441. *Steroids derived from Hecogenin. Part I. 12-Methyl-steroids.*

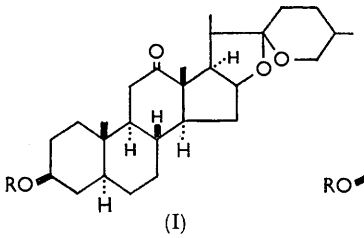
By Peter Bladon and W. McMeekin.

The preparation of several 12-methyl-steroids from hecogenin and $\Delta^{9(11)}$-dehydrohecogenin is described.

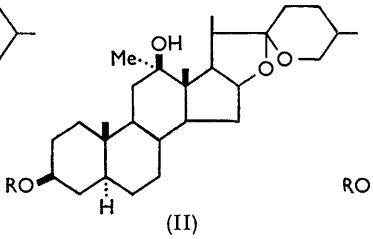
There is considerable current interest in steroid-hormone analogues containing alkyl (and other) substituents in the steroid nucleus,[1] but hitherto 12-alkylated steroids have not been prepared. The 12-carbonyl groups of hecogenin and $\Delta^{9(11)}$-dehydrohecogenin offered a route to such compounds by reaction with lithium alkyls and some exploratory work on this approach is described in this paper.

The reaction between hecogenin acetate (I; R = Ac) and methyl-lithium gave only small yields of the desired 12-methyl-alcohol. Accordingly, attention was directed towards derivatives of hecogenin in which the hydroxyl group is adequately protected.

The 3β-(tetrahydro-2-pyranyl) ether (I; R = C_5H_9O) of hecogenin reacted smoothly with methyl-lithium, to furnish a good yield of the intermediate tetrahydropyranyl ether of the secondary-tertiary glycol (II; R = C_5H_9O). Hydrolysis of this by aqueous-ethanolic hydrogen chloride and acetylation of the intermediate glycol gave 3β-acetoxy-12α-methyl-5α,25*D*-spirostan-12β-ol (II; R = Ac), together with a small amount of 12-methylenetigogenin acetate (III; R = Ac). This last compound on hydrolysis furnished the known [2] 12-methylenetigogenin (III; R = H).



(I)



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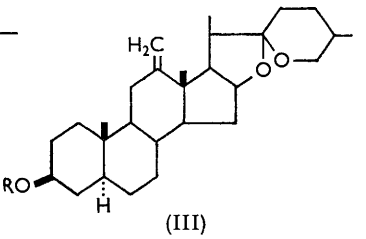


(III)

It was originally thought that the 12-methylenetigogenin was derived from an epimeric tertiary alcohol (IV) by dehydration during the hydrolysis of the corresponding tetrahydropyranyl ether by mineral acid. That this was not so, was shown by the failure to isolate either 12-methylenetigogenin (as acetate) or the epimeric tertiary alcohol when the hydrolysis was conducted in aqueous-ethanolic acetic acid.

The main product formed by either method of hydrolysis was the tertiary alcohol (II; R = H), since extensive chromatography of the acetate (II; R = Ac) failed to reveal any substantial inhomogeneity. Further, a sample of this material, treated with hot aqueous ethanolic hydrochloric acid, developed low-intensity ultraviolet-light absorption with a maximum at 205 mμ, presumably due to a small amount of 12-methylenetigogenin acetate. The olefinic substance was not isolated. The conclusion is drawn that the reaction of the 12-ketone group with methyl-lithium leads to a single product.

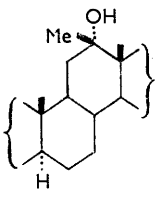
The formulation of this product as the 12β-hydroxy-12α-methyl compound (II) is based on the well-known propensity [3] of steroids to " rear attack," particularly in the region of ring C. In this case the attacking group is the methyl anion. No direct proof of this formulation is adduced, but the reactions described below are consistent with it. In the case of the 11-carbonyl group, Fonken and Hogg [4] and Ringold, Batres, and Zderic [5]

[1] Fried and Borman, *Vitamins and Hormones*, 1958, **16**, 303.
[2] Sondheimer and Mechoulam, *J. Amer. Chem. Soc.*, 1957, **79**, 5029.
[3] Fieser, *Experientia*, 1950, **6**, 312.
[4] Fonken and Hogg, *Tetrahedron*, 1958, **2**, 365; Fonken, *J. Org. Chem.*, 1958, **23**, 1075; Fonken, Hogg, and McIntosh, *ibid.*, 1959, **24**, 1600.
[5] Ringold, Batres, and Zderic, *Tetrahedron*, 1958, **2**, 164.

have shown recently that methyl-lithium gives a single product formulated as the 11β-hydroxy-11α-methyl compound.

