Steroids and Walden Inversion. Part XIV. 5-Hydroxycholestane-3β-carboxylic Acid and Related Compounds.*

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Cholest-5-ene-3β-carboxylic acid and its methyl ester react with perbenzoic acid to give principally a 5α : 6α-epoxide. This, by reduction with lithium aluminium hydride and oxidation of the resulting diol, is converted into 5-hydroxycholestane-3β-carboxylic acid, which furnishes a 5α-hydroxy-anhydride with acetic anhydride and regenerates cholest-5-ene-3β-carboxylic acid by ionic dehydration.

Oxidation of methyl cholest-5-ene-3β-carboxylate with peracetic acid, or acetolysis of the above 5α : 6α-epoxide, gave methyl 6β-acetoxy-5-hydroxycholestane-3β-carboxylate, smoothly dehydrated to methyl 6β-acetoxycholest-4-ene-3β-carboxylate. This ester with perbenzoic acid gave only a single epoxide, shown to be the 4α : 5α-epoxide by conversion into 5-hydroxy-6-oxocholestane-3β-carboxylic acid, which by treatment with acetic anhydride yielded a 5α-hydroxy-anhydride.

CHOLEST-5-ENE-3β-CARBOXYLIC ACID (IV; R = H) reacts with perbenzoic acid, to give apparently a single epoxy-acid (VI; R = H), characterised as the methyl ester. Methyl cholest-5-ene-3β-carboxylate (IV; R = Me), however, and perbenzoic acid furnish a mixture of epimeric epoxy-esters. The major component, readily obtained pure by chromatography, is formulated as methyl 5 : 6α-epoxycholestane-3β-carboxylate (VI; R = Me) on the basis of optical rotatory evidence and by analogy with the major products of peroxidation of cholesterol (Plattner, Petrzilka, and Lang, *Helv. Chim. Acta*, 1944, **27**, 513, 1872) and *epi*cholesterol (Fudge, Shoppee, and Summers, *J.*, 1954, 958; Plattner, Fürst, Koller, and Kuhn, *Helv. Chim. Acta*, 1954, **37**, 258). Reduction of the epoxy-acid (VI; R = H) or of the epoxy-ester (VI; R = Me) with lithium aluminium hydride gave the diol (III), of which only the primary hydroxyl group was oxidised by chromium trioxide to yield 5-hydroxycholestane-3β-carboxylic acid (II; R = H). The methyl ester (II; R = Me) by dehydration with thionyl chloride–pyridine at 20° regenerated methyl cholest-5-ene-3β-carboxylate (IV; R = Me); the acid (II; R = H) by dehydration with acetic anhydride at 140° gave the anhydride (I), which by alkaline hydrolysis regenerated the acid (II; R = H).

The structure (I) is supported by infra-red spectroscopic evidence. The spectrum of a Nujol mull of the acid (II; R = H) shows peaks at 3560 (free hydroxyl), 3300—2500 (associated hydroxyl group of carboxylic acid) and 1702 cm.$^{-1}$ (carbonyl group of carboxylic acid); the spectrum of the methyl ester (II; R = Me) in carbon disulphide solution shows peaks at 3570 (free hydroxyl) and at 1735 and 1165 cm.$^{-1}$ (carboxylic ester). The anhydride

