Synthesis of sulfonate analogs of bile acids

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Sulfonate analogs of C_{23} and C_{24} bile acids were synthesized from norcholic, norchenodeoxycholic, norursodeoxycholic, nordeoxycholic, norhyodeoxycholic, cholic, deoxycholic, hyodeoxycholic, and lithocholic acids. The principal reactions used were (1) reduction of the bile acids with $NaBH_4$ to the corresponding bile alcohols, (2) selective tosylation of the terminal hydroxyl group, (3) iodination of the tosyl esters with NaI, and (4) treatment of the iodides with Na_2SO_3 to form the sulfonate analogs of the bile acids. The sulfonate analogs showed polarity similar to that of taurine-conjugated bile acids on thin-layer chromatography. The carbon 13 nuclear magnetic resonance spectral data for the sulfonate analogs were tabulated. (*Steroids* **57**:193–198, 1992)

Keywords: sulfonate analogs; bile acids; nor-bile acids; cholesterol gallstones; sterols

Introduction

Chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA) are currently used as therapeutic agents for the dissolution of cholesterol gallstones.[1,2] These compounds are absorbed, transformed by the liver into glycine and taurine conjugates, and undergo enterohepatic circulation along with the endogenous bile acids. However, during enterohepatic circulation the conjugates are hydrolyzed to form unconjugated CDCA and UDCA; the latter are then 7-dehydroxylated by intestinal microorganisms to form lithocholic acid.[3] This monohydroxy bile acid is a potential hepatotoxin[4] and also acts as a promoter of colon cancer.[5] It is known that bacterial 7-dehydroxylation takes place mainly with unconjugated bile acids.[6] We hypothesized that the presence of the highly polar sulfonic acid group at the end of the bile acid side chain would render these analogs resistant to bacterial dehydroxylation. In a previous paper we reported the synthesis of the sulfonate analogs of CDCA and UDCA.[7] These compounds correspond to C_{25} homologs of CDCA and UDCA with respect to the position of the negative charge. Such analogs are unhydrolyzable and would be expected to act as gallstone-dissolving agents with greater efficacy and reduced hepatotoxicity, according to the hypothesis. In hamsters the sulfonate analog of UDCA showed complete resistance to bacterial 7-dehydroxylation.[8] This finding suggests the potential usefulness of the sulfonate analogs of certain bile acids for the medical therapy of cholesterol gallstones.

We have now synthesized sulfonate analogs from norcholic acid (NCA), norchenodeoxycholic acid (NCDCA), norursodeoxycholic acid (NUDCA), nordeoxycholic acid (NDCA), and norhyodeoxycholic acid (NHDCA), which possess a negative charge at the same position as the common C_{24} bile acids. In order to investigate the properties of the sulfonate analogs in more detail, four sulfonates of the C_{24} bile acids, cholic acid (CA), deoxycholic acid (DCA), hyodeoxycholic acid (HDCA), and lithocholic acid (LCA), were also synthesized to supplement the data obtained with the CDCA and UDCA sulfonates.[7]

Experimental

General

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. The IR spectra were obtained on a JASCO IRA-1 spectrometer (KBr discs). Proton nuclear magnetic resonance (^{1}H NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra were measured on a JEOL JMN-FX-400 spectrometer (400 MHz) using CD_3OD as a solvent and tetramethylsilane as internal standard. ^{13}C-NMR spectra were measured on both proton noise decoupling and distortionless enhancement by polarization transfer modes. Negative ion, high-resolution mass

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spectra in fast atom bombardment mode (FABMS) were obtained on a JEOL D-300 mass spectrometer and polyethyleneglycol 600 was used as standard (resolution 5,000). Thin-layer chromatography (TLC) was performed using precoated silica gel G plates (0.25 mm thickness; Merck & Co., USA) and the spots were visualized by spraying with a 10% solution of phosphomolybdic acid in ethanol and heating at 110 C for 5 minutes.

Bile acids

CA, CDCA, DCA, UDCA, HDCA, LCA, cholyl taurine, and chenodeoxycholyl taurine were commercial products. NCA, NCDCA, NDCA, NUDCA, and NHDCA were prepared according to the method reported previously.[9]

Reduction of bile acids to bile alcohols

24-Nor-5β-cholane-3α,7α,12α,23-tetrol (2a). A typical experimental procedure was as follows. NCA (**1a,** 1.5 g) was dissolved in THF (40 ml). To this solution triethylamine (2.0 ml) was added, followed by ethyl chloroformate (dropwise 1.2 ml). After stirring for 2 hours, a solution containing 1.6 g of $NaBH_4$ in 16 ml of water was added, and the reaction mixture was stirred for 3 hours. The mixture was diluted with water (300 ml), acidified with 1 N HCl, and extracted with ethyl acetate (300 ml). After washing with water and drying over anhydrous Na_2SO_4, the solvent was evaporated to dryness. The resulting residue was crystallized from ethyl acetate to yield **2a** (662 mg, 46%). mp 215–220 C. ^{1}H NMR (CD_3OD)δ: 0.72 (3H, s, H-18), 0.92 (3H, s, H-19), 1.02 (3H, d, J = 6.6 Hz, H-21), 3.36 (1H, m, H-3β), 3.55 and 3.63 (each 1H, m, H-23), 3.79 (1H, m, H-7β), 3.95 (1H, m, H-12β).

