Synthesis of potential cholelitholytic agents: 3α,7α,12α-trihydroxy-7β-methyl-5β-cholanoic acid, 3α,7β,12α-trihydroxy-7α-methyl-5β-cholanoic acid, and 3α,12α-dihydroxy-7ξ-methyl-5β-cholanoic acid

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Abstract This report describes the chemical synthesis of six new bile acid analogs, namely, 3α,7α,12α-trihydroxy-7β-methyl-5β-cholanoic acid (7β-methyl-cholic acid), 3α,7β,12α-trihydroxy-7α-methyl-5β-cholanoic acid (7α-methyl-ursocholic acid), 3α,12α-dihydroxy-7ξ-methyl-5β-cholanoic acid (7ξ-methyl-deoxycholic acid), 3α,12α-dihydroxy-7-methyl-5β-chol-7-en-24-oic acid, 3α,12α-dihydroxy-7-methyl-5β-chol-6-en-24-oic acid, and 3α,12α-dihydroxy-7-methylene-5β-cholan-24-oic acid. The carboxyl group of the starting material 3α,12α-dihydroxy-7-oxo-5β-cholanoic acid was protected by conversion to its oxazoline derivative. A Grignard reaction of the bile acid oxazoline with CH_3MgI followed by acid hydrolysis gave two epimeric trihydroxy-7-methyl-cholanoic acids and three dehydration products. The latter were purified by silica gel column chromatography and silica gel-$AgNO_3$ column chromatography of their methyl ester derivatives. Catalytic hydrogenation of 3α,12α-dihydroxy-7-methyl-5β-chol-6-en-24-oic acid and 3α,12α-dihydroxy-7-methylene-5β-cholan-24-oic acid gave 3α,12α-dihydroxy-7ξ-methyl-5β-cholanoic acid. The configuration of the 7-methyl groups and the position of the double bonds were assigned by proton nuclear magnetic resonance spectroscopy and the chromatographic and mass spectrometric properties of the new compounds. These compounds were synthesized for the purpose of exploring new and potentially more effective cholelitholytic agents. The hydrophilic bile acids 7β-methyl-cholic acid and 7α-methyl-ursocholic acid are of particular interest because they should be resistant to bacterial 7-dehydroxylation. —**Kuroki, S., M. Une, and E. H. Mosbach.** Synthesis of potential cholelitholytic agents: 3α,7α,12α-trihydroxy-7β-methyl-5β-cholanoic acid, 3α,7β,12α-trihydroxy-7α-methyl-5β-cholanoic acid, and 3α,12α-dihydroxy-7ξ-methyl-5β-cholanoic acid. *J. Lipid Res.* 1985. **26:** 1205–1211.

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Chenodeoxycholic acid (CDA) and ursodeoxycholic acid (UDA) are currently in use as therapeutic agents for the dissolution of cholesterol gallstones (1, 2). However, these compounds are potentially hepatotoxic because they are 7-dehydroxylated by intestinal anaerobes to form lithocholic acid (LCA) (3). We hypothesized that the 7-methyl analogs of CDA and UDA should be more resistant to bacterial dehydroxylation than the parent substances. Reduced bacterial 7-dehydroxylation should result in a slower biological turnover, lessened hepatotoxicity and, perhaps, greater cholelitholytic efficacy. We have already demonstrated in this laboratory that 3α,7α-dihydroxy-7β-methyl-5β-cholanoic acid (7-Me-CDA) and 3α,7β-dihydroxy-7α-methyl-5β-cholanoic acid (7-Me-UDA) (4) are rapidly absorbed from the intestine, extracted by the liver, and secreted into the bile fully conjugated with glycine and taurine. They were considerably more resistant to bacterial dehydroxylation than CDA (5). Moreover, 3α-hydroxy-7ξ-methyl-5β-cholanoic acid (7-Me-LCA) was shown not to be cholestatic in the hamster at doses that would have produced severe cholestasis in the case of LCA (6). We have further shown that in the prairie dog model of cholesterol cholelithiasis 7-Me-CDA prevented the formation of gallstones more effectively than CDA (Kuroki, S., M. Une, and E. H. Mosbach, unpublished observations).

Abbreviations: CA, cholic acid; UCA, 3α,7β,12α-trihydroxy-5β-cholan-24-oic acid; CDA, chenodeoxycholic acid; UDA, ursodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; 7-Me-CA, 3α,7α,12α-trihydroxy-7β-methyl-5β-cholan-24-oic acid; 7-Me-UCA, 3α,7β,12α-trihydroxy-7α-methyl-5β-cholan-24-oic acid; 7-Me-CDA, 3α,7α-dihydroxy-7β-methyl-5β-cholan-24-oic acid; 7-Me-UDA, 3α,7β-dihydroxy-7α-methyl-5β-cholan-24-oic acid; 7-Me-DCA, 3α,12α-dihydroxy-7ξ-methyl-5β-cholan-24-oic acid; 7-Me-LCA, 3α-hydroxy-7ξ-methyl-5β-cholan-24-oic acid; 7ξ, mixture of 7α and 7β epimers; PMR, proton nuclear magnetic resonance; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; GLC–MS, gas–liquid chromatography–mass spectrometry; HPLC, high-pressure liquid chromatography; RRT, relative retention time.