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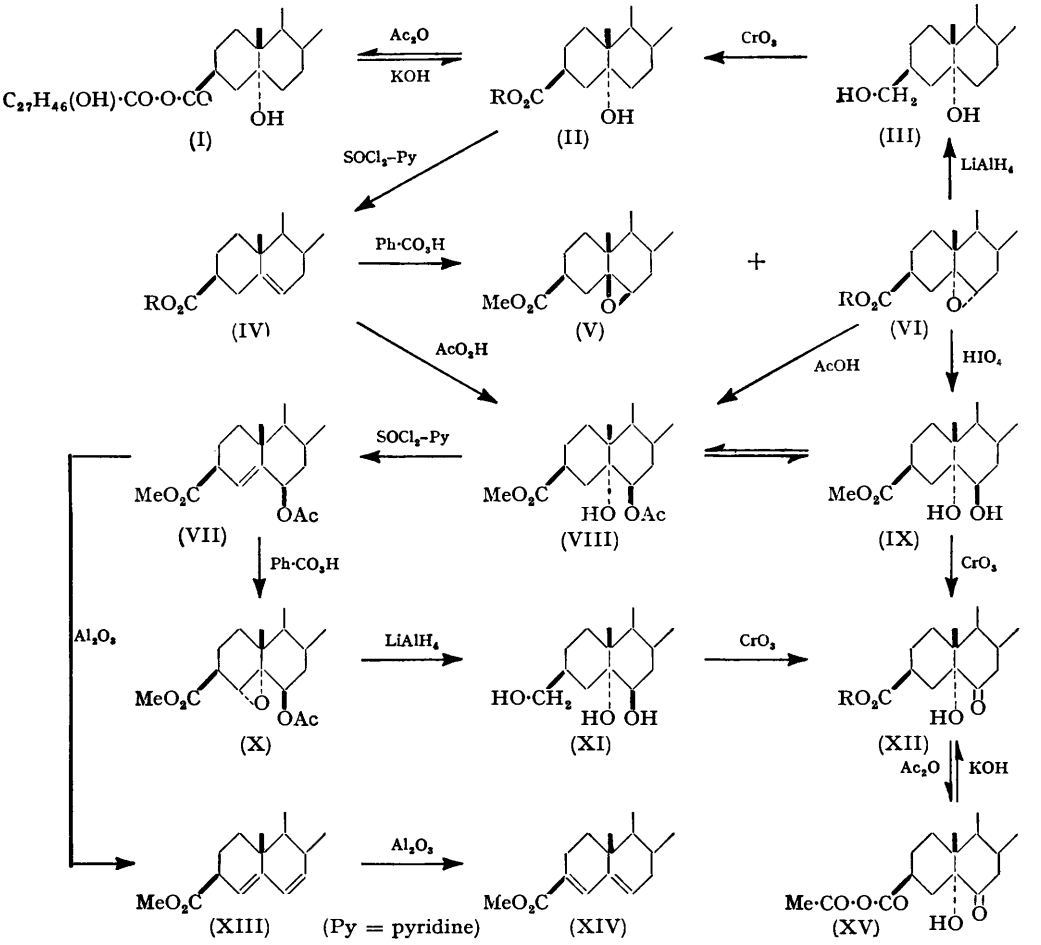
(I) in carbon disulphide solution exhibited peaks at 3560 (free hydroxyl) and at 1810 and 1743 cm.$^{-1}$ (carboxylic anhydride). In Nujol, the peaks appeared at 3540, 1787, and 1730 cm.$^{-1}$ respectively.

The formation of the anhydride (I) demonstrates the *trans*-relationship of the carboxyl and the hydroxyl group in the acid (II; R = H), and confirms the β-configuration assigned to the $C_{(3)}$-carboxyl group in Marker's acid (IV; R = H) (Shoppee and Stephenson, *J.*, 1954, 2230; Roberts, Shoppee, and Stephenson, *ibid.*, p. 2705), because a *cis*-arrangement would have furnished a γ-lactone (cf. Shoppee, *J.*, 1948, 1032). It seemed of interest in



this connection to attempt to prepare 5-hydroxycoprostane-3β-carboxylic acid and to examine its behaviour on dehydration.

The preparation of 5β-hydroxy-steroids has been achieved (*a*) in 20% yield by reduction of 5β : 6β-epoxysteroids with lithium aluminium hydride (Plattner, Heusser, and Feurer, *Helv. Chim. Acta*, 1949, **32**, 587) and (*b*) smoothly and in excellent yield by reduction of 4β : 5β-epoxy-steroids with the same reagent (Plattner *et al.*, *ibid.*, 1948, **31**, 1822, 1888; 1949, **32**, 266, 1070). Since the 5β : 6β-epoxy-ester (V) was formed in small quantity only, and was not isolated in a state of purity, a Δ^4-steroid suitable for preparation of a 4β : 5β-epoxide was obtained as follows. Methyl cholest-5-ene-3β-carboxylate (IV; R = Me) was treated with peracetic acid to give mainly the diol monoacetate (VIII) with some of the diol (IX); alternatively, acetolysis of the mixture of epoxy-esters (V + VI; R = Me) gave the diol monoacetate (VIII), whilst hydration of the pure epoxy-ester (VI; R = Me) with

periodic acid yielded the diol (IX), converted by treatment with acetic anhydride into the diol monoacetate (VIII). Subsequently, it was found that performic acid was more effective, and that the mixture of diol monoformate (as VIII) and diol (IX) could be separated chromatographically or hydrolysed with methanolic hydrogen chloride to the diol (IX). Dehydration of the diol monoacetate (VIII) with thionyl chloride–pyridine gave methyl 6β-acetoxycholest-4-ene-3β-carboxylate (VII).

