Steroids. Part VII. Some 5-Hydroxy-derivatives of Cholestane.*

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[Reprint Order No. 4803.]

The course of ionic dehydration of some 5α-hydroxy-steroids has been examined; a new route to *epi*cholesteryl chloride has been discovered, and the 6-epimeric cholestane-3α : 5 : 6-triols have been prepared. The mildest conditions for oxidation of 3β : 5α-dihydroxy-steroids to 5α-hydroxy-3-oxo-steroids and for dehydration of the latter to Δ^4-3-oxo-steroids have been investigated. 5-Acetoxycholestan-3β-ol resists oxidation with chromium trioxide in acetic acid at 20°; this resistance appears to arise from steric hindrance at $C_{(3)}$ by the 5α-acetoxy-group and may be a general property of 5α-acyloxy-steroids.

THE ready ionic dehydration of 6β-hydroxy-steroids of the cholestane series [5α-H(polar)/6β-OH(polar); *trans*], which appears to be independent of configuration at $C_{(3)}$, has been used previously for the preparation of the cholesteryl and *epi*cholesteryl halides (Shoppee and Summers, *J.*, 1952, 1786, 1790). It appeared of interest to examine the inverse case of the ionic dehydration of 5-hydroxy-steroids of the cholestane series [5α-OH(polar)/4β- or 6β-H(polar); *trans*] and the dependence or otherwise of its course on the presence and configuration of a substituent at $C_{(3)}$.

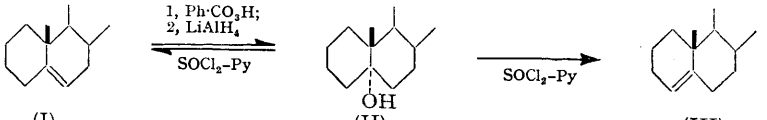
Cholestan-5-ol (II), prepared from cholest-5-ene (I) by conversion into the 5 : 6α-epoxide (Ruzicka, Furter, and Thomann, *Helv. Chim. Acta*, 1933, **26**, 333) and reduction of this with lithium aluminium hydride (cf. Plattner, Petrzilka and Lang, *ibid.*, 1944, **27**, 513), by dehydration with thionyl chloride–pyridine gave a mixture of cholest-5- (I) and -4-ene (III) in approximately equal proportions.† This behaviour may be compared with that of 5-chlorocholestane (cf. Barton and Miller, *J. Amer. Chem. Soc.*, 1950, **72**, 1066, footnote 28) which by dehydrochlorination with ethanolic potassium acetate gives chiefly cholest-4-ene,

* Part VI, *J.*, 1953, 3683.

† The production, by pinacolic rearrangement, of 5β-methyl-19-norcoprost-9-ene is not excluded.

probably accompanied by some cholest-5-ene (cf. Mauthner, *Monatsh.*, 1907, **28**, 1113; Wettstein, Miescher, *et al.*, *Helv. Chim. Acta*, 1946, **29**, 627, especially 629).

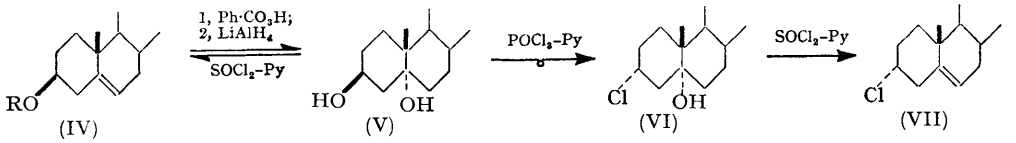
Cholestane-3β : 5-diol (V), prepared from cholesterol (IV; R = H) by reduction of the 5 : 6α-epoxide with lithium aluminium hydride (Plattner, Heusser, and Feurer, *Helv. Chim. Acta*, 1949, **32**, 587; cf. Plattner, Petrzilka, and Lang, *loc. cit.*), by treatment as the 3-monoacetate with thionyl chloride–pyridine gave cholesteryl acetate (IV; R = Ac) which appeared to be unaccompanied by 3β-acetoxycholest-4-ene. This result may be compared with the dehydrochlorination with potassium acetate of 5-chlorocholestan-3β-ol [≡cholesterol hydrochloride] to a mixture of cholesterol and cholest-4-en-3β-ol (Windaus, *Annalen*,



(I) (II) (III)

(Py = pyridine.)

