

LABELLED BILE ACIDS III:  
 DEUTERIUM LABELLED CHENODEOXYCHOLIC AND URSODEOXYCHOLIC ACIDS (1)

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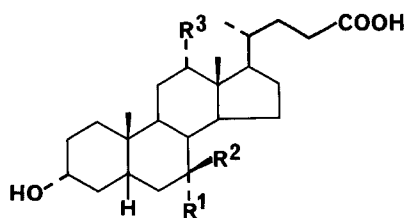
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SUMMARY

Synthetic routes to some deuterium labeled bile acids are described. The conjugated methyl esters of chenodeoxycholic 15 and cholic 23 acids are the key intermediates to these syntheses.

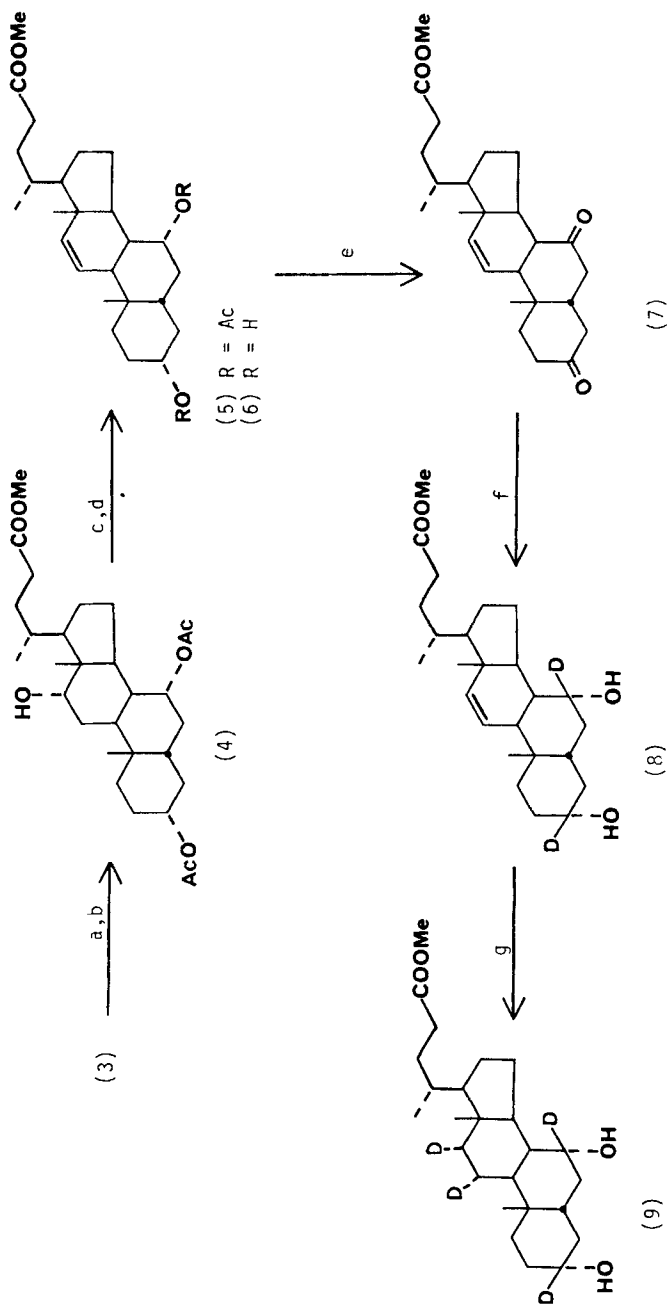
Key Words: Bile acids, Chenodeoxycholic, Ursodeoxycholic, Deuterium-labeled.



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

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Scheme I



(a)  $\text{TsOH/MeOH}$ , (b)  $\text{Ac}_2\text{O/Py}/\phi\text{H}$ , (c)  $\text{POCl}_3/\text{Py}$ , (d)  $\text{KOH/MeOH}$ , (e) Jones Reagent, (f)  $\text{NaBD}_4/\text{EtOD}$ ,  
 (g)  $\text{Pt-c/D}_2/\text{MeOD}$

## INTRODUCTION

Labeled metabolites and potential metabolites which are involved in the catabolism of cholesterol to the bile acids are necessary in order to elucidate the qualitative and quantitative aspects of biosynthetic pathways. These bile acids are labeled with a deuterium enrichment of at least M+3 in order to be significant (2) in our studies on bile acid treatment of cholestasis, bile duct atresia and giant cell hepatitis.

## DISCUSSION

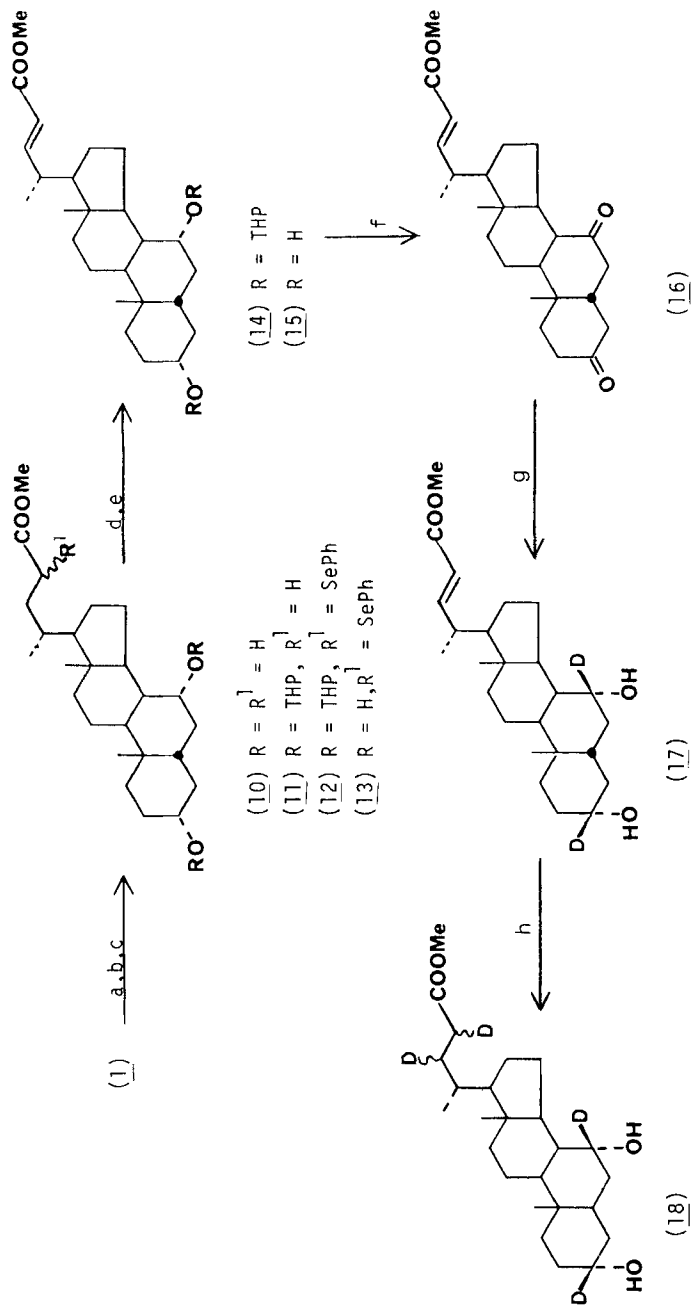
The starting materials utilized are the commercially available chenodeoxycholic 1 and cholic 3 acids. We will concentrate on the syntheses of deuterium labeled chenodeoxycholic 1 and ursodeoxycholic 2 acids in this publication.

