Synthesis of new bile salt analogues, sodium 3α,7α-dihydroxy-5β-cholane-24-sulfonate and sodium 3α,7β-dihydroxy-5β-cholane-24-sulfonate

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Abstract This report describes the chemical synthesis of two new bile salt analogues, namely sodium 3α,7α-dihydroxy-5β-cholane-24-sulfonate and sodium 3α,7β-dihydroxy-5β-cholane-24-sulfonate from chenodeoxycholic acid and ursodeoxycholic acid, respectively. Each common bile acid was converted into the corresponding 5β-cholane-3,7,24-triol by treatment with ethyl chloroformate in the presence of triethylamine followed by sodium borohydride reduction. Reaction of the cholanetriol with *p*-toluenesulfonyl chloride at 4°C afforded the partially tosylated product, 24-*p*-toluenesulfoxy-5β-cholane-3,7-diol, which was then treated with sodium iodide to produce 24-iodo-5β-cholane-3,7-diol. The 24-iodide was refluxed with sodium sulfite in aqueous ethanol to give the desired sulfonate analogue of the naturally occurring bile acid, chenodeoxycholic acid or ursodeoxycholic acid. —**Kihira, K., M. Yoshii, A. Okamoto, S. Ikawa, H. Ishii, and T. Hoshita.** Synthesis of new bile salt analogues, sodium 3α,7α-dihydroxy-5β-cholane-24-sulfonate and sodium 3α,7β-dihydroxy-5β-cholane-24-sulfonate. *J. Lipid Res.* 1990. **31:** 1323–1326.

Supplementary key words bile acids • cholesterol gallstones • 7-dehydroxylation • sulfonate analogue

Chenodeoxycholic acid (CDC) and ursodeoxycholic acid (UDC) are currently in use as therapeutic agents for dissolution of cholesterol gallstones (1, 2). These compounds are absorbed and transformed by the liver into glycine and taurine conjugates and undergo enterohepatic circulation. However, during enterohepatic cycling the conjugates are hydrolyzed and metabolized by intestinal microorganisms to lithocholic acid (3). It is known that lithocholic acid is a potentially hepatotoxic compound (4) that also acts as a promoter of colon cancer (5).

In our attempts to develop new cholesterol gallstone-dissolving agents with greater efficacy and reduced hepatotoxicity, we synthesized the sulfonic acid analogues of CDC and UDC, sodium 3α,7α-dihydroxy-5β-cholane-24-sulfonate (CDC-SUL) and sodium 3α,7β-dihydroxy-5β-cholane-24-sulfonate (UDC-SUL). The new sulfonic acid analogues may act potentially as nonhydrolyzable taurine derivatives. If they are efficiently transported by the ileum they may participate in the enterohepatic circulation, but since they resemble taurine conjugates they may not undergo 7-dehydroxylation.

MATERIALS AND METHODS

Chenodeoxycholic and ursodeoxycholic acids used were commercial products.

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected.

Infrared (IR) spectra were obtained on a JASCO IRA-1 spectrometer as KBr disks. Absorption frequencies are described in reciprocal centimeters.

Proton nuclear magnetic (PMR) spectra were measured at 400 MHz on a JEOL JMN-FX-400 spectrometer in CD_3OD solution using tetramethylsilane as an internal standard. The chemical shifts are given in δ (ppm) from the internal standard.

Fast atom bombardment–high resolution mass spectra (FAB-HR-MS) were obtained on a JEOL D-300 mass spectrometer.

Thin-layer chromatography (TLC) was carried out using precoated silica gel G plates (0.25 mm thickness, Merck) and the spots were visualized by spraying with 10% solu-

Abbreviations: CDC, chenodeoxycholic acid; UDC, ursodeoxycholic acid; CDC-SUL, sodium 3α,7α-dihydroxy-5β-cholane-24-sulfonate; UDC-SUL, sodium 3α,7β-dihydroxy-5β-cholane-24-sulfonate; Ts, *p*-toluenesulfonyl; IR, infrared; PMR, proton nuclear magnetic resonance; FAB-HR-MS, fast atom bombardment–high resolution mass spectrometry; TLC, thin-layer chromatography; PHP-LH-20, piperidinohydroxypropyl Sephadex LH-20; TCDC, taurochenodeoxycholic acid; TUDC, tauroursodeoxycholic acid; GCDC, glycochenodeoxycholic acid; GUDC, glycoursodeoxycholic acid.

tion of phosphomolybdic acid in ethanol and heating at 110°C for 5 min.

