154. *The Structure of Ruscogenin.*

By D. Burn, B. Ellis, and V. Petrow.

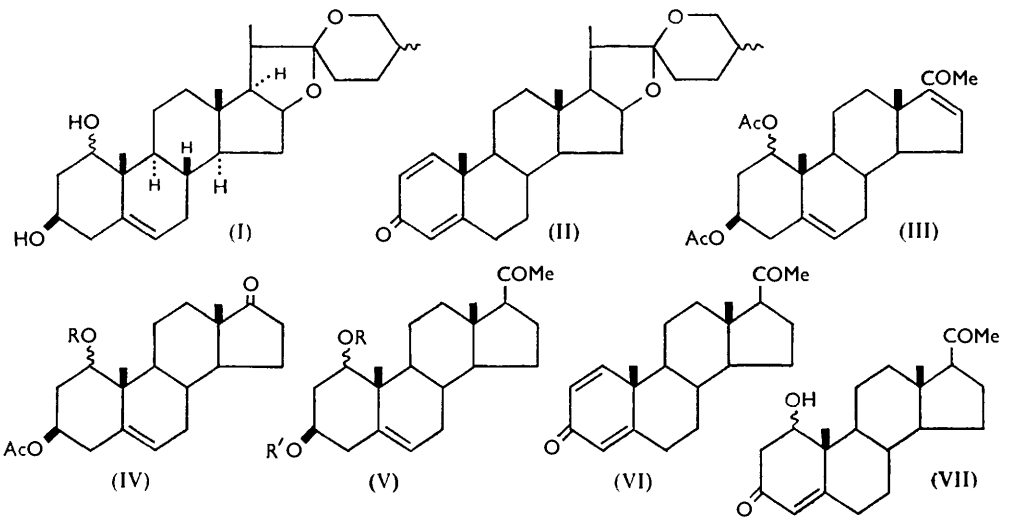
Ruscus aculeatus L., derived from Belgian sources, yields a *ca.* 1 : 1 mixture of ruscogenin (25D) and *neo*ruscogenin (25L). Degradation of this mixture gives an excellent yield of 1ξ : 3β-diacetoxypregna-5 : 16-dien-20-one (III), the structure of which follows from its transformations. The constitutions 25D- and 25L-spirost-5-ene-1ξ : 3β-diol (I) are consequently assigned to ruscogenin and *neo*ruscogenin, respectively.

In 1955 Lapin and Sannié [1] reported the presence in *Ruscus aculeatus* L. of a new sapogenin, ruscogenin, to which they assigned [2] the constitution of a 19-hydroxydiosgenin. In view of the potential importance of such a raw material for the preparation of the 19-nor-steroid hormones, we obtained a supply of *Ruscus aculeatus* L. from Belgian sources and isolated therefrom a genin with physical constants similar to those reported for ruscogenin. In contrast to the French results, degradation showed that our genin was a 1ξ- (I) and not 19-hydroxydiosgenin. In addition, infrared data furnished presumptive evidence that our product was a mixture of 25D- and 25L-forms. Our conclusions, published in preliminary form,[3,4] are now reported in full.

Whilst in Paris, one of us was told by Dr. H. Lapin that his independent findings [5] now led him to favour the 1ξ-hydroxy-structure (I). Our " ruscogenin," as anticipated in the preliminary communication, is a *ca.* 1 : 1-mixture of the 25D- (ruscogenin) and what

[1] Lapin and Sannié, *Bull. Soc. chim. France*, 1955, 1552.
[2] Sannié and Lapin, *ibid.*, p. 1556.
[3] Burn, Ellis, and Petrow, *Proc. Chem. Soc.*, 1957, 119.
[4] Petrow, Burn, and Ellis, XVIth Internat. Congr. Pure Appl. Chem., Résumés des Communications, Vol. II, p. 264.
[5] Lapin and Delépine, *Compt. rend.*, 1957, **244**, 3065.

