THE REACTION OF DIKETENE WITH STEROID ALCOHOLS

and

STEROIDS DERIVED FROM HECOGENIN

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T H E S I S

submitted to

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in fulfilment of the

requirements for the

DEGREE OF DOCTOR OF PHILOSOPHY

by

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September, 1962.

Summary

Part I of this thesis describes the preparation of the acetoacetates of steroid alcohols by reaction with diketene, and the examination of their pyrolysis products.

Acetoacetates of 3-alcohols were very stable, little or no decomposition to the original alcohol being noticeable. The same was true of other secondary and tertiary alcohols studied.

Alcohols in the 17-position having an adjacent 20-ketone gave $\alpha\beta$-unsaturated lactones having an acetyl side-chain. Attempts were made to remove this side-chain and thus obtain a lactone ring similar to that found in the cardenolides.

Hydrolysis using acid and alkali, and the Haleform Reaction were unsuccessful. The 23-oxime was prepared with the intention of carrying out Beckmann Rearrangement to the amide, followed by hydrolysis and rearrangement to the required product. This was also unsuccessful. The oxime, on treatment with acid or base gave an interesting compound formulated as 4-(3'β,17'α-dihydroxy-11'-oxo-20'-pregnenylidene)-3-methyl-5-isoxazolone.

At the other end of the molecule the 3-acetate was hydrolysed, oxidised, and a 1,4-diene system inserted by reaction of the saturated 3-ketone with 2,3-dichloro-5,6-dicyanoquinone.

The second part of the thesis deals with an attempted prepar-

ation of an 18-oxygenated steroid from hecogenin acetate cyanhydrin.
The latter compound on treatment with thionyl chloride gave 3β-
acetoxy-13β-cyano-17a-methylene-C-nor-D-homo-5α,25D-spirostane
which gave the 17a-ketone on ozonolysis. Hydrolysis of the nitrile
group did not give the β-keto acid expected.

3β-Acetoxy-17a-oxo-C-nor-D-homo-5α,25D-spirostane was prepared,
and its cyanhydrin, when treated with thionyl chloride, gave an
elimination and not the rearrangement expected.

The 17a-methylene compound obtained above was reduced to the
13β-aminomethyl steroid with lithium aluminium hydride. Nitrous
acid treatment of the amine gave the alcohol and not the ring
enlarged compound.

C-Homo-hecogenin was obtained by reduction of hecogenin acetate
cyanhydrin with lithium aluminium hydride and treatment of the crude
product with nitrous acid. Separation of the ketonic fraction with
Girard Reagent T gave the required product. The amine from the
reduction was characterised as the acetate. This acetate, on treat-
ment with nitrous acid gave a ketone, thought to be the isomeric
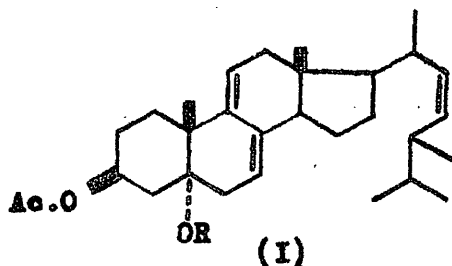C-homo ketone.

CONTENTS

PART I

THE REACTION OF DIKETENE WITH STEROID ALCOHOLS

INTRODUCTION

The observation by Clayton[1] that pyrolysis of 5a-acetoacetoxy-3β- acetoxyergosta-7:9(11):22-triene (I; R=OCOCH$_2$COCH$_3$) gave the 5a-hydroxy compounds prompted an investigation into the value of the acetoacetate group as a protecting group.

(I)

That this was unsuccessful was rather overshadowed by the discovery that side-chain a-ketols when treated with diketene gave lactones similar to those found in the cardiac aglycones.

The cardiac aglycones fall into two main groups, the cardenolides (II) and the bufadienolides (III).

(II)   (III)
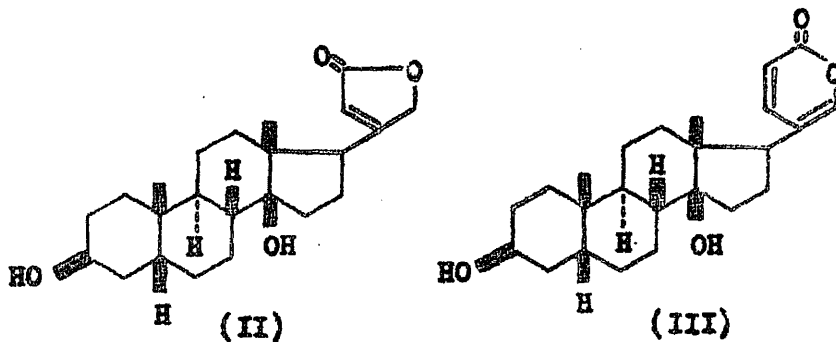
The more prevalent cardenolides, are C_{23} steroids having a β-side-chain which consists of an αβ-unsaturated five-membered lactone ring. In the bufadienolides, the side-chain is a doubly unsaturated six-membered lactone ring. Both series carry a 14β-hydroxyl group, rings C and D being trans-fused.

The stereochemistry of the ring system invariably corresponds to that of a sterol or bile acid at C_8, C_9, C_{10}, C_{13}, and C_{17}, the main differences between the two series being at C_3 and C_5 and the presence or absence of oxygen functions at other positions in the molecule.

The cardenolides occur as plant glycosides, while bufadien-olides occur in both plant and animal organisms. Hydrolysis of the glycosides gives the aglycone and a sugar residue. About twenty such sugars have been isolated from hydrolysis products of cardiac glycosides, most of which are not obtainable from other plant sources.

The lactone (IV) described below, prepared by reaction of a 21-hydroxy-20-oxo-steroid with diketene was similar to the cardenolide lactone, except for an additional acetyl side-chain.

(IV)   (V)
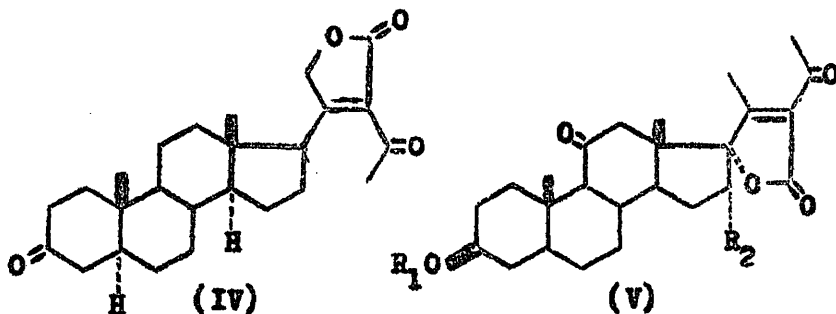
Removal of this acetyl side-chain would obviously give the true cardenolide lactone ring. In our attempts to carry this out, the more readily available lactones (V; R=H or CH_3), from 17α-hydroxy-20-oxo-steroids were used. These efforts were unsuccessful.

Pharmacological screening of the lactones (V; R=H or CH_3) showed anticorticoid activity; in other words, the lactones could nullify the sodium and water retaining effects of compounds such as deoxycorticosterone (VI) and aldosterone (VII).

(VII)
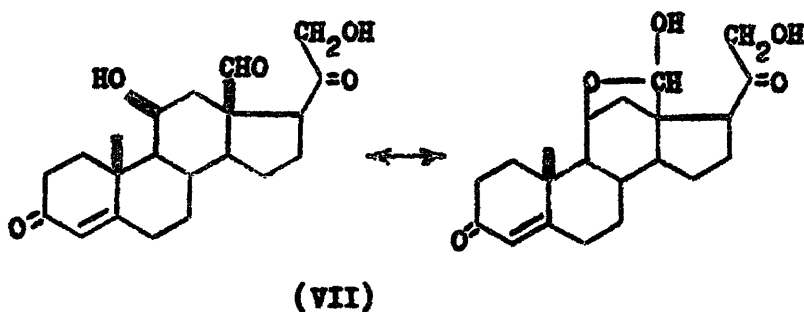
Similar, but unsubstituted saturated lactones (VIII; R=H or CH_3) have been synthesised by Cella and Kagawa[2] and shown to be aldosterone antagonists.

(VI)   (VIII)
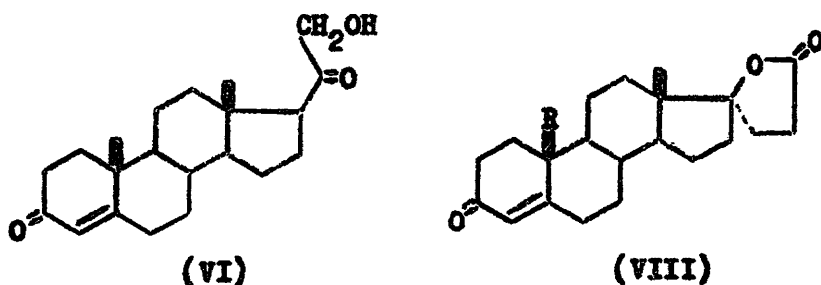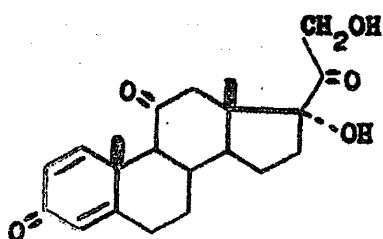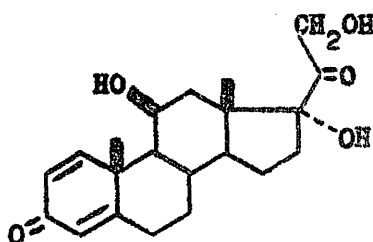
(IX)   (X)

It has been reported[3] that $\Delta^{1,4}$-3-ones (IX) and (X) have 3 times the activity of the 1,2-saturated steroids in the treatment arthritis. For this reason, the lactones (V) prepared below, were converted to $\Delta^{1,4}$-diene-3-ones. This was achieved by treatment of the saturated 3-ketone with 2,3-dichloro-5,6-dicyanoquinone. The use of this reagent to obtain $\Delta^{1,4}$-diene-3-ones from Δ^{4}-3-ones has been described by Burn, Kirk, and Petrow[4]. However, the same reagent can also be used with saturated 3-ketones[5] to give $\Delta^{1,4}$-diene-3-ones. The results of screening tests on these compounds are not yet available.

THEORETICAL

The following study of steroid acetoacetates and the products derived from them was prompted by a report[1] that pyrolysis of 5α-acetoacetoxy-3β-acetoxyergosta-7,9(11),22-triene (I; R=COCH$_2$COCH$_3$) and related compounds resulted in decomposition to the original alcohols (I; R=H). If this is generally true of steroid acetoacetates, a very convenient protecting group will have been found.

OR   (I)
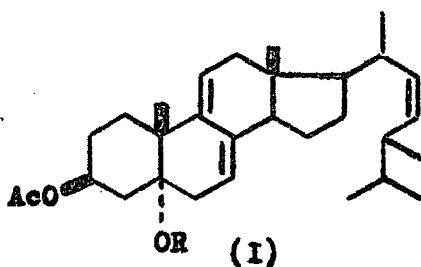
The acetoacetates of cholesterol (XI; R=H), ergosterol (XII; R=H), hecogenin (XIII; R=H), and diosgenin (XIV; R=H) were prepared by refluxing with diketene in a suitable solvent, and in the presence of a basic catalyst, triethylamine.

(XI)

(XII)
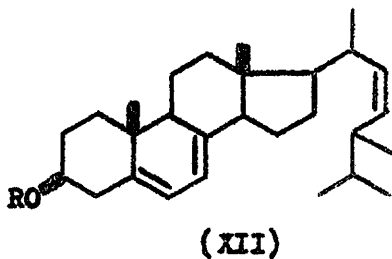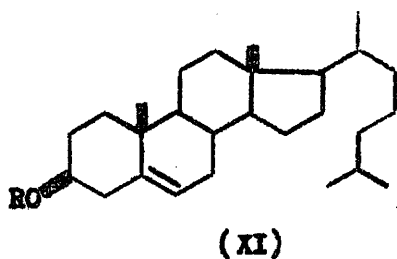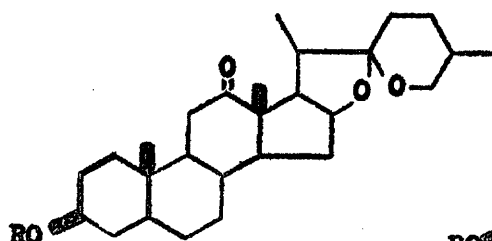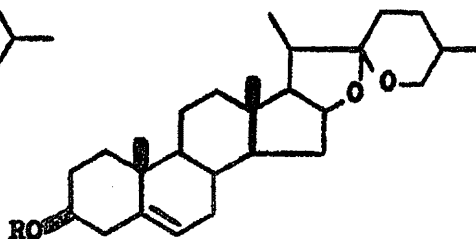
(XIII)   (XIV)

At the same time, a polymer of diketene was formed, and chromatography was necessary to isolate the products. The polymer appears to be identical with a compound examined by Steel, Boese, and Dull[6] and which they claim to be 2,6-bis-(6-methyl-4-oxo-2-pyranylmethyl)-pyrone (XV).
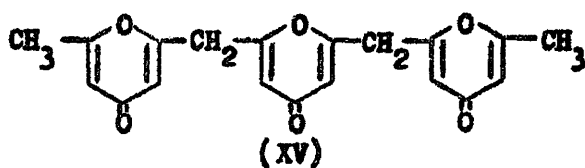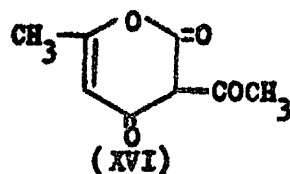
(XV)   (XVI)

Cholesteryl[7] and ergosteryl[8] acetoacetates (XI and XII; $R=COCH_2COCH_3$) are known, and the physical constants recorded below are in agreement with the published values. The acetoacetates of hecogenin and diosgenin (XIII and XIV; $R=COCH_2COCH_3$) are claimed in a patent[9] but the compounds themselves are not described.

Pyrolysis of cholesteryl acetoacetate was attempted under vacuum at 200°. The product was mainly unchanged acetoacetate. That some decomposition to the original alcohol did occur, was shown by the presence of a little dehydroacetic acid (XVI), a dimer of diketene, at the top of the pyrolysis tube.

The other acetoacetates mentioned above gave similar results, thus establishing the stability of acetoacetates of 3β-hydroxy steroids.
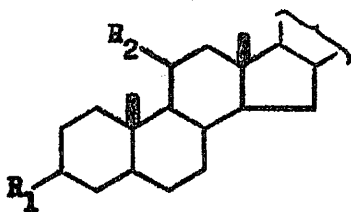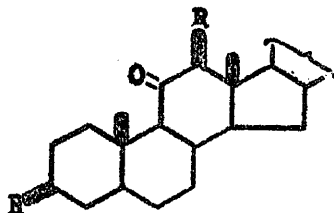
(XVII)   (XVIII)

Other secondary alcohols treated with diketene were 11α-hydroxy-tigogenin (XVII; R_1=β-OH, R_2=α-OH), 11β-hydroxytigogenin (XVII; R_1=R_2=β-OH), and its 3-acetate (XVII; R_1=β-OAc, R_2=β-OH), and 3β,12β-dihydroxy-11-oxo-5α,25D-spirostane (XVIII; R=β-OH) all but the last of which, were non-crystalline.

Attention was now turned to tertiary hydroxyl groups since it appeared that any instability may be due to the steric hindrance at

these points. In particular, two 5α-hydroxy steroids were treated with diketene and examined as before. These were 3β-acetoxy-5α-hydroxycholestane (XIX; R=H) and 3β-acetoxy-5α-hydroxy-11-keto-ergostane (XX; R=H).

(XIX)   (XX)
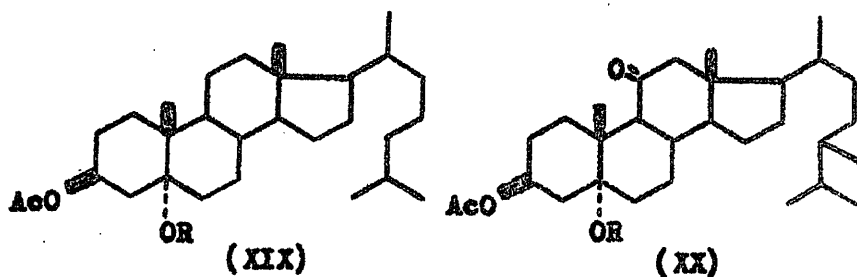
The former compound (XIX; R=COCH$_2$COCH$_3$) was heated under vacuum in a pyrolysis tube which was connected to a U-tube containing aniline. By this means, any ketene or diketene formed as a result of the pyrolysis could be characterised as acetanilide or acetoacet-anilide respectively. Once again, the only decomposition product found was a small quantity of dehydroacetic acid (XVI).

By this time, it was realised that the acetoacetates of steroids were, in general, stable compounds. Any instability in the compounds reported[1] must be due to the diene system. Since ergosterol (XII; R=H) has a similar diene system, and does not give an unstable acetoacetate, it is concluded that decomposition must be associated

with a _tertiary_ hydroxyl group, β to a conjugated diene system.

It was found in a later experiment, however, that treatment of cholesteryl acetoacetate (XI; R=COCH₂COCH₃) with hydroxylamine hydrochloride gave cholesterol (XI; R=H).

(XXI)
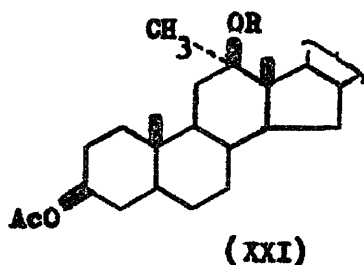
An attempt to prepare the acetoacetate of 3β-acetoxy-12β-hydroxy-12α-methyl-5α,25D-spirostane (XXI; R=H) resulted in the formation of a gum. Infrared and ultraviolet spectra showed that reaction had taken place, but no crystalline material could be isolated. Hydrolysis of a sample of the gum gave the 3β,12β-diol which was crystalline.

(XXII)   (XXIII)
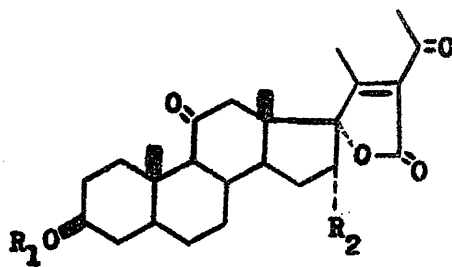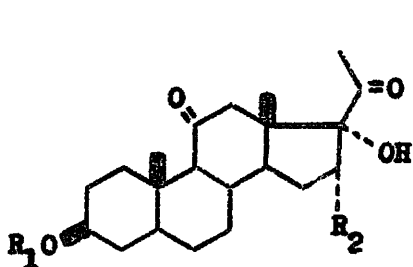
The third type of tertiary hydroxyl group to be studied was that in the 17α-position in the pregnane series. The compounds[10] of this type used were 3β-acetoxy-17α-hydroxy-11,20-dioxo-5α-pregnane (XXII; R_1=Ac, R_2=H) and its 16α-methyl analogue (XXII; R_1=Ac, R_2=CH$_3$). When treated with diketene as before, these compounds formed $\Delta^{\alpha\beta}$-lactones having an α-acetyl substituent (XXIII).

A similar but unsubstituted αβ-unsaturated lactone structure is present in the cardiac aglycones, which have an ultraviolet absorption at 220 mμ. This difference can only be due to the presence of the acetyl group. The compounds obtained also gave positive Legal[11,12] tests, indicative of $\Delta^{\alpha\beta}$-lactones.

With the formation of the $\Delta^{\alpha\beta}$-lactone system in mind, 21-hydroxy-3,20-dioxo-5α-pregnane (XXIV; R=H) was treated with diketene, giving a compound (VI) having the α-acetyl cardenolide lactone structure. As this reaction with 21-hydroxy steroids has already been described by Ruschig, Fritsch, and Lindler,[9] attention was confined to the 17α-hydroxy-20-oxo-steroids described above.

(XXIV)   (IV)
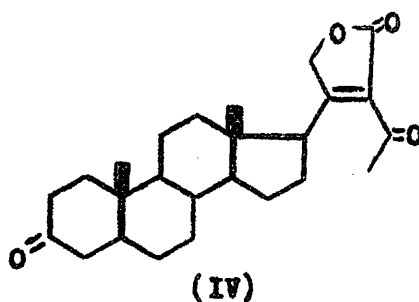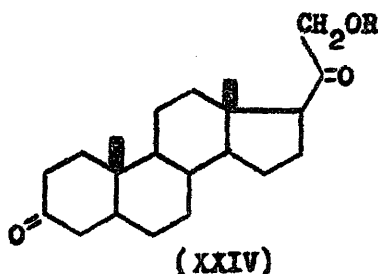
One significant difference between the compounds described below and those described by the German workers is that the latter claim to obtain the open chain acetoacetates which are then cyclised by means of sodium in methanol, while the 17α-acetoacetoxy compounds apparently cyclise spontaneously. Since all diketene products obtained in the work presently described were chromatographed on deactivated alumina, however, it may be that this is sufficient to cause cyclisation. Rushig et al.[9] report that the αα'-acetone-dicarboxylic acid ethyl ester derivative of 21-hydroxy-3,20-dioxo-5α-pregnane (XXIV; R=COCH$_2$COCH$_2$CO$_2$Et) is cyclised by allowing to stand on an alumina column in 1:1 petroleum ether-benzene for 2-3 hours.

The $\Delta^{\alpha\beta}$-lactones (XXIII) were pharmacologically screened and shown to have some anticorticoid activity i.e. they reduce the sodium retaining effects of the mineralocorticoids. It was felt therefore that removal of the α-acetyl side-chain should give rise to interesting compounds having increased activity.

Of the two starting materials the 16α-methyl steroid (XXIII; R$_1$=Ac, R$_2$=CH$_3$) was more readily obtainable, hence most of the reactions were carried out using this compound. Some, but not all, of the reactions repeated using 3β-acetoxy-17α-hydroxy-11,23-dioxochol-20(22)-ene-22-carboxylic acid 22→17 lactone (XXIII; R$_1$=Ac, R$_2$=H). The results in both series were almost exactly parallel, no

difference due to the 16α-methyl group being noticeable.

Perhaps the most obvious method of removing the 22-acetyl group is by means of the haloform reaction which should give the 22-carboxylic acid (XXV) and hence by decarboxylation the required product (XXVI). However, treatment of 3β-acetoxy-17α-hydroxy-11,23-dioxochol-20(22)-ene-22-carboxylic acid 22→17 lactone (XXIII; R_1=Ac, R_2=H) in alkaline solution with iodine in potassium iodide solution[13] gave no recognisable product. Sodium hypobromite[14] and sodium hypochlorite[15] also gave disappointing results when reacted with the corresponding 16α-methyl derivative (XXIII; R_1=Ac, R_2=CH$_3$). In the latter case, starting material was obtained. The failure of the iodoform reaction may be due to the proximity of the double bond to the methyl ketone.

(XXV)   (XXVI)
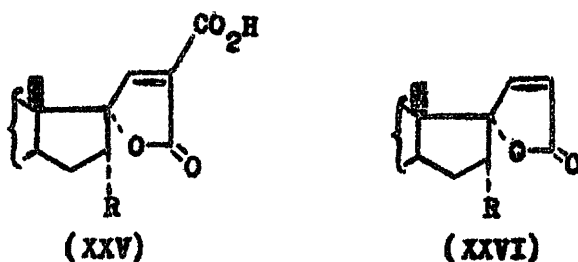
Attention was now turned to hydrolysis using strong acid or alkali. Cold methanolic potassium hydroxide had no effect other than to remove the 3-acetyl group giving the 3β-hydroxy compounds (XXIII; R_1=H, R_2=H or CH$_3$). A similar result was obtained with potassium

hydroxide in refluxing methanol. Hydrolysis of 3β-acetoxy-17a-
hydroxy-16a-methyl-11,23-dioxochol-20(22)-ene-22-carboxylic acid
22→17 lactone (XXIII; R_1=Ac, R_2=CH$_3$) using potassium hydroxide in
refluxing ethylene glycol resulted in extensive decomposition. The
crude product gave a negative Legal test.

Hydrochloric acid in acetic acid gave back unchanged starting
material. The same acid in methanol at 65° and 140° resulted in the
formation of the 3β-hydroxy derivative (XXIII; R_1=H, R_2=CH$_3$), while
ethylene glycol at 190° gave a black tar from which no steroid
could be isolated.

The use of hydrochloric acid in acetic acid was due to Lacey[16]
who has made a thorough study of the reaction of diketene with low
molecular weight, 1,2 and 1,3 ketols (XXVII).
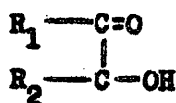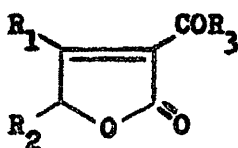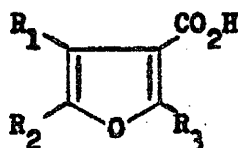
(XXVII)   (XXVIII)   (XXIX)

The products of these reactions cyclised spontaneously, or on
treatment with alkali to give lactones of the type shown (XXVIII).
These lactones on treatment with hydrochloric acid in acetic acid,
gave β-furoic acids (XXIX). Clearly, the structures of the steroid

lactones described above do not permit this type of rearrangement. Thus, it appears that the lack of success with hydrolysis attempts was due to the inability of the 20(22)-double bond to move into the 17(20)-position. For this reason it was decided to hydrogenate the double bond, and attempt to hydrolyse the product.

