648. *Steroids and Walden Inversion. Part VIII.* The Epimeric 6-Hydroxy-2 : 3-secocholestane-2 : 3-dicarboxylic Acids.*

By C. W. Shoppee and G. H. R. Summers.

The configurations at $C_{(6)}$ of the epimeric 6-hydroxy-2 : 3-*seco*cholestane-2 : 3-dicarboxylic acid 3→6-lactones have been established and these compounds have been related to the epimeric cholestan-6-ols, whereby the configurations of the latter are confirmed. The formation of 3→6-lactones is shown not to be by itself diagnostic of the configuration of steroid 6-hydroxyl groups.

In connexion with work on the stereochemistry of the 3 : 5-*cyclo*steroid rearrangement,* we desired completely to establish the configurations of the epimeric cholestan-6-ols. Standard methods for the determination of configuration involve (i) molecular-rotation differences, (ii) steric-compression differences influencing (*a*) reaction rates and (*b*) equilibria, (iii) differences in molecular geometry influencing the facility of (*a*) ionic and (*b*) thermal elimination reactions, (iv) formation of cyclic compounds, and (v) fission of cyclic compounds. We have already utilised methods (i), (ii*a* and *b*), (iii*a* and *b*). An attempt to apply method (v) to the solution of our configurational problem was made incidentally by Plattner, Petrzilka, and Lang (*Helv. Chim. Acta*, 1944, **27**, 513); they examined the fission by catalytic reduction of 5α : 6α-epoxycholestane (Ruzicka, Furter, and Thomann, *ibid.*, 1933, **16**, 327) and obtained an inseparable mixture of cholestan-5α-ol and cholestan-6β-ol in which the presence of the latter could be established only by oxidation to cholestan-6-one. It seemed therefore of interest to examine method (iv).

Participation in lactone formation has enabled proof to be given of the β-configuration

* Part VII, preceding paper.

PLATE I.

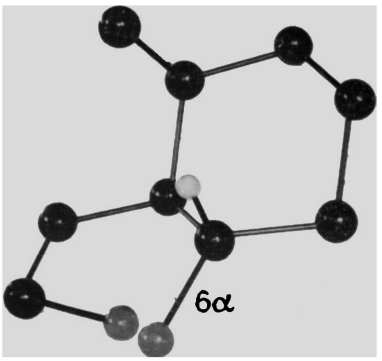
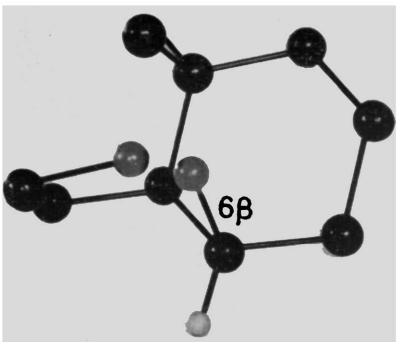


PLATE II.

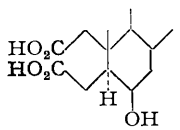


The white sphere represents the $C_{(6)}$*-hydrogen atom, and the grey spheres represent the oxygen atoms of the hydroxyl groups attached to* $C_{(6)}$ *and of the carboxyl group attached to* $C_{(3)}$.

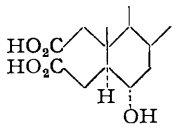
[*To face p.* 3375.

of $C_{(3)}$-hydroxyl group in cholesterol (Shoppee, *J.*, 1948, 1032), and of the α-configuration of the $C_{(12)}$-hydroxyl group in deoxycholic acid (Reichstein and Sorkin, *Helv. Chim. Acta*, 1946, **29**, 1218); we decided therefore to examine the capacity for 3→6-lactonisation of the epimeric 6-hydroxy-2 : 3-*seco*cholestane-2 : 3-dicarboxylic acids (I, II).

The literature already contains some evidence relating to analogous cases. Windaus (*Annalen*, 1926, **477**, 233) found that 6-keto-2 : 3-*seco*-5α-cholane-2 : 3 : 24-tricarboxylic acid (hyodeoxy*iso*bilianic acid, "oxo-Stadensäure") by hydrogenation with platinum–acetic acid gives the 3→6β-lactonic acid and not the expected 6β-hydroxy-2 : 3-dicarboxylic acid, dehydration accompanying or immediately succeeding reduction, and that the 3→6β-lactonic acid is converted by treatment with sodium ethoxide at 190° for 10 hours into an isomeride. This is clearly the 6α→3-lactonic acid and arises by inversion of the $C_{(6)}$–O orientation from the β-configuration (polar) to the more thermodynamically stable α-configuration (equatorial).