Prolonged treatment of the tertiary alcohol (III; R = Ac) with phosphorus pentachloride gave 12α-chloro-12β-methyltigogenin acetate (V). This reaction is assumed to involve an inversion characteristic of the reactions of phosphorus pentachloride with saturated alcohols.[6] Treatment of the chloro-compound (V) with sodium methoxide in methanol yielded 12-methylenetigogenin (III; R = H).

The reverse transformation of 12-methylenetigogenin into the tertiary alcohol (II) was achieved in two steps: first, epoxidation of the acetyl derivative (III; R = Ac) gave the epoxide (VI) together with unchanged starting material; then reduction with lithium aluminium hydride followed by re-acetylation gave the acetate (II; R = Ac).



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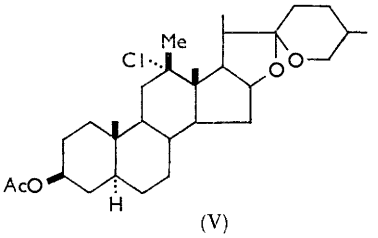

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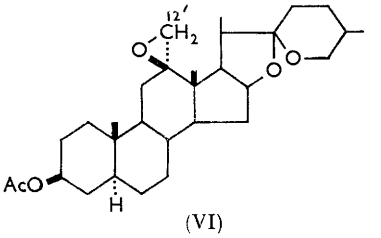
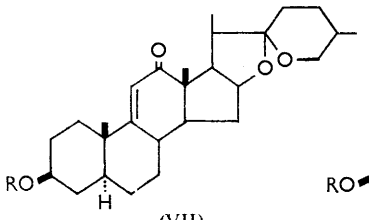


(VI)

To account for the formation of the 12β-hydroxyl group on reduction of the epoxide with lithium aluminium hydride, it is necessary to assume that the epoxide having the β-configuration is formed by attack on the front (β) side of the steroid nucleus by the OH^+ ion. Such an attack would be expected to centre on the exocyclic 12'-carbon atom (Markownikoff's rule) which is relatively unhindered. The alternative supposition, in which the alcohol has the 12α-hydroxy-12β-methyl configuration (front attack of Me^-) and the epoxide necessarily has the α-configuration (rear attack of OH^+), is considered unlikely.

The 3β-tetrahydropyranyl ether (VII; R = C_5H_9O) of 9(11)-dehydrohecogenin with methyl-lithium gave the tetrahydropyranyl ether (VIII; R = C_5H_9O) of the tertiary allylic alcohol. Even under the mild conditions of hydrolysis (aqueous-ethanolic acetic



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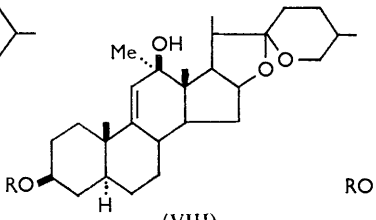

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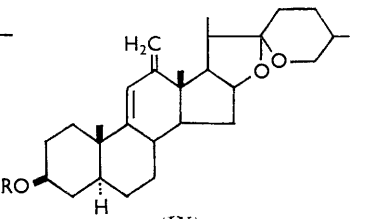

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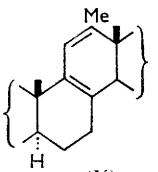

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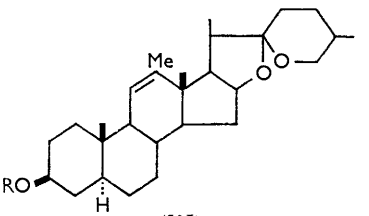

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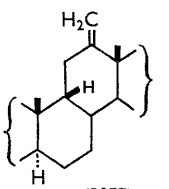

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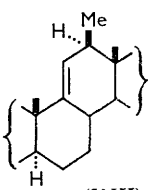


