Potential bile acid metabolites. XVIII. Synthesis of stereoisomeric 3,6,12α-trihydroxy-5β-cholanoic acids

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Abstract Two new 6-hydroxylated bile acids, 3β,6α,12α- and 3β,6β,12α-trihydroxy-5β-cholanoic acids, were synthesized from deoxycholic acid. In addition, their C-3 epimers, 3α,6α,12α- and 3α,6β,12α-trihydroxy acids, were prepared by a new route. The principal reactions used were *1*) 6β-hydroxylation of 3-methoxy-3,5-dienes with *m*-chloroperbenzoic acid in aqueous dioxane; *2*) catalytic hydrogenation of the resulting 6β-hydroxy-3-oxo-4-enes to the 6β-hydroxy-3-oxo-5β compounds with palladium on calcium carbonate catalyst in ethanol; and *3*) stereoselective reduction of appropriate 3-oxo derivatives with potassium *tri-sec*-butylborohydride and *tert*-butylamine-borane complex. The thin-layer chromatographic, gas–liquid chromatographic, and high performance liquid chromatographic mobilities, and ^{1}H- and ^{13}C-nuclear magnetic resonance spectroscopic data of the four stereoisomers are presented. With this work all the 6-hydroxylated derivatives of lithocholic, deoxycholic, chenodeoxycholic, ursodeoxycholic, and cholic acids in the 5β series are now known and have been synthesized. — **Iida, T., T. Tamaru, F. C. Chang, J. Goto, and T. Nambara.** Potential bile acid metabolites. XVIII. Synthesis of stereoisomeric 3,6,12α-trihydroxy-5β-cholanoic acids. *J. Lipid Res.* 1991. **32:** 649–658.

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The 6-hydroxylated derivatives of lithocholic (3α-hydroxy-5β-cholanoic), chenodeoxycholic (3α,7α-dihydroxy-5β-cholanoic), ursodeoxycholic (3α,7β-dihydroxy-5β-cholanoic), deoxycholic (3α,12α-dihydroxy-5β-cholanoic), and cholic (3α,7α,12α-trihydroxy-5β-cholanoic) acids are of substantiated interest in biosynthetic and metabolic studies of bile acids (1–8). Although these bile acids, excreted in human biological fluids from patients with hepatobiliary diseases and in fetuses and newborn infants, have been exclusively identified by gas–liquid chromatographic and gas–liquid chromatographic–mass spectrometric analyses, their stereochemical configuration of hydroxyl groups often remains uncertain.

As part of a program of synthesis of potential bile acid metabolites for use as authentic specimens, we have recently reported the preparation of new and uncommon 3,6-dihydroxy- (9), 3,6,7-trihydroxy- (10), and 3α,6,7,12α-tetrahydroxy-5β-cholanoic acids (11). In this report we describe the synthesis of the two remaining hitherto unreported stereoisomers (3β,6α,12α[**3**] and 3β,6β,12α[**4**]) of the four possible 3,6,12α-trihydroxy-5β-cholanoic acids (**Chart 1**) and, in addition, present an alternative route to the known two stereoisomers (3α,6α,12α[**1**] (12, 13) and 3α,6β,12α[**2**] (14)). Since all the 6-hydroxylated derivatives of lithocholic, chenodeoxycholic, ursodeoxycholic, and cholic acids have already been synthesized (9–11), with the publication of this work the entire set of the theoretically possible 5β-stereoisomers of the five prominent naturally occurring acids have now been prepared, characterized, and recorded in the literature.

EXPERIMENTAL PROCEDURES AND RESULTS

Melting points (mp) were determined on an electric micro hot stage and are uncorrected. Infrared (IR) spectra were obtained on a Perkin-Elmer 1600 Series FTIR as KBr disks. ^{1}H nuclear magnetic resonance (NMR) spectra were obtained on a JEOL FX-90Q instrument at 90 MHz with $CDCl_3$ containing 1% Me_4Si as the solvent,

Abbreviations: deoxycholic acid, 3α,12α-dihydroxy-5β-cholanoic acid; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; HPLC, high performance liquid chromatography; NMR, nuclear magnetic resonance; MS, mass spectrum; IR, infrared; NPM, 4-nitrophthalimidemethyl; Me-TMS, methyl ester-trimethylsilyl; Me-DMES, methyl ester-dimethylethylsilyl; K-Selectride, potassium *tri-sec*-butylborohydride. The various compounds in Chart 1 and Schemes 1–4 are designated by **bold face** numbers. The corresponding methyl esters at C-24 are designated "a" after the compound numbers.

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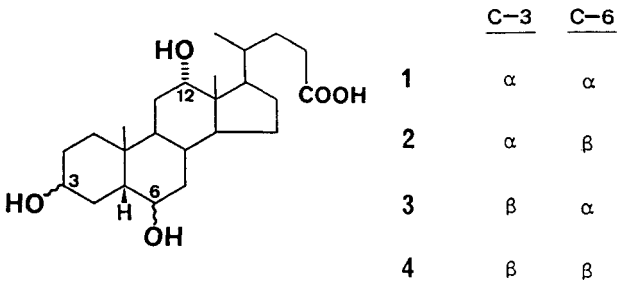


	C-3	C-6
1	α	α
2	α	β
3	β	α
4	β	β

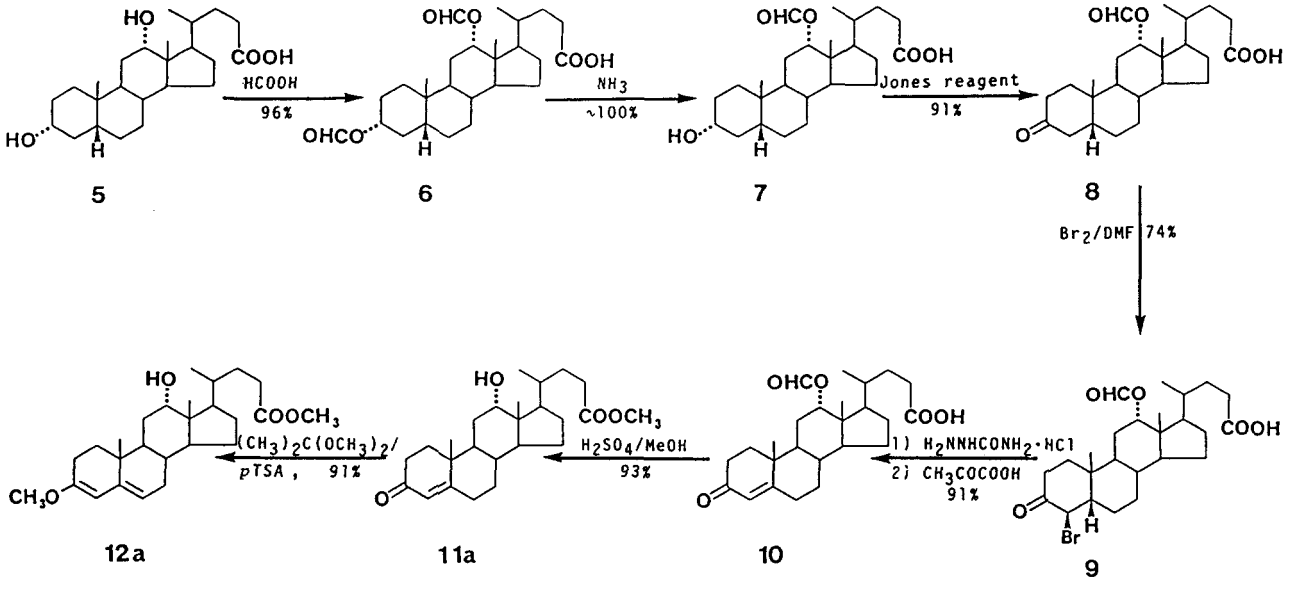
Chart 1.