24-Nor-5β-cholane-3α,12α,23-triol (2b). The reaction was performed by a procedure similar to that used in the preparation of **1a.** Compound **2b** was obtained from NDCA (**1b,** 6.9 g) as colorless crystals (4.1 g, 62%). mp 210–212 C. ^{1}H NMR (CD_3OD)δ: 0.72 (3H, s, H-18), 0.93 (3H, s, H-19), 1.01 (3H, d, J = 6.6 Hz, H-21), 3.54 (2H, m, H-3β and 23-H), 3.61 (1H, m, H-23), 3.96 (1H, m, H-12β).

24-Nor-5β-cholane-3α,7α,23-triol (2c). The reaction was performed by a procedure similar to that used in the preparation of **1a.** Compound **2c** was obtained from NCDCA (**1c,** 7.0 g) as colorless crystals (4.5 g, 67%). mp 247–249 C. ^{1}H NMR (CD_3OD)δ: 0.70 (3H, s, H-18), 0.93 (3H, s, H-19), 0.96 (3H, d, J = 6.6 Hz, H-21), 3.35 (1H, m, H-3β), 3.54 and 3.61 (each 1H, m, H-23), 3.79 (1H, m, H-7β).

24-Nor-5β-cholane-3α,7β,23-triol (2d). The reaction was performed by a procedure similar to that used in the preparation of **1a.** Compound **2d** was obtained from NUDCA (**1d,** 5.1 g) as colorless crystals (4.0 g 81%). mp 179–181 C. ^{1}H NMR (CD_3OD)δ: 0.72 (3H, s, H-18), 0.96 (3H, s, H-19), 0.96 (3H, d, J = 6.6 Hz, H-21), 3.48 (2H, m, H-3β and 7α), 3.54 and 3.61 (each 1H, m, H-23).

24-Nor-5β-cholane-3α,6α,23-triol (2e). The reaction was performed by a procedure similar to that used in the preparation of **1a.** Compound **2e** was obtained from NHDCA (**1e,** 3.1 g) as colorless crystals (2.4 g, 62%). mp 212–215 C. ^{1}H NMR (CD_3OD)δ: 0.70 (3H, s, H-18), 0.93 (3H, s, H-19), 0.96 (3H, d, J = 6.6 Hz, H-21), 3.51 (2H, m, H-3β and 23), 3.61 (2H, m, H-23), 4.00 (1H, m, H-6β).

5β-Cholane-3α,7α,12α,24-tetrol (2f). The reaction was performed by a procedure similar to that used in the preparation of **1a.** Compound **2f** was obtained from CA (**1f,** 10.0 g) as colorless crystals (8.6 g, 89%). mp 231.5–234 C (231–233 C, reported).[10] ^{1}H NMR (CD_3OD)δ: 0.71 (3H, s, H-18), 0.92 (3H, s, H-19), 1.02 (3H, d, J = 6.6 Hz, H-21), 3.37 (1H, m, H-3β), 3.51 (2H, m, H-24), 3.79 (1H, m, H-7β), 3.96 (1H, m, H-12β).

5β-Cholane-3α,12α,24-triol (2g). The reaction was performed by a procedure similar to that used in the preparation of **1a.** Compound **2g** was obtained from DCA (**1g,** 10.0 g) as colorless crystals (8.0 g, 83%). mp 118–120 C (123 C, reported).[9] ^{1}H NMR (CD_3OD)δ: 0.71 (3H, s, H-18), 0.93 (3H, s, H-19), 1.01 (3H, d, J = 6.4 Hz, H-21), 3.51 (3H, m, H-3β and H-24), 3.96 (1H, m, H-12β).

5β-Cholane-3α,6α,24-triol (2h). The reaction was performed by a procedure similar to that used in the preparation of **1a.** Compound **2h** was obtained from HDCA (**1h,** 10.0 g) as colorless crystals (8.1 g, 85%). mp 150–155 C. ^{1}H NMR (CD_3OD)δ: 0.69 (3H, s, H-18), 0.93 (3H, s, H-19), 0.95 (3H, d, J = 6.6 Hz, H-21), 3.50 (3H, m, H-3β and H-24), 4.00 (1H, m, H-6β-H).