In the hydrogenation of 3β-acetoxy-17α-hydroxy-11,23-dioxochol-20(22)-ene-22-carboxylic acid 22→17 lactone (XXIII; R_1=Ac, R_2=H) and its 16α-methyl homologue (XXIII; R_1=Ac, R_2=CH$_3$) it was found that two products could be obtained, depending on whether palladium on charcoal or platinum oxide was used as catalyst. The latter tended to take the reaction a stage further.
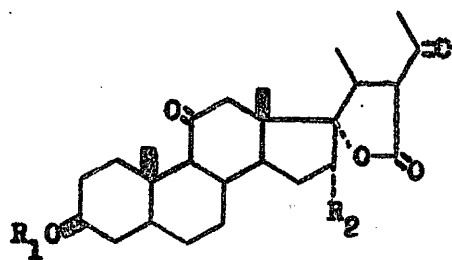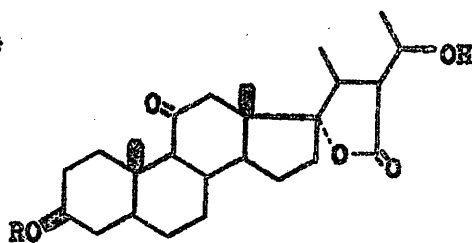
(XXX)   (XXXI)

Thus, with palladium on charcoal, 3β-acetoxy-17α-hydroxy-11,23-dioxochol-20(22)-ene-22-carboxylic acid 22→17 lactone (XXIII; R_1=Ac, R_2=H) gave 3β-acetoxy-17α-hydroxy-11,23-dioxocholan-22-carboxylic acid 22→17 lactone (XXX; R_1=Ac, R_2=H) while platinum

oxide yielded 3β-acetoxy-17,23-dihydroxy-11-oxocholan-22-carboxylic acid 22→17 lactone (XXXI; R=Ac).

With the more powerful platinum oxide, the 23-carbonyl group has been reduced. This can occur either by direct attack on the carbonyl group, or by enolisation, followed by reduction of the double bond. Reduction of the 23-carbonyl in preference to the 11-carbonyl is postulated since the infrared spectrum shows that the hydroxyl group formed is hydrogen bonded. This can only happen at the 23-position.

Hydrogenation of the 16α-methyl derivative (XXIII; R_1=Ac, R_2=CH$_3$) using palladium on charcoal as catalyst followed an identical pattern to that indicated above, the products being 3β-acetoxy-17α-hydroxy-16α-methyl-11,23-dioxocholan-22-carboxylic acid 22→17 lactone (XXX; R_1=Ac, R_2=CH$_3$) and 3β-acetoxy-17,23-dihydroxy-16α-methyl-11-oxocholan-22-carboxylic acid 22→17 lactone (XXXII; R=Ac).
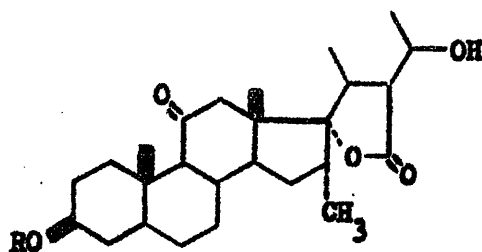
(XXXII)

All hydrogenation products gave a negative Legal test, showing
the absence of the $\Delta^{\alpha\beta}$-lactone system. Both palladium products have
ultraviolet absorption maxima at about 255 mμ in ethanol, which is
raised to 282 mμ in alkaline solution. This behaviour is similar
to that of ethyl acetoacetate[17] (245→274 mμ). The platinum oxide
reduction products showed no such absorption, which indicates that the
23-carbonyl has been affected.

Hydrolysis of the saturated lactones (XXX; R_1=Ac, R_2=H or CH_3)
gave non-crystalline products. Infrared spectra, however, indicated
that the lactone had remained intact.
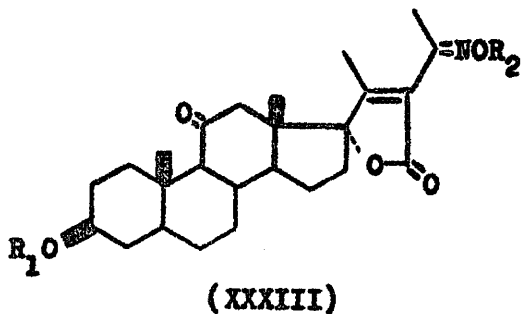
(XXXIII)

The next approach to removal of the α-acetyl side-chain was to
prepare the 23-oxime (XXXIII; R_1=Ac, R_2=H) by treatment of 3β- acetoxy
-17α-hydroxy-11,23-dioxochol-20(22)-ene-22-carboxylic acid 22→17
lactone (XXIII; R_1=Ac, R_2=H) with hydroxylamine. It was planned to
rearrange the oxime to the amide (XXXIV) which should be capable of

hydrolysis to the acid (XXXV). This on decarboxylation should give the required $\Delta^{\alpha\beta}$-lactone (XXVI) with no 22-substituent.
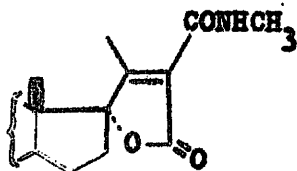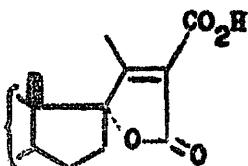
(XXXIV)　　　　　(XXXV)　　　　　(XXVI)
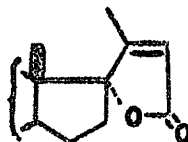
Beckmann rearrangement, however, did not occur, the only products being starting material and a small quantity of a compound formulated as 4-(3'β-acetoxy-17'α-hydroxy-11'-oxo-20'-pregnenylidene)-3-methyl-5-isoxazolone (XXXVI; R=Ac).

The oxime was hydrolysed with both hydrochloric acid and potassium hydroxide in an attempt to regenerate the 23-ketone. The product in both cases was 4-(3'β,17'α-dihydroxy-11'-oxo-20'-pregnenylidene)-3-methyl-5-isoxazolone (XXXVI; R=H).
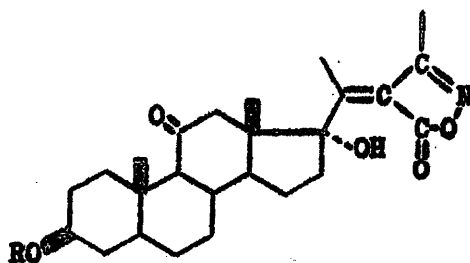
(XXXVI)

Acetylation of this diol gave a diacetate, shown to be 3β-acetoxy-23-acetoximino-17a-hydroxy-11-oxochol-20(22)-ene-22-carboxylic acid 22→17 lactone (XXXIII; $R_1 = R_2 = Ac$) and which gave a mixture of the oxime (XXXIII; $R_1 = Ac$, $R_2 = H$) and its hydrolysis product (XXXVI; R=H) on chromatography.

Acetylation of the oxime (XXXIII; $R_1 = Ac$, $R_2 = H$) gave, on repeated crystallisation, starting material. It was found that the infrared spectrum of the acetylation product, before recrystallisation was identical with the spectrum (observed under similar conditions) of the product obtained on acetylation of the 3β-ol (XXXVI; R=H).

It would appear that the oxime side-chain can exist in lactone or isoxazolone forms; which form is isolated depends on whether the group at C_3 is hydroxyl or acetoxyl. When the 3-position bears an acetoxy group, the product is isolated as the oxime (XXXIII; $R_1 = Ac$, $R_2 = H$), while the 3β-ol exists mainly as the isoxazolone (XXXVI; R=H).

Attempted hydrogenations of the oxime and its hydrolysis product in the presence of acetic acid were unsuccessful. In the former case, however, a small quantity of the isoxazolone (XXXVI; R=Ac) was obtained.

The diacetate (XXXIII; $R_1 = R_2 = Ac$) had a triple melting point, with apparent decomposition after the second melting point. Pyrolysis of this diacetate at 225° did not give a crystalline product.

3β-Acetoxy-17α-hydroxy-11,23-dioxochol-20(22)-ene-22-carboxylic

acid 22→17 lactone (XXIII; R_1=Ac, R_2=H) was treated with urea,

phenylhydrazine, hydrazine hydrate, and semicarbazide hydrochloride

in attempts to prepare derivatives analagous to the isoxazolone

described above. Urea was without effect and starting material was

recovered, while the phenylhydrazone was non-crystalline. In the case

of the last two reagents crystalline products were obtained.
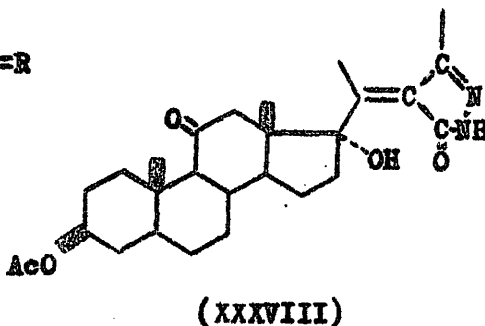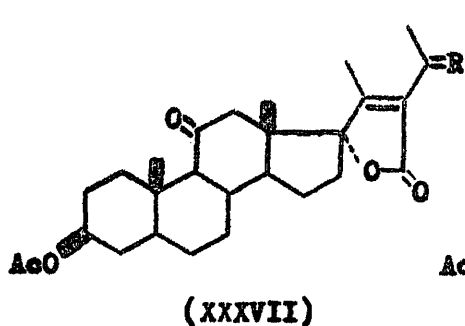
(XXXVII)   (XXXVIII)

Hydrolysis of the hydrazone (XXXVII; R = N.NH) gave material

thought to be 4-(3'β,17'α-dihydroxy-11'-oxo-20'-pregnenylidene)-3-

methyl-5-oxo-pyrazoline (XXXVIII; R=H). The semicarbazone (XXXVII;

R = N.NH.CO.NH$_2$) on the other hand, gave 3β,17α-dihydroxy-11,23-dioxo-

chol-20(22)-ene-22-carboxylic acid 22→17 lactone (XXIII; R_1=R_2=H).

Insufficient material was available to permit a full examination of

the hydrolysis products.

Treatment of 3β-acetoxy-17α-hydroxy-11,23-dioxocholan-22-

carboxylic acid 22→17 lactone (XXX; R_1=Ac, R_2=H) with urea gave
starting material, but reaction with hydroxylamine hydrochloride gave
the 23-oxime (XXXIX).

(XXXIX)
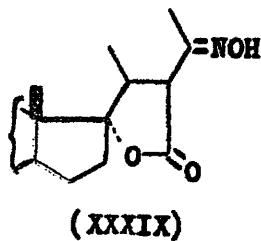
All attempts at removing the side-chain having been unsuccessful,
attention was now turned to the other end of the molecule. In
particular to the insertion of a 1,4-diene-3-one system in ring A.
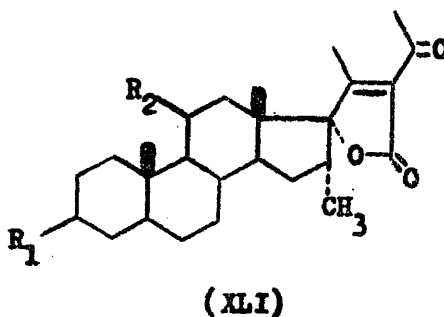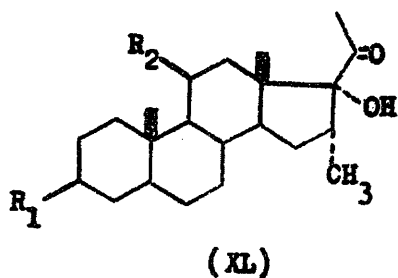
(XL)   (XLI)

To this end, 3β,11α-diacetoxy-17α-hydroxy-16α-methyl-20-oxo-
5α-pregnane (XL; R_1=β-OAc, R_2=α-OAc) was treated with diketene. The
product was chromatographed to give the required lactone, (XLI;
R_1=β-OAc, R_2=α-OAc) which was amorphous. This was hydrolysed to the

amorphous diol (XLI; $R_1=\beta$-OH, $R_2=\alpha$-OH) which on oxidation, gave crystalline 17a-hydroxy-16a-methyl-3,11,23-trioxochol-20(22)-ene-22-carboxylic acid 22→17 lactone (XLII).

(XLII)   (XLIII)
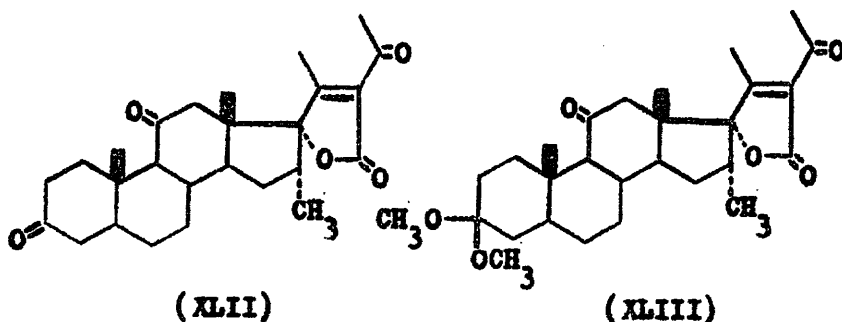
From the mother liquors of the oxidation product (XXIX) was obtained a second product which had almost identical infrared and ultraviolet spectra. The only difference between the two was a peak in the infrared at 1100 cm^{-1}. which could not be assigned to any of the known functional groups in the molecule. Such absorption, however, is known to be typical of ethers,[18] and hydrolysis of the second product gave the 3-ketone. Thus the second product is thought to be the acetal of the 3-ketone (XLIII).

Oxidation of 3β,17a-dihydroxy-16a-methyl-11,23-dioxochol-20(22) -ene-22-carboxylic acid 22→17 lactone (XXIII; R_1=H, R_2=CH_3) gave the 3-ketone (XLII) which was identical with the high melting product. Once again, the mother liquors yielded the acetal (XLIII).
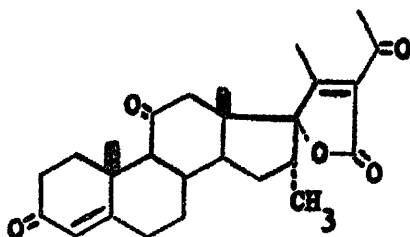
(XLIV)

The oxidation product (XLII) was treated with 2,3-dichloro-5,6-dicyanoquinone[5] (D.D.Q.) in an attempt to introduce a 1,4-diene system. The product, however, was shown to be 17a-hydroxy-16a-methyl-3,11,23-trioxochol-4,20(22)-diene-22-carboxylic acid 22→17 lactone (XLIV).

3β,11a-Diacetoxy-17a-hydroxy-16a-methyl-20-oxo-5a-pregnane (XL; R_1=β-OAc, R_2=a-OAc) was converted by successive use of the reagents potassium hydroxide, chromic acid in acetone, and 2,3-dichloro-5,6-dicyanoquinone, into 17a-hdroxy-16a-methyl-3,11,20-trioxo-5a-pregna-1,4-diene (XLV) which, with diketene gave the required lactone (XLVI).
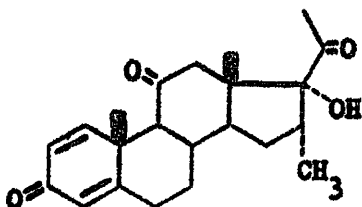
(XLV)
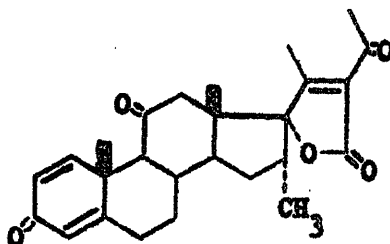
(XLVI)

$3\beta,17\alpha$-Dihydroxy-11,23-dioxochol-20(22)-one-22-carboxylic acid
22→17 lactone (XXIII; $R_1=R_2=H$) was likewise oxidised to the 3-ketone
(XLVII) which was treated with D.D.Q. in an attempt to prepare the
analagous diene-one (XLVIII) to the one prepared above. The product,
however, was an oil from which no steroid could be isolated.
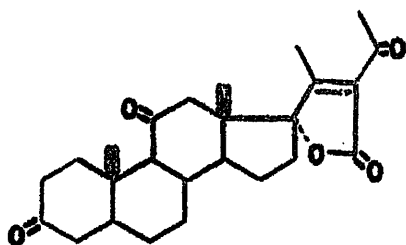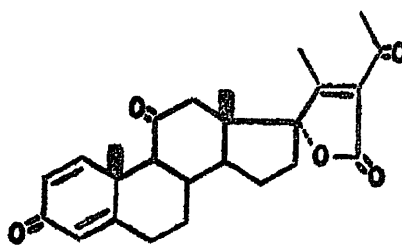
(XLVII)   (XLVIII)

21-Acetoxy-17α-hydroxy-3,11,20-trioxo-5α-pregnane[10] (XLIX) was
treated with diketene, as before, to give a gum which did not
crystallise.
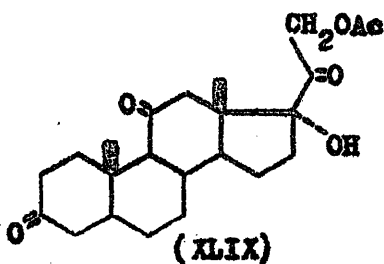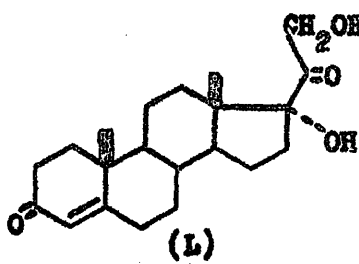
(XLIX)   (L)

Likewise, compound S[10] (L), treated with diketene followed by
sodium methoxide according to the method of Rushig, Fritsch and
Lindler[9] gave non-crystalline material.

EXPERIMENTAL

General Experimental Techniques.- Unless otherwise stated, all melting points were done on a Kofler apparatus and are therefore corrected values; specific rotations were determined in chloroform solution in a 10 cm. micro tube; infrared spectra were obtained from potassium chloride discs; ultraviolet spectra were done on ethanolic solutions; extracts were dried with anhydrous sodium sulphate; alumina was deactivated by shaking the slurry in 60-80° petroleum ether with 5 ml. of 10% aqueous acetic acid for each 100 g. alumina;[19] petroleum ether had b.p. 60-80°.

Cholesteryl Acetoacetate $(XI; R=COCH_2COCH_3)$.- Cholesterol $(XI; R=H)$ (2.55 g.) was dissolved in chloroform (50 ml.) and treated with diketene (4.4 ml.) and triethylamine (0.1 ml.). After a one hour reflux, the solution was evaporated to dryness under reduced pressure. A solid was obtained which was dissolved in benzene and chromatographed on deactivated alumina (100 g.).

Benzene eluted material (1.71 g.) which, after three recrystallisations from chloroform-methanol was shown to be cholesteryl acetoacetate $(XI; R=COCH_2COCH_3)$, m.p. 93-96°, $[\alpha]_D$ -24.3° (c, 1.26) λ_{max} 204 (ε 3460) and 245 mμ (ε 1690), v_{max} 1745 ($>C=O$), 1732 ($>C=O$), 1648 ($>C=C<$), and 1163 cm^{-1}, (acetoacetate). c.f. Bader, Cummings, and Vogel[7] who give m.p. 94°, $[\alpha]_D$ -33°.

9:1 Benzene:ether eluted material which, after three recryst-

allisations from chloroform-methanol had m.p. 235-236°, $[\alpha]_D$ 0°
(Found:C,67.05; H,5.05. Calc. for $C_{19}H_{16}O_6$: C,67.05; H,4.75%),
λ_{max} 210 (ε 26,900) and 302 mμ (ε 25,600), ν_{max} 3448w, 1710s,
1623s, 1597s, 1569s, 1549s, 1418m, 1308s, 1123s, 1037s, 956s, 882s,
784s, and 760s cm^{-1}.

This material appears to be identical with a polymer of diketene
examined by Steel, Boese, and Dull[6] and which they claim to be
2,6-bis-(6-methyl-4-oxo-2-pyranylmethyl)-pyrone (XV).

Pyrolysis of Cholesteryl Acetoacetate.- Cholesteryl acetoacetate
(XI; R=COCH$_2$COCH$_3$) (100 mg.) was heated at 200° for 45 min. in an
evacuated pyrolysis tube. The product was dissolved in benzene and
poured through a column of alumina (10 g.).

The material obtained on elution with benzene had m.p. 80-140°.
Two recrystallisations from methylene chloride-methanol gave
m.p. 115-143°, ν_{max} 3400 (OH), 1724 and 1680 cm^{-1}. (weak aceto-
acetate). It would seem that the acetoacetate has partially
decomposed, giving a mixture of cholesterol (Lit. m.p. 149) and its
acetoacetate. Evidence to support this was obtained from the form-
ation of some dehydroacetic acid (XVI) in the pyrolysis tube. This
is a dimer of diketene, and could only have come from the decomp-
osition of cholesteryl acetoacetate.

Ergosteryl Acetoacetate (XII; R=COCH$_2$COCH$_3$).- Ergosterol (XII;
R=H) (1.2 g.) in benzene (50 ml.) was treated with diketene (1.2 ml.)

and triethylamine (0.2 ml.). The crude product, isolated as above, was chromatographed on deactivated alumina (100 g.).

9:1 Benzene:ether eluted material which, after three recrystallisations from chloroform-methanol was shown to be ergosteryl acetoacetate (XII; $R=COCH_2COCH_3$), m.p. 122.5-132°, $[\alpha]_D$ -82° ($\underline{c}$, 0.39), λ_{max} 205 (ϵ 5830), 265 (ϵ 9000), 272 (ϵ 12,000), 282 (ϵ 12,400), and 294 mμ (ϵ 6880), ν_{max} 1745 ($>$C=O), 1706 ($>$C=O), 1650 ($>$C=C$<$), 1592 ($>$C=C$<$), 1152 (acetoacetate), and 833 cm^{-1}.(=C-H). c.f. Bader and Vogel[8] who give m.p. 124-5°, $[\alpha]_D$ -79.3°.

Pyrolysis gave unchanged acetoacetate and only minute traces of dehydroacetic acid.

Hecogenin Acetoacetate (XIII; $R=COCH_2COCH_3$).- Hecogenin (XIII; R=H) (2.09 g.) in chloroform (50 ml.) was treated with diketene (4.0 ml.) and triethylamine (0.2 ml.) as before. The crude product obtained on evaporation of the chloroform solution was dissolved in benzene and chromatographed on deactivated alumina (90 g.).

Benzene eluted material which, after four recrystallisations from methylene chloride-methanol was shown to be hecogenin acetoacetate (XIII; $R=COCH_2COCH_3$), m.p. 198-201°, $[\alpha]_D$ -1.7° ($\underline{c}$, 1.19) (Found: C,71.9; H,8.7. $C_{31}H_{46}O_6$ requires C,72.3; H,9.0%), λ_{max} 204 (ϵ 2780) and 240 mμ (ϵ 2000), ν_{max} 1754 ($>$C=O), 1718 ($>$C=O), 1653 ($>$C=C$<$), and 1154 cm^{-1}. (acetoacetate).

Pyrolysis as before gave a mixture of hecogenin acetoacetate,
hecogenin, and dehydroacetic acid.

<u>Diosgenin Acetoacetate</u> (XIV; R=COCH$_2$COCH$_3$).- Diosgenin (XIV;
R=H) (2.08 g.) in chloroform (40 ml.) was treated with diketene
(4.0 ml.) and triethylamine (0.1 ml.) as before. Isolation of the
crude product was followed by chromatography on deactivated alumina
(90 g.).

Benzene eluted material which, after three recrystallisations
from chloroform-methanol gave <u>diosgenin acetoacetate</u> (XIV; R=COCH$_2$-
COCH$_3$), m.p. 169-171°, [a]$_D$ -102° (c, 1.08)   (Found: C,74.4; H,9.35.
C$_{31}$H$_{46}$O$_5$ requires C,74.7; H,9.3%), λ_{max} 204 (ϵ 4420)and 247 mμ
(ϵ 1840), ν_{max} 1729 ($>$C=O), 1714 ($>$C=O), 1651 ($>$C=C$<$), and 1168 cm^{-1}.
(acetoacetate).

Pyrolysis as before gave the three materials, diosgenin,
diosgenin acetoacetate, and dehydroacetic acid.

<u>5α-Acetoacetoxy-3β-acetoxy-cholestane</u> (XIX; R=COCH$_2$COCH$_3$).-
3β-Acetoxy-5α-hydroxy-cholestane (XIX; R=H) (0.49 g.) in benzene
(25 ml.) was treated with diketene (0.5 ml.) and triethylamine
(0.1 ml.) and refluxed for 2 hours. Evaporation of the solvent
under reduced pressure gave the crude product which was chromato-
graphed on deactivated alumina (40 g.).

Benzene eluted material which, after three recrystallisations

from methanol gave 5α-<u>acetoacetoxy</u>-3β-<u>acetoxy-cholestane</u> (XIX; R=CO-
CH_2COCH_3), m.p. 133-138°, $[\alpha]_D$ +19.7° (c, 0.78) (Found: C,74.7;
H,10.5. $C_{33}H_{54}O_5$ requires C,74.7; H,10.3%), λ_{max} 208 (ε 9750)
and 250 mμ (ε 4200), ν_{max} 1751 ($\supset$C=O), 1672 ($\supset$C=C$\subset$), 1247 (OAc),
and 1151 cm^{-1}. (acetoacetate).

Pyrolysis was carried out under high vacuum. Any gases formed
in the pyrolysis were made to pass through a cooled tube containing
aniline. Thus any ketene or diketene formed could be characterised
as the corresponding amide. The temperature of the pyrolysis tube
was raised to 180° and kept there for 5 min.. The tube containing
the aniline, still under vacuum, was allowed to come to room temper -
ature, in order to aid any amide formation. The aniline was washed
out with ether and the ether solution washed several times with
dilute hydrochloric acid. Evaporation gave no product. The sublimate
at the top of the pyrolysis tube melted at 98-109° and was thus
dehydroacetic acid. Both the lower sublimate and the residual gum
were recrystallised from methanol to give acetoacetate. In neither
case was any trace of the 5α-hydroxy compound found.

<u>5α-Acetoacetoxy-3β-acetoxy-11-keto-ergostane (XX; R=COCH$_2$COCH$_3$)</u>-
3β-Acetoxy-5α-hydroxy-11-keto-ergostane (XX; R=H) (100 mg.) was
dissolved in chloroform (10 ml.) and treated with diketene (0.13 ml.)
and triethylamine (0.1 ml.). After refluxing the solution for 3 hrs.

the crude product was isolated in the usual manner and chromato-
graphed on deactivated alumina (10 g.).

