[Chem. Pharm. Bull. 34(5)1934—1938(1986)]

Potential Bile Acid Metabolites. XI. Syntheses of Stereoisomeric 7,12-Dihydroxy-5α-cholanic Acids[1)]

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(Received September 17, 1985)

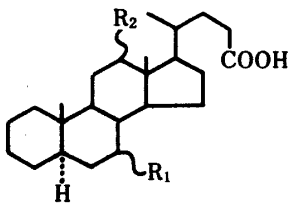
Three new compounds, 7α,12β-, 7β,12α-, and 7β,12β-dihydroxy-5α-cholanic acids, were synthesized. The principal reactions employed were 1) selective acylation at C-7 of a 7α,12α-dihydroxy ester with the propionic anhydride–4-dimethylaminopyridine system, 2) potassium superoxide-18-crown-6 ether inversion of the 7α-hydroxyl group, and 3) stereoselective reduction of the 12-ketones with the sodium borohydride–palladium chloride system and *tert*-butylamine–borane complex. High-performance liquid chromatography was of key importance in characterizing the compounds and determining their purity.

Keywords——bile acid; allo bile acid; 7,12-dihydroxy-5α-cholanoic acid; selective acylation; potassium superoxide-18-crown-6 ether reaction; sodium borohydride–palladium chloride reduction; *tert*-butylamine–borane complex reduction; HPLC

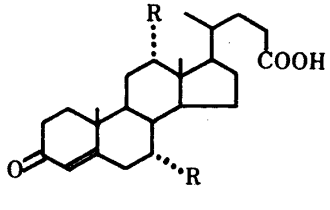
Of the eight stereoisomeric 7,12-dihydroxy bile acids in the 5α ("allo") and 5β ("normal") series, all four isomers in the latter series have been recently synthesized and characterized by two groups.[2,3)] In the allo series, the 7α,12α-dihydroxy isomer (**1**) has been synthesized,[4)] but the three remaining stereoisomers, 7α,12β- (**2**), 7β,12α- (**3**), and 7β,12β-dihydroxy compounds (**4**), are new. This paper deals with syntheses of these three stereoisomeric 7,12-dihydroxy bile acids from cholic acid (**5**).

3-Oxo-7α,12α-dihydroxy-4-cholenic acid (**6**), a key intermediate for allomerization of 5β bile acids, was prepared from **5** by using procedures similar to those reported in the previous paper.[1)] Treatment of **6** with lithium in liquid ammonia[5)] followed by Huang–Minlon reduction of the resulting allo 3-oxo-7α,12α-dihydroxy ester (**7a**)[4)] yielded **1**, the starting compound in the synthesis of the remaining allo 7,12-stereoisomers.

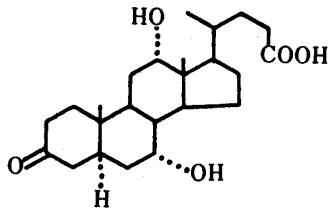
Attempts at partial acetylation of **1a**, which was employed in the 5β-sereis,[3)] failed under various conditions, giving a mixture of **1a**, mono- and diacetates, from which the desired 7-*O*-acetate (**8a**) was obtained in only poor yield after chromatography. However, with a change of the acylating reagent to propionic anhydride and combined use of 4-dimethylaminopyridine as a catalyst,[6)] partial acylation of **1a** proceeded smoothly. The reaction was completed within a few minutes at room temperature to give the 7α-monopropionate (**8a**) quantitatively. Compound **8a** was then oxidized with potassium chromate to give the 12-oxo-7α-propionyloxy derivative (**9a**), which in turn was hydrolyzed with methanolic potassium hydroxide to afford the 7α-hydroxy-12-oxo acid (**10**) in good yield (80% from **1a**). The ester (**10a**), when subjected to potassium superoxide (KO_2)-18-crown-6 ether treatment in dimethyl sulfoxide (DMSO) underwent inversion at C-7 *via* the 7α-mesylate (**11a**), as previously reported,[7)] accompanied by hydrolysis of the C-24 ester group to give the 7β-hydroxy-12-oxo acid (**12**) in 76% yield.



