T H E S I S

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STUDIES ON HECOGENIN

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

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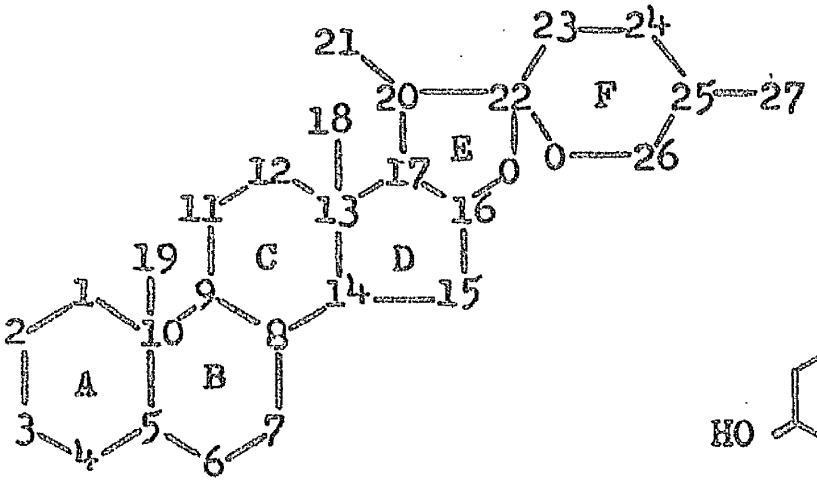
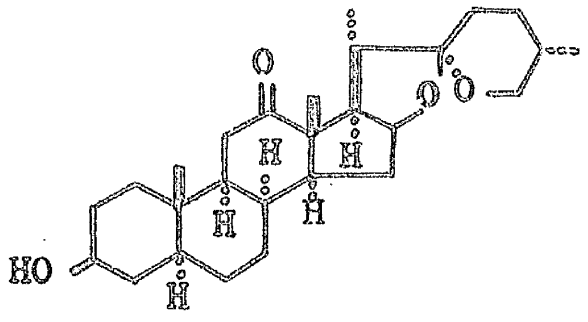
INTRODUCTION

Hecogenin (II) is a steroid sapogenin which was isolated by Marker and his co-workers[1a] in 1943 from Hechtia texensis, and it has since been shown to be present in many plants of the Agave species.[2] Extraction of the waste material from sisal[3] (Agave sisalana) now provides the large quantities of hecogenin necessary for the production of cortisone and its analogues. Detailed accounts of the experimental evidence from which the structures of the sapogenins were deduced have been presented by Fieser and Fieser,[4] and by Shoppee.[5]

The basic steroid sapogenin structure, with the system of enumeration, is shown in (I). Variations in the naturally



(I)

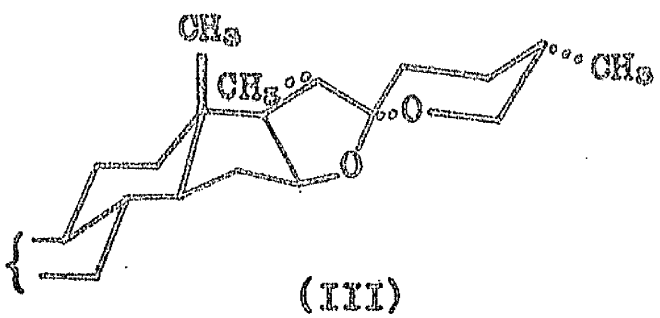


(II)

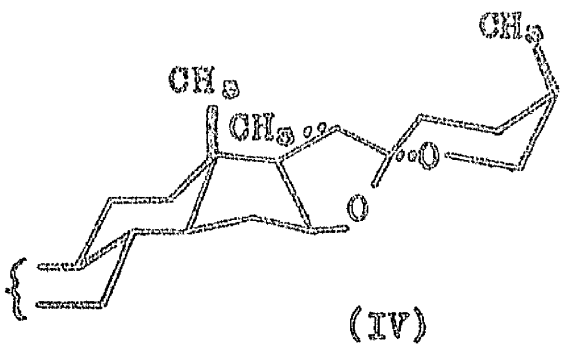
occurring steroid sapogenins arise from (a) the number, position and configuration of nuclear hydroxyl groups, (b) the occasional presence of a carbonyl group at C-(12), (c) the mode of union cis (5β-H), trans (5α-H), or involving a Δ^5 double bond, of rings A and B, and (d) the configuration at C-(25). The

spiroketal nature of the side-chain (rings E and F) was first recognised by Marker and Rohrmann[6] and provides the name "spirostan" for the parent system.[7] Hecogenin (II) is therefore 3β-hydroxy-12-oxo-5α,25D-spirostan.

The stereochemistry of the side-chain of the steroid sapogenins has been the subject of many publications and it is now established that isomerism occurs only at C-(25) and not, as was formerly thought, at C-(22). The isomerism at C-(25) divides the sapogenins into two main classes which are named 25D (sometimes isosapogenins) and 25L (sometimes normal or neo sapogenins). The letters D and L are derived from the absolute configuration of the α-methyl glutaric acids obtained from carbon atoms C-(22) to C-(27) on degradation. Hecogenin belongs to the 25D-series, in which the methyl group at C-(25) is equatorial, as in the partial formula (III). The corresponding 25L isomer, sisalagenin, is a companion of hecogenin and has the partial structure (IV), with the methyl group at C-(25) in the axial configuration. In 1939 Marker and Rohrmann[8] discovered that sarsasapogenin (side-chain corresponding to sisalagenin), on being refluxed with



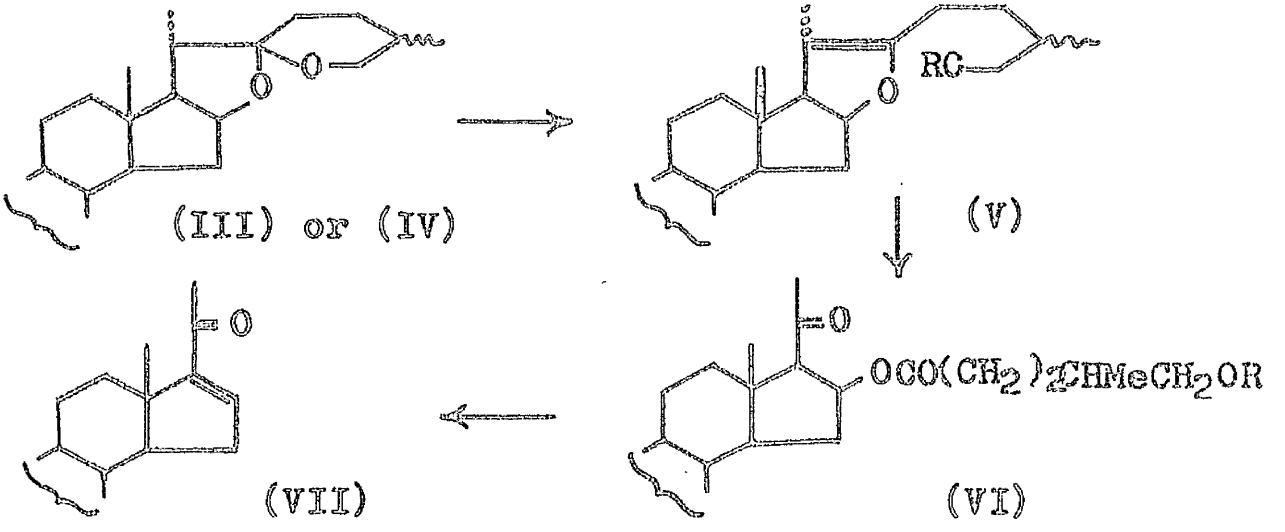
(III)



(IV)

ethanolic hydrochloric acid, was converted mainly to the isomeric smilagenin (side-chain corresponding to hecogenin). Since then other pairs of sapogenins isomeric at C-(25) have been identified. Recently the sapogenin side-chain has been synthesised,[9] a final cyclisation stage providing a mixture of the 25L and 25D compounds, with the latter predominating.

When sapogenins of both the 25D and 25L series are heated at 200° with acetic anhydride[1b], or with similar reagents,[9] esters of the corresponding isomeric pseudosapogenins are formed (V) in which ring-F has opened, with formation of a 20(22)-double bond. These are important intermediates in the conversion of sapogenins to pregnane derivatives since, upon oxidation with chromic acid, ring-E is split, with formation of a 16-acyloxy-20-oxo steroid (VI). This in turn is readily transformed by brief treatment with acetic acid (or other reagents) to the Δ^{16}-20-oxo pregnane derivative (VII), which is a well known intermediate in hormone synthesis.



(III) or (IV) (V) (VII) (VI)

It seems likely that the products of modification of ring-C of hecogenin which are described herein could be degraded in a similar manner to provide potentially valuable hormone intermediates.[x]

When the pseudosapogenins are subjected to mild acid conditions the 20-iso(cyclopseudo- or ana-) sapogenins are formed. In these ring-F has reformed, with the methyl group at C-(20) in the axial configuration. These are unstable and can readily be transformed, with mineral acid, to the corresponding sapogenins.

The work described in this thesis is concerned solely with reactions of the carbonyl group of hecogenin and not with the side-chain.

[x] Mazur[10] has already effected this side chain degradation with the aza-steroids described.

THEORETICAL

PART I

12-Methyl Steroids

Interest in methods for the introduction of methyl (and other alkyl) groups into the steroid nucleus has been stimulated by the discovery of the enhanced physiological activity of certain methylated hormone derivatives,[11] and also by the isolation of naturally occurring steroids containing "extra" methyl groups.[12] Many procedures have been devised[35] and one of the simplest of these is the reaction of oxo-steroids with Grignard reagents. Steroid derivatives with methyl groups at C-(3), C-(6), C-(7), C-(11), C-(12), and C-(17) have been obtained by this procedure and some examples are collected in Table I.

A study of the results of these Grignard reactions reveals that substitution normally takes place on the relatively unhindered α-face of the steroid molecule, in agreement with the rule of rear attack.[13]

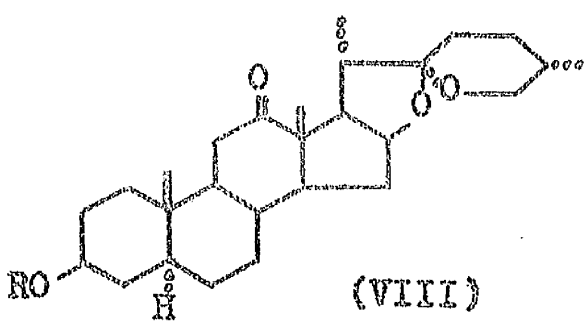
Recently Fonken and his associates[14] extended the use of methyl-lithium as a Grignard-type reagent by preparing 11α-methyl-11β-hydroxy derivatives from C-(11) ketones of the 5α- and 5β-pregnane series, in particular 11α-methylhydrocortisone acetate. With the same reagent Ringold et al.[31] obtained 11α-methyl-11β-hydroxytestosterone (the configuration [35] Toromanoff[16] has written a review on this subject

TABLE I

Reference	Position of Carbonyl group	Configuration at C-(5)	Yield of	
			α-OH, β-Me	β-OH, α-Me
16	C-(3)	5α-H	58%	38%
17	"	5α-H	54%	40%
18	"	5α-H(+4α-Me)	70%	30%
18	"	5α-H(+4α-Me)	80%	20%
19	C-(6)	5α-H	-	79%
20	"	5α-H	-	91%
21	C-(7)	Δ^5	-	62%
22	"	Δ^5	-	69%
23	"	Δ^5	33%	-
24	C-(16)	Δ^5	-	70%
25	C-(17)	Δ^5	-	65%
26	"	Δ^5	-	60%
27	C-(11)	5α-H	-	83%
15	"	5α-H	-	80%
15	"	5β-H	-	65%
28	C-(12)	5α-H	-	74%
29	"	5α-H	9%	86%
30	"	5β-H	60%	25%
30	"	5β-H	73%	-

of the C-(11) methyl group in these compounds has not been
or the C-(11) methyl group in these compounds has not been rigidly established). Methyl-lithium attacks the relatively hindered C-(11) carbonyl group only under certain conditions. These include protection of any hydroxyl groups, or groups readily hydrolysed by alkali (e.g. acetate) since methyl-lithium generally reacts with these to form the alcohol lithium salt, the insolubility of which precludes further reaction. One exception to this rule has been reported.[81] Elks[87] has used methyl-lithium to form 11α-methyl derivatives in the sapogenin series, and similar results have been obtained by Kirk and Petrow,[88] using methylmagnesium bromide.

It was decided to subject a suitable derivative of hecogenin to the action of methyl-lithium in order to obtain a new class of methyl-sapogenins and hence, by degradation of the side-chain, 12-methyl allopregnane derivatives. The first attempt was made using hecogenin acetate (VIII; R = Ac) but this gave, after acetylation and chromatography, a very poor yield of the desired 12α-methyl,12β-hydroxy-5α,25D-spirostan-3β-yl acetate (IX; R = Ac), along with starting material and an amorphous product. Apparently the reagent had attacked



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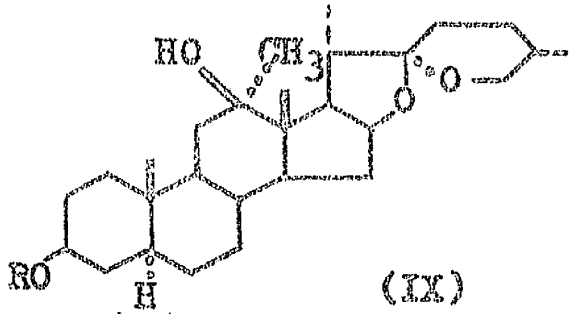
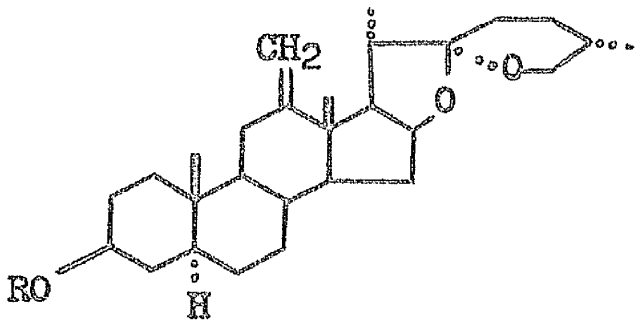


(IX)

both the carbonyl and acetate groups; the former was no longer present since the amorphous material from the reaction did not form a derivative with Girard's reagent-T. The tetrahydropyranyl derivative of hecogenin (VIII; R = C_5H_9O) described by Hirschmann et al.[33] was therefore prepared and found to be a more suitable starting material.

Reaction proceeded smoothly using excess of reagent in a benzene-ether solution and the protective group in the product was removed with mineral acid. The product was not homogenous and was acetylated and chromatographed. The first material isolated was eluted with benzene and was impure, but several recrystallisations from methanol gave a small amount (5% yield) of a monounsaturated acetate, m.p. 182-184°, of formula $C_{30}H_{46}O_4$. This was 12-methylenetigogenin acetate (X; R = Ac), since hydrolysis gave the corresponding alcohol (X; R = H), which had physical constants in close agreement with those recorded by Sondheimer and Mechoulam.[34] In order to verify

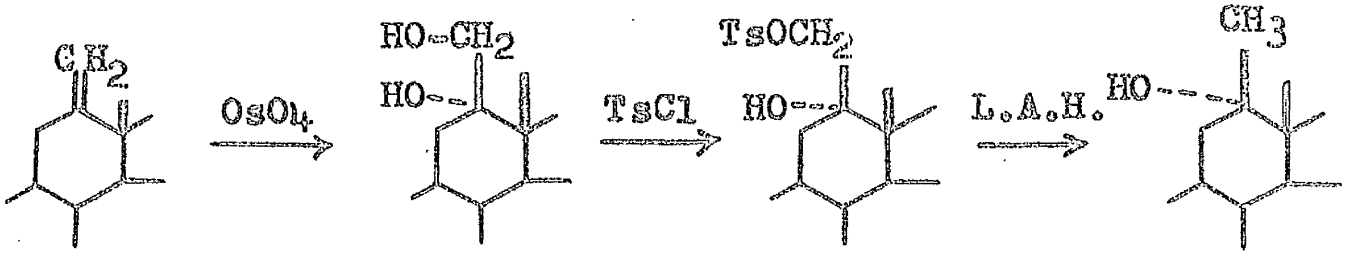


(X)

this identity several attempts were made to prepare authentic 12-methylenetigogenin by the method described by the above workers,[84] which involved the use of methylenetriphenylphosphorane. In each case starting material was recovered, but when phenyl-lithium was substituted for butyl-lithium (cf. Joska et al[85]) and hecogenin tetrahydropyranyl ether for hecogenin acetate, reaction took place to some extent. The product was a mixture, which after being hydrolysed and acetylated, was separated by chromatography. Much starting material was recovered, along with 12-methylenetigogenin acetate, which was identical with the unsaturated acetate, m.p. 182-184°, obtained previously.

The next material isolated by chromatography was a saturated acetate, $C_{30}H_{48}O_5$, which was eluted in fractions of increasing purity by benzene-ether mixtures of increasing elutory power. The purest material, obtained in the ether eluates, had m.p. 226-228°, and $[\alpha]_D$ - 47°. It is formulated as 12α-methyl-12β-hydroxy-5α,25D-spirostan-3β-yl acetate (IX; R = Ac). The corresponding alcohol, 12α-methyl-3β,12β-dihydroxy-5α,25D-spirostan (IX; R = H) was prepared by alkaline hydrolysis of the acetate (IX; R = Ac) and had the interesting characteristic of crystallising only in the presence of a small amount of pyridine. The results of analysis showed that a steroid-pyridine complex was formed.

When this work was completed it was not certain that the 12-methyl carbinol formed by the methyl-lithium reaction had the 12α-methyl, 12β-hydroxy structure (IX). This was merely assumed, on the basis of the normal mode of rear attack by bulky groups, such as the methyl anion, on the steroid nucleus.[18] However, in view of the recent work of Levine and Wall[29] which duplicates this work to some extent, it seems certain that the above assignment of configuration to the carbinol acetate is correct. These workers prepared from 12-methylenetigogenin acetate, by the sequence indicated below, the carbinol (XIII) epimeric with the product of the Grignard reaction.



(X, R=Ac) (XI) (XII) (XIII)

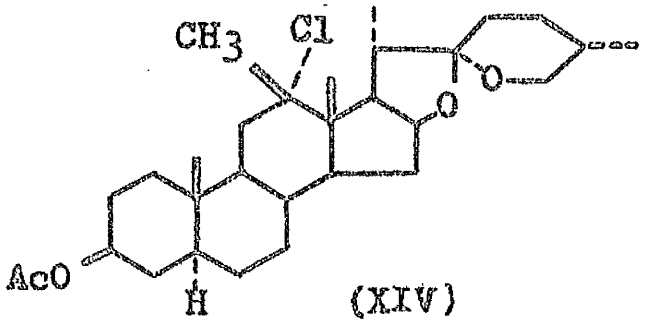
Attack by osmium tetroxide must be from the rear of the molecule, providing the 12β-hydroxymethyl compound (XI) which is then transformed, without rearrangement, into 12β-methyl,12α-hydroxytigogenin (XIII). Levine and Wall considered that this compound is a minor product of the Grignard reaction.

but were unable to isolate it. Similarly, we isolated only one pure isomer, but it is possible, in view of the similarity of the physical constants of (IX; R = Ac) (m.p. 226-228°, $[\alpha]_D$ - 47°) and (XIII; R = Ac) (m.p. 212-213°, $[\alpha]_D$ - 47°) that the earlier fractions of the chromatogram contained some of the isomeric acetate (XIII; R = Ac). It seems possible then that 12-methylenetigogenin acetate is formed by acid dehydration of the less stable isomer during hydrolysis of the ether group at C-(3).

When hecogenin tetrahydropyranyl ether was treated with methyl-lithium as before, and the product hydrolysed under mild conditions, using ethanolic acetic acid, three products were isolated (after acetylation). The acetate (IX; R = Ac) was the major product, along with small quantities of tigogenin acetate and 12-methylene-5α,25D-spirost-9(11)-en-3β-yl acetate (XXV; R = Ac). This last compound is derived from the 9(11)-dehydrohecogenin present, along with tigogenin, in the starting material. The presence of the unsaturated acetate was confirmed by the presence of a maximum in the ultraviolet spectrum of a sample of hecogenin acetate at 238 mμ. (ε 400). The occurrence of 9(11)-dehydrohecogenin in a commercial preparation of hecogenin from Agave deserti Engelm has been demonstrated by Wagner et al.[36] Since no 12-methylenetigogenin acetate (X; R = Ac) was formed in the reaction it appears

likely that only the more stable isomer (IX; R = Ac) is formed in the Grignard-type reaction. On the other hand some lower melting fractions (m.p. 210-220°) were obtained in the chromatogram and these may consist of the isomeric carbinol, unaffected by the mild acid conditions. There is also some evidence that 12α-methyl-12β-hydroxytigogenin acetate may be the source of 12-methylenetigogenin acetate, since, when it was refluxed with ethanolic hydrochloric acid for 40 minutes the product showed end absorption in the ultraviolet at 250 mμ.

Reaction of the carbinol (IX; R = Ac) with phosphorus oxychloride had little effect, substantially unchanged starting material being recovered. Phosphorus pentachloride provided 12-chloro-12-methyltigogenin acetate. Since this reagent normally substitutes with inversion of configuration[4a] the chloro compound has been formulated as (XIV). In contrast reaction of the acetate with thionyl chloride in pyridine produced much amorphous material, along with a small amount of a compound containing chlorine. This may be 12β-chloro-12α-methyltigogenin acetate (XV), since thionyl chloride usually substitutes with retention of configuration.



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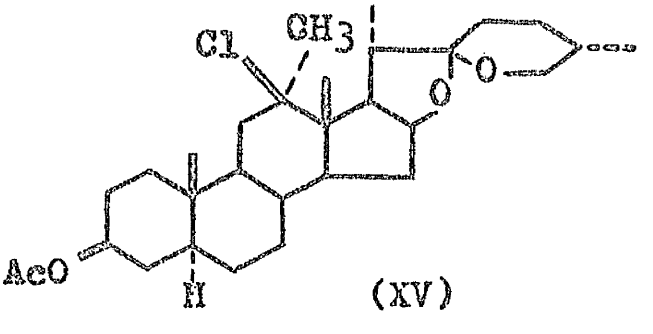
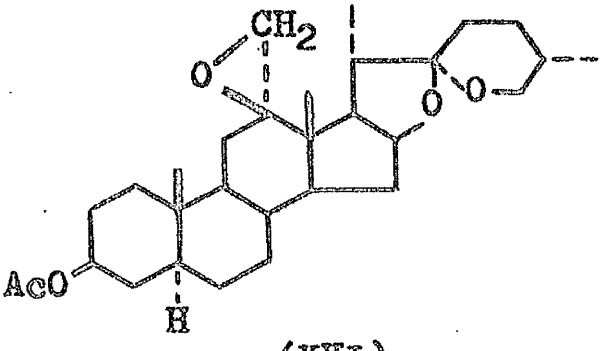


(XV)

Alkaline hydrolysis of the chloro compound (XIV) gave a halogen-free mixture which, on acetylation and chromatography, provided a small amount of unidentified crystalline material. Next the chloro compound was treated with sodium methoxide in methanol. Crystallisation of the product gave 12-methylene-tigogenin (X; R = H) in 60% yield. The mother liquor, on addition of water, provided impure material containing no chlorine.