appears to be the newly isolated [6] 25L-form (*neo*ruscogenin). This follows from (i) the identity of the infrared spectra of our " ruscogenin " with those of the authentic 25D- and 25L-forms (kindly supplied by Dr. H. Lapin) in all regions, except for the intensities of the bands near 920 and 900 cm.$^{-1}$ which are diagnostic of configuration at $C_{(25)}$, (ii) the preparation, after extensive fractionation, of pure ruscogenin diacetate from our genin



mixture (see p. 797), and (iii) the isolation, in considerably greater than 50% yield, of a single pregnane derivative by standard side-chain degradation of our sample. Slight differences in experimental findings between the two groups arose from the different proportions of the two forms present in our crude genin and in that available to Dr. H. Lapin, which had been derived from French sources and was markedly richer in the 25D-isomer. Our degradative studies had moreover been performed on the *ca.* 1 : 1 mixture of the 25D- and 25L-forms, as we had been interested primarily in pregnane and androstane derivatives obtainable from either.

Oppenauer oxidation of our ruscogenin (25D + 25L) gave, in low yield, a ketone to which the constitution 25L-spirosta-1 : 4-dien-3-one (II) is assigned, the 1 : 4-dien-3-one system being established by its ultraviolet spectrum [7] ($\lambda_{max.}$ 244·5 mμ) and that of the derived 2 : 4-dinitrophenylhydrazone [8] ($\lambda_{max.}$ 402 mμ). The 25L-configuration follows from comparison of the infrared spectrum with that of authentic 25D-spirosta-1 : 4-dien-3-one (II) (with which it is not chemically identical, see p. 797), revealing virtually identical bands except in the region 920—900 cm.$^{-1}$ (see above).[9] In addition, the 25D- and 25L-dienones have nearly identical optical rotations, in accord with the generalisation [10] that epimerisation at $C_{(25)}$ has a negligible effect on optical rotation. Fractionation of the mother-liquors from the Oppenauer oxidation did not yield the expected 25D-dienone, probably owing to the unsatisfactory nature of the oxidation which gave only the 25L-isomer in very low yield after exhaustive purification.

Degradation of the sapogenin side-chain of " ruscogenin " by standard procedures furnished 1ξ : 3β-diacetoxypregna-5 : 16-dien-20-one (III), which was further degraded by Beckmann rearrangement of its oxime and subsequent mild reacetylation to 3β-acetoxy-1ξ-hydroxyandrost-5-en-17-one (IV; R = H); more vigorous acetylation gave the diacetate (IV; R = Ac).

[6] Sannié and Lapin, XVIth Internat. Congr. Pure Appl. Chem., Résumés des Communications, Vol. II, p. 261.
[7] Cf. Dorfman, *Chem. Rev.*, 1953, **53**, 68.
[8] Djerassi and Ryan, *J. Amer. Chem. Soc.*, 1949, **71**, 1000.
[9] Wall, Eddy, McClennan, and Klumpp, *Analyt. Chem.*, 1952, **24**, 1337.
[10] Wall, *Experientia*, 1955, **11**, 340.

Partial hydrogenation of the diene (III) gave 1ξ : 3β-diacetoxypregn-5-en-20-one (V; R = R′ = Ac), which differed from the 3β : 19-diacetoxypregn-5-en-20-one previously obtained from strophanthidin.[11] The diacetate (V; R = R′ = Ac) with methanolic potassium hydroxide afforded the diol (V; R = R′ = H) which passed into 1-dehydroprogesterone[12,13] (VI) on Oppenauer oxidation, albeit in low yield. The same end-product was obtained by microbiological oxidation of the diol (V; R = R′ = H) with *Corynebacterium mediolanum* to 1ξ-hydroxyprogesterone (VII), followed by dehydration with methanolic alkali.

The two hydroxyl groups in the diol (V; R = R′ = H) differ in reactivity. Treatment of the diacetate (V; R = R′ = Ac) with aqueous methanolic potassium hydrogen carbonate leads to a monoacetate, regarded as 1ξ-acetoxy-3β-hydroxypregn-5-en-20-one (V; R = Ac; R′ = H) as it readily gives a sparingly soluble digitonin complex. Mild acetylation of the diol (V; R = R′ = H) furnished the isomeric 3β-acetoxy-1ξ-hydroxypregn-5-en-20-one (V; R = H; R′ = Ac) which failed to react with digitonin. These observations are analogous to those reported for 1α : 3β-diols of the cholestane[14] and 5α-etiocholanic acid[15] series. However, in the absence of experimental results relating to the behaviour of isomeric 1β : 3β-diols, our findings do not justify an assignment of configuration to the 1-hydroxyl group of (V; R = R′ = H) and of ruscogenin (I). The 1β-configuration is attractive on biogenetic grounds.