5β-Cholane-3α,24-diol (2i). The reaction was performed by a procedure similar to that used in the preparation of **1a.** Compound **2i** was obtained from LCA (**1i,** 5 g) as colorless crystals (3.2 g, 66%). mp 177–178 C (177–180 C reported).[10] ^{1}H NMR (CD_3OD)δ: 0.69 (3H, s, H-18), 0.94 (3H, s, H-19), 0.96 (3H, d, J = 6.2 Hz, H-21), 3.51 (3H, m, H-3β and H-24).

Preparation of tosyl esters of bile alcohols

23-*p*-Toluenesulfoxy-24-nor-5β-cholane-3α,7α,12α-triol (3a). A typical experimental procedure was as follows. To a THF solution (20 ml) of **2a** (500 mg), triethylamine (3 ml) and *p*-toluenesulfonyl chloride (500 mg, 2.0 eq.) was added subsequently at 0 C. Reaction mixture was kept at 4 C for 72 hours. After dilution with 5 vol of 1 N HCl, reaction product was extracted with ether and purified by silica gel column chromatograpyhy to yield **3a** (213 mg, 30%) as an oily residue. ^{1}H NMR (CD_3OD)δ: 0.66 (3H, s, H-18), 0.91 (3H, s, H-19), 0.94 (3H, d, J = 6.4 Hz, H-21), 2.46 (3H, s, phenyl-Me), 3.36 (1H, m, H-3β), 3.79 (1H, m, H-7β), 3.91 (1H, m, H-12β), 4.00 (2H, m, H-23), 7.44 and 7.78 (each 2H, m, *p*-disubstituted phenyl).

23-*p*-Toluenesulfoxy-24-nor-5β-cholane-3α,12α-diol (3b). In a procedure similar to that used in the typical experiment, **3b** was obtained from **2b** (4.1 g) as an oily residue (2.7 g, 46%). ^{1}H NMR (CD_3OD)δ: 0.65 (3H, s, H-18), 0.92 (3H, s, H-19), 0.93 (3H, d, J = 7.1 Hz, H-21), 2.45 (3H, s, phenyl-Me), 3.51 (1H, m, H-3β), 3.92 (1H, m, H-12β), 4.00 (2H, m, H-23), 7.44 and 7.78 (each 2H, m, *p*-disubstituted phenyl).

23-*p*-Toluenesulfoxy-24-nor-5β-cholane-3α,7α-diol (3c). In a procedure similar to that used in the typical experiment, **3c** was obtained from **2c** (4.5 g) as an oily residue (2.0 g, 31%). ^{1}H NMR (CD_3OD)δ: 0.63 (3H, s, H-18), 0.83 (3H, d, J = 6.6 Hz, H-21), 0.91 (3H, s, H-19), 2.45 (3H, s, phenyl-Me), 3.37 (1H, m, H-3β), 3.78 (1H, m, H-7β), 4.07 (2H, m, H-23), 7.44 and 7.78 (each 2H, m, *p*-disubstituted phenyl).

23-*p*-Toluenesulfoxy-24-nor-5β-cholane-3α,7β-diol (3d). In a procedure similar to that used in the typical experiment, **3d** was obtained from **2d** (4.0 g) as an oily residue (3.0 g, 53%). ^{1}H NMR (CD_3OD)δ: 0.65 (3H, s, H-18), 0.95 (3H, s, H-19), 0.84 (3H, d, J = 6.6 Hz, H-21), 2.46 (3H, s, phenyl-Me), 3.48 (1H, m, H-3β and 7α), 4.07 (2H, m, H-23), 7.45 and 7.78 (each 2H, m, *p*-disubstituted phenyl).

23-*p*-Toluenesulfoxy-24-nor-5β-cholane-3α,6α-diol (3e). In a procedure similar to that used in the typical experiment, **3e** was obtained from **2e** (2.4 g) as an oily residue (2.2 g, 64%). ^{1}H NMR (CD_3OD)δ: 0.63 (3H, s, H-18), 0.92 (3H, s, H-19), 0.83 (3H, d, J = 6.6 Hz, H-21), 2.46 (3H, s, phenyl-Me), 3.51 (1H, m, H-3β), 4.01 (1H, m, H-6β), 4.09 (2H, m, H-23), 7.45 and 7.78 (each 2H, m, *p*-disubstituted phenyl).