(XIII)

acid) necessary to effect removal of the protecting group, this compound was dehydrated, to yield (after acetylation) 3β-acetoxy-5α,25*D*-spirosta-9(11),12(12')-diene (IX; R = Ac). This had the expected light absorption with a maximum at 240 mμ. Hydrolysis by

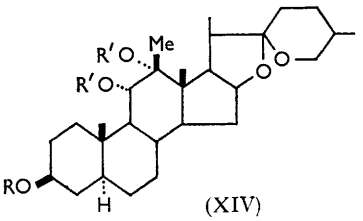
[6] Cf. Fieser and Fieser, " Steroids," Reinhold, New York, 1959, p. 322.

mineral acid caused a further partial rearrangement of the double bonds, to give a mixture of this diene and the isomeric 8(9),11-diene (X). Attempts to isolate the latter diene by crystallisation and by chromatography of the free alcohols and the 3β-acetates failed. The homoannular diene (X) in the mixture did not yield a crystalline product with maleic anhydride. Treatment of the diene mixture with hydrogen chloride in chloroform at low temperature (cf. ref. 7) caused inefficient conversion into the 9(11),12(12′)-diene (IX).

On hydrogenation under neutral conditions with a platinum catalyst, the 9(11),12(12′)-diene (IX; R = Ac) took up one mol. of hydrogen, to yield 3β-acetoxy-12-methyl-5α,25*D*-spirost-11-ene (XI; R = Ac). The corresponding alcohol (IX; R = H) behaved in the same way, to afford an alcohol (XI; R = H), which was also produced by hydrolysis of the acetate (XI; R = Ac).

This structure with the 11,12-double bond is favoured for these compounds for the following reasons. Under conditions comparable to those in which the diene was hydrogenated, 12-methylenetigogenin acetate (III; R = Ac) took up no hydrogen and was recovered unchanged, making hydrogenation of the methylene group in the diene (IX) unlikely. Hydrogenation of 9(11)-double bonds is known [8] to occur under these conditions, but in this particular case it would lead to 12-methylenetigogenin (acetate), and the monounsaturated compounds are quite different from 12-methylenetigogenin and its acetate. The unlikely addition of hydrogen to the front of the 9(11)-double bond would give 9β-12-methylenetigogenin derivatives (XII). Such a structure can be ruled out on chemical evidence (see below). There remains only the possibility of 1,4-addition of hydrogen at positions 9 and 12′, to give an 11,12-double bond. Subsequent migration of the double bond to the 9(11)-position (XIII) is improbable, since the isomerisation of 7-enes to 8(14)-enes proceeds only slowly,[9] if at all,[10] with a platinum catalyst under neutral conditions.

Successive treatment of 3β-acetoxy-12-methyl-5α,25*D*-spirost-11-ene with osmium tetroxide and lithium aluminium hydride gave 12β-methyl-5α,25*D*-spirostane-3β,11α,12α-triol (XIV; R = R′ = H) (cf. ref. 11).



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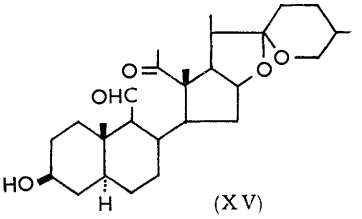

(XV)

Cleavage of the triol with lead tetra-acetate gave an amorphous compound (XV) having a peak in its infrared spectrum at 2878 cm.$^{-1}$ which indicates an aldehyde group. No formaldehyde was formed. These facts exclude structure (XII) for the olefin but do not distinguish between structures (XI) and (XIII).

Acetylation of the triol with acetic anhydride and pyridine at 100° gave a mixture of the 3β-monoacetate (XIV; R = Ac, R′ = H) and the 3β,11α,12α-triacetate (XIV; R = R′ = Ac).

Comparison of the molecular-rotation differences of the mono-olefin (XI) and its derivatives with the corresponding differences of the 9(11)- and 11-unsaturated compounds lacking the 12-methyl group does not allow of an unequivocal proof of the structure (XI) (cf. refs. 12 and 11 respectively).

