Stereoselective Introduction of Hydroxy Groups into the Cholesterol Side Chain. Preparation of (24*R*)- and (24*S*)-24,25-Dihydroxy- and (25*R*)- and (25*S*)-25,26-Dihydroxyvitamin D_3 by Asymmetric Synthesis

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24,25-Epoxy-26-hydroxy-3β-tetrahydropyranyloxycholest-5-enes (7a) and (8a), prepared by asymmetric epoxidation of the allylic alcohol (4), and 24-hydroxy-3β-tetrahydropyranyloxycholesta-5,25-dienes (11) and (12), synthesized by asymmetric reduction of the enone (6), were stereoselectively converted into 25,26- and 24,25-dihydroxycholesterol derivatives, which could be transformed into 25,26- and 24,25-dihydroxyvitamin D_3. The highly stereoselective epoxide cleavage of 26-benzoyloxy-24,25-epoxides (7b), (8b), (25), and (26) was found to proceed with retention at C-24.

The biological activities of various physiologically active steroids which have functional groups in the side chain depend on the stereochemical configuration of those functional groups.[1] For the synthesis of those steroids, such as vitamin D metabolites, ecdysteroids, bile alcohols, and other biologically active steroids, the stereoselective introduction of hydroxy groups into the sterol side chain is one of the most important steps. Among a number of vitamin D_3 metabolites, 24,25-dihydroxyvitamin D_3[2] and 25,26-dihydroxyvitamin D_3[3] were isolated by DeLuca *et al.* in 1972 and 1970, respectively. The former was shown by our group to have *R* configuration at C-24,[4] whereas the latter is proposed to have *S* configuration at C-25.[5-7] In our previous synthesis of the two metabolites,[8,9] both epimers at C-24 or C-25 were synthesized by a route which involves a rather tedious separation of the epimers of the synthetic intermediates. Recently, several stereoselective syntheses of these metabolites using chiral synthons have been reported.[5,6,10]

Our continuing interest in the synthesis of biologically active steroids led us to explore more convenient methods for the stereoselective introduction of hydroxy groups at C-24, -25, and -26. Many examples are reported of the stereoselective introduction of a hydroxy group at C-22 of the steroid side chain[11] because it is easy to employ the chiral environment of the steroid nucleus as well as the C-20 position in such synthetic reactions. However, it is more difficult to achieve high stereoselectivity at C-23, -24, and -25, because these positions are remote from this influence, and application of an asymmetric reagent therefore seemed to be a rational approach to a chiral centre at C-24 or -25. The chirality generated in such an initial asymmetric reaction could then be transferred to an appropriate position,[12] if necessary.

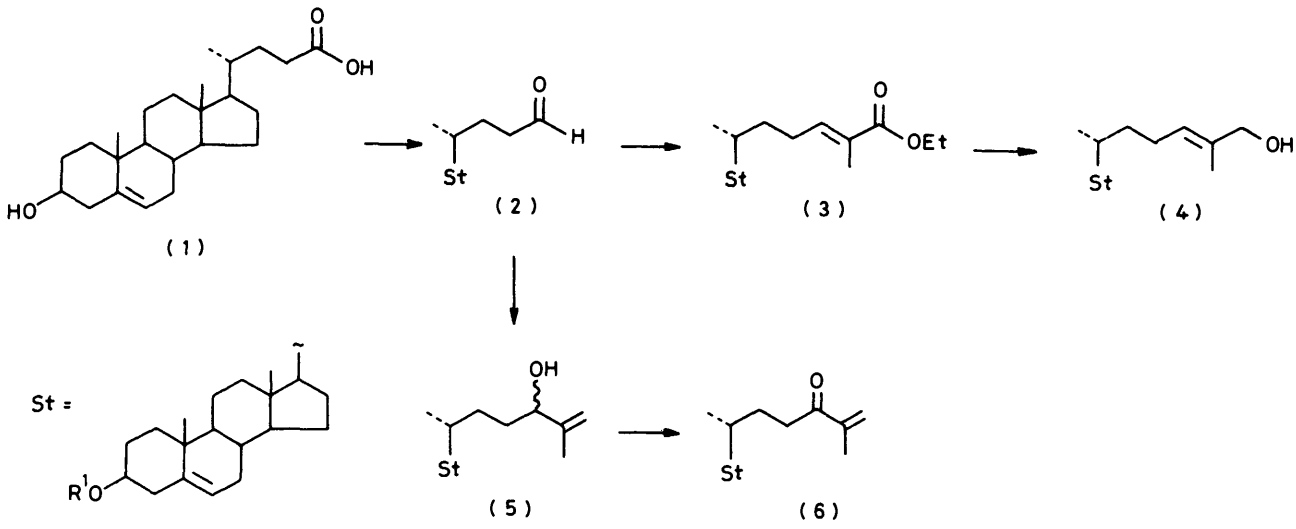
This approach was accomplished by application of asymmetric epoxidation, developed by Sharpless *et al.*,[13] to the 24-en-26-ol (4), and of asymmetric reduction reported by Noyori *et al.*[14,15] to the 25-en-24-one (6).[16] The products obtained by these procedures were subsequently converted into (24*R*)- and (24*S*)-24,25-dihydroxyvitamin D_3 and (25*R*)- and (25*S*)-25,26-dihydroxyvitamin D_3. These results are described in this paper with full experimental details.

The readily available 3β-hydroxychol-5-enoic acid (1) was treated with 2,3-dihydropyran (2.8 equiv.) in benzene–tetrahydrofuran, and the reaction mixture was directly reduced with lithium aluminium hydride under reflux in the same solvent, to afford 3β-tetrahydropyranyloxychol-5-en-24-ol in 92% yield. Treatment of the 24-ol with pyridinium chlorochromate in dichloromethane gave 3β-tetrahydropyranyloxychol-5-en-24-al (2) in 73% yield. Wittig reaction of the 24-aldehyde (2) with α-ethoxycarbonylethylidenetriphenylphosphorane afforded the (24*E*)-ester (3) in 97% yield.[9] Compound (3) was converted into (24*E*)-3β-tetrahydropyranyloxy-26-hydroxycholesta-5,24-diene (4) with aluminium hydride in 75% yield. Treatment of the allylic alcohol (4) with t-butyl hydroperoxide, titanium tetraisopropoxide and D-(−)-diethyl tartarate afforded (24*R*,25*R*)-24,25-epoxy-26-hydroxy-3β-tetrahydropyranyloxycholest-5-ene (7a) in 70% yield. By the same procedure, (24*S*,25*S*)-24,25-epoxy-26-hydroxy-3β-tetrahydropyranyloxycholest-5-ene (8a) was also synthesized in 86% yield with L-(+)-diethyl tartarate in place of the D-(−)-antipode. The configuration at C-24 and -25 of the epoxy alcohols (7a) and (8a) was tentatively assigned by Sharpless' prediction,[13] and this was confirmed later. Both epoxides (7a) and (8a) were converted into (25*S*)-25,26-dihydroxycholesterol (9b) and (25*R*)-25,26-dihydroxycholesterol (10b), respectively, by lithium aluminium hydride reduction in refluxing tetrahydrofuran followed by acid hydrolysis of the tetrahydropyranyl group.

The stereoselectivity of the asymmetric epoxidation was revealed by high pressure liquid chromatography (h.p.l.c.) analysis of the 3,26-bis-(+)-α-methoxy-α-trifluoromethyl-α-phenylacetates (MTPA) (9c) and (10c) of the 25,26-dihydroxycholesterols (9b) and (10b), as shown in Figure 1, and proved to be over 98%. The MTPA derivatives (9c) and (10c) could also be distinguished by their ^{1}H n.m.r. spectra. The signal of the C-26 methylene protons for the (25*S*)-isomers was observed as a quartet at δ 4.17, whereas that for the (25*R*)-compound appeared as a singlet, as shown in Figure 2.

Compounds (9b) and (10b), whose stereochemical purities were over 99% after recrystallization, were converted into the diacetates (9d) and (10d) with excess of acetic anhydride in pyridine. According to a single crystal *X*-ray analysis, the diacetate (10d), derived from the epoxidation product in the presence of L-(+)-diethyl tartarate, has space group $P2_1$, with $a = 14.862$ (6) Å, $b = 7.498$ (3) Å, $c = 13.492$ (5) Å, $\beta = 105.03$ (7)°, and $Z = 2$. The number of unique reflections and final value of *R* are 2 791 and 5.1%, respectively. The perspective view represented in Figure 3 clearly indicated that the compound (10d) has a (25*R*)-configuration. Thus, the stereochemistry of the epoxy alcohols, (7a) and (8a), was revealed, and the asymmetric epoxidation of the 24-en-26-ol (4) therefore proceeds as predicted by Sharpless *et al.*

The diacetates (9d) and (10d) were brominated with *N*-bromosuccinimide in carbon tetrachloride and the resulting 7-bromo compounds were dehydrobrominated with s-collidine in refluxing xylene. (25*S*)-3β,26-Diacetoxy-25-hydroxycholesta-5,7-diene (26%) and (25*R*)-3β,26-diacetoxy-



Scheme 1. R^1 = Tetrahydropyranyl (THP)

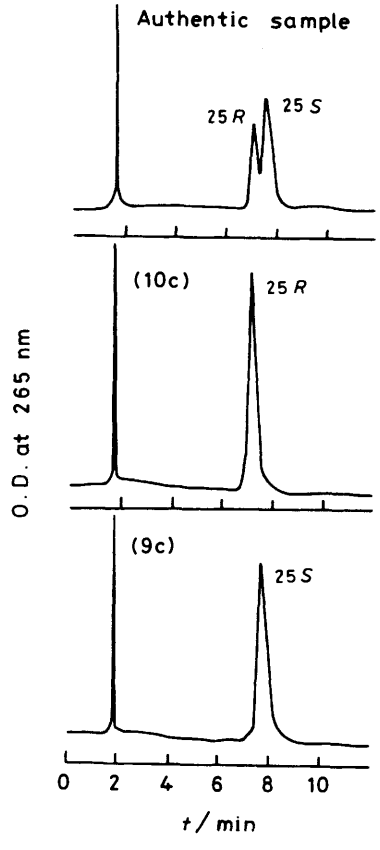


Figure 1. H.p.l.c. analysis of MTPA derivatives of 25,26-dihydroxycholesterol. Column: Zorbax CN(4.6 mm × 25 cm); eluant, hexane–dichloromethane (5 : 1); flow rate, 2.0 ml/min