Scheme I is utilized as the model study for our syntheses. Selective acetylation of the  $3\alpha,7\alpha$  alcohol of the methyl cholate 19 (3) followed by dehydration of the  $12\alpha$ -hydroxy group gave the  $\Delta^{11}$ -olefin 5. Although many dehydration procedures of the  $12\alpha$ -hydroxy group of sterols have been described (4), phosphorus oxychloride was found to be most convenient. Hydrolysis followed by Jones oxidation yielded the desired diketone 7.

Wigfield (5) and Chang (6) have found that sodium borohydride reduction of the unhindered 3-oxo and the relatively hindered 7-oxo bile acids gave predominantly the  $\alpha$ -hydroxy epimers, i.e., the equatorial and axial alcohol respectively. Following this procedure with sodium borodeuteride on the diketone 7, the corresponding  $3\alpha,7\alpha$ -diol 8 was obtained as the major product. Saturation of the  $\Delta^{11}$  double bond with deuterium aided by platinum catalyst yielded the desired tetradeuterated species 9.

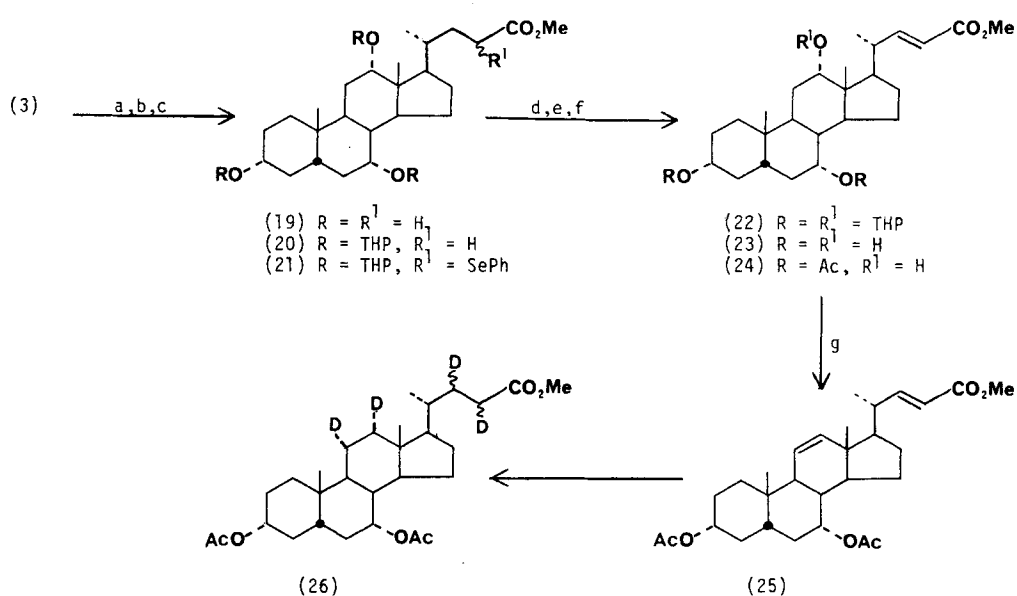
A similar procedure to Scheme I is followed for Scheme II and III. In both cases the conjugated methyl ester of chenodeoxycholic 14 and cholic 22 acids are key intermediates.

Scheme II



(a) MeOH/TsOH/ $\Delta$ , (b) Dihydropyran/TsOH, (c) LDA/PhSeCl, (d) m-CPBA/CH<sub>2</sub>Cl<sub>2</sub>/0°C, (e) TsOH/MeOH, (f) Jones Reagent, (g) NaBD<sub>4</sub>/EtOH, (h) Pt-C/D<sub>2</sub>/EtOH

Scheme III

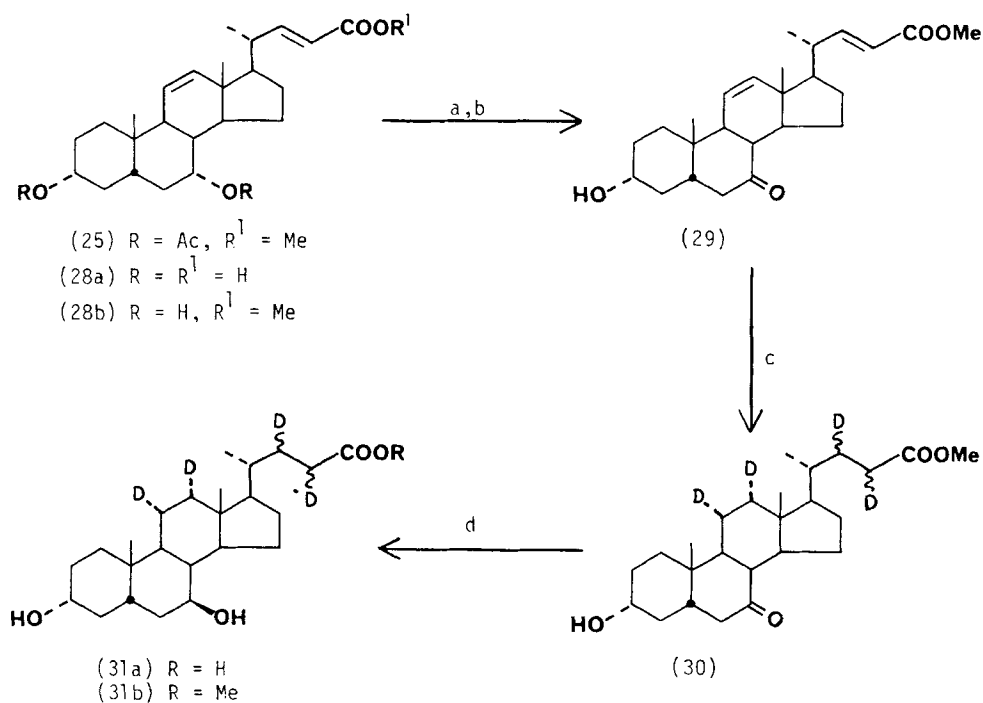


(a) MeOH/TsOH/ $\Delta$ , (b) Dihydropyran/TsOH, (c) LDA/PhSeCl, (d) *m*-CPBA, (e) TsOH/MeOH, (f) Ac<sub>2</sub>O/PhH/Py, (g) POCl<sub>3</sub>/Py, (h) Pt-C/D<sub>2</sub>/EtOD

Generation of the  $\Delta^{22}$  olefins utilized the well-known selenenylation procedures described by Sharpless *et al* (7). In both cases, only the (E)-olefin is isolated. The coupling constant value (16 Hz) is comparable to that acceptable for the (E) configuration (8). Although both (R)- and (S)-isomers of the selenenylated ester 13 are isolated (structure not proven, see Experimental Section), the (E)-olefins 14 and 22 are postulated to have been derived from the oxidative syn-elimination of an intermediate with a pseudo-threo configuration (7,9).

Hydrolysis, oxidation and reduction with deuteride followed by hydrogenation with deuterium (Scheme II) in an analogous manner to Scheme I yielded the tetradeuterated methyl ester of chenodeoxycholic acid 18.

