1α-Hydroxycholesterol

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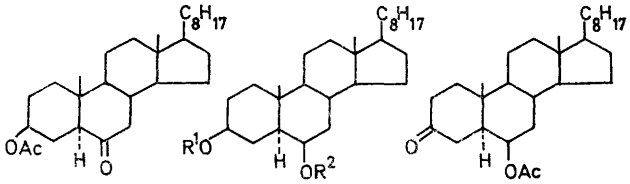
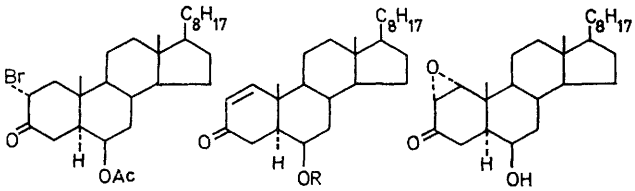
1α-Hydroxycholesterol has been synthesised *via* 6β-hydroxycholest-1-en-3-one

For the preparation of 1α-hydroxyvitamin D_3, a model compound of a possible metabolite of 25-hydroxycholecalciferol,[1] 1α-hydroxycholesterol could be an important intermediate. We have prepared this compound from 3β-acetoxy-5α-cholestan-6-one (I). Reduction with lithium tri-t-butoxyaluminium hydride [2] or sodium borohydride afforded the 6β-hydroxy-derivative (II) [3,4] in high yield. The hydroxy-derivative was acetylated with acetic anhydride under reflux to give the 3β,6β-diacetoxy-derivative (III), identical with material prepared by Shoppee and Summers [5] by another route. The method of Shoppee and Summers [5] for preparation of 6β-acetoxy-5α-cholestan-3β-ol (IV) from the diacetate was not satisfactory on a larger scale. A modification of the alkaline hydrolysis [potassium hydroxide (1·1 mol) in ether–methanol at 0—5° overnight] afforded compound (IV) in high yield. Oxidation of (IV) to the 3-ketone (V) with sodium dichromate in acetic acid at 50° improved the purity and the results.[5]



(I) (II) R^1 = Ac, R^2 = H (III) R^1 = R^2 = Ac (IV) R^1 = H, R^2 = Ac (V)

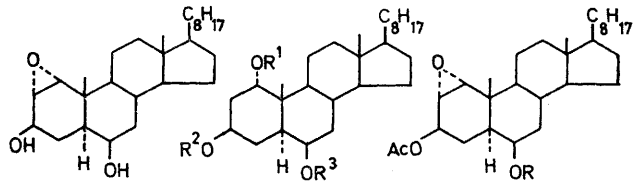
Bromination of the ketone (V) with bromine in acetic acid afforded the 2α-bromo-3-ketone (VI), which was converted into the unsaturated compound (VII) by treatment with calcium carbonate in dimethylformamide.[6] The u.v. spectrum ($\lambda_{max.}$ 230 nm.) confirmed the Δ^1-3-ketone structure of the compound. After



(VI) (VII) R = Ac (VIII) R = H (IX)

alkaline hydrolysis the 6β-hydroxy-derivative (VIII) was isolated; epoxidation with hydrogen peroxide in alkaline medium [7] then afforded the 1α,2α-epoxy-derivative (IX). Reduction of this compound with lithium aluminium hydride [8] led to the 1α,3β,6β-triol (XI).

Attempts to prepare the pure 1α,3β-diacetoxy-6β-hydroxy-compound (XII), which could be a useful intermediate for the preparation of a Δ^5-derivative (XX) by dehydration with phosphoryl chloride [9] were not successful. Theoretically the formation of this diacetate is favoured, since the steric hindrance (by the 10-methyl group) favours acetylation in positions 1α and 3β.[10] Acetylation of the triol with acetic anhydride in pyridine afforded a mixture of monoacetates, diacetates, and a triacetate. Chromatographic purification did not lead to any crystalline material. An approach from the non-crystalline triacetate (XIII), by alkaline saponification, afforded two crystalline substances, a diacetate (XV) and a monoacetate (XIV). Their structures can be deduced from the relative rates of saponification of the equatorial (3β) and axial (1α,6β) acetates (equatorial acetates are known [10] to hydrolyse more quickly). With reference to the two axial acetates, the 1α-position is less sterically hindered (three 1,3-interactions with hydrogen atoms in position 3α,5α, and 9α) than the 6β-position (two 1,3-interactions with hydrogen atoms in position 4β and 8β and one interaction with the 10-methyl group). The isolated monoacetate is therefore probably a 6β-acetoxy-derivative (XIV), and the diacetate the 1α,6β-diacetoxy-derivative (XV). I.r. spectra of both compounds show acetoxy- (1725, 1270, and 1040 $cm.^{-1}$) and hydroxy-absorptions (3520—3550 $cm.^{-1}$).

