157. 3(β) : 6(β)-*Dihydroxycholanic Acid, an Epimeride of Hyodeoxycholic Acid.*

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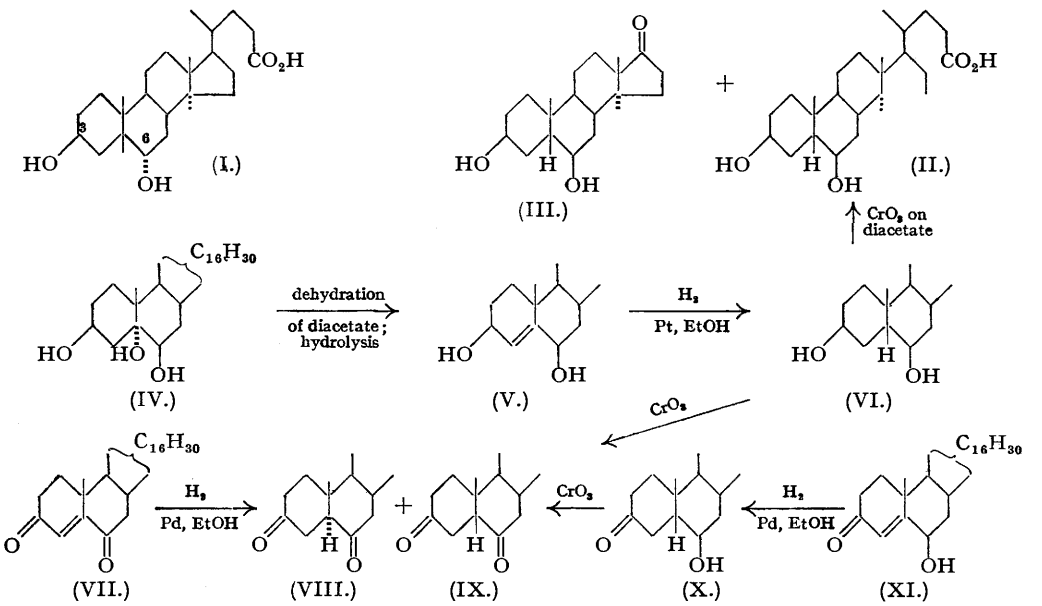
The oxidative degradation of coprostan-3(β) : 6(β)-diol (VI), by means of which 3(β) : 6(β)-dihydroxycholanic acid (II) and 3(β) : 6(β)-dihydroxyætiocholan-17-one (III) were obtained, is described. The acid (II) does not correspond in its properties to either of the known, naturally occuring 3 : 6-dihydroxycholanic acids, and hence " β "-hyodeoxycholic acid is 3(β) : 6(α)-dihydroxycholanic acid (I). Some preliminary experiments in connection with a possible route to coprostane-3(α) : 6(β)-diol from cholesterol are also described.

THE main structural features of " α "-hyodeoxycholic acid, isolated from pigs' bile, have been determined (Windaus and Bohne, *Annalen*, 1923, **433**, 278; Windaus, *ibid.*, 1926, **447**, 233; Wieland, Dane, and Martius, *Z. physiol. Chem.*, 1933, **215**, 18), and its formulation as a 3(α) : 6-dihydroxycholanic acid leaves only the configuration of the hydroxyl group at C_6 to be settled. Kimura (*ibid.*, 1937, **248**, 280) has reported the isolation, from the same natural source, of an epimeric 3 : 6-dihydroxycholanic acid (" β "-hyodeoxycholic acid) to which, by applying a series of degradation experiments analogous to those employed by Wieland *et al.* (*loc. cit.*) in the case of the " α "-isomer, he was able to assign the structure of a 3(β) : 6-dihydroxycholanic acid. The work now described is in continuation of a project to obtain 3 : 6-dihydroxycholanic acids from cholesterol in order to study the configurational relationships between " α "-hyodeoxycholic acid and its three theoretically possible epimerides (compare Paige, *J.*, 1943, 437), and shows that " β "-hyodeoxycholic acid is 3(β) : 6(α)-dihydroxycholanic acid (I).