Experimental

Optical rotations refer to $CHCl_3$ solutions. Ultraviolet (in EtOH) and infrared (in CS_2) absorption spectra were kindly determined by Mr. M. T. Davies, B.Sc., of this laboratory and by Dr. A. E. Kellie of the Courtauld Institute of Biochemistry. Alumina of B.D.H. chromatography grade was used.

Genin Mixture.—As isolated from *R. aculeatus* and crystallised from aqueous methanol, the genin mixture (*ca.* 1 : 1 25D and 25L) formed plates, m. p. 197—199°, $[\alpha]_D^{21}$ −106° (*c* 0·58), $\nu_{max.}$ 898, 922 cm.$^{-1}$ (approximately equal intensity) (Found: C, 71·7; H, 9·5. Calc. for $C_{27}H_{42}O_4,H_2O$: C, 72·3; H, 9·9%). Lapin and Sannié [1] give m. p. 197—202°, $[\alpha]_D^{16}$ −119°, and, after extensive drying, m. p. 205—210°, $[\alpha]_D^{19}$ −127°.

Ruscogenin Diacetate.—The genin mixture (1·8 g.) in acetic anhydride (120 ml.) containing toluene-*p*-sulphonic acid (0·05 g.) was kept at room temperature for 2 hr. The product was isolated with ether and repeatedly crystallised from methanol. Ruscogenin diacetate formed needles, m. p. 190—192°, $[\alpha]_D^{20}$ −68° (*c* 0·33), $\nu_{max.}$ 899 > 921 cm.$^{-1}$ (Found: C, 71·7; H, 9·2. Calc. for $C_{31}H_{46}O_6$: C, 72·3; H, 9·0%). Lapin and Sannié [1] give m. p. 192—194°. The constants of *neo*ruscogenin diacetate (kindly supplied by Dr. H. Lapin), as determined by us, were m. p. 132—134°, $[\alpha]_D$ −64°, $\nu_{max.}$ 921 > 900 cm.$^{-1}$.

Hydrolysis of the 25D-diacetate afforded ruscogenin hydrate, m. p. 210—213°, $[\alpha]_D^{25}$ −116° (*c* 0·76) (Found: C, 72·9; H, 10·0%).

25L-*Spirosta*-1 : 4-*dien*-3-*one*.—A solution of the genin mixture (0·3 g.) in toluene (18 ml.) and *cyclo*hexanone (3 ml.) was distilled to remove water, aluminium *iso*propoxide in toluene (25% w/v; 1 ml.) was added, and the mixture was refluxed for 4 hr. The product was chromatographed in benzene on silica (20 g.). Elution with benzene–ether (9 : 1) gave a gum which crystallised from aqueous methanol, to give 25L-*spirosta*-1 : 4-*dien*-3-*one* as needles, m. p. 206—209°, $[\alpha]_D^{23}$ −77·2° (*c* 0·32), $\lambda_{max.}$ 245 mμ (ε 15,174), $\nu_{max.}$ 1658, 1624, 1605 cm.$^{-1}$ (1 : 4-dien-3-one) (in $CHCl_3$), 922 > 897 cm.$^{-1}$ (Found: C, 78·4; H, 8·5. $C_{27}H_{38}O_3$ requires C, 79·0; H, 9·3%); the mixed m. p. with the 25D-isomer (see below) was 177—186°. The 2 : 4-*dinitrophenylhydrazone* (prepared in ethanol–phosphoric acid) formed dark red needles, m. p. 243—244° (decomp.), $\lambda_{max.}$ 402 mμ (ε 34,200) (Found: C, 67·1; H, 6·85; N, 9·25. $C_{33}H_{42}O_6N_4$ requires C, 67·1; H, 7·2; N, 9·5%), after crystallisation from acetone–ethanol.