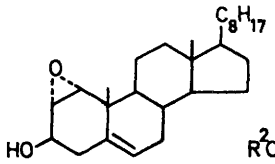


(X) (XI) R^1 = R^2 = R^3 = H (XII) R^1 = R^2 = Ac, R^3 = H (XIII) R^1 = R^2 = R^3 = Ac (XIV) R^1 = R^2 = H, R^3 = Ac (XV) R^1 = R^3 = Ac, R^2 = H (XVI) R = H (XVII) R = Mes

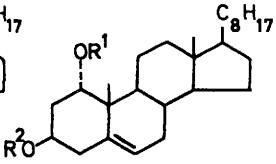
When the route to 1α-hydroxycholesterol (XIX) *via* the triol (XI) failed, attention was turned to the conversion of the epoxy-derivative (X) into (XIX). The crude

[1] D. E. M. Lawson, P. W. Wilson, E. Kodicek, *Nature*, 1969, **222**, 171.
[2] H. C. Brown and C. J. Shoaf, *J. Amer. Chem. Soc.*, 1964, **86**, 1079.
[3] Pl. A. Plattner, Th. Petrzilka, and W. Lang, *Helv. Chim. Acta*, 1944, **27**, 513.
[4] Pl. A. Plattner and W. Lang, *Helv. Chim. Acta*, 1944, **27**, 1872.
[5] C. W. Shoppee and G. H. R. Summers, *J. Chem. Soc.*, 1952, 3361.
[6] J. A. Zderic, H. Carpio, A. Bowers, and C. Djerassi, *Steroids*, 1963, **1**, 233.
[7] W. M. Hoehn, *J. Org. Chem.*, 1958, **23**, 929.
[8] F. Salmann and C. Tamm, *Helv. Chim. Acta*, 1956, **39**, 1340.
[9] H. Reich and A. Lardon, *Helv. Chim. Acta*, 1946, **29**, 671.
[10] L. F. Fieser and M. Fieser, ' Steroids,' Reinhold, New York, 1959, p. 216.

diol (X) was acetylated with acetic anhydride in pyridine for 4 hr.,[9] and the 3β-acetoxy-derivative (XVI) was isolated by chromatography. Methylsulphonylation in pyridine gave the mesyl derivative (XVII), which failed



(XVIII)



(XIX) $R^1 = R^2 = H$
(XX) $R^1 = R^2 = Ac$

to crystallise. Hydrolysis of the 3-acetoxy-group and a diaxial elimination of methanesulphonic acid (positions 5 and 6) by hydrochloric acid in methanol afforded the non-crystalline unsaturated compound (XVIII) [$\nu_{max.}$ 3500 cm.$^{-1}$ (OH)], which on reduction with lithium aluminium hydride [8] gave 1α-hydroxycholesterol (XIX).

EXPERIMENTAL

M.p.s were determined with a Gallenkamp apparatus. I.r. and u.v. spectra were taken with Unicam SP 200 and SP 800 spectrometers. Optical rotations were measured for solutions in chloroform (*c* 0·5—2%) unless otherwise stated, at 20°.

3β-*Acetoxy*-5α-*cholestan*-6β-*ol* (II).—(*a*) The oxo-acetate (I) [11] (1 g.) in dry ether (20 ml.) was added dropwise to a solution of lithium tri-t-butoxyaluminium hydride (2 g.) in ether (100 ml.) and the mixture was stirred and refluxed for 5 hr. The cooled mixture was poured on ice and acetic acid (3 ml.) was added. The product was extracted with benzene and after the usual work-up was crystallised from ether–light petroleum (b.p. 60—80°), m.p. 155—157°. The mother liquor was evaporated and the residue was dissolved in benzene and filtered through a short column of alumina (activity II). After evaporation of the solvent a second crop of compound (II) was obtained, m.p. 153—155° (lit.,[3,4] 143—144°).

(*b*) The 6-oxo derivative (I) (6·8 g.) in ether (50 ml.) and methanol (50 ml.) was stirred and sodium borohydride (700 mg.) was added without external cooling. After 3 hr. acetic acid (1 ml.) was added and the mixture was diluted with water. The ether layer was worked up, the ether was evaporated almost to dryness, and light petroleum (b.p. 60—80°; 50 ml.) was added. The collected crystals of (II) had m.p. 153—157° (6·1 g.).