1927, **453**, 101; Schoenheimer and Evans, *J. Biol. Chem.*, 1936, **114**, 567), and with the dehydrochlorination with dimethylaniline of 3β-acetoxy-5-chlorochola-20(22) : 23-diene and its 22-bromo-derivative to mixtures of chola-4 : 20(22) : 23- and -5 : 20(22) : 23-triene in which the latter predominated (Wettstein, Miescher, *et al.*, *loc. cit.*). When the diol (V) was treated with thionyl chloride–pyridine cholesteryl sulphite was produced, but use of phosphorus oxychloride–pyridine gave 3α-chlorocholestan-5-ol (VI) with inversion at $C_{(3)}$ by a normal S_N2 replacement, or, less probably, by an internal S_N2 replacement (cf. Ingold, "Structure and Mechanism in Organic Chemistry," Bell, London 1953, p. 383) involving attack at $C_{(3)}$ by a phosphorus oxychloride ester derived from the 5-hydroxyl group (cf. Plattner, Petrzilka, and Lang, *loc. cit.*; Fieser *et al.*, *J. Amer. Chem. Soc.*, 1953, **75**, 4377, 4423). Dehydration of 3α-chlorocholestan-5-ol (VI) with thionyl chloride–pyridine gave *epi*cholesteryl chloride (VII).



(IV) (V) (VI) (VII)

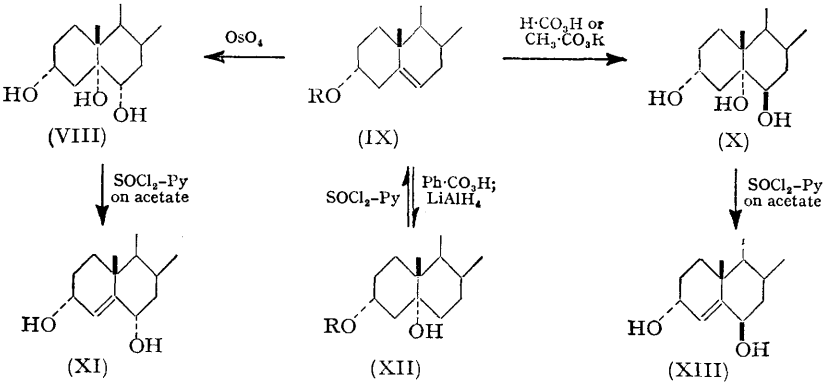
Cholestane-3α : 5-diol (XII; R = H) has been obtained previously by hydrogenation with nickel of 5-hydroxycholestan-3-one, by acid or alkaline hydrolysis (S_N2) of the 3-methanesulphonate of cholestane-3β : 5α-diol * (V) (Plattner, Fürst, Heusser, and Lang, *Helv. Chim. Acta*, 1948, **31**, 1455), and by reduction of the 4α : 5-epoxide of 3α-acetoxycholest-4-ene with lithium aluminium hydride (Plattner, Heusser, and Kulkarni, *ibid.*, 1949, **32**, 1070); we have prepared it more simply from *epi*cholesterol (IX; R = H) by conversion into the 5 : 6α-epoxide and reduction of this with lithium aluminium hydride. By treatment as the 3-monoacetate (XII; R = Ac) with thionyl chloride–pyridine, cholestane-3α : 5-diol gave *epi*cholesteryl acetate (IX; R = Ac) which appeared to be unaccompanied by 3α-acetoxycholest-4-ene.

Hydroxylation of *epi*cholesterol (IX; R = H) with osmium tetroxide furnished cholestane-3α : 5α : 6α-triol (VIII) whilst use of performic or peracetic acid yielded cholestane-3α : 5α : 6β-triol (X). Dehydration of the triols with thionyl chloride–pyridine afforded, after alkaline hydrolysis, cholest-4-ene-3α : 6α- (XI) and -3α : 6β-diol (XIII) respectively; these allylic alcohols give no colour with tetranitromethane–chloroform (cf. Ruzicka *et al.*, *Annalen*, 1929, **471**, 25; Mancera, Rosenkranz, and Djerassi, *J. Org. Chem.*, 1951, **16**, 192).

In view of work proceeding at the University of Manchester and involving 5α-hydroxysteroids with or without a $C_{(17)}$-dihydroxyacetone side-chain (Jones, Henbest, *et al.*, *J.*,

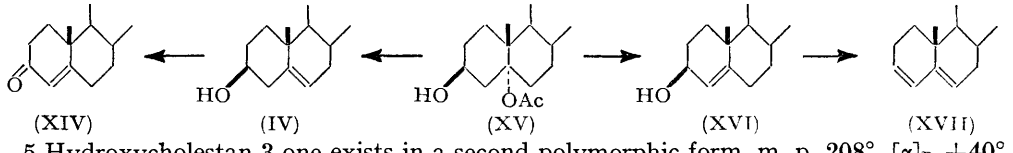
* Under alkaline conditions it would be expected that, in addition to a replacement of the 3β-methanesulphonyl group by an external hydroxyl anion with inversion at $C_{(3)}$, attack by the 5α-hydroxyl anion at the α-face of $C_{(3)}$ should lead by an internal S_N2 process to a 3α : 5α-epoxide. 3α : 5-Epoxycholestane has in fact recently been isolated from the hydrolysis product by Dr. R. B. Clayton at the University of Manchester.

