Synthesis of (25*R*)-[26-$^2H_1$]Cholesterol and ^1H N.m.r. and H.p.l.c. Resolution of (25*R*)- and (25*S*)-26-Hydroxycholesterol

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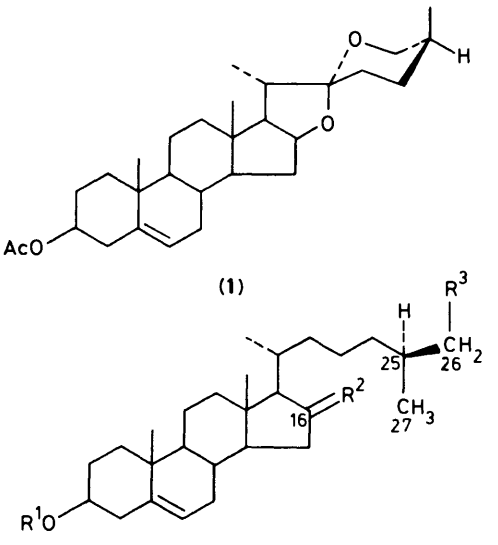
Yamogenin acetate (**1**) was isolated from crude diosgenin acetate and converted into (25*S*)-26-hydroxycholesterol (**6a**). The absolute configuration at C-25 of (**6a**) was determined by *X*-ray crystallography. (25*R*)-[26-$^2H_1$]Cholesterol (**10**) was prepared by reduction of the 26-tosyloxy group by $LiAl^2H_4$. Reverse-phase h.p.l.c. resolution without derivatization was developed for the diastereoisomers, (25*R*)- and (25*S*)-26-hydroxycholesterol. The (+)- or (−)-MTPA esters of these diastereoisomers showed distinctive ^1H n.m.r. signals for 26-H.

Recently we found by ^1H n.m.r. spectroscopy that the isopropyl methyl groups of cholesterol are magnetically inequivalent and assigned their signals by preparing stereospecifically deuteriated (25*S*)-[26-$^2H_1$]cholesterol.[1] Now we have prepared (25*R*)-[26-$^2H_1$]cholesterol (**10**) *via* (25*S*)-26-hydroxycholesterol (**6a**) and confirmed the n.m.r. assignments for 26-H and 27-H (*pro-R* and *pro-S* methyl groups, respectively, at C-25) of cholesterol. The 26-hydroxylated steroid is a potentially important compound as an intermediate in the biosynthesis of bile acids and probably pavoninins (shark repellent),[2] as an inhibitor of cholesterol synthesis *in vivo*,[3] and also as an intermediate in steroidal sapogenin biosynthesis in plants.[4] For these studies, facile methods are essential for the preparation, resolution, and differentiation of (25*S*)- and (25*R*)-26-hydroxycholesterol, (**6a**) and (**6b**). Although the (25*R*)-epimer has been synthesized,[1,5] the (25*S*)-isomer has been less easy to prepare, Byon's method being rather complex.[6]

Chromatographic resolution of the diastereoisomers has been achieved in a multiple recycling process[7] of the acetates of (**6a**) and (**6b**) and recently in a single path as the *p*-bromobenzoates of these compounds.[8] Here we report a facile preparation of (25*S*)-26-hydroxycholesterol (**6a**) from yamogenin acetate (**1**), which was isolated easily from crude diosgenin and a simple method for resolving (25*S*)- and (25*R*)-26-hydroxycholesterol (**6a**) and (**6b**) by reverse-phase high-performance liquid chromatography (h.p.l.c.) without derivatization. Also described is the efficient differentiation of these diastereomeric isomers by ^1H n.m.r. spectroscopy as their 3,26-bis-(+)- or (−)-methoxy(trifluoromethyl)phenylacetyl esters (MTPA); only one isomer is required to determine the configuration at C-25.



(1)

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

(3) R^1 = *p*-$NO_2C_6H_4CO$, R^2 =  , R^3 = *p*-$NO_2C_6H_4COO$

(4) R^1 = *p*-$NO_2C_6H_4CO$, R^2 = O, R^3 = *p*-$NO_2C_6H_4COO$

(5) R^1 = H, R^2 = O, R^3 = OH

(6a) R^1 = H, R^2 = H_2, R^3 = OH

(7) R^1 = Ac, R^2 = H_2, R^3 = $OCPh_3$

(8) R^1 = Ac, R^2 = H_2, R^3 = OH

(9) R^1 = Ac, R^2 = H_2, R^3 = O-*p*-Ts

(10) R^1 = H, R^2 = H_2, R^3 = D

Results and Discussion

We chose yamogenin acetate (**1**) as a starting material for (25*S*)-26-hydroxycholesterol (**6a**) because it has the required stereochemistry at C-25. Yamogenin is usually present in commercially available diosgenin sometimes in a concentration of up to 15%. Silica gel chromatography of crude diosgenin acetate gave good resolution, affording yamogenin acetate (**1**). The purity was >99.5% measured by reverse-phase h.p.l.c. after recrystallization (Figure 4 in Experimental section).

Clemmensen reduction of yamogenin acetate (**1**) gave (25*S*)-3β,16β,26-trihydroxycholesterol (**2**) (75%), the 3- and 26-hydroxy groups of which were protected selectively as *p*-nitrobenzoyl derivatives; subsequent oxidation of the 16-hydroxy group followed by deprotection of the 3- and 26-positions gave the dihydroxy-16-oxo compound (**5**). This upon Huang-Minlon reduction afforded (25*S*)-26-hydroxycholesterol (**6a**).

