Steroids. Part XII. The Structures of Cholegenin and* iso*Cholegenin.*

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Cholegenin, obtained from ox-bile, is shown to be spirostane-3α : 27-diol (III); *iso*cholegenin differs from it in configuration at $C_{(25)}$.

THE preceding paper describes the isolation of cholegenin from ox-bile; a simplified method for this extraction is described in the Experimental section of this paper. Cholegenin, $C_{27}H_{44}O_4$, $[\alpha]_D$ −27°, forms a monoacetate and a diacetate; it is not unsaturated since it does not give a colour with tetranitromethane and does not show selective absorption between 2000 and 4000 Å. It does not contain a carbonyl group since it does not show low intensity selective absorption in the neighbourhood of 2800 Å or show carbonyl bands in the infra-red,† and it is not reduced by lithium aluminium hydride. Treatment of cholegenin with hydrochloric acid in ethanol or with hydrogen chloride in chloroform converts it into *iso*cholegenin without change in the function of the four oxygens. Cholegenin thus appears to contain two hydroxyl groups and two ether-like oxygen atoms. Its origin, lævorotation, molecular formula, and general properties all suggested that it may be a steroid sapogenin, and this view has been confirmed.

Treatment of *iso*cholegenin diacetate with acetic anhydride at 200—220° gives an oily product presumed to be a *pseudo*sapogenin acetate since in contrast to *iso*cholegenin diacetate it shows strong selective absorption at 2100 Å. Mild oxidation of the *pseudo*-sapogenin acetate with chromic acid followed by alkaline hydrolysis of the product gives a crystalline compound, $C_{21}H_{32}O_2$, which shows the characteristic ultra-violet absorption spectrum of an αβ-unsaturated ketone and was identified as 3α-hydroxypregn-16-en-20-one (I) (Marker, *J. Amer. Chem. Soc.*, 1940, **62**, 3350) by comparison with a specimen obtained by hydrolysis of a sample of its acetate kindly given to us by Dr. C. Djerassi of Wayne University, Detroit. A similar degradation of cholegenin also gave 3α-hydroxypregn-16-en-20-one.

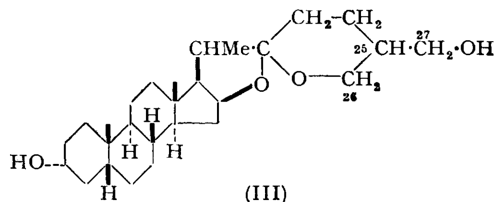
Confirmation of the sapogenin-like nature of cholegenin was obtained by oxidation of its diacetate with chromic acid to an acetate-lactone, $C_{24}H_{36}O_4$, identified as 3α-acetoxy-16β-hydroxybisnorcholanic lactone (II) by comparison with a lactone obtained by similar oxidation of 3α-acetoxy*iso*spirostan (*epi*smilagenin acetate).

The conversion of cholegenin into 3α-hydroxypregn-16-en-20-one (I) and 3α-acetoxy-16β-hydroxybisnorcholanic lactone (II) shows that it is derived from spirostan-3α-ol by introduction of a second hydroxyl group into the fragment C_{23}—C_{27}. Evidence that the second hydroxyl group is primary and consequently attached to $C_{(27)}$ was adduced from the behaviour of cholegenin on oxidation with chromic acid. When mild reaction conditions are used the product is a keto-acid, $C_{27}H_{40}O_5$, characterised as its methyl ester. Reduction of the keto-acid with lithium aluminium hydride converted it into cholegenin. It follows that cholegenin is spirostane-3α : 27-diol (III).‡ Cholegenin and *iso*cholegenin differ in configuration at $C_{(25)}$. The latter is therefore termed, specifically, 25-*iso*cholegenin.

The infra-red absorption spectra of cholegenin and its derivatives have been examined

* Part XI, preceding paper.
† We are indebted to Professor E. R. H. Jones, F.R.S., and Dr. G. D. Meakins for this information.
‡ Configuration at $C_{(22)}$ is not implied by the designation or formula used for the spiro-ketal chain in (III).

in some detail by Professor H. H. Günthard, Zürich, to whom we express our best thanks for the following report. In addition to a strong band at 3410 cm.$^{-1}$ (hydroxyl) the spectrum of cholegenin shows a number of intense bands between 850 and 1300 cm.$^{-1}$ attributable to the spiro-ketal group; these include bands at 1009 and 1042 cm.$^{-1}$. Of the other bands, those at 920 and 900 cm.$^{-1}$ correspond to the ψ- and ω-bands of normal sapogenins (Jones, Katzenellenbogen, and Dobriner, *ibid.*, 1953, **75**, 158) both in location and in relative intensity, the former being more intense than the latter. The spectrum of



