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Potential Bile Acid Metabolites. X. Syntheses of Stereoisomeric 3,7-Dihydroxy-5α-cholanic Acids[1)]

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New synthetic routes to allochenodeoxycholic (3α,7α-dihydroxy-5α-cholanic) and alloursodeoxycholic (3α,7β-dihydroxy-5α-cholanic) acids, and their stereoisomers are described. Treatments of allo 7α-hydroxy-3β-tosyloxy ester with *N,N*-dimethylformamide and of allo 7α-mesyloxy-3β-tosyloxy and 3β-cathyloxy-7α-mesyloxy esters with potassium superoxide-crown ether afforded the desired 3α,7α-, 3α,7β-, and 3β,7β-dihydroxy stereoisomers, respectively, in high yield. High-performance liquid chromatography was of key importance in characterizing the compounds and determining their purity.

Keywords——bile acid; allo bile acid; 3,7-dihydroxy-5α-cholanic acid; allochenodeoxycholic acid; alloursodeoxycholic acid; *N,N*-dimethylformamide reaction; potassium superoxide-18-crown-6 ether reaction; HPLC

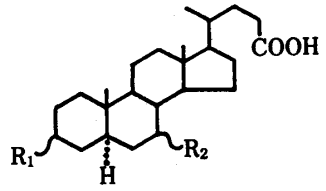
Allo (5α) cholanic acids are minor components of the bile of mammals including the human.[2)] As part of a program of synthesis of potential bile acid metabolites, we previously reported the preparation of the four isomers of allo 3,12-dihydroxycholanic acids.[1)] The present paper describes stereoselective syntheses of the four possible 3,7-dihydroxy allo acids (**1**—**4**) *via* 3-oxo-7α-hydroxy-4-cholenic acid (**5**).

7α-Hydroxy-3-oxo-4-cholenic acids, which would serve as important intermediates in the preparation of the allo bile acids,[3,4)] are accessible by two published procedures.[4,5)] Therefore, we initially applied the precedent methods to the preparation of **5** from chenodeoxycholic acid (**6**). The first, dehydrogenation with selenium dioxide[4)] of 7α-hydroxy-3-oxo-5β-cholanic acid, derived from **6**, was found to be impractical because tedious chromatographic purification was required for isolating the desired **5** and only a low yield was obtained. Our attempts to apply the second method recently introduced by Leppik[5)] failed at the dehydrobromination step with lithium carbonate and lithium bromide of 4β-bromo-7α-formyloxy-3-oxo-5β-cholanic acid (**7**), derived from **6**, to yield 3-oxo-4,6-choladienic acid as the major product. However, we have been able to obtain the desired 7α-formyloxy-3-oxo-4-cholenic acid (**8**) in 42% yield by carrying out the dehydrobromination with the use of semicarbazide and pyruvic acid.[1)] Alkaline hydrolysis of **8** under mild conditions[5)] afforded **5**. The overall yield of **5** from **6** was 31%. Treatment of **5** with lithium in liquid ammonia in the presence of methanol as a proton source led to a satisfactory yield (52%) of 3β,7α-dihydroxy-5α-cholanic acid (**3**).[4)]

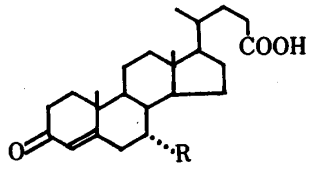
Previous methods for the preparation of allochenodeoxycholic acid (**1**) involved reduction of the corresponding 3-keto derivative with various agents known to favor axial hydroxy products: a) catalytic hydrogenation in acidic media[6,7)]; b) trimethyl-phosphite and iridium chloride[4)]; and c) K-Selectride (potassium *tri-sec*-butylborohydride).[8)] However, with all these procedures, **1a** (as the ester) had to be isolated by chromatographic separation from

the resulting mixture of epimers and other by-products. Therefore, we performed the synthesis of **1a** by inversion of an equatorial hydroxyl group at C-3, the method described in a previous paper.[9] The 3β-hydroxy ester (**3a**) readily yielded the 3β-monotosylate (**9a**), which underwent inversion with *N*,*N*-dimethylformamide (DMF) to give **1a** in good yield (82%). This product was easily hydrolyzable to the acid (**1**).