The configuration assigned to the triol obtained by hydrogen peroxide oxidation of cholesterol (Pickard and Yates, *J.*, 1908, **93**, 1678) as cholestane-3(β) : 5 : 6(β)-triol (IV) (Ellis and Petrow, *J.*, 1939, 1078) has received further confirmation from the work of Plattner and Lang (*Helv.*



Chim. Acta, 1944, **27**, 1872). Hydrogenation, with a platinum catalyst, of Δ^4-cholesten-3(β) : 6(β)-diol (V), obtained by hydrolysis of 3(β) : 6(β)-diacetoxy-Δ^4-cholestene, the product of

Darzens dehydration of the diacetate of (IV), was reported to give cholestane-3(β) : 6(β)-diol (Petrow, Rosenheim, and Starling, *J.*, 1938, 679). However, Prelog and Tagmann (*Helv. Chim. Acta*, 1944, **27**, 1880) have reinvestigated the hydrogenation and shown that the saturated diol (VI) obtained in good yield, by using an alcoholic medium, does in fact belong to the coprostane series, their evidence being based mainly on their observation that oxidation of the diol gave a saturated diketone (IX) which was isomeric with, but distinct from the known cholestane-3 : 6-dione (VIII), and was readily allomerised to the latter by dilute acid. In further support for the coprostane configuration of the saturated diol (VI), it is now found that catalytic hydrogenation of Δ^4-cholestene-3 : 6-dione (VII) in alcoholic solution with palladium (cf. Bretschneider, *Ber.*, 1941, **74**, 1361) gives a mixture from which are isolated cholestan-3 : 6-dione (VIII), in about 40% yield, and also about 20% of coprostane-3 : 6-dione (IX) identical with a specimen prepared according to Prelog and Tagmann.

3(β) : 6(β)-*Dihydroxycholanic acid* (II)* and 3(β) : 6(β)-*dihydroxyætiocholan*-17-*one* (III) were isolated from the products of oxidation of the diacetate of 3(β) : 6(β)-dihydroxycoprostane (VI). The dihydroxycholanic acid was characterised by oxidation to a diketo-acid (m. p. 161—162°),† which crystallised with one molecule of water from dilute ethanol, and further by isomerisation of this acid to the corresponding diketo-acid (m. p. 205°) of the C_5-*allo*-series. The identity of the last two acids with " α "-dehydrohyodeoxycholic acid monohydrate (m. p. 161·5—162°) and " β "-dehydrohyodeoxycholic acid (m. p. 209°), respectively, which were obtained from " α "-hyodeoxycholic acid (Windaus, *Annalen*, 1926, **447**, 233), appears certain. Since the properties of 3(β) : 6(β)-dihydroxycholanic acid (m. p. 250°) do not correspond to those of " β "-hyodeoxycholic acid (m. p. 189—190°), the latter acid must be 3(β) : 6(α)-dihydroxycholanic acid (I). It does not necessarily follow, however, that " α "-hyodeoxycholic acid has the configuration, 3(α) : 6(α)-dihydroxycholanic acid (compare Kimura, *loc. cit.*).

In order to examine the possibility of obtaining coprostane-3(α) : 6(β)-diol from cholesterol, catalytic hydrogenation of Δ^4-cholesten-6(β)-ol-3-one (XI), was studied. With a palladium catalyst, absorption of hydrogen ceased after about one molecule had been taken up, and the resulting gum gave in good yield, *coprostan*-6(β)-*ol*-3-*one semicarbazone*. The product obtained by hydrolysis of the semicarbazone was also a gum, but would appear to consist essentially of coprostan-6(β)-ol-3-one (X), since on oxidation it gave pure coprostane-3 : 6-dione (IX) in good yield. A possibly more convenient route to the saturated ketol (X) lies in partial hydrolysis of the diacetate of 3(β) : 6(β)-dihydroxycoprostane (VI) and subsequent oxidation. Hydrolysis yielded a crystalline hydroxyacetoxycoprostane which, by analogy with other partial hydrolyses of 3 : 6-diacetoxy-steroids previously reported in the literature (see, *e.g.*, Plattner and Lang, *loc. cit.*; Marker and Krueger, *J. Amer. Chem. Soc.*, 1940, **62**, 79), was presumed to be 6(β)-*acetoxycoprostan*-3(β)-*ol*. Circumstances have made it impracticable to complete further work which might be expected to lead to the projected transformation of this into 3(α) : 6(β)-dihydroxycholanic acid.