Benzene eluted material which, after three recrystallisations
from methanol was shown to be 5α-acetoacetoxy-3β-acetoxy-11-keto-
ergostane (XX; R=COCH$_2$COCH$_3$), m.p. 110-112°, [α]$_D$ +44° (c, 1.0)
(Found: C,73.4; H,9.8. C$_{34}$H$_{54}$O$_6$ requires C,73.1; H, 9.7%),
λ$_{max}$ 247 mμ (ε 840), ν$_{max}$ 1724 (>C=O), 1704 (>C=O), 1248 (OAc),
and 1152 cm^{-1}. (acetoacetate).

Reaction of 11α-Hydroxy-tigogenin (XVII; R$_1$=β-OH, R$_2$=α-OH)
with Diketene.- 11α-Hydroxy-tigogenin (XVII; R$_1$=β-OH, R$_2$=α-OH)
(2.02 g.)was dissolved in benzene (50 ml.) and treated with diketene
(2.0 ml.) and triethylamine (0.2 ml.). The solution was refluxed
for one hour, reduced in volume to 20 ml. and petroleum ether (20 ml.)
added. Chromatography on alumina gave a gum which contained some
diketenepolymer (XV). Further chromatography gave a gum, probably
the di-acetoacetate (XVII; R$_1$=β-OCOCH$_2$COCH$_3$, R$_2$=α-OCOCH$_2$COCH$_3$)
which appeared to be almost free of polymer, [α]$_D$ -52.7°, λ$_{max}$ 208
(E$_{1cm.}^{1\%}$ 93) and 250 mμ (E$_{1cm.}^{1\%}$ 129), ν$_{max}$ 1748 (>C=O), 1727 (>C=O),
1642 (>C=C<), 1161 (acetoacetate), 1054, 980, and 901 cm^{-1}. (spiro-
stane side chain).

A sample of the gum so obtained was hydrolysed to the dihydroxy
compound (XVII; R$_1$=β-OH, R$_2$=α-OH) showing that reaction had taken
place.

Reaction of 11β-Hydroxy-tigogenin (XVII; $R_1=R_2=\beta-OH$) with
Diketene.- 11β-Hydroxy-tigogenin ($R_1=R_2=\beta-OH$) (1.42 g.) was
dissolved in benzene (50 ml.) and treated with diketene (2.0 ml.)
and triethylamine (0.2 ml.) as before. The product, isolated as
above, was chromatographed on deactivated alumina to give a gum,
thought to be the required di-acetoacetate (XVII; $R_1=R_2=\beta-OCOCH_2-$
$COCH_3$), $[\alpha]_D$ -21.5°, λ_{max} 206 ($E^{1\%}_{1cm.}$ 59) and 232 mμ ($E^{1\%}_{1cm.}$ 119),
ν_{max} 1742 ($>C=O$), 1721 ($>C=O$), 1642 ($>C=C<$), 1155 (acetoacetate),
1045, 978, and 896 cm^{-1}. (spirostane side chain).

A sample of the gum was hydrolysed to give the diol (XVII;
$R_1=R_2=\beta-OH$).

Reaction of 11β-Hydroxy-tigogenin acetate (XVII; $R_1=\beta-OAc$,
$R_2=\beta-OH$) with Diketene.- 11β-Hydroxy-tigogenin acetate (XVII;
$R_1=\beta-OAc$, $R_2=\beta-OH$) (278 mg.) formed by acetylation of the 3β,11β-
diol, in chloroform (25 ml.) was treated with diketene (0.5 ml.)
and triethylamine (0.1 ml.). The crude product was chromatographed
on deactivated alumina, as before, to give a clear gum, thought to
be the 11β-acetoacetate (XVII; $R_1=\beta-OAc$, $R_2=\beta-OCOCH_2COCH_3$) $[\alpha]_D$ -4.4°
(c, 0.69) λ_{max} 229 ($E^{1\%}_{1cm.}$ 127) and 255 mμ ($E^{1\%}_{1cm.}$ 52), ν_{max} 1752
(OAc and $>C=O$), 1631 ($>C=C<$), 1236 (OAc), and 1140 cm^{-1}. (aceto-
acetate).

Hydrolysis, as above gave the 3β,11β-diol.

<u>Reaction of 3β-Acetoxy-12β-hydroxy-12α-methyl-5α,25D-spirostane</u>
<u>XXI; R=H) with Diketene.</u>- 3β-Acetoxy-12β-hydroxy-12α-methyl-5α,25D-
spirostane (XXI; R=H) (2.05 g.) was dissolved in chloroform (50 ml.)
and treated with diketene (4.0 ml.) and triethylamine (0.1 ml.).
After refluxing the solution for 1 hour, the product was obtained
as before and chromatographed on de$_a$ctivated alumina((90 g.).

3:1 Petroleum ether-benzene eluted material which was a gum
and could not be crystallised. This was shown to be 12β-acetoacetoxy-
3β-acetoxy-12α-methyl-5α,25D-spirostane (XXI; R=COCH$_2$COCH$_3$). λ_{max}208
(ε 7700), 260 (ε 6450) and 347 (ε 3380), ν_{max} 1739 ($>$C=0), 1639
($>$C=C$<$), 1238 (OAc), 1153 (acetoacetate), 1052, 978 and 899 cm^{-1}.
(spirostane side chain).

A sample of the acetoacetate obtained above (115 mg.) was
dissolved in methanol (5 ml.) and treated with potassium hydroxide
(2.0 g.) in aqueous methanol (15 ml.). The solution was refluxed
for 6 hours, diluted with water (100 ml.) and the product extracted
into ether. Evaporation gave a froth (76 mg.) which was recrystall-
ised from ether containing a little pyridine to give 3β,12β-
dihydroxy-12α-methyl-5α,25D-spirostane as a pyridine solvate,
m.p. 205-212.5^o with change of form at 130° (due to loss of pyridine).
c.f. Bladon and McMeekin,[20] who give m.p. (130, 190) 210-213°.

<u>3β,12β-Diacetoacetoxy-11-keto-5α,25D-spirostane (XVIII; R=OCOCH$_2$-</u>
<u>COCH$_3$).</u>- 3β,12β-Dihydroxy-11-keto-5α,25D-spirostane (XVIII; R$_1$=R$_2$=

OH) (1.03 g.) in chloroform (50 ml.) was treated with diketene (3.2 ml.) and triethylamine (0.2 ml.). The crude product was chromatographed on deactivated alumina (150 g.).

Ether eluted material which after four recrystallisations from methylene chloride-methanol was shown to be 3β,12β-diacetoacetoxy-11-keto-5α,25D-spirostane (XVIII; R=OCOCH$_2$COCH$_3$), m.p. 158-162°, $[\alpha]_D$ -53.4° (c, 0.2) (Found: C,67.85; H,8.35. C$_{35}$H$_{50}$O$_9$·½CH$_3$OH requires C,67.6; H,8.3%), λ_{max}246 mµ (ε 3000), ν_{max} 1751 ($>$C=O), 1724 ($>$C=O), 1157 (acetoacetate), 1053, 981, and 898 cm^{-1}. (spirostane side chain).

<u>3β-Acetoxy-17α-hydroxy-16α-methyl-11,23-dioxochol-20(22)-ene 22-carboxylic acid 22→17 lactone (XXIII; R$_1$=Ac, R$_2$=CH$_3$).</u>- Acetylation of 3β,17α-dihydroxy-16α-methyl-11,20-dioxo-5α-pregnane (XXII; R$_1$=H, R$_2$=CH$_3$) (4.96 g.) gave the 3β-acetate (XXII; R$_1$=Ac, R$_2$=CH$_3$) which was dissolved in chloroform (100 ml.) and treated with diketene (4.5 ml.) and triethylamine (0.2 ml.) as before.

From the chromatogram of the crude product, benzene eluted material which after four recrystallisations from methylene chloride-methanol was shown to be 3β-<u>acetoxy</u>-17α-<u>hydroxy</u>-16α-<u>methyl</u>-11,23-<u>dioxochol</u>-20(22)-<u>ene</u>-22-<u>carboxylic acid</u> 22→17 <u>lactone</u> (XXIII; R$_1$=Ac, R$_2$=CH$_3$), m.p. 277-281.5°, $[\alpha]_D$ -35° (c, 0.74) (Found: C,71.5; H,8.1. C$_{28}$H$_{38}$O$_6$ requires C,71.5; H,8.1%), λ_{max} 204 (ε 4380) and 246 mµ

(ε 9150), ν_{max} 1761 (unsaturated lactone), 1721 (OAc), 1686 ($>$C=O),

1615 ($>$C=C$<$), and 1236 cm^{-1}. (OAc).

The ultraviolet and infrared spectra show that an $\alpha\beta$-unsat-

urated lactone has been formed. Such a structural feature is

typical of the cardenolides, all of which give the Legal test.[11,12]

A few milligrams of the lactone obtained above were dissolved in

pyridine and a few drops of sodium nitroprusside-sodium hydroxide

solution added. The solution turned deep red-violet in colour,

showing the presence of a $\Delta^{\alpha\beta}$-lactone structure.

Treatment of 3β-Acetoxy-17α-hydroxy-16α-methyl-11,23-dioxochol-

20(22)-ene-22-carboxylic acid 22→17 lactone (XXIII; R_1=Ac, R_2=CH$_3$)

with :-

(a) Sodium hypochlorite.[14] The steroid (XXIII; R_1=Ac, R_2=CH$_3$) (60 mg.)

was dissolved in dioxan (10 ml.) and sodium hypochlorite (0.2 ml.)

added. The mixture was stirred for 3 hours, acidified with sodium

sulphite solution, water (20 ml.) added and the solution allowed to

stand overnight. Extraction into chloroform and work up as usual

gave starting material, m.p. 265-277°. This is supported by infrared

and ultraviolet spectra.

(b) Sodium hypobromite.[15] The steroid (XXIII; R_1=Ac, R_2=CH$_3$)

(100 mg.) was dissolved in methanolic sodium hydroxide (0.5 g. in

4 ml.) and the solution refluxed for 2 hours. Evaporation of the

methanol accompanied by slow addition of water precipitated the

steroid which redissolved on further addition of water (100 ml.).
The solution was cooled to 2-3° and a solution (4 ml.) of bromine
(2.5 g.) and sodium hydroxide (5 g.) in water (100 ml.) added. The
reaction mixture went cloudy at once and was left overnight at 2-3°.

Extraction into ether gave a neutral product (96 mg.) which
was non-crystalline, slightly soluble in ether, and soluble in
methylene chloride and methanol, ν_{max} 3450 (OH), 1771 (lactone),
1711 ($>$C=O),1618 ($>$C=C$<$), and 1019 cm^{-1}. (OH).

A sample of this product did not sublime when heated at 160°
for 10 min. under vacuum but gave amorphous material having an
infrared spectrum identical with that obtained above.

The neutral product was shown to be impure 3β,17α-dihydroxy-
16α-methyl-11,23-dioxochol-20(22)-ene-22-carboxylic acid 22→17
lactone (XXIII; R_1=H, R_2=CH$_3$) (see below).

(c) <u>Cold concentrated potassium hydroxide solution</u>. The steroid
(XXIII; R_1=Ac, R_2=CH$_3$) (50 mg.) was treated with aqueous potassium
hydroxide solution (25 ml.; 50%) in a nitrogen atmosphere and
stirred for three hours. The solid did not dissolve as the sodium
salt. Ethanol (25 ml.) was added to effect solution. After 30 min.
a sample was taken for ultraviolet examination, λ_{max} 258 (ε 11,300)
and 307 mμ (ε 11,800). Further samples taken after 2 hours and 3½
hours respectively showed no change in absorption characteristics.

Extraction of the reaction mixture with chloroform gave 3.4 mg.
material. The solution was acidified and re-extracted to give
material (49 mg.) m.p. 250-260°. One recrystallisation from
methylene chloride-methanol gave material which was shown to be
3β,17a-dihydroxy-16a-methyl-11,23-dioxochol-20(22)-ene-22-carboxylic
acid 22→17 lactone (XXIII; R_1=H, R_2=CH$_3$), m.p.255-261°, λ_{max} 204
(ε 2830) and 245 mμ (ε 8570), ν_{max} 3355 (OH), 1758 (lactone), 1703
($\rangle$C=O), and 1623 cm^{-1}. ($\rangle$C=C$\langle$).

(d) <u>Hot potassium hydroxide solution</u>. The steroid (XXIII; R_1=Ac,
R_2=CH$_3$) (55 mg.) in methanol (10 ml.) was added to potassium
hydroxide (20 g.) dissolved in water (10 ml.). This gave a
homogeneous solution which precipitated a white solid when the
methanol was allowed to evaporate off. After refluxing the solution
for 2 hours, water (100 ml.) was added and the solution extracted
with chloroform to give 5.1 mg. of material.

Acidification with hydrochloric acid precipitated material
which was extracted into chloroform and worked up to give a solid
(42.3 mg.) whose infrared spectrum was identical with that obtained
above for the 3β-hydroxy compound (XXIII; R_1=H, R_2=CH$_3$). Three
recrystallisations from methanol gave material, m.p. 258-260.5°,
$[\alpha]_D$ -37.5° (c, 0.5) (Found: C,70.4; H,8.6. $C_{26}H_{36}O_5 \cdot CH_3OH$ requires
C,70.4; H,8.75%), λ_{max} 204 (ε 2830) and 245 mμ (ε 9570), ν_{max} 3445

(OH), 1755 (lactone), 1704 ($\sum$C=O), 1623 ($\sum$C=C$\sum$), and 1041 cm^{-1}. (OH).

The product also gave a positive Legal test, showing that the lactone was still intact.

Acetylation of a sample of this material gave the 3β-acetate, m.p.275-280°, identical with starting material.

(e) <u>Potassium hydroxide in refluxing ethylene glycol</u>. Potassium hydroxide (5 g.) in ethylene glycol (25 ml.) was added to the steroid (XXIII; R_1=Ac, R_2=CH$_3$) (88 mg.) dissolved in ethylene glycol (25 ml.). The solution was refluxed for $4\frac{1}{2}$ hours, left overnight, and worked up by addition of water (100 ml.) and extraction with ether. This gave a neutral product which could not be crystallised. ν_{max} 1742 ($\sum$C=O), 1709 ($\sum$C=O), and 1603 cm^{-1}. ($\sum$C=C$\sum$).

The extracted reaction mixture was acidified with hydrochloric acid and re-extracted with ether to give an amorphous acid fraction. λ_{max} 209 (ϵ 15,900), 236 infl. (ϵ 11,470) and 262 mμ (ϵ 8250). ν_{max} 3422 (acid), 1711 ($\sum$C=O), 1669, 1626 ($\sum$C=C$\sum$), and 1031 cm^{-1}. (OH).

This material also gave a negative Legal test.

Acetylation with acetic anhydride in pyridine gave material which was still non-crystalline, ν_{max} 3450 (acid), 1739 ($\sum$C=O), 1712 ($\sum$C=O), 1667, 1634 ($\sum$C=C$\sum$), 1248 (OAc), and 1029 cm^{-1}. (OH).

The material obtained above seems to contain a carboxylic acid

group (broad OH band) and was treated with diazomethane in ether to give non-crystalline material, ν_{max} 1748 ($>$C=O), 1742 ($>$C=O), 1675, 1629 ($>$C=C$<$), and 1244 cm^{-1}. (OAc).

(f) <u>Hydrochloric acid in acetic acid</u>. The steroid (XXIII; R_1=Ac, R_2=CH$_3$) (96 mg.) was dissolved in glacial acetic acid (6 ml.) and concentrated hydrochloric acid (6 ml.) added. The solution was refluxed for 2 hours, and water (75 ml.) added. Extraction with ether gave a white crystalline product which was shown to be starting material.

(g) <u>Hydrochloric acid in refluxing methanol</u>. The steroid (XXIII; R_1=Ac, R_2=CH$_3$) (60 mg.) was dissolved in methanol (10 ml.) and concentrated hydrochloric acid (3 ml.) added. After a 2 hour reflux, water (50 ml.) was added and the solution extracted with ether to give material (59 mg.) which after four recrystallisations from acetone-petroleum ether was shown to be 3β,17α-dihydroxy-16α-methyl-11,23-dioxochol-20(22)-ene-22-carboxylic acid 22$\rightarrow$17 lactone (XXIII; R_1=H, R_2=CH$_3$), m.p. 253-257°,$[\alpha]_D$ -27.4° ($\underline{c}$, 0.35) (Found: C,72.5, H,8.6. $C_{26}H_{36}O_5$ requires C,72.85; H,8.5%), λ_{max} 206 (ε 4460) and 246 mμ (ε 9500), ν_{max} 3460 (OH), 3400 (OH), 1755 (lactone), 1696 ($>$C=O), 1617 ($>$C=C$<$), and 1040 cm^{-1}. (OH).

(h) <u>Hydrochloric acid in methanol at 140°</u>. The steroid (XXIII; R_1=Ac, R_2=CH$_3$) (103 mg.), methanol (25 ml.) and concentrated

hydrochloric acid (8 ml.) were placed in a Carius tube which was then sealed and maintained at a temperature of 140° for 20 hours. On cooling and releasing the pressure, a precipitate was formed which after filtration and one recrystallisation from methylene chloride-methanol gave material m.p. $230-245^o$, λ_{max} 206 (ϵ 6860) and 245 mμ (ϵ 13,840), ν_{max} 3510 (OH), 1764 (lactone), 1704 ($>$C$=$O), 1623 ($>$C$=$C$<$), and 1024 cm^{-1}. (OH). In spite of the low melting point, this was thought to be the 3β-hydroxy compound (XXIII; $R_1=$H, $R_2=$CH$_3$).

The remaining reaction mixture was extracted with ether to give a syrup which crystallised on trituration with methanol, m.p. $200-225^o$. Recrystallisation from methylene chloride-methanol gave material m.p. $230-245^o$ with change of form at $185-200^o$. This material had an infrared spectrum identical with that obtained above and was concluded to be the same compound.

(i) <u>Hydrochloric acid in ethylene glycol at 190°</u>. The steroid (XXIII; $R_1=$Ac, $R_2=$CH$_3$) (118 mg.) was dissolved in ethylene glycol (25 ml.) and concentrated hydrochloric acid (8 ml.) added. This solution was placed in a Carius tube which was then sealed and kept at a temperature of 196° for 20 hours. The product was a black tar from which no steroid could be isolated.

(j) <u>Hydrogen and palladium-charcoal catalyst</u>. The steroid (XIII; R_1=Ac, R_2=CH$_3$) (104 mg.) was dissolved in glacial acetic acid (25 ml.) and palladium-charcoal (110 mg.; 10%) added as catalyst. The solution was stirred in a hydrogen atmosphere until uptake of hydrogen ceased (3 hours) and then filtered. Addition of water (100 ml.) and extraction with ether gave a crystalline solid (93 mg.) which after three recrystallisations from methylene chloride-methanol was shown to be <u>3β-acetoxy- 17α-hydroxy-16α-methyl-11,23-dioxocholan-22-carboxylic</u> acid <u>22→17 lactone</u> (XXX; R_1=Ac, R_2=CH$_3$), m.p. 236-238°, $[\alpha]_D$ -6° (c, 0.47) (Found: C,71.65; H,8.9. $C_{28}H_{40}O_6$ requires C,71.3; H,8.9%), λ_{max} 255 mμ (ε 2000) (in NaOH) 225 (ε 12,900) and 288 mμ (ε 25,600), v_{max} 1783 (saturated lactone), 1727 (OAc), 1706 (>C=O), and 1250 cm^{-1}. (OAc). The product gave a negative Legal test.

(k) <u>Hydrogen and platinum oxide as catalyst</u>. The steroid (XXIII; R_1=Ac, R_2=CH$_3$) (98 mg.) was dissolved in glacial acetic acid (25 ml.) and platinum oxide (104 mg.) added. The solution was stirred under hydrogen until uptake of hydrogen ceased (5 hours) and the product obtained by filtration and extraction as before. Four recrystallisations from methylene chloride-methanol gave material shown to be <u>3β-acetoxy-17α,23-dihydroxy-16α-methyl-11-oxocholan-22-carboxylic</u> acid <u>22→17 lactone</u> (XXXII; R=Ac), m.p.236-240.5°, $[\alpha]_D$ -47.8°

($\underline{c}$, 0.93) (Found: C,71.1; H, 8.85. $C_{28}H_{42}O_6$ requires C,70.85;
H,8.9%), λ_{max} 226 mμ (ϵ 7600) (in NaOH) 226 mμ (ϵ 7480),
ν_{max} 3520 (OH), 1754 (lactone), 1736 (OAc), 1709 ($>$C=O), and 1250
cm^{-1}, (OAc).

$\underline{17\alpha\text{-Hydroxy-16}\alpha\text{-methyl-3,11,20-trioxochol-20(22)-ene-22-carbox-}}$
$\underline{\text{ylic acid 22}\rightarrow17\text{ lactone (XLII).}}$– 3$\beta$,11$\alpha$-Diacetoxy-17$\alpha$-hydroxy-16$\alpha$-
methyl-20-oxo-5α-pregnane (XL; R_1=β-OAc, R_2=α-OAc) (5.58 g.) was
dissolved in benzene (150 ml.) and treated with diketene (5.5 ml.)
and triethylamine (0.3 ml.). This solution was refluxed for three
hours and evaporated to dryness under reduced pressure. The result-
ing gum was dissolved in benzene (30 ml.), petroleum ether added,
and the solution chromatographed on deactivated alumina (200 g.).

9:1 Benzene-ether eluted material which did not crystallise
but which was shown to be 3β,11α-diacetoxy-17α-hydroxy-16α-methyl-
23-oxochol-20(22)-ene-22-carboxylic acid 22$\rightarrow$17 lactone (XLI;
R_1=β-OAc, R_2=α-OAc), $[\alpha]_D$ –107.6° ($\underline{c}$, 0.87), λ_{max} 243 mμ (ϵ 9300),
ν_{max} 1754 (unsaturated lactone), 1730 (OAc), 1692 ($>$C=O), 1610
($>$C=C$<$), and 1241 cm^{-1}, (OAc).

The product from the above reaction (3.17 g.) was treated with
methanolic potassium hydroxide (15 g. in 150 ml.) and refluxed for
8 hours. The product (1.97 g.), which was amorphous was obtained
by addition of water, acidification, and extraction into chloroform.

This was the required diol (XLI; $R_1 = \beta$-OH, $R_2 = \alpha$-OH), $[\alpha]_D$ -71.5°
(c, 0.49), λ_{max} 245 mµ (ε 6720), ν_{max} 3425 (OH), 1761 (unsaturated
lactone), 1692 ($\supset$C=O), 1610 ($\supset$C=C$\subset$), and 1029 cm^{-1}, (OH).

The diol (1.65 g.) was dissolved in acetone (100 ml.) and
stirred with 8N chromic acid (6.0 ml.) for 10 min. at room tempera-
ture. Excess reagent was destroyed using dilute hydrochloric acid
and excess sodium sulphite. Addition of water and extraction into
ether yielded the crude product which was purified by chromatography
on deactivated alumina (70 g.).

9:1 Benzene-ether gave material m.p. (228-231) 238-240°, which
after three recrystallisations from chloroform-methanol was shown
to be 17α-hydroxy-16α-methyl-3,11,23-trioxochol-20(22)-ene-22-
carboxylic acid 22→17 lactone (XLII), m.p. 243-245°, $[\alpha]_D$ -39.6°
(c, 0.6) (Found: C,72.9; H,8.0. $C_{26}H_{34}O_5$ requires C,73.2, H,8.0%)
λ_{max} 210 (ε 4190) and 246 mµ (ε 10,440), ν_{max} 1754 (lactone),
1742 ($\supset$C=O), 1701 ($\supset$C=O), 1689 ($\supset$C=O), and 1616 cm^{-1}, ($\supset$C=C$\subset$).

From the mother liquors of this recrystallisation a second
compound was obtained, shown to be 17α-hydroxy-16α-methyl-3,11,23-
trioxochol-20(22)-ene-22-carboxylic acid 22→17 lactone-3-acetal
(XLIII) m.p. 197-204°, $[\alpha]_D$ -33.1° (c, 0.72) (Found: C,70.7; H,8.75.
$C_{28}H_{40}O_6$ requires C,71.15; H,8.5%), λ_{max} 204 (ε 5880) and 244 mµ
(ε 9540), ν_{max} 1761 (lactone), 1709 ($\supset$C=O), 1695 ($\supset$C=O), 1616

$(\supset C = C\subset)$, and 1103 cm^{-1}. (acetal).

Crystallisation of the high and low melting products from the same solvents gave no change in either melting point. Both products were heated in a pyrolysis tube under vacuum. The higher melting product sublimed unchanged, while the other material did not sublime, but gave a clear gum which crystallised with methanol, yielding the original material.

A sample of the low melting product, on hydrolysis with hydrochloric acid (0.1 ml.) in 10% aqueous dioxan (4 ml.) gave 17αhydroxy-16α-methyl-3,11,23-trioxochol-20(22)-ene-22-carboxylic acid 22→17 lactone (XLII). Hence the low melting product is an acetal, probably of the 3-ketone. (XLIII).

<u>Oxidation of 3β,17α-Dihydroxy-16α-methyl-11,23-dioxochol20(22)-ene-22-carboxylic acid 22→17 lactone (XXIII; R_1=H, R_2=CH$_3$).</u>
The steroid (XXIII; R_1=H, R_2=CH$_3$) (54 mg.) was dissolved in acetone (10 ml.) and treated with 8N chromic acid (0.5 ml.) for 5 min. at room temperature. Excess reagent was destroyed by addition of 1:1 hydrochloric acid (1 ml.) followed by sodium sulphite in excess. Addition of water(30 ml.) and extraction into ether gave material m.p.(231-232) 242-245°, ν_{max}1754 (lactone), 1715 ($\supset$C=O), 1692 ($\supset$C=O), and 1610 cm^{-1}. ($\supset$C=C$\subset$), which is therefore identical with the higher melting product obtained above (XLII).

The mother liquors yielded a second product, m.p.180-187°, ν_{max} 1761 (lactone), 1712 ($>C=O$), 1695 ($>C=O$), 1613 ($>C=C<$), and 1101 cm^{-1}. (acetal), which is identical with the acetal obtained above.