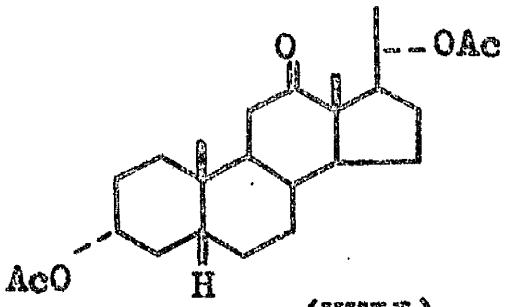
On treating 12-methylenetigogenin acetate with perbenzoic acid a single oxide was formed in reasonable yield. This must be the β-oxide (XVI) since lithium aluminium hydride opened the oxide ring to give 12α-methyl,12β-hydroxytigogenin (IX; R = H recognised by its characteristic of crystallising only in the presence of pyridine. Acetylation provided authentic



(XVI)

12α-methyl,12β-hydroxytigogenin acetate (IX; R = Ac). The peracid must therefore have attacked the exocyclic methylene group from the β-face.

Recently Just and Nagarajan[30] reported on the reaction between 12-oxo compounds of the 5β-pregnane series with excess of methylmagnesium bromide. For 3α,20α-diacetoxy-5β-pregnan-12-one (XVII) the reaction proceeded to give a single C-(12)-methyl carbinol which they considered to have the unexpected 12β-methyl,12α-hydroxy structure (XVIII). In the case of 3α,20-diacetoxy-5β-pregnan-12-one (XIX) both isomeric carbinols



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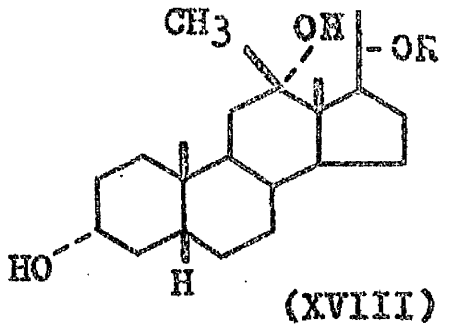
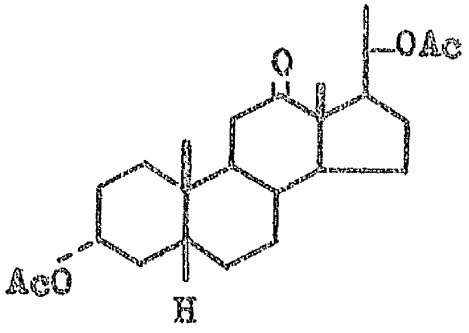


(XVIII)

(XX) and (XXI) were isolated, the former predominating. These



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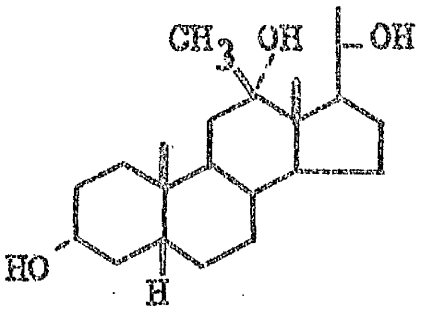

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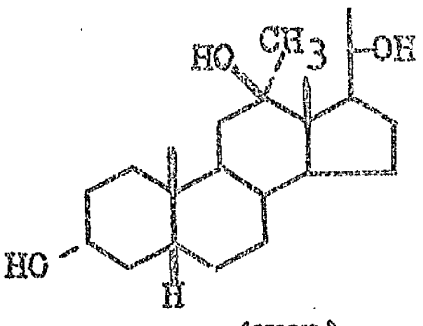
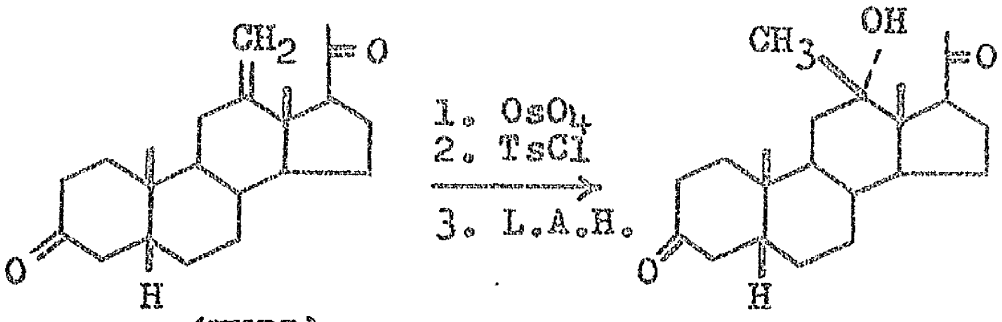


(XXI)

workers found that the minor product of the reaction, on oxidation at C-(3) and C-(20), gave material which absorbed at 3450 $cm.^{-1}$ in the infrared, due to intramolecular bonding of the hydroxyl group with the C-(20) carbonyl function. Examination of the models indicates that this can only take

place when the hydroxyl group is equatorial (β). In addition they found that successive treatment of admittedly impure unsaturated acetate (XXII) with osmium tetroxide, toluene-p-sulphonyl chloride, and lithium aluminium hydride, afforded material identical with their major reaction product, thus confirming the axial nature of the hydroxyl group in (XX). The Canadian workers results contrast with those of Levine and



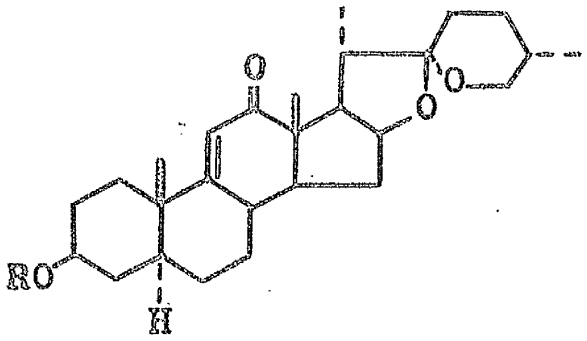
(XXII)

Wall, who obtained, by the above sequence, from pure 12-methyle tigogenin acetate, a carbinol epimeric with their major product. These facts can be reconciled only by assuming that the mode of attack by Grignard-type reagents differs in the 5β-pregnane and 5α-sapogenin series. This may be due to subtle long-range effects (see Barton et al.[37]) which alter the relative rates of reaction on the α- and β- faces.

In their report Just and Najarajan state that our results[28] conflict with those of Levine and Wall.[29] This is not so; the properties of our compounds, with one exception, agree closely with those of the latter workers. Nor did we,

or Ruzicka et al.[39], osmylate comparable monounsaturated compounds.

Since the dienyl acetate (XXV; R = Ac) had been formed by the reaction of methyl-lithium with the trace amount of 9(11)-dehydrohecogenin acetate present in hecogenin acetate it was decided to prepare the αβ-unsaturated ketone (XXIV; R = H)



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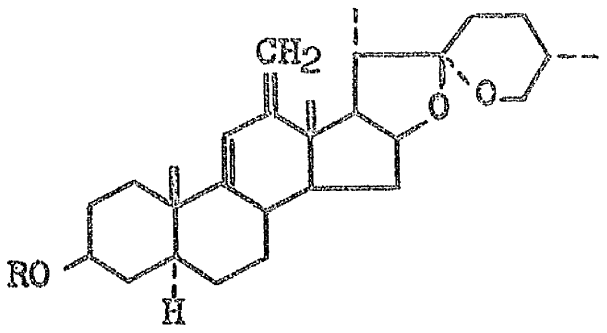


(XXV)

for a reaction on a larger scale. The method of preparation of (XXIV) was that of Djerassi, Martinez and Rosenkranz,[39] and involved dibromination of hecogenin acetate at C-(11) and C-(23), followed by dehydrobromination with refluxing collidine.[M] The latter stage was not very efficient, so that after removal of side chain bromine with zinc and ethanol, the product was a mixture of hecogenin acetate and 9(11)-dehydrohecogenin acetate. Chromatographic separation of these was not completely successful

[M] Conversion of hecogenin acetate to 9(11)-dehydrohecogenin acetate in 89% yield by oxidation with selenium dioxide has been reported by a Syntex group.[40]

and afforded only a moderate yield of the αβ-unsaturated ketone (XXIV; R = Ac).[35]

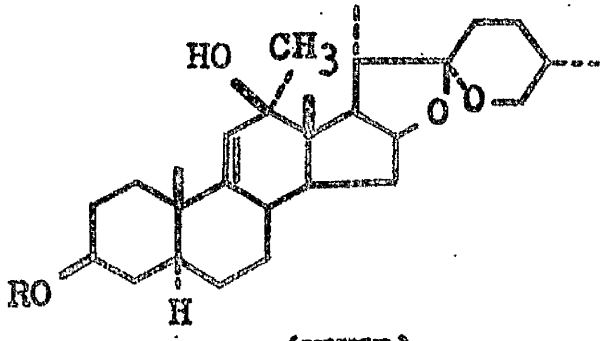
It was thought that the Baeyer-Villiger oxidation of the mixture of hecogenin acetate (VIII; R = Ac) and 9(11)-dehydrohecogenin acetate (XXIV; R = Ac) might provide, after saponification, hecolic acid (XLVI; R = R' = H) and 9(11)-dehydrohecogenin (XXIV; R = H), since 9(11)-dehydrohecogenin had been isolated from the neutral fraction in the preparation of hecolic acid.[35] Although the unsaturated acetate was not oxidised under the Baeyer-Villiger conditions used it apparently inhibited the complete reaction of hecogenin acetate. The products were hecolic acid and a mixture containing 65% of 9(11)-dehydrohecogenin.[36]

9(11)-Dehydrohecogenin acetate was hydrolysed and the tetrahydropyranyl ether (XXIV; R = C_5H_9O) was formed. Treatment of this with methyl-lithium provided a tetrahydropyranyl ether (or rather a mixture of ethers due to asymmetry at C-2') corresponding to the formula $C_{33}H_{52}O_5$, with a $\lambda_{max.}$ at 204 mμ. There was no carbonyl absorption in the infrared so the material must be the 12α-methyl-12β-hydroxy derivative (XXVI; R = C_5H_9O). The protective group was then removed by acetic or hydrochloric acid, and since these gave quite

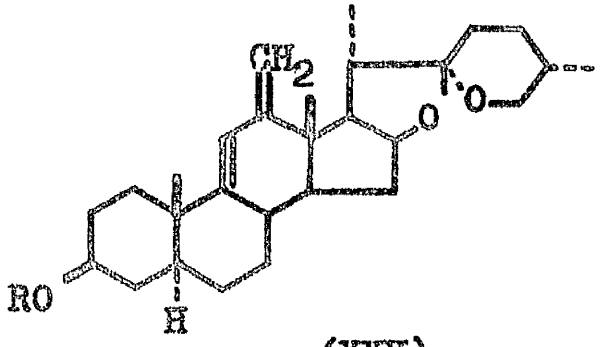
[36] An efficient separation procedure which employs Girard reagent-T has been described by Mueller et al.[41]

different results the hydrolyses will be described separately.

When the tetrahydropyranyl ether (XXVI; R = C_5H_9O) was refluxed with ethanolic acetic acid a single product was obtained in excellent yield. This was a compound $C_{32}H_{48}O_3$, which crystallised from methanol as a hemimethanolate, m.p. 204-205°, and showed a single high intensity ultraviolet absorption maximum at 238 mμ. There was infrared absorption at 1640 $cm.^{-1}$, but no carbonyl peak, and the side-chain was unaltered. This material must be 12-methylene-9(11)-dehydrotigogenin (XXV; R = H). This should have a $\lambda_{max.}$ of 239 mμ.



(XXVI)



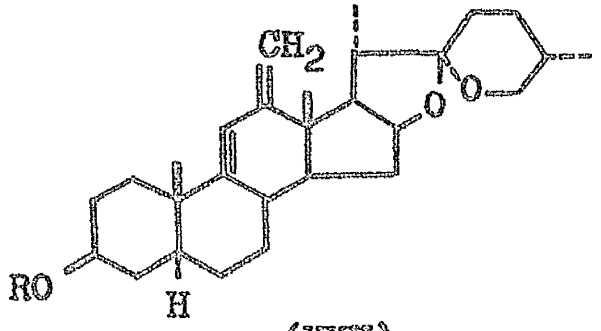
(XXV)

calculated from Woodward's rules. The basic structural formula was confirmed by analysis of the corresponding acetate (XXV; R = Ac) and benzoate (XXV; R = Bz). Evidently the allylic double bond in (XXVI; R = C_5H_9O) promotes acid catalysed dehydration under very mild conditions.

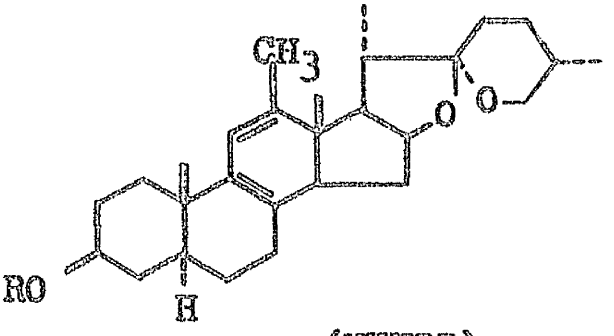
When the tetrahydropyranyl ether (XXVI) was heated with ethanolic hydrochloric acid for a short time the product was

a mixture of alcohols, m.p. 177-183°. This mixture showed ultraviolet absorption maxima at 204, 240 and 270 mμ. and could not be purified by chromatography. Acetylation of the mixture produced material with similar ultraviolet absorption, again not separable by chromatography. The same mixture of acetates was produced when 12-methylene-9(11)-dehydrohecogenin acetate (XXV; R = Ac) was refluxed in ethanolic hydrochloric acid for a short time, although mineral acid in the cold had no effect. It is probably that during mineral acid hydrolysis (XXV) is formed first and then changes to an equilibrium mixture which probably contains ~40% of the cisoid diene (XXVII) which would have a calculated $\lambda_{max.}$ at 278 mμ.

That the acid catalysed isomerisation of the dienyl acetate



(XXV)

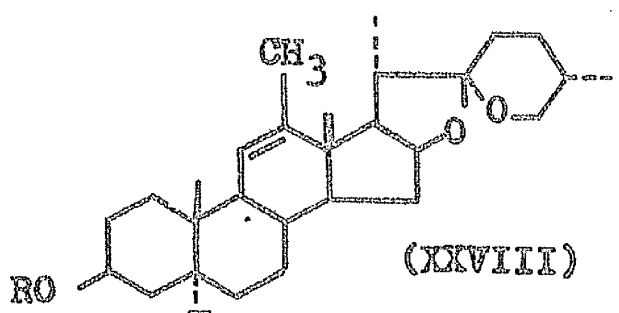


(XXVII)

is a reversible one was demonstrated when a sample of the dienyl acetate mixture in dry chloroform was treated with dry hydrogen chloride at low temperature.[42] The product, although not sharp-melting, had a single ultraviolet maximum at 238 mμ.

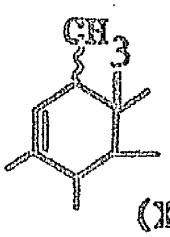
It was thought that a separation of the two acetates might be achieved by treatment with maleic anhydride, which should only react with the homoannular diene system (XXVII). Accordingly samples of the mixture were refluxed with the dienophile in several solvents, but no adduct was formed. It must be assumed that steric factors prevent the approach of the anhydride to both faces of the molecule. When the mixture was treated with perbenzoic acid some reaction took place, since the product lacked the absorption maximum at 270 mμ., but no pure material could be isolated. Since the mixture could not be purified, attention was directed to the pure dienol (XXV; R = H) and dienyl acetate (XXV; R = Ac).

Catalytic reduction of the acetate (XXV; R = Ac) under neutral conditions was quickly effected with uptake of only one molar equivalent of hydrogen. The single product, m.p. 175-177° absorbed in the ultraviolet at 206 mμ., indicating the presence of an isolated double bond, and this was confirmed by the infrared maximum at 1654 cm.$^{-1}$ The analytical results for the acetate and the corresponding alcohol supported the view that a monounsaturated compound had been formed, and this has been assigned the Δ^{11}-structure (XXVIII; R = Ac) for the

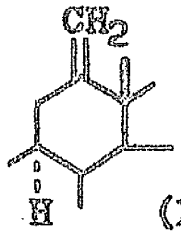


(XXVIII)

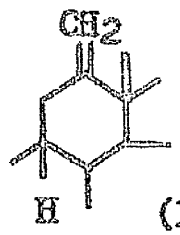
following reasons. There appeared to be four possible alternatives, the $\Delta^{9(11)}$- (XXIX), 12-methylene-(9α or 9β) (XXX and XXXI), or Δ^{11} - (XXVIII) structures. The first of these was rejected, because under the conditions of catalytic



(XXIX)



(XXX)



(XXXI)

hydrogenation employed 12-methylenetigogenin failed to absorb hydrogen, suggesting that this group requires more vigorous treatment for reduction (cf. Levine and Wall[20]). The second possibility was also eliminated since the reduction product had different physical constants from 12-methylenetigogenin acetate, which was obtained previously. Moreover, these two acetates gave a melting point depression on admixture, and the infrared spectra were different. The next structure to be considered was 12-methylene-9β-tigogenin acetate (XXXI) which would be formed by addition of hydrogen to the β-face of the molecule. This unlikely formulation was eliminated when fission of the cis diol (XXXII) formed by the action of osmium tetroxide on the reduction product, with lead tetracetate, afforded amorphous material with infrared absorption characteristic of a keto-aldehyde. The 12-methylene structure would

have afforded a ketone isomeric with hecogenin under these conditions. Also the formation of a compound possessing both carbonyl and aldehyde groups agrees with the Δ^{11}-position for the double bond, as in (XXVIII).

Attack by the hydrogen on the diene system present in (XXV) must be envisaged as 1,4-addition from the unhindered α-face, with formation of the 11,12-double bond. Although this is usually formed only with difficulty, for instance in the bile acid series by pyrolysis of C-(12) esters, the additional C-(12) methyl group may have a stabilising effect. Levine and Wall[29] report the formation of a supposed Δ^{11}-12-methyltigogenin acetate, with different physical constants from our compound, and it seems likely that their material is composed of equimolar quantities of (XXVIII; R = Ac) and (X, R = Ac).

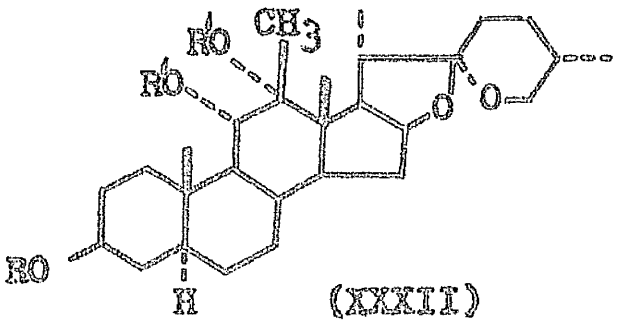
12-Methyl-5α,25D-spirost-11-en-3β-ol (XXVIII; R = H) was prepared either by catalytic reduction of the dienol (XXV; R = H) or by hydrolysis of the reduced acetate. The latter method was preferred, since the dienol is less soluble than the dienyl acetate in ethyl acetate.

Treatment of 12-methylene-9(11)-dehydrotigogenin acetate with mercuric acetate failed to extend the unsaturation to ring B. The inactivity of the acetate (XXV; R = Ac) to this reagent is comparable with that of steroids containing an

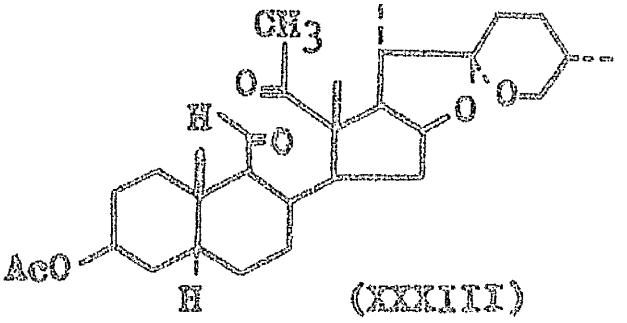
isolated $\Delta^{9(11)}$-double bond. In contrast steroids having a Δ^{7}-double bond provide $\Delta^{7,9(11)}$-dienes when treated with mercuric acetate.[35]

Catalytic reduction of the acetate (XXV; R = Ac) in acetic acid was incomplete, the amorphous product showing slight ultraviolet absorption.

Successive treatment of 3β-acetoxy-12-methyl,5α,25D-spirost-11-ene (XXVIII; R = Ac) with osmium tetroxide and lithium aluminium hydride provided crystalline material, from which were separated by chromatography, starting material (35%) and a new, highly crystalline product, m.p. 265-269°. This crystallised slowly from methanol and was shown by elementary analysis to be the monomethanolate of a triol $C_{28}H_{48}O_5$. It was transparent to ultraviolet light and possessed strong hydroxyl absorption in the infrared. The probable structure, 12β-methyl-5α,25D-spirostan-3β,11α,12α-triol (XXXII; R = R' = H) was confirmed by acetylation with acetic anhydride and pyridine at 100°. The product on chromatography, provided two compounds



(XXXII)



(XXXIII)

with similar melting points. One of these, eluted with benzene, analysed correctly for a monoacetate of the triol, and must be 3β-acetoxy-12-methyl-5α,25D-spirostan-11α,12α-diol (XXXII ; R = Ac, R' = H). The other, eluted with light petroleum-benzene (9:1), possessed no hydroxyl absorption maxima in the infrared and analysed correctly for a triacetate of the triol. Since, in steroids possessing no substituents at C-(12) the 11α-hydroxyl group acetylates readily, whereas an 11β-hydroxyl group required very vigorous conditions, by analogy, the triacetate must be 12-methyl-3β,11α,12α-triacetoxy-5α,25D-spirostan (XXXII; R = R' = Ac). The triol from which these derivatives are formed must therefore be the 3β,11α,12α-triol (XXXII; R = R' = H).

As indicated above, treatment of the triol (XXXII; R = R' = H) with lead tetracetate provided amorphous material absorbing in the infrared at 2878 and 1700 cm.$^{-1}$ The former maximum is characteristic of the aldehyde group as in the keto-aldehyde (XXXIII) and supports the view that the structure of the triol is (XXXII) [although the 12-methyl-$\Delta^{9(11)}$-compound (XXIX), which was rejected for previously described reasons, would also give a keto-aldehyde when treated with osmium tetroxide and lead tetracetate].