unless otherwise specified; chemical shifts are expressed in δ (ppm) relative to Me_4Si. The high resolution ^1H- and ^{13}C-NMR spectra were also recorded on a JEOL GSX-500 instrument at 500 and 125.65 MHz, respectively. The signal assignments in ^{13}C-NMR were carried out by measuring the distortionless enhancement by polarization transfer (DEPT) spectra. High and low resolution mass spectra (MS) were recorded on JEOL JMS-AX 500 and DX-303 mass spectrometers at 70 eV, respectively. A Shimadzu GC-14A gas chromatograph equipped with a flame ionization detector was used isothermally at 270°C; it was fitted with a chemically bonded aluminum-clad flexible fused silica capillary column (HiCap-CBPM1 (equivalent to OV-101); 25 m × 0.25 mm i.d.; film thickness, 0.1 μm; Shimadzu Co.); bile acid samples were analyzed as their methyl ester-trimethylsilyl (Me-TMS) and methyl ester-dimethylethylsilyl (Me-DMES) ether derivatives (15). High performance liquid chromatography (HPLC) was carried out on a Waters Associates system (M-45 pump; U6K sample loop injector) using a Nova-Pak C_{18} reversed-phase column (15 cm × 3.9 mm i.d., 5 μm) with methanol-water 7:3 (v/v) as the mobile phase; bile acid samples were derivatized as their 4-nitrophthalimidemethyl esters (16) and monitored at 254 nm using a Shimadzu SPD-2A UV detector. Analytical thin-layer chromatography (TLC) was performed on precoated silica gel (20 cm × 20 cm, 0.25 mm layer thickness; Merck) using hexane-ethyl acetate-acetic acid 10:40:2 (v/v/v) as the developing solvent.

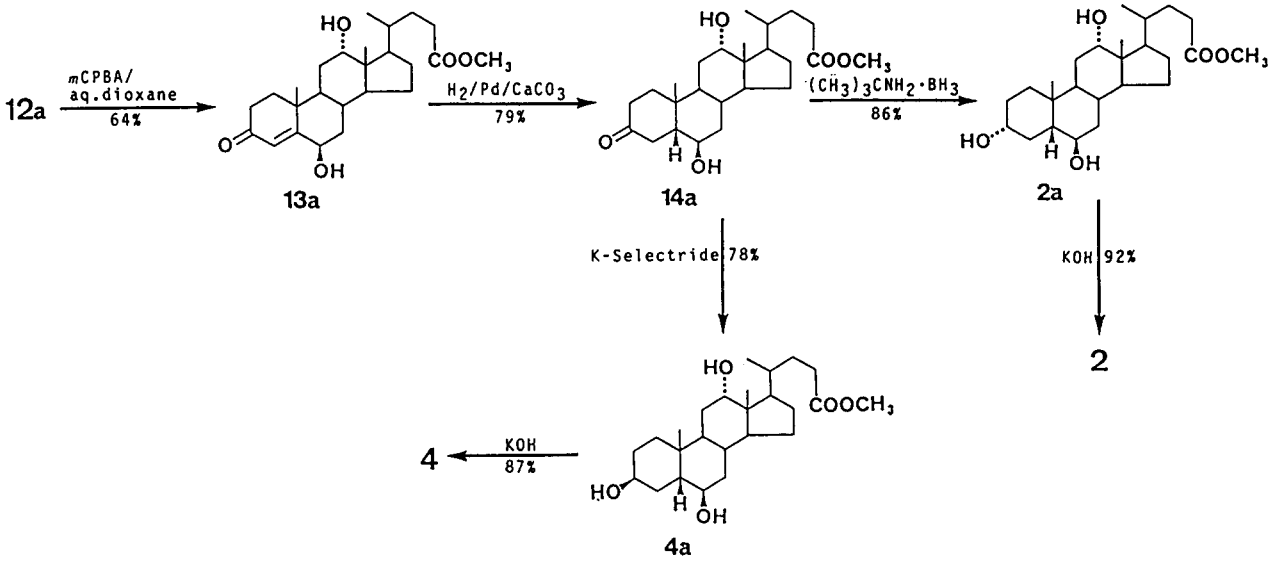
The four stereoisomeric 3,6,12α-trihydroxy acids **1-4** were synthesized from deoxycholic acid according to the routes shown in **Schemes 1-4**. The individual steps were carried out as follows.

12α-Formyloxy-3-oxochol-4-enoic acid [10]

A solution of semicarbazide hydrochloride (20 g) and sodium acetate (10 g) in water (70 ml) was added to a solution of 4β-bromo-12-formyloxy-3-oxo-5β-cholanoic acid (**9**) (35 g), prepared in four steps from **5** (17-19), in acetic acid (600 ml). The mixture was stirred for 30 min at 60°C under N_2 and then 1 h at room temperature. Water was added to the mixture to precipitate the semicarbazone of **10**, which was collected by filtration and washed with water. A solution of pyruvic acid (55 ml) in water (70 ml) was added to a stirred suspension of the crude semicarbazone in acetic acid (500 ml) under N_2, and stirring of the mixture at room temperature was continued until a homogeneous solution was obtained (ca. 12 h). The reaction product was extracted with CH_2Cl_2, and the combined extracts were washed with water, dried over Drierite, and evaporated. The oily residue was crystallized gradually from acetone-hexane as colorless needles;



Scheme 1. *p*TSA = *p*-toluenesulfonic acid.

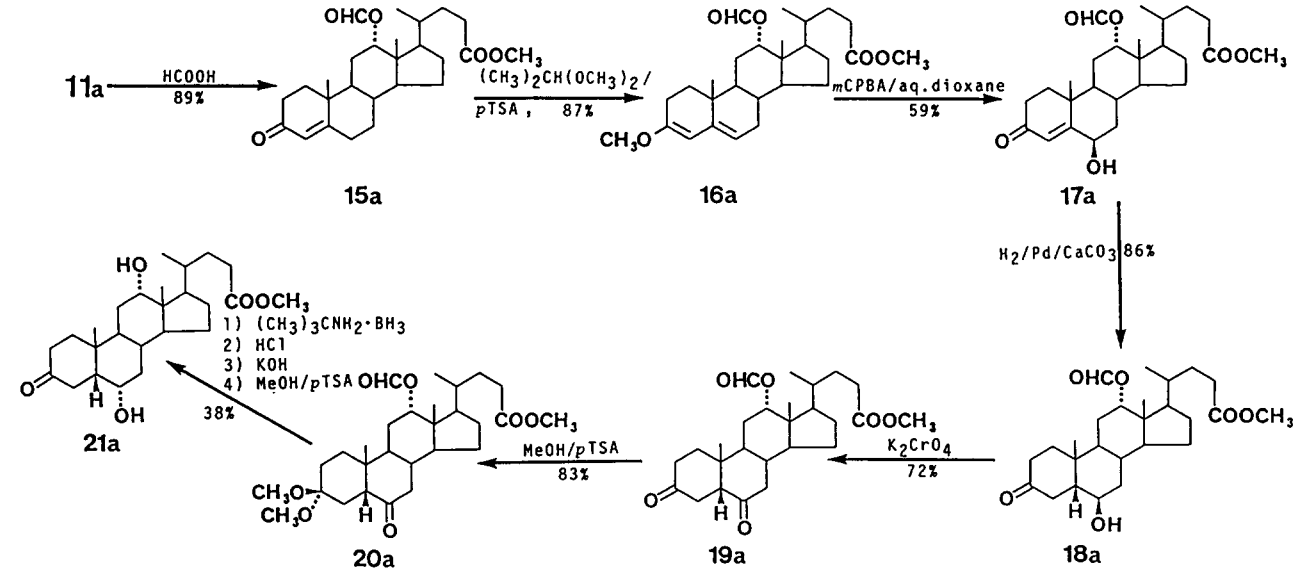


Scheme 2. *m*CPBA = *m*-chloroperbenzoic acid.