By contrast with the results of Plattner *et al.* (*loc. cit.*), the unsaturated ester (VII) with perbenzoic acid gave only a single 4 : 5-epoxide, which was shown to be methyl 6β-acetoxy-4α : 5α-epoxycholestane-3β-carboxylate (X). By reduction with lithium aluminium hydride, the 4α : 5α-epoxide (X) gave the triol (XI), converted by oxidation with chromium trioxide into the keto-hydroxy-acid (XII; R = H), characterised as the methyl ester which was identical with the product obtained by oxidation of the diol (IX) with chromium trioxide. Wolff–Kishner reduction of the keto-hydroxy-acid (XII; R = H) caused concomitant dehydration to Marker's acid (IV; R = H).

The infra-red absorption spectrum of a Nujol mull of the acid (XII; R = H) showed peaks at 3430 cm.$^{-1}$ (free hydroxyl; the shift from 3550 cm.$^{-1}$ being due to vicinal action of the carbonyl group at $C_{(6)}$), 2990 and 2820 (associated hydroxyl group of carboxylic acid), 1735 and 1250 (carbonyl of carboxylic acid), and 1695 cm.$^{-1}$ (ketone). The methyl ester (XII; R = Me) in carbon disulphide solution shows maxima at 3550 and 3440 (hydroxyl), 1735 and 1165 (carboxylic ester), and 1710 cm.$^{-1}$ (ketone). The anhydride (XV) in a Nujol mull exhibited peaks at 3500 and 3404 (hydroxyl), 1710 (ketone), 1812 and 1730 cm.$^{-1}$ (carboxylic anhydride); weak peaks at 3000 and 2820 cm.$^{-1}$ are ascribed to slight hydrolysis of the anhydride.

It was observed that chromatography of the unsaturated acetoxy-ester (VII), $\lambda_{max.}$ 208 mμ in EtOH (log ε 3·32), led to loss of acetic acid with production of a doubly unsaturated ester, $\lambda_{max.}$ 274 mμ (log ε 4·26). We believe that the allyl acetate (VII) undergoes an elimination reaction (*E*1) on the column of aluminium oxide to give the 4 : 6-diene ester (XIII) which is isomerised to methyl cholesta-3 : 5-diene-3-carboxylate (XIV). This structure is consistent with the position of the ultra-violet absorption maximum (calc. for an αγδ-substituted conjugated dienone: $\lambda_{max.}$ 266 mμ), and appears to be supported by the infra-red absorption spectrum in carbon disulphide solution. This shows a carbonyl maximum at 1712 cm.$^{-1}$ displaced from the frequency 1735 cm.$^{-1}$ common to methyl esters. No data for unsaturated esters of the type RO·CO·C:C·C:C appear to be available, but it seems appropriate to compare the above displacement with that observed in passing from steroid 3-ketones to the Δ^4-analogues (1718 ⟶ 1675 cm.$^{-1}$).

Experimental

For general details see *J.*, 1953, 243. Neutralised alumina (*J.*, 1953, 543) was used where stated. $[\alpha]_D$ are in chloroform except where noted; ultra-violet spectra were determined in ethanol on a Unicam SP 500 spectrophotometer, with a corrected scale, and infra-red spectra were examined in carbon disulphide on a Perkin–Elmer double-beam instrument.

Methyl 5 : 6α-*Epoxycholestane*-3β-*carboxylate*.—(*a*) Methyl cholest-5-ene-3β-carboxylate (2 g.) (Roberts, Shoppee, and Stephenson, *loc. cit.*) was treated with a solution of perbenzoic acid (1·1 mol.) in chloroform (50 c.c.) for 48 hr. at 15°. The solution was diluted with ether, and excess of perbenzoic acid destroyed by repeated washing with sodium iodide solution; after further washing with solutions of sodium thiosulphate and chloride, the product was isolated in the usual way and chromatographed on a column of aluminium oxide (60 g.) prepared in pentane. Elution with benzene–pentane (1 : 9, 11 × 200 c.c.; 2 : 3, 2 × 200 c.c.) gave a solid (total 1·4 g.); all fractions were identical. *Methyl* 5 : 6α-*epoxycholestane*-3β-*carboxylate*, recrystallised from methanol, had m. p. 80°, $[\alpha]_D$ −30° (*c*, 2·0) [Found (after drying at 15°/0·01 mm. for 15 hr.) : C, 78·3; H, 10·9. $C_{29}H_{48}O_3$ requires C, 78·3; H, 10·9%]. Further elution with benzene–pentane (2 : 3, 2 × 200 c.c.) furnished solid material, m. p. 62—80° (from methanol).