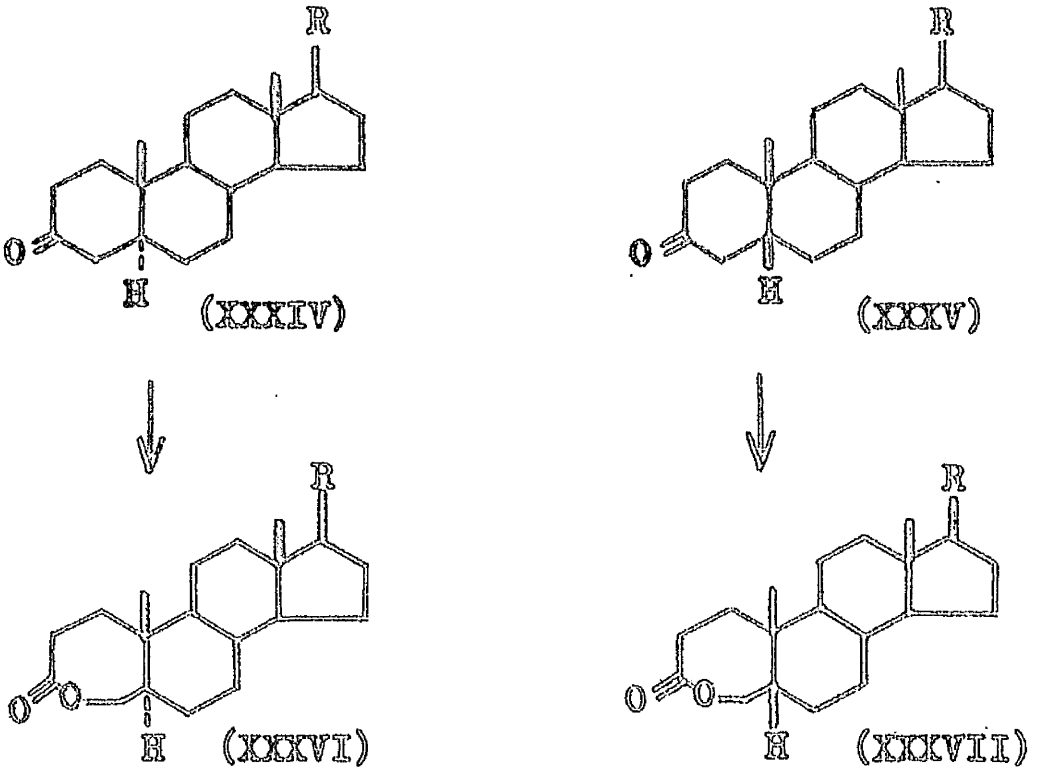
PART II

Ring-C Seco Steroids

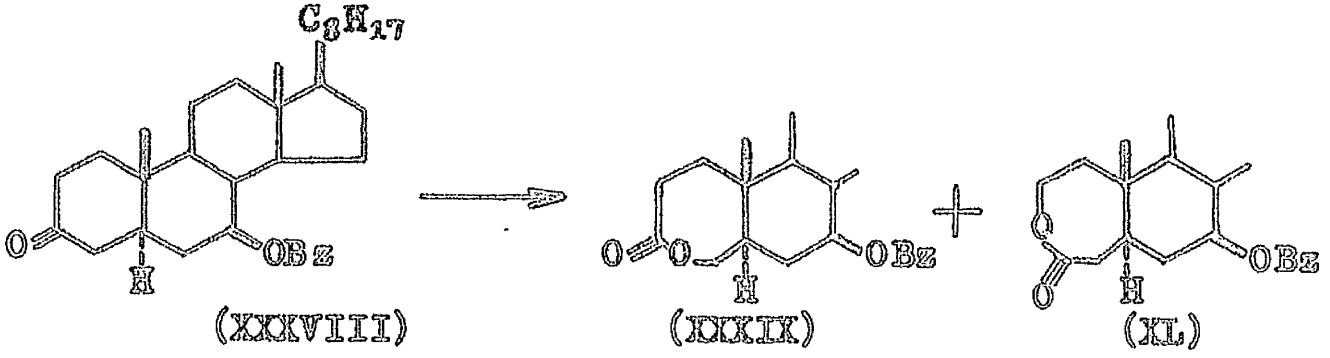
The Baeyer-Villiger Oxidation of Hecogenin Acetate.

In 1899 Baeyer and Villiger[43] showed that the oxidation of menthone and camphor with monopersulphuric acid led to the formation of lactones. Further studies, using a variety of ketones or aldehydes, and hydrogen peroxide or peracids, in different media, have established the wide applicability of the reaction.[44] In the steroid series this procedure has been applied successfully to compounds with carbonyl groups at C-(3), C-(7), C-(17), C-(20) and C-(12).

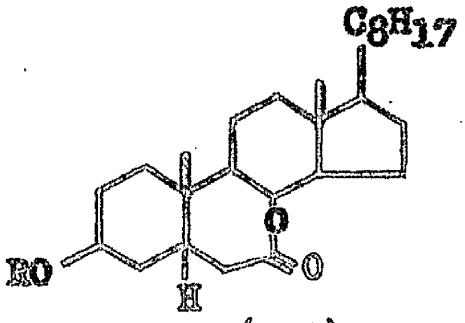
Most of the reports of oxidation of 3-oxo steroids with peracids describe the isolation of a single lactonic product, obtained by fission of the C-(3)- C-(4) bond. Thus Prelog and his colleagues[45] obtained from 5α-androstan-3-one (XXXIV; R = H) and 5β-androstan-3-one (XXXV; R = H), by treatment with perbenzoic acid, the isomeric lactones (XXXVI; R = H) and (XXXVII; R = H) respectively. Cholestanone and coprostanone are reported[46] to give the analogous products (XXXVI; R = C_8H_{17}) and (XXXVII; R = C_8H_{17}). However, Heymann and Fieser[47] obtained the two isomeric lactones (XXXIX) and (XL) by treating the 7β-benzoyloxy derivative of cholestanone (XXXVIII) with perbenzoic acid and Fieser[4b] suggested that the lactones



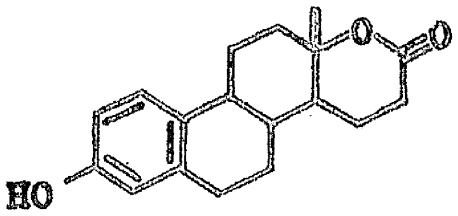
that have been isolated are not necessarily the only ones formed



The lactones formed by oxidation of several 7-oxo cholestanyl derivatives have been shown[49] to possess the structure (XLI) but the possibility of the isomeric lactone being formed as a minor product cannot be excluded.



(XLI)

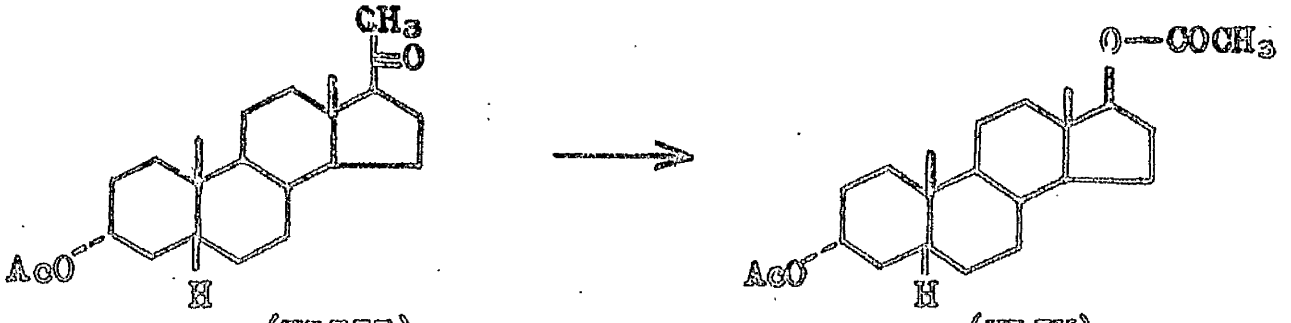


(XLII)

Westerfield[49] observed that oestrone, on treatment with alkaline hydrogen peroxide, produced a lactone, oestrololactone, and assigned to it the structure (XLII), since the corresponding hydroxy acid was easily esterified. Jacobsen et al.[50] found that oxidation of 17-oxo steroids proceeds best with peracetic acid containing a little toluene-p-sulphonic acid, and extended the reaction to the androstane and equilenin series. The fact that it is the C-(13)-C-(17) bond which is preferentially split during the oxidation was demonstrated by Wendler and his colleagues[51] and Murray et al.[52] Again, more recent work[53] has shown that the isomeric lactone, formed by fission of the C-(16)-C-(17) bond, is produced in small quantity.

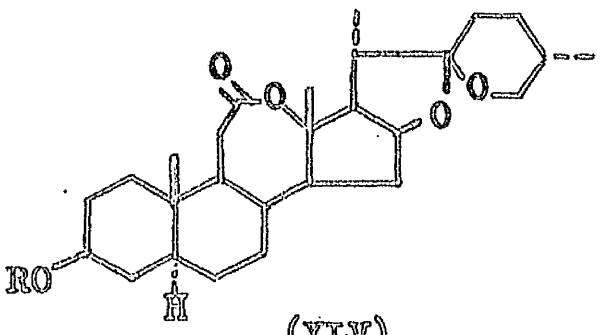
Oxidation of a 20-oxo steroid (with a 17β side-chain) was reported by Marker[6] to give, after hydrolysis, the corresponding 17α-hydroxy compound. However, Gallagher and Kritchevsky[13] and others, studied several such oxidations, and concluded that the configuration of the C-(17)-acetoxy or -hydroxy group

formed was the same as that of the original side chain. Thus 3α-acetoxy-5β-pregnan-20-one (XLIII) affords 3α,17β-diacetoxy-5β-pregnane (XLIV).

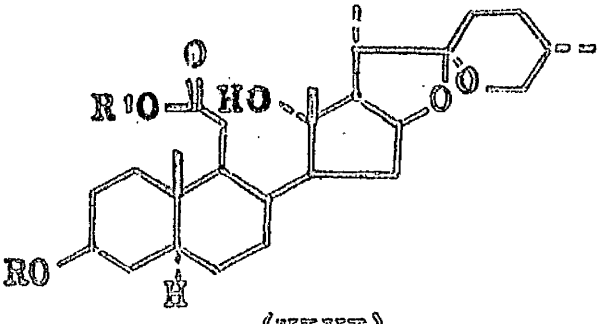


(XLIII) (XLIV)

Prior to the work of Rothman, Wall, and Eddy[54], 12-oxo steroids had been reported[55] to be inert to peracids, but the American workers, by lengthy treatment of hecogenin acetate and methyl 3α-acetoxy-12-oxocholanate, with peracetic acid, obtained a good yield of crystalline lactone in each case. The product from hecogenin acetate, hecololactone acetate, was hydrolysed to the corresponding dihydroxy acid, hecolic acid. The transformations which the lactone acetate and the acid underwent led these workers to ascribe to them the undoubtedly correct structures 3β-acetoxy-13α-hydroxy-12,13-seco-5α,25D-spirostan-12-oic-acid-12,13-lactone (XLV; R = Ac) and 3β,13α-dihydroxy-12,13-seco-5α,25D-spirostan-12-oic acid (XLVI; R = R' = H). According to Rothman and his colleagues hecololactone was the only product of the oxidation, but it is now shown that an isomeric lactone acetate is formed in low yield.



(XLV)



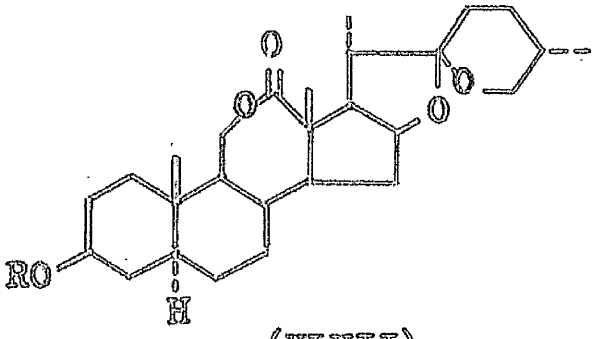
(XLVI)

This is not surprising in view of the results of other oxidations described above.

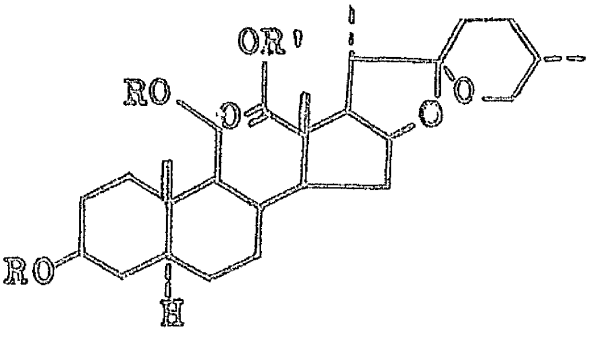
Methyl hecolate (XLVI; R = H, R' = Me) seemed a suitable starting material for the preparation of the corresponding nor-acid by the Barbier-Wieland method (see below). Accordingly the preparation of hecolic acid was repeated using the second of the two published methods.[54] This involved oxidation of hecogenin acetate with peracetic acid in a two-phase system in the presence of sulphuric acid. The resulting crude lactone mixture was not purified but was hydrolysed directly to give a mixture of acids. This gave, on crystallisation, 82% of hecolic acid, and 5% of a new acid which has been named isohecolic acid. The separation of the two acids was facilitated by the sparing solubility of hecolic acid in chloroform. The higher melting isohecolic acid was more soluble and was purified by crystallisation from acetone.

Isohecolic acid formed a methyl ester (methyl isohecolate) on being treated with diazomethane. This in turn, on treatment with acetic anhydride and pyridine at room temperature, gave a diacetate which no longer had absorption in the infrared characteristic of a hydroxyl group. This suggests that isohecolic acid is 3β,11-dihydroxy-11,12-seco-5α,25D-spirostan-12-oic acid (XLVIII; R = R' = H), formed by rupture of ring-C of hecogenin between C-(11) and C-(12). The methyl ester and the methyl ester diacetate must then have the structures (XLVIII; R = H, R' = Me) and (XLVIII; R = Ac, R' = Me) respectively.

Isohecolic acid readily lactonised on being heated <u>in vacuo</u> above the melting point. The product, isohecololactone (XLVII; R = H), was also characterised as its acetate (XLVII; and benzoate (XLVII; R = Bz).



(XLVII)



(XLVIII)

The isomeric structure (XLVIII; R = R' = H) for isohecolic acid was originally discarded, since high C-values

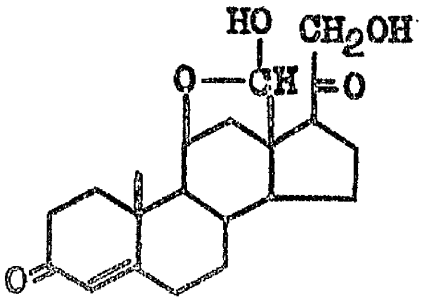
were frequently obtained on analysis of the acid and its derivatives. These agreed with a C_{29} formula for the acid. At the time this work was first carried out, reports of the isolation of various naturally occurring methyl steroids had just appeared.[12] The possibility that isohecolic acid had extra carbon atoms at C-(4), C-(14), or in the side chain, and was derived from a persistent contaminant of hecogenin, had to be considered. These possibilities were discounted since: (a) determination of the equivalent weight of isohecolic acid by microtitration against alkali gave results very near the calculated value for an acid of the same molecular weight as hecolic acid, and differing from a homologue; (b) triangular crystallisation of hecogenin benzoate failed to separate any homologous material; (c) Kuhn-Roth estimation of C-CH_3 groups provided a result of 7.9%. For four C-CH_3 groups, as in hecolic acid, the theoretical result is 12.8%. However, for a sample of hecolic acid the result was 7.4% and in general, complicated molecules such as steroids, give results lower than the theoretical.[56] The calculated values for 4,14-dimethy 4,4-dimethyl-, and 24-ethyl derivatives are respectively 18.4, 15.4, and 15.4%; (d) repeated analysis of the acid and its derivatives afforded results in agreement with the C-(27) formula for the acid. Accordingly the isomeric structures (XLVII and XLVIII) are considered correct.

Some of the derivatives of isohecolic acid show a superficial resemblance to those of hecolic acid in having double melting points, but there are significant differences between corresponding members of the two series. As expected, the final melting points of the acids in each series are the sam as those of the corresponding lactones.

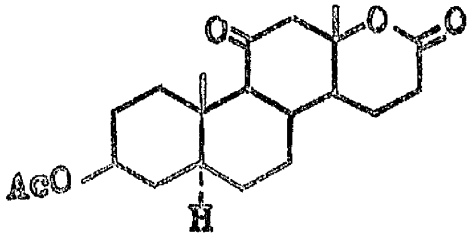
On comparing specific rotations it is seen that the "iso" seco compounds have more positive values than the normal seco compounds, while the opposite is the case for the cyclised structures. Comparison of infrared spectra shows that in the "iso" series maximal carbonyl absorption occurs at lower frequencies.

Barbier-Wieland Degradation of Methyl Hecolate.

Methyl hecolate (XLVI; R = H, R' = Me) was prepared (see above) to provide an intermediate for a projected synthesis of aldosterone (XLIX). This active adrenocortical hormone has been totally synthesised by several groups since its isolation in 1953.[4c] Barton and his associates[57] introduced an aldehyde function at C-(13) by a sequence of reactions starting from a six-membered ring-D lactone (L), and it appeared likely that a

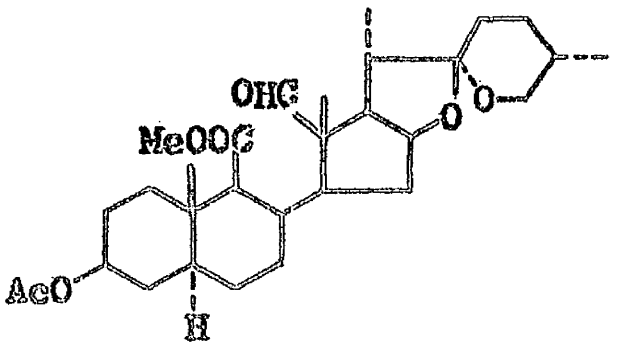


(XLIX)

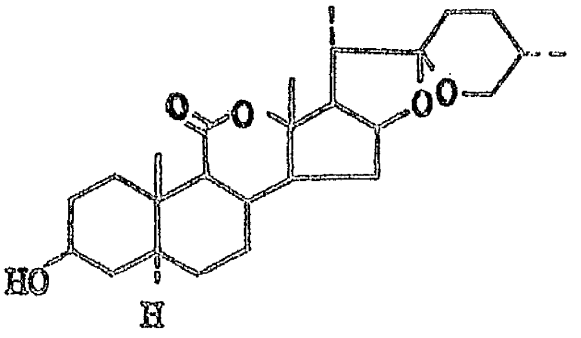


(L)

similar result could be achieved by suitable treatment of a six-membered ring-C lactone. It was intended to prepare such a lactone by Barbier-Wieland degradation[58] of hecolic acid to 12-nor hecolic acid, followed by lactonisation. It is interesting to note that the desired lactone (LII) has recently been prepared by Zderic et al.[59] by the Baeyer-Villiger oxidation of methyl 3β-acetoxy-11,12-seco-12-oxo-5α,25D-spirostanoate (LI), followed by hydrolysis and acidification.

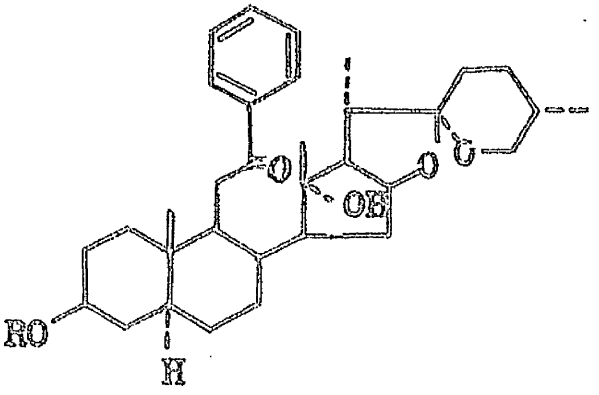


(LI)



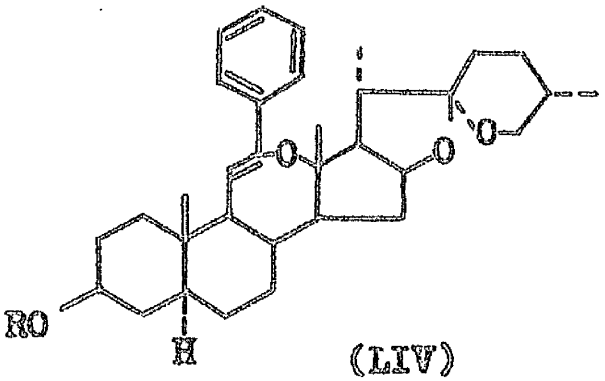
(LII)

Treatment of methyl hecolate with excess of phenyl-magnesium bromide under the usual Barbier-Wieland conditions resulted in the uptake of only one mole of reagent. A similar result was obtained using phenyl-lithium. The product, which in neither case was obtained crystalline, had an ultraviolet absorption maximum at 242 mμ , similar to that of propiophenone ($\lambda_{max.}$ 242 mμ) and consistent with its formulation as the phenyl ketone (LIII).



(LIII)

On acetylation, followed by brief treatment with boiling acetic acid, this compound was dehydrated to give a crystalline solid with ultraviolet absorption maxima at 205, 218, and 263 mμ Basic hydrolysis of this acetate provided an alcohol with similar absorption characteristics, and from this the corresponding benzoate was obtained. The analyses of these compounds were in agreement with a $C_{35}H_{48}O_5$ formula for the acetate, which can therefore be assigned the structure 3β-acetoxy-12α-oxa-12-phenyl-C-homo-5α,25D-spirost-11-ene (LIV; R = Ac).



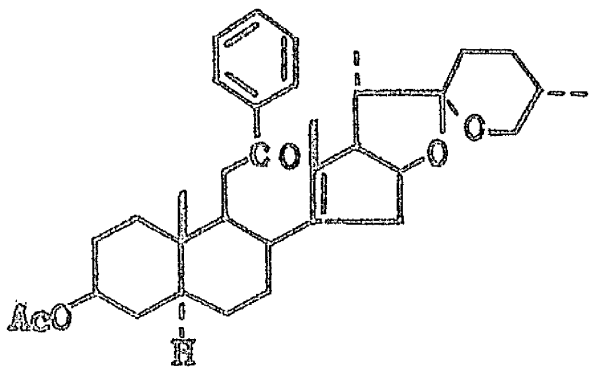
(LIV)

The ultraviolet spectrum was fairly consistent with this formulation, the difference between it and that of the analogous β-methylstyrene[60] ($\lambda_{max.}$ 251 mμ.) being attributable to the influence of the ethereal oxygen atom.

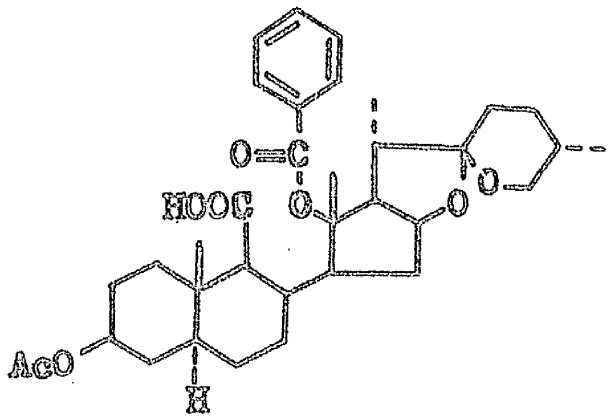
Good yields of this compound were obtained only under anhydrous conditions, since, when it was heated with aqueous acetic acid ring-C was opened and 3β-acetoxy-12-phenyl-12,13-

seco-5α,25D-spirost-13-en-12-one (LV) was formed. This contrasts with the action of alkali on the enol ether system, which gave the free alcohol (LIV; R = H).

