Synthesis of [26,27-$^2H_6$]Cholesterol and Derivatives substituted in the Side chain

David N. Kirk * and Michael J. Varley
Medical Research Council Steroid Reference Collection, Chemistry Department, Westfield College, Hampstead, London NW3 7ST
Hugh L. J. Makin and David J. H. Trafford
Department of Chemical Pathology, The London Hospital Medical College, Whitechapel, London E1 2AD

The carbanion derived from 24-phenylsulphonylchol-5-en-3β-ol 3-tetrahydropyranyl ether (6) reacted with [$^2H_6$]acetone to give the 24-phenylsulphonyl-25-hydroxy[26,27-$^2H_6$]cholesterol derivative (7a), which was reduced by sodium amalgam to a separable mixture of the labelled 25-hydroxycholesterol and cholest-5,24-dien-3β-ol (desmosterol) derivatives. [26,27-$^2H_6$]Cholesterol has been obtained *via* a selective reduction of the Δ^{24}-unsaturation in [26,27-$^2H_6$]desmosteryl benzoate with di-imide, or more efficiently by reducing 25-hydroxy[26,27-$^2H_6$]cholesteryl 3,25-diacetate with lithium in ethylamine, without significant loss of label. The labelled 24,25-dihydroxycholesterols were also prepared from [26,27-$^2H_6$]desmosteryl benzoate.

Deuterium labelled steroids are now used extensively as standards in the assay and analysis of steroids by mass spectrometry.[1] In order to develop an isotope-dilution assay for vitamin D_3 and its hydroxylated metabolites (1), we required a general synthesis of cholesterol derivatives bearing at least three non-exchangeable deuterium atoms, at high isotopic purity. The most accessible site(s) for incorporation of several deuterium atoms appeared to be the two terminal methyl groups (C-26 and C-27) of the cholesterol side chain. Cholesta-5,24-dien-3β-ol (desmosterol) (2a) was selected as the key intermediate in this work since it may be converted by standard methods into derivatives variously hydroxylated at C-24, -25, and -26.

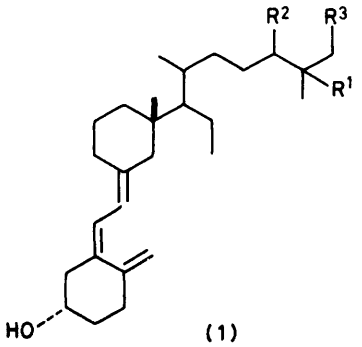
[26,27-$^2H_6$]*Desmosterol and* 25-*Hydroxy*[26,27-$^2H_6$]*cholesterol*.—Initial attempts to prepare desmosterol labelled with deuterium in one or both of the terminal methyl groups were not encouraging. Many of the reported syntheses [2-6] of desmosterol involve dehydration of 25-hydroxycholesteryl acetate (3a). In our hands this reaction always resulted in a mixture of the 5,24-diene (2c) and the Δ^{25}-isomer (4), separable only by preparative t.l.c. on silver nitrate-impregnated silica. Some methods [5,6] of dehydration of the 25-alcohol (3a), reported to give mainly the diene (2c), relied upon the acid-catalysed isomerisation of the larger part of any Δ^{25}-isomer (4) formed, to convert it into the more stable Δ^{24}-compound. This isomerisation would clearly lead to loss of label in the preparation of deuteriated desmosterol from 25-hydroxy[26(27)-$^2H_3$]cholesterol.[7]

Desmosterol derivatives have been prepared [8] by the Wittig reaction, from reaction of ylides generated from steroidal phosphonium salts (5) with trifluoro- and hexafluoro-acetone. Attempts to use this method to prepare desmosterol, with acetone (or hexadeuterioacetone) as the ketonic component, met with only limited success. A 26% yield of desmosterol was obtained from the phosphonium salt (5; R = H, X = Br) and acetone. Protection of the 3β-hydroxy group did not improve yields. Other known routes [9,10] to desmosterol were not conveniently adaptable to allow incorporation of deuterium. However, a modification of the Julia [11] olefin synthesis, as used by Kobayashi [12a] to prepare 26,26,26-trifluorodesmosterol proved more successful.