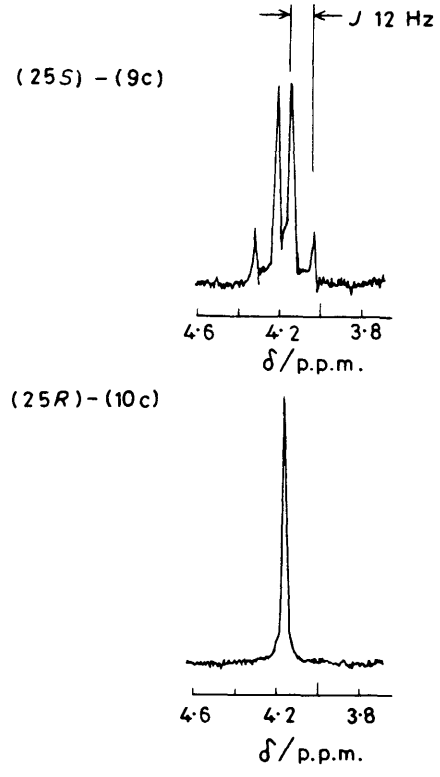


Figure 2. ^1H N.m.r. spectra of MTPA derivatives of 25,26-dihydroxycholesterol

25-hydroxycholesta-5,7-diene (28%) were isolated by preparative t.l.c. Irradiation of the 5,7-dienes in benzene–ethanol with a medium-pressure mercury lamp afforded crude precalciferols, which were refluxed to effect thermal isomerization into vitamin D_3. The desired (25*S*)-25,26-dihydroxyvitamin D_3 (19) (18%), and (25*R*)-25,26-dihydroxyvitamin D_3 (20) (22%) were isolated by alkaline hydrolysis followed by preparative h.p.l.c.*

The key intermediates for the preparation of (24*R*)- and (24*S*)-24,25-dihydroxyvitamin D_3, (24*R*)- and (24*S*)-24,25-dihydroxycholesterols (17b) and (18b), were readily synthesized by another asymmetric reaction. The 3-tetrahydropyranyloxy 24-aldehyde (2) was treated with propenylmagnesium bromide (1.4 equiv.), to give 24-hydroxy-3β-tetrahydropy-

* (25*S*)- and (25*R*)-25,26-Dihydroxyvitamin D_3 were easily separable as the 3,26-bis-MTPA ester by h.p.l.c., and co-chromatography with radiolabelled natural metabolite was accomplished. The result will be presented in a forthcoming paper.

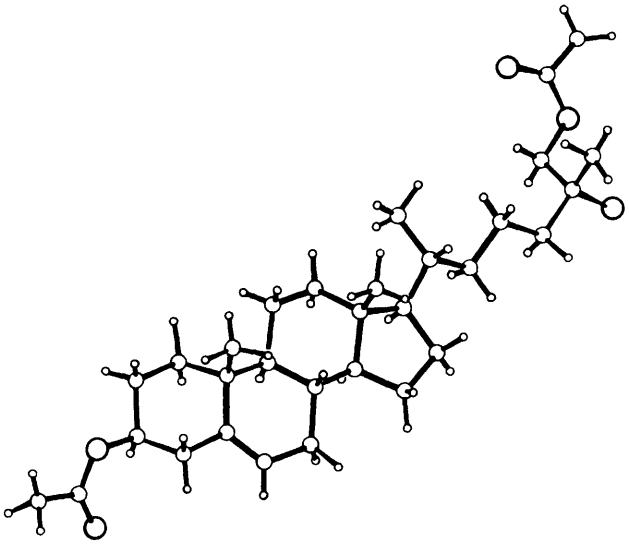


Figure 3. Perspective view of the molecular structure of (24*R*)-3,26-diacetoxy-25-hydroxycholesterol (10d)

ranyloxycholesta-5,25-diene (5) in 88% yield. Oxidation of the allylic alcohol (5) with pyridinium dichromate [17] gave 24-oxo-3β-tetrahydropyranyloxycholesta-5,25-diene (6) in 90% yield. When the 24-oxo compound (6) was treated with 3 equiv. of the reducing reagent, prepared from lithium aluminium hydride, ethanol, and (*R*)-(+)-2,2′-dihydroxy-1,1′-binaphthyl, which was developed by Noyori *et al.*,[14,15] (24*R*)-24-hydroxy-3β-tetrahydropyranyloxycholesta-5,25-diene (11) was obtained in 75% yield. The enone (6) was similarly converted into (24*S*)-24-hydroxy-3β-tetrahydropyranyloxycholesta-5,25-diene (12) with (*S*)-(−)-2,2′-dihydroxy-1,1′-binaphthyl in place of the (*R*)-(+)-compound. Compounds (13) and (14) were formed from the asymmetric reduction products (11) and (12) by a benzoylation followed by hydrogenation and the stereochemistry was determined by a comparison with authentic (24*R*)- and (24*S*)-3β,24-dibenzoyloxycholest-5-enes [18] (13) and (14) with respect to the mobility on h.p.l.c. The chromatography of (13) and (14) also showed that the stereoselectivity in the each reduction was 97 : 3.

The allylic alcohols (11) and (12), obtained by the asymmetric reduction, were epoxidized with t-butyl hydroperoxide and $VO(acac)_2$ [19] in benzene, to give respectively (24*R*,25*S*)-25,26-epoxy-24-hydroxy-3β-tetrahydropyranyloxycholest-5-ene (15) (70%) and (24*S*,25*R*)-25,26-epoxy-24-hydroxy-3β-tetrahydropyranyloxycholest-5-ene (16) (72%). The configuration at C-25 was determined by the conversion of those respective epoxides into the isomeric epoxy alcohols (7a) and (8a). Treatment of (24*R*)- and (24*S*)-25,26-epoxy-24-alcohols (15) and (16) with potassium carbonate in refluxing propan-2-ol gave (24*R*,25*R*)- and (24*S*,25*S*)-24,25-epoxy-26-hydroxy-3β-tetrahydropyranyloxycholest-5-enes (7a) and (8a) in *ca.* 85% yield, respectively. The synthesis of (7a) and (8a) by this procedure provides an alternative route for the synthesis of 25,26-dihydroxycholesterol. As the stereochemistry at C-25 in the epoxide rearrangement is expected to be complete inversion, (24*R*)- and (24*S*)-25,26-epoxy-24-alcohols (15) and (16) should be the (24*R*,25*S*)- and (24*S*,25*R*)-compounds, respectively. Therefore, the stereochemistry of the epoxidation coincides with the results reported by Sharpless *et al.*[19] The stereoselectivity of the Sharpless epoxidation (>96 : 4) was deduced from the analysis by h.p.l.c. of the 3,26-bis-MTPA esters (9c) and (10c), as described before.

Treatment of the epoxy alcohols (15) and (16) with lithium aluminium hydride afforded (24*R*)- and (24*S*)-24,25-dihydroxy-3β-tetrahydropyranyloxycholest-5-enes (17a) and (18a) quantitatively, which were converted into the (24*R*)- and (24*S*)-24,25-dihydroxycholesterols [8] (17b) and (18b), respectively, by acid hydrolysis. By essentially the same method as described for the 25,26-dihydroxy compounds (9b) and (10b), the individual 24,25-diols (17b) and (18b) can be converted into the corresponding (24*R*)- and (24*S*)-24,25-dihydroxyvitamin D_3 [8] (21) and (22).