1952, 4883, 4890, 4894; 1953, 2009, 2015; *Chem. and Ind.*, 1953, 945) we have investigated the mildest conditions for oxidation of 3β : 5α-diols to 5α-hydroxy-3-ketones and for dehydration of the latter to Δ^4-ketones. For this purpose cholestane-3β : 5-diol and 3β : 5 : 17α-trihydroxy*allo*pregnan-20-one were envisaged as model substances.



For oxidation of 3-hydroxy-steroids *N*-bromoacetamide appears to be the reagent of choice (Reich and Reichstein, *Helv. Chim. Acta*, 1943, **26**, 583; Gallagher *et al.*, *J. Amer. Chem. Soc.*, 1949, **71**, 3262; 1951, 73, 184; Casanova, Shoppee, and Summers, *J.*, 1953, 2983). Cholestane-3β : 5-diol resists oxidation at 20° by *N*-bromosuccinimide in *tert.*-butanol–pyridine–water and by *N*-bromoacetamide in acetone–pyridine–water (cf. Stavely, *Fed. Proc.*, 1950, **9**, 233); but *N*-bromoacetamide in *tert.*-butanol–pyridine–water, potassium chromate in aqueous acetic acid–sodium acetate or in aqueous acetic acid, and chromium trioxide–acetic acid at 20° caused oxidation to 5-hydroxycholestan-3-one in order of increasing intensity. A similar series has been reported in a contemporaneous comparative study of oxidation of the 3α-, 7α-, and 12α-hydroxyl groups of the bile acids (Fieser and Rajagopalan, *J. Amer. Chem. Soc.*, 1950, **72**, 2307; 1951, **73**, 118).

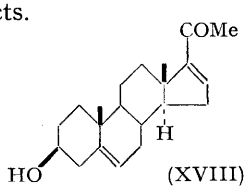
5-Acetoxycholestan-3β-ol (XV) exhibited unexpected resistance to oxidation. It was unaffected by 1% chromium trioxide–acetic acid and by potassium chromate–acetic acid at room temperature; *N*-bromoacetamide in *tert.*-butanol–pyridine–water at 25° gave cholesterol (IV) and cholesta-3 : 5-diene (XVII), whilst Oppenauer oxidation gave cholest-4-en-3-one (XIV). The resistance to oxidation appears to result from hindrance by the 5α-acetoxyl group of attack on the 3α-hydrogen atom by a proton acceptor (cf. Westheimer *et al.*, *J. Amer. Chem. Soc.*, 1949, **71**, 25); the products obtained by use of *N*-bromoacetamide appear to arise by base-catalysed 4 : 5- or 5 : 6-ionic *trans*-elimination of acetic acid, with subsequent dehydration of the allylic alcohol (XVI) and rearrangement.



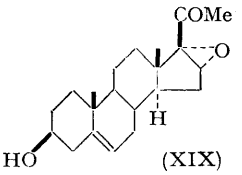
5-Hydroxycholestan-3-one exists in a second polymorphic form, m. p. 208°, $[\alpha]_D$ +40°, in addition to that of m. p. 226°, $[\alpha]_D$ +41°, described by Plattner, Fürst, Koller, and Lang (*Helv. Chim. Acta*, 1948, **31**, 1461; cf. Urushibara and Chuman, *Bull. Chem. Soc. Japan*, 1949, **22**, 69). Both forms are stable to thermal dehydration at 120°/0·01 mm. [although the epimeric 5-hydroxycoprostan-3-one undergoes dehydration at 110°/0·01 mm. (Plattner and Kulkarni, *Helv. Chim. Acta*, 1948, **31**, 1822)], but readily undergo 4 : 5-ionic *trans*-elimination to give cholest-4-en-3-one. 5α-Hydroxy-3-keto-steroids readily furnish Δ^4-3-ketones on treatment with acids (Fernholz, *Annalen*, 1934, **508**, 215; Ehrenstein *et al.*, *J. Org. Chem.*, 1941, **6**, 626, 908; 1951, **16**, 1050; 1952, **17**, 713; Plattner and Lang, *Helv. Chim. Acta*, 1944, **27**, 513; Lardon, *ibid.*, 1949, **32**, 1577) and with alkali (Plattner *et al.*, *ibid.*, 1947, **30**, 1432, 1441; 1948, **31**, 1455; 1952, **35**, 665, 2080). We have found