Treatment of the sulphone [12b] (6) with lithium di-isopropylamide (3 equiv.) at 0 °C, followed by addition of hexadeuterioacetone (6 equiv.) gave the hydroxysulphone (7a) in 81% yield. Reductive desulphonation of the crude hydroxy sulphone with a 30-fold excess of 6% sodium amalgam in methanol–tetrahydrofuran (1 : 1) at 20 °C led to a mixture of the deuteriated 5,24-diene (2e) and the 25-hydroxycholesterol derivative (3b), in 48—52 and 28% yields [overall from sulphone (6)], respectively after preparative h.p.l.c. When a lower excess (10-fold) of sodium amalgam was used, the yield of diene (2e) was reduced (42%); the yield of alcohol (3b) was almost unchanged (25%). Both the diene (2e) and the alcohol (3b) contained little (if any) labelled species other than the hexadeuteriated compounds (mass spectrometry). Mild acid hydrolysis of the tetrahydropyranyl (THP) ether (3b) gave 25-hydroxy[26,27-$^2H_6$]cholesterol (3d) in 85% yield.

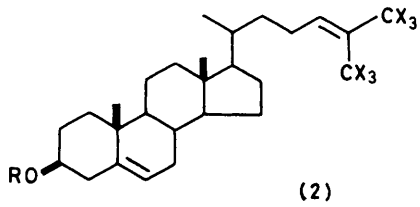
Attempts were made to improve the yield of 5,24-diene (2e) by derivatising the 25-hydroxy group of the hydroxy sulphone (7a). Trimethylsilylimidazole reacted with the non-deuteriated hydroxysulphone (7b) to give the trimethylsilyl derivative (7c) which, without purification, was treated with sodium amalgam to give the diene (2d) and the alcohol (3c) in 55 and 31% yields respectively. The slight improvement hardly justifies the additional step. The diacetate (7d) [prepared by acid-catalysed acetylation [13] of (7b)] did not give improved yields of desmosterol when treated with sodium amalgam.

[26,27-$^2H_6$]*Cholesterol*.—Some difficulties were encountered in converting the deuteriated desmosterol derivatives into cholesterol without loss of label. The tetrahydropyranyl group was removed from the diene (2e) under mild acidic conditions and the resultant desmosterol (2b) was benzoylated. Selective reduction of the 24,25-double bond of the benzoate (2f) was achieved by slow catalytic hydrogenation over palladium on calcium carbonate in ethyl acetate. However, the mass spectrum of the product revealed extensive scrambling and loss of deuterium. Slightly less scrambling occurred when Adams platinum oxide was used as catalyst (1.5 h; ethyl acetate) but the reduced product contained substantial amounts of 5α-cholestan-3β-ol benzoate. Attempted homogeneous hydrogenation with tris(triphenylphosphine)rhodium chloride [14] led only to recovery of starting material. Satisfactory selective reduction of [26,27-$^2H_6$]desmosteryl benzoate (2f) was ultimately achieved with di-imide, generated [15] *in situ* by thermolysis of toluene-*p*-sulphonohydrazide (8 equiv.) in boiling diglyme, to give, after preparative h.p.l.c., a moderate yield (46%) of deuteriated cholesteryl benzoate (8a) with only slight loss (2%) of the deuterium label. Alkaline hydrolysis of the benzoate (8a) completed the synthesis of [26,27-$^2H_6$]cholesterol (8b).



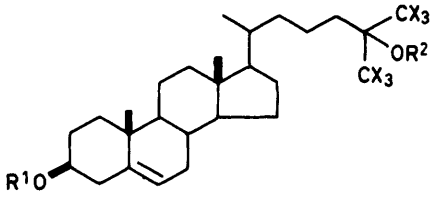
(1)

R^1 = OH, R^2 = R^3 = H
R^1 = R^2 = OH, R^3 = H
R^1 = R^3 = OH, R^2 = H



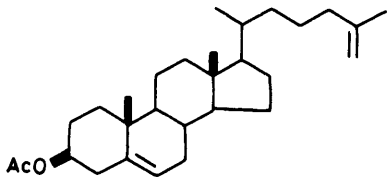
(2)

a; R = H, X = H
b; R = H, X = D
c; R = Ac, X = H
d; R = THP, X = H
e; R = THP, X = D
f; R = PhCO, X = D

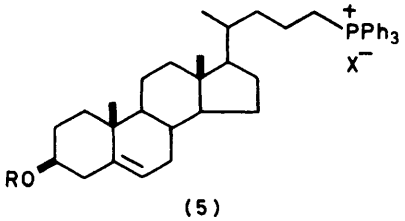


(3)