In a repetition, methyl 5 : 6α-epoxycholestane-3β-carboxylate was isolated by crystallisation from methanol–ethyl acetate. The mother-liquor material was chromatographed on aluminium oxide (30 g.) prepared in pentane. Elution with pentane afforded a trace of oil whilst benzene–pentane (2 : 3, 100 c.c.) gave a solid (66 mg.), which was crystallised from methanol, m. p. 76—86°. A further eluate (67 mg.) melted at 80—88° whilst use of ether (100 c.c.) afforded a

solid (374 mg.) which, recrystallised from methanol, had m. p. 76—85°, $[\alpha]_D$ +0·2° (*c*, 2·4), the change in optical rotation being due to the presence of methyl 5 : 6β-epoxycoprostane-3β-carboxylate.

(*b*) Cholest-5-ene-3β-carboxylic acid (2 g.) was treated with an excess of perbenzoic acid in chloroform at 15° for 40 hr. Working up gave a product which was esterified with ethereal diazomethane and chromatographed on aluminium oxide (30 g.) prepared in pentane. Elution with pentane (100 c.c.) furnished a trace of oil whilst further elution with pentane (400 c.c.), benzene–pentane (2 : 3, 500 c.c.), and benzene (200 c.c.) furnished methyl 5 : 6α-epoxycholestane-3β-carboxylate (total 1·64 g.); crystallised from methanol, this had m. p. 80°.

3β-*Hydroxymethylcholestan-5-ol.*—Methyl 5 : 6α-epoxycholestane-3β-carboxylate (2·5 g.) in ether (150 c.c.) was added to a solution of lithium aluminium hydride (2·5 g.) in ether (300 c.c.). After 2 hours' refluxing, excess of lithium aluminium hydride was destroyed by water, and the product isolated in the usual manner. The *diol*, crystallised several times from acetone, had m. p. 138—139° [Found (after sublimation) : C, 80·2; H, 11·9. $C_{28}H_{50}O_2$ requires C, 80·3; H, 12·0%].

5-*Hydroxycholestane*-3β-*carboxylic Acid.*—The foregoing product (1 g.) in acetic acid (50 c.c.) was treated with a 2% solution of chromium trioxide in 98% acetic acid (50 c.c.) at 15° for 16 hr.; the solution was diluted, acidified with 2N-sulphuric acid, and extracted with ether. Washing the ether extracts with 2N-sodium carbonate removed acetic acid and precipitated sodium 5-hydroxycholestane-3β-carboxylate; this was filtered off and the free acid obtained by shaking the sodium salt with ether–2N-sulphuric acid. The acid was esterified with ethereal diazomethane, and the ester was introduced on to a column of aluminium oxide (25 g.) in pentane. Elution with benzene–pentane (1 : 4, 2 × 100 c.c.) afforded only a trace of material, whilst further elution with benzene–pentane (1 : 1, 4 × 100 c.c.), benzene (100 c.c.), and ether (100 c.c.) gave *methyl* 5-*hydroxycholestane*-3β-*carboxylate* (1 g.), which was crystallised from methanol as plates, m. p. 113°, $[\alpha]_D$ +18° (*c*, 2·9), +21° (*c*, 1·0) [Found (after drying at 20°/0·01 mm. for 16 hr.) : C, 77·8; H, 11·4. $C_{29}H_{50}O_3$ requires C, 77·95; H, 11·3%]. 5-*Hydroxycholestane*-3β-*carboxylic acid*, obtained by hydrolysis with methanolic N-potassium hydroxide and recrystallised from methanol, had m. p. 211—213°, $[\alpha]_D$ +19° (*c*, 1·0) [Found (after drying at 60°/0·01 mm. for 6 hr.) : C, 77·2; H, 11·1. $C_{28}H_{48}O_3$ requires C, 77·7; H, 11·2%].