Experimental.

Hydrogenation of Δ^4-*Cholestene*-3 : 6-*dione* (VII).—A solution of the dione (1·0 g.) (Windaus, *Ber.*, 1906, **39**, 2249) in warm absolute ethanol (25 c.c.) was cooled quickly to room temperature, and the resulting suspension of small crystals shaken with palladium black (0·1 g.) in an atmosphere of hydrogen. Uptake reached a maximum (*ca.* 1·3 mols.) after 1 hour, and shaking was continued for a further hour. The mixture was warmed and filtered from catalyst, and the crystalline material which separated on cooling was combined with a further amount obtained by evaporation of the mother-liquors. Fractional

* *Added, May* 31*st*, 1947.—Since this paper was submitted for publication, it has been found that the same dihydroxycholanic acid was described in papers not hitherto accessible, and not abstracted in this country. Tukamoto (*J. Biochem. Japan*, 1941, **32**, 451, 467) obtained the four theoretically possible dihydroxycholanic acids by aluminium *iso*propoxide reduction of " α "-dehydrohyodeoxycholic acid prepared from natural " α "-hyodeoxycholic acid. Of these four stereoisomeric 3 : 6-dihydroxycholanic acids, the isomer, m. p. 258°, which gave 3(β)-hydroxy-6-ketocholanic acid, m. p. 189°, on partial oxidation (cf. Kimura, *Z. physiol. Chem.*, 1937, **248**, 280, who reported that the hydroxyketo-acid had m. p. 154°), was assigned the structure 3(β) : 6(β)-dihydroxycholanic acid, apparently arbitrarily in respect to the configuration of the hydroxyl group at C_6. The properties of 3(β) : 6(β)-dihydroxycholanic acid obtained by oxidation of the diacetate of coprostane-3(β) : 6(β)-diol, as described above, correspond to those of the dihydroxycholanic acid, m. p. 258°, described by Tukamoto. Further support for their identity has now been obtained by partial oxidation, with chromium trioxide, of the former acid under conditions similar to those employed by the Japanese author. The 3(β)-hydroxy-6-ketocholanic acid thus obtained formed prismatic needles from ether–pentane, m. p. 188° (Found : C, 74·2; H, 9·4. Calc. for $C_{24}H_{38}O_4$: C, 73·8; H, 9·7%). The author is indebted to Dr. R. B. Moffett who kindly presented micro-photostat copies of these Japanese publications.

† *Added, May* 31*st*, 1947.—The m. p. of the diketo-acid was not depressed by admixture with an authentic sample of " α "-dehydrohyodeoxycholic acid, prepared from natural " α "-hyodeoxycholic acid and supplied by Dr. R. B. Moffett.

crystallisation of the material yielded as less soluble product, needles (0·4 g.), m. p. 163—170°, $[\alpha]_D^{19°}$ + 10° (*c* = 0·902 in chloroform), of cholestane-3 : 6-dione which gave no depression in m. p. on admixture with an authentic sample (Windaus, *loc. cit.*), and as more soluble product, long needles (0·2 g.), m. p. 175—179°, $[\alpha]_D^{19°}$ — 82° (*c* = 0·268 in chloroform), of coprostane-3 : 6-dione. A sample of coprostane-3 : 6-dione prepared according to Prelog and Tagmann (*loc. cit.*) had m. p. 175—179°, $[\alpha]_D^{19°}$ — 79° (*c* = 0·33 in chloroform), and showed no depression in m. p. on admixture with the latter product.