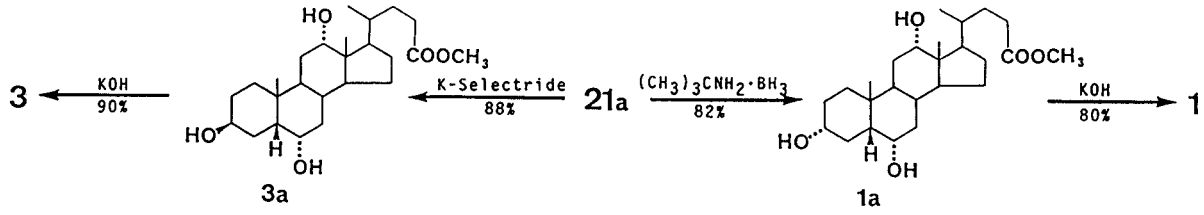
yield, 26.73 g (74%); mp, 195–197°C. IR $V_{max}cm^{-1}$: 1749,1719, 1679 (C=O), 1618 (C=C), 1192,1170 (formate). ^{1}H-NMR δ: 0.82 (s,3H,18-Me), 0.85 (d,3H, J = 5.4 Hz,21-Me), 1.18 (s,3H,19-Me), 5.29 (m,1H,12-H), 5.75 (brs,1H, 4-H), 8.09 (s,1H,12-CHO). Anal. calcd for $C_{25}H_{36}O_5$: C,72.08; H,8.71. Found: C,71.82; H,8.66.

Methyl 12α-hydroxy-3-oxochol-4-enoate [11]

To the acid **10** (7.5 g) dissolved in methanol (50 ml) was added conc. H_2SO_4 (1.5 ml). After standing for 12 h at room temperature, the solution was diluted with water to near turbidity, and allowed to stand until crystallization was complete. The precipitated solid was filtered and washed with cold methanol; yield, 6.72 g (93%); mp, 152–154°C (colorless thin plates from aqueous acetone) (lit. mp, 150–151.5°C (19)). IR $V_{max}cm^{-1}$: 3517,1008 (OH), 1721,1669 (C=O), 1611 (C=C). ^{1}H-NMR δ; 0.74 (s,3H,18-Me), 0.98 (d,3H, J = 5.4 Hz,21-Me), 1.17 (s,3H,19-Me), 3.66 (s,3H,COOMe), 4.04 (m, 1H,12-H), 5.71 (brs,1H,4-H).



Scheme 3.



Scheme 4.

Methyl 12α-hydroxy-3-methoxychola-3,5-dienoate [12a]

A mixture of the ester **11a** (6 g) and *p*-toluenesulfonic acid (400 mg) in methanol (7 ml), 2,2-dimethoxypropane (35 ml), and DMF (35 ml) was refluxed for 1.5 h. After cooling the mixture at room temperature, 5% $NaHCO_3$ solution (50 ml) was added, and the reaction product was extracted with ethyl acetate. The combined extract was washed with water, dried over Drierite, and evaporated to an oily residue which crystallized from aqueous acetone as colorless thin plates; yield 5.65 g (91%); mp, 115–117°C. IR $V_{max}cm^{-1}$: 3528,1039,980 (OH), 1738,1716 (C=O), 1655,1630 (C=C). ^{1}H-NMR δ; 0.74 (s,3H,18-Me), 0.96 (s,3H,19-Me), 0.99 (d,3H, J = 5.4 Hz,21-Me), 3.57 (s,3H,3-OMe), 3.66 (s,3H,COOMe), 4.05 (m,1H, 12-H), 5.13 (brs,1H,3-H), 5.24 (m,1H,5-H). Anal. calcd for $C_{26}H_{40}O_4$: C,74.96; H,9.68. Found: C,75.08; H,9.58.

Methyl 6β,12α-dihydroxy-3-oxochol-4-enoate [13a]

To a stirred solution of the ester **12a** (4 g) in 80% dioxane (200 ml) was added gradually (ca. 30 min) *m*-chloroperbenzoic acid (3 g), and the mixture was further stirred for 1 h at room temperature. The solution was poured onto water, and extracted with CH_2Cl_2. The combined extract was washed with 5% sodium thiosulfate, 5% $NaHCO_3$, and water, dried over Drierite, and evaporated to an oily residue. Chromatography of the oil on a column of silica gel (120 g) and elution with benzene–ethyl acetate 1:4 (v/v) afforded the title compound **13a** which crystallized from aqueous methanol as colorless thin plates; yield, 2.58 g (64%); mp, 184–186°C. IR $V_{max}cm^{-1}$: 3500,1039,980 (OH), 1734,1691 (C=O), 1615 (C=C). ^{1}H-NMR δ; 0.77 (s,3H,18-Me), 0.98 (d,3H, J = 5.4 Hz,21-Me), 1.36 (s,3H,19-Me), 3.66 (s,3H,COOMe), 4.06 (m,1H,12-H), 4.34 (m,1H,6-H), 5.80 (brs,1H,4-H). Anal. calcd for $C_{25}H_{38}O_5$: C,71.74; H,9.15. Found: C,71.53; H,8.90.

Methyl 6β,12α-dihydroxy-3-oxo-5β-cholanoate [14a]

The ester **13a** (3 g) dissolved in absolute ethanol (60 ml) was catalytically hydrogenated with palladium on calcium carbonate ($Pd/CaCO_3$) (400 mg) at a slight positive pressure. After the hydrogen uptake ceased, the catalyst was filtered off, and the solvent was evaporated. The oily residue was chromatographed on silica gel column (120 g) and eluted with benzene–ethyl acetate 1:4 (v/v). Recrystallization of the product from acetone–hexane gave the desired ester **14a** as colorless prisms; yield, 2.37 g (79%); mp, 138–139°C. IR $V_{max}cm^{-1}$: 3555,3373,1028,974 (OH), 1721 (C=O). ^{1}H-NMR δ; 0.75 (s,3H, 18-Me), 0.98 (d,3H, J = 5.4 Hz, 21-Me), 3.66 (s,3H,COOMe), 3.71 (m,1H, 6-H), 4.06 (m,1H,12-H). Anal. calcd for $C_{25}H_{40}O_5$: C,71.39; H,9.59. Found: C,71.61; H,9.44.

