The Hydroxylation of Cholest-4-en-7β-ol

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Hydroxylation of cholest-4-en-7β-ol with osmium tetroxide gives principally 4β,5,7β-trihydroxy-5β-cholestane accompanied by some 4α,5,7β-trihydroxy-5α-cholestane, whereas peracids give mainly 4β,5,7β-trihydroxy-5α-cholestane and a little 4α,5,7β-trihydroxy-5β-cholestane. The four triols were related by oxidation to the corresponding 5-hydroxy-4,7-diketones and the orientation at C-5 was established by optical rotatory dispersion and elimination studies.

FEW examples are available of *cis*-hydroxylation of the Δ^4-ethylenic linkage in steroids; most recent cases cited show the formation of a mixture of the 4α,5α- and 4β,5β-diols.[1] Treatment of cholest-4-en-7β-ol (I; R = H) (*ψ*-cholesterol) with osmium tetroxide gave a mixture of 4α,5,7β-trihydroxy-5α-cholestane (II; R = H) (22%) and 4β,5,7β-trihydroxy-5β-cholestane (III; R = H) (60%) which were separated by chromatography of their 4,7-diacetates.

Oxidation of triols (II and III; R = H), to the diketones (IV) and (V), respectively, by chromium trioxide (2%) in acetic acid presented some unexpected difficulty. 4α,5,7β-Trihydroxy-5α-cholestane (II; R = H) was recovered unchanged after reaction with this reagent for 20 hours, oxidation nevertheless occurred in low yield (40%) with chromium trioxide in pyridine. Neither of the above reagents gave the diketone (V) from the triol (III; R = H), but it was successfully oxidised, albeit in very low yield (15%), by brief treatment with Jones reagent. The difficulty of oxidising the triol (III; R = H) is clearly a reflection of the steric hindrance of the 4α- and 7α-protons.

Hydroxylation of cholest-4-en-7β-yl acetate (I; R = Ac) with peracetic acid, or of the parent alcohol (I; R = H) with performic acid, followed by acetylation, gave a mixture of the diacetates (VI; R = Ac) (68%) and (VII; R = Ac) (15—18%), which was successfully separated by chromatography on alumina.

Oxidation of the triol (VI; R = H) by chromium trioxide in acetic acid gave 5-hydroxy-5α-cholestane-4,7-dione in high yield, and similar treatment of the triol (VII; R = H) gave the 5β-isomer (V). The formation of the hydroxy-diketones (IV) and (V), from triols (II and VI; R = H) and of (IV) from triols (III and VI; R = H) show that these pairs have the same hydroxyl orientation at C-5. The structure of diketone (IV) was confirmed by *trans*-dehydration to the known [2] cholest-5-ene-4,7-dione (VIII).

The 5-hydroxyl orientation of the four triols (I, II, III, and IV; R = H) was inferred from elimination reactions of the diacetates and confirmed by optical rotatory dispersion studies of the two 5,7-dihydroxy-4-ketone 7-acetates (IX) and (X) derived from cholest-4-en-7β-yl acetate (I; R = Ac) by reaction with monoperphthalic acid.

Cholest-4-en-7β-yl acetate (I; R = Ac), on treatment with monoperphthalic acid in ether solution for 6 days at 0°, gave a mixture of starting material and the two 4,5-epoxides. Chromatography on alumina produced mainly fission of the epoxide rings yielding the 4β,5α- and 4α,5β-dihydroxy-7β-acetoxy compounds (XI) and (XII) and small amounts of the two epoxides (XIII) and (XIV). Hydrolysis [3] of the two epoxy-acetates (XIII and XIV; R = Ac) with perchloric acid in tetrahydrofuran gave, respectively, 4β,5-dihydroxy-5α-cholestan-7β-yl acetate (XI; R = Ac) and, surprisingly, a mixture of this compound with the 4α,5β-dihydroxy-isomer (XII; R = Ac) which was separated by chromatography of their diacetates (VI and VII; R = Ac). Thus, acid hydrolysis of the 4β,5-epoxide occurs in two ways, giving predominantly the 4α,5β-disubstituted derivative (by attack of water at the less-hindered 4α-position), but with a significant proportion (about one-quarter) of the