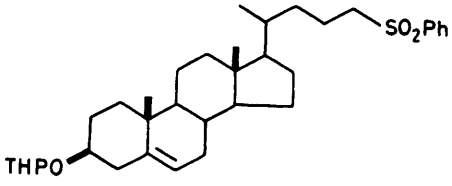
a; R^1 = Ac, R^2 = X = H
b; R^1 = THP, R^2 = H, X = D
c; R^1 = THP, R^2 = X = H
d; R^1 = R^2 = H, X = D
e; R^1 = R^2 = Ac, X = D



(4)



(5)



(6)

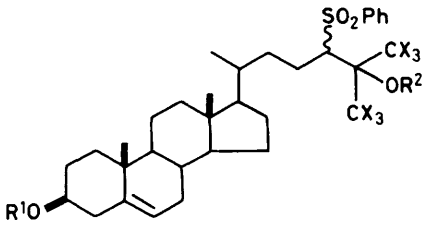
An alternative route to deuteriated cholesterol was developed in order to circumvent the difficulties encountered in the hydrogenation of the 24,25-double bond of the diene (2f). Treatment of [26,27-$^2H_6$]cholest-5-ene-3β,25-diol 3-tetrahydropyranyl ether (3b) with acetic anhydride containing toluene-*p*-sulphonic acid [13] gave the 3,25-diacetate (3e) in 80% yield. This diacetate reacted [16] with lithium in ethylamine to give [26,27-$^2H_6$]cholesterol (8b) in 72% yield without loss of deuterium. 25-Hydroxy[26(27)-$^2H_3$]cholesterol [7] was also converted by this method into [26(27)-$^2H_3$]cholesterol.

(24R,S)-24,25-*Dihydroxy*-[26,27-$^2H_6$]*cholesterol*.—[26,27-$^2H_6$]Desmosteryl benzoate (2f) was treated [17] with *m*-chloroperbenzoic acid at 0 °C to give selectively the (24*R*,*S*)-24,25-epoxide (9), in 64% yield after preparative h.p.l.c. Further chromatography of the epoxide (9) on the more efficient semi-preparative h.p.l.c. columns (see Experimental section) achieved separation of the (24*R*)- and (24*S*)-isomers (10) and (11). Opening of the epoxide (9) with perchloric acid in aqueous tetrahydrofuran [18] at 20 °C, followed by alkaline hydrolysis of the benzoate group gave (24*R*,*S*)-24,25-dihydroxy-[26,27-$^2H_6$]cholesterol (12) in 88% yield.

The [26,27-$^2H_6$]cholesterol (8b) and the 25-hydroxy- and (24*R*,*S*)-24,25-dihydroxy derivatives, (3d) and (12) respectively, were converted by standard methods [19] into the corresponding labelled vitamin D_3 (cholecalciferol) derivatives (1).

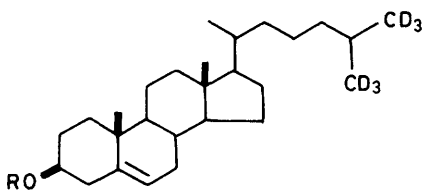
Experimental

Melting points were determined on a Reichert hot-stage apparatus. I.r. spectra refer to KBr discs. N.m.r. spectra were recorded at 100 MHz for solutions in deuteriochloroform with tetramethylsilane as internal standard. Deuterium contents were measured by mass spectrometry on an LKB 2091 G-C.-M-S. instrument, accelerating voltage 20 eV, 100—150 °C, 50 mA filament current, with direct insertion of the sample. Preparative h.p.l.c. was carried out with a Waters Associates Prep LC/System 500 equipped with Preppak-500/Silica cart-



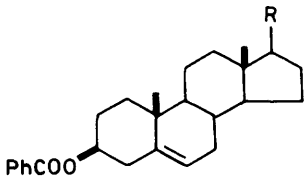
(7)

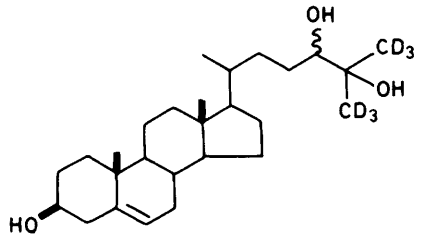
a; R^1 = THP, R^2 = H, X = D
b; R^1 = THP, R^2 , X = H
c; R^1 = THP, R^2 = Me_3Si, X = H
d; R^1 ,R^2 = Ac, X = H



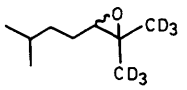
(8)

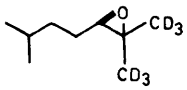
a; R = PhCO
b; R = H

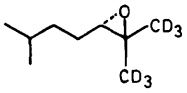




(12)

(9) R = 

(10) R = 

(11) R = 

ridges. Semi-preparative h.p.l.c. was carried out on columns (25 cm, 10 mm i.d.) packed with Nucleosil-50 (5 μm, normal phase) and Spherisorb ODS (5 μm, reverse phase). All solvents were distilled before use. Light petroleum refers to the fraction, b.p. 60—80 °C. Tetrahydrofuran was dried by distillation from lithium aluminium hydride. Solutions of organic products were dried over anhydrous sodium sulphate and evaporated under reduced pressure below 40 °C.