[7] Cornforth, Gore, and Popjak, *Biochem. J.*, 1957, **65**, 94.
[8] Ruyle, Chamberlin, Chemerda, Sita, Aliminosa, and Erickson, *J. Amer. Chem. Soc.*, 1952, **74**, 5929.
[9] Nes and Mosettig, *J. Org. Chem.*, 1953, **18**, 276.
[10] Barton, Cox, and Holness, *J.*, 1949, 1771.
[11] Elks, Phillipps, Walker, and Wyman, *J.*, 1956, 4330.
[12] Hirschmann, Snoddy, Hiskey, and Wendler, *J. Amer. Chem. Soc.*, 1954, **76**, 4013.

Experimental

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 100 g.).

The hecogenin acetate which was the starting material for this work had $\lambda_{max.}$ 236 mμ (ε 363). This peak is attributed to the presence of about 3% of 9(11)-dehydrohecogenin acetate.

Extracts were dried over anhydrous sodium sulphate before evaporation unless stated otherwise.

3β-(*Tetrahydro-2-pyranyloxy*)-5α,25D-*spirostan*-12-*one* (I; R = C_5H_9O).—Hecogenin (I; R = H) (48 g.), suspended in dry benzene (400 ml.), was treated with 2,3-dihydropyran (40 ml.) and phosphorus oxychloride (1·5 ml.). After 10 min. at room temperature brief warming gave a homogeneous mixture. This was set aside for 1 hr. 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 (56 g., 99%) as needles (from methanol containing a little pyridine), m. p. 195—200°. Hirschmann *et al.*[12] record m. p. 209—213° and 51% yield.

12α-*Methyl*-3β-(*tetrahydro-2-pyranyloxy*)-5α,25D-*spirostan*-12β-*ol* (II; R = C_5H_9O).—The foregoing tetrahydropyranyl ether (I; R = C_5H_9O) (50 g.) in benzene (400 ml.) was treated with ethereal methyl-lithium, prepared from lithium (15 g.), methyl iodide (60 ml.), and ether (500 ml.), and the mixture was refluxed for 1·5 hr. 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 (51·9 g.) which was used directly for the next step. A portion of similar material, recrystallised twice from methylene chloride–methanol containing pyridine, gave the *tetrahydropyranyl ether* (II; R = C_5H_9O), m. p. 205—207°, $[\alpha]_D$ −38·2° (*c* 0·872) (Found: C, 72·8; H, 10·4. $C_{33}H_{54}O_5,CH_3{\cdot}OH$ requires C, 72·6; H, 10·4%).

Hydrolysis of the Tetrahydropyranyl Ether (II; R = C_5H_9O).—(a) *With mineral acid.* The ether (51·2 g.) was refluxed with 6N-hydrochloric acid (140 ml.) in ethanol (1200 ml.) for 40 min. Water (2800 ml.) was added and the product isolated by ether-extraction. The extracts were washed with aqueous potassium hydrogen carbonate, dried, and evaporated, to yield a yellowish semi-solid residue (38·4 g.). This was treated with pyridine (300 ml.) and acetic anhydride (200 ml.), and left overnight. Working up in the usual way by ether-extraction afforded a semi-solid residue (42 g.). This was chromatographed in light petroleum–benzene (1 : 6; 350 ml.) on alumina (2·8 Kg.). Benzene (3 l.) eluted material, m. p. 152—172° (8·1 g.). Several recrystallisations from methylene chloride–methanol afforded 12-*methylene*-5α,25D-*spirostan*-3β-*yl acetate* (III; 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%), $\lambda_{max.}$ 200 mμ (ε 3000), $\nu_{max.}$ 1739 (OAc) 1653 (>C=CH_2), 1242 (OAc), and 978, 914, 897, 861 $cm.^{-1}$ (spirostan system). This material gave a light yellow colour with tetranitromethane in chloroform.

Benzene–ether and ether eluted 3β-*acetoxy*-12α-*methyl*-5α,25D-*spirostan*-12β-*ol* (II; R = Ac) (34 g.), 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 1230 $cm.^{-1}$ (OAc), and the typical spirostan bands.