that 5-hydroxycholestan-3-one is unaffected by 1% hydrogen chloride in methanol at 15°, but is dehydrated to cholest-4-en-3-one by 2% hydrogen chloride in chloroform at 15°, and by larger concentrations of hydrogen chloride in methanol. Dehydration is slowly effected at 15° by aqueous methanolic 0·05N-potassium carbonate, and more rapidly by ethanolic 0·01N-sodium hydroxide or 0·015N-sodium ethoxide, and by 0·2N-potassium *tert.*-butoxide. Aluminium *tert.*-butoxide in acetone–dioxan–benzene at 15° and dimethylaniline at 194° were ineffective but hot acetic anhydride and thionyl chloride–pyridine afforded smooth and rapid dehydration. 2 : 4-Dinitrophenylhydrazine hydrochloride in hot ethanol gave the 2 : 4-dinitrophenylhydrazone of cholest-4-en-3-one (cf. Reich, Walker, and Collins, *J. Org. Chem.*, 1951, **16**, 1753), whilst semicarbazide acetate in methanol at 15° gave the semicarbazone of cholest-4-en-3-one. Use of the Girard T reagent in methanol containing 5% acetic acid also led to dehydration (cf. Prelog and Häfliger, *Helv. Chim. Acta*, 1949, **32**, 2088).

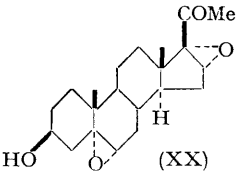
Attempts to prepare the second model substance 3β : 5 : 17α-trihydroxy*allo*pregnan-20-one were unsuccessful, and had to be discontinued owing to external circumstances. 3β-Hydroxypregna-5 : 16-dien-20-one (XVIII) was quantitatively converted by alkaline hydrogen peroxide into the 16α : 17α-epoxide (XIX), which by treatment with excess of perbenzoic acid in benzene at 15° gave the 5 : 6α-16α : 17α-diepoxide (XX) accompanied by an unidentified substance. A crystalline 20-ketal of the diepoxide (XX) could not be obtained; direct reduction of the bisepoxide with lithium aluminium hydride, or by use of hydrogen bromide followed by debromination with Raney nickel, failed to give crystalline products.



(XVIII)



(XIX)



(XX)

An alternative approach involving peroxidation of the 17 : 20-enol acetate of 3β-hydroxypregn-5-en-20-one was unsatisfactory.

Experimental

For general experimental details see *J.*, 1953, 3683. $[\alpha]_D$ are in $CHCl_3$; ultra-violet absorption spectra were determined in EtOH on a Unicam SP. 500 with corrected scale, and infra-red absorption spectra on a Perkin-Elmer double-beam instrument.

Dehydration of Cholestan-5-ol.—Cholest-5-ene was converted by treatment with perbenzoic acid into a mixture of 5 : 6α-epoxycholestane and 5 : 6β-epoxycoprostane, which by reduction with lithium aluminium hydride gave a mixture of cholestan-5- and -6β-ol. This was acetylated (acetic anhydride–pyridine at 20°) and then by chromatography furnished (pentane as eluant) cholestan-5-ol, m. p. 107—109°, $[\alpha]_D$ +12·5° (*c*, 2·1), and [benzene–pentane (1 : 9)] cholestan-6β-yl acetate, which by alkaline hydrolysis gave cholestan-6β-ol, m. p. 79—80° (cf. Shoppee and Summers, *J.*, 1952, 3361). Dehydration of cholestan-5-ol (100 mg.) with thionyl chloride (1 c.c.) in pyridine (5 c.c.) at 20° furnished an oil, $[\alpha]_D$ +3·5°, giving an intense yellow colour with tetranitromethane–chloroform. The value of $[\alpha]_D$ corresponds to an approximately equimolecular mixture of cholest-4-ene ($[\alpha]_D$ +65°) and -5-ene ($[\alpha]_D$ −56°); filtration of a pentane solution through a column of aluminium oxide and a single elution of the column with pentane gave two fractions with $[\alpha]_D$ +8° and +1° respectively. A repetition gave a similar result.

Dehydration of Cholestane-3β : 5-diol and its 3-Monoacetate.—The monoacetate (500 mg.) in pyridine (15 c.c.) was treated at 0° with thionyl chloride (5 c.c.); the solution became deep red, and after 10 min. at 15° was poured into ice-water. The product by chromatography gave cholesteryl acetate, m. p. 112—115°, mixed m. p. 114°, as the sole crystalline product. The diol (1 g.) by similar treatment gave cholesteryl sulphite (720 mg.), m. p. 190—192° after crystallisation from dioxan.