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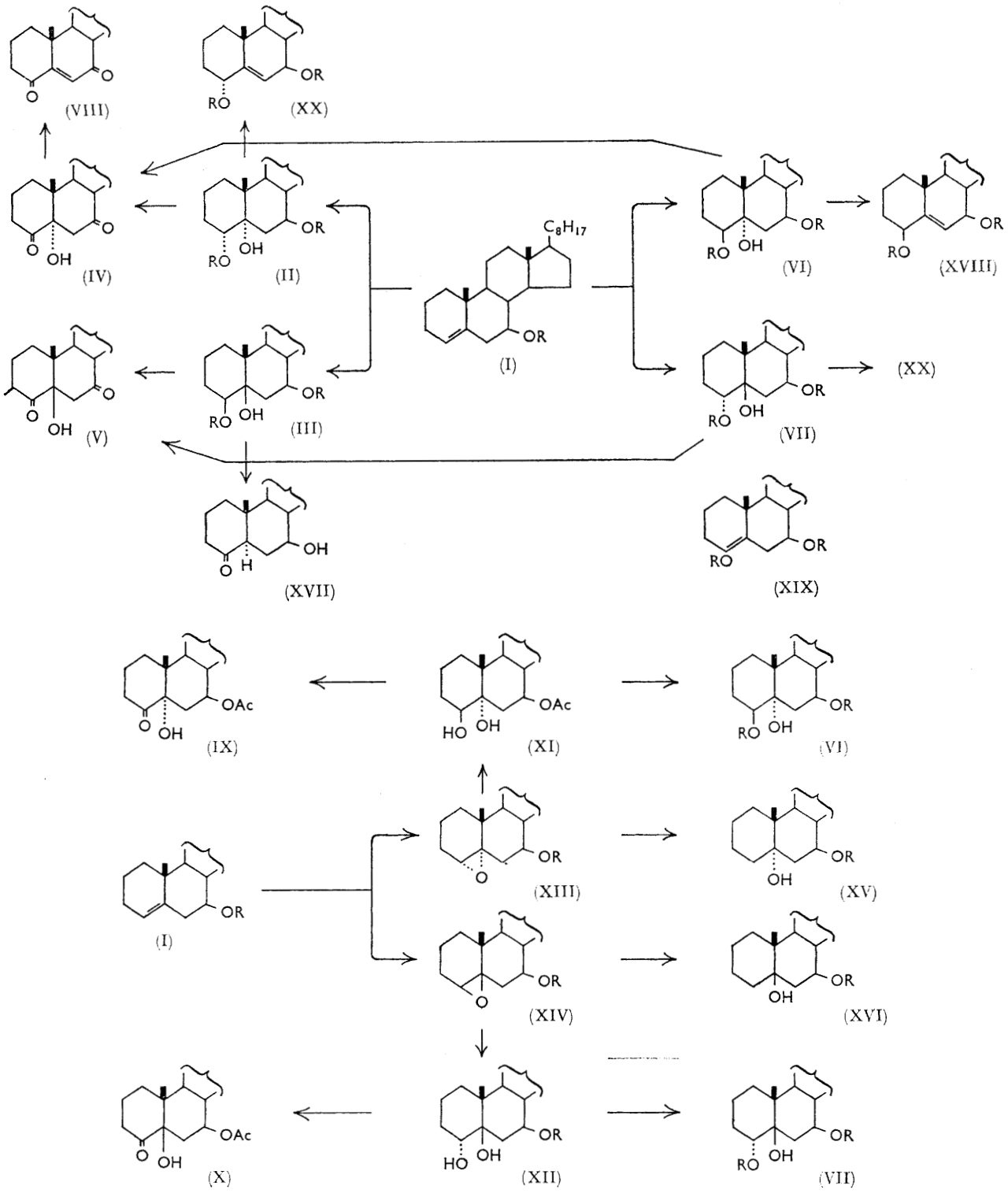
[2] J. C. Eck and E. W. Hollingsworth, *J. Amer. Chem. Soc.*, 1941, **63**, 107.

[3] I. Heilbron, S. Law, and F. S. Spring, *Rec. Trav. chim.*, 1938, **57**, 532; B. Camerino, B. Pattelli, and A. Vercellone, *J. Amer. Chem. Soc.*, 1951, **79**, 1540; M. Tomoeda, M. Ishizake, H. Kobayastri, S. Kanatomo, Koga, M. Inuzuka, and T. Furuta, *Tetrahedron*, 1965, **21**, 733.



4β,5α-isomer. Reduction of the 4α,5-epoxy-7-acetate (XIII; R = Ac) with lithium aluminium hydride in dioxan yielded cholestane-5α,7β-diol (XV; R = H) which on acetylation gave a monoacetate (XV; R = Ac). Reduction of the 4β,5-epoxy-7-acetate (XIV; R = Ac) yielded similarly a single diol (XVI; R = H), which established the homogeneity of the starting material. The mixture of 7β-acetoxy-diols (XI) and (XII) was oxidised by chromium trioxide in pyridine to a mixture of 5-hydroxy-4-oxo-5β-cholestan-7β-yl acetate (X) and the 5α-hydroxy-isomer (IX) which was resolved by chromatography on alumina. Neither hydroxy-ketones (IX) nor (X) gave 4-oxo-5α-cholestan-7β-yl acetate by direct reduction with zinc and acetic acid,[4] but did so after initial chlorination with hydrogen chloride in chloroform. The 5α-hydroxy-4-ketone (IX) was also prepared in high yield by preferential oxidation of the 4β-hydroxyl group in the triol (VI; R = H) with *N*-bromosuccinimide in aqueous ether,[5] followed by acetylation.

The axial nature of the hydroxyl groups in both α-hydroxy-ketones (X) and (IX) was demonstrated[6] by their ultraviolet spectra, $\lambda_{max.}$ 305 mμ (ε 41·5) and $\lambda_{max.}$ 305 mμ (ε 39·5), respectively, a shift of +23 mμ relative to the parent ketone.

The optical rotatory dispersion curve of the 5α-hydroxy-ketone (IX) ($10^{-2}a = -46$)[7] agreed with the value ($10^{-2}a = -45$) obtained by Djerassi *et al.*[8] for an unsubstituted 5α-hydroxy-5α-cholestan-4-one. The optical rotatory dispersion curve of the 5β-hydroxy-ketone (X) ($10^{-2}a = -22$) is of a new type with no analogy, but is consistent with predictions based on the Octant rule[9] for a 5β-hydroxy-4-ketone.

Treatment of the diacetate (VI; R = Ac) with thionyl chloride in pyridine gave cholest-5-ene-4β,7β-diyl diacetate (XVIII; R = Ac) which was shown to contain a trisubstituted double bond by its nuclear magnetic resonance spectrum, which exhibited a doublet centred at τ 4·35, and by its infrared spectrum ($\nu_{max.}$ 828 cm.). Hydroxy-5β-cholestane-4β,7β-diyl diacetate (III; R = Ac) on dehydration under identical conditions gave a mixture of two unsaturated diacetates which, on alkaline hydrolysis, gave 7β-hydroxy-5α-cholestan-4-one (XVII) (70%) and cholest-5-ene-4β,7β-diol (XVIII; R = H) (21%). Thus, *trans*-diaxial elimination of the 5β-hydroxyl group with the 4α-proton to give the enol acetate (XIX) was the preferred mode of reaction. Both 5β- (VII; R = Ac) and 5α-hydroxycholestane-4α,7β-diyl diacetate (II; R = Ac) gave the same Δ^5-4,7-diacetate. No trace of enol acetate was found in either case. Dehydration under acid conditions involving rearrangement will be discussed in a later Paper.

EXPERIMENTAL

For general directions see preceding Paper.