Treatment of 17α-Hydroxy-16α-methyl-3,11,23-trioxochol-20(22)-ene-22-carboxylic acid 22→17 lactone (XLII) with 2,3-Dichloro-5,6-dicyanoquinone.- The steroid (XLII) (0.22 g.) in dioxan (15 ml.) was treated with 2,3-dichloro-5,6-dicyanoquinone (D.D.Q.) (0.49 g.) and the mixture refluxed for 6½ hours. At the end of this time the suspension of the hydroquinone was taken to dryness under reduced pressure. Benzene was added and the mixture again taken to dryness. A further addition of benzene gave a solution which was poured on to an alumina column (10 g.). Elution with ether gave the product which, after four recrystallisations from chloroform-methanol-di-isopropyl ether was shown to be 17α-hydroxy-16α-methyl-3,11,23-trioxochol-4,20(22)-diene-22-carboxylic acid 22→17 lactone (XLIV), m.p. 227.5-234.5°, $[\alpha]_D$ +35.7° (c, 0.66) (Found: C,73.4, 73.45; H,7.5, 7.6. $C_{26}H_{32}O_5$ requires C,73.55; H,7.6%), λ_{max} 234 mμ (ϵ 17,000), ν_{max} 1761 (lactone), 1698 ($>C=O$), 1681 ($>C=O$), 1667 ($>C=C<$), and 1613 cm^{-1}. ($>C=C<$).

Treatment of the Acetal (XLIII) with 2,3-Dichloro-5,6-dicyanoquinone.- The steroid (XLIII) (0.275 g.) in dioxan (2 ml.) was

added to a solution of D.D.Q. (0.55 g.) in dioxan (10 ml.) which

had been refluxing for 5 min. but which was not homogeneous. After

10 min. the material in the flask started " bumping " and this was

taken as an indication that some hydroquinone had been formed. The

mixture was kept refluxing for one hour longer to ensure complete

reaction, and the product isolated as before.

This product was a yellow amorphous material, $[\alpha]_D$ -119°

($\underline{c}$, 0.65), λ_{max} 205 ($E_{1cm.}^{1\%}$ 279), 221 ($E_{1cm.}^{1\%}$ 408), 242 ($E_{1cm.}^{1\%}$ 439),

and 278 mμ ($E_{1cm.}^{1\%}$ 324), ν_{max} 1757s, 1709sh, 1692s, 1613s, 1592sh,

1555 and 278 mμ ($E_{1cm.}^{1\%}$ 324), ν_{max} 1757s, 1

1555sh, 1499w, 1235s, and 1028s cm^{-1}. <u>with Diketene</u>.- Cholesta-

1:4-diene-3-one (47 mg.) in benzene (15 ml.) was refluxed with

diketene (0.2 ml.) and triethylamine (1 drop) for one hour.

Isolation of the product by evaporation followed by chromatography

gave, after one recrystallisation , unchanged starting material,

m.p. 111-114°. This shows the stability of the 1,4-diene-3-one

system to diketene.

<u>17α-Hydroxy-16α-methyl-3,11,20-trioxo-5α-pregnane (XL; $R_1 \equiv R_2 = 0$)</u>.-

3β,11α-Diacetoxy-17α-hydroxy-16α-methyl-20-oxo-5α-pregnane (XL;

$R_1 = \beta$-OAc, $R_2 = \alpha$-OAc) (5.06 g.) was treated with potassium hydroxide

(5 g.) in methanol (50 ml.) and the resulting solution refluxed for

3 hours. The reaction mixture was acidified, diluted with water

(500 ml.), and extracted ten times with ethyl acetate (250 ml. in all). The extracts were washed with saturated sodium chloride solution and evaporated to give 4.88 g. of the required triol (crude) m.p. 200 -245°. c.f. Heusler, Kebrle, Meystre, Ueberwasser, Anner, Wieland, and Wettstein[21] who give m.p. 248-252°.

This product was dissolved in acetone (180 ml.) and treated with 8N chromic acid (16 ml.) at room temperature for 10 min.. The reaction mixture was worked up as before to give a crude product (3.87 g.) m.p. 208-245°, which was chromatographed on deactivated alumina (160 g.).

1:1 Benzene-ether and ether eluted material which had the same melting range as the material put on the column. (The German workers give the melting point as 235-238°).

Acetylation of one of the fractions gave material m.p. 225-238°, but which had an identical infrared spectrum to that of the crude product. It was therefore assumed that the melting point difference was due to a crystalline modification, and that the product obtained was 17α-hydroxy-16α-methyl-3,11,20-trioxo-5α-pregnane (XL; $R_1=R_2=0$).

17α-Hydroxy-16α-methyl-3,11,23-trioxochol-20(22)-ene-22-carboxylic acid 22→17 lactone (XLII).- The product obtained above (XL; $R_1=R_2=0$) (0.4 g.) in benzene (25 ml.) was treated with diketene

(0.5 ml.) and triethylamine (0.1 ml.) and refluxed for one hour.
Isolation of the product by chromatography gave material m.p. 225-
240° with some change of form at 230°. This is identical with the
high melting lactone obtained previously (XLII).

17a-Hydroxy-16a-methyl-3,11,20-trioxopregna-1,4-diene (XLV).-
The oxidation product (XLII) obtained above (3.07 g.) was treated
with D.D.Q. (6.0 g.) and dioxan (60 ml.) as before. The product
was again isolated by eluting from an alumina column using ether
and ether-methanol mixtures. Three recrystallisations from chloroform
-methanol gave material which was shown to be 17a-hydroxy-16a-
methyl-3,11,20-trioxopregna-1,4-diene (XLV), m.p. 260-267°,
$[\alpha]_D$ +80.6° ($\underline{c}$, 0.73) (Found: C,74.0; H,8.0. $C_{22}H_{28}O_4$ requires
C,74.1; H,7.9%), λ_{max} 209 (ϵ 7860), 236 (ϵ 12,660), and 290 mμ
(ϵ 1500), ν_{max} 3472 (OH), 1709 ($>$C=O), 1667 (-C=C-C=O), 1626, and
1605 ($>$C=C$<$) cm^{-1}.

17a-Hydroxy-16a-methyl-3,11,23-trioxochol-1,4,20(22)-triene-22-
carboxylic acid 22→17 lactone (XLVI).- 17a-Hydroxy-16a-methyl-3,11,
20-trioxopregna-1,4-diene (XLV) (0.576 g.) in benzene (30 ml.) was
treated with diketene (0.5 ml.) and triethylamine (0.1 ml.). The
product was chromatographed as before to give material which, after
three recrystallisations from chloroform-methanol was shown to be
17a-hydroxy-16a-methyl-3,11,23-trioxochol-1,4,20(22)-triene-22-

carboxylic acid 22→17 lactone (XLVI), m.p. 127-133°, $[\alpha]_D$ +103.8°

(c, 0.34) (Found: C,71.6; H,8.1. $C_{26}H_{30}O_5 \cdot CH_3OH$ requires C,71.35;

H,7.55%), λ_{max} 204 (ε 8560), 235 (ε 12,640), and 288 mμ (ε 2740),

ν_{max} 1754 (lactone), 1704 ($\supset$C=O), 1692 ($\supset$C=O), 1667 (-C=C-C=O),

1621 (lactone $\supset$C=C$\subset$), and 1605 cm^{-1}, ($\supset$C=C$\subset$).

3β-Acetoxy-17α-hydroxy-11,23-dioxochol-20(22)-ene-22-carboxylic

acid 22→17 lactone (XXIII; R_1=Ac, R_2=H).- 3β,17α-Dihydroxy-11,20-

dioxo-5α-pregnane (XXII; R_1=R_2=H) (4.65 g.) was acetylated by

means of acetic anhydride in pyridine to give the 3β-acetate (XXII;

R_1=Ac, R_2=H). This latter compound (1.06 g.) was dissolved in chlor-

oform (25 ml.) and treated with diketene (2 ml.) and triethylamine

(0.1 ml.). The solution was refluxed for one hour and evaporated

to dryness under reduced pressure. The crude product, which also

contained some diketene polymer (XI), was dissolved in benzene

(20 ml.) to which petroleum ether (20 ml.) was added before chrom-

atography on deactivated alumina (50 g.).

2:3 Petroleum ether-benzene eluted material (0.55 g.) which,

after five recrystallisations from methylene chloride-petroleum

ether was shown to be 3β-acetoxy-17α-hydroxy-11,23-dioxochol-

20(22)-ene-22-carboxylic acid 22→17 lactone (XXIII; R_1=Ac, R_2=H),

m.p. 271-276°, $[\alpha]_D$ -44.2° (c, 1.11) (Found: C,70.7; H,8.0,

$C_{27}H_{36}O_6$ requires C,71.0; H,7.95%), λ_{max} 203 (ε 3620) and 243 mμ

(ε 9720), ν_{max} 1765 (unsaturated lactone), 1732 (OAc), 1703 ($\supset$C=O), 1620 ($\supset$C=C$\subset$), and 1245 cm^{-1}. (OAc).

This compound also gave the Legal test, thus confirming the $\Delta^{\alpha\beta}$-lactone structure assigned to it.

Treatment of 3β-Acetoxy-17α-hydroxy-11,23-dioxochol-20(22)-ene-22-carboxylic acid 22$\rightarrow$17 lactone (XXIII; R_1=Ac, R_2=H) with:-
(a) Sodium hypoiodite (Iodoform reaction).[13] The steroid (XXIII; R_1=Ac, R_2=H) (0.483 g.) was dissolved in dioxan (15 ml.) and to this was added 1.25 ml. of a solution consisting of iodine (10 g.) and potassium iodide (20 g.) in water (80 ml.). Sodium bicarbonate was added to the stirred solution which was then warmed for two min.. The solution turned pale yellow, followed by an almost immediate change to a dark-brown colour. After one hours stirring at room temperature, water (100 ml.) was added and the solution extracted into chloroform. The extracts, taken to dryness, yielded a dark brown froth (0.474 g.) which could not be crystallised and which did not improve on treatment with charcoal.

The mother liquors from the chloroform extraction were acidified with concentrated hydrochloric acid (5 ml.) and re-extracted to give gummy material. Esterification with diazomethane in ether solution gave a white non-crystalline solid which could not be identified.

(b) _Potassium hydroxide._ The steroid (XXIII; R_1=Ac, R_2=H) (316 mg.) was treated with methanolic potassium hydroxide (5.0 g. in 20 ml.) and the solution heated on the steam-bath for two hours. Addition of water (150 ml.) and concentrated hydrochloric acid (25 ml.) was followed by extraction to give a yellow froth (300 mg.) which was dissolved in benzene and chromatographed on silica gel (20 g.)

1:1 Ether-benzene eluted material which, after three recrystallisations from methanol was shown to be 3β,17α-_dihydroxy-11,23-dioxochol-20(22)-ene-22-carboxylic acid_ 22→17 _lactone_ (XXIII; R_1=R_2=H) m.p.(185-188) 212.5-216°, $[\alpha]_D$ -45.8° (_c_, 0.43) (Found: C, 70.25; H, 8.9. $C_{25}H_{34}O_5 \cdot CH_3OH$ requires C, 69.9; H, 8.6%), λ_{max} 204 (ε 5550) and 242 mμ (ε 11,450), ν_{max} 3448 (OH), 1770 (unsaturated lactone), 1709 ($\supset$C=O), 1618 ($\supset$C=C$\subset$), and 1042 cm^{-1}. (OH).

The product gave a positive Legal test, and on re-acetylation gave starting material m.p. 267-274°,

(c) _Hydrogen and palladium-charcoal catalyst._ The steroid (XXIII; R_1=Ac, R_2=H) (200 mg.) was dissolved in glacial acetic acid (25 ml.) and palladium-charcoal catalyst (128 mg.; 10%) added. The solution was stirred under hydrogen until uptake of hydrogen ceased (2 hrs.), filtered and the product obtained by addition of water and extraction with ether. After three recrystallisations from methylene

chloride-methanol was obtained 3β-acetoxy-17α-hydroxy-11,23-dioxo-cholan-22-carboxylic acid 22→17 lactone (XXX; R_1=Ac, R_2=H), m.p. 266-270°, $[\alpha]_D$ -39° (c, 0.51) (Found: C,70.7; H, 8.2. $C_{27}H_{38}O_6$ requires C,70.7; H,8.35%), λ_{max} 253 mµ (ε 3300) (in NaOH) 284 mµ (ε 23,400), ν_{max} 1783 (saturated lactone), 1733 (OAc), 1715 ($>$C=O), and 1250 cm^{-1}. (OAc). The product gave a negative Legal test.

(d) <u>Hydrogen and platinum oxide catalyst</u>. The steroid (XXIII; R_1=Ac, R_2=H) (95 mg.) was dissolved in glacial acetic acid (25 ml.) as before and platinum oxide added (100 mg.). The solution was stirred under hydrogen for three hours until uptake of hydrogen ceased. The product was isolated as above to give material (95 mg.), which, after four recrystallisations from methylene chloride-methanol was shown to be 3β-acetoxy-17α,23-dihydroxy-11-oxocholan-22-carboxylic acid 22→17 lactone (XXXI; R=Ac), m.p. 313-314°, $[\alpha]_D$ -2.4° (c, 0.45) (Found: C,70.6; H,9.0. $C_{27}H_{40}O_6$ requires C,70.4; H,8.75%), λ_{max} 284 (ε 3280) (in NaOH) 284 mµ (ε 3300), ν_{max} 3413 (OH), 1779 (saturated lactone), 1730 (OAc), 1709 ($>$C=O), 1253 (OAc), and 1027 cm^{-1}. (OH). The product gave a negative Legal test.

In a larger scale reduction with platinum oxide it was found that both the above compounds were formed. Separation by chromatography on silica gel gave 60% product (c) and 40% product (d).

(e) <u>Hydroxylamine hydrochloride</u>. The steroid (XXIII; R_1=Ac, R_2=H)
(0.505 g.) was dissolved in pyridine (20 ml.) and treated with a
solution of hydroxylamine hydrochloride (0.4 g.) in the same solvent
(20 ml.). The solution was heated on the steam-bath for $5\frac{1}{2}$ hours
and worked up by the addition of water (1 litre) followed by
extraction with chloroform. The extracts were washed several times
with dilute hydrochloric acid, washed with water, dried, and evapor-
ated under reduced pressure. After one recrystallisation from
chloroform-methanol, the product, 3β-<u>acetoxy-17α-hydroxy-11,23-</u>
<u>dioxochol-20(22)-ene-22-carboxylic</u> acid <u>22→17 lactone-23-oxime</u>
(XXXIII; R_1=Ac, R_2=H), was in 50% yield, m.p. 283.5-291°. Two
further recrystallisations from the same solvents gave analytically
pure material, m.p. 288-294°, $[\alpha]_D$ +6° (<u>c</u>, 0.55) (Found: C,68.9;
H,7.8; N,3.2. $C_{27}H_{37}O_6N$ requires C,68.8; H,7.8; N,3.0%), λ_{max} 210
(ε 14,260) and 224 mμ (ε 12,490), ν_{max} 3378 (OH), 1767 (unsaturated
lactone), 1704 ($>$C=O), 1653 ($>$C=C$<$), 1274 (OAc), and 1039 cm^{-1}. (OH).

Recrystallisation of the mother liquors of the analytical
sample gave a second product, m.p. 302-306°. The same material was
also obtained by acid treatment of the oxime (see below).

The oxime does not give the Legal test. It is also soluble in
hot aqueous potassium hydroxide. A few milligrams of the oxime
were dissolved in hot aqueous potassium hydroxide and the solution

filtered. On acidification a gelatinous precipitate was obtained
m.p. 285-295°, and which had an infrared spectrum identical with
the product (m.p. 302-306°) obtained above.

(f) <u>Hydrazine hydrate</u>. The steroid (XXIII; R_1=Ac, R_2=H) (120 mg.),
hydrazine hydrate (0.5 ml.) and ethanol (12 ml.) were refluxed
together on the steam-bath for 4 hours. The reaction mixture was
worked up by addition of water and extraction to give an amorphous
product (125 mg.) which crystallised on trituration with ether and
methanol.

Two recrystallisations from methylene chloride-methanol gave
material m.p. 279-283°, $[\alpha]_D$ -64.8° ($\underline{c}$, 0.23) (Found: C,66.45; H,8.3;
N,5.6. $C_{27}H_{34}O_5N_2 \cdot CH_3OH$ requires C,66.9; H,8.4; N,5.6%),
λ_{max} 207 mμ (ε 3950), ν_{max} 3356, 3279 (NH), 1773 (lactone), 1733
(OAc), 1689 ($\supset$C=O), 1672 ($\supset$C=N-), 1653 ($\supset$C=C$\subset$), 1541, 1241 (OAc),
and 1033 cm^{-1}. and which is therefore 3β-<u>acetoxy</u>-17α-<u>hydroxy</u>-11,23-
<u>dioxochol</u>-20(22)-<u>ene</u>-22-<u>carboxylic acid</u> 22→17 <u>lactone</u>-23-<u>hydrazone</u>
(XXXVII; R=N.NH).

Hydrolysis of a sample of the hydrazone with potassium hydro-
xide gave a product, m.p. 275-285°, ν_{max} 3597m, 1742s, 1634m cm^{-1}.,
which may be 4-(3'β,17'α-dihydroxy-11'-oxo-20'-pregnenylidene)-3-
methyl-5-oxo-pyrazoline (XXXVIII; R=H).

(g) <u>Semicarbazide hydrochloride</u>. The steroid (XXIII; R_1=Ac, R_2=H) (100 mg.) was treated with semicarbazide hydrochloride (150 mg.) in pyridine (10 ml.) and heated at $100°$ for $3\frac{1}{2}$ hours, then left over-night at room temperature. Water, (150 ml.) was added and the reaction mixture worked up as before to give crystalline material (94 mg.) which, after three recrystallisations from methylene chloride-methanol had m.p. $259-266.5°$, $[\alpha]_D$ $-60.4°$ (c, 0.47) (Found: C,64.1; H,8.0; N,7.95. $C_{28}H_{39}O_6N_3.CH_3OH$ requires C,63.8; H,7.9; N,7.7%), λ_{max} 220 (ε 17,000) and 280 mμ (ε 10,250), ν_{max} 3448, 3257, 3205 (NH), 1757 (lactone), 1733 (OAc), 1695 ($\supset$C=O), 1570 (-N=N-), 1247 (OAc), and 1029 cm^{-1}. (NH), and which is therefore 3β-acetoxy-17α-hydroxy-11,23-dioxochol-20(22)-ene-22-carboxylic acid 22→17 lactone-23-semicarbazone (XXXVII; R=N.NH.CO.NH$_2$).

Hydrolysis of a sample of the semicarbazone with methanolic potassium hydroxide gave a small quantity of crystalline material m.p. $189-201°$, which may be 3β,17α-dihydroxy-11,23-dioxochol-20(22)-ene-22-carboxylic acid 22→17 lactone (XXIII; R_1=R_2=H). Insufficient material was obtained to permit further examination.

(h) <u>Phenylhydrazine</u>. The steroid (XXIII; R_1=Ac, R_2=H) (105 mg.), phenylhydrazine (0.5 ml.), and acetic acid (10 ml.) were heated together on the steam-bath for 6 hours. The product, which was obtained by addition of water (100 ml.) and extraction as before, was a dark oil (0.26 g.)

This oil was dissolved in chloroform, the solution washed with hydrochloric acid and evaporated. The product was then taken up in methanol and boiled with activated charcoal for a few minutes, giving a pale yellow solution. This gave a solid, non-crystalline material λ_{max} 208 (ε 13,040), 235 (ε 12,830), and 346 mμ (ε 5000), ν_{max} 1757 (lactone), 1738 (OAc), 1706 ($>$C=O), 1603, 1497, 1245 (OAc), and 696 cm^{-1}. (aromatic ring).

The same material precipitated from ethanol by addition of water had m.p. 235-261°.

(i) Urea. The steroid (XXIII; R_1=Ac, R_2=H) (106 mg.) in ethanol (15 ml.) was treated with urea(0.3 g.) in ethanol(10 ml.). This solution was heated at 100° for 6½ hours, then left overnight at room temperature. Some material which precipitated out on cooling was filtered off and shown to starting material m.p. 269-273.5^{o}.

To the remainder, water (100 ml.) was added and the solution extracted with chloroform. The product (86 mg.) m.p. 255-269°, ν_{max} 1764 (lactone), 1727 (OAc), 1701 ($>$C=O), 1623 ($>$C=C$<$), and 1245 cm^{-1}. (OAc), was starting material.

(j) Chromic acid. The steroid (XXIII; R_1=Ac, R_2=H) (73 mg.) was dissolved in acetone (15 ml.) and treated with 8N chromic acid (0.5 ml.) for 8 min. at room temperature. The product, isolated as before had m.p. 270-276° and was thus starting material.

17α-Hydroxy-3,11,23-trioxochol-20(22)-ene-22-carboxylic acid 22→17 lactone (XLVII).- 3β,17α-Dihydroxy-11,23-dioxochol-20(22)-ene-22-carboxylic acid 22→17 lactone (XXIII; R_1=R_2=H) (0.48 g.) was dissolved in acetone (50 ml.) and oxidised with 8N chromic acid (1.0 ml.) for 10 min.. The reaction mixture was worked up as before to give material, m.p. 268-275°, which was chromatographed on deactivated alumina (30 g.).

9:1 Benzene-ether eluted material which, after two recrystallisations from chloroform-methanol was shown to be 17α-hydroxy-3,11,23-trioxochol-20(22)-ene-22-carboxylic acid 22→17 lactone (XLVII), m.p. 270-274°, $[\alpha]_D$ -29.1° (c, 1.15) (Found: C,71.6; H,7.6. $C_{25}H_{32}O_5 \cdot \frac{1}{2}CH_3OH$ requires C,71.5; H,8.0%), λ_{max} 204 (ε 4250) and 244 mμ (ε 9850), ν_{max} 1761 (lactone), 1712 ($>$C=O), 1695 ($>$C=O), and 1621 cm^{-1}. ($>$C=C$<$).

Reaction of 17α-Hydroxy-3,11,23-trioxochol-20(22)-ene-22-carboxylic acid 22→17 lactone (XLVII) with D.D.Q..- The steroid (0.1 g.) was dissolved in dioxan (10 ml.) containing D.D.Q. (0.243 g.) and the mixture refluxed for 6 hours. Isolation of the product as before gave a brown solid which was chromatographed on deactivated alumina (20 g.). No steroid was found, the only identifiable product being the hydroquinone.

Miscellaneous Reactions of 3β-Acetoxy-17α-hydroxy-11,23-dioxo-
chol-20(22)-ene-22-carboxylic acid 22→17 lactone-23-oxime (XXXIII;
R_1=Ac, R_2=H) and subsequent products.-

Attempted acetylation of the oxime. The oxime (XXXIII; R_1=Ac, R_2=H)
(50 ml.) in pyridine (3 ml.) was treated with acetic anhydride (3 ml.)
overnight at room temperature. The product, obtained by addition of
water (30 ml.) and filteration, followed by recrystallisation from
methylene chloride-methanol was shown to be starting material,
m.p. 273-279.5°, λ_{max} 210 (ε 9360) and 232 mμ (ε 6700), ν_{max} 3356
(OH), 1767 (lactone), 1704 ($>$C=O), 1653 ($>$C=C$<$), 1277 (OAc), and
1037 cm^{-1}. (OH).

Attempted Beckmann rearrangement of the oxime. The oxime (XXXIII;
R_1=Ac, R_2=H) (164 mg.) in pyridine (3 ml.) was treated with a
pyridine solution of p-toluenesulphonyl chloride (180 mg. in 3 ml.)
and the resulting solution heated at 100° for 4½ hours. Water (5 ml.)
was added followed by excess 2N hydrochloric acid. The product,
isolated by filtration had m.p. 265-275°. Recrystallisation from
methylene chloride-methanol gave starting material, m.p. 265-270°
with resolidification and remelting at 278-283°, mixed m.p. 275-283°.

The recrystallised product was again treated with p-toluene-
sulphonyl chloride in pyridine. The reaction was carried out at
reflux temperature for four hours as indicated by Mazur[22] in the

rearrangement of hecogenin acetate oxime.

The product, isolated as before, had m.p. 250-280°, after one recrystallisation from methanol-ether, α_D +15.8° (c, 0.19) λ_{max} 209 (ϵ7130) and 232 infl. mμ (ϵ 6020) and is thus starting material.

Treatment of the oxime with hydrochloric acid. The oxime (XXXIII; R_1=Ac, R_2=H) (0.33 g.) was dissolved in methanol (20 ml.) by refluxing for 15 min.. A sample taken after complete solution showed, by infrared examination, that no change had occured. This solution was then treated with concentrated hydrochloric acid (2 ml.). After 5 min. a white solid separated out, and after a further 10 min. heating, the solution was filtered. The crystalline product (0.24 g.) had m.p. 308-312° (decomp.). Recrystallisation from chloroform-methanol gave 4-(3'β,17'α-dihydroxy-11'-oxo-20'-pregnenylidene)-3-methyl-5-isoxazolone (XXXVIII; R=H), m.p. 312-314.5° (decomp), $[\alpha]_D$-28.2° (c, 0.4 in pyridine) (Found: C,69.7; H,8.0; N,3.4. $C_{25}H_{35}O_5N$ requires C,69.9; H,8.2; N,3.3%), λ_{max} 208 (ϵ 8300) and 224 infl. mμ (ϵ 5680), ν_{max} 3546, 3322 (OH), 1739 (isoxazolone), 1715 ($\supset$C=O), 1647 ($\supset$C=C$\subset$), and 1037 cm^{-1}.. The product also gave a negative Legal test.

Treatment of the oxime with potassium hydroxide. The oxime (XXXIII; R_1=Ac, R_2=H) (120 mg.) in methanol (10 ml.) was treated with

potassium hydroxide (1.0 g.) in methanol (10 ml.). The solution was refluxed for four hours, then left overnight at room temperature. Addition of water and extraction with chloroform gave a neutral product (24 mg.) m.p. 302-305°.

