Potential bile acid metabolites. 17. Synthesis of 2β-hydroxylated bile acids

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The 2β-hydroxylated derivatives of lithocholic, chenodeoxycholic, deoxycholic, and cholic acids were synthesized from the respective parent bile acids by established procedures. The principal reactions involved were (1) bromination of 3-oxo formylated bile acids in N,N-*dimethylformamide, (2) rearrangement and substitution of the resulting 4β-bromo-3-oxo derivatives to the 2β-acetoxy-3-oxo compounds with potassium acetate, and (3) reduction to the 2β-acetoxy-3α-hydroxy compounds with* tert-*butylamine-borane complex. As for the prepared 2β-hydroxylated bile acids with a diequatorial* trans-*glycol structure, proton and carbon-13 nuclear magnetic resonance spectroscopic and gas-liquid chromatographic/mass spectrometric properties are discussed. (Steroids* **56:**114–122, 1991)

Keywords: steroids; bile acids; 2β,3α-dihydroxy-5β-cholanoic acid; 2β,3α,7α-trihydroxy-5β-cholanoic acid; 2β,3α,12α-trihydroxy-5β-cholanoic acid; 2β,3α,7α,12α-tetrahydroxy-5β-cholanoic acid; GC/MS; ^{1}H NMR; ^{13}C NMR; cholanoic acids

Introduction

In recent years, considerable attention has been focused on the difference in biosynthesis and metabolism of bile acids between the fetus and adult. We have recently reported that two new 4β-hydroxylated bile acids were isolated from human fetal bile,[1] and their structures were unambiguously characterized by chemical syntheses as 3α,4β,7α-trihydroxy- and 3α,4β,7α,12α-tetrahydroxy-5β-cholanoic acids having a diequatorial *trans*-3,4-glycol structure.[2]

On the other hand, analogous 2-hydroxylated bile acids with a vicinal 2,3-glycol structure have also recently been identified in the biologic samples from fetuses and neonates by gas-liquid chromatography/mass spectrometry (GC/MS). Clayton et al.[3] isolated 2β,3α,7α,12α-tetrahydroxy-5β-cholanoic acid from the gastric contents of neonates with intestinal obstruction. The presence of this acid in the amniotic fluid of pregnant women has also been recently reported by Nakagawa and Setchell.[4] Subsequently, 2β,3α,6α,7α-tetrahydroxy-5β-cholanoic acid has been identified in the urine of healthy newborns by Strandvik and Wikstrom.[5] In more recent work, Gustafsson et al.[6] investigated the biotransformation of [24-^{14}C]lithocholic acid by the microsomes from human fetal liver and identified the formation of a 2-hydroxylated lithocholic acid (2ξ,3α-dihydroxy-5β-cholanoic acid). These findings indicate the existence of new metabolic pathways in the fetus and neonate, namely, that of 2-hydroxylation. Although the occurrence of several C_{27} 2β-hydroxylated bile acid and bile alcohol metabolites in the samples from osteoglossid fishes, Arapaima gigas (Cuvier; family Osteoglossidae), has already been disclosed,[7] analogous 2-hydroxylated bile acids in human biologic samples have not hitherto been reported.

For a series of our studies on new and unusual bile acid metabolites, 2β-hydroxylated bile acids with a diequatorial *trans*-glycol structure are required as authentic specimens. This paper describes the synthesis of the four 2β-hydroxylated derivatives (**1a–d**; Scheme 1) of lithocholic, chenodeoxycholic, deoxycholic, and cholic acids (and their corresponding methyl esters), starting from the respective parent bile acids (**2a–d**), and presents their ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopic and GC/MS properties.

Experimental

Melting points (mp) were determined on a micro hot stage apparatus and are uncorrected. Infrared (IR) spectra were obtained on a Perkin-Elmer 1600 Series

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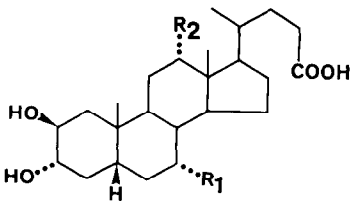


	R_1	R_2
1a	H	H
1b	OH	H
1c	H	OH
1d	OH	OH

Scheme 1

FTIR. ^{1}H nuclear magnetic resonance spectra were obtained on a JEOL FX-90Q at 90 MHz with $CDCl_3$ containing tetramethylsilane (TMS) as an internal standard. The high resolution ^{1}H (400 MHz) and ^{13}C (125.65 MHz) NMR spectra were also recorded on JEOL GSX-400 and GSX-500 instruments, respectively. A Shimadzu GC-14A gas chromatograph equipped with a flame ionization detector was used isothermally (270 C); it was fitted with an aluminum-clad fused-silica capillary column (HiCap CBPM1 [equivalent to OV-101], 25 m × 0.25 mm ID, film thickness, 0.1 μm; Shimadzu Corp., Kyoto, Japan). A Hitachi M-80B GC/MS system equipped with a data processing system was used under the following conditions: GC, 15 m × 0.25 mm ID DB-1 chemically bonded fused silica capillary column at 250 C using helium (linear velocity, 40 cm/sec) as carrier gas; column temperature, 250 C; ionization temperature, 250 C; ionization energy, 20 eV; and acceleration voltage, 3.0 kV. Bile acid samples were analyzed as their methyl ester-trimethylsilyl (Me-TMS) ether derivatives, using bis(trimethylsilyl)trifluoroacetamide as a derivatizing reagent. Analytic thin-layer chromatography (TLC) was performed on pre-coated silica gel plates (20 cm × 20 cm, 0.25 mm layer thickness; E. Merck AG) using EtOAc/hexane (6:4, v/v) or EtOAc/hexane/acetic acid (50:50:1 to 10:40:2, v/v/v) as the developing solvent. All compounds were dried by azeotropic distillation (benzene or benzene/CH_2Cl_2, CH_2Cl_2, or CH_2Cl_2/MeOH) before use in reactions.