Methyl Cholest-5-ene-3β-*carboxylate.*—Methyl 5-hydroxycholestane-3β-carboxylate (43 mg.), dissolved in pyridine (1 c.c.), was treated at 0° with thionyl chloride (0·2 c.c.). After 10 min., the mixture was poured into water and the product (39 mg.) isolated in the usual manner. Chromatography on aluminium oxide (6 g.) with pentane as eluant furnished only traces of oil, whilst use of benzene–pentane (1 : 4, 100 c.c.) gave methyl cholest-5-ene-3β-carboxylate (38 mg.), m. p. and mixed m. p. 100—101° (from methanol).

5-*Hydroxycholestane*-3β-*carboxylic Anhydride.*—5-Hydroxycholestane-3β-carboxylic acid (200 mg.) was refluxed with acetic anhydride (12 c.c.) for 30 min. Removal of solvent under reduced pressure gave 5-*hydroxycholestane*-3β-*carboxylic anhydride* which, recrystallised from methanol–ethyl acetate, had m. p. 228—230°, $[\alpha]_D$ +15° (*c*, 0·8) [Found (after drying at 15°/0·01 mm.) : C, 79·3; H, 11·0. $C_{56}H_{94}O_5$ requires C, 79·4; H, 11·1%]. Hydrolysis with methanolic N-potassium hydroxide gave, after acidification and the usual extraction, 5-hydroxycholestane-3β-carboxylic acid, m. p. and mixed m. p. 210—213°.

Methyl 5 : 6β-*Dihydroxycholestane*-3β-*carboxylate.*—(*a*) Methyl cholest-5-ene-3β-carboxylate (10 g.) was suspended in formic acid (95%; 100 c.c.) and benzene (100 c.c.). Hydrogen peroxide (100-vol.; 100 c.c.) was added and the mixture stirred at 45° for 1 hr. Benzene was removed and, after cooling slowly during 2 hr., the solution was diluted with water, and the product extracted with ether, to furnish after the usual working up, an oil, a portion of which (1·8 g.) was chromatographed on aluminium oxide (40 g.) prepared in benzene. Elution with benzene (5 × 100 c.c.) gave an oil (214 mg.), whilst further elution with benzene (1 × 100 c.c.) and ether–benzene (1 : 19, 4 × 100 c.c.) gave a solid (1·1 g.) which was recrystallised from methanol to give *methyl* 6β-*formoxy*-5-*hydroxycholestane*-3β-*carboxylate* as prisms, m. p. 134—135°, $[\alpha]_D$ −17° (*c*, 1·7) [Found (after drying at 90°/0·03 mm. for 3 hr.) : C, 73·1; H, 10·2. $C_{30}H_{50}O_5$ requires C, 73·4; H, 10·3%]. Further elution with ether–benzene (1 : 4, 100 c.c.; 1 : 1, 100 c.c.) furnished a solid (350 mg.), which was recrystallised from methanol to give *methyl* 5 : 6β-*dihydroxycholestane*-3β-*carboxylate*, double m. p. 134°/151°, $[\alpha]_D$ +3° (*c*, 1·7) [Found (after sublimation at 180°/0·01 mm.) : C, 75·3; H, 10·8. $C_{29}H_{50}O_4$ requires C, 75·3; H, 10·9%]. The mixture of diol and its 6β-formyl derivative obtained from methyl cholest-5-ene-3β-carboxylate (10 g.) was partially hydrolysed by refluxing it with methanol (400 c.c.) and concentrated hydrochloric acid (15 c.c.). After 1 hr., the solution was diluted with water, and the product extracted with

ether, washed, dried, and evaporated to furnish methyl 5 : 6β-dihydroxycholestane-3β-carboxylate, double m. p. 134°/151°, after recrystallisation from methanol.

(*b*) Methyl 5 : 6α-epoxycholestane-3β-carboxylate (100 mg.) was refluxed for 45 min. with acetone (3 c.c.) and water (1 c.c.) containing periodic acid (64 mg.); the solution was diluted with water and extracted with ether, and the product isolated in the usual manner. Crystallisation from methanol gave methyl 5 : 6β-dihydroxycholestane-3β-carboxylate, double m. p. 133—134°/151°.