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Recently, some investigators have considered the possibility of using the relatively hydrophilic trihydroxy bile acids as cholelitholytic agents. For example, Carulli et al. (7) showed that in man cholic acid can lower the saturation index of bile, provided the formation of deoxycholic acid (DCA) is inhibited by the simultaneous administration of the antibiotic, ampicillin. 3α,7β,12α-Trihydroxy-5β-cholanoic acid (UCA) is highly hydrophilic (8) and could conceivably dissolve cholesterol gallstones via formation of a mesophase, as has been observed with UDA. However, since both CA and UCA are readily dehydroxylated by the intestinal microflora to yield DCA (9, 10), it is difficult to evaluate the effect of these trihydroxy bile acids directly. It seemed possible to preserve the 7-hydroxy group of CA and UCA by introducing a 7-methyl group into the molecule, thus preventing the 7-dehydroxylation while maintaining the hydrophilic nature of the molecule.

The present report deals with the synthesis of 3α,7α,12α-trihydroxy-7β-methyl-5β-cholanoic acid (7-Me-CA), 3α,-7β,12α-trihydroxy-7α-methyl-5β-cholanoic acid (7-Me-UCA), and 3α,12α-dihydroxy-7ξ-methyl-5β-cholanoic acid (7-Me-DCA), starting from cholic acid. Availability of these compounds will make it possible to investigate the physiological properties and therapeutic possibilities of these new bile acid analogs.

MATERIALS AND METHODS

Melting points were determined on a Thermolyne melting point apparatus and are uncorrected.

Proton nuclear magnetic resonance (PMR) spectra, given in δ ppm, were measured on a Hitachi model R-40 spectrometer at 90 MHz using tetramethylsilane as an internal standard.

Thin-layer chromatography (TLC) was performed on precoated silica gel G plates (Merck, 0.2 mm thickness) or silica gel G plates impregnated with 15% silver nitrate (Analtech, Inc., 0.25 mm thickness). The spots were made visible by spraying with a 10% ethanolic solution of phosphomolybdic acid followed by heating at 120°C for 3 min.

Gas–liquid chromatography (GLC) was carried out on a Hewlett-Packard 5830A gas chromatograph using an SE-30 column. The bile acid analogs were analyzed as their methyl ester-trimethylsilyl (TMS) ether derivatives. All retention times are described relative to that of the TMS ether of methyl cholate (1.00).

Gas–liquid chromatography–mass spectrometry (GLC–MS) was carried out on a Hewlett-Packard 5992B spectrometer under the following conditions: column, 3% SP-2250; column temperature, 260°C; ion source temperature, 140°C; source pressure, 2×10^{-6} torr.

High-pressure liquid chromatography (HPLC) was performed with a Varian model 5000 liquid chromatograph equipped with a variable-wavelength UV detector (195 nm, Varichrom 50, Varian Associates, Palo Alto, CA). A reversed-phase column, Radial PAK C_{18} cartridge (Waters Associates, Milford, MA), 100 mm × 8 mm I.D., particle size 5 μ, in a Waters Z-module was used for analyzing bile acid analogs as their methyl ester derivatives. The column was operated at ambient temperature and the eluting solvent was methanol–water (80:20, v/v) at a flow rate of 2.0 ml/min.

EXPERIMENTAL

3α,12α-Diformyloxy-7-oxo-5β-cholan-24-oic acid (IIb)

Cholic acid (**Fig. 1**, I, 80 g) was recrystallized twice from methanol and treated with N-bromosuccinimide as described by Fieser and Rajagopalan (11). The resulting 3α,12α-dihydroxy-7-oxo-5β-cholan-24-oic acid (IIa, 40 g) was dissolved in 100 ml of 97% formic acid and the solution was heated at 65°C overnight. Evaporation of the solvent gave an oily residue, which was crystallized from aqueous ethanol. Recrystallizations from aqueous ethanol yielded 30 g of colorless needles of 3α,12α-diformyloxy-7-oxo-5β-cholan-24-oic acid (IIb), melting at 208–211°C [reported, 204–208°C (12)]. PMR (δ ppm): 0.68 (3H, s, 18–CH_3), 0.93 (3H, d, J = 6Hz, 21–CH_3), 1.08 (3H, s, 19–CH_3), 4.73 (1H, m, 3β–H), 5.24 (1H, m, 12β–H), 8.03 and 8.18 (2H, s, 2 × –COH).