Although the desired Barbier-Wieland reaction had not been obtained it was thought that the double bond in the phenyl enol ether (LIV; R = Ac) might be susceptible to oxidation, to give the keto-acid (LVI). This would then furnish, on hydrolysis, the required nor-acid. Several oxidising agents were employed, but in each case only small, amorphous acid products were formed (except with chromium trioxide, when benzoic acid was obtained).



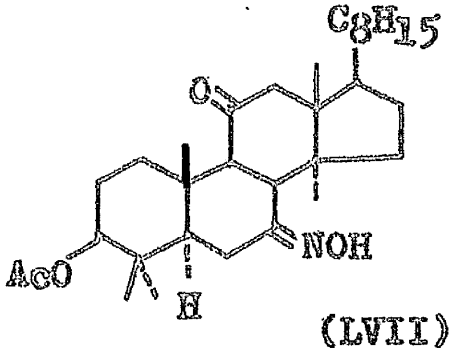
(LV)



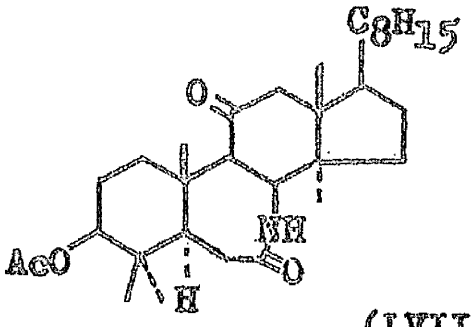
(LVI)

Beckmann Rearrangement of Hecogenin Acetate Oxime.

The Beckmann rearrangements of steroid C-(7),[61,62] C-(17)[63,64] and C-(12)[10] oximes have been reported. Barnes and his associates[61] and Falco et al.[62] found that rearrangement of the lanosterol derivative (LVII) yielded only one amide, to which they assigned the 7α-aza structure (LVIII). However, the related Δ^8-7-mono-oxime (steroid numbering) afforded the corresponding Δ^9-unsaturated 7-aza and 7α-aza compounds.

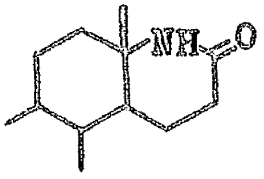


(LVII)

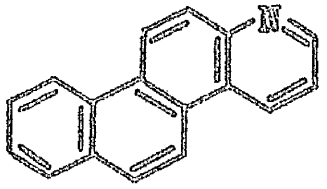


(LVIII)

Kaufmann[63] discovered that some 17-oximes of the androstane series were transformed to single amides, which he considered possessed the 17α-aza-D-homo structure (LIX) since the product of selenium dehydrogenation was 1-aza chrysene

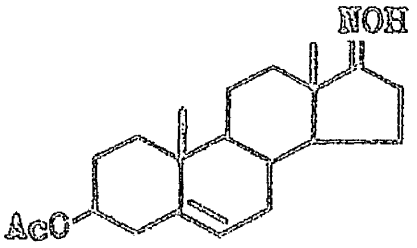


(LIX)

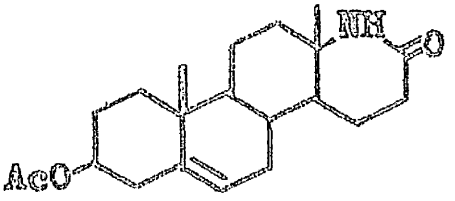


(LX)

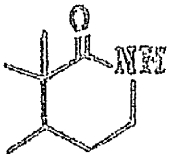
(LX). In contrast Heusser's group[64] reported that Beckmann reaction of 3β-acetoxy-androst-5-ene-17-oxime (LXI) provided both isomeric amides (LXII) and (LXIII) as a sharp-melting mixture.



(LXI)

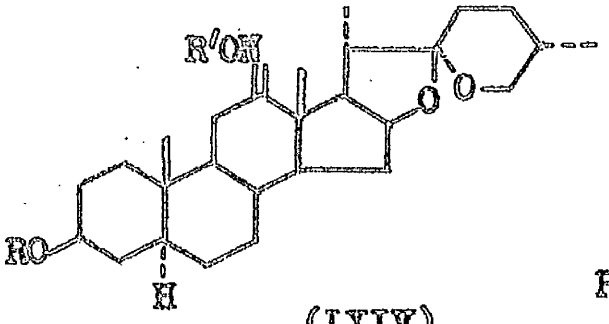


(LXII)

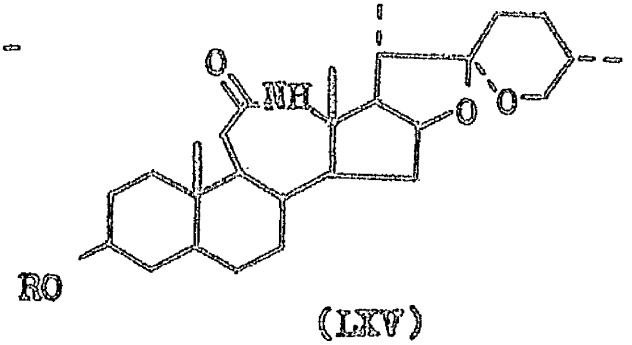


(LXIII)

In 1959 Mazur[10] obtained a single lactam (hecololactam acetate) by rearrangement of hecogenin acetate oxime (LXIV; R = Ac, R' = H) and tentatively formulated it as the 12a-aza-



(LXIV)



(LXV)

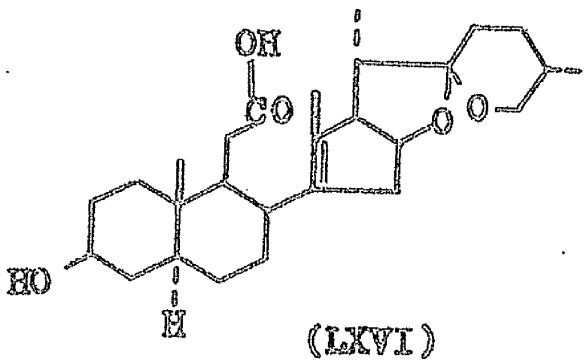
lactam acetate (LXV), since the related 9(11)-dehydrohecololactam acetate (LXXI; R = Ac) derived from 9(11)-dehydrohecogenin acetate oxime (LXX; R = Ac, R' = H) had a $\lambda_{max.}$ at 220 mμ, and was converted by hydrogenation to hecololactam acetate.

However, the possibility of the product being the isomeric 12-aza-lactam acetate , or a mixture, remained. Since the two isomeric lactones derived from hecogenin were now available Mazur's hypothesis could be tested by conversion of hecololactam into the corresponding lactone. According to White[65] the N-nitroso derivative of a substituted lactam or amide readily loses nitrogen on being heated gently, to yield a lactone or an ester. This reaction has already been used successfully in the steroid series by Sato and Latham[66] in the conversion of O,N-diacetyldihydrotomatidine and O,N-diacetyl-dihydrosolasodine into dihydroneotigogenin and dihydrotigogenin respectively.

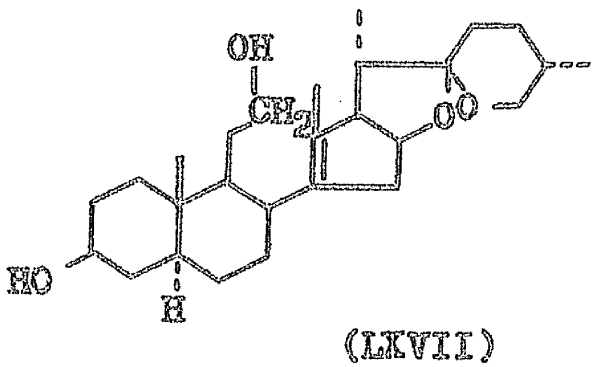
Hecogenin acetate oxime (LXIV; R = Ac, R' = H) was prepared under the usual conditions. Anliker et al.[67] previously obtained this compound but incorrectly assigned to it the structure of the corresponding alcohol (LXIV; R = R' = H Acetylation of either the oxime or oxime acetate produced a diacetate (LXIV; R = R' = Ac) also prepared by the Swiss workers. The Beckmann rearrangement of (LXIV; R = Ac, R' = H) with toluene-p-sulphonyl chloride in pyridine then provided hecololactam acetate.

Attempted nitrosation of hecololactam acetate by dinitrogen tetroxide in carbon tetrachloride (the reagent used

by Sato and Latham) was unsuccessful, starting material being recovered. However, treatment of the lactam acetate in acetic acid and acetic anhydride with sodium nitrite at 0° gave directly, without isolating the nitroso derivative, and without heating, hecololactone acetate (LXV; R = Ac) in 8-10% yield. The material in the mother liquors failed to crystallise, but when it was heated with methanolic potassium hydroxide, and the solution acidified, a crystalline acid was formed in 90% yield. This is formulated as 3β-hydroxy-12,13-seco-5α,25D-spirost-13-ene-12-oic acid (LXVI) (anhydrohecolic acid), since on reduction with lithium aluminium hydride, it gave the known 12,13-seco-5α,25D-spirost-13-ene-3β,12-diol (LXVII) (anhydrohecolyl alcohol).[54]



(LXVI)

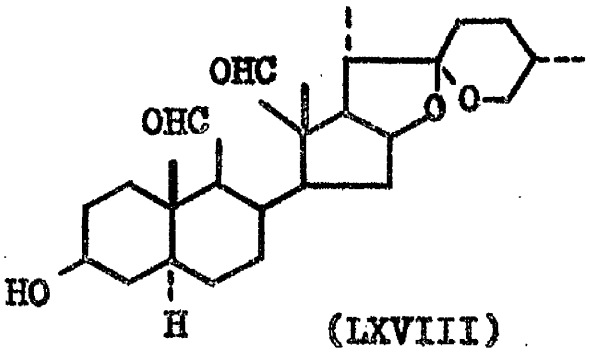


(LXVII)

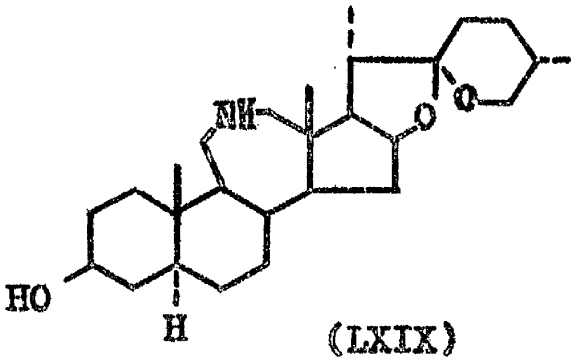
Since both the products of this simple denitrosation are derived (in a total 100% yield) from hecolic acid and not iso-hecolic acid, the C-(11)-C-(12) bond must remain intact in the reaction. It necessarily follows that hecololactam acetate has

the 12-aza structure (LXV; R = Ac). The possibility that the lactam was a mixture is also eliminated.

Recently a Syntex group[59] also proved that the 12a-aza structure is the correct one by reducing hecololactam acetate with lithium aluminium hydride for 10 days (also attempted in these laboratories, but for a much shorter time). This gave a C-homo-azatigogenin which was not identical with C-homo-12-azatigogenin (LXIX) obtained by reduction of the seco-dialdehyde (LXVIII) in ethanolic ammonia.



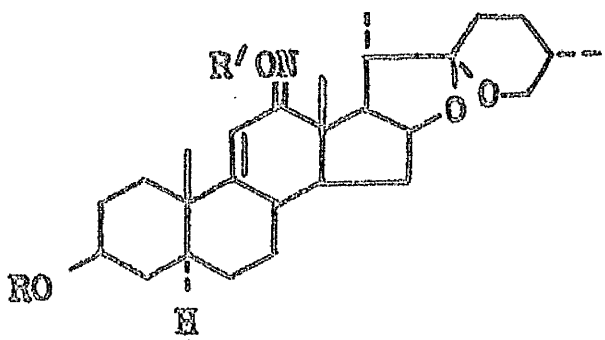
(LXVIII)



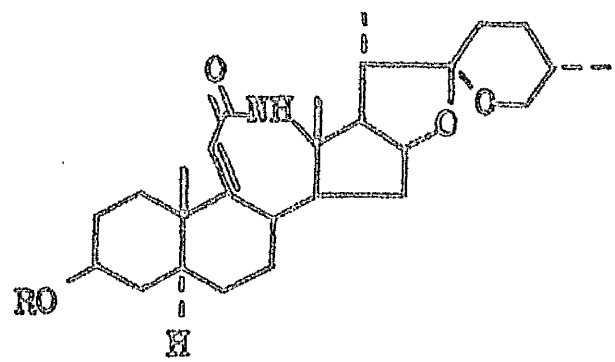
(LXIX)

The oxime of 9(11)-dehydrohecogenin acetate (LXX; R = Ac, R' = H) was rearranged in an analogous manner to give 9(11)-dehydrohecololactam acetate (LXXI; R = Ac), but in this case some of the intermediate oxime toluene-p-sulphonate (LXX; R = Ac R' = p-$C_6H_4Me.SO_2$) was isolated. On being heated with acidified ethanol this was readily converted to 9(11)-dehydro-hecololactam acetate (LXXI; R = Ac). Treatment of this with

the nitrosating reagent used previously caused no reaction.



(LXX)



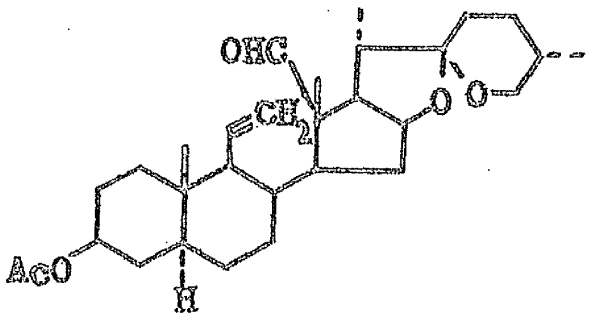
(LXXI)

Action of Ultraviolet Light on Hecogenin Acetate.

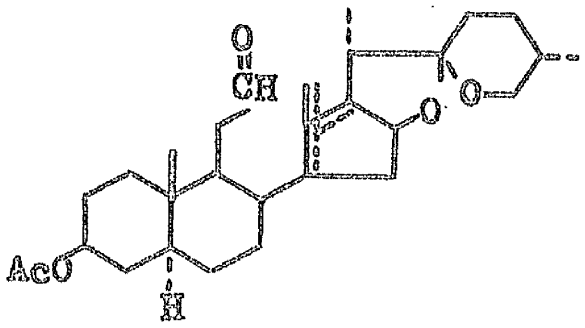
Ultraviolet irradiation of a solution of hecogenin acetate in dioxan, under nitrogen, produced a change in the specific rotation. After periods of 20-28 hours this was constant at a value of -40° and evaporation, then crystallisation, provided a new compound, lumihecogenin acetate, in good yield. This was shown to be homogenous by chromatography and recrystallisation.

The results of analyses agreed with those of the starting material, indicating isomerism, and the infrared spectrum (see Fig.I), with maxima at 1739, 1709 and 1240 cm.$^{-1}$ showed that the acetate and carbonyl functions were present. The fingerprint region possessed the peaks characteristic of a 25D-sapogenin, proving that the side-chain was unaltered, and there was a low intensity peak at 2740 cm.$^{-1}$, which indicated that the carbonyl function was aldehydic. Lumihecogenin acetate had an isolated double bond, as proved by the $\lambda_{max.}$ at 204 mμ and the yellow colour obtained with tetranitromethane in chloroform.

It was apparent that ring-C of hecogenin acetate had been split, forming either the 11,12-seco compound (LXXII) or the isomer (LXXIII) (cf. Rothman *et al.*[54]). The first structure can be rejected, because reduction of lumihecogenin acetate with lithium aluminium hydride provided, as the sole product,

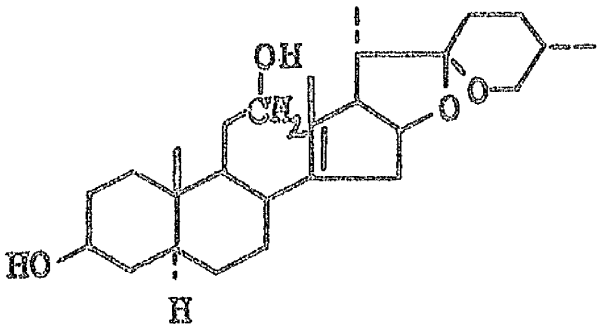


(LXXII)

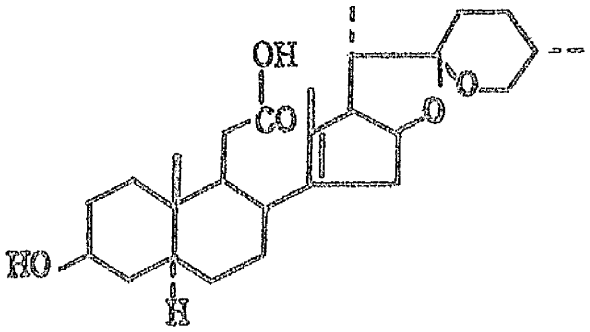


(LXXIII)

anhydrohecolyl alcohol, which has been proved[54] to be the 12,13-seco compound (LXVII). Furthermore, oxidation of lumihecogenin acetate with chromium trioxide in sulphuric acid, followed by hydrolysis of the product, provided (in part) anhydrohecolic acid (LXVI).



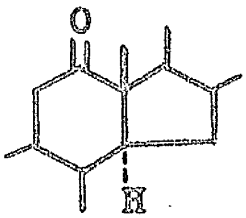
(LXVII)



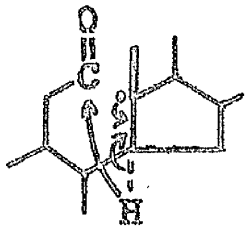
(LXVI)

Lumihecogenin acetate displayed a plain optical rotatory dispersion curve. This is more consistent with structure (LXXI than with structure (LXXII), in which the aldehyde group is directly attached to an asymmetric centre.[55]

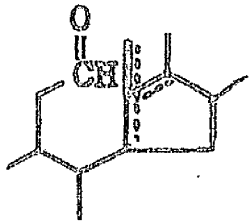
This type of photochemical reaction, in which a cyclic ketone is transformed to a seco unsaturated aldehyde, has not been reported in the steroid series. In this case it must involve the formation of the biradical (LXXIV) which can, by abstraction of the hydrogen atom attached to C-(14), rearrange



(VIII)

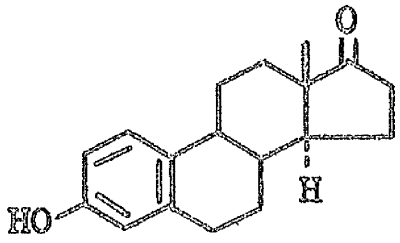


(LXXIV)

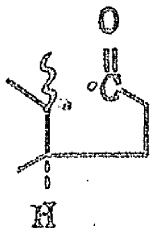


(LXXV)

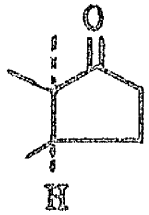
to form the aldehyde (LXXV). Study of a Dreiding model of (LXXIV) shows that the distance from C-(12) to the hydrogen atom attached to C-(14) is only 1.24Å, i.e. very nearly the normal C-H bond distance (1.09Å). In the analogous case of the irradiation of oestrone[69] (LXXVI) to form lumioestrone (LXXVIII) the ends of the intermediate biradical (LXXVII) merely rejoin, producing inversion at C-(13). This may be because



(LXXVI)



(LXXVII)

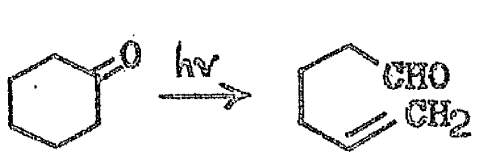


(LXXVIII)

the distance from C-(17) to the hydrogen at C-(14) is 1.84Å (measured from the Dreiding model) and it cannot easily be

captured.

Examples of seco unsaturated aldehyde formation from simpler cyclic ketones are given by the formation of hex-5-enal (LXXX) from cyclohexanone (LXXIX),[70] and campholenaldehyde (LXXXII) from camphor (LXXXI).[71] It will be noticed that in (LXXIV) and (LXXXII) fission has occurred between the carbonyl

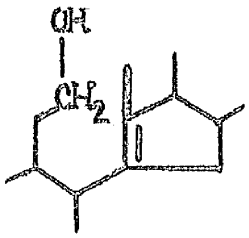


(LXXIX) (LXXX)

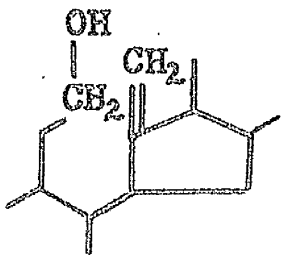
(LXXXI) (LXXXII)

group and the carbon having more substituents.

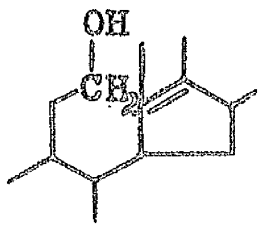
The double bond in lumihecogenin acetate must be in the same position as it is in other members of the anhydrohecolic series. Rothman and his colleagues considered that there were three possible structures for anhydrohecolyl alcohol, viz. (LXVII) (Δ^{13}-double bond), (LXXXIII) ($\Delta^{13(18)}$-double bond), or LXXXIV ($\Delta^{13(17)}$-double bond). In view of the resistance of



(LXVII)



(LXXXIII)



(LXXXIV)

anhydrohecolyl alcohol to hydrogenation and oxidation and the fact that there was no infrared absorption due to an exocylic methylene group, they rejected structure (LXXXIII). In addition since the double bond formed in both ionic and photochemical reactions is in the same position, it is reasonable to exclude other possible positions for it, e.g. $\Delta^{9(14)}$ [Ξ] and Δ^{14}, which would require two separate stages for both reactions.

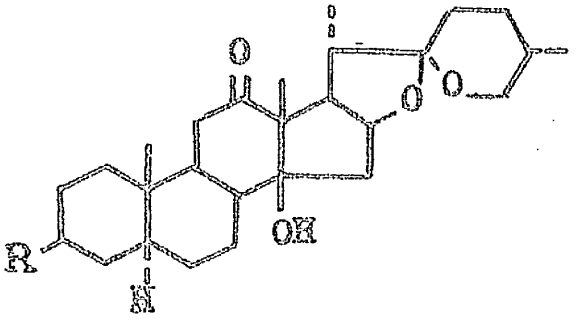
Some additional evidence which helps to distinguish between the remaining alternative structures (LXVII) and (LXXXIV) was obtained from the oxidation of lumihecogenin acetate with chromium trioxide. The major product was neutral, contained no double bond and had an elementary analysis corresponding to an empirical formula $C_{29}H_{44}O_6$. The infrared spectrum (in carbon tetrachloride) showed peaks at 3500 (hydrogen bonded hydroxyl group), 1739 (3β-acetate) and 1706 cm.$^{-1}$ (carbonyl group in open chain, or in a 6- or 7-membered ring) and was very similar to that of hecogenin acetate. The hydroxyl group was tertiary, as proved by the recovery of the material after attempted acetylation. It is reasonable to assume that the compound is hecogenin acetate, or a steroisomer, with an additional hydroxyl

[Ξ] Anhydrohecolyl alcohol does not give a colour in the Tortelli-Jaffe test [54], which is usually positive for this double bond position.