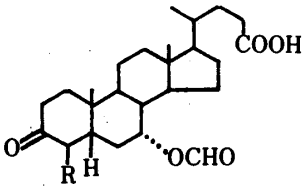
Alloursodeoxycholic acid (**2**) has previously been prepared from methyl 3α-carboethoxy-6-bromo-7,12-diketo-5β-cholanate.[10] Our new synthesis is an application of a direct stereospecific reaction discovered in the 5β-series.[11-13] 7α-Mesyloxy-3β-tosyloxy ester (**10a**), when subjected to treatment with potassium superoxide (KO_2)-18-crown-6 ether in dimethyl sulfoxide (DMSO), underwent simultaneous inversion at both C-3 and C-7, accompanied by hydrolysis of the C-24 ester group to give **2** in 59% isolated yield. In the 5β-series, the 3α-tosyloxy group required 22 h for complete inversion in the KO_2-crown ether reaction.[12,13] In contrast, under identical conditions, the equatorial 3β-tosylate in the 5α-series underwent complete inversion in 8 h.



1: $R_1=R_2=\alpha$-OH
2: $R_1=\alpha$-OH, $R_2=\beta$-OH
3: $R_1=\beta$-OH, $R_2=\alpha$-OH
4: $R_1=R_2=\beta$-OH
9: $R_1=\beta$-$OSO_2C_6H_4CH_3(p)$, $R_2=\alpha$-OH
10: $R_1=\beta$-$OSO_2C_6H_4CH_3(p)$, $R_2=\alpha$-OSO_2CH_3
11: $R_1=\beta$-$OCOOC_2H_5$, $R_2=\alpha$-OH
12: $R_1=\beta$-$OCOOC_2H_5$, $R_2=\alpha$-OSO_2CH_3



5: R=OH
8: R=OCHO



7: R=Br
13: R=H

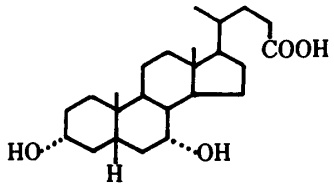


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Chart 1

TABLE I. TLC and HPLC Data for 3,7-Dihydroxy Stereoisomers of Bile Acid Esters[a]

Configuration of hydroxyls	TLC[b] (*Rf*-Values)		HPLC[c] (k'_r-Values)	
	5α	5β	5α	5β
3α,7α	0.21	0.27	1.13	1.00
3α,7β	0.25	0.23	0.36	0.31
3β,7α	0.18	0.30	0.44	0.51
3β,7β	0.17	0.23	0.39	0.34

a) The designation 5α refers to allocholanates, and 5β to 5β-cholanates. *b*) In TLC on silica gel, the samples were analyzed as the C-24 methyl esters and developed in hexane–EtOAc–acetic acid (50:50:1, v/v/v). *c*) The samples were analyzed as the C-24 4-nitrophthalimidemethyl esters under the following conditions: column, Nova-Pak C_{18}; detector, UV at 254 nm; mobile phase, MeOH–water (75:25, v/v); flow rate, 0.7 ml/min. Capacity factors (k') were expressed relative to that of 3α,7α-dihydroxy-5β-cholanic acid ester.

The fourth 3β,7β-dihydroxy stereoisomer (**4**) had previously been isolated as the ester by the Raney nickel reduction of methyl 6-oxo-3α,7β-dihydroxy-5α-cholanate 6,6-ethylenethioketal.[14,15] The present synthesis is analogous to the conversion of **10a** to **2**. The 3β-

cathyloxy-7α-mesyloxy ester (**12a**), derived from the 3β-cathyloxy-7α-hydroxy ester (**11a**), was directly converted by means of the KO_2-crown ether reaction to **4** in excellent yield (77%).

Table I shows the *Rf*-values on thin layer chromatography (TLC) and the k'_r-values (relative capacity factors) on high-performance liquid chromatography (HPLC) for the C-24 esters of stereoisomeric 3,7-dihydroxy-5α-cholanic acids, together with the data for the corresponding esters of the 5β-series.[12] The *Rf*- and k'_r-values were obtained for the methyl and 4-nitrophthalimidemethyl[16] esters, respectively. As can be seen, two of the allo 3,7-diol isomers and some isomeric pairs of the 5α- and 5β-series exhibit very similar mobilities on TLC. However, all eight 3,7-dihydroxy stereoisomers were well resolved by HPLC on a C'_{18} reversed-phase column. It should be noted that the 3α,7α-diols of both the 5α- and 5β-series are eluted much more slowly than the others.