The diol (1 g.) in pyridine (25 c.c.) was treated gradually with phosphorus oxychloride (10 c.c.) at 15°. After 1 hr. at 15°, the excess of reagent and the solvent were removed in a vacuum at 40°. The product was worked up in the usual way, and crystallised when rubbed with methanol; recrystallisation from ether–methanol gave 3α-*chlorocholestan-5-ol* (680 mg.), m. p. 118—119°

[Found (after drying at 60°/0·03 mm. for 3 hr.): C, 76·7; H, 11·1. $C_{27}H_{47}OCl$ requires C, 76·6; H, 11·1%]; it gave no colour with tetranitromethane, and furnished a red colour in the Rosenheim test only after 0·5 hr. 3α-Chlorocholestan-5-ol (100 mg.) in pyridine (2·5 c.c.) was treated at 0° with thionyl chloride (0·5 c.c.); after 10 min. at 15°, the mixture was poured on ice and worked up in the usual way. The resultant yellow oil (94 mg.) by chromatography on neutralised aluminium oxide (3 g.) and elution with pentane gave 3α-chlorocholest-5-ene (*epi*cholesteryl chloride) (59 mg.), m. p. and mixed m. p. 105—107·5°. The diol, by treatment with phosphorus pentachloride in chloroform at 0° in presence of calcium carbonate gave unsaturated material which failed to crystallise despite attempted chromatographic purification. By use of anhydrous copper sulphate (5 g.) in boiling benzene (100 c.c.) for 3·5 hr., the diol (1 g.) gave an oil (875 mg.), which crystallised when rubbed with methanol; recrystallisation from methanol–ether gave cholesta-3 : 5-diene, m. p. 80°, $\lambda_{max.}$ 235, log ε 4·25, giving an immediate positive reaction in the Rosenheim test.

5 : 6α-*Epoxycholestan*-3α-*ol*.—*epi*Cholesterol (1·1 g.) in chloroform (25 c.c.) was treated with perbenzoic acid (1·1 mol.) in chloroform overnight at −10°. The solution was washed successively with potassium iodide, 0·01N-sodium thiosulphate, sodium hydrogen carbonate solution, and water, and the product (1 g.) crystallised from acetone–methanol, to give 5 : 6α-*epoxycholestan*-3α-*ol* in needles, m. p. 125—128°, $[\alpha]_D$ −44° (*c*, 0·58) [Found (after drying at 60°/0·01 mm. for 3 hr.): C, 80·4; H, 11·9. $C_{27}H_{46}O_2$ requires C, 80·5; H, 11·5%].

Cholestane-3α : 5-*diol*.—5 : 6α-Epoxycholestan-3α-ol (246 mg.) was reduced with lithium aluminium hydride in ether under reflux. Treatment with ice-cold N-sulphuric acid and working up gave cholestane-3α : 5-diol (227 mg.) as shining plates (from methanol), m. p. 199°, $[\alpha]_D$ +18° (*c*, 1·00). Acetylation with acetic anhydride–pyridine at 20° gave the 3α-monoacetate, m. p. 132—133° (cf. Plattner *et al.*, *Helv. Chim. Acta*, 1948, **31**, 1454).

Dehydration of Cholestane-3α : 5-*diol* 3-*Monoacetate*.—The 3α-monoacetate (500 mg.) in pyridine (15 c.c.) was treated with thionyl chloride (5 c.c.) at 0°; the solution became deep red and, after 10 min. at 15°, was poured into ice-water, and worked up. The resulting oil, dissolved in pentane, was chromatographed on aluminium oxide, and gave *epi*cholesteryl acetate, plates (from methanol), m. p. 82°, undepressed by admixture with a genuine specimen, as the sole crystalline product.

Cholestane-3α : 5 : 6α-*triol*.—Cholest-5-en-3α-ol (*epi*cholesterol) (500 mg.) in ether (10 c.c.) was treated with a solution of osmium tetroxide (300 mg.) in ether (50 c.c.) containing pyridine (0·5 c.c.). After 14 days at 15°, ether was removed by evaporation and the residue shaken with 2N-potassium hydroxide. The product was extracted with ether, and the extract washed with aqueous sodium hydrogen sulphite and with water, dried, and evaporated to give a gel. This crystallised on trituration with solvents, and recrystallisation from acetic acid gave *cholestane*-3α : 5 : 6α-*triol* (381 mg.), m. p. 168—172°, $[\alpha]_D$ +13° (*c*, 1·50) [Found (after drying at 70°/0·02 mm. for 6 hr.): C, 77·0; H, 11·55. $C_{27}H_{48}O_3$ requires C, 77·1; H, 11·5%]. The acetate was prepared by using acetic anhydride–pyridine at 15°, but could not be obtained crystalline after chromatographic purification and sublimation.

Cholestane-3α : 5 : 6β-*triol*.—Cholest-5-en-3α-ol (*epi*cholesterol) (1·62 g.) in acetic acid (50 c.c.) was treated with hydrogen peroxide (7 c.c. of 100-vol.), and the mixture heated at 70° for 2·5 hr. After dilution with water, the product was extracted with ether and worked up to give an oil, which was hydrolysed with N-methanolic potassium hydroxide under reflux for 1 hr. The product (1·2 g.), isolated in the usual way, failed to crystallise; attempted crystallisation from a large variety of solvents gave gels. Acetylation with acetic anhydride–pyridine at 15° gave *cholestane*-3α : 5 : 6β-*triol* 3-*monoacetate*, which crystallised from ethyl acetate–methanol without difficulty, having m. p. 188—190°, $[\alpha]_D$ −22° (*c*, 1·10) [Found (after drying at 70°/0·02 mm. for 6 hr.): C, 75·3; H, 10·8. $C_{29}H_{50}O_4$ requires C, 75·3; H, 10·8%]. This acetate was also obtained from *epi*cholesterol by use of performic acid, hydrolysis, and reacetylation.