Acidification of the extracted solution and re-extraction with chloroform gave further material (80 mg.) m.p. 307-310°. Infrared examination showed both products to be the same, and two recrystallisations of the combined products from chloroform-methanol gave material shown to be identical with the hydrochloric acid product (XXXVIII; R=H), m.p. 311-314° (decomp.), $[\alpha]_D$ -22.1° ($\underline{c}$, 0.49 in pyridine), λ_{max} 210 (ε 8000) and 228 infl. mμ (ε 4970), ν_{max} 3484, 3300 (OH), 1736 (isoxazolone), 1712 ($>$C=O), 1645 ($>$C=C$<$), and 1036 cm^{-1}.. It also gave a negative Legal test.

of the hydrolysis product. The oxime (XXXIII; R_1=Ac, ~~Chromatogram of the hydrolysis product. The oxime (XXXIII; R_1=Ac,~~ R_2=H) (0.30 g.) was treated with potassium hydroxide (1.5 g.) in methanol (25 ml.) for three hours as before. The product was dissolved in prior to chromatography on silica gel. Some material which would not dissolve was filtered off (103 mg.) and shown to be identical with the hydrolysis product obtained above, m.p. 309-314° $[\alpha]_D$ -33.3° ($\underline{c}$, 0.48 in pyridine), ν_{max} 3497, 3289 (OH), 1733 (isoxazolone), 1712 ($>$C=O), 1645 ($>$C=C$<$), and 1037 cm^{-1}..

The filtrate was chromatographed. Elution of the column in

100 ml. fractions gave only trace amounts in each fraction. Ether

containing 3% methanol eluted material (27 mg.) shown to be the

hydrolysis product (XXXIII; R=H). Elution was continued with more

polar solvents until 90% ether-10% acetic acid, with similar results,

i.e. only trace amounts in each fraction.

Acetylation of the hydrolysis product. 4-(3'β,17'α-Dihydroxy-11'-

oxo-20'-pregnenylidene)-3-methyl-5-isoxazolone (XXXVIII; R=H)

(103 mg.) was treated overnight with acetic anhydride (1 ml.) in

pyridine (1 ml.). Addition of water and extraction in the usual

manner gave a solid which crystallised with difficulty from methanol.

Two recrystallisations gave 3β-acetoxy-23-acetyloximino-17α-

hydroxy-11,23-dioxochol-20(22)-ene-22-carboxylic acid 22→17 lactone

(XXXIII; $R_1 = R_2 = Ac$) m.p. (120) (175-180) 274-288° (decomp.), $[\alpha]_D$ +5.1°

(c, 0.94) (Found: C,67.5; H,7.3; N,3.0. $C_{29}H_{39}O_7N$ requires

C,67.8; H,7.6; N,2.7%), λ_{max} 208 (ε 10,760) and 243 mμ (ε 8110),

ν_{max} 1786 (N-OAc), 1767 (lactone), 1739 (OAc), 1658 ($>C=C<$), 1253

(OAc), 1221 (N-OAc), and 1035 cm^{-1}.

The apparent impurity of this compound indicated by the melting

point prompted a chromatographic examination.

The hydrolysis product (XXXIII; R=H) (0.286 g.) was acetylated

with acetic anhydride (2 ml.) in pyridine (2 ml.) overnight. After

working up in the usual manner, the product was chromatographed on

deactivated alumina (20 g.).

Ether eluted material m.p. 304-310°, ν_{max} 3472, 3289 (OH), 1736 (isoxazolone), 1712 ($\supset$C=O), 1645 ($\supset$C=C$\subset$), and 1035 cm^{-1}., after one recrystallisation from chloroform-methanol. This is identical with starting material (XXXVIII; R=H).

Ether containing 3% methanol eluted a small quantity of material m.p. 284-290°, $[\alpha]_D$ +6.6°, ν_{max} 3378 (N-OH), 1761 (lactone), 1698 ($\supset$C=O), 1656 ($\supset$C=C$\subset$), 1277 (OAc), and 1039 cm^{-1}. This is identical with the oxime (XXXIII; R_1=Ac, R_2=H).

No trace of the diacetate was found, although the fractions containing the hydrolysis product turned orange in colour during recrystallisation before the physical properties were examined. This seems to indicate that the hydrolysis product is formed by decomposition.

A further acetylation of the hydrolysis product was carried out as before and the product chromatographed on silica gel.

4:1 Benzene-ether eluted material (51 mg.) m.p. 269-281°. The infrared spectrum verified that this was the oxime (XXXIII; R_1=Ac, R_2=H). The same solvent also eluted material (39 mg.) m.p. (120) (180) 220-298°, apparently the required diacetate, which after one recrystallisation from chloroform-methanol had m.p. 275-280° (decomp.) and which had an infrared spectrum identical with that of the oxime.

The oxime (5 mg.) and its hydrolysis product (5 mg.) were each treated with acetic anhydride (0.6 ml.) in pyridine (0.3 ml.) over-night at room temperature. The solvents were evaporated off without heating, a little water was added to each, and the contents of the flasks taken to dryness once more. Both products gave identical infrared spectra which were very similar to that of the diacetate (XXXIII; $R_1=R_2=Ac$).

Beckmann rearrangement of the oxime (chromatogram). The oxime (XXXIII; $R_1=Ac$, $R_2=H$) (0.255 g.) was dissolved in pyridine (5 ml.) and treated with a solution of p-toluenesulphonyl chloride (0.20 g.) in pyridine (10 ml.). The solution was refluxed for three hours and left overnight at room temperature. The product, which was isolated as before was light brown in colour and was chromatographed on deactivated alumina (20 g.).

1:1 Benzene-ether eluted material (162 mg.) m.p. 270-282° (decomp.), $[\alpha]_D$ +7.9°, ν_{max} 3401 (OH), 1764 (isoxazolone), 1736 (OAc), 1709 ($>C=O$), 1645 ($>C=C<$), 1575 ($>C=N-$), 1244 (OAc), and 1026 cm^{-1}. (OH). After one recrystallisation, however, the infrared spectrum changed to that of the hydrolysis product (XXXVIII; R=H).

Ether containing 5% methanol eluted material (55 mg.) m.p. 240-259.5° (after one recrystallisation from acetone-petroleum ether), $[\alpha]_D$ -3.8° (c, 0.37), λ_{max} 210 (ε 10,130) and 229 infl. mμ (ε 6400),

v_{max} 3483 (OH), 1764 (isoxazolone),1733 (OAc), 1695 ($\supset$C=O), 1667 ($\supset$C=C$\subset$), 1575 ($\supset$C=N-), 1245 (OAc), and 1030 cm^{-1}.. This is 4-(3'β-acetoxy-17'α-hydroxy-11'-oxo-20'-pregnenylidene)-3-methyl-5-isoxazo-lone (XXXVIII; R=Ac). (see below).

Platinum oxide hydrogenation of the oxime. The oxime (XXXIII; R_1=Ac, R_2=H) (0.107 g.) in ethanol (25 ml.) containing acetic acid (3 ml.) was added to 124 mg. of pre-reduced platinum oxide catalyst and the mixture stirred in a hydrogen atmosphere for two hours. There was no apparent uptake of hydrogen. Filtration of the solution was followed by addition of water and extraction as usual. One recrystallisation gave material m.p. 248-254°, which was not the expected oxime. Further recrystallisation showed, by infrared examination, that the product was changing to the oxime.

The experiment was repeated in order to obtain an analytical sample, m.p. 264-273°, $[\alpha]_D$ -4.2^o (c, 0.36) (Found: C,69.0; H,7.8; N,3.1. $C_{27}H_{37}O_6N$ requires C,68.8; H,7.9; N,3.0%), λ_{max} 210 (ε 9180) and 226 infl. mμ (ε 6000), v_{max} 3460 (OH), 1761 (isoxaz-olone), 1727 (OAc), 1689 ($\supset$C=O), 1664 ($\supset$C=C$\subset$), 1245 (OAc), and 1029 cm^{-1}.. This material is identical with that obtained in the attempt-ed Beckmann rearrangement above, and is therefore 4-(3'β-acetoxy-17'α-hydroxy-11'-oxo-20'-pregnenylidene)-3-methyl-5-isoxazolone (XXXVIII; R=Ac).

<u>Platinum oxide hydrogenation of the hydrolysis product</u> (XXXVIII;
R=Ac). The hydrolysis product (XXXVIII; R=Ac) (56 mg.) was dissolved in glacial acetic acid (20 ml.) and added to pre-reduced
platinum oxide catalyst (70 mg.) in glacial acetic acid (15 ml.).
After 5 hours stirring in a hydrogen atmosphere, the reaction mixture was worked up as before. The product was shown to be starting
material m.p. 281-297°, λ_{max} 208 (ε 9150) and 234 infl. mμ (ε 5650),
ν_{max} 3497, 3289 (OH), 1739 (isoxazolone), 1715 ($\supset$C=O), 1645 ($\supset$C=C$\supset$),
and 1037 cm^{-1}..

<u>Attempted pyrolysis of the diacetate</u> (XXXIII; R_1=R_2=Ac). The diacetate (XXXIII; R_1=R_2=Ac) (98 mg.) was heated at atmospheric pressure
to 210° for 1 min.. The material melted but did not sublime. On
cooling, a sample was taken and crystallised from methanol. Infrared
examination showed that no change had taken place. The original
material was reheated and maintained at a temperature of 224-226°
for 5 min.. From the resulting dark brown gum, no crystalline
material could be isolated. This gum had an infrared spectrum
ν_{max} 1770, 1740, 1715, 1680, and 1248 cm^{-1}. which is very similar to
that of starting material.

<u>Treatment of Cholesteryl Acetoacetate with Hydroxylamine Hydrochloride</u>.- Cholesteryl acetoacetate (XI; R=COCH$_2$COCH$_3$) (154 mg.) was
heated with hydroxylamine hydrochloride (141 mg.) in pyridine (12 ml.)

for 6 hours. The reaction mixture, worked up in the usual manner, gave a crystalline product (113 mg.) m.p. 145-149°, ν_{max} 3425 (OH) and 1057 cm^{-1}.(OH), which is therefore cholesterol (XI; R=H).

<u>Treatment of Cholesteryl Acetate with Hydroxylamine Hydrochloride.-</u> Cholesteryl acetate (XI; R=Ac) (0.617 g.) was heated at 100° with hydroxylamine hydrochloride (1.03 g.) in pyridine (25 ml.) for 5 hours. The product, obtained on working up in the usual manner was shown to be starting material (0.574 g.), m.p. 114-115°, ν_{max} 1733 (OAc), 1250 (OAc), and 1038 cm^{-1}..

<u>Treatment of 3β-Acetoxy-17a-hydroxy-11,23-dioxocholan-22-carboxylic acid 22→17 lactone (XXX; R_1=Ac, R_2=H) with Potassium Hydroxide.-</u> The steroid (XXX; R_1=Ac, R_2=H) (68 mg.) was treated with methanolic potassium hydroxide (2.0 g. in 20 ml.) and the solution refluxed for 5 hours. On allowing the methanol to evaporate, a crystalline solid was deposited. This solid was soluble in hot water, and on ignition left a deposit, m.p. 200-215°, ν_{max} 3390s, 1695sh, 1658s, and 1039 cm^{-1}..

The mother liquors, on extraction yielded no neutral product. On acidification and re-extraction, an acid fraction was obtained as an amorphous solid, ν_{max} 3413 (OH), 1770 (saturated lactone), 1706 (⊃C=O), 1161, and 1041 cm^{-1}. (OH), which may be the 3β-hydroxy compound (XXX; R_1=R_2=H).

<u>Treatment of 3β-Acetoxy-17,23-dihydroxy-11-oxocholan-22-</u>

<u>carboxylic acid 22→17 lactone (XXXI; R=Ac) with Potassium hydroxide.-</u>
The steroid (XXXI; R=Ac) (65 mg.) in methanol (5 ml.) was treated
with potassium hydroxide (1.0 g.) in methanol (10 ml.). The solution
was refluxed for 3 hours and some of the solvent allowed to evap-
orate off. A white solid was precipitated m.p. 215-225° (with residue).

Acidification of the mother liquors and re-extraction gave a
gum which did not crystallise.

<u>3β-Acetoxy-17α-hydroxy-11-oxocholan-22-carboxylic acid 22→17</u>

<u>lactone-23-oxime (XXXIX).-</u> The steroid (XXX; R_1=Ac, R_2=H) (68 mg.)
was heated with hydroxylamine hydrochloride (100 mg.) in pyridine
(4 ml.) for 5 hours at 100°. Extraction as described previously
gave a non-crystalline product (80 mg.). This was precipitated
twice from methanol by addition of water to give the 23-oxime,
(XXXIX), m.p. 160-165° (amorphous), $[a]_D$ -10.5° ($\underline{c}$, 0.21) (Found:
C,66.4; H,8.2; N,4.0. $C_{27}H_{39}O_6N.CH_3OH$ requires C,66.5; H,8.6;
N,2.8%), λ_{max} 206 (ε 5050), 258 (ε 4020) and 264 mμ (ε 4070),
v_{max} 3367 (OH), 1773 (saturated lactone), 1736 (>C=0), 1642 (>C=C<),
1613 (>C=N-), and 1250 cm^{-1} (OAc).

An attempt to sublime this product gave charred material at the
bottom of the tube and some sublimate as a clear gum which would
not crystallise.

Treatment of 3β-Acetoxy-17α-hydroxy-11,23-dioxocholan-22-carboxylic acid 22→17 lactone (XXX; R_1=Ac, R_2=H) with Urea.- The steroid (XXX; R_1=Ac, R_2=H) (100 mg.) was heated with urea (200 mg.) in refluxing ethanol (20 ml.) for 3 hours and left overnight at room temperature. The product, (101 mg.) obtained as above, was recrystallised once from chloroform-methanol to give material, m.p. 263-269°, λ_{max} 206 (ε 1400) and 252 mμ (ε 2800), ν_{max} 1779 (saturated lactone), 1736 (OAc), 1712 (>C=O), and 1250 cm^{-1}.(OAc), which is therefore starting material.

22-Acetyl-3-oxo-5α-card-20(22)-enolide (VI).- 21-Hydroxy-3,20-dioxo-5α-pregnane-21-hemisuccinate (XXIV; R=COCH$_2$CH$_2$CO$_2$H) (2.48 g.) was hydrolysed by dissolving in aqueous potassium bicarbonate and leaving overnight under an atmosphere of nitrogen.[23] The product, 21-hydroxy-3,20-dioxo-5α-pregnane (XXIV; R=H) was dissolved in chloroform and treated with diketene (2.5 ml.) and triethylamine (0.2 ml.) as before. Chromatography on alumina gave material, which, after three recrystallisations from methylene chloride-methanol was shown to be 22-acetyl-3-oxo-5α-card-20(22)-enolide (VI), m.p. 234-238°, $[\alpha]_D$ -21.5° (c, 0.79) (Found: C,75.45; H,8.6. $C_{25}H_{34}O_4$ requires C,75.3; H,8.6%), λ_{max} 206 (ε 4450) and 245 mμ (ε 9860), ν_{max} 1766 (lactone), 1700 (>C=O), and 1613 cm^{-1}. (>C=C<).

This compound also gave a positive Legal test, showing the

presence of the $\Delta^{\alpha\beta}$-lactone system. In this case, the product is related to the cardiac aglycones, which absorb in the ultraviolet at 220 mμ.

Reaction of 21-Acetoxy-17α-hydroxy-3,11,20-trioxo-5α-pregnane (XLIX) with Diketene.- The steroid (XLIX) (1.044 g.) in benzene (50 ml.) was treated, as before, with diketene (1.5 ml.) and triethyl-amine (0.2 ml.) and the solution refluxed for one hour. After evaporation under reduced pressure, the gum produced was redissolved in benzene (20 ml.) and chromatographed on silica gel (20 g.).

1:4 Ether-benzene eluted material as a gum which could not be crystallised, but the infrared spectrum of which, indicated that it may be the required compound, $[\alpha]_D$ +28.5°, ν_{max} 3390, 1764 (lactone), 1730 (OAc), 1712 ($>$C=O), and 1608 cm^{-1}, ($>$C=C$<$).

Reaction of Diketene with Compound S (L)[9].- Compound S (L) (0.61 g.) was dissolved in benzene (40 ml.) and heated to 60°. Triethylamine (0.2 ml.) was added with stirring, followed by diketene (0.3 ml.) in benzene (10 ml.) added slowly. The solution was stirred for 30 min. at 60° and allowed to come to room temper-ature (1 hour). Washing with 2N hydrochloric acid, water, followed by drying and evaporation under reduced pressure gave a red oil. This was dissolved in methanol (10 ml.) and treated with sodium methoxide (0.3 g. sodium in 3 ml. methanol) with stirring for 10

min.. The solution was again washed with 2N hydrochloric acid and worked up as before to give a froth which did not crystallise, v_{max} 3425 (OH), 1761 (lactone), 1721 ($>$C=O), 1681 ($>$C=O), and 1623 cm^{-1}. ($>$C=C$<$).

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PART II

STEROIDS DERIVED FROM HECOGENIN

In 1952, Grundy, Simpson, and Tait[1] presented evidence for a
potent mineral factor, different from the known hormones, in the
mother liquors obtained in the crystallisation of the more water
soluble steroids. This substance was remarkably effective in
maintaining the life of adrenalectomised animals.

The same group of workers, in co-operation with a Swiss group
later isolated the active constituent,[2] aldosterone (I), and
elucidated its structure.[3]

(I)
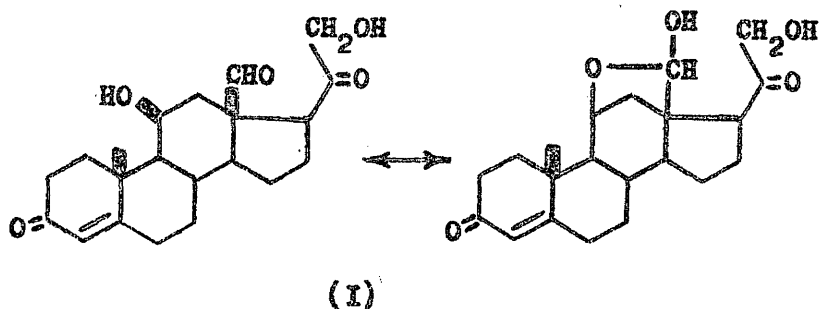
Aldosterone was shown to have high mineralocorticoid activity,
being 100 times more active than deoxycorticosterone[3] (II) in
promoting the retention of sodium, and to be effective in the
treatment[4] of Addisons disease.[5]

Following the elucidation of the structure of aldosterone,
several groups set out to synthesise an 18-oxygenated steroid. One
approach was total synthesis and this was achieved by Wettstein and

(II)

(III)
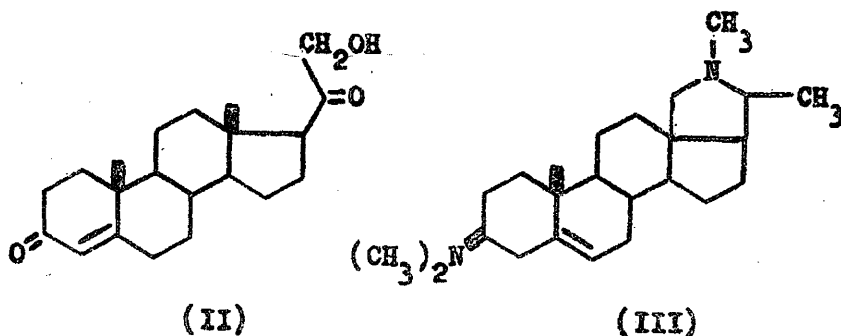
his co-workers, and Johnston and his co-workers.[6] The alkaloid
conessine (III) provided a second method of approach to the 18-
oxygen function since it already contains a nitrogen atom attached
to C_{18} and can readily be degraded to an 18-oxygenated steroid.
Extension of this work to 3β-hydroxy-11-oxoconanine (IV) gave
aldosterone (I)[7].

(IV)   (V)   (VI)
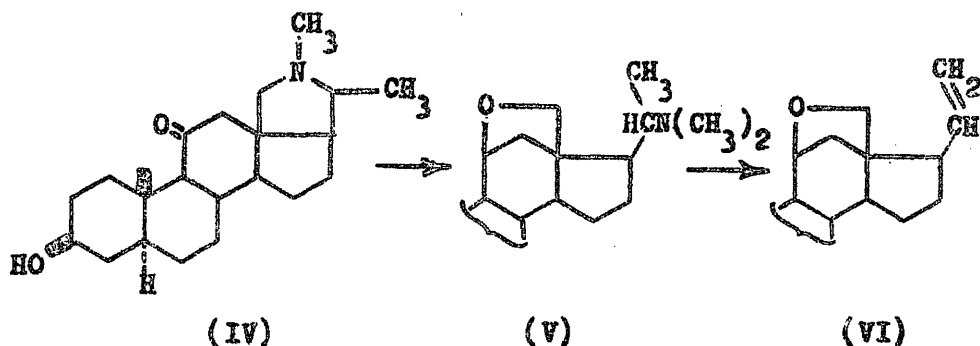
Thus, 11-oxoconanine (IV) was treated with lithium aluminium
hydride, and the product subjected to Hofmann elimination, giving the
lactol (V). Reaction of the lactol with methyl iodide followed by

sodium methoxide gave the 20-olefin (VI) which was converted to the 20,21-diacetoxy compound (VII). This lactol, on treatment with
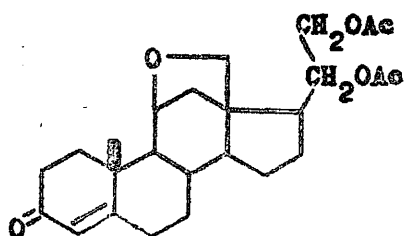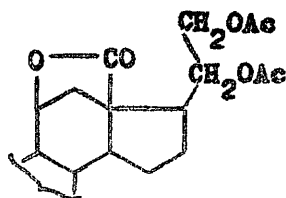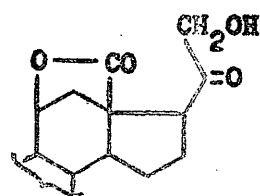

(VII)   (VIII)   (IX)

ruthenium oxide gave the lactone (VIII) and hence by hydrolysis, protection of the 21-hydroxyl group, and oxidation, the lactone (IX). This product has been converted by Von Euw, Neher, and Reichstein[8] to aldosterone (I). The carbonyl groups at C_3 and C_{20} were protected by formation of the ketal (X) which was then reduced with lithium aluminium hydride to the product (XI) which gave aldosterone (I) on hydrolysis.
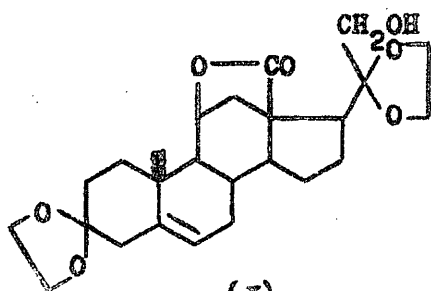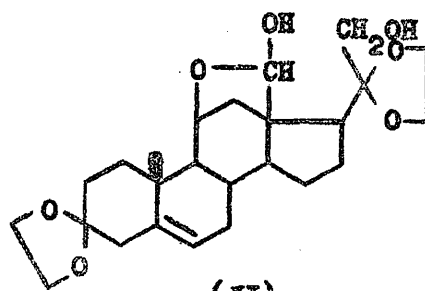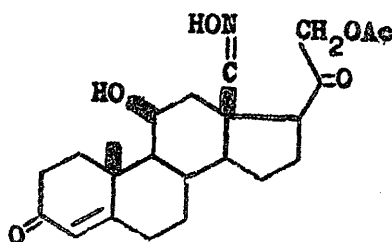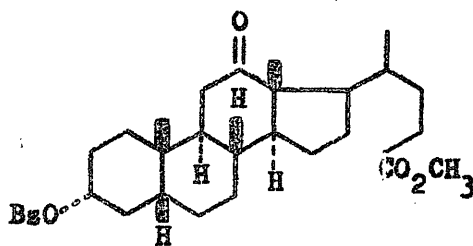

(X)   (XI)
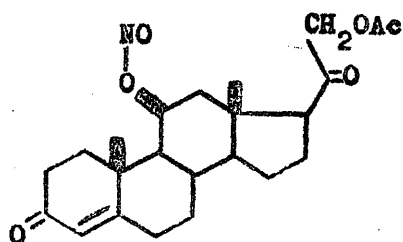
(XII) (XIII)

Barton, Beaton, Geller, and Pechet,[9] have described a very
elegant synthesis of aldosterone by irradiation of the 11-nitrite
of corticosterone acetate (XII). The immediate product of this
reaction was aldosterone acetate oxime (XIII) which gave aldosterone
(I) on hydrolysis.

(XIV)

In a patent, Ruzicka and Jeger,[10] have described the preparation
of a 13-cyano-steroid. Starting with the cyanhydrin of methyl 3α-
benzoyloxy-12-oxocholanate (XIV), the steroid nucleus is made to

undergo C-nor-D-homo rearrangement giving the unsaturated nitrile (XV). The ketone obtained on ozonolysis followed by hydrolysis was converted to its cyanhydrin, which, it is claimed, undergoes retrogression to the Δ^{11}-13-cyano steroid (XVI).
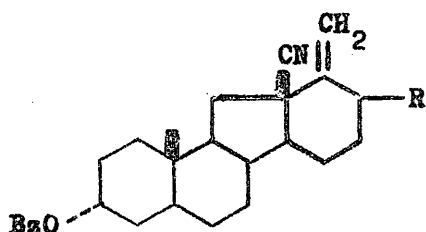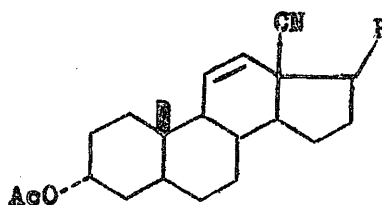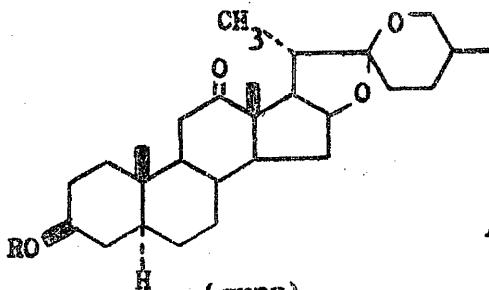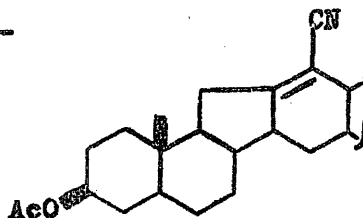

(XV)   (XVI)

The present work is an attempt to apply these reactions to the readily available sapogenin hecogenin (XVII; R=H). It has been shown that in this series, reverse rearrangement to the angular nitrile group does not occur, instead, elimination takes place to give the αβ-unsaturated nitrile (XVIII).
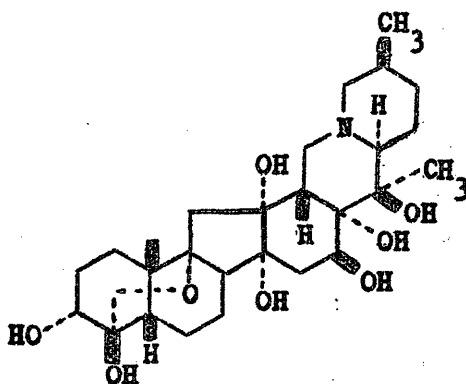

(XVII)   (XVIII)

This compound is interesting in that it has the C-nor-D-homo structure and angular nitrogen function, and may be useful as a starting material in the synthesis of the ceveratrum alkaloids, the best known of which is cevine (XIX).