Scheme IV



(a)  $\text{MeOH}/\text{KOHaq}/\Delta$ , (b)  $\text{K}_2\text{CrO}_4/\text{NaOAc}$ , (c)  $\text{Pd-C}/\text{H}_2$ , (d)  $\text{Na}/n\text{-BuOH}/120^\circ\text{C}$

Scheme III uses cholic acid as starting material. The diene 25 is formed in a similar manner as described above (Scheme I and II). In this case, tetradeuteration is incorporated in a single step by complete saturation of the two double bonds ( $\Delta^{11}$  and  $\Delta^{22}$ ) with deuterium and platinum catalyst.

Labeling is normally done in the last step of a sequence of transformations. However, we find it advantageous in the synthesis of tetradeuterated ursodeoxycholic acid 31a to start from the common intermediate, the diene 25, and insert the deuterium before inversion of the  $7\alpha$ -hydroxy group (Scheme IV). Several

procedures (10,11,12) are available for the inversion of the 7 $\alpha$ -hydroxy function to the 7 $\beta$ -epimer. However, oxidation to the 7-ketone, followed by sodium/n-butanol (11) reduction stands out for its ease and higher yield.

Selective oxidation of the hydrolyzed diene 28b with potassium dichromate gave the 7-keto compound 29. Saturation of the double bonds with deuterium in the usual manner gave the tetradeuterated compound 30. Reduction of the 7-ketone with sodium in boiling n-butanol, followed by esterification yielded the tetra-deuterated methyl ursodeoxycholate 31b in an overall 52% from the diene 25.

Isotope purity of all labeled compounds was determined by mass spectra.

#### EXPERIMENTAL

Melting points were determined on a Kofler melting point apparatus and are uncorrected. The UV spectra were determined in acetonitrile solutions on a Perkin-Elmer 202 spectrophotometer. The IR spectra of crystals were determined as KBr pellets and of oils, as a film, on sodium chloride windows. The NMR spectra were obtained in deuteriochloroform solution, using tetramethylsilane as an internal reference and were recorded on a 90 MHz Varian EM-390 spectrometer. Mass spectra were recorded on a Nuclide 1290 G spectrometer using a direct insertion probe and a Hewlett Packard 5992A GC/MS system, where applicable.

Methyl 3 $\alpha$ ,7 $\alpha$ -diacetoxy-12 $\alpha$ -hydroxy-5 $\beta$ -cholan-24-oate (4). The cholic acid 3 was methylated with methanol and *p*-toluenesulphonic acid according to the procedure of Dayal et al. (14) and then selectively acetylated with a 1:1 acetic anhydride-pyridine mixture in benzene (3). The alcohol 4 (overall yield 60%), was crystallized from aqueous methanol, mp 171-173 $^{\circ}$ C [Lit. (3) 185-187 $^{\circ}$ C]. IR  $\nu_{\max}$  3500 (OH), 1710 (C=O), 1250 (C-O)  $\text{cm}^{-1}$ ; NMR  $\delta$  0.72 (3H, s, 18-Me), 0.95 (3H, s, 19-Me), 0.97 (3H, d,  $J$  = 7 Hz, 21-Me), 2.02 (3H, s, 3-acetate Me), 2.05 (3H, s, 7-acetate Me), 3.60 (3H, s, 24-COOMe), 3.92 (1H, m,  $w/2$  = 7 Hz, 12-H), 4.43 (1H, br.m.,  $w/2$  = 15 Hz, 3-H), 4.82 (1H, m,  $w/2$  = 7 Hz, 7-H).

Methyl 3 $\alpha$ ,7 $\alpha$ -diacetoxy-5 $\beta$ -chol-11-en-24-oate (5). The 12 $\alpha$ -alcohol 4 (760 mg, 1.49 mmol) in anhydrous pyridine (5 mL) was cooled in ice. The system was flushed with nitrogen and sealed with a rubber septum. Phosphorus oxychloride (0.18 mL, 1.94 mmol) was added dropwise. The reaction mixture was kept in a refrigerator overnight and then heated over a steam bath for 2 hr. The mixture was poured into ice cold 10% hydrochloric acid (20 mL) and extracted with ether.

Work-up in the usual manner and purification on preparative TLC (30% ethyl acetate/hexane) yielded the  $\Delta^{11}$ -olefin 5 (520 mg, 70%). An analytical sample was recrystallized in aqueous methanol to give colorless needles, mp 122-125 $^{\circ}$ C [Lit. (15) 139-141 $^{\circ}$ C]. IR  $\nu$  1720 (C=O), 1250 (C-O), 770, 735, 720, 695 (C=C)  $\text{cm}^{-1}$ ; NMR  $\delta$  0.75 (3H, s, 18-Me), 0.92 (3H, s, 19-Me), 1.00 (3H, d,  $\underline{J}$  = 7 Hz, 21-Me), 2.02 (3H, s, 3-acetate Me), 2.04 (3H, s, 7-acetate Me), 3.62 (3H, s, 24-COOMe), 4.53 (1H, br.m., w/2 = 18 Hz, 3-H), 4.90 (1H, m, w/2 = 9 Hz, 7-H), AB system  $H_A$  6.07 (1H, dd,  $\underline{J}$  = 3 Hz,  $\underline{J}_{AB}$  = 10 Hz, 12-H),  $H_B$  5.39 (1H, dd,  $\underline{J}$  = 2 Hz,  $\underline{J}_{AB}$  = 10 Hz, 11-H).

Methyl 3 $\alpha$ ,7 $\alpha$ -dihydroxy-5 $\beta$ -chol-11-en-24-oate (6). The ester 5 (500 mg) was hydrolyzed according to the Nakada procedure (15) with 5% methanolic potassium hydroxide. Recrystallization of the free acid from ethyl acetate gave colorless blades, mp 202-204 $^{\circ}$ C [Lit. (15) 204-206 $^{\circ}$ C]. The acid was esterified with a catalytic amount of *p*-toluenesulphonic acid in methanol (14) to give the ester 6, (370 mg, 89% overall) as an oil which was shown to be homogeneous by TLC and NMR, but resisted crystallization attempts: IR  $\nu_{\text{max}}$  3400 (OH), 1720 (C=O), 1150, 1080 (C-O)  $\text{cm}^{-1}$ ; NMR  $\delta$  0.73 (3H, s, 18-Me), 0.84 (3H, s, 19-Me), 1.00 (3H, d,  $\underline{J}$  = 7 Hz, 21-Me), 3.50 (1H, br.m., w/2 = 18 Hz, 3-H), 3.67 (3H, s, 24-COOMe), 3.97 (1H, m, w/2 = 9 Hz, 7-H), AB system  $H_A$  6.14 (1H, dd,  $\underline{J}$  = 3 Hz,  $\underline{J}_{AB}$  = 10 Hz, 12-H),  $H_B$  5.49 (1H, dd,  $\underline{J}$  = 2 Hz,  $\underline{J}_{AB}$  = 10 Hz, 11-H).