Cholest-4-*ene*-3α : 6α-*diol*.—The amorphous 3α : 5 : 6α-triol acetate by treatment with thionyl chloride in pyridine at 0° in the usual manner furnished a product which was hydrolysed with hot N-methanolic potassium hydroxide for 1 hr. Crystallisation from acetone gave *cholest*-4-*ene*-3α : 6α-*diol*, m. p. 166—167°, $[\alpha]_D$ +81° (*c*, 1·4), giving no colour with tetranitromethane [Found (after drying at 70°/0·02 mm. for 6 hr.): C, 80·8; H, 11·4. $C_{27}H_{46}O_2$ requires C, 80·55; H, 11·5%].

Cholest-4-*ene*-3α : 6β-*diol*.—The 3α : 5 : 6β-triol acetate, m. p. 188° (500 mg.), in pyridine (15 c.c.) was treated with thionyl chloride (5 c.c.) at 0°; the solution at once became deep red, and after 15 min. at 15° was poured into ice-cold 2N-hydrochloric acid. The product, isolated in the usual way, was hydrolysed with hot N-methanolic potassium hydroxide for 1 hr. Crystal-

lisation from ethyl acetate afforded *cholest-4-ene-3α : 6β-diol*, m. p. 220—223°, insoluble in chloroform, giving no colour with tetranitromethane [Found (after sublimation at 190°/0·005 mm.) : C, 80·4; H, 11·4%].

Oxidation of Cholestane-3β : 5-diol.—Owing to the proximity of the m. p. of the diol and of the two forms of 5-hydroxycholestan-3-one, the identity of material was confirmed by treatment with methanolic 2 : 4-dinitrophenylhydrazine which rapidly converts 5-hydroxycholestan-3-one into the red dinitrophenylhydrazone, m. p. 235°, of cholest-4-en-3-one.

(*a*) The diol (1 g.), dissolved in *tert.*-butanol (75 c.c.) and water (25 c.c.), was treated with *N*-bromosuccinimide at 10° for 20 hr.; the product was unaltered diol, m. p. and mixed m. p. 222—224°, $[\alpha]_D$ +17°, after crystallisation from ethyl acetate.

(*b*) The diol (0·5 g.), dissolved in acetone (100 c.c.) and pyridine (2·5 c.c.), was treated with *N*-bromoacetamide (0·4 g.) at 25° for 50 hr.; the product was unchanged diol, m. p. 218—222°, after crystallisation from acetone.

(*c*) The diol (1 g.), dissolved in *tert.*-butanol (150 c.c.), pyridine (2·5 c.c.), and water (2·5 c.c.), was treated with *N*-bromoacetamide (0·75 g.) at 25° for 72 hr.; the product was 5-hydroxycholestan-3-one, which crystallised from acetone in two forms: m. p. 208—210°, and m. p. 229—231°.

(*d*) (*e*) (*f*) (*g*). The diol (1 g.) by oxidation (*d*) with a solution of potassium chromate (3·2 g. in 10 c.c. of water) in acetic acid (200 c.c.) containing sodium acetate trihydrate (2 g.) at 15° for 20 hr., (*e*) with a solution of potassium chromate (2 g. in 5 c.c. of water) in acetic acid (100 c.c.) at 15° for 20 hr., (*f*) with a 1% solution of chromium trioxide in 98% acetic acid at 18° for 17 hr., or (*g*) in anhydrous acetic acid gave 5-hydroxycholestan-3-one, m. p. 208°, $[\alpha]_D$ +42°; in experiment (*f*), the second form, m. p. 229—231°, $[\alpha]_D$ +41°, was also encountered.

(*h*) The diol (0·5 g.), dissolved in dioxan (20 c.c.) and acetone (30 c.c.), was treated with a solution of aluminium *tert.*-butoxide (2 g.) in benzene (70 c.c.) under reflux for 20 hr. The product, after chromatographic purification, afforded cholest-4-en-3-one (416 mg.), m. p. 81—82° (dinitrophenylhydrazone, m. p. 235°).

Oxidation of 5-Acetoxycholestan-3β-ol.—This was obtained from cholestane-3β : 5-diol, by conversion with acetyl chloride–dimethylaniline in boiling chloroform into the 3β : 5-diacetate, m. p. 140—141°, and partial hydrolysis with N-ethanolic potassium hydroxide at 10° for 20 hr.; it had m. p. 160—161°, $[\alpha]_D$ +30° (*c*, 1·1) (cf. Plattner *et al.*, *Helv. Chim. Acta*, 1944, **27**, 513, 1872).