(XIX)

THEORETICAL

The steroid skeleton has been subjected to many alterations in its structure. Among the most common of these is ring expansion and contraction, some of which are described by Fieser and Fieser[11]. A,[12] B,[13] and D[11]-homo steroids are known, as are A,[14] B,[15] C,[16] and D[17]-nor steroids. The rearrangement of particular interest was that involving the C and D rings of the steroid nucleus, giving C-nor-D-homo compounds. Several reactions of this type are known, generally with derivatives of hecogenin (XVII; R=H) or rockogenin (XX; $R_1=R_2=H$).

(XX)
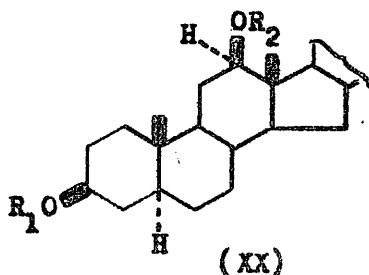
In 1952, Bamford and Stevens[18] published the results of studies on the alkaline decomposition of toluene-p-sulphonylhydrazones. The product of this type of decomposition is usually an olefin, sometimes formed by rearrangement of the carbon skeleton.

Thus, Hirschmann, Snoddy, Hiskey, and Wendler[19] found that hecogenin acetate toluene-p-sulphonylhydrazone (XXI) gave an olefin

when treated with sodium in ethylene glycol. An examination of this product showed that it could not have the normal steroid skeleton, and hence, rearrangement to the C-nor-D-homo structure was postulated.


(XXI)　　　　　　　(XXII)　　　　　　　(XXIII)
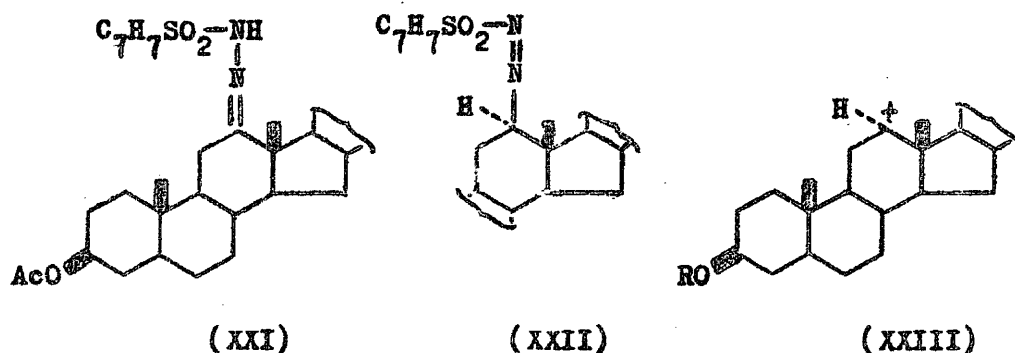
This is feasible only if decomposition takes place to give the carbonium ion (XXIII) by means of the intermediate compound (XXII) which breaks up to give nitrogen and a sulphinic acid derivative as the other products.

A prerequisite of the C-nor-D-homo rearrangement is formation of the carbonium ion which, in its transitory state can be said to have 12β-configuration. Substituents in the 12β-position are coplanar with the carbon atoms at 12, 13, and 14. Thus rearrangement takes place by the breaking of the 13-14 carbon bond and rear attack on the 12-position by C_{14}. This in turn, gives a second carbonium ion (XXIV) which loses H^+ to form an olefin.
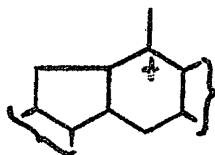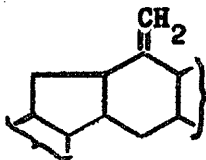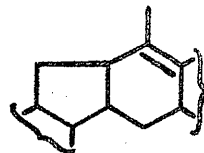
(XXIV)   (XXV)   (XXVI)

Hirschmann et al.[19] have shown that in the decomposition describ-
ed above, the major product is the endocyclic olefin (XXVI). The
exocyclic olefin is also formed, however, as a minor product.

These two olefins were also obtained by the same workers from
the solvolysis of the 12-mesyl derivative of rockogenin (XX; R_1=Ac,
R_2=CH$_3$SO$_2$-) with methanolic potassium hydroxide, the exocyclic
olefin (XXV) being in slight excess over the other. Elks, Phillipps,
Taylor, and Wyman,[20] on the other hand showed that the exocyclic
olefin (XXV) could be obtained in almost quantitative yield when
potasssium in tert.-butanol was used as solvolysing agent.
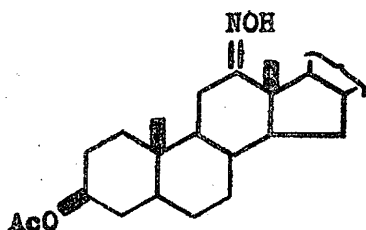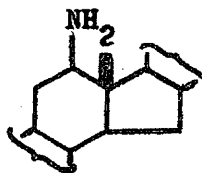
(XXVII)   (XXVIII)

Reduction of hecogenin acetate oxime (XXVII) and treatment of the resulting amine (XXVIII) with sodium nitrite has been shown by Anliker, Rohr, and Heusser[21] to give two olefins, identical with those obtained by Hirshmann and co-workers.

In the reactions described above, the carbonium ion (XXIII) is a predictable intermediate. The same is also true of the reaction between thionyl chloride and hecogenin acetate cyanhydrin (XXIX) which gives the exocyclic methylene compound (XXX) in high yield. This work was carried out by Hirshmann and co-workers.[19]

(XXIX)   (XXX)
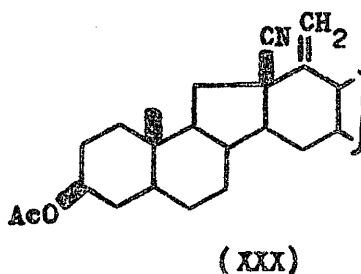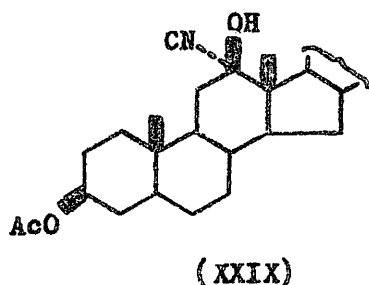
Ruzicka and Jeger[10] used the last method above to prepare a steroid in the cholanic acid series having a similar angular nitrile group. Methyl 3α-benzoyloxy-12-oxocholanate (XIV) was treated with potassium cyanide in acetic acid and the resulting cyanhydrin (XXXI) rearranged to give an olefin, methyl 3α-benzoyloxy-13-cyano-17a-methylene-C-nor-D-homo-cholanate (XXXII).
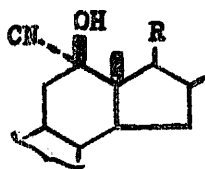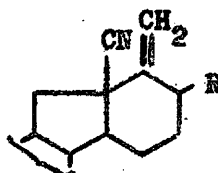
(XXXI)　　　　　　　　(XXXII)
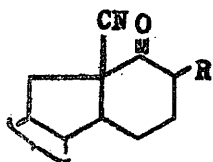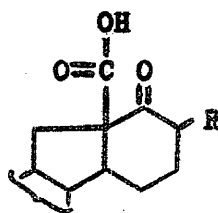
(XXXIII)　　　　　　　(XXXIV)

Ozonolysis removed the methylene group, giving a ketone
(XXXIII) which, was hydrolysed with methanolic potassium hydroxide
to the acid (XXXIV).　Decarboxylation was followed by esterification
and acetylation, giving methyl 3a-acetoxy-17a-oxo-C-nor-D-homo-18-
nor-cholanic acid (XXXV).
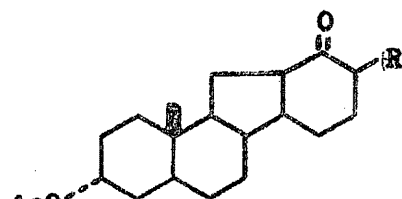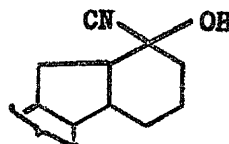
(XXXV)　　　　　　　　(XXXVI)

The cyanhydrin of this ketone (XXXVI) was prepared by
treatment with liquid hydrogen cyanide in chloroform, using

triethylamine as catalyst, and the original steroid structure was

regained by a reverse rearrangement with thionyl chloride, giving

methyl 3α-acetoxy-13-cyano-11-cholenate (XXXVII).
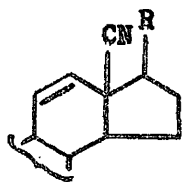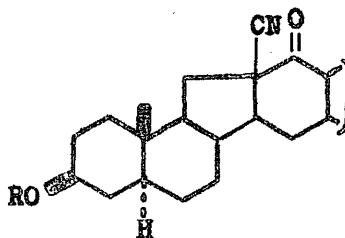
(XXXVII)   (XXXVIII)

A similar series of reactions had led Hirschmann et al.[19] to the

cyanoketone (XXXVIII), and it was planned in the work described

below, to extend this series of reactions in the manner outlined

by the Swiss workers. It was forseen that this could lead to the

aldosterone (I) type of compound.

Thus, hecogenin acetate cyanhydrin (XXIX) was prepared by the

reaction of potassium cyanide in acetic acid with hecogenin acetate

(XVII; R=Ac). The melting point of this product was 30° lower than

that quoted by Hirschmann[19] when carried out on the Kofler block.

A sample sealed in a capillary tube under vacuum, however, had a

melting point approaching the published value. The low melting

point is thought to be due to a decomposition to hecogenin acetate

and hydrogen cyanide when the compound is on the point of melting.

Rearrangement of the cyanhydrin with thionyl chloride proceeded smoothly to give 3β-acetoxy-13β-cyano-17a-methylene-C-nor-D-homo-5α,25D-spirostane (XXXIX). A sample of the cyanhydrin mother liquors treated in the same way gave the Δ^{11}-12-cyano-steroid (XL), which had an ultraviolet absorption in agreement with that quoted for a similar compound.[22]
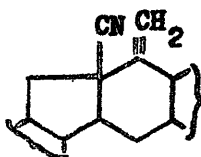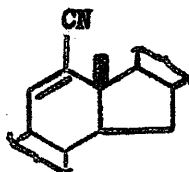
(XXXIX)   (XL)

This obviously comes from dehydration of the epimeric cyanhydrin which has an 11α-hydroxyl group. Ozonolysis of the exocyclic methylene compound (XXXIX) gave the cyanoketone (XXXVIII) in good yield, hydrolysis of which did not give the expected product (XLI). Instead, a series of hydrolysis attempts gave mainly an acidic product which was not the keto acid (XLII; R=H) which might be expected, since the infrared spectrum showed that the nitrile group was substantially untouched. It seems from this that "acid

(XLI)

(XLII)

(XLIII)
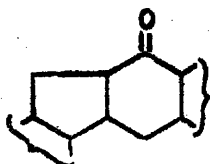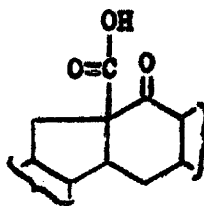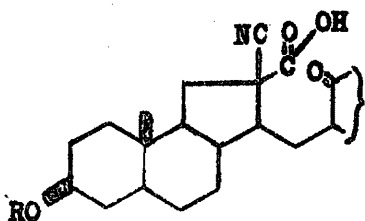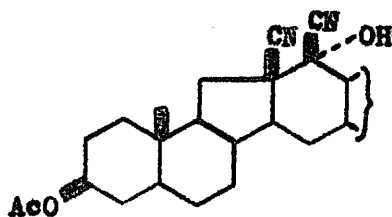
(XLIV)

cleavage" is taking place, giving rise to an acid (XLIII; R=H) in which ring D has been opened. All attempts to decarboxylate this acid were unsuccesful.

Despite the presence of the nitrile group, the ketone (XXXVIII) was treated with hydrogen cyanide to give the cyanhydrin (XLIV). However, rearrangement with thionyl chloride to the unsaturated nitrile (XLV) did not occur.
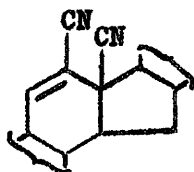
(XLV)
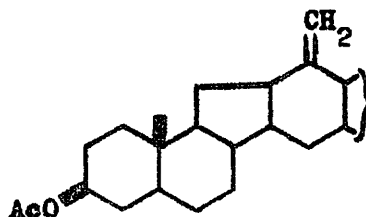
(XLVI)

This may be due to the presence of the nitrile group at C_{13} and so it was decided to prepare the known ketone (XLI). To this end, 3β-acetoxy-17a-methylene-C-nor-D-homo-5a,25D-spirostane (XLVI) was prepared by solvolysis of rockogenin-3-acetate-12-mesylate (XX; R_1=Ac, R_2=CH_3SO_2-) according to the method of Elks et al.[20]. This olefin was treated with osmium tetroxide [19] and the resultant glycol (XLVII) cleaved by periodic acid to give the required product (XLI).
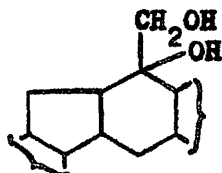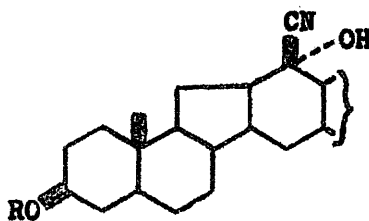
(XLVII)　　　　　　(XLVIII)

It was also found, that the 17a-methylene compound (XLVI) did not stand up well to ozonolysis, the product on two occasions being obtained in only 5% yield.

The ketone (XLI) was treated with potassium cyanide in acetic acid to give the impure cyanhydrin (XLVIII; R Ac) which contained starting material. Attempted recrystallisation resulted in the regeneration of ketone, hence the crude reaction mixture was treated with thionyl chloride. The product was shown to be 3β-acetoxy-17a -
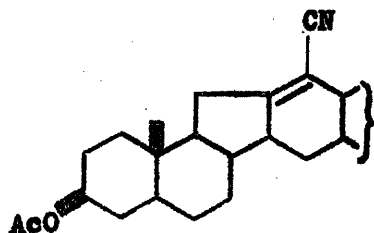
(XLIX)   (L)
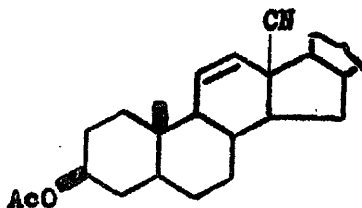
cyano-5α,25D-spirost-13(17a)-ene (XLIX).

If the hydroxyl group at carbon-17a in the cyanhydrin has the β-configuration, rearrangement is expected to take place to give the nitrile (L) since the atoms at (17aβ)-(17a)-(13)-(14) are coplanar. In fact, elimination occurs, since the (17aα)-(17a)-(13)-(13β) atoms are also coplanar. This means that the nitrile group of the cyanhydrin must have the β-configuration, and the same must be true of the cyanhydrin of the cyanoketone (XLIV). In the latter case the angular group acts as a blocking group to elimination, resulting in unchanged cyanhydrin being obtained. Also, it is now possible to state the stereochemistry of the compounds obtained.

The formation of a β-nitrile in the cyanhydrin indicates that the cyano group attacks from the top face of the molecule. The reverse is to be expected since the β-side-chain must cause some steric hindrance at the 17a-position.

Thus, elimination has taken place giving the αβ-unsaturated nitrile. Evidence for this is obtained from the infrared and ultraviolet spectra. In the former, nitrile absorption at 2237 cm^{-1}. indicates conjugation while this is supported by an absorption in the ultraviolet at 229 mμ (ε 10,100).

An examination of the 17a-methylene compound (XXXIX) proved interesting. For example, treatment with lithium aluminium hydride gave the 13-aminomethyl compound (LI; R=H) and the unexpected 13-methyl compound (LII; R=H). The structure of this latter compound
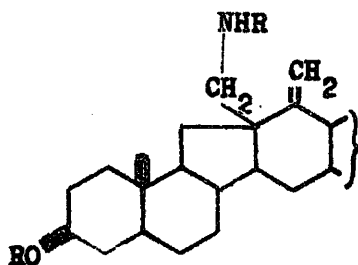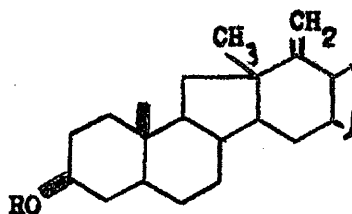
(LI)   (LII)

was arrived at on the basis of the analysis obtained for it, and the fact that its melting point was very different from that of the 13-nor compound (XLVI) obtained by Hirschmann[19] and Elks[20].

On nitrosation, the amine (LI; R=H) gave the primary alcohol (LIII), instead of the ring enlarged product (LIV) which had been hoped for. Also obtained from this reaction was an amorphous

material, thought to be the nitrite ester (LV). Hydrolysis however gave a product, also amorphous, which was not the alcohol (LIII).

(LIII)

(LIV)

(LV)

(LVI)
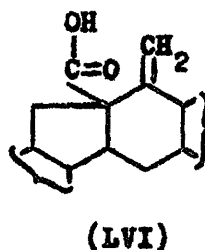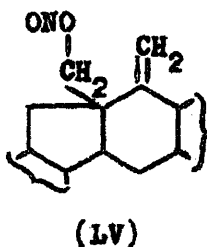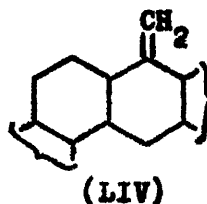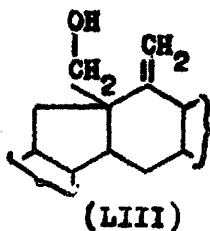
That a primary alcohol (LIII) was obtained above was shown by its oxidation to a carboxylic acid (LVI). It was hoped to correlate this compound with the material obtained by Hirschmann and Elks by ozonolysis to the β-keto acid (LVII) followed by decarboxylation to the diketone (LVIII). Ozonolysis, however, resulted in complete break-up of the molecule.

(LVII)

(LVIII)
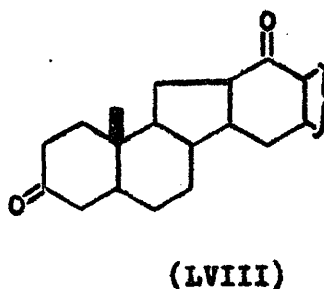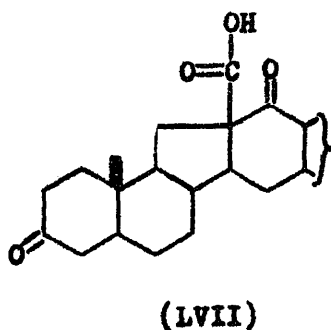
Since homo-steroids are known in which rings A, B, and D have been increased in size, it was proposed to prepare a C-homo-steroid.

In the enlargement of ring A (LX), Nelson and Schut[12] treated cholestan-3-one (LIX) with diazomethane prepared _in situ_. The reaction also works with 3-oxo-Δ^4-cholestene[23] giving a homo-steroid containing a double bond in ring A. Hecogenin, however, treated in this way gave starting material.

(LIX)   (LX)
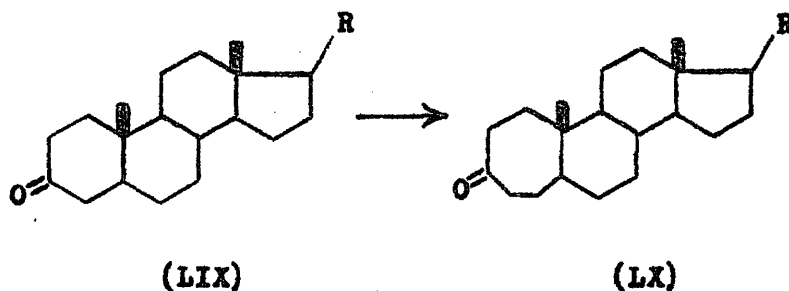
In 1960, Ringold[13] prepared B-homo-androstane (LXIII) by catalytic reduction of the cyanhydrin of 7-keto-androstane (LXI) and treatment of the amine (LXII) obtained with nitrous acid.

(LXI)   (LXII)   (LXIII)
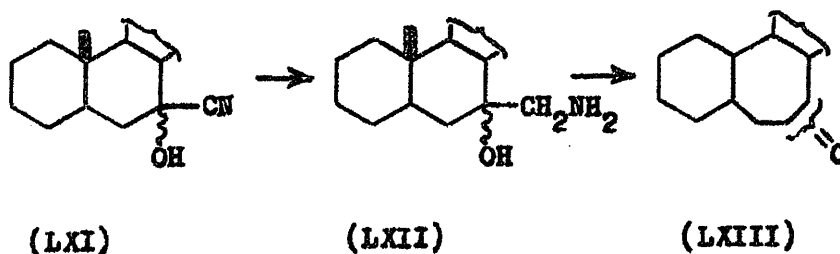
Using the method of Ringold[13] since supplies of hecogenin acetate
cyanhydrin (XXIX) were available, attempts were made to reduce the
cyanhydrin to the aminomethyl compound. All methods attempted
involving catalytic hydrogenation were unsuccessful. Similar results
were obtained with sodium borohydride, with or without aluminium
chloride, the products being hecogenin acetate (XVII; R=Ac) and
rockogenin-3-acetate (XX; R_1=Ac, R_2=H).

Lithium aluminium hydride on the other hand, tended to take the
reaction too far, resulting in the formation of rockogenin (XX;
R_1=R_2=H), epirockogenin (LXIV; R_1=R_2=H) and a small amount of an
amine (LXV; R_1=R_2=H) which crystallised with difficulty, and could
only be properly characterised as the N-acetate (LXV; R_1=R_2=Ac).
This N-acetate was treated with potassium hydroxide but did not
yield the parent amine, the 3β-hydroxy compound being the only
product (LXV; R_1=H, R_2=Ac).
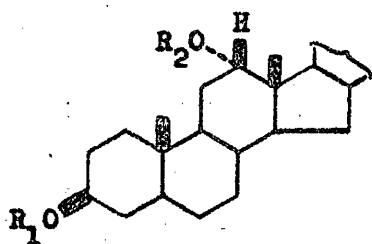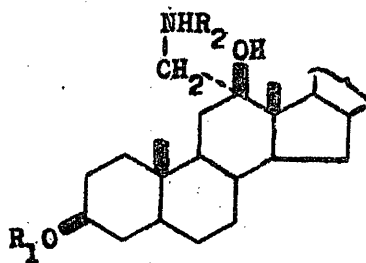
(LXIV)   (LXV)

The amide (LXV; $R_1 = R_2 = Ac$) with hydrochloric acid gave what may

be the hydrochloride (LXV; $R_1 = H$, $R_2 = H \cdot HCl$) and which on treatment

with nitrous acid gave a product showing carbonyl absorption in

the infrared spectrum. This may be the required C-homo-steroid.

(LXVI; R=H)
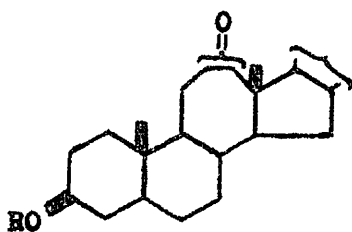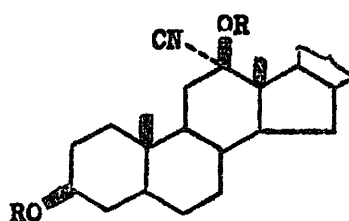
(LXVI)   (LXVII)

The reaction between the cyanhydrin and lithium aluminium hydride

was studied in order to improve the yield of amine. The maximum

yield which could be obtained, however, was 38.6% (estimated as the

acetate). Formation of rockogenin and its 12-epimer is probably due

to decomposition of the cyanhydrin, under the alkaline conditions of

the reaction, to give hecogenin which is then further reduced to the

12-alcohols.

Roberts and Goreham[24] reported improved yields of the aminomethyl

compound by prior acetylation. Thus, hecogenin acetate cyanhydrin

(XXIX) was acetylated with acetic anhydride in pyridine at 100° to

give the 12β-acetate (LXVII; R=Ac). Acetyl chloride resulted in

incomplete reaction, while refluxing acetic anhydride in pyridine was too strong. Reduction with lithium aluminium hydride gave yields similar to those obtained before.