(I)



(II)

Similarly the 3β : 6α-dihydroxysapogenin chlorogenin by oxidation furnishes 6-keto-2 : 3-*seco*-5α : 22α-spirostane-2 : 3-dicarboxylic acid (Noller, *J. Amer. Chem. Soc.*, 1937, **59**, 1092), which by hydrogenation with platinum–acetic acid gives directly the 3→6β-lactonic acid (Marker *et al.*, *ibid.*, 1947, **69**, 2183).

If ring B possesses the chair-conformation in the 6-hydroxy-acids (I, II), the $C_{(4)}$–$C_{(6)}$ bond has a fixed β-orientation but is capable of "free" rotation, so that the $C_{(3)}$–$C_{(4)}$ bond can traverse the surface of a cone of angle 120°. Examination of molecular models so constructed as to permit free rotation about the bond axes suggests that there should be little, if any, difference in the ease of formation of 3→6-lactonic acids with C–C and C–O bonds of normal length by the 6β- (I; Plate I *) and the 6α-hydroxy-acid (II; Plate II *). The following experimental evidence shows this to be the case, and that the formation of 6→3-lactones is not by itself diagnostic of the configuration of steroid 6-hydroxyl groups.

Windaus and Hossfeld (*Z. physiol. Chem.*, 1925, **145**, 175) by oxidation of the diacetate (VI) (m. p. 107°, $[\alpha]_D$ +39°) of cholestane-3β : 6α-diol (IX) (m. p. 217°, $[\alpha]_D$ +38°) with a concentrated solution of chromium trioxide in acetic acid at 95° obtained a small quantity of an acid, m. p. 196°. This acid gave a monomethyl ester, m. p. 99°, and titrated as a monobasic acid at 20° but as a dibasic acid at 95°; it was clearly a lactonic acid, and, as we shall show, it is the 3→6α-lactonic acid (III; R = H). We have repeated the work of Windaus and Hossfeld; in three repetitions, we obtained a small yield of the lactonic acid only once, the main oxidation product being a neutral substance, m.p. 223°. The production of the lactonic acid appears to depend on the presence of traces of water, and it may be noted that the oxidation of 3β : 6β-diacetoxy-5β-cholestane under similar drastic conditions leads to fission of the side-chain only with formation of 3β : 6β-diacetoxycholanic acid (Moffatt, *J.*, 1947, 812).

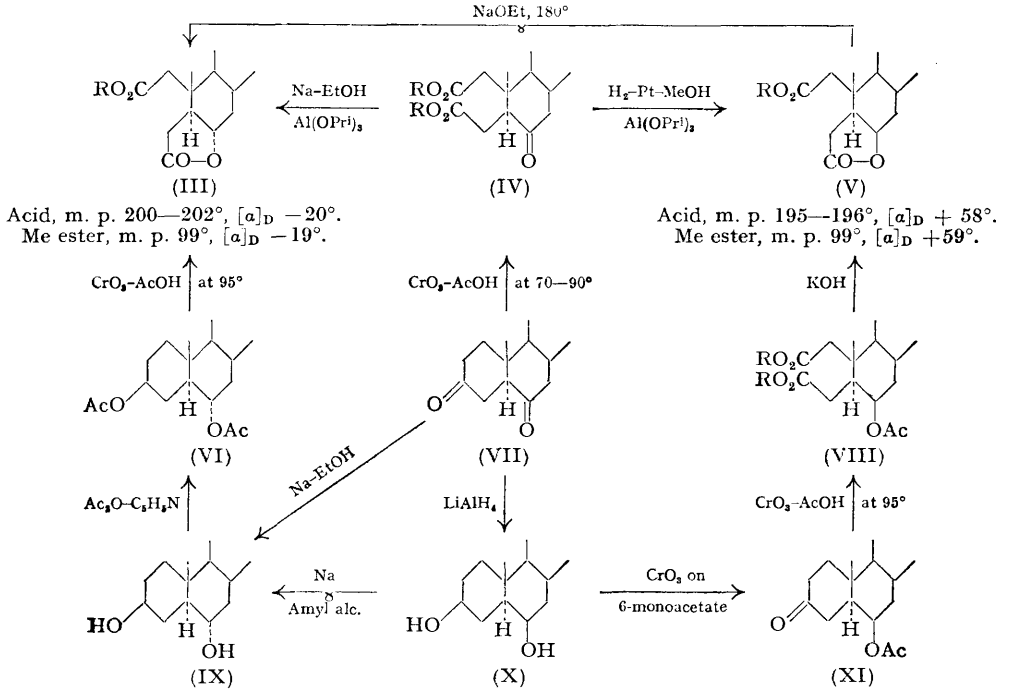
When 6-keto-2 : 3-*seco*cholestane-2 : 3-dicarboxylic acid (IV; R = H), obtained by oxidation of cholestane-3 : 6-dione (VII) (Windaus, *Ber.*, 1903, **36**, 3752), is reduced with sodium in ethanol + *iso*propyl alcohol, the 6α→3-lactonic acid, m. p. 202—204° (III; R = H) (methyl ester m. p. 99°, $[\alpha]_D$ −19°), of Windaus and Hossfeld is produced; the formation of the more thermodynamically stable 6α(equatorial)-configuration of the hydroxyl group in the precursor (II) of the lactonic acid by this reducing agent is to be expected.