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
8 : $R_1=\alpha$-$OCOC_2H_5$, $R_2=\alpha$-OH



6 : R=OH
15 : R=OCHO



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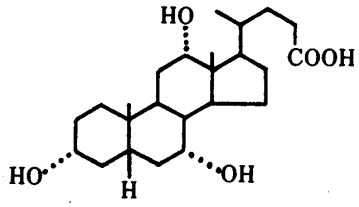

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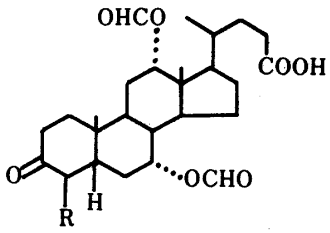
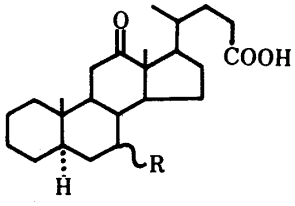


13 : R=Br
14 : R=H



9 : R=α-$OCOC_2H_5$
10 : R=α-OH
11 : R=α-OSO_2CH_3
12 : R=β-OH

Chart 1

TABLE I. TLC and HPLC Data for 7,12-Dihydroxy Stereoisomers of Bile Acid Esters[a]

Configuration of hydroxyls	TLC[b] (*Rf*-Values)		HPLC[c] (k_r'-Values)	
	5α	5β	5α	5β
7α,12α	0.31	0.76	4.06	3.72
7α,12β	0.69	0.76	1.02	1.00
7β,12α	0.59	0.63	0.90	0.83
7β,12β	0.61	0.65	0.80	0.68

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 (6:4, 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') are expressed relative to that of 7α,12β-dihydroxy-5β-cholanic acid ester.

As expected, reduction of **10a** and **12a** with *tert*-butylamine–borane reagent[8] yielded the corresponding 12β-hydroxy compounds as the major products, in essentially the same 12β/12α ratios, 3.2 for **2a** and 3.4 for **4a**. On the other hand, a practical route to axial 12α-hydroxy compounds in the 5β-series has been attained by sodium borohydride ($NaBH_4$) reduction.[9] A recent publication on the utility of the $NaBH_4$–palladium chloride system[10] prompted us to apply it to **10a** and **12a**. The reduction reaction proceed faster and more stereoselectively than with $NaBH_4$ alone, yielding **1a** and **3a** in 97% and 94% purities, respectively. Separation of the two 12-epimers was efficiently achieved by fractional crystallization or alumina column chromatography. Alkaline hydrolysis of **2a**, **3a**, and **4a** afforded quantitatively the desired acids, **2**, **3**, and **4**, respectively.

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 the stereoisomeric 7,12-dihydroxy-5α-cholanic acids, together with the data for the corresponding esters of the 5β-series.[3] The *Rf*- and k'_r-values were obtained as the methyl and 4-nitrophthalimidemethyl[11] esters, respectively. While some pairs of isomers exhibited very similar mobilities on TLC, all eight 7,12-dihydroxy stereoisomers were resolved by HPLC. It should be noted that the 7α,12α-diols in 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 for KBr tablets on a JASCO IRA-II double-beam spectrometer. Ultraviolet (UV) spectra were determined in ethanol solution using a Shimadzu 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, unless 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 × 20 cm, 0.25 mm layer thickness; E. Merck AG) using hexane–EtOAc (6:4, v/v) as the developing solvent for methyl esters.

4β-Bromo-3-oxo-7α-,12α-diformyloxy-5β-cholanic Acid (13)——Prepared from 3-oxo-7α,12α-diformyloxy-5β-cholanic acid (**14**) (obtained from **5**[12]) by the bromine-*N*,*N*-dimethylformamide method described in the previous paper.[13] Yield 82%. mp 198—200 °C (chloroform–ethyl ether) (lit. mp 192—193 °C).[12] IR ν_{max} cm^{-1}: 1715 (C=O), 1180 (C–O), 555 (C–Br). ^{1}H-NMR δ: 0.81 (3H, s, 18-H), 1.10 (3H, s, 19-H), 5.19 (1H, m, 7-H), 5.31 (1H, m, 12-H), 5.32 (1H, d, *J* = 10.8 Hz, 4-H), 8.11 and 8.14 (each 1H, s, CHO).