Ion exchange column chromatography on piperidinohydroxypropyl Sephadex LH-20 (PHP-LH-20) was performed as described previously (6).

'The usual work-up' refers to dilution with water, extraction with an organic solvent, washing to neutrality, drying over anhydrous Na_2SO_4, filtration, and evaporation of the organic solvent under reduced pressure to dryness.

EXPERIMENTAL

Chemical synthesis

5β-Cholane-3α,7α,24-triol (IIa) **Fig. 1.** Chenodeoxycholic acid (Ia, 5 g) was dissolved in 100 ml of tetrahydrofuran. To this solution 3 ml of triethylamine was added, followed by the dropwise addition of 2 ml of ethyl chloroformate. After stirring for 2 h at room temperature, 6 g of $NaBH_4$ dissolved in 35 ml of water was added to the reaction mixture. The reaction mixture was stirred for 12 h at room temperature and treated with 1 N HCl (100 ml). The usual work-up with ethyl acetate gave an oily residue. The residue was crystallized from ethyl acetate to give 3.9 g of 5β-cholane-3α,7α,24-triol (IIa) as colorless needles. Mp: 99–100°C; PMR: 0.69 (3H, s, 18-C$\underline{H}_3$), 0.93 (3H, s, 19-C$\underline{H}_3$), 0.97 (3H, d, J = 6.6 Hz, 21-C$\underline{H}_3$), 3.38 (1H, m, 3β-$\underline{H}$), 3.51 (2H, m, 24-C$\underline{H}_2$-OH), 3.79 (1H, m, 7β-$\underline{H}$).

24-p-Toluenesulfoxy-5β-cholane-3α,7α-diol (IIIa). 5β-Cholane-3α,7α,24-triol (IIa, 0.5 g) was dissolved in anhydrous tetrahydrofuran (20 ml). To this solution were added 3 ml of triethylamine and then 0.6 g of *p*-toluenesulfonyl chloride. The reaction mixture was allowed to stand at 4°C for 1 week. The usual work-up resulted in a residue which was chromatographed on a silica gel (50 g) column and eluted with increasing concentrations of ethyl acetate in benzene. Monitoring by TLC allowed combination of appropriate fractions. Evaporation of the solvent gave an oily residue of chromatographically pure 24-*p*-toluenesulfoxy-5β-cholane-3α,7α-diol (IIIa, 309 mg), which could not be crystallized. PMR: 0.64 (3H, s, 18-C$\underline{H}_3$), 0.92 (3H, s, 19-C$\underline{H}_3$), 0.88 (3H, d, J = 6.6 Hz, 21-C$\underline{H}_3$), 2.45 (3H, s, C$\underline{H}_3$-phenyl), 3.37 (1H, m, 3β-$\underline{H}$), 3.79 (1H, m, 7β-$\underline{H}$), 4.01 (2H, m, 24-C$\underline{H}_2$-O-Ts), 7.44 and 7.77 (4H, phenyl protons).

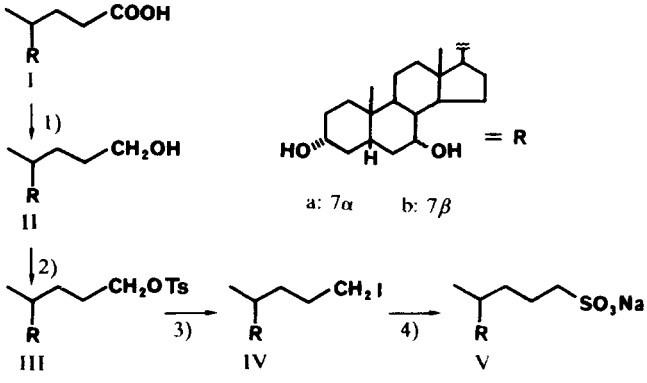

Fig. 1. Synthesis of sodium 3α,7α-dihydroxy-5β-cholane-24-sulfonate and sodium 3α,7β-dihydroxy-5β-cholane-24-sulfonate. Ia: chenodeoxycholic acid; Ib: ursodeoxycholic acid; IIa: 5β-cholane-3α,7α,24-triol; IIb: 5β-cholane-3α,7β,24-triol; IIIa: 24-*p*-toluenesulfoxy-5β-cholane-3α,7α-diol; IIIb: 24-*p*-toluenesulfoxy-5β-cholane-3α,7β-diol; IVa: 24-iodo-5β-cholane-3α,7α-diol; IVb: 24-iodo-5β-cholane-3α,7β-diol; Va: sodium 3α,7α-dihydroxy-5β-cholane-24-sulfonate; Vb: sodium 3α,7β-dihydroxy-5β-cholane-24-sulfonate; 1) ethyl chloroformate, $NaBH_4$; 2) *p*-toluenesulfonyl chloride; 3) NaI; 4) Na_2SO_3.