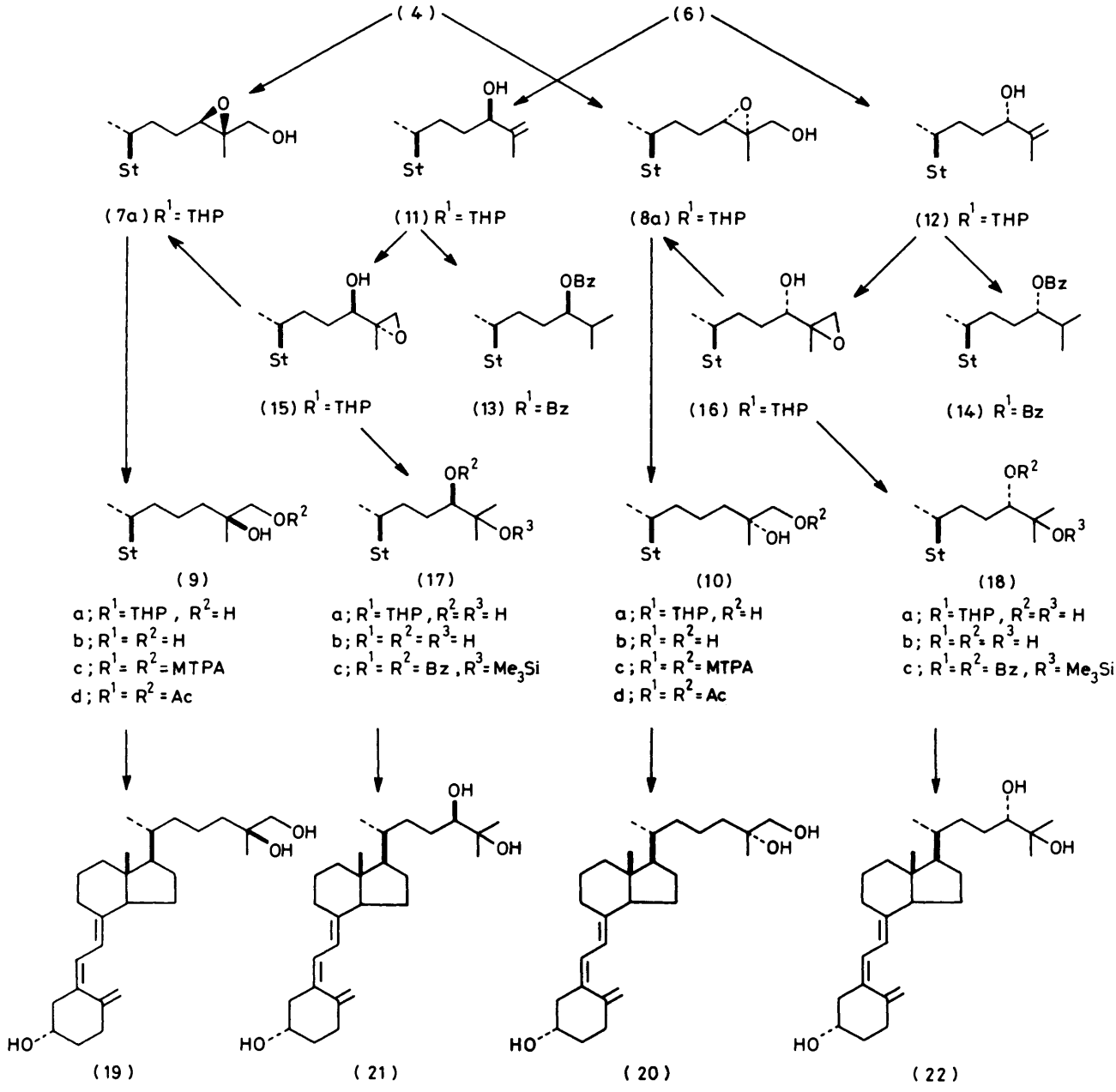
In our earlier synthesis of 25,26-dihydroxyvitamin D_3,[9] the 24,25-epoxy-26-benzoates (25) and (26) were key intermediates which were obtained from the allylic alcohol (4) by OsO_4 oxidation followed by 24-monotosylation and base treatment. According to the reaction mechanism, the 24,25-epoxy-26-benzoates (25) and (26) should be epimeric with (7a) and (8a). Reduction of the more polar 24,25-epoxy-26-benzoate (25) and the less polar isomer (26) with lithium aluminium hydride gave [9] diols which have been identified above as (25*R*)- and (25*S*)-25,26-dihydroxycholesterols (10b) and (9b). The structures previously formulated [9] for (25) and (26) are therefore in error and they are now assigned (24*R*,25*S*)- and (24*S*,25*R*)-configurations, respectively.

These compounds (25) and (26) could be correlated to the (24*R*)- and (24*S*)-24,25-dihydroxycholesterols (17b) and (18b) by the following procedure, as shown in Scheme 4. Treatment of the more polar epoxy-benzoate (25) with aqueous perchloric acid in tetrahydrofuran afforded a mixture of 25,26-dihydroxy-24-benzoate (27a) and 24,25-dihydroxy-26-benzoate (27b) in the ratio 2 : 3, respectively. As the individual isomer (27a) or (27b) gave a similar mixture of (27a) and (27b) under the same acidic conditions, these compounds were concluded to be equilibrating in these conditions. Monotosylation of (27a) followed by lithium aluminium hydride reduction gave (24*R*)-24,25-dihydroxycholesterol (17b). Similar treatment of the less polar epoxy benzoate (26) afforded (24*S*)-24,25-dihydroxycholesterol (18b). Identification of (17b) and (18b) with authentic samples of (24*R*)- and (24*S*)-isomers, respectively, was carried out by h.p.l.c. analysis of their 3,24-dibenzoyloxy-25-trimethylsilyl derivatives (17c) and (18c).[8] Thus, it is concluded that the epoxide opening reaction proceeds with retention at C-24 and with inversion at C-25 * (Scheme 3). Therefore, the reaction mechanism which involves inversion at C-24, reported before,[9] has to be revised. The mechanism of the reaction was also independently confirmed by experiments using ^{18}O compounds.[20]

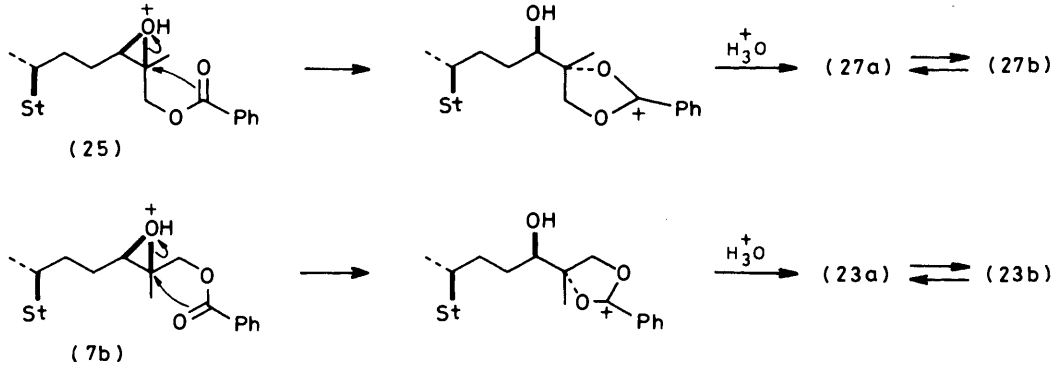
Analogously, reaction of the (24*R*,25*R*)- and (24*S*,25*S*)-24,25-epoxy-26-benzoates (7b) and (8b) with aqueous perchloric acid yielded a equilibrating mixture of (23a) and (23b), and of (24a) and (24b), in the ratio 2 : 3, respectively (Scheme 4). Subsequently, the 25,26-dihydroxy-24-benzoates (23a) and (24a) could also be converted into 24,25-dihydroxycholesterols (17b) and (18b), respectively, *via* deoxygenation of the 26-toluene-*p*-sulphonates (23c) and (24c). This route constitutes an alternative and highly stereoselective synthesis of (24*R*)- and (24*S*)-24,25-dihydroxycholesterol derivatives.

The present investigation has demonstrated the utility of asymmetric reaction to construct the chiral steroidal side chain. Both asymmetric epoxidation of the allylic alcohol (4) and asymmetric reduction of the enone (6) facilitated the highly stereoselective synthesis of 25,26- and 24,25-dihydroxycholesterol, which were converted into their vitamin D_3 derivatives.

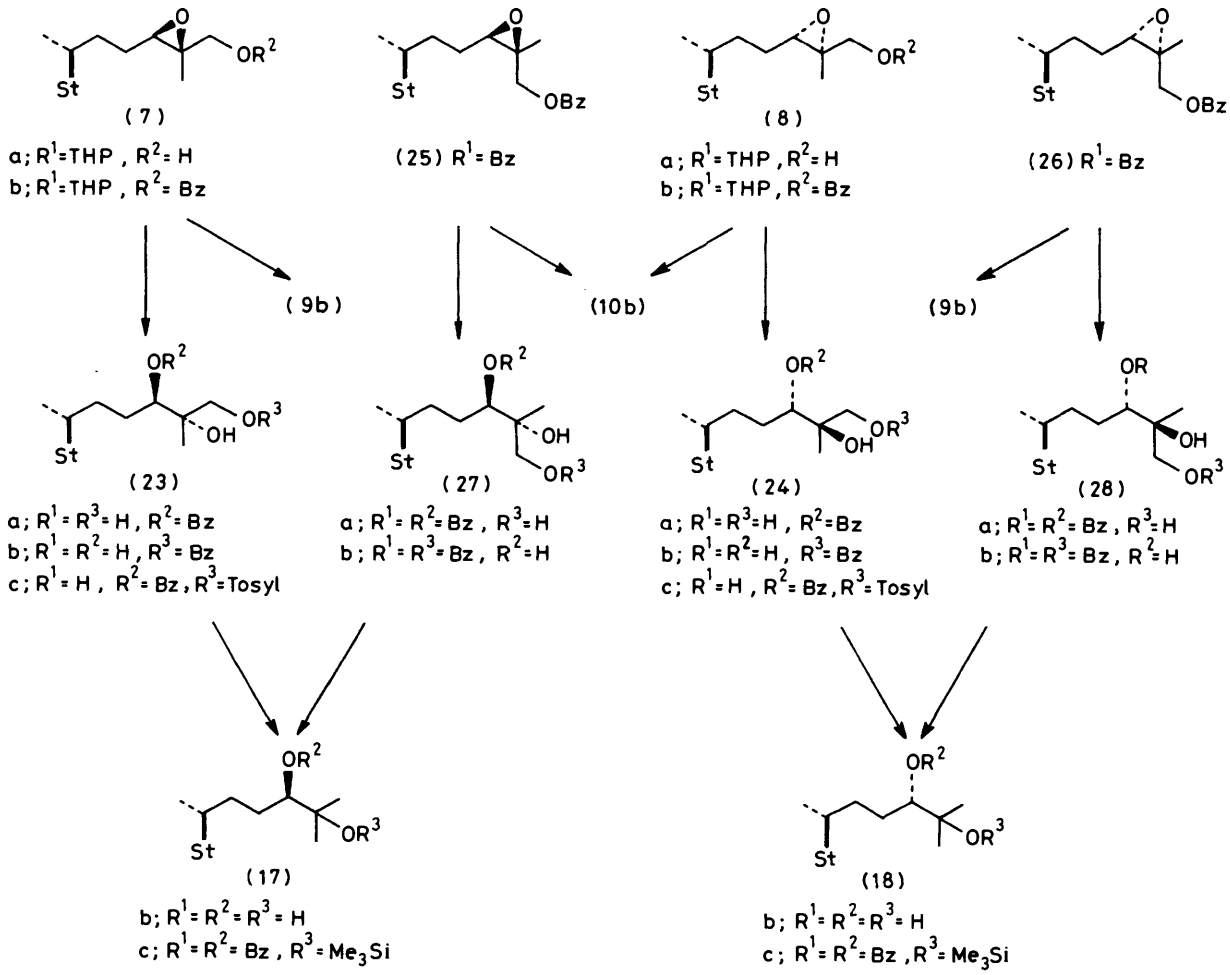
* The configuration at C-25 of (23), (24), (27), and (28) was tentatively assigned based on the inversion at C-25 during the epoxide opening reaction.