3-Oxo-7α,12α-diformyloxy-4-cholenic Acid (15)——A solution of semicarbazide (3.45 g) and sodium acetate (2.25 g) in water (13 ml) was added to a solution of **13** (5.8 g) in acetic acid (65 ml). The mixture was stirred for 30 min at 60 °C under N_2 and then for 1 h at room temperature. Water was gradually added to the mixture to precipitate the semicarbazone of **15**, which was collected by filtration and washed with water. A solution of pyruvic acid (15 ml) in water (30 ml) was added to a stirred suspension of the crude semicarbazone in acetic acid (120 ml) under N_2, and vigorous stirring of the mixture at room temperature was continued until a homogeneous solution was obtained (*ca.* 24 h). The solution was extracted with EtOAc three times, and the combined extracts were washed with water, dried over Drierite, and evaporated. The pale yellow oily residue was crystallized from EtOAc–hexane to provide 2.36 g (48%) of **15**. Concentration of the mother liquor yielded 2.21 g of a pale yellow oil, which was chromatographed on a column of silica gel (95 g). Elution with benzene–EtOAc (8:2, v/v) gave 0.76 g (17%) of a solid, which was characterized as 3-oxo-12α-formyloxy-4,6-choladienic acid. mp 210—212 °C (CH_2Cl_2–hexane) (lit. mp 211—212 °C).[12]

Continuous elution with benzene–EtOAc (1:1, v/v) gave an additional 1.04 g (21%) of **15** (combined yield, 69%). mp 156—157 °C (lit. mp 159—160 °C).[12] IR ν_{max} cm^{-1}: 1718 (C=O), 1663 (4-ene), 1180 (C–O). UV λ_{max} nm (ε): 237 (18300). ^{1}H-NMR δ: 0.82 (3H, s, 18-H), 1.21 (3H, s, 19-H), 5.20 (1H, m, 7-H), 5.29 (1H, m, 12-H), 5.72 (1H, s, 4-H), 8.07 and 8.10 (each 1H, s, CHO).

3-Oxo-7α,12α-dihydroxy-4-cholenic Acid (6)——Prepared from **15** according to the procedure of Leppik.[12] Yield 97%. mp 235—237 °C (aq. MeOH) (lit. mp 234—236 °C).[12] IR ν_{max} cm^{-1}: 1708 (C=O), 1620 (4-ene). UV λ_{max} nm (ε): 244 (18000). ^{1}H-NMR ($CDCl_3$ + 20% DMSO-d_6) δ: 0.72 (3H, s, 18-H), 0.99 (3H, d, *J* = 5.4 Hz, 21-H), 1.17 (3H, s, 19-H), 3.93 (2H, m, 7- and 12-H), 5.72 (1H, s, 4-H).

Methyl 3-Oxo-7α,12α-dihydroxy-5α-cholanate (7a)——Obtained by Li–NH_3 reduction of **6** followed by methyl esterification.[5] Yield 57%. mp 153—155 °C (lit. mp 156—157 °C[4] and 152—154 °C[5]). IR ν_{max} cm^{-1}: 1735, 1710 (C=O), 3400, 1033 (OH). ^{1}H-NMR δ: 0.71 (3H, s, 18-H), 0.99 (3H, s, 19-H), 3.66 (3H, s, COOMe), 3.85 (1H, m, 7-H), 3.98 (1H, m, 12-H).

7α,12α-Dihydroxy-5α-cholanic Acid (1)——Prepared by Huang–Minlon reduction of **7a**.[4] mp 225—227 °C (aq. acetone) (lit. mp 236—237 °C).[4] IR ν_{max} cm^{-1}: 1719 (C=O), 3400, 1028 (OH). ^{1}H-NMR ($CDCl_3$ + 20% DMSO-d_6) δ: 0.67 (3H, s, 18-H), 0.77 (3H, s, 19-H), 0.99 (3H, d, *J* = 5.4 Hz, 21-H), 3.74 (1H, m, 7-H), 3.89 (1H, m, 12-H).