If however the keto-dibasic acid (IV; R = H) is reduced catalytically by hydrogenation with platinum–ethanol or platinum–acetic acid (cf. Marker, Turner, and Ulshafer, *J. Amer. Chem. Soc.*, 1942, **64**, 1843) the isomeric 3→6β-lactonic acid, m. p. 195—196° (V; R = H), is formed; the reduction product, after esterification with diazomethane, by

* If ring B has the boat conformation, the acid (I) can readily form a 6β→3-lactonic acid, but the acid (II) cannot furnish a 6α→3-lactonic acid.

chromatographic analysis yielded the 3→6β-lactonic methyl ester (V; R = Me), m. p. 99°, $[\alpha]_D$ +59°. This ester, mixed with the isomeric 3→6α-lactonic methyl ester, m. p. 99°, in approximately equal proportion, gives a depression of ~12°; alkaline hydrolysis of the ester (V; R = Me) gives the 3→6β-lactonic acid (V; R = H).

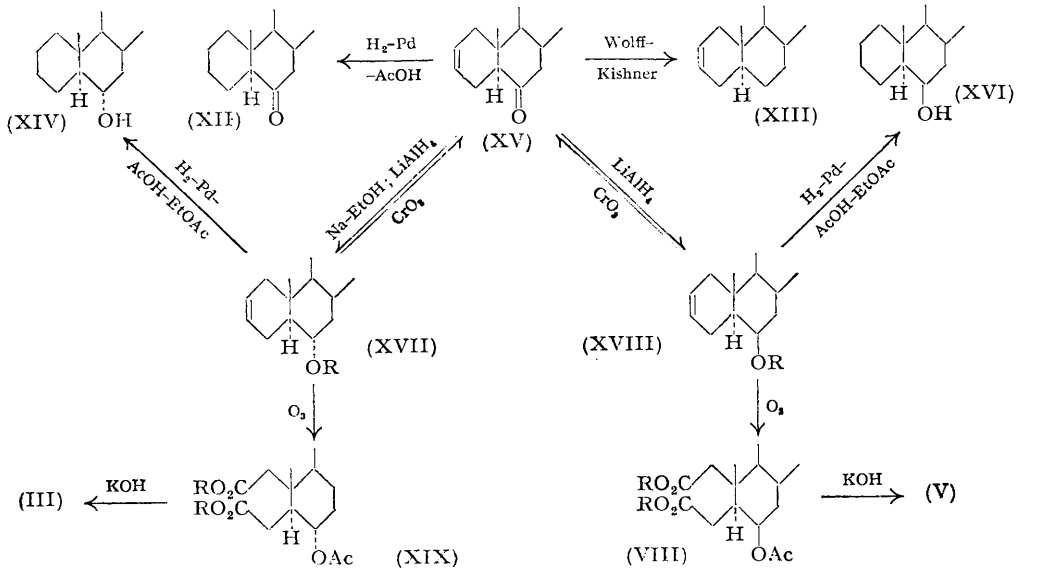
Reduction of the keto-dimethyl ester (IV; R = Me) with aluminium *iso*propoxide–*iso*propyl alcohol affords a mixture of the lactonic methyl esters (III and V; R = Me), partly separable by fractional crystallisation and completely separable by chromatography. Here lactonisation occurs by spontaneous elimination of a molecule of methanol, and may be compared with the ready 17→12-lactonisation of methyl 3α : 12α-dihydroxy-5β-androstane-17α-carboxylate (17-*isoætio*deoxycholic acid) (Sorkin and Reichstein, *loc. cit.*).



The 6β→3-lactonic acid (V; R = H) has also been obtained by partial synthesis. Reduction of cholestane-3 : 6-dione (VII) with lithium aluminium hydride gives cholestane-3β : 6β-diol (X) (m. p. 192°, $[\alpha]_D$ +13°), which by prolonged treatment with sodium–amyl alcohol at 180° undergoes partial conversion into the thermodynamically more stable cholestane-3β : 6α-diol (IX); the 3β : 6β-diol by acetylation gives the diacetate (m. p. 138°, $[\alpha]_D$ −23°), converted by partial hydrolysis into the 6β-monoacetate (Shoppee and Summers, *J.*, 1952, 1790) which by oxidation with chromium trioxide at 20° yields 6β-acetoxycholestan-3-one (XI). Further oxidation under more drastic conditions cleaves ring A, to afford 6β-acetoxy-2 : 3-*seco*cholestane-2 : 3-dicarboxylic acid, m. p. 238—240° (VIII; R = H), characterised as the dimethyl ester, m. p. 74—76°. Alkaline hydrolysis of the 6β-acetoxy-acid furnishes the 3→6β-lactonic acid (V; R = H).