24-*p*-Toluenesulfoxy-5β-cholane-3α,7α,12α-triol (3f). In a procedure similar to that used in the typical experiment, **3f** was obtained from **2f** (1.0 g) as an oily residue (0.9 g, 65%). ^{1}H NMR (CD_3OD)δ: 0.66 (3H, s, H-18), 0.91 (3H, s, H-19), 0.94 (3H, d, J = 6.4 Hz, H-21), 2.46 (3H, s, phenyl-Me), 3.36 (1H, m, H-3β), 3.79 (1H, m, H-7β), 3.91 (1H, m, H-12β), 4.00 (2H, m, H-24), 7.44 and 7.78 (each 2H, m, *p*-disubstituted phenyl).

24-*p*-Toluenesulfoxy-5β-cholane-3α,12α-diol (3g). In a procedure similar to that used in the typical experiment, **3g** was obtained from **2g** (5.5 g) as an oily residue (4.0 g, 52%). ^{1}H NMR (CD_3OD)δ: 0.65 (3H, s, H-18), 0.92 (3H, s, H-19), 0.93 (3H, d, J = 7.2 Hz, H-21), 2.45 (3H, s, phenyl-Me), 3.51 (1H, m, H-3β), 3.92 (1H, m, H-12β), 4.00 (2H, m, H-24), 7.44 and 7.77 (each 2H, m, *p*-disubstituted phenyl).

24-*p*-Toluenesulfoxy-5β-cholane-3α,6α-diol (3h). In a procedure similar to that used in the typical experiment, **3h** was obtained from **2h** (8.0 g) as an oily residue (7.4 g, 67%). ^{1}H NMR (CD_3OD)δ: 0.64 (3H, s, H-18), 0.92 (3H, s, H-19), 0.86 (3H, d, J = 6.6 Hz, H-21), 3.51 (1H, m, H-3β), 4.01 (3H, m, H-6β and H-24), 7.44 and 7.78 (each 2H, m, *p*-disubstituted phenyl).

24-*p*-Toluenesulfoxy-5β-cholane-3α-ol (3i). In a procedure similar to that used in the typical experiment, **3i** was obtained from **2i** (3.0 g) as an oily residue (2.1 g, 50%). ^{1}H NMR (CD_3OD)δ: 0.63 (3H, s, H-18), 0.86 (3H, d, J = 6.6 Hz, H-21), 0.93 (3H, s, H-19), 2.45 (3H, s, phenyl-Me), 3.54 (1H, m, H-3β), 4.00 (1H, m, 24-H), 7.43 and 7.77 (each 2H, m, *p*-disubstituted phenyl).

Preparation of iodide

23-Iodo-24-nor-5β-cholane-3α,7α,12α-triol (4a). A typical experimental procedure was as follows. 23-*p*-Toluenesulfoxy-24-nor-5β-cholane-3α,7α,12α-triol (**3a**, 210 mg) was dissolved in acetone (30 ml) containing 1.0 g of NaI and refluxed for 4 hours. The reaction mixture was diluted with water and extracted with ether (200 ml). After washing with water, drying over Na_2SO_4, and evaporating the solvent, the resulting residue was crystallized from MeOH to yield **4a** (144 mg, 75%) as colorless crystals. mp 183–185 C. ^{1}H NMR (CD_3OD)δ: 0.73 (3H, s, H-18), 0.92 (3H, s, H-19), 1.01 (3H, d, J = 6.4 Hz, H-21), 3.16 (1H, m, H-23), 3.36 (2H, m, H-3β and H-23), 3.79 (1H, m, H-7β), 3.95 (1H, m, H-12β).

23-Iodo-24-nor-5β-cholane-3α,12α-diol (4b). In a procedure similar to that used in the typical experiment, **4b** was obtained from **3b** (2.7 g) as colorless crystals (2.0 g, 81%). mp 181–183 C. ^{1}H NMR (CD_3OD)δ: 0.72 (3H, s, H-18), 0.93 (3H, s, H-19), 0.99 (3H, d, J = 6.2 Hz, H-21), 3.15 and 3.36 (each 1H, m, H-23), 3.52 (1H, m, H-3β), 3.96 (1H, m, H-12β).

23-Iodo-24-nor-5β-cholane-3α,7α-diol (4c). In a procedure similar to that used in the typical experiment, **4c** was obtained from **3c** (2.0 g) as colorless crystals (727 mg, 40%). mp 88–89 C. ^{1}H NMR (CD_3OD)δ: 0.71 (3H, s, H-18), 0.93 (3H, s, H-19), 0.95 (3H, d, J = 6.2 Hz, H-21), 3.14 (1H, m, H-23), 3.36 (2H, m, H-3β and H-23), 3.80 (1H, m, H-7β).