N-Acetyl-3β-acetoxy-12a-aminomethyl-12β-hydroxy-5a,25D-spiro-stane (LXV; $R_1=R_2=Ac$) when treated with nitrous acid gave a product formulated as 3β-acetoxy-12-oxo-C-homo-5a,25D-spirostane (LXVIII; R=Ac).
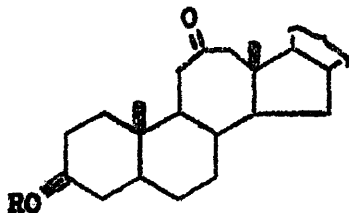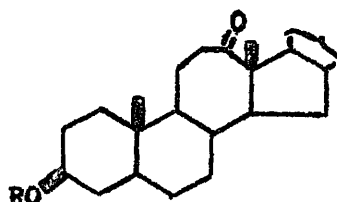
(LXVIII)   (LXIX)

It was also found, however, that a crude reduction product of hecogenin acetate cyanhydrin when reacted with nitrous acid, and the reaction product treated with Girard reagent, gave on acetylation, a ketone, 3β-acetoxy-12a-oxo-C-homo-5a,25D-spirostane (LXIX; R=Ac)

A mechanistic examination is not helpful in distinguishing between the two isomers, since the carbonium ion shown (LXX) seems to be an essential intermediate in any route to the C-homo-steroid. The infrared spectra show that the product from the amide has a peak at 1714 cm^{-1}. while the ketone obtained from the amine absorbs at
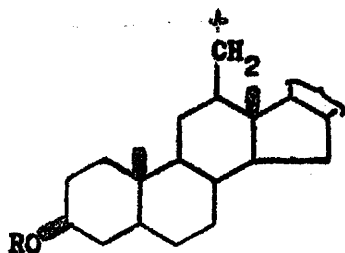
(LXX)

1697 cm^{-1}.. The former absorption is typical of a ketone in a
six or seven membered ring adjacent to two methylene groups. On
the other hand, a 12-ketone in the normal steroid absorbs at about
1700 cm^{-1}.. The lowering of the frequency is probably due to the
neighbouring quaternary carbon atom, c.f. steroid and triterpenoid
3-ketones which absorb at 1709 and 1700 cm^{-1}. respectively.[25] Hence
the C-homo ketone having the lower infrared absorption frequency is
taken to be the 12a-ketone (LXIX), and the product obtained from
the amide is therefore the 12-ketone (LXVIII).

EXPERIMENTAL

General Experimental Techniques.– Unless otherwise stated,
all melting points were done on a Kofler apparatus and are there-
fore corrected values; specific rotations were determined in
chloroform solution in a 10 cm. micro tube; infrared spectra were
obtained from potassium chloride discs; ultraviolet spectra were
done on ethanolic solutions; extracts were dried with anhydrous
sodium sulphate; alumina was deactivated by shaking the slurry in
60–80° petroleum ether with 5 ml. of 10% aqueous acetic acid for
each 100 g. alumina;[19] petroleum ether had b.p. 60–80°.

Hecogenin Acetate Cyanhydrins.– a) Hecogenin acetate (XVII;
R=Ac) (60 g.) in chloroform (480 ml.) and acetic acid (170 ml.) was
cooled to 0° and a suspension of potassium cyanide (200 g.) in
methanol (720 ml.) was added with stirring. The mixture was stirred
for $2\frac{1}{2}$ hours at 0°, treated with water (2 litres) and the mixture
shaken. The chloroform layer was washed with water containing acetic
acid and evaporated under reduced pressure. On trituration with
chloroform the residue obtained (34 g.) was shown to be 3β–acetoxy–
12a–cyano–12β–hydroxy–5a,25D–spirostane (XXIX). Recrystallisation
from chloroform–methanol gave a white powder, m.p. 242–244°,
$[a]_D$ –41.2° (c, 1.07) (Found: C,72.0; H,8.95;N,2.8. Calc. for
$C_{30}H_{45}O_5N$: C,72.1; H,9.1; N,2.8%), v_{max} 3300 (OH), 1700 (OAc), and
1270 cm^{-1}. (OAc). m.p. 265° in a sealed capillary tube under vacuum.
Hirschmann et al.[19] give m.p. 271–275° (decomp.) with previous

sintering.

The mother liquors on evaporation and addition of methanol yielded (a) 6.8 g. m.p. 239-244°, $[\alpha]_D$ -40°. (b) 18 g. m.p. 236-241°, $[\alpha]_D$ -39.1°. (c) 1.5 g. m.p. 225° and 237-242°, $[\alpha]_D$ -43.8°. (d) 0.5 g. m.p. 212-216°, $[\alpha]_D$ -42.6°, as successive crops.

A sample of the cyanhydrin was heated to 260° under vacuum. Very little sublimation took place, and the residue was unchanged cyanhydrin (which had not even melted). On heating to 300° under the same conditions as above, the cyanhydrin melted and sublimation occurred. The sublimate was very pure hecogenin acetate (XVII; R=Ac).

b) Hecogenin acetate (XVII; R=Ac) (20 g.) was dissolved in pyridine (200 ml.) and treated with liquid hydrogen cyanide (100 ml.) and triethylamine (2 ml.). The solution which quickly turned dark was left overnight at room temperature. The reaction mixture was then diluted with 3.5N hydrochloric acid (800 ml.) and extracted with chloroform. Successive washing of the extracts with dilute hydrochloric acid and water, followed by drying and evaporation to lower bulk gave the cyanhydrin (XXIX) (8.2 g.), m.p. 235-239°

The mother liquors yielded a second crop (10.8 g.).

3β-Acetoxy-13β-cyano-17a-methylene-C-nor-D-homo-5α,25D-spirostane (XXXIX).- 3β-Acetoxy-12a-cyano-12β-hydroxy-5α,25D-spirostane (XXIX) (7 g.) was dissolved in pyridine (140 ml.). The solution was cooled to 0° and treated with thionyl chloride (5.6 ml.)

purified by the method described by Vogel.[26] The solution was left

standing overnight at room temperature, then poured on to an excess

of crushed ice. The precipitate was collected and dissolved in

chloroform which was then washed with dilute hydrochloric acid,

water, aqueous sodium bicarbonate, and water again, dried, and

evaporated under reduced pressure. Crystallisation of the residue

(6.9 g.) from methanol gave 3β-acetoxy-13β-cyano-17a-methylene-C-nor

-D-homo-5α,25D-spirostane (XXXIX). Two recrystallisations from

methylene chloride-methanol gave material as needles m.p. 216-220°,

$[α]_D$ -32.7° (c, 0.94) (Found: C,73.6; H,8.8; N,2.8. Calc. for

$C_{30}H_{43}O_4N.\frac{1}{2}CH_3OH$: C,73.6; H,9.1; N,2.8%), $ν_{max}$ 3400 (OH from MeOH),

2230 (-C≡N), 1725 (OAc), 1640 (⊃C=C⊂), and 1255 cm^{-1}, (OAc).

A sample recrystallised twice from acetone-chloroform had

m.p. 216-220°, (Found: C,74.8; H,8.8; N,2.7. Calc. for $C_{30}H_{43}O_4N$:

C,74.8; H,9.0; N,2.9%). Hirschmann et al.[19] give m.p. 220-221.5°

with previous sintering.

An attempt to carry out this rearrangement using phosphorus

oxychloride was unsuccessful, the product being unchanged cyanhydrin.

3β-Acetoxy-Δ^{11}-12β-cyano-5α,25D-spirostene (XL).- A portion

(1 g.) of the material from crop (b) from the mother liquor of

hecogenin acetate cyanhydrin was treated with thionyl chloride

(2.0 ml.) in pyridine (15 ml.) as above. Extraction by similar

methods gave a brown chloroform solution which was boiled with animal charcoal, filtered and on addition of methanol and cooling yielded slightly coloured needles (0.3 g.). These were chromato-graphed on deactivated alumina (20 g.).

1:1 Petroleum ether-benzene eluted material (0.17 g.) which, after two recrystallisations from chloroform-methanol, was shown to be 3β-acetoxy-Δ^{11}-12β-cyano-5α,25D-spirostene (XL), m.p. 195-198°, $[\alpha]_D$ -51.6° (c, 0.97) (Found: C,74.8; H,9.1; N,2.9. $C_{30}H_{43}O_4N$ requires C,74.8; H,9.0; N,2.9%), λ_{max} 216 mμ (ε 12,950), ν_{max} 2220 (-C≡N), 1725 (OAc), 1240 (OAc), and 870 cm^{-1}. ($\supset$C=C$\subset$).

The ultraviolet was similar to that of a similar nitrile described by Bladon, Henbest, Jones, Lovell, and Woods.[22]

3β-Acetoxy-13β-cyano-17a-oxo-C-nor-D-homo-5α,25D-spirostane (XXXVIII; R=Ac).- 3β-Acetoxy-13β-cyano-17a-methylene-C-nor-D-homo-5α,25D-spirostane (XXXIX) (5.015 g.) was dissolved in methylene chloride (1200 ml.) and cooled in a bath of acetone and crushed solid carbon dioxide, to a temperature of -65°. Ozone was passed in until the solution turned blue. The solvent was evaporated on the steam-bath under reduced pressure, the last 50 ml. being removed in the cold under vacuum. The ozonide was dissolved in hot glacial acetic acid and zinc (5 g.) added. The solution was heated on the steam-bath for 1½ hours, filtered, and the zinc washed with acetic

acid. Water (1 litre) was added to the filtrate and the steroid isolated in the usual way. The crude material (4.47 g.) on recrystallisation from chloroform-methanol gave 3β-acetoxy-13β-cyano-17a-oxo-C-nor-D-homo-5α,25D-spirostane (XXXVIII) (1.7 g.) as plates, m.p. 265-267°, $[\alpha]_D$ -16.9° (c, 0.68) (Found: C,70.9; H,8.9; N,2.8. $C_{29}H_{41}O_5N.\frac{1}{2}CH_3OH$ requires C,70.95; H,8.7; N,2.8%), λ_{max} 202 mμ (ε 1070) (in dioxan) λ_{max} 282 mμ (ε 84.5), ν_{max} 2230 (-C≡N), 1760 (OAc), and 1250 cm^{-1}. (OAc).

The ketone did not form a 2,4-dinitrophenylhydrazone.

An attempt to form the ketone obtained above by reaction of the methylene group with osmium tetroxide and sodium metaperiodate[27] was unsuccessful.

Attempted hydrolysis of 3β-acetoxy-13β-cyano-17a-oxo-C-nor-D-homo-5α,25D-spirostane (XXXVIII). Several attempts were made to hydrolyse the cyanoketone (XXXVIII). These are summarised below. In each case the reaction mixture was poured into water, made alkaline if necessary, and extracted to give a neutral product. Acidification and re-extraction then yielded any acid product.

Reagents	Conditions	Wt. of starting material	Product		Notes
			Neutral	Acid	
KOH (5 g.) MeOH (50 ml.) Dioxan (30 ml.)	Reflux for 4 hrs on the steam-bath.	0.6 g.	0.038 g.	0.423 g.	1

Reagents	Conditions	Wt. of starting material	Product Neutral	Acid	Notes
HCl conc.(2 ml.) MeOH (20 ml.) Dioxan (60 ml.)	Reflux for 2 hrs. on steam-bath	0.5 g.	0.513 g.	0.002 g.	2
KOH (5 g.) H_2O_2 (2 ml.) MeOH (300 ml.) C_6H_6 (50 ml.)	Stand for 1 hr. then reflux 1 hr. on steam-bath.	0.5 g.	0.166 g.	0.061 g.	3
KOH (5 g.) H_2O_2 (2 ml.) MeOH (140 ml.) C_6H_6 (25 ml.)	"	0.5 g.	0.062 g.	0.390 g.	
KOH (10 g.) H_2O_2 (4 ml.) MeOH (250 ml.) C_6H_6 (50 ml.)	"	1.0 g.	0.092 g.	0.799 g.	4
KOH (5 g.) H_2O_2 (2 ml.) MeOH (150 ml.) C_6H_6 (25 ml.)	Overnight in cold reflux for 8 hrs. on steam-bath	0.52 g.	0.058 g.	0.399 g.	4
KOH (5 g.) MeOH (100 ml.) H_2O_2 (2.8 ml.) C_6H_6 (25 ml.)	Left several days in cold No heating.	0.64 g.	0.026 g.	0.218 g.	
KOH (5 g.) H_2O_2 (2 ml.) MeOH (70 ml.) C_6H_6 (25 ml.)	Left cold [a] taken continuously over 4 hrs.	0.51 g.	0.173 g.	0.284 g.	5

Reagents	Conditions	Wt. of starting material	Product		Notes
			Neutral	Acid	
KOH (5 g.) H₂O₂ (0.2 ml.) MeOH (70 ml.) C₆H₆ (15 ml.)	Left in cold for 4 hrs. (room temp.)	0.16 g.	0.008 g.	0.146 g.	
KOH (20 g.) (CH₂OH)₂ (200 ml.)	Refluxed until no more ammonia evolved.	0.49 g.	0.123 g.	0.408 g.	

Note 1. The neutral product appears to be a mixture of the required product (XLI) and the amide (LXXII).

(XLI)   (LXXII)
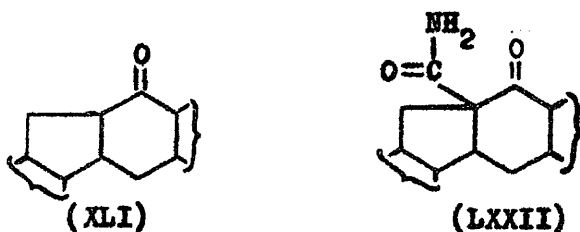
Note 2. The neutral product is 3β-hydroxy-13β-cyano-17a-oxo-C-nor-D-homo-5α,25D-spirostane (XXXVIII; R=H) which recrystallised as needles from chloroform-methanol, m.p. 235-236.5°, $[\alpha]_D$ -28.7° (c, 0.67) (Found: C, 73.3; H, 9.0; N, 3.0. Calc. for $C_{27}H_{39}O_4N$: C, 73.5; H, 8.9; N, 3.2%), ν_{max} 3420 (OH), 2220 (-C≡N), and 1725 cm^{-1} (>C=O). Hirschmann et al.[19] give m.p. 237-238°

Note 3. The low yield is due to the use of ether alone in the extraction pprocess. It was found that ether and chloroform should

be used.

Note 4. The neutral product appeared to be the desired compound 3β-hydroxy-17a-oxo-C-nor-D-homo-5α,25D-spirostane (XLI). It had m.p. 179-184°, $[a]_D$ -89.5°, v_{max} 3400 (OH), and 1700 cm^{-1}. ($>$C=O). c.f. Hirschmann et al.[19] who give m.p. 180-183°, $[a]_D$ -93.8°.

Note 5. The $[a]_D$ did not change during the reaction.

Examination of the acid products.- The acid products all showed the presence of the nitrile band in the infrared spectrum. Thus there seems to have been acid cleavage instead of ketone cleavage.

A portion of the acid was treated with diazomethane[28] but did not give a crystalline derivative.

Attempts to decarboxylate the acid using copper chromite[29,30] as catalyst gave dark brown gums.

3β-Acetoxy-13β,17aβ-dicyano-C-nor-D-homo-5α,25D-spirostan-17aα-ol (XLIV).- (a) 3β-Acetoxy-13β-cyano-17a-oxo-C-nor-D-homo-5α,25D-spirostane (XXXVIII; R=Ac) (0.46 g.) was dissolved in chloroform (10 ml.), a large excess of liquid hydrogen cyanide added (5 ml.), followed by triethylamine (0.2 ml.). The solution was left over-night, poured into 3.5N hydrochloric acid (100 ml.) and the solution extracted with chloroform. The extracts were washed with water, dried and evaporated under reduced pressure in the presence of a little acetic acid to prevent loss of hydrogen cyanide by hydrolysis. This

yielded light brown material (0.49 g.) which after four recrystall-
isations from chloroform-petroleum ether, gave 3β-acetoxy-13β,17aβ-
dicyano-C-nor-D-homo-5a,25D-spirostan-17aa-ol (XLIV), m.p. 268-269.5°,
$[a]_D$ -50° (c, 1.10) (Found: C,70.3; H,8.2; N,5.5. $C_{30}H_{42}O_5N_2$
requires C,70.55; H,8.3; N,5.5%), v_{max} 3200 (OH), 2190 (-C≡N),
1680 (OAc), and 1237 cm^{-1}. (OAc).

(b) 3β-Acetoxy-13β-cyano-17a-oxo-C-nor-D-homo-5a,25D-spirostane
(XXXVIII; R=Ac) (0.52 g.) was dissolved in chloroform (12 ml.) and
methanol (6 ml.) and acetic acid (1.5 ml.) added. The solution was
cooled to 0° and potassium cyanide (1.7 g.) added with stirring.
The mixture was stirred for one hour at 0° and then one hour at
room temperature. Water (100 ml.) was added and the solution
extracted with chloroform. The extracts were washed with water,
dried, and evaporated under reduced pressure to give material (0.51 g.)
m.p. 265-269.5°. The infrared spectrum was very similar to that of
material obtained by method (a), but the optical rotation showed that
it was a mixture of starting material and cyanhydrin. Because of this,
method (a) was preferred.

The melting point of the cyanhydrin is the same as that of the
starting material. This is due to the evolution of hydrogen cyanide
on heating, to give the cyanoketone. Since considerable sublimation
takes place above 200°, evolution of hydrogen cyanide occurs

without any other noticeable change in crystal structure. A sample of the cyanhydrin in a tube was placed in a heated metal block (235°) for 45 sec.. A piece of benzidine-copper acetate test paper held at the mouth of the tube turned blue showing the presence of hydrogen cyanide. The infrared spectrum of the residue was identical with that of the cyanoketone.

3β-Acetoxy-17a-methylene-C-nor-D-homo-5α,25D-spirostane[20] (XXV).- Hecogenin acetate (XVII; R=Ac) (20 g.) was dissolved in methylene chloride (60 ml.) and ethanol (300 ml.). To the stirred, cloudy, solution was added sodium borohydride (1.2 g.) in water (5 ml.). The solution was stirred until it went clear (2 hours) and left ½ hour longer. Water (500 ml.) was added and the solution acidified with hydrochloric acid. Extraction with chloroform gave the crude rockogenin-3-monoacetate (XX; R_1=Ac, R_2=H) which contained some of the 12α-epimer. One recrystallisation from chloroform-methanol gave reasonably pure material (11.22 g.), m.p. 215-220°. This product was dissolved in pyridine (30 ml.), cooled to 0°, and treated with methane sulphonyl chloride (10 ml.) overnight at room temperature. The reaction mixture was poured on to ice and allowed to stand for 30 min.. Extraction with ether and work up by washing with acid several times, bicarbonate, and water, gave a gum, which was dissolved in tert.-butanol (150 ml.) containing potassium (3.2 g.) and

refluxed for 6 hours.

The product, isolated by addition of water and extraction was acetylated by heating on the steam-bath for one hour with acetic anhydride in pyridine. The crystalline product of the acetylation (2.24 g.) after one recrystallisation from chloroform-methanol was 3β-acetoxy-17a-methylene-C-nor-D-homo-5α,25D-spirostane (XXV) (1.87 g.) m.p. 213.5-220°, c.f. Elks et al.[20] who give m.p. 218-223°.

<u>3β-Acetoxy-17a-oxo-C-nor-D-homo-5α,25D-spirostane (XLI)</u>.- The 17a-methylene compound (XXV) obtained above (1.87 g.) in dry benzene (50 ml.) containing pyridine (1.2 ml.) was treated with osmium tetroxide (1.23 g.) and left at room temperature for 6 days. (c.f. Hirschmann et al.[19])

The solution was diluted with benzene and the pyridine removed by acid washing. Some steroid which precipitated out as the osmate ester was filtered off. The filtrate was washed with more acid, followed by water, then evaporated to give a black residue to which the filtered product was added. This residue was dissolved in ethanol (400 ml.) and sodium thiosulphate (4.4 g.) in water (100 ml.) added. After refluxing 1½ hours, the reaction mixture, which contained a black precipitate, was treated with sodium hydroxide (1.0 g.)in water (3 ml.) and refluxed a further 1½ hours.

The triol, obtained by chloroform extraction (1.34 g.) m.p. 191-

200°, was allowed to stand overnight with periodic acid (0.7 g.) in ethanol (200 ml.). The solvent was removed _in vacuo_ without heating and the residue, in ether, washed with sodium bicarbonate, water, and saturated sodium chloride solution. Evaporation gave the required product (XLI) (1.24 g.) m.p. $225-230^\circ$ after one recrystallisation from chloroform-methanol. c.f. Hirschmann et al.[19] who give m.p. 227 -232°.

Note: Ozonolysis of the 17a-methylene compound (XXV) (0.7 g.) gave very little of the required product (40 mg.).

The steroid (XXV) (0.7 g.) was dissolved in methylene chloride (40 ml.) which was then cooled to -68° and ozone passed in until the solution became deep blue in colour. The solution was allowed to come to room temperature and again cooled to low temperature. When the solution again became blue, the ozone flow was stopped and the solvent evaporated under reduced pressure. Zinc (1.0 g.) was added to the ozonide in acetic acid (20 ml.) which was then heated on the steam-bath for one hour. Isolation of the product by filtration, evaporation, and chromatography gave material (40 mg.) m.p. 220- 227°.

Cyanhydrin of 3β-Acetoxy-17a-oxo-C-nor-D-homo-5a,25D-spiro-stane.-

The 17a-ketone (XLI) (0.92 g.) was dissolved in chloroform (8 ml.) and acetic acid (3 ml.). To this solution (cooled to 0°) was

added potassium cyanide (3 g.) as a slurry in methanol (12 ml.).
The reaction was allowed to come to room temperature, and stirred for
4 hours. Water (150 ml.) was added and the solution extracted with
chloroform. The extracts were washed with water containing a little
acetic acid, dried, and evaporated. The infrared spectrum of the
crude product showed that some cyanhydrin had been formed. However,
three recrystallisations from chloroform-methanol gave starting
material m.p. 228-231° (sublimation in needles).

The whole product was treated once again with potassium cyanide
as above, a larger amount of methanol being used to give complete
solution of the cyanide. The reaction was not cooled and was kept
at room temperature for $7\frac{1}{2}$ hours. The product was isolated as before
but was still a mixture of the cyanhydrin and starting material,
m.p. 198-204° with recrystallisation and remelt m.p. 222-230°,
v_{max} 3378 (OH), 1727 (OAc), 1709 ($>$C=O), 1259 and 1235 cm^{-1}, (OAc).

Treatment of the Crude Cyanhydrin with Thionyl Chloride in
Pyridine.- The cyanhydrin (XLVIII) obtained above (0.87 g.) was
dissolved in pyridine (15 ml.) and treated carefully with thionyl
chloride (0.5 ml.), the solution being kept below 5°. The reaction
mixture was allowed to stand overnight. Excess of thionyl chloride
was destroyed by addition of water (200 ml.) and the solution
extracted into chloroform. The extracts were washed with dilute

hydrochloric acid and water, dried, and evaporated under reduced
pressure. The product obtained after two recrystallisations from
chloroform-methanol was shown to be the 17a-ketone (XLI), m.p. 220-
228°, v_{max} 1724 (OAc), 1704 ($>$C=O), and 1236 cm^{-1} (OAc).

Examination of the mother liquors yielded a second material,
which, after three recrystallisations from chloroform-methanol was
shown to be 3β-acetoxy-17a-cyano-5a,25D-spirost-13(17a)-ene (XLIX),
m.p. 178-182°,$[a]_D$ -91.1° (c, 0.43) (Found: C,74.3; H,8.7; N,3.0.
$C_{29}H_{43}O_4$ N requires C,74.2; H,9.2; N,3.0%) λ_{max} 229 mμ (ε 10,100),
v_{max} 2237 (-C≡N), 1736 (OAc), 1650 ($>$C=C$<$), 1252 (OAc), 1058, 981,
and 896 cm^{-1}. (spirostane side-chain).

Reaction of 3β-Acetoxy-13β-cyano-17a-methylene-C-nor-D-homo-
5a,25D-spirostane (XXXIX) with Lithium Aluminium Hydride.- Lithium
aluminium hydride (0.74 g.) was refluxed with tetrahydrofuran (65 ml.)
and the steroid (XXXIX) (0.52 g.) added dropwise to the stirred
solution. The funnel was washed down with more tetrahydrofuran and
the mixture refluxed for 6 hours. The reaction mixture was then cool-
ed, excess reagent destroyed with water, and the alumina filtered
off. The filter pad was shaken up with some tetrahydrofuran and
filtered again. On taking the combined filtrates to dryness, a gum
was obtained which, on trituration with ether gave material m.p. 183-
191°, shown to be 3β-hydroxy-13β-aminomethyl-17a-methylene-C-nor-D-

homo-5α,25D-spirostane (LI; R=H). Three recrystallisations from ether gave an analytical sample, m.p. 193-197°, $[\alpha]_D$ -48.4° ($\underline{c}$, 0.31) (Found: C,75.8; H,9.8; N,3.3. $C_{28}H_{43}O_3N$ requires C,75.5; H,10.1; N,3.3%), λ_{max} 209 (ε 1940) and 276 mμ (ε 2030), ν_{max} 3344, 3300, 3205, 3106 (NH and OH), 1637 ($\supset$C=C$\subset$), 1613 (amine), 1057, 982, 890 (spirostane side-chain), and 898 cm^{-1}, ($\supset$C=C$\subset$). Positive tetra-nitromethane test.

Acetylation of this material gave an amorphous product (LI; R=Ac), λ_{max} 206 (ε 5400) and 274 mμ (ε 1440), ν_{max} 3311 (amide NH), 3049 (=CH$_2$), 1730 (OAc), 1656 (amide, band I), 1637 ($\supset$C=C$\subset$), 1534 (amide, band II),[31] and 1239 cm^{-1}. (OAc).

The mother liquors (0.25 g.) of the above reaction were chrom-atographed on deactivated alumina (20 g.).

Benzene eluted material shown to be 3β-hydroxy-13β-methyl-17a-methylene- C-nor-D-homo-5α,25D-spirostane (LII; R=H), m.p. 107.5-116°, $[\alpha]_D$ -57.2° ($\underline{c}$, 0.94) (Found: C,76.7; H,10.3. $C_{28}H_{44}O_3$, $\frac{1}{2}$CH$_3$OH requires C,77.0; H,10.4%), λ_{max} 211 mμ (ε 7050), ν_{max} 3390 (OH), 1639 ($\supset$C=C$\subset$), 1055 (OH), 1041, 979, and 899 cm^{-1}. (spirostane side-chain). The compound also gave a positive tetranitromethane test.