(24R,S)-24-*Phenylsulphonyl*[26,27-$^2H_6$]*cholest*-5-*ene*-3β,25-*diol* 3-*Tetrahydropyran*-2-*yl Ether* (7a).—A solution of methyllithium in ether (1.2M; 18.8 ml, 24 mmol MeLi) was added to a stirred solution of di-isopropylamine (3.36 ml, 24 mmol, distilled from CaH_2) in dry tetrahydrofuran (40 ml) at 0 °C under dry nitrogen. After 10 min at 0 °C a solution of the sulphone [12b] (6) (4.54 g, 8 mmol) in dry tetrahydrofuran (80 ml) was added during 30 min. The yellow solution was stirred at 0 °C for 15 min and then treated rapidly with a solution of [$^2H_6$]acetone (3.89 ml, 48 mmol; 99.8% $^2H_6$) in dry tetrahydrofuran (8 ml). After a further 30 min at 0 °C the solution was treated with ether (100 ml) and water (20 ml) and then diluted further with ether (300 ml). The aqueous phase was separated and re-extracted with ether. The combined organic phases were washed with water, dried, and evaporated. Preparative h.p.l.c. (ethyl acetate–light petroleum, 1 : 6, as mobile phase) of the residue gave (24R,S)-24-*phenylsulphonyl*-[26,27-$^2H_6$]*cholest*-5-*ene*-3β,25-*diol* 3-*tetrahydropyran*-2-*yl ether* (7a) (4.17 g, 81%), m.p. 150—162 °C (ethyl acetate–hexane); $\nu_{max.}$ 2 220 cm^{-1} (C–D); no detectable signal at δ 1.56 and 1.58 due to 26- and 27-$^1H_3$.

The corresponding non-deuteriated material (7b) had $\nu_{max.}$ 3 520, 3 490, 1 295, and 1 140 cm^{-1}; δ 0.54 and 0.58 (s,s, 18-H_3), 0.74 (d, *J* 7 Hz, 21-H_3), 0.98 (s, 19-H_3), 1.56 and 1.58 (s,s 26- and 27-H_3), 3.02 (m, 24-H), 3.3—4.1 (m, 8 H, THP), 4.24 (s, 25-OH), 4.7 (br s, 1 H, THP), *ca.* 5.3 (m, 6-H), 7.4—7.17 (m, 3 H, aromatic protons), and 7.8—8.0 (m, 2 H, aromatic protons) (Found: C, 72.85; H, 9.2. $C_{38}H_{55}O_5S$ requires C, 72.8; H, 9.3%).

[26,27-$^2H_6$]*Cholesta*-5,24-*dien*-3β-*ol* 3-*Tetrahydropyran*-2-*yl Ether* (2d) *and* [26,27-$^2H_6$]*Cholest*-5-*ene*-3β,25-*diol* 3-*Tetrahydropyran*-2-*yl Ether* (3b).—The crude hydroxy sulphone (7a) (*i.e.* before preparative h.p.l.c.) from the sulphone (6) (5.68 g, 10 mmol), was dissolved in dry tetrahydrofuran (50 ml). The stirred solution was diluted with methanol (50 ml, distilled from CaH_2) and treated at room temperature with sodium amalgam (6%; 116 g, 0.3 g-atom Na). After 4 h the mixture was filtered. The filtrate was diluted with ether (500 ml), washed with water, dried, and evaporated. Preparative h.p.l.c. (ethyl acetate–light petroleum, 1 : 6) of the residue (4.38 g) gave [26,27-$^2H_6$]cholesta-5,24-dien-3β-ol 3-tetrahydropyran-2-yl ether (2d) (2.26 g, 48%), m.p. 119—122 °C (acetone) (lit.,[10] 112—116 °C for non-deuteriated material); 98% $^2H_6$; $\nu_{max.}$ 2 230, 2 190, 2 110, and 2 070 cm^{-1} (C–D); no detectable signal at δ 1.60 and 1.68 for 26- and 27-$^1H_3$.