Although recent developments in h.p.l.c. have made it possible to separate (25*S*)- (**6a**) and (25*R*)-hydroxycholesterol (**6b**),[7,8] there subsequent identification without a pair of authentic samples is impossible. Therefore we determined the configuration at C-25 of (**6a**) as *S* by means of *X*-ray crystallography (see Figure 1).† This (25*S*)-epimer (**6a**) was compared by reverse-

† Yamogenin (**1**) has the same nuclear configuration as that of its (25*R*)-epimer, diosgenin, since (**1**) and diosgenin are chemically correlated; the absolute configuration of diosgenin was determined by *X*-ray crystallography (W. Kline and J. Buckingham, 'Atlas of Stereochemistry,' Chapman and Hall, London, 1974, p. 126).

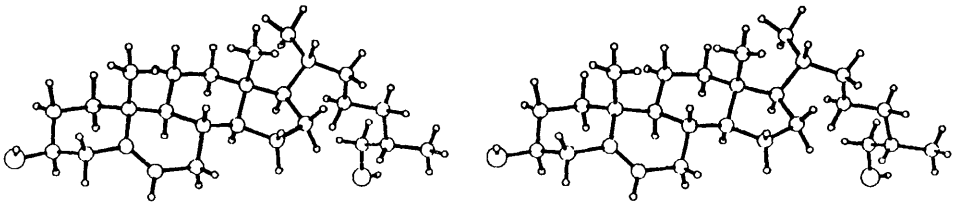


Figure 1. A stereoview of compound (**6a**)

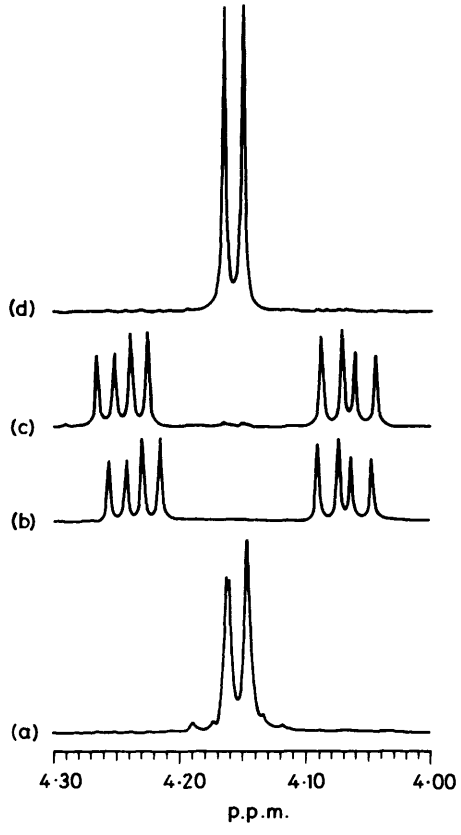


Figure 2. Portion of 400 MHz ^{1}H n.m.r. spectra of 26-H of (a) (−)-MTPA diester of (25*R*)-26-hydroxycholesterol (**6b**), (b) (+)-MTPA diester of (25*R*)-26-hydroxycholesterol (**6b**), (*c*) (−)-MTPA diester of (25*S*)-26-hydroxycholesterol (**6a**), and (d) (+)-MTPA diester of (25*S*)-26-hydroxycholesterol (**6a**) recorded on a Varian XL-400 instrument at 23 °C in $CDCl_3$

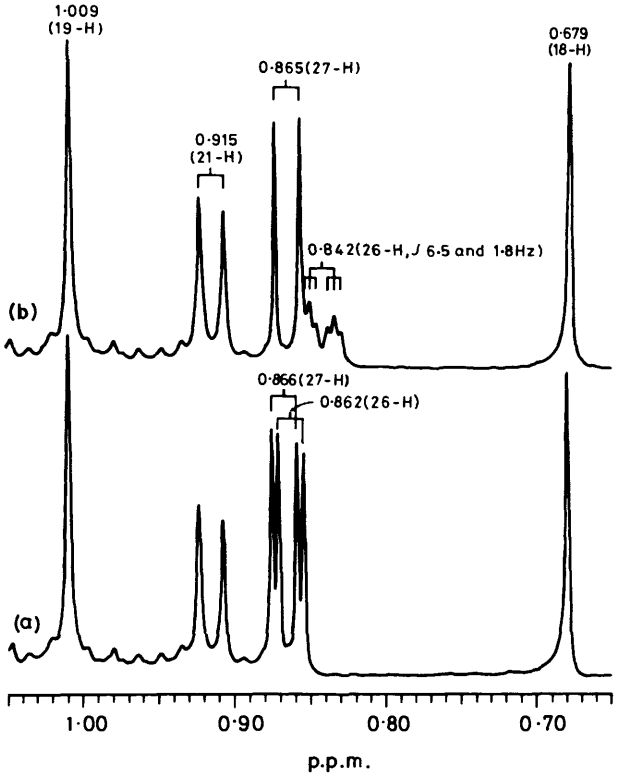


Figure 3. Portion of 400 MHz ^{1}H n.m.r. spectra of (a) cholesterol and (b) (25*R*)-[26-^{2}H]cholesterol (**10**) recorded on a Varian XL-400 instrument at 23 °C in $CDCl_3$

phase h.p.l.c. with its (25*R*)-epimer, which was synthesized from diosgenin.[1] As shown in Figure 5 (see Experimental section), (25*S*)- (**6a**) and (25*R*)-26-hydroxycholesterol (**6b**) could be reasonably resolved, without derivatization by a single passage through a TSK gel ODS-120T column developed with methanol–water (93:7). The (25*S*)-epimer (**6a**) showed no peak due to the (25*R*)-epimer (**6b**). Although Clemmensen reduction of the 1,5-ketol system has been reported to cause isomerization at C-25 by a 1,5-hydride shift,[5b] this was avoided in our experiment by slow addition of hydrochloric acid and also by acetal protection at C-22.