Each of the following general procedures was used for the preparation of **1a–d** and their methyl esters. The key intermediates **5e–h** were prepared from the respective parent bile acids **2a–d** in three steps via the compounds **3f–h** through **4f–h**.[8,9] The results are compiled in Tables 1 and 2.

*Bromination of 3-oxo derivatives (**5e–h**) to the 4β-bromo-3-oxo compounds (**6e–h**)*

The 3-oxo derivative (**5e–h**) (20 mmol), prepared from the respective parent bile acids **2a–d**, was dissolved in *N,N*-dimethylformamide (DMF) (60 ml) containing *p*-toluenesulfonic acid (550 mg), and added in one portion with a solution of bromine (26 mmol) in DMF (20 ml). Stirring was continued overnight at room temperature, and ice-water was gradually added to the mixture. The solid products (**6f** and **6g**) were collected by filtration, washed with water, and recrystallized from the solvent indicated in Table 1. The oily products (**6e** and **6h**) were extracted from the mixture with CH_2Cl_2. The combined extract was washed with 5% $Na_2S_2O_3$ solution and then with water, and was dried over Drierite. The solvent was evaporated, and the remaining product was recrystallized from the solvent indicated in Table 1.

*Rearrangement and substitution of 4β-bromo-3-oxo derivatives (**6e–h**) to the 2β-acetoxy-3-oxo compounds (**7e–h**)*

A mixture of the 4β-bromo-3-oxo ester (**6e–h**) (10 mmol) and anhydrous potassium acetate (90 mmol) in acetic acid (45 ml) was refluxed for 30 minutes. The solution was cooled at room temperature, diluted with water until near turbidity, and allowed to stand in the refrigerator. The solid product (**6e**) was collected by filtration, washed with water, and recrystallized from MeOH. In the case of compounds **6f–h**, the oily product was extracted with CH_2Cl_2, and the combined extract was washed with water, dried over Drierite, and evaporated. Chromatography of the oily residue on a column of silica gel (120 g) and elution with benzene/EtOAc (6:4, v/v) afforded the desired compounds, which were recrystallized from the solvent shown in Table 1.

*Reduction of 2β-acetoxy-3-oxo derivatives (**7e–h**) to the 2β-acetoxy-3α-hydroxy compounds (**8e–h**)*

tert-Butylamine-borane complex (8 mmol) was added to a stirred solution of the 2β-acetoxy-3-oxo ester (**7e–h**) (3.5 mmol) in CH_2Cl_2 (30 ml). The mixture was stirred at room temperature for 2 hours, then acidified with 10% HCl. The CH_2Cl_2 layer was washed with water, dried over Drierite, and evaporated to give an oil, which consisted essentially of a single component as judged by TLC. Chromatography of the oil on a silica gel column (230 to 400 mesh, 60 g) and elution with benzene/EtOAc (4:1 to 1:1, v/v) afforded the desired compounds (**8e–g**), which were homogeneous according to TLC and ^{1}H NMR but failed to crystallize. The product **8h** was recrystallized from acetone/hexane.

*Hydrolysis of 2β-acetoxy-3α-hydroxy derivatives (**8e–h**) to the corresponding free acids (**1a–d**)*

The 2β-acetoxy-3α-hydroxy ester (**8e–h**) (300 mg) was refluxed in 5% methanolic potassium hydroxide (9 ml) for 1 hour. MeOH was evaporated off, and the residue was dissolved in water. The solution was cooled in an ice-bath, and acidified with 10% H_2SO_4 with stirring. The precipitate was filtered, washed with saturated

Table 1 4β-Bromo-3-oxo (6e–h), 2β-acetoxy-3-oxo (7e–h), and 2β-acetoxy-3α-hydroxy (8e–h) compounds prepared