Scheme 2. St as in Scheme 1



Scheme 3. St as in Scheme 1



Scheme 4. St as in Scheme 1

Experimental

M.p.s were determined with a hot stage microscope apparatus. Specific rotation was determined with a Karl Zeiss photometric polarimeter. I.r. spectra were recorded with a Hitachi 260-10 spectrometer and u.v. spectra with a Shimadzu UV-200 instrument. N.m.r. spectra were obtained with a Hitachi R-24A, a JEOL PS-100, or a JEOL FX-400 spectrometer with tetramethylsilane as internal standard. Mass spectra were run on a Shimadzu LKB-9000S spectrometer. Column chromatography was performed with silica gel (E. Merck silica gel 60). T.l.c. was carried out on pre-coated plates of silica gel (E. Merck). The usual work-up refers to dilution with water, extraction with an organic solvent, washing to neutrality, drying ($MgSO_4$), filtration, and evaporation under reduced pressure. Ether refers to diethyl ether.

3β-*Tetrahydropyranyloxychol-5-en*-24-*al* (2).—A mixture of cholenoic acid (1) (50 g), dihydropyran (40 ml), toluene-*p*-sulphonic acid (100 mg) and benzene (1.5 l) was stirred at room temperature for 2 h, and then tetrahydrofuran (THF) (50 ml) was added, followed by lithium aluminium hydride (5 g) with stirring. After further stirring for 1 h, a saturated solution of water in ether (100 ml) and then water (30 ml) were carefully added. The resulting precipitate was filtered off and washed with ethyl acetate. The combined organic fraction was dried ($MgSO_4$) and evaporated to dryness to give 24-hydroxy-3β-tetrahydropyranyloxychol-5-ene (50 g), which was used without further purification in the following reaction.

Pyridinium chlorochromate (4.39 g) was added to a solution of the 24-alcohol (4.49 g) in dichloromethane (100 ml) at room temperature, and the mixture was stirred at room temperature for 3 h. The reaction mixture diluted with anhydrous ether (500 ml) was passed through a column of Florisil (50 g), and was then purified by column chromatography on silica gel (42 g) (solvent, benzene), to give the 24-*aldehyde* (2) (3.26 g); δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.93 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H, 10-Me), 4.70 (m, 1 H, acetal H), 5.34 (m, 1 H, 6-H), and 9.74 (s, 1 H, CHO); $\nu_{max.}$ ($CHCl_3$) 1 720 cm^{-1} (Found: C, 78.6; H, 10.8. $C_{29}H_{46}O_3$ requires C, 78.68; H, 10.47%).

Ethyl (24E)-3β-*Tetrahydropyranyloxycholesta*-5,24-*dien*-26-*oate* (3).—3β-Tetrahydropyranyloxychol-5-en-24-al (2) (5.09 g) was dissolved in dry benzene (30 ml) and α-ethoxycarbonylethylidenetriphenylphosphorane (7.26 g) was added. The reaction mixture was stirred at room temperature under argon for 2 days. The usual work-up (ether extraction) gave a crude product (12.89 g) which was chromatographed on silica gel (100 g). Elution with benzene–ethyl acetate (100 : 1) gave the *ester* (3) (5.88 g), m.p. 105—107 °C (hexane–ether); δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H, 10-Me), 1.28 (t, 3 H, *J* 7 Hz, $CO_2CH_2CH_3$), 1.83 (s, 3 H, 25-Me), 4.18 (q, 2 H, *J* 7 Hz, $CO_2CH_2CH_3$),

4.70 (m, 1 H, acetal H), 5.34 (m, 1 H, 6-H), and 6.74 (m, 1 H, 24-H); $\nu_{max.}$ ($CHCl_3$) 1 690 cm^{-1} (Found: C, 80.2; H, 10.3. $C_{34}H_{52}O_3$ requires C, 80.26; H, 10.30%).

(24E)-26-*Hydroxy*-3β-*tetrahydropyranyloxycholesta*-5,24-*diene* (4).—To a stirred mixture of aluminium chloride (443 mg) and THF (5 ml), was added lithium aluminium hydride (380 mg) under argon at room temperature, and the mixture was stirred at room temperature for 40 min. A solution of the ester (3) (263 mg) in THF (5 ml) was added, and the reaction mixture was stirred at room temperature for 45 min under argon. The usual work-up (ethyl acetate extraction) gave a crude product (245 mg), which was applied to a column of silica gel (5 g). Elution with benzene–ethyl acetate (100 : 1) afforded the *alcohol* (4) (181 mg), m.p. 124—125 °C (acetone); δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.97 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H, 10-Me), 1.68 (s, 3 H, 25-Me), 4.70 (m, 1 H, acetal H), and 5.20—5.50 (m, 2 H, 6- and 24-H) (Found: C, 79.15; H, 11.05. $C_{32}H_{52}O_3$ requires C, 79.28; H, 10.81%).

24-*Hydroxy*-3β-*tetrahydropyranyloxycholesta*-5,25-*diene* (5).—To a solution of propenylmagnesium bromide prepared with magnesium (109 mg) and propenyl bromide (0.45 ml) in THF (7 ml), was added the 24-aldehyde (2) (1.5 g) in THF (4 ml) at room temperature, and stirring of the reaction mixture was continued at room temperature for 3 h. After addition of ether and aqueous ammonium chloride solution, the usual work-up (ether extraction) was performed to give the 24ξ-*alcohol* (5) (1.4 g), m.p. 120—126 °C (acetone); $[\alpha]_D^{25}$ −32.4° (*c* 1, $CHCl_3$); δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.96 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H, 10-Me), 1.72 (s, 3 H, 25-Me), 4.70 (m, 1 H, acetal H), 4.82 (br s, 1 H, 26-H), 4.91 (br s, 1 H, 26-H), and 5.34 (m, 1 H, 6-H) (Found: C, 79.0; H, 11.15. $C_{32}H_{52}O_3$ requires C, 79.28; H, 10.81%).

3β-*Tetrahydropyranyloxycholesta*-5,25-*dien*-24-*one* (6).—To a solution of the allylic alcohol (5) (129 mg) in dichloromethane (2 ml) and *N*,*N*-dimethylformamide (2 ml) was added pyridinium dichromate (238 mg). The reaction mixture was stirred at room temperature for 2 h, and the usual work-up (ether extraction) afforded a crude product (143 mg). Recrystallization from methanol–acetone–dichloromethane gave the *enone* (6) (115.4 mg), m.p. 136—138 °C; $[\alpha]_D^{25}$ −30.5° (*c* 1, $CHCl_3$); δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.93 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H, 10-Me), 1.87 (s, 3 H, 25-Me), 4.70 (m, 1 H, acetal H), 5.32 (m, 1 H, 6-H), 5.68 (s, 1 H, 26-H), and 5.88 (s, 1 H, 26-H); $\nu_{max.}$ ($CDCl_3$) 1 670 cm^{-1} (Found: C, 79.6; H, 10.45. $C_{32}H_{50}O_3$ requires C, 79.62; H, 10.44%).

(24R,25R)-24,25-*Epoxy*-26-*hydroxy*-3β-*tetrahydropyranyloxycholest*-5-*ene* (7a).—To a solution of titanium tetraisopropoxide (0.49 ml) and D-(−)-diethyl tartarate (0.28 ml) in dry dichloromethane (15 ml), were added a solution of the allylic alcohol (4) (800 mg) in dry dichloromethane (15 ml) and anhydrous t-butyl hydroperoxide (0.33 ml) at −20 °C. The mixture was kept at −25 °C for 18 h, and an aqueous solution (10%) of tartaric acid (4.2 ml) was then added with stirring at −20 °C. The mixture was stirred at −20 °C for 30 min and at room temperature for 1 h. The reaction product was extracted with dichloromethane, washed with water, dried over $MgSO_4$, and concentrated under reduced pressure to afford a colourless oil (1.154 g), which was applied to a column of silica gel (40 g). Elution with benzene–ethyl acetate (20 : 1) gave the *epoxy alcohol* (7a) (652 mg), m.p. 135—138 °C (acetone); $[\alpha]_D^{25}$ −4.8° (*c* 1, $CHCl_3$); δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H, 10-Me), 1.28 (s, 3 H, 25-Me), 3.00 (m, 1 H, 24-H), 4.70 (m, 1 H, acetal H), and 5.34 (m, 1 H, 6-H) (Found: C, 76.65; H, 10.5. $C_{32}H_{52}O_4$ requires C, 76.75; H, 10.47%).

(24S,25S)-24,25-*Epoxy*-26-*hydroxy*-3β-*tetrahydropyranyloxycholest*-5-*ene* (8a).—The allylic alcohol (4) (805 mg), L-(+)-diethyl tartarate (0.28 ml), titanium tetraisopropoxide (0.49 ml) and t-butyl hydroperoxide (0.33 ml) in dry dichloromethane (30 ml) were treated similarly, to give (24S,25S)-*epoxide* (8a) (717 mg), m.p. 130—132 °C (acetone); $[\alpha]_D^{15}$ −38.4° (*c* 1, $CHCl_3$); δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H, 10-Me), 1.28 (s, 3 H, 25-Me), 3.00 (m, 1 H, 24-H), 4.70 (m, 1 H, acetal H), and 5.34 (m, 1 H, 6-H) (Found: C, 76.6; H, 10.6. $C_{32}H_{52}O_4$ requires C, 76.75; H, 10.47%).