^{1}H N.m.r. spectra of the two epimers at 200 MHz were so similar that differentiation between (**6a**) and (**6b**) was difficult. However, when the two isomers were esterified with (+)-or (−)-MTPA chloride,[9] the hydrogens at C-26 showed diagnostic signals. As shown in Figure 2, the (−)-MTPA diester of the (25*R*)-epimer (**6b**) (Figure 2a) and the (+)-MTPA diester of the (25*S*)-epimer (**6a**) (Figure 2d) show the two hydrogens at C-26 almost as a doublet signal. In contrast, these hydrogens of the (+)-MTPA diester of the (25*R*)-epimer (**6b**) (Figure 2b) and the (−)-MTPA diester of the (25*S*)-epimer (**6a**) (Figure 2c) appeared as a well separated pair of double doublets; even at 90 MHz n.m.r., these two epimers could be identified by their 26-H signals. This method may be generally applicable to a determination of the configuration of a hydroxymethyl group.[10] Only some signals in the ^{13}C n.m.r. spectra of (**6a**) and (**6b**) differ: C-20 to C-27, by 0.02—0.21 p.p.m.

In a previous study, we assigned the ^{1}H n.m.r. signals of 26-H and 27-H of cholesterol using (25*S*)-[26-$^2H_1$]cholesterol.[1] In order to confirm the assignments (25*S*)-26-hydroxycholesterol (**6a**) was transformed to (25*R*)-[26-$^2H_1$]cholesterol (**10**) according to a known method.[1] As shown in Figure 3, the ^{1}H n.m.r. spectrum of (**10**) shows 27-H as a doublet ($J_{H,H}$ 6.5 Hz) at δ_H 0.865 and 26-H as a doublet of triplets at δ_H 0.842 which couples to 25-H ($J_{H,H}$ 6.5 Hz) and 26-^{2}H ($J_{H,^2H}$ 1.8 Hz). This confirms the signal assignments at δ_H 0.862 and 0.866 to 26-H (*pro-R* methyl group at C-25) and 27-H (*pro-S* methyl group at C-25) of cholesterol, respectively. In the ^{13}C n.m.r. spectrum of compound (**10**), C-26 appeared at δ_C 22.26 as a triplet ($^1J_{C,^2H}$ 19 Hz, $^1\Delta\delta_{C(^2H)}$ −0.3 p.p.m.) and C-27 at δ_C 22.80 as a singlet, which agrees with the assignments[11] originally made by Popják on the basis of biosynthetic findings.[12] We therefore conclude that our findings confirm a *si*-face reduction of the 24(25)-double

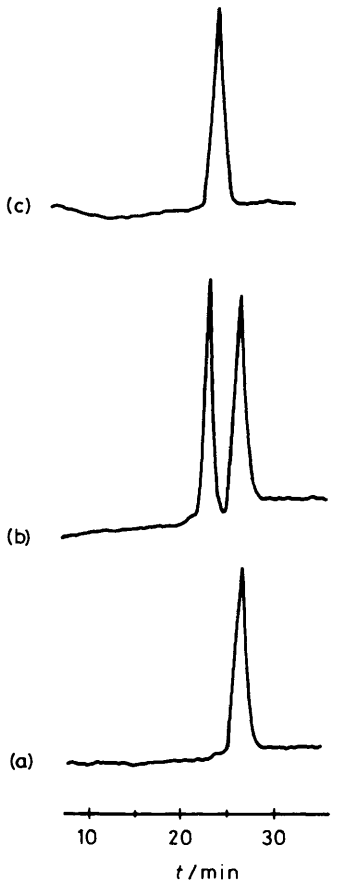


Figure 4. H.p.l.c. analysis of (a) yamogenin acetate (**1**), (b) a mixture of yamogenin and diosgenin, and (c) diosgenin. Conditions: column, TSKgel ODS-120T 250 × 4 mm i.d.; solvent, 7% water in methanol, 1 ml/min; detector, UVILOG-5IIIA at 208 nm

bond of lanosterol in the biosynthesis of cholesterol proposed by Caspi,[13] because the *Z*-methyl group (C-27) at C-25 of lanosterol originating from C-3′ of mevalonate turns into one of the methyl groups at C-25 of cholesterol, which appeared at δ_C 22.9.[12] We confirmed the signal to correspond to C-27 (*pro-S* methyl group at C-25).

Experimental

M.p.s were taken on a hot plate and are uncorrected. ^{1}H N.m.r. spectra were recorded on a Varian XL-200 (200.057 MHz) or an XL-400 (399.948 MHz) spectrometer in [^{2}H]chloroform unless otherwise stated using a 5-mm spinning tube. ^{13}C N.m.r. spectra were determined on a Varian XL-100-12A spectrometer operating at 25.16 MHz in [^{2}H]chloroform using a 10-mm spinning spherical tube. Chemical shifts are given in p.p.m. downfield from internal tetramethylsilane. Acquisition times of ^{1}H and ^{13}C measurements were 5 and 1.6 s, respectively. Optical rotation was determined on a Perkin-Elmer 241 Polarimeter and mass spectra were recorded on a Hitachi RMU-8GN spectrometer. H.p.l.c. was performed with a Waters 600 multisolvent delivery system equipped with a U6K injector and UVILOG-5IIIA u.v. detector.