23-Iodo-24-nor-5β-cholane-3α,7β-diol (4d). In a procedure similar to that used in the typical experiment, **4d** was obtained from **3d** (3.0 g) as an oily residue (2.5 g, 91%). ^{1}H NMR (CD_3OD)δ: 0.73 (3H, s, H-18), 0.95 (3H, d, J = 6.2 Hz, H-21), 0.96 (3H, s, H-19), 3.16 and 3.36 (each 1H, m, H-23), 3.48 (2H, m, H-3β and H-7α).

23-Iodo-24-nor-5β-cholane-3α,6α-diol (4e). In a procedure similar to that used in the typical experiment, **4e** was obtained from **3e** (2.2 g) as colorless crystals (1.6 g, 80%). mp 185–187 C. ^{1}H NMR (CD_3OD)δ: 0.71 (3H, s, H-18), 0.93 (3H, s, H-19), 0.94 (3H, d, J = 6.4 Hz, H-21), 3.18 and 3.35 (each 1H, m, H-23), 3.51 (1H, m, H-3β), 4.01 (1H, m, H-6β).

24-Iodo-5β-cholane-3α,7α,12α-triol (4f). In a procedure similar to that used in the typical experiment, **4f** was obtained from **3f** (1.8 g) as colorless crystals (1.4 g, 84%). ^{1}H NMR (CD_3OD)δ: 0.71 (3H, s, H-18), 0.92 (3H, s, H-19), 1.00 (3H, d, J = 6.4 Hz, H-21), 3.18 (2H, m, H-24), 3.35 (1H, m, H-3β), 3.80 (1H, m, H-7β), 3.95 (2H, m, H-12β).

24-Iodo-5β-cholane-3α,12α-diol (4g). In a procedure similar to that used in the typical experiment, **4g** was obtained from **3g** (4.0 g) as an oily residue (3.4 g, 92%). ^{1}H NMR (CD_3OD)δ: 0.71 (3H, s, H-18), 0.93 (3H, s, H-19), 1.00 (3H, d, J = 6.6 Hz, H-21), 3.20 (2H, m, H-24), 3.52 (1H, m, H-3β), 3.95 (1H, m, H-12β).

24-Iodo-5β-cholane-3α,6α-diol (4h). In a procedure similar to that used in the typical experiment, **4h** was obtained from **3h** (7.4 g) as an oily residue (7.2 g, quantitative). ^{1}H NMR (CD_3OD)δ: 0.69 (3H, s, H-18), 0.93 (3H, s, H-19), 0.95 (3H, d, J = 6.6 Hz, H-21), 3.20 (2H, m, H-24), 3.51 (1H, m, H-3β), 4.00 (1H, m, H-6β).

24-Iodo-5β-cholan-3α-ol (4i). In a procedure similar to that used in the typical experiment, **4i** was obtained from **3i** (2.1 g) as colorless crystals (1.5 g, 77%). mp 131–134 C. ^{1}H NMR (CD_3OD)δ: 0.68 (3H, s, H-18), 0.94 (3H, s, H-19), 0.94 (3H, d, J = 6.6 Hz, H-21), 3.18 (2H, m, H-24), 3.56 (1H, m, H-3β).

Preparation of sulfonate analogs

Sodium 3α,7α,12α-trihydroxy-24-nor-5β-cholane-23-sulfonate (5a). A typical experimental procedure was as follows. 23-Iodo-24-nor-5β-cholane-3α,7α,12α-triol (**4a**, 144 mg) was dissolved in ethanol (30 ml) and to the solution 25 ml of 5% aqueous Na_2SO_3 was added. The mixture was heated at 100 C for 12 hours and concentrated under reduced pressure to remove ethanol. The resulting solution was applied to a column of MCI-Gel HP-20 (100 ml, Mitsubishi Kasei Co., Japan; polymer of styrene and divinylbenezene, reversed-phase resin). After washing with 400 ml of water to remove inorganic ions, the column was eluted with 70% aqueous MeOH. The methanolic eluant was evaporated to dryness and crystallized from EtOAc/MeOH to yield **5a** (128 mg, 94%) as colorless crystals. mp >300 C. IR: 1,175, 1,045 (−SO_3), 3,400 (−OH). ^{1}H NMR (CD_3OD)δ: 0.72 (3H, s, H-18), 0.92 (3H, s, H-19), 1.03 (3H, d, J = 6.6 Hz, H-21), 2.70 and 2.89 (each 1H, m, H-23), 3.37 (1H, m, H-3β), 3.80 (1H, m, H-7β), 3.95 (1H, m, H-12β). Negative ion high resolution FABMS: calculated for $[C_{24}H_{39}O_6S]^-$: 457.2624. Found: 457.2658.