Product	Yield[a] (%)	Isolation method[b]	Molecular formula[c]	mp[d] (C)	IR (KBr)[e] ν(cm^{-1})	^{1}H NMR ($CDCl_3$/TMS)[f] δ(ppm)
Methyl 4β-bromo-3-oxo-5β-cholanoate (**6e**)	67	A	$C_{25}H_{39}O_3Br$	99–100 (aq. acetone)	1,739 (C=O), 556 (C—Br)	0.69 (s, 3H, 18-Me), 0.93 (d, 3H, J = 6.3 Hz, 21-Me), 1.08 (s, 3H, 19-Me), 3.67 (s, 3H, COOMe), 4.98 (d, 1H, J = 11.7 Hz, 4-H),
4β-Bromo-7α-formyloxy-3-oxo-5β-cholanoic acid (**6f**)	78	A	$C_{25}H_{37}O_5Br$	164–167 (EtOAc/hexane)	1,743, 1,705 (C=O), 1,180, 1,159 (formate), 557 (C—Br)	0.70 (s, 3H, 18-Me), 0.94 (d, 3H, J = 5.4 Hz, 21-Me), 1.10 (s, 3H, 19-Me), 5.15 (m, 1H, 7-H), 5.33 (d, 1H, J = 11.7 Hz, 4-H), 8.08 (s, 1H, 7-CHO)
4β-Bromo-12α-formyloxy-3-oxo-5β-cholanoic acid (**6g**)	74	A	$C_{25}H_{37}O_5Br$	182–184 (Et_2O/hexane)	1,718 (C=O), 1,181 (formate), 558 (C—Br)	0.80 (s, 3H, 18-Me), 0.84 (d, 3H, J = 6.3 Hz, 21-Me), 1.07 (s, 3H, 19-Me), 4.95 (d, 1H, J = 11.7 Hz, 4-H), 5.29 (m, 1H, 12-H), 8.11 (s, 1H, 12-CHO)
4β-Bromo-7α,12α-diformyloxy-3-oxo-5β-cholanoic acid (**6h**)	80	A	$C_{26}H_{37}O_7Br$	197–199 ($CHCl_3$/Et_2O)	1,720 (C=O), 1,186 (formate), 554 (C—Br)	0.81 (s, 3H, 18-Me), 0.84 (d, 3H, J = 6.3 Hz, 21-Me), 1.10 (s, 3H, 19-Me), 5.21 (m, 1H, 7-H), 5.31 (m, 1H, 12-H), 5.33 (d, 1H, J = 11.7 Hz, 4-H), 8.11 and 8.15 (s, each 1H, 7- and 12-CHO)
Methyl 2β-acetoxy-3-oxo-5β-cholanoate (**7e**)	61	A	$C_{27}H_{42}O_5$	168–170 (MeOH)	1,748, 1,728 (C=O), 1,264, 1,040 (acetate)	0.68 (s, 3H, 18-Me), 0.93 (d, 3H, J = 6.3 Hz, 21-Me), 1.05 (s, 3H, 19-Me), 2.14 (s, 3H, 2-OCOMe), 3.66 (s, 3H, COOMe), 5.22 (dd, 1H, J = 13.5 and 6.3 Hz, 2-H)
2β-Acetoxy-7α-formyloxy-3-oxo-5β-cholanoic acid (**7f**)	53	B	$C_{27}H_{40}O_7$	197–200 (acetone/hexane)	1,749 (C=O), 1,257, 1,038 (acetate)	0.69 (s, 3H, 18-Me), 0.95 (d, 3H, J = 4.5 Hz, 21-Me), 1.08 (s, 3H, 19-Me), 2.16 (s, 3H, 2-OCOMe), 5.11 (m, 1H, 7-H), 5.30 (dd, 1H, J = 13.5 and 6.3 Hz, 2-H), 8.07 (s, 1H, 7-CHO)
2β-Acetoxy-12α-formyloxy-3-oxo-5β-cholanoic acid (**7g**)	56	B	$C_{27}H_{40}O_7$	169–173 (acetone/hexane)	1,716 (C=O), 1,231 (acetate) 1,182 (formate)	0.79 (s, 3H, 18-Me), 0.85 (d, 3H, J = 5.4 Hz, 21-Me), 1.04 (s, 3H, 19-Me), 2.13 (s, 3H, 2-OCOMe), 5.13 (dd, 1H, J = 13.5 and 6.3 Hz, 2-H), 5.33 (m, 1H, 12-H), 8.13 (s, 1H, 7-CHO)