Isolation of Yamogenin Acetate (**1**).—Diosgenin (22 g; purchased from Wako Pure Chemical Industries, Ltd.) was acetylated with acetic anhydride (80 ml) and pyridine (60 ml) to obtain the crude acetate (24 g). Chromatography of the acetate (1 g) on silica gel [2 × Lobar B, hexane–chloroform–ethyl acetate (15:1:1), 2.5 ml/min] gave diosgenin acetate (200—250 ml fraction, 917 mg) and raw yamogenin acetate (250—300 ml fraction, 83 mg), which when recrystallized from methanol, gave yamogenin acetate (**1**), m.p. 180—182 °C, $[\alpha]_D^{24}$ −130.8° (*c* 0.655 $CHCl_3$); δ_H (C_5D_5N) 0.844 (3 H, s, 18-H), 0.975 (3 H, s, 19-H), 1.086 (3 H, d, *J* 7.2 Hz, 27-H), 1.161 (3 H, d, *J* 6.8 Hz, 21-H), 2.069 (3 H, s, Ac), 3.38 (1 H, d, *J* 11 Hz, 26β-H), 4.07 (1 H, dd, *J* 11 and 3 Hz, 26α-H), 4.52 (1 H, dt, *J* 8 and 7 Hz, 16-H), 4.82 (1 H, m, 3-H), and 5.35 (1 H, d, *J* 5 Hz, 6-H); δ_C 37.01 (C-1), 27.78 (C-2), 73.92 (C-3), 38.14 (C-4), 139.75 (C-5), 122.38 (C-6), 32.08 (C-7), 31.45 (C-8), 50.02 (C-9), 36.77 (C-10), 20.86 (C-11), 39.79 (C-12), 40.27 (C-13), 56.48 (C-14), 31.83 (C-15), 80.92 (C-16), 62.01 (C-17), 16.30 (C-18), 19.35 (C-19), 42.18 (C-20), 14.36 (C-21), 109.75 (C-22), 26.02 (C-23), 25.83 (C-24), 27.12 (C-25), 65.14 (C-26), 16.08 (C-27), and 21.40 and 170.5 (CH_3CO). Reverse-phase h.p.l.c. is shown in Figure 4.

(25S)-*Cholest-5-ene*-3β,16β,26-*triol* (**2**).—Zinc amalgam [freshly prepared from zinc powder (30 g) and $HgCl_2$ (3 g)] was added to yamogenin acetate (1 g) dissolved in 90% ethanol (100 ml). Concentrated hydrochloric acid (30 ml) was then added over 2.5 h to the above mixture whilst it was stirred vigorously and heated under reflux; the heating was then continued for a further 30 min. The mixture was cooled and the inorganic material filtered off. The filtrate was concentrated under reduced pressure to 30 ml and extracted with chloroform (200 ml); the extract was then washed successively with water (100 ml), 3% aqueous sodium hydrogen carbonate (100 ml), and water (100 ml) and evaporated. Chromatography of the residue (1 g) on silica gel eluted with hexane–chloroform–ethyl acetate–isopropyl alcohol (7:7:7:1) gave (25*S*)-cholest-5-ene-3β,16β,26-triol (**2**) (721 mg), m.p. 177—178 °C (from methanol), $[\alpha]_D^{23}$ −26.5° (*c* 1.02 $CHCl_3$–MeOH, 3:1) (Found: C, 76.9; H, 11.0%; M^+, 418. $C_{27}H_{46}O_3$ requires C, 77.5; H, 11.1%; *M*, 418); δ_H ($CDCl_3$–CD_3OD 3:1) 0.887 (3 H, s, 18-H), 0.914 (3 H, d, *J* 6.7 Hz, 27-H), 0.982 (3 H, d, *J* 6.7 Hz, 21-H), 1.022 (3 H, s, 19-H), 3.37 and 3.43 (2 H, AB part of ABX, *J* 10.5, 6.5, and 6 Hz, 26-H), 3.4 (1 H, m, 3-H), 4.36 (1 H, m, 16-H), and 5.36 (1 H, d, *J* 5 Hz, 6-H); δ_C 37.38 (C-1), 31.19 (C-2), 72.36 (C-3), 42.94 (C-4), 142.20 (C-5), 122.24 (C-6), 32.21 (C-7), 32.75 (C-8), 51.58 (C-9), 36.92 (C-10), 21.80 (C-11), 38.40 (C-12), 43.26 (C-13), 55.69 (C-14), 41.22 (C-15), 72.77 (C-16), 62.73 (C-17), 13.46 (C-18), 19.86 (C-19), 37.61 (C-20), 18.80 (C-21), 34.84 (C-22), 24.92 (C-23), 32.90 (C-24), 37.91 (C-25), 68.40 (C-26), and 17.32 (C-27).

(25S)-3β,26-*Bis*-p-*nitrobenzoyloxycholest-5-en*-16β-*ol* (**3**).—*p*-Nitrobenzoyl chloride (529 mg) was added to the triol (**2**) (596 mg) dissolved in dried dichloromethane (27 ml) and pyridine (170 mg) at 0 °C. The mixture was stirred at room temperature for 2 h. Methanol (2 ml) was added to destroy the excess of chloride and the solution was stirred at room temperature for 20 min, and then diluted with chloroform (100 ml), washed successively with water, 1% hydrochloric acid, and water, and evaporated. Chromatography on silica gel eluted with hexane–chloroform–ethyl acetate (7:2:2) gave (25*S*)-3β,26-bis-*p*-nitrobenzoyloxycholest-5-en-16β-ol (**3**) (499 mg), m.p. 190—190.5 °C (from methanol), $[\alpha]_D^{23}$ 0.0° (*c* 1.009 $CHCl_3$ + 1 drop of MeOH) (Found: C, 68.5; H, 7.4. Calc. for $C_{41}H_{52}N_2O_9$: C, 68.7; H, 7.3); δ_H ($CDCl_3$ + 1 drop of CD_3OD) 0.909 (3 H, s, 18-H), 0.999 (3 H, d, *J* 6.4 Hz, 21-H), 1.045 (3 H, d, *J* 6.7 Hz, 27-H), 1.093 (3 H, s, 19-H), 2.50 (2 H, d, *J* 7 Hz, 4-H), 4.19 and 4.30 (2 H, AB part of ABX, *J* 10.5, 7, and 6 Hz, 26-H), 4.35 (1 H, m, 16-H), 4.92 (1 H, m, 3-H), 5.45 (1 H, d, *J* 5 Hz, 6-H), 8.21 and 8.28 (4 H, A_2B_2, *J* 9 Hz, ArH), and 8.21 and 8.29 (4 H, A_2B_2, *J* 9 Hz, ArH).