Methyl 6β-*Acetoxy*-5-*hydroxycholestane*-3β-*carboxylate*.—(*a*) Methyl cholest-5-ene-3β-carboxylate (26 g.) was dissolved in acetic acid (500 c.c.), and hydrogen peroxide (100-vol.; 50 c.c.) added to the solution maintained at 95°; a further addition of hydrogen peroxide (50 c.c.) was made after 0·5 hr. After a total period of 1·5 hr. at 95°, the mixture was poured into water, the product extracted with benzene and, after the usual washing, drying, and removal of solvent, the oil obtained was dissolved in pyridine (50 c.c.) and acetic anhydride (25 c.c.). After being kept at 15° for 15 hr., the mixture was diluted with water and extracted with ether, and after the usual washing, drying, and removal of solvent the product was crystallised from methanol to furnish *methyl* 6β-*acetoxy*-5-*hydroxycholestane*-3β-*carboxylate*, m. p. 173—175°, $[\alpha]_D$ −24° (*c*, 1·7) [Found (after drying at 15°/0·01 mm. for 15 hr.): C, 73·7; H, 10·2. $C_{31}H_{52}O_5$ requires C, 73·8; H, 10·4%].

(*b*) Methyl 5 : 6α-epoxycholestane-3β-carboxylate (2·2 g.) was refluxed in acetic acid (150 c.c.) with anhydrous potassium acetate (4 g.) for 2 hr.; the mixture was cooled and diluted with water, and the product isolated in the usual manner. Crystallisation from methanol furnished methyl 6β-acetoxy-5-hydroxycholestane-3β-carboxylate (2·1 g.), m. p. 174—176°, identical with the previous preparation. A similar experiment using the αβ-epoxide mixture gave analogous results.

(*c*) Methyl 5 : 6β-dihydroxycholestane-3β-carboxylate (11·5 g.), in benzene (100 c.c.) and pyridine (20 c.c.), was treated with acetic anhydride (20 c.c.) at 15° for 16 hr. The solvents were removed in a vacuum, the product was dissolved in ether, and the ethereal solution washed, dried, and evaporated to furnish methyl 6β-acetoxy-5-hydroxycholestane-3β-carboxylate, m. p. 173—175° (11·5 g.).

Methyl 6β-*Acetoxycholest*-4-*ene*-3β-*carboxylate*.—Methyl 6β-acetoxy-5-hydroxycholestane-3β-carboxylate (10·5 g.) in pyridine (100 c.c.) was treated with thionyl chloride (12 c.c.) at 0° for 15 min. Excess of thionyl chloride was destroyed by ice. Further dilution furnished a solid which was filtered off, washed, and crystallised from methanol to give *methyl* 6β-*acetoxycholest*-4-*ene*-3β-*carboxylate*, m. p. 122—123°, $[\alpha]_D$ +14° (*c*, 2·0), $\lambda_{max.}$ 208 mμ in EtOH (log ε 3·32) [Found (after drying at 15°/0·01 mm. for 15 hr.): C, 76·2; H, 10·3. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·4%].

A solution of methyl 6β-acetoxycholest-4-ene-3β-carboxylate in pentane was introduced on to a column of aluminium oxide (Spence Type H, activity ~II). After 24 hr., elution with benzene–pentane (1 : 4) gave *methyl cholesta*-3 : 5-*diene*-3-*carboxylate* which, crystallised from methanol, had m. p. 88°, $[\alpha]_D$ −140° (*c*, 1·5), $\lambda_{max.}$ 274 mμ in EtOH (log ε 4·26) [Found (after drying at 20°/0·01 mm.): C, 81·2; H, 10·9. $C_{29}H_{46}O_2$ requires C, 81·6; H, 10·9%].

Methyl 6β-*Acetoxy*-4α : 5-*epoxycholestane*-3β-*carboxylate*.—Methyl 6β-acetoxycholest-4-ene-3β-carboxylate (7 g.) in chloroform (20 c.c.) was treated with perbenzoic acid (1·2 mol.) in chloroform (20 c.c.). After 36 hr. at 15°, the solution was diluted with ether, and the product isolated in the usual way to furnish *methyl* 6β-*acetoxy*-4α : 5-*epoxycholestane*-3β-*carboxylate*, which crystallised from methanol and then from ethyl acetate as needles, m. p. 141—143°, $[\alpha]_D$ +28° (*c*, 1·2) [Found (after drying at 15°/0·01 mm. for 15 hr.): C, 74·3; H, 10·3. $C_{31}H_{50}O_5$ requires C, 74·1; H, 10·1%].