Experimental

Melting points were determined on a micro hot stage apparatus and are uncorrected. Infrared (IR) spectra were obtained on a JASCO IRA-II double beam spectrometer as KBr tablets. Ultraviolet (UV) spectra were determined in ethanol solution using a Shimadzu UV-200 double-beam spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained on a JEOL FX-90Q instrument with $CDCl_3$ containing 1% Me_4Si as the solvent except where otherwise indicated; chemical shifts are expressed in δ (ppm) relative to Me_4Si. Mass spectra (MS) were recorded on a Hitachi RMU-7M mass spectrometer under the following conditions: ion source temperature, 180 °C; ionizing voltage, 70 eV. HPLC was carried out on a Waters Associates system (M-45 pump; U6K sample loop injector; R401 differential refractometer or Shimadzu SPD-2A UV detector) using a Nova-Pak C_{18} reversed-phase column (15 cm × 3.9 mm i.d., 5 μm; Waters Associates) with MeOH–water mixture (75 : 25, v/v) as the mobile phase. Analytical TLC was performed on pre-coated silica gel (20 cm × 20 cm, 0.25 mm layer thickness; E. Merck AG) using hexane–EtOAc–acetic acid mixture (50 : 50 : 1, v/v/v) as the developing solvent for methyl esters.

4β-Bromo-7α-formyloxy-3-oxo-5β-cholanic Acid (7)——Prepared from 7α-formyloxy-3-oxo-5α-cholanic acid (**13**) (obtained from **6**)[17] by the bromination procedure described in the previous paper.[1] Crystallization of the oily product from EtOAc–hexane gave **7** as colorless needles. mp 159—161 °C. IR ν_{max} cm^{-1}: 1720, 1708 (C=O), 1178 (C–O), 560 (C–Br). ^{1}H-NMR δ: 0.70 (3H, s, 18-H), 0.94 (3H, d, J=5.4 Hz, 21-H), 1.10 (3H, s, 19-H), 5.17 (1H, m, 7-H), 5.33 (1H, d, J=11.7 Hz, 4-H), 8.08 (1H, s, CHO). *Anal.* Calcd for $C_{25}H_{37}BrO_5$: C, 60.36; H, 7.50. Found: C, 59.93; H, 7.65.

7α-Formyloxy-3-oxo-4-cholenic Acid (8)——A solution of semicarbazide (2.6 g) and sodium acetate (1.7 g) in water (10 ml) was added to a solution of **7** (4.4 g) in acetic acid (150 ml). The mixture was stirred for 30 min at 60 °C under N_2 and then for 1 h at room temperature. Water was added gradually to the mixture to cause precipitation of the semicarbazone of **8**, which was filtered off and washed with water. A solution of pyruvic acid (9 ml) in water (22 ml) was added to a solution of the crude semicarbazone in acetic acid (90 ml). The mixture was stirred overnight at room temperature under N_2, and the precipitated solid was filtered off. The filtrate was extracted with EtOAc three times, and the combined extracts were washed with water to neutrality, dried over Drierite, and evaporated. The pale yellow residue (2.69 g) was chromatographed on a column of silica gel (120 g). Elution with benzene–EtOAc (9 : 1, v/v) gave 0.82 g (25%) of a solid which was characterized as 3-oxo-4,6-choladienic acid. mp 197—198 °C (CH_2Cl_2–hexane). IR ν_{max} cm^{-1}: 1710 (C=O), 1628, 1608 (4,6-diene). UV λ_{max} nm (ε): 284 (30500). ^{1}H-NMR δ: 0.77 (3H, s, 18-H), 0.95 (3H, d, J=5.4 Hz, 21-H), 1.11 (3H, s, 19-H), 5.69 (1H, s, 4-H), 6.12 (2H, s, 6- and 7-H). *Anal.* Calcd for $C_{24}H_{34}O_3$: C, 77.80; H, 9.25. Found: C, 77.97; H, 9.18.

Further elution with benzene–EtOAc (7 : 3, v/v) and crystallization of the eluate from acetone–hexane gave **8** (1.53 g; 42%) as colorless fine needles. mp 199—200 °C. IR ν_{max} cm^{-1}: 1730 (C=O), 1650 (4-ene), 1180, 1160 (C–O). UV λ_{max} nm (ε): 248 (17000). ^{1}H-NMR δ: 0.72 (3H, s, 18-H), 0.94 (3H, d, J=5.4 Hz, 21-H), 1.22 (3H, s, 19-H), 5.16 (1H, m, 7-H), 5.70 (1H, s, 4-H), 8.05 (1H, s, CHO). *Anal.* Calcd for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71. Found: C, 71.91; H, 8.63.