Table 1 *(cont'd.)*

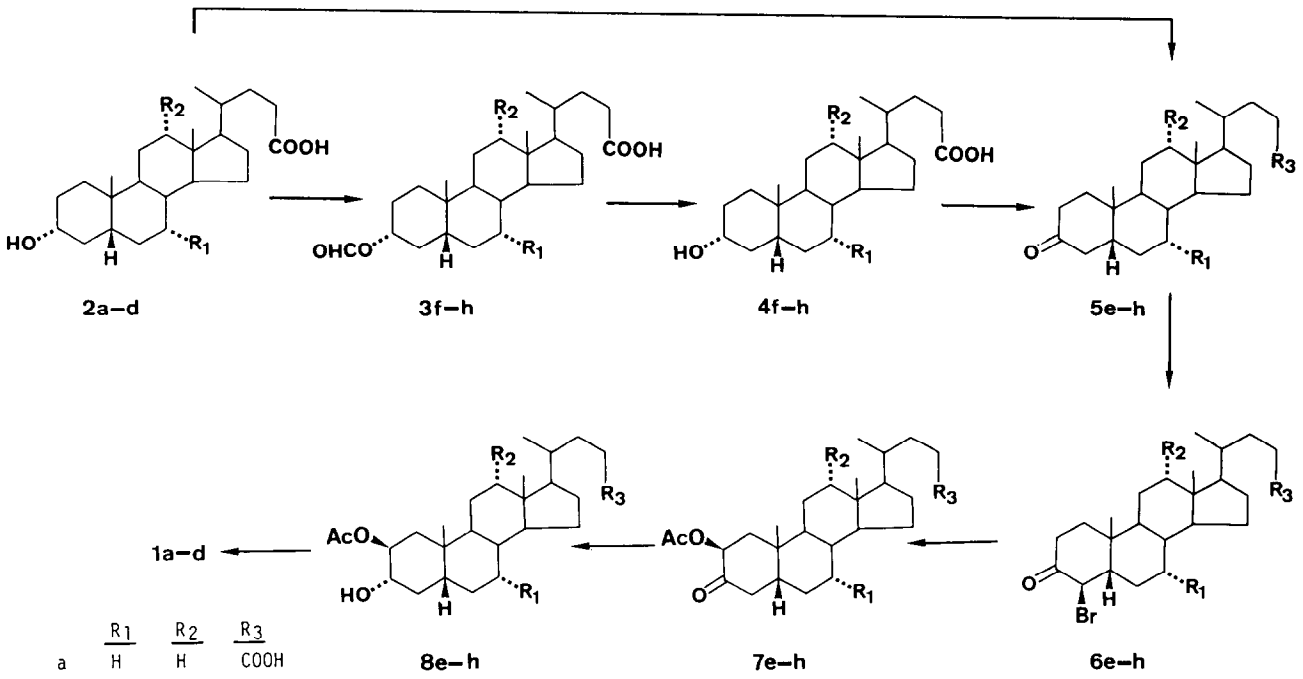
2β-Acetoxy-7α,12α-diformyloxy-3-oxo-5β-cholanoic acid (**7h**)	48	B	$C_{28}H_{40}O_9$	231–233 (acetone/hexane)	1,724 (C=O), 1,236 (acetate), 1,183 (formate)	0.80 (s, 3H, 18-Me), 0.86 (d, 3H, J = 6.3 Hz, 21-Me), 1.07 (s, 3H, 19-Me), 2.14 (s, 3H, 2-OCOMe), 5.14 (m, 1H, 7-H), 5.18 (dd, 1H, J = 13.5 and 6.3 Hz, 2-H), 5.35 (m, 1H, 12-H), 8.11 and 8.15 (s, each 1H, 7- and 12-CHO)
Methyl 2β-acetoxy-3α-hydroxy-5β-cholanoate (**8e**)	91	B	$C_{27}H_{44}O_5$	—	1,740 (C=O), 3,502 (OH), 1,247, 1,035 (acetate)	0.64 (s, 3H, 18-Me), 0.94 (d, 3H, J = 6.3 Hz, 21-Me), 0.95 (s, 3H, 19-Me), 2.07 (s, 3H, 2-OCOMe), 3.59 (brm, 1H, 3-H), 3.66 (s, 3H, COOMe), 4.70 (brm, 1H, 2-H)
2β-Acetoxy-7α-formyloxy-3α-hydroxy-5β-cholanoic acid (**8f**)	85	B	$C_{27}H_{42}O_7$	—	1,719 (C=O), 3,500 (OH), 1,248, 1,035 (acetate), 1,181 (formate)	0.65 (s, 3H, 18-Me), 0.94 (d, 3H, J = 6.3 Hz, 21-Me), 0.97 (s, 3H, 19-Me), 2.08 (s, 3H, 2-OCOMe), 3.51 (brm, 1H, 3-H), 4.70 (brm, 1H, 2-H), 5.02 (m, 1H, 7-H), 8.07 (s, 1H, 7-CHO)
2β-Acetoxy-12α-formyloxy-3α-hydroxy-5β-cholanoic acid (**8g**)	80	B	$C_{27}H_{42}O_7$	—	1,724 (C=O), 3,446 (OH), 1,244, 1,048 (acetate), 1,173 (formate)	0.74 (s, 3H, 18-Me), 0.85 (d, 3H, J = 4.5 Hz, 21-Me), 0.93 (s, 3H, 19-Me), 2.04 (s, 3H, 2-OCOMe), 3.63 (brm, 1H, 3-H), 4.63 (brm, 1H, 2-H), 5.28 (m, 1H, 12-H), 8.14 (s, 1H, 12-CHO)
2β-Acetoxy-7α,12α-diformyloxy-3α-hydroxy-5β-cholanoic acid (**8h**)	76	B	$C_{28}H_{42}O_9$	214–218 (acetone/hexane)	1,720 (C=O), 3,504, 1,019, 992 (OH), 1,249 (acetate), 1,180 (formate)	0.75 (s, 3H, 18-Me), 0.86 (d, 3H, J = 5.4 Hz, 21-Me), 0.96 (s, 3H, 19-Me), 2.06 (s, 3H, 2-OCOMe), 3.53 (brm, 1H, 3-H), 4.62 (brm, 1H, 2-H), 5.05 (m, 1H, 7-H), 5.30 (m, 1H, 12-H), 8.10 and 8.17 (s, each 1H, 7- and 12-CHO)

[a] Yield of isolated, pure product.
[b] Purified by (A) direct crystallization or by (B) column chromatography on silica gel, C_6H_6-EtOAc (4 : 1 to 1 : 1, v/v).
[c] The microanalyses were in good agreement with the calculated values: C ± 0.26; H ± 0.14.
[d] Uncorrected; compounds **8e–g** were homogeneous according to TLC and ^1H NMR, but failed to crystallize (EtOAc, ethyl acetate; Et_2O, ethyl ether; MeOH, methanol).
[e] Recorded on a Perkin-Elmer 1600 Series FTIR spectrometer.
[f] Recorded at 90 MHz on a JEOL FX-90Q spectrometer: s, singlet; d, doublet (J in Hz); m, multiplet; brm, broad multiplet; dd, double doublet.

