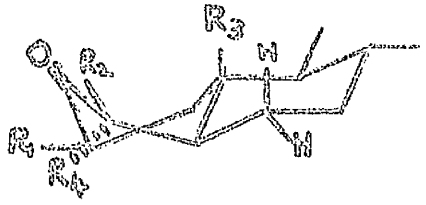
(CXI)

When ring A is highly substituted, the chair conformation (CXII) has unfavourable homoannular 1,3 interactions between substituents R_1, R_2, and R_3, and heteroannular interactions between R_2 and R_4 and the 6α hydrogens respectively. The homoannular interactions may be relieved by a lateral displacement, but the only means of relieving the heteroannular interactions is by rotation about

the 4-6 axis, thus converting ring A to the twist boat conformation[78,79] (CXIII).



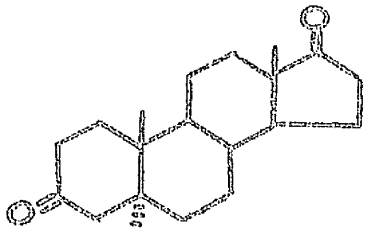
(CXII)



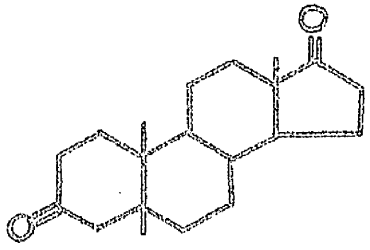
(CXIII)

A twist boat or boat conformation has often been inferred to rationalise apparently anomalous physical and chemical properties of ring A substituted steroids.

From a comparison of the dipole moments of 5α-(CXIV) and 5β-(CXV) androstan-3,17-diones, Nace and Turner[80] suggested that 16% of the latter exists with ring A as a boat.



(CXIV)

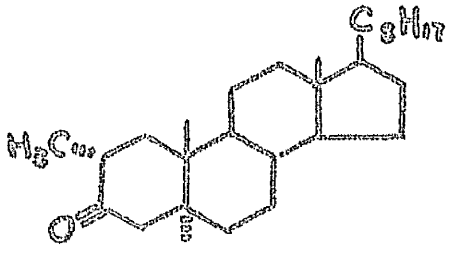


(CXV)

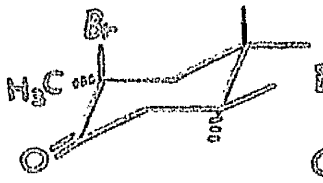
In the past six years, the combination of infrared spectroscopy

and optical rotatory dispersion has detected the existence of ring A in the boat conformation in a number of ring A substituted steroids.

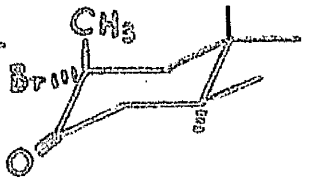
Bromination of 2α-methyl-5α-cholestan-3-one (CXVI) gives a 2-bromoketone which does not show a change in the frequency of the infrared carbonyl absorption[81], and on this basis the bromine was assigned the 2β-axial configuration[82]. The optical rotatory dispersion curve[83] does not agree with this assignment, since, according to the axial halo-ketone rule[84], this substance, with ring A as a chair (CXVII) should show a positive Cotton effect, instead of the observed negative value. The Cotton effect and infrared spectrum are also inconsistent with 2α-bromo-2β-methyl-5α-cholestan-3-one if it exists with ring A in the chair conformation (CXVIII). The only conformation which agrees with the physical measurements is 2α-bromo-2β-methyl with ring A as a boat (CXIX). In this way the unfavourable methyl-methyl interaction in (CXVIII) is relieved.



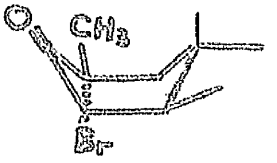
(CXVI)



(CXVII)



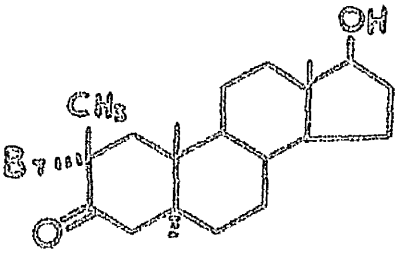
(CXVIII)



(CXIX)

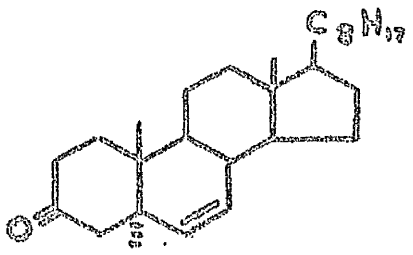
Mauli, Ringold, and Djerassi[85] have shown by similar reasoning that

2α-bromo-2β-methyl-5α-androstan-17β-ol (CXX) exists with ring A in the boat conformation

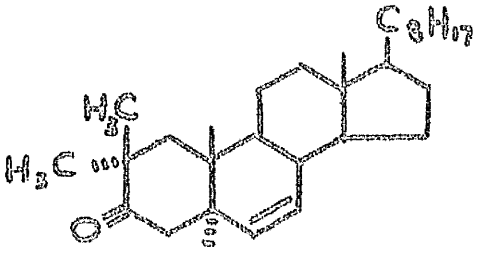


(CXX)

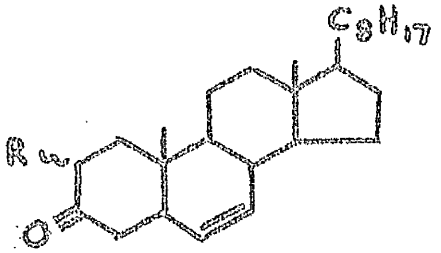
Methylation of 5α-cholest-6-en-3-one (CXXI) yields three products which have been identified as the 2,2-dimethyl (CXXI) and the 2α-(CXXIIIa) and 2β-methyl (CXXIIIb) derivatives respectively[26]. It has previously been shown[27] that 2β-methyl-3-oxo-steroids readily epimerise to the 2α-equatorial epimer, but in this instance epimerisation attempts failed and it was inferred that ring A exists as a boat (CXXIV), in which the methyl group is equatorial, rather than as the chair conformation (CXXV). Optical rotatory dispersion again provided evidence in favour of the boat conformation, the octant rule[27] predicting a positive Cotton effect for (CXXIIIb) in the chair conformation (CXXV) whereas a strong negative value is observed which is compatible with the boat conformation.



(CXXI)



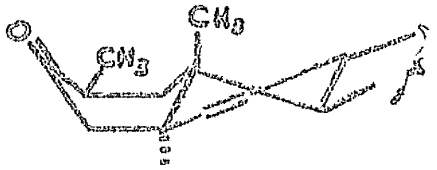
(CXXII)



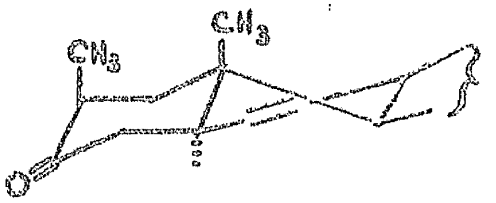
(CXXIII)

a, R = α-CH_3

b, R = β-CH_3

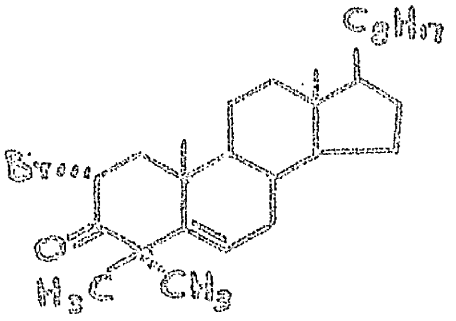


(CXXIV)

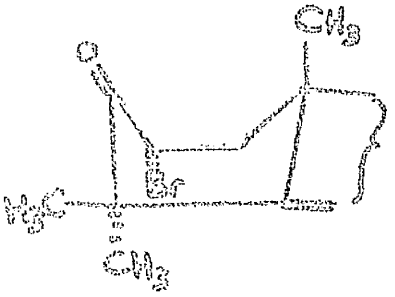


(CXXV)

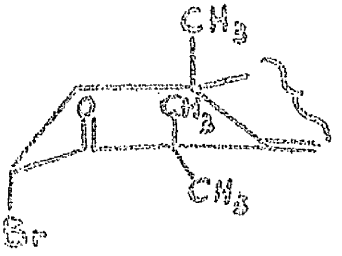
In theexample quoted, ring A exists in the boat conformation as a consequence of steric factors associated with the ring and the presence of a double bond in ring B. A double bond at $C_{(5)}$ has similarly been shown to influence the conformation of ring A. Cropp, Dewhurst and Holker[88] consider that ring A of 2a-bromo-4,4-dimethyl-cholest-5-en-3-one (CXXVI) has a shape intermediate between (CXXVII) and (CXXVIII) whereas the saturated analogue has ring A in the chair conformation.



(CXXVI)

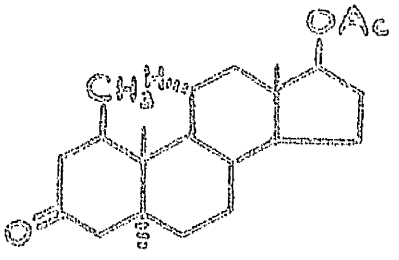


(CXXVII)



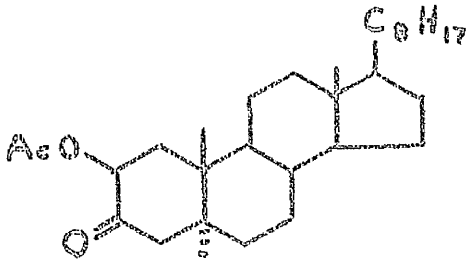
(CXXVIII)

Ring B of 1β-methyl-5α-androstan-17β-ol-3-one 17-acetate (CXXIX) is fully saturated but the steric interference of the 11α-hydrogen atom in ring C with the equatorial 1-methyl group causes a distortion of ring A which is reflected in the optical rotatory dispersion curve[89].

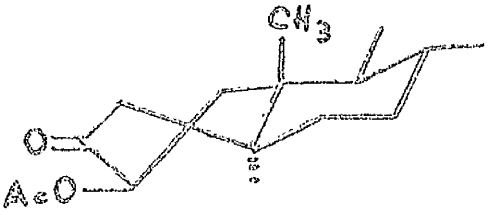


(CXXIX)

Further examples of the boat conformation of ring A have been detected by nuclear magnetic resonance spectroscopy. Williamson and Johnson[90] calculated the dihedral angles of 5α-cholestan-2β-ol-3-one 2β-acetate (CXXX) from the nuclear magnetic resonance coupling constants and the Karplus equation[91], and deduced that ring A exists in a twist boat conformation (CXXV). The chair form of ring A is unstable owing to the 1,3-diaxial interaction between the 2β-acetyl group and the methyl group at $C_{(10)}$.



(CXXX)



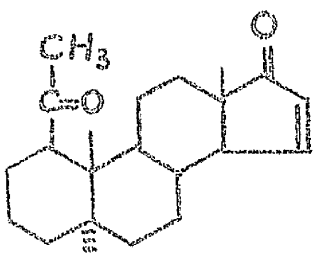
(CXXXI)

Kuriyama, Kondo, and Tori[92] similarly demonstrated that ring A in a number of 2β-hydroxy- and 2β-acetoxy-Δ4-3-oxo-steroids is in the boat conformation. Karplus[93], however, has pointed out that this method must be used with reservation, and other aspects of molecular environment must be taken into account.

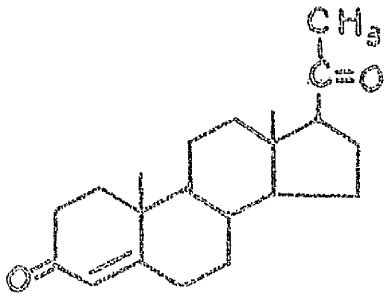
Many other examples of boat and twist boat conformationsof ring A are recorded in the literature, but the examples quoted above illustrate the principal techniques used in their detection.

DISCUSSION

There is no record of the preparation of 1-acetyl-5α-androst-16-en-17-one (CXXXII) which would have a structure analogous to the important sex hormone progesterone (CXXXIII), (CXXXIII), and would perhaps possess biological activity. As a preliminary step in the synthesis of (CXXXII) it was decided to investigate possible routes to the introduction of an acetyl group at $C_{(1)}$, using 5α-cholestane derivatives as model compounds.



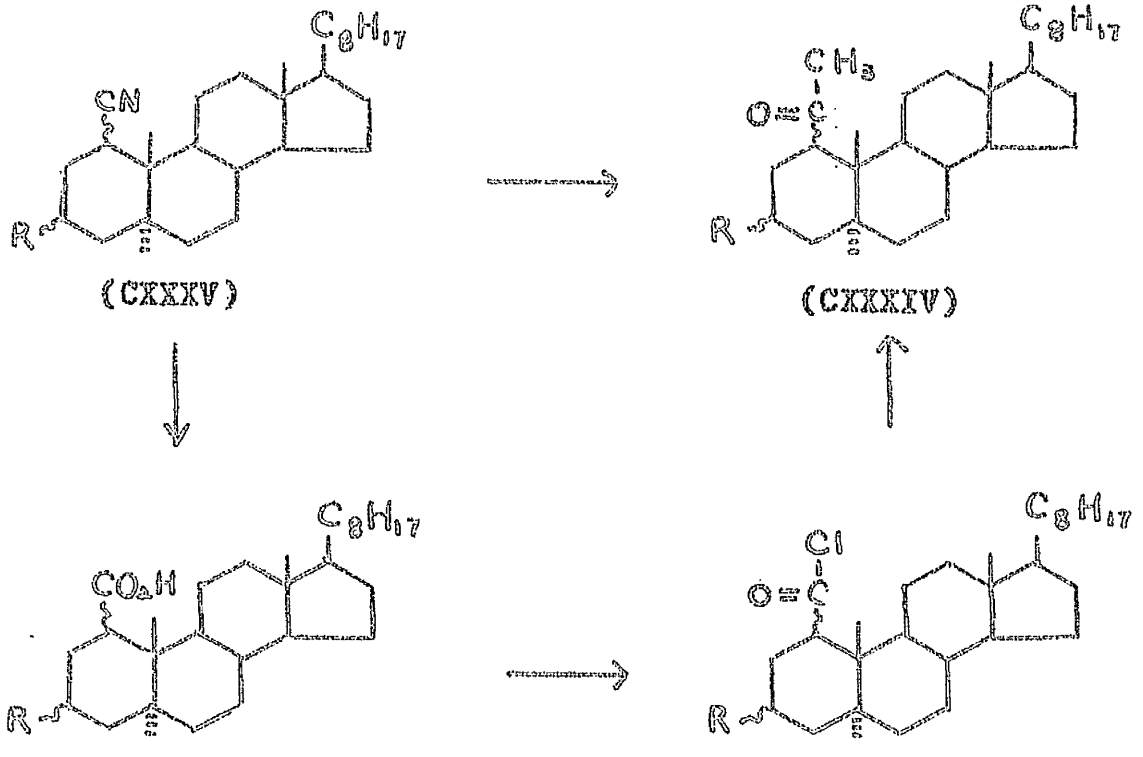
(CXXXII)



(CXXXIII)

At the start of this investigation, the only carbon containing substituent which had been introduced at $C_{(1)}$ was a methyl group, and it was felt that a functional group with a greater diversity of chemical activity would be required before the desired 1-acetyl-5α-cholestane (CXXXIV) could be synthesised. A 1-cyano-steroid (CXXV) was considered to be particularly suitable since it could serve as an

intermediate in several possible routes to (CXXXIV). Two of the routes considered involved either a Grignard reaction to give the methyl ketone (CXXXIV) directly, or hydrolysis to the 1-carboxylic acid (CXXXVI), the acid chloride (CXXXVII) of which, on treatment with cadmium methyl should also give (CXXXIV).



(CXXXV) (CXXXIV)

(CXXXVI) (CXXXVII)

A number of groups[5,74,75,77,84] had reported the addition of the elements of hydrogen cyanide to steroidal α,β-unsaturated ketones but none had been concerned with cyanation at $C_{(1)}$.

After much of the work described here had been completed three publications reported cyanation at this position.[21,22]

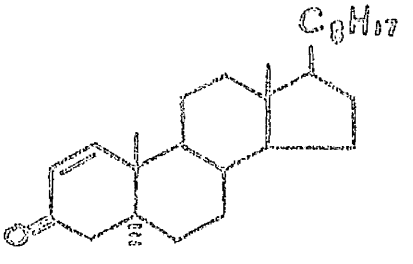
The addition of the elements of hydrogen cyanide to α,β-unsaturated ketones was first studied by Lapworth and Jones[95] who showed that the cyano group always goes to the β-position, and that little reaction takes place when hydrogen cyanide itself is used, a cyanide, such as potassium cyanide is necessary and the rate of reaction is directly proportional to the concentration of free cyanide ions. The rate controlling step is thus -

$$CN^{-} + RCH = CH - \overset{|}{C} = O \longrightarrow \underset{|}{RCH} - \underset{CN}{CH^{-}} - \overset{|}{C} = O$$

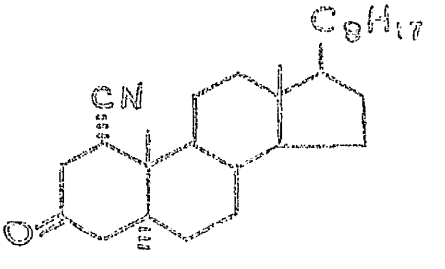
The use of potassium cyanide in aqueous methanol for the cyanation of steroids promotes side reactions such as hydrolysis and dimerisation, whereas potassium cyanide and ammonium chloride in aqueous dimethylformamide were shown to give only cyano derivatives[74,75]. Meyer and Wolfe[96] observed similar effects in the preparation of cyano-indanones.

Treatment of 5α-cholest-1-en-3-one (VIIIb) with potassium cyanide and ammonium chloride in aqueous dimethylformamide for 8 hr. at 100° gave, in 60% yield, a product whose infrared spectrum showed absorption at 2245 and 1722 cm.$^{-1}$ consistent with the presence of a nitrile group and a carbonyl group in a

six membered ring respectively, and since attack of cyanide ion was more likely to take place on the less hindered α-face, the product was considered to be 1α-cyano-5α-cholestan-3-one (XXI). Chromatography of the mother liquors yielded a further 5% of the same cyanoketone and 10% of 5α-cholestan-3-one (XXXV), which was probably present as an impurity in the 5α-cholest-1-en-3-one (VIIIb) which had been prepared by dehydrobromination of 2α-bromo-5α-cholestan-3-one[97]. Warnhoff[98] has shown that 2α-bromo-5α-cholestan-3-one, when purified by recrystallisation still contains 5 - 15% of 5α-cholestan-3-one which is not detectable by melting point or optical rotation, and although the unsaturated ketone was chromatographed before use, its infrared carbonyl absorption band at 1675 cm.$^{-1}$ showed a slight shoulder of low intensity at 1712 cm.$^{-1}$, the frequency expected for 5α-cholestan-3-one (XXXV). The yield of cyanoketone (XXI) was not improved by increasing the concentrations of potassium cyanide and ammonium chloride or by carrying out the reaction at reflux temperature.

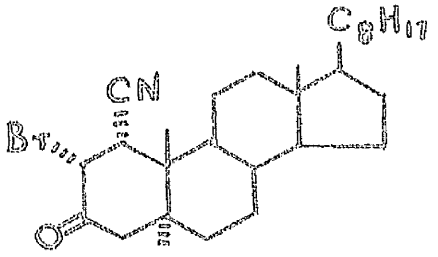


(VIIIb)



(XXI)

When 1α-cyano-5α-cholestan-3-one (XXI) in acetic acid was treated with one equivalent of bromine in acetic acid, in the presence of hydrogen bromide[99], a crystalline monobromoketone was obtained whose infrared spectrum still showed nitrile absorption at 2245 cm.$^{-1}$. The carbonyl band, however, now appeared at 1740 cm.$^{-1}$, a shift of +18 cm.$^{-1}$, which indicated that the bromine was equatorial[81], thus the major product of bromination was 2α-bromo-1α-cyano-5α-cholestan-3-one (CXXXVIII).



(CXXXVIII)

Dehydrobromination of (CXXXVIII) with calcium carbonate in dimethylacetamide[97], or better with lithium chloride in dimethylformamide[55], gave an α,β-unsaturated ketone showing nitrile absorption at 2237 cm.$^{-1}$, carbonyl absorption at 1690 cm.$^{-1}$, and ethylenic absorption at 1595 cm.$^{-1}$. The ultraviolet spectrum showed maximum absorption at 236 mμ

(ϵ = 11,000), and from the above evidence the unsaturated ketone appeared to be 1-cyano-5α-cholest-1-en-3-one (CXXXIX), although the ultraviolet absorption might have been expected at a slightly higher wavelength. Woodward's rules[100] predict a wavelength of 239 mμ for an α,β-unsaturated ketone with two β-substituents and it was felt that the nitrile group would extend the conjugation and move the absorption to an even higher wavelength. The other possible location for the double bond was in the 4,5 position (CXL) which could have resulted from isomerisation, or from the starting material being the 4-bromoketone and not the 2-bromoketone.

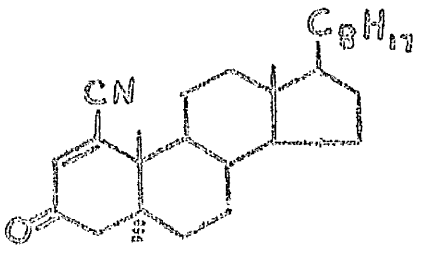
Preparation of an enol-acetate of the unsaturated ketone would help to decide its structure, since the enol acetate of (CXXXIX) would be expected to have a homoannular diene system (CXLI), while that of (CXL) would have a heteroannular diene system[101] (CXLII), and these dienes would be readily distinguishable by their ultraviolet spectra.[100] Attempts at enol-acetylation by the usual methods[102] were however unsuccessful.

The mother liquors from the preparation of the bromoketone (CXXVIII) were concentrated and dehydrobrominated with lithium chloride in dimethylformamide[55]. Chromatography of the product yielded small amounts of two more unsaturated cyanoketones,

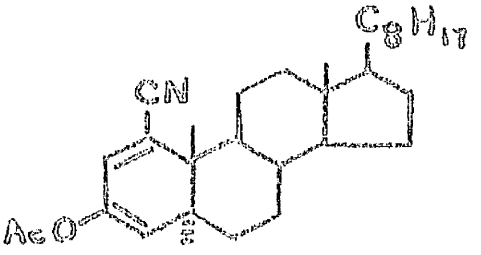
one of which showed nitrile absorption at 2237 cm.$^{-1}$, carbonyl absorption at 1665 cm.$^{-1}$, and double bond absorption at 1621 cm.$^{-1}$ and 1587 cm.$^{-1}$, with an ultraviolet maximum absorption at 250 mμ (ϵ = 13,500). The other unsaturated ketone showed nitrile absorption at 2255 cm.$^{-1}$ carbonyl absorption at 1678 cm.$^{-1}$ and double bond absorption at 1612 cm.$^{-1}$ with maximum ultraviolet absorption at 242 mμ (ϵ = 15,000). These sets of spectral characteristics are compatible with the expected data for 1-cyano-cholesta-1,4-dien-3-one (CXLIII) and 1α-cyano-cholest-4-en-3-one (CXL) respectively, thus strengthening the evidence that the first unsaturated ketone isolated was 1-cyano-5α-cholest-1-en-3-one (CXXXIX).

The proposed structures for the three unsaturated ketones were confirmed by their nuclear magnetic resonance spectra. The spectrum of the suspected Δ^1-3-ketone (CXXXIX) showed a singlet at 3.54 τ (area = 1H) corresponding to anolefinic proton at $C_{(2)}$ and the suspected Δ^4-3-ketone (CXL) showed a barely resolved triplet centred at 4.4τ (area = 1H) corresponding to an olefinic proton at $C_{(4)}$, the signal appearing as a triplet as a result of coupling with protons at $C_{(6)}$ and probably $C_{(2)}$, while the remaining substance (CXLIII) showed two doublets, the first centred at 3.17τ (area = 1H) attributable to a proton at $C_{(2)}$ being coupled with another at $C_{(4)}$, and the second, which

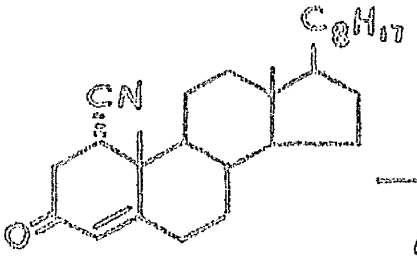
was more complex, centred at 3.81τ (area = 1H) attributable to a proton at $C_{(6)}$ showing coupling with protons at $C_{(2)}$ and $C_{(6)}$. It is possible that the assignments of the doublets could be reversed, but the presence of two olefinic protons is still confirmed.