Methyl 7α,12α-Dihydroxy-5α-cholanate (1a)——Prepared from **1** by the usual procedure of methyl esterification, and crystallized from aq. MeOH as colorless crystals. mp 165—166 °C (lit. mp 170—172 °C). IR ν_{max} cm^{-1}: 1735 (C=O), 3460, 1028 (OH). ^{1}H-NMR δ: 0.69 (3H, s, 18-H), 0.78 (3H, s, 19-H), 0.98 (3H, d, *J* = 5.4 Hz, 21-H), 3.66 (3H, s, COOMe), 3.81 (1H, m, 7-H), 3.96 (1H, m, 12-H). MS *m/z* (relative intensity): 388 (7, M−H_2O), 370 (73, M−2H_2O), 355 (16, M−CH_3−2H_2O), 273 [15, M−H_2O−side chain (SC)], 255 (100, M−2H_2O−SC).

Methyl 12α-Hydroxy-7α-propionyloxy-5α-cholanate (8a)——Propionic anhydride (3.0 g) was added in one portion to a solution of **1a** (3.7 g) and 4-*N*,*N*-dimethylaminopyridine (1.15 g) in dry benzene (90 ml). The mixture was stirred for 5 min at room temperature and poured into water. The organic layer was washed with 3 N HCl and water, dried over Drierite, and evaporated to dryness. The residual oil (4.20 g), although homogeneous according to TLC and ^{1}H-NMR analyses, resisted crystallization. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1722 (C=O), 3525, 1020 (OH), 1195, 1078 (C–O). ^{1}H-NMR δ: 0.68 (3H, s, 18-H), 0.79 (3H, s, 19-H), 0.98 (3H, d, J=4.5 Hz, 21-H), 1.16 (3H, t, J=7.2 Hz, $OCOCH_2\underline{CH_3}$), 2.35 (2H, q, J=10.8 Hz, $OCO\underline{CH_2}CH_3$), 3.66 (3H, s, COOMe), 3.96 (1H, m, 12-H), 4.90 (1H, m, 7-H). *Anal.* Calcd for $C_{28}H_{46}O_5$: C, 72.69; H, 10.02. Found: C, 72.35; H, 9.70.

Methyl 12-Oxo-7α-propionyloxy-5α-cholanate (9a)——A solution of K_2CrO_4 (2.5 g) in water (8 ml) was added to a stirred solution of **8a** (4.0 g) in acetic acid (80 ml). The mixture was stirred overnight at room temperature, then water was added to precipitate a solid, which was filtered off and washed with water. Recrystallization from aq. acetone afforded **9a** (3.2 g; 80%) as colorless fine needles. mp 159.5—160.5 °C. IR ν_{max} cm^{-1}: 1720, 1698 (C=O), 1215, 1060 (C–O). ^{1}H-NMR δ: 0.85 (3H, d, J=6.3 Hz, 21-H), 0.89 (3H, s, 18-H), 1.02 (3H, s, 19-H), 1.12 (3H, t, J=8.1 Hz, $OCOCH_2\underline{CH_3}$), 2.30 (2H, q, J=11.7 Hz, $OCO\underline{CH_2}CH_3$), 3.65 (3H, s, COOMe), 3.97 (1H, m, 7-H). *Anal.* Calcd for $C_{28}H_{44}O_5$: C, 73.00; H, 9.63. Found: C, 73.05; H, 9.49.