(25S)-3,26-*Bis*-p-*nitrobenzoyloxycholest*-5-*en*-16-*one* (**4**).—Jones' reagent (1.0 ml) was added to bis-*p*-nitrobenzoate (**3**)

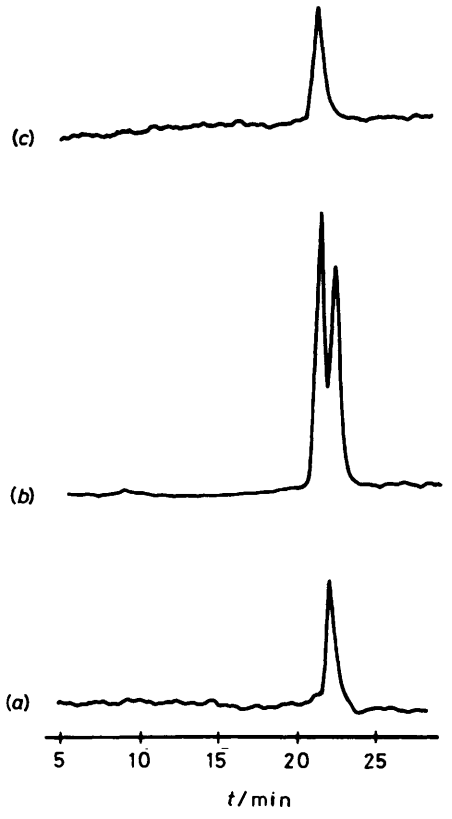


Figure 5. H.p.l.c. analysis of (a) (25*S*)-26-hydroxycholesterol (**6a**), (b) a mixture of (**6a**) and (**6b**), and (c) (25*R*)-26-hydroxycholesterol (**6b**). Conditions: column, TSKgel ODS-120T 250 × 4 mm i.d.; solvent, 7% water in MeOH, 1 ml/min; detector, UVILOG-5IIIA at 205 nm

(635 mg) in acetone (40 ml) at 0 °C and stirred at 0 °C for 30 min. The reaction mixture was diluted with water (100 ml) and extracted with dichloromethane (150 ml), and the extract washed with water, and evaporated to give (25*S*)-3β,26-bis-*p*-nitrobenzoyloxycholest-5-en-16-one (**4**) (624 mg), m.p. 157—159 °C (from methanol), $[\alpha]_D^{23.5}$ − 80.6 °C (*c* 1.021 $CHCl_3$) (Found: C, 68.7; H, 7.0; N, 4.0. Calc. for $C_{41}H_{50}N_2O_9$: C, 68.9; H, 7.1; N, 4.0); δ_H 0.846 (3 H, s, 18-H), 0.990 (3 H, d, *J* 6.2 Hz, 21-H), 1.042 (3 H, d, *J* 6.8 Hz, 27-H), 1.109 (3 H, s, 19-H), 2.31 (2 H, d, *J* 6.6 Hz, 4-H), 4.17 and 4.28 (2 H, AB part of ABX, *J* 10.5, 6.5, and 6.0 Hz, 26-H), 4.92 (1 H, m, 3-H), 5.45 (1 H, d, *J* 5 Hz, 6-H), and 8.21 and 8.28 (4 H, A_2B_2, *J* 9 Hz, ArH), and 8.21 and 8.29 (4 H, A_2B_2, *J* 9 Hz, ArH).

(25S)-3β,26-*Dihydroxycholest-5-en-16-one* (**5**).—A solution of 16-oxo-bis-*p*-nitrobenzoate (**4**) (500 mg) in 1% methanolic potassium hydroxide (40 ml) was refluxed for 50 min. The reaction mixture was diluted with water (150 ml) and extracted with chloroform (400 ml), and the extract washed with water (300 ml) and evaporated to give (25*S*)-3β,26-dihydroxycholest-5-en-16-one (**5**) (280 mg), m.p. 166—167.5 °C (from methanol), $[\alpha]_D^{23.5}$ − 171.6° (*c* 0.87 $CHCl_3$) (Found: C, 77.3; H, 10.6. Calc. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.7); δ_H 0.837 (3 H, s, 18-H), 0.924 (3 H, d, *J* 6.7 Hz, 27-H), 0.976 (3 H, d, *J* 6.5 Hz, 21-H), 1.040 (3 H, s, 19-H), 3.40 and 3.52 (2 H, AB part of ABX, *J* 12, 6.5, and 6 Hz, 26-H), 3.5 (1 H, m, 3-H), and 5.36 (1 H, d, *J* 5 Hz, 6-H).

(25S)-*Cholest-5-en*-26-*ol* (**6a**).—A solution of the 16-oxo diol (**5**) (199 mg) in triethylene glycol (10.4 ml) was heated at 130 °C for 1.5 h with hydrazine hydrochloride (360 mg) and 80% hydrazine hydrate (1.8 g). Potassium hydroxide (900 mg) was added to the mixture which was then heated at 210 °C for 3 h with distillation of the water. The mixture was cooled, diluted with water (100 ml), and extracted with chloroform (450 ml). The extract was washed with water (100 ml) and then evaporated. Purification of the residue (187 mg) by reverse-phase h.p.l.c. (Figure 5) gave (25*S*)-cholest-5-en-26-ol (**6a**) (141 mg), m.p. 177—178 °C (from methanol) (lit.,[6] 171—174 °C). $[\alpha]_D^{23}$ −44.0° (*c* 1.00 $CHCl_3$) (Found: C, 80.7; H, 11.6%; M^+, 402. $C_{27}H_{46}O_2$ requires C, 80.5; H, 11.5%; *M*, 402); δ_H 0.679 (3 H, s, 18-H), 0.920 (6 H, d, *J* 6.5 Hz, 21-H and 27-H), 1.009 (3 H, s, 19-H), 3.43 and 3.52 (2 H, AB part of ABX *J* 10.5, 6.5, and 6 Hz, 26-H), 3.50 (1 H, m, 3-H), and 5.36 (1 H, d, *J* 5 Hz, 6-H); δ_C 37.30 (C-1), 31.71 (C-2), 71.82 (C-3), 42.36 (C-4), 140.82 (C-5), 121.70 (C-6), 31.94 (C-7 and C-8), 50.19 (C-9), 36.54 (C-10), 21.12 (C-11), 39.83 (C-12), 42.36 (C-13), 56.81 (C-14), 24.31 (C-15), 28.26 (C-16), 56.18 (C-17), 11.89 (C-18), 19.41 (C-19), 35.85 (C-20*), 18.77 (C-21), 36.30 (C-22), 23.50 (C-23), 33.71 (C-24), 35.80 (C-25*), 68.37 (C-26), and 16.74 (C-27). (*These assignments may be reversed.)