3-Oxo-7α-hydroxy-4-cholenic Acid (5)——Prepared from **8** by a slight modification of the procedure of Leppik.[5] Recrystallization from aq. MeOH gave **5** (94%) as colorless fine needles. mp 227—229 °C (lit. mp 231—233 °C).[4] IR ν_{max} cm^{-1}: 1718 (C=O), 1630 (4-ene), 3500 (OH). UV λ_{max} nm (ε): 242 (15900). ^{1}H-NMR ($CDCl_3$ + 20% DMSO-d_6) δ: 0.71 (3H, s, 18-H), 0.93 (3H, d, J=5.4 Hz, 21-H), 1.19 (3H, s, 19-H), 3.91 (1H, m, 7-H), 5.70 (1H, s, 4-H).

Methyl 3β,7α-Dihydroxy-5α-cholanate (3a)——The acid (**5**) was reduced with Li–NH_3[4] and the reaction was quenched by adding MeOH. Treatment of the residual bile salts by acidification followed by methyl esterification, and chromatographic purification of the product, gave **3a** as the main product (52%). mp 160—161 °C (acetone–hexane) (lit. mp 159—160 °C[4] and 160—161 °C[7]). IR ν_{max} cm^{-1}: 1713 (C=O), 3530, 1033, 1015 (OH). ^{1}H-NMR δ: 0.66 (3H, s, 18-H), 0.80 (3H, s, 19-H), 0.92 (3H, d, J=6.3 Hz, 21-H), 3.58 (1H, br m, 3-H), 3.66 (3H, s, COOMe), 3.84

(1H, m, 7-H). MS *m/z* (relative intensity): 406 (2, M^+), 388 (100, $M-H_2O$), 373 (25, $M-H_2O-CH_3$), 273 [83, $M-H_2O$ – side chain (SC)], 264 (20, M – SC–part of ring D), 249 (38, M – SC – ring D), 246 (46, $M-H_2O$ – part of ring D).

3β,7α-Dihydroxy-5α-cholanic Acid (3)——Prepared from **3a** by the usual method with 5% methanolic KOH. Recrystallization of the product from aq. MeOH gave **3** as colorless needles. mp 271—272 °C (lit. mp 273—274.5 °C).[2] IR ν_{max} cm^{-1}: 1715 (C=O), 3550, 1042, 1024 (OH). ^{1}H-NMR ($CDCl_3$ + 20% DMSO-d_6) δ: 0.65 (3H, s, 18-H), 0.78 (3H, s, 19-H), 0.92 (3H, d, J=5.4 Hz, 21-H), 3.53 (1H, br m, 3-H), 3.74 (1H, m, 7-H).

Methyl 7α-Hydroxy-3β-tosyloxy-5α-cholanate (9a)——Prepared from **3a** by the tosyl chloride–pyridine method[18] in quantitative yield. Crystallization from benzene–hexane gave **9a** as colorless prisms. mp 157—158 °C. IR ν_{max} cm^{-1}: 1733 (C=O), 1333, 1175, 920, 868 (SO_2), 3600, 1032, 1016 (OH). ^{1}H-NMR δ: 0.64 (3H, s, 18-H), 0.77 (3H, s, 19-H), 0.90 (3H, d, J=5.4 Hz, 21-H), 2.44 (3H, s, ArMe), 3.65 (3H, s, COOMe), 3.79 (1H, m, 7-H), 4.39 (1H, br m, 3-H), 7.32 and 7.78 (each 2H, d, J=9.0 Hz, *para*-disubstituted phenyl). *Anal.* Calcd for $C_{32}H_{48}O_6S$: C, 68.54; H, 8.63. Found: C, 68.78; H, 8.36.

Methyl 3α,7α-Dihydroxy-5α-cholanate (1a)——A solution of **9a** (320 mg) in DMF (15 ml) was kept at 80±1 °C for 65 h. The reaction mixture was diluted with water and the product was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with water, dried over Drierite and evaporated. The residual oily product was dissolved in benzene and poured onto a column of neutral alumina (activity II, ratio 50 : 1). After 18 h, the column was eluted with benzene–EtOAc (1 : 1, v/v) and recrystallization of the eluate from aq. acetone gave **1a** (190 mg; 82%) as colorless thin plates. mp 126.5—127.5 °C (lit. mp 116—118 °C[4] and 125—126 °C[6]). IR ν_{max} cm^{-1}: 1735, 1713 (C=O), 3380, 1033 (OH). ^{1}H-NMR δ: 0.66 (3H, s, 18-H), 0.78 (3H, s, 19-H), 0.93 (3H, d, J=6.3 Hz, 21-H), 3.66 (3H, s, COOMe), 3.83 (1H, m, 7-H), 4.05 (1H, m, 3-H). MS *m/z* (relative intensity): 406 (4, M^+), 388 (100, $M-H_2O$), 373 (27, $M-H_2O-CH_3$), 370 (25, $M-2H_2O$), 355 (17, $M-2H_2O-CH_3$), 273 (93, $M-H_2O$–SC), 264 (24, M – SC – part of ring D), 249 (38, M – SC – ring D), 246 (42, $M-H_2O$ – SC – part of ring D).