A correlation of the lactonic acids (III and V; R = H) with the epimeric cholestan-6-ols, which confirms the configurations assigned to the latter, has been achieved in the following way. Cholest-2-en-6-one (XV), prepared by the method of Blunschy, Hardegger, and Simon (*Helv. Chim. Acta*, 1946, **29**, 199), was characterised by conversion into, and regeneration from, the dibromide, m. p. 132°, and its structure was confirmed by hydrogenation with palladium–acetic acid to cholestan-6-one (XII) and by Wolff–Kishner reduction to cholest-2-ene (XIII) (Fürst and Plattner, *ibid.*, 1949, **32**, 179; Barton and Rosenfelder, *J.*, 1949, 2359; 1951, 1048).

Reduction of cholest-2-en-6-one (XV) with lithium aluminium hydride gave a mixture of cholest-2-en-6α-ol (XVII; R = H), m. p. 138°, and cholest-2-en-6β-ol (XVIII; R = H), m. p. 90°, containing ~95% of the latter; the epimerides were separated chromatographically and both furnished cholest-2-en-6-one (XV) by mild oxidation with chromium



trioxide. Cholest-2-en-6β-ol (XVIII; R = H) was characterised as the dibromide, m. p. 146°, and was converted by hydrogenation with palladium–ethyl acetate–acetic acid into cholestan-6β-ol (XVI), m. p. 81°. Acetylation gave the acetate (XVIII; R = Ac), m. p. 75°, which by ozonolysis, followed by oxidation with chromium trioxide and esterification with diazomethane, gave the 6β-acetoxy-dimethyl ester (VIII; R = Me); this result provides further evidence of configuration at $C_{(6)}$ in cholestan-6β-ol (XVI).

Cholest-2-en-6α-ol (XVII; R = H) is formed in ~5% yield by reduction of cholest-2-en-6-one (XV) with lithium aluminium hydride, but by use of sodium in ethanol it becomes the sole product. It was converted by hydrogenation in the presence of palladium–ethyl acetate–acetic acid into cholestan-6α-ol (XIV), m. p. 129°. Acetylation yielded the acetate (XVII; R = Ac), m. p. 91—93°, which by ozonolysis, subsequent oxidation, and esterification with diazomethane gave the 6α-acetoxy-dimethyl ester (XIX; R = Me). This by alkaline hydrolysis furnished the 6α→3-lactonic acid (III; R = H), a result which confirms the configuration at $C_{(6)}$ assigned to cholestan-6α-ol (XIV) and, by exclusion, that assigned to cholestan-6β-ol (XVI).

Lastly, the direct conversion of the less thermodynamically stable 3→6β-lactonic acid (V) into the more thermodynamically stable 3→6α-lactonic acid (III) by treatment with sodium ethoxide at 180° supplies final confirmation of the correctness of the configurations assigned at $C_{(6)}$.

Experimental

The general notes on p. 3369 apply also to this paper.

6-*Keto*-2 : 3-seco*cholestan*-2 : 3-*dicarboxylic Acid* (IV).—This was prepared from cholestane-3 : 6-dione by a modification of the methods described by Windaus (*Ber.*, 1903, **36**, 3752) and Marker (*J. Amer. Chem. Soc.*, 1942, **64**, 1843). A solution of cholestane-3 : 6-dione (m. p. 170—173°; 80 g.) in glacial acetic acid (1600 c.c. distilled over CrO_3) was heated to 65—70° and treated with an aqueous solution of chromium trioxide (64 g. in 80 c.c. of water) added dropwise to the vigorously stirred solution during 0·5 hour. Depending on the rate of addition of the chromic acid, the reaction temperature increased but was not allowed to exceed 95°. After 2 hours the reaction mixture was diluted with hot water (1000 c.c.) and left overnight. The reaction product was filtered off, washed with water, and dried on porous porcelain.

Unchanged starting material (37 g.) was removed by heating the product with a 10% aqueous potassium hydroxide (300 c.c.) for 0·5 hour, followed by filtration of the insoluble cholestane-3 : 6-dione. The alkaline filtrate, after cooling in ice, gave by treatment with 4N-sulphuric acid a voluminous precipitate of 6-keto-2 : 3-*seco*cholestane-2 : 3-dicarboxylic acid. The acid was converted into its dimethyl ester by methanol (500 c.c.) and concentrated sulphuric acid (50 c.c.) (2 hours). The sulphuric acid was neutralised with ammonia and the methanol partly removed under reduced pressure. The dimethyl ester was precipitated from solution by dilution with water and extracted with benzene–ether. The extract was washed with water, dried, and evaporated, to yield a thick brown oil (14·3 g.), which was further dried by repeated azeotropic distillation with benzene. The product was purified by chromatography on alumina (700 g.) prepared in pentane. Elution with pentane (5 × 1000 c.c.) gave a colourless oil (11·7 g.) which crystallised. Recrystallisation from acetone–methanol gave dimethyl 6-keto-2 : 3-*seco*cholestane-2 : 3-dicarboxylate as prismatic needles, m. p. 113—114°, $[\alpha]_D$ +20°±1° (*c*, 1·829).