(III)

25-*iso*cholegenin on the other hand includes bands at 3290 cm.$^{-1}$ (hydroxyl) and a number of bands in the region 850—1300 cm.$^{-1}$, of which that at 923 cm.$^{-1}$ (T-band) is less intense than that at 895 cm.$^{-1}$ (U-band). In these respects cholegenin shows the characteristics of a normal sapogenin, and 25-*iso*cholegenin those of an *iso*sapogenin. A remarkable feature of the infra-red absorption spectrum of 25-*iso*cholegenin diacetate is worthy of comment. Although absorption bands at approximately 1730 and 1250 cm.$^{-1}$ attributable to the acetate group are present in the spectra of cholegenin diacetate and *iso*cholegenin monoacetate and diacetate, the last compound differs from the other two in that the 1250 cm.$^{-1}$ band is complex (peaks at 1222, 1250, 1253, and 1264 cm.$^{-1}$). This effect has been observed in a number of steroid acetates (Jones, Humphries, Herling, and Dobriner, *ibid.*, 1951, **73**, 3215; Fürst, Kuhn, Scotoni, and Günthard, *Helv. Chim. Acta*, 1952, **35**, 951) and, in all but one case, the acetoxy-group involved has the axial conformation, the corresponding epimeric (equatorial) acetate having an unresolved 1250-cm.$^{-1}$ band. Acetylation of 25-*iso*cholegenin with pyridine and acetic anhydride at room temperature yields mainly the monoacetate whereas similar treatment of cholegenin yields the diacetate. It is concluded that 25-*iso*cholegenin monoacetate is the 3-acetate and that the resolution of the 1250 cm.$^{-1}$ band of 25-*iso*cholegenin diacetate arises from a relatively hindered acetoxy group in the side chain.

Experimental

For general instructions see preceding paper. Ultra-violet absorption spectra were measured in ethanol with a Unicam S.P. 500 spectrophotometer.

Simplified Method for the Isolation of Cholegenin.—The neutral fraction (380 g.) from ox-bile (80 gallons), prepared as described in the preceding paper, was refluxed with ether (3 l.) for 24 hr. The ether-insoluble fraction (30 g.) was not examined. The ether-soluble fraction was boiled under reflux with ethanolic potassiumhydroxide (5%; 1·5 l.) for 3 hr. and the non-saponifiable fraction isolated by means of ether. This fraction was refluxed with light petroleum (2 l.) for 30 min., and set aside at room temperature overnight. The solid was collected and extracted with boiling light petroleum (500 c.c. × 5) and the insoluble residue A (32·6 g.) treated as described below. The combined hot light petroleum extracts were concentrated in vacuum, and the separating solid was collected and recrystallised from methanol, to yield cholesterol (49 g., m. p. and mixed m. p. (148—149°). Concentration of the methanol mother-liquor gave more cholesterol (5 g.), evaporation of the mother-liquor from which gave a residue B (2·4 g.). The light petroleum mother-liquor was evaporated, the residue (40 g.) combined with residues A and B, and the mixture chromatographed in benzene (150 c.c.) on alumina (1500 g.). Benzene (2 l.) and benzene–ether (4 : 1, 2·5 l.; 1 : 1, 2 l.; 2 : 3, 2 l.; 1 : 4, 1·75 l.) eluted a gum (4·26 g.). Continued elution with benzene–ether (2 : 3, 0·75 l.; 1 : 9, 1·7 l.) eluted a resin (6·5 g.) which, after trituration with methanol, gave cholesterol (3 g.), m. p. and mixed m. p. 148—149°. The next fraction (24·3 g.), which was eluted with benzene–ether (1 : 9, 6 l.), also gave cholesterol (20 g.), m. p. and mixed m. p. 148—149° after crystallisation from methanol. Continued washing of the column with the same solvent mixture (3·5 l.) gave a fraction (6·6 g.; m. p. 125—154°) which was triturated with ether (100 c.c.), crystallisation of the ether-soluble fraction (4·25 g.) from methanol giving