Hydroxylation of Cholest-4-ene-7β-ol.—(*a*) *With peracetic acid.* A solution of cholest-4-en-7β-ol (7·5 g.) in glacial acetic acid (350 ml.) was stirred vigorously at 35—40°. Hydrogen peroxide (100-vol.; 15 ml.) was added immediately and the mixture maintained at 35—40° for 4 hr. and then allowed to cool to room temperature. More hydrogen peroxide (60 ml.) was added over 20 hr., the reaction mixture was stirred at room temperature for another 16 hr., and then poured into a saturated solution of sodium hydrogen carbonate. The solid precipitate formed was collected by filtration, dissolved in ether, and the ether solution worked up in the usual way to give a white glassy solid (8 g.). This was acetylated in pyridine with acetic anhydride and the resulting diacetate was chromatographed on alumina (250 g.). Elution with benzene–ether (9 : 1; 17 × 900 ml.) gave a white solid (6·2 g.) which crystallised from acetone–methanol to give *5-hydroxy-5α-cholestane-4β,7β-diyl diacetate* (VI; R = Ac) as prisms, m. p. 171—173°, $[\alpha]_D$ +74° (*c* 1·13), $\nu_{max.}$ 3400, 1023 (OH), 1720, 1250 cm.$^{-1}$ (7-acetate)[10] (Found: C, 73·3; H, 10·25. $C_{31}H_{52}O_5$ requires C, 73·75; H, 10·4%).

Further elution with benzene–ether (9 : 1; 6 × 900 ml.) gave an oil (730 mg.) which was a mixture of two compounds. Elution with benzene–ether (4 : 1, 10 × 900 ml.) gave a white solid (1·42 g.) which was crystallised from acetone–methanol to yield *5-hydroxy-5β-cholestane-4α,7β-diyl diacetate* (VII; R = Ac) as long needles, m. p. 194·5—195·5°, $[\alpha]_D$ −0·2° (*c* 0·94), $\nu_{max.}$ 3500, 1025 (OH), 1730, 1255 cm.$^{-1}$ (acetate) (Found: C, 73·8; H, 10·4%).

(*b*) *With performic acid.* A suspension of cholest-4-en-7β-ol (2 g.) in formic acid (88%; 40 ml.) was heated to 75° with stirring for 25 min. when an oily layer separated. The mixture was cooled to room temperature, treated with hydrogen peroxide (100-vol.; 4 ml.), and then maintained at 30° for 3 hr. The solid formed on cooling did not dissolve in the formic acid–hydrogen peroxide mixture after 3 hr. and ether (200 ml.) was added until a clear solution was obtained. More formic acid (40 ml.) and hydrogen peroxide (14 ml.) were added and the mixture was left at room temperature for 16 hr. and then poured into a large excess of water. The product was extracted with ether and the ether solution worked up in the usual way to give a white solid (2·2 g.). The solid was dissolved in methanol (200 ml.), sodium hydroxide (5 g.) was added, and the mixture heated under reflux for 3 hr. Carbon dioxide was passed into the solution until the precipitation of sodium carbonate was complete and the methanol was then distilled off under reduced pressure. The residue was dissolved in ether and water, the ether solution was separated and worked up in the usual way to give a white solid (2·03 g.). The solid was dissolved in pyridine (20 ml.), acetic anhydride (20 ml.) was added, and the mixture left at room temperature for 12 hr., then poured into water. Working up in the usual way gave a colourless oil (2·3 g.) which was chromatographed on alumina (70 g.). Benzene–ether (9 : 1; 9 × 250 ml.) eluted the (VI; R = Ac) (1·39 g.) which was crystallised

[4] D. H. R. Barton and C. H. Robinson, *J. Chem. Soc.*, 1954, 3045.

[5] P. C. Cookson, *J. Chem. Soc.*, 1954, 282; G. Baumgertner and C. H. Tamm, *Helv. Chim. Acta*, 1955, **38**, 441.

[6] L. F. Fieser and S. Rajagopalun, *J. Amer. Chem. Soc.*, 1949, **71**, 3938.

[7] For nomenclature see C. Djerassi and W. Klyne, *Proc. Chem. Soc.*, 1957, 55.

[8] C. Djerassi, W. Clossen, and A. E. Lippman, *J. Amer. Chem. Soc.*, 1956, **78**, 3163.

[9] W. Moffitt, R. B. Woodward, A. Moscowitz, W. Klyne, and C. Djerassi, *J. Amer. Chem. Soc.*, 1961, **83**, 4013.

[10] R. N. Jones, P. Humphries, F. Herling, and K. Dobriner, *J. Amer. Chem. Soc.*, 1951, **73**, 3215.

from acetone–methanol, m. p. and mixed m. p. 171—173°, $[\alpha]_D$ +71° (*c* 0·89).

Further elution with benzene–ether (9 : 1; 4 × 250 ml.) gave an oil which was a mixture of two compounds. Elution with benzene–ether (4 : 1, 6 × 250 ml.) gave the isomer (VIII; R = Ac) (354 mg.), which crystallised from acetone–methanol, m. p. and mixed m. p. 194—195·5°, $[\alpha]_D$ −1° (*c* 1·10).

Hydrolysis of the Diacetate (VI; R = Ac).—A suspension of lithium aluminium hydride (690 mg.) in anhydrous ether (25 ml.) was added dropwise to a solution of the diacetate (1·86 g.) in anhydrous ether (75 ml.) and the mixture heated under reflux for 1 hr. The excess of reagent was destroyed by addition of acetone and the reaction worked up in the usual way to give a white solid which was recrystallised from acetone to give *5α-cholestane-4β,5,7β-triol* as plates, double m. p. 185—187°/231—234·5°, $[\alpha]_D$ +96·2° (*c* 0·52) (Found: C, 77·35; H, 11·3. $C_{27}H_{48}O_3$ requires C, 77·1; H, 11·5%).