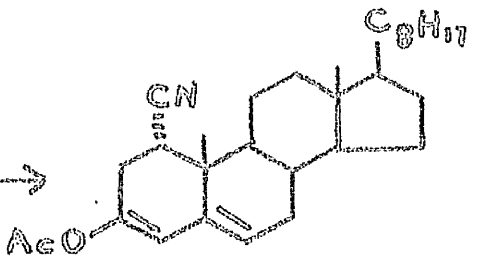
(CXXXIX)



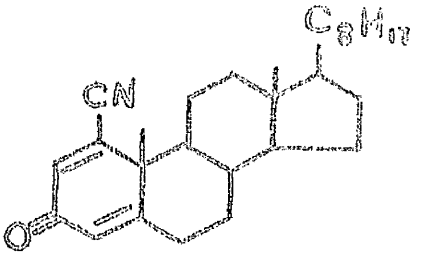
(CXLI)



(CXL)



(CXLII)

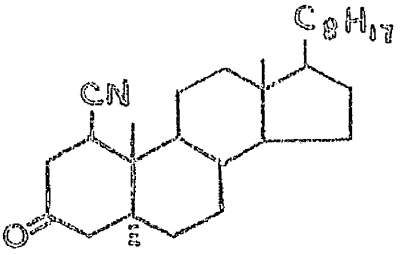


(CXLIII)

When steroids with an A/B trans ring junction are catalytically hydrogenated they are considered to be adsorbed on the catalyst surface on the less hindered α-face and so hydrogenation occurs from that side[103]. Thus 1-cyano-5α-cholest-1-en-3-one (CXXXIX) would be expected to add hydrogen at the 1α and 2α positions to give

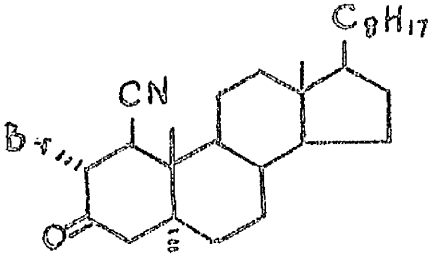
1β-cyano-5α-cholestan-3-one (CXLIV), epimeric at $C_{(1)}$ with (XXI). Hydrogenation of (CXXXIX) in ethyl acetate in the presence of 10% palladium-charcoal gave a cyanoketone with an infrared spectrum almost identical to that of 1α-cyano-5α-cholestan-3-one (XXI) but with a different melting point, optical rotation, and nuclear magnetic resonance spectrum and was formulated as 1β-cyano-5α-cholestan-3-one (CXLIV). The nuclear magnetic resonance spectra of the epimeric 1-cyanoketones were of interest in that 1α-cyano-5α-cholestan-3-one showed a doublet centred at 7.36τ (J = 4.1 c.p.s.) and a triplet centred at 6.83τ (J = 4 c.p.s.). These values were consistent with the $C_{(1)}$ β-hydrogen being equally coupled to both $C_{(2)}$ hydrogens. While the 1β-cyano-5α-cholestan-3-one showed a single peak at 7.31τ (area = 3H), due to the 1α, 2α and 2β hydrogens having an identical chemical shift and so no measurable coupling could be observed.

Dehydrogenation of 3-oxo-steroids with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (D.D.Q.) has been shown to involve abstraction of a 1α hydrogen[104] and it was thought that further proof of the stereochemistry at $C_{(1)}$ of the epimeric cyanoketones (XXI) and (CXLIV) could be afforded by use of this reagent, since only the 1β-epimer should be dehydrogenated. In point of fact, neither epimer could be dehydrogenated, due, probably, to a combination of steric and electronic factors associated with the cyano group.[105]



(CXLIV)

Bromination of 1β-cyano-5α-cholestan-3-one (CXLIV) by the method[99] which was used for the 1α-epimer gave a monobromo-ketone which showed bands at 2240 and 1739 cm.$^{-1}$ in the infrared. The shift in the carbonyl absorption (+17 cm.$^{-1}$) implied that the bromine atom had the equatorial configuration[81] and the bromoketone appeared to be 2α-bromo-1β-cyano-5α-cholestan-3-one (CXLV). The location of the bromine at $C_{(2)}$ was confirmed by dehydrobromination with lithium chloride in dimethylformamide[59] to give 1-cyano-5α-cholest-1-on-3-one (CXXXIX) in 80% yield, compared with a yield of 60% obtained by dehydrobromination of 2α-bromo-1α-cyano-5α-cholestan-3-one (CXXXVIII).



(CXLV)

Schmitz and Johnson[106] have shown that dehydrobromination of 1α-deutero-2α-bromo-3-oxo-steroids proceeds with loss of deuterium, but they were unable to say whether the elimination was cis, involving the 2α-bromine, or trans diaxial involving preliminary conversion of the bromine to 2β; and Wendler, Taub, and Kuo[107] have shown by dehydrobromination of β-deuterated epimeric α-bromoketones that trans diaxial elimination is favoured. The more ready dehydrobromination of 1β-cyano-2α-bromo-5α-cholestan-3-one (CXLV) may be rationalised by a mechanism in which the bromine is converted to the 2β-axial configuration followed by trans diaxial elimination with the 1α-hydrogen. 2α-Bromo-1α-cyano-5α-cholestan-3-one (CXXXVIII) does not have a 1α-axial hydrogen atom and so it dehydrobrominates more slowly since the reaction cannot proceed by the preferred route.

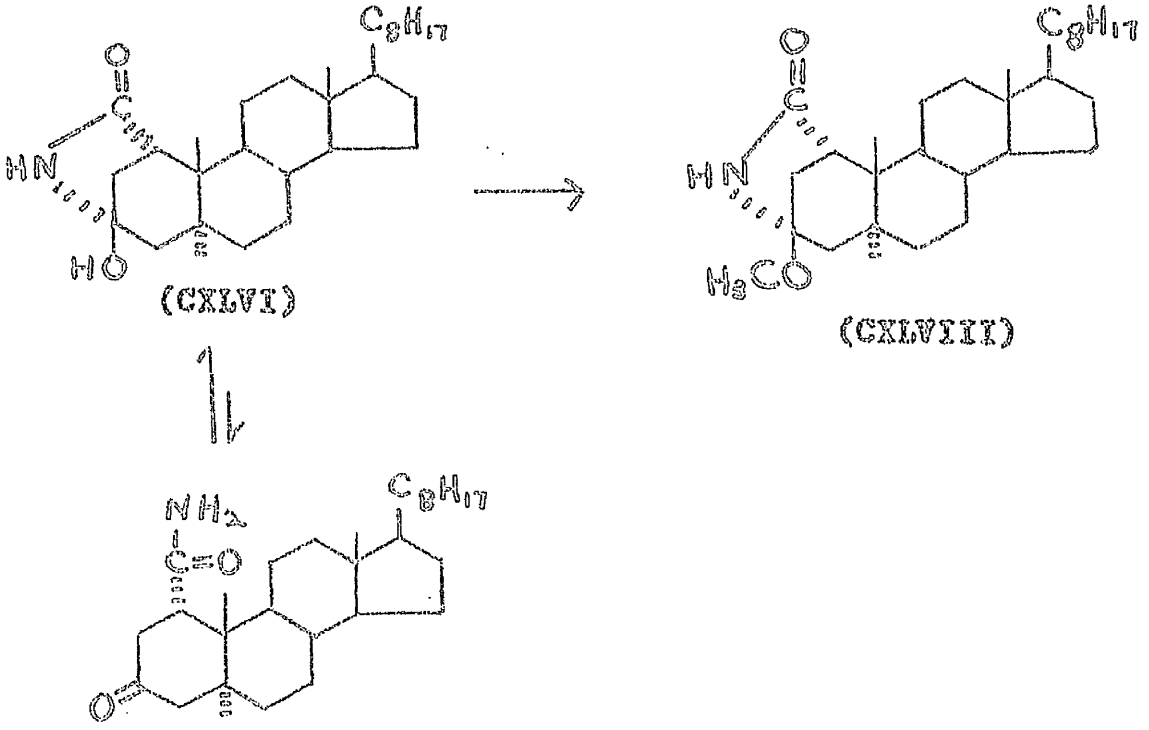
Cyanosteroids have been hydrolysed to the corresponding carboxylic acids by treatment with aqueous ethanolic potassium hydroxide[108], but in some cases forcing conditions, such as alkali in refluxing ethylene glycol[44,109], are necessary. A neighbouring functional group may react with the initial hydrolysis product, and Nagata[74] and Bowers[75] found that the amides formed by hydrolysis of 5-cyano-3-oxo-steroids existed mainly as 3,5-lactams, such as (CII) and (CIII). Nagata[110] also obtained an analogous lactam by hydrolysis of a 13-cyano-16-oxo-D-homosteroid.

When 1α-cyano-5α-cholestan-3-one (XXI) was treated with 5% aqueous ethanolic potassium hydroxide or with 75% aqueous sulphuric acid, a substance was obtained, in 66% yield, whose infrared spectrum measured as a nujol mull or in chloroform solution had bands at 3500, 3390 and 3215 $cm.^{-1}$ which could be attributed to a hydroxyl group and primary or secondary amino groups. The spectrum in nujol showed three bands in the carbonyl region at 1712, 1672, and 1626 $cm.^{-1}$ which could be attributed to a carbonyl group in a six membered ring and primary and secondary amide carbonyl groups respectively, while the solution spectrum showed bands at 1708 and 1677 $cm.^{-1}$, which suggested amide or lactam and lactam carbonyl groups respectively, and a band at 1590 $cm.^{-1}$ which was probably due

to the NH deformation mode of an amide. These spectral features were similar to those shown by the 3,5 amide-lactam obtained by Nagata[74] and the hydrolysis product was considered to be an analogous equilibrium mixture of a 1-amide and a 1,3-lactam with the lactam form predominating. Treatment of the lactam with dry methanol containing dry hydrogen chloride resulted in the isolation by chromatography of two products along with unchanged starting material. The first product, which was obtained in too small a quantity to permit full investigation showed infrared absorption bands at 3390 and 1724 cm.$^{-1}$, the latter of which suggested a six membered cyclic ketone. The second product had bands in the infrared at 3320 and 3140 cm.$^{-1}$ which were attributed to NH stretching frequencies. A single band in the carbonyl region at 1695 cm.$^{-1}$ was consistent with a lactam carbonyl group and the substance was formulated as the O-methyl derivative (CXLVIII) of the 1,3 lactam

1β-Cyano-5α-cholestan-3-one (CXLIV) on treatment with 5% aqueous ethanolic potassium hydroxide afforded the same hydrolysis product as its 1α-epimer, hence hydrolysis of one of the epimers had obviously been accompanied by an epimerisation. The stereochemistry of the lactam could not be decided until it was ascertained which cyanoketone had

epimerised. Solutions of each, in 1% ethanolic potassium hydroxide, were allowed to stand at room temperature, and at intervals samples were examined by thin layer chromatography. By this means it was possible to follow the course of hydrolysis of each epimer. The 1β-cyanoketone epimerised before lactam formation and the 1α-cyanoketone went directly to the lactam. After two days, however, a little of the unchanged 1α-cyanoketone had also epimerised. These observations established that the axial configuration at $C_{(1)}$ is preferred, with respect to 1-cyano-5α-cholestan-3-ones. Usually the preferred configuration of substituents in cyclohexane rings is equatorial, but the presence of special steric and electronic factors may result in the axial epimer predominating[111]. In the present case conversion of the cyano group to the axial configuration probably alleviates interaction of the cyano group with the 11α-hydrogen and presents a more favourable electronic arrangement with the $C_{(3)}$ carbonyl group. The product of hydrolysis could now be assigned the structure of 3α-amino-1α-carboxy-3β-hydroxy-5α-cholestane lactam (CXLVI) with some of the 1α-amide form (CXLVII) also present.



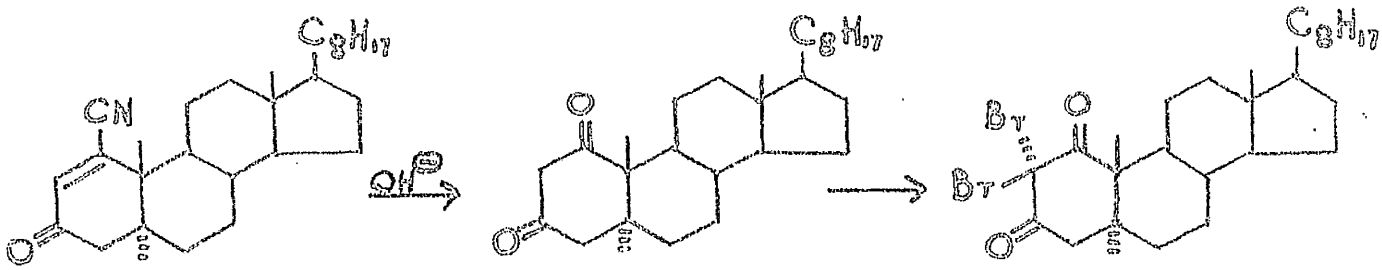
(CXLVI)

(CXLVIII)

(CXLVII)

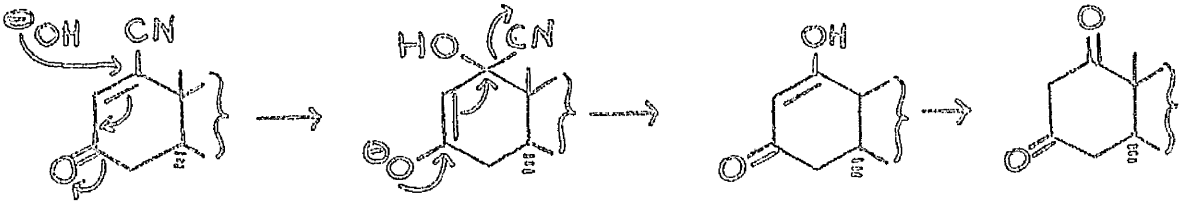
When 1-cyano-5α-cholest-1-en-3-one (CXXXIX) was treated with aqueous ethanolic potassium hydroxide, an odour of cyanide was detected during the working up of the reaction, and the product m.p. 166 - 168, $[\alpha]_D$ + 100°, did not contain nitrogen. Its ultraviolet spectrum, when measured in n-hexane showed very low absorption at about 300 mμ, while in ethanol there was high absorption at 255 mμ (ϵ = 16,000), and in ethanol containing 10% of decinormal aqueous sodium hydroxide, maximum absorption occurred at 285 mμ (ϵ = 19,000). The infrared spectrum measured in carbon tetrachloride showed bands at 1736 and

1710 cm.$^{-1}$ These physical constants were in good agreement with those of 5α-cholestan-1,3-dione[112] (CXLIX). Treatment with bromine in chloroform-methanol gave a dibromoketone whose properties, m.p. 164 - 5°, $[\alpha]_D$-16°, and infrared absorption at 1724 cm.$^{-1}$ with a shoulder at 1739 cm.$^{-1}$ were in agreement with those of 2,2-dibromo-5α-cholestan-1,3-dione[112a] (CL), and thus confirmed that 1-cyano-5α-cholest-1-en-3-one (CXXXIX) with base yielded 5α-cholestan-1,3-dione (CXLIX).



(CXXXIX) (CXLIX) (CL)

A probable mechanism for the reaction is formation of the cyanohydrin (CLI) by attack of hydroxyl ion at $C_{(1)}$ followed by loss of cyanide ion to give the enol (CLII) which subsequently ketonises.



(CLI) (CLII)

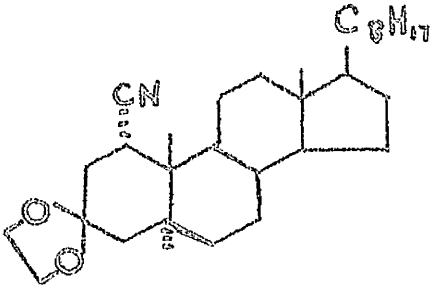
The unsaturated ketone (CXXXIX) was unaffected by treatment with hydrochloric acid in ethanol.

It was apparent that the $C_{(1)}$ cyano group could not be hydrolysed directly to a carboxyl group while a carbonyl group was present at $C_{(3)}$, although the 1-carboxylic acid might have been obtained from the lactam (CXLVI) by Nagata's[76] procedure of O-methylation and then N-mesylation followed by ring cleavage with alkali, but since the first step had only proceeded in 40% yield the procedure was not considered to be of synthetic value.

If the influence of the carbonyl group were removed by ethylene ketal formation it was possible that hydrolysis might yield the carboxylic acid.

Accordingly, the ketal (CLIII) was prepared by treatment of 1α-cyano-5α-cholestan-3-one (XXI) with ethylene glycol and a trace of boron trifluoride etherate at room temperature[113]. The ethylene ketal group was not hydrolysed by treatment with

ether containing a trace of concentrated hydrochloric acid[114], but the ketone was readily regenerated by refluxing with p-toluene sulphonic acid in acetone[115]. Attempts to hydrolyse the cyano group of (CLIII) with aqueous ethanolic potassium hydroxide were unsuccessful, starting material being recovered in each case.

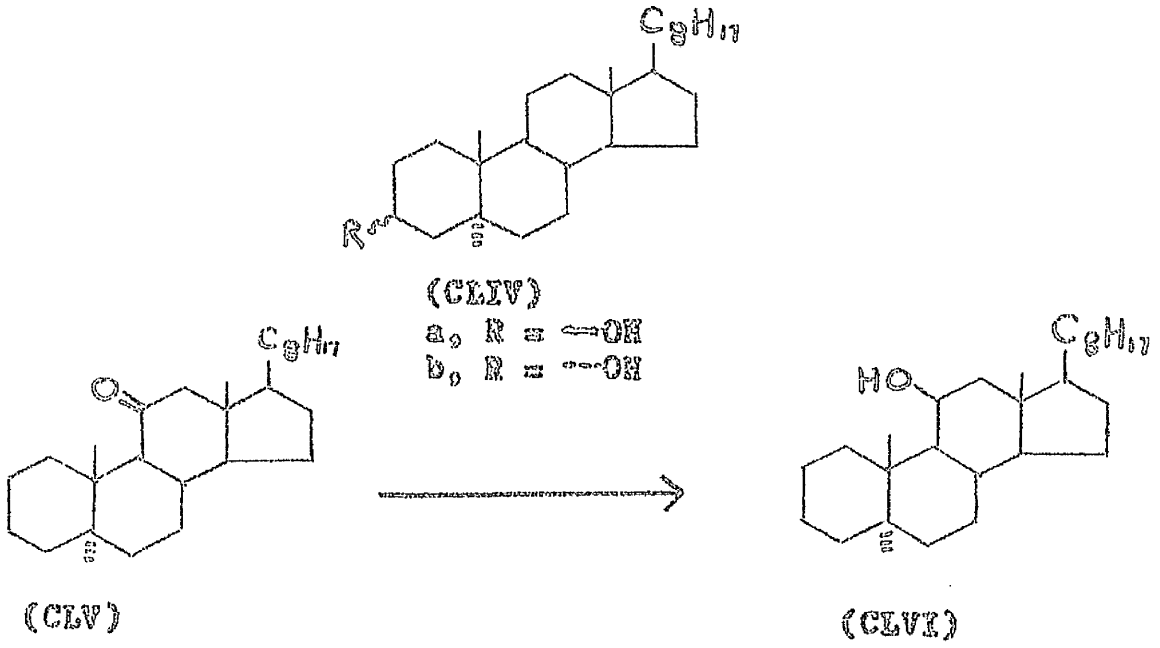


(CLIII)

Another means of eliminating the influence of the carbonyl group at $C_{(3)}$ consists of conversion to a hydroxyl group, using a selective reducing agent which would not attack the nitrile group. It could be envisaged that a 1-carboxylic acid would readily form a γ-lactone with a $C_{(3)}$ hydroxyl group and so yield a cyclic hydrolysis product, which is considered undesirable in the route to the 1-methyl-ketone (CXXXII), but since the proposed Grignard reaction on the $C_{(1)}$ nitrile group necessitates modification of the carbonyl group beforehand and

since interesting effects might result from the presence of a hindering group at $C_{(1)}$ on the reduction of the carbonyl group, attempts to prepare the 1α-cyano-5α-cholestan-3-ols (CLXI) and (CLXII) were justified.

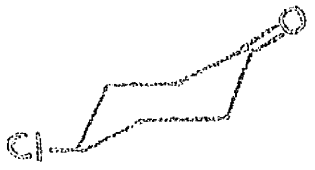
A review of the reduction of cyclic ketones by lithium aluminium hydride and by sodium borohydride[115] shows that an unhindered ketone gives the equatorial alcohol, while a hindered ketone generally gives the axial epimer. For example 5α-cholestan-3-one (XXXV), which is unhindered, yields 5α-cholestan-3β-ol (CLIVa) and a small amount (10%) of the axial epimer 5α-cholestan-3α-ol (CLIVb),[117] while 5α-cholestan-11-one (CLV), containing a hindered carbonyl group gives, on reduction, the 11β axial alcohol (CLVI) only[118].



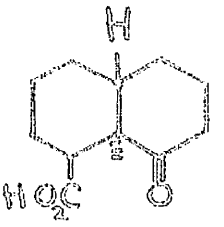
The steric course of reduction of carbonyl groups with complex metal hydrides has been interpreted by Dauben[119] in terms of steric approach control and product development control. The former concerns the first step in the reduction process, the approach of the reducing agent to the carbonyl group, and in the case of an unhindered ketone such as 5α-cholestan-3-one (XXXV) approach is equally easy from either side of the molecule. The factor which determines the configuration of the final product is product development control, which concerns the equilibration of the two possible intermediates and gives in this instance the equatorial (CLIVa) and axial (CLIVb) alcohols in a 9:1 ratio. The more favourable direction of approach to an 11-one group is on the α-face, and this results in the intermediate complex being β-orientated, and hence the exclusive formation of the 11β-alcohol (CLVI). The important factor in the reduction of unhindered ketones is thus product development control, while with hindered ketones steric approach control is more important.

Another factor which has recently been considered to affect the approach of the reducing agent is electrostatic repulsion by neighbouring functional groups. This effect has been inferred to account for the unexpected ratios of epimers obtained by sodium borohydride reduction of 4-chloro-

cyclohexanone[120] (CLVII) and the carboxy-decalone[121] (CLVIII)



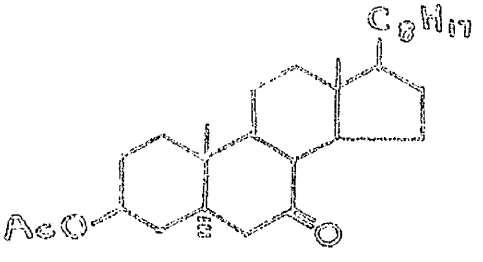
(CLVII)



(CLVIII)

More detailed investigations have been carried out into the effects of a number of complex metal hydrides and changes of solvent on the proportion of axial and equatorial alcohols obtained.

Dauben and his co-workers[119b] found that reduction of 5α-cholestan-3β-ol-7-one acetate (CLIX) with sodium borohydride gives a larger proportion of the axial alcohol than does lithium aluminium hydride, and account for this on a basis of the larger size and solvation of the former.



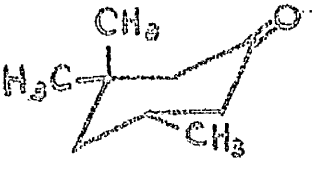
(CLIX)

Wheeler and Huffman[122] have considered the mechanism and manner of dissociation of the complex hydrides in relation of the ratio of epimeric alcohols formed.