(*a*) 5-Acetoxycholestan-3β-ol (550 mg.), dissolved in acetic acid (25 c.c.), was treated with a 2% solution of chromium trioxide in 98% acetic acid (25 c.c.) at 20° for 20 hr. The product (500 mg.), isolated in the usual way, crystallised from methanol in needles, m. p. 160—161°, and consisted of unchanged material; the alkaline washings by acidification gave traces of a solid acid. Potassium chromate in acetic acid at 20° was likewise ineffective.

(*b*) 5-Acetoxycholestan-3β-ol (1 g.), dissolved in *tert.*-butanol (100 c.c.), pyridine (5 c.c.), and water (5 c.c.), was treated with *N*-bromoacetamide (1 g.) at 20° for 24 hr. The pale yellow solution was diluted with ether, washed with sodium thiosulphate solution, and worked up in the usual way. The product was chromatographed on a column of neutralised aluminium oxide (30 g.) prepared in pentane. Elution with pentane and benzene–pentane (1 : 9) gave cholesta-3 : 5-diene (143 mg.) (possibly containing traces of cholesta-2 : 4-diene), m. p. 78°, mixed m. p. 78—79°, $[\alpha]_D$ −102° (*c*, 1·32), giving an immediate red colour in the Rosenheim test. Use of benzene and ether–benzene mixtures gave only oils, but elution with ether and chloroform–ether (1 : 1) gave cholesterol (215 mg.), m. p. 148°, characterised as cholesteryl acetate, m. p. 115°.

(*c*) 5-Acetoxycholestan-3β-ol (400 mg.), dissolved in dioxan (18 c.c.) and acetone (25 c.c.), was heated with a solution of aluminium *tert.*-butoxide (2 g.) in benzene (70 c.c.) for 22 hr. The product (352 mg.), by chromatography on neutralised aluminium oxide (12 g.) and elution with benzene–pentane mixtures, gave cholest-4-en-3-one (256 mg.), m. p. 79—82° (2 : 4-dinitrophenylhydrazone, m. p. 235°), and by use of ether–benzene mixtures starting material (70 mg.), m. p. 161°.

5-Hydroxycholestan-3-one.—Both forms, m. p. 208—212°, $[\alpha]_D$ +40° (*c*, 1·5), and m. p. 229—231°, $[\alpha]_D$ +41° (*c*, 1·4) [Found (after drying at 100°/0·01 mm. for 2 hr.) : C, 80·3; H, 11·2. Calc. for $C_{27}H_{46}O_2$: C, 80·5; H, 11·5%], were unchanged after 6 hr. at 120°/0·01 mm; sublimation occurred at 185°/0·01 mm. to give a product melting over the range 206—231°, $[\alpha]_D$ +41° (*c*, 1·9). Both forms by treatment with methanolic 2 : 4-dinitrophenylhydrazine hydrochloride gave the red dinitrophenylhydrazone, m. p. 238°, of cholest-4-en-3-one. Attempts to prepare the 5-acetate by use of keten or of the acetyl chloride–dimethylaniline procedure were unsuccessful (cf. Plattner *et al.*, *Helv. Chim. Acta*, 1948, **31**, 1461).

Dehydration of 5-Hydroxycholestan-3-one.—(*a*) The ketol (m. p. 208°; 435 mg.) was dissolved in methanol (100 c.c.) containing 1% of hydrogen chloride at 20° and set aside for 20 hr. After neutralisation with solid sodium hydrogen carbonate, methanol was removed in a vacuum, and the residue crystallised from acetone, to give the unchanged ketol (400 mg.), m. p. 208°.

(*b*) A solution of the ketol (m. p. 208°; 230 mg.) in pure dry chloroform (10 c.c., free from ethanol) containing 2% of hydrogen chloride was allowed to stand at 15° for 20 hr. The product, isolated in the usual way, was a colourless oil (200 mg.), which crystallised when rubbed with methanol and consisted of cholest-4-en-3-one, m. p. and mixed m. p. 80—82°.

(*c*) The ketol (m. p. 231°; 500 mg.), dissolved in acetone (20 c.c.) and dioxan (10 c.c.), was treated with a solution of aluminium *tert.*-butoxide (2·5 g.) in benzene (70 c.c.) at 15° for 18 hr. The product (490 mg.) was recrystallised from ethyl acetate, to give the starting material, m. p. and mixed m. p. 230—232°. The ketol was unchanged by refluxing dimethylaniline for 6 hr., but dehydration to cholest-4-en-3-one was effected at 15° by aqueous-methanolic 0·05N-potassium carbonate, by 0·01N-sodium hydroxide and 0·015N-sodium ethoxide in ethanol, and by 0·2N-potassium *tert.*-butoxide in *tert.*-butanol.