7α-Hydroxy-12-oxo-5α-cholanic Acid (10)——A solution of **9a** (3.27 g) in 10% methanolic KOH (80 ml) was refluxed for 12 h. After addition of water (150 ml), the ice-cooled solution was acidified with 5 N H_2SO_4. The precipitated solid was filtered off and washed with water. Recrystallization from aq. MeOH gave **10** (2.89 g; 100%) as colorless thin plates. mp 185—186.5 °C. IR ν_{max} cm^{-1}: 1710, 1692 (C=O), 3400, 1022 (OH). ^{1}H-NMR ($CDCl_3$+20% DMSO-d_6) δ: 0.84 (3H, d, J=5.4 Hz, 21-H), 0.87 (3H, s, 18-H), 1.02 (3H, s, 19-H), 3.82 (1H, m, 7-H). *Anal.* Calcd for $C_{24}H_{38}O_4 \cdot 3/4H_2O$: C, 71.87; H, 9.93. Found: C, 71.76; H, 9.68.

Methyl 7α-Hydroxy-12-oxo-5α-cholanate (10a)——Prepared from **10** (2.5 g) by the general esterification method. Crystallization from aq. MeOH gave **10a** (2.18 g; 84%) as colorless thin plates. mp 122—123 °C. IR ν_{max} cm^{-1}: 1735, 1690 (C=O), 3480, 1028, 993 (OH). ^{1}H-NMR δ: 0.85 (3H, d, J=4.5 Hz, 21-H), 0.87 (3H, s, 18-H), 1.02 (3H, s, 19-H), 3.66 (3H, s, COOMe), 3.90 (1H, m, 7-H). *Anal.* Calcd for $C_{25}H_{40}O_4$: C, 74.21; H, 9.97. Found: C, 74.00; H, 9.76.

Methyl 7α-Mesyloxy-12-oxo-5α-cholanate (11a)——**10a** was mesylated with methanesulfonyl chloride and processed as described in the previous paper[1] to give an oily product. The oil was crystallized from CH_2Cl_2–isopropyl ether to afford **11a** (2.42 g; 96%) as colorless fine needles. mp 135—136 °C. IR ν_{max} cm^{-1}: 1730, 1702 (C=O), 1358, 1175 (SO_2), 968, 948, 893 (mesylate). ^{1}H-NMR δ: 0.85 (3H, d, J=6.3 Hz, 21-H), 0.90 (3H, s, 18-H), 1.03 (3H, s, 19-H), 2.99 (3H, s, SO_2Me), 3.66 (3H, s, COOMe), 4.95 (1H, m, 7-H). *Anal.* Calcd for $C_{26}H_{42}O_6S$: C, 64.70; H, 8.77. Found: C, 64.84; H, 8.47.

7β-Hydroxy-12-oxo-5α-cholanic Acid (12)——**11a** (2.40 g) in DMSO-1,2-dimethoxyethane (2 : 1, v/v) (30 ml) was added to a solution of powdered KO_2 (1.3 g) and 18-crown-6 (0.8 g) in DMSO (60 ml), and the mixture was stirred for 1.5 h at room temperature under N_2. The mixture was processed,[1] and the resulting product was crystallized from aq. MeOH to give **12** (1.53 g; 76%) as colorless prisms. mp 219—220 °C. IR ν_{max} cm^{-1}: 1710, 1682 (C=O), 3300, 1030, 990 (OH). ^{1}H-NMR ($CDCl_3$+20% DMSO-d_6) δ: 0.83 (3H, d, J=6.3 Hz, 21-H), 0.89 (3H, s, 18-H), 1.04 (3H, s, 19-H), 3.55 (1H, br m, 7-H). *Anal.* Calcd for $C_{24}H_{38}O_4$: C, 73.80; H, 9.81. Found: C, 73.57; H, 9.65.

Methyl 7β-Hydroxy-12-oxo-5α-cholanate (12a)——Prepared from **12** by the usual esterification method and crystallized from acetone–hexane as colorless fine needles (96%). mp 107—108 °C. IR ν_{max} cm^{-1}: 1735, 1685 (C=O), 3490, 1028 (OH). ^{1}H-NMR δ: 0.86 (3H, d, J=6.3 Hz, 21-H), 0.90 (3H, s, 18-H), 1.05 (3H, s, 19-H), 3.34 (1H, br m, 7-H), 3.66 (3H, s, COOMe). *Anal.* Calcd for $C_{25}H_{40}O_4$: C, 74.21; H, 9.97. Found: C, 74.10; H, 9.74.