2-(3α,12α-Diformyloxy-7-oxo-5β-cholan-24-amido)-2-methyl-1-propanol (IIIa)

To a stirred solution of 3α,12α-diformyloxy-7-oxo-5β-cholan-24-oic acid (IIb, 19 g) in 400 ml of ethyl acetate were added 8.0 ml of triethylamine, a solution of 6.0 ml of 2-amino-2-methyl-1-propanol in 50 ml of ethyl acetate, and 15 g of N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline and the mixture was refluxed for 5 hr. After cooling to room temperature, the reaction mixture was washed successively with water (50 ml × 1), 1 N HCl solution (50 ml × 4), water (50 ml × 1), 5% $NaHCO_3$ solution (50 ml × 2), and then water to neutrality (50 ml × 3). The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated in vacuo to give an oily residue (21.5 g) which could not be crystallized. PMR (δ ppm): 0.66 (3H, s, 18–CH_3), 0.85 (3H, d, J = 6Hz, 21–CH_3), 1.08 (3H, s, 19–CH_3), 1.53 (6H, s, –$C(CH_3)_2$–), 3.88 (2H, s, –CH_2OH), 4.71 (1H, m, 3β–H), 5.22 (1H, m, 12β–H), 7.58 (1H, s, –NH–), 8.03 and 8.18 (2H, s, 2 × –COH).

For characterization of the free amide, the diformyl amide (IIIa, 2 g) was hydrolyzed with 5% methanolic KOH (reflux, 1 hr). Dilution with water and extraction with ethyl acetate gave chromatographically pure 2-(3α,12α-dihydroxy-7-oxo-5β-cholan-24-amido)-2-methyl-1-propanol (IIIb, 1.8 g) which was recrystallized from ethyl acetate (colorless prisms), melting at 229–231°C.