Hydrolysis of 5-Hydroxy-5β-cholestane-4α,7β-diyl Diacetate (VIII; R = Ac).—A suspension of lithium aluminium hydride (1·0 g.) in ether (25 ml.) was added to a solution of the diacetate (893 g.) in ether (75 ml.) and the mixture heated under reflux for 1 hr. Acetone was added to destroy excess of reagent and the reaction was worked up in the usual way to give a white solid which was crystallised from acetone–methanol to give *5β-cholestane-4α,5,7β-triol* as needles, m. p. 150—152°, $[\alpha]_D$ −39·1° (*c* 0·62) (Found: C, 76·8; H, 11·45%).

Reaction of Cholest-4-en-7β-ol with Osmium Tetroxide.—Osmium tetroxide (1·36 g.) was added to a solution of cholest-4-en-7β-ol (2·1 g.) in anhydrous ether (200 ml.) containing pyridine (5 ml.) and the mixture left at room temperature for 5 days. Lithium aluminium hydride (700 mg.) was then added and the mixture heated under reflux for 12 hr. Acetone was added to destroy excess of reagent, followed by sulphuric acid, and the black precipitate of osmium dioxide removed by filtration. The ether solution was separated and worked up in the usual way to give a white solid. The solid was dissolved in pyridine (20 ml.) and acetic anhydride (10 ml.) and the solution left at room temperature for 12 hr. Working up in the usual way gave a colourless oil (2·5 g.) which was chromatographed on alumina (120 g.). Pentane (3 × 300 ml.) eluted cholest-4-en-7β-yl acetate (373 mg.) which was crystallised from acetone, m. p. and mixed m. p. 96—98°, $[\alpha]_D$ +72° (*c* 0·93). Elution with benzene (3 × 300 ml.) gave a white foam (442 mg.) which could not be recrystallised from a variety of solvents but from the evidence of thin-layer chromatography it appeared to be pure 5-hydroxy-5α-cholestane-4α,7β-diyl diacetate (II; R = Ac) $[\alpha]_D$ +65° (*c* 1·1). $\nu_{max.}$ (in $CHCl_3$) 3500, 1070 (OH); 1720, 1250 cm.$^{-1}$ (acetate) (Found: C, 73·9; H, 10·25. $C_{31}H_{52}O_5$ requires C, 73·75, H, 10·4%). Further elution with benzene (9 × 300 ml.) gave *5-hydroxy-5β-cholestane-4β,-7β-diyl diacetate* (III; R = Ac) (1·18 g.) which was crystallised from acetone at 0°, double m. p. 56—60°/105—106°, $[\alpha]_D$ +54·7° (*c* 1·06); $\nu_{max.}$ (in $CHCl_3$) 3550, 1029; 1720, 1250 cm.$^{-1}$ (acetate) (Found: C, 74·0; H, 10·5%).

Hydrolysis of the Diacetate (II; R = Ac).—A suspension of lithium aluminium hydride (350 mg.) in anhydrous ether (10 ml.) was added to a solution of the diacetate (430 mg.) in anhydrous ether (10 ml.) and the reaction mixture was heated under reflux for 1 hr. Working up in the usual way gave *5α-cholestane-4α,5α,7β-triol* (390 mg.) as needles after crystallisation from acetone, m. p. 193—195·5° $[\alpha]_D$ +47° (*c* 0·8) (Found: C, 77·0; H, 11·4. $C_{27}H_{48}O_3$ requires C, 77·1; H, 11·5%).

Hydrolysis of the Diacetate (III; R = Ac).—A suspension of lithium aluminium hydride (1·0 g.) in anhydrous ether (25 ml.) was added to a solution of the diacetate (1·2 g.) in anhydrous ether (100 ml.) and the reaction mixture heated under reflux for 1 hr. Working up in the usual way gave *5β-cholestane-4β,5,7β-triol* (800 mg.) as fine needles after crystallisation from ethyl acetate, m. p. 160—162°, $[\alpha]_D$ +44° (*c* 0·6) (Found: C, 76·7; H, 11·25%).

Oxidation of 5α-Cholestane 4β,5,7β triol (VI; R = OH).—A solution of chromium trioxide (930 mg.) in water (1 ml.) and acetic acid (11 ml.) was added to a solution of the triol (547 mg.) in acetic acid (20 ml.) and the mixture left at room temperature for 3 days. The solution was then poured into water and the precipitate formed was collected by filtration and dissolved in ether. The ether solution was worked up in the usual way to give an oil which was crystallised from acetone–methanol to yield *5-hydroxy-5α-cholestane-4,7-dione* (IV) (350 mg.) as needles, m. p. 190—191°, $[\alpha]_D$ −9·5°, $\nu_{max.}$ 3400 (OH); 1730, 1710 cm.$^{-1}$ (C=O) (Found: C, 77·4; H, 10·6. $C_{27}H_{44}O_3$ requires C, 77·8; H, 10·65%).

Dehydration of the Dione (IV).—Thionyl chloride (1 ml.) was added to a solution of the dione (183 mg.) in pyridine (5 ml.) at 0°. The mixture was left at 0° for 30 min. and then at room temperature for a further 20 min. Excess of reagent was destroyed by the addition of water and the reaction mixture was worked up in the usual way to give a yellow oil (77 mg.). The product was chromatographed on alumina (3 g.). Benzene–ether (7 : 3) eluted a yellow solid which was crystallised from acetone–methanol to give cholest-5-ene-4,7-dione (VIII) (27 mg.) as plates, m. p. 159—161°, $[\alpha]_D$ −51° (*c* 0·75); $\nu_{max.}$ (in CCl_4) 1685, 1677 cm.$^{-1}$ (αβ-unsaturated diketone); $\lambda_{max.}$ 249 mμ (log ε 0·88) (lit.,[2] m. p. 160—161°, $[\alpha]_D$ −51·7°).