Methyl 3α,6β,12α-trihydroxy-5β-cholanoate [2a]

tert-Butylamine-borane complex (450 mg) was added to a stirred solution of **14a** (1 g) in CH_2Cl_2 (60 ml). The mixture was allowed to stand at room temperature for 2 h and then acidified with 10% HCl (20 ml). After stirring for 30 min at room temperature, the CH_2Cl_2 layer was washed with 5% $NaHCO_3$ and water, dried over Drierite, and evaporated. The oily residue, which consisted essentially of a single spot on TLC, was chromatographed on a column of silica gel (60 g). Elution with ethyl acetate–methanol 98:2 (v/v) gave a homogenous oil which was characterized as the desired **2a**; yield, 864 mg (86%). IR $V_{max}cm^{-1}$: 3416,1046,962 (OH), 1722 (C=O). ^{1}H-NMR δ: 0.71 (s,3H,18-Me), 0.97 (d,3H, J = 5.4 Hz,21-Me), 1.10 (s,3H,19-Me), 3.60 (brm,1H,3-H), 3.66 (s,3H,COOMe), 3.76 (m,1H,6-H), 4.00 (m,1H,12-H). Low resolution MS, *m/z* (relative intensity): 404 (4%, M-H_2O), 386 (28%,M-2H_2O), 368 (12%,M-3H_2O), 289 (19%, M-H_2O-Side Chain (S.C.)), 271 (100%, M-2H_2O-S.C.), 253 (34%, M-3H_2O-S.C.). High resolution MS: 404.2909 (M^+-H_2O, $C_{25}H_{40}O_4$ requires 404.2927).

3α,6β,12α-Trihydroxy-5β-cholanoic acid [2]

The ester **2a** (300 mg) was refluxed in 5% methanolic KOH (9 ml) for 1 h. Most of the solvent was evaporated off, and the residue was dissolved in water and acidified with 10% H_2SO_4 with stirring and ice-bath cooling. The precipitate was collected by filtration, washed with water, and recrystallized from ethyl acetate–hexane as colorless crystals; yield, 267 mg (92%); mp, 137–139°C (lit. mp, 137–139°C (14)). IR $V_{max}cm^{-1}$: 3401,1045 (OH), 1711 (C=O). ^{1}H-NMR δ: 0.71 (s,3H,18-Me), 0.98 (d,3H, J = 6.3 Hz,21-Me), 1.09 (s,3H,19-Me), 3.62 (brm, 1H,3-H), 3.74 (m,1H,6-H), 3.98 (m,1H,12-H). Anal. calcd for $C_{24}H_{40}O_5$: C,70.55; H,9.87. Found: C,70.28; H,9.75.

Methyl 3β,6β,12α-trihydroxy-5β-cholanoate [4a]

To a stirred solution of the ester **14a** (600 mg) in dry tetrahydrofuran (THF) (6 ml), at −20°C under N_2, was added slowly a 1 M solution of potassium *tri-sec*-butylborohydride (K-Selectride) in THF (3 ml). After the mixture was further stirred under N_2 at −20°C for 3 h, 3 N NaOH solution (2 ml) and then 30% H_2O_2 (2 ml) were added dropwise to the solution, and the reaction product was extracted with CH_2Cl_2. The combined extract was washed with 10% HCl and water, dried over Drierite, and evaporated. The oily residue was chromatographed on a silica gel column (35 g). Elution with ethyl acetate–methanol 98:2 (v/v) afforded the desired ester **4a** which was crystallized from acetone as colorless prisms; yield, 468 mg (78%); mp, 196–197°C. IR $V_{max}cm^{-1}$: 3566,3383,1033 (OH), 1721 (C=O). ^{1}H-NMR δ: 0.72 (s,3H, 18-Me), 0.97 (d,3H, J = 5.4 Hz,21-Me), 1.14 (s,3H,19-Me), 3.66 (s,3H,COOMe), 3.70 (m,1H,6-H), 4.01 (m,1H, 12-H), 4.06 (m,1H,3-H). Low resolution MS, *m/z* (relative intensity): 404 (2%, M−H_2O), 386 (18%, M−2H_2O), 289 (14%,M−H_2O-S.C.), 271 (100%, M−2H_2O-S.C.), 253 (19%, M−3H_2O-S.C.). Anal. calcd for $C_{25}H_{42}O_5$: C,71.05; H,10.02. Found: C,70.83; H,9.92.

3β,6β,12α-Trihydroxy-5β-cholanoic acid [4]

The ester **4a** (350 mg), hydrolyzed with 5% methanolic KOH and processed as described for the preparation of **2a**, yielded the crude acid. Recrystallization from ethyl acetate–hexane gave **4** as colorless crystals; yield, 294 mg (87%); mp, 131–133°C. IR $V_{max}cm^{-1}$: 3408,1023 (OH), 1709 (C=O). ^{1}H-NMR ($CDCl_3$ + 20% DMSO-d_6) δ: 0.70 (s,3H,18-Me), 0.98 (d,3H, J = 5.4 Hz,21-Me), 1.11 (s,3H,19-Me), 3.66 (m,1H,6-H), 3.99 (m,2H,3- and 12-H). Anal. calcd for $C_{24}H_{40}O_5 \cdot 1/2\ H_2O$: C,69.03; H,9.90. Found: C,69.30; H,9.89.

Methyl 12α-formyloxy-3-oxochol-4-enoate [15a]

The ester **11a** (12 g) was formylated with 99% formic acid by the procedure of Tserng and Kelin (17). The curde product was recrystallized from acetone–hexane as colorless needles; yield, 11.38 g (89%); mp, 178–179°C (colorless needles from acetone–hexane). IR $V_{max}cm^{-1}$: 1739, 1720,1668 (C=O), 1614 (C=C), 1166 (formate). ^{1}H-NMR δ: 0.81 (s,3H,18-Me), 0.84 (d,3H, J = 5.4 Hz,21-Me), 1.17 (s,3H,19-Me), 3.66 (s,3H,COOMe), 5.29 (m, 1H,12-H), 5.73 (brs,1H,4-H), 8.08 (s,1H,12-CHO). Anal. calcd for $C_{26}H_{38}O_5$: C,72.52; H,8.90. Found: C,72.63; H,8.76.

Methyl 12α-formyloxy-3-methoxychola-3,5-dienoate [16a]

This compound was prepared from the ester **15a** (10 g) by the isomerization method as described in the preparation of **12a**; yield, 8.96 g (87%); mp, 136–138°C (colorless needles from acetone–hexane). IR $V_{max}cm^{-1}$: 1737,1708 (C=O), 1654,1628 (C=C), 1179 (formate). ^{1}H-NMR δ; 0.80 (s,3H,18-Me), 0.83 (d,3H, J = 5.4 Hz,21-Me), 0.96 (s,3H,19-Me), 3.57 (s,3H, 3-OMe), 3.66 (s,3-H,COOMe), 5.13 (brs,1H,3-H), 5.28 (m,2H,5- and 12-H), 8.10 (s,1H, 12-CHO). Anal. calcd for $C_{27}H_{40}O_5$: C,72.94; H,9.07. Found: C,72.87; H,8.91.

Methyl 12α-formyloxy-6β-hydroxy-3-oxochol-4-enoate [17a]

The ester **16a** (8 g), subjected to the hydroxylation reaction with *m*-chloroperbenzoic acid in aqueous dioxane and processed as described for the preparation of **13a**, gave an oily residue. Chromatography of the oil on a column of silica gel (180 g) and elution with benzene–ethyl acetate 3:2 (v/v) afforded the desired compound **17a** which crystallized from acetone–hexane as colorless needles; yield, 4.76 g (59%); mp, 173–174°C. IR $V_{max}cm^{-1}$: 3471,1029 (OH), 1743,1717,1680 (C=O), 1619 (C=C), 1179 (formate). ^{1}H-NMR δ; 0.84 (s,3H,18-Me), 1.37 (s,3H,19-Me), 3.66 (s,3H,COOMe), 4.36 (m,1H,6-H), 5.31 (m,1H,12-H), 5.81 (brs,1H,4-H), 8.08 (s,1H,12-CHO). Anal. calcd for $C_{26}H_{38}O_6$: C,69.93; H,8.58. Found: C,69.88; H,8.51.

