Sulfonate analogues of chenodeoxycholic acid: metabolism of sodium 3α,7α-dihydroxy-25-homo-5β-cholane-25-sulfonate and sodium 3α,7α-dihydroxy-24-nor-5β-cholane-23-sulfonate in the hamster

Shigeo Miki,[1] Erwin H. Mosbach,[2] Bertram I. Cohen, Michiko Yoshii,[3] Nariman Ayyad, and Charles K. McSherry

Departments of Surgery, Beth Israel Medical Center and the Mount Sinai School of Medicine of the City University of New York, New York, NY

Abstract This report describes the chemical synthesis of a new bile acid analogue, namely, sodium 3α,7α-dihydroxy-25-homo-5β-cholane-25-sulfonate from homochenodeoxycholic acid. The structure of the new compound was assigned by proton magnetic resonance and infrared spectrometry. Its metabolism was studied in the hamster in comparison with sodium 3α,7α-dihydroxy-24-nor-5β-cholane-23-sulfonate and sodium taurochenodeoxycholate. After intraduodenal administration of the ^{3}H-labeled analogues into bile fistula hamsters, both sulfonates were absorbed from the intestine and nearly 80% of the radioactivity was secreted into bile within 8 h. Intra-ileal administration revealed that these compounds resembled taurochenodeoxycholate in that they were much more rapidly absorbed from the ileum than from the proximal small intestine: more than 85% of the radioactivity was recovered in bile within 1 h. After intravenous infusion the sulfonates were efficiently extracted by the liver at rates similar to that of sodium taurochenodeoxycholate. Chromatographic analysis of the bile showed that, regardless of the route of administration, most (> 95%) of the sulfonates were not biotransformed and they became major biliary bile acids. Sodium 3α,7α-dihydroxy-25-homo-5β-cholane-25-sulfonate and, to a lesser extent, sodium 3α,7α-dihydroxy-24-nor-5β-cholane-23-sulfonate induced cholestasis at infusion rates at which sodium taurochenodeoxycholate produced choleresis.—**Miki, S., E. H. Mosbach, B. I. Cohen, M. Yoshii, N. Ayyad, and C. K. McSherry.** Sulfonate analogues of chenodeoxycholic acid: metabolism of sodium 3α,7α-dihydroxy-25-homo-5β-cholane-25-sulfonate and sodium 3α,7α-dihydroxy-24-nor-5β-cholane-23-sulfonate in the hamster. *J. Lipid Res.* 1992. **33:** 1629–1637.

Supplementary key words *Mesocricetus auratus* • biliary fistula • intestinal absorption • organic synthesis • bacterial 7-dehydroxylation • biotransformation

Chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA) have been in use clinically for a number of years to dissolve cholesterol gallstones (1–4). However, the efficacy of these bile acids as gallstone-dissolving agents has not been entirely satisfactory. CDCA (at a dose of 750 mg/day, as used in the National Gallstone Cooperative Study) produced complete dissolution in only 13.5% of the patients after 2 years of treatment (5). Orally administered CDCA and UDCA are 7-dehydroxylated by intestinal bacteria to form lithocholic acid (LCA) (6–8), which is a known hepatotoxin (9–11) and considered by some to promote intestinal carcinogenesis (12, 13). Therefore, there exists a need to develop new cholelitholytic agents with greater efficacy and less potential hepatotoxicity.

It is known that side chain conjugation prevents bacterial 7-dehydroxylation (14). The intestinal bacteria deconjugate glycine and taurine conjugates prior to 7-dehydroxylation (15). The studies of Coleman et al. (16) confirmed that a free terminal carboxyl group is required for 7-dehydroxylation. We considered that certain side chain-modified bile acid analogues should be resistant to 7-dehydroxylation and might prove to be potentially useful in cholesterol gallstone dissolution. It is also known

Abbreviations: CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid; CDC-sul, 3α,7α-dihydroxy-5β-cholane-24-sulfonate; UDC-sul, 3α,7β-dihydroxy-5β-cholane-24-sulfonate; homoCDC-sul, 3α,7α-dihydroxy-25-homo-5β-cholane-25-sulfonate; norCDC-sul, 3α,7α-dihydroxy-24-nor-5β-cholane-23-sulfonate; CDC-tau, chenodeoxycholyl taurine; PMR, proton magnetic resonance; IR, infrared; TLC, thin-layer chromatography; HPLC, high performance liquid chromatography.