Oxidation of 5β-cholestane-4α,5,7β-triol.—A 2% solution of chromium trioxide in acetic acid (8 ml.) was added to a solution of the triol (450 mg.) in acetic acid (5 ml.). The mixture was left at room temperature for 40 hr., methanol was then added to destroy the excess of reagent, and the mixture was poured into water. The product was extracted with ether and worked up in the usual way to give a white foam (280 mg.) which was chromatographed on alumina (10 g.). Benzene (9 × 30 ml.) eluted an oil (63 mg.) which could not be crystallised and was shown by thin-layer chromatography to be a mixture of several compounds. Elution with benzene–ether (9 : 1; 16 × 30 ml.) gave an oil (128 mg.) which was crystallised from ethyl acetate to yield *5-hydroxy-5β-cholestane-4,7-dione* (V) as cubes, m. p. 148—151·5°, $[\alpha]_D$ +12·2° (*c* 1·04), $\nu_{max.}$ 3450 (OH); 1718, 1695 cm.$^{-1}$ (C=O) (Found: C, 77·1; H, 10·6. $C_{27}H_{44}O_3$ requires C, 77·8; H, 10·65%).

Oxidation of 5α-Cholestane-4α,5,7β-triol.—(*a*) *With chromium trioxide in acetic acid.* A solution of chromium trioxide (50 mg.) in acetic acid (2 ml.) was added to a solution of the triol (161 mg.) in acetic acid (10 ml.). The mixture was left at room temperature for 60 hr., methanol was added to destroy the excess of reagent, and the mixture was diluted with water. The product was extracted with ether and worked up in the usual way to give a white solid (170 mg.). Crystallisation from acetone yielded unchanged starting material, m. p. and mixed m. p. 192—196°, $[\alpha]_D$ +46° (*c* 0·6).

(*b*) *With chromium trioxide in pyridine.* A mixture of the triol (250 mg.) and chromium trioxide (309 mg.) in pyridine (7 ml.) was left at room temperature for 15 hr. Pentane was then added and the precipitate removed by filtration through a pad of Super-Cel. The filtrate was washed with ether–pentane which was worked up in the usual way to give a white crystalline solid (152 mg.) which was chromatographed on alumina (5 g.). Pentane (23 × 10 ml.) eluted an oil (31 mg.) which was shown to be a mixture of several compounds by thin-layer chromatography. Elution with pentane–ether (3 : 1; 32 × 10 ml.) gave pure 5-hydroxy-5α-cholestane-4,7-dione (98 mg.) as needles after crystallisation from acetone–methanol, m. p. and mixed m. p. 190—191°, $[\alpha]_D$ −6° (*c* 0·83).

Oxidation of 5β-cholestane-4β,5,7β-triol.—(*a*) *With chromium trioxide in acetic acid.* A solution of the triol (251 mg.) in acetic acid (7 ml.) was added to a solution of chromium trioxide (469 mg.) in water (1 ml.) and acetic acid (14 ml.) at such a rate that the temperature did not exceed 16°. The reaction mixture was then left at room temperature for 24 hr., methanol was added to destroy the excess of reagent, followed by water. The product was extracted with ethyl acetate which was worked up in the usual way to give a yellow oil (27 mg.). Thin-layer chromatography showed this oil to be a mixture of many compounds. The sodium hydrogen carbonate washings were combined and hydrogen chloride was added until the solution was acid to litmus and the resulting mixture was extracted with ether. The ether solution was worked up in the usual way to give a white solid (190 mg.) which was crystallised from methanol–water, m. p. 194—196°, $[\alpha]_D$ +21° (*c* 0·94), $\nu_{max.}$ 1698 cm.$^{-1}$ (C=O) (Found: C, 69·3; H, 9·75. $C_{27}H_{46}O_6$ requires C, 69·5; H, 9·95%).

(*b*) *With chromium trioxide in pyridine.* A solution of the triol (160 mg.) in pyridine (1·6 ml.) was added to a mixture of chromium trioxide (340 mg.) and pyridine (3·5 ml.). The reaction mixture was left at room temperature for 24 hr., diluted with benzene, and filtered through a pad of Super-Cel. The filtrate was extracted with ether–benzene which was worked up in the usual way to give a white solid (120 mg.). The solid was crystallised from acetone–methanol to yield unchanged starting material, m. p. and mixed m. p. 160—162°, $[\alpha]_D$ +46° (*c* 0·55).

(*c*) *With chromium trioxide–sulphuric acid in acetone.* Standard chromate reagent * (0·4 ml.) was added to a solution of the triol (120 mg.) in acetone (16 ml.). The mixture was left at room temperature for 2 min. and then poured into a saturated aqueous solution of sulphur dioxide. A saturated aqueous solution of potassium carbonate was added and the product extracted with ether. The ether solution was worked up in the usual way to give a colourless oil (44 mg.) which was chromatographed on alumina (1·5 g.). Ether eluted an oil (21 mg.) which was crystallised from methanol to give 5-hydroxy-5β-cholestane-4,7-dione as cubes, m. p. and mixed m. p. 149—151°, $[\alpha]_D$ +10° (*c* 0·81).

Oxidation of Cholest-4-en-7β-yl Acetate with Monoperphthalic Acid.—The solution of monoperphthalic acid in ether was prepared by the method described by Fieser [11] and was standardised against 0·1N-sodium thiosulphate. The solution of monoperphthalic acid in ether (0·23N; 58 ml.) was added to a solution of cholest-4-en-7β-yl acetate (2·35 g.) in ether and the mixture stored at 0° for 13 days. Excess of potassium iodide was then added and the ether solution was washed with 0·1N-sodium thiosulphate solution, water, saturated aqueous sodium hydrogen carbonate, and water, dried over anhydrous magnesium sulphate, and the ether distilled off under reduced pressure. A white foam was obtained and thin-layer chromatography showed the presence of two compounds of very similar R_F distinguishable only by their slightly different colour reactions with antimony trichloride. The product was chromatographed on alumina (95 g.). Pentane (10 × 250 ml.) eluted unchanged cholest-4-en-7β-yl acetate (416 mg.), m. p. and mixed m. p. 96—98°, $[\alpha]_D$ +75° (*c* 0·81), after crystallisation from methanol–acetone.