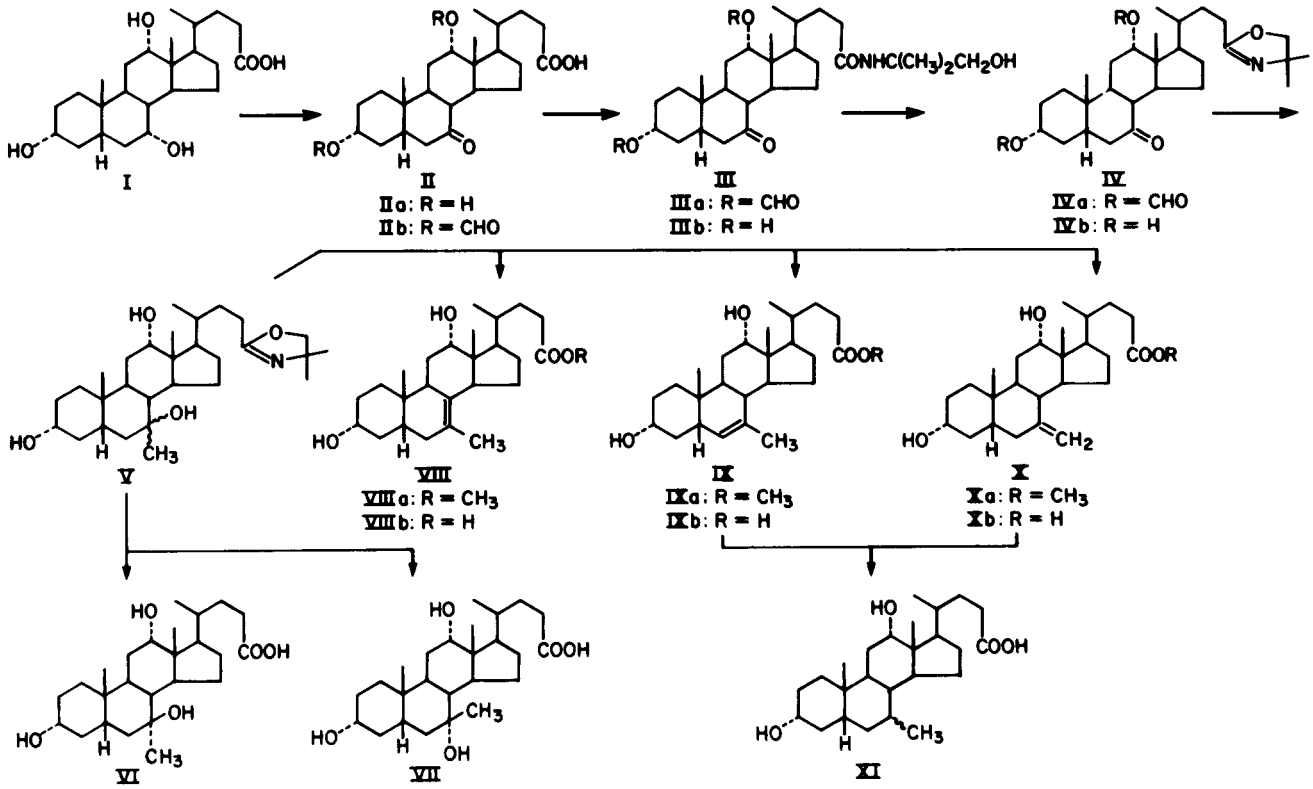

Fig. 1. Synthesis of bile acid analogs. I, cholic acid; II, 3α,12α-dihydroxy-7-oxo-5β-cholan-24-oic acid; III, 2-(3α,12α-dihyroxy-7-oxo-5β-cholan-24-amido)-2-methyl-1-propanol; IV, 2-(3α,12α-dihydroxy-7-oxo-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline; V, 2-(3α,7ξ,12α-trihydroxy-7ξ-methyl-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline; VI, 3α,7β,12α-trihydroxy-7α-methyl-5β-cholan-24-oic acid; VII, 3α,7α,12α-trihydroxy-7β-methyl-5β-cholan-24-oic acid; VIII, 3α,12α-dihydroxy-7-methyl-5β-chol-7-en-24-oic acid; IX, 3α,12α-dihydroxy-7-methyl-5β-chol-6-en-24-oic acid; X, 3α,12α-dihydroxy-7-methylene-5β-cholan-24-oic acid; XI, 3α,12α-dihydroxy-7ξ-methyl-5β-cholan-24-oic acid.

PMR (δ ppm): 0.70 (3H, s, 18-CH_3), 1.09 (3H, d, J = 6Hz, 21-CH_3), 1.16 (3H, s, 19-CH_3), 1.52 (6H, s, -$C(CH_3)_2$-), 3.65 (1H, m, 3β-H), 3.86 (2H, s, -CH_2OH), 4.09 (1H, m, 12β-H), 7.54 (1H, s, -NH-).

2-(3α,12α-Dihydroxy-7-oxo-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (IVb)

2-(3α,12α-Diformyloxy-7-oxo-5β-cholan-24-amido)-2-methyl-1-propanol (IIIa, 11.2 g) was dissolved in 60 ml of tetrahydrofuran. Freshly distilled thionyl chloride (7.0 ml) was added dropwise to the stirred ice-cooled solution and the reaction mixture was stirred further for 1 hr. The solution was added slowly to 300 ml of stirred, ice-cooled diethyl ether and the white precipitate that formed was collected by filtration and quickly dissolved in a mixture of saturated $NaHCO_3$-diethyl ether (1:1, v/v). The ethereal extract was washed with water until neutral, dried over anhydrous Na_2SO_4, and evaporated to dryness. The colorless oily residue was chromatographically pure 2-(3α,12α-diformyloxy-7-oxo-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (IVa, 10.4 g), which could not be crystallized. PMR (δ ppm): 0.67 (3H, s, 18-CH_3), 0.88 (3H, d, J = 6Hz, 21-CH_3), 1.08 (3H, s, 19-CH_3), 1.23 (6H, s, -$C(CH_3)_2$-), 3.80 (2H, s, -OCH_2-), 4.72 (1H, m, 3β-H), 5.23 (1H, m, 12β-H), 8.01 and 8.17 (2H, s, 2 × -COH).

For further purification, the diformyloxyoxazoline (IVa, 9.4 g) was hydrolyzed as described above and the resulting 2-(3α,12α-dihydroxy-7-oxo-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (IVb, 7.9 g) was crystallized from ethyl acetate. Repeated crystallizations from ethyl acetate yielded colorless prisms of pure oxazoline, melting at 174-175°C. PMR (δ ppm): 0.73 (3H, s, 18-CH_3), 1.12 (3H, d, J = 6Hz, 21-CH_3), 1.16 (3H, s, 19-CH_3), 1.23 (6H, s, -$C(CH_3)_2$-), 3.65 (1H, m, 3β-H), 3.80 (2H, s, -OCH_2-), 4.10 (1H, m, 12β-H).