The ester (3 g.) was hydrolysed by ethanolic potassium hydroxide (100 c.c. of 10% solution) under reflux for 1 hour. After concentration the diacid was precipitated at 0° by 4N-sulphuric acid, filtered off, washed with water, and dried on porous porcelain. Crystallisation from 60% acetic acid gave 6-keto-2 : 3-*seco*cholestane-2 : 3-dicarboxylic acid (2·6 g.) as glistening plates, m. p. 219—220° (decomp. and gas evolution).

6α-*Hydroxy*-2 : 3-seco*cholestane*-2 : 3-*dicarboxylic Acid* 3→6α-*Lactone* (III).—(*a*) This was prepared by oxidation of cholestane-3β : 6α-diol diacetate according to Windaus and Hossfeld's directions (*Z. physiol. Chem.*, 1925, **145**, 175). Once in three attempts was a lactonic acid isolated, the main product being a neutral substance, m. p. 223°, which was difficult to recrystallise since it tended to form a gel. It eventually recrystallised in plates from methanol containing a trace of hydrochloric acid. The lactonic acid, isolated in very small yield, crystallised from ether–methanol in plates, m. p. 200—202°. Treatment of the lactonic acid with diazomethane at 0° gave the lactonic methyl ester, which crystallised from pentane in long prismatic needles, m. p. 99°, $[\alpha]_D$ −19°±1° (*c*, 2·61).

(*b*) 6-Keto-2 : 3-*seco*cholestane-2 : 3-dicarboxylic acid (2·6 g.) in *iso*propyl alcohol (200 c.c.) was treated with sodium (5 g.). The sodium salt of the above acid separated immediately, ethanol (200 c.c.) was added, and the mixture refluxed with sodium (5·3 g.) for 2 hours. The solution, on concentration, dilution with water, and acidification with 4N-sulphuric acid, gave a brown semi-solid precipitate which solidified on cooling. The reaction product was taken up in ether and the ethereal extract washed with water, dried, and evaporated, to yield a solid which crystallised from ether–methanol in plates, m. p. 200—202°, $[\alpha]_D$ −20°±2° (*c*, 1·847) (yield, after three crystallisations : 1·95 g.). The lactonic acid so obtained did not depress the m. p. of that prepared by the method of Windaus and Hossfeld (*loc. cit.*). Titration with 0·01N-potassium hydroxide at 20° confirmed the monobasic nature of the compound [Found : *M*, 437. Calc. for $C_{27}H_{44}O_4$: *M* (monobasic), 432·6].

6β-*Hydroxy*-2 : 3-seco*cholestane*-2 : 3-*dicarboxylic Acid* 6β→3-*Lactone* (V).—6-Keto-2 : 3-*seco*cholestane-2 : 3-dicarboxylic acid (500 mg.) in absolute ethanol (110 c.c.) was hydrogenated in the presence of platinum oxide (200 mg.). The catalyst was reduced immediately but the hydrogenation proceeded very slowly, one mol. of hydrogen being absorbed only after 8 hours. The catalyst was filtered off and the solution evaporated to dryness. The white solid lactonic acid obtained was converted with ethereal diazomethane into its methyl ester, which was purified by chromatography on alumina (20 g.) prepared in pentane. Elution with pentane (50-c.c. eluates : fractions 1—5) yielded a colourless oil which crystallised from methanol in needles, to give *methyl* 6β-*hydroxy*-2 : 3-seco*cholestane*-2 : 3-*dicarboxylate* 3→6β-*lactone*, m. p. 99—100°, $[\alpha]_D$ +59°±2° (*c*, 0·886) (Found, after drying at 70°/0·02 mm. for 1 hour : C, 75·7; H, 10·5. $C_{28}H_{46}O_4$ requires C, 75·3; H, 10·4%). Further pentane eluates (fractions 6 and 7) gave oils which could not be induced to crystallise, but which by rechromatography were resolved into the above 3→6β-lactonic ester and a little dimethyl 6-keto-2 : 3-*seco*cholestane-2 : 3-dicarboxylate (IV; R = Me). A mixture of the 3→6β-lactonic methyl ester, m. p. 99°, with the 3→6α-lactonic methyl ester, m. p. 99°, melted at 88°.