Table 2 2β-Hydroxylated bile acids (**1a–d**) and their methyl esters prepared[a]

Product	Yield (%)	Isolation method	Molecular formula	mp (C)	IR (KBr) ν(cm⁻¹)	¹H NMR ($CDCl_3$/TMS) δ(ppm)[b]
2β,3α-Dihydroxy-5β-cholanoic acid (**1a**)	86	A	$C_{24}H_{40}O_4$	193–196 (EtOAc/MeOH)	1,715 (C=O), 3,364, 1,001 (OH)	0.64 (s, 3H, 18-Me), 0.94 (s, 3H, 19-Me), 3.33 (brm, 2H, 2- and 3-H)
Methyl 2β-3α-dihydroxy-5β-cholanoate		A	$C_{25}H_{42}O_4$	164/166 (aq. MeOH)	1,734 (C=O), 3,429, 1,005 (OH)	0.64 (s, 3H, 18-Me), 0.92 (d, 3H, J = 6.3 Hz, 21-Me), 0.96 (s, 3H, 19-Me), 3.45 (brm, 2H, 2- and 3-H), 3.66 (s, 3H, COOMe)
2β,3α,7α-Trihydroxy-5β-cholanoic acid (**1b**)	83	A	$C_{24}H_{40}O_5$	132–134 (EtOAc)	1,700 (C=O), 3,350, 1,008, 975 (OH)	0.65 (s, 3H, 18-Me), 0.92 (s, 3H, 19-Me), 3.30 (brm, 2H, 2- and 3-H), 3.75 (m, 1H, 7-H)
Methyl 2β,3α,7α-trihydroxy-5β-cholanoate		A	$C_{25}H_{42}O_5$	125–128 (acetone/hexane)	1,740 (C=O), 3,420, 974 (OH)	0.66 (s, 3H, 18-Me), 0.93 (d, 3H, J = 4.5 Hz, 21-Me), 0.95 (s, 3H, 19-Me), 3.40 (brm, 2H, 2- and 3-H), 3.66 (s, 3H, COOMe), 3.84 (m, 1H, 7-H)
2β,3α,12α-Trihydroxy-5β-cholanoic acid (**1c**)	89	A	$C_{24}H_{40}O_5$	234–235 (acetone/hexane)	1,723 (C=O), 3,368, 1,004 (OH)	0.65 (s, 3H, 18-Me), 0.92 (s, 3H, 19-Me), 3.45 (brm, 2H, 2- and 3-H), 3.92 (m, 1H, 12-H)
Methyl 2β,3α,12α-trihydroxy-5β-cholanoate		A	$C_{25}H_{42}O_5$	191–192 (acetone/hexane)	1,738 (C=O), 3,392, 1,004 (OH)	0.67 (s, 3H, 18-Me), 0.93 (s, 3H, 19-Me), 3.45 (brm, 2H, 2- and 3-H), 3.66 (s, 3H, COOMe), 3.99 (m, 1H, 12-H)
2β,3α,7α,12α-Tetrahydroxy-5β-cholanoic acid (**1d**)	82	A	$C_{24}H_{40}O_6$	230–232 (aq. MeOH)	1,702 (C=O), 3,436, 978 (OH)	0.65 (s, 3H, 18-Me), 0.90 (s, 3H, 19-Me), 0.99 (d, 3H, J = 5.4 Hz, 21-Me), 3.40 (brm, 2H, 2- and 3-H), 3.88 (m, 1H, 7-H), 4.03 (m, 1H, 12-H)
Methyl 2β,3α,7α,12α-tetrahydroxy-5β-cholanoate		A	$C_{25}H_{42}O_6$	197–199 (aq. MeOH)	1,725 (C=O), 3,361, 1,016, 976 (OH)	0.69 (s, 3H, 18-Me), 0.93 (s, 3H, 19-Me), 3.45 (brm, 2H, 2- and 3-H), 3.66 (s, 3H, COOMe), 3.83 (m, 1H, 7-H), 3.99 (m, 1H, 12-H)

[a] The microanalyses were in good agreement with the calculated values: C ± 0.18; H ± 0.18; see footnotes to Table 1 for other explanations.
[b] For reasons of solubility, the free acids (**1a-d**) were measured in $CDCl_3$ containing 20% DMSO-d_6.



	R1	R2	R3
a	H	H	COOH
b	OH	H	COOH
c	H	OH	COOH
d	OH	OH	COOH
e	H	H	$COOCH_3$
f	OCHO	H	COOH
g	H	OCHO	COOH
h	OCHO	OCHO	COOH

Scheme 2

brine, and recrystallized from the solvent given in Table 2.