6β-*Acetoxy*-5α-*cholestan*-3β-*ol* (IV).—The diol acetate (II) (5 g.) in acetic anhydride (10 ml.) was refluxed for 90 min. The solution was poured on ice and the product was extracted with ether. The ether solution was washed with cold 1% sodium hydroxide and with water. The dried solution was cooled to 0—5° and a chilled solution of potassium hydroxide (700 mg.) in methanol (20 ml.) was added. The mixture was kept overnight in a refrigerator, then acetic acid (0·5 ml.) was added and the solution was concentrated. Crystallisation gave the 3β-hydroxy-derivative (IV), m.p. 123—125° (3·99 g.) (lit.,[5] 70—128°; lit.,[4] 124—125°).

6β-*Acetoxy*-5α-*cholestan*-3-*one* (V).—A solution of the 3β-hydroxy-derivative (IV) (34·8 g.) in acetic acid (350 ml.) was heated to 40° and sodium dichromate dihydrate (13·5 g.) was added with stirring. The temperature rose to 53° and stirring was continued for a total of 30 min. A solution of sodium sulphite (5 g.) was added, most of the acetic acid was evaporated off *in vacuo*, and water was added. The product was filtered off with addition of Celite 545, and the dried product was extracted with ether. Crystallisation from methanol afforded the 3-ketone (V), m.p. 94—97° in two crops (27 g.) (lit.,[5] m.p. 85—97°).

6β-*Acetoxy*-2α-*bromo*-5α-*cholestan*-3-*one* (VI).—A solution of the ketone (V) (30 g.) in acetic acid (600 ml.) was treated with hydrogen bromide in acetic acid (30%; 1 ml.) and brominated with bromine (11 g.) in acetic acid (100 ml.) during 10 min. Methanol (50 ml.) was added and the solution was kept in a refrigerator overnight. The product was filtered off and washed with methanol and with ether. The *bromo-derivative* (VI) (27·2 g.) yielded crystals, m.p. 196—198° (from dichloromethane–acetone), $[\alpha]_D$ +16° (Found: C, 67·0; H, 9·1; Br, 15·0. $C_{29}H_{45}BrO_3$ requires C, 66·8; H, 8·7; Br, 15·3%).

6β-*Acetoxy*-5α-*cholest*-1-*en*-3-*one* (VII).—A solution of the bromo-derivative (VI) (27 g.) in dimethylformamide (200 ml.) was refluxed with calcium carbonate (25 g.) for 1 hr. The unchanged carbonate was filtered off and washed with two portions (100 ml.) of benzene and water. The benzene layer was worked up and after evaporation gave material (15·7 g.), m.p. 95—98° (from methanol). Recrystallisation gave the *product*, m.p. 102—103°, $[\alpha]_D$ +2°, $\lambda_{max.}$ (MeOH) 230 nm. (ε 8300) (Found: C, 78·6; H, 10·3. $C_{29}H_{46}O_3$ requires C, 78·7; H, 10·5%).

6β-*Hydroxy*-5α-*cholest*-1-*en*-3-*one* (VIII).—A solution of the acetoxy-derivative (VII) (3 g.) in methanol (30 ml.) and benzene (15 ml.) was refluxed with sodium hydroxide (1 g.) in water (5 ml.) for 1 hr. After neutralisation with acetic acid (0·5 ml.) the solution was kept in a refrigerator overnight and the 6β-*hydroxy-derivative* (2·2 g.) (VIII) was collected by suction, m.p. 184—185° (from methanol), $[\alpha]_D$ +3°, $\lambda_{max.}$ (MeOH) 230 nm. (ε 8400) (Found: C, 81·0; H, 11·2. $C_{27}H_{44}O_2$ requires C, 81·0; H, 11·1%).

1α,2α-*Epoxy*-6β-*hydroxy*-5α-*cholestan*-3-*one* (IX).—The ketone (VIII) (11·4 g.) in chloroform (50 ml.) and methanol (100 ml.) was treated with sodium hydroxide (500 mg.) in water and stirred at 15—20° during the addition of hydrogen peroxide (30%; 11 ml.). After a further 2 hr. stirring the solution was kept in a refrigerator overnight. Water was added and the chloroform layer was worked up to yield the *epoxy-ketone* (IX) (8·9 g.), m.p. 176—178° (from acetone–methanol), $[\alpha]_D$ +8°, no u.v. absorption (Found: C, 78·1; H, 10·8. $C_{27}H_{44}O_3$ requires C, 77·8; H, 10·7%).