3α,7α-Dihydroxy-5α-cholanic Acid (1)——Prepared from **1a** by the usual hydrolysis procedure. Recrysrallization of the product from EtOAc gave **1** as colorless needles. mp 245—247 °C (lit. mp 245—246 °C[7] and 238—240 °C[19]). IR ν_{max} cm^{-1}: 1700 (C=O), 3400, 1027, 1000 (OH). ^{1}H-NMR ($CDCl_3$ + 20% DMSO-d_6) δ: 0.64 (3H, s, 18-H), 0.75 (3H, s, 19-H), 0.91 (3H, d, J=5.4 Hz, 21-H), 3.72 (1H, m, 7-H), 3.95 (1H, m, 3-H).

Methyl 7α-Mesyloxy-3β-tosyloxy-5α-cholanate (10a)——Methanesulfonyl chloride (0.8 ml) was slowly added dropwise to a stirred solution of **9a** (700 mg) in dry pyridine (10 ml). Stirring was continued for 1 h and the mixture was then allowed to stand overnight at room temperature. The dark brown solution was dripped into cold water and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed successively with water, 3 N HCl, and water, decolorized with Norite, and evaporated. The residual oil (740 mg), although apparently homogeneous on TLC and ^{1}H-NMR analyses, could not be crystallized. IR ν_{max} cm^{-1}: 1732 (C=O), 1350, 1177 (SO_2), 930, 892, 863 (mesylate). ^{1}H-NMR δ: 0.64 (3H, s, 18-H), 0.80 (3H, s, 19-H), 0.90 (3H, d, J=6.3 Hz, 21-H), 2.43 (3H, s, ArMe), 3.00 (3H, s, SO_2Me), 3.65 (3H, s, COOMe), 4.39 (1H, br m, 3-H), 4.80 (1H, m, 7-H), 7.30 and 7.76 (each 2H, d, J=8.1 Hz, *para*-disubstituted phenyl). *Anal.* Calcd for $C_{33}H_{50}O_8S_2$: C, 62.05; H, 7.89. Found: C, 61.96; H, 7.63.

3α,7β-Dihydroxy-5α-cholanic Acid (2)——A suspension of powdered KO_2 (160 mg) in dry DMSO (12 ml) was stirred under N_2 for 10 min, then 18-crown-6 (90 mg) was added. The suspension was further stirred at room temperature until most of the KO_2 was dissolved (*ca.* 2 h). A solution of **10a** (320 mg) dissolved in DMSO (5 ml) was then added to the above solution and the mixture was stirred under N_2 for an additional 8 h. The flask was immersed in an ice bath, and saturated NaCl solution (15 ml) was added gradually. The resulting solution was extracted with benzene. The aq. layer was cooled in an ice bath, acidified with 3 N HCl, and extracted with EtOAc. The EtOAc extract was washed with water, dried over Drierite, and evaporated to dryness. The residue, when treated with aq. MeOH, afforded 115 mg (59%) of **2** as colorless needles. mp 243.5—245.0 °C (lit. mp 223—224 °C).[10] IR ν_{max} cm^{-1}: 1677 (C=O), 3300, 1032, 1003 (OH). ^{1}H-NMR ($CDCl_3$ + 20% DMSO-d_6) δ: 0.68 (3H, s, 18-H), 0.78 (3H, s, 19-H), 0.93 (3H, d, J=5.4 Hz, 21-H), 3.40 (1H, br m, 7-H), 3.95 (1H, m, 3-H).