Oxidation of the Diacetate of 3(β) : 6(β)-*Dihydroxycoprostane* (VI).—A mechanically stirred solution of the diacetoxy-compound (22·9 g.) (Prelog and Tagmann, *loc. cit.*) in glacial acetic acid (900 c.c.) was heated on the water-bath and treated dropwise during 6 hours with a solution of chromium trioxide (50 g.) in glacial acetic acid (150 c.c.) and water (28 c.c.). Heating was continued for a further 4 hours. After remaining overnight at room temperature, the mixture was treated with ethanol (25 c.c.), and then evaporated in a vacuum to small bulk. The residue was treated with 2N-sulphuric acid (750 c.c.) and extracted with ether, and the ethereal layer washed thrice with water and then extracted with 2N-sodium hydroxide (110 c.c.). The alkaline extract was saponified by heating on the water-bath for 2 hours. The sparingly soluble sodium salt thus obtained was separated (centrifuge), washed with a little water, dissolved in hot water, and the free acid precipitated by addition of concentrated hydrochloric acid. The crude material was filtered off and purified by extraction with boiling ethyl acetate (Soxhlet), followed by crystallisation from dilute ethanol, giving colourless plates (870 mg.), m. p. 250° of 3(β) : 6(β)-*dihydroxycholanic acid* (II) (Found : C, 73·4; H, 10·1. $C_{24}H_{40}O_4$ requires C, 73·5; H, 10· %). The ethereal solution from which acid substances had been removed was washed with water and evaporated. The residual oil was dissolved in warm methanol (45 c.c.), the solution allowed to cool, and seeded with a crystal of starting material. The unchanged diacetoxycoprostane which crystallised (1·6 g.) was filtered off, and the filtrate evaporated. The residue was freed from volatile, pleasant-smelling substances by distilling in steam. The residue was extracted with ether, the ethereal solution washed with water, and evaporated. The residual gum was dissolved in warm ethanol (30 c.c.), the solution treated with semicarbazide hydrochloride (0·85 g.), and sodium acetate (1·02 g.), and the mixture boiled under reflux on the water-bath for 2 hours. After cooling, ether and water were added. The ethereal layer was separated and washed repeatedly with small portions of water, until solid material started to separate. When separation was complete, the material was filtered off and washed with a little ether. Crystallisation from methanol gave microscopic, white needles (170 mg.), m. p. 218—220° (decomp.), of 3(β) : 6(β)-*diacetoxyætiocholan*-17-*one semicarbazone* (Found : C, 64·3; H, 8·0; N, 9·3. $C_{24}H_{37}O_5N_3$ requires C, 64·4; H, 8·3; N, 9·4%). This semicarbazone (149 mg.) was dissolved in warm ethanol (7·5 c.c.), the solution treated with a mixture of concentrated sulphuric acid (0·75 c.c.) and water (1·5 c.c.), and boiled under reflux for 30 minutes. After cooling, the mixture was treated with water and ether, and the ethereal layer washed with water and then evaporated. The residue was boiled under reflux with 2% methanolic potassium hydroxide (4·5 c.c.) for 1 hour. The solution was treated with water and extracted with ether. The ethereal layer was washed with water and then treated with pentane. Recrystallisation of the precipitate from ether–pentane gave colourless rhombs, m. p. 209—210°, of 3(β) : 6(β)-*dihydroxyætiocholan*-17-*one* (III) (Found : C, 74·8; H, 9·5. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·8%).

Oxidation of 3(β) : 6(β)-*Dihydroxycholanic Acid.*—A solution of the dihydroxy-acid (200 mg.) in warm acial acetic acid (7 c.c.) was cooled to room temperature and treated gradually, with shaking, with a solution of 80% acetic acid (3 c.c.) which contained chromium trioxide (128 mg.). After remaining at room temperature for 3½ hours, the solution was treated carefully with dilute sulphuric acid. The crystalline product was well washed with water and recrystallised from a small amount of glacial acetic acid. Further recrystallisation from dilute ethanol gave rectangular leaflets, m. p. 161—162°, of "α"-dehydrohyodeoxycholic acid monohydrate (Found : C, 71·2; H, 9·1. Calc. for $C_{24}H_{36}O_4,H_2O$: C, 70·9; H, 9·4%).