[1]Permanent address: Department of Surgery I, Kyushu University Faculty of Medicine, Fukuoka 812, Japan.

[2]To whom correspondence should be addressed at: Department of Surgery, Beth Israel Medical Center, First Avenue at 16th Street, New York, NY 10003.

[3]Present address: Institute of Pharmaceutical Science, Hiroshima University School of Medicine, Hiroshima 743, Japan.

that the length of the side chain affects the ability of the intestinal bacteria to hydrolyze conjugated bile acids. Taurohomochenodeoxycholic acid, glyco- and tauro-homocholic acids were hydrolyzed less than 10% after incubation with excess cholylglycine hydrolase for 24 h, whereas glyco- and taurocholic acids were completely hydrolyzed (17).

Recently, Kihira et al. (18) synthesized the sulfonate analogues of CDCA and UDCA, namely, sodium 3α,7α-dihydroxy-5β-cholane-24-sulfonate (CDC-sul) and sodium 3α,7β-dihydroxy-5β-cholane-24-sulfonate (UDC-sul), hypothesizing that the presence of a highly polar functional group at the end of the side chain should hinder bacterial 7-dehydroxylation. In experiments in hamsters, CDC-sul was not hydrolyzed and showed complete resistance to bacterial dehydroxylation (19).

In order to define whether side-chain length influences the biological properties of sulfonate analogues of CDCA, we synthesized the sulfonates of nor-chenodeoxycholic acid (norCDC-sul) (**Fig. 1, III**) and homochenodeoxycholic acid (homoCDC-sul) (Fig. 1, IV). Because these compounds have a polar functional group at the end of the side chain instead of a free carboxyl group, we postulated that they should also resist bacterial 7-dehydroxylation.

The following report describes the synthesis of homoCDC-sul and compares the biological properties of homoCDC-sul, norCDC-sul, and CDC-tau in the hamster.

MATERIALS AND METHODS

General

Melting points were determined on a Thermolyne melting point apparatus and are not corrected. Proton magnetic resonance (PMR) spectra, in ppm, were determined at 400 MHz on a JEOL JMN-FX-400 spectrometer in CD_3OD solution using tetramethylsilane as an internal standard. Infrared (IR) spectra were obtained on a JASCO IRA-I spectrometer as KBr discs. Radioactivity was measured using Aquasol-2 (NEN Research Products, Boston, MA) on a Beckman LS 3801 liquid scintillation system (Beckman Instruments, Fullerton, CA) with automatic quench correction.

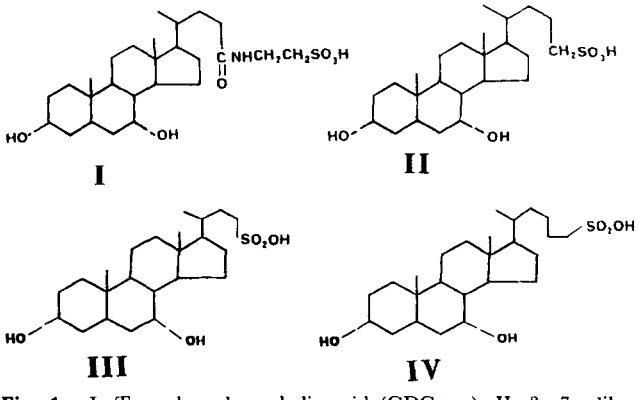

Fig. 1. I, Taurochenodeoxycholic acid (CDC-tau); II, 3α,7α-dihydroxy-5β-cholane-24-sulfonate (CDC-sul); III, 3α,7α-dihydroxy-24-nor-5β-cholane-23-sulfonate (norCDC-sul); IV, 3α,7α-dihydroxy-25-homo-5β-cholane-25-sulfonate (homoCDC-sul).