The more polar fraction gave [26,27-$^2H_6$]*cholest*-5-*ene*-3β,25-*diol* 3-*tetrahydropyran*-2-*yl ether* (3b) (1.37 g, 28%), m.p. 150—154 °C (ethyl acetate–hexane); $\nu_{max.}$ 2 230 cm^{-1} (C–D); no detectable signal at δ 1.22 due to 26- and 27-$^1H_3$; [see preparation of the diol (3d) for deuterium content].

The corresponding non-deuteriated alcohol (3c) had $\nu_{max.}$ 3 340 cm^{-1}; δ 0.68 (s, 18-H_3), 0.92 (d, *J* 6 Hz, 21-H_3), 1.02 (s, 19-H_3), 1.22 (s, 26- and 27-H_3), 3.3–4.4 (m, 8 H, THP), 4.7 (br s, 1 H, THP), and *ca.* 5.3 (m, 6-H) (Found: C, 79.0; H, 11.2. $C_{32}H_{54}O_3$ requires C, 78.95; H, 11.2%).

[26,27-$^2H_6$]*Cholest-5-ene*-3β,25-*diol* (3d).—Dilute hydrochloric acid (4.8 ml; conc. HCl–water, 1 : 4) was added to a suspension of the tetrahydropyranyl ether (3b) (1.36 g, 2.76 mmol) in acetone (84 ml) and water (8.4 ml). The suspension was stirred at room temperature for 24 h and then diluted with ether (500 ml). The solution was washed with saturated aqueous sodium hydrogen carbonate and water, dried, and evaporated. Crystallisation of the residue from acetone gave [26,27-$^2H_6$]cholest-5-ene-3β,25-diol (3d) (962 mg, 85%), m.p. 177—180 °C (lit.,[5] 177—179 °C for non-deuteriated material); 98% $^2H_6$; $\nu_{max.}$ 3 300, 2 240 (C–D), and 1 065 cm^{-1}; no detectable signal at δ 1.22 due to 26- and 27-$^1H_3$.

Cholesta-5,24-*dien*-3β-*ol* 3-*Tetrahydropyran*-2-*yl Ether* (2d) *and Cholest*-5-*ene*-3β,25-*diol* 3-*Tetrahydropyran*-2-*yl Ether* (3c) via *the Trimethylsilyl Ether* (7c).—*N*-Trimethylsilylimidazole (2.5 ml) was added to the hydroxy sulphone (7b) (319 mg, 0.5 mmol) and the suspension was stirred at room temperature for 3.5 h. The solution was then diluted with ether (50 ml), washed with water, dried, and evaporated to give an oil (7c). This product was treated with sodium amalgam in the same manner as for the hydroxy sulphone (7a) to give, after semi-preparative h.p.l.c., cholesta-5,24-dien-3β-ol 3-tetrahydropyran-2-yl ether (2d) (128 mg, 55%), m.p. 110—123 °C (acetone) (lit.,[10] 112—116 °C) and cholest-5-ene-3β,25-diol 3-tetrahydropyran-2-yl ether (3c) (76 mg, 31%), m.p. 151—155 °C (ethyl acetate–hexane).

(24R,S)-24-*Phenylsulphonylcholest*-5-*ene*-3β,25-*diol Diacetate* (7d).—Toluene-*p*-sulphonic acid (38 mg, monohydrate, 0.2 mmol) was added to a suspension of the hydroxy sulphone (7b) (128 mg, 0.2 mmol) in acetic anhydride (1 ml). The suspension was stirred at room temperature. After 21 h the brown solution was treated with ice, left at room temperature for 5 h, and then extracted with ether. The extracts were washed with saturated aqueous sodium hydrogen carbonate and water, dried, and evaporated. Semi-preparative h.p.l.c. (ethyl acetate–light petroleum, 1 : 4) of the oily residue (156 mg) gave (24R,S)-24-*phenylsulphonylcholest*-5-*ene*-3β,25-*diol diacetate* (7d) (107 mg, 84%), m.p. 128—131 °C (methanol); $\nu_{max.}$ 1 730 cm^{-1}; δ 0.58 and 0.60 (s,s, 18-H_3), 0.74 (d, *J* 6 Hz, 21-H_3), 1.00 (s, 19-H_3), 1.58 and 1.60 (s,s 26- and 27-H_3), 1.82 and 1.84 (s,s 25-OAc), 2.02 (s, 3-OAc), 4.08 (m, 24-H), *ca.* 4.5 (m, 3-H), *ca.* 5.34 (m, 6-H), 7.4—7.65 (m, 3 H, aromatic protons), and 7.75—8.0 (m, 2 H, aromatic protons) (Found: C, 70.7; H, 8.5. $C_{37}H_{54}O_6S$ requires C, 70.9; H, 8.7%).