Methyl 12α-formyloxy-6β-hydroxy-3-oxo-5β-cholanoate [18a]

The ester **17a** (6 g), subjected to the catalytic hydrogenation with Pd/$CaCO_3$ in ethanol and processed as described for the preparation of **14a**, afforded an oily residue. Chromatography of the oil on a silica gel column (240 g) and elution with benzene–ethyl acetate 3:2 (v/v) gave the title compound **18a** which crystallized from aqueous acetone as colorless needles; yield, 5.16 g (86%); mp, 82–84°C. IR $V_{max}cm^{-1}$: 3478,1024,1005,991 (OH), 1720 (C=O), 1179 (formate). ^{1}H-NMR; 0.82 (s,3H,18-Me), 0.85 (d,3H, J = 5.4 Hz,21-Me), 1.21 (s,3H,19-Me), 3.66 (s,3H,COOMe), 3.73 (m,1H,6-H), 5.30 (m,1H,12-H), 8.13 (s,1H,12-CHO). Anal. calcd for $C_{26}H_{40}O_6$: C,69.61; H,8.99. Found: C,69.38; H,9.04.

Methyl 3,6-dioxo-12α-formyloxy-5β-cholanoate [19a]

A solution of potassium chromate (2.4 g) in water (6 ml) was added to a stirred solution of the ester **18a** (4 g) in acetic acid (70 ml), and the mixture was further stirred at room temperature for 6 h. The dark brown solution was diluted with water to near turbidity, and on standing, crystals separated. A second crop was obtained on further dilution with water of the mother liquor. The combined product recrystallized from acetone–hexane as colorless needles; yield, 2.85 g (72%); mp, 186–188°C. IR $V_{max}cm^{-1}$: 1706 (C=O), 1182 (formate). ^{1}H-NMR; 0.80 (s,3H,18-Me), 0.85 (d,3H, J = 5.4 Hz, 21-Me), 0.96 (s,3H,19-Me), 3.66 (s,3H,COOMe), 5.35 (m,1H,12-H), 8.16 (s,1H, 12-CHO). Anal. calcd for $C_{26}H_{38}O_6$: C,69.93; H,8.58. Found: C,69.83; H,8.52.

Methyl 3,3-dimethoxy-12α-formyloxy-6-oxo-5β-cholanoate [20a]

To the ester **19a** (3 g) dissolved in CH_2Cl_2 (10 ml) and methanol (60 ml) were added *p*-toluenesulfonic acid (1 g) and molecular sieve (20 g), and the mixture was stirred vigorously for 2 h at room temperature. Molecular sieve was filtered off, and the mother liquor was diluted with water and extracted with CH_2Cl_2. The combined extract was washed with water to neutrality, dried over Drierite, and evaporated to afford the desired **20a** which crystallized gradually from benzene–hexane as colorless thin plates; yield, 2.74 g (83%); mp, 55–56°C. IR $V_{max}cm^{-1}$: 1719 (C=O), 1178 (formate). ^{1}H-NMR δ: 0.76 (s,3H,18-Me), 0.85 (s,3H,19-Me), 3.12 and 3.19 (s, each 3H,3,3-OMe), 3.66 (s,3H,COOMe), 5.30 (m,1H,12-H), 8.16 (s,1H,12-CHO). Anal. calcd for $C_{28}H_{44}O_7$: C,68.26; H,9.00. Found: C,67.96; H,8.77.

Methyl 6α,12α-dihydroxy-3-oxo-5β-cholanoate [21a]

The ester **20a** (5 g), reduced with *tert*-butylamine-borane complex and processed as described for the preparation of **2a**, yielded an oily residue. The oil was hydrolyzed with 5% methanolic KOH, followed by esterification with methanol and *p*-toluenesulfonic acid (10). The product, which consisted of a mixture of two components by TLC, was chromatographed on a column of neutral alumina (350 g; activity III). Elution with benzene–ethyl acetate 3:7–1:9 (v/v) provided two well-separated fractions. The less polar fraction (2.28 g, 53%) was identified with **14a**, according to TLC and ^{1}H-NMR.

The more polar fraction was recrystallized from ethyl acetate–hexane to give the desired ester **21a** as colorless needles; yield, 1.61 g (38%); mp, 153–155°C. IR $V_{max}cm^{-1}$: 3450,1045,1015 (OH), 1732,1708 (C=O). ^{1}H-NMR δ; 0.71 (s,3H,18-Me), 0.99 (s,3H,19-Me), 3.66 (s,3H,COOMe), 4.04 (m,1H,12-H), 4.16 (brm,1H,6-H). Anal. calcd for $C_{25}H_{40}O_5 \cdot 3/4H_2O$: C,69.18; H,9.29. Found: C,69.02; H,9.38.

Methyl 3α,6α,12α-trihydroxy-5β-cholanoate [1a]

The ester **21a** (600 mg) was subjected to the reduction reaction with *tert*-butylamine-borane complex and processed as described for the preparation of **2a** to yield an oily residue. The oil was purified by a column of neutral alumina (35 g; activity III). Elution with ethyl acetate–methanol 97:3 (v/v) gave the title compound **1a**, which was homogeneous according to TLC and ^{1}H-NMR but failed to crystallize; yield, 495 mg (82%). IR $V_{max}cm^{-1}$: 3400,1041 (OH), 1740 (C=O). ^{1}H-NMR δ: 0.67 (s,3H, 18-Me), 0.89 (s,3H,19-Me), 0.96 (d,3H, J = 5.4 Hz,21-Me), 3.54 (brm, 1H,3-H), 3.66 (s,3H,COOMe), 3.98 (m,1H,12-H), 4.05 (brm,1H,6-H). Low resolution MS, *m/z* (relative intensity): 404 (7%, M–H_2O), 386 (81%, M–$2H_2O$), 368 (23%, M–$3H_2O$), 289 (20%, M–H_2O–S.C.), 271 (100%, M–$2H_2O$–S.C.), 253 (67%, M–$3H_2O$–S.C.). High resolution MS: 404.2905 (M^+–H_2O, $C_{25}H_{40}O_4$ requires 404.2927).

3α,6α,12α-Trihydroxy-5β-cholanoic acid (1)

The ester **1a** (300 mg), hydrolyzed by the usual method, recrystallized from ethyl acetate–hexane as colorless crystals; yield, 233 mg (80%); mp, 135–137°C (lit. oil (13)). IR $V_{max}cm^{-1}$: 3401,1037 (OH), 1709 (C=O). ^{1}H-NMR δ: 0.67 (s,3H,18-Me), 0.89 (s,3H,19-Me), 0.99 (d,3H, J = 5.4 Hz,21-Me), 3.58 (brm, 1H,3-H), 4.03 (m and brm,2H, 6- and 12-H). Anal. calcd for $C_{24}H_{40}O_5 \cdot H_2O$: C,67.57; H,9.93. Found: C,67.32; H,9.79.