(24R,25R)-24,25-*Epoxy*-26-*hydroxy*-3β-*tetrahydropyranyloxycholest*-5-*ene* (7a) *from the Epoxy Alcohol* (15).—A mixture of the epoxy alcohol (15) (25 mg) and potassium carbonate (25 mg) in propan-2-ol (2 ml) was refluxed for 2 h. The usual work-up (ether extraction) afforded a crude product (49 mg). Purification by preparative t.l.c. [solvent, benzene–ethyl acetate (1 : 1)] gave the 24,25-epoxy-26-alcohol (7a) (21 mg).

(24S,25S)-24,25-*Epoxy*-26-*hydroxy*-3β-*tetrahydropyranyloxycholest*-5-*ene* (8a) *from the Epoxy Alcohol* (16).—The epoxy alcohol (16) (20 mg) and potassium carbonate in propan-2-ol were treated similarly to give (8a) (17 mg).

(25S)-25,26-*Dihydroxy*-3β-*tetrahydropyranyloxycholest*-5-*ene* (9a).—To a solution of the epoxy alcohol (7a) (489 mg) in THF (50 ml), was added lithium aluminium hydride (112 mg), and the mixture was refluxed for 2 h. A saturated solution of water in ether (100 ml) was added to the mixture, which was filtered. Usual work-up (ethyl acetate extraction) of the filtrate afforded the 25,26-*diol* (9a) (367 mg), m.p. 169—171 °C (acetone–methanol); $[\alpha]_D^{20}$ −36.2° (*c* 1, $CHCl_3$); δ 0.68 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H, 10-Me), 1.17 (s, 3 H, 25-Me), 3.43 (m, 2 H, 26-H), 4.70 (m, 1 H, acetal H), and 5.34 (m, 1 H, 6-H) (Found: C, 76.15; H, 10.7. $C_{32}H_{54}O_4$ requires C, 76.44; H, 10.83%).

(25R)-25,26-*Dihydroxy*-3β-*tetrahydropyranyloxycholest*-5-*ene* (10a).—The epoxy alcohol (8a) (600 mg) in dry THF was similarly reduced with lithium aluminium hydride (137 mg), to give the 25,26-*diol* (10a) (534 mg), m.p. 148—151 °C (acetone–methanol); $[\alpha]_D^{17}$ −14.4° (*c* 1, $CHCl_3$); δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.93 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H, 10-Me), 1.18 (s, 3 H, 25-Me), 3.42 (m, 2 H, 26-H), 4.68 (m, 1 H, acetal H), and 5.34 (m, 1 H, 6-H) (Found: C, 76.35; H, 10.75. $C_{32}H_{54}O_4$ requires C, 76.44; H, 10.83%).

(25S)-3β,25,26-*Trihydroxycholest*-5-*ene* (9b).—The tetrahydropyranyl ether (9a) (267 mg) was stirred in methanol (20 ml) and 2M-aqueous hydrochloric acid (60 μl) at room temperature for 1 h. Usual work-up (ethyl acetate extraction) provided the *triol* (9b) (215 mg), m.p. 181—183 °C (methanol); $[\alpha]_D^{25}$ −28° (*c* 0.1, methanol); *m*/*z* 418 (M^+), 400, 382, 273, and 255 (Found: C, 77.25; H, 11.35. $C_{27}H_{46}O_3$ requires C, 77.46; H, 11.08).

(25R)-3β,25,26-*Trihydroxycholest*-5-*ene* (10b).—The tetrahydropyranyl ether (10a) (403 mg) was treated similarly with hydrochloric acid in methanol to give the *triol* (10b) (306 mg), m.p. 192—194 °C (methanol); $[\alpha]_D^{21}$ −40° (*c* 0.1, methanol); *m*/*z* 418 (M^+), 400, 382, 273, and 255 (Found: C, 77.4; H, 11.1. $C_{27}H_{46}O_3$ requires C, 77.46; H, 11.08%).

(25S)-25-*Hydroxy*-3β,26-*bis*(α-*methoxy*-α-*trifluoromethyl*-α-*phenylacetoxy*) (9c).—The triol (9b) (34 mg) was stirred with

(+)-α-methoxy-α-trifluoromethyl-α-phenylacetyl chloride (120 mg) in pyridine (2 ml) for 18 h. Usual work-up (ethyl acetate extraction) and purification with preparative t.l.c. [solvent, benzene–ethyl acetate (10 : 1)] gave the 3β,26-*diMTPA ester* (9c) (56 mg), δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.90 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H, 10-Me), 1.20 (s, 3 H, 25-Me), 3.56 (s, 6 H, OMe), 4.09 (d, 1 H, *J* 12 Hz, 26-H), 4.26 (d, 1 H, *J* 12 Hz, 26-H), 4.68—5.08 (m, 1 H, 3α-H), 5.38 (m, 1 H, 6-H), and 7.28—7.68 (m, 10 H, Ph) (Found: C, 66.15; H, 7.1. $C_{47}H_{60}F_6O_7$ requires C, 66.33; H, 7.11%).

(25R)-25-*Hydroxy*-3β,26-*bis*(α-*methoxy*-α-*trifluoromethyl*-α-*phenylacetoxy*) (10c).—Similar treatment of the triol (10b) (40 mg) yielded 3β,26-diMTPA ester (10c) (71 mg); δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.90 (d, 3 H, *J* 6 Hz, 20-Me), 1.00 (s, 3 H, 10-Me), 1.18 (s, 3 H, 25-Me), 3.56 (s, 6 H, OMe), 4.17 (s, 2 H, 26-H), 4.68—5.08 (m, 1 H, 3α-H), 5.39 (m, 1 H, 6-H), and 7.24—7.68 (m, 10 H, Ph).

(25S)-3β,26-*Diacetoxy*-25-*hydroxycholest*-5-*ene* (9d).—A solution of the triol (9b) (49 mg) in pyridine (2 ml) and acetic anhydride (1 ml) was stirred at room temperature for 18 h. After the usual work-up (ethyl acetate extraction), the crude product was chromatographed on silica gel. Elution with benzene–ethyl acetate (100 : 1) provided the *diacetate* (9d) (48 mg), m.p. 116—118 °C (methanol–acetone); $[\alpha]_D^{25}$ −38.5° (*c* 0.68, $CHCl_3$); δ(400 MHz, CCl_4) 0.68 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H, 10-Me), 1.12 (s, 3 H, 25-Me), 1.95 (s, 3 H, Ac), 2.04 (s, 3 H, Ac), 3.849 (d, 1 H, *J* 1.2 Hz, 26-H), 3.852 (d, 1 H, *J* 1.2 Hz, 26-H), 4.48 (m, 1 H, 3α-H), and 5.34 (m, 1 H, 6-H); $\nu_{max.}$ ($CHCl_3$) 1 725 cm^{-1} (Found: C, 74.15; H, 10.0. $C_{31}H_{50}O_5$ requires C, 74.06; H, 10.03%).

(25R)-3β,26-*Diacetoxy*-25-*hydroxycholest*-5-*ene* (10d).—The triol (10b) (162 mg) was acetylated similarly with acetic anhydride (1 ml) in pyridine (2 ml) to give the *diacetate* (10d) (185 mg), m.p. 151—153 °C (methanol–acetone); $[\alpha]_D^{14}$ −31.1° (*c* 0.88, $CHCl_3$); δ(400 MHz; CCl_4) 0.68 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H, 10-Me), 1.12 (s, 3 H, 25-Me), 1.95 (s, 3 H, Ac), 2.04 (s, 3 H, Ac), 3.826 (d, 1 H, *J* 11.2 Hz, 26-H), 3.871 (d, 1 H, *J* 11.2 Hz, 26-H), 4.48 (m, 1 H, 3-H), and 5.34 (m, 1 H, 6-H); $\nu_{max.}$ ($CHCl_3$) 1 725 cm^{-1} (Found: C, 76.15; H, 10.0. $C_{31}H_{50}O_5$ requires C, 76.06; H, 10.03%).

(24R)-24-*Hydroxy*-3β-*tetrahydropyranyloxycholesta*-5,25-*diene* (11).—A 2M-solution of ethanol (0.15 ml) in THF was added to lithium aluminium hydride (11.4 mg) in THF (0.2 ml) at 0 °C. After 30 min, a solution of (*R*)-(+)-2,2′-dihydroxy-1,1′-binaphthyl (85.8 mg) in THF (0.7 +)was added at 90 °C and the mixture was then stirred at room temperature for 1 h. The enone (6) (482 mg) in (7 ml) was added dropwise at −90 °C, and the reaction mixture was then stirred at −90 °C for 20 h. Treatment of the mixture with aqueous ammonium chloride solution and the usual work-up (ethyl acetate extraction) provided the crude product, which was purified by column chromatography on silica gel [solvent; benzene–ethyl acetate (100 : 1)], to give the *alcohol* (11) (36.4 mg), m.p. 130—132 °C (acetone); $[\alpha]_D^{25}$ −19° (*c* 1, $CHCl_3$); δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H, 10-Me), 1.72 (s, 3 H, 25-Me), 4.70 (m, 1 H, acetal H), 4.82 (br s, 1 H, 26-H), 4.91 (br s, 1 H, 26-H), and 5.32 (m, 1 H, 6-H) (Found: 79.2; H, 10.8. $C_{32}H_{52}O_3$ requires C, 79.28; H, 10.81%).