Methyl 3,7-dioxo-5 $\beta$ -chol-11-en-24-oate (7). The diol 6 (180 mg, 0.44 mmol) was dissolved in acetone (1 mL) and cooled in an ice bath. Jones reagent was added dropwise until a permanent orange color persisted. This was then stirred at room temperature for 1/2 hr. Crushed ice was added until precipitation was



complete. After 1/2 hr the precipitate was filtered, washed with water and dried to give the diketone 7 (165 mg, 93%). An analytical sample was recrystallized from acetone/hexane to give white granules, mp 162-164°C: IR  $\nu_{\max}$  1720, 1700 (C=O), 1200, 1160, 1100 (C-O), 725, 700 (C=C)  $\text{cm}^{-1}$ ; NMR  $\delta$  0.79 (3H, s, 18-Me), 1.03 (3H, d,  $\underline{J}$  = 6 Hz, 21-Me), 1.23 (3H, s, 19-Me), 3.68 (3H, s, 24-COOMe), AB system  $H_A$  6.32 (1H, dd,  $\underline{J}$  = 3 Hz,  $\underline{J}_{AB}$  = 10 Hz, 12-H),  $H_B$  5.41 (1H, dd,  $\underline{J}$  = 2 Hz,  $\underline{J}_{AB}$  = 10 Hz, 11-H); MS m/e 398 ( $M^+$ ), 285 ( $M^+$  - side chain).

Anal. Calcd for  $C_{25}H_{36}O_4$ : C, 74.96; H, 9.06. Found: C, 74.95; H, 9.10.

[3 $\beta$ ,7 $\beta$ - $^2H_2$ ]-Methyl 3 $\alpha$ ,7 $\alpha$ -dihydroxy-5 $\beta$ -chol-11-en-24-oate (8). To the diketone 7 (200 mg, 0.50 mmol) in deuterated ethanol (OD) (5 mL, 99.5 + atom % D) was added sodium borodeuteride (42 mg, 0.96 mmol, 98 atom % D). This was stirred under  $N_2$  atmosphere at room temperature for 4 hr. The reaction was then slowly quenched with cold 10% hydrochloric acid (20 mL) and then extracted with ethyl acetate. The ethyl acetate extract was washed successively with water, saturated sodium bicarbonate and water. The solvent was evaporated and the compound dried by azeotropic distillation with benzene/ethanol to yield a crude oil. This was chromatographed on TLC (5 x 30% acetone/hexane) to give the diol 8 (152 mg, 75%) as an oil which was shown to be homogeneous by TLC and HPLC. IR  $\nu_{\max}$  3400 (OH), 2100 (C-D), 1720 (C=O), 1170, 1110 (C-O)  $\text{cm}^{-1}$ ; NMR  $\delta$  0.73 (3H, s, 18-Me), 0.84 (3H, s, 19-Me), 1.01 (3H, d,  $\underline{J}$  = 6 Hz, 21-Me), 3.67 (3H, s, 24-COOMe), AB system  $H_A$  6.15 (1H, dd,  $\underline{J}$  = 3 Hz,  $\underline{J}_{AB}$  = 10 Hz, 12-H),  $H_B$  5.48 (1H, dd,  $\underline{J}$  = 2 Hz,  $\underline{J}_{AB}$  = 10 Hz, 11-H); MS m/e 388 [ $(M+2)^+ - H_2O$ ], 370 [ $(M+2)^+ - 2H_2O$ ]; Isotope purity 82% ( $M+2$ ), 16% ( $M+1$ ), 2% ( $M$ ).

[3 $\beta$ ,7 $\beta$ ,11 $\alpha$ ,12 $\alpha$ - $^2H_4$ ]-Methyl 3 $\alpha$ ,7 $\alpha$ -dihydroxy-5 $\beta$ -cholan-24-oate (9). The hydroxy olefin 8 (100 mg, 0.25 mmol) was suspended in deuterated ethanol (OD) (2 mL). 5% Platinum-on-carbon (100 mg) was added and the vessel placed in a hydrogenator under a deuterium atmosphere. The mixture was stirred vigorously overnight and then filtered through a pad of celite. The residues were

washed with ethyl acetate. Evaporation of the solvents gave an oily tetra-deuterated ester 9 (89 mg, 89%), IR  $\nu_{\max}$  3350 (OH), 2100 (C-D), 1725 (C=O), 1160, 1080 (C-O)  $\text{cm}^{-1}$ ; NMR  $\delta$  0.68 (3H, s, 18-Me), 0.91 (3H, s, 19-Me), 0.94 (3H, d,  $J$  = 7 Hz, 21-Me), 3.68 (3H, s, 24-COOMe); MS  $m/e$  374  $[(M+4)^+-2H_2O]$ ; Isotope purity: 70% (M+4), 15% (M+3), 7% (M+2), 5% (M+1), 3% (M).

Methyl 3 $\alpha$ ,7 $\alpha$ -dihydroxy-5 $\beta$ -cholestan-24-oate (10). To chenodeoxycholic acid 1 (3.9 g, 9.95 mmol) in absolute methanol (10 mL) was added *p*-toluene-sulfonic acid (0.36 g) and the solution refluxed for 20 min. The reaction mixture was concentrated, diluted with ethyl acetate and washed with saturated sodium bicarbonate and water. The ethyl acetate solution was evaporated and azeotroped to dryness with benzene to yield the methyl 3 $\alpha$ ,7 $\alpha$ -dihydroxy-cholestan-24-oate (10) (3.8 g, 94%), identical when compared to an authentic sample (TLC, IR, NMR).

Methyl 3 $\alpha$ ,7 $\alpha$ -Di[(tetrahydro-2H-pyran-2-yl)oxy]-5 $\beta$ -cholestan-24-oate (11). The diol 10 (5 g, 12.3 mmol) in dry benzene (20 mL) was cooled in an ice bath. Dihydropyran (3.4 mL) was added, followed by a catalytic amount of *p*-toluene-sulfonic acid (23 mg). After 10 min the ice bath was removed and the solution stirred at room temperature. After 45 min it was worked up by diluting with ether and washed successively with saturated sodium bicarbonate, water, brine and dried over anhydrous magnesium sulfate. Evaporation of solvent yielded an oil which was kept in high vacuum overnight to give the syrupy 3 $\alpha$ ,7 $\alpha$ -ditetrahydropyranyl ether 11 (6.9 g, 98%). An analytical sample was purified by HPLC. IR  $\nu_{\max}$  1720 (C=O), 1180, 1150, 1100, 1070 (C-O), 1020, 980 (THP ether)  $\text{cm}^{-1}$ ; NMR  $\delta$  0.67 (3H, s, 18-Me), 0.93 (3H, s, 19-Me), 0.94 (3H, d,  $J$  = 6 Hz, 21-Me), 3.50 (6H, br.m.,  $w/2$  = 31 Hz, O-CH), 3.63 (3H, s, 24-COOMe), 4.63 (2H, m,  $w/2$  = 10 Hz, O-CH-O).

Anal. Calcd for  $C_{35}H_{58}O_6$ : C, 73.13; H, 10.17. Found: C, 73.06; H, 10.18.