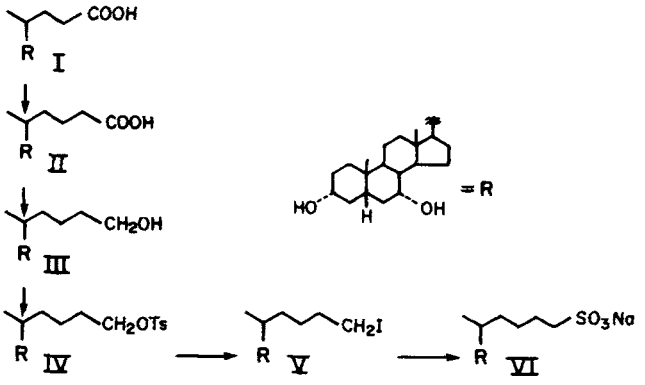

Fig. 2. Synthesis of sodium 3α,7α-dihydroxy-25-homo-5β-cholane-25-sulfonate. I, chenodeoxycholic acid; II, homochenodeoxycholic acid; III, 25-homo-5β-cholane-3α,7α,25-triol; IV, 25-homo-25-*p*-toluenesulfoxy-5β-cholane-3α,7α-diol; V, 25-homo-25-iodo-5β-cholane-3α,7α-diol; VI, 3α,7α-dihydroxy-25-homo-5β-cholane-25-sulfonate.

Chromatography

Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F_{254} plates (0.2 mm thickness, EM Science, Darmstadt, West Germany) using a 10% ethanolic solution of phosphomolybdic acid to visualize the spots. High performance liquid chromatography (HPLC) was carried out on a Varian model 5020 liquid chromatograph with a refractive index detector (HP1047A, Hewlett-Packard, Avondale, PA) and a UV detector (Waters 480 variable wavelength detector, Millipore, Milford, MA) and using a NOVA-C_{18} 4 μ column (Waters Associates, Milford, MA) in a Waters Z-module (solvent, methanol–water 75:25, pH adjusted to 5.37 with phosphoric acid, flow rate 0.9 ml/min).

Synthesis

NorCDC-sul was prepared from norchenodeoxycholic acid by the method of Kihira et al. (20). The synthesis of homoCDC-sul (**Fig. 2, VI**) used homoCDCA (Fig. 2) which was prepared from CDCA (Fig. 2, I) via the Arndt-Eistert reaction (21). HomoCDC-sul was synthesized as described below.

25-Homo-5β-cholane-3α,7α,25-triol (Fig. 2, III)

To a cooled solution (O°C) of 2.96 g of homochenodeoxycholic acid (Fig. 2, II) in dry tetrahydrofuran (100 ml) were added 1.5 ml of triethylamine and 1.5 ml of ethyl chloroformate. The solution was stirred at 0°C for 3 h. Twenty ml of a 10% aqueous solution of $NaBH_4$ was added and stirring was continued at room temperature for 2 h. The solution was diluted with

water, acidified with 1 N HCl, and extracted with ethyl acetate. The residue was crystallized from methanol–ethyl acetate, and colorless crystals of 25-homo-5β-cholane-3α,7α,25-triol (Fig. 2, III) were obtained; yield 2.40 g, mp 187–189°C, PMR 0.69(3H,-s,18-CH_3), 0.93(3H,s,19-CH_3), 0.95(3H,d,J=6.6 Hz,21-CH_3), 3.31(1H,m,3β-H), 3.79(1H,m,7β-H), 3.53(2H,t,J=6.5 Hz,25-CH_2-OH).

25-Homo-25-*p*-toluenesulfoxy-5β-cholane-3α,7α-diol (Fig. 2, IV)

To 25-homo-5β-cholane-3α,7α,25-triol (Fig. 2, III) (685 mg) in anhydrous tetrahydrofuran (40 ml) were added *p*-toluenesulfonyl chloride (0.7 g) and triethylamine (4.2 ml); this solution was stirred at 4°C for 7 days. The reaction mixture was diluted with water, acidified with 1 N HCl, and extracted with ethyl acetate. The residue was purified on a silica gel 60 column (50 g, 35–70 mesh ASTM, Merck, Germany) which was eluted with increasing amounts of ethyl acetate in benzene. The fractions containing 25-homo-25-*p*-toluenesulfoxy-5β-cholane-3α,7α-diol (Fig. 2, IV) were collected and evaporated to dryness (yield, 360 mg). PMR 0.66(3H,s,18-CH_3), 0.88(3H,d, J=6.4 Hz,21-CH_3), 0.92(3H,s,19-CH_3), 2.46(3H,s,CH_3-phenyl), 3.37(1H,m,3β-H), 3.79(1H,m,7β-H), 4.02(2H,t,J=6.2 Hz,25-CH_2OTS), 7.78 and 7.44(4H, phenyl protons).