3β-*Hydroxymethylcholestane*-5 : 6β-*diol*.—Methyl 6β-acetoxy-4α : 5-epoxycholest-4-ene-3β-carboxylate (2·2 g.) was refluxed with lithium aluminium hydride (2 g.) in ether (250 c.c.) for 1·5 hr., water was added, and the product isolated in the usual manner. Crystallisation from ether–pentane gave the *triol*, m. p. 188—189°, $[\alpha]_D$ +5° (*c*, 1·0) [Found (after sublimation at 200°/0·01 mm.): C, 77·4; H, 11·6. $C_{28}H_{50}O_3$ requires C, 77·4; H, 11·6%].

5-*Hydroxy*-6-*oxocholestane*-3β-*carboxylic Acid*.—(*a*) The triol (1 g.) in acetic acid (10 c.c.) was treated with chromium trioxide (0·67 g.) in acetic acid (33 c.c.) for 16 hr. at 25°, then the excess of chromium trioxide was destroyed by adding methanol. Dilution with water followed by extraction with ether, extraction of the acid with 2N-potassium hydroxide, and acidification and ether-extraction of the alkaline extracts furnished 5-*hydroxy*-6-*oxocholestane*-3β-*carboxylic acid* which, crystallised from pentane, had m. p. 246—250°, $[\alpha]_D$ −35° (*c*, 1·1) [Found (after drying at 40°/0·01 mm. for 15 hr.): C, 75·5; H, 10·3. $C_{28}H_{46}O_4$ requires C, 75·3; H, 10·4%]. The *methyl* ester, prepared by using ethereal diazomethane and crystallised from pentane,

had m. p. 152°, $[\alpha]_D$ −39° (*c*, 1·2) [Found (after drying at 15°/0·01 mm. for 15 hr.) : C, 75·4; H, 10·5. $C_{29}H_{48}O_4$ requires C, 75·6; H, 10·5%].

(*b*) Methyl 5 : 6β-dihydroxycholestane-3β-carboxylate (3 g.) was oxidised in acetic acid (100 c.c.) with chromium trioxide (420 mg.) in acetic acid (10 c.c.) at 25° for 16 hr. Methanol was then added, the solution diluted with water, and the product isolated in the usual manner. Chromatography of the product on aluminium oxide (90 g.), using benzene (300 c.c.) as eluant, furnished some oily material, whilst use of ether–benzene (1 : 19; 5 × 300 c.c.) gave methyl 5-hydroxy-6-oxocholestan-3β-carboxylate (2·2 g.), which, crystallised from pentane, had m. p. and mixed m. p. with the previous preparation 151—152°.

Cholest-5-ene-3β-carboxylic Acid.—Methyl 5-hydroxy-6-oxocholestane-3β-carboxylate (600 mg.) was refluxed in diethylene glycol (25 c.c.) with potassium hydroxide (800 mg.) and hydrazine hydrate (2 c.c.) for 45 min.; the temperature was allowed to rise to 210° and refluxing continued for 12 hr. Dilution with water, acidification, and ether extraction gave, after the usual washing, drying, and evaporation, a product, which was extracted with pentane to leave cholest-5-ene-3β-carboxylic acid (220 mg.), m. p. and mixed m. p. 215—219°. Esterification (diazomethane) gave methyl cholest-5-ene-3β-carboxylate, m. p. and mixed m. p. 101° (from methanol).

Anhydride of 5-Hydroxy-6-oxocholestane-3β-carboxylic Acid and Acetic Acid.—5-Hydroxy-6-oxocholestane-3β-carboxylic acid (200 mg.) was refluxed in acetic anhydride (12 c.c.) for 45 min., solvent was removed under reduced pressure, and the mixed *anhydride* of 5-hydroxy-6-oxocholestane-3β-carboxylic and acetic acid was obtained by crystallisation from ethyl acetate; it had m. p. 209—211°, $[\alpha]_D$ −37° (*c*, 0·8) [Found (after drying at 80°/0·001 mm. for 5 hr.) : C, 74·2; H, 10·4. $C_{30}H_{48}O_5$ requires C, 73·7; H, 9·9%]. Hydrolysis of the anhydride with methanolic N-potassium hydroxide regenerated the original acid.

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