(E)-Methyl 3 $\alpha$ ,7 $\alpha$ -di[(tetrahydro-2H-pyran-2-yl)oxy]-5 $\beta$ -cholestan-22-en-24-oate (14). The methyl ester 11 (5 g, 8.7 mmol) in anhydrous tetrahydrofuran was added dropwise to a freshly prepared lithium diisopropylamide solution

(11.8 mL, 1.3 M *n*-butyllithium, 2.15 mL diisopropylamine, 10 mL tetrahydrofuran at  $-78^{\circ}\text{C}$  in a nitrogen atmosphere). After 1/2 hr, a tetrahydrofuran solution (10 mL) of phenylselenenyl chloride (2.16 g, 11 mmol) was added. The reaction mixture was kept at  $-78^{\circ}\text{C}$  for 1/2 hr and then allowed to warm to  $0^{\circ}\text{C}$ . Work-up in the usual manner yielded a crude orange oil. This was dissolved in methylene chloride (20 mL) and placed in an ice bath. *m*-Chloroperbenzoic acid (3 g, 17 mmol) was added all at once. This was allowed to stir for 1/2 hr at  $0^{\circ}\text{C}$ , then calcium hydroxide powder (1.5 g, 20 mmol) was added. The slurry was stirred at room temperature for 1/2 hr and then filtered. The residues were washed with methylene chloride and the combined solvent was evaporated to yield a crude oil. This was chromatographed in a Waters Prep LC/500 with 5% ethyl acetate/hexane at a flow rate of 150 mL/min. The conjugated methyl ester 14 was isolated as a pale yellow oil (2.1 g, 42%) which was homogeneous according to TLC, HPLC and NMR. IR  $\nu_{\text{max}}$  1720 (C=O), 1640 (C=C), 1170, 1110, 1070 (C-O), 1020, 980 (THP ether)  $\text{cm}^{-1}$ ; UV (acetonitrile)  $\lambda_{\text{max}}$  215 nm ( $\epsilon$  10,000), NMR  $\delta$  0.69 (3H, s, 18-Me), 0.83 (3H, s, 19-Me), 1.03 (3H, d,  $\underline{J} = 7$  Hz, 21-Me), 3.53 (4H, br.m.,  $w/2 = 33$  Hz, -O-CH), 3.68 (3H, s, 24-COOMe), 4.63 (2H, m,  $w/2 = 10$  Hz, O-CH-O), AB system  $H_A$  6.80 (1H, dd,  $\underline{J} = 9$  Hz,  $\underline{J}_{AB} = 16$  Hz, 22-H),  $H_B$  5.68 (1H, d,  $\underline{J}_{AB} = 16$  Hz, 23-H).

(E)-Methyl 3 $\alpha$ ,7 $\alpha$ -dihydroxy-5 $\beta$ -chol-22-en-24-oate (15). To the THP ether 14 (1.55 g, 2.7 mmol) in absolute methanol (33 mL) at  $0^{\circ}\text{C}$  was added *p*-toluenesulfonic acid (0.53 g, 2.8 mmol). The reaction mixture was allowed to stir at room temperature for 1 hr. Work-up in the usual manner yielded the diol 15 (1.1 g, 100%) as an oil which was shown to be homogeneous by TLC, HPLC and NMR, but resisted crystallization attempts: IR 3400 (OH), 1700 (C=O), 1640 (C=C), 1210, 1170, 1160, 1070 (C-O)  $\text{cm}^{-1}$ ; UV (acetonitrile)  $\lambda_{\text{max}}$  221 nm ( $\epsilon$  15,900), NMR  $\delta$  0.70 (3H, s, 18-Me), 0.91 (3H, s, 19-Me), 1.10 (3H, d,  $\underline{J} = 7$  Hz, 21-Me), 3.47 (1H, br.m.,  $w/2 = 18$  Hz, 3-H), 3.73 (3H, s, 24-COOMe), 3.83 (1H, m,  $w/2 = 8$  Hz, 7-H), AB system  $H_A$  6.87 (1H, dd,  $\underline{J} = 9$  Hz,  $\underline{J}_{AB} = 16$  Hz,

22-H),  $H_B$  5.76 (1H, d,  $J_{AB}$  = 16 Hz, 23-H); MS  $m/e$  404 ( $M^+$ ), 386 ( $M-H_2O$ )<sup>+</sup>, 368 ( $M-2H_2O$ )<sup>+</sup>.

Anal. Calcd for  $C_{25}H_{40}O_4$ : C, 74.21; H, 9.97. Found: C, 74.65; H, 10.17.

(23 R/S)-Methyl 3 $\alpha$ ,7 $\alpha$ -dihydroxy-23-phenylselenyl-5 $\beta$ -cholan-24-oate (13).

To the crude selenide 12 (559 mg, 0.77 mmol) in absolute methanol (10 mL) was added *p*-toluenesulphonic acid (150 mg, 0.79 mmol). This was allowed to stir at room temperature for 1/2 hr. Work-up as before and chromatography on preparative TLC (3 x 30% acetone/hexane) gave as the higher  $R_f$  product, 13a (125 mg, 29%) as an oil; IR  $\nu_{max}$  3400 (OH), 1730 (C=O), 1250, 1125, 1075 (C-O)  $cm^{-1}$ ; NMR  $\delta$  0.62 (3H, s, 18-Me), 0.90 (3H, s, 19-Me), 0.90 (3H, d,  $J$  = 7 Hz, 21-Me), 3.33 (1H, br.m.,  $w/2$  = 15 Hz, 3-H), 3.53 (3H, s, 24-COOMe), 3.73 (1H, m,  $w/2$  = 7 Hz, 7-H), 7.10-7.53 (5H, m, phenyl-H); and the lower  $R_f$  product 13b (227 mg, 53%): IR 3400 (OH), 1730 (C=O), 1250, 1125, 1075 (C-O)  $cm^{-1}$ ; NMR  $\delta$  0.67 (3H, s, 18-Me), 0.90 (3H, s, 19-Me), 0.90 (3H, d,  $J$  = 7 Hz, 21-Me), 3.38 (1H, br.m.,  $w/2$  = 15 Hz, 3-H), 3.60 (3H, s, 24-COOMe), 3.77 (1H, m,  $w/2$  = 7 Hz, 7-H), 7.12-7.57 (5H, m, phenyl-H).

(E)-Methyl 3,7-dioxo-5 $\beta$ -chol-22-en-24-oate (16). The diol 15 (1.1 g, 2.7 mmol) was dissolved in acetone (10 mL) and cooled in an ice bath. Jones reagent was added dropwise until a permanent orange color persisted. This was then allowed to stir at room temperature for 1/2 hr. The reaction mixture was diluted with ice water and extracted with ethyl acetate (3 x 100 mL). The organic layer was washed successively with water, saturated bicarbonate and water. The solvent was evaporated and azeotroped to dryness with the aid of a benzene/ethanol mixture to give the diketone 16 (1.0 g, 92%) as a white crystalline solid. An analytical sample was prepared by recrystallization from ether: mp 176-178<sup>o</sup>C; IR  $\nu_{max}$  1700 (C=O), 1250, 1220, 1060 (C-O), 990, 980 (olefinic C-H)  $cm^{-1}$ ; UV (acetonitrile)  $\lambda_{max}$  213 nm ( $\epsilon$  9300); NMR  $\delta$  0.73 (3H, s, 18-Me), 1.12 (3H, d,  $J$  = 7 Hz, 21-Me), 1.33 (3H, s, 19-Me), 3.68 (3H, s, 24-COOMe), AB system  $H_A$  6.78 (1H, dd,  $J$  = 9 Hz,  $J_{AB}$  = 16 Hz, 22-H);

$H_B$  5.70 (1H, d,  $J_{AB} = 16$  Hz, 23-H); MS  $m/e$  400 ( $M^+$ ), 287 ( $M^+$  - side chain), 269 [ $M^+$  - ( $H_2O$  + side chain)].