Wheeler and Vail[123] studied the reduction of 5α-cholestan-3-one (XXXV) by a number of complex metal hydrides in different solvents and showed that the proportion of axial (3α) alcohol obtained by sodium borohydride reduction increases as solvent is changed from iso-propyl alcohol to ethanol and then methanol. They suggest, as do Beckett and his co-workers[124] from their investigation of the reduction of tropinone, that in methanol, sodium borohydride forms bulky alkoxyborohydrides which attack from the less hindered side and increase the tendency to axial alcohol formation. Jones and Wise[125] reported that in the initial stages of the reduction, before borohydride-solvent interaction has had time to take place a smaller proportion of axial alcohol is present. Wheeler and Vail[123], in summing up, agree with Dauben[119b] that the size of the reducing species is important and also stress the importance of the ionic or covalent character of the reducing agent.

Haubenstock and Eliel[126] investigated the reduction of dihydroisophorone (CLX) and came to the conclusion that lithium aluminium alkoxyhydrides disproportionate to tetra-alkoxy compounds and lithium aluminium hydride, the latter

being the effective reducing species. An exception to this, however, was lithium aluminium tri-tert-butoxyhydride, since formation of lithium tetra-tert-butoxyaluminium was thermodynamically difficult. Those workers were not of the opinion that the results obtained with sodium borohydride in methanol could be explained on a basis of alkoxyborohydride formation[123,124] and showed that the use of methanol as solvent increased the stereoselectivity of the reagent, but agreed that Dauben's[119b] theory of solvation was probably the reason for solvent effects. As an alternative, they suggested differential solvation of the transition states such as to change their steric requirements.



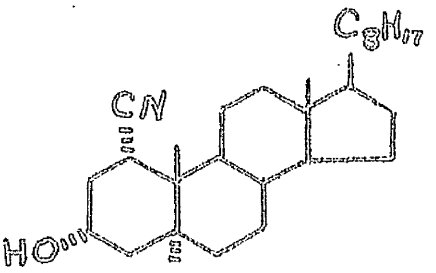
(CLX)

Despite the amount of work that has been carried out, no clear picture of the mechanism of reduction with complex metal hydrides has yet emerged, and further study is necessary. Some

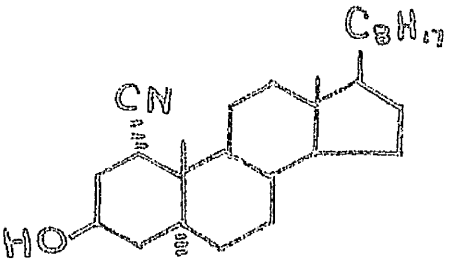
of the discrepancies described here may be attributed to the fact that in some cases, different ketones were used for the reduction studies, thus, although the reaction conditions were the same, the actual environment of the reduction process was not.

Reduction of 1α-cyano-5α-cholestan-3-one (XXI) with sodium borohydride in aqueous methanol for 1 hr. at room temperature gave a product which showed no carbonyl absorption in the infrared but showed bands at 3400 and 2245 $cm.^{-1}$ indicative of hydroxyl and nitrile groups respectively, and which was expected to be an epimeric mixture of 1α-cyano-5α-cholestan-3α-ol (CLXI) and 1α-cyano-5α-cholestan-3β-ol (CLXII), although it was apparently homogeneous when subjected to thin layer chromatography. Column chromatography, moreover, did not result in the separation of epimers. All fractions eluted were recrystallised with difficulty from aqueous methanol and each melted gradually between 90 and 126°, these properties were characteristic of an epimeric mixture. Acetylation with acetic anhydride and pyridine yielded material which also appeared homogeneous on thin layer and column chromatography but could not be crystallised to give a sharp melting substance. The same results were obtained when the time for the reduction was extended to 30 hr., and when the reaction was carried out

by the addition of solid sodium borohydride to the ketone in methanol, showing that no significant equilibration was taking place and that inverse addition does not alter the composition of the final product[123].



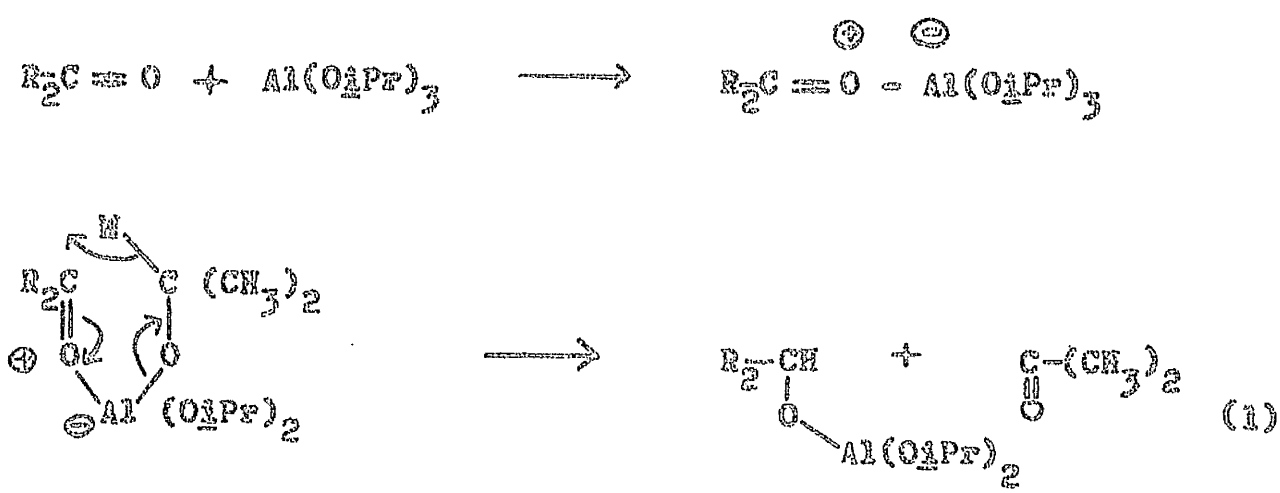
(CLXI)



(CLXII)

When 1α-cyano-5α-cholestan-3-one (XXI) was reduced with sodium borohydride in iso-propyl alcohol, a product was obtained, which had an infrared spectrum consistent with that expected for a 1α-cyano-5α-cholestan-3β-ol, (CLXI) or (CLXII), was homogeneous on thin layer and column chromatography, and could be recrystallised from petrol as needles m.p. 153 - 155°, it readily formed an acetate m.p. 129 - 131° on treatment with acetic anhydride and pyridine. This substance appeared to be a pure 1α-cyano-5α-cholestan-3-ol but the configuration at $C_{(3)}$ was unknown. Rapid Meerwein-Ponndrof reduction of 1α-cyano-5α-cholestan-3-one (XXI), was carried out by distillation of the ketone and aluminium iso-propoxide in iso-propyl alcohol for 1 hour and

a product was obtained in 80% yield, identical in all respects with that obtained by reduction with sodium borohydride in iso-propyl alcohol. Nace and O'Conner have shown that when 5α-cholestan-3-one (XXXV) is reduced in this way a relatively large (28%) proportion of the axial alcohol is formed, the reasons for this, they suggest, are the size of the reducing agent and the mechanism (1) of the reduction which requires a cyclic transition state[128]



(1)

When 1α-cyano-5α-cholestan-3-one (XXI) was reduced with lithium aluminium tri-tert-butoxyhydride in tetrahydrofuran at 0°, conditions known to yield 98.5% of 5α-cholestan-3β-ol (CLIV) from 5α-cholestan-3-one (XXXV),[129] material with infrared absorption at 3400 and 2245 cm.$^{-1}$ and no carbonyl absorption was obtained, which was recrystallised from aqueous

methanol to give (in 50% yield) a product of m.p. 120-122°, unaffected by further recrystallisations. The mother liquors were concentrated and a second product was obtained, which after repeated recrystallisation gave (in 5% yield) a 1α-cyano-5α-cholestan-3-ol (CLXI) or (CLXII) identical with that obtained by sodium borohydride reduction in iso-propyl alcohol and by Meerwein-Ponndorf reduction, partial separation of epimers having been achieved. The first crop was acetylated and the crude product, when examined by thin layer chromatography, showed the presence of two substances of very similar polarity, the less polar of which corresponded to the acetate of the alcohol of m.p. 153 - 155°, but the mixture could not be separated by column chromatography. It had been shown, however, that the material of m.p. 120 - 122 was an epimeric mixture although homogeneous on thin layer chromatography. The epimers were apparently of such similar polarity that they could not be resolved by the chromatographic methods employed, or else they existed as a molecular complex[130], the components of which are formed in the correct proportions when the reduction is carried out with sodium borohydride in aqueous methanol, whereas an excess of the epimer of m.p. 153 - 155° is formed on reduction with lithium aluminium tri-tert-butoxyhydride in tetrahydrofuran.

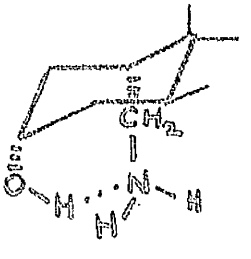
Reduction of 1α-cyano-5α-cholestan-3β-ol (CLXI) or (CLXII) with an excess of lithium aluminium hydride in ether yielded 1α-aminomethyl-5α-cholestan-3β-ol whose infrared spectrum showed bands at 3390 (-OH), 3311, 3205 $cm.^{-1}$ (-NH stretch) and 1590 $cm.^{-1}$ (-NH deformation), and no nitrile absorption. The amine formed a salt with hydrochloric acid, and a diacetate which showed infrared absorption at 3280 and 3080 $cm.^{-1}$ (-NH), 1740 (acetate C = O), 1653 (amideC = O), and 1553 cm (-NH deformation). Hydrolysis of the diacetate with 0.5 mol. of methanolic sodium carbonate at room temperature[131] yielded a monoacetate, preferential hydrolysis of the $C_{(3)}$ acetyl group having taken place, since the infrared spectrum now showed hydroxyl absorption at 3460 $cm.^{-1}$ and no acetate carbonyl absorption at 1740 $cm.^{-1}$. The amide bands were present as before.

Reduction of 1α-cyano-5α-cholestan-3-one (XXI) with an excess of lithium aluminium hydride in ether gave an amine identical to that obtained by reduction of the hydroxy-nitrile (CLXI) or (CLXII), thus showing that this method of reduction of the carbonyl group followed the same stereochemical path as occurred with sodium borohydride in iso-propyl alcohol or aluminium iso-propoxide.

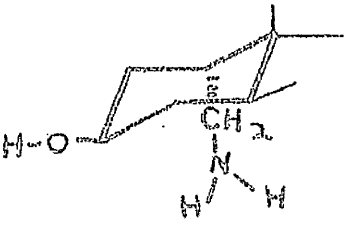
The infrared spectrum of the hydroxy-nitrile (CLXI), or (CLXII), in dilute solution in carbon tetrachloride, showed

hydroxyl absorption at 3663 cm.$^{-1}$. Under the same conditions, the hydroxy-amine showed hydroxyl absorption at 3650 cm.$^{-1}$ the decrease in frequency (13 cm.$^{-1}$)[132] being indicative of intramolecular hydrogen bonding between the hydrogen of the hydroxyl group and the nitrogen of the amino group. The diacetate showed -NH absorption at 3472 and 3448 cm.$^{-1}$ which altered in the mono-acetate to a broad band from 3322 to 3175 cm.$^{-1}$, with the hydroxyl group absorbing at 3640 cm.$^{-1}$ ($\Delta\nu$=23 cm.$^{-1}$). These absorption bands were characteristic of the hydrogen attached to nitrogen being intramolecularly bonded to the oxygen of the hydroxyl group, and the hydroxyl hydrogen being intramolecularly hydrogen bonded to the oxygen of the N-acetyl group respectively.

The stereochemistry at $C_{(1)}$ was known to be the axial, since lithium aluminium hydride would not alter the configuration in the course of reduction, while the stereochemistry at $C_{(3)}$ could be either axial (3α) (CLXIII)or equatorial (3β) (CLXIV). The interatomic distances (> 3.5 Å)[133] in the latter epimer are too great for intramolecular hydrogen bonding to occur, but this would be possible in the diaxial epimer.

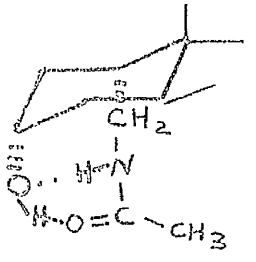


(CLXIII)

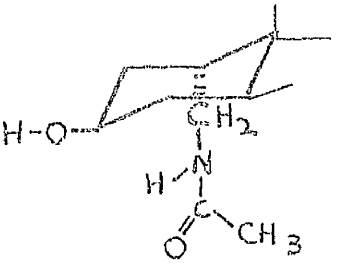


(CLXIV)

A similar situation would prevail in the two possible monoacetates (CLXV) and (CLXVI) epimeric at $C_{(3)}$.

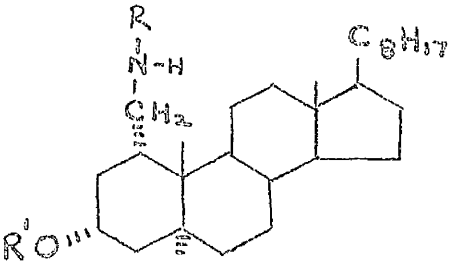


(CLXV)



(CLXVI)

From this evidence it was concluded that the hydroxy-nitrile was 1α-cyano-5α-cholestan-3α-ol (CLXI) and the amine was consequently 1α-aminomethyl-5α-cholestan-3α-ol (CLXVIIa) while the mono- and diacetates had structures (CLXVIIb) and (CLXVIIc) respectively.



(CLXVII)

a, R = R' = H

b, R = Ac, R' = H

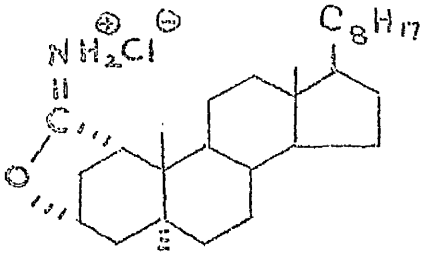
c, R = R' = Ac

Nitriles react with alcohols in the presence of hydrogen chloride to form imino-ether hydrochlorides[134] (2)

$$RC{\equiv}N \;+\; R'OH \xrightarrow{HCl} RC(OR')=NH_2^{+}\,Cl^{-} \qquad (2)$$

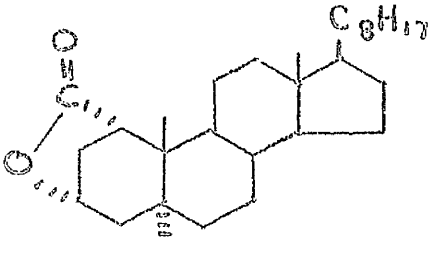
Hydroxy-nitriles yield cyclic imino-ethers on treatment with hydrogen chloride if the hydroxyl group is suitably orientated. Three examples of steroidal cyclic imino-ether formation are recorded in the literature[135] and in each case a 1,3-cis diaxial relationship was required. Examination of molecular models of the four possible 1-cyano-5α-cholestan-3-ols showed that only a 1α,3α arrangement would readily permit cyclic imino-ether formation, a 1β,3β arrangement would perhaps form an imino-ether but would involve considerable ring strain. Neither of the trans compounds 1α,3β and 1β,3α would be able to react.

Treatment of the 1α-cyano-5α-cholestan-3α-ol (CLXI) in ether with hydrogen chloride under anhydrous conditions gave 5α-cholestan-1α,3α-imino-ether hydrochloride (CLXVIII) with characteristic C=N absorption at 1690 $cm.^{-1}$ in the infrared.



(CLXVIII)

The free imino-ether was obtained as an oil by shaking its hydrochloride with sodium bicarbonate solution, followed by extraction with ether and evaporation of the solvent. Hydrolysis[135] of the imino-ether or its hydrochloride (CLXVIII) would be expected to give the lactone (CLXIX), and in practice hydrolysis of the hydrochloride with hydrochloric acid did show evidence of lactone formation in a band in the infrared spectrum at 1770 $cm.^{-1}$. Other products obtained included a nitrile, indicated by a band at 2245 $cm.^{-1}$, (probably 1α-cyano-5α-cholestan-3α-ol from rearrangement of the imino-ether), and some unchanged imino-ether indicated by a band at 1685 $cm.^{-1}$. Thin layer chromatography showed the presence of two other substances in trace amounts but column chromatography failed to effect a separation.



(CLXXIX)

The isolation of the axial (3α) alcohol (CLXI) as the major product of reduction of the ketone (XXI) with sodium borohydride in iso-propyl alcohol or lithium aluminium hydride in ether can be explained in terms of the theories of metal hydride reduction outlined earlier; 1α-cyano-5α-cholestan-3-one (XXI) is a hindered ketone, and so steric approach control is important. The approach of a reducing agent on the α-face is sterically hindered by the axial nitrile group at $C_{(1)}$, there may also be electrostatic repulsion by the cyano group, and so attack occurs from the β-side forming an α-orientated intermediate complex, which gives the axial (3α) alcohol. The increase in the proportion of equatorial alcohol obtained by reduction with sodium borohydride in aqueous methanol probably results from solvation of the reducing agent or formation of complex alkoxyborohydrides thus rendering the intermediate complex rather large for complete stability in the 3α-configuration and so product development control comes into effect. This theory is borne out by the mixture of epimers obtained by reduction with lithium aluminium tri-tert-butoxyhydride which is very bulky, and although it would attack from the β-side to give an α-orientated intermediate, the steric crowding by the nitrile group would encourage conversion to the

equatorial configuration. Meerwein-Ponndorf reduction must have resulted in β-face attack by aluminium iso-propoxide with subsequent ring formation on that face leading to the axial hydroxyl group.

After the completion of this work Julia, Lonares and Simon[22] reported the preparation of four of the compounds described herein, 1α-cyano-5α-cholestan-3-one (XXI), was prepared from 5α-cholest-1-en-3-one (VIIIb) and acetone cyanohydrin. Reduction of the cyanoketone (XXI) with potassium borohydride yielded a compound which Julia considers to be 1α-cyano-5α-cholestan-3β-ol (CLXII), but from its constants and those of its acetate it is obviously what has been shown in this work to be the 3α-alcohol. Julia compared the rotation difference on acetylation with the corresponding difference shown by 5α-cholestan-1α,3β-diol and assigned the 3β-configuration to the reduction product, but later he mentions that 5α-cholestan-1α-ol-3one is reduced to the 1α,3α-alcohol exclusively, although it is recognised that 3-oxo-steroids normally give the 3β-alcohol.

Table 1 compares the constants found by Julia's group with those found by the author.

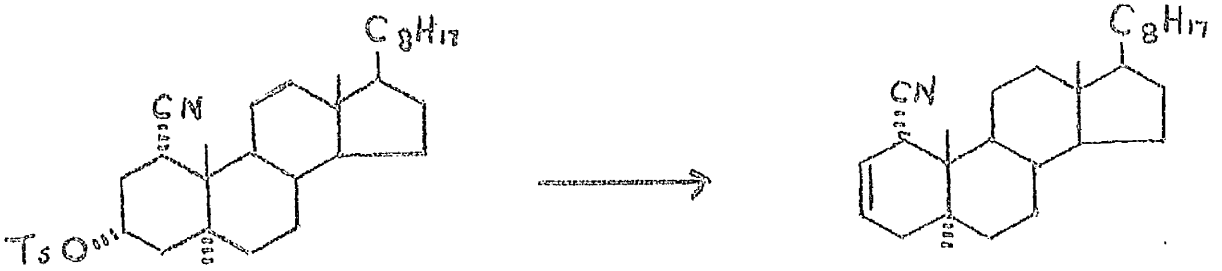
TABLE 1

	Author		Julia et. al.	
	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$
1α-cyano-5α-cholestan-3-one	168 - 169°	+ 56°	164°	+ 58°
1α-cyano-3-ethylene-dioxy-5α-cholestane	182 - 183°	+ 40°	175 - 177°	+ 42°
1α-cyano-5α-cholestan-3α-(3β)ol	153 - 155°	+ 53°	150 - 151.5°	+ 56°
1α-cyano-5α-cholestan-3α-(3β)yl acetate	129 - 131°	+ 29°	134°	+ 31°

Since 1α-cyano-5α-cholestan-3β-ol (CLXII) had not been prepared pure by reduction experiments, it was decided to attempt epimerisation of the 3α-alcohol (CLXI) by hydrolysis of its tosyl ester. This derivative was prepared from the alcohol by treatment with p-toluene sulphonyl chloride in pyridine at 0°. Repeated recrystallisation of the crude product from acetone did not furnish a pure substance, but the infrared spectrum with bands at 2257 (CN), 1600 (aromatic)

and 815 cm.$^{-1}$ (1,4-disubstituted benzene ring) indicated that the desired cyano-tosylate had been prepared. Purification by chromatography was not attempted since tosylates are unstable to such treatment[136]. Chang and Blickenstaff[137] heated 5α-cholestan-3β-yl tosylate in dimethylformamide and obtained a mixture of 5α-cholest-2-ene and 5α-cholestan-3α-yl formate, chromatography of which hydrolysed the latter to 5α-cholestan-3α-ol (CLIVb). A modification[138] of this method was used in an attempt to epimerise the crude 1α-cyano-5α-cholestan-3α-yl tosylate (CLXX). The ester was heated at 100-105° for 5 hr. in aqueous dimethylformamide in the presence of sodium acetate. The crude product was chromatographed to give (in 20% yield) a substance which was shown by analysis to contain carbon, hydrogen, and nitrogen only, and whose infrared spectrum showed peaks at 2250 (CN) and 1658 cm.$^{-1}$ (C = C). On this evidence it was concluded that the substance was 1α-cyano-5α-cholest-2-ene (CLXXI). The next material (15%) to be eluted was shown by thin layer chromatogtaphy to be a mixture of two substances, the infrared spectrum of which showed the presence of a nitrile group, and peaks characteristic of the starting tosylate were also present; in addition there was a band at 1740 cm.$^{-1}$ which was suggestive of an acetate group. The mixture showed ultraviolet absorption at 226 mμ. Finally 1α-cyano-5α-cholestan-3α-ol (CLXI) was

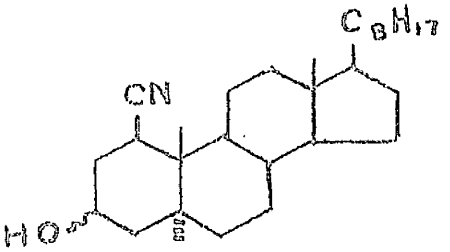
eluted, which could have resulted from hydrolysis of the tosylate (CLXX) or from initial incomplete tosylation. The mixture was rechromatographed on silica gel but still no separation was achieved. It was then treated with ethanolic potassium hydroxide and the band at 1740 $cm.^{-1}$ disappeared, being replaced by hydroxyl absorption at 3450 $cm.^{-1}$, thin layer chromatography showed that one component of the mixture had become more polar, ultraviolet absorption was still at 226 $m\mu$. Thus it appeared that one component of the mixture had been an acetate or perhaps a formate. A further chromatography resulted in the isolation of a small quantity of material which was homogeneous and had infrared and ultraviolet absorption similar to the starting tosylate, the melting point, however was some 30° lower. The material must have been the 3α- or 3β-tosylate, but was obtained in too small a quantity for satisfactory identification. Continued elution yielded only gummy material which was a mixture probably containing unchanged tosylate and some alcohol which had resulted from hydrolysis of the tosylate as it passed through the chromatographic column, along with the alcohol whose ester was present in the original mixture.



(CLXX) (CLXXI)

When 1β-cyano-5α-cholestan-3-one (CXLIV) was reduced with sodium borohydride in aqueous methanol, and the crude product examined by thin layer chromatography, it was found that reduction had resulted in the formation of one major product and a trace of another of very similar polarity. The major product was obtained exclusively when the reduction was conducted in iso-propyl alcohol. Its infrared spectrum was consistent with that expected for 1β-cyano-5α-cholestan-3ξ-ol (CLXXII) with bands at 3500 and 2245 $cm.^{-1}$ indicative of hydroxyl and nitrile groups respectively. The alcohol readily formed an acetate on treatment with acetic anhydride and pyridine. When the alcohol, in ether, was treated with hydrogen chloride, under anhydrous conditions, no reaction occurred. This does not unequivocally establish the configuration at $C_{(3)}$ since a 1β,3β-imino-ether could form with difficulty. If ring A is in a chair conformation, then any hindrance by the cyano group to the approach of the reducing agent would be on the

β-face of the molecule thus encouraging α-face attack and subsequent formation of the 3β-alcohol. Ring A, however, probably does not exist as a true chair, and there may be some slight distortion of the ring as a result of the interaction of the 1β-cyano group with the 11α hydrogen[89]. Thus the configuration at $C_{(3)}$ in 1β-cyano-5α-cholestan-3β-ol remains unknown. Further work, such as reduction to the amine followed by a study of hydrogen bonding effects would perhaps provide the answer.