X-*Ray Structure Determination of* (**6a**).—Crystals were grown from ethyl acetate solution by slow evaporation.

Crystal data. $C_{27}H_{46}O_2$, $M = 402.7$. Orthorhombic, $a = 11.887(1)$, $b = 32.635(3)$, $c = 6.297(1)$ Å, $V = 2\,442.8(4)$ Å^3 (by least-squares refinement of diffractometer angles for 24 centred reflections, $20 < \theta < 28°$, $\lambda = 1.541\,78$ Å), space group $P2_12_12_1$, $Z = 4$, $D_x = 1.10$ g cm^{-3}; colourless needles; crystal dimensions 0.10 × 0.15 × 0.40 mm, μ (Cu-K_α) = 0.51 mm^{-1}.

Data collection and processing. Rigaku AFC-5 diffractometer, $\omega/2\theta$ scan, graphite-monochromated Cu-K_α, 2 420 unique reflections measured ($\theta \leqq 65.5°$, $+h,k,l$), no absorption correction, giving 2 236 with $I > \sigma(I)$.

Structure analysis and refinement. H Atoms were located from difference map by direct methods.[14] Block diagonal least-squares refinement was carried out with anisotropic temperature factors for non-H atoms and isotropic for H atoms; there was no correction for secondary extinction. $\Sigma\omega\Delta^2$Minimized, $\Delta = |F_o| - |F_c|$, $\omega = 1/\sigma^2(F_o)$ for $|F_c| > 2\sigma(F_o)$, $\omega = 0$ for $|F_c| \leqq 2\sigma(F_o)$ or $|\Delta| > 5\sigma(F_o)$, $\sigma(F_o) = [\sigma_1{}^2(F_o) + 0.002\,47\,|F_o|^2]^{1/2}$, $\sigma_1(F_o)$ = e.s.d. based on counting errors;[15] $R = 0.058$, $R_w = 0.080$. Atomic scattering factors from ref. 16. Calculations performed on a FACOM-M340R computer. Atomic co-ordinates are given in the Table. Bond lengths and angles are anisotropic and isotropic temperature factors, are available on request from the Cambridge Crystallographic Data Centre.†

(+)- *and* (−)-*MTPA Esters of* (25S)- *and* (25R)-26-*Hydroxycholesterol* (**6a**) *and* (**6b**).—A solution of (25*S*)-26-hydroxycholesterol (**6a**) (5 mg) and (+)-MTPA chloride[9] (3 drops) in pyridine–dichloromethane (1:1) (1 ml) was left at room temperature for 15 h. Water was added to destroy the excess of chloride after which the product was extracted with ether (30 ml) and the extract evaporated. Chromatography of the residue on silica gel t.l.c. (hexane–chloroform–ethyl acetate, 15:1:1) gave the (+)-MTPA diester of (**6a**) (5 mg), δ_H 0.666 (3 H, s, 18-H), 0.895 (3 H, d, *J* 6.7 Hz, 21-H), 0.921 (3 H, d, *J* 6.4 Hz, 27-H), 1.003 (3 H, s, 19-H), 3.553 (3 H, q, $^5J_{H,F}$ 1.2 Hz, OMe), 3.566 (3 H, q, $^5J_{H,F}$ 1.2 Hz, OMe), 4.157 (2 H, d, *J* 6.0 Hz, 26-H), 4.88 (1 H, m, 3-H), 5.40 (1 H, d, *J* 4 Hz, 6-H), 7.40 (6 H, m, ArH), and 7.53 (4 H, m, ArH).

Treatment of (**6a**) with (−)-MTPA using the same procedure as above yielded the (−)-MTPA diester of (**6a**) (5 mg), δ_H 0.667 (3 H, s, 18-H), 0.898 (3 H, d, *J* 6.5 Hz, 21-H), 0.913 (3 H, d, *J* 6.4 Hz, 27-H), 1.002 (3 H, s, 19-H), 3.553 (3 H, q, $^5J_{H,F}$ 1.2 Hz, OMe),

† See Instructions to Authors (1987), *J. Chem. Soc., Perkin Trans. 1*, 1987, Issue 1.