2-(3α,7ξ,12α-Trihydroxy-7ξ-methyl-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (V)

To a solution of 2-(3α,12α-dihydroxy-7-oxo-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (IVb, 5.0 g) dissolved in 200 ml of dry benzene was added dropwise with stirring a 3.0 M ethereal solution of methyl magnesium iodide (20 ml) and benzene (20 ml). The reaction mixture was refluxed for 2 hr and then stirred at room temperature for 2 hr. A saturated NH_4Cl solution (200 ml) was added with vigorous stirring. The benzene layer was separated and

the aqueous layer was then extracted twice with ethyl acetate. The organic extracts were combined, washed successively with water (50 ml × 1), 10% $Na_2S_2O_3$ solution (50 ml × 1), and water to neutrality (50 ml × 3), dried over anhydrous Na_2SO_4, and evaporated to dryness. Repeated crystallizations from acetone and then from ethyl acetate of the residue (5.1 g) gave colorless prisms of 2-(3α,7ξ,12α-trihydroxy-7ξ-methyl-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (V). Melting point, 103–104.5°C. PMR (δ ppm): 0.80 (3H, s, 18-CH_3), 0.94 (3H, s, 19-CH_3), 1.18 (3H, d, J = 6Hz, 21-CH_3), 1.22 (6H, s, -$C(CH_3)_2$-), 1.37 (3H, s, 7ξ-CH_3), 3.66 (1H, m, 3β-H), 3.81 (2H, s, -OCH_2-), 4.10 (1H, m, 12β-H).

3α,7β,12α-Trihydroxy-7α-methyl-5β-cholan-24-oic acid (VI) and 3α,7α,12α-trihydroxy-7β-methyl-5β-cholan-24-oic acid (VII)

2-(3α,7ξ,12α-Trihydroxy-7ξ-methyl-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (V, 2.8 g) was dissolved in 400 ml of 0.1 N HCl solution and warmed at 37°C for 3 days. The clear solution was decanted and the oily precipitate was dissolved in ethyl acetate (400 ml). The ethyl acetate solution was washed to neutrality, dried, and evaporated to dryness. The mixture of free acids (1.8 g), which showed three major spots on silica gel TLC (**Table 1**, solvent system A-1), was treated with diazomethane and the resulting methyl ester derivatives were placed on a column of silica gel (150 g, silica gel 60, 35–70 mesh, Merck, Darmstadt, West Germany) and eluted with increasing concentrations of acetone in benzene. Fifteen percent acetone in benzene (3 l) eluted a mixture of unsaturated dihydroxy compounds (VIIIa, IXa, and Xa, 730 mg, described below). The 7β-hydroxy isomer (methyl ester of VI, 320 mg) and the 7α-hydroxy isomer (methyl ester of VII, 400 mg) were eluted with 25% acetone in benzene (4 l) and 30% acetone in benzene (4 l), respectively. Alkaline hydrolysis of the two isomers with 5% methanolic KOH afforded the corresponding free acids, which were recrystallized from methanol–ethyl acetate: 3α,7β,12α-trihydroxy-7α-methyl-5β-cholan-24-oic acid (VI), colorless prisms from methanol–ethyl acetate, mp 141–142°C. PMR (δ ppm): 0.82 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 1.20 (3H, d, J = 6Hz, 21-CH_3), 1.46 (3H, s, 7α-CH_3), 3.66 (1H, m, 3β-H), 4.14 (1H, m, 12β-H); 3α,7α,12α-trihydroxy-7β-methyl-5β-cholan-24-oic acid (VII), colorless prisms from methanol–ethyl acetate, mp 221–223°C. PMR (δ ppm): 0.81 (3H, s, 18-CH_3), 0.94 (3H, s, 19-CH_3), 1.21 (3H, d, J = 6Hz, 21-CH_3), 1.37 (3H, s, 7β-CH_3), 3.64 (1H, m, 3β-H), 4.09 (1H, m, 12β-H).

TABLE 1. Chromatographic properties of synthetic bile acid analogs

Compound	CA	VII	UCA	VI	DCA	VIII	IX	X	XI
TLC[a] (R_f values)									
A-1	0.37	0.39	0.45	0.49	0.67	0.67	0.69	0.69	0.69
N-1	0.11	0.13	0.24	0.29	0.53	0.58	0.56	0.58	0.55
N-2						0.65	0.50	0.45	0.65
N-3						0.45	0.39	0.30	0.43
GLC (RRT)[b]									
SE-30	1.00	1.21	1.16	1.33	0.91	0.95	1.07	0.97	0.97
HPLC (RRT)[c]									
RP-C_{18}	1.00	1.27	0.41	0.39	1.99	1.99	2.21	1.80	2.26

For experimental details see text. The Roman numerals (VI–XI) refer to the bile acid analogs described in Fig. 1. CA, cholic acid; UCA, 3α,7β-12α-trihydroxy-5β-cholanoic acid; DCA, deoxycholic acid.

[a]Solvent systems: A-1 (free acids), benzene–isopropanol–acetic acid 30:10:1 (v/v/v); N-1 (methyl esters), benzene–acetone 60:40 (v/v); N-2 (methyl esters, silica gel G impregnated with 15% $AgNO_3$), chloroform–acetone 60:40 (v/v); N-3 (methyl esters, $AgNO_3$ plates), benzene–ethyl acetate 30:70 (v/v).