(CLXXII)

The hope that 1α-cyano-5α-cholestan-3α-ol (CLXI) would hydrolyse was not realised. Attempted hydrolyses with varying concentrations of potassium hydroxide in aqueous ethanol, potassium hydroxide in ethylene glycol, potassium hydroxide and hydrogen peroxide in dioxan, and ethanolic hydrogen chloride all resulted in almost quantitative

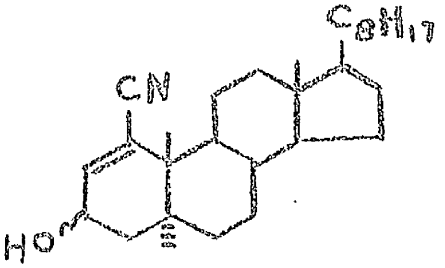
recovery of starting material, which in some cases was contaminated with a substance which showed a very small peak in the carbonyl region of the infrared, but it was never large enough to merit isolation of the substance or the use of the reaction as a synthetic procedure.

Cyano-steroids have been converted to methyl ketones in good yield by means of the Grignard reaction (3) by several groups. For example, Butenandt[139] and Burn and Petrow[140] prepared 17-acetyl-steroids and Nathanson and his co-workers[53] prepared 3-acetyl-steroids.

$$R\text{-}C\equiv N \;\uparrow\; CH_3MgX \longrightarrow R\text{-}\underset{\underset{NMgX}{\|}}{C} \xrightarrow{H_2O} R\text{-}\underset{\underset{O}{\|}}{C}\text{-}CH_3 \qquad (3)$$

When 1α-cyano-5α-cholestan-3α-ol (CLXI) and 1α-cyano-3-ethylene-dioxy-5α-cholestane (CLIII) were treated with methyl magnesium iodide or methyl magnesium bromide in ether or in ether-benzene, little or no reaction took place although reaction times of up to 65 hr. were allowed. Attack by the bulky Grignard reagent was probably prevented by the hindered nature of the nitrile group and it was felt that the use of a smaller organometallic reagent would perhaps overcome the steric hindrance. Methyl lithium[141], in ether and in tetrahydrofuran, was tried without any appreciable reaction taking place. Although its use in

the former solvent gave material with a small carbonyl peak in the infrared, no pure crystalline carbonyl compound, however could be isolated by chromatography of the crude product or by formation of a 2,4-dinitrophenylhydrazone. The reported successful Grignard reactions[53,139,140] had involved the use of α,β-unsaturated nitriles and so it was decided to attempt the Grignard reaction on a 1-cyano-Δ^1-steroid. 1-Cyano-5α-cholest-1-en-3-one (CXXXIX) was reduced with sodium borohydride in methanol to give 1-cyano-5α-cholest-1-en-3β-ol (CLXXIII), characterised by the hydroxyl, cyano, and double bond peaks at 3450, 2237, and 1684 cm.$^{-1}$ respectively, and by its absorption at 220 mμ (ϵ = 9300) in the ultraviolet caused by the α,β-unsaturated nitrile system.

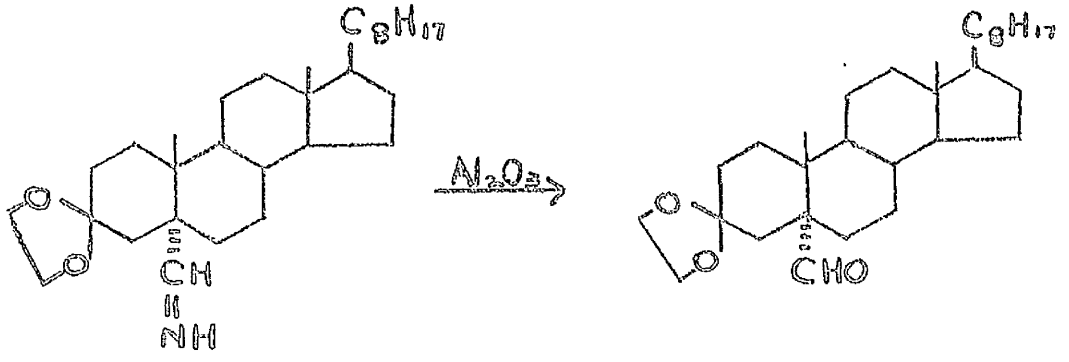


(CLXXIII)

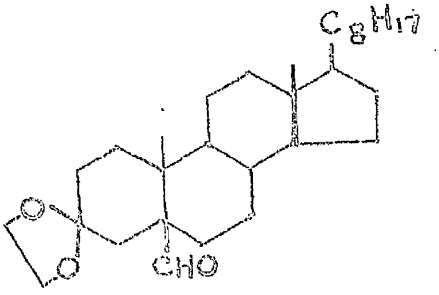
The unsaturated cyano-alcohol (CLXXIII) proved to be resistant to hydrolysis with aqueous ethanolic potassium

hydroxide and to treatment with methylmagnesium iodide and with methyl lithium.

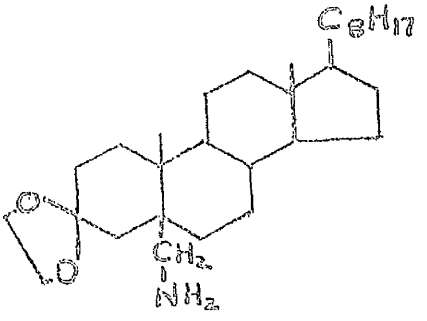
Another possible approach to a 1-carboxylic acid would be oxidation of an aldehyde group. While reduction of nitriles with an excess of lithium aluminium hydride leads to amines many examples are recorded of partial reduction to aldehydes[142] by the use of small amounts of the reducing agent. Brown and his co-workers[143] have also found lithium aluminium tri-ethoxy-hydride a convenient reagent for the conversion of nitriles to aldehydes in the aromatic series. Nagata et. al.[76] reduced 5α-cyano-3-ethylenedioxy-cholestane with lithium aluminium hydride or lithium aluminium tri-ethoxyhydride and obtained in good yield, the imine (CLXXIV), which on contact with alumina was converted to the aldehyde (CLXXV), while the epimeric 5β-cyano-3-ethylenedioxy-cholestane gave a mixture of the aldehyde (CLXXVI), the amine (CLXXVII), and the dimeric anil (CLXXVIII).



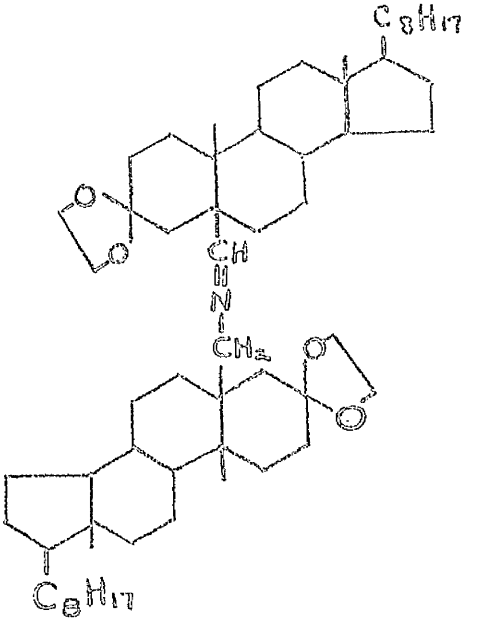
(CLXXIV) (CLXXV)



(CLXXVI)



(CLXXVII)

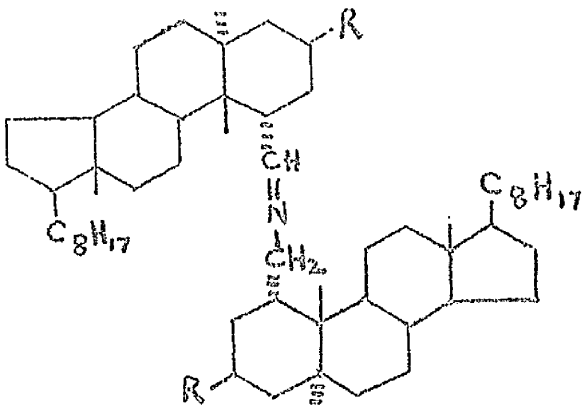


(CLXXVIII)

Treatment of 1α-cyano-5α-cholestan-3α-ol (CLXI) with one molar equivalent of lithium aluminium hydride gave (in 25% yield) a substance of doubtful homogenity which showed infrared absorption at 3640 and 1670 $cm.^{-1}$ attributable to hydroxyl and C = N functions respectively. The substance was found to contain nitrogen in a percentage consistent with its being the dimeric anil (CLXXIXa) or a 1:1 mixture of the imine (CLXXXa) and the aldehyde (CLXXXIa). The presence of aldehyde was discounted, since it would be very unlikely for the aldehyde

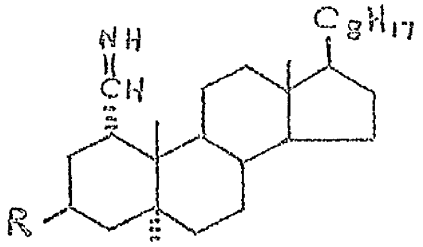
carbonyl absorption in the infrared to be so low as to coincide with the C = N absorption, and since no typical aldehydic derivatives could be obtained. The carbon and hydrogen analyses were not consistent with either of the above suggestions regarding the reduction product.

Treatment of 1α-cyano-3-ethylenedioxy-5α-cholestane (CLIII) with an excess of lithium aluminium hydride in ether afforded, in very low yield, a substance with infrared absorption at 1710 $cm.^{-1}$ and which gave a precipitate with 2,4-dinitrophenylhydrazine solution and so appeared to be 3-ethylenedioxy-5α-cholestan-1α-al (CLXXXIb), and a substance with C = N absorption in the infrared at 1650 $cm.^{-1}$ in too low a yield (3%) to be characterised. When the ketal (CLIII) was treated with one equivalent of lithium aluminium hydride in ether, the only crystalline material isolated was a small yield (8%) of a substance with a band in the infrared at 1680 $cm.^{-1}$ and which was probably 3-ethylenedioxy-1α-imino-5α-cholestane (CLXXXb). Julia[22] obtained 3-ethylenedioxy-5α-cholestan-1α-ol (CLXXXII) by reduction of the ketal (CLIII) with lithium aluminium hydride in tetrahydrofuran but could not isolate any pure materials or interpret the results by reduction in ether. Treatment of the alcohol (CLXI) and the ketal (CLIII) with lithium aluminium tri-ethoxyhydride in ether resulted in recovery of unchanged starting material.



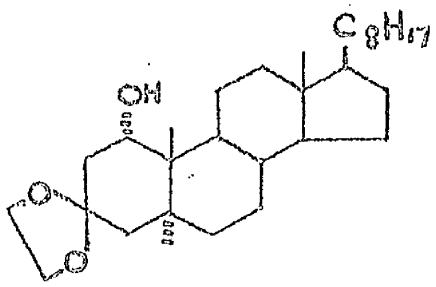
(CLXXIX)

a, R = ...OH
b, R = 

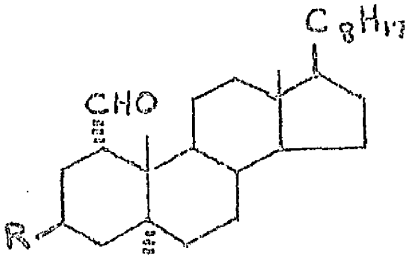


(CLXXX)

a, R = ...OH
b, R = 



(CLXXXII)



(CLXXXI)

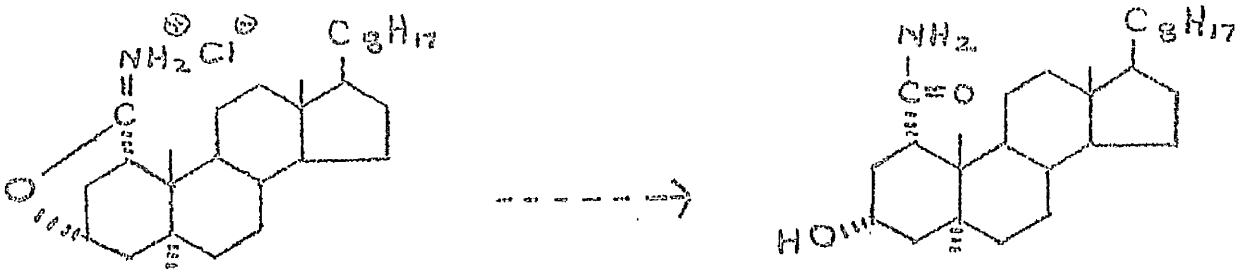
a, R = ...OH
b, R = 

Another method for conversion of nitriles into aldehydes is the Stephens reduction[144], with stannous chloride in ether in the presence of hydrogen chloride. An aldimine hydrochloride-stannic chloride complex is formed which on hydrolysis yields an aldehyde. The reaction proceeds in high yield in many cases, but there are some unaccountable instances in which it does not go at all[144]. Treatment of 1α-cyano-5α-cholestan-3α-ol (CLXI) with stannous chloride in ether saturated with hydrogen chloride and subsequent hydrolysis of the product with boiling water afforded the amide (CLXXXIII) (9%) with

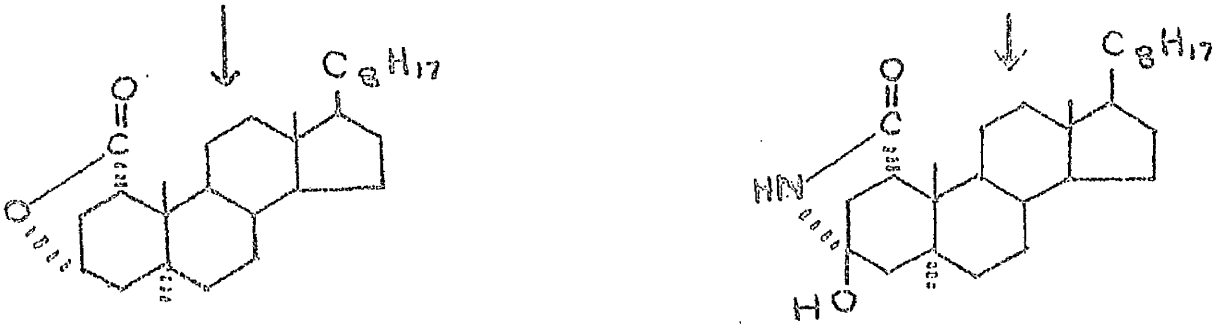
infrared absorption at 1670 and 1630 $cm.^{-1}$ corresponding to an amide carbonyl group and N - H deformation respectively. The $C_{(3)}$-hydroxyl group of the amide was oxidised to a carbonyl and the stable lactam form (CXLVI) of the keto-amide (CXLVII), which had already been prepared by another route was isolated, thus confirming the structure of the reduction product.

The isolation of an amide from an attempted Stephen reduction is unusual; it has already been shown that in the absence of stannous chloride an imino-ether hydrochloride (CLXVIII) is formed which shows evidence of lactone formation on hydrolysis with hydrochloric acid. Another property of imino-ether hydrochlorides is their ability to lose alkyl chloride on heating to give the amide[134] (4). With cyclic imino-ether hydrochlorides the chlorine atom would be expected to appear at the position of the original hydroxyl group. If this reaction had occurred in the present work, a chlorine substituent would have been expected at $C_{(3)}$ instead of the hydroxyl group which was found. The amide (CLXXXIII) was the only identifiable product of the reaction and further study of the reaction on a larger scale would be necessary to clarify the mechanism of amide formation.

$$R - \underset{\underset{OR'}{|}}{C} = NH_2^{\oplus}Cl^{\ominus} \longrightarrow R - \underset{\underset{O}{\|}}{C} - NH_2 + R'Cl \qquad (4)$$



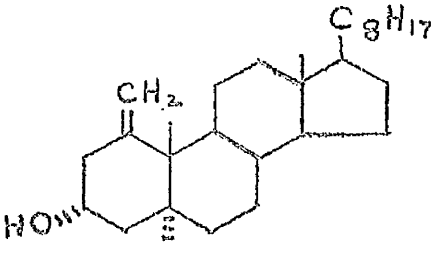
(CLXVIII) (CLXXXIX)



(CLXIX) (CXLVI)

A typical reaction of aliphatic primary amines, is their deamination by the action of nitrous acid to form alcohols and olefins[145]. This reaction has been carried out on steroidal amines[112b,146,147] but in some cases the reaction was slow and the yield poor. When 1α-aminomethyl-5α-cholestan-3α-ol (CLXVIIa) was treated with nitrous acid at room temperature for 24 hr., the main product was the ether insoluble acetic acid salt of the amine, characterised by bands in the infrared at 1621 ($NH_3^{\oplus}$) and carboxylate anion bands at 1540, 1408, and 1379 $cm.^{-1}$ and by its conversion to the parent amine by treatment with sodium hydroxide. The minor, ether soluble product was a mixture of substances which showed a small peak at 1740 $cm.^{-1}$ in the infrared indicating partial acetylation. No pure crystalline material was obtained either after

hydrolysis or after acetylation, and the material was still a mixture. Infrared absorption at 1637 and 885 cm.$^{-1}$ indicated that the olefin (CLXXXIV) was present.



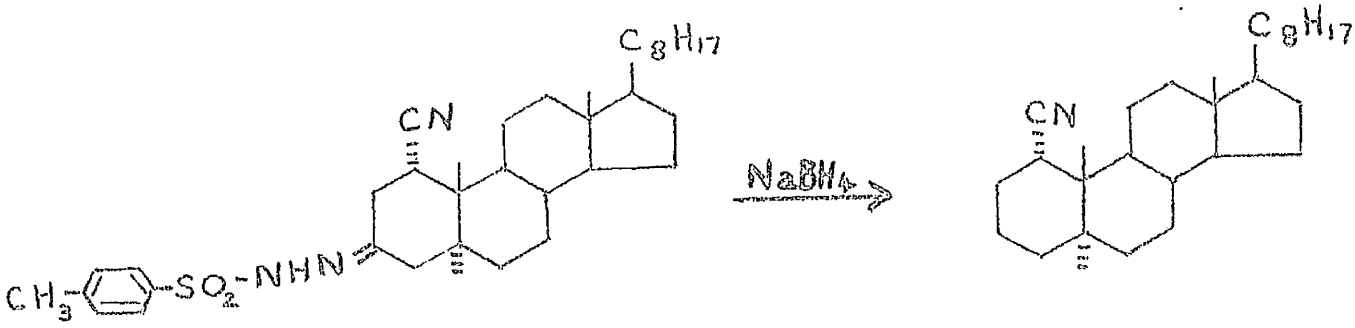
(CLXXXIV)

Bladon and McMeekin[148] were able to convert a steroid lactam to a lactone and then to a hydroxy acid by treatment with nitrous acid in acetic anhydride and acetic acid. An analogous reaction was attempted on 3β-hydroxy-3a-amino-1a-carboxy-5a-cholestane lactam (CXLVI) but starting material was recovered, nor did the lactam react with aqueous nitrous acid.

Since the substituent at $C_{(3)}$ had in many cases participated in reactions with the $C_{(1)}$ nitrile group, it was decided to investigate the properties of compounds possessing only hydrogen substituents at $C_{(3)}$.

The tosylhydrazone (CLXXXV) of 1a-cyano-5a-cholestan-3-one (XXI) was readily prepared by treatment of the ketone in methanol with p-toluenesulphonylhydrazine. The infrared spectrum of the tosylhydrazone showed nitrile absorption at

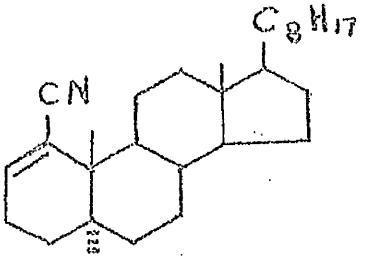
2245 and aromatic absorption at 1597 and 813 cm.$^{-1}$. Reduction of the tosylhydrazone (CLXXXV) with sodium borohydride in dioxan[149] followed by chromatography of the crude product afforded (in 60% yield) a substance which contained only carbon, hydrogen, and nitrogen, and had infrared absorption at 2252 cm.$^{-1}$ (CN), showing it to be 1α-cyano-5α-cholestane (CLXXXVI). Continued elution of the column gave a small quantity of unchanged tosylhydrazone (CLXXXV), and finally 1α-cyano-5α-cholestan-3α-ol (CLXI). The isolation of the latter compound was unexpected and it was considered to have arisen from unchanged ketone in the tosylhydrazone or from the hydrolysis of some of the tosylhydrazone in the course of the reaction to give the ketone which was subsequently reduced. Reduction of the tosylhydrazone with sodium borohydride in methanol was less successful, only a 15% yield of impure 1α-cyano-5α-cholestane (CLXXXVI) was obtained despite a longer reaction time. The impurity appeared, from examination of the mixture by thin layer chromatography, to be 1α-cyano-5α-cholest-2-ene (CLXXI). The infrared spectrum showed very weak absorption at 1665 cm.$^{-1}$ indicating the presence of a double bond. Unchanged tosylhydrazone was the only other material isolated from the reaction.



(CLXXXV) (CLXXXVI)

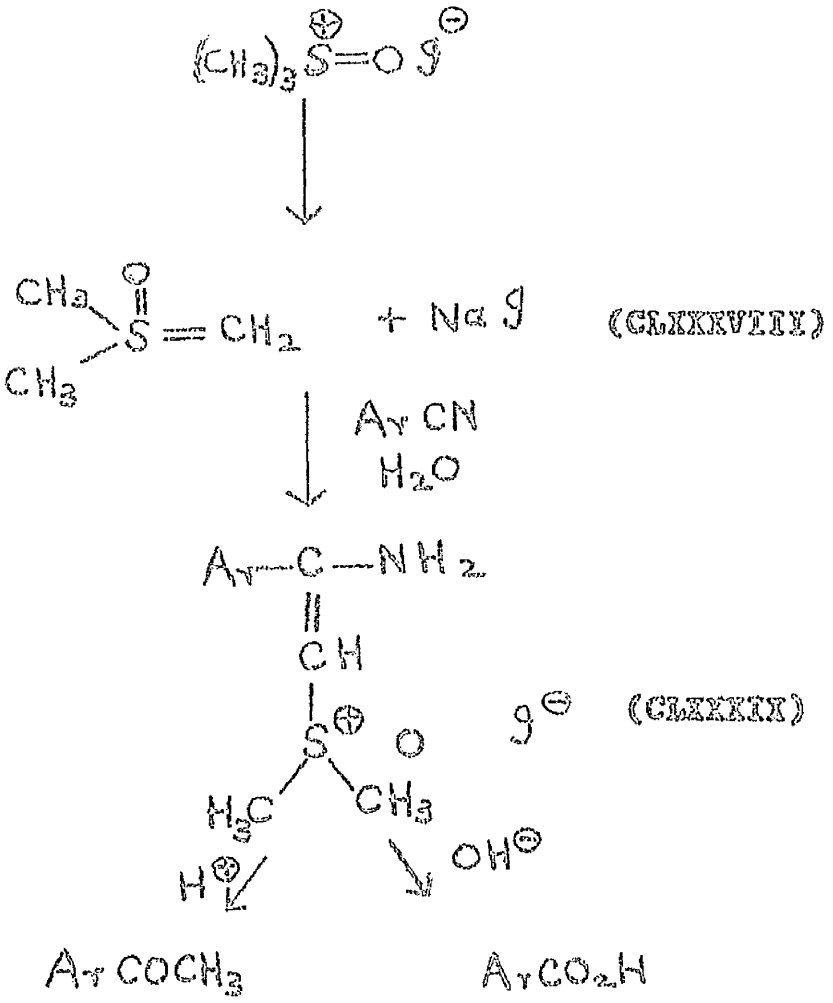
Aqueous ethanolic potassium hydroxide did not hydrolyse 1α-cyano-5α-cholestane (CLXXXVI), and it was unaffected by treatment with methyl magnesium iodide.

When 1α-cyano-5α-cholest-2-ene (CLXXI) was refluxed with aqueous ethanolic potassium hydroxide, a gum was obtained which still showed nitrile absorption in the infrared but the olefinic absorption band at 1659 $cm.^{-1}$ had become more complex. Examination of the product by thin layer chromatography showed it to be a mixture of two substances of similar polarity, one of which was starting material. There was new strong ultra-violet absorption at 217 mμ which suggested that the double bond had moved into conjugation with the nitrile group to give 1-cyano-5α-cholest-1-ene(CLXXXVII). A successful separation of the two materials by column chromatography was not achieved.