(b) *With aqueous acetic acid.* The tetrahydropyranyl ether (II; R = C_5H_9O) (5·5 g.) was refluxed with ethanol (180 ml.), acetic acid (40 ml.), and water (20 ml.) for 45 min. Water (1·5 l.) was added, and the precipitated solid was filtered off, then washed with water, dried, and acetylated with acetic anhydride (10 ml.) in pyridine (20 ml.) for 1 hr. at 100°. The product (4·8 g.) was isolated by addition of water and extraction with ether in the usual way, and chromatographed on alumina (500 g.). Light petroleum (750 ml.) eluted material (430 mg.), m. p. 145—182°, which was rechromatographed (see below). Light petroleum–benzene and benzene eluted 3β-acetoxy-12α-methyl-5α,25*D*-spirostan-12β-ol (II; R = Ac) (3·8 g.), m. p. 218—233°, $[\alpha]_D$ −43° (*c* 1·0). The material in the light petroleum eluates was rechromatographed on alumina (40 g.). Light petroleum–benzene (7 : 3) eluted tigogenin acetate (84 mg.), m. p. and mixed m. p. 204—207° (no ultraviolet absorption). Light petroleum–benzene (1 : 1) eluted

material (85 mg.) which crystallised from methanol to give needles, m. p. 150—152°, $\lambda_{max.}$ 238 mμ (ε 12,000). These gave no m. p. depression with 12-methylene-9(11)-dehydrotigogenin acetate (IX; R = Ac see below). This material is derived from 9(11)-dehydrohecogenin acetate, which is present together with tigogenin acetate in the hecogenin acetate as an impurity.

Action of Methyl-lithium on Hecogenin Acetate.—Hecogenin acetate (I; R = Ac) (5 g.) in ether (100 ml.) and benzene (80 ml.) was treated with a solution of methyl-lithium [prepared from lithium (1·6 g.), methyl iodide (6 ml.) and ether (100 ml.)] and refluxed for 3 hr. The product, isolated by ether-extraction in the usual way (5·65 g.), was acetylated with acetic anhydride and pyridine at room temperature overnight. Chromatography on alumina (750 g.) allowed the isolation of hecogenin acetate (110 mg.), m. p. 243—247°, and 3β-acetoxy-12α-methyl-5α,25*D*-spirostan-12β-ol (II; R = Ac) (100 mg.), m. p. 220—222°, as the only crystalline products. The remaining products from the reaction failed to react with Girard's reagent-T, showing that a carbonyl group was no longer present.

Treatment of Tetrahydropyranyl Ether of Hecogenin with Methylenetriphenylphosphorane.—The preparation of 12-methylenetigogenin by Sondheimer and Mechoulam's method [2] failed. The following modification was therefore devised. A solution of the Wittig reagent was prepared by stirring together methyltriphenylphosphonium bromide (1 g.) and 0·45N-phenyl-lithium in ether (6·5 ml.) for 0·5 hr. Then the tetrahydropyranyl ether of hecogenin (I; R= C_5H_9O) (300 mg.) 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 (III; R = C_5H_9O), m. p. 170—172°. This was refluxed with ethanol (40 ml.) and 6N-hydrochloric acid (4 ml.) for 50 min. Dilution with water and extraction with ether gave a solid, m. p. 215—240°, $\lambda_{max.}$ 202 mμ (ε 1000). This was acetylated with acetic anhydride (4 ml.) and pyridine (6 ml.) overnight at room temperature. Working up in the usual way afforded a semi-solid product (430 mg.) which was chromatographed on alumina (25 g.). Light petroleum–benzene (5 : 1) eluted 12-methylenetigogenin acetate (III; R = Ac) as platelets (from methylene chloride–methanol), m. p. 178—181°, $\lambda_{max.}$ 203 mμ (ε 3000) (35 mg.). 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° (170 mg.).

12-*Methylene*-5α,25D-*spirostan*-3β-*ol.*—12-Methylenetigogenin acetate (III; R = Ac) obtained by the methyl-lithium method (200 mg.) was hydrolysed by potassium hydroxide (2 g.) in boiling methanol (25 ml.) and dioxan (25 ml.) for 30 min. Extraction with ether gave 12-methylene-5α,25*D*-spirostan-3β-ol (III; 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 $C_{28}H_{44}O_3$,0·5CH_3·OH: C, 77·0; H, 10·4%), $\lambda_{max.}$ 202 mμ (ε 1700) $\nu_{max.}$ 3250 (OH), 1640 cm.$^{-1}$ (>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* (III; 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* (II; R = Ac).—(*a*) The acetate (150 mg.) in pyridine (3 ml.) was treated with phosphorus oxychloride (0·3 ml.) 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° (120 mg.).