[b]Retention times relative to methyl ester-trimethylsilyl ether derivative of cholic acid (RRT = 1.00, RT = 5.12 min).

[c]Retention times relative to methyl cholate (RRT = 1.00, RT = 3.79 min).

3α,12α-Dihydroxy-7-methyl-5β-chol-7-en-24-oic acid (VIIIb), 3α,12α-dihydroxy-7-methyl-5β-chol-6-en-24-oic acid (IXb), and 3α,12α-dihydroxy-7-methylene-5β-cholan-24-oic acid (Xb)

2-(3α,7ξ,12α-Trihydroxy-7ξ-methyl-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (V, 2.5 g) was dissolved in a solution of concentrated HCl (2 ml) and methanol (200 ml). The solution was refluxed for 6 hr, diluted with water, and extracted with ethyl acetate. The extract was washed with water, dried, and evaporated to dryness to give methyl ester derivatives of three dehydration products (1.9 g, VIIIa, IXa, and Xa). The residue was combined with the unsaturated dihydroxy fraction described above (total 2.6 g) and placed on a column of Merck silica gel (150 g) impregnated with 15 g of $AgNO_3$. Elution with increasing proportions of acetone in chloroform yielded two major fractions. *Fraction 1*, eluted with 7% acetone in chloroform (3 l), gave 480 mg of an oily residue. Alkaline hydrolysis of the residue and recrystallizations from ethyl acetate yielded colorless prisms of 3α,12α-dihydroxy-7-methyl-5β-chol-7-en-24-oic acid (VIIIb). Melting point, 160–163°C. PMR (δ ppm): 0.79 (3H, s, 18-CH_3), 0.86 (3H, s, 19-CH_3), 1.16 (3H, d, J = 6Hz, 21-CH_3), 1.73 (3H, s, 7-CH_3), 3.70 (1H, m, 3β-H), 4.13 (1H, m, 12β-H). This compound could not be hydrogenated under the conditions described below. *Fraction 2*, eluted with 10% acetone in chloroform (4 l), yielded approximately a 1:1 mixture of two unsaturated compounds (1.52 g) on $AgNO_3$-TLC, GLC and GLC–MS (see Table 1 and **Table 2**). These two compounds were separated on a column of silica gel (150 g) impregnated with 15 g $AgNO_3$ using increasing concentrations of ethyl acetate in benzene. Elution with 3 l of 35% ethyl acetate in benzene (*Fraction 2A*), followed by alkaline hydrolysis, afforded 640 mg of 3α,12α-dihydroxy-7-methyl-5β-chol-6-en-24-oic

TABLE 2. Mass spectral data of synthetic bile acid analogs

Ion	m/z	VI	VII	m/z	VIII	IX	X	m/z	XI
M^+	652	5.4	4.4	562	0	0	0	564	0
M − 15	637	3.8	6.7	547	2.4	1.9	4.1	549	18.8
M − 90	562	2.1	2.2	472	37.3	23.1	21.0	474	22.7
M − (90 + 15)	547	2.1	1.3	457	4.7	1.3	1.7	459	1.9
M − (90 + 31)	531	0.7	0.7	441	1.7	1.0	1.5	443	5.6
Fragment C[a]	492	6.4	6.6						
M − (2 × 90)	472	17.7	16.2	382	14.3	22.6	44.3	384	87.4
M − (2 × 90 + 15)	457	1.4	1.5	367	9.7	15.2	14.2	369	7.6
M − (side chain + 90)	447	1.0	0.2	357	41.3	4.9	12.1	359	41.9
M − (2 × 90 + 31)	441	1.5	0.9						
M − (3 × 90)	382	6.9	8.6						
M − (3 × 90 + 15)	367	2.2	3.4						
M − (side chain + 2 × 90)	357	10.5	6.2	267	100.0	100.0	100.0	269	100.0
M − (side chain + 2 × 90 + 15)	342	3.5	3.7						
M − (side chain + 3 × 90)	267	17.1	9.7						
Fragment A[b]	257	100.0	100.0						
Fragment B[c]	143	82.6	80.6						

All the bile acid analogs were analyzed as their methyl ester trimethylsilyl ether derivatives. The Roman numerals (VI–XI) refer to the bile acid analogs described in Fig. 1.

[a]Fragment C (m/z, 492) = M − (C4–C7 + OTMS + 7-CH_3).

[b]Fragment A (m/z, 257) = C3–C7 + 2 × OTMS + 7-CH_3.