Sodium 3α,12α-dihydroxy-24-nor-5β-cholane-23-sulfonate (5b). In a procedure similar to that used in the typical experiment, **5b** was obtained from **4b** (2.0 g) as colorless crystals (1.4 g, 74%). mp >300 C. IR: 1,190, 1,045, (−SO_3), 3,400 (−OH). ^{1}H NMR (CD_3OD)δ: 0.72 (3H, s, H-18), 0.93 (3H, s, H-19), 1.02 (3H, d,

J = 6.4 Hz, H-21), 2.69 and 2.88 (each 1H, m, H-23), 3.51 (1H, m, H-3β), 3.95 (1H, m, H-12β). Negative ion high resolution FABMS: calculated for $[C_{23}H_{39}O_5S]^-$: 427.2518. Found: 427.2556.

Sodium 3α,7α-dihydroxy-24-nor-5β-cholane-23-sulfonate (5c). In a procedure similar to that used in the typical experiment, **5b** was obtained from **4c** (727 mg) as colorless crystals (446 mg, 65%). mp >300 C. IR: 1,190, 1,045 (−SO_3), 3,400 (−OH). ^{1}H NMR (CD_3OD)δ: 0.70 (3H, s, H-18), 0.93 (3H, s, H-19), 0.97 (3H, d, J = 6.3 Hz, H-21), 2.68 and 2.87 (each 1H, m, H-23), 3.36 (1H, m, H-3β), 3.79 (1H, m, H-7β). Negative ion high resolution FABMS: calculated for $[C_{23}H_{39}O_5S]^-$: 427.2518. Found: 427.2515.

Sodium 3α,7β-dihydroxy-24-nor-5β-cholane-23-sulfonate (5d). In a procedure similar to that used in the typical experiment, **5d** was obtained from **4d** (2.5 g) as colorless crystals (1.8 g, 76%). mp >300 C. IR: 1,190, 1,045 (−SO_3), 3,400 (−OH). ^{1}H NMR (CD_3OD)δ: 0.72 (3H, s, H-18), 0.96 (3H, s, H-19), 0.97 (3H, d, J = 7.1 Hz, H-21), 2.70 and 2.85 (each 1H, m, H-23), 3.47 (2H, m, H-3β and H-7α). Negative ion high resolution FABMS: calculated for $[C_{23}H_{39}O_5S]^-$: 427.2518. Found: 427.2509.

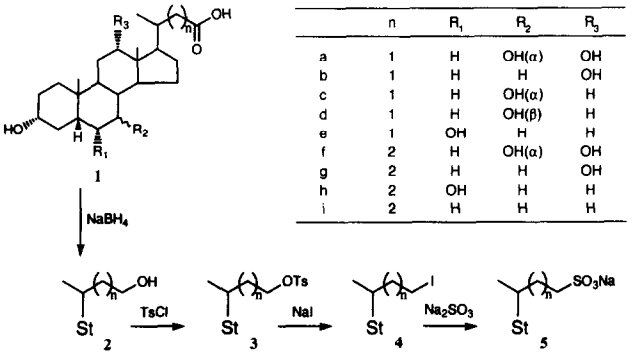
Sodium 3α,6α-dihydroxy-24-nor-5β-cholane-23-sulfonate (5e). In a procedure similar to that used in the typical experiment, **5e** was obtained from **4e** (1.6 g) as colorless crystals (1.3 g, 72%). mp >300 C. IR: 1,180, 1,050 (−SO_3), 3,400 (−OH). ^{1}H NMR (CD_3OD)δ: 0.68 (3H, s, H-18), 0.93 (3H, s, H-19), 0.97 (3H, d, J = 6.2 Hz, H-21), 2.67 and 2.86 (each 1H, m, H-23), 3.50 (1H, m, H-3β), 4.00 (1H, m, H-6β). Negative ion high resolution FABMS: calculated for $[C_{23}H_{39}O_5S]^-$: 427.2518. Found: 427.2548.

Sodium 3α,7α,12α-trihydroxy-5β-cholane-24-sulfonate (5f). In a procedure similar to that used in the typical experiment, **5f** was obtained from **4f** (900 mg) as colorless crystals (350 mg, 41%). mp >300 C. IR: 1,180, 1,045 (−SO_3), 3,400 (−OH). ^{1}H NMR (CD_3OD)δ: 0.72 (3H, s, H-18), 0.92 (3H, s, H-19), 1.04 (3H, d, J = 6.4 Hz, H-21), 2.74 (2H, m, H-24), 3.34 (1H, m, H-3β), 3.78 (1H, m, H-7β), 3.96 (1H, m, H-12β). Negative ion high resolution FABMS: calculated for $[C_{24}H_{41}O_6S]^-$: 443.2467. Found: 443.2498.