Methyl 7β,12α-Dihydroxy-5α-cholanate (3a)——$NaBH_4$ (560 mg) was added to a suspension of **12a** (560 mg) and $PdCl_2$ (450 mg) in MeOH (30 ml) over a period of 30 min at room temperature. The suspension became dark brown on each addition of $NaBH_4$. After further stirring for 2 h, the precipitated Pd was removed by filtration and washed with MeOH. The filtrate and washings were combined and evaporated, and the residue was extracted with EtOAc–ethyl ether (1 : 1, v/v). The combined extract was washed with water, dried over Drierite, and evaporated down. The oily residue (510 mg) was estimated by HPLC to be a 94 : 6 mixture of the epimers (**3a** and **4a**).

The oil (490 mg) was chromatographed on neutral alumina (activity II, ratio 70 : 1). Elution with benzene–EtOAc (8 : 2, v/v) and recrystallization of the eluate from acetone–hexane gave **3a** (420 mg) as colorless thin plates. mp 133.5—134.0 °C. IR ν_{max} cm^{-1}: 1743 (C=O), 3375, 1030, 995, 984 (OH). ^{1}H-NMR δ: 0.71 (3H, s, 18-H), 0.80 (3H, s, 19-H), 0.99 (3H, d, J=5.4 Hz, 21-H), 3.38 (1H, br m, 7-H), 3.66 (3H, s, COOMe), 3.98 (1H, m, 12-H). MS *m/z* (relative intensity): 388 (3, M−H_2O), 370 (20, M−$2H_2O$), 355 (6, M−$2H_2O$−CH_3), 273 (78, M−H_2O−SC), 255 (100, M−$2H_2O$−SC). *Anal.* Calcd for $C_{25}H_{42}O_4$: C, 73.85; H, 10.41. Found: C, 73.61; H, 10.26.

7β,12α-Dihydroxy-5α-cholanic Acid (3)——**3a** was hydrolyzed by the usual method with methanolic KOH. Recrystallization of the product from EtOAc–hexane gave **3** as colorless fine needles. mp 207.5—208.5 °C. IR ν_{max} cm^{-1}: 1685 (C=O), 3300, 1028, 990 (OH). ^{1}H-NMR ($CDCl_3$+20% DMSO-d_6) δ: 0.68 (3H, s, 18-H), 0.78 (3H, s, 19-H), 0.99 (3H, d, J=6.3 Hz, 21-H), 3.26 (1H, br m, 7-H), 3.90 (1H, m, 12-H). *Anal.* Calcd for $C_{24}H_{40}O_4$: C, 73.43; H, 10.27. Found: C, 73.19; H, 10.12.

Methyl 7α,12β-Dihydroxy-5α-cholanate (2a)——*tert*-Butylamine–borane complex (260 mg) was added to a stirred solution of **10a** (550 mg) in CH_2Cl_2 (30 ml). The mixture was allowed to stand at room temperature for 3 h and

then acidified with 3 N HCl. The CH_2Cl_2 layer was shaken with 10% $NaHCO_3$ and water, dried over Drierite, and evaporated down. The oily residue (540 mg) was estimated by HPLC to be a 16 : 5 mixture of the epimers (**2a** and **1a**). The oil (520 mg) was chromatographed on neutral alumina (activity II, ratio 70 : 1). Elution with benzene–EtOAc (8 : 2, v/v) provided two well-separated fractions. The less polar fraction was recrystallized from acetone–hexane to give **2a** (360 mg) as colorless thin plates. mp 147.5—148.0 °C. IR ν_{max} cm^{-1}: 1738 (C=O), 3375, 1030, 1013 (OH). ^{1}H-NMR δ: 0.72 (3H, s, 18-H), 0.79 (3H, s, 19-H), 1.01 (3H, d, J=6.3 Hz, 21-H), 3.39 (1H, br m, 12-H), 3.66 (3H, s, COOMe), 3.83 (1H, m, 7-H). MS *m/z* (relative intensity): 388 (5, M−H_2O), 370 (18, M−2H_2O), 355 (8, M−2H_2O−CH_3), 273 (35, M−H_2O−SC), 255 (100, M−2H_2O−SC). *Anal.* Calcd for $C_{25}H_{42}O_4$: C, 73.85; H, 10.41. Found: C, 73.98; H, 10.30.