(24S)-24-*Hydroxy*-3β-*tetrahydropyranyloxycholesta*-5,25-*diene* (12).—The enone (6) (48.4 mg) was treated similarly with a reducing reagent prepared from lithium aluminium hydride, ethanol and (*S*)-(−)-2,2′-dihydroxy-1,1′-binaphthyl to give the *alcohol* (12) (35.8 mg), m.p. 135—137 °C (acetone); $[\alpha]_D^{25}$ −27.8° (*c* 1, $CHCl_3$); δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H, 10-Me), 1.72 (s, 3 H, 25-Me), 4.70 (m, 1 H, acetal H), 4.83 (br s, 1 H, 26-H), 4.92 (br s, 1 H, 26-H), and 5.34 (m, 1 H, 6-H) (Found: C, 79.15; H, 10.9. $C_{32}H_{52}O_3$ requires C, 79.28; H, 10.81%).

(24R)-3β,24-*Dibenzoyloxycholest*-5-*ene* (13) *and* (24S)-3β,24-*Dibenzoyloxycholest*-5-*ene* (14).—The crude product (20 mg) of asymmetric reduction (11) or (12) was treated with 2M-aqueous hydrochloric acid (0.1 ml) in THF (1 ml) and methanol (1 ml) at room temperature for 1 h. The usual *work-up* (ethyl acetate extraction) afforded crude 3β,24-dihydroxycholesta-5,25-diene (16 mg), which was treated with benzoyl chloride (23 μl) at room temperature for 12 h. After the usual work-up (ethyl acetate extraction), the resulting 3β,24-dibenzoyloxycholesta-5,25-diene (24 mg) was hydrogenated in ethanol (10 ml) and THF (10 ml) in the presence of 5% Pd–C (20 mg) at room temperature for 5 h to give crude 3β,24-dibenzoyloxycholest-5-ene (13) or (14). Retention times of (13) and (14) were 8.5 and 9.6 min, respectively, when analyzed with a Shimadzu LC-3A Liquid Chromatograph; Column, Zorbax SIL (25 cm × 2.1 mm); mobile phase, 10% dichloromethane in hexane; flow rate 0.3 ml/min.

(24R,25S)-25,26-*Epoxy*-24-*hydroxy*-3β-*tetrahydropyranyloxycholest*-5-*ene* (15).—To a solution of the allylic alcohol (11) (66 mg) in dry benzene (0.2 ml), were added t-butyl hydroperoxide (37 mg) in dry benzene (0.2 ml) and $VO(acac)_2$ (0.5 mg) under argon. The reaction mixture was stirred at room temperature under argon for 3 h. The usual work-up (benzene extraction) afforded the crude product (68 mg), which was purified with preparative t.l.c. [solvent, benzene–ethyl acetate (10 : 1)], to give the *epoxy alcohol* (15) (47.8 mg), m.p. 145—147 °C (acetone); $[\alpha]_D^{25}$ −28° (*c* 1, $CHCl_3$); δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.92 (d, 3 H, *J* 6 Hz, 20-Me), 1.00 (s, 3 H, 10-Me), 1.34 (s, 3 H, 25-Me), 2.58 (d, 2 H, *J* 6 Hz, 26-H), 2.88 (d, 2 H, *J* 6 Hz, 26-H), 4.70 (m, 1 H, acetal H), and 5.34 (m, 1 H, 6-H) (Found: C, 76.55; H, 10.5. $C_{32}H_{52}O_4$ requires C, 76.75; H, 10.47%).

(24S,25R)-25,26-*Epoxy*-24-*hydroxy*-3β-*tetrahydropyranyloxycholest*-5-*ene* (16).—The allylic alcohol (12) (66 mg) was similarly epoxidized with t-butyl hydroperoxide (37 mg) and $VO(acac)_2$ (0.5 mg) in dry benzene (0.5 ml), to give the *epoxy alcohol* (16) (49.2 mg), m.p. 148—150 °C (acetone); $[\alpha]_D^{25}$ −26.8° (*c* 1, $CHCl_3$); δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.92 (d, 3 H, *J* 6 Hz, 20-Me), 1.00 (s, 3 H, 10-Me), 1.35 (s, 3 H, 25-Me), 2.60 (d, 1 H, *J* 6 Hz, 26-H), 2.89 (d, 1 H, *J* 6 Hz, 26-H), 4.70 (m, 1 H, acetal H), and 5.34 (m, 1 H, 6-H) (Found: C, 76.65; H, 10.55. $C_{32}H_{52}O_4$ requires C, 76.75; H, 10.47%).

(24R)-24,25-*Dihydroxy*-3β-*tetrahydropyranyloxycholest*-5-*ene* (17a).—To a solution of the epoxy alcohol (15) (11.8 mg) in THF (1 ml), was added lithium aluminium hydride (1.5 mg), and the mixture was refluxed for 3.5 h. A saturated solution of water in ether (15 ml) was added to the reaction mixture, the precipitate was filtered off and the filtrate was dried ($MgSO_4$). Evaporation of the solvent provided a crude product (12.1 mg), which was purified by preparative t.l.c. [solvent, benzene–ethyl acetate (1 : 1)], to give the *diol* (17a) (11.6 mg), m.p. 159—161 °C (hexane–acetone); $[\alpha]_D^{25}$ −21.1° (*c* 0.18, $CHCl_3$); δ($CDCl_3$) 0.68 (s, 3 H, 25-Me), 0.93 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H, 10-Me), 1.16 (s, 3 H, 25-Me), 1.20 (s, 3 H, 25-Me), 4.70 (m, 1 H, acetal H), and 5.34 (m,

1 H, 6-H) (Found: C, 76.3; H, 10.9. $C_{32}H_{54}O_4$ requires C, 76.44; H, 10.83%).

(24S)-24,25-*Dihydroxy*-3β-*tetrahydropyranyloxycholest*-5-*ene* (18a).—Similarly, reduction of the epoxy alcohol (16) (11 mg) with lithium aluminium hydride (2 mg) in THF afforded the *diol* (18a) (11 mg), m.p. 160—162 °C (hexane–acetone); $[\alpha]_D^{25}$ −50.0° (*c* 0.2, $CHCl_3$); δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.93 (d, 3 H, *J* 6 Hz, 20-Me), 1.00 (s, 3 H, 10-Me), 1.16 (s, 3 H, 25-Me), 1.20 (s, 3 H, 25-Me), 4.70 (m, 1 H, acetal H), and 5.34 (m, 1 H, 6-H) (Found: C, 76.2; H, 10.95. $C_{32}H_{54}O_4$ requires C, 76.44; H, 10.83%).

(24R)-3β,24-*Dibenzoyloxy*-25-*trimethylsilyloxycholest*-5-*ene* (17c).—The tetrahydropyranyl ether (17a) (160 mg) was stirred in methanol (5 ml) and 2M-aqueous hydrochloric acid (60 μl) at room temperature for 1 h. The usual work-up (ethyl acetate extraction) afforded the triol (17b) (130 mg), which was treated with benzoyl chloride (140 mg) in pyridine (2 ml) at room temperature for 12 h. After the usual work-up (ethyl acetate extraction), a crude product of (24*R*)-3β,24-dibenzoyloxy-25-hydroxycholest-5-ene (191 mg) in hexane (0.2 ml) was treated with *N*-trimethylsilylimidazole (180 mg) at 50 °C for 1 h. Usual work-up (ethyl acetate extraction), followed by preparative t.l.c. [solvent, benzene–hexane (1 : 1)] gave the 25-*trimethylsilyl ether* (17c) (203 mg), m.p. 155—156 °C (hexane); δ($CDCl_3$) 0.10 (s, 9 H, Me_3Si), 0.65 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.06 (s, 3 H, 10-Me), 1.24 (s, 6 H, 25-Me), 4.90 (m, 2 H, 3α- and 24-H), 5.45 (m, 1 H, 6-H), and 7.2—8.2 (m, 10 H, Ph) (Found: C, 75.5; H, 9.0. $C_{44}H_{62}O_5Si$ requires C, 75.60; H, 8.94%).

(24S)-3β,24-*Dibenzoyloxy*-25-*trimethylsilyloxycholest*-5-*ene* (18c).—Similar treatment of the tetrahydropyranyl ether (18a) (48 mg) with 2M-hydrochloric acid, benzoyl chloride and trimethylsilylimidazole yielded the *silyl ether* (18c) (61 mg), m.p. 159—160 °C (hexane); δ($CDCl_3$) 0.10 (s, 9 H, Me_3Si), 0.60 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.06 (s, 3 H, 10-Me), 1.24 (s, 6 H, 25-Me), 4.90 (m, 2 H, 3α- and 24-H), 5.45 (m, 1 H, 6-H), and 7.2—8.2 (m, 10 H, Ph) (Found: C, 75.55; H, 8.9. $C_{44}H_{62}O_5Si$ requires C, 75.60; H, 8.94%).

(25S)-25,26-*Dihydroxyvitamin* D_3 (19).—To a refluxing solution of the diacetate (9d) (50 mg) in carbon tetrachloride (3 ml), was added *N*-bromosuccinimide (25 mg). The reaction mixture was refluxed for 20 min under argon, and then filtered. The filtrate, evaporated under reduced pressure, was dissolved in xylene (2 ml) and added dropwise to a refluxing solution of s-collidine (0.5 ml) in xylene (3 ml) under argon and refluxed for 10 min. Usual work-up (ethyl acetate extraction) gave a crude compound, which was treated with toluene-*p*-sulphonic acid (*ca.* 2 mg) in acetone (20 ml) at room temperature for 15 h under argon in the dark. The usual work-up (ethyl acetate extraction) followed by preparative t.l.c. [solvent, hexane–ethyl acetate (2 : 1)] afforded (25*S*)-3β,26-diacetoxy-25-hydroxycholesta-5,7-diene (13 mg); δ($CDCl_3$) 0.65 (s, 3 H, 13-Me), 0.94 (s, 3 H, *J* 6 Hz, 20-Me), 0.98 (s, 3 H, 10-Me), 1.24 (s, 3 H, 25-Me), 2.08 (s, 3 H, Ac), 2.16 (s, 3 H, Ac), 4.00 (s, 2 H, 26-H), 4.40—4.90 (m, 1 H, 3α-H), and 5.45 (ABq, 2 H, 6- and 7-H).