Methyl 3α,7β-Dihydroxy-5α-cholanate (2a)——Prepared from **2** by the usual esterification method. The ester, although apparently homogeneous on TLC and ^{1}H-NMR analyses, could not be crystallized. IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 1735 (C=O), 3430, 1032, 1003 (OH). ^{1}H-NMR δ: 0.68 (3H, s, 18-H), 0.79 (3H, s, 19-H), 0.93 (3H, d, J=5.4 Hz, 21-H), 3.34 (1H, br m, 7-H), 3.65 (3H, s, COOMe), 4.05 (1H, m, 3-H). MS *m/z* (relative intensity): 388 (64, $M-H_2O$), 373 (14, $M-H_2O-CH_3$), 370 (100, $M-2H_2O$), 355 (36, $M-2H_2O-CH_3$), 255 (64, $M-2H_2O$–SC), 249 (25, M – SC – ring D), 246 (22, $M-H_2O$ – SC – part of ring D). *Anal.* Calcd for $C_{25}H_{42}O_4$: C, 73.85; H, 10.41. Found: C, 73.58; H, 10.32.

Methyl 3β-Cathyloxy-7α-hydroxy-5α-cholanate (11a)——Prepared from **3a** (700 mg) by the cathylation method reported previously.[11] Crystallization from acetone–hexane gave **11a** (610 mg; 74%) as colorless needles. mp 120—121 °C (lit. mp 122—123 °C).[15] IR ν_{max} cm^{-1}: 1738 (C=O), 3560 (OH). ^{1}H-NMR δ: 0.66 (3H, s, 18-H), 0.81 (3H, s, 19-H), 0.91 (3H, d, J=6.3 Hz, 21-H), 1.30 (3H, t, J=7.2 Hz, $OCOOCH_2\underline{CH_3}$), 3.65 (3H, s, COOMe), 3.80 (1H, m, 7-H), 4.15 (2H, q, J=6.3 Hz, $OCOO\underline{CH_2}CH_3$), 4.59 (1H, br m, 3-H).

Methyl 3β-Cathyloxy-7α-mesyloxy-5α-cholanate (12a)——Prepared from **11a** (500 mg) by the mesylation procedure described above. The oily product (530 mg), although apparently homogeneous on TLC and ^{1}H-NMR

analyses, could not be crystallized. IR ν_{max} cm^{-1}: 1735 (C=O), 1332, 1175 (SO_2), 890 (mesylate). ^{1}H-NMR δ: 0.66 (3H, s, 18-H), 0.84 (3H, s, 19-H), 0.92 (3H, d, J=5.4 Hz, 21-H), 1.30 (3H, t, J=7.2 Hz, OCOOCH$_2$CH$_3$), 3.02 (3H, s, SO_2Me), 3.66 (3H, s, COOMe), 4.17 (2H, q, J=6.3 Hz, OCOOCH$_2$CH$_3$), 4.60 (1H, br m, 3-H), 4.90 (1H, m, 7-H). *Anal.* Calcd for $C_{29}H_{48}O_8S$: C, 62.57; H, 8.69. Found: C, 62.38; H, 8.85.

3β,7β-Dihydroxy-5α-cholanic Acid (4)——The ester (**12a**) (280 mg), subjected to the KO_2-18-crown-6 ether inversion procedure as described above, required 1.5 h for complete reaction. The mixture was processed, and the resulting product was crystallized from aq. MeOH to give **4** (152 mg; 77%) as colorless crystals. mp 245.0—246.5 °C (lit. mp 240—241 °C).[2] IR ν_{max} cm^{-1}: 1690 (C=O), 3400, 1034 (OH), ^{1}H-NMR ($CDCl_3$+20% DMSO-d_6) δ: 0.67 (3H, s, 18-H), 0.91 (3H, s, 19-H), 0.93 (3H, d, J=6.3 Hz, 21-H), 3.35 (2H, br m, 3- and 7-H).

Methyl 3β,7β-Dihydroxy-5α-cholanate (4a)——Prepared from **4** by the usual esterification procedure. Crystallization from EtOAc gave **4a** as colorless needles. mp 159—160 °C (lit. mp 158—159 °C).[15] IR ν_{max} cm^{-1}: 1735 (C=O), 3340, 1037 (OH). ^{1}H-NMR δ: 0.69 (3H, s, 18-H), 0.83 (3H, s, 19-H), 0.93 (3H, d, J=6.3 Hz, 21-H), 3.41 (2H, br m, 3- and 7-H), 3.66 (3H, s, COOMe). MS *m/z* (relative intensity): 406 (2, M^+), 388 (100, M−H_2O), 373 (35, M−H_2O−CH_3), 273 (62, M−H_2O−SC), 264 (20, M−SC−part of ring D), 255 (27, M−2H_2O−SC), 249 (29, M−SC−ring D), 246 (35, M−H_2O−SC−part of ring D).

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References and Notes

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