Anal. Calcd for  $C_{25}H_{36}O_4$ : C, 74.96; H, 9.06. Found: C, 75.16; H, 9.22.

(E)-Methyl 3,7-dioxo-5 $\beta$ -chol-22-en-24-oate (16) from 13a and 13b. The dihydroxyselenide 13a (129 mg, 0.23 mmol) was treated with Jones reagent. Work-up in the usual manner gave the olefinic diketone 16 (88 mg, 96%) as white crystals, identical (mp, TLC, NMR) to an authentic sample.

The dihydroxyselenide 13b (226 mg, 0.40 mmol) was treated with Jones reagent, and work-up as above yielded the olefinic diketone 16 (160 mg, 99%) as white crystals, identical (mp, TLC, NMR) with an authentic sample.

[3 $\beta$ ,7 $\beta$ - $^2H_2$ ]-(E)-Methyl 3 $\alpha$ ,7 $\alpha$ -dihydroxy-5 $\beta$ -chol-22-en-24-oate (17). To the diketone 16 (230 mg, 0.58 mmol) in deuterated ethanol (OD) (5 mL) was added sodium borodeuteride (48 mg, 1.1 mmol). This was stirred at room temperature for 4 hr. The reaction was then slowly quenched with 10% hydrochloric acid and extracted with ethyl acetate. Work-up in the usual manner yielded a crude oil (272 mg). This was chromatographed on TLC (5 x 30% acetone/hexane) to give the diol 17 (181 mg, 78%) as a white crystalline compound, mp 169-172°C; IR  $\nu_{max}$  3400 (OH), 2100 (C-D), 1700 (C=O), 1640 (C=C), 1160, 1120, 1080, 1000 (C-O)  $cm^{-1}$ ; UV (acetonitrile)  $\lambda_{max}$  215 nm ( $\epsilon$  12,000); NMR  $\delta$  0.70 (3H, s, 18-Me), 0.90 (3H, s, 19-Me), 1.08 (3H, d,  $J = 7$  Hz, 21-Me), 3.67 (3H, s, 24-COOMe), AB system  $H_A$  6.74 (1H, dd,  $J = 8$  Hz,  $J_{AB} = 16$  Hz, 22-H),  $H_B$  5.66 (1H, d,  $J_{AB} = 16$  Hz, 23-H); MS  $m/e$  [( $M+2$ ) $^+$ - $H_2O$ ], 370 [( $M+2$ ) $^+$ -2 $H_2O$ ]; Isotope purity: 79% ( $M+2$ ), 15% ( $M+1$ ) and 6% ( $M$ ).

Anal. Calcd for  $C_{25}H_{40}O_4$ : C, 74.21; H, 9.97. Found: C, 74.36; H, 10.22.

[3 $\beta$ ,7 $\beta$ ,22 $\xi$ ,23 $\xi$ - $^2H_4$ ]-Methyl 3 $\alpha$ ,7 $\alpha$ -dihydroxy-5 $\beta$ -cholan-24-oate (18). The hydroxy olefin 17 (85 mg, 0.21 mmol) was suspended in deuterated ethanol (OD) (2 mL). 5% Platinum-on-carbon (180 mg) was added and the vessel placed in a hydrogenator under a deuterium atmosphere. The mixture was stirred vigorously overnight and then filtered through a pad of celite. The residues were washed

with ethyl acetate. Evaporation of the solvents gave the tetradeuterated ester 18 (78 mg, 91%) as an oil, IR  $\nu_{\max}$  3400 (OH), 2100 (CD), 1730 (C=O), 1250, 1180, 1160, 1100, 1080, 1000 (C-O)  $\text{cm}^{-1}$ ; NMR  $\delta$  0.67 (3H, s, 18-Me), 0.90 (3H, s, 19-Me), 0.93 (3H, d,  $J = 7$  Hz, 21-Me), 3.67 (3H, s, 24-COOMe); MS  $m/e$  374 ( $M+4$ )<sup>+</sup>-2H<sub>2</sub>O; Isotope purity: 69% ( $M+4$ ), 22% ( $M+3$ ) and 9% ( $M$ ).

Anal. Calcd for C<sub>25</sub>H<sub>42</sub>O<sub>4</sub>: C, 73.85; H, 10.41. Found: C, 73.97; H, 10.70.

(E)-Methyl 3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ -tri[(tetrahydro-2H-pyran-2-yl)oxy]-5 $\beta$ -chol-22-en-24-oate (22) was prepared from 20 (18.3 g, 27.1 mmol) by the general selenenylation procedure described for 14. Work-up in the usual manner and purified by chromatography on a Waters Prep LC/500 (5% ethyl acetate/hexane; flow rate 150 ml/min) to give the conjugated ester 22 (12.5 g, 69%) as an oil which was homogeneous according to TLC, HPLC and NMR: IR  $\nu_{\max}$  1700 (C=O), 1640 (C=C), 1180, 1120, 1070 (C-O), 1020, 980 (THP ether)  $\text{cm}^{-1}$ ; UV (acetonitrile)  $\lambda_{\max}$  212 nm ( $\epsilon$  10,200); NMR  $\delta$  0.70 (3H, s, 18-Me), 0.92 (3H, s, 19-Me), 1.05 (3H, d,  $J = 7$  Hz, 21-Me), 3.67 (3H, s, 24-COOMe), 3.67 (9H, m,  $w/2 = 31$  Hz, -O-CH), 4.67 (3H, m,  $w/2 = 10$  Hz, O-CH-O), AB system H<sub>A</sub> 6.81 (1H, dd,  $J = 8$  Hz,  $J_{AB} = 16$  Hz, 22-H), H<sub>B</sub> 5.63 (1H, d,  $J_{AB} = 16$  Hz, 23-H), MS  $m/e$  570 ( $M^+$ -THPOH), 468 ( $M^+$ -2THPOH), 366 ( $M^+$ -3THPOH). An analytical sample was prepared as the hydrolyzed compound, methyl 3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ -trihydroxy-5 $\beta$ -chol-22-en-24-oate (23),

(E)-Methyl 3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ -trihydroxy-5 $\beta$ -chol-22-en-24-oate (23) was prepared from the THP ether 22 (222 mg, 0.37 mmol) by the general hydrolysis procedure described for 15. Work-up in the usual manner yielded a crude oil (166 mg). Purification on preparative TLC (50% acetone/hexane) yielded the triol 23 (150 mg, 96%) as white crystals. An analytical sample was prepared by recrystallization from ether/hexane to give white needle-shaped crystals: mp 181-182<sup>o</sup>C; UV (acetonitrile)  $\lambda_{\max}$  213 nm ( $\epsilon$  8800); IR  $\nu_{\max}$  3300 (OH), 1720 (C=O), 1640 (C=C), 1240, 1080 (C-O)  $\text{cm}^{-1}$ ; NMR  $\delta$  0.71 (3H, s, 18-Me), 0.90 (3H, s, 19-Me), 1.13 (3H, d,  $J = 7$  Hz, 21-Me), 3.47 (1H, br.m.,  $w/2 = 15$  Hz, 3-H), 3.73 (3H, d, 24-COOMe), 3.87 (1H, m,  $w/2 = 7$  Hz, 7-H), 3.98 (1H, m,

w/2 = 7 Hz, 12-H), AB system  $H_A$  6.92 (1H, dd,  $J = 8$  Hz,  $J_{AB} = 16$  Hz, 23-H),  $H_B$  5.78 (1H, d,  $J_{AB} = 16$  Hz, 23-H); MS m/e 420 ( $M^+$ ), 402 ( $M^+ - H_2O$ ), 384 ( $M^+ - 2H_2O$ ), 366 ( $M^+ - 3H_2O$ ).