25-Homo-25-iodo-5β-cholane-3α,7α-diol (Fig. 2, V)

To a solution of 25-homo-25-*p*-toluenesulfoxy-5β-cholane-3α,7α-diol (Fig. 2, IV) (360 mg) in acetone, NaI (1.2 g) was added and the mixture was allowed to stand at 55°C for 3 h. The solution was diluted with water and extracted with ethyl acetate. Evaporation gave an oily residue of 25-homo-25-iodo-5β-cholane-3α,7α-diol (Fig. 2, V) (331 mg). PMR 0.69(3H,s,18-CH_3), 0.93(3H,s,19-CH_3), 0.96(3H,d,J=6.4 Hz,21-CH_3), 3.23(2H,sep,J_1=6.9 Hz,J_2=2.4 Hz,25-CH_2-I), 3.37(1H,m,3β-H), 3.79(1H,m,7β-H).

Sodium 3α,7α-dihydroxy-25-homo-5β-cholane-25-sulfonate (Fig. 2, VI)

25-Homo-25-iodo-5β-cholane-3α,7α-diol (Fig. 2, V) (331 mg) in 15 ml of ethanol and 30 ml of 20% aqueous Na_2SO_3 were refluxed for 16 h. The ethanol was evaporated; the residue was taken up in methanol and filtered. The filtrate was evaporated, and the residue was purified on an XAD-2 column (30 g). The column was washed with water (150 ml) to remove Na_2SO_3 and was then eluted with 50% methanol–water. The fractions containing sodium 3α,7α-dihydroxy-25-homo-5β-cholane-25-sulfonate (Fig. 2, VI) were evaporated, and (VI) (248 mg) was crystallized from methanol–ethyl acetate. MP 273–275°C, IR, cm^{-1}, 1050, 1190 (-SO_3), 3400 (-OH). PMR 0.69(3H,s,18-CH_3), 0.92(3H,s,19-CH_3), 0.95(3H, d,J=6.6 Hz,21-CH_3), 2.79(2H,m,25-CH_2-SO_3Na), 3.35(1H,m,3β-H), 3.79(1H,m,7β-H).

Labeled compounds

[11,12-^{3}H]norCDC-sul (sp act 9.8 × 10^5 dpm/mg) was prepared from [11,12-^{3}H]norchenodeoxycholic acid as described previously (20). The starting material was a gift from Dr. A. F. Hofmann.

[11,12-^{3}H]homoCDC-sul (sp act 5.3 × 10^5 dpm/mg) was prepared as described above starting with [11,12-^{3}H]CDCA which was prepared from [11,12-^{3}H]norchenodeoxycholic acid by the Arndt-Eistert reaction (21).

[24-^{14}C]CDC-tau (sp act 9.5 × 10^4 dpm/mg) was synthesized from [24-^{14}C]CDCA by the method of Tserng, Hachey, and Klein (22).

Radiochemical purity of the labeled compounds was better than 95% as determined by radio-TLC (see Analytical techniques for TLC details). Each labeled compound was dissolved in 0.9% aqueous NaCl solution.

Animal experiments

Male golden Syrian hamsters (Sasco, Omaha, NE), weighing 108 ± 11 g, were fed Purina chow (Purina, St. Louis, MO) and water ad libitum, and kept under a controlled 12-h light/dark cycle for 2 weeks prior to surgery. All animals were operated on between 8:30 and 10:00 AM. The animals were anesthetized by intramuscular injection of ketamine (Ketaset, Bristol Labs, Syracuse, NY) with a dose of 20 mg/animal. Intramuscular injections of ketamine (5–10 mg/dose) were used to maintain anesthesia as required.

A polyethylene catheter (PE-10, 0.28 mm ID, Clay Adams, Parsippany, NJ) was inserted into the left or right femoral vein and 0.9% NaCl solution was infused at a rate of 1.5 ml/h using a Harvard syringe pump (Harvard Apparatus, Mills, MA). The abdomen was opened by a midline incision, the cystic duct was ligated with a hemostatic clip (Hemo Clip, Edward Weck & Co., Inc., Research Triangle Park, NC), and an external biliary fistula was constructed using PE-10 polyethylene tubing. The urethra was ligated with a hemostatic clip to allow urine to accumulate in the bladder.