Table. Fractional atomic co-ordinates for compound (**6a**) with e.s.d.s in parentheses

	x	*y*	*z*
C(1)	0.219 0(3)	0.147 5(1)	0.461 3(5)
C(2)	0.208 2(3)	0.101 8(1)	0.524 2(6)
C(3)	0.301 6(3)	0.090 0(1)	0.675 4(5)
C(4)	0.294 8(3)	0.116 2(1)	0.874 9(5)
C(5)	0.299 4(2)	0.161 5(1)	0.821 7(4)
C(6)	0.371 6(2)	0.185 4(1)	0.920 4(5)
C(7)	0.383 6(2)	0.231 0(1)	0.886 1(5)
C(8)	0.283 6(2)	0.248 9(1)	0.763 3(5)
C(9)	0.252 3(2)	0.220 4(1)	0.576 4(4)
C(10)	0.216 6(2)	0.176 7(1)	0.654 2(4)
C(11)	0.165 8(2)	0.239 7(1)	0.425 2(5)
C(12)	0.194 6(3)	0.284 3(1)	0.357 9(5)
C(13)	0.214 4(2)	0.311 6(1)	0.552 4(4)
C(14)	0.310 8(2)	0.291 4(1)	0.678 3(4)
C(15)	0.350 5(3)	0.323 9(1)	0.837 6(5)
C(16)	0.333 5(3)	0.364 3(1)	0.715 9(5)
C(17)	0.267 0(2)	0.354 9(1)	0.508 2(4)
C(18)	0.107 3(2)	0.315 9(1)	0.683 3(5)
C(19)	0.097 9(2)	0.177 0(1)	0.751 6(7)
C(20)	0.186 3(3)	0.389 7(1)	0.443 8(6)
C(21)	0.127 0(3)	0.382 6(1)	0.234 0(7)
C(22)	0.245 4(3)	0.432 1(1)	0.441 1(6)
C(23)	0.349 9(3)	0.436 1(1)	0.307 4(7)
C(24)	0.390 0(3)	0.481 1(1)	0.305 9(7)
C(25)	0.511 0(3)	0.488 0(1)	0.235 0(6)
C(26)	0.531 8(3)	0.472 9(1)	0.012 9(7)
C(27)	0.543 5(3)	0.532 6(1)	0.263 0(9)
O(3)	0.298 4(2)	0.047 0(1)	0.7272(4)
O(26)	0.646 8(2)	0.474 0(1)	−0.043 8(5)

3.568 (3 H, q, $^5J_{H.F}$ 1.2 Hz, OMe), 4.070 (1 H, dd, *J* 10.4 and 7.2 Hz, 26-H_a), 4.239 (1 H, dd, *J* 10.4 and 5.6 Hz, 26-H_b), 4.88 (1 H, m, 3-H), 5.42 (1 H, d, *J* 6 Hz, 6-H), 7.40 (6 H, m, ArH), and 7.53 (4 H, m, ArH).

Treatment of (**6b**) with (+)-MTPA using the same procedure as above yielded the (+)-MTPA diester of (**6b**) (5 mg), δ_H 0.666 (3 H, s, 18-H), 0.899 (3 H, d, *J* 6.5 Hz, 21-H), 0.910 (3 H, d, *J* 6.7 Hz, 27-H), 1.003 (3 H, s, 19-H), 3.553 (3 H, q, $^5J_{H.F}$ 1.2 Hz, OMe), 3.566 (3 H, q, $^5J_{H.F}$ 1.2 Hz, OMe), 4.072 (1 H, dd, *J* 10.4 and 7.2 Hz, 26-H_a), 4.230 (1 H, dd, *J* 10.4 and 5.6 Hz, 26-H_b), 4.88 (1 H, m, 3-H), 5.403 (1 H, d, *J* 4 Hz, 6-H), 7.40 (6 H, m, ArH), and 7.53 (4 H, m, ArH).

Treatment of (**6b**) with (−)-MTPA using the above procedure yielded the (−)-MTPA diester of (**6b**) (5 mg), δ_H 0.666 (3 H, s, 18-H), 0.899 (3 H, d, *J* 6.8 Hz, 21-H), 0.916 (3 H, d, *J* 6.8 Hz, 27-H), 1.002 (3 H, s, 19-H), 3.553 (3 H, q, $^5J_{H.F}$ 1.2 Hz, OMe), 3.568 (3 H, q, $^5J_{H.F}$ 1.2 Hz, OMe), 4.154 (1 H, dd, *J* 11 and 5.5 Hz, 26-H_a), 4.155 (1 H, dd, *J* 11 and 6.5 Hz, 26-H_b), 4.88 (1 H, m, 3-H), 5.42 (1 H, d, *J* 5 Hz, 6-H), 7.53 and 7.40 (6 H, m, ArH), and 7.53 (4 H, m, ArH).

(25S)-3β-*Acetoxy*-26-*trityloxycholest*-5-*ene* (**7**).—Trityl chloride (134 mg) was added to the diol (**6a**) (97.5 mg) dissolved in dried pyridine (1.2 ml) and the mixture was stirred at 80 °C for 2 h; acetic anhydride (0.8 ml) was then added and the mixture stirred at 80 °C for a further 30 min. Methanol (3 ml) was added to the reaction mixture to destroy the excess of reagents and then ice–water (50 ml) was added. The product was extracted with ethyl acetate (210 ml), washed successively with 0.5% hydrochloric acid (120 ml), water (120 ml), 1% sodium carbonate (120 ml), and water (120 ml), and then evaporated. Chromatography of the residue (300 mg) on silica gel (hexane–chloroform–ethyl acetate, 18:1:1) gave (25*S*)-3-acetoxy-26-trityloxycholest-5-ene (**7**) (118 mg) (together with a small amount of 3,26-bis-tritylate), *m/z* 626 (M^+), δ_H 0.665 (3 H, s, 18-H), 0.865 (3 H, d, *J* 6.5 Hz, 21-H), 0.950 (3 H, d, *J* 6.7 Hz, 27-H), 1.018 (3 H, s, 19-H), 2.031 (3 H, s, Ac), 2.85 and 2.96 (2 H, AB part of ABX, *J* 9, 6.5, and 6 Hz, 26-H), 4.61 (1 H, m, 3-H), 5.38 (1 H, d, *J* 5 Hz, 6-H), and 7.2—7.5 (15 H, m, ArH).