Anal. Calcd for  $C_{25}H_{40}O_5$ : C, 71.39; H, 9.59. Found: C, 71.33; H, 9.68.

(E)-Methyl 3 $\alpha$ ,7 $\alpha$ -diacetoxy-12 $\alpha$ -hydroxy-5 $\beta$ -chol-22-en-24-oate (24). A

solution of the methylcholate 23 (6.6 g, 15.7 mmol), in dry benzene (31.5 mL), anhydrous pyridine (7.9 mL) and acetic anhydride (7.9 mL) was allowed to stand at room temperature overnight. It was then poured into ice water, the benzene layer was separated, and the aqueous phase extracted with ethyl acetate. The combined organic solvents were washed with water, brine and dried over anhydrous magnesium sulfate. Evaporation of solvents gave a crude oil (8.15 g) which was purified by preparative liquid chromatography (20% ethyl acetate/hexane-flow rate 150 mL/min) yielded the 12 $\alpha$ -alcohol 24 (5.42 g, 68%) as white crystals. An analytical sample was prepared by recrystallization from ether: mp 181-182 $^{\circ}$ C; IR  $\nu_{max}$  3550 (OH), 1725 (C=O), 1650 (C=C), 1250, 1025 (C-O)  $cm^{-1}$ ; UV (acetonitrile)  $\lambda_{max}$  216 nm ( $\epsilon$  8800); NMR  $\delta$  0.73 (3H, s, 18-Me), 0.95 (3H, s, 19-Me), 1.17 (3H, d,  $J = 7$  Hz, 21-Me), 2.05 (3H, s, 3-acetate Me), 2.07 (3H, s, 7-acetate Me), 3.73 (3H, s, 24-COOMe), 4.00 (1H, m, w/2 = 7 Hz, 12-H), 4.53 (1H, br.m., w/2 = 15 Hz, 3-H), 4.90 (1H, m, w/2 = 7 Hz, 7-H), AB system  $H_A$  6.87 (1H, dd,  $J = 9$  Hz,  $J_{AB} = 16$  Hz, 22-H),  $H_B$  5.77 (1H, d,  $J_{AB} = 16$  Hz, 23-H); MS m/e 504 ( $M^+$ ), 486 ( $M^+ - H_2O$ ), 444 ( $M^+ - HOAc$ ), 426 [ $M^+ - (H_2O + HOAc)$ ], 384 ( $M^+ - 2HOAc$ ), 366 [ $M^+ - 2HOAc + H_2O$ ].

Anal. Calcd for  $C_{29}H_{44}O_7$ : C, 69.02; H, 8.79. Found: C, 69.29; H, 8.98.

(E)-Methyl 3 $\alpha$ ,7 $\alpha$ -diacetoxy-5 $\beta$ -cholan-11,22-dien-24-oate (25). The 12 $\alpha$ -

alcohol 24 (320 mg, 0.63 mmol) in anhydrous pyridine (5 mL) was cooled in ice. It was treated with phosphorous oxychloride (0.08 mL, 0.86 mmol) according to the procedure described for compound 5. Work-up in the usual manner and purification on preparative TLC (30% ethyl acetate/hexane) yielded the  $\Delta^{11}$ -olefin 25 (216 mg, 68%) as an oil. This was crystallized in ethyl acetate/hexane to

give mp 114-116<sup>o</sup>C; IR  $\nu_{\max}$  1720 (C=O), 1650 (C=C), 1250, 1100, 1030 (C-O)  $\text{cm}^{-1}$ ; UV (acetonitrile)  $\lambda_{\max}$  207 nm ( $\epsilon$  11,700); NMR  $\delta$  0.77 (3H, s, 18-Me), 0.90 (3H, s, 19-Me), 1.17 (3H, d,  $J$  = 7 Hz, 21-Me), 2.02 (3H, s, 3-acetate Me), 2.03 (3H, s, 7-acetate Me), 3.67 (3H, s, 24-COOMe), 4.53 (1H, br.m.,  $w/2$  = 18 Hz, 3-H), 4.90 (1H, m,  $w/2$  = 9 Hz, 7-H), AB system  $H_A$  6.07 (1H, dd,  $J$  = 3 Hz,  $J_{AB}$  = 10 Hz, 12-H),  $H_B$  5.43 (1H, dd,  $J$  = 2 Hz,  $J_{AB}$  = 10 Hz, 11-H),  $H_A$  6.75 (1H, dd,  $J$  = 8 Hz,  $J_{A'B'}$  = 16 Hz, 22-H),  $H_B$  5.68 (1H, d,  $J_{A'B'}$  = 16 Hz, 23-H); MS  $m/e$  426 ( $M^+$ -HOAc), 366 ( $M^+$ -2HOAc).

Anal. Calcd for  $C_{29}H_{42}O_6$ : C, 71.57; H, 8.70. Found: C, 71.64; H, 8.82.

[11 $\alpha$ ,12 $\alpha$ ,22 $\xi$ ,23 $\xi$ -<sup>2</sup>H<sub>4</sub>]-Methyl 3 $\alpha$ ,7 $\alpha$ -diacetoxo-5 $\beta$ -cholan-24-oate (26). To the

diene 25 (380 mg, 0.79 mmol) dissolved in deuterated ethanol (OD) (5 mL) was added 10% platinum-on-carbon (400 mg). This was stirred vigorously under a deuterium atmosphere at room temperature overnight. Filtration and evaporation of the solvent gave a crude oil which was purified on preparative TLC (5 x 30% ether/hexane) to yield the tetradeuterated ester 26 (238 mg, 60%) as white crystals; mp 124-125<sup>o</sup>C [Lit. (16) 130-132<sup>o</sup>C]; IR  $\nu_{\max}$  2120 (C-D), 1730 (C=O), 1230, 1160 (C-O)  $\text{cm}^{-1}$ ; NMR  $\delta$  0.66 (3H, s, 18-Me), 0.90 (3H, d,  $J$  = 7 Hz, 21-Me), 0.93 (3H, s, 19-Me), 2.03 (3H, s, 3-OAc Me), 2.05 (3H, s, 7-OAc Me), 3.67 (3H, s, 24-COOMe), 4.57 (1H, br.m.,  $w/2$  = 18 Hz, 3-H), 4.90 (1H, m,  $w/2$  = 8 Hz, 7-H); MS  $m/e$  434 [(M+4)<sup>+</sup>-HOAc], 374 [(M+4)<sup>+</sup>-2HOAc]; Isotope purity: 76% (M+4), 24% (M+3).

Anal. Calcd for  $C_{29}H_{46}O_6$ : C, 70.98; H, 9.45. Found: C, 70.80; H, 9.39.