In the intravenous infusion studies, saline was infused into the femoral vein for 20 min prior to the administration of the labeled compound; the labeled compound was then infused for 20 min at a dose of 50 μg/min (total amount administered, 1 mg); the infusion of saline was then resumed until the end of the experiment. In control experiments, saline was infused throughout the experimental period. Bile samples were collected in weighed tubes at 20-min intervals for a total period of 4 h. The samples obtained from 0–120 min were used to determine the biliary bile acid composition (see the Analytical techniques section and Table 1). At the end of the experi-

ments, urine was obtained by aspiration from the urinary bladder.

In the intraduodenal injection experiments, after cannulation of the bile duct and the femoral vein as described above, the labeled compounds (0.5 mg) were injected as a bolus into the duodenum. In the intra-ileal injection studies, after cannulation of the bile duct and the femoral vein, the end of the ileum was ligated, and the labeled compounds (0.5 mg) were injected as a bolus into the terminal ileum. Bile samples were collected every 20 min for 8 h (intraduodenal injection), or every 20 min for 4 h (intra-ileal injection).

In order to estimate their effects on bile flow, unlabeled homoCDC-sul, norCDC-sul, and CDC-tau were infused intravenously into biliary fistula hamsters at a dose of 3 μmol/min per kg for 2 h. Saline was infused for 20 min during an initial control period and at the end of the bile acid infusion.

Dose response data were obtained as follows. After a 2-h control period, during which saline was infused intravenously, homoCDC-sul or norCDC-sul was administered by the same route at increasing rates (0.1, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, and 3.0 μmol/min per kg). The doses were infused for consecutive 40-min periods; bile samples were collected at 20-min intervals. All biological specimens were stored at −20°C.

Analytical techniques

In the metabolic experiments, bile samples were collected every 20 min. Three aliquots were taken from each sample to determine the fate of the administered labeled compounds as follows. *1)* The radioactivity of the first set of aliquots (10 μl) was determined by liquid scintillation counting. *2)* The second set of aliquots was analyzed by TLC to detect the metabolites of the administered labeled compounds using solvent system A (butanol–acetic acid–water 85:10:5) for norCDC-sul and solvent system B (chloroform–methanol–acetic acid–water 65:20:10:5) for homoCDC-sul. Five μl of bile was spotted directly on a TLC plate along with reference compounds. After development (16 cm) and detection of the spots, each TLC plate was cut into segments (1 cm) from the origin to the solvent front and each segment was put into a scintillation vial. After addition of 1 ml of methanol, radioactivities were determined by liquid scintillation counting. *3)* The third set of aliquots (50 μl) was analyzed for bile acids by HPLC (Table 1): to each aliquot there was added 20 μg of internal standard (7α,12α-dihydroxy-5β-cholanoyl glycine sodium salt) followed by 10 volumes of ethanol to precipitate protein. The sample was centrifuged and the supernatant solution was evaporated to dryness. The residue was dissolved in methanol and filtered with a disposable syringe filter (22 μm, Alltech Associates Inc., Deerfield, IL). The samples were then analyzed for bile acids by HPLC. (See section on chromatography in Materials and Methods.) Aliquots of urine were taken for determination of radioactivity.

Total bile acid concentration was measured using 3α-hydroxysteroid dehydrogenase (Sigma Chemical, St. Louis, MO), as described earlier (23).

Calculations

Numerical data are expressed as mean ± SEM.

RESULTS

Synthesis of homoCDC-sul

The synthetic route of homoCDC-sul is shown in Fig. 2. HomoCDCA (Fig. 2, II) was treated with ethyl chloroformate and reduced with $NaBH_4$ to form 25-homo-5β-cholane-3α,7α,25-triol (Fig. 2, III). The terminal hydroxyl group of the bile alcohol (Fig. 2, III) was selectively tosylated in anhydrous tetrahydrofuran with *p*-toluenesulfonyl chloride and triethylamine to give 25-homo-25-*p*-toluenesulfoxy-5β-cholane-3α,7α-diol (Fig. 2, IV). Selective tosylation of the terminal hydroxyl group was confirmed by PMR. The signal of the terminal protons of the bile alcohol (Fig. 2, III) was shifted 0.49 ppm downfield by tosylation. The signals of the 18- and 21-methyl protons were shifted upfield by 0.03 and 0.07 ppm, respectively. Iodination of the tosyl ester (Fig. 2, IV) was carried out with sodium iodide to form 25-homo-25-iodo-5β-cholane-3α,7α-diol (Fig. 2, V). In the 25-iodide (Fig. 2, V), the terminal protons were observed at 3.23 ppm. The 25-iodide was refluxed with sodium sulfite to yield the desired homoCDC-sul (Fig. 2, VI). The presence of the sulfonic acid at the end of the