Both forms of the ketol were dehydrated by hot acetic anhydride (5 min.), and by thionyl chloride–pyridine at 15° (30 min.). Use of 2 : 4-dinitrophenylhydrazine hydrochloride in ethanol gave cholest-4-en-3-one 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 238—241°, whilst semicarbazide acetate in methanol at 50° for 2 min. and then at 15° for 1·5 hr. gave an 80% yield of cholest-4-en-3-one semicarbazone, m. p. 250—251° (decomp.), after recrystallisation from acetone–methanol [Found (after drying at 50°/0·01 mm. for 3 hr.) : N, 9·65. Calc. for $C_{28}H_{47}ON_3$: N, 9·5%] [Diels and Abderhalden, *Ber.*, 1904, **37**, 3092, give m. p. 234° (decomp.)].

(*d*) The ketol (m. p. 231°; 500 mg.) in ethanol (50 c.c.) was refluxed with Girard's reagent T (600 mg.) and acetic acid (2·5 c.c.) for 1·5 hr. The mixture was cooled to 15° by addition of ice, a solution of anhydrous sodium carbonate (1 g.) added, and the whole extracted quickly twice with ether. The combined extracts were washed with ice-water, dried, and evaporated, to give the unchanged ketol (50 mg.), m. p. 230—231° after recrystallisation from acetone. The alkaline liquor, together with the aqueous washings, were acidified with 2N-sulphuric acid, warmed to 40°, and set aside at 20° for 3 hr. Extraction with ether gave an oil (350 mg.) which crystallised, and by chromatographic purification furnished cholest-4-en-3-one (300 mg.), m. p. 81—82°, $[\alpha]_D$ +89° (*c*, 2·5).

16α : 17α-*Epoxy*-3β-*hydroxypregn*-5-*en*-20-*one*.—3β-Hydroxypregna-5 : 16-dien-20-one (3 g.; $\lambda_{max.}$ 238 mμ, log ε 4·03; $\lambda_{max.}$ 208 mμ, log ε 3·69), dissolved in methanol (300 c.c.), was treated with 4N-sodium hydroxide (6 c.c.) followed by 30% hydrogen peroxide (12 c.c.). The mixture was warmed to 30° for 0·5 hr., and then kept at 5° until examination of the ultra-violet absorption of a sample showed that the band at 238 mμ had disappeared (20 hr.). The product by recrystallisation from methanol gave 16α : 17α-epoxy-3β-hydroxypregn-5-en-20-one (2·5 g.), m. p. 190°, $\lambda_{max.}$ 208 mμ, log ε 3·87 [2 : 4-dinitrophenylhydrazone, yellow needles, m. p. 179—182°] (cf. Julian *et al.*, *J. Amer. Chem. Soc.*, 1950, **72**, 5145).

5 : 6α-16α : 17α-*Diepoxy*-3β-*hydroxy*allo*pregnan*-20-one.—The 16α : 17α-epoxide (5 g.), dissolved in benzene (200 c.c.), was treated with a solution of perbenzoic acid (0·0317 mg./c.c.) in benzene (80 c.c.) at 15° for 1 week. During this period the band at 208 mμ became reduced in intensity but did not disappear; an excess of perbenzoic was always present, and crystals (2·96 g.) gradually separated. Recrystallisation from acetone gave 5 : 6α-16α : 17α-*diepoxy*-3β-*hydroxy*allo*pregnan*-20-*one*, m. p. 214—216°, $\lambda_{max.}$ 207 mμ, log ε 3·42, with bands in the infra-red at 3610 and 3480 cm.$^{-1}$ (OH), 1694 cm.$^{-1}$ (CO), and 1648, 1300, and 912 (two epoxide groups), which appeared tenaciously to retain solvent [Found (after drying at 100°/0·01 mm. for 6 hr. : C, 72·5; H, 8·8. $C_{21}H_{30}O_4$ requires C, 72·8; H, 8·7%] (2 : 4-dinitrophenylhydrazone, yellow prisms, m. p. 195—197°). A second unidentified substance, m. p. 148—149° (Found : C, 69·7; H, 8·8%) with broad bands in the infra-red at 3570 and 3420 (>1OH), 1694 (CO), and at 1635, 1300, and 900 cm.$^{-1}$ (one epoxide group), obtained from the mother liquors, cannot be a 16α : 17α-epoxy-3β : 5 : 6-triol 6-monobenzoate since it was unchanged by hot 5% methanolic potassium hydroxide.

Two of us (A. J. F. and G. H. R. S.) acknowledge the financial support of Glaxo Laboratories Ltd. and of the Department of Scientific and Industrial Research respectively. We thank Glaxo Laboratories Ltd. for a gift of cholesterol, British Drug Houses Ltd. for a gift of pregnenolone, and Dr. G. Rosenkranz of Syntex Ltd., Mexico City, for a gift of 3β-hydroxypregna-5 : 16-dien-20-one.

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