(CLXXXVIII)

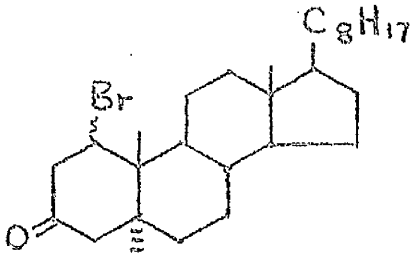
König and Metzger[150] found that aromatic nitriles react with dimethylsulphoxonium methylide[159] (CLXXXVIII) (prepared from trimethylsulphoxonium iodide[152] and sodium hydride) to form a complex salt (CLXXXIX), which is isolable and on treatment with alkali gives a carboxylic acid, while treatment with acid gives a methyl ketone. This reaction was repeated, and although the intermediate could not be isolated, favourable yields of acetophenone (isolated as its 2,4-dinitrophenylhydrazone) and benzoic acid were obtained from benzonitrile. The use of a sodium hydride dispersion in oil was probably a contributory factor in the failure to isolate the intermediate. When the reaction was attempted on 1α-cyano-5α-cholestan-3α-ol (CLXI) starting material was quantitatively recovered.



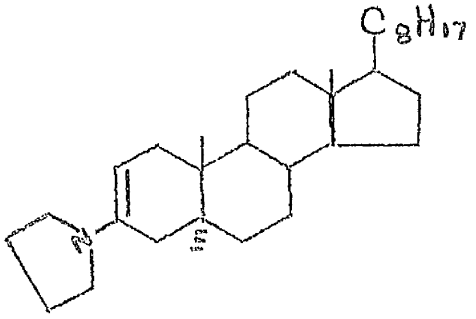
Although Grignard reagents had failed to attack the cyano group at $C_{(1)}$, it was possible that a 1-halo-steroid would itself form a Grignard reagent. If this could be achieved, then subsequent treatment with carbon dioxide would afford the desired 1-carboxylic acid (CKXXVI). Since 1-halo-steroids in the 5α-cholestane series appear to be unknown, this type of compound would first have to be synthesised.

Although the chemistry of enamines has been highly developed in recent years[153], there is no record of allylic

bromination of an enamine with N-bromosuccinimide. Treatment of an enamine of 5α-cholestan-3-one (XXXV) with this reagent would, it was hoped, afford 1β-bromo-5α-cholestan-3-one (CXC). When 5α-cholestan-3-one pyrrolidine enamine[154] (CXCI) was treated with N-bromosuccinimide followed by decomposition of the product in refluxing ethanol, no pure β-bromoketone was isolated. The product gave a positive Beilstein test but could not be purified and appeared to consist mainly of 5α-cholestan-3-one (XXXV).



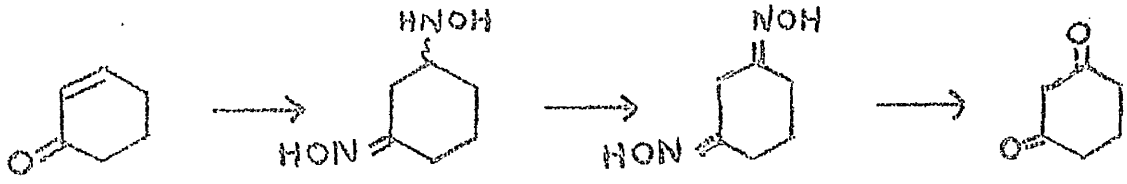
(CXC)



(CXCI)

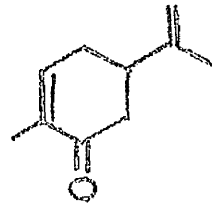
In 1909, Kötz and Grethe reported that when cyclohexenone (CXCII) is treated with two molar equivalents of hydroxylamine, the reagent, in addition to attacking the carbonyl group in the usual way, also adds to the double bond to give the compound (CXCIII), which when refluxed in water with

mercuric oxide is dehydrogenated to the dioxime (CXCIV) of cyclohexan-1,3-dione (CXCV) from which the ketone (CXCV) is readily obtained by treatment with 10% sulphuric acid.



(CXCII) (CXCIII) (CXCIV) (CXCV)

Baddeley and Brocklehurst[156] recently studied the reaction of carvone (CXCVI) with hydroxylamine and found that α,β- addition of hydroxylamine proceeds thirty times faster than oxime formation in neutral media. These workers did not conduct any dehydrogenation experiments.



(CXCVI)

Application of Kötz and Grethe's reaction sequence to 5α-cholest-1-en-3-one (VIIIb) would provide a new route to

5α-cholestan-1,3-dione (CXLIX), but in actual fact, treatment of (VIIIb) with hydroxylamine resulted in the isolation of a substance which was shown by analysis to contain only one nitrogen atom. Ultraviolet absorption at 233 mμ (ϵ = 15,000) indicated that the product was an α,β-unsaturated oxime, and so no α,β-addition had taken place.

Many of the reactions carried out in this work merit further investigation to determine the exact nature of the products, but since the problem was of a synthetic nature, it was considered more useful to explore as many approaches to the desired compounds as possible rather than to study reactions which went in low yield.

Nuclear Magnetic Resonance

Much of the interest in the nuclear magnetic resonance spectra of steroids has been concerned with the chemical shifts of the $C_{(18)}$ and $C_{(19)}$[157,158,159] methyl groups, and the way in which the shifts are affected by other functional groups in the molecule. The studies have shown that the changes are constant for a given functional group and they have been attributed mainly to the anisotropy in the magnetic molecular susceptibility of the group.

Table 2 shows the results obtained for some 1-cyano-steroids compared with the corresponding cholestane derivatives which were all calculated from the contributions reported by Zürcher,[159b] with the exception of those of 3-ethylenedioxy-5α-cholestane, which were calculated from a combination of data given by Cross and Harrison[160] and Zürcher.[159b] The chemical shifts can be considered to be accurate to within ±.02 τ.

TABLE 2

Parent Compound	Chemical shift (τ)		Cyano-derivative	Chemical shift (τ)		Δ_{A-B} (τ)	
(A)	$C_{(19)}$	$C_{(18)}$	(B)	$C_{(19)}$	$C_{(18)}$	$C_{(19)}$	$C_{(18)}$
5α-cholestane	9.22	9.36	1α	9.12	9.36	.10	.00
5α-cholestan-3-one	8.98	9.32	1α	8.90	9.31	.08	.01
5α-cholestan-3α-ol	9.22	9.35	1α	9.09	9.35	.14	.00
2α-bromo-5α-cholestan-3-one	8.91	9.32	1α	8.80	9.32	.11	.00
3-ethylenedioxy-5α-cholestane	9.18	9.34	1α	9.08	9.34	.10	.00
cholest-4-en-3-one	8.81	9.28	1α	8.72	9.28	.09	.00
5α-cholest-1-en-3-one	8.97	9.31	1	8.80	9.30	.17	.01
cholesta-1,4-dien-3-one	8.77	9.26	1	8.47	9.36	.30	.00
5α-cholestan-3-one	8.98	9.32	1β	8.73	9.32	.25	.00
2α-bromo-5α-cholestan-3-one	8.91	9.32	1β	8.65	9.32	.26	.00
5α-cholestan-3α-ol	9.22	9.35	1β	8.91	9.34	.31	-.01
5α-cholestan-3β-ol	9.18	9.25				.27	-.01

A 1-cyano group has no significant effect on the chemical shift of the $C_{(18)}$ methyl group, but does, however, induce a low field shift in the $C_{(19)}$ methyl signal especially when the cyano group is in the 1β-configuration and is consequently nearer to the $C_{(19)}$ methyl group. There is fair agreement between the 1α-cyano-steroids and between the 1β-cyano-steroids studied, but 1-cyano-5α-cholest-1-en-3-one and 1-cyano-cholesta-1,4-dien-3-one do not show agreement with either set of values, since in calculations of this nature the whole unsaturated system has to be taken as a single entitity and the Δ^1 double bond will alter the position of the nitrile group relative to the $C_{(19)}$ protons.

Levisalles and his co-workers[156] have shown the additive shift of the 5α-cyano group in 5α-cyano-cholestan-3-one (XCVIIa) to be 0.125 τ. Cross and Harrison[160] in a more extensive study involving six 5α-cyano-steroids and three 5β-cyano-steroids calculated the value for the long range shielding of the $C_{(19)}$ methyl protons by the C≡N triple bond, and the calculations predicted a shift to higher field. Experiment, however, showed a low field shift, and they attributed the discrepancy to the anisotropy of the $C_{(5)}$ - C≡N single bond and to the distortion of the magnetic environment of the methyl group by the $C_{(5)}$- C≡N dipole. The combined effect of these

factors was opposite in sign to and greater in magnitude than the long range shielding by the C≡N triple bond. The overall contribution of a 5α-cyano substituent to the chemical shift of the $C_{(19)}$ protons was between 0.12 and 0.18 τ, while in the case of a 5β-cyano substituent it was between 0.23 and 0.25 τ. Neither a 5α- nor a 5β-cyano group had any significant effect on the chemical shift of the $C_{(18)}$ methyl group.

TABLE 1

Compound	Solvent	Conc.[a] (mg./ml.)	Peak	Trough	Amplitude
1α-cyano-5α-cholestan-3-one (XXI)	n-heptane	0.11	$[\phi]_{324}$ + 2160°	$[\phi]_{292}$ - 100°	+23
	methanol	0.27	$[\phi]_{312}$ + 2654°	$[\phi]_{275}$ - 838°	+35
1β-cyano-5α-cholestan-3-one (CXLIV)	n-heptane	0.16	$[\phi]_{320}$ + 1206°	$[\phi]_{274}$ - 1935°	+31
	methanol	0.33	$[\phi]_{311}$ + 883°	$[\phi]_{266}$ - 1456°	+23
2α-bromo-1α-cyano-5α-cholestan-3-one (CXXXVIII)	methanol	0.51	$[\phi]_{316}$ + 2000°	$[\phi]_{268}$ - 2540°	+45
2α-bromo-1β-cyano-5α-cholestan-3-one (CXLV)	methanol	0.64	$[\phi]_{310}$ + 2180°	$[\phi]_{258}$ - 1069°	+32

Optical Rotatory Dispersion.

The results of optical rotatory dispersion measurements on some 1-cyano-steroids are shown in table 3.

The curves of (XXI) and (CXLIV) show positive Cotton effects typical of 3-oxo-5α-steroids[161]. The fact that the curve of 1β-cyano-5α-cholestan-3-one (CXLIV) agrees with the octant rule[87] suggests that ring A is in the normal chair form and thus the 1β-cyano group is not large enough to cause ring distortion through interaction with the 11α-hydrogen as in the case of 1β-methyl steroids[89]. The amplitudes of the Cotton effects of 1α-cyano5α-cholestan-3-one (XXI) in n-heptane and methanol are +35 and +23 respectively, compared with +54 and +42 for 5α-cholestan-3-one (XXXV) in the same solvents[162], thus the contribution of the 1α-cyano group to the amplitude of the Cotton effect is -19, and is independent of solvent polarity. This relatively large contribution is in contrast to that of the cyano group in 5α-cyano-cholestan-3-one and 8β-cyano-3,7-diethylenedioxy-androst-5-en-11-one where it is -3 and -6 respectively, and is attributed to disymmetric solvation rather than to dipole interaction between the carbonyl and nitrile groups[162].

Both bromo-ketones (CXXXVIII) and (CXLV) show a positive Cotton effect and agree with the axial halo-ketone rule[84] thus confirming the 2α-configuration assigned to each bromine atom on the basis of infrared carbonyl frequency shifts.

EXPERIMENTAL

All melting points are uncorrected. Specific rotations were determined in chloroform solution in a 1 dm. tube at room temperature. Ultraviolet absorption spectra were measured in ethanol solution with a Perkin - Elmer 137 U.V. spectrophotometer. Infrared absorption spectra were measured as potassium chloride discs, (unless otherwise stated) with a Grubb Parsons S.4 double beam spectrophotometer with sodium chloride optics. Nuclear magnetic resonance spectra were measured with a Perkin Elmer 40 M.c. spectrometer, in deuterochloroform solution with tetramethylsilane as an internal standard. Spence grade H alumina was used for column chromatography, (unless otherwise stated), and Merck's Kieselgel G was used for thin layer chromatography. 'Petrol' refers to petroleum-ether b.p.60 - 80°.

1α-Cyano-5α-cholestan-3-one. - (A) 5α-Cholest-1-en-3-one (3.86 g.), potassium cyanide (1.30 g.), and ammonium chloride (0.79 g.) in dimethylformamide (80 ml.) and water (10 ml.) were stirred at 100° for 8 hr. After removal of the solvent *in vacuo*, water was added and the product was extracted with ether. The ethereal extract was washed 3 times with water, dried over anhydrous sodium sulphate, and evaporated to dryness to give a pale yellow solid (3.46 g.) which was recrystallised several times from chloroform-methanol to furnish 1α-cyano-5α-cholestan-3-one (2.60 g.) as needles m.p. 168 - 169°. $[\alpha]_D$ + 56° (C, 1.3); $\nu_{max.}$ 2245 (CN) and 1722 cm.$^{-1}$ (C = O). (Found: C, 81.4; H, 11.1; N, 3.3. $C_{28}H_{45}ON$ requires C, 81.7; H, 11.0; N, 3.4%).

The mother liquors from the crystallisation were evaporated to dryness to give a light brown, solid gum (750 mg.) which was chromatographed on alumina (25 g.) Elution with petrol-benzene (2:1) gave 5α-cholestan-3-one (400 mg.) m.p. 125 - 126°, (undepressed by mixture with an authentic specimen and showing identical infrared absorption). Elution with benzene gave a further 200 mg. of 1α-cyano-5α-cholestan-3-one m.p. 167 - 168°.

1α-Cyano-5α-cholestan-3-one. - (B) 5α-cholest-1-en-3-one (500 mg.), potassium cyanide (160 mg.), and ammonium chloride (100 mg.) were heated under reflux in dimethylformamide (10 ml.) and water (1.5 ml.) for 4.5 hr. The solvent was evaporated *in vacuo*.

water was added, and the product extracted with ether and worked up in the usual way. The residue (420 mg.), after evaporation of the ether, was chromatographed on alumina (15 g.) Elution with petrol-benzene (2:1) gave 5α-cholestan-3-one (45 mg.) m.p. 123 -125° and elution with benzene gave 1α-cyano-5α-cholestan-3-one (210 mg.) m.p. 167 - 168°.

2α-Bromo-1α-cyano-5α-cholestan-3-one.- 1α-Cyano-5α-cholestan-3-one (1g.) in glacial acetic acid (45 ml.) was treated with bromine in acetic acid (2.5 ml; 1M) and 45% hydrogen bromide in acetic acid (1 ml.) with stirring at room temperature. The bromine solution was added dropwise over 30 m 2α-bromo-1α-cyano-5α- 4 hr., the colourless crystalline product (1.05 gm.) was collected and recrystallised from chloroform-petrol to give 2α-bromo-1α-cyano-5α-cholestan-3-one (720 mg.), as fine needles m.p. 210 - 212° (dec.); $[\alpha]_D$ + 14° (C, 0.9); $\nu_{max.}$ 2245, (CN), 1740 cm.$^{-1}$ (C = O). (Found: C, 68.7; H, 8.8; N, 2.9; Br, 16.4. $C_{28}H_{44}ONBr$ requires C, 68.5; H, 9.0; N, 2.9; Br, 16.3%).

1-Cyano-5α-cholest-1-en-3-one.- (A) 2α-Bromo-1α-cyano-5α-cholestan-3-one (500 mg.) and lithium chloride (5 g.) in dimethylformamide (75 ml.) were refluxed for 2 hr. The reaction mixture was poured into water, extracted with ether and worked up in the usual way. Evaporation of the ether yielded a pale yellow solid (300 mg.) which was recrystallised from chloroform-methanol to give 1-cyano-5α-cholest-1-en-3-one, 1740 cm. $C_{28}H_{44}ON$ 1-Cyano- 1-cyano-5α-cholest-1-en-3

as pale yellow plates m.p. 188-190°, $[\alpha]_D$ + 28° (C, 1.9); $\lambda_{max.}$ 236 mμ (ε = 11,000), $\nu_{max.}$ 2237, (CN), 1690, (C = O), 1575 $cm.^{-1}$ (C = C). (Found: C,82.5; H,10.9; N,3.6. $C_{28}H_{43}ON$ requires C,82.2; H,10.6; N,3.6%).

1-Cyano-5α-cholest-1-en-3-one. - (B) 2α-Bromo-1α-cyano-5α-cholestan-3-one (700 mg.) was added to a stirred, refluxing mixture of calcium carbonate (600 mg.)and dimethylformamide (7 ml.). The reaction mixture was refluxed for 30 min., and then most of the solvent was distilled off under reduced pressure. The residue was extracted several times with ether, the ether extracts were combined and washed with dilute hydrochloric acid and then water, and dried over anhydrous sodium sulphate. Evaporation of the solvent gave a dark brown gum (380 mg.) which was chromatographed on alumina (12 g.). Elution with petrol-benzene (1:9) gave 1-cyano-5α-cholest-1-en-3-one (125 mg.) m.p. 184 - 186°; showing infrared and ultraviolet identity with the material obtained by lithium chloride dehydrobromination. Elution with benzene yielded impure 1-cyano-5α-cholest-1-en-3-one (110 mg.) m.p. 160 - 180°, and elution with benzene-ether (5:1) yielded non-crystalline material (90 mg.). When the above reaction was attempted with gentle refluxing for only 5 min., starting material was recovered almost quantitatively.

Treatment of 1-cyano-5α-cholest-1-en-3-one with acetic anhydride. - 1-Cyano-5α-cholest-1-en-3-one (125 mg.) and p-toluenesulphonic acid (60 mg.) were dissolved in acetic anhydride (12 ml.), and the latter was slowly distilled over 4 hr. After the addition of water, the residue was extracted with ether and the ethereal extract was washed with aqueous sodium hydroxide (1N) and then water and dried over anhydrous sodium sulphate. Evaporation of the ether gave a brown solid (124 mg.) which was chromatographed on neutral alumina (5 g.). Elution with petrol-benzene (3:1) gave starting material (95 mg.), m.p. 187-189°, confirmed by comparison of its infrared spectrum and its mixed melting point with an authentic sample.

Treatment of 1-cyano-5α-cholest-1-en-3-one with isopropenyl acetate. - 1-Cyano-5α-cholest-1-en-3-one (125 mg.) and p-toluenesulphonic acid (20 mg.) were dissolved in isopropenyl acetate (5 ml.). The solvent was slowly distilled over 8 hr., more isopropenyl acetate was added from time to time to keep the volume at about 4 ml. After cooling, sodium bicarbonate (100 mg.) was added to the solution and the remaining isopropenyl acetate was distilled off under reduced pressure and below 30°. The residue was shaken with ether and ice water. After working up in the usual way, the ether was evaporated to yield a pale yellow solid (110 mg.) which was chromatographed on neutral alumina (4 g.). Elution with petrol-

benzene (3:1) yielded starting material (90 mg.), m.p. 188 - 190°, showing infrared identity with an authentic specimen.

1-Cyano-cholesta-1,4-dien-3-one and 1α-cyano-5α-cholest-4-en-3-one. The material (2 g.) obtained by concentration of the mother liquors from several bromination reactions of 1α-cyano-5α-cholestan-3-one was dehydrobrominated with lithium chloride (20 g.) in dimethylformamide (300 ml.) under reflux for 2 hr. Dilution with water, extraction with ether and working up in the usual way gave a solid gum (1.4 g.) which was crystallised from chloroform-methanol to give 1-cyano-5α-cholest-1-en-3-one (350 mg.), m.p. 186 - 188°. The mother liquor was evaporated to dryness to give a very viscous oil (1 g.) which was chromatographed on neutral alumina (40 g.)

Elution with petrol-benzene (2:1) gave 1-cyano-5α-cholest-1-en-3-one (60 mg.) m.p. and mixed m.p. 186 - 188°. Further elution with the same solvent mixture gave a mixture (50 mg.) of the previous compound and a more polar substance (shown by thin layer chromatography) and then material (220 mg.) which after several recrystallisations from aqueous methanol gave 1-cyano-cholesta-1,4-dien-3-one m.p. 95 - 97°, $[\alpha]_D$ -74° (C, 1.3); $\lambda_{max.}$ 214 (ϵ = 12,100), 250 mμ (13,500), $\nu_{max.}$ 2237 (CN), 1665 (C = O), 1621 and 1587 cm.$^{-1}$ (C = C). (Found: C, 81.9; H, 9.8; N, 3.6. $C_{28}H_{41}ON$ requires C, 82.5; H, 10.1; N, 3.4%).

Continued elution with petrol-benzene (2:1) gave a mixture (50 mg.) of 1-cyano-cholesta-1,4-dien-3-one and a more polar substance, (shown by thin layer chromatography) and a colourless gum (120 mg.), which after several recrystallisations from aqueous methanol gave 1α-cyano-cholest-4-en-3-one (60 mg.), as fine needles m.p. 55 - 57°, $[\alpha]_D$ +92° (C, 1.1); $\lambda_{max.}$ 242 mμ (ϵ = 15,000), $\nu_{max.}$ 2255 (CN), 1670 cm.$^{-1}$ (C = O). (Found: C, 80.1; H, 10.3; N, 3.7. $C_{28}H_{43}ON.\frac{1}{2}H_2O$ requires C, 80.4; H, 10.6 N, 3.3%).

1β-Cyano-5α-cholestan-3-one. - 1-Cyano-5α-cholest-1-en-3-one (500 mg.) and 10% palladised charcoal (50 mg.) in ethyl acetate (100 ml.) were hydrogenated at room temperature for 48 hr. Thin layer chromatography of a sample of the solution showed one major spot, and a minor one which corresponded to starting material. The catalyst was filtered off and the filtrate evaporated to dryness. Chromatography of the residue (490 mg.) on alumina (15 g.) yielded 1-cyano-5α-cholest-1-en-3-one (40 mg.) on elution with petrol-benzene (2:1). Elution with benzene yielded a colourless solid (410 mg.) which was recrystallised from aqueous methanol to give 1β-cyano-5α-cholestan-3-one, as plates m.p. 144 - 145°, $[\alpha]_D$ +17° (C, 1.3); $\nu_{max.}$ 2247 (CN), 1724 cm.$^{-1}$ (C = O). (Found: C, 81.7; H, 11.3; N, 3.6%. $C_{28}H_{45}ON$ requires C, 81.7; H, 11.0, N, 3.4%).

Treatment of 1α- and 1β-cyano-5α-cholestan-3-ones with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (D.D.Q.) - (A.). 1α-Cyano-5α-cholestan-3-one (40 mg.) and recrystallised D.D.Q. (24 mg., 1.1 equiv.) in dry benzene (4 ml.) were refluxed for 12 hr. The reaction mixture was chromatographed on alumina (2 g.). Elution with benzene gave a white solid (36 mg.), which was homogeneous on thin layer chromatography, and on recrystallisation gave 1α-cyano-5α-cholestan-3-one, (30 mg.) m.p. and mixed m.p. 165 - 167°.

(B.) 1β-Cyano-5α-cholestan-3-one (40 mg.) was treated in a similar fashion. No reaction took place and starting material was recovered.

(C.) 1β-Cyano5α-cholestan-3-one (40 mg.) and D.DQ (24 mg.) in dry dioxan (4 ml.) were allowed to stand at room temperature for 24 hr. after dry hydrogen chloride had been bubbled into the solution for a few seconds. Thin layer chromatography showed no evidence of any reaction having taken place.

Bromination of 1β-cyano-5α-cholestan-3-one. -1β-Cyano-5α-cholestan-3-one (250 mg.) in glacial acetic acid (10 ml.) was treated with 45% hydrogen bromide in acetic acid and bromine in acetic acid (0.7 ml; 1M) was added dropwise with stirring at room temperature. A colourless crystalline precipitate was formed within 15 min. of the first addition of bromine.