[26,27-$^2H_6$]*Cholesta*-5,24-*dien*-3β-*ol Benzoate* (2f).—The tetrahydropyranyl ether (2e) (2.19 g, 4.6 mmol) was dissolved in tetrahydrofuran (50 ml). The solution was diluted with methanol (45 ml) and dilute hydrochloric acid (5 ml; conc. HCl–water, 1 : 4) and then stirred at room temperature. After 17 h the solution was evaporated to *ca.* 50 ml, diluted with ethyl acetate (200 ml), washed with saturated aqueous sodium hydrogen carbonate and water, dried, and evaporated. The residue in pyridine (20 ml, distilled from CaH_2) was treated with benzoyl chloride (1.6 ml, 13.7 mmol). After 20 h the mixture was poured into ice–water and extracted with ether. The extracts were washed with dilute hydrochloric acid and water, dried, and evaporated. The residue in light petroleum was adsorbed onto a short column of alumina (6 × 3 cm, activity 1). Elution with ethyl acetate–light petroleum (1 : 9) gave [26,27-$^2H_6$]cholesta-5,24-dien-3β-ol benzoate (2f) (2.14 g, 94%), m.p. 127—130 °C (acetone–methanol) (lit.,[20] 131 °C for non-deuteriated material); 98% $^2H_6$; $\nu_{max.}$ 2 230, 2 200, 2 120 and 2 070 (C–D), 1 710, 1 275, and 720 cm^{-1}; no detectable signal at δ 1.60 and 1.68 due to 26- and 27-$^1H_3$.

Catalytic Hydrogenation of the Diene (2f).—A solution of the diene (2f) (49 mg, 0.1 mmol) in ethyl acetate (5 ml) containing palladium on calcium carbonate (10%, 12 mg) was stirred and hydrogenated at room temperature and atmospheric pressure for 8 h. The suspension was filtered, the residue was washed with ethyl acetate, and the filtrate and washings were evaporated to give deuteriated cholesteryl benzoate (*ca.* 100%) containing *ca.* 3% of starting material (2f) (h.p.l.c., see later). The mass spectrum of the product showed $[M - 122]^+$ peaks (loss of benzoic acid; no molecular ion observed), corresponding to $^2H_6$, 12.7; $^2H_5$, 10.7; $^2H_4$, 14.2; $^2H_3$, 17.6; $^2H_2$, 19.8; $^2H_1$, 6.8; and $^2H_0$, 8.1%.

[26,27-$^2H_6$]*Cholesterol* (8b).—(a) *From the labelled diene* (2f). A solution of the diene (2f) (494 mg, 1 mmol) and toluene-*p*-sulphonohydrazide (1.49 g, 8 mmol) in diglyme (20 ml, distilled from CaH_2 and $LiAlH_4$, b.p. 84—86 °C/70 mmHg) was stirred and heated under reflux under nitrogen for 2 h. The mixture was then cooled, diluted with ether (200 ml), washed with water (4 × 50 ml), dried, and evaporated. Reverse-phase semi-preparative h.p.l.c. (ethyl acetate–methanol, 2 : 3) gave [26,27-$^2H_6$]cholesteryl benzoate (8a) (229 mg, 46%), m.p. 148—150 °C (acetone) (lit.,[21] 150—151 °C for non-deuteriated material); 94—96% $^2H_6$; $\nu_{max.}$ 2 210, 2 130 and 2 080 (C–D), and 1 710 cm^{-1}; no detectable signal at δ 0.84 and 0.91 due to 26- and 27-$^1H_3$.

A suspension of the benzoate (8a) (50 mg, 0.1 mmol) in methanol (5 ml) and methanolic 5% potassium hydroxide (0.23 ml; 0.2 mmol KOH) was stirred and heated under reflux for 4 h. The solution was cooled, diluted with ether (30 ml), washed with water, dried, and evaporated. Crystallisation of the residue from methanol gave [26,27-$^2H_6$]-cholesterol (8b) (32 mg, 82%), m.p. 145—147 °C (lit.,[21] 148.5 °C for non-deuteriated material) without loss of deuterium; $\nu_{max.}$ 3 420, 2 220, 2 130, 2 080 (C–D), and 1 065 cm^{-1}; no detectable signal at δ 0.82 and 0.89 due to 26- and 27-$^1H_3$.