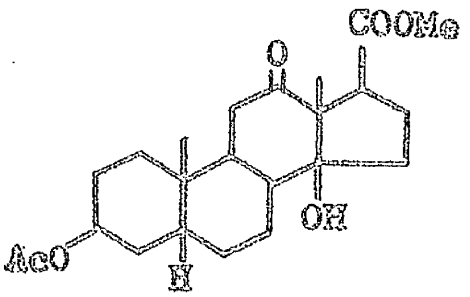
group.

Bearing in mind the two possibilities for the position of the double bond in lumihecogenin acetate there are four possible positions for the hydroxyl group, viz. 14α, 14β, 17α, and 17β. The infrared spectra of the oxidation product and the corresponding 3-ketone in carbon tetrachloride show that the hydroxyl group is weakly hydrogen bonded. However, this bond cannot involve the carbonyl group, which has its frequency in the normal position. The bonding must therefore be to the ring-E oxygen atom of the sapogenin side-chain, and this is possible only if the hydroxyl group is in the 14β-configuration. Accordingly, the oxidation product is 3β,14β-dihydroxy-12-oxo-5α,25D-spirostan-3β-yl acetate (LXXXV ; R= OAc)

The optical rotatory dispersion curve of this substance showed a positive Cotton effect (amplitude + 158). This is consistent with the formula (LXXXV;R=OAc) since Djerassi et al.[72] obtained a similar curve (amplitude + 130) for the 14β-hydroxy-12-oxo steroid (LXXXVI).

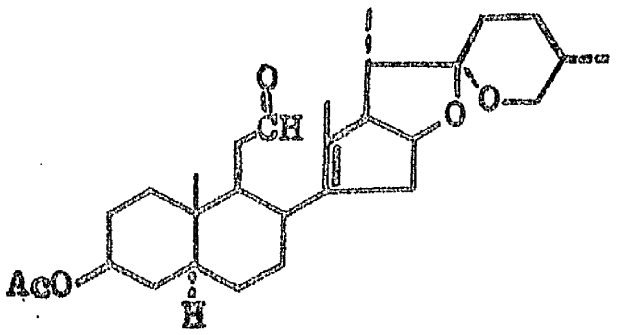


(LXXXV)



(LXXXVI)

Lumihecogenin acetate must then have the structure (LXXXVII).

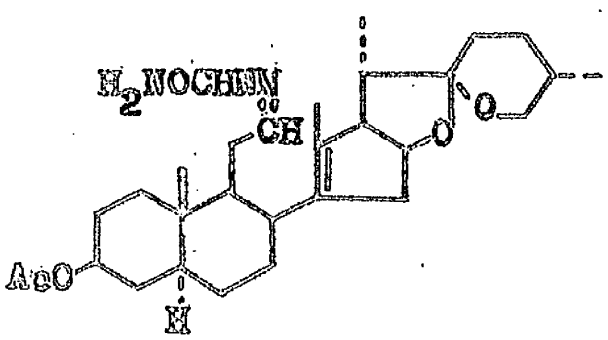


(LXXXVII)

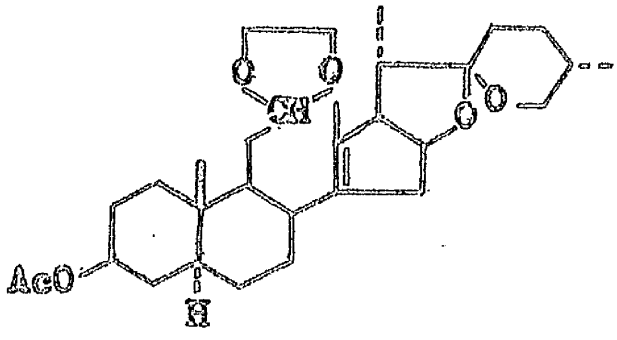
The nuclear magnetic resonance spectrum of lumihecogenin acetate (Fig.II) confirms the presence of the aldehyde group, with a distinctive peak at 0.7τ . Reference to the integrate curve indicates that the multiplets with centres at 5.5τ and 6.7τ are each derived from two protons. The absence of a sharp band at 5.3τ rules out a structure containing an exo-cyclic methylene group, and also a Δ^{14}- structure. If, as is thought, lumihecogenin acetate possesses a Δ^{13}-double bond there should be a peak (C-18 CH_3 group) near 8.3τ, and this is probably incorporated in the acetate peak at 8.0τ . The maximum at 9.2τ represents the protons of the C-19 methyl grou along with the side-chain methyl protons.

Lumihecogenin acetate was sensitive to alkali, hydrolysis providing an intractable gum, and attempted formation of the oxime, using pyridine as solvent, was unsuccessful. However, in slightly acid media it was more stable and the semicarbazon

(LXXXVIII) and ethylene acetal (LXXXIX) were prepared.



(LXXXVIII)



(LXXXIX)

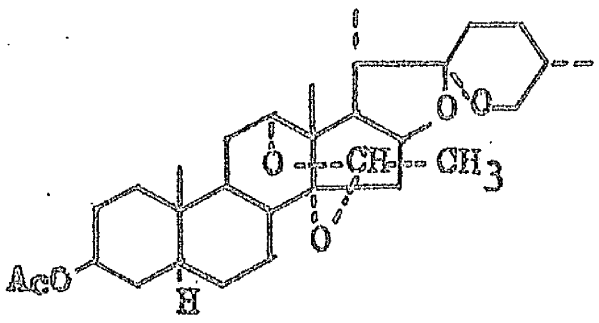
When the period of irradiation of hecogenin acetate was extended to 40-48 hours the specific rotation of the solution remained almost constant at its previous value. However, the neutral material obtained after solvent removal provided, on addition of methanol, a new crystalline substance, m.p. 203-206°. This compound, photohecogenin acetate, was isolated in 25% yield and usually the mother liquors contained a little lumihecogenin acetate.

The results of analyses of photohecogenin acetate agreed with an empirical formula $C_{31}H_{48}O_6$, which requires the addition of a C_2H_4O moiety to hecogenin acetate. It was saturated and the infrared spectrum had peaks at 1740 and 1250 cm.$^{-1}$, representing the acetate group, and the usual spirostan side-chain maxima (see Fig.I). Alkaline hydrolysis proceeded smoothly to furnish photohecogenin, which exists in two crystalline forms.

It was reasonable to assume that lumihecogenin acetate was the precursor of photohecogenin acetate, and this was confirmed by irradiating a dioxan solution of the former compound for 20 hours, whereby a good yield of photohecogenin acetate was obtained. However, a shorter period of irradiation did not effect any change.

It is assumed that photohecogenin acetate is formed from an activated form of the seco-aldehyde, which then attacks the solvent or its normally occurring impurity, 2-methyl-1,3-dioxolan It seems likely that it is the latter which react to provide photohecogenin acetate, since irradiation of hecogenin acetate in specially purified dioxan produced an exceptionally low yield of photohecogenin acetate, compatible with the calculated amount of 2-methyldioxolan present.

The structure of this new substance is at the moment uncertain, but the formula (XC) is tentatively put forward as satisfying the available experimental evidence. Zeisel estimation on photohecogenin acetate produced two molar equivalen



(XC)

of ethyl iodide, one of which is derived from the acetate group at C-(3). The other would be formed from the acetal grouping. Contrary to expectation, however, hydrolysis of photohecogenin acetate with mineral acid did not provide any acetaldehyde.

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

Unless otherwise stated, optical rotations were determined for chloroform solutions, ultraviolet spectra were obtained with ethanolic solutions, and infrared spectra with Nujol mulls. M.p.s were determined on a Kofler block.

The alumina used for chromatography was neutralised and deactivated with 10% aqueous acetic acid (5 ml. per 100g.), and the solvents used for elution were not previously dried. Light petroleum refers to that fraction of b.p. range 60-80°.

"Working up in the usual way" means addition of water and extraction with ether. The ethereal extracts are then washed with 3N-hydrochloric acid, water, aqueous potassium hydrogen carbonate and water, before being dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure.

12-Methyl Steroids

3β-(Tetrahydro-2-pyranyloxy)-5α,25D-spirostan-12-one
3β-(Tetrahydro-2-pyranyloxy)-5α,25D-spirostan-12-one (VIII;R=C_5H_9O). Hecogenin acetate (VIII;R=Ac) (57g.) was refluxed with potassium hydroxide (35g.) in methanol (250 ml.) and dioxan (500ml.) for 1.5hr. Water (3 l.) was added and the precipitated hecogenin (VIII;R=H) was filtered off and dried at 60° (55g., m.p.260-262°). Part of this (48g.), suspended in dry benzene (400ml.), was treated with 2,3-dihydropyran (40ml.) and phosphorus oxychloride (1.5ml.). After 10 min. with slight warming the mixture became clear and was set aside for 1hr. Ether (1 l.) was added and the mixture was washed with potassium hydrogen carbonate solution, dried, and evaporated under reduced pressure to give the tetrahydropyranyl ether (56g., 99%) as needles (from methanol containing a little pyridine), m.p.195-200°. Hirschmann et al.[33] record m.p.209-213°.

Preparation of methyl-lithium.- A typical preparation used lithium (10g.) cut in fine slices, in ether (30ml.) contained in a round bottomed flask fitted with a dropping funnel and a reflux condenser. The mixture was magnetically stirred during the addition of methyl iodide (40ml.) in ether (450ml.) over 30 min., and was then refluxed for 30 min. and allowed to cool. The strength of the solution was determined by adding 5ml. portions to water and titrating

the lithium hydroxide formed against standard hydrochloric acid with methyl orange as indicator. The reagent was freshly prepared for the reaction and transferred to the reaction flask by decantation. It was possible to use a solution which had been kept overnight in the refrigerator but after longer periods it became too weak.

12α-Methyl-3β-(tetrahydro-2-pyranyloxy)-5α,25D-spirostan-12β-ol(IX; R=C_5H_9O).- The tetrahydropyranyl ether of hecogenin(VIII; R=C_5H_9O) (50g.) in benzene (400ml.) was treated with an ethereal solution of methyl-lithium prepared from lithium (15g.), methyl iodide (60ml.) and ether (500ml.) and the mixture was refluxed for 1.5hr. Excess of reagent was destroyed with methanol, dilute hydrochloric acid was added, and the organic layer separated. The aqueous layer was extracted with ether. The combined extracts were washed with water and aqueous potassium hydrogen carbonate, dried, and evaporated to yield a white solid (51.9g.) which was used directly for the next step. A portion of similar material was recrystallised twice from methylene chloride-methanol containing pyridine to give the tetrahydropyranyl ether (IX; R=C_5H_9O), m.p.205-207°,$[\alpha]_D$-38.2°(c 0.872) (Found: C, 72.8; H, 10.4. $C_{33}H_{54}O_5.CH_3OH$ requires C, 72.55; H, 10.4%).

Hydrolysis of the Tetrahydropyranyl Ether (IX; R=C_5H_9O).-(a) With mineral acid. The ether (51.2g.) was refluxed

with 6N-hydrochloric acid (140ml.) in ethanol (1200ml.) for 40 min. Water was added and the product isolated by ether extraction. The extracts were washed with aqueous potassium hydrogen carbonate, dried, and evaporated to yield a yellow semi-solid residue (38.4g.). This was treated with pyridine (300ml.) and acetic anhydride (200ml.) and left overnight. Working up in the usual way by ether extraction gave a semi-solid residue (42g.) which on addition of methanol provided crystalline material m.p.210-218° (28g.). Further crops were obtained by concentration of the mother liquors but these were wide melting and the entire material was chromatographed in light petroleum-benzene (1:6; 350ml.) on alumina (2.5 Kg.).

Benzene eluted material, m.p.152-172° (8.1g.). Several recrystallisations from methylene chloride-methanol afforded 12-methylene-5α,25D-spirostan-3β-yl acetate (X; R=Ac) as plates, m.p.182-184°, $[\alpha]_D$-28°(c 1.0) (Found: C, 76.8; H, 9.6. $C_{30}H_{46}O_4$ requires C, 76.55; H, 9.85%), $\lambda_{max.}$ 202mμ(ε 3000), $\nu_{max.}$ 1739(OAc), 1653(>C=CH_2), 1242(OAc) and 978, 914, 897, 861cm.$^{-1}$(spirostan system). This material gave a light yellow colour with tetranitromethane in chloroform. Levine and Wall[29] report m.p.178-180°, $[\alpha]_D$-24.1°. The non-crystalline mother liquor material (3.5g.) on chromatography gave amorphous fractions with $\lambda_{max.}$ 202(ε 2000) and 240mμ(ε 1500).

Benzene-ether, and ether, eluted 3β-acetoxy-12α-methyl-

5α,25D-spirostan-12β-ol (IX; R=Ac) (34g.), m.p. in the range 215-224°. A pure sample, recrystallised from chloroform-methanol, formed rods, m.p.226-228°, $[\alpha]_D$-47°(c 1.0) (Found: C, 73.7; H, 9.7. $C_{30}H_{48}O_5$ requires C, 73.7; H, 9.9%), $\nu_{max.}$ 1750 and 1230cm.$^{-1}$(OAc) and the typical spirostan bands. Levine and Wall[29] record m.p.220°, $[\alpha]_D$-47°.

(b) With aqueous acetic acid. The tetrahydropyranyl ether (IX; R=C_5H_9O) (5.5g.) was refluxed with ethanol (180ml.), acetic acid (40ml.) and water (20ml.) for 45 min. Water (1.5 l.) was added and the precipitated solid was filtered off, washed with water, dried, and acetylated with acetic anhydride (10ml.) in pyridine (20ml.) at 100° for 1 hr. The product (4.8g.) was isolated by the addition of water and extraction with ether in the usual way, and chromatographed on alumina (500g.). Light petroleum eluted material (430mg.), m.p.145-182°, which was rechromatographed (see below). Light petroleum-benzene, and benzene, eluted 3β-acetoxy-12α-methyl-5α,25D-spirostan-12β-ol (IX; R=Ac) (3.8g.), m.p.218-223°, $[\alpha]_D$-43°(c 1.0). The material in the light petroleum eluates was rechromatographed on alumina (40g.). Light petroleum-benzene (7:3) eluted tigogenin acetate (84mg.), m.p. and mixed m.p.204-207° (no ultraviolet absorption). Light petroleum-benzene (1:1) eluted material (85mg.) which crystallised from methanol to give needles, m.p.150-152°, $\lambda_{max.}$ 238mμ(ε 12,000). These gave

no m.p. depression with 12-methylene-9(11)-dehydrotigogenin acetate (XXV; R=Ac) (see below). This material is derived from 9(11)-dehydrohecogenin acetate, which is present along with tigogenin acetate in the hecogenin acetate as an impurity (cf. [36]).

Action of Methyl-lithium on Hecogenin Acetate.- Hecogenin acetate (VIII; R=Ac) (5g.) in ether (100ml.) and benzene (80ml.) was treated with a solution of methyl-lithium prepared from lithium (1.6g.), methyl iodide (6ml.) and ether (100ml.), and refluxed for 3 hr. The product, isolated by ether extraction (5.6g.), was acetylated at room temperature overnight. Chromatography on alumina (750g.) allowed the isolation of hecogenin acetate (110mg.), m.p.243-247°, and 3β-acetoxy-12α-methyl-5α,25D-spirostan-12β-ol, (IX; R=Ac), m.p.220-222°, as the only crystalline products. A portion of the remainder (2g.) was refluxed in ethanol (80ml.) and acetic acid (8ml.) with Girard's reagent-T (1g.) for 1 hr., but was recovered on pouring the solution into aqueous potassium hydrogen carbonate.

Treatment of the Tetrahydropyranyl Ether of Hecogenin with Methylenetriphenylphosphorane.- The preparation of 12-methylenetigogenin by Sondheimer and Mechoulam's method[34] failed. The following modification was therefore devised.

A solution of the Wittig reagent was prepared by stirring

methyltriphenylphosphonium bromide (1g.) and an ethereal solution of phenyl-lithium (0.45N; 6.5ml.) for 0.5 hr. Then the tetrahydropyranyl ether (VIII; R=C_5H_9O) (300mg.) was added. Next morning the ether was distilled off while an equal volume of dry tetrahydrofuran was added. The solution was then refluxed for 6 hr. Working up in the usual way afforded crude tetrahydropyranyl ether of 12-methylenetigogenin (X; R=C_5H_9O), m.p.170-172°. This was refluxed with ethanol (40ml.) and 6N-hydrochloric acid (4ml.) for 50 min. Dilution with water and extraction with ether gave a solid, m.p.215-240°, $\lambda_{max.}$ 202mμ(ε 1000). This was acetylated with acetic anhydride (4ml.) and pyridine (6ml.) overnight at room temperature. Working up in the usual way afforded a semi-solid product (430mg which was chromatographed on alumina (25g.). Light petroleum-benzene (5:1) eluted 12-methylenetigogenin acetate (X; R=Ac) as platelets (from methylene chloride-methanol), m.p.178-181°, $\lambda_{max.}$ 203mμ(ε 3000) (35mg.). This material showed no depression in m.p. on admixture with the material obtained by the action of methyl-lithium on the tetrahydropyranyl ether of hecogenin. Light petroleum-benzene (1:1) eluted hecogenin acetate, m.p. and mixed m.p.244-247° (170mg.). The ether eluates provided material (85mg.) containing phosphorus (detected by the ammonium molybdate-benzidine test).

12-Methylene-5α,25D-spirostan-3β-ol.- 12-Methylenetigogenin

acetate (X; R=Ac) obtained by the methyl-lithium method (200mg.) was hydrolysed by potassium hydroxide (2g.) in boiling methanol (25ml.) and dioxan (25ml.) for 30 min. Extraction with ether gave 12-methylene-5α,25D-spirostan-3β-ol (X; R=H) as needles (from methylene chloride-methanol), m.p.231-233°, $[\alpha]_D$+25°(c 1.6) (Found: C, 76.9; H, 10.2. Calc. for $C_{28}H_{44}O_3$, $CH_3.OH$: C, 77.0; H, 10.4%), $\lambda_{max.}$202mμ(ε 1700), $\nu_{max.}$3250(OH), 1640cm.$^{-1}$(>C=CH_2). Sondheimer and Mechoulam record m.p.233-235°, $[\alpha]_D$+20°. Treatment of this material with benzoyl chloride in pyridine gave the benzoate (X; R=Bz) as prisms (from methanol), m.p.214-216°, $[\alpha]_D$-22.8°(c 0.78) (Found: C, 78.0; H, 9.1. $C_{35}H_{48}O_4$,0.5H_2O requires C, 77.6; H, 9.1%).

Attempts to dehydrate 3β-Acetoxy-12α-methyl-5α,25D-spirostan-12β-ol (IX; R=Ac).- (a) The acetate (150mg.) in pyridine (3ml.) was treated with phosphorus oxychloride (0.3ml.) and left for 24 hr. Addition of water and extraction with ether in the usual way gave starting material, m.p. and mixed m.p.225-227°(120mg.).

(b) The acetate (500mg.) in pyridine (20ml.) was treated with purified thionyl chloride (2ml.). After 3 hr. the brown solution had deposited a solid. Addition of water and working up by ether-extraction in the usual way gave a light yellow resin (450mg.). This was chromatographed on alumina (30g.). Light petroleum eluted crystalline material (50mg.) which on

recrystallisation from methanol gave needles, m.p.164-167°. This material contained chlorine and had no ultraviolet light absorption (Found: C, 75.95; H, 9.7%).

(c) The acetate (50mg.) in ethanol (15ml.) containing 6N-hydrochloric acid (1.5ml.) was refluxed for 40 min. Addition of water and filtration gave largely unchanged starting material which, however, had $\lambda_{max.}$ 205mμ(ε 700).

Hydrolysis of 3β-Acetoxy-12α-methyl-5α,25D-spirostan-12β-ol.- The acetate (IX; R=Ac) (700mg.) in dioxan (25ml.) and methanol (25ml.) was treated with potassium hydroxide (2g.), and the mixture refluxed for 2 hr. The product isolated by dilution with water and ether-extraction was a colourless syrup (675mg.) which crystallised when triturated with pyridine. Recrystallisation from ether containing a little pyridine afforded the pyridine solvate of 12α-methyl-5α,25D-spirostan-3β,12β-diol (IX; R=H) as needles, m.p.210-213° with changes of crystalline form at 130° and 190° approx., $[\alpha]_D$-37.1°(c 0.57) (Found, in material dried in vacuo at room temperature: C, 75.4; H, 10.2; loss in weight at 130° in vacuo, 15.3. $C_{28}H_{46}O_4.C_5H_5N$ requires C, 75.4; H, 9.8; loss in weight, 15.2% . Found, in material dried at 130° in vacuo for 4 hr.: C, 75.3; H, 10.2. $C_{28}H_{46}O_4$ requires C, 75.3; H, 10.4%), $\lambda_{max.}$ 252(ε 2000), 257mμ(ε 2180). Compare the light absorption of pyridine: $\lambda_{max.}$ 251(ε 2460), 257(ε 2670) and 263mμ(ε 1800). Levine and

Wall quote m.p.197-198°, $[\alpha]_D$-36.8° for the unsolvated material. Barton *et al.*[37] give m.p.197-199°, $[\alpha]_D$-37°.

Benzoylation of this material gave the 3β-benzoate (IX; R=Bz) as needles (from methylene chloride-methanol), m.p.209-212°, $[\alpha]_D$-35.5° (*c* 0.743) (Found: C, 76.0; H, 9.4. $C_{35}H_{50}O_5$ requires C, 76.3; H, 9.15%). Levine and Wall report m.p.192-194°, $[\alpha]_D$-38°.

12α-Chloro-12β-methyl-5α,25D-spirostan-3β-yl Acetate (XIV).- 12β-Hydroxy-12α-methyltigogenin acetate (IX; R=Ac) (5g.) in dry ether (500ml.) was treated with phosphorus pentachloride (5.2g.) with intermittent stirring for 48 hr. The solution was washed with 5% aqueous potassium hydroxide, dried, and evaporated. The residue (5g.) was adsorbed on alumina (250g.). Elution with light petroleum-benzene (5:1) gave 12α-chloro-12β-methyl-5α,25D-spirostan-3β-yl acetate (XIV) (2g.), as needles (from methanol), m.p.202-204°, $[\alpha]_D$-40° (*c* 1.0) (Found: C, 70.9; H, 9.5; Cl, 5.8. $C_{30}H_{47}ClO_4$ requires C, 71.0; H, 9.3; Cl, 7.0%), $\nu_{max.}$ 1739 and 1242cm.$^{-1}$ (OAc) (no hydroxyl band). In another preparation, worked up after 1.5 hr., material m.p.217-218° was isolated [Found: C, 73.1; H, 9.9; Cl, 0.6. Calc. for a 9:1 mixture of $C_{30}H_{48}O_5$ (IX; R=Ac) and $C_{30}H_{47}O_4Cl$ (XIV): C, 73.45; H, 9.85; Cl, 0.7%]. Other mixtures of the chloro compound (XIV) and starting material were sometimes obtained and could not be separated

by chromatography . Levine and Wall report m.p.189-194°, $[\alpha]_D$-40° for a 12-chloro,12-methyltigogenin acetate.