Methyl 3β,6α,12α-trihydroxy-5β-cholanoate [3a]

The ester **21a** (600 mg) was subjected to the reduction reaction with K-Selectride and processed as described for the preparation of **4a** to yield an oily residue. The oil was chromatographed on a column of neutral alumina (35 g; activity III) and eluted with ethyl acetate–methanol 95:5 (v/v). Crystallization of the product from CH_2Cl_2–hexane gave **3a** as colorless crystals; yield, 530 mg (88%); mp, 115–117°C. IR $V_{max}cm^{-1}$: 3355,1037,976 (OH), 1721 (C=O). ^{1}H-NMR δ: 0.68 (s,3H,18-Me), 0.93 (s,3H,19-Me), 0.96 (d,3H, J = 5.4 Hz,21-Me), 3.66 (s,3H,COOMe), 3.99 (m,1H,12-H), 4.02 (brm,1H,6-H), 4.14 (m,1H,3-H). Low resolution MS, *m/z* (relative intensity): 404 (6%, M–H_2O), 386 (42%, M–$2H_2O$), 289 (79%, M–H_2O–S.C.), 271 (100%, M–$2H_2O$–S.C.), 253 (39%, M–$3H_2O$–S.C.). Anal. calcd for $C_{25}H_{42}O_5 \cdot 1/2H_2O$: C,69.57; H,9.81. Found C,69.64; H,9.89.

3β,6α,12α-Trihydroxy-5β-cholanoic acid [3]

The ester **3a** (300 mg), hydrolyzed by the usual method, recrystallized from ethyl acetate–hexane as colorless crystals; yield, 262 mg (90%); mp, 135–137°C. IR $V_{max}cm^{-1}$: 3412, 1036,976 (OH), 1709 (C=O). ^{1}H-NMR ($CDCl_3$ + 20% DMSO-d_6) δ: 0.67 (s,3H,18-Me), 0.92 (s,3H,19-Me), 0.96 (d,3H, J = 7.2 Hz, 21-Me), 3.96 (m,1H,12-H), 4.00 (brm, 1H,6-H), 4.08 (m,1H,3-H). Anal. calcd for $C_{24}H_{40}O_5 \cdot 1/5H_2O$: C,69.94; H,9.78. Found: C,70.21; H,10.04.

RESULTS AND DISCUSSION

The two stereoisomers of 3,6,12α-trihydroxy acids were synthesized some years ago in fairly low total yield processes starting from cholic acid. The acid **1**, one of the earliest 3,6,12-trihydroxy acids known (5), has been prepared by *a*) Huang-Minlon reduction of methyl 7-oxo-3α,6α,12α-triacetoxy-5β-cholanoate (13), or by *b*) hydrogenolysis of the 7-ethylenedithioketal derivative with

TABLE 1. Chromatographic data for 3,6,12α-trihydroxy stereoisomers

Configuration of Hydroxyls	TLC[a] (R_f Values)	HPLC[b] (rk' Values)	GLC[c] (MU Values)	
			Me-TMS	Me-DMES
3α, 6α, 12α [1]	0.47	1.00	33.13	36.21
3α, 6β, 12α [2]	0.62	0.40	32.61	35.81
3β, 6α, 12α [3]	0.34	0.71	32.87	35.91
3β, 6β, 12α [4]	0.57	0.32	32.61	35.81

[a]The samples were analyzed as the methyl esters and developed in hexane–ethyl acetate–acetic acid 10:40:2 (v/v/v).
[b]The samples were analyzed as the C-24 4-nitrophthalimidemethyl ester (25) under the following conditions: column, Nova-Pak C_{18}; detector, UV at 254 nm; mobile phase, methanol-water 7:3 (v/v); flow rate, 0.8 ml/min. Capacity factors (k') were expressed relative to that of the 3α,6α,12α-trihydroxy ester.
[c]The samples were analyzed as the methyl ester-trimethylsilyl (Me-TMS) and methyl ester-dimethylethylsilyl (Me-DMES) ethers (26) under the following conditions: column, HiCap-CBPM1 (25 m × 0.25 mm i.d.); column temp., 270°C; carrier gas flow rate, 1.5 ml/min (helium); splitting ratio, 1:50. Retention times were expressed as the methylene unit (MU) values.

Raney nickel (12, 13).[2] Partial synthesis of the acid **4** has also been reported by treatment of methyl 3α,12α-diacetoxy-5β-chol-6-enoate with silver acetate/bromine and subsequent debromination of the resulting bromohydrin acetate with Raney nickel (14).

In exploring procedures for preparing the four new and known 3,6,12α-trihydroxy stereoisomers (Chart 1) we recognized that by reduction of appropriate 3-oxo-6α,12α- and 3-oxo-6β,12α-dihydroxy intermediates with the two stereoselective reducing agents, K-Selectride (20) and *tert*-butylamine-borane complex (21), in the final steps, the desired acids, **1** to **4**, might be prepared. Accordingly, we have devised such a procedure which, through essentially a single key intermediate prepared from commercially available deoxycholic acid **5**, does result in the four acids.

As summarized in Scheme 1, the key intermediate **12a**, methyl 12α-hydroxy-3-methoxychola-3,5-dienoate, was prepared by established procedures from **5**, as follows: **5** was converted to its diformate **6**; selective hydrolysis at C-3 with NH_3 afforded the 12α-formyloxy-3α-hydroxy acid **7**; oxidation with Jones reagent yielded the 12α-formyloxy-3-oxo acid **8** (17, 18); bromination in *N*,*N*-dimethylformamide (DMF) gave the 4β-bromo-12α-formyloxy-3-oxo acid **9**; semicarbazide hydrochloride, then pyruvic acid in acetic acid treatment (19) yielded the 12α-formyloxy-3-oxo-4-ene acid **10**; simultaneous esterification at C-24 and hydrolysis at C-12 gave the 12α-hydroxy-3-oxo-4-ene ester **11a**; enol etherification with 2,2-dimethoxypropane and *p*-toluenesulfonic acid in DMF (22) gave **12a** in isolated yield of 50% from **5**.

The desired esters **2a** (3α,6β,12α) and the new **4a** (3β,6β,12α) were obtained from **12a** by a process consisting of *a*) 6β-hydroxylation of **12a**, *b*) reduction of the resulting 6β,12α-dihydroxy-3-oxo-4-ene ester **13a** to the 6β,12α-dihydroxy-3-oxo-5β ester **14a**, and *c*) reduction of **14a** by the two stereoselective reagents as detailed in the Experimental Section (Scheme 2). Noteworthy is the clean 6β-hydroxylation step with *m*-chloroperbenzoic acid in aqueous dioxane (23, 24) in which no epimeric 6α-hydroxy product was obtained, and the absence of allomerization at C-5 in the catalytic hydrogenation with $Pd/CaCO_3$ catalyst in ethanol (25, 26).

Esters **1a** and **3a** were prepared via **16a**, the 12-formate of **12a**, which might be expected to be easily obtained from the acid **10** by methyl esterification at C-24 followed by enol etherification of the resulting 12α-formyloxy-3-oxo-4-ene ester **15a**. However, attempts to prepare **15a** by the usual treatment of **10** either with methanol–HCl or methanol–*p*-toluenesulfonic acid (10) yielded mixtures which required column chromatography to obtain pure product.