(*b*) The acetate (510 mg.) in pyridine (20 ml.) was treated with purified thionyl chloride (2 ml.). 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 (450 mg.). This was chromatographed on alumina (30 g.). Light petroleum eluted crystalline material (50 mg.) 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 (50 mg.) in ethanol (15 ml.) containing 6N-hydrochloric acid (1·5 ml.) was refluxed for 40 min. Addition of water and filtration gave largely unchanged starting material which, however, had $\lambda_{max.}$ 205 mμ (ε 700).

Hydrolysis of 3β-*Acetoxy*-12α-*methyl*-5α,25D-*spirostan*-12β-*ol.*—The acetate (II; R = Ac) (700 mg.) in dioxan (25 ml.) and methanol (25 ml.) was treated with potassium hydroxide (2 g.), and the mixture refluxed for 2 hr. The product isolated by dilution with water and ether-extraction was a colourless syrup (675 mg.) which crystallised when triturated with pyridine.

Recrystallisation from ether containing a little pyridine afforded the *pyridine solvate* of 12α-methyl-5α,25*D*-spirostan-3β,12β-diol (II; 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, on material dried *in vacuo* at room temperature: C, 75·4; H, 10·2; loss in wt. at 130° *in vacuo*, 15·3. $C_{28}H_{46}O_4,C_5H_5N$ requires C, 75·4; H, 9·8; loss in wt., 15·2%. Found, on 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), 257 mμ (ε 2180). Compare the light absorption of pyridine: $\lambda_{max.}$ 251 (ε 2460), 257 (ε 2670) 263 mμ (ε 1800).

Benzoylation of this material gave the 3β-*benzoate* (II; 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%).

12α-*Chloro*-12β-*methyl*-5α,25D-*spirostan*-3β-*yl Acetate* (V).—12β-Hydroxy-12α-methyltigogenin acetate (II; R = Ac) (5 g.) in dry ether (500 ml.) was treated with phosphorus pentachloride (5·2 g.) with intermittent stirring for 48 hr. The solution was washed with 5% aqueous potassium hydroxide, dried, and evaporated. The residue (5 g.) was adsorbed on alumina (250 g.). Elution with light petroleum–benzene (5 : 1) gave 12α-*chloro*-12β-*methyl*-5α,25D-*spirostan*-3β-*yl acetate* (V) (2 g.), 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 1242 cm.$^{-1}$ (OAc) (no hydroxyl band).

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

12β,12′-*Epoxy*-12α-*methyl*-5α,25D-*spirostan*-3β-*yl Acetate* (VI).—12-Methylenetigogenin acetate (800 mg.) in dry benzene (45 ml.) was treated with perbenzoic acid (500 mg.) in benzene (8·3 ml.), 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 chromatographed on alumina (50 g.). Light petroleum–benzene (7 : 3) eluted impure starting material (320 mg.), m. p. 166—170°, $\lambda_{max.}$ 202 mμ (ε 500). Light petroleum–benzene (1 : 1) eluted material (180 mg.), m. p. 185—206°, which was not examined further. Benzene and ether eluted 12β,12′-*epoxy*-12α-*methyl*-5α,25D-*spirostan*-3β-*yl acetate* (VI) (156 mg.), 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 1250 cm.$^{-1}$ (OAc). This compound showed no light absorption in the ultraviolet region.

Reduction of the Epoxide (VI) *with Lithium Aluminium Hydride.*—The epoxide (VI) (50 mg.) in dry tetrahydrofuran (30 ml.) was refluxed with lithium aluminium hydride (100 mg.) 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,25*D*-spirostan-3β,12β-diol (II; 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 sample. A portion (20 mg.) 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,25*D*-spirostan-12β-ol, m. p. and mixed m. p. 223—226°.

3β-(*Tetrahydro*-2-*pyranyloxy*)-5α,25D-*spirost*-9(11)-*en*-12-*one* (VII; R = C_5H_9O).—9(11)-Dehydrohecogenin acetate (VII; R = Ac) was prepared according to the method of Djerassi, Martinez, and Rosenkranz,[13] and had m. p. 218—220°, $\lambda_{max.}$ 238 mμ (ε 11,500). Hydrolysis gave the free alcohol, m. p. 223—225°. This material (4·5 g.) was suspended in benzene (30 ml.) and treated with 2,3-dihydropyran (3 ml.) and phosphorus oxychloride (0·5 ml.). 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* (VII; 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%), $\lambda_{max.}$ 238 mμ (ε 13,300).