[c]Fragment B (m/z, 143) = C5–C7 + OTMS + 7-CH_3.

acid (IXb). Recrystallization from ethyl acetate gave colorless needles melting at 144–148°C. PMR (δ ppm): 0.79 (3H, s, 18-CH_3), 0.89 (3H, s, 19-CH_3), 1.22 (3H, d, J = 6Hz, 21-CH_3), 1.69 (3H, s, 7-CH_3), 3.70 (1H, m, 3β-H), 4.13 (1H, m, 12β-H), 5.36 (1H, m, 6-H). *Fraction 2B*, eluted with 40% ethyl acetate in benzene (3 l), gave a second compound (450 mg) which, after alkaline hydrolysis, was recrystallized from ethyl acetate to give colorless needles of 3α,12α-dihydroxy-7-methylene-5β-cholan-24-oic acid (Xb), mp 199–202°C. PMR (δ ppm): 0.79 (3H, s, 18-CH_3), 1.05 (3H, s, 19-CH_3), 1.20 (3H, d, J = 6Hz, 21-CH_3), 3.75 (1H, m, 3β-H), 4.16 (1H, m, 12β-H), 4.61 and 4.74 (2H, m, C=CH_2).

3α,12α-Dihydroxy-7ξ-methyl-5β-cholan-24-oic acid (XI)

Compounds IXb and Xb (320 mg) were dissolved in 50 ml of methanol and hydrogenated at 40 psi with 50 mg of PtO_2. The reaction mixture was filtered and the solvent was evaporated under reduced pressure. Crystallization from ethyl acetate yielded colorless needles of 3α,12α-dihydroxy-7ξ-methyl-5β-cholan-24-oic acid (XI, 210 mg). Melting point, 177–179°C. PMR (δ ppm): 0.74 (3H, s, 18-CH_3), 0.95 (3H, s, 19-CH_3), 1.03 (3H, d, J = 6Hz, 7ξ-CH_3), 1.17 (3H, d, J = 6Hz, 21-CH_3), 3.63 (1H, m, 3β-H), 4.11 (1H, m, 12β-H).

RESULTS AND DISCUSSION

This report describes the synthesis of the following new bile acids: 3α,7α,12α-trihydroxy-7β-methyl-5β-cholan-24-oic acid (7-Me-CA, VII), 3α,7β,12α-trihydroxy-7α-methyl-5β-cholan-24-oic acid (7-Me-UCA, VI), and 3α,12α-dihydroxy-7ξ-methyl-5β-cholan-24-oic acid (7-Me-DCA, XI) (Fig. 1). These compounds are 7-methyl analogs of the known bile acids, CA, UCA, and DCA, respectively. Some of the dehydration products obtained, for example, 7-methylene deoxycholic acid, might also be of interest. The 7-methyl bile acids should be more resistant to bacterial 7-dehydroxylation or possibly, in case of the 7-methylene bile acid, should inhibit the 7-dehydroxylation of the primary bile acids. It will be of interest to use the new compounds in studies designed to produce more effective and safer cholelitholytic agents.

In order to perform a Grignard reaction with methyl magnesium iodide, the carboxyl group of 3α,12α-dihydroxy-7-oxo-5β-cholan-24-oic acid (IIa) must be protected as the oxazoline derivative (13). In our previous study (13, 14) dealing with the conjugation of diformyloxycholanoic acids and 2-amino-2-methyl-1-propanol, silica gel column chromatography using increasing concentrations of acetone in chloroform was required to obtain a formyloxy amide derivative in pure form. In the present experiment, we used a modified method that was reported by Tserng, Hachey, and Klein (15) to synthesize taurine and glycine conjugated bile acids. The reaction was almost quantitative and we obtained a chromatographically pure formyloxyamide derivative (IIIa) without column chromatography. Unfortunately, the formyloxy derivatives, i.e., 2-(3α,12α-diformyloxy-7-oxo-5β-cholan-24-amido)-2-methyl-1-propanol (IIIa) and 2-(3α,12α-diformyloxy-7-oxo-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (IVa), were not crystalline. However, after alkaline hydrolysis to remove the formyl groups, we could easily purify the intermediate, 2-(3α,12α-dihydroxy-7-oxo-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (IVb), by recrystallizations from ethyl

acetate. The Grignard reaction of IVb with methyl magnesium iodide resulted in a quantitative yield of 2-(3α,7ξ,12α-trihydroxy-7ξ-methyl-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (V), which was also easily purified by recrystallizations.

Mild acid hydrolysis of the key intermediate (V) gave a mixture of unsaturated compounds (VIIIb, IXb, and Xb), 7-Me-UCA (VI), and 7-Me-CA (VII) in the ratio of 3:2:2. Much milder hydrolysis conditions designed to reduce the formation of unsaturated compounds required longer reaction times and resulted in increased amounts of unhydrolyzed oxazoline derivatives. It is possible that the presence of the 12α-hydroxyl group in V makes the 7ξ-hydroxyl group more susceptible to dehydration than in the case of 7-Me-CDA and 7-Me-UDA (4).