24-Iodo-5β-cholane-3α,7α-diol (IVa). 24-*p*-Toluenesulfoxy-5β-cholane-3α,7α-diol (IIIa, 300 mg) was added to a solution of acetone containing 1 g of sodium iodide. The reaction mixture was kept at 40°C for 2 h. The usual work-up with ether gave a residue which was crystallized repeatedly from methanol to give colorless crystals of 24-iodo-5β-cholane-3α,7α-diol (IVa, 255 mg). Mp: 85–87°C; PMR: 0.70 (3H, s, 18-C$\underline{H}_3$), 0.92 (3H, s, 19-C$\underline{H}_3$), 0.96 (3H, d, J = 6.6 Hz, 21-C$\underline{H}_3$), 3.20 (2H, m, 24-C$\underline{H}_2$-I), 3.37 (1H, m, 3β-$\underline{H}$), 3.79 (1H, m, 7β-$\underline{H}$).

Sodium 3α,7α-dihydroxy-5β-cholane-24-sulfonate (Va, CDC-SUL). A solution of 24-iodo-5β-cholane-3α,7α-diol (IVa, 250 mg) dissolved in 10 ml of ethanol was added to 20 ml of a 20% aqueous Na_2SO_3 solution. After refluxing for 12 h, ethanol was evaporated from the reaction mixture. The resulting aqueous solution was applied to a column packed with 100 ml of DIAION (similar to Sep-Pak C-18) (Mitsubishi Kasei Co., Japan). After washing with 300 ml of water to remove inorganic ions, the column was eluted with 100 ml of methanol. The solvent was evaporated and the residue was crystallized from ethyl acetate to give crystals of CDC-SUL (Va, 205 mg). Mp: >300°C; IR: 1050, 1190 ($-SO_3^-$), 3400 (hydroxyl); PMR: 0.70 (3H, s, 18-C$\underline{H}_3$), 0.93 (3H, s, 19-C$\underline{H}_3$), 0.99 (3H, d, J = 6.6 Hz, 21-C$\underline{H}_3$), 2.74 (2H, m, 24-C$\underline{H}_2$-SO_3Na), 3.36 (1H, m, 3β-$\underline{H}$), 3.79 (1H, m, 7β-$\underline{H}$); FAB-HR-MS: calcd. for $C_{24}H_{41}O_5SNa + H^+$ = 465.2651; found, 465.2655.

5β-Cholane-3α,7β,24-triol (IIb). Ursodeoxycholic acid (Ib, 5 g) was treated as described above for preparation of 5β-cholane-3α,7α,24-triol (IIa) and afforded 2.4 g of 5β-cholane-3α,7β,24-triol (IIb) as colorless crystals (ethyl acetate). Mp: 163–164°C; IR: 3400 (hydroxyl); PMR: 0.71 (3H, s, 18-C$\underline{H}_3$), 0.96 (3H, s, 19-C$\underline{H}_3$), 0.97 (3H, d, J = 6.6 Hz, 21-C$\underline{H}_3$), 3.50 (4H, m, 3β-$\underline{H}$, 7α-$\underline{H}$, and 24-C$\underline{H}_2$-OH).

24-p-Toluenesulfoxy-5β-cholane-3α,7β-diol (IIIb). 5β-Cholane-3α,7β,24-triol (IIb, 1 g) was treated with *p*-toluenesulfonyl chloride as described above to give an oily residue (717 mg) of 24-*p*-toluenesulfoxy-5β-cholane-3α,7β-diol (IIIb). PMR: 0.66 (3H, s, 18-C$\underline{H}_3$), 0.95 (3H, s, 19-C$\underline{H}_3$), 0.88 (3H, d, J = 6.6 Hz, 21-C$\underline{H}_3$), 2.45 (3H, s, C$\underline{H}_3$-phenyl), 3.48 (2H, m, 3β-$\underline{H}$ and 7α-$\underline{H}$), 4.01

(2H, m, 24-C$\underline{H}_2$-O-Ts), 7.44 and 7.78 (4H, phenyl protons).