Hydrolysis of the 3→6β-lactonic methyl ester, with a dioxan–methanolic solution of potassium hydroxide gave the lactonic acid (V), which crystallised from methanol in needles, m. p. 195—196°, $[\alpha]_D$ +58·5°±2° (*c*, 1·95).

6β-*Acetoxy*-2 : 3-seco*cholestane*-2 : 3-*dicarboxylic Acid* (VIII).—A solution of 6β-acetoxycholestan-3-one (m. p. 101°; 3·2 g.) in 90% acetic acid (90 c.c.) was treated at 60—65° with chromium trioxide (3 g.) in 90% acetic acid (30 c.c.) added dropwise during 0·5 hour to the

stirred solution. Stirring was continued for 1 hour; excess of chromium trioxide was then destroyed by addition of methanol (20 c.c.), and the solvent removed under reduced pressure. The green sludge obtained was dissolved in ether, the ethereal extract washed until colourless with 2N-sulphuric acid, to neutrality with water, and dried, and the ether evaporated, to yield an oil. This was extracted with three portions of hot 2N-sodium carbonate solution, and the extracts were filtered. The combined alkaline filtrates by acidification yielded a white precipitate, which was filtered off, dissolved in ether, and purified in the usual way. The oil obtained crystallised when rubbed with acetic acid and on recrystallisation from acetic acid gave 6β-acetoxy-2 : 3-*seco*cholestane-2 : 3-dicarboxylic acid (0·7 g.), m. p. 238—240°. Treatment with ethereal diazomethane at 0° for 0·25 hour yielded after purification a *dimethyl* ester, which crystallised from methanol as needles, m. p. 74—76° (Found, after drying at 40°/0·02 mm. for 3 hours and 20°/0·02 mm. for 5 hours: C, 71·3; H, 10·35. $C_{31}H_{52}O_6$ requires C, 71·5; H, 10·1%).

Hydrolysis was effected by 5% ethanolic potassium hydroxide for 0·5 hour. Acidification gave a white precipitate which was filtered off; it was readily soluble in ether and the solution by evaporation gave an oil which crystallised spontaneously and yielded needles, m. p. 195°, from methanol, undepressed by admixture with 6β-hydroxy-2 : 3-*seco*cholestane-2 : 3-dicarboxylic acid 3→6β-lactone (V).

Formation of the 3→6β- *and* 3→6α-*Lactonic Methyl Esters by Use of Aluminium* iso-*Propoxide*.—Methyl 6-keto-2 : 3-*seco*cholestane-2 : 3-dicarboxylate (5·3 g.) in absolute *iso*propyl alcohol (30 c.c.) was treated with aluminium *iso*propoxide (4 g.). The reduction as indicated by acetone formed and distilled off required 4 hours for completion. *iso*Propyl alcohol was removed under reduced pressure, the residual oil, after treatment with water and ice-cold 2N-sulphuric acid, was extracted with ether, and the extract worked up in the usual manner, to yield a colourless oil. This crystallised when rubbed with methanol, and recrystallisation from the same solvent gave thin needles, m. p. 95—99°, which on recrystallisation from pentane yielded even thinner needles, m. p. 99° (3·4 g.) undepressed on admixture with methyl 6β-hydroxy-2 : 3-*seco*cholestane-2 : 3-dicarboxylate 3→6β-lactone (V; R = Me). The mother-liquors from these crystallisations by evaporation gave an oil (2 g.), which was chromatographed on neutral alumina (80 g.) prepared in pentane, with pentane elution (100-c.c. fractions). The product (1·1 g.), m. p. 99° (from methanol), from fractions 6—9 consisted of the 3→6β-lactonic methyl ester (V; R = Me) and showed no m. p. depression when mixed with a genuine specimen. The product (0·7 g.), m. p. 98—99°, from fractions 12—15 consisted of the 6α→3-lactonic methyl ester (III; R = Me) and gave no m. p. depression by admixture with this. The material (0·29 g.; m. p. 81—92°) from fractions 10 and 11 consisted of a mixture of the isomeric lactonic methyl esters.

Alkaline hydrolysis of the pure lactonic esters furnished the respective lactonic acids (III, V; R = H), which by treatment with ethereal diazomethane regenerated the respective lactonic esters.

Conversion of the 3→6β-*Lactonic Acid* (V) *into the* 3→6α-*Lactonic Acid* (III).—6β-Hydroxy-2 : 3-*seco*cholestane-2 : 3-dicarboxylic acid 3→6β-lactone (m. p. 195—196°; 547 mg.) was heated at 189—200° for 15 hours with a solution of sodium (0·5 g.) in absolute ethanol (5 c.c.). The reaction product was separated by precipitation from dilute aqueous-alcoholic solution with 2N-sulphuric acid, and crystallisation from methanol gave plates, m. p. 200—202°, which showed no depression on admixture with 6α-hydroxy-2 : 3-*seco*cholestane-2 : 3-dicarboxylic acid 3→6α-lactone.