Elution with benzene–pentane (1 : 3; 21 × 250 ml.) gave an oil (699 mg.) which could not be crystallised and which appeared to be a mixture of the 4β,5- and 4α,5-epoxy-7-acetates. Further elution with benzene–pentane (1 : 3; 15 × 250 ml.) gave an oil (496 mg.) which crystallised from methanol to yield 4α,5-*epoxy*-5α-*cholestan*-7β-*yl acetate* (XIII; R = Ac) as small prisms, m. p. 110—112°, $[\alpha]_D$ +85·5° (*c* 1·07), $\nu_{max.}$ 1739 cm.$^{-1}$ (7-acetate) (Found: C, 78·7; H, 10·6. $C_{27}H_{48}O_3$ requires C, 78·3; H, 10·9%).

Elution with benzene–ether (1 : 1; 8 × 250 ml.) gave a white solid (1·22 g.) which was crystallised twice from methanol to yield 4β,5-*dihydroxy*-5α-*cholestan*-7β-*yl acetate* (XV) (540 mg.) as small needles, m. p. 170—172°, mixed m. p. with 5-hydroxy-5α-cholestane-4β,7β-diyl diacetate, 114—162°, $[\alpha]_D$ +64·7° (*c* 1·39), $\nu_{max.}$ 1718 cm.$^{-1}$ (acetate) (Found: C, 75·2; H, 10·9. $C_{29}H_{50}O_4$ requires C, 75·3; H, 10·9%).

The mixture of 4α,5- and 4β,5-epoxy-acetates was rechromatographed on alumina. Pentane–benzene (17 : 3; 12 × 25 ml.) eluted an oil (386 mg.) which could not be crystallised but seemed to be homogenous 4β,5-epoxy-5β-cholestan-7β-yl acetate, $[\alpha]_D$ +21° (*c* 1·40), $\nu_{max.}$ (in $CHCl_3$) 1739 cm.$^{-1}$ (7-acetate)(Found: C, 78·75; H, 10·55. $C_{29}H_{48}O_3$ requires C, 78·3; H, 10·9%). Further elution with pentane–benzene (17 : 3; 14 × 25 ml.) gave a mixture of the two epoxides (93·7 mg.). Elution with benzene gave 4α,5-epoxy-5α-cholestan-7β-yl acetate (59 mg.), m. p. and mixed m. p. 113—114°, $[\alpha]_D$ +84° (*c* 1·3). Ether (5 × 25 ml.) eluted a white solid which was crystallised twice from methanol to give 4β,5-dihydroxy-5α-cholestane-7β-yl acetate, m. p. and mixed m. p. 170—172°, $[\alpha]_D$ +66° (*c* 1·0).

Reaction of 4α,5-*Epoxy*-5α-*cholestan*-7β-*yl Acetate* (XIII; R = Ac) *with Acid.*—Perchloric acid (60% w/w; 1·0 ml.) was added to a solution of the epoxy-acetate (57 mg.) in tetrahydrofuran. The solution was left at room temperature for 6 hr. then diluted with water and extracted with ether. The ether solution was worked up in the usual way to give 4β,5-dihydroxy-5α-cholestane-7β-yl acetate as needles on crystallisation from methanol, m. p. and mixed m. p. 169—172°, $[\alpha]_D$ +85° (*c* 0·91).

Treatment in the usual way with acetic anhydride in pyridine gave 5-hydroxy-5α-cholestane-4β,7β-diyl diacetate, m. p. and mixed m. p. 171—173°, $[\alpha]_D$ +76° (*c* 1·01).

Reduction of 4α,5-*Epoxy*-5α-*cholestan*-7β-*yl Acetate* (XIII; R = Ac) *with Lithium Aluminium Hydride.*—A mixture of the epoxy-acetate (140 mg.) and lithium aluminium hydride (193 mg.) in anhydrous dioxan (25 ml.) was heated under reflux for 3 hr. Acetone was then added, followed by dilute

* Standard chromate solution: 26·72 g. of chromium trioxide in 23 ml. of concentrated sulphuric acid, diluted with water to 100 ml.

[11] L. F. Fieser, "Experiments in Organic Chemistry," Heath and Co., Boston, 1957, p. 329.

sulphuric acid, and the mixture extracted with ether. The ether solution was worked up in the usual way to give *5α-cholestane-5,7β-diol* (95 mg.) as needles after crystallisation from acetone, m. p. 197—198°, $[\alpha]_D$ 0° (*c* 0·57) (Found: C, 80·65; H, 12·05. $C_{27}H_{48}O_2$ requires C, 80·15; H, 11·95%).

5-Hydroxy-5α-cholestane-7β-yl Acetate (XV; R = Ac).—A solution of the foregoing diol (69·5 mg.) in pyridine (5 ml.) and acetic anhydride (5 ml.) was left at room temperature for 16 hr. and then diluted with water. The product was extracted with ether and worked up in the usual way to give the *hydroxy-acetate* (26 mg.) as fine needles after crystallisation from methanol, m. p. 108—110°, $[\alpha]_D$ +50° (*c* 1·20) (Found: C, 77·9; H, 11·1. $C_{29}H_{50}O_3$ requires C, 77·95; H, 11·3%).

Oxidation of 4β,5-Dihydroxy-5α-cholestane-7β-yl Acetate (XI) (*with Chromium Trioxide in Pyridine*.—A mixture of the dihydroxy-acetate (350 mg.) and chromium trioxide (399 mg.) in pyridine (7·5 ml.) was left at room temperature for 20 hr. and then diluted with benzene. The precipitate was removed by filtration and washed thoroughly with ether–benzene. The washings were combined and worked up in the usual way to yield *5-hydroxy-4-oxo-5α-cholestan-7β-yl acetate* (IX) (175 mg.), double m. p. 90—91°/164—166°, $[\alpha]_D$ +80° (*c* 0·50), after crystallisation 3 times from methanol, $\nu_{max.}$ 1718 (C=O), 1250 cm.$^{-1}$ (acetate), $\lambda_{max.}$ 305 mμ ($\varepsilon_{max.}$ 41·5). Optical rotatory dispersion: trough, +115 (320 mμ); peak, +4750 (278 mμ), amplitude −46 (Found: C, 76·1; H, 10·9. $C_{29}H_{48}O_4$ requires C, 75·6; H, 10·5%).