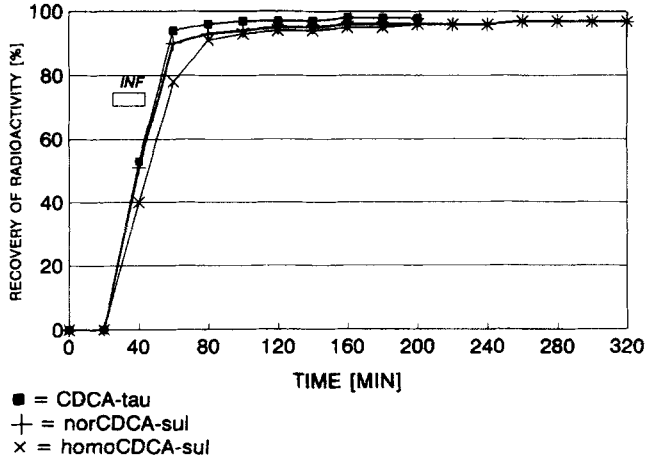

Fig. 3. Biliary secretion of radioactivity in bile fistula hamsters after intravenous infusion of ^{3}H-labeled homoCDC-sul and norCDC-sul, and ^{14}C-labeled CDC-tau. After a 20-min control period, the bile acid analogues were infused at a dose of 50 μg/min for 20 min. Each point represents the average from four male hamsters.

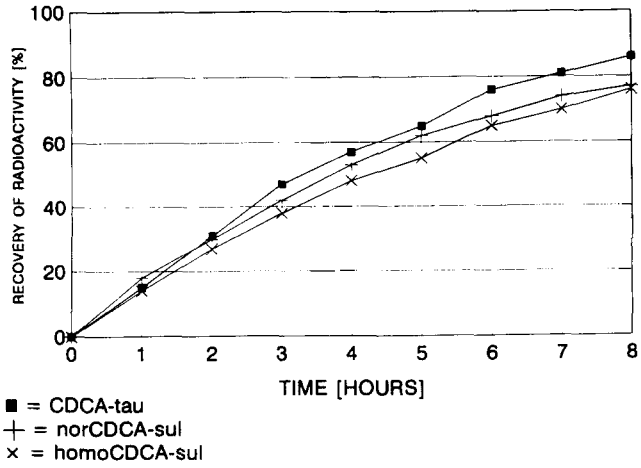

Fig. 4. Biliary secretion of radioactivity in bile fistula hamsters after intraduodenal injection of ^{3}H-labeled homoCDC-sul and norCDC-sul, and ^{14}C-labeled CDC-tau. After a 20-min control period, the compounds were injected as a bolus (0.5 mg). Each point represents the average from four male hamsters.

side chain was confirmed by the PMR signal of the protons of the C-25 methylene group observed at 2.79 ppm. The IR spectrum showed bands at 1050 and 1190 cm^{-1}, indicative of the presence of a sulfonic acid moiety in the compound (18).

Recovery of radioactivity

Fig. 3 shows the cumulative biliary excretion of radioactivity after intravenous infusion of the labeled compounds (total dose 1 mg). HomoCDC-sul and norCDC-sul appeared rapidly in the bile, similar to the naturally occurring bile acid, CDC-tau. Ninety % of the infused