5α-*Cholestane*-1α,3β,6β-*triol* (XI).—The ketone (IX) (140 mg.) in ether (20 ml.) was added dropwise to lithium aluminium hydride (50 mg.) in ether (10 ml.) and the mixture was refluxed for 3 hr. The usual work-up gave the *triol* (XI), m.p. 206—208° (from ethyl acetate–acetone), $[\alpha]_D$ +2° (in MeOH) (Found: C, 76·8; H, 11·1. $C_{27}H_{48}O_3$ requires C, 77·1; H, 11·5%).

6β-*Acetoxy*-5α-*cholestane*-1α,3β-*diol* (XIV) *and* 1α,6β-*Diacetoxy*-5α-*cholestan*-3-*ol* (XV).—The triol (XI) (2·4 g.) was refluxed in acetic anhydride (8 ml.) for 2 hr. Water was added and the triacetate (XIII) was isolated by extraction with ether. It failed to crystallise and its i.r. spectrum showed no OH absorption. To the solution of triacetate in ether (30 ml.) a solution of potassium hydroxide (690 mg.) in methanol (20 ml.) was added, and the mixture was kept overnight at room temperature. Water was added and after the usual work-up the product (2·5 g.) was chromatographed on alumina (activity II; 70 g.). Fractions

[11] J. Mauthner and W. Suida, *Monatsh.*, 1894, **15**, 85; 1903, **24**, 648.

eluted with ethyl acetate gave the *diacetate* (XV) (330 mg.), m.p. 186—188° (from ether), $[\alpha]_D$ +10° (Found: C, 74·1; H, 10·4. $C_{31}H_{52}O_5$ requires C, 73·8; H, 10·4%). Elution with ethyl acetate–acetone (1 : 1) afforded the *monoacetate* (XIV) (740 mg.), m.p. 175—176° (from ether), $[\alpha]_D$ +2° (Found: C, 75·0; H, 10·8. $C_{29}H_{50}O_4$ requires C, 75·3; H, 10·9%).

3β-*Acetoxy*-1α,2α-*epoxy*-5α-*cholestan*-6β-*ol* (XVI).—The ketone (IX) (13 g.) in methanol (250 ml.) and ether (100 ml.) was reduced with sodium borohydride (1·3 g.) overnight. Acetic acid (1·3 ml.) was added, followed by water, and the ether layer was worked up. The dried product was dissolved in pyridine (10 ml.) and dry benzene (100 ml.). Acetic anhydride (13 ml.) was added and the mixture was stirred for 4 hr. at room temperature. Water was added and the benzene layer was worked up. The material was purified on an alumina column (260 g.). Elution with light petroleum–benzene (1 : 1) afforded a mixture of the 3,6-diacetate and the 3-monoacetate; further elution with the same mixture and with ether gave almost pure 3β-*acetoxy-derivative* (XVI), m.p. 145—148° (from methanol) (6·9 g.); further crystallisation from acetone gave material, m.p. 149—151°, $[\alpha]_D$ 0 ± 1° (Found: C, 75·9; H, 10·7. $C_{29}H_{48}O_4$ requires C, 75·6; H, 10·5%). Elution with ethyl acetate and methanol gave unchanged starting material (IX).

1α,2α-*Epoxycholest*-5-*en*-3β-*ol* (XVIII).—The acetoxy-derivative (XVI) (2 g.) was dissolved in pyridine (5 ml.), methanesulphonyl chloride (1 ml.) was added dropwise, and the mixture was set aside overnight. Water and ether were added, and after work-up the product was dissolved in ether (10 ml.) and methanol (30 ml.). Conc. hydrochloric acid (5 ml.) was added and the mixture was refluxed for 1 hr. Water and ether were added and the ether layer was worked up. The ether was evaporated off, a solution of potassium hydroxide (500 mg.) in methanol (30 ml.) was added, the mixture was refluxed for 1 hr., water was added, and the ether layer was worked up. After evaporation of the solvent, the product failed to crystallise, though it showed only one spot on t.l.c. (silica gel).

Cholest-5-*ene*-1α,3β-*diol* (XIX).—The epoxy-derivative (XVIII) (1·3 g.) in tetrahydrofuran (40 ml.) was added to lithium aluminium hydride (200 mg.) in tetrahydrofuran (20 ml.). The mixture was refluxed for 2 hr., ethyl acetate and water were added, and the ethyl acetate layer was worked up. The product (1·6 g.) was chromatographed on alumina (activity II; 50 g.). The *diol* (XIX) was eluted with acetone–methanol (1 : 1), m.p. 185—192° (from acetone) (1 g.); further recrystallisation gave material, m.p. 195—200°, $[\alpha]_D$ 0 ± 1° (in MeOH) (Found: C, 76·2; H, 11·1. $C_{27}H_{46}O_2,0{\cdot}5H_2O$ requires C, 75·9; H, 11·1%).

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