12α-*Methyl*-3β-(*tetrahydro*-2-*pyranyloxy*)-5α,25D-*spirost*-9(11)-*en*-12β-*ol* (VIII; R = C_5H_9O). —The foregoing tetrahydropyranyl ether (VII; R = C_5H_9O) (1·2 g.) in ether (50 ml.) was

[13] Djerassi, Martinez, and Rosenkranz, *J. Org. Chem.*, 1951, **16**, 303.

refluxed with 0·42N-ethereal methyl-lithium (32 ml.) 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* (VIII; R = C_5H_9O) as prisms, m. p. 182—186°, $[\alpha]_D$ −61° (*c* 1·43) (Found: C, 72·55; H, 9·75. $C_{33}H_{52}O_5$ requires C, 75·0; H, 9·9%) $\lambda_{max.}$ 204 mμ (ε 4000).

12-*Methylene*-5α,25D-*spirost*-9(11)-*en*-3β-*ol* (IX; R = H).—The tetrahydropyranyl ether (VIII; R = C_5H_9O) (1·1 g.) was refluxed for 2 hr. with acetic acid (10 ml.) in ethanol (90 ml.). Addition of water and extraction in the usual way with ether gave 12-*methylene*-5α,25D-*spirost*-9(11)-*en*-3β-*ol* (IX; R = H) (940 mg.) as needles (from methylene chloride–methanol), m. p. 204—205°, $[\alpha]_D$ +41° (*c* 1·33) (Found: C, 76·8; H, 10·1. $C_{28}H_{44}O_3,0{\cdot}5CH_3{\cdot}OH$ requires C, 77·0; H, 10·4%), $\lambda_{max.}$ 240 mμ (ε 20,000), $\nu_{max.}$ 3250 (OH), 1638 cm.$^{-1}$ (>C=C–C=CH_2). This material gave a deep yellow colour with tetranitromethane in chloroform.

The corresponding *acetate* (IX; 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%), $\lambda_{max.}$ 238 mμ (ε 19,700), $\nu_{max.}$ 1736 (OAc), 1626 (>C=C–C=CH_2), and 1250 cm.$^{-1}$ (OAc).

The corresponding *benzoate* (IX; 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%), $\lambda_{max.}$ 238 mμ (ε 29,000).

Hydrolysis of 12α-*Methyl*-3β-(*tetrahydro*-2-*pyranyloxy*)-5α,25D-*spirost*-9(11)-*en*-12β-*ol with Mineral Acid.*—The ether (VIII; R = C_5H_9O) (1·4 g.) was refluxed with 4N-hydrochloric acid (12 ml.) in ethanol (80 ml.) for 40 min. Dilution with water and extraction with ether gave a white solid (1·2 g.). This crystallised from methanol as needles, m. p. 164—174°, $[\alpha]_D$ +10°, $\lambda_{max.}$ 202 (ε 4300), 242 (ε 8800), 270 mμ (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), 272 mμ (ε 4200), which could not be purified by chromatography.

Action of Mineral Acid on 12-*Methylene*-9(11)-*dehydrotigogenin Acetate.*—The diene acetate (IX; R = Ac) (50 mg.) in ethanol (25 ml.) and 4N-hydrochloric acid (2 ml.) 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), 272 mμ (ε 3000).

Miscellaneous Experiments on the Dienyl Acetate Mixture (A).—(a) *Action of maleic anhydride.* The mixture (100 mg.) was refluxed in dry benzene (10 ml.) with maleic anhydride (120 mg.) 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), 272 mμ (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 (200 mg.) in dry chloroform (5 ml.) 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 acetate.

12-*Methyl*-5α,25D-*spirost*-11-*en*-3β-*yl Acetate* (XI; R = Ac).—12-Methylene-9(11)-dehydrotigogenin acetate (IX; R = Ac) (120 mg.) in ethyl acetate (15 ml.) was stirred with pre-reduced platinum oxide (120 mg.) in hydrogen [uptake 4·3 ml. (1 mol.) in 30 min.]. Filtration followed by evaporation gave 12-*methyl*-5α,25D-*spirost*-11-*en*-3β-*yl acetate* (XI; 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%), $\lambda_{max.}$ 206 mμ (ε 2800), $\nu_{max.}$ 1730 (OAc), 1654 (>C=CH–), 1238 cm.$^{-1}$ (OAc). This compound gave a pale yellow colour with tetranitromethane in chloroform.