Reaction of 4β,5-epoxy-5β-cholestan-7β-yl Acetate (XIV; R = Ac) *with Acid*.—A solution of the epoxy-acetate (250 mg.) in tetrahydrofuran (30 ml.) containing perchloric acid (60% w/w; 4 ml.) was left at room temperature for 6 hr. and then poured into water. The product was extracted with ether and the ether solution worked up in the usual way. The oil obtained could not be crystallised and a portion of the product (50 mg.) was acetylated with acetic anhydride in pyridine. Water was added to the pyridine solution and the product extracted with ether. The ether solution was worked up in the usual way and the product chromatographed on alumina (2 g.). Benzene–ether (9 : 1; 10 × 5 ml.) eluted 5-hydroxy-5α-cholestane-4β,7β-diyl (VI; R′ = Ac) diacetate (13 mg.), m. p. and mixed m. p. 171—173°, $[\alpha]_D$ +78 (*c* 0·51), after crystallisation from methanol–acetone. Further elution with benzene–ether (9 : 1; 5 × 5 ml.) gave a mixture of the two diacetates (11 mg.) and then (12 × 5 ml.) the isomer (VII; R = Ac) (26 mg.), m. p. and mixed m. p. 193—196°, $[\alpha]_D$ −3° (*c* 1·11).

Reduction of 4β,5-Epoxy-5β-cholestan-7β-yl Acetate (XIV; R = Ac) *with Lithium Aluminium Hydride*.—A mixture of the epoxide (165 mg.) and lithium aluminium hydride (200 mg.) in anhydrous ether (20 ml.) was heated under reflux for 1 hr. Excess of reagent was destroyed by the addition of acetone and the reaction worked up in the usual way. The product obtained was shown to be one compound by thin-layer chromatography and was crystallised from ether to give *5β-cholestane-5,7β-diol* as fine needles, m. p. 129—131°, $[\alpha]_D$ +63° (*c* 0·98) (Found: C, 79·8; H, 11·95. $C_{27}H_{48}O_2$ requires C, 80·15; H, 11·95%).

5-Hydroxy-5β-cholestan-7β-yl Acetate (XIV; R = Ac).—A solution of the foregoing diol (75 mg.) in pyridine (2·5 ml.) and acidic anhydride (2·5 ml.) was left at room temperature for 48 hr. and then diluted with water. The product was extracted with ether and worked up in the usual way to give the *acetate* (36 mg.) as needles after crystallisation from acetone–methanol, m. p. 118—120°, $[\alpha]_D$ +70° (*c* 1·05) (Found: C, 77·95; H, 11·4. $C_{29}H_{50}O_3$ requires C, 77·95; H, 11·3%).

5-Hydroxy-4-oxo-5β-cholestan-7β-yl Acetate (X).—Chromium trioxide (1·6 g.) in pyridine (15 ml.) was added to a solution of the mixture (1·6 g.) of the acetates (XI) and (XII; R = Ac) in pyridine (15 ml.). After 24 hr. benzene was added and the precipitate removed by filtration and washed thoroughly with benzene–ether. The washings and filtrate were combined and worked up in the usual way. The oil obtained was chromatographed on alumina (50 g.). Benzene eluted an oil (101 mg.) which could not be crystallised and was shown by thin-layer chromatography to be a mixture of several compounds. Benzene–ether (4 : 1; 4 × 150 ml.) eluted the keto-ester (IX) (355 mg.), m. p. and mixed m. p. 90—91°/164—166°, $[\alpha]_D$ +73° (*c* 1·1). Further elution with benzene–ether (4 : 1; 6 × 150 ml.) gave a mixture of the two ketones which yielded pure *5-hydroxy-4-oxo-5β-cholestan-7β-yl acetate* (X) (751 mg.) on crystallisation from methanol, m. p. 190—192°, $[\alpha]_D$ +53° (*c* 0·85), $\nu_{max.}$ 1724, 1709 (C=O), 1255 cm.$^{-1}$ (acetate), $\lambda_{max.}$ 305 mμ ($\varepsilon_{max.}$ 39·5). Optical rotatory dispersion: trough, −405 (323 mμ); peak, +1810 (282 mμ), amplitude −22 (Found: C, 75·9; H, 10·7. $C_{29}H_{48}O_4$ requires C, 75·6; H, 10·5%).

Reduction of the Ketone (IX).—Dry hydrogen chloride gas was passed through a solution of the ketone (213 mg.) in chloroform (10 ml.) for 2 hr. The chloroform was evaporated off under reduced pressure and the residue was dissolved in acetic acid (15 ml.). The acetic acid solution was heated to reflux and zinc dust was added portionwise over a period of 1·5 hr. The zinc dust was removed by filtration and washed thoroughly with chloroform; the washings and filtrate were combined and the solvent was evaporated off under reduced pressure. The solid remaining was dissolved in ether and dilute sulphuric acid and the ether solution worked up in the usual way to give 4-oxo-5α-cholestan-7β-yl acetate as long needles after crystallisation from acetone–methanol, m. p. and mixed m. p. 101—103°/110—112°, $[\alpha]_D$ +63° (*c* 1·31) (lit.,[12] m. p. 101—103°, $[\alpha]_D$ +75°; m. p. 102—103°, $[\alpha]_D$ +75°).

Reduction of the Ketone (X).—Dry hydrogen chloride gas was passed through a solution of the ketone (100 mg.) in chloroform (5 ml.) for 3 hr. The chloroform was distilled off under reduced pressure and the residue was heated under reflux with acetic acid (5 ml.) and zinc dust (2 g.) for 1·5 hr. The mixture was worked up in the usual way and the oil obtained (95 mg.) was crystallised from acetone–methanol to give 4-oxo-5α-cholestan-7β-yl acetate, m. p. and mixed m. p. 100—101°/110—112°, $[\alpha]_D$ +66° (*c* 1·25).