The 5,7-diene (7 mg), in benzene (90 ml) and ethanol (40 ml), was irradiated by a medium-pressure mercury lamp using a Vicor filter under argon with ice-cooling for 2.5 min. Then the solution was refluxed for 1 h under argon, the solvent was removed under reduced pressure, and the crude product was subjected to preparative t.l.c. [solvent, hexane–ethyl acetate (2 : 1)], to give (25*S*)-25,26-dihydroxyvitamin D_3 3β,26-diacetate (3.69 mg); $\lambda_{max.}$ (EtOH) 265 nm (ε 18 000).

The 3,26-diacetate (3.69 mg) was treated with 5% KOH–MeOH (3 ml) at room temperature under argon for 14 h. Usual work-up (ethyl acetate extraction) and purification by preparative t.l.c. [solvent, benzene–ethyl acetate (1 : 1)] gave the triol (19) (1.05 mg); δ($CDCl_3$) 0.54 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.20 (s, 3 H, 25-Me), 3.43 (d, 1 H, *J* 12 Hz, 26-H), 3.48 (d, 1 H, *J* 12 Hz, 26-H), 3.95 (m, 1 H, 3α-H), 4.86 (d, 1 H, *J* 3 Hz, 19-H), 5.09 (d, 1 H, *J* 3 Hz, 19-H), 6.03 (d, 1 H, *J* 12 Hz, 6- or 7-H), and 6.25 (d, 1 H, *J* 12 Hz, 6- or 7-H); $\lambda_{max.}$ (EtOH) 265 nm (ε 18 000); *m/z* 416 (M^+), 398, 383, 271, 253, 136, and 118; high resolution mass spectrum, *m/z* 416.3279 (M^+, calc. for $C_{27}H_{44}O_3$: *m/z* 416.3279 M^+, calc. for $C_{27}H_{44}O_3$: *m/z* 416.3291).

(25R)-25,26-*Dihydroxyvitamin* D_3 (20).—The diacetate (10d) (48 mg) was treated similarly, to give (20) (2.45 mg); δ($CDCl_3$) 0.54 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.20 (s, 3 H, 25-Me), 3.43 (d, 1 H, *J* 12 Hz, 26-H), 3,48 (d, 1 H, *J* 12 Hz, 26-H), 3.95 (m, 1 H, 3α-H), 4.86 (d, 1 H, *J* 3 Hz, 19-H), 5.09 (d, 1 H, *J* 3 Hz, 19-H), 6.03 (d, 1 H, *J* 12 Hz, 6- or 7-H), and 6.25 (d, 1 H, *J* 12 Hz, 6- or 7-H); $\lambda_{max.}$ (EtOH) 265 nm (ε 18 000); *m/z* 416 (M^+), 398, 383, 271, 253, 136, 118; high resolution mass spectrum *m/z* 416.3282 (M^+; calc. for $C_{27}H_{44}O_3$: *m/z* 416.3291).

(24R,25R)-16-*Benzoyloxy*-24,25-*epoxy*-3β-*tetrahydropyranyloxycholest*-5-*ene* (7b).—Benzoyl chloride (70 mg) was added to a solution of the 26-alcohol (7a) (140 mg) in pyridine (2 ml) at 0 °C. The reaction mixture was stirred at room temperature for 4 h. Usual work-up (ethyl acetate extraction) and preparative t.l.c. [solvent, benzene–ethyl acetate (20 : 1)] gave the *epoxy-benzoate* (7b) (155 mg) as an amorphous powder; δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H, 10-Me), 1.39 (s, 3 H, 25-Me), 2.91 (m, 1 H, 24-H), 4.12 (d, 1 H, *J* 12 Hz, 26-H), 4.40 (d, 1 H, *J* 12 Hz, 26-H), 4.70 (m, 1 H, acetal H), 5.34 (m, 1 H, 6-H), and 7.2—8.2 (m, 5 H, Ph).

(24S,25S)-26-*Benzoyloxy*-24,25-*epoxy*-3β-*tetrahydropyranyloxycholest*-5-*ene* (8b).—Benzoylation of the 26-alcohol (8a) (160 mg) as described above yielded the *epoxy-benzoate* (8b) (180 mg), m.p. 163.0—164.4 °C (acetone); δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H, 10-Me), 1.39 (s, 3 H, 25-Me), 2.91 (m, 1 H, 24-H), 4.12 (d, 1 H, *J* 12 Hz, 26-H), 4.40 (d, 1 H, *J* 12 Hz, 26-H), 4.70 (m, 1 H, acetal H), 5.34 (m, 1 H, 6-H), and 7.2—8.2 (m, 5 H, Ph); *m/z* 502, 398, 380, 271, and 105 (Found: C, 77.5; H, 9.4. $C_{39}H_{59}O_5$ requires C, 77.44; H, 9.33%).

(24R,25S)-24-*Benzoyloxy*-3β,25,26-*trihydroxycholest*-5-*ene* (23a) *and* (24R, 25S)-26-*Benzoyloxy*-3β,24,25-*trihydroxycholest*-5-*ene* (23b).—A solution of the epoxy-benzoate (7b) (115 mg) in THF (4 ml), 70% aqueous perchloric acid (0.1 ml), and water (0.8 ml) was stirred at room temperature for 48 h. Usual work-up (ethyl acetate extraction) and purification by preparative t.l.c. [solvent, benzene–ethyl acetate (3 : 1)] afforded the more polar benzoate (41 mg) and the less polar benzoate (64 mg). The more polar compound was the 24-*benzoate* (23a); δ($CDCl_3$) 0.66 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H, 10-Me), 1.16 (s, 3 H, 13-Me), 3.40 (m, 3 H, 3α- and 26-H), 5.01 (m, 1 H, 24-H), 5.34 (m, 1 H, 6-H), and 7.2—8.2 (m, 5 H, Ph); *m/z* 538, 520, 358, 340, 273, 271, 122, and 105; high resolution mass spectrum, *m/z* 538.3644 (M^+; calc. for $C_{34}H_{50}O_5$: *m/z* 538.3658). The less polar compound was the 26-*benzoate* (23b); δ($CDCl_3$) 0.68 (s, 3 H, 25-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H,

10-Me), 1.28 (s, 3 H, 25-Me), 3.40 (m, 2 H, 3α- and 24-H); 4.22 (d, 1 H, *J* 12 Hz, 26-H), 4.53 (d, 1 H, *J* 12 Hz, 26-H), 5.34 (m, 1 H, 6-H), and 7.2—8.2 (m, 5 H, Ph); *m/z* 538, 520, 358, 340, 273, 271, 122, and 105; high resolution mass spectrum, *m/z* 538.3641 (M^+; $C_{34}H_{50}O_5$ requires *m/z* 538.3658).

(24S,25R)-24-*Benzoyloxy*-3β,25,26-*trihydroxycholest*-5-*ene* (24a) *and* (24S,25R)-26-*Benzoyloxy*-3β,24,25-*trihydroxycholest*-5-*ene* (24b).—The epoxy-benzoate (8b) (140 mg) was treated similarly with aqueous perchloric acid in THF to give the 24-*benzoate* (24a) (49 mg) and the 26-*benzoate* (24b) (76 mg); (24a) had m.p. 164.5—165.8 °C (acetone); δ($CDCl_3$) 0.62 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H, 10-Me), 1.16 (s, 3 H, 25-Me), 3.4 (m, 3 H, 3- and 26-H), 5.01 (m, 1 H, 24-H), 5.34 (m, 1 H, 6-H), and 7.2—8.2 (m, 5 H, Ph) (Found: C, 75.6; H, 9.35. $C_{34}H_{50}O_5$ requires C, 75.80; H, 9.36%). Compound (24b) had m.p. 153.0—155.8 °C (acetone); δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H, 10-Me), 1.28 (s, 3 H, 25-Me), 3.4 (m, 2 H, 3α- and 24-H), 4.22 (d, 1 H, *J* 12 Hz, 26-H), 4.53 (d, 1 H, *J* 12 Hz, 26-H), 5.34 (m, 1 H, 6-H), 7.2—8.2 (m, 5 H, Ph); high resolution mass spectrum *m/z* 538.3645 (M^+; $C_{34}H_{50}O_5$ requires *m/z* 538.3658).

(24R)-3β,24-*Dibenzoyloxy*-25-*trimethylsilyloxycholest*-5-*ene* (17c) *from* (24R,25S)-24-*Benzoyloxy*-3β,25,26-*trihydroxycholest*-5-*ene* (23a).—The 24-benzoate (23a) (200 mg), dissolved in pyridine (4 ml), was treated with toluene-*p*-sulphonyl chloride (80 mg) at 0 °C. After 24 h at room temperature, the reaction mixture was worked up in the usual manner (ethyl acetate extraction) and the crude product was purified by preparative t.l.c. [solvent, benzene–ethyl acetate (4 : 1)] to give the 26-toluene-*p*-sulphonate (23c) (162 mg). Compound (23c) (160 mg) was refluxed with lithium aluminium hydride (70 mg) in THF (20 ml) for 2 h. Usual work-up (ethyl acetate extraction) afforded the 24,25-diol (17b) (87 mg). Benzoyl chloride (70 mg) was added to a solution of the diol (17b) (87 mg) in pyridine (1.5 ml) at 0 °C and the reaction mixture was stirred at room temperature for 12 h. Usual work-up (ethyl acetate extraction) gave a crude 3β,24-dibenzoate, which was treated with trimethylsilyl imidazole (80 mg) in hexane (0.1 ml) at 50 °C for 1 h. Usual work-up (ethyl acetate extraction) and purification by preparative t.l.c. [solvent, benzene–hexane (1 : 1)] afforded the 3β,24-dibenzoyloxy-25-trimethylsilyl ether (17c) (129 mg).