Sodium 3α,12α-dihydroxy-5β-cholane-24-sulfonate (5g). In a procedure similar to that used in the typical experiment, **5g** was obtained from **4g** (3.2 g) as colorless crystals (1.7 g, 56%). mp >300 C. IR: 1,180, 1,045 (−SO_3), 3,400 (−OH). ^{1}H NMR (CD_3OD)δ: 0.71 (3H, s, H-18), 0.93 (3H, s, H-19), 1.03 (3H, d, J = 6.6 Hz, H-21), 2.74 (2H, m, H-24), 3.51 (1H, m, H-3β), 3.96 (1H, m, H-12β). Negative ion high resolution FABMS: calculated for $[C_{24}H_{41}O_5S]^-$: 441.2675. Found: 441.2708.

Sodium 3α,6α-dihydroxy-5β-cholane-24-sulfonate (5h). In a procedure similar to that used in the typical experiment, **5h** was obtained from **4h** (1.0 g) as colorless crystals (0.8 g, 84%). mp >300 C. IR: 1,180, 1,050 (−SO_3), 3,400 (−OH). ^{1}H NMR (CD_3OD)δ: 0.69 (3H, s, H-18), 0.93 (3H, s, H-19), 0.97 (3H, d, J = 6.4 Hz, H-21), 2.74 (2H, m, H-24), 3.50 (1H, m, H-3β), 4.00 (1H, m, H-6β). Negative ion high resolution FABMS: calculated for $[C_{24}H_{41}O_5S]^-$: 441.2675. Found: 441.2662.

Sodium 3α-hydroxy-5β-cholane-24-sulfonate (5i). In a procedure similar to that used in the typical experiment, **5i** was obtained from **4i** (246 mg) as colorless crystals (117 mg, 50%). mp >300 C. IR: 1,180, 1,045 (−SO_3), 3,400 (−OH). ^{1}H NMR (CD_3OD)δ: 0.69 (3H, s, H-18), 0.94 (3H, s, H-19), 0.97 (3H, d, J = 6.9 Hz, H-21), 2.74 (2H, m, H-24), 3.53 (1H, m, H-3β). Negative ion high resolution FABMS: calculated for $[C_{24}H_{41}O_4S]^-$: 425.2726. Found: 425.2743.



	n	R_1	R_2	R_3
a	1	H	OH(α)	OH
b	1	H	H	OH
c	1	H	OH(α)	H
d	1	H	OH(β)	H
e	1	OH	H	H
f	2	H	OH(α)	OH
g	2	H	H	OH
h	2	OH	H	H
i	2	H	H	H

Scheme 1

Results and discussion

The synthetic route of the sulfonate analogs **5a–i** from C_{23} and C_{24} bile acids **1a–i** is outlined in Scheme 1. The bile acids **1a–i** were treated with ethyl chloroformate to form acid anhydrides and were then reduced with $NaBH_4$ to yield the corresponding C_{23} and C_{24} bile alcohols **2a–i.** This reduction seems to be superior to the reduction with $LiAlH_4$.[10] Selective tosylation of the bile alcohols **2a–i** was performed in THF using triethylamine as a catalytic base. Tosylation of alcoholic compounds is usually performed in pyridine, but in the present case the use of pyridine as a solvent resulted in substantial amounts of less polar side products, probably ditosylates. Selective tosylation of the terminal hydroxyl group was confirmed by ^{1}H NMR spectroscopy. In the compounds **3a–i** the signals of the terminal methylene protons were shifted downfield by 0.45–0.58 ppm by tosylation. The chemical shifts of the 18- and 21-methyl protons were significantly shifted upfield by

Table 1 R_f value of sulfonate analog

Compound	A	B
TCA	0.23	0.20
TCDC	0.31	0.34
5a	0.23	0.25
5b	0.34	0.39
5c	0.30	0.38
5d	0.34	0.41
5e	0.33	0.39
5f	0.27	0.29
5g	0.39	0.40
5h	0.36	0.39

The solvent systems used were (A) n-butanol/acetic acid/water = 17:2:1 and (B) chloroform/methanol/acetic acid/water = 13:4:2:1.
Abbreviations: TCA, cholyltaurine; TCDC, chenodeoxycholyltaurine.