*Esterification of free acids (**1a–d**) to the corresponding methyl esters*

p-Toluenesulfonic acid (15 mg) was added to the free acid (**1a–d**) (150 mg) in MeOH (5 ml), and the mixture was allowed to stand overnight at room temperature. MeOH was evaporated and the residue was extracted with CH_2Cl_2. The organic extract was washed with 5% $NaHCO_3$ and water, dried over Drierite, and evaporated to give the corresponding methyl ester, which was recrystallized from the solvent shown in Table 2.

Results and discussion

3-1 Synthesis of 2β-hydroxylated bile acids

Partial synthesis of **1d**, starting from methyl 7α,12α-diacetoxy-3-oxo-5β-cholanoate, has been previously reported by Haslewood and Tokes.[7] The principal reactions used were bromination of the 3-oxo derivative in acetic acid, rearrangement in the substitution reaction of the resulting 4β-bromo-3-oxo ester with potassium acetate, and subsequent reduction of the 2β-acetoxy-3-oxo derivative with sodium borohydride ($NaBH_4$). It seemed very likely that the successful synthesis of **1a–d** would depend on improving the methods. We have been able to do this by adapting known procedures for several steps.

In our synthesis, the 3-oxo formylated bile acids **5f–h** (with the exception of **5e**) were chosen as the starting 3-ketones (Scheme 2) because formylated bile acids obviate the need for prior esterification of the carboxyl group in reactions and subsequent chromatographic purification, and are readily hydrolyzed to the free acids and crystallized more easily compared with the corresponding acetylated bile acid esters. Compounds **5f–h** were thus prepared in total yield of over 75% from the respective parent bile acids (**2b–d**) in three steps, with a slight modification of the procedures of Tserng and Klein[8] and Leppik.[9] The procedures consist of formylation (**3f–h**) with 99% formic acid, selective deformylation (**4f–h**) of the 3-formyloxy group with saturated ammonia in methanol (MeOH), and then oxidation of the 3α-hydroxy products with Jones reagent. Methyl 3-oxo-5β-cholanoate (**5e**) was obtained directly from **2a** via the methyl ester **2e**.

By carrying out the bromination of **5e–h** in DMF,[10,11] instead of the commonly used acetic acid, in the presence of a catalytic amount of *p*-toluenesulfonic acid, an analytically pure sample of the 4β-bromo-3-oxo

compounds (**6e–h**) was obtained in reasonable yields (67% to 80%). These compounds essentially show a single spot on TLC and their ^{1}H NMR exhibit for the 4α-hydrogen a characteristic doublet signal (J = 11.7 Hz) at 4.95 to 4.98 ppm for **6e** and **6g** and at 5.33 ppm for **6f** and **6h**. The difference in chemical shift is probably due to the proximity of the axial 7α-formyloxy group.

Treatment of the 4β-bromo-3-oxo compounds (**6e–h**) with anhydrous potassium acetate in boiling acetic acid led to a substitution accompanied by the rearrangement of C-4 (4β-Br) to C-2 (2β-OCOMe), a method introduced by Satoh et al.[12] and modified by Haslewood and Tokes.[7] The rearrangement and substitution proceeded smoothly, and the sterically pure 2β-acetoxy-3-oxo intermediates (**7e–h**) were formed in isolated yield of 48% to 61% by direct crystallization or after chromatographic purification. The structure of **7e–h** was confirmed by the ^{1}H NMR signal, appearing at 2.13 to 2.16 ppm as a sharp singlet due to the acetoxy group and at 5.13 to 5.30 ppm as a double doublet (J = 13.5 and 6.3 Hz) due to coupling of the 2α-hydrogen with 1α- and 1β-hydrogens.[13,14]

The high equatorial selectivity found in our previous reduction of the analogous 3-keto bile acids with *tert*-butylamine-borane complex[2] suggested its use for an improved preparation of the 2β-acetoxy-3α-hydroxy acids (**8e–h**). Indeed, when the reduction reaction was carried out on **7e–h**, the desired equatorial 3α-hydroxylated products (**8e–h**) were obtained with isolated yields of 76% to 91% after chromatographic purification on silica gel; in each reaction, no isolated amount of the 3β-epimer was recovered. The equatorial configuration of the 2β-acetoxy and 3α-hydroxyl groups in **8e–h** was confirmed by the two broad multiplet signals (^{1}H NMR) appearing at 4.62 to 4.70 ppm (axial 2α-hydrogen) and at 3.51 to 3.63 ppm (axial 3β-hydrogen).

Usual alkaline hydrolysis of **8e–h** with 5% methanolic potassium hydroxide followed by acidification afforded the desired 2β-hydroxylated bile acids (**1a–d**). Esterification of **1a–d** by the usual method yielded the corresponding methyl esters.

3-2 Characterization of compounds by gas-liquid chromatography/mass spectrometry and ^{1}H and ^{13}C nuclear magnetic resonance

Chemical evidence for the 1,2-diol structure, the stereochemical configuration of hydroxyls, and the purity of **1a–d** were further confirmed by GC/MS and high-resolution ^{1}H and ^{13}C NMR spectral data.