16a was subsequently prepared from **15a**, through **11a**, the penultimate intermediate in the synthesis of **12a** (Scheme 3). **16a** was hydroxylated to the 12α-formyloxy-6β-hydroxy-3-oxo-4-ene ester **17a**, hydrogenated to the 12α-formyloxy-6β-hydroxy-3-oxo-5β ester **18a**, oxidized to the 3,6-dioxo-12-α-formyloxy ester **19a**, and selectively protected at C-3 to the 3,3-dimethoxy-12-α-formyloxy-6-oxo ester **20a**. Following the procedure used in our previous synthesis of 3β,6α-dihydroxy-5β-chonanoic acid (9), **20a** underwent reduction with the aminoborane reagent to an epimeric mixture of 6-hydroxylated product without allomerization at C-5. The ketal mixture was successively cleaved with HCl, hydrolyzed at C-12 and C-24, reesterified at C-24, and chromatographed on neutral

[2]Yields reported by Takeda and Igarashi (13) by procedures *a* and *b* were 50% and 20%, respectively. However, our attempt to repeat procedure *a* was discouraging; Huang-Minlon reduction of methyl 7-oxo-3α,6α,12α-trihydroxy-5β-cholanoate (11) was less straightforward than indicated in the literature, and a low yield (16%) of the desired ester la, together with numerous by-products (e.g., methyl deoxycholate, 35%) was obtained only after a laborious column chromatographic purification (Iida et al., unpublished results).

TABLE 2. High resolution ^{1}H-NMR spectral data for stereoisomeric methyl 3,6,12α-trihydroxy-5β-cholanoates (**1a–4a**)[a]

	18-Me[b]	19-Me[b]	21-Me[c]	COOMe[b]	3-H[c]	6-H[c]	12-H[c]
1a (3α,6α,12α)	0.666	0.890	0.967 (d, 6.5)	3.663	3.583 (brm, 28.5)	4.007 (brm, 23.4)	3.977 (m, 7.1)
2a (3α,6β,12α)	0.712	1.099	0.976 (d, 6.0)	3.665	3.598 (brm, 27.9)	3.776 (m, 6.8)	4.006 (m, 6.6)
3a (3β,6α,12α)	0.680	0.929	0.973 (d, 6.5)	3.664	4.156 (m, 8.8)	4.119 (brm, 25.3)	3.993 (m, 6.9)
4a (3β,6β,12α)[d]	0.686	1.101	0.983 (d, 6.5)	3.640	3.973 (m, 7.9)	3.602 (m, 7.9)	3.925) (m, 5.5)

[a]In ppm downfield from Me_4Si; measured at 500 MHz.
[b]Singlet.
[c]Values in parentheses refer to signal multiplicity and coupling constant (J in Hz) or width at half-height ($W_{1/2}$ in Hz): d, doublet; m, multiplet (or broad singlet); brm, broad multiplet.
[d]For solubility reason, the sample was measured in $CDCl_3$ containing 20% DMSO-d_6.

alumina to separate efficiently the epimeric 6,12α-dihydroxy-3-oxo esters **14a** and **21a** in the approximate ratio of 1.4 to 1.

Reduction of **21a** with K-Selectride gave the new 3β,6α,12α-trihydroxy ester **3a**, and with aminoborane complex yielded the known 3α,6α,12α-trihydroxy ester **1a** (Scheme 4). The ester **14a** can be used for the reductions to **2a** and **4a** (see above).

Esters **1a** to **4a** were readily hydrolyzed to the free acids in the usual manner. All the four acids **1–4** could be easily crystallized from ethyl acetate-hexane. In the earlier preparation (13) acid **1** was an oil.

Characterization of compounds by TLC, HPLC, GLC, ^{1}H- and ^{13}C-NMR

Table 1 shows the R_f values on TLC, the rk' (relative capacity factor) values on HPLC, and the MU (methylene unit) values on capillary gas-liquid chromatography (GLC) for the C-24 esters of stereoisomeric 3,6,12α-trihydroxy-5β-cholanoic acids (**1–4**). The R_f, rk', and

TABLE 3. ^{13}C-NMR spectral data for stereoisomeric methyl 3,6,12α-trihydroxy-5β-cholanoates (**1a–4a**)[a]

	1a	**2a**	**3a**	**4a**[b]
Carbon	3α,6α,12α	3α,6β,12α	3β,6α,12α	3β,6β,12α
1	35.66	35.76	30.08	29.43
2	30.08	30.12	27.43	26.50[c]
3	71.53	71.30	66.09	64.44
4	29.18	36.44	26.25	32.24
5	48.53	48.68	43.05	42.23
6	68.08	73.13	67.85	71.38
7	34.81	34.45	34.37	33.42
8	35.10	30.96	34.93	29.91
9	32.97	34.06	32.33	32.86
10	35.60	33.97	36.05	33.48
11	28.62	28.51	28.93	28.04
12	72.95	73.13	73.04	71.58
13	46.69	46.63	46.75	45.57
14	47.87	48.00	48.12	47.05
15	23.81	23.70	23.67	22.86
16	27.51	27.51	27.43	26.64[c]
17	47.27	47.41	47.42	45.84
18	12.73	12.86	12.78	11.91
19	23.28	25.41	23.84	24.99
20	35.27	35.16	35.13	34.37
21	17.32	17.43	17.40	16.36
22	31.18	31.17	31.12	30.09
23	30.96	30.96	30.93	29.91
24	174.85	174.78	174.78	173.65
25	51.59	51.59	51.59	50.49

[a]In ppm downfield from Me_4Si; measured at 125.65 MHz.
[b]For solubility reason, the sample was measured in $CDCl_3$ containing 20% DMSO-d_6.
[c]Assignments may be interchanged.

MU values were obtained for the methyl ester, 4-nitrophthalimidemethyl ester (16), and methyl ester-silyl ether (15) derivatives, respectively. While two of the triols (3α,6β,12α and 3β,6β,12α) exhibit the identical retention times on GLC, the four stereoisomers are well resolved by TLC on a silica gel plate and by HPLC on a C_{18} reversed-phase column.

The high resolution ^{1}H- and ^{13}C-NMR chemical shift data are shown in **Table 2** and **Table 3**. As expected, the 19-methyl proton signal (1.10 ppm) in **2a** and **4a** resonates at lower field than the corresponding signals (0.89–0.93 ppm) in **1a** and **3a** due to its 1,3-diaxial interaction with the 6β-hydroxy group. Axial 3β-H in **1a** and **2a** appears at 3.58–3.60 ppm as a broad multiplet, while the corresponding equatorial 3α-H in **3a** and **4a** occurs at 3.97–4.16 ppm as a multiplet. On the other hand, axial 6β-H in **1a** and **3a** and equatorial 6α-H in **2a** and **4a** resonate at 4.01–4.12 ppm as a broad multiplet and at 3.60–3.78 ppm as a multiplet, respectively.

In ^{13}C-NMR, the shielding data of the α-carbon absorption occurring in the region of 64–73 ppm in each spectrum are of particular importance in characterizing the position and stereochemical nature of 3-, 6-, and 12-hydroxyls. A comparison of the shielding data for **1a–4a** with those reported for the corresponding 3α,12α- and 3β,12α-dihydroxy-5β-cholanoates (27) further revealed that the presence and stereochemistry of 6-hydroxyl groups markedly affects the resonance position of the C-4, C-8, C-10, and C-19 carbon signals. In **1a** and **3a**, the C-4 methylene carbon, which has a γ-*gauche* relationship to the 6α-hydroxyl group, is shielded by 6.7–7.1 ppm, while the corresponding carbon in **2a** and **4a** is either barely shifted or shielded slightly (0.5–1.1 ppm) because of its γ-*trans* relationship to the 6β-hydroxyl group (28). The reverse is true for the C-8 methine carbon, which suffers a large up-field shift (5.2–5.8 ppm) in **2a** and **4a** owing to the γ-*gauche* effect. Furthermore, the C-10 quaternary carbon signal in **1a** and **3a** is shifted to up-field by 1.6–1.7 ppm, and the C-19 methyl carbon signal in **2a** and **4a** is shifted to down-field by 1.5–2.5 ppm due to a *syn*-diaxial δ OH-CH_3 interaction (29).