Oxidation of 5α-Cholestane-4β,5,7β-triol (VII; R = H) *with* N-*Bromosuccinimide*.—*N*-Bromosuccinimide (628 mg.) in methanol–water (1 : 1, 8 ml.) was added to a solution of the triol (450 mg.) in ether (50 ml.) and the mixture shaken until a deep red colour developed (2 hr.). Ether was then added and the solution was washed with water, aqueous sodium sulphite solution (10%), water, saturated aqueous sodium hydrogen carbonate solution, and water, and dried over anhydrous magnesium sulphate. The ether was evaporated off under reduced pressure and the white solid obtained was acetylated with acetic anhydride in pyridine to give a colourless oil (420 mg.) which was chromatographed on alumina (12 g.). Benzene (2 × 40 ml.) eluted an

[12] G. H. R. Summers, *Proc. Chem. Soc.*, 1960, 24; Q. R. Petersen, *J. Amer. Chem. Soc.*, 1960, **82**, 3677.

unidentifiable oil (98 mg.) which was a mixture of several compounds. Further elution with benzene (22 × 40 ml.) gave an oil (320 mg.) which was crystallised from methanol to yield the ketone (IX) as needles, double m. p. and mixed m. p. 90—91°/164—166°, $[\alpha]_D$ +78·5° (*c* 1·02).

Dehydration of the Diacetate (VI; R = Ac).—Thionyl chloride (1 ml.) was added to a solution of the diacetate (280 mg.) in pyridine (5 ml.) at 0°. The mixture was left at 0° for 30 min., at room temperature for a further 30 min. and then poured into water. The product was extracted with ether and the ether solution was worked up in the usual way to give *cholest-5-ene-4β,7β-diyl diacetate* (XIX; R = Ac) (180 mg.) as fine needles after crystallisation from acetone–methanol, m. p. 160—162°, $[\alpha]_D$ +53° (*c* 0·90) (Found: C, 76·55; H, 10·25. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·35%).

Cholest-5-ene-4β,7β-diol.—A mixture of the foregoing diacetate (120 mg.) and lithium aluminium hydride (120 mg.) in anhydrous ether (20 ml.) was heated under reflux for 1 hr. Acetone was added to destroy the excess of reagent and the reaction was worked up in the usual way to give a white solid. Crystallisation from ethyl acetate gave the *diol* (XIX; R = H) (82 mg.) as needles, m. p. 172—174°, $[\alpha]_D$ +5° (*c* 1·21) (Found: C, 80·6; H, 11·45; $C_{27}H_{46}O_2$ requires C, 80·55; H, 11·5%).

Dehydration of the Diacetate (III; R = Ac).—Thionyl chloride (1·75 ml.) was added to a solution of the diacetate (190 mg.) in pyridine (10 ml.) at 0°. The mixture was left at 0° for 30 min. and at room temperature for a further 30 min., and then poured into water. The mixture was worked up in the usual way to give an oil (160 mg.) which could not be crystallised, but from thin-layer chromatography it seemed to be one compound of the same R_F as cholest-5-ene-4β,7β-diyl diacetate. The oil was dissolved in methanol (20 ml.) containing potassium hydroxide (1 g.) and the solution was heated under reflux for 1·5 hr., poured into water, and extracted with ether. The ether solution was worked up in the usual way to give an oil (138 mg.) which was chromatographed on alumina (5 g.). Pentane–ether (1 : 1; 10 × 15 ml.) eluted 7β-hydroxy-5α-cholestan-4-one (85 mg.) which was crystallised from methanol, m. p. and mixed m. p. 153—153·5°, $[\alpha]_D$ +64° (*c* 0·72) (lit.,[12] m. p. 151—153°, $[\alpha]_D$ +62°; m. p. 152—153°, $[\alpha]_D$ +50°). Ether (2 × 15 ml.) eluted cholest-5-ene-4β,7β-diol (26 mg.) which was crystallised from ethyl acetate, m. p. and mixed m. p. 172—174° $[\alpha]_D$ +7° (*c* 1·03).

Dehydration of the Diacetate (VII; R = Ac).—Thionyl chloride (1 ml.) was added to a solution of the diacetate (111 mg.) in pyridine (5 ml.) at 0°. The reaction mixture was left at 0° for 1 hr. and at room temperature for a further 1 hr. The reaction was worked up in the usual way and the oil obtained crystallised from acetone–methanol to give *cholest-5-ene-4α,7β-diyl diacetate* (XV; R = Ac), m. p. 144—146°, $[\alpha]_D$ +70° (*c* 0·95) (Found: C, 77·0; H, 10·25. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·35%).

Hydrolysis of the Diacetate (XX; R = Ac).—A mixture of the diacetate (130 mg.) and potassium hydroxide (50 mg.) in methanol (10 ml.) was heated under reflux for 2 hr. and then worked up in the usual way to give *cholest-5-ene-4α,7β-diol* (XX; R = H) (64 mg.) as needles after crystallisation from methanol–ether, m. p. 148—151°, $[\alpha]_D$ +58° (*c* 0·90). The compound could only be crystallised in the presence of methanol, and the analytical results would only agree with the predicted value by assuming 1 mole of methanol of crystallisation (Found: C, 77·55; H, 11·6. $C_{27}H_{46}O_2,CH_3OH$ requires C, 77·35; H, 11·6%).

Dehydration of the 4,7-*Diacetate* (II; R = Ac).—Thionyl chloride (1 ml.) was added to a solution of the diacetate (423 mg.) in pyridine (5 ml.) at 0° and the mixture left at 0° for 1 hr. and at room temperature for a further 1 hr. The mixture was worked up in the usual way to give cholest-5-ene-4α,7β-diyl diacetate (220 mg.) as needles after crystallisation from methanol, m. p. and mixed m. p. 114—146°, $[\alpha]_D$ +72° (*c* 0·92).

The diacetate was hydrolysed in refluxing methanolic potassium hydroxide to give cholest-5-ene-4α,7β-diol (XX; R = H), m. p. and mixed m. p. 148—151°, $[\alpha]_D$ +60 (*c* 1·01).

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