[11] Ehrenstein and Dünnenberger, *J. Org. Chem.*, 1956, **21**, 774.
[12] Vischer, Meystre, and Wettstein, *Helv. Chim. Acta*, 1955, **38**, 835.
[13] Sondheimer, Velasco, and Rosenkranz, *J. Amer. Chem. Soc.*, 1955, **77**, 5673.
[14] Striebel and Tamm, *Helv. Chim. Acta*, 1954, **37**, 1094.
[15] Sallmann and Tamm, *ibid.*, 1956, **39**, 1340.

25D-*Spirosta*-1 : 4-*dien*-3-*one*.—To 5α : 25D-spirostan-3-one (tigogenone) (3·65 g.) in acetic acid (150 ml.), bromine (4·1 g., 3 equiv.) in acetic acid (25 ml.) was added dropwise with stirring. The resulting purple solution was kept at room temperature overnight and then poured into water. The precipitated solids crystallised from dichloromethane–methanol, to yield moderately pure 2α : 4α : 23ξ-tribromo-5α : 25D-spirostan-3-one as needles, m. p. 192° (decomp.). No attempt was made to separate the $C_{(23)}$ isomers. The total product (1·55 g.) was heated with collidine (15 ml.) under reflux for 2 hr. The precipitated hydrobromide (0·93 g.) was removed and the product was isolated with ether. Purification from methanol yielded 23ξ-bromo-25D-spirosta-1 : 4-dien-3-one as needles, m. p. 192—195°. The last compound (1·8 g.) was heated under nitrogen with acetic acid (60 ml.) containing sodium iodide [16] (6 g.) on the steam-bath for 30 hr. and the product was isolated with ether–benzene. Purification from aqueous acetone gave 25D-spirosta-1 : 4-dien-3-one as needles, m. p. 198—200°, $[\alpha]_D^{24}$ −70·4° (*c* 0·64), $\lambda_{max.}$ 244·5 mμ (ε 15,280), $\nu_{max.}$ 1659, 1621, 1604 cm.$^{-1}$ (1 : 4-dien-3-one) (in $CHCl_3$), 921 < 898 cm.$^{-1}$ (Found: C, 78·4; H, 9·25%). Miki and Hara [17] give m. p. 186° and no other constants.

Degradation of the Genin Mixture.—The genin (15 g.) was heated under reflux for 2·5 hr. with acetic anhydride (30 ml.) and pyridine (15 ml.) containing methylamine hydrochloride (5 g.). The mixture was poured into water and extracted with ether. The ethereal extract was washed with dilute acid, aqueous sodium carbonate, and water, dried (Na_2SO_4), and evaporated under reduced pressure. The residual gum was purified from methanol, to yield *ψ-ruscogenin triacetate* as laths, m. p. 96—98°, $[\alpha]_D^{22}$ ±0° (*c* 1·0) (Found: C, 70·9; H, 8·7. $C_{33}H_{48}O_7$ requires C, 71·2; H, 8·7%).

A solution of this triacetate (10 g.) in acetic acid (100 ml.) was treated slowly, with stirring, with chromium trioxide (2·9 g.) in acetic acid (30 ml.). After 1 hr. the mixture was poured into water, and the product was isolated with ether and boiled with acetic acid (50 ml.) under reflux for 2 hr. The residue obtained on evaporation under reduced pressure was dissolved in ether, the ethereal solution was washed with aqueous sodium carbonate and water, dried (Na_2SO_4), and evaporated. Chromatography of the residue in benzene solution on alumina (150 g.) gave 1ξ : 3β-*diacetoxypregna*-5 : 16-*dien*-20-*one* (III) as needles, m. p. 127—129°, $[\alpha]_D^{23}$ ±0° (*c* 0·8), $\lambda_{max.}$ 239·5 mμ (ε 9,250) (Found: C, 72·8; H, 8·5. $C_{25}H_{34}O_5$ requires C, 72·4; H, 8·3%), after purification from acetone–hexane.