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REFERENCES

1. Almé, B., A. Bremmelgaard, J. Sjövall, and P. Thomassen. 1977. Analysis of metabolic profiles of bile acids in urine using a lipophilic anion exchanger and computerized gas–liquid chromatography–mass spectrometry. *J. Lipid Res.* **18:** 339–362.
2. Bremmelgaard, A., and J. Sjövall. 1979. Bile acid profiles in urine of patients with liver diseases. *Eur. J. Clin. Invest.* **9:** 341–348.
3. Thomassen, P. A. 1979. Urinary bile acids in late pregnancy and in recurrent cholestasis of pregnancy. *Eur. J. Clin. Invest.* **9:** 425–432.
4. Bremmelgaard, A., and J. Sjövall. 1980. Hydroxylation of cholic, chenodeoxycholic, and deoxycholic acids in patients with intrahepatic cholestasis. *J. Lipid Res.* **21:** 1072–1080.
5. Almé, B., and J. Sjövall. 1980. Analysis of bile acid glucuronides in urine. Identification of 3α,6α,12α-trihydroxy-5β-cholanoic acid. *J. Steroid Biochem.* **13:** 907–916.
6. Batta, A. K., R. Arora, G. Salen, G. S. Tint, D. Eskreis, and S. Katz. 1989. Characterization of serum and urinary bile acids in patients with primary biliary cirrhosis by gas–liquid chromatography–mass spectrometry: effect of ursodeoxycholic acid treatment. *J. Lipid Res.* **30:** 1953–1962.
7. Nakagawa, M., and K. D. R. Setchell. 1990. Bile acid metabolism in early life: studies of amniotic fluids. *J. Lipid Res.* **31:** 1089–1098.
8. Shoda, J., N. Tanaka, T. Osuga, K. Matsuura, and H. Miyazaki. 1990. Altered bile acid metabolism in liver disease: concurrent occurrence of C-1 and C-6 hydroxylated bile acid metabolites and their preferential excretion into urine. *J. Lipid Res.* **31:** 249–259.
9. Iida, T., T. Momose, T. Tamura, T. Matsumoto, F. C. Chang, J. Goto, and T. Nambara. 1988. Potential bile acid metabolites. 13. Improved routes to 3β,6β- and 3β,6α-dihydroxy-5β-cholanoic acids. *J. Lipid Res.* **29:** 165–171.
10. Iida, T. T. Momose, T. Tamura, T. Matsumoto, F. C. Chang, J. Goto, and T. Nambara. 1989. Potential bile acid metabolites. 14. Hyocholic and muricholic acid stereoisomers. *J. Lipid Res.* **30:** 1267–1279.
11. Iida, T., I. Komatsubara, S. Yoda, J. Goto, T. Nambara, and F. C. Chang. 1990. Potential bile acid metabolites. 16. Synthesis of stereoisomeric 3α,6,7,12α-tetrahydroxy-5β-cholanoic acids. *Steroids.* **55:** 530–539.
12. Haslewood, G. A. D. 1958. Comparative studies of bile salts. 11. 3α,6α,12α-Trihydroxycholanic acid and related substances. *Biochem. J.* **70:** 551–558.
13. Takeda, K., and K. Igarashi. 1959. Bile acids and steroids. XI. Synthesis of 3α,6α,12α-trihydroxycholanic acid and its oxidation products. *J. Biochem.* **46:** 1313–1322.
14. Ratliff, R. L., J. T. Matschiner, E. A. Doisy, Jr., S. L. Hsia, S. A. Thayer, W. H. Elliott, and E. A. Doisy. 1961. Bile acids. XV. Partial synthesis of the new metabolite of deoxycholic acid, 3α,6β,12α-trihydroxycholanic acid. *J. Biol. Chem.* **236:** 685–687.
15. Iida, T., T. Momose, T. Tamura, T. Matsumoto, J. Goto, T. Nambara, and F. C. Chang. 1987. Capillary gas chromatography of hydroxylated bile acid stereoisomers of the *allo* and normal series. *J. Chromatogr.* **389:** 155–164.
16. Iida, T., Y. Ohnuki, F. C. Chang, J. Goto, and T. Nambara. 1985. High performance liquid chromatographic separation of stereoisomeric bile acids as their UV-sensitive esters. *Lipids.* **20:** 187–194.
17. Tserng, K-Y., and P. D. Klein. 1977. Formylated bile acids: improved synthesis, properties, and partial deformylation. *Steroids.* **29:** 635–648.
18. Leppik, R. A. 1983. Improved synthesis of 3-keto, 4-ene-3-keto, and 4,6-diene-3-keto bile acids. *Steroids.* **41:** 475–484.
19. Iida, T., T. Tamura, T. Matsumoto, and F. C. Chang. 1985. Potential bile acid metabolites. 9. 3,12-Dihydroxy- and 12β-hydroxy-5α-cholanoic acids. *J. Lipid Res.* **26:** 874–881.
20. Contreras, R., and L. Mendoza. 1979. The reduction of

5α-cholestan-3-one and 5β-cholestan-3-one by some borane and hydroborates. *Steroids.* **34:** 121–124.

21. Iida, T., T. Momose, F. C. Chang, J. Goto, and T. Nambara. 1989. Potential bile acid metabolites. XV. Synthesis of 4β-hydroxylated bile acids; unique bile acids in human fetal bile. *Chem. Pharm. Bull.* **37:** 3323–3329.
22. Nussbaum, A. L., E. Yuan, D. Dincer, and E. P. Oliveto. 1961. Enol ethers of steroidal Δ^4-3-ketones. *J. Org. Chem.* **26:** 3925–3928.
23. Kirk, D. N., and J. M. Wiles. 1970. The reaction of *m*-chloroperbenzoic acid with 3-acetoxy-steroidal 3,5-diene. *J. Chem. Soc. Chem. Commun.* 518.
24. Kirk, D. N., and J. M. Wiles. 1970. Competing reactions in the peroxy-acid oxidation of 3-alkoxy-steroidal 3,5-dienes. *J. Chem. Soc. Chem. Commun.* 1015–1016.
25. Combe, M. G., H. B. Henbest, and W. R. Jackson. 1967. Aspects of stereochemistry. Part XXI. Hydrogenation of 3-oxo-Δ^4-steroids over a palladium–calcium carbonate catalyst. *J. Chem. Soc. (C).* 2467–2471.
26. Herz, J. E., and R. Ocampo. 1982. Synthesis of 1-hydroxylated bile acids: Methyl 1α,3α-dihydroxy-5β-cholan-24-oate. *Steroids.* **40:** 661–664.
27. Iida, T., T. Tamura, T. Matsumoto, and F. C. Chang. 1983. Carbon-13 NMR spectra of hydroxylated bile acid steroisomers. *Org. Magn. Reson.* **21:** 305–309.
28. Eggert, H., C. L. VanAntwerp, N. S. Bhacca, and C. Djerassi. 1976. Carbon-13 nuclear magnetic resonance spectra of hydroxy steroids. *J. Org. Chem.* **41:** 71–78.
29. Blunt, J. W. 1975. Carbon-13 NMR spectra of 6β-substituted-5α-cholestane-3β,5-diols: γ- and δ-effects. *Aust. J. Chem.* **28:** 1017–1021.