The electron impact ionization mass spectra of **1a–d** as their Me-TMS ether derivatives are shown in Figure 1, and the methylene unit value[15] observed for those compounds on a nonselective capillary column, HiCap CBPM1 (equivalent to OV-101), are as follows: **1a**, 32.82; **1b**, 33.36; **1c**, 34.15; and **1d**, 34.55. In each mass spectrum, a molecular ion peak (M^+) of the Me-TMS ethers (**1b** and **1c**, 638 amu; **1d**, 726 amu) is very small or absent, except for **1a** (550 amu). Major peaks are

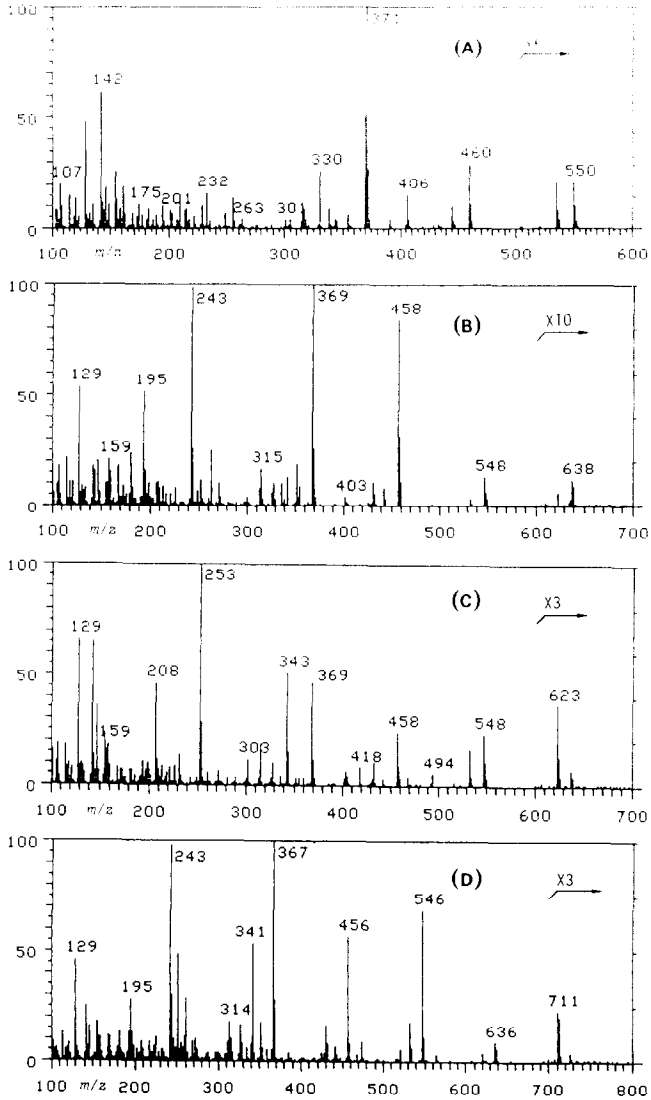


Figure 1 Electron impact ionization (20 eV) mass spectra of the Me-TMS ether derivatives of (A) 2β,3α-dihydroxy-5β-cholanoic acid (**1a**), (B) 2β,3α,7α-trihydroxy-5β-cholanoic acid (**1b**), (C) 2β,3α,12α-trihydroxy-5β-cholanoic acid (**1c**), and (D) 2β,3α,7α,12α-tetrahydroxy-5β-cholanoic acid (**1d**). In each panel, the intensities, from the tail of the arrow onward, have been multiplied by the scaling factor indicated above the arrow.

ions arising from one to four sequential losses of trimethylsilanol (TMSOH, 90 amu) from M^+ and the related ions due to further loss of side chain (115 amu). However, a more diagnostic ion that characterizes a vicinal 2β,3α-glycol structure involves loss of -OTMS (89 amu) rather than TMSOH.[16] Thus, the base peaks for the Me-TMS ether of **1a**, **1b**, and **1d** are the ions at *m/z* 371 (M-90-89), 369 (M-90-90-89), and 367 (M-90-90-90-89), respectively. In addition, the relative intensity of the ion at *m/z* 369 in **1c** is much higher than that of the ion at *m/z* 368 (M-90-90-90).

Tables 3 and 4 show the high-resolution ^{1}H and ^{13}C NMR data for the methyl esters of **1a–d**. In the 400 MHz ^{1}H NMR spectra, particularly noteworthy is the complete separation of axial 2α- and 3β-protons, which overlap each other in the 90-MHz spectra. In **1a** and

Table 3 High-resolution ^1H nuclear magnetic resonance spectral data for the methyl ester derivatives of 2β-hydroxylated bile acids (**1a–d**)[a]