(b) *From the tetrahydropyranyl ether* (3b) *of labelled* 25-*hydroxycholesterol*. Toluene-*p*-sulphonic acid (247 mg, monohydrate, 1.3 mmol) was added to a suspension of the mono-tetrahydropyranyl ether (3b) (640 mg, 1.3 mmol) in acetic anhydride (6 ml). The suspension was stirred at room temperature for 2 h, treated with ice and, after 2 h, extracted with ether. The extracts were washed with saturated aqueous sodium hydrogen carbonate and water, dried, and evaporated. Semi-preparative h.p.l.c. (ethyl acetate–light petroleum, 4 : 96) of the residue gave [26,27-$^2H_6$]cholest-5-ene-3β,25-diol diacetate (3e) (510 mg, 80%), m.p. 122—124 °C (methanol) (lit.,[3] 119—120.5 °C for non-deuteriated material).

Lithium (448 mg, 64 mg-atom, small pieces) was added to a solution of the diacetate (3e) (394 mg, 0.8 mmol) in anhydrous ethylamine (40 ml) at 0 °C. After 5 min at 0 °C the suspension was stirred and heated under reflux. After *ca.* 2 h a deep blue colour developed and after a further 1 h t-butyl alcohol (10 ml) was added during 5 min. The mixture was diluted with ether (100 ml), washed with water (3 × 40 ml), dried, and evaporated. Semi-preparative h.p.l.c. (ethyl acetate–light petroleum, 1 : 3) of the residue gave [26,27-$^2H_6$]cholesterol (225 mg, 72%), m.p. 146—148 °C (lit.,[21] 148.5 °C for non-deuteriated material); 97% $^2H_6$.

The more polar fraction gave [26,27-$^2H_6$]cholest-5-ene-3β,25-diol (3d) (30 mg, 9%), identical with material prepared as in the preceding pages.

24,25-*Epoxy*[26,27-$^2H_6$]*cholest-5-en*-3β-*ol Benzoates* (9), (10), *and* (11).—*m*-Chloroperbenzoic acid (406 mg, 85%, 2 mmol) was added to a stirred solution of the diene (2f) (988 mg, 2 mmol) in dichloromethane (80 ml) at 0 °C. After 40 min at 0 °C further *m*-chloroperbenzoic acid (81 mg, 0.4 mmol) was added. After a further 30 min the solution was diluted with ethyl acetate (250 ml), washed with aqueous sodium carbonate (5%, 2 × 50 ml) and saturated aqueous sodium chloride (2 × 50 ml), dried, and evaporated. Preparative h.p.l.c. (ethyl acetate–light petroleum, 5 : 95) of the residue gave (24*R*,*S*)-24,25-epoxy[26,27-$^2H_6$]cholest-5-en-3β-ol benzoate (9) (653 mg, 64%), m.p. 145—148 °C (acetone); $\nu_{max.}$ 2 250 and 2 230 cm^{-1} (C–D). Further chromatography of the mixed diastereoisomers (9) (200 mg) by semi-preparative h.p.l.c. (ethyl acetate–light petroleum, 4 : 96) and crystallisation from acetone gave (24*S*)-24,25-epoxy[26,27-$^2H_6$]cholest-5-en-3β-ol benzoate (10) (79 mg), m.p. 155—158 °C (lit.,[18] 150—152 °C for non-deuteriated material) and (24*R*)-24,25-epoxy[26,27-$^2H_6$]cholest-5-en-3β-ol benzoate (11) (88 mg), m.p. 169—172 °C (lit.,[18] 164—165 °C for non-deuteriated material).

(24R,S)-[26,27-$^2H_6$]*Cholest-5-ene*-3β,24,25-*triol* (12).—Perchloric acid (72% w/w; 50 µl, 0.6 mmol) was added to a solution of the epoxides (9) (102 mg, 0.2 mmol) in tetrahydrofuran (10 ml) and water (2 ml). The solution was stirred at room temperature for 1 h, diluted with ethyl acetate (50 ml), washed with saturated aqueous sodium hydrogen carbonate and water, dried, and evaporated. A suspension of the residue in methanol (8 ml) and methanolic 5% potassium hydroxide (0.24 ml, 0.22 mmol KOH) was stirred and heated under reflux for 3 h. The solution was cooled, diluted with water (30 ml), and extracted with ethyl acetate (3 × 20 ml). The extracts were washed with water, dried, and evaporated. Crystallisation of the residue from acetone–hexane gave (24*R*,*S*)-[26,27-$^2H_6$]cholest-5-ene-3β,24,25-triol (12) (75 mg, 88%), m.p. 196—199 °C (lit.,[22] 196—198 °C for non-deuteriated material); $\nu_{max.}$ 3 390, 2 230 (C–D), and 1 065 cm^{-1}; 96% $^2H_6$ (measured for the 3-benzoate).