cholesterol (m. p. and mixed m. p.). The ether-insoluble fraction was not examined. The column was next washed with benzene–ether (1 : 9; 4 l.), ether (22·25 l.), and ether–methanol (99 : 1; 1 l.), to give a fraction (11·3 g.), m. p. 172—195°, which was triturated with cold ether (2 × 100 c.c.). The ether-soluble fraction was recrystallised (5 times) from ether–light petroleum (b. p. 60—80°), to yield cholegenin (4·1 g.) as needles, m. p. and mixed m. p. 192°, $[\alpha]_D$ −27° (*c*, 1·6). From the mother-liquors a further quantity of impure cholegenin (1·1 g.) was obtained, m. p. and mixed m. p. 185—187°. The fraction insoluble in ether, when crystallised four times from chloroform–ethyl acetate, gave *iso*cholegenin (0·3 g.) as fine needles, m. p. 249—254°, $[\alpha]_D$ −65° (*c*, 0·5); the m. p. of a mixture with an authentic specimen of m. p. 256° was undepressed.

*3α-Hydroxypregn-16-en-20-one from 25-*iso*Cholegenin.*—25-*iso*Cholegenin diacetate (200 mg.) in acetic anhydride was heated in a sealed tube at 200° for 10 hr. The oily product, isolated by means of ether, was chromatographed in light petroleum (5 c.c.) on neutral alumina (8 g.) prepared as described by Mancera, Barton, Rosenkranz, and Djerassi (*J.*, 1952, 1021). A fraction (oil; 45 mg.) eluted with light petroleum (150 c.c.) showed intense ultra-violet absorption with a maximum at 2100 Å (ε 7000). This was dissolved in acetic acid (80%; 10 c.c.) and treated dropwise during 10 min. at 15° with chromic acid (45 mg.) in acetic acid (2 c.c.; 80%). After 2 hr. at room temperature, the solution was treated with methanol; the neutral product was isolated by means of ether and refluxed for 35 min. with a solution of potassium hydrogen carbonate (2·4 g.) in methanol (70%; 50 c.c.). The hydrolysed neutral product (39 mg.) in light petroleum–benzene (1 : 1, 5 c.c.) was filtered through alumina (8 g.). Benzene–ether (50 : 1; 150 c.c.) eluted a solid which was sublimed in a high vacuum at 130°, crystallised from ether, and sublimed once more, to give 3α-hydroxypregn-16-en-20-one as prisms, m. p. 194—196°, $[\alpha]_D$ +57° (*c*, 0·5). Light absorption : Max. at 2390 Å (ε 8900) (Found : C, 79·7; H, 10·6. Calc. for $C_{21}H_{32}O_2$: C, 79·7; H, 10·2%).

An authentic sample of 3α-hydroxypregn-16-en-20-one was obtained by hydrolysis of its acetate (m. p. 103—106°) with aqueous potassium hydroxide in tetrahydrofuran. After chromatography on alumina, it separated from ether as prisms, m. p. 198—199°, $[\alpha]_D$ +60° (*c*, 0·9). Light absorption : Max. at 2390 Å (ε 9300). It was undepressed in m. p. when mixed with the specimen obtained from 25-*iso*cholegenin.

3α-Hydroxypregn-16-en-20-one from Cholegenin.—Cholegenin (0·46 g.) in acetic anhydride (10 c.c.) was kept at 220° for 16 hr. (sealed tube). The anhydride was decomposed with water, and the amorphous product isolated by means of ether. A solution of the product in acetic acid (80%; 10 c.c.) was treated dropwise during 10 min., with stirring, with chromic acid (0·4 g.) in acetic acid (80%; 5 c.c.) at 10—15°. After 2 hr. at room temperature the solution was treated with methanol. The neutral oxidation product (0·21 g.) was refluxed for 2 hr. with methanolic potassium hydroxide (3%; 50 c.c.), to give neutral (128 mg.) and acidic (86 mg.) fractions. A solution of the former in light petroleum–benzene (9 : 1; 10 c.c.) was filtered through alumina (8 g.). Benzene–ether (20 : 1, 150 c.c.) eluted a crystalline fraction which, after recrystallisation from ether and sublimation in a high vacuum at 120° (bath-temp.), gave 3α-hydroxypregn-16-en-20-one as prisms (from ether), m. p. 194—196°. Light absorption : Max. at 2390 Å (ε 8800). Mixtures with the specimens described above were undepressed in m. p.