'β'-*Dehydrohyodeoxycholic Acid.*—The above diketo-acid (90 mg.) was boiled under reflux with glacial acetic acid (1·8 c.c.) containing one drop of concentrated hydrochloric acid for 1½ hours. The hot solution was carefully treated with water until it was faintly turbid, and the material which crystallised on cooling was repeatedly recrystallised from ethanol, giving minute, colourless leaflets, m. p. 205° (Found : C, 73·9; H, 9·4. Calc. for $C_{24}H_{36}O_4$: C, 74·2; H, 9·3%).

Hydrogenation of Δ⁴-*Cholesten*-6(β)-*ol*-3-*one* (XI).—A solution of the unsaturated hydroxy-ketone (400 mg.) (Ellis and Petrow, *J.*, 1939, 1081) in absolute ethanol (20 c.c.) was hydrogenated with palladium black (40 mg.) at room temperature. Absorption of hydrogen ceased after 20 minutes when approximately 1·1 mols. had been taken up. After filtration of catalyst, removal of the solvent in a vacuum left a hard, brittle gum which could not be obtained crystalline either by solvent treatment or by absorption from light petroleum (b. p. 60—80°) solution on a column of activated alumina followed by elution of the column with chloroform–methanol. The gum (330 mg.), dissolved in ethanol (10 c.c.), was treated with semicarbazide hydrochloride (137 mg.) and sodium acetate (167 mg.), and the mixture boiled under reflux on the water-bath for 2 hours; the solid material which had separated was filtered off and well washed with water. Crystallisation from ethanol gave minute leaflets (240 mg.), m. p. 202—203°, of *coprostan*-6(β)-*ol*-3-*one semicarbazone* (Found : C, 72·7; H, 10·2; N, 9·3. $C_{28}H_{49}O_2N_3$ requires C, 73·1; H, 10·7; N, 9·2%). Hydrolysis of this semicarbazone (147 mg.) with boiling 3N-aqueous-ethanolic sulphuric acid in the usual way regenerated a brittle gum. This (76 mg.) was dissolved in glacial acetic acid (4 c.c.), and the solution treated dropwise, with shaking, with 90% acetic acid (3·71 c.c.) containing chromium trioxide (23·6 mg.). After standing at room temperature for 15 hours, the solution was treated with a few drops of methanol, poured into water, and extracted with ether. The ethereal extract, after being washed with dilute sodium carbonate solution then with water and evaporated, left a crystalline residue which, on recrystallisation from methanol, gave long needles (60 mg.), m. p. 175—179°, $[\alpha]_D^{19°}$ — 76° (*c* = 0·332 in chloroform), of coprostane-3 : 6-dione, the m. p. of which was not depressed on admixture with an authentic sample.

6(β)-*Acetoxycoprostan*-3(β)-*ol.*—A solution of 3(β) : 6(β)-diacetoxycoprostane (488 mg.) in warm methanol (13·5 c.c.) was boiled under reflux on the water-bath, and the boiling solution treated dropwise,

during 30 minutes, with 0·189N-methanolic potassium hydroxide solution (5·56 c.c. = 1·05 mols.). Boiling was continued for a further 10 minutes, and the solution set aside for 12 hours. After acidification with a few drops of glacial acetic acid, the solution was evaporated to small bulk on the water-bath. The *substance* which separated was filtered off and triturated with water, giving a crystalline solid (300 mg.; m. p. 125—132°) which, after repeated recrystallisation from methanol, gave long, colourless leaflets, m. p. 143°, strongly depressed on admixture with starting material (Found: C, 78·2; H, 11·2. $C_{29}H_{50}O_3$ requires C, 78·0; H, 11·2%).

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