Cholest-2-en-6-one (XV).—This was prepared by the method of Blunschy, Hardegger, and Simon (*Helv. Chim. Acta*, 1946, **29**, 199); it had m. p. 104—105°, and was characterised as the dibromide, prepared in chloroform: the oil (55 mg.) obtained crystallised from acetone–methanol, to give 2 : 3-*dibromocholestan*-6-*one* in fine needles, m. p. 132°, $[\alpha]_D$ +48°±1° (*c*, 0·737) (Found, after drying at 20°/0·01 mm. for 20 hours: C, 59·4; H, 8·1. $C_{27}H_{44}OBr_2$ required C, 59·65; H, 8·0%). Treatment of the dibromide with zinc dust in acetic acid followed by the usual purification regenerated cholest-2-en-6-one, m. p. 102°. Its structure was confirmed as follows: (*a*) Cholest-2-en-6-one (515 mg.) in triethylene glycol (7 c.c.) was heated at 150° with potassium hydroxide (0·5 g.) and pure hydrazine hydrate (3 c.c.) for 0·5 hour and then at 220° for a further 2·5 hours. The reaction mixture, after cooling, was poured into water and extracted with pentane (50 c.c.), and the extract washed with water, dried, and evaporated. The oil so obtained was purified by filtration of its solution in pentane through alumina, to give cholest-2-ene (369 mg.), which crystallised from ether–methanol in needles, m. p. 74°. (*b*) Cholest-2-en-6-one (90 mg.) was hydrogenated in acetic acid (5 c.c.) in the presence of palladium oxide (30 mg.).

After the theoretical uptake of hydrogen the solution was filtered and evaporated, to yield an oil which was purified in the usual manner. The oil (80 mg.) crystallised from methanol in plates, m. p. 98°, giving no depression with genuine cholestan-6-one.

Cholest-2-en-6α- (XVII) *and -6β-ol* (XVIII).—Cholest-2-en-6-one (m. p. 104—105°; 3·7 g.) in ether (200 c.c.) was heated under reflux for 0·5 hour with finely powdered lithium aluminium hydride (1·0 g.). The mixture was cooled in ice and excess of lithium aluminium hydride destroyed with ice-water and 4N-sulphuric acid. The ethereal solution, worked up in the usual manner, yielded an oil (3·5 g.) which was dried by repeated evaporation with benzene under reduced pressure and chromatographed on alumina (120 g.) prepared in pentane; 200-c.c. eluates were collected. The substance (3·1 g.) from fractions 9—17 (eluant, 1 : 4→1 : 1 benzene–pentane), crystallised from acetone–methanol, had m. p. 89—90°, $[\alpha]_D$ +39·5°±2° (*c*, 4·01), +43°±2° (*c*, 1·193) and proved to be *cholest-2-en-6β-ol* (XVIII) (Found, after sublimation at 90°/0·05 mm.: C, 83·95; H, 11·9. $C_{27}H_{46}O$ requires C, 83·8; H, 12·0%), characterised as the *dibromide* (prepared in chloroform), needles (from acetone–methanol), m. p. 146°, $[\alpha]_D$ +64°±1° (*c*, 1·891) (Found, after drying at 60°/0·01 mm. for 2 hours: C, 59·2; H, 7·9. $C_{27}H_{46}OBr_2$ requires C, 59·3; H, 8·4%). The alcohol was converted into the *acetate* (acetic anhydride–pyridine at 20°), prisms (from aqueous acetone), m. p. 75°, $[\alpha]_D$ +19·5°±1° (*c*, 1·767) (Found, after drying at 40°/0·01 mm. for 6 hours: C, 81·3; H, 11·3. $C_{29}H_{48}O_2$ requires C, 81·2; H, 11·3%). By oxidation with 2% chromium trioxide–acetic acid (0·5 c.c.) at 20°, cholest-2-en-6β-ol (24 mg.) yielded cholest-2-en-6-one (21 mg.) as needles (from acetone), m. p. 102—104°, undepressed by admixture with a genuine specimen.

The compound (0·11 g.) from fractions 20 and 21 (eluant, 1 : 1 benzene–pentane) crystallised from pentane in prisms, m. p. 138°, $[\alpha]_D$ +72°±4° (*c*, 2·915); it was the minor reduction product, *cholest-2-en-6α-ol* (XVII) (Found, after drying at 40°/0·01 mm. for 6 hours: C, 83·8; H, 11·5%); it gave an oily acetate (see below). By oxidation with chromium trioxide at 20°, it gave cholest-2-en-6-one, m. p. 102—104°.