Acetate-lactone $C_{24}H_{36}O_4$.—(*a*) The acidic fraction obtained by oxidation of cholegenin as described above was refluxed for 3 hr. with acetic acid (50 c.c.) containing hydrochloric acid (15%; 5 c.c.). The product was isolated by means of ether, and the neutral fraction (70 mg.) acetylated with acetic anhydride and pyridine at room temperature. The acetylated product in light petroleum (5 c.c.) was chromatographed on alumina (8 g.). Light petroleum–benzene (1 : 1; 150 c.c.) eluted a crystalline solid (35 mg.) which after two recrystallisations from ether–light petroleum gave the *acetate-lactone* as needles, m. p. 158°, $[\alpha]_D$ +3° (*c*, 0·4) (Found : C, 74·0; H, 9·6. $C_{24}H_{36}O_4$ requires C, 74·2; H, 9·3%).

(*b*) Cholegenin diacetate (350 mg.) in acetic acid (25 c.c.) was treated dropwise, with stirring, with chromic acid (380 mg.) in acetic acid (25 c.c.; 90%) at 80—90°. The mixture was kept at 90° for 1 hr., then treated with methanol, and the neutral product (53 mg.) was isolated with ether, and chromatographed in light petroleum (10 c.c.) on alumina (8 g.). The fraction eluted with light petroleum–benzene (2 : 1, 150 c.c.; 1 : 1, 150 c.c.) was twice recrystallised from ether–light petroleum (60—80°) and sublimed in a high vacuum at 140°, to give the lactone (15 mg.) as needles, m. p. 156—157°, $[\alpha]_D$ +2° (*c*, 0·9), undepressed in m. p. when mixed with the specimen described in (*a*).

(*c*) *epi*Smilagenin acetate (1 g.) (Djerassi, Yashin, and Rosenkranz, *J. Amer. Chem. Soc.*,

1952, **74**, 422) in acetic acid (40 c.c.) was treated dropwise during 45 min., with stirring, with chromic acid (1·2 g.) in acetic acid (80%; 10 c.c.) at 85—95° and the reaction mixture kept for 2 hr. at the same temperature. The neutral product (207 mg.) was recrystallised thrice from ether–light petroleum (b. p. 60—80°), to give the lactone, m. p. and mixed m. p. 157—159°, $[\alpha]_D$ −2° (*c*, 0·5) (Found : C, 74·1; H, 9·5%). The identity of the two specimens prepared by methods (*b*) and (*c*) was confirmed by a comparison of their infra-red absorption spectra kindly made by Professor H. H. Günthard. These were identical and each specimen showed bands (in Nujol) at 1770 (γ-lactone), 1721 and 1253 cm.$^{-1}$ (acetate).

Chromic Acid Oxidation of Cholegenin.—Cholegenin (500 mg.) in acetic acid was treated dropwise with chromic acid (550 mg.) in acetic acid (80%, 20 c.c.) with constant stirring at room temperature. After 4 hr. at room temperature, the excess of chromic acid was decomposed with methanol, and the product isolated by means of ether and separated into neutral (200 mg.) and acidic (300 mg.) fractions. The acidic fraction was recrystallised four times from aqueous acetone, to yield the *acid* (250 mg.) as plates, m. p. 197—201°, $[\alpha]_D$ −36° (*c*, 0·5) (Found: C, 72·2, 72·2; H, 9·0, 9·2. $C_{27}H_{40}O_5$ requires C, 72·9; H, 9·1. $C_{27}H_{40}O_5,\frac{1}{4}H_2O$ requires C, 72·1; H, 9·1%). The acid (200 mg.) was treated with ethereal diazomethane, and the neutral product thrice crystallised from light petroleum (b. p. 60—80°), to give the *methyl ester* as needles, m. p. 156°, $[\alpha]_D$ −67° (*c*, 0·8) (Found: C, 73·0; H, 9·5. $C_{28}H_{42}O_5$ requires C, 73·3; H, 9·2%). 2 : 4-Dinitrophenylhydrazine yields a crystalline precipitate.

Reduction of the Acid, $C_{27}H_{40}O_5$, *with Lithium Aluminium Hydride.*—The acid (30 mg.) in ether (40 c.c.) was refluxed with lithium aluminium hydride (100 mg.) for 30 min. After addition of ice and dilute acetic acid, the product was isolated with ether. A solution of the product in benzene was chromatographed on alumina (8 g.). The solid (24 mg.) eluted with benzene–ether (2 : 1, 150 c.c.; 1 : 1, 225 c.c.) and ether (75 c.c.) was recrystallised twice from ether–light petroleum (b. p. 60—80°), to yield cholegenin as needles, m. p. and mixed m. p. 191—192°, $[\alpha]_D$ −26° (*c*, 0·6).

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