7-Me-UCA and 7-Me-CA were purified as their methyl ester derivatives by silica gel chromatography. Their configurations were determined by PMR and they were further characterized by TLC, GLC, HPLC (Table 1), and GLC–MS (Table 2). Each PMR spectrum showed 7-methyl protons as a singlet and 3β- and 12β-protons. The chemical shift of the 7α-methyl protons of 7-Me-UCA (1.46 ppm) shifted to a lower magnetic field than that of the 7β-methyl protons of 7-Me-CA (1.37 ppm) just as previously observed with the dihydroxy acids [7α-methyl protons of 7-Me-UDA (1.40 ppm) and 7β-methyl protons of 7-Me-CDA (1.32 ppm)] (4).

On TLC, 7-Me-UCA and 7-Me-CA were slightly less polar than UCA and CA, respectively. Upon GLC, the C_{25} 7-methyl derivatives had longer retention times on SE-30 columns than the C_{24} bile acids. The structures suggested by theoretical calculations from the RRTs of the known compounds (4, 16) were consistent with the conclusions based upon the PMR and the TLC data, above. As reported before (4), the 7β-hydroxyl group imparts greater hydrophilicity to the steroidal molecule than a 7α-hydroxyl group. It was also clearly shown by HPLC, using a reversed phase column, that the elution volume of the 7β-hydroxyl analog is smaller than that of the 7α-hydroxyl compound. It is worth mentioning that 7-Me-UCA had a lower elution volume than UCA, whereas all other 7-methyl analogs (7-Me-CA, 7-Me-DCA, 7-Me-CDA, 7-Me-UDA, and 7-Me-LCA) were less hydrophilic than the original bile acids.

The mass spectral data showed a base peak at m/z 257 for both 7-methyl analogs. This ion was also observed in the case of the methyl ester-trimethylsilyl ether derivatives of 7-Me-CDA and 7-Me-UDA and reveals that they have hydroxyl substituents at C-3 and C-7 and a methyl group at C-7 (4). The prominent ion at m/z 143 probably originates from scissions between C-4 and C-5 and between C-7 and C-8. High intensities of this fragment were also detected with the methyl ester-trimethylsilyl ether derivatives of 7-Me-CDA and 7-Me-UDA [although this was not reported in the previous paper (4)]. All other fragment ions were consistent with the structures of trihydroxycholanoic acids possessing a methyl group in the nucleus.

As expected, a vigorous acidic hydrolysis of the key intermediate (V) resulted in almost complete conversion to a mixture of dehydrated compounds. These were purified by $AgNO_3$–silica gel chromatography using two columns with two different solvent systems. Elution with increasing proportions of acetone in chloroform clearly separated methyl 3α,12α-dihydroxy-7-methyl-5β-chol-7-en-24-oate (fraction 1, VIIIa) from the other two isomers (fraction 2, IXa and Xa). After alkaline hydrolysis and recrystallizations, the PMR data of VIIIb showed a methyl group signal at 1.83 ppm as singlet and no olefinic proton, indicating the presence of a double bond between C-7 and C-8. This compound could not be hydrogenated under the condition which resulted in the catalytic reduction of IXb and Xb. Next, fraction 2 was subjected to $AgNO_3$–silica gel chromatography with increasing concentrations of ethyl acetate in benzene which separated the other two isomers. After alkaline hydrolysis, PMR of the more rapidly eluted compound (fraction 2A) showed a methyl signal at 1.69 ppm as singlet and an olefinic proton at 5.36 ppm. This suggests that compound IXb has a double bond between C-6 and C-7. The PMR spectrum of Xb (the more slowly eluted compound, fraction 2B) showed two proton signals at 4.61 and 4.74 ppm, indicating the structure of a 7-methylene derivative of DCA. The mass spectral data of the three compounds were consistent with structures of dihydroxycholanoic acids with a nuclear methyl group and a double bond in the nucleus (Table 2).

IXb and Xb were completely hydrogenated with PtO_2 as catalyst to give 7-Me-DCA (XI). Utilizing the first solvent system (chloroform–acetone) and $AgNO_3$–silica gel chromatography followed by catalytic hydrogenation of the more slowly eluted compounds (fraction 2), we could synthesize 7-Me-DCA in good yield. As the catalytic hydrogenation is not stereospecific, the 7-Me-DCA formed must be a mixture of 7α- and 7β-methyl isomers. However, they could not be resolved by either GLC (SE-30, HiEff-8BP, and SP-2250), TLC with four different systems, or HPLC. The isomeric mixture may nevertheless be useful in investigations of the metabolism of 7-Me-CA and 7-Me-UCA, as reference compounds, and of 7-Me-DCA itself.

In summary, we synthesized and characterized 7-methyl derivatives of CA, UCA, and DCA and certain unsaturated derivatives of these bile acid analogs. Some of these compounds are potentially useful as cholelitholytic agents and further studies with these compounds are now in progress.

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