Catalytic reduction of the dienyl acetate in acetic acid with platinum as catalyst resulted in the uptake of 2 mols. of hydrogen. The product was amorphous and had $\lambda_{max.}$ 210 mμ (ε 500).

12-*Methyl*-5α,25D-*spirost*-11-*en*-3β-*ol*.—(*a*) The dien-3β-ol (IX; R = H) (200 mg.) in ethyl acetate (20 ml.) was hydrogenated in the presence of pre-reduced platinum oxide (50 mg.). The product, isolated in the aforementioned way, had m. p. 198—201°. Recrystallisation from aqueous methanol gave 12-*methyl*-5α,25D-*spirost*-11-*en*-3β-*ol* (XI; R = H) as the *hemihydrate*, m. p. 206—208°, $[\alpha]_D$ −63° (*c* 1·11) (Found: C, 76·8; H, 10·1. $C_{28}H_{44}O_3,0{\cdot}5H_2O$ requires C, 76·9; H, 10·4%), $\lambda_{max.}$ 208 mμ (ε 2800), $\nu_{max.}$ 3220 (OH), 1639 cm.$^{-1}$ (>C=CH–).

(*b*) The same compound was obtained by hydrolysis of the acetate (XI; R = Ac), and had m. p. 206—208° undepressed by material prepared as in (*a*).

12-*Methyl*-5α,25D-*spirost*-11-*en*-3β-*yl benzoate* (XI; 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* (XIV; R = R′ = H).—12-Methyl-5α,25*D*-spirost-11-en-3β-yl acetate (XI; R = Ac) (2 g.) was treated in benzene (10 ml.) with osmium tetroxide (750 mg.) and pyridine (0·5 ml.) 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 (3 g.) for 1 hr. Excess of hydride was destroyed by methanol, followed by 4N-hydrochloric acid. The steroids were isolated by several extractions with chloroform (total 500 ml.). The extracts were washed with aqueous potassium hydrogen carbonate and water and evaporated without being dried. The residue (2·2 g.), containing a small amount of osmium compounds, was dissolved in benzene and chloroform (100 ml., 1 : 1) and adsorbed on alumina (150 g.). Benzene eluted material B (1·1 g.), m. p. 215—218°. Benzene–ether and ether eluted 12β-*methyl*-5α,25D-*spirostan*-3β,11α,12α-*triol* (XIV; 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. $C_{28}H_{46}O_5,0{\cdot}5CH_3{\cdot}OH$ requires C, 71·55; H, 10·1%).

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 (40 g.). Benzene eluted 12-methyl-5α,25*D*-spirost-11-en-3β-yl acetate (XI; R = Ac), m. p. and mixed m. p. 173—176° (700 mg.). Ether–methanol eluted the 3-monoacetate of the triol (XIV; R = Ac, R′ = H), m. p. 246—249° (70 mg.) (see below).

Acetylation of the Triol (XIV; R = R′ = H).—The triol (105 mg.) was heated with pyridine (2·5 ml.) and acetic anhydride (1·5 ml.) on the steam-bath for 90 min. Working up in the usual way gave a mixture which was chromatographed on alumina (20 g.). Light petroleum–benzene (9 : 1) eluted 12-*methyl*-3β,11α,12α-*triacetoxy*-5α,25D-*spirostan* (XIV; R = R′ = Ac), 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%). Benzene eluted 12-*methyl*-3β-*acetoxy*-5α,25D-*spirostan*-11α,12α-*diol* (XIV; R = Ac, R′ = H), 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 (OAc), 1270 cm.$^{-1}$ (OAc).

Treatment of the Triol (XIV; R = R′ = H) *with Lead Tetra-acetate*.—The triol (100 mg.) in t-butyl alcohol (10 ml.) and acetic acid (10 ml.) was treated with lead tetra-acetate (500 mg.) for 18 hr. at room temperature. Testing a portion of the mixture with chromotropic acid reagent [14] 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).

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[14] Feigl, " Spot Tests in Organic Analysis," Elsevier, Amsterdam, 1956, p. 331.