Stirring was continued for 3.5 hr. and the precipitate was filtered off. Recrystallisation from chloroform-petrol gave material m.p. 196 - 198°(dec.). Concentration of the mother liquor gave a further 60 mg. of crystals m.p. 193 - 195°(dec.) Recrystallisation of the first crop from chloroform-petrol gave 2α-bromo-1β-cyano-5α-cholestan-3-one, as plates m.p. 197-199°(dec.); $[\alpha]_D$ +56.5° (C 1.4); $\nu_{max.}$ 2240 (CN), 1739 cm.$^{-1}$ (C = O). (Found: C,68.7; H,8.7; N,3.0; Br,16.5 $C_{28}H_{44}ONBr$ requires C,68.5; H,9.0; N,2.9; Br,16.3%).

Dehydrobromination of 2α-bromo-1β-cyano-5α-cholestan-3-one. - 2α-Bromo-1β-cyano-5α-cholestan-3-one (50 mg.) and lithium chloride (500 mg.) in dimethylformamide (7.5 ml.) were refluxed for 2 hr. After addition of water and working up by ether extraction in the usual way, the ether was evaporated to yield a pale yellow solid which on recrystallisation from chloroform-methanol gave 1-cyano-5α-cholest-1-en-3-one (33 mg.), m.p. 186 - 188° undepressed by mixture with an authentic specimen and having an identical infrared spectrum.

3α-Amino-1α-carboxy-3β-hydroxy-5α-cholestane lactam. - (A) 1α-Cyano-5α-cholestan-3-one (360 mg.) and potassium hydroxide (1.4 g.) in ethanol (24 ml.) and water (5 ml.) were refluxed for 1 hr. The reaction mixture was poured into water, acidified with 2 N hydrochloric acid and extracted with ether in the usual way.

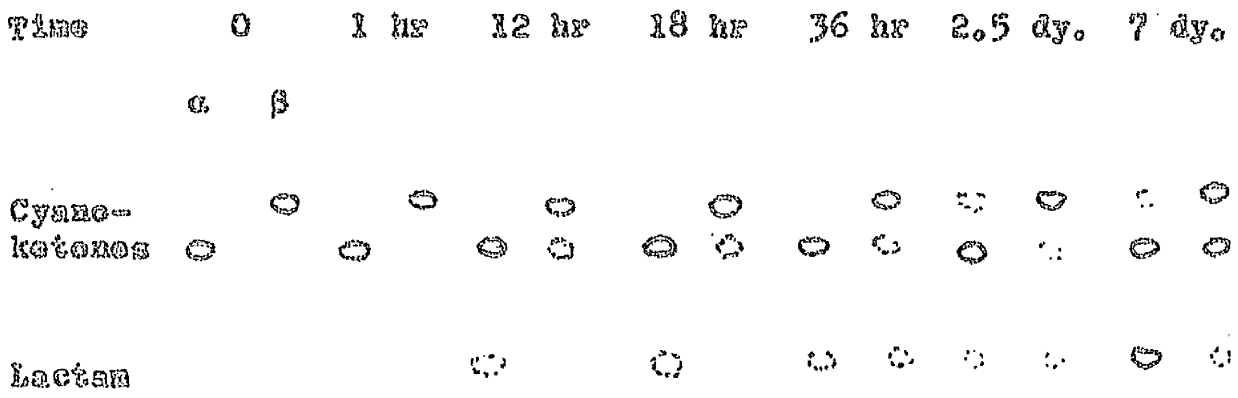
Evaporation of the ether gave a white solid (310 mg.) which on crystallisation from methanol gave 3α-amino-1α-carboxy-3β-hydroxy-5α-cholestane lactam as plates m.p. 247 - 249° raised by a further recrystallisation from chloroform-methanol to 252 - 254°, $[\alpha]_D$ +82° (C 0.7); $\nu_{max.}^{nujol}$ 3500 (-OH), 3390 and 3215 cm.$^{-1}$ (NH_2 or NH), 1712, 1672, and 1626 cm.$^{-1}$ (C = O), $\nu_{max.}^{CHCl_3}$ 3500 (-OH), 3390 and 3330 (NH_2 or NH), 1708 and 1677 cm.$^{-1}$ (C =O), and 1590 cm.$^{-1}$ (NH). (Found: C,78.6, H,10.7; N,3.55. $C_{28}H_{47}ON$ requires C,78.3; H,11.0; N,3.7%)

(B.) - 1α-Cyano-5α-cholestan-3-one (50 mg.) and 75% sulphuric acid were heated on the steam bath for 4 hr., when slight charring occurred. The reaction mixture was diluted with water and filtered, the residue being washed with water and crystallised from methanol to give 3α-amino-1α-carboxy-3β-hydroxy-5α-cholestane lactam (33 mg.) m.p. 247 - 249°, identical in all respects with the product of alkaline hydrolysis.

(C.) - 1β-Cyano-5α-cholestan-3-one (100 mg.) and potassium hydroxide (400 mg.) in ethanol (7 ml.) and water (1.3 ml.) were refluxed for 1 hr. After dilution with water and acidification with 2 N hydrochloric acid, the product was extracted with ether and worked up in the usual way. After evaporation of the ether the residue was crystallised from methanol to give 3α-amino-1α-carboxy-3β-hydroxy-5α-cholestane lactam (65 mg.)

identical (m.p., mixed m.p. and infrared spectrum) with the product obtained by hydrolysis of 1α-cyano-5α-cholestan-3-one.

Epimerisation of 1β-cyano-5α-cholestan-3-one. Solutions of 1α-cyano-5α-cholestan-3-one (50 mg.) and 1β-cyano-5α-cholestan-3-one (50 mg.) in 1% ethanolic potassium hydroxide (5 ml.) were allowed to stand at room temperature. Samples from each solution were examined by thin layer chromatography using hexane-ethyl acetate (2:1) as the developing solvent. The results are shown in the diagram below, which shows that 1α- and 1β-compounds undergo equilibration in the presence of alkali and that the 1α-compound hydrolyses before it epimerises.



Methylation of 3α-amino-1α-carboxy-3β-hydroxy-5α-cholestane lactam. - Dry methanol was saturated with dry hydrogen chloride at 0° and 3 ml. of this solution were added to the lactam (200 mg.) in dry methanol (40 ml.). The solution was refluxed for 2.5 hr. and then allowed to stand at room temperature for 18 hr. Most of the methanol was distilled off under reduced pressure and ether was added. The ether solution was washed with water, saturated aqueous sodium bicarbonate, and water again, and dried over anhydrous sodium sulphate. Evaporation of the ether gave a gum (170 mg.) which was chromatographed on alumina (5 g.). Elution with benzene gave a solid (20 mg.) m.p. 118 - 120° raised by recrystallisation from aqueous methanol to 122 - 124°; $\nu_{max.}$ 3390, 1724 cm.$^{-1}$. In view of the small quantity available, this substance was not characterised further. Elution with benzene-chloroform (4:1) gave non-crystalline material (30 mg.) and elution with benzene-chloroform (2:1) gave a gum (40 mg.) which after several recrystallisations from aqueous methanol gave 3α-amino-1α-carboxy-3β-methoxy-5α-cholestane lactam, m.p. 141 - 143°, $[\alpha]_D$ +28° (C, 1.9); $\nu_{max.}$ 3320, 3140 cm.$^{-1}$ (NH) and 1695 cm.$^{-1}$ (C = O), $\nu_{max.}^{CHCl_3}$ 3448 cm.$^{-1}$ (NH) 1692 cm.$^{-1}$ (C = O). (Found: C, 77.0; H, 11.1; $C_{29}H_{49}O_2N$ requires C, 78.4; H, 11.1%). Elution with chloroform methanol (50:1) gave starting material (65 mg.) identical in all respects with an authentic specimen.

5α-Cholestan-1,3-dione.- 1-Cyano-5α-cholest-1-en-3-one (500 mg.) and potassium hydroxide (2 g.) in ethanol (35 ml.) and water (6.5 ml.) were heated under reflux for 1 hr. and then poured into water. On acidification with 2 N hydrochloric acid a strong odour of hydrogen cyanide was observed. The product was extracted with ether and worked up in the usual manner. Evaporation of the ether gave a colourless solid (410 mg.) which was recrystallised twice from ether-pentane and once from ethyl acetate to give 5α-cholestan-1,3-dione as plates m.p. 166 - 168°, $[\alpha]_D$ +100° (C, 1.0); $\lambda_{max.}$ 255 mμ (ε 16,000), $\lambda_{max.}^{EtOH/N\ NaOH}$ 285 mμ (ε 19,000), $\nu_{max.}^{CCl_4}$ 1733, 1698 cm.$^{-1}$ (C = O), $\nu_{max.}$ 1736, 1710 cm.$^{-1}$ (C = O). (Literature values[112a] m.p. 173 - 174°; $[\alpha]_D^{26}$ +105±2°; $\lambda_{max.}^{EtOH/NaOH}$ 255 mμ (ε 12,000), $\lambda_{max.}$ 285 mμ (ε 27,000), $\nu_{max.}$ 1727, 1695 cm.$^{-1}$

2,2-Dibromo-5α-cholestan-1,3-dione.- 5α-Cholestan-1,3-dione (80 mg.) in chloroform (2.4 ml.) and methanol (2.4 ml.) was treated with bromine in chloroform (0.8 ml.;0.5M), added dropwise with stirring. Water was added and the mixture concentrated under reduced pressure. The aqueous suspension was extracted with chloroform, washed with water, and dried over anhydrous sodium sulphate. The chloroform was evaporated to give the crude product (88 mg.) which was recrystallised several times from chloroform-methanol yielding 2,2-dibromo-5α-cholestan-1,3-dione as needles m.p. 164 - 165°, $[\alpha]_D$ - 16° (C, 1.0); $\nu_{max.}^{CHCl_3}$

1724 cm.$^{-1}$(C = O). (Literature values[112a] m.p. 157 - 161°, $[\alpha]_D^{22}$ - 15.5±1°, $\nu_{max.}^{CNCl_2}$ 1721 cm.$^{-1}$

Treatment of 1-cyano-5α-cholest-1-en-3-one with acid. - 1-Cyano-5α-cholest-1-en-3-one (100 mg.) was refluxed for 2 hr. in ethanol (12 ml.) containing concentrated hydrochloric acid (2 ml.). The solution was diluted with water and extracted with ether. After being washed with water, saturated aqueous sodium bicarbonate, and water again, the ether extract was dried over anhydrous sodium sulphate and evaporated to give an almost quantitative recovery of starting material.

1α-Cyano-3-ethylenedioxy-5α-cholestane. - 1α-Cyano-5α-cholestan-3-one (550 mg.) in ethylene glycol (6 ml.) and borontrifluoride etherate (1 ml.) was allowed to stand at room temperature for 3 days, and then diluted with chloroform and washed three times with water. After being dried over anhydrous sodium sulphate, the chloroform was evaporated and the colourless crystalline residue was recrystallised from methanol to give 1α-cyano-3-ethylenedioxy-5α-cholestane as needles m.p. 182 - 183°, $[\alpha]_D$ + 40° (C, 0.8); $\nu_{max.}$ 2245 cm.$^{-1}$ (CN), 1067 cm.$^{-1}$(ether link) (Found: C, 79.0; H, 11.0; N, 3.0. $C_{30}H_{49}O_2N$ requires C, 79.1; H, 10.8; N, 3.1%)

Regeneration of 1α-cyano-5α-cholestan-3-one from 1α-cyano-3-ethylenedioxy-5α-cholestane. - 1α-Cyano-3-ethylenedioxy-5α-cholestane (60 mg.) and p-toluenesulphonic acid (15 mg.) in acetone (10 ml.) were refluxed for ½ hr. The reaction mixture was poured into water and worked up with ether in the usual way. After evaporation of the ether, the residue was crystallised from chloroform-methanol to give 1α-cyano-5α-cholestan-3-one (40 mg.), m.p. 168 - 169° identical in all respects with an authentic specimen.

Treatment of 1α-cyano-3-ethylenedioxy-5α-cholestane with ether and hydrochloric acid. - 1α-Cyano-3-ethylenedioxy-5α-cholestane (100 mg.) in ether (10 ml.) and concentrated hydrochloric acid (0.2 ml.) was stirred at room temperature for 20 min. More ether was added and the solution was washed with water, 5% aqueous sodium bicarbonate, and twice more with water. The ether was dried over anhydrous sodium sulphate and was then evaporated off. Recrystallisation of the residue gave unchanged starting material (94 mg.).

Treatment 1α-cyano-3-ethylenedioxy-5α-cholestane with alkali. - 1α-Cyano-3-ethylenedioxy-5α-cholestane (100 mg.) and potassium hydroxide (400 mg.) in ethanol (7 ml.) and water (1.3 ml.) were refluxed for 1 hr. The product was isolated by means of ether in the usual manner, and after recrystallisation from

methanol was found to be identical in all respects with starting material.

Reduction of 1α-cyano-5α-cholestan-3-one with sodium borohydride in aqueous methanol. - 1α-Cyano-5α-cholestan-3-one (950 mg.) in methanol (90 ml.) was added to sodium borohydride (115 mg.) in methanol (10 ml.) and water (2 ml.) and the reaction allowed to stand for 1 hr. at room temperature. The reaction mixture was diluted with water and the product isolated with ether in the usual way and recrystallised from aqueous methanol to give a crystalline mixture of the 3α- and 3β-epimers of 1α-cyano-5α-cholestan-3-ol (650 mg.), m.p. 123 - 126° (softened 90°). $[\alpha]_D$ +58° (c, 1.0); $\nu_{max.}$ 3440 (-OH) and 2245 cm.$^{-1}$ (CN) and no absorption in the carbonyl region (Found: C, 81.6; H, 11.5; N, 3.5. $C_{28}H_{47}ON$ requires C, 81.4; H, 11.5; N, 3.4%). The material was not separated by chromatography on alumina.

The procedure was repeated allowing a reaction time of 30 hr. and a product identical in all respects with the above was obtained.

Reduction of 1α-cyano-5α-cholestan-3-one with sodium borohydride in methanol. - 1α-Cyano-5α-cholestan-3-one (100 mg.) in methanol (10 ml.) was treated with solid sodium borohydride (10 mg.). The solution was allowed to stand at room temperature for 1 hr. and then the product was isolated by means of ether in the usual way to give a colourless gum (90 mg.) which on crystallisation

from aqueous methanol yielded material (75 mg.) identical with that obtained in the previous reductions, namely, an epimeric mixture of 1α-cyano-5α-cholestan-3α and 3β-ols with the 3α-epimer predominating.

Acetylation of the epimeric mixture. - The mixture (290 mg.) in acetic anhydride (3 ml.) and pyridine (4.5ml.) was heated on the steam bath for 2 hr. The solution was diluted with water and the product was extracted with ether and the ether was washed with dilute hydrochloric acid and water. Evaporation of the dried ether extract gave a gum (315 mg.) which was chromatographed on neutral alumina (10 g.). Elution with benzene gave a mixture of the epimeric 3-acetates m.p. 120 - 125°; $[\alpha]_D$ +28° (C, 0.9); $\nu_{max.}$ 2245 (CN), 1740 (C = O), 1250 cm.$^{-1}$ (C - O).

1α-Cyano-5α-cholestan-3α-ol - 1α-Cyano-5α-cholestan-3-one (900 mg.) in iso-propyl alcohol (75 ml.) was treated with sodium borohydride (200 mg.) in iso-propyl alcohol (75 ml.) and the reaction mixture allowed to stand at room temperature for 24 hr. After dilution with water and extraction with ether in the usual way, evaporation of the ether yielded a colourless gum (899 mg.) which was chromatographed on alumina (30 g.). Elution with benzene-ether (1:1) yielded 1α-cyano-5α-cholestan-3α-ol (205 mg.), m.p. 151 - 153°, raised by recrystallisation from petrol to 153 - 155°. $[\alpha]_D$ +53° (C, 0.9); $\nu_{max.}$ 3440 (OH), 2245 cm.$^{-1}$ (CN). (Found: C,81.3; H,11.3; N,3.6. $C_{28}H_{47}ON$

requires C,81.3; H,11.5; N,3.4%). Elution with benzene-ether (1:2) and ether yielded material (50 mg.) which could not be crystallised, and elution with ether-methanol (50:1) yielded a white solid (470 mg.) m.p. 125 - 145° which was rechromatographed on alumina (15 g.) Elution with benzene-ether (2:1) yielded 1α-cyano-5α-cholestan-3α-ol (450 mg.) identical in all respects with that already obtained.

Meerwein-Ponndorf reduction of 1α-cyano-5α-cholestan-3-one. - 1α-Cyano-5α-cholestan-3-one (300 mg.) and aluminium iso-propoxide (360 mg.) in iso-propyl alcohol (6 ml.) were heated, and the solvent distilled. The presence of acetone in the distillate was shown with 2,4-dinitrophenylhydrazine solution. Distillation was continued for 1 hr., by which time the distillate was acetone-free. The solvent was evaporated under reduced pressure and the residue taken up in ether and dilute hydrochloric acid, the organic layer was separated, and the aqueous layer extracted twice more with ether. The ether extracts were combined and washed twice with water, and the ether dried over anhydrous sodium sulphate. Evaporation of the ether yielded a white solid (270 mg.) which was recrystallised from petrol to give 1α-cyano-5α-cholestan-3α-ol as fine needles m.p. 151 - 153°, identical in all respects with the product obtained by reduction with sodium borohydride in iso-propyl alcohol.

1α-Cyano-5α-cholestan-3α-ol 3-acetate. - 1α-Cyano-5α-cholestan-3α-ol (100 mg.) in acetic anhydride (1 ml.) and pyridine (1.5 ml.) was heated on the steam bath for 2 hours. After dilution with water and extraction with ether, the organic layer was washed with dilute hydrochloric acid and then water and dried over anhydrous sodium sulphate. Evaporation of the ether gave a colourless gum (75 mg.) which was recrystallised several times from methanol to give 1α-cyano-5α-cholestan-3α-ol 3-acetate, as needles m.p. 129 - 131°, $[\alpha]_D$ +29° (C, 0.9); $\nu_{max.}$ 2247 (CN), 1740 (C = O), and 1250 cm.$^{-1}$ (CO). (Found: C,79.1; H,10.9; N,3.2. $C_{30}H_{49}O_2N$ requires C,79.1; H,10.8; N,3.1%).

Reduction of 1α-cyano-5α-cholestan-3-one with lithium aluminium tri- tert- butoxyhydride. - The reducing agent was prepared by slow addition of tert-butyl alcohol (3.2 ml.) to lithium aluminium hydride (640 mg.) in anhydrous tetrahydrofuran (50 ml.) at 0°. 1α-Cyano-5α-cholestan-3-one (1.6 g.) in anhydrous tetrahydrofuran (50 ml.) was added to the reducing agent solution and the reaction was kept at 0° for 30 min., and then at room temperature for 1.5 hr. The reaction mixture was poured into excess dilute hydrochloric acid, extracted with ether and worked up in the usual way. After evaporation of the ether, the residue was recrystallised from aqueous methanol to give a mixture of 1α-cyano-5α-cholestan-3α-ol and 1α-cyano-

5α-cholestan-3β-ol (1 g.) m.p. 118 - 120° raised by further recrystallisation to 120 - 122°. $\nu_{max.}$ 3450 (OH), 2245 cm.$^{-1}$ (CN), and homogeneous on thin layer chromatography. Concentration of the mother liquors gave a further 300 mg. of material m.p. 120 - 126°, which after two more recrystallisations from aqueous methanol and one from petrol gave 1α-cyano-5α-cholestan-3α-ol m.p. 151 - 153°, identical in all respects with an authentic sample.

Acetylation of the mixture of α-cyano-5α-cholestan-3α and 3β-ol. - The epimeric mixture (600 mg.) was heated on the steam bath for 2 hr. with acetic anhydride (1.5 ml.) and pyridine (2.5 ml.) The product (610 mg.), isolated by means of ether in the usual way, showed two spots, close together, on thin layer chromatography, and was chromatographed on neutral alumina (18 g.). All fractions eluted showed two spots also.

1α-Aminomethyl-5α-cholestan-3α-ol. - (A) 1α-Cyano-5α-cholestan-3α-ol (300 mg.) and lithium aluminium hydride (75 mg.) in anhydrous ether (30 ml.) were refluxed for 3 hr. The excess of lithium aluminium hydride was decomposed with ethyl acetate and the reaction mixture was diluted with ether. The ether was washed several times with water and dried over anhydrous sodium sulphate. Evaporation of the ether gave a gum (230 mg.) which was crystallised several times from ether to give

1α-aminomethyl-5α-cholestan-3α-ol, m.p. 185 - 186°, $[\alpha]_D$ +38° (C, 1.0); $\nu_{max.}$ 3390 (OH), 3311, 3205 cm.$^{-1}$ (NH stretch), 1590 cm.$^{-1}$ (NH def.). (Found: C, 80.35; H, 11.85; N, 3.25. $C_{28}H_{51}ON$ requires C, 80.6; H, 12.3; N, 3.4%).

(B.) 1α-Cyano-5α-cholestan-3-one (1 g.) in anhydrous ether (35 ml.) was treated with lithium aluminium hydride (500 mg.) in anhydrous ether (40 ml.) and the reaction mixture refluxed for 3 hr. The excess of lithium aluminium hydride was decomposed with ethyl acetate and the product isolated as before. Recrystallisation from ether or from ethyl acetate gave 1α-aminomethyl-5α-cholestan-3α-ol (320 mg.) m.p. 181 - 184°, identical in all respects with the sample prepared in the preceeding experiment.

1α-Aminomethyl-5α-cholestan-3α-ol 1α,3α-diacetate. - 1α-Amino-methyl-5α-cholestan-3α-ol (100 mg.) and acetic anhydride (0.5 ml.) were heated on the steam bath for 1.5 hr. Water was added to the solution and the product was extracted with ether. The ether extract was washed with dilute sodium hydroxide solution and with water, and then dried over anhydrous sodium sulphate. Evaporation of the ether gave a gum (104 mg.) which was recrystallised from aqueous methanol to give 1α-aminomethyl-5α-cholestan-3α-ol 1α,3α-diacetate (80 mg.), m.p. 181 - 183°, $[\alpha]_D$ +55° (C, 1.2); $\nu_{max.}$ 3280, 3080 (NH), 1740 (C = O), 1653 (amide C = O), 1553 (NH def.), and 1250 cm.$^{-1}$ (CO). (Found: C, 76.6;

H,10.9; N,2.9. $C_{32}H_{55}O_3N$ requires C,76.6; H,11.05; N,2.8%). An identical product was obtained when the acetylation was carried out at room temperature.

1α-Aminomethyl-5α-cholestan-3α-ol 1α-acetate. - The 1α,3α-diacetate (80 mg.) in methanol (20 ml.) was treated with sodium carbonate (8 mg.) in water (0.5 ml.) and the solution was allowed to stand at room temperature for 24 hr. Water was added and some of the methanol was distilled off at room temperature under reduced pressure. The product was isolated by means of ether in the usual way and recrystallised from aqueous methanol to give 1α-aminomethyl-5α-cholestan-3α-ol 1α-acetate (45 mg.), as needles m.p. 184 - 186°, $[\alpha]_D^{CHCl_3}$ +32° (C, 1.1); $\nu_{max.}$ 3460 (OH), 3356 3247 (NH), 1647 (amide C = O), 1558 cm.$^{-1}$ (NH def.). (Found: C,78.6; H,11.6; N,3.3. $C_{30}H_{53}O_2N$ requires C,78.4; H,11.6; N,3.05%).

Treatment of 1α-cyano-5α-cholestan-3α-ol with hydrogen chloride in ether. - Dry hydrogen chloride was bubbled into a solution of 1α-cyano-5α-cholestan-3α-ol in dry ether (100 ml.) for 1 hr. at room temperature and the reaction mixture was allowed to stand at room temperature overnight. Hydrogen chloride was then bubbled in for a further 1 hr. The solution was evaporated to dryness under reduced pressure. A little dry ether was added

and the insoluble material filtered off and recrystallised from methanol-ethyl acetate to give 5α-cholestan-1α,3α-imino-ether hydrochloride (250 mg.) m.p. 133 - 140°, positive Beilstein test; $\nu_{max.}$ 1690 cm.$^{-1}$ (N = H). The hydrochloride (100 mg.) was suspended in ether and the suspension shaken with saturated sodium bicarbonate solution whereupon the solid went into solution. The aqueous layer was separated, and after neutralisation with excess of dilute nitric acid gave a positive test for chloride ions with silver nitrate solution. After being washed with water, the ether layer was dried over anhydrous sodium sulphate and the ether was evaporated to give a very viscous oil (80 mg.) which could not be crystallized $\nu^{thin\ film}_{max.}$ 3279, 3226 cm.$^{-1}$ (NH), 1680 cm.$^{-1}$ (C = N).