Acknowledgements

We thank the Medical Research Council for financial support (to D. N. K., for the Steroid Reference Collection), and the Special Trustees of the London Hospital for their help in the purchase of the mass spectrometer. N.m.r. spectra were provided by Dr. R. D. Farrant.

References

1 A. M. Lawson, C. K. Lim, W. Richmond, D. M. Somson, K. D. R. Setchell and A. C. S. Thomas, in 'Current Developments in the Clinical Applications of HPLC, GC, and MS,' A. M. Lawson, C. K. Lim, and W. Richmond (ed.), Academic Press, London, 1980, p. 135.
2 A. I. Reyer, W. H. Grebert, and N. M. Murrill, *J. Am. Chem. Soc.*, 1950, **72**, 4247.
3 W. G. Dauben and H. L. Bradlow, *J. Am. Chem. Soc.*, 1950, **72**, 4248.
4 D. R. Idler and U. H. M. Fagerlund, *J. Am. Chem. Soc.*, 1957, **79**, 1989.
5 W. Bergmann and J. P. Dusza, *J. Org. Chem.*, 1958, **23**, 459.
6 R. Ikan, A. Markus, and Z. Goldschmidt, *J. Chem. Soc., Perkin Trans. 1*, 1972, 2423.
7 I. Björkhem and I. Holmberg, *Clin. Chim. Acta*, 1976, **68**, 215.
8 J. E. Herz and S. C. Montalvo, *Steroids*, 1971, **17**, 649; *J. Chem. Soc., Perkin Trans. 1*, 1973, 1233.
9 M. A. Apfel, *J. Org. Chem.*, 1978, **43**, 2284; K. Ochi, I. Matsunaga, M. Shindo, and C. Kaneko, *Steroids*, 1977, **30**, 795.
10 S. K. Dasgupta, D. R. Crump, and M. Gut, *J. Org. Chem.*, 1974, **39**, 1658.
11 M. Julia and J. M. Paris, *Tetrahedron Lett.*, 1973, 4833; P. J. Kocienski, *Chem. Ind.*, 1981, 548.
12 (*a*) Y. Kobayashi, T. Taguchi, N. Kanuma, N. Ikekawa, and J. Oshida, *Tetrahedron Lett.*, 1981, **22**, 4309; (*b*) *J. Chem. Soc., Chem. Commun.*, 1980, 459.
13 Huang-Minlon, E. Wilson, N. L. Wender, and M. Tishler, *J. Am. Chem. Soc.*, 1952, **74**, 5394.
14 C. Djerassi and J. Gutzwiller, *J. Am. Chem. Soc.*, 1966, **88**, 4537.
15 R. S. Dewey and E. E. van Tamelen, *J. Am. Chem. Soc.*, 1961, **83**, 3729.
16 R. B. Boar, L. Joukhadar, J. F. McGhie, S. C. Misra, A. G. M. Barrett, D. H. R. Barton, and P. A. Prokopiou, *J. Chem. Soc., Chem. Commun.*, 1978, 68.
17 *Cf.* S. C. Eyley and D. H. Williams, *J. Chem. Soc., Perkin Trans. 1*, 1976, 731.
18 M. Seki, N. Koizumi, M. Morisaki, and N. Ikekawa, *Tetrahedron Lett.*, 1975, 15.
19 H. Jones and G. H. Rasmusson, *Fortschr. Chem. Org. Naturst.*, 1980, **39**, 63; P. E. Georghiou, *Chem. Soc. Rev.*, 1977, **6**, 83.
20 U. H. M. Fagerlund and D. R. Idler, *J. Am. Chem. Soc.*, 1957, **79**, 6473.
21 I. Heilbron and H. M. Bunbury, 'Dictionary of Organic Compounds,' Eyre and Spottiswoode, London, 1953, vol. 1, p. 576.
22 J. J. Partridge, V. Toome, and M. R. Uskokovic, *J. Am. Chem. Soc.*, 1976, **98**, 3739.

Received 11*th April* 1983; *Paper* 3/559