The more polar fraction (120 mg) was identified as **1a** by TLC, HPLC, and ^{1}H-NMR comparisons.

7α,12β-Dihydroxy-5α-cholanic Acid (2)——Prepared from **2a** by the usual hydrolysis procedure. Recrystallization of the product from EtOAc–hexane gave **2** as colorless crystals. mp 168—170 °C. IR ν_{max} cm^{-1}: 1708 (C=O), 3425, 1032, 1015 (OH). ^{1}H-NMR ($CDCl_3$+20% DMSO-d_6) δ: 0.68 (3H, s, 18-H), 0.77 (3H, s, 19-H), 1.02 (3H, d, J=6.3 Hz, 21-H), 3.27 (1H, br m, 12-H), 3.74 (1H, m, 7-H). *Anal.* Calcd for $C_{24}H_{40}O_4 \cdot 1/4H_2O$: C, 72.59; H, 10.28. Found: C, 72.57; H, 10.02.

Methyl 7β,12β-Dihydroxy-5α-cholanate (4a)——**12a** (650 mg) was reduced with *tert*-butylamine–borane complex and processed as described above to give 640 mg of an oil. The oily residue was estimated by HPLC to be a 3.4 : 1 mixture of the epimers (**4a** and **3a**). Similar column chromatographic purification afforded **4a** (410 mg) and **3a** (140 mg). The less polar compound was recrystallized from acetone–hexane to give **4a** as colorless prisms. mp 154.5—156.0 °C. IR ν_{max} cm^{-1}: 1736 (C=O), 3325, 1028, 1003 (OH). ^{1}H-NMR δ: 0.76 (3H, s, 18-H), 0.81 (3H, s, 19-H), 1.00 (3H, d, J=6.3 Hz, 21-H), 3.35 (2H, br m, 7- and 12-H), 3.66 (3H, s, COOMe). MS *m/z* (relative intensity): 388 (5, M−H_2O), 370 (23, M−2H_2O), 355 (7, M−2H_2O−CH_3), 273 (100, M−H_2O−SC), 255 (65; M−2H_2O−SC). *Anal.* Calcd for $C_{25}H_{42}O_4$: C, 73.85; H, 10.41. Found: C, 73.99; H, 10.29.

7β,12β-Dihydroxy-5α-cholanic Acid (4)——**4a** was hydrolyzed and recrystallized from aq. MeOH to give **4** as colorless fine needles. mp 208—210 °C. IR ν_{max} cm^{-1}: 1677 (C=O), 3275, 1036, 1008, 990 (OH). ^{1}H-NMR ($CDCl_3$+20% DMSO-d_6) δ: 0.72 (3H, s, 18-H), 0.80 (3H, s, 19-H), 1.00 (3H, d, J=6.3 Hz, 21-H), 3.26 (2H, br m, 7- and 12-H). *Anal.* Calcd for $C_{24}H_{40}O_4 \cdot 1/4H_2O$: C, 72.59; H, 10.28. Found: C, 72.72; H, 10.05.

Methyl 7α,12α-Dihydroxy-5α-cholanate (1a)——Prepared from **10a** (100 mg) by the $NaBH_4$–$PdCl_2$ method as described for the preparation of **3a**. The crude product, which was estimated by HPLC to be a 97 : 3 mixture of the epimers (**1a** and **2a**), was recrystallized from aq. MeOH to afford **1a** (70 mg; 70%).

Acknowledgement This work was supported in part by a grant from the Japan Association of Chemistry (T. Iida). We thank the Department of Pharmacy, College of Science and Technology, Nihon University, for IR, MS, and elemental analyses. We are indebted to Dr. J. Goto and Dr. Y. Noda for helpful discussions.

References and Notes

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