Hydrolysis of crude imino-ether hydrochloride. - 1α-Cyano-5α-cholestan-3α-ol (400 mg.) in anhydrous ether (30 ml.) was treated with anhydrous hydrogen chloride as in the preceding experiment and the residue obtained after evaporation of the ether was refluxed with hydrochloric acid (30 ml.; 2N) for 8 hr. The product was isolated by means of ether in the usual way and was a gum (340 mg.), $\nu_{max.}$ 2245 (CN), 1770 (lactone C = O), 1685 cm.$^{-1}$ (C = N), which could not be separated into its components by chromatography. Thin layer chromatography showed the presence of five substances of very similar polarity.

Attempted epimerisation of 1α-cyano-5α-cholestan-3α-ol and preparation of 1α-cyano-5α-cholest-2-ene. 1α-Cyano-5α-cholestan-3α-ol (2 g.) and p-toluenesulphonyl chloride (1.75 g.) were dissolved in the minimum quantity of pyridine and kept in the refrigerator for 3 days. Water was added dropwise and the product was precipitated. The precipitate was dried to give crude 1α-cyano-5α-cholestan-3α-ol 3α-tosylate (2.3 g.). A sample was recrystallised several times from acetone but no sharp melting material was obtained, the m.p. was always indefinite in the range 150 - 170°. $\nu_{max.}$ 2257 (CN), 1600 (aromatic), 815 cm.$^{-1}$ (1,4-disubstituted benzene ring. The remainder of the crude tosylate was stirred at 100 - 105° for 5 hr. with potassium acetate (5 g.) in dimethylformamide (14 ml.) and water (1.5 ml.). After the addition of 50% acetic acid (3 ml.) the product was worked up in the usual manner with ether. Evaporation of the ether gave a solid gum (1.7 g.) which was chromatographed on alumina (50 g.). Elution with petrol-benzene (20:1) gave a gum (310 mg.) which on recrystallisation from aqueous methanol afforded 1α-cyano-5α-cholest-2-ene, as needles m.p. 86 - 87°; $[\alpha]_D$ +60° (C, 1.1); $\nu_{max.}^{CHCl_3}$ 2250 (CN), 1658 cm.$^{-1}$ (C = C). (Found: C, 85.3; H, 11.7; N, 3.2. $C_{28}H_{47}N$ requires C, 85.0; H, 11.5; N, 3.5%).

Elution with benzene yielded a gum (200 mg.) which was shown

by thin layer chromatography to be a mixture of two substances, $\lambda_{max.}$ 226 mμ, $\nu_{max.}$ 2250 (CN), 1740 (C = O), 1600, (aromatic), 1250, 1230 cm.$^{-1}$ (C - O). Elution with ether-methanol (100:1) gave 1α-cyano-5α-cholestan-3α-ol (650 mg.), m.p. 148 - 151° undepressed by mixture with an authentic sample.

The benzene eluate was rechromatographed on silica gel (6 g.) but separation was not achieved.

The mixture was refluxed for 1 hr. with 5% ethanolic potassium hydroxide, and after neutralisation, the product was isolated in the usual way, by means of ether. Thin layer chromatography showed two spots; one of the components of the mixture had been unaffected by treatment with alkali and the other had become more polar. The mixture (150 mg.), which still showed ultra-violet absorption at 226 mμ, was chromatographed on silicagel (5 g.). Elution with benzene gave a gum (30 mg.) which was recrystallised with difficulty from aqueous acetone to give a colourless solid (20 mg.) m.p. 112 - 114, $\lambda_{max.}$ 226 mμ, $\nu_{max.}$ 2245 (CN), 1600 cm.$^{-1}$ (aromatic) and complex absorption in the range 700 - 900 cm.$^{-1}$ The substance was homogeneous on thin layer chromatography, but was not characterised further since only a small quantity was obtained. Further elution with solvents of increasing polarity yielded fractions of gummy materials which were not homogeneous.

1β-Cyano-5α-cholestan-3β-ol - (A.) 1β-Cyano-5α-cholestan-3-one (100 mg.) in methanol (2.5 ml.) was added to sodium borohydride (12 mg.) in water (0.2 ml.) and methanol (1 ml.). After being left at room temperature for 1 hr., the reaction mixture was diluted with water and the product extracted with ether in the usual way. After evaporation of the ether, a gum (93 mg.) was obtained which was shown by thin layer chromatography to be a mixture of two products, one in very large excess. Two recrystallisations from aqueous methanol and one from ether-pentane gave material (43 mg.) m.p. 150 - 152°, $[\alpha]_D$-10° (C, 1.1), which was not homogeneous, $\nu_{max.}$ 3450 cm.$^{-1}$ (-OH) 2245 cm.$^{-1}$ (-CN) and no carbonyl absorption.

(B.) 1β-Cyano-5α-cholestan-3-one (200 mg.) in iso-propyl alcohol (15 ml.) was mixed with sodium borohydride (40 mg.) in iso-propyl alcohol (15 ml.) and the solution allowed to stand at room temperature for 48 hr. The product was isolated in the usual way with ether and recrystallised twice from ether-pentane to give 1β-cyano-5α-cholestan-3β-ol (130 mg.), homogeneous on thin layer chromatography, as plates m.p. 156 - 158°; $[\alpha]_D$-15.5° (C, 1.0), ν_{max} 3450 cm.$^{-1}$ (OH), 2245 cm.$^{-1}$ (CN). (Found C81.5; H,11.5 ; N,3.6. $C_{28}H_{45}ON$ requires C,81.3; H,11.5; N,3.4%).

1β-Cyano-5α-cholestan-3β-ol 3-acetate. - 1β-Cyano-5α-cholestan-

3β-ol (40 mg.) in pyridine (1.5 ml.) and acetic anhydride (1 ml.) was heated on the steam bath for 2 hr. The solution was diluted with water and extracted with ether. After washing with dilute hydrochloric acid and water and drying over anhydrous sodium sulphate, the ether was evaporated, and the residue crystallised from aqueous methanol to give 1β-cyano-5α-cholestan-3β-ol 3β-acetate (33 mg.), as needles m.p. 96 - 98°; $[\alpha]_D$ 3° (C, 0.9); $\nu_{max.}$ 2250 (CN), 1733 (C = O), 1240 $cm.^{-1}$ (C - O). (Found: C, 79.4; H, 11.0; N, 3.4. $C_{30}H_{49}O_2N$ requires C, 79.1; H, 10.8; N, 3.1%).

Treatment of 1β-cyano-5α-cholestan-3β-ol with hydrogen chloride. - Anhydrous hydrogen chloride was passed into a solution of 1β-cyano-5α-cholestan-3β-ol (25 mg.) in anhydrous ether (4 ml.) for 1 hr. and the solution left at room temperature for 24 hr. Evaporation of the ether gave a white solid which was identical with starting material in all respects.

Attempted hydrolyses of 1α-cyano-5α-cholestan-3α-ol. - 1α-Cyano-5α-cholestan-3α-ol was refluxed with a number of hydrolysing agents under the conditions shown in the table following. Each reaction was worked up in the usual manner, basic solutions being first neutralised with 2N hydrochloric acid. Starting material was recovered in each case.

Hydrolysing Agent	Solvent	Time (hr.)
KOH (5%)	EtOH - H_2O (5:1)	2.5
KOH (50%)	EtOH - H_2O (5:1)	2
KOH (5%)	Ethyleneglycol-H_2O (4:1)	22
KOH (5%)-H_2O_2 (100 vols.) (10%)	Dioxan	1
HCl (10%) equal vol.	EtOH	2
HCl (conc.) (25%)	EtOH	0.25

Reaction of 1α-cyano-5α-cholestan-3α-ol with methyl Grignard reagent.-(A.) 1α-Cyano-5α-cholestan-3α-ol (300 mg.) in dry benzene (11 ml.) was added to methyl magnesium iodide (6 molar equivs.) made from magnesium turnings (110 mg.) and methyl iodide (0.35 ml.) in dry ether (6 ml.) and the reaction mixture was refluxed for 24 hr. Acetic acid (18 ml.) and water (6 ml.) were added and refluxing was continued for a further 30 min. Most of the solvent was distilled off under reduced pressure and the residue was diluted with water and extracted with ether. The ether layer was reddish brown owing to the presence of iodine.

which was removed by shaking with aqueous sodium thiosulphate solution. After being washed with water, the ether layer was dried over anhydrous sodium sulphate, and the ether was evaporated to give a gum (280 mg.), $\nu_{max.}$ 3410 (OH), 2247 cm.$^{-1}$ (CN) and a very small peak at 1695 cm.$^{-1}$ (C = O), which was recrystallised from aqueous methanol to give 1α-cyano-5α-cholestan-3α-ol (230 mg.).

(B.) The reaction was repeated using methyl magnesium bromide (6 molar equivs.) and extending the period of refluxing to 30 hr. with similar results.

Reaction of 1α-cyano-3-ethylenedioxy-5α-cholestane with methyl Grignard reagent.-(A)The ketal (300 mg.) in dry ether (10 ml.) was added to methyl magnesium iodide made from magnesium turnings (110 mg.) and methyl iodide (0.35 ml.) and the reaction mixture was refluxed for 65 hours. After being worked up as described in the previous experiment and the solvent evaporated, the residue was crystallised from methanol to yield unchanged starting material (240 mg.) m.p. 180 - 181°.

(B.) The ketal (600 mg.) in dry benzene (30 ml.) and dry ether (30 ml.) was added to methyl magnesium iodide prepared from magnesium (220 mg.) and methyl iodide (0.7 ml.) and the reaction was refluxed for 6 hr. The reaction mixutre was cooled and poured on to a mixture of crushed ice (15 g.) and ammonium chloride (1.5 g.). After 1 hr., the organic layer was separated and washed with water and dried over anhydrous sodium sulphate.

The ether was evaporated and the residue was recrystallised from methanol to give starting material (370 mg.). The mother liquor was evaporated to dryness, and its infrared spectrum showed a peak at 2245 cm.$^{-1}$ (CN) and a very small peak at 1700 cm.$^{-1}$ (C = O)

Treatment of 1α-cyano-5α-cholestan-3α-ol with methyl lithium

(A.) Lithium (280 mg.) was hammered flat and cut in small pieces into ether (10 ml.) under nitrogen, and methyl iodide (2.2 g.) was then added with stirring. After 2 hr., a sample (2 ml.) of the methyl lithium solution was removed, and after hydrolysis with water (2 ml.), was titrated with N hydrochloric acid to determine the methyl lithium concentration. Three molar equivalents of methyl lithium solution were added to 1α-cyano-5α-cholestan-3α-ol (550 mg.) in dry ether (15 ml.) and the solution was stirred for 18 hr. at room temperature under nitrogen. The excess of methyl lithium was destroyed with methanol (1 ml) and the reaction mixture was poured into water and extracted with ether. The ether extract was washed with dilute hydrochloric acid and then water and dried over anhydrous sodium sulphate. Evaporation of the ether gave a gum (450 mg.) which was chromatographed on alumina (15 g.). Elution with benzene-ether (9:1 and 7:3) gave a gum (220 mg.), $\nu_{max.}$ 3400

(OH), 2245 (CN), 1690 cm.$^{-1}$ (C = O), and elution with ether-methanol (100:1) gave starting material (180 mg.), m.p. 149 - 152°, confirmed by comparison with an authentic sample. 2,4-Dinitrophenylhydrazine solution was added to a portion (30 mg.) of the gum which had shown carbonyl absorption, but no precipitate was obtained at room temperature, on warming, or on standing overnight. The remainder (140 mg.) of the gum was rechromatographed on alumina (5 g.) but all fractions eluted were mixtures showing both nitrile and carbonyl absorption in the infrared.

(B.) The procedure described above was repeated using tetrahydrofuran as solvent for 1α-cyano-5α-cholestan-3α-ol and refluxing the reaction for 18 hr. under nitrogen. Similar results were obtained.

1-Cyano-5α-cholest-1-en-3β-ol. - 1-Cyano-5α-cholest-1-en-3-one (100 mg.) in methanol (20 ml.) was treated with sodium borohydride (15 mg.) in methanol (1 ml.) and water (0.2 ml.). The reaction mixture was allowed to stand at room temperature for 2 hr., was diluted with water, and the product isolated by means of ether in the usual way. Evaporation of the ether gave a gum (95 mg.), which after several recrystallisations from aqueous methanol gave 1α-cyano-5α-cholest-1-en-3β-ol (50 mg.) as an amorphous solid which melted and resolidified

as needles at 75 - 77°, melting again at 120 - 121°, $[\alpha]_D$ +5° (C, 1.0); $\lambda_{max.}$ 220 mμ (ε = 9,300), $\nu_{max.}$ 3450 (OH), 2237 (CN), 1684, 811 cm.$^{-1}$ (C = C). (Found: C, 81.4; H, 10.9; N, 3.9. $C_{28}H_{45}ON$ requires C, 81.7; H, 11.0; N, 3.4%).

Treatment of 1-cyano-5α-cholest-1-en-3β-ol with alkali. - 1-Cyano-5α-cholest-1-en-3β-ol (100 mg.) and potassium hydroxide (400 mg.) in ethanol (7 ml.) and water (1.3 ml.) were refluxed for 1 hr. After neutralisation with 2 N hydrochloric acid, the reaction mixture was worked up with ether in the usual way. Infrared examination of the gum (93 mg.) obtained after the ether had been evaporated showed that no reaction had taken place.

Treatment of 1-cyano-5α-cholest-1-en-3β-ol with methyl magnesium iodide. - 1-Cyano-5α-cholest-1-en-3β-ol (300 mg.) in dry benzene (10 ml.) was added to methyl magnesium iodide made from magnesium (110 mg.) and methyl iodide (0.35 ml.) in dry ether (6 ml.). After refluxing for 24 hr., acetic acid (18 ml.) and water (6 ml.) were added and refluxing was continued for a further 30 min. More water was added, and the ether layer was separated, washed with water, and dried over anhydrous sodium sulphate. Evaporation of the ether gave a gum (280 mg.) whose infrared spectrum showed that no reaction had occurred.

Reduction of 1α-cyano-5α-cholestan-3α-ol with one molar equivalent of lithium aluminium hydride - 1α-cyano-5α-cholestan-

3α-ol (200 mg.) in dry ether (7 ml.) was added to lithium aluminium hydride (20 mg.) in dry ether (4 ml.). After being refluxed for 1.5 hr., any excess of lithium aluminium hydride was destroyed by the addition of ethyl acetate (0.2 ml.), and then water (20 ml.). More ether was added and the organic layer was separated, washed with water, and dried over anhydrous sodium sulphate. Evaporation of the ether gave a colourless solid (170 mg.) which after several recrystallisations from petrol gave material (55 mg.) m.p. 204 - 207°, $[\alpha]_D$ +52° (C, 1.2), $\nu_{max.}$ 3640 (OH), 3210 (NH) and 1670 cm.$^{-1}$ (C = NH). (Found: C,81.3; H,10.8; N,1.95. $C_{28}H_{49}ON$ requires C,80.9; H,11.9, N,3.4%; $C_{56}H_{97}O_2N$ requires C,82.4; H,11.9; N,1.7%, $C_{28}H_{49}ON + C_{28}H_{48}O_2$ (1:1) requires C,80.9; H,11.7; N,1.7%). No pure material was obtained by chromatography of the mother liquors.

Reaction of 1α-cyano-3-ethylenedioxy-5α-cholestane with lithium aluminium hydride. - 1α-Cyano-3-ethylenedioxy-5α-cholestane (300 mg.) in dry ether (10 ml.) was added to lithium aluminium hydride, (100 mg.) in dry ether (30 ml.) and the solution was refluxed for 3 hr. The excess of lithium aluminium hydride was destroyed by the addition of ethyl acetate (1 ml.) and then water (25 ml.). More ether was added and the organic layer was separated, washed with water and dried over anhydrous sodium

sulphate. The residue obtained after evaporation of the ether was chromatographed on alumina (15 g.). Elution with benzene gave a gum (20 mg.) which was recrystallised several times from aqueous methanol to give a solid (10 mg.), m.p. 97 - 99° $\nu_{max.}$ 1710 cm.$^{-1}$ (C = O) which gave a precipitate with 2,4-dinitrophenylhydrazine solution and was probably 3-ethylene-dioxy-5α-cholestan-1α-al. Elution with benzene containing increasing amounts of ether yielded a series of gums (170 mg.) which thin layer chromatography showed to be complex mixtures, and elution with ether-methanol (50:1) gave a white solid (25 mg.) which on recrystallisation from ether gave material (12 mg.) m.p. 156 - 158°; $\nu_{max.}$ 1650 cm.$^{-1}$ which was probably an imine or a dimeric anil, the poor yield of this material prevented further investigation.

Reaction of 1α-cyano-3-ethylenedioxy-5α-cholestane with one molar equivalent of lithium aluminium hydride -

(A.) The ketal (400 mg.) in dry ether (25 ml.) was added to lithium aluminium hydride (40 mg.) in dry ether (10 ml.) and the reaction was refluxed for 1.5 hr. The mixture was worked up as in previous reductions of this nature. Evaporation of the ether gave a gum (360 mg.) which showed four spots on thin layer chromatography. The gum was chromatographed on alumina (15 g.), elution with petrol-benzene (1:1) gave a gum (320 mg.) which was a mixture of the same four substances.

The gum was rechromatographed on silica gel (12 g.), elution with petrol gave a white solid which was recrystallised from acetone to give material (30 mg.) as needles m.p. 153 - 155° which did not give a precipitate with 2,4-dinitrophenylhydrazine solution, had infrared absorption at 1680 cm.$^{-1}$, and was probably 3-ethylenedioxy-1α-imino-5α-cholestane. Elution with solvents of increasing polarity gave only gummy material which thin layer chromatography showed to be mixtures of several components.

(B.) The procedure was repeated, with stirring at room temperature for 2 hr. using the same quantities of reagents. Starting material was quantitatively recovered.

Attempted reductions with lithium aluminium triethoxyhydride. - (A.) Ethyl acetate (0.13 ml.) was added to lithium aluminium hydride (83 mg.) in dry ether (15 ml.) at 0° with stirring. 1α-Cyano-5α-cholestan-3α-ol (1 g.) in dry ether (35 ml.) was added dropwise to the solution with stirring at 0°. The reaction was stirred at 0° for 3 hr. and then a mixture of aqueous potassium sodium tartrate (35 ml; 1M) and aqueous tartaric acid (7 ml.; 0.25M) was added, the temperature being maintained at 0°. The ether layer was separated and the aqueous layer was extracted twice with ether. The ether extracts were combined, washed with water, and dried over

anhydrous sodium sulphate. Evaporation of the ether yielded starting material (900 mg.) identical in all respects with an authentic specimen.

(B.) The procedure was repeated using 1α-cyano-3-ethylene-dioxy-5α-cholestane and starting material was again obtained in quantitative yield.

Stephen reduction of 1α-cyano-5α-cholestan-3α-ol. - Stannous chloride dihydrate (4.5 g.) was slowly added to acetic anhydride (4 ml.) with stirring. After being allowed to cool to room temperature, the suspension was filtered and the residue of anhydrous stannous chloride (3.6 g.) was washed with ether and stored in a desiccator.

Anhydrous stannous chloride (1.66 g.) was suspended in dry ether (5 ml.) and dry hydrogen chloride was passed in until a clear solution was obtained. 1α-Cyano-5α-cholestan-3α-ol (600 mg.) was added and the mixture was shaken. A heavy white precipitate was formed almost immediately, and the reaction mixture was allowed to stand at room temperature for 20 hr. The mixture was filtered and the residue was washed with dry ether and refluxed in water (10 ml.) for 2 hr. The product was isolated by means of ether in the usual way, and the ether was evaporated to give a white solid which was recrystallised from aqueous ethanol to give 5α-cholestan-3α-ol 1α-amide (70 mg.),

m.p. 128 - 132°; $[\alpha]_D$ +90° (C, 0.4); $\nu_{max.}$ 3360 (OH), 3185 (NH_2), 1670 (C = O), 1613 $cm.^{-1}$ (NH def.). (Found: C,77.5; H,11.1; N,3.45. $C_{28}H_{49}O_2N$ requires C,77.9; H,11.4; N,3.2%).

Oxidation of 5α-cholestan-3α-ol 1α-amide. - The amide (50 mg.) in benzene (2 ml.) was added to a solution of sodium dichromate (68 mg.) in water (0.5 ml.), acetic acid (0.1 ml.), and concentrated sulphuric acid (0.2 ml.). The reaction mixture was shaken for 6 hr. at room temperature and the layers were allowed to settle, the organic layer was separated, and the aqueous layer was extracted with benzene. The benzene solutions were combined and washed with water, 5% sodium hydroxide, and water again. Evaporation of the benzene and recrystallisation of the residue from petrol gave 3α-amino-1α-carboxy-3β-hydroxy-5α-cholestane lactam (22 mg.), m.p. and mixed m.p. 252 - 254°, and identical in all respects with an authentic specimen.

Deamination of 1α-aminomethyl-5α-cholestan-3α-ol. - Sodium nitrite (700 mg.) in 50% aqueous acetic acid (7 ml.) was added to 1α-aminomethyl-5α-cholestan-3α-ol (350 mg.) in 50% aqueous acetic acid (14 ml.) and the reaction was left at room temperature for 24 hr. More water was added, and the mixture was extracted with ether. An insoluble crystalline solid remained at the interface. The ether layer was separated, washed with water,

and dried over anhydrous sodium sulphate. Evaporation of the ether gave a light brown gum (80 mg.) which was refluxed for 1 hr. with 5% methanolic potassium hydroxide (8 ml.). The solution was diluted with water and the product was isolated with ether in the usual way. Evaporation of the ether gave a gum (70 mg.), which could not be crystallised, and was shown by thin layer chromatography to consist of at least three substances. The mixture was heated on the steam bath for 2 hr. with acetic anhydride (1 ml.) and pyridine (1.5 ml.) and the reaction was worked up with ether in the usual way. Evaporation of the ether gave a very viscous oil which could not be crystallised and gave a positive test with tetranitromethane, $\nu_{max.}^{thin\ film}$ 1730 cm.$^{-1}$ (C = O), 1637 cm.$^{-1}$ (C = C), 1248 cm.$^{-1}$, 1022 cm.$^{-1}$ (C - O), and 885 cm.$^{-1}$ ($=CH_2$).

The ether insoluble portion (190 mg.) was recrystallised from ethanol to give the acetate salt of 1α-aminomethyl-5α-cholestan-3α-ol as needles m.p. 210 - 212° (dec.), $[\alpha]_D^{MeOH}$ +27 (C, 0.4); $\nu_{max.}$ 3330 (-OH), 3175; 3115 ($-NH_3^+$), 1621 (NH), 1540, 1408, 1379 ($-CO_2^-$) and 1030 cm.$^{-1}$ (C - O). (Found: C,75.25; H,11.3; N,3.2. $C_{30}H_{55}O_3N$ requires C,75.4; H,11.6; N,2.9%).

Regeneration of 1α-aminomethyl-5α-cholestan-3α-ol from its acetate salt. - The salt (50 mg.) was shaken with aqueous 2 N

sodium hydroxide and the solid material was collected and recrystallised from ether to give 1α-aminomethyl-5α-cholestan-3α-ol (30 mg.), identical in all respects with an authentic specimen.

Treatment of 3α-amino-1α-carboxy-3β-hydroxy-5α-cholestane lactam with nitrous acid. - (A.) Saturated sodium nitrite solution (1 ml.) and 50% aqueous acetic acid (1 ml.) were added to a suspension of finely ground lactam (30 mg.) in water (3 ml.) at 0°. The reaction was left at room temperature for 18 hr. and then diluted with water. The solid was collected and recrystallised from methanol to give starting material (22 mg.)

(B.) The lactam (300 mg.) in acetic anhydride (8 ml.) and acetic acid (1.5 ml.) was cooled to 0°, treated with sodium nitrite (2 g.), stirred at 0 - 5° for 5 hr., and left in the refrigerator overnight. After being stirred for a further 5 hr. at 0 - 5°, the mixture was diluted with water and the product extracted with ether in the usual way. The residue, after evaporation of the ether, was recrystallised from methanol to give starting material (140 mg.), identical in all respects with an authentic sample. Thin layer chromatography showed the mother liquor to consist of starting material and traces of two other substances.