The foregoing compound (5 g.) in ethanol (100 ml.) was treated at room temperature with hydroxylamine hydrochloride (5 g.) and sodium acetate (7·5 g.) in water (20 ml.) for 48 hr. Dilution with water precipitated the crystalline *oxime*, stout needles, m. p. 188—192°, $[\alpha]_D^{25}$ +5·1° (*c* 0·78), $\lambda_{max.}$ 236 mμ (ε 14,930) (Found: C, 69·6; H, 8·0; N, 3·3. $C_{25}H_{35}O_5N$ requires C, 69·9; H, 8·2; N, 3·25%), after crystallisation from aqueous methanol.

3β-*Acetoxy*-1ξ-*hydroxyandrost*-5-*en*-17-*one* (IV; R = H).—A solution of the foregoing oxime (4·75 g.) in dry pyridine (50 ml.) containing toluene-*p*-sulphonyl chloride (5 g.) was kept at 0° for 18 hr. The solution was then poured slowly into ice-cold 10% sulphuric acid (300 ml.), and the resulting suspension was kept at room temperature for a further 3 days. The product was isolated with ether as a gum which was reacetylated with acetic anhydride–pyridine on the steam-bath for 1 hr. The product precipitated with water was chromatographed in benzene on alumina (40 g.); elution with the same solvent gave a gum which, purified from acetone–hexane, gave 3β-*acetoxy*-1ξ-*hydroxyandrost*-5-*en*-17-*one*, needles, m. p. 94—98°, $[\alpha]_D^{21}$ +17·85° (*c* 0·62), $\lambda_{max.}$ 294 mμ (ε 34) (Found: C, 72·3; H, 9·15. $C_{21}H_{30}O_4$ requires C, 72·8; H, 8·7%).

1ξ : 3β-*Diacetoxyandrost*-5-*en*-17-*one* (IV; R = Ac).—The foregoing monoacetate (0·35 g.) was treated with acetic anhydride (5 ml.) containing toluene-*p*-sulphonic acid (0·05 g.) at room temperature for 2 hr. Dilution with water and recrystallisation of the product from acetone–hexane gave 1ξ : 3β-*diacetoxyandrost*-5-*en*-17-*one* as needles, m. p. 149—151°, $[\alpha]_D^{20}$ +20·7° (*c* 0·99), $\lambda_{max.}$ 294 mμ (ε 41) (Found: C, 71·6; H, 8·6. $C_{23}H_{32}O_5$ requires C, 71·1; H, 8·3%).

1ξ : 3β-*Diacetoxypregn*-5-*en*-20-*one* (V; R = R′ = Ac).—The corresponding diene (III) (2 g.) was hydrogenated in ethanol (100 ml.) in the presence of 2% palladium–barium carbonate (0·4 g.) at room temperature and pressure. After removal of the catalyst, dilution with water gave a solid which was recrystallised from aqueous methanol, to yield 1ξ : 3β-diacetoxypregn-5-en-20-one as needles, m. p. 166—168°, $[\alpha]_D^{22}$ +45·4° (*c* 0·74), $\lambda_{max.}$ 286 mμ (ε 45) (Found: C, 71·8; H, 8·7. Calc. for $C_{25}H_{36}O_5$: C, 72·1; H, 8·7%). Lapin and Delépine [5] give m. p. 160—162° $[\alpha]_D$ +54°.

[16] Kirk, Patel, and Petrow, *J.*, 1957, 1046.
[17] Miki and Hara, *Pharm. Bull.* (*Japan*), 1956, **4**, 421.

1ξ : 3β-*Dihydroxypregn*-5-*en*-20-*one* (V; R = R′ = H).—The diacetate (V; R = R′ = Ac) (1·7 g.) in methanol (50 ml.) was treated with potassium hydroxide (4·6 g.) in water (15 ml.) for 18 hr. at room temperature. Dilution with water gave solids which, purified from aqueous methanol, yielded 1ξ : 3β-dihydroxypregn-5-en-20-one as laths, m. p. 187—190°, $[\alpha]_D^{19}$ +26·2° (*c* 0·81) (Found: C, 75·4; H, 9·3. Calc. for $C_{21}H_{32}O_3$: C, 75·8; H, 9·7%). Lapin and Delépine [5] give m. p. 186—188°, $[\alpha]_D$ +34°.