(24S)-3β,24-*Dibenzoyloxy*-25-*trimethylsilyloxycholest*-5-*ene* (18c) *from* (24S,25R)-24-*Benzoyloxy*-3β,25,26-*trihydroxycholest*-5-*ene* (24a).—The benzoate (24a) (26 mg) was treated by the similar procedure to give the 3β,26-dibenzoyloxy-25-trimethylsilyl ether (18c) (14 mg).

Analysis of (24R)-3β,24-*Dibenzoyloxy*-25-*trimethylsilyloxycholest*-5-*ene* (17c) and (24S)-3β,24-*Dibenzoyloxy*-25-*trimethylsilyloxycholest*-5-*ene* (18c) *with H.P.L.C.*—The reaction products, before purification, were analyzed to elucidate the stereoselectivity and stereochemistry. The retention times of (17c) and (18c) were 7.8 and 9.5 min, respectively, when analyzed with a Shimadzu LC-3A Liquid Chromatograph; Column, Zobax SIL (25 cm × 2.1 mm); 10% dichloromethane in hexane; flow rate 0.4 ml/min.

(24R,25S)-3β,26-*Dibenzoyloxy*-24,25-*epoxycholest*-5-*ene* (25) *and* (24S,25R)-3β,26-*Dibenzoyloxy*-24,25-*epoxycholest*-5-*ene* (26).—The allylic alcohol (4) (370 mg) in methanol (7 ml), THF (4 ml) and 2M-hydrochloric acid (0.1 ml) was stirred at room temperature for 2 h. Usual work-up (dichloromethane extraction) and recrystallization from benzene provided (24E)-3β,26-*dihydroxycholesta*-5,24-*diene* (240 mg), m.p. 178 °C; δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.01 (s, 3 H, 10-Me), 1.66 (s, 3 H, 25-Me), 3.60 (m, 1 H, 3α-H), 3.97 (br s, 2 H, 26-H), and 5.34 (m, 2 H, 6- and 24-H) (Found: C, 80.85; H, 11.1. $C_{27}H_{44}O_2$ requires C, 80.94; H, 11.07%).

The 3β,26-diol (42 mg) in pyridine (1 ml) was treated with benzoyl chloride (38 mg) at room temperature for 12 h. Usual work-up (ethyl acetate extraction) gave (24E)-3β,26-*dibenzoyloxycholesta*-5,24-*diene* (60 mg); δ($CDCl_3$) 0.70 (s, 3 H, 13-Me), 0.96 (d, 3 H, *J* 6 Hz, 20-Me), 1.07 (s, 3 H, 10-Me), 1.77 (s, 3 H, 25-Me), 4.73 (br s, 2 H, 26-H), 4.85 (m, 1 H, 3α-H) 5.48, (m, 1 H, 6-H), 5.61 (m, 1 H, 24-H), and 7.2—8.2 (m, 10 H, Ph).

The 3β,26-dibenzoate (60 mg) was stirred with osmium tetraoxide (4 mg) and *N*-methylmorpholine oxide (100 mg) in THF (5 ml), t-butyl alcohol (8 ml), and water (0.8 ml) at room temperature for 12 h. Usual work-up (ethyl acetate extraction) afforded 3β,26-dibenzoyloxy-24,25-dihydroxycholest-5-ene (61 mg); δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.06 (s, 3 H, 10-Me), 1.36 (s, 3 H, 25-Me), 3.60 (m, 1 H, 24-H), 4.22 (d, 1 H, *J* 12 Hz, 26-H), 4.48 (d, 1 H, *J* 12 Hz, 26-H), 4.85 (m, 1 H, 3α-H), 5.47 (m, 1 H, 6-H), and 7.2—8.2 (m, 10 H, Ph).

The resulting 24,25-diol (61 mg) in pyridine (1.5 ml) was treated with toluene-*p*-sulphonyl chloride (60 mg) at room temperature for 12 h. Usual work-up yielded a crude product of 24-toluene-*p*-sulphonate (64 mg); δ($CDCl_3$) 0.62 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.06 (s, 3 H, 10-Me), 1.27 (s, 3 H, 25-Me), 2.41 (s, 3 H, $SO_2C_6H_4CH_3$), 4.30 (br s, 2 H, 26-H), 4.85 (m, 2 H, 3α- and 24-H), 5.47 (m, 1 H, 6-H), and 7.2—8.2 (m, 14 H, aromatic).

The crude 24-toluene-*p*-sulphonate (64 mg) was stirred with potassium carbonate (40 mg) in THF (1 ml), methanol (0.4 ml), and water (0.1 ml) at room temperature for 10 min. The usual work-up (ethyl acetate extraction) and preparative t.l.c. (solvent, benzene) gave the 24,25-epoxides (25) and (26). The more polar compound was (24R,25S)-3β,26-*dibenzoyloxy*-24,25-*epoxycholest*-5-*ene* (25) (16 mg); δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.06 (s, 3 H, 10-Me), 1.46 (s, 3 H, 25-Me), 2.88 (m, 1 H, 24-H), 4.31 (d, 1 H, *J* 12 Hz, 26-H), 4.48 (d, 1 H, *J* 12 Hz, 26-H), 4.85 (m, 1 H, 3α-H), 5.47 (m, 1 H, 6-H), and 7.2—8.2 (m, 10 H, Ph); *m/z* 502, 380, 365, 253, and 122; high resolution mass spectrum, *m/z* 502.3426 (M^+ − $PhCO_2H$; $C_{34}H_{46}O_3$ requires *m/z* 502.3447). The less polar epoxide was (24S,25R)-3β,26-*dibenzoyloxy*-24,25-*epoxycholest*-5-*ene* (26) (17 mg); δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.06 (s, 3 H, 10-Me), 1.46 (s, 3 H, 25-Me), 2.88 (m, 1 H, 24-H), 4.36 (br s, 2 H, 26-H), 4.85 (m, 1 H, 3α-H), 5.47 (m, 1 H, 6-H), and 7.2—8.2 (m, 10 H, Ph); *m/z* 502, 380, 365, 253, and 122; high resolution mass spectrum, *m/z* 502.3223 (M^+ − $PhCO_2H$; $C_{34}H_{46}O_3$ requires *m/z* 502.3447).

(24R,25R)-3β,24-*Dibenzoyloxy*-25,26-*dihydroxycholest*-5-*ene* (27a), (24R,25R)-3β,26-*Dibenzoyloxy*-24,25-*dihydroxycholest*-5-*ene* (27b), (24S,25S)-3β,24-*Dibenzoyloxy*-25,26-*dihydroxycholest*-5-*ene* (28a), *and* (24S,25S)-3β,26-*Dibenzoyloxy*-24,25-*dihydroxycholest*-5-*ene* (28b).—Similar treatment of (24*R*,25*S*)-epoxy-benzoate (25) (14 mg) with aqueous perchloric acid in THF as described for (23a) and (23b) afforded the more polar diol (27a) (4 mg) and the less polar compound (27b) (7 mg). Compounds (28a) (5 mg) and (28b) (7 mg) were obtained from (24*S*,25*R*)-epoxy-benzoate (26) (14 mg) by the same procedure. *Compound* (27a); δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.05 (s, 3 H, 10-Me), 1.24 (s, 3 H, 25-Me), 3.52 (br s, 2 H, 26-H), 4.85 (m, 1 H, 3α-H), 5.24 (m, 1 H, 24-H), 5.45 (m, 1 H, 6-H), and 7.2—8.2 (m, 10 H, Ph); *m/z* 520, 502, 398, 380, 368, and 340; high

resolution mass spectrum, m/z 520.3532 ($M^+ - PhCO_2H$; $C_{34}H_{48}O_4$ requires m/z 520.3553).

Compound (27b); δ($CDCl_3$) 0.68 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.06 (s, 3 H, 10-Me), 1.25 (s, 3 H, 25-Me), 3.58 (m, 1 H, 24-H), 4.24 (d, 1 H, *J* 12 Hz, 26-H), 4.44 (d, 1 H, *J* 12 Hz, 26-H), 4.85 (m, 1 H, 3α-H), 5.45 (m, 1 H, 6-H), and 7.2—8.2 (m, 10 H, Ph); m/z 520, 502, 398, 380, 368, and 340.

Compound (28a); δ($CDCl_3$) 0.63 (s, 3 H, 13-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.05 (s, 3 H, 10-Me), 1.24 (s, 3 H, 25-Me), 3.52 (br s, 2 H, 26-H), 4.85 (m, 1 H, 3α-H), 5.24 (m, 1 H, 24-H), 5.45 (m, 1 H, 6-H), and 7.2—8.2 (m, 10 H, Ph); m/z 520, 502, 398, 380, 368, and 340.

Compound (28b); δ($CDCl_3$) 0.68 (s, 3 H, 25-Me), 0.94 (d, 3 H, *J* 6 Hz, 20-Me), 1.06 (s, 3 H, 10-Me), 1.25 (s, 3 H, 25-Me), 3.58 (m, 1 H, 24-H), 4.24 (d, 1 H, *J* 12 Hz, 26-H), 4.44 (d, 1 H, *J* 12 Hz, 26-H), 4.85 (m, 1 H, 3α-H), 5.45 (m, 1 H, 6-H), and 7.2—8.2 (m, 10 H, Ph); m/z 520, 502, 398, 380, 368, and 340.

Acknowledgements

The authors thank Drs. Y. Iitaka and A. Itai, University of Tokyo, for the *X*-ray analysis, and also the Ministry of Education, Science, and Culture, Japan, for financial support (No. 5670026).

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Received 6th December 1982; *Paper* 2/1790