(25S)-3β-*Acetoxycholest*-5-*en*-26-*ol* (**8**).—A solution of 26-*O*-tritylate (**7**) (115 mg, without further purification) in ethanol (7 ml) and hydrochloric acid (0.3 ml) was stirred at room temperature for 2 h. The reaction mixture was diluted with water (70 ml), extracted with ethyl acetate, and the extract washed successively with water, 1% aqueous sodium carbonate, and water, and then evaporated. Chromatography of the residue on silica gel with hexane–chloroform–ethyl acetate (2:1:1) as eluant gave (25*S*)-3-acetoxycholest-5-en-26-ol (**8**) (58 mg), m.p. 132—133 °C (from methanol), $[\alpha]_D^{25}$ −46.6° (*c* 1.00 $CHCl_3$) (Found: C, 78.1; H, 10.7. Calc. for $C_{29}H_{48}O_3$: C, 78.3; H, 10.9); δ_H 0.676 (3 H, s, 18-H), 0.922 (6 H, d, *J* 6.5 Hz, 21-H and 27-H), 1.018 (3 H, s, 19-H), 2.032 (3 H, s, Ac), 3.42 and 3.52 (2 H, AB part of ABX, *J* 10.5, 6.5, and 6 Hz, 26-H), 4.60 (1 H, m, 3-H), and 5.37 (1 H, d, *J* 5 Hz, 6-H); δ_C 37.01 (C-1), 27.78 (C-2), 73.99 (C-3), 38.13 (C-4), 139.69 (C-5), 122.62 (C-6), 29.72 (C-7), 31.88 (C-8), 50.06 (C-9), 36.60 (C-10), 21.03 (C-11), 39.75 (C-12), 42.34 (C-13), 56.70 (C-14), 24.27 (C-15), 28.23 (C-16), 56.12 (C-17), 11.86 (C-18), 19.30 (C-19), 35.85 (C-20*), 18.74 (C-21), 36.27 (C-22), 23.48 (C-23), 33.69 (C-24), 35.78 (C-25*), 68.36 (C-26), 16.71 (C-27), and 21.40 and 170.51 (CH_3CO). (*These assignments may be reversed.)

(25S)-3-*Acetoxy*-26-*tosyloxycholest*-5-*ene* (**9**).—A mixture of the monohydroxy acetate (**8**) (30 mg) and toluene-*p*-sulphonyl chloride (46 mg) in dried pyridine (0.8 ml) was stirred at room temperature for 5 h after which water was added. The product was extracted with ether (210 ml) and the extract washed successively with 0.3% hydrochloric acid (150 ml), water (50 ml), 1% aqueous sodium carbonate (50 ml), and water (150 ml), and then evaporated. Chromatography of the residue (51 mg) on silica gel with hexane–chloroform–ethyl acetate (8:1:1) as eluant gave (25*S*)-3-acetoxy-26-tosyloxycholest-5-ene (**9**) (40 mg); δ_H 0.665 (3 H, s, 18-H), 0.871 (3 H, d, *J* 6.2 Hz, 21-H), 0.881 (3 H, d, *J* 6.5 Hz, 27-H), 1.018 (3 H, s, 19-H), 2.032 (3 H, s, Ac), 2.33 (2 H, d, *J* 8 Hz, 4-H), 2.450 (3 H, s, ArMe), 3.81 and 3.89 (2 H, AB part of ABX, *J* 9, 6.5, and 6 Hz, 26-H), 4.61 (1 H, m, 3-H), 5.38 (1 H, d, *J* 5 Hz, 6-H), and 7.36 and 7.80 (4 H, A_2B_2, *J* 8 Hz, ArH).

(25R)-[26-$^2H_1$]*Cholesterol* (**10**).—Lithium aluminium tetradeuteride ($LiAl^2H_4$) (80 mg) was added to the 26-*O*-tosylate (**9**) (40 mg) in dried ether (4 ml) and stirred at room temperature for 20 h. The excess of reagent was destroyed with 0.5% aqueous sodium hydrogen carbonate and the mixture filtered. The filtrate was then diluted with ether (300 ml), washed with water (300 ml), and then evaporated. Chromatography of the residue (20 mg) on silica gel with hexane–chloroform–ethyl acetate (4:1:1) as eluant gave crude (25*R*)-[26-^{2}H] cholesterol (20 mg), which was purified with reverse-phase h.p.l.c. (column TSKgel-ODS-120T 250 × 20 mm i.d. developed with methanol equipped with a refractometric detector), m.p. 148—148.5 °C (from methanol), $[\alpha]_D^{23.5}$ −40.8° (*c* 0.77 $CHCl_3$); *m/z* 387 (M^+ + ^{1}H 99%), 386 (1%); δ_H 0.679 (3 H, s, 18-H), 0.842 (2 H, dt, $J_{H.H}$ 6.5 and $J_{H.^2H}$ 1.8 Hz, $^1\Delta\delta_{^2H}$ −0.020 p.p.m. 26-H), 0.865 (3 H, d, *J* 6.6 Hz, 27-H), 0.915 (3 H, d, *J* 6.6 Hz, 21-H), 1.009 (3 H, s, 19-H), 3.525 (1 H, m, 3-H), and 5.353 (1 H, d, *J* 5.5 Hz, 6-H); δ_C 37.27 (C-1), 31.69 (C-2), 71.82 (C-3), 42.32 (C-4), 140.76 (C-5), 121.72 (C-6), 31.92 (C-7 and C-8), 50.15 (C-9), 36.52 (C-10), 21.10 (C-11), 39.80 (C-12), 42.32 (C-13), 56.78 (C-14), 24.30 (C-15), 28.24 (C-16), 56.16 (C-17), 11.87 (C-18), 19.41 (C-19), 35.79 (C-20), 18.73 (C-21), 36.20 (C-22), 23.83 (C-23), 39.50

(C-24), 27.94 ($^2\Delta\delta_{C(^2H)}$ −0.06 p.p.m. C-25), 22.26 (t, $^1J_{C,^2H}$ 19 Hz, $^1\Delta\delta_{C(^2H)}$ −0.3 p.p.m., C-26), and 22.80 (C-27).

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