Bile acid	18-Me[b]	19-Me[b]	21-Me[c]	COOMe[b]	2α-H[c]	3β-H[c]	7β-H[c]	12β-H[c]
2β,3α-(OH)$_2$ (**1a**)	0.64	0.96	0.91 (d, 6.5)	3.66	3.52 (brm, 25.9)	3.42 (brm, 26.5)		
2β,3α,7α-(OH)$_3$ (**1b**)	0.62	0.91	0.88 (d, 6.5)	3.62	3.47 (brm, 23.9)	3.21 (brm, 26.3)	3.78 (m, 7.4)	
2β,3α,12α-(OH)$_3$ (**1c**)	0.64	0.90	0.93 (d, 6.5)	3.63	3.60 (brm)[d]	3.35 (brm, 26.7)		3.96 (m, 6.3)
2β,3α,7α,12α-(OH)$_4$ (**1d**)	0.63	0.88	0.93 (d, 6.0)	3.63	3.58 (brm, 24.1)	3.24 (brm, 25.6)	3.79 (m, 6.3)	3.92 (m, 7.0)

[a] In parts per million downfield from TMS; recorded at 400 MHz on a JEOL GSX-400 spectrometer.
[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; brm, broad multiplet.
[d] The width at half-height of this signal could not be determined due to overlapping with the methyl ester signal.

Table 4 ^{13}C nuclear magnetic resonance spectral data for the methyl ester derivatives of 2β-hydroxylated bile acids (**1a–d**)[a]

Carbon	2β,3α-(OH)$_2$ (**1a**)	2β,3α,7α-(OH)$_3$ (**1b**)	2β,3α,12α-(OH)$_3$ (**1c**)	2β-3α-7α,12α-(OH)$_4$ (**1d**)
1	43.27	43.05	43.60	43.14
2	71.53	71.27	70.75	70.55
3	76.85	76.94	76.37	76.54
4	34.18	37.33	34.50	37.26
5	42.10[b]	41.51	42.04	41.39[b]
6	26.50[c]	33.97	26.49[b]	33.87
7	26.41[c]	68.27	26.16[b]	68.33
8	35.90	39.41	36.07	39.46
9	42.07[b]	34.26	35.06	27.67
10	37.23	37.53	36.58	37.16
11	21.12	20.92	28.60	28.43
12	40.13	39.68	73.21	72.99
13	42.76	42.66	46.61	46.44
14	56.44	50.36	47.98	41.64[b]
15	24.21	23.65	23.73	23.22
16	28.23	28.26	27.57	27.62
17	55.99	55.95	47.20	47.03
18	12.10	11.80	12.77	12.50
19	23.41	22.89	23.01	22.43
20	35.43	35.44	35.34	35.48
21	18.33	18.35	17.35	17.34
22	31.14	31.10	31.32	31.29
23	31.04	31.03	31.07	31.04
24	174.88	174.88	174.80	174.86
25[d]	51.58	51.59	51.54	51.54

[a] In parts per million downfield from TMS; recorded at 125.65 MHz on a JEOL GSX-500 spectrometer.
[b,c] Assignments in each column may be interchanged.
[d] The methyl group of the methyl ester.

1c,the axial 3β-hydrogen appears at 3.35 to 3.42 ppm as a broad multiplet ($W_{1/2}$, 26.5 to 26.7 Hz), while the corresponding signal in **1b** and **1d** is slightly shielded, probably due to the 7α-hydroxyl group, and occurs at 3.21 to 3.24 ppm ($W_{1/2}$, 25.6 to 26.3 Hz). On the other hand, the axial 2α-hydrogen, also appearing as a broad multiplet ($W_{1/2}$, 23.9 to 25.9 Hz), resonates at lower field (3.47 to 3.60 ppm) than the corresponding 3β-hydrogen. The remaining proton signals, equatorial 7β- and 12β-hydrogens, appear at 3.78 to 3.79 ppm ($W_{1/2}$, 6.3 to 7.4 Hz) and at 3.92 to 3.96 ppm ($W_{1/2}$, 6.3 to 7.0 Hz) as multiplet, respectively.

The assignment of each carbon signal of the methyl ester of **1a–d** in the ^{13}C NMR was made on the basis of previous reports.[17,18] The signal assignments were further confirmed by the distortionless enhancement by polarization transfer (DEPT) spectra, which allows the differentiation of the signals of methine (CH), methylene (CH_2), methyl (CH_3), and quaternary (C) carbons; C, CH, and CH_2 versus CH_3 signals were easily

differentiated from the DEPT 1, 2, and 3 spectra, respectively. A comparison of the shielding data for **1a–d** with those for the parent compounds **2a–d**[18] revealed that introduction of a 2β-hydroxyl group causes a downfield shift of C-1 (7.8 to 8.5 ppm), C-2 (40.5 to 41.4 ppm), C-3 (4.8 to 5.9 ppm), C-9 (1.5 to 2.0 ppm), and C-10 (2.5 to 3.0 ppm), while C-4 is shielded by 1.4 to 2.3 ppm. Remaining carbon signals are either little affected or shifted slightly. Since these sharp signals are unequivocally identified and separated completely from each other, the shielding data allow a straightforward identification of each compound as well as an estimation of its purity.

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Names

Lithocholic acid	3α-hydroxy-5β-cholanoic acid
Chenodeoxycholic acid	3α,7α-dihydroxy-5β-cholanoic acid
Deoxycholic acid	3α,12α-dihydroxy-5β-cholanoic acid
Cholic acid	3α,7α,12α-trihydroxy-5β-cholanoic acid.

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