Action of alkali on 12α-chloro-12β-methyl-5α,25D-spirostan-3β-yl acetate.- (a) The acetate (XIV) (95mg.) in methanol was refluxed with potassium hydroxide (200mg.) for 16 hr. Water was added and the product isolated in the usual way as a white solid containing no chlorine, m.p.212-220° (85mg.). This was acetylated overnight and the product chromatographed on alumina. Light petroleum-benzene eluted material (22mg.) which was crystallised from methanol to give a substance m.p.160-170° (Found: OMe, 1.55%). This may be attributed to methanol of crystallisation,

(b) Sodium Methoxide.- The chloro-compound (XIV) (500mg.) was refluxed with sodium methoxide (1g.) in methanol for 24 hr. Addition of water and ether-extraction gave a white solid. Crystallisation of this from methanol gave 12-methylenetigogenin (X; R=H) (200mg.), m.p. and mixed m.p.230-232°. Acetylation of several crops abtained from the mother-liquors gave 12-methylenetigogenin acetate (X; R=Ac) (20mg.), m.p. and mixed m.p.180-182°.

12β,12'-Epoxy-12α-methyl-5α,25D-spirostan-3β-yl Acetate (XVI).- 12-Methylenetigogenin acetate (800mg.) in dry benzene (45ml.) was treated with perbenzoic acid (500mg.) in benzene (8.3ml.), and the solution was kept at room temperature for

46 hr. The solution was washed with aqueous sodium hydroxide and water, dried, and evaporated. The product was chromatographe on alumina (50g.). Light petroleum-benzene (7:3) eluted impure starting material (320mg.), m.p.166-170°, $\lambda_{max.}$202mμ(ε 500). Light petroleum-benzene (1:1) eluted material (180mg.), m.p. 195-208°, which was not examined further. Benzene, and ether, eluted 12β,12′-epoxy-12α-methyl-5α,25D-spirostan-3β-yl acetate (XVI) (156mg.), which crystallised as needles (from methanol), m.p.240-242°, $[\alpha]_D$-24°(c 1.2) (Found: C, 74.3; H, 9.75. $C_{30}H_{46}O_5$ requires C, 74.0; H, 9.5%), $\nu_{max.}$1739 and 1250cm.$^{-1}$ (OAc). This compound showed no light absorption in the ultra-violet region.

Reduction of the Epoxide (XVI) with Lithium Aluminium Hydride.- The epoxide (XVI) (50mg.) in dry tetrahydrofuran (30ml.) was refluxed with lithium aluminium hydride (100mg.) for 3.5 hr. Cautious addition of water, followed by hydrochloric acid, and extraction with ether gave a syrup which crystallised only on addition of a drop of pyridine. Crystallisation from methanol containing pyridine gave crystals of 12α-methyl-5α,25D-spirostan-3β,12β-diol (IX; R=H) as the pyridine solvate, m.p. and mixed m.p.210-212° (change of form at 130°). The infrared spectrum was identical with that of an authentic specimen. A portion (20mg.) was acetylated with acetic anhydride and pyridine at 100° for 1 hr. After working up in the usual

way with ether there resulted 3β-acetoxy-12α-methyl-5α,25D-spirostan-12β-ol, m.p. and mixed m.p.223-226°.

3βAcetoxy-5α,25D-spirost-9(11)-en-12-one (XXIV; R=Ac).- This was prepared according to the method of Djerassi et al.[39] Hecogenin acetate (30g.) in acetic acid (900ml.) containing hydrobromic acid (1ml.) was heated to 35° and bromine (8ml.) in acetic acid (100ml.) was added with stirring over 10 min. After 2 hr. at room temperature water (3 l.) was added and the dibromo derivative was isolated by ether-extraction in the usual way. This was a yellow semi-solid (33g.) and it was refluxed in γ-collidine (350ml.) for 1 hr. and left overnight. The product was obtained by ether-extraction and the combined extracts were washed several times with dilute hydrochloric acid. Evaporation of the ether provided a brown solid (31g.) which was dissolved in ethanol (1.3l.) and refluxed with activated zinc dust (180g.) for 65 hr. The zinc was filtered off and the steroid isolated by adding water and extracting with ether. The material obtained after solvent evaporation (30g.) was dissolved in benzene (100ml.) and adsorbed on alumina (1200g.). Benzene, and benzene-ether, eluted mixtures of the saturated and unsaturated acetates. Ether eluted 9(11)-dehydrohecogenin acetate (XXIV; R=Ac), m.p.218-220°, $\lambda_{max.}$ 238mμ(ε 11,500). Djerassi's group[39] reports m.p. 218-220°, $\lambda_{max.}$ 238mμ(ε 12,000).

In an attempt to separate the mixture of hecogenin acetate and 9(11)-dehydrohecogenin acetate obtained above a sample (5g.) in chloroform (75ml.) and acetic acid (30ml.) was treated with hydrogen peroxide (3ml.; 30% $^{v}/_{v}$) and concentrated sulphuric acid (0.1ml.) for 8 days at room temperature. The chloroform was washed with water and then steam-distilled. The solid obtained was roughly dried and refluxed with 4% methanolic potassium hydroxide (150ml.) and water (10ml.) for 1.5 hr. Water was added and the neutral steroidal material was extracted with ether in the usual way. This had m.p.205-218°, $\lambda_{max.}$ 238mμ(ε 8000) (1.11g.). The acid product of the saponification was recovered by acidification of the aqueous layer, followed by filtration. This was reasonably pure hecolic acid, m.p.(188-195)250-258°, $\lambda_{max.}$ 206mμ(ε 260) (2.6g.).

3β-(Tetrahydro-2-pyranyloxy)-5α,25D-spirost-9(11)-en-12-one (XXIV; R=C_5H_9O).- Saponification of 9(11)-dehydrohecogenin acetate provided the free alcohol, m.p.223-225°. This material (4.5g.) was suspended in benzene (30ml.) and treated with 2,3-dihydropyran (3ml.) and phosphorus oxychloride (0.5ml.). After 1 hr. at 20°, the product was isolated by extraction with ether in the usual way. Crystallisation from ether-isopentane gave platelets of 3β-(tetrahydro-2-pyranyloxy)-5α,25D-spirost-9(11)-en-12-one (XXIV; R=C_5H_9O), m.p.174-178°, $[\alpha]_D$-7°(c 1.01) (Found: C, 74.7; H, 9.7. $C_{32}H_{48}O_5$ requires

C, 75.0; H, 9.4%), $\lambda_{max.}$ 238mμ(ε 13,330).

12α-Methyl-3β-(tetrahydro-2-pyranyloxy)-5α,25D-spirost-9(11)-en-12β-ol (XXVI; R=C_5H_9O). - The foregoing tetrahydropyranyl ether (XXIV; R=C_5H_9O) (1.2g.) in ether (50ml.) was refluxed with 0.42N-ethereal methyl-lithium (32ml.) for 6 hr. and then left overnight. Addition of methanol, water, and dilute acid, and extraction with ether in the normal way, afforded a yellowish syrup. Recrystallisation from methylene chloride-methanol gave 12α-methyl-3β-(tetrahydro-2-pyranyloxy)-5α,25D-spirost-9(11)-en-12β-ol (XXVI; R=C_5H_9O) as prisms, m.p.182-186°, $[\alpha]_D$-61°(c 1.43) (Found: C, 74.3; H, 9.8. $C_{33}H_{52}O_5$ requires C, 75.0; H, 9.9%).

12-methylene-5α,25D-spirost-9(11)-en-3β-ol (XXV; R=H).- The tetrahydropyranyl ether (XXVI; R=C_5H_9O) (1.1g.) was refluxed for 2 hr. with acetic acid (10ml.) in ethanol(90ml.). Addition of water and extraction with ether in the usual way gave 12-methylene-5α,25D-spirost-9(11)-en-3β-ol (XXV; R=H) (940mg.) as needles (from methylene-chloride-methanol), m.p. 204-205°, $[\alpha]_D$+41°(c 1.33) (Found: C,76.8; H, 10.1. $C_{28}H_{44}O_3$, $0.5CH_3OH$ requires C, 77.0; H, 10.4%), $\lambda_{max.}$ 240mμ(ε 20,000), $\nu_{max.}$ 3250(OH), 1638cm.$^{-1}$(>C=C-C=CH_2). This material gave a deep yellow colour with tetranitromethane in chloroform.

The corresponding acetate (XXV; R=Ac) obtained by acetylation with acetic anhydride and pyridine formed needles,

m.p.158-160°, $[\alpha]_D$ +50°(c 1.46), from methylene chloride-methanol (Found: C,77.0; H, 9.4. $C_{30}H_{44}O_4$ requires C, 76.9; H, 9.5%), $\lambda_{max.}$ 238mμ(ε 19,700), $\nu_{max.}$ 1736(OAc), 1626 (>C=C-C=CH_2), and 1250cm.$^{-1}$(OAc).

The corresponding benzoate (XXV; R=Bz) obtained with benzoyl chloride and pyridine formed long needles (from methylene chloride-methanol), m.p.205-206°, $[\alpha]_D$+37°(c 1.35) (Found: C, 79.2; H, 9.0. $C_{35}H_{46}O_4$ requires C, 79.2; H, 8.7%), $\lambda_{max.}$ 238mμ(ε 29,000).

Hydrolysis of 12α-Methyl-3β-(tetrahydro-2-pyranyloxy)-5α,25D-spirost-9(11)-en-12β-ol with Mineral Acid.- The ether (XXVI; R=C_5H_9O) (1.4g.) was refluxed with 4N-hydrochloric acid (12ml.) in ethanol (80ml.) for 40 min. Dilution with water and extraction with ether gave a white solid (1.2g.). This crystallised from methanol as needles, m.p.164-174°, $[\alpha]_D$+10°, $\lambda_{max.}$ 202(ε 4300), 242(ε 8800), 270mμ(ε 4100). A portion of this material, chromatographed on alumina, gave a series of fractions all similar to the original material in m.p. and light absorption. Acetylation in the usual way gave a mixture of dienyl acetates (A) crystallising from methanol as needles, m.p.140-146°, $\lambda_{max.}$ 202(ε 3600), 240(ε 6200), 272mμ (ε 4200), which could not be purified by chromatography.

Action of Mineral Acid on 12-Methylene-9(11)-dehydro-tigogenin Acetate.- The diene acetate (XXV; R=Ac) (50mg.) in

ethanol (25ml.) and 4N-hydrochloric acid (2ml.) was refluxed for 40 min. The product isolated in the usual way formed needles (from methanol), m.p.122-152°, $\lambda_{max.}$ 202(ε 4000), 242 (ε 6000), 272mμ(ε 3000).

Miscellaneous Experiments on the Dienyl Acetate Mixture (A).- (a) Action of maleic anhydride. The mixture (100mg.) was refluxed in dry benzene (10ml.) with maleic anhydride (120mg for 7 hr. The solution was washed with aqueous sodium hydroxide, dried, and evaporated. The product crystallised from methanol as needles, m.p.139-143°, $\lambda_{max.}$ 202(ε 3300), 240(ε 8300), 272mμ(ε 3700). Similar results were obtained with toluene as solvent, but with refluxing xylene the product was a resin.

(b) Catalytic Hydrogenation. The dienyl acetate mixture in ethyl acetate was shaken with pre-reduced Adams platinum oxide in hydrogen. No uptake occurred, and starting material was recovered (m.p.146-152°).

(c) Action of hydrogen chloride. The dienyl acetate mixture (200mg.) in dry chloroform (5ml.) was cooled to -63° (melting-chloroform bath), and dry hydrogen chloride was bubbled through it for 1 hr. Most of the dissolved hydrogen chloride was then removed under reduced pressure, and the solution was washed with aqueous potassium hydrogen carbonate and water. After drying of the solution and evaporation, the yellow resin obtained was triturated with methanol. Recrystallisation of the

solid from methanol gave needles, m.p.147-153°, $[\alpha]_D$ +33° (c 1.2), not depressed in m.p. on admixture with 12-methylene-9(11)-dehydrotigogenin.

(d) Action of perbenzoic acid. The mixture (468mg.) in benzene (12ml.) was treated with perbenzoic acid (414mg.) in benzene (5.3ml.) at 20° for 140 hr. Ether was added and the solution was washed with aqueous sodium hydroxide and water before being evaporated. The product was amorphous, with $\lambda_{max.}$ 208(ε 5700) and 234mμ(ε 3900).

12-Methyl-5α,25D-spirost-11-en-3β-yl acetate (XXVIII; R=Ac) - 12-Methylene-9(11)-dehydrotigogenin acetate (XXV; R=Ac) (120mg.) in ethyl acetate (15ml.) was stirred with pre-reduced platinum oxide (120mg.) in hydrogen [uptake 4.3ml. (1 mol.) in 30 min.]. Filtration followed by evaporation gave 12-Methyl-5α,25D-spirost-11-en-3β-yl acetate (XXVIII; R=Ac) as needles (from methylene chloride-methanol), m.p.175-177°, $[\alpha]_D$-61° (c 0.915) (Found: C, 76.25; H, 10.25. $C_{30}H_{46}O_4$ requires C, 76.55; H, 9.85%), $\lambda_{max.}$206mμ(ε 2800), $\nu_{max.}$1730(OAc), 1654(>C=CH-), 1238cm.$^{-1}$(OAc). This compound gave a pale yellow colour with tetranitromethane in chloroform. Levine and Wall[29] record m.p.162.5-164.5°, $[\alpha]_D$-45° for this compound. It seems likely that they obtained an equimolar mixture of this (XXVIII; R=Ac) and the exocyclic isomer (X; R=Ac). When such a mixture was crystallised from methanol it had m.p.153-15

$[\alpha]_D$-45°.

Catalytic reduction of the dienyl acetate in acetic acid with platinum as catalyst resulted in the uptake of two mols. of hydrogen. The product was amorphous and had $\lambda_{max.}$ 210mμ (ε 500).

12-Methyl-5α,25D-spirost-11-en-3β-ol (XXVIII; R=H).- (a) The dien-3β-ol (XXV; R=H) (200mg.) in ethyl acetate (20ml.) was hydrogenated in the presence of pre-reduced platinum oxide (50mg.). The product, isolated in the aforementioned way, had m.p.198-201°. Recrystallisation from aqueous methanol gave feathery needles of 12-methyl-5α,25D-spirost-11-en-3β-ol (XXVIII; R=H) as the hemihydrate, m.p.206-208°, $[\alpha]_D$-63° (c 1.11) (Found: C, 76.8; H, 10.1. $C_{28}H_{44}O_3$, $0.5H_2O$ requires C, 76.9; H, 10.4%), $\lambda_{max.}$ 208mμ (ε 2800), $\nu_{max.}$ 3220(OH), 1639cm.$^{-1}$ (>C=CH-).

(b) The same compound was obtained by hydrolysis of the acetate (XXVIII; R=Ac) and had m.p.206-208° undepressed by material prepared as in (a). This method is better since the dienol (XXV; R=H) is less soluble in ethyl acetate than the dienyl acetate (XXV; R=Ac).

12-Methyl-5α,25D-spirost-11-en-3β-yl benzoate (XXVIII; R=Bz) prepared in the usual way and crystallised from methylene chloride-methanol, had m.p.183-185° (change of form at 168°), $[\alpha]_D$-49.1° (c 0.548) (Found: C, 79.0; H, 9.0. $C_{35}H_{48}O_4$ requires

C, 78.9; H, 9.1%).

12-Methyl-5α,25D-spirostan-3β,11α,12α-triol (XXXII; R=R′=H) - 12-Methyl-5α,25D-spirost-11-en-3β-yl acetate (XXVIII; R=Ac) (2g.) was treated in benzene (10ml.) with osmium tetroxide (750mg.) and pyridine (0.5ml.) and left for 5 days at room temperature. The mixture was evaporated to dryness under reduced pressure and the residue, suspended in dry ether, was refluxed with lithium aluminium hydride (3g.) for 1 hr. Excess of hydride was destroyed with methanol, followed by 4N-hydrochloric acid. The steroids were isolated by several extractions with chloroform (total 500ml.). The extracts were washed with aqueous potassium hydrogen carbonate and water and evaporated without being dried. The residue (2.2g.), containing a small amount of osmium compounds, was dissolved in benzene and chloroform (100ml., 1:1) and adsorbed on alumina (150g.). Benzene eluted material B (1.1g.), m.p.215-218°. Benzene-ether and ether eluted 12β-methyl-5α,25D-spirostan-3β,11α,12α-triol (XXXII; R=R′=H), which crystallised from methanol slowly as prisms, m.p.265-269°, $[\alpha]_D$-43°(c 0.9 in dioxan) (Found: C,71.5; H, 10.2. $C_{28}H_{46}O_5$,0.5CH_3OH requires C, 71.55; H, 10.1%), $\nu_{max.}$3500, 3300, 3230cm.$^{-1}$(OH). This material was transparent to ultraviolet light.

The material B in the benzene eluates was acetylated with acetic anhydride and pyridine in the usual way, and the product

was chromatographed on alumina (40g.). Light petroleum eluted 12-methyl-5α,25D-spirost-11-en-3β-yl acetate (XXVIII; R=Ac), m.p. and mixed m.p.173-176° (700mg.). Ether eluted the 3-monoacetate of the triol (XXXII; R=Ac, R′=H), m.p.246-249° (70mg.) (see below).

Acetylation of the Triol (XXXII; R=R′=H).- The triol (105mg.) was heated with pyridine (2.5ml.) and acetic anhydride (1.5ml.) on the steam-bath for 90 min. Working up in the usual way gave a mixture which was chromatographed on alumina (20g.). Light petroleum-benzene (9:1) eluted 12β-methyl-3β,11α,12α-triacetoxy-5α,25D-spirostan (XXXII; R=R′=Ac) as needles (from methanol), m.p.253-255°, $[\alpha]_D$-48°(c 0.858) (Found: C, 69.5; H, 9.3. $C_{34}H_{52}O_8$ requires C, 69.4; H, 8.9%), $\nu_{max.}$ 1724, 1242, 1227cm.$^{-1}$(OAc). Benzene eluted 12-methyl-3β-acetoxy-5α,25D-spirostan-11α,12α-diol (XXXII; R=Ac, R′=H) as needles (from methanol), m.p.251-254°, $[\alpha]_D$-57°(c 0.58) (Found: C, 71.1; H, 9.6. $C_{30}H_{48}O_5$ requires C, 71.4; H, 9.6%), $\nu_{max.}$ 3500(OH), 1720, 1270cm.$^{-1}$(OAc).

Treatment of the Triol (XXXII; R=R′=H) with Lead Tetra-Acetate.- The triol (100mg.) in t-butyl alcohol (10ml.) and acetic acid (10ml.) was treated with lead tetra-acetate (500mg.) for 18 hr. at room temperature. Testing a portion of the mixture with chromotropic acid reagent[73] showed that no formaldehyde was present. The remaining solution was treated with ethylene

glycol and water. The solution was extracted with ether, and the extracts were washed with dilute acetic acid, water, and aqueous potassium hydrogen carbonate, dried, and evaporated. The amorphous residue had $\nu_{max.}$ (in CCl_4) 2878 (C-H stretching in CHO) and 1700 $cm.^{-1}$ (carbonyl groups) (calcium fluoride prism used).

Baeyer-Villiger Oxidation of Hecogenin Acetate.- Hecogenin acetate (100g.) in chloroform (1500ml.) and acetic acid (600ml.) was treated with hydrogen peroxide (60ml. of 30% aq. solution) and sulphuric acid (22ml. of 10% v/v solution in acetic acid). The mixture was allowed to stand for 12 days at room temperature and was shaken occasionally. The chloroform layer was washed with water several times, mixed with a large volume of water, and steam-distilled until all the solvent was removed. The crude mixture of lactones was filtered off, washed with water, and roughly dried (m.p. 290-295°).

This mixture was saponified by refluxing with potassium hydroxide (100g.) in methanol (1 l.) and water (100ml.) for 2 hr. Dilution with water (total volume 3 l.), and filtration, afforded neutral material (A), m.p. 228-230°(1.9g.). The filtrate was acidified with concentrated hydrochloric acid (to Congo Red) and the precipitated acids were filtered off, washed with water, and dried in vacuo below 40°[yield 100g., m.p. (197) 255-258°].

The acids were continuously extracted with hot acetone (the small insoluble residue being discarded), and the extracts were concentrated under reduced pressure until crystals formed. These, which were filtered off after cooling, and three further crops obtained by further concentrat-

ing the mother-liquors and progressively adding chloroform, had respectively, m.p.(195-201) 251-254°, (197-203) 255-259°, (193-197) 250-254°, and (187) 250-254°,(total weight 82g.). These were substantially pure hecolic acid (XLVI; R=R'=H). A pure sample of the hemihydrate obtained by recrystallisation from acetone-chloroform had m.p.(195) 260-264°, $[\alpha]_D$-67.9°(c 0.8 in dioxan) (Found, on sample dried at 50°/0.01mm.: C, 68.0; H, 9.8%; equiv., 470, 473; loss on drying at 105°/0.01mm., 1.3. $C_{27}H_{44}O_6$, $0.5H_2O$ requires C, 68.5; H, 9.6%; equiv., 474; loss on drying, 1.9%. Found, on sample dried at 105°/0.01mm.: C, 69.8; H, 9.7. Calc. for $C_{27}H_{44}O_6$: C, 69.8; H, 9.6%), $\nu_{max.}$ 1724 cm.$^{-1}$ and hydroxyl band. Rothman, Wall, and Eddy[54] record m.p.(187) 253-255°, $[\alpha]_D$-66.3°(dioxan) for the anhydrous material.

The chloroform mother liquors from the fourth crop, on standing overnight gave a fifth crop, m.p. (177-200) 265-270° (5g.). Recrystallisation from acetone gave 3β,11-dihydroxy-11,12-seco-5α,25D-spirostan-12-oic acid (isohecolic acid) (XLVIII; R=R'=H), as prisms, m.p. (236-238),275-280° (3.2g.). A pure sample had m.p. (236-238) 286-288°,$[\alpha]_D$ -62.9° (c 1.02 in dioxan) (Found on sample dried at room temperature in vacuo : C,69.7; H,9.8; Equiv., 465,468. $C_{27}H_{44}O_6$ requires C,69.8; H,9.6%; Equiv., 465), $\nu_{max.}$ 1724 cm.$^{-1}$ and hydroxyl band.

Methyl Isohecolate.- Isohecolic acid (XLVIII; R=R'=H)

(400mg.) in methanol (50ml.) was treated with an excess of ethereal diazomethane. After a few minutes glacial acetic acid was added to discharge the yellow colour. After filtration to remove polymethylene, the solution was evaporated. The residue was crystallised from acetone to give methyl 3β,11-dihydroxy-11,12-seco-5α,25D-spirostan-12-oate (methyl isohecolate) (XLVIII; R=H, R′=Me) as fine needles, m.p.(136-138)198-199°, $[\alpha]_D$-55.4° (c 0.85) (Found: C, 70.4; H, 9.9. $C_{28}H_{46}O_6$ requires C, 70.3; H, 9.7%), $\nu_{max.}$ (nujol) 1724, (CS_2) 1721cm.$^{-1}$

Acetylation of a portion with acetic anhydride and pyridine at room temperature overnight gave the 3β,11-diacetate (XLVIII; R=Ac, R′=Me) as hard prisms from ether-isopentane, m.p.117-119°, $[\alpha]_D$-51° (c 0.925) (Found: C, 68.5; H, 9.1. $C_{32}H_{50}O_8$ requires C, 68.3; H, 9.0%), $\nu_{max.}$1724, (CCl_4) 1739cm.$^{-1}$ and no absorption in the infrared region.