24-Iodo-5β-cholane-3α,7β-diol (IVb). 24-*p*-Toluenesulfoxy-5β-cholane-3α,7β-diol (IIIb, 400 mg) was treated as described above to give 370 mg of 24-iodo-5β-cholane-3α,7β-diol (IVb) as a colorless oily residue. PMR: 0.71 (3H, s, 18-C$\underline{H}_3$), 0.96 (3H, s, 19-C$\underline{H}_3$), 0.98 (3H, d, J = 6.6 Hz, 21-C$\underline{H}_3$), 3.20 (2H, m, 3.36, 24-C$\underline{H}_2$-I), 3.48 (2H, m, 3β-$\underline{H}$ and 7α-$\underline{H}$).

Sodium 3α,7β-dihydroxy-5β-cholane-24-sulfonate (Vb, UDC-SUL). 24-Iodo-5β-cholane-3α,7β-diol (IVb, 330 mg) was treated as described above to give 236 mg of UDC-SUL (Vb) as colorless crystals. Mp: >300°C; IR: 1050, 1190 (-SO_3^-), 3400 (hydroxyl); PMR: 0.71 (3H, s, 18-C$\underline{H}_3$), 0.96 (3H, s, 19-C$\underline{H}_3$), 0.98 (3H, d, J = 6.6 Hz, 21-C$\underline{H}_3$), 2.74 (2H, m, 24-C$\underline{H}_2$-SO_3Na), 3.48 (2H, m, 3β-$\underline{H}$ and 7α-$\underline{H}$); FAB-HR-MS: calcd. for $C_{24}H_{41}O_5SNa+H^+$ = 465.2651; found, 465.2628.

PHP-LH-20 ion-exchange column chromatography

Five mg of CDC-SUL or UDC-SUL dissolved in 1 ml of 90% aqueous ethanol was applied on a column packed with 1 ml of PHP-LH-20. The column was eluted with 8-ml portions of 90% aqueous ethanol, 0.1 M acetic acid in 90% aqueous ethanol, 0.2 M formic acid in 90% aqueous ethanol, and finally 1% ammonium carbonate in 70% aqueous ethanol. This eluted neutral compounds, free bile acids, glycine-conjugated bile acids, and taurine-conjugated bile acids (and also sulfated bile acids) in that order. CDC-SUL and UDC-SUL were eluted with 1% ammonium carbonate in 70% aqueous ethanol.

RESULTS AND DISCUSSION

Agents designed to dissolve cholesterol gallstones must be absorbed actively from the ileum and participate efficiently in the enterohepatic circulation. A single negative charge on the side chain is required for the active transport of bile salts through the ileum (7). Because sodium 3α,7α-dihydroxy-5β-cholane-24-sulfonate (CDC-SUL) and sodium 3α,7β-dihydroxy-5β-cholane-24-sulfonate (UDC-SUL) have a single negative charge on the side chain, these compounds should be transported actively and participate in the enterohepatic circulation.

It is known that bacterial 7-dehydroxylation takes place mainly with unconjugated bile acids (8). Kimura et al. (9) demonstrated the suppression of the formation of lithocholic acid from chenodeoxycholic acid (CDC) and ursodeoxycholic acid (UDC) by conjugation with sarcosine (N-methylglycine), which rendered them resistant to hydrolysis by intestinal anaerobes. We hypothesized that the resistance of the conjugated bile acids to bacterial dehydroxylation should be dependent on the presence of highly polar functional groups at the end of the side chain. According to our hypothesis, CDC-SUL and UDC-SUL would also resist the bacterial 7-dehydroxylation because of their high polarity.

It is hypothesized that active transport and resistance to bacterial 7-dehydroxylation of CDC-SUL and UDC-SUL should result in a slower turnover, lower hepatotoxicity, and, hopefully, greater efficacy of cholelitholysis.