Table 2 Carbon 13 NMR data for sulfonate analogs of bile acids[a]

Carbon	Sulfonate analog[b] of										
	NCA **5a**	NDC **5b**	NCDC **5c**	NUDC **5d**	NHDC **5e**	CA **5f**	DC **5g**	HDC **5h**	LC **5i**	CDC	UDC
1	36.6	36.5	36.6	36.2	36.8	36.6	36.5	36.8	36.5	36.6	36.2
2	31.2	31.1	31.4	31.1	31.2	31.2	31.1	31.2	31.2	31.4	31.1
3	72.9	72.6	72.9	72.0	72.4	73.0	72.6	72.4	72.5	72.9	72.2
4	40.5	37.2	40.5	38.6	30.0	40.5	37.2	30.1	37.2	40.5	38.7
5	43.3	43.7	43.3	44.6	50.0	43.3	43.7	49.9	43.6	43.3	44.6
6	35.8	28.4	35.9	38.1	68.7	35.9	28.5	68.7	28.4	35.9	38.1
7	69.1	27.5	69.1	72.2	35.6	69.1	27.5	35.6	27.7	69.1	72.0
8	41.1	37.5	40.9	44.1	36.2	41.1	37.5	36.2	37.3	40.9	44.1
9	27.9	34.9	34.1	40.7	41.3	27.9	34.9	41.1	41.9	34.1	40.8
10	35.9	35.3	36.3	35.2	37.0	36.0	35.3	36.2	35.7	36.3	35.2
11	29.6	29.9	21.8	22.4	21.9	29.6	29.9	21.9	22.0	21.8	22.4
12	74.0	74.0	41.1	41.6	41.4	74.1	74.1	41.4	41.6	41.1	41.6
13	47.6	47.6	43.7	44.8	44.0	47.6	47.6	44.0	43.9	43.7	44.8
14	43.0	49.3	51.5	56.5	57.4	43.0	49.3	57.5	58.0	51.6	56.7
15	24.4	24.9	24.6	27.9	25.3	24.3	24.9	25.3	25.3	24.7	28.0
16	28.7	28.6	29.2	29.6	29.2	28.8	28.8	29.3	29.4	29.4	29.8
17	48.0	48.0	57.3	57.5	57.6	48.4	48.4	57.7	57.6	57.5	57.6
18	13.0	13.2	12.2	12.7	12.5	13.0	13.2	12.5	12.5	12.2	12.7
19	23.2	23.7	23.4	24.0	24.1	23.2	23.7	24.1	24.0	23.4	24.0
20	36.5	36.4	36.5	36.4	36.4	37.2	37.1	37.0	37.0	37.1	37.0
21	17.9	17.8	19.1	19.2	19.0	18.0	17.9	19.1	19.1	19.1	19.3
22	32.0	32.0	32.1	32.1	32.0	36.3	36.3	36.2	36.2	36.3	36.3
23	50.0	50.0	49.9	49.9	50.0	23.1	23.0	22.7	22.7	22.8	22.8
24						53.2	53.2	53.2	53.2	53.2	53.3

[a] The spectra were measured as CD_3OD solution.
[b] Sulfonate analogs derived from NCA, norcholic acid; NDC, nordeoxycholic acid; NCDC, norchenodeoxycholic acid; NUDC, norursodeoxycholic acid; NHDC, norhyodeoxycholic acid; CA, cholic acid; DC, deoxycholic acid; HDC, hyodeoxycholic acid; LC, lithocholic acid; CDC, chenodeoxycholic acid; UDC, ursodeoxycholic acid.

0.05–0.07 ppm and 0.08–0.13 ppm, respectively. The tosyl esters **3a–i** were converted almost quantitatively into the corresponding iodides **4a–i** by treatment with NaI. In the C_{23} iodide derivatives, terminal methylene protons were observed at 3.14–3.18 ppm and 3.36–3.61 ppm. In the C_{24} group they were observed at 3.18–3.20 ppm. The iodide derivatives **4a–i** were finally converted into the sulfonate derivatives **5a–i** by treatment with ethanolic Na_2SO_3. These compounds **(5a–i)** were extracted and purified by a column chromatography using MCI-Gel HP-20.

The mobilities of the sulfonate analogs **(5a–i)** on TLC are listed in Table 1; they correspond to those of taurine-conjugated bile acids. In the ^{13}C-NMR spectra the carbon atoms in the steroid nucleus showed chemical shifts similar to those of the parent bile acids,[11] as shown in Table 2. The terminal carbons, which are bonded directly to the sulfur atom, were observed at 50.0 and 53.2 ppm in the C_{23} and C_{24} sulfonate analogs, respectively.[12]

Studies on the metabolism and physiological properties of the sulfonate analogs are being conducted in these laboratories and the details will be reported elsewhere in the near future.

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Appendix

CA cholic acid
DCA deoxycholic acid
CDCA chenodeoxycholic acid
UDCA ursodeoxycholic acid
HDCA hyodeoxycholic acid
LCA lithocholic acid
NCA norcholic acid
NDCA nordeoxycholic acid
NCDCA norchenodeoxycholic acid
NUDCA norursodeoxycholic acid
NHDCA norhyodeoxycholic acid
TCA cholyl taurine
TCDC chenodeoxycholyl taurine
IR infrared
NMR nuclear magnetic resonance
FABMS fast atom bombardment mass spectrometry
TLC thin-layer chromatography

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