Cholest-2-en-6α-ol (XVII).—This substance was more satisfactorily prepared as follows: Cholest-2-en-6-one (XV) (133 mg.) in boiling ethanol (5 c.c.) was treated with sodium (*ca.* 0·5 g.). The cold reaction mixture was diluted with water and the greater part of the ethanol removed under reduced pressure; working up in the usual way gave a product which was treated in pyridine (1·5 c.c.) with acetic anhydride (1·0 c.c.) at 15° for 12 hours. The resultant oil was filtered in pentane through alumina (4 g.) prepared in pentane; evaporation of the filtrate gave an oil which crystallised partly on standing and completely when moistened with methanol. *Cholest-2-en-6α-yl acetate* (XVII; R = Ac) was obtained by recrystallisation from acetone–methanol as prismatic needles, m. p. 91—93°, $[\alpha]_D$ +105°±3° (*c*, 1·200) (Found, after drying at 80°/0·02 mm. for 1 hour: C, 81·3; H, 11·3%).

Cholestan-6α-ol (XIV).—Cholest-2-en-6α-ol (50 mg.) was hydrogenated with palladium oxide (50 mg.) in acetic acid–ethyl acetate (1 : 1; 30 c.c.). After removal of the catalyst, complete evaporation of the filtrate in a vacuum gave a solid, which by crystallisation from acetone yielded cholestan-6α-ol in plates, m. p. 128—129°.

Cholestan-6β-ol (XVI).—Cholest-2-en-6β-ol (150 mg.) was hydrogenated as above. The oil obtained tended to crystallise from methanol but the crystals did not appear to be homogeneous. After being dried by azeotropic distillation with benzene, the oil (141 mg.) was chromatographed on alumina (5 g.) prepared in pentane. Elution with pentane (7 × 15 c.c.) gave cholestane, m. p. 78—80° (33 mg.), and subsequent elution with ether furnished an oil (96 mg.) which gave no colour with tetranitromethane in chloroform and on inoculation with authentic cholestan-6β-ol crystallised immediately in prisms, m. p. 75—80°. Further recrystallisation from methanol gave cholestan-6β-ol, m. p. 80—81°.

Ozonolysis of Cholest-2-en-6β-yl Acetate.—Cholest-2-en-6β-yl acetate (250 mg.) in glacial acetic acid (10 c.c.) was treated with ozonised oxygen for 0·5 hour. The solution was then diluted with water (2 c.c.) and warmed on a steam-bath for 2 hours. To the cold (0°) mixture, chromium trioxide (5 c.c. of a 1% solution in acetic acid) was added and the whole left overnight. Excess of chromic acid was destroyed with methanol, and the solution evaporated completely in a vacuum. The residue (114 mg.) was dissolved in ether, and the solution washed with ice-cold N-sulphuric acid, and with water, dried and evaporated. Ethereal diazomethane was added and the mixture left at 0° for 0·5 hour; an oil was isolated in the usual way which crystallised from methanol in needles, m. p. 73—76°, and was identical with dimethyl 6β-acetoxy-2 : 3-*seco*cholestane-2 : 3-dicarboxylate (VIII).

Ozonolysis of Cholest-2-en-6α-yl Acetate.—This acetate (450 mg.) similarly gave dimethyl 6α-acetoxy-2 : 3-*seco*cholestane-2 : 3-dicarboxylate (XIX) which did not crystallise. A portion

was hydrolysed with hot 4% methanolic potassium hydroxide for 0·5 hour to 6α-hydroxy-2 : 3-*seco*cholestane-2 : 3-dicarboxylic acid 3→6α-lactone, plates, m. p. 200—202° (from ether–methanol), which did not depress the m. p. of a genuine specimen and furnished by treatment with ethereal diazomethane the methyl ester, m. p. 99°.

Conversion of Cholestane-3β : 6β-*diol* (X) *into Cholestane*-3β : 6α-*diol* (IX).—Cholestane-3β : 6β-diol (1 g.) was heated with sodium (1 g.) and amyl alcohol (10 c.c.) in a sealed tube at 200° for 40 hours. After cooling, the solution was poured into excess of 2N-hydrochloric acid and extracted with ether. The ethereal extract was washed with water and saturated sodium carbonate solution, dried, and evaporated, to give a dark brown solid. This was acetylated with acetic anhydride and worked up in the usual way, to give an oil (617 mg.), which was chromatographed on alumina (25 g.) prepared in pentane and repeatedly eluted with pentane (150 c.c.). Fractions 1—3 gave an oil which crystallised from methanol and had m. p. 138° undepressed on admixture with authentic cholestane-3β : 6β-diol diacetate. Fractions 4 and 5 could not be crystallised, but fractions 6—8 by inoculation with cholestane-3β : 6α-diol diacetate gave a solid, m. p. 85—101°. Alkaline hydrolysis of these last fractions yielded a solid which crystallised from acetone in plates, m. p. 200—211°, which gave no depression with genuine cholestane-3β : 6α-diol, m. p. 217°.

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