1-*Dehydroprogesterone* (VII). —A solution of the foregoing diol (1·15 g.) in toluene (40 ml.) and *cyclo*hexanone (10 ml.) was distilled to remove 10 ml. A solution of aluminium *iso*propoxide in toluene (25% w/v; 4 ml.) was added and the mixture heated under reflux for 0·5 hr. The product crystallised from aqueous methanol, unchanged diol (0·3 g.; m. p. and mixed m. p. 188—190°) separating. Chromatography of the solids left in the mother-liquors in benzene on alumina (30 g.) afforded 1-dehydroprogesterone, prisms, m. p. 155—157°, $[\alpha]_D^{20}$ +131·7° (*c* 0·88), $\lambda_{max.}$ 244 mμ (ε 16,740) (Found: C, 80·9; H, 8·8. Calc. for $C_{21}H_{28}O_2$: C, 80·7; H, 9·0%), after crystallisation from acetone–hexane. The m. p. was not depressed on admixture with an authentic sample kindly provided by Dr. A. Wettstein.

1ξ-*Hydroxyprogesterone* (VIII) (with Mrs. I. A. Stuart-Webb, B.Sc., and Mrs. A. Carmichael, B.Sc.).—The diol (V; R = R′ = H) (0·2 g.) was oxidised microbiologically with *Corynebacterium mediolanum*. The product, isolated with chloroform, crystallised from acetone–hexane to give 1ξ-*hydroxyprogesterone* as needles, m. p. 153°, $[\alpha]_D^{23}$ +192° (*c* 0·11), $\lambda_{max.}$ 240·5 mμ (ε 14,500) (Found: C, 76·3; H, 9·2. $C_{21}H_{30}O_3$ requires C, 76·4; H, 9·1%).

Treatment of this material (8 mg.) with methanolic potassium hydroxide for 1 hr. under reflux yielded 1-dehydroprogesterone, m. p. 138—143°, $\nu_{max.}$ 1660 (1 : 4-dien-3-one), 1707 cm.$^{-1}$ (20-ketone), mixed m. p. with an authentic sample 144—147°.

1ξ-*Acetoxy*-3β-*hydroxypregn*-5-*en*-20-*one* (V; R = Ac, R′ = H).—To a solution of the diacetate (V; R = R′ = Ac) (2 g.) in methanol (200 ml.) was added potassium hydrogen carbonate (5 g.) in water (100 ml.), and the mixture was kept at room temperature for 18 hr. Dilution with water and extraction with ether afforded a gum which was crystallised from aqueous methanol, to give 1ξ-*acetoxy*-3β-*hydroxypregn*-5-*en*-20-*one* as needles, m. p. 161—163°, $[\alpha]_D^{21}$ +50° (*c* 0·93) (Found: C, 73·3; H, 9·05. $C_{23}H_{34}O_4$ requires C, 73·7; H, 9·15%).

3β-*Acetoxy*-1ξ-*hydroxypregn*-5-*en*-20-*one* (V; R = H, R′ = Ac).—A solution of the diol (V; R = R′ = H) (0·7 g.) in acetic acid (3 ml.) and acetic anhydride (1 ml.) was kept for 2 hr. at 60° and a further 2 days at room temperature. The product was isolated with ether and crystallised from acetone–hexane, unchanged diol (0·3 g.; m. p. and mixed m. p. 187—190°) separating. Chromatography of the solids left in the mother-liquors in benzene on alumina (20 g.) afforded 3β-*acetoxy*-1ξ-*hydroxypregn*-5-*en*-20-*one* as plates, m. p. 188—190°, $[\alpha]_D^{24}$ +31·2° (*c* 0·63) (Found: C, 73·6; H, 9·6. $C_{23}H_{34}O_4$ requires C, 73·7; H, 9·15%), after crystallisation from aqueous methanol.

The authors thank Dr. J. Forrest, Ph.D., for assistance in the isolation of the genins and the Directors of The British Drug Houses Ltd., for permission to publish this work.

Chemical Research Laboratories,
The British Drug Houses Ltd., London, N.1. [*Received, September 16th*, 1957.]