Isohecololactone (XLVII; R=H).- Isohecolic acid (250mg.) was heated to 240° at a pressure of 0.01mm., when it melted, evolved water, and resolidified. When the temperature was raised to 260-280° the lactone was formed, subliming on the cooler parts of the tube. A second sublimation, followed by two recrystallisations from dichloromethane-ether, gave 3β,11-dihydroxy-11,12-seco-5α,25D-spirostan-12-oic acid 11,12-lactone (isohecololactone) (XLVII; R=H), m.p. 285°, $[\alpha]_D$-82°

(c 0.8) (Found: C, 72.7; H, 9.4. $C_{27}H_{42}O_5$ requires C, 72.6; H, 9.5%), $\nu_{max.}$ 1720cm.$^{-1}$, (CS_2) 1724cm.$^{-1}$

Acetylation of the lactone overnight with acetic anhydride and pyridine gave the acetate (XLVII; R=Ac) as needles from dichloromethane-acetone, m.p.(250)292-294°, $[\alpha]_D$-81° (c 1.3) (Found: C,71.55; H, 9.1. $C_{29}H_{44}O_6$ requires C, 71.3; H, 9.1%).

Benzoylation of the lactone with benzoyl chloride and pyridine overnight gave the benzoate (XLVII; R=Bz) as needles from methanol, m.p. 297-299°, $[\alpha]_D$-76° (c 0.802) (Found: C, 74.65; H, 8.7. $C_{34}H_{46}O_6$ requires C, 74.15; H, 8.4%), $\nu_{max.}$(KCl) 1724cm.$^{-1}$

Derivatives of Hecolic Acid.- The following were made for comparison with the isohecolic acid derivatives.

Methyl hecolate (XLVI; R=H, R′=Me), m.p.(78-80)163-164°, $[\alpha]_D$-67° (c 1.45), $\nu_{max.}$ 1733cm.$^{-1}$, (KCl) 1724cm.$^{-1}$

Hecololactone (XLV; R=H), from dichloromethane-ether, m.p. 259-261°, $[\alpha]_D$-56.5° (c 1.29), $\nu_{max.}$ 1741cm.$^{-1}$, (CS_2) 1736cm.$^{-1}$

Hecololactone acetate (XLV; R=Ac), prisms from dichloromethane-ether, m.p.(250)298-300°, $[\alpha]_D$-65° (c 0.98), $\nu_{max.}$ 1740cm.$^{-1}$, (CS_2) 1739cm.$^{-1}$

Hecololactone benzoate (XLV; R=Bz), needles from dichloromethane-acetone, m.p.307-310°, $[\alpha]_D$-63.2° (c 0.95)

(Found: C, 74.4; H, 8.2. $C_{34}H_{46}O_6$ requires C, 74.2; H, 8.4%), $\nu_{max.}$(KCl) 1739, 1718cm.$^{-1}$

Methyl hecolate 3-acetate (XLVI; R=Ac, R =Me), m.p.120-124°, $[\alpha]_D$-73.2° (c 1.34) (Found: C, 69.4; H, 9.7. Calc. for $C_{30}H_{48}O_7$: C, 69.2; H, 9.3%), $\nu_{max.}$1730cm.$^{-1}$, (CCl_4) 1739cm.$^{-1}$.

With the exception of the last compound these constants are in close agreement with those recorded by Rothman, Wall, and Eddy[54]; viz.

Methyl hecolate, m.p.(79)164-165°, $[\alpha]_D$-63.6°.

Hecololactone, m.p. 256-258°, $[\alpha]_D$-52°.

Hecololactone acetate, m.p.292-292.5°, $[\alpha]_D$-65.1°.

Methyl hecolate 3-acetate, m.p.99-101°, $[\alpha]_D$-62.7°.

Reaction between Methyl Hecolate and Phenylmagnesium Bromide.- Phenylmagnesium bromide was prepared from magnesium turnings (10g.), bromobenzene (45ml.), and ether (500ml.) in the usual way. Methyl hecolate (12g.) in benzene [600ml.; dried by distilling off a portion of the solvent (100ml.)] was added. The mixture was refluxed with stirring for 6 hr., and some solvent was distilled off. Dilute hydrochloric acid was added and the product isolated by extraction with ether in the usual way. The residual oil was steam-distilled for 4.5 hr. until 4 litres of distillate had collected, and removal of biphenyl was assisted by the occasional addition of toluene to the distillation flask. The residue in the flask was dissolved in ether and the extract was washed with water, dried, and evaporated. This gave a yellow froth (12.7g.), $\lambda_{max.}$ 206(ϵ 17,500) and 242mμ (ϵ 9750), which was treated with pyridine (60ml.) and acetic anhydride (50ml.) overnight at room temperature. Working up in the usual way with ether gave a froth (13.4g.), $\lambda_{max.}$205 (ϵ 16,000) and 242mμ (ϵ 9030). This was boiled in Analar acetic acid (25ml.). After 1 min. a large amount of solid separated. Most of the acetic acid was evaporated under reduced pressure and di-isopropyl ether (50ml.) was added to the cooled residue. The solid obtained was filtered off, the filtrate evaporated, and the residue treated again with boiling acetic acid (10ml.)

to give a second crop of solid (weight of first two crops 7.0g.; m.p.255-257°). The material in the mother-liquor was treated with boiling acetic acid for a third time, and the material was worked up by dilution with ether and washing with aqueous potassium hydrogen carbonate. Crystallisation of the product from di-isopropyl ether gave a third crop of solid, m.p.250-252°(0.85g.). The material in the mother-liquors did not yield any more solid and had $\lambda_{max.}$206 (ε 23,700) and 244mμ(ε 11,300).

Further crystallisation of the solid gave 3β-acetoxy-12a-oxa-12-phenyl-C-homo-5α,25D-spirost-11-ene (LIV; R=Ac) as needles, m.p.257-259°(from chloroform-methanol), m.p. 254-256°(from dichloromethane-ether), $[\alpha]_D$-70.5°(c 0.64) (Found: C,76.7; H, 8.6. $C_{35}H_{48}O_5$ requires C, 76.6; H, 8.8%), $\lambda_{max.}$205(ε 11,910), 218(ε 10,250) and 263mμ(ε 13,100), $\nu_{max.}$ 1739, 1647, 1605, 1582, 1240, 772cm.$^{-1}$

3β-Hydroxy-12a-oxa-12-phenyl-C-homo-5α,25D-spirost-11-ene (LIV; R=H), obtained by hydrolysis of the acetate with boiling methanolic potassium hydroxide, formed needles (from methanol), m.p.192-194°, $[\alpha]_D$-75° (c 0.8) (Found; C,78.6; H, 9.5. $C_{33}H_{46}O_4$ requires C, 78.2; H, 9.15%), $\lambda_{max.}$212 (ε 11,300), 263mμ(ε 13,000), $\nu_{max.}$ 3390, 1647, 1626, 1605, 1580, 770cm.$^{-1}$

The benzoyl derivative, formed by overnight treatment

of the alcohol (LIV; R=H) with benzoyl chloride and pyridine, formed needles (from chloroform-methanol), m.p. 274-277°, $[\alpha]_D$-76° (c 0.6) (Found; C, 78.8; H, 8.5. $C_{40}H_{50}O_5$ requires C, 78.65; H, 8.25%), $\lambda_{max.}$ 214 (shoulder) (ε 18,000), 224 (ε 22,000), and 264mμ (ε 10,400), $\nu_{max.}$ 1730, 1644, 1608, 1275, and 764cm.$^{-1}$

Treatment of Methyl Hecolate with Phenyl-lithium.- Methyl hecolate (1g.) in ether (100ml.) was refluxed with ethereal 0.78M-phenyl-lithium (15ml, 6 mol.) for 3 hr. Working up in the usual way (with steam-distillation to remove biphenyl) gave a yellow froth (0.96g.), $\lambda_{max.}$ 209 (ε 13,000), 242mμ (ε 8500). A portion of this (150mg.) was treated with acetic anhydride and pyridine, then with boiling acetic acid. The product was chromatographed on alumina, to yield 3β-acetoxy-12a-oxa-12-phenyl-C-homo-5α,25D-spirost-11-ene, m.p. 258-260° (eluted with 4:1 light petroleum-benzene) and hecololactone acetate, m.p. and mixed m.p. 294-296° (eluted with ether).

3β-Acetoxy-12-phenyl-12,13-seco-5α,25D-spirost-13-en-12-one (LV).- The phenyl enol ether (LIV; R=Ac) (501mg.) was heated on the steam-bath with acetic acid (10ml.) and water (1ml.) for 3 hr. It dissolved except for a trace of amorphous material, which was filtered off. The filtrate was diluted with water and the precipitate filtered off, dissolved

in acetic acid, and reprecipitated with water. 3β-Acetoxy-12-phenyl-12,13-seco-5α,25D-spirost-13-en-12-one (LV) was amorphous and had m.p.86-91°, $[\alpha]_D$-40°(c 1.175) (Found: C, 76.5; H, 8.9. Calc. for $C_{35}H_{48}O_5$: C, 76.6; H, 8.8%), $\lambda_{max.}$206(ε 20,000), 241mμ(ε 16,300), $\nu_{max.}$(KCl) 1727(OAc), 1678(Ph ketone), 1590 and 1570(conjugated benzene ring), 1447(benzene ring), 1239(OAc), 758 and 697cm.$^{-1}$(monosubstituted benzene ring).

Action of Chromium Trioxide on the Phenyl Ketone.- The amorphous phenyl ketone (LIII) (250mg.) was acetylated overnight and the product was dissolved in acetic acid (15ml.) and treated with chromium trioxide (200mg.) at room temperature for 40 hr. After working up with ether in the usual way an acid fraction (50mg.) and a neutral fraction (190mg.) were obtained, both non-crystalline. Chromatography of the latter provided hecololactone acetate (eluted with ether), m.p. and mixed m.p.295-298°(60mg.), probably by cyclisation of unreacted hecolic acid.

Oxidation of the Phenyl Enol Ether (LIV; R=Ac).- The following reagents were used in attempts to oxidise the phenyl enol ether (LIV; R=Ac): ozone, chromium trioxide in acetic acid, chromium trioxide in aqueous sulphuric acid, potassium permanganate in acetone, osmium tetroxide, perbenzoic acid, and performic acid. In most cases there resulted

neutral and acidic fractions (the latter very small) from which no crystalline material could be obtained (except benzoic acid, m.p.120°, formed by chromium trioxide in acetic acid).

Beckmann Rearrangement of Hecogenin Acetate Oxime.

Hecogenin Acetate Oxime (LXIV; R=Ac, R′=H).- Hecogenin acetate (4.72g.) and hydroxylamine hydrochloride (2.1g.) in pyridine (50ml.) were heated at 100° for 3 hr., by which time an oil had separated. Ethanol (10ml.) was added to dissolve the oil, and after a further 30 min. an excess of water was added. The precipitated solid was filtered off (4.66g., m.p.318-320°). An analytical specimen of this oxime, obtained by recrystallisation from chloroform-acetone, had m.p.318-320°, $[\alpha]_D$-1.7°(c 0.875) (Found: C, 71.5; H, 9.0; N, 3.15. Calc. for $C_{29}H_{45}NO_5$: C,71.4; H, 9.3; N, 2.9%). Mazur[10] records m.p.318-321°, $[\alpha]_D$-2.4°.

Hecogenin Oxime (LXIV; R=R′=H).- Saponification of the foregoing acetate gave hecogenin oxime (LXIV; R=R′=H), as plates from aqueous methanol, m.p.256-260°, $[\alpha]_D$+3°(c 1.0) (Found: C, 72.8; H, 9.35; N, 2.9. $C_{27}H_{43}NO_4$ requires C, 72.8; H, 9.7; N, 3.1%). Anliker, Rohr and Heusser[67] record m.p. 317-318°, $[\alpha]_D$±0° for a hemihydrate prepared in an unspecified manner. Their m.p. is similar to that of the 3β-acetate and the analytical figures quoted agree with this formulation. Acetylation of hecogenin oxime or its 3β-acetate with acetic anhydride and pyridine at room temperature gave the diacetate (LXIV; R=R′=Ac), m.p.194-196°, $[\alpha]_D$+16°(c 1.1) (Found: C, 70.2; H, 8.7; N, 2.3. Calc. for $C_{31}H_{47}NO_6$: C, 70.3; H, 8.95; N, 2.65%)

Anliker *et al.*[67] record m.p. 185°, $[\alpha]_D$ +7.9°.

Hecololactam Acetate.- Hecogenin acetate oxime (5g.) in pyridine (100ml.) was treated with toluene-p-sulphonyl chloride (5g.) at 100° for 3.5 hr. Water was added till a slight cloudiness appeared, kieselguhr was added, and the solution filtered. Acidification of the filtrate precipitated the lactam (4.72g.). Crystallisation from aqueous acetone gave 3β-acetoxy-12a-aza-C-homo-5α,25D-spirostan-12-one (hecolo-lactam acetate) (LXV;R=Ac) as needles, m.p.234-236°, $[\alpha]_D$-72° (c 1.19) (Found: C, 71.6; H, 8.8; N, 3.0. Calc. for $C_{29}H_{45}NO_5$: C, 71.4; H, 9.3; N, 2.9%). When the reaction was carried out at room temperature for 96 hr. a similar yield was obtained, but after 20 hr. the product consisted of a mixture of the lactam acetate (2.9g.) and unchanged oxime (1.9g.); these were easily separated since the former is soluble in aqueous pyridine. Mazur[10] records m.p.231-234°, $[\alpha]_D$-70°.

Hecololactam.- Hecololactam acetate (LXV; R=Ac) (500mg.) in methanol (300ml.) was refluxed with potassium hydroxide (1.5g.) in a little water for 2 hr. Addition of an excess of water precipitated 3β-hydroxy-12a-aza-C-homo-5α,25D-spirostan-12-one (hecololactam) (LXV; R=H), which crystallised from aqueous methanol as prisms, becoming opaque at 100°, m.p. (150-160)202-205°, $[\alpha]_D$-61.7° (c 0.94)((Found, in material dried at 100°/0.01mm.: C, 70.4; H, 9.5; N, 3.15. $C_{27}H_{43}NO_4,H_2O$ requires

C, 69.95; H, 9.8; N, 3.0. Found, in material dried at 132°/0.01mm.: C, 71.55; H, 9.75. $C_{27}H_{43}NO_4,0.5H_2O$ requires C, 71.4; H, 9.8. Found, in material sublimed at 270-290°/0.01mm.: C, 72.8; H, 8.25; N, 3.6. $C_{27}H_{43}NO_4$ requires C, 72.8; H, 9.7; N, 3.15%). The sublimed amorphous anhydrous material, on crystallisation from aqueous methanol, gave back the hydrate, m.p.(140-150)200-202°. Mazur records m.p. over the range 145-215° and gives no analysis.

Action of Lithium Aluminium Hydride on Hecololactam Acetate.- The lactam acetate (LXV; R=Ac) (500mg.) was refluxed with lithium aluminium hydride (800mg.; =16 molar equivalents) in tetrahydrofuran (100ml.) for 7 hr.(cf.[59]). The product, obtained by ether extraction, was refluxed with 5% methanolic potassium hydroxide for 30 min. Addition of water provided hecololactam (LXV; R=H), which after crystallisation from aqueous acetone had m.p.(145-160)200-202°, undepressed on admixture with an authentic specimen.

Action of Dinitrogen Tetroxide on Hecololactam Acetate.- The lactam acetate (150mg.) in carbon tetrachloride (5ml.) was treated with dinitrogen tetroxide in carbon tetrachloride (5ml., 2%V/V solution), followed by the addition of anhydrous sodium acetate (125mg.). The mixture was stirred for 20 min. and added to crushed ice. The organic material was isolated by ether as a solid which on crystallisation from aqueous

methanol gave starting material, m.p. and mixed m.p.232-234°.

Action of Nitrous Acid on Hecololactam Acetate.- Hecololactam acetate (1.89g.) in acetic anhydride (40ml.) and acetic acid (8ml.) was cooled to 0° and treated with sodium nitrite (12g.) for 22 hr. Water was added and the organic material isolated by ether-extraction. This was a syrup, which on addition methanol afforded crystals (140mg.), m.p.270-279°. These, after recrystallisation from dichloromethane-methanol, gave hecololactone acetate, m.p.298-301°, $[\alpha]_D$-63°, which did not depress the m.p. of an authentic sample and had an identical infrared spectrum.

The material in the mother liquors was not crystalline and was refluxed with 5% methanolic potassium hydroxide (50ml.) for 1 hr. Addition of water caused, on one occasion only, the precipitation of hecololactam, m.p.(145)200-205°. In other cases a clear solution was obtained which on acidification with 6N-hydrochloric acid gave crystals (1·56 g.), m.p.205-215°, $[\alpha]_D$-37°. Recrystallisation from aqueous acetone gave 3β-hydroxy-12,13-seco-5α,25D-spirost-13-en-12-oic acid (anhydrohecolic acid) (LXVI), m.p.220-223°, $[\alpha]_D$-39° (c 1.0) (Found: C, 72.8; H, 9.4. $C_{27}H_{42}O_5$ requires C, 72.6; H, 9.5%), $\lambda_{max.}$208mμ(ε 4400), giving a yellow colour with tetranitromethane in chloroform.

Anhydrohecolyl Alcohol.- (a) Anhydrohecolic acid (310mg.)

was treated with lithium aluminium hydride (220mg.) in refluxing tetrahydrofuran (80ml.) for 3.25 hr. After decomposition of the excess of reagent and addition of dilute mineral acid, the product was isolated with ether. The product (340mg.), on crystallisation from acetone, gave 12,13-seco-5α,25D-spirost-13-en-3β,12-diol (anhydrohecolyl alcohol) (LXVII), m.p.176-178°, $[\alpha]_D$-43° (c 1.05) (Found: C, 75.25; H, 10.5. Calc. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25%), $\lambda_{max.}$ 205mμ (ε 4400).

(b) Methyl hecolate (1.03g.) was treated with lithium aluminium hydride (450mg.) in refluxing tetrahydrofuran (80ml.) for 2 hr. The steroid was isolated with ether as before. Addition of chloroform to the product provided hecolyl alcohol, m.p.140-142°, $[\alpha]_D$-65° (c 0.8 in acetone). Rothman *et al.*[54] record m.p.141°, $[\alpha]_D$-71.4°.

This material (100mg.) was dissolved in methanol (5ml.) and treated with 60% aqueous perchloric acid (0.1ml.) at 20° for 17 hr. Addition of water precipitated anhydrohecolyl alcohol, which after crystallisation from acetone had m.p. 178-181°, undepressed on admixture with material prepared by method (a) and having an identical infrared spectrum. Rothman *et al.* give m.p.174-176°, $[\alpha]_D$-46.1°.

9(11)-Dehydrohecogenin Acetate Oxime. (LXX; R=Ac, R'=H).- 9(11)-Dehydrohecogenin acetate (3.1g.) in pyridine (40ml.)

was refluxed with hydroxylamine hydrochloride (1.5g.) for 2 hr. The yellow solution was poured into water (1 l.) and the precipitate was collected, washed with water, and dried at 100°. The crude material, m.p.273-278°, was crystallised from dichloromethane-methanol and provided the oxime acetate (LXX; R=Ac, R′=H) as plates, m.p.286-289°, $[\alpha]_D$ +26°, $\lambda_{max.}$ 238mμ(ε 11,000). Mazur[10] records m.p.282-284°, $[\alpha]_D$ +36°, $\lambda_{max.}$ 238mμ(ε 13,600).

Beckmann Rearrangement of 9(11)-Dehydrohecogenin Acetate Oxime.- The oxime (5.18g.) in pyridine (80ml.) was treated with toluene-p-sulphonyl chloride (4g.) at 20° for 64 hr. Water (1 l.) was added and the precipitated pink solid was filtered off, washed, and dried (m.p.168-175°; 3.55g.; 52%). Crystallisation from aqueous acetone gave needles of 12-toluene-p-sulphonyloxyimino-5α,25D-spirost-9(11)-en-3β-yl acetate (LXX; R=Ac, R′=p-Me.C_6H_4.SO_2-), m.p.184-186°, $[\alpha]_D$-46°(c 1.46) (Found: C, 67.1; H, 7.4; N, 2.3; S, 5.1. $C_{36}H_{49}NO_7S$ requires C, 67.6; H, 7.7; N, 2.2; S, 5.2%), $\lambda_{max.}$204(ε 21,000), 227mμ (ε 21,600), $\nu_{max.}$(KCl)3422, 1736,1666, 1629, 1600, 1250cm.$^{-1}$

The aqueous pyridine filtrates were filtered again to remove a small quantity of solid and then acidified to pH 3 with hydrochloric acid. The precipitate which formed was filtered off (m.p.220-227°; 2.2g.; 40%). Recrystallisation from aqueous acetone gave 3β-acetoxy-12a-aza-C-homo-5α,25D-

spirost-9(11)-en-12-one [9(11)-dehydrohecololactam acetate] (LXXI; R=Ac), m.p.230-232°. From acetone-light petroleum (b.p.60-80°) it separated as prisms, m.p.255-258°, $[\alpha]_D$-69.4° (c 1.19) (Found: C, 71.5; H, 9.2; N, 3.0. Calc. for $C_{29}H_{43}NO_5$: C, 71.7; H, 8.9; N, 2.9%), $\lambda_{max.}$220mμ(ε 17,200). Drying at 100°/0.01mm. did not cause a loss in weight. The two forms of this substance had identical infrared spectra. Mazur[10] gives m.p.228-231°, $[\alpha]_D$-72°, $\lambda_{max.}$220mμ(ε 15,800).

Treatment of Dehydrohecololactam Acetate with Nitrous Acid.- The lactam acetate (206mg.) in acetic anhydride (4ml.) and acetic acid (0.08ml.) was treated with sodium nitrite (1.1g) at 0° for 68 hr. Addition of water, and extraction with ether, gave neutral material (200mg). Crystallisation from acetone-light-petroleum gave starting material, m.p. and mixed m.p. 254-257° (60mg.). Further crops (120mg.) had m.p.252-255°.

Formation of Dehydrohecololactam Acetate from the Oxime Toluene-p-sulphonate(LXX; R=Ac, R'=p-$C_6H_4Me.SO_2$-).- (a) The oxime toluene-p-sulphonate (160mg.) in ethanol (8ml.), acetic acid (4ml.) and a few drops of water, was refluxed for 30 min. Addition of water precipitated the lactam acetate, which after crystallisation from acetone-light petroleum had m.p.252-255°.

(b) On attempted recrystallisation of the oxime toluene-p-sulphonate (3.5g.) from chloroform-methanol, a syrup was obtained. Prolonged standing of this dissolved in ether-isopentane gave the lactam acetate, m.p.222-226°(1.5g.).

Irradiation of Hecogenin Acetate with Ultraviolet Light.- The ultraviolet lamp used was a Hanovia 1906/G with intensity stabiliser U.V.S. 50 Model XII. Irradiation was effected using either of the two methods described below.