The chemical synthesis of CDC-SUL and UDC-SUL was performed as shown in Fig. 1. CDC or UDC were converted to the corresponding 5β-cholane-3,7,24-triols by the treatment with ethyl chloroformate in the presence of triethylamine, followed by sodium borohydride reduction. Although lithium aluminum hydride reduction of bile acids to corresponding alcoholic compounds has been reported (10), the handling in the present method is more convenient than the lithium aluminum hydride reduction. The yields of this step were 78% (IIa) and 48% (IIb). The hydroxyl group at C24 of the cholanetriol was selectively converted to *p*-toluenesulfonyl ester by standing at 4°C with *p*-toluenesulfonyl chloride in the presence of triethylamine in an anhydrous tetrahydrofuran solution. The yields of this step were 62% (IIIa) and 72% (IIIb). The resulting 24-*p*-toluenesulfoxy-5β-cholane-3,7-diol was then treated with sodium iodide to produce 24-iodo-5β-cholane-3,7-diol. The yields of this step were 85% (IVa) and 93% (IVb). The 24-iodide was refluxed with sodium

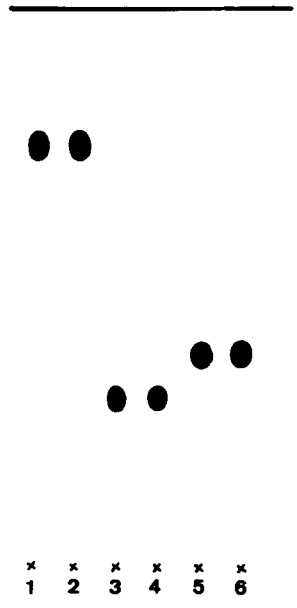

Fig. 2. Thin-layer chromatogram of sodium 3α,7α-dihydroxy-5β-cholane-24-sulfonate and sodium 3α,7β-dihydroxy-5β-cholane-24-sulfonate. 1: glycochenodeoxycholic acid; 2: glycoursodeoxycholic acid; 3: taurochenodeoxycholic acid; 4: tauroursodeoxycholic acid; 5: sodium 3α,7α-dihydroxy-5β-cholane-24-sulfonate; 6: sodium 3α,7β-dihydroxy-5β-cholane-24-sulfonate; solvent system: chloroform–methanol–acetic acid–water 65:20:10:5.

TABLE 1. R_f values of sulfonic acid analogues of bile acids on TLC

Bile Salts	Solvent A	Solvent B
GCDC	0.76	0.62
GUDC	0.76	0.64
TCDC	0.31	0.34
TUDC	0.31	0.36
CDC-SUL	0.38	0.40
UDC-SUL	0.38	0.42

The solvent systems used were A: chloroform–methanol–acetic acid–water 65:20:10:5 and B: n-butanol–acetic acid–water 85:10:5. Abbreviations: GCDC, glycochenodeoxycholic acid; GUDC, glycoursodeoxycholic acid; TCDC, taurochenodeoxycholic acid; TUDC, tauroursodeoxycholic acid; CDC-SUL, sodium 3α,7α-dihydroxy-5β-cholane-24-sulfonate; UDC-SUL, sodium 3α,7β-dihydroxy-5β-cholane-24-sulfonate.

sulfite in aqueous ethanol to give the desired sulfonate analogue, CDC-SUL or UDC-SUL. The yields of this step were 82% (Va) and 72% (Vb). The overall yields were satisfactory for both CDC-SUL and UDC-SUL and were 30% and 23%, respectively. The tosylation step gave a relatively lower yield than the other steps, which were almost quantitative. To obtain better yields, an improvement of the tosylation conditions would be required.

In the IR spectra of CDC-SUL and UDC-SUL, the bands at 1050 and 1190 cm^{-1} indicate that a sulfonic acid moiety is present in the molecule. In the PMR spectra, the signals of the methyl groups of the steroid nucleus were almost in the same positions as those of the corresponding bile acids. The signal of the hydrogens on the C-24 methylene bond observed at 2.74 ppm as multiplet indicate the presence of the sulfonic acid at the terminus of the side chain.

The behaviors on TLC of the sulfonic acid derivatives are shown in **Fig. 2** and **Table 1.** The mobilities of CDC-SUL and UDC-SUL with the two solvent systems used were very close, but slightly less than those of the corresponding taurine-conjugated bile acids. On ion exchange column chromatography the behavior of the sulfonic acid analogues was the same as those of taurine-conjugated bile acids and sulfated bile acids. Although the distance of sulfonic acid moiety from steroid nucleus is not equal to the corresponding taurine-conjugated bile acids, the polarities of the synthetic CDC-SUL and UDC-SUL were shown to be similar to those of the taurine conjugates of their parent bile acids on TLC and ion exchange chromatography.

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