Acetylation gave the 3-acetate (LII; R=Ac), m.p.138-142°, $[\alpha]_D$ -58.8° ($\underline{c}$, 1.02) (Found: C,76.2; H,9.9. $C_{30}H_{46}O_4$ requires

C,76.6; H,9.85%), λ_{max} 210 mμ (ϵ 9550), ν_{max} 1736 (OAc), 1653

($\supset$C=C$\subset$), 1028, 978, and 896 cm^{-1}. (spirostane side chain).

Reaction of 3β-Hydroxy-13β-aminomethyl-17a-methylene-C-nor-D-homo-5α,25D-spirostane (LI; R=H) with Nitrous Acid.- The steroid

(LI; R=H) (0.39 g.) was dissolved in acetic acid (60 ml.), cooled to

0°, and the stirred solution treated with sodium nitrite (3.0 g.) in

water (10 ml.). The reaction mixture was kept overnight at 2° and

the product obtained by addition of water and extraction into ether.

The ethereal extracts were washed with water (3 times), bicarbonate

(3 times), and water again, dried, and evaporated. Three recrystall-

isations from methanol-isopropyl ether gave 3β-hydroxy-13β-hydroxy-

methyl-17a-methylene-C-nor-D-homo-5α,25D-spirostane (LIII), m.p. 248-

252°, $[\alpha]_D$ -59.4^{o} (c, 1.04) (Found: C,75.1; H,9.95. $C_{28}H_{44}O_4$

requires C,75.6; H,10.0%), λ_{max} 205 mμ (ϵ 1680), ν_{max} 3333 (OH),

1634 ($\supset$C=C$\subset$), 1057, 980, and 898 cm^{-1}. (spirostane side chain).

Since only 200 mg. of crystalline product were obtained, the

mother liquors were chromatographed on deactivated alumina (20 g.).

Benzene eluted material which was amorphous, $[\alpha]_D$ -51.7^{o}

(c, 0.92), λ_{max} 204 mμ ($E_{1cm.}^{1\%}$ 173), ν_{max} (CCl$_4$) 1736, 1631 ($\supset$C=C$\subset$),

1538, 1058, 979, 899 (spirostane side-chain), 800, and 712 cm^{-1}..

The substance gives a positive tetranitromethane test. A nitrogen

analysis was carried out on the amorphous material (Found: N,3.1%),

which showed that only one nitrogen atom is present.

Hydrolysis of a sample with hydrochloric acid (0.3 ml.) in methanol gave a similar product which did not crystallise, λ_{max} 210 ($E_{1cm.}^{1\%}$ 129) and 218 mμ ($E_{1cm.}^{1\%}$ 128), ν_{max} 3448 (OH), 1712, 1634 ($\supset$C=C$\subset$), 1567, 1541, and 1497 cm^{-1}..

Acetylation gave another amorphous product, thought to be the 3-acetate, $[\alpha]_D$ -52.3° ($\underline{c}$, 1.03), λ_{max} 203 mμ ($E_{1cm.}^{1\%}$ 81.5), ν_{max} (CCl$_4$) 1739 (OAc), 1631 ($\supset$C=C$\subset$), 1534, 1238 (OAc), 1057, 979, 899 (spirostane side-chain), 798, and 711 cm^{-1}..

Oxidation of the original reduction product with chromic acid gave an amorphous product thought to be the 3-ketone $[\alpha]_D$ -53.7° ($\underline{c}$, 0.86), λ_{max} 203 mμ ($E_{1cm.}^{1\%}$ 107), ν_{max} (CCl$_4$) 1739, 1718 ($\supset$C=O), 1626 ($\supset$C=C$\subset$), 1550, 1058, 979, 899 (spirostane side chain) and 710 cm^{-1}..

Oxidation of 3β-Hydroxy-13β-hydroxymethyl-17a-methylene-C-nor-D-homo-5α,25D-spirostane (LIII).— The steroid (LIII) (0.55 g.) in acetone (50 ml.) was treated dropwise with 8N chromic acid (1.8 ml.) for 10 min. at room temperature. Excess reagent was destroyed with sodium sulphite and dilute hydrochloric acid. Water (50 ml.) was added and the solution extracted. The product obtained in the usual manner, was shown to be 17a-methylene-3-oxo-C-nor-D-homo-5α,25D-spirostane-13β-carboxylic acid (LVI). Three recrystallisations

from methanol gave material, m.p. 198-201°, $[\alpha]_D$ -24.8° (c, 0.23) (Found: C,70.2; H,8.7. $C_{28}H_{40}O_5 \cdot H_2O$ requires C,70.8; H,8.9%), ν_{max} 3401 (OH of acid), 1721 ($\supset$C=O), 1650 ($\supset$C=C$\subset$), 1064, 979, 877 (spirostane side-chain) and 870 cm^{-1}. ($\supset$C=C$\subset$).

Ozonolysis of 17a-Methylene-3-oxo-C-nor-D-homo-5a,25D-spirostane -13β-carboxylic acid (LVI).- The steroid (LVI) (110 mg.) was dissolved in methylene chloride (25 ml.) and cooled to -70°. Ozone was passed in until the solution turned blue, indicating the presence of excess reagent. The solvent was removed in vacuo without heating. The ozonide was dissolved in acetic acid (20 ml.) and zinc (0.5 g.) added. After one hour at 100 ° the reaction mixture was filtered, and worked up to give a brown gum (140 mg.) which did not crystallise, λ_{max} 209 mμ (ε 3620), ν_{max} 3436 (acid), 1681, 1603 ($\supset$C=C$\subset$), 1055, 977, and 895 cm^{-1}. (spirostane side-chain).

Attempted Hydrogenation of Hecogenin Acetate Cyanhydrin (XXIX) using Platinum Oxide as Catalyst.- Hecogenin acetate cyanhydrin (XXIX) (0.47 g.) was dissolved in acetic acid (10 ml.) and added to pre-reduced platinum oxide in the same solvent (10 ml.). After 4 hours at room temperature, water was added and the solution extracted with ether. The ethereal extracts were washed with dilute hydrochloric acid to remove any basic material. Treatment of the acid washings with base and re-extraction gave no basic product.

The original extracts were washed with water, dried and evaporated to give a solid m.p. 230-245°, shown by its infrared spectrum to be starting material.

Note: The same result was obtained in other hydrogenations using Raney nickel and palladium-charcoal as catalysts.

Attempted Reduction of Hecogenin Acetate Cyanhydrin (XXIX) with Sodium Borohydride.- The steroid (XXIX) (0.514 g.) in ethanol (130 ml.) and ether (30 ml.) was treated with sodium borohydride (0.214 g.) in ethanol (5 ml.) overnight at room temperature. Water (100 ml.) was then added and the product, isolated by acidification and ether extraction, was shown by its infrared spectrum to be a mixture of hecogenin acetate (XVII; R=Ac) and rockogenin-3-mono-acetate (XX; R_1=Ac, R_2=H).

Note: A similar experiment using sodium borohydride and aluminium chloride gave a mixture of hecogenin acetate and unchanged cyanhydrin.

Lithium Aluminium Hydride Reduction of 3β-Acetoxy-12α-cyano-12β-hydroxy-5α,25D-spirostane (Hecogenin Acetate Cyanhydrin) (XXIX).-The steroid (XXIX) (0.504 g.) was dissolved in tetrahydrofuran (60 ml.) and lithium aluminium hydride (0.152 g.) added as a slurry with 10 ml. of solvent. The solution was refluxed for 6 hours, allowed to cool, and water was added carefully until no more reaction occured, then 10% more water was added and the solution

filtered. The alumina was washed with a little tetrahydrofuran and brought to dryness under reduced pressure to give amorphous material (0.431 g.), m.p. below 100°.

The product was dissolved in ether which was then washed with 3.5N hydrochloric acid several times. This should remove basic material from the ether solution. The acid solution was basified with ammonia and extracted with ether to give material (0.049 g.). The original ether solution was washed well with water, dried and evaporated under reduced pressure to give material (0.294 g.) which had an infrared spectrum similar to the material obtained from the acid extract.

Acetylation of the Crude Reduction Product.- The reduction product (105 mg.) was dissolved in pyridine (2.0 ml.) and acetic anhydride (1.0 ml.) added. The solution was left overnight, and worked up in the usual manner to give a gum (99 mg.). This was crystallised from methanol-water, but the solid formed was still partially amorphous, m.p. 130-180°.

Treatment of the Reduction Product with Methanolic Potassium Hydroxide.- The reduction product (106 mg.) was dissolved in 10% methanolic potassium hydroxide (10 ml.) and the solution refluxed for 3 hours. The steroid product was precipitated with water and the solution filtered to give material, m.p. 196-207°, with

previous partial melting. An infrared spectrum showed great simil-arity with starting material. This means that the reduction product is stable to hydrolysis and is not a simple derivative of hecogenin.

Treatment of the Reduction Product with Nitrous Acid.— The reduction product (106 mg.) was dissolved in glacial acetic acid (15 ml.) and water (15 ml.) added. The solution was cooled to 0° and treated with sodium nitrite (73 mg.). After being kept at 0° for one hour, the solution was allowed to stand at room temperature for one hour. This was followed by gentle heating on the steam-bath for 5 min.. Extraction and washing in the usual manner gave a froth (86 mg.) which showed slight carbonyl and double bond absorption in its infrared spectrum.

The product obtained above was retreated with nitrous acid, left for 5 hours at 0°, and then overnight at room temperature. Extraction as before gave material (74 mg.) which had increased absorption in the carbonyl region of its infrared spectrum. This gave an indication that the required reaction to a C-homo steroid may be taking place, but obviously not to a very great extent. It was decided to determine the exact nature of the reduction product.

Reduction of Hecogenin Acetate Cyanhydrin (XXIX) followed by Acetylation and Chromatography of the Product.— Reduction of hecogenin acetate cyanhydrin (1.0 g.) was carried out as above, to

give a gum (0.94 g.) which was acetylated with acetic anhydride (8 ml.) in pyridine (4 ml.). This gave a froth which was chromatographed on deactivated alumina (40 g.).

1:1 Petroleum ether-benzene eluted 3β,12α-diacetoxy-5α,25D-spirostane (epi-rockogenin acetate) (LXIV; $R_1=R_2=Ac$), m.p. 150-153°, $[α]_D$ -25.3° (c, 1.19), $ν_{max}$ 1755 (OAc), 1243 (OAc), 1060, 982, and 905 cm^{-1}. (spirostane side-chain). c.f. Elks et al.[20] who give m.p. 156-159°, $[α]_D$ -17°.

1:2 Petroleum ether-benzene and benzene alone eluted 3β,12β-diacetoxy-5α,25D-spirostane (rockogenin acetate) (XX; $R_1=R_2=Ac$), m.p. 208-211°, $[α]_D$ -77.6° (c, 0.90), $ν_{max}$ 1735 (OAc), 1248 (OAc), 1060, 990, and 909 cm^{-1}. (spirostane side-chain). c.f. Elks et al.[20] who give m.p. 202-206.5°, $[α]_D$ -65.1°.

1:1 Benzene-ether eluted material m.p. 245-246°, $[α]_D$ -39.9° (c, 0.92) (Found: C,68.6; H,9.5; N,2.5. $C_{32}H_{51}O_5N.CH_3OH$ requires C,68.5; H,9.6; N,2.6%), $ν_{max}$ 3280 (OH), 1735 (OAc), 1650 (amide, band I), 1545 (amide, band II)[31] 1248 (OAc), 1060, 989, and 908 cm^{-1}. (spirostane side-chain). This would appear to be the required N-acetyl-3β-acetoxy-12α-aminomethyl-12β-hydroxy-5α,25D-spirostane (LXV; $R_1=R_2=Ac$).

99:1 Ether-methanol eluted material similar to the above, m.p. 247.5-249°, $[α]_D$ -35.9° (c,0.75) (Found: C,70.4; H,9.5; N,2.5.

$C_{32}H_{51}O_5N$ requires C,70.4; H,9.4; N,2.6%), ν_{max} 3330 (OH), 1725 (OAc), 1650 (amide, band I), 1540 (amide, band II)[31] 1248 (OAc), 1060, 989, and 909 cm^{-1}. (spirostane side-chain).

In this chromatogram, the quantity of acetylated amine obtained was only about 25% of the material put on the column. The experiment was repeated several times under varying conditions and methods of extraction. In each case, with one exception, the cyanhydrin was reduced with lithium aluminium hydride and the product acetylated before chromatography. The results are summarised below:-

Conditions	Material on column (g)	Recovery (%)	Acetylated amine (%)	Note.
1.Straight addition of LiAlH$_4$ to material and 5 hr. reflux in tetrahydrofuran	0.939	88.1	24.8	a
2.As above, 6 hr. reflux	0.621	Complete	38.6	b
3.As above.	0.880	Complete	29.5	c,d
4.As above.	5.50	94.8	21.9	c
5.Straight addition of LiAlH$_4$ and left overnight in cold tetrahydrofuran.	1.194	94.5	29.1	c
6.Dropwise inverse addition of steroid in ether (stir), and 2 hr. reflux	1.151	87.4	34.1	c
7.As above using T.H.F.	10.3	90.3	36.8	
8.Soxhlet extraction of steroid into T.H.F.	2.25	92.9	24.9	c
9.As above	1.54	Complete	26.0	

Notes: (a) The starting material came from the mother liquors of a hecogenin acetate cyanhydrin preparation.

(b) The starting material was obtained by two recrystallis-ations of a sample of reasonably pure hecogenin acetate cyanhydrin.

(c) The starting material was from the same preparation as (b) without any recrystallisation.

(d) The reduction product was not acetylated before chromatography. (see text)

From these results it would appear that the cyanhydrin samples contained impurities since purification led to an improved yield (see No.2). Also, slow addition of the steroid to the reducing agent, as in Nos. 6 and 7 gave improved yields.

Chromatogram of Reduction Product before Acetylation.-

1:1 Benzene-ether eluted $3\beta,12\beta$-dihydroxy-5α,25D-spirostane (rockogenin) (XX; $R_1=R_2=H$), m.p. 210-212°, $[\alpha]_D$ -65.6^o. c.f. Elks et al.[20] who give m.p. 210-213°, $[\alpha]_D$ -63.7^o.

3:2 Ether-benzene and ether alone eluted $3\beta,12\alpha$-dihydroxy-5α,25D-spirostane (LXIV; $R_1=R_2=H$), m.p. 219-222.5^o, $[\alpha]_D$ -40.0^o. c.f. Elks et al.[20] who give m.p. 216-220°, $[\alpha]_D$ -34.4^o.

19:1 Ether-methanol eluted material which was difficult to crystallise and was obtained as a froth initially from aqueous

methanol. Crystallisation from chloroform-ether gave material

m.p.196-200°, $[\alpha]_D$ -33.6° (c, 0.21) (Found: C,69.2; H,9.7; N,2.5. $C_{28}H_{48}O_4N.H_2O$ requires: C,70.0; H,10.5; N,2.9%), ν_{max} 3340 (OH and NH), 1662, 1058, 984, and 902 cm^{-1}. (spirostane side-chain). This material is therefore 3β,12β-dihydroxy-12α-aminomethyl-5α,25D-spirostane (LXV; $R_1=R_2=H$).

19:1 Chloroform-acetic acid eluted a small amount of material which had properties similar to the above amine. Acetylation gave material which had an infrared spectrum identical with that of the amide (LXV; $R_1=R_2=Ac$).

Treatment of N-Acetyl-3β-acetoxy-12α-aminomethyl-12β-hydroxy-5α,25D-spirostane (LXV; $R_1=R_2=Ac$) with methanolic potassium hydroxide.- N-Acetyl-3β-acetoxy-12α-aminomethyl-12β-hydroxy-5α,25D-spirostane (LXV; $R_1=R_2=Ac$) (0.139 g.) was dissolved in methanol (10 ml.) containing potassium hydroxide (0.5 g.). The solution was refluxed for 2 hours, cooled, and left overnight. Addition of water, filtration, and washing gave material (0.10 g.) m.p. 285-289°. Two recrystallisations from methanol-ether gave material, m.p. 291-292.5°,$[\alpha]_D$ -56.2° (c,0.58) (Found: C,69.4; H,9.9; N,2.5. $C_{30}H_{49}O_5N.CH_3OH$ requires C,69.3; H,10.1; N,2.6%), ν_{max} 3450, 3355 (OH), 1640 (amide, band I), 1549 (amide, band II),[31] 1242 (N-OAc), 1057, 982, and 900 cm^{-1}. (spirostane side-chain), which is therefore

N-acetyl-3β,12β-dihydroxy-12α-aminomethyl-5α,25D-spirostane (LXV;
R₁=H, R₂=Ac).

Treatment of N-Acetyl-3β-acetoxy-12α-aminomethyl-12β-hydroxy-
5α,25D-spirostane (LXV; R₁=R₂=Ac) with Hydrochloric Acid.- N-Acetyl-
3β-acetoxy-12α-aminomethyl-12β-hydroxy-5α,25D-spirostane (LXV; R₁=R₂
=Ac) (0.112 g.) was dissolved in dioxan (7 ml.) and concentrated
hydrochloric acid (1.0 ml.) added dropwise. The mixture was refluxed
for 2 hours, water (12 ml.) added, and the solution left overnight.
A crystalline solid was formed, m.p. 227-233°, ν_{max} 3372 (OH), 1680
(amide, band I), 1500 (amide, band II), 1240 (N-OAc), 1052, 983,
and 899 cm⁻¹, (spirostane side-chain). This shows that the 3β-acetate
has been removed, but the amide link, although altered, appears to be
still present.

The total solution was diluted with water (50 ml.) and extract-
ed into ether. The material obtained on evaporation was treated with
methanol (7 ml.) and concentrated hydrochloric acid (1.0 ml.) and
refluxed as before. Addition of water and extraction into ether gave
gummy material (25 mg.) which was shown to contain a quantity of
starting material.

About 70 mg. of material were unaccounted for, and must there-
fore remain in the extracted solution. This solution, when reduced
in bulk, precipitated crystalline material, m.p. 223-229°, (mixed

m.p. with starting material caused depression), ν_{max} 3330 (OH), 1678 (amide, band I), 1499 (amide, band II), 1241 (N-OAc), 1056, 984, and 901 cm^{-1}. (spirostane side-chain). This product has a similar infrared spectrum to that of the free amine (LXV; $R_1=R_2=H$) except for a broadening of the OH band and the appearance of the band at 1499 cm^{-1}. and hence may be the hydrochloride of the amine (LXV; $R_1=H$, $R_2=H.HCl$).

A sample of this material (4 mg.) was dissolved in acetic acid and sodium nitrite added. This solution was left overnight and diluted with water. Extraction with chloroform gave material which had a peak in the infrared spectrum at 1735 cm^{-1}., thus showing ketonic character. This may be the required C-homo ketone (LXVI; R=H). (see below)

Treatment of N-Acetyl-3β-acetoxy-12a-aminomethyl-12β-hydroxy-5a,25D-spirostane (LXV; $R_1=R_2=Ac$) with Nitrous Acid.- N-Acetyl-3β-acetoxy-12a-aminomethyl-12β-hydroxy-5a,25D-spirostane (LXV; $R_1=R_2=Ac$) (1.052 g.) was dissolved in glacial acetic acid (100 ml.) and sodium nitrite (7.5 g.) dissolved in water (15 ml.) added slowly. The solution was left overnight at room temperature, heated on the steam-bath for 2 min. and extracted into ether. The extracts were worked up in the usual manner to give material (0.935 g.) which was chromatographed on neutral alumina.

4:1 Benzene-ether eluted material (0.094 g.) which, after two recrystallisations from methylene chloride-methanol gave 3β-acetoxy-12-oxo-C-homo-5α,25D-spirostane (LXVII; R=Ac), m.p. 244-247.5°, $[a]_D$ -55.5° (c, 0.47) (Found: C,72.6; H,10.2. $C_{30}H_{46}O_5 \cdot \frac{1}{2}H_2O$ requires C,72.7; H,9.6%) v_{max} 1733 (OAc), 1714 ($>$C=O), 1240 (OAc), 1045, 975, and 892 cm^{-1}. (spirostane side-chain). The melting point was depressed with hecogenin acetate.

Reduction of Hecogenin Acetate Cyanhydrin and Treatment of the Product with Nitrous Acid.- The steroid (XXIX) (7.0 g.) was dissolved in tetrahydrofuran (230 ml.) and lithium aluminium hydride (3.56 g.) added as a slurry with some of the solvent. The solution was refluxed for 5 hours, and worked up as before to give a gummy product (5.675 g.) which was dissolved in glacial acetic acid (100 ml.) and water (10 ml.) added. This solution was cooled and sodium nitrite (38 g.) in water (80 ml.) added slowly. After 24 hours at room temperature the solution was heated on the steam-bath for 10 min.. This was followed by addition of water (800 ml.) and extraction into ether to give 5.67 g. material. Acetylation of this with acetic anhydride (44 ml.) in pyridine (22 ml.) gave 5.69 g. material.

Girard Separation of Ketonic Product.- The acetylated product obtained above was dissolved in absolute ethanol (150 ml.) containing glacial acetic acid (15 ml.). Girard T reagent (7.5 g.) was added,

and the mixture heated on the steam-bath for 30 min., during which time, a homogeneous solution was formed. The solution was poured on to a mixture of ice (250 g.) and sodium bicarbonate (25 g.) and the whole diluted to 800 ml.. Extraction into ether gave 3.65 g. of material, m.p. 190-218°. This is the non-ketonic fraction.

The water residue (plus the washings of the ethereal extracts) was made acid with hydrochloric acid and heated on the steam-bath for several hours before being left overnight. This solution was extracted with ether to give the ketonic fraction (0.397 g.). After four recrystallisations from methylene chloride-methanol was obtained material, m.p. 215-222°, $[\alpha]_D$ +4.5° ($\underline{c}$, 0.33) (Found: C,74.1; H,9.8. $C_{30}H_{46}O_5$ requires C,74.0; H,9.5%), ν_{max} 1736 (OAc), 1697 ($\supset$C=O), 1241 (OAc), 1059, 984, and 900 cm^{-1}. (spirostane side-chain). This is believed to be 3β-acetoxy-12a-oxo-C-homo-5α,25D-spirostane (LXIX; R=Ac), i.e. the isomer of the C-homo compound previously isolated.

<u>Acetylation of Hecogenin Acetate Cyanhydrin (XXIX)</u>.- Hecogenin acetate cyanhydrin (XXIX) (2.0 g.) in pyridine (4 ml.) was treated with acetic anhydride (8 ml.). The solution was heated at 100° for 6 hours and worked up in the usual manner to give a crude product m.p. 222-288°.

Chromatography on deactivated alumina (80 g.) with 4:1 benzene-

petroleum ether gave 3β,12β=diacetoxy-12α-cyano-5α,25D-spirostane

(LXVII; R=Ac) (0.9 g.). Three recrystallisations from chloroform-

methanol gave the analytical sample, m.p. 299-301°, $[\alpha]_D$ -40.2°

(c, 0.60) (Found: C,70.5; H,8.7; N,2.7. $C_{32}H_{47}O_6N$ requires C,70.9;

H,8.75; N,2.6%), ν_{max} 1767 (OAc), 1730 (OAc), 1238 (OAc), 1224 (OAc),

1049, 979, and 900 cm^{-1}. (spirostane side-chain).

7:3 Benzene-ether eluted material shown to be hecogenin acetate,

m.p. 237-248°.

An acetylation using the method of Roberts and Gorham[24] gave

mainly unchanged cyanhydrin. The steroid (2.0 g.) in chloroform

(22.4 ml.) was treated with acetyl chloride (20.5 ml.) and dimethyl-

aniline((26.5 ml.). After refluxing the solution for 8 hours, the

product was isolated by addition of water and extraction in the usual

manner. This was shown to be starting material m.p. 220-242° and

identical infrared spectrum.

Treatment of 3β,12β-Diacetoxy-12α-cyano-5α,25D-spirostane with

Methanolic Potassium Hydroxide.- The steroid (LXVII; R=Ac) (0.12 g.)

was treated with potassium hydroxide (1.0 g.) in methanol (12 ml.)

and the solution refluxed for 3 hours. Cooling the reaction mixture

gave a crystalline product which was filtered off and shown to be

hecogenin (XVII; R=H), m.p. 267-268°. Addition of water to the mother

liquors followed by extraction gave a second crop m.p. 261-263°.

<u>Reaction of 3β,12β-Diacetoxy-12α-cyano-5α,25D-spirostane (LXVII;</u>

<u>R≡Ac) with Lithium Aluminium Hydride.</u>— The steroid (LXVII; R≡Ac)

(0.265 g.) in ether (50 ml.) was added dropwise to a stirred solution

of lithium aluminium hydride (0.5 g.) in the same solvent (100 ml.).

The mixture was allowed to stand overnight at room temperature and

worked up by the method of Nace and Smith.[32] A 20% solution of

sodium hydroxide (100 ml.) was added and the solution extracted with

ether. The ethereal extracts were washed with water, dilute hydro-

chloric acid, potassium bicarbonate, and water, dried, and evaporated.

Acetylation of the product with acetic anhydride in pyridine

gave material (0.12 g.) which was chromatographed on deactivated

alumina (10 g.). The poor recovery of amide (17%) was undoubtedly

due to the loss of material in the isolation process.

<u>Treatment of Hecogenin Acetate with Diazomethane.</u>[12]— Hecogenin

acetate (XVII; R≡Ac) (1.0 g.) was dissolved in a mixture of ether

(60 ml.) and methanol (100 ml.). Potassium hydroxide (1.2 g.) was

added and when this had dissolved, the solution was cooled to 0°.

Nitrosomethylurea (1.2 g.) was added to the stirred solution and

the reaction mixture kept at room temperature for 5 hours. The solution

was then treated with dilute hydrochloric acid (20 ml.), filtered,

and the residue washed with ether and chloroform. The filtrate was

diluted with water and extracted to give a crude product which was

chromatographed on deactivated alumina (40 g.).

19:1 Benzene-ether eluted material shown to be tigogenin, (present as an impurity in the starting material), m.p. 196-205°.

3:1 Benzene-ether and subsequent mixtures eluted hecogenin, m.p. 258-261°, mixed m.p. showed no depression.

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