Tosylhydrazone of 1α-cyano-5α-cholestan-3-one. - 1α-Cyano-5α-

cholestan-3-one (200 mg.) in methanol (8 ml.) was heated under reflux and p-toluenesulphonyl hydrazine (110 mg.) was added. Refluxing was continued for 2 hr., and the reaction mixture was left at room temperature for 18 hr. The crude product was isolated by filtration and recrystallised from chloroform-methanol to give the tosylhydrazone of 1α-cyano-5α-cholestan-3-one (240 mg.), m.p. 215 - 217° (dec.), $[\alpha]_D$-22° (C,1.3); $\lambda_{max.}$ 233 mμ (ε =13,900), $\nu_{max.}$ 3175 (NH), 2245 (CN), 1645 (C = N), 1597, 1493 (aromatic), 813 cm.$^{-1}$ (1,4-disubstituted benzene ring). (Found: N,7.2; S,5.1. $C_{35}H_{53}O_2N_3S$ requires N,7.25; S,5.5%).

Reduction of the tosylhydrazone of 1α-cyano-5α-cholestan-3-one with sodium borohydride. - (A.) The tosylhydrazone (500 mg.) and sodium borohydride (1 g.) in dioxan (25 ml.) were refluxed for 10 hr. The reaction mixture was poured into water and the product was extracted with ether, and the ether washed with saturated sodium bicarbonate and water. Evaporation of the ether extract gave a gum (430 mg.) which was chromatographed on alumina (15 g.). Elution with petrol-benzene (50:1) gave a gum (200 mg.), which was recrystallised from methanol to give 1α-cyano-5α-cholestane (170 mg.) as needles m.p. 95 - 96°, $[\alpha]_D$+70° (C,0.7); $\nu_{max.}$ 2252 cm.$^{-1}$ (CN). (Found: C,84.55;

N,12.13; N,3.8. $C_{28}H_{47}N$ requires C,84.6; H,11.9. N,3.5%). Elution with benzene gave a gum (60 mg.), which could not be crystallised and showed several spots on thin layer chromatography, and appeared to consist mainly of unchanged tosylhydrazone. Elution with ether gave 1α-cyano-5α-cholestan-3α-ol (110 mg.), identical in all respects with an authentic specimen.

(B.) The tosylhydrazone (500 mg.) and sodium borohydride (1 g.) in methanol (50 ml.) were refluxed for 6 hr. The product was isolated as described in the preceding experiment, and the residue (460 mg.) obtained after evaporation of the ether, was shaken with petrol (100 ml.). The suspension was filtered and the filtrate chromatographed on alumina (5 g.). Elution with petrol and petrol-benzene (50:1) gave a mixture of 1α-cyano-5α-cholestane and 1α-cyano-5α-cholest-2-ene (60 mg.), $\nu_{max.}$ 2245 (CN) and 1665 $cm.^{-1}$ (C = C). Thin layer chromatography showed two spots almost fused into one.

Treatment of 1α-cyano-5α-cholestane with alkali. - 1α-Cyano-5α-cholestane (200mg.) was refluxed for 2 hr. with ethanol (1.5 ml.) containing potassium hydroxide (100 mg.) and water (0.3 ml.). The solution was acidified with 2 N hydrochloric acid and the product was isolated in the usual way. Starting material was quantitatively recovered.

Treatment of 1α-cyano-5α-cholest-2-ene with alkali. - 1α-Cyano-5α-cholest-2-ene was refluxed for 6 hr. with ethanol (4 ml.) and water (1 ml.) containing potassium hydroxide (350 mg.). After being neutralised with 2 N hydrochloric acid, the reaction mixture was worked up with ether in the usual way. Evaporation of the ether gave a gum (45 mg.) $\lambda_{max.}$ 216 mμ, $\nu_{max.}$ 2247 cm.$^{-1}$ (CN) and 1664 cm.$^{-1}$ with a shoulder at 1670 cm.$^{-1}$ (C = C). Thin layer chromatography showed the presence of two substances of very similar polarity, one corresponding to starting material. A separation was not effected by column chromatography.

Treatment of 1α-cyano-5α-cholestane with methyl magnesium iodide. - 1α-Cyano-5α-cholestane (100 mg.) in dry benzene (15 ml.) was added to methyl magnesium iodide made from magnesium (110 mg.) and methyl iodide (0.4 ml.) in ether (12 ml.). The solution was refluxed for 4 hr., and after being allowed to cool, was poured on to crushed ice and solid ammonium chloride (250 mg.). After 30 min., water was added and the organic layer was separated. The aqueous layer was extracted with ether and the two organic extracts were combined, washed with water, and dried over anhydrous sodium sulphate. After evaporation of the ether, the residue (93 mg.) was crystallised from methanol

to give 1α-cyano-5α-cholestane (54 mg.). Thin layer chromatography of the mother liquor showed only starting material and traces of two other substances.

Acetophenone from benzonitrile - Sodium hydride (750 mg. of a 50% dispersion in oil) was added to trimethylsulphoxonium iodide (3.3 g.) in dimethylsulphoxide (30 ml.) (purified by drying over calcium hydride for 48 hr. and redistilling under high vacuum) and the reaction was stirred for ½ hr. at room temperature under nitrogen, by which time evolution of hydrogen had ceased. Benzonitrile (1.5 g.) was added and the mixture was allowed to stand at room temperature for 48 hr. and then poured on to crushed ice. When the ice had melted an excess of 2,4-dinitrophenylhydrazine solution and dilute sulphuric acid was added. The precipitate was collected and chromatographed on silica gel (50 g.). Elution with benzene gave the 2,4-dinitrophenylhydrazone of acetophenone (700 mg.), m.p. 236 - 238°, identical in all respects with an authentic specimen.

Benzoic acid from benzonitrile. - The procedure described above was followed up to the stage of the addition of the 2,4-dinitrophenylhydrazine solution. Instead of the hydrazine, an excess of 5% aqueous sodium hydroxide was added and the

reaction was heated on the steam bath for 1.5 hr. The alkaline solution was extracted with ether and the aqueous layer was acidified with 2 N hydrochloric acid and then extracted with ether. The ether solution was washed with water and dried over anhydrous sodium sulphate. Evaporation of the ether gave a white solid which was recrystallised from water to give benzoic acid (990 mg.) as needles m.p. 119 - 121°, identical in all respects with an authentic specimen.

Reaction of 1α-cyano-5α-cholestan-3α-ol with trimethylsulphoxonium methylide. - Sodium hydride (82 mg. of a 50% dispersion in oil) was added to trimethylsulphoxonium iodide (374 mg.) in pure dry dimethylsulphoxide (15 ml.) with stirring under nitrogen. After 1.5 hr., 1α-cyano-5α-cholestan-3α-ol (700 mg.) in pure dry dimethylsulphoxide (7 ml.) was added and stirring under nitrogen was continued for 1 hr. at room temperature. The reaction was left at room temperature for 48 hr. and then poured on to crushed ice, when the ice had melted, the resulting suspension was filtered and the residue was refluxed for 1 hr. with 5% methanolic hydrochloric acid and the reaction worked up in the usual way. Evaporation of the ether gave a gum (660 mg.) which was crystallised from aqueous methanol to give starting material (410 mg.), identical in all respects with an

authentic specimen. The mother liquor was examined by thin layer chromatography and was found to consist almost entirely of starting material, there was no carbonyl absorption in its infrared spectrum.

Reaction of 5α-cholest-1-en-3-one with hydroxylamine. - Hydroxylamine hydrochloride (45 mg.) and sodium acetate (53 mg.) were dissolved in the minimum quantity of methanol and the precipitated sodium chloride was removed by filtration. 5α-Cholest-1-en-3-one (100 mg.) in methanol (3 ml.) was added to the hydroxylamine solution and the mixture was allowed to stand at room temperature for 8 days. The mixture was filtered and the residue was recrystallised several times from methanol to give 5α-cholest-1-en-3-one oxime (35 mg.), m.p. 152 - 154°, $[\alpha]_D$ +93.5° (C, 0.7); $\lambda_{max.}$ 233 mμ (ε = 15,000) and no carbonyl absorption in the infrared. (Found: N, 3.40. $C_{27}H_{45}ON$ requires N, 3.5%).

Reaction of 5α-cholestan-3-one pyrrolidine enamine with N-bromosuccinimide. - The enamine (1 g.) and N-bromosuccinimide (410 mg.) were refluxed in carbon tetrachloride (30 ml.) for 5 min. The precipitated succinimide was removed by filtration, and the filtrate was evaporated to dryness in a rotatory evaporator, and then refluxed for 5 min. with ethanol to decompose any unchanged enamine. The solution was diluted

with water and extracted with ether in the usual way, the ether solution being washed with dilute hydrochloric acid. Evaporation of the ether gave a dark brown gum (600 mg.), which could not be crystallised and showed several spots on thin layer chromatography, one of which corresponded to 5α-cholestan-3-one. The gum was chromatographed on neutral alumina (18 g.), but no pure crystalline material was obtained, the purest fractions from the chromatogram had m.p. 114-118°, undepressed by mixture with 5α-cholestan-3-one, $\nu_{max.}$ 1713 cm.$^{-1}$ (C = O).

REFERENCES

REFERENCES

1. Djerassi, Krakower, Lemin, Liu, Mills, and Villotti, J. Amer. Chem. Soc., 1958, 80, 6284.
2. Barton, Experientia, 1950, 6, 316.
3. Ringold, 'Mechanism of Action of Steroid Hormones', Pergamon Press, New York, 1961, p. 200.
4. Fried and Salbo, J. Amer. Chem. Soc., 1953, 75, 2273; 1954, 76, 1455.
5. Bowers, Denot, Sánchez, Sánchez-Hidalgo and Ringold, J. Amer. Chem. Soc., 1959, 81, 5233.
6. Inhoffen and Huang-Minlon, Ber., 1941, 74, 604.
7. Dreiding and Voltman, J. Amer. Chem. Soc., 1954, 76, 537.
8. Dreiding and Pummer, J. Amer. Chem. Soc., 1954, 76, 3162.
9. Ringold, Rosenkranz, and Sondheimer, J. Amer. Chem. Soc., 1956, 78, 2477.
10. Djerassi, Lipman, and Grossman, J. Amer. Chem. Soc., 1956, 78, 2479.
11. Bowers, Ringold, and Denot, J. Amer. Chem. Soc., 1958, 80, 6115.
12. Djerassi, R. Riniker, and B. Riniker, J. Amer. Chem. Soc., 1956, 78, 6377.
13. Popper, G.P. 1023764 (Chem. Abs., 1960, 55, 5756.)

14. Wiechert, G.P. 1135900. (Chem. Abs., 1962, 57, 15191.)

15. Wiechert and Kaspar, Ber., 1960, 93, 1710.

16. Wiechert, G.P. 1122944 (Chem. Abs., 1962, 57, 5994.)

17. Wiechert, Krob, and Schultz, Naturwiss., 1964, 51, 86.

18. Kharasch and Tawney, J. Amer. Chem. Soc., 1941, 63, 2308.

19. Mori, Chem. Pharm. Bull. (Tokyo), 1962, 10, 386.

20. Wechter, J. Org. Chem., 1964, 29, 163.

21. Bowers and Ringold, U.S.P. 3054809 (Chem. Abs., 1963, 58, 9174; 59, 14074).

22. Julia, Lenares, and Simon, Bull. Soc. chim. France, 1963, 2471.

23. Glen and McLean, Tetrahedron Letters, 1964, 1387.

24. C.K. Ingold, 'Structure and Mechanism in Organic Chemistry', Cornell University Press, New York, 1953, p. 690.

25. Ringold and Rosenkranz, J. Org. Chem., 1956, 21, 1333.

26. Stiller and Rosenheim, J., 1938, 353.

27. Ringold, Batres, Halpern, and Necoechea, J. Amer. Chem. Soc., 1959, 81, 427.

28. Knox and Volarde, J. Org. Chem., 1962, 27, 3925.

29. Edwards, Orr, and Bowers, J. Org. Chem., 1962, 27, 3378.

30. Tsuda and Nozoe, Chem. Pharm. Bull. (Tokyo), 1959, 7, 232.

31. Clinton, Clarke, Skanner, Manson, Jennings, and Phillips, J. Org. Chem., 1962, 27, 2800.

32. Wald and Taurins, Canad. J. Chem., 1960, 38, 1983.

33. Winternitz, Monon and Arnal, Bull. Soc. chim. France, 1960, 505.

34. Kissman, Hoffman, and Weiss, J. Org. Chem., 1962, 27, 3168.

35. Bolt and Backer, Rec. Trav. chim., 1937, 56, 1139.

36. Farmer and Ken, J., 1937, 414.

37. Kuwada and Miyazaka J. Pharm. Soc. Japan, 1938, 58, 540. (Chem. Abs., 1938, 32, 7474.)

38. Barton, Campos-Neves, and Cookson, J., 1956, 3500.

39. Bohlman and Rufer, Ber., 1964, 97, 1770.

40. Wittig and Schollkopf, Ber., 1954, 87, 1318.

41. Corey, Chaykovsky, and Greenwald, J. Org. Chem., 1963, 28, 1128.

42. Marker, Oakwood, and Crooks, J. Amer. Chem. Soc., 1936, 58, 481.

43. Baker and Squire, J. Amer. Chem. Soc., 1948, 70, 1487.

44. Roberts, Shoppee, and Stephenson, J., 1954, 2705.

45. Kaiser and Swarz, J. Amer. Chem. Soc., 1945, 67, 1309.

46. Shoppee and Stephenson, J., 1954, 2230.

47. Ralls, J. Amer. Chem. Soc., 1953, 75, 2123.

48. Corey and Sneen, J. Amer. Chem. Soc., 1953, 75, 6234.

49. Bose, Tetrahedron Letters, 1963, 959.

50. Milas and Priesing, J. Amer. Chem. Soc., 1958, 80, 2189.

51. Henbest and Jackson, J., 1962, 954.

52. Pohorylos, Gat, and Sarel, J., 1962, 3261.

53. Nathanson and Ribaldone, Gazzetta, 1959, 89, 1218, 1884; Nathanson and Pirola, ibid., 1960, 90, 407; Nathanson, Casu, and Ribaldone, ibid., 1960, 90, 1147.

54. Kagan, Bull. Soc. chim. France, 1960, 535; Kagan, Marquet, Dvolaitzky, Lemaire, and Jaques, ibid., 1960, 539; Kagan, Marquet and Jaques, ibid., 1960, 1079.

55. Masur and Sondheimer, J. Amer. Chem. Soc., 1958, 80, 5220.

56. Woodward, Patchett, Barton, Ives, and Kelly, J., 1957, 1131.

57. Beton, Halsall, Jones, and Phillips, J., 1957, 753.

58. Atwater, J. Amer. Chem. Soc., 1957, 79, 5315.

59. Turner, J. Amer. Chem. Soc., 1950, 72, 581.

60. Meakins and Rodig, J. 1956, 4679.

61. Hartman, Tomasewski, and Dreiding, J. Amer. Chem. Soc., 1956, 78, 5662.

62. Ringold and Rosenkranz, J. Org. Chem., 1957, 22, 602.

63. Kirk and Petrow, J., 1962, 1091.

64. Bell, Ph.D. Thesis, R.C.S.T. Glasgow, 1961.

65. Julia and Lavaux, Compt. rend., 1960, 251, 733.

66. Julia and Moutonnier, Bull Soc. chim. France, 1964, 321.

67. Westphalen, Ber., 1915, 48, 1064.

68. Lettré and Muller, Ber., 1937, 70, 1947.

69. Bladon, Nembest, and Wood, J., 1952, 2737.

70. Ellis and Petrow, J., 1952, 2246.

71. Urushibara and Chuman, Bull. Chem. Soc. Japan, 1949, 22, 69.

72. Birch and Smith, Proc. Chem. Soc., 1962, 356.

73. Burgstahler and Nordin, J. Amer. Chem. Soc., 1959, 89, 3151.

74. Nagata, Hirai, Itazaki, and Takeda, J. Org. Chem., 1961, 26, 2413.

75. Bowers, J. Org. Chem., 1961, 26, 2043.

76. Nagata, Hirai, Itazaki, and Takeda, Annalen, 1961, 641, 184, 196.

77. Torrigoe and Fishman, Tetrahedron Letters, 1963, 1251.

78. Johnson, Bauer, Margrove, Frisch, Dreger, and Hubbard, J. Amer. Chem. Soc., 1961, 83, 610.

79. Holker and Whalley, Proc. Chem. Soc., 1961, 464.

80. Naco and Turner, J. Amer. Chem. Soc., 1953, 75, 4063.

81. Jones, Ramsay, Herling, and Dobrinor, J. Amer. Chem. Soc., 1952, 74, 2828.

82. Mazur and Sondheimer, J. Amer. Chem. Soc., 1958, 80, 5220.

83. Djerassi, Finch, Cookson, and Bird, J. Amer. Chem. Soc., 1960, 82, 5488.

84. Djerassi and Klyne, J. Amer. Chem. Soc., 1957, 79, 1506.

85. Mauli, Ringold, and Djerassi, J. Amer. Chem. Soc., 1960, 82, 5494.

86. Sondheimer, Klibansky, Haddad, Summers, and Klyne, J., 1961, 768.

87. Moffit, Woodward, Moscowitz, Klyne, and Djerassi, J. Amer. Chem. Soc., 1961, 83, 4013.

88. Cropp, Dewhurst, and Holker, Chem. and Ind., 1961, 209, 1667.

89. Djerassi, Lund, and Akhrem, J. Amer. Chem. Soc., 1962, 84, 1249.

90. Williamson and Johnson, J. Amer. Chem. Soc., 1961, 83, 4623.

91. Karplus, J. Chem. Phys., 1959, 30, 11.

92. Kuriyama, Kondo, and Tori, Tetrahedron Letters, 1963, 1485.

93. Karplus, J. Amer. Chem. Soc., 1963, 85, 2870.

94a. Rome, Tetrahedron, 1958, 2, 37.

b. Nagata, Kikkawa, and Takeda, Chem. Pharm. Bull. (Tokyo), 1961, 9, 79;

c. Marshall and Johnson, J. Amer. Chem. Soc., 1962, 84, 1485;

d. Nagata, Teresawa and Aoki, Tetrahedron Letters, 1963, 865.

95. Lapworth, J., 1903, 995; 1904, 1214; 1906, 945; Jones, ibid., 1914, 1547.

96. Meyer and Wolfe, J. Org. Chem., 1964, 29, 170.

97. Green and Long, J., 1961, 2532.

98. Warnhoff, J. Org. Chem., 1962, 27, 4587.

99. Butenandt and Wolff, Ber., 1935, 68, 2093.

100a. Woodward, J. Amer. Chem. Soc., 1942, 64, 72;

b. L.F. Fieser and M. Fieser, 'Steroids', Rheinhold, New York, 1959, p. 15.

101. Westphal, Ber., 1937, 70, 2128.

102. Dauben and Eastham, J. Amer. Chem. Soc., 1951, 73, 3261; Moffet and Weisblat, ibid., 1952, 74, 2185.

103. Ref. 100b, p.271

104. Ringold and Turner, Chem. and Ind., 1962, 211; Ringold, Gut, Hayano, and Turner, Tetrahedron Letters, 1962, 835.

105. Dr. A.B. Turner, private communication.

106. Schmitz and Johnson, Tetrahedron Letters, 1962, 647.

107. Wendler, Taub, and Kuo, J. Amer. Chem. Soc., 1960, 82, 5701.

108. Romo, Tetrahedron, 1963, 19, 25.

109. Beal and Pike, J. Org. Chem., 1961, 26, 3887.

110. Nagata, Tetrahedron, 1961, 13, 278.

111. Barton and Morrison, Fortschr. Chem. org. Naturstoffe, 1961, 19, 172.

112a. Tamm and Albrecht, Helv. Chim. Acta, 1960, 43, 768;

b. Shoppee, Roy, and Goodrich, J., 1961, 1586.

113. Adams, Kirk, Patel, Petrow, and Stuart-Webb, J., 1954, 2298.

114. Tadanier and Colo, J. Org. Chem., 1962, 27, 4624.

115. Loewenthal, Tetrahedron, 1959, 6, 269.

116. Barton, J., 1953, 1027.

117. Dauben, Micheli, and Eastham, J. Amer. Chem. Soc., 1952, 74, 3852.

118. Bernstein, Lenhard, and Williams, J. Org. Chem., 1953, 18, 1166.

119a. Dauben, Fonken, and Noyce, J. Amer. Chem. Soc., 1956, 78, 2579;

b. Dauben, Blanz, Jui, and Micheli, ibid., 1956, 78, 3752.

120. Combe and Henbest, Tetrahedron Letters, 1961, 404.

121. D.M.S. Wheeler and M.M. Wheeler, J. Org. Chem., 1962, 27, 3798.

122. D.M.S. Wheeler and Huffman, Experientia, 1960, 16, 516.

123. D.M.S. Wheeler and Vail, J. Org. Chem., 1962, 27, 3803.

124. Beckett, Harper, Balon, and Watts, Tetrahedron, 1959, 6, 319.

125. Jones and Wise, J. Amer. Chem. Soc., 1962, 84, 997.

126. Haubenstock and Eliel, J. Amer. Chem. Soc., 1962, 84, 2363, 2368.

127. Naco and O'Connor, J. Amer. Chem. Soc., 1951, 73, 5824.

128. Dewar, 'Electronic Theory of Organic Chemistry', Oxford University Press, London, 1949, p.136.

129. Mateos and O.M. Wheeler, Canad. J. Chem., 1958, 36, 1431.

130. Barter and Spring, J., 1943, 613.

131. Clark, Ph.D. Thesis, R.C.S.T. Glasgow, 1962.

132. Henbest and Lovell, J., 1957, 1965.

133. Pimental and McLellan 'The Hydrogen Bond', Freeman, San Francisco, 1960, p.293.

134. Taylor and Baker, 'Sidgwicks Organic Chemistry of Nitrogen', Oxford University Press, London, 1937, p. 154.

135. Barton, Beaton, Geller, and Pechet, J. Amer. Chem. Soc., 1961, 83, 4076; Krok and Wolff, J. Org. Chem., 1963, 28, 423; Wolff and Muñoz, Chem. and Ind., 1964, 1312.

136. Chang and Blickenstaff, Chem. and Ind., 1958, 590.

137. Chang and Blickenstaff, J. Amer. Chem. Soc. 1958, 80, 2906.

138. Dr. D. Savage, Organon Laboratories Ltd., private communication.

139. Butenandt, Mamoli, and Heusner, Ber., 1939, 72, 1614.

140. Burn and Petrow, J., 1962, 365.

141. Gilman, 'Organic Reactions', Wiley, New York, 1954, Vol. 8 p. 258.

142. Gaylord, 'Reduction with Complex Metal Hydrides', Interscience, New York, 1956, p. 748.

143. Brown, Sheaf and Garg, Tetrahedron Letters, 1959, (No. 3), 9.

144. Ref. 141, p. 246.

145. Ref. 134, p. 23.

146. Shoppee, Evans, and Summers, J., 1957, 97; Evans and Summers, ibid., 906; Shoppee, Cromlyn, Evans, and Summers, ibid., 4364.

147. Tadanier and Cole, J. Org. Chem., 1962, 27, 4615.

148. Bladon and McMeekin, J., 1961, 3504.

149. Caglioti and Grasselli, Chem. and Ind., 1964, 153.

150. König and Metzger, Z. Naturforsch., 1963, 18 b, 976.

151. Corey and Chaykovsky, J. Amer. Chem. Soc., 1962, 84, 867.

152. Major and Hess, Annalen, 1958, 611, 117.

153. Szmuszkovicz, Advances in Organic Chemistry, 1963, 4, 1.

154. Hoyl and Horr, J. Amer. Chem. Soc., 1953, 75, 1918.

155. Kötz and Grethe, J. Prakt. Chem., 1909, 80, 501.

156. Baddeley and Brocklehurst, Proc. Chem. Soc., 1962, 145.

157. Shoolery and Rogers, J. Amer. Chem. Soc., 1958, 80, 5121.

158. Jacquesy, Lehn, and Lovicalles, Bull. Soc. chim. France, 1961, 2444.

159. Zürcher, Helv. Chim. Acta, (a) 1961, 44, 1380; (b) 1963, 46, 2054.

160. Cross and Harrison, J. Amer. Chem. Soc., 1963, 85, 3223.

161. Djerassi, 'Optical Rotatory Dispersion', McGraw-Hill, New York, 1960, p. 42.

162. Kuriyama and Tori, private communication.