(E)-Methyl 3 $\alpha$ ,7 $\alpha$ -dihydroxy-5 $\beta$ -chola-11,22-dien-24-oate (28b). To the diene 25 (1.67 g, 3.43 mmol) was added a methanolic potassium hydroxide solution (36 mL, 5 g potassium hydroxide, 75 mL methanol, 24 mL water), refluxed for 4 hr, then concentrated, cooled and acidified with cold 10% hydrochloric acid. The precipitate was filtered, washed with water and dried to give the diol 28a (1.34 g, 100%), as white crystals, mp 125-128<sup>o</sup>C. The acid 28a was esterified in the usual manner with methanol and *p*-toluenesulfonic acid and chromatographed to



give the methyl ester 28b as an oil (1.30 g, 93%) which was shown to be homogeneous by TLC, HPLC and NMR: IR  $\nu_{\max}$  3400 (OH), 1710 (C=O), 1640 (C=C), 1230, 1190, 1170 (C-O)  $\text{cm}^{-1}$ ; UV (acetonitrile)  $\lambda_{\max}$  217 nm ( $\epsilon$  11,300); NMR  $\delta$  0.77 (3H, s, 18-Me), 0.86 (3H, s, 19-Me), 1.17 (3H, d,  $\underline{J}$  = 6 Hz, 21-Me), 3.43 (1H, br.m.,  $w/2$  = 18 Hz, 3-H), 3.71 (3H, s, 24-COOME), 3.97 (1H, m,  $w/2$  = 8 Hz, 7-H), AB system  $H_A$  6.12 (1H, dd,  $\underline{J}$  = 3 Hz,  $\underline{J}_{AB}$  = 10 Hz, 12-H),  $H_B$  5.53 (1H, dd,  $\underline{J}$  = 2 Hz,  $\underline{J}_{AB}$  = 10 Hz, 11-H),  $H_A$ , 6.86 (1H, dd,  $\underline{J}$  = 9 Hz,  $\underline{J}_{A'B'}$  = 16 Hz, 22-H),  $H_B$ , 5.77 (1H, d,  $\underline{J}_{A'B'}$  = 16 Hz, 23-H).

Anal. Calcd for  $C_{25}H_{38}O_4$ : C, 74.59; H, 9.52. Found: C, 74.63; H, 9.70.

(E)-Methyl 3 $\alpha$ -hydroxy-7-oxo-5 $\beta$ -chola-11,22-dien-24-oate (29). To the diol 28b (420 mg, 1.08 mmol) in glacial acetic acid (4.2 mL) was added anhydrous sodium acetate (840 mg). Potassium dichromate (270 mg, 1.4 mmol) in water (0.5 mL) was added dropwise. The bath temperature was maintained between 10-20°C throughout the period. After 6 hr, the reaction mixture was quenched with ice. Worked-up as before gave the 7-oxo product 29 (328 mg, 78%) as an oil which exhibited IR  $\nu_{\max}$  3400 (OH), 1700 (C=O), 1640 (C=C), 1170, 1075 (C-O), 980 (trans-olefin)  $\text{cm}^{-1}$ ; UV (acetonitrile)  $\lambda_{\max}$  209 nm ( $\epsilon$  10,700); NMR  $\delta$  0.73 (3H, s, 18-Me), 1.10 (3H, s, 19-Me), 1.13 (3H, d,  $\underline{J}$  = 7 Hz, 21-Me), 3.63 (1H, br.m.,  $w/2$  = 18 Hz, 3-H), 3.69 (3H, s, 24-COOME), AB system  $H_A$  6.17 (1H, dd,  $\underline{J}$  = 3 Hz,  $\underline{J}_{AB}$  = 10 Hz, 12-H),  $H_B$  5.38 (1H, dd,  $\underline{J}$  = 2 Hz,  $\underline{J}_{AB}$  = 10 Hz, 11-H),  $H_A$ , 6.79 (1H, dd,  $\underline{J}$  = 9 Hz,  $\underline{J}_{A'B'}$  = 16 Hz, 22-H),  $H_B$ , 5.74 (1H, d,  $\underline{J}_{A'B'}$  = 16 Hz, 23-H); MS  $m/e$  400 ( $M^+$ ), 382 ( $M^+ - H_2O$ ).

Anal. Calcd for  $C_{25}H_{36}O_4$ : C, 74.96; H, 9.06. Found: C, 74.81; H, 8.78.

[11 $\alpha$ ,12 $\alpha$ ,22 $\xi$ ,24 $\xi$ - $^2H_4$ ]-Methyl 3 $\alpha$ -hydroxy-7-oxo-5 $\beta$ -cholane-24-oate (30). The diene 29 (120 mg, 0.30 mmol) was treated with platinum-carbon and deuterated methanol (OD) in a deuterium atmosphere according to the procedure described for compound 26. Work-up according to that procedure gave the tetradeuterated ester 30 (100 mg, 82%), as colorless needles, mp (MeOH) 104-106°C [Lit. (16) 107-109°C]; IR  $\nu_{\max}$  3400 (OH), 2150 (C-D), 1725, 1690 (C=O), 1250, 1190,

1160 (C=O)  $\text{cm}^{-1}$ ; NMR  $\delta$  0.63 (3H, s, 18-Me), 0.93 (3H, d,  $J = 6$  Hz, 21-Me), 1.18 (3H, s, 19-Me), 3.50 (1H, br.m.,  $w/2 = 17$  Hz, 3-H), 3.60 (3H, s, 24-COOMe); MS  $m/e$  390  $[(M+4)^+ - H_2O]$ , 372  $[(M+4)^+ - 2H_2O]$ ; Isotope purity: 74% (M+4), 21% (M+3), 5% (M+2).

$[11\alpha, 12\alpha, 22\epsilon, 23\epsilon - 2H_4]$ -Methyl  $3\alpha, 7\beta$ -dihydroxy-5 $\beta$ -cholan-24-oate (31b). The hydroxyketone 30 (80 mg, 0.2 mmol) was dried by azeotropic distillation from toluene and kept overnight under vacuum. Then it was dissolved in anhydrous *n*-butanol (1 mL) and heated to 120°C in a nitrogen atmosphere. Sodium pieces (105 mg, 4.6 mmol) were added, producing immediately the yellow color of the enolate. The reaction was terminated when the yellow color disappeared. The alcoholate formed was decomposed by addition of water and the butanol was removed by vacuum distillation. The alkaline solution was neutralized by addition of a solution of a 10% hydrochloric acid. The precipitate was filtered, washed with water (3 x 10 mL) and dried to give the deuterated acid 31a (75 mg, 97%). The acid 31a was esterified with methanol *p*-toluenesulfonic acid in the usual manner and after preparative TLC (5 x 30% ethyl acetate/hexane) yielded the deuterated methyl ursodeoxycholate 31b (58 mg, 77% from the acid 31a). An analytical sample was recrystallized from ether/hexane as fine colorless needles, mp 151-153°C [Lit (17) 152°C]. IR  $\nu_{\text{max}}$  3350 (OH), 2180, 2100 (C-D), 1720 (C=O), 1190, 1160, 1080, 1060, 1030 (C-O)  $\text{cm}^{-1}$ , NMR  $\delta$  0.69 (3H, s, 18-Me), 0.96 (3H, s, 19-Me), 3.67 (3H, s, 24-COOMe); MS  $m/e$  398  $[(M+4)^+ - H_2O]$ , 380  $[(M+4)^+ - 2H_2O]$ ; Isotope purity: 69% (M+4), 20% (M+3), 9% (M+2), 2% (M+1)

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