In method A the solution of the steroid was placed in a quartz flask fitted with a reflux condenser. Nitrogen was purified by passage through Fieser's solution and concentrated sulphuric acid, and then passed through glass tubing down the condenser to just above the level of the refluxing liquid. The ultraviolet lamp was placed directly below the flask and the rays were reflected on to the flask by aluminium foil.

In method B the flask was fitted with a three-way stopcock. Air was removed by alternately evacuating the flask and then admitting unpurified ("white spot") nitrogen. This process was repeated several times and finally the flask was partially evacuated. It was then placed under cold running water on a level with, and a few inches distant from the lamp. The joints of the stopcock were lubricated with Apiezon-L high vacuum grease and during the reaction were shielded with aluminium foil.

Solvents.

(a) Dioxan.- This was normally purified by extensive refluxing over sodium, followed by distillation. The fraction b. p. 100-101° was used for the reaction. This solvent, on vapour

phase chromatography, was found to contain 1% of 2-methyl-1,3-dioxolan.

Specially purified dioxan was obtained by Vogel's method.[74] Technical dioxan (3 l.) was refluxed with concentrated hydrochloric acid (42ml.) and water (300ml.) for 10 hr. A slow stream of nitrogen was bubbled through the solution to remove the acetaldehyde formed. The solution was allowed to cool and potassium hydroxide pellets were added until some remained undissolved, and the aqueous layer was removed. The dioxan was then allowed to stand over potassium hydroxide pellets for 24 hr. and refluxed with sodium for 12 hr. The liquid was then fractionally distilled and each fraction was analysed by vapour phase chromatography.

(b) 2-Methyl-1,3-dioxolan.- Paraldehyde (598g.), ethylene glycol (820g.) and toluene-p-sulphonic acid (5g.) were refluxed for 18 hr. Sodium hydrogen carbonate (50g.) was added and the mixture filtered. The filtrate was distilled through a 24" column of Fenske helices and fractions (100ml.) of b. ps. in the range 72-100° were collected. These were examined by gas chromatography and those which contained only small amounts of acetaldehyde were combined and treated repeatedly with potassium hydroxide pellets. When no more aqueous layer separated and no further resification occurred the liquid was allowed to stand over sodium wire for 2 hr.

before being fractionated. The fraction b.p. 80-82° (390g.) was pure 2-methyl-1,3-dioxolan and gave a single peak in a gas chromatogram.

(c) Tetrahydrofuran was purified by refluxing over potassium until the metal remained bright, followed by fractional distillation.

Gas Chromatographic Determination of 2-Methyldioxolan in Dioxan.- The chromatograms were run at 68° using a Griffin and George Mark IIB instrument fitted with a 6ft.×¼mm. column of 30% silicon grease (E 301) on celite. A flow of 1.45 l./ hr. of nitrogen was used, the inlet pressure being 48¼mm. and the outlet pressure 195mm. The bridge current was 100m.amps. Under these conditions 2-methyldioxolan gave a peak at 8 min. and dioxan at 13.5 min. For the determination 10 to 12 drops of the sample were injected and the peak due to 2-methyldioxolan was recorded at attenuation×1. Just before the dioxan peak the attenuation was changed to ×10. The concentration was calculated by comparing the relative areas of the curves, the attenuation factor being taken into account.

The following samples were tested. (1) A standard mixture of pure dioxan with 0.96% by weight of 2-methyl-dioxolan added. (2) Dioxan purified by acid treatment and then by refluxing over sodium. This contained 0.11% of

2-methyldioxolan. (3) Dioxan refluxed over sodium. This contained 0.92% of 2-methyldioxolan.

Lumihecogenin Acetate.- (a) Hecogenin acetate (VIII; R=Ac) (20g.) in dioxan purified over sodium (400ml.) was irradiated with ultraviolet light for 28 hr. using method A. Most of the solvent was evaporated under reduced pressure, and water (1 l.) was added. The organic material was separated by several extractions with ether. The combined ethereal extracts were washed with 5% aqueous potassium hydroxide, then with water, and finally dried with sodium sulphate. The solvent was evaporated in vacuo to give a light yellow gum (20g.) which on trituration with methanol provided 3β-acetoxy-12,13-seco-12-oxo-5α,25D-spirost-13-ene (lumihecogenin acetate) (LXXXVII) as prisms, m.p. 141-145° (12.8g.). A pure sample, obtained by recrystallisation from dichloromethane-methanol, had m.p.146-150°(in a sealed evacuated tube 157-159°), $[\alpha]_D$ -46° (c 1.0) (Found: C, 73.7; H, 9.7. $C_{29}H_{44}O_5$ requires C, 73.7; H, 9.4%), $\lambda_{max.}$ 204mμ (ε 4800), $\nu_{max.}$ 2740 (-CH=O), 1739(OAc), 1709(-CH=O), 1240 cm.$^{-1}$ (OAc) (see fig. I). This material gave a pale yellow colour with tetranitromethane in chloroform.

The alkaline washings (see above) were combined, acidified, and extracted with ether. The ethereal extracts were washed with water, dried, and evaporated to give

amorphous material (30mg.).

(b) Lumihecogenin acetate was recovered after being irradiated in 2-methyldioxolan according to method E for 2.5 hr.

3β-Acetoxy-12,13-seco-12,12-ethylenedioxy-5α,25D-spirost-13-ene (LXXXIX).- Lumihecogenin acetate (215mg.) in 2-methyldioxolan (15ml.) was refluxed with toluene-p-sulphonic acid (50mg.) for 22 hr. Ether was added and the solution was washed with aqueous potassium hydrogen carbonate, dried, and evaporated. The residue (280mg.) was crystallised from methanol to give prisms, m.p.180-185°. Further crystallisation provided a pure sample of the ethylene acetal (LXXXIX) m.p.182-185°, $[\alpha]_D$-36.2°(c 1.27) (Found: C, 72.25; H, 9.35. $C_{31}H_{48}O_6$ requires C, 72.1; H, 9.35%), $\lambda_{max.}$206mμ (ε 4480), $\nu_{max.}$(in CCl_4) 1730(OAc), 1240cm.$^{-1}$(OAc) with the fingerprint region different from that of photohecogenin acetate. This compound gave a pale yellow colour with tetranitromethane in chloroform.

Lumihecogenin acetate 12-semicarbazone (LXXXVIII).- The acetate (LXXXVII) (195mg.) in ethanol (10ml.) was treated with semicarbazide hydrochloride (200mg.) and sodium acetate (300mg.) at 100 for 15 min. Water was added and the precipitated solid was collected and dried. Recrystallisation from aqueous methanol provided the semicarbazone (LXXXVIII) as

needles m.p. 193-196°, $[\alpha]_D$-13° (c 1.23) (Found: C, 68.1; H, 9.0; N, 7.65. $C_{30}H_{47}O_5N_3$ requires C, 68.0; H, 8.95; N, 7.9%).

Other Reactions of Lumihecogenin Acetate.- (a) The acetate (LXXXVII)(200mg.) in methanol (15ml.) was refluxed with potassium hydroxide (800mg.) for 1.5 hr. Excess of water was added and the product isolated by extraction with ether, as a pale yellow solid.

(b) The acetate (200mg.) in pyridine (2ml.) was treated with hydroxylamine hydrochloride (100mg.) at 100° for 2 hr. The product, isolated with ether, was amorphous.

Photohecogenin Acetate.- (a) Hecogenin acetate (VIII; R=Ac) (20g.) in dioxan (400ml.) was irradiated with ultra-violet light for 38 hr. using method A. The solvent was evaporated under reduced pressure and the residual gum was dissolved in hot methanol (100ml.). When the solution was cooled crystalline material appeared and this was collected and dried (m.p. 197-202°; 4.03g.) A second crop, m.p. 191-197° (2.07g.) was obtained by concentrating the mother-liquor. The higher melting material was recrystallised several times from methanol and provided photohecogenin acetate, as plates, m.p. 203-206°, $[\alpha]_D$-38.7° (c 1.61) [Found: C, 72.1; H, 9.15; OEt, 19.4. $C_{31}H_{48}O_6$ requires C, 72.05; H, 9.35; OEt(for 1 $CH_3.CO$ and 1 CH_3-CH$\overset{O-}{-O-}$) 17.8%], $\nu_{max.}$ 1739, 1242cm.$^{-1}$(OAc) (see fig. I). This material was transparent to ultraviolet

light and gave no colour with tetranitromethane in chloroform.

(b) When hecogenin acetate (1g.) in dioxan (50ml.) was irradiated with ultraviolet light for 46 hr., using method B, impure photohecogenin acetate (0.45g.) was isolated.

(c) Hecogenin acetate (5g.) in specially purified dioxan (100ml.) was irradiated for 45 hr., using method B. The solvent was evaporated and the residue, on addition of methanol, provided photohecogenin acetate, m.p. and mixed m.p. 198-204° (430mg. =8% yield).

(d) Lumihecogenin acetate (506mg.) in dioxan (20ml.) was irradiated using method B for 20 hr. The solvent was evaporated and the residue afforded photohecogenin acetate, m.p. and mixed m.p. 183-196° (also identified by its infrared spectrum).

(e) Lumihecogenin acetate (185mg.) in 2-methyldioxolan (10ml.) was irradiated for 21 hr., employing method B. Crystallisation of the product from methanol gave authentic photohecogenin acetate, m.p. and mixed m.p. 194-199°.

Photohecogenin.- (a) Photohecogenin acetate (525mg.) in methanol (30ml.) and water (2ml.) was refluxed with potassium hydroxide (1g.) for 1 hr. The product was isolated by addition of water and ether extraction in the usual way. Crystallisation from acetone provided photohecogenin as

diamond shaped crystals, m.p.166-170°, $[\alpha]_D$-41°(c 1.4) (Found: C, 73.2; H, 9.5. $C_{29}H_{46}O_5$ requires C, 73.4; H, 9.8%), $\nu_{max.}$ 3330(OH), 1720(w)(acetone?) cm.$^{-1}$ (see fig. I). Crystallisation from aqueous methanol gave a different crystalline form, viz. needles m.p.204-208°. The infrared spectrum was almost identical to that of the lower melting form, but lacked the maximum at 1720cm.$^{-1}$

(b) Photohecogenin acetate (112mg.) in anhydrous ether (30ml.) was refluxed with lithium aluminium hydride (150mg.) for 35 min. Hydrochloric acid was added and the sterol isolated by extraction with ether. The product was crystallised from acetone to give photohecogenin, m.p.166-170°. Its identity was confirmed by a mixed m.p. and by comparison of infrared spectra.

A portion of photohecogenin (91mg.), on treatment overnight with acetic anhydride (3ml.) and pyridine (5ml.) provided photohecogenin acetate, m.p.195-202°. This did not depress the m.p. of an authentic sample and the infrared spectra were identical.

Action of Acid on Photohecogenin Acetate.- The acetate (200mg.) in methanol (20ml.) was refluxed with 6N-hydrochloric acid (2ml.) for 20 min. The condenser was arranged so that any low boiling product formed (e.g. acetaldehyde) would pass through an aqueous solution of 2,4-dinitrophenylhydrazine.

The methanol was then slowly distilled, water being added to maintain the volume. No precipitate formed in the reagent and it was extracted several times with ether. The extracts were combined, dried, and evaporated to give a red solid (400mg.) which was adsorbed on alumina. Benzene eluted 2,4-dinitrophenylhydrazine, m.p.194°, as the sole crystalline material. The precipitated material in the distillation flask was isolated by ether extraction in the usual way. It was amorphous (200mg.).

Action of Lithium Aluminium Hydride on Lumihecogenin Acetate.- The acetate (280mg.) in dry ether (40ml.) was refluxed with lithium aluminium hydride (220mg.) for 5 min. Excess of reagent was destroyed with water and the product was isolated by extraction with ether. It was a clear gum (260mg.) which on trituration with acetone-light petroleum yielded anhydrohecolyl alcohol (LXVII), m.p.175-178° (200mg.). A pure sample had m.p. 180-182°, $[\alpha]_D$-53°(c 0.83) (Found: C, 74.9; H, 10.4. Calc. for $C_{27}H_{44}O_4$: C, 74.95; H,10.25%), $\lambda_{max.}$ 205mμ(ε 4400). The infrared spectrum was identical to that of a previously prepared sample and there was no mixed m.p. depression. Rothman et al. [54] report m.p.174-176°.

Action of Chromium Trioxide on Lumihecogenin Acetate.- Lumihecogenin acetate(LXXXVII)(155mg.) in acetone (10ml.) was stirred with chromium trioxide (480mg.) in sulphuric

acid (8N; 1.8ml.) at 35° for 5 min. The excess of oxidant was reduced with sulphur dioxide and the steroidal material was extracted with ether. The ethereal solution was washed with dilute aqueous potassium hydroxide, then with water, after which it was dried and evaporated. The residue (100mg.), on crystallisation from ether-isopentane, gave 3β,14β-dihydroxy-12-oxo-5α,25D-spirostan-3β-yl acetate (LXXXV ; R=OAc), m.p. 231.5-234°, $[\alpha]_D$-6° (c 0.74) (Found: C, 70.8; H, 8.8. $C_{29}H_{44}O_6$ requires C, 71.3; H, 9.1%), $\nu_{max.}$ 3540(bonded OH), 1739(OAc), 1706(>C=O) and 1250cm.$^{-1}$(OAc). This was transparent to ultraviolet light and gave no colour with tetranitromethane in chloroform.

The alkali washings from the extraction were acidified and extracted with ether in the usual way. After the solvent was evaporated the syrup obtained was refluxed in methanol (20ml.) with potassium hydroxide (1g.) for 30 min. The product was isolated by acidifying the solution, then extracting with ether in the usual way. It was a gum(60mg.) which, on crystallisation from aqueous acetone, provided anhydrohecolic acid (LXVI), m.p. and mixed m.p. with an authentic specimen 223-226°. The infrared spectra were superimposable.

3β,14β-Dihydroxy-5α,25D-spirostan-12-one (LXXXV ; R=OH) was obtained by refluxing the acetate (LXXXV ; R=OAc) (160mg.) in methanol (25ml.) with potassium hydroxide (1g.)

for 30 min. Water was added and the precipitate filtered off. The filtrate gave no precipitate on acidification. The neutral material was dried and crystallised from acetone-isopentane as prisms, m.p.270-272°, $[\alpha]_D$+16.4° (c 0.94) (Found: C, 72.15; H, 9.2. $C_{27}H_{42}O_5$ requires C, 72.6; H, 9.5%), $\nu_{max.}$ 3520(OH), 1708cm.$^{-1}$(>C=O).

14β-Hydroxy-5α,25D-spirostan-3,12dione (LXXXV ; R=O), was obtained by the action of chromium trioxide (53mg.) in sulphuric acid (8N; 2ml.) on anhydrohecolyl alcohol (LXVII) (130mg.) in acetone (10ml.). After maintaining the solution at 35° for 5 min. the product was obtained by extracting with ether in the usual way and separated into neutral and acid fractions. The former (102mg.) was crystallised from acetone isopentane to give 14β-Hydroxy-5α,25D-spirostan-3,12-dione, m.p.259-261°, $[\alpha]_D$+31.5° (c 1.08) (Found: C, 72.5; H, 8.95. $C_{27}H_{40}O_5$ requires C, 72.95; H, 9.1%), ν_{max} 3510(bonded OH), 1712cm.$^{-1}$(>C=O).

FIGURE I

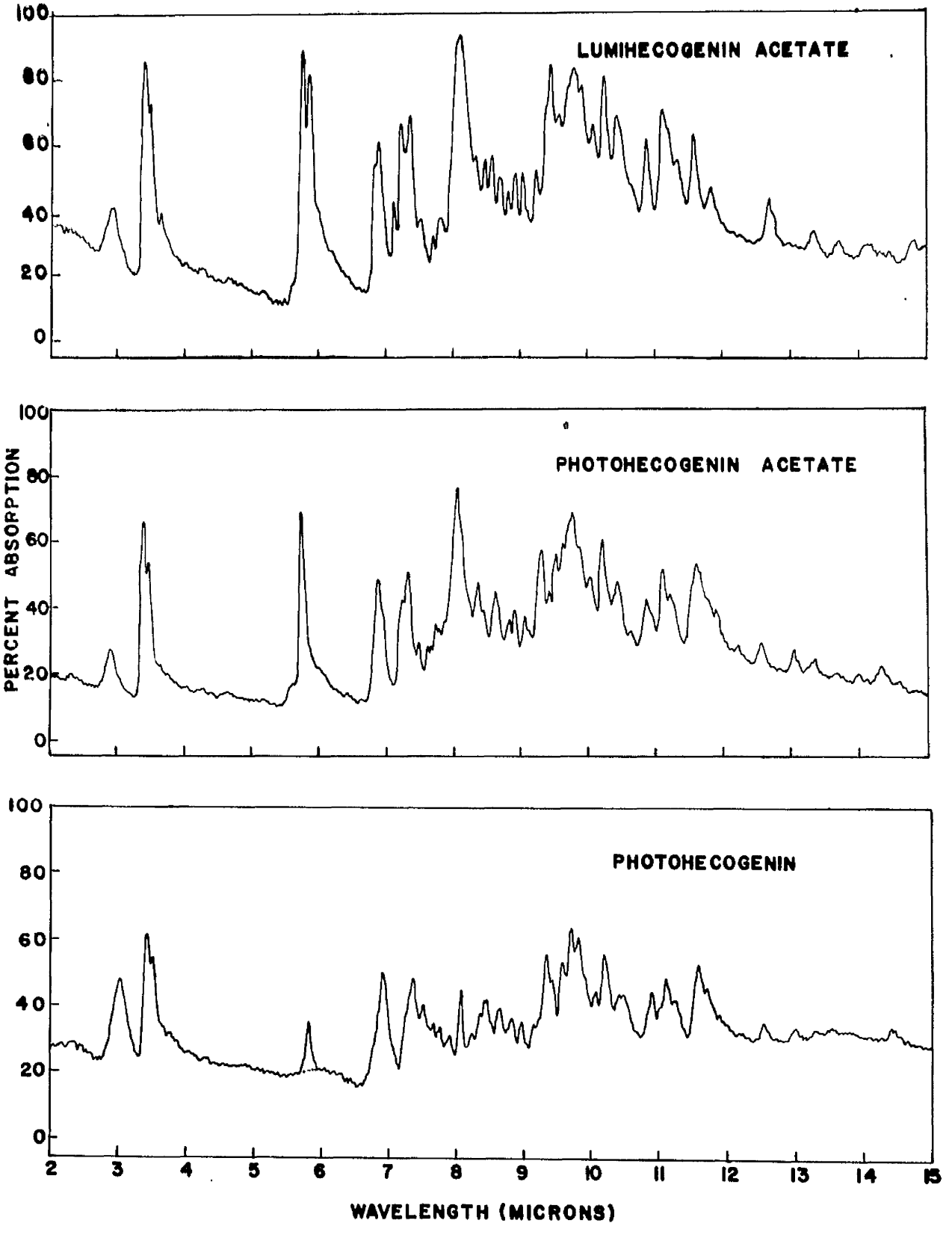
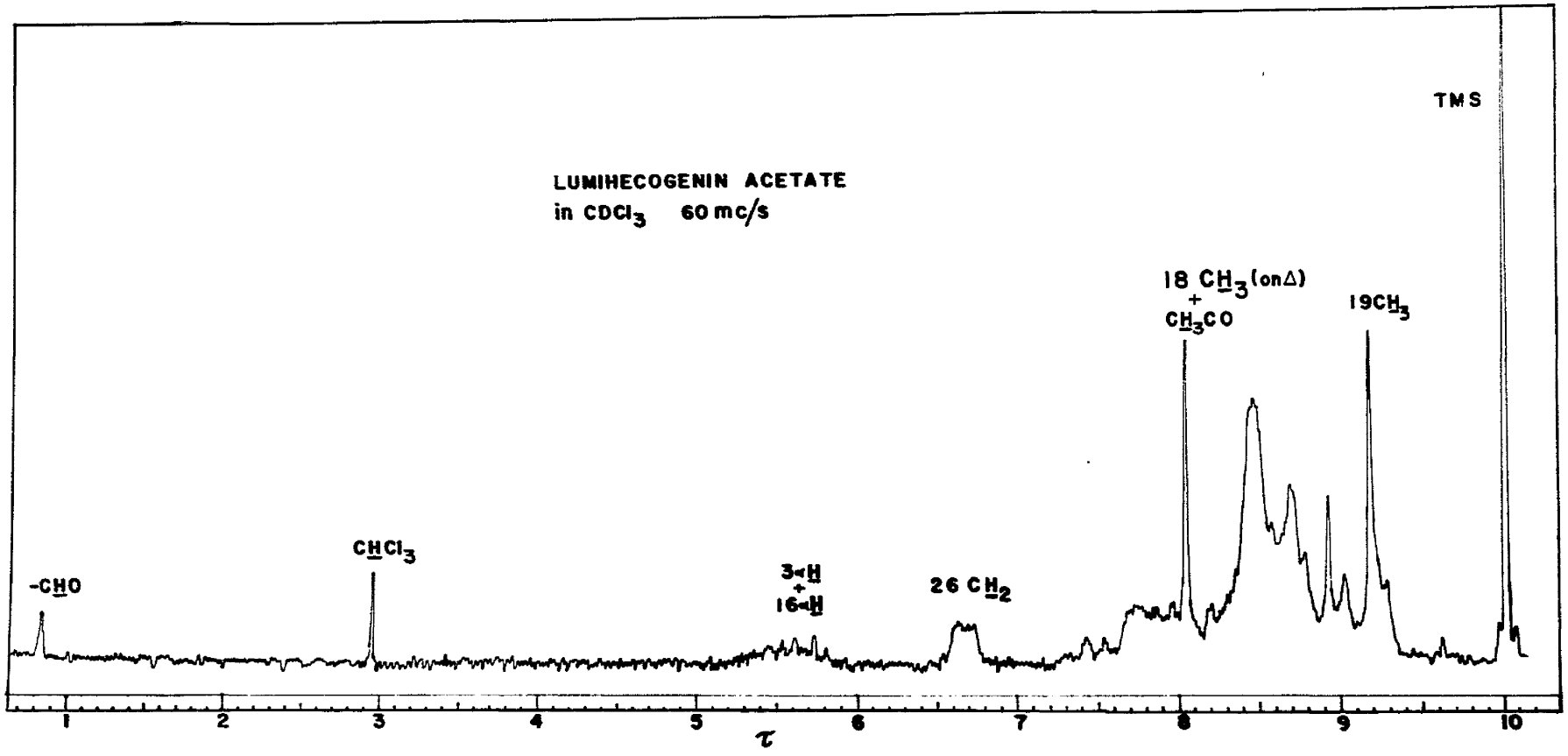

LUMIHECOGENIN ACETATE
PHOTOHECOGENIN ACETATE
PHOTOHECOGENIN
PERCENT ABSORPTION
100
80
60
40
20
0
2
3
4
5
6
7
8
9
10
11
12
13
14
15
WAVELENGTH (MICRONS)

FIGURE II


LUMIHECOGENIN ACETATE
in CDCl3 60 mc/s
TMS
18 CH3 (on Δ)
+
CH3CO
19CH3
-CHO
CHCl3
3-H
+
16-H
26 CH2
1
2
3
4
5
6
7
8
9
10
τ

B I B L I O G R A P H Y

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