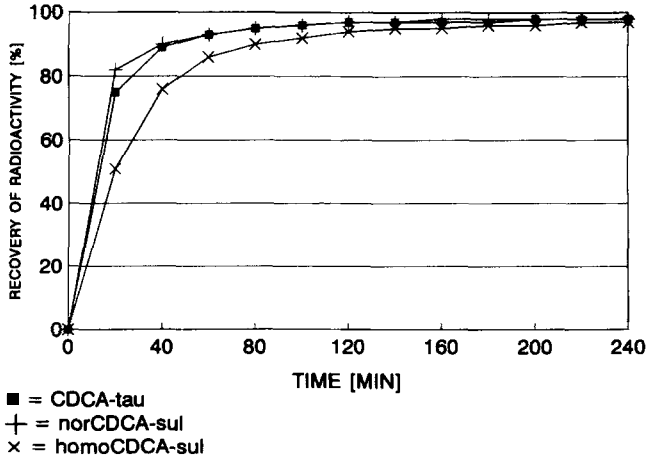

Fig. 5. Biliary secretion of radioactivity in bile fistula hamsters after intra-ileal injection of ^{3}H-labeled homoCDC-sul and norCDC-sul, and ^{14}C-labeled CDC-tau. After a 20-min control period, the compounds were injected as a bolus (0.5 mg). Each point represents the average from four male hamsters.

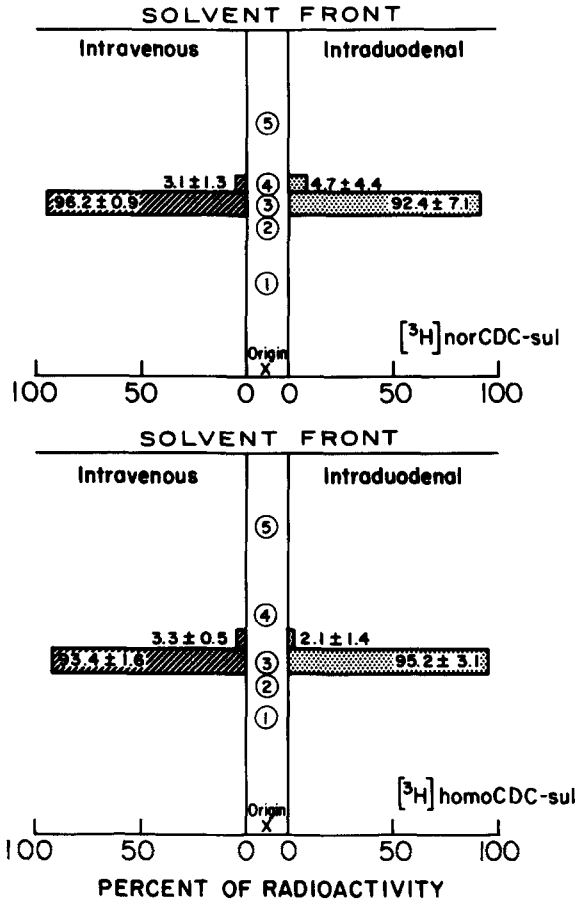

Fig. 6. Thin-layer chromatographic analysis of radioactivity in the bile from four bile fistula hamsters after intravenous or intraduodenal administration of labeled norCDC-sul (upper) and homoCDC-sul (lower). Bile samples were analyzed by solvent system A (n-butanol–acetic acid–water 85:10:5) or by solvent system B (chloroform–methanol–acetic acid–water 65:20:10:5). Reference compounds were as follows: 1, taurocholate; 2, taurochenodeoxycholate; 3 (upper), sodium 3α,7α-dihydroxy-24-nor-5β-cholane-23-sulfonate (norCDC-sul); 3 (lower), sodium 3α,7α-dihydroxy-25-homo-5β-cholane-25-sulfonate (homoCDC-sul); 4, glycocholate; 5, glycochenodeoxycholate.

radioactivity was secreted within 1 h. The half-times ($t_{1/2}$) of biliary recovery were 19 min (CDC-tau and norCDC-sul) and 25 min (homoCDC-sul). In the case of both analogues, less than 0.5% of the administered radioactivity was excreted into the urine.

Fig. 4 depicts the biliary recoveries of radioactivity after injection of 0.5 mg of homoCDC-sul, norCDC-sul, and CDC-tau into the duodenum. The time course of the recoveries of homoCDC-sul and norCDC-sul resembled that of CDC-tau. About 80% of the radioactivity of each compound was recovered within 8 h, and more than 96% at the end of 24 h.

Biliary excretion of the radioactivity after intra-ileal injection of a 0.5 mg bolus is shown in **Fig. 5.** Each compound appeared in bile much more rapidly compared to

intraduodenal administration; more than 85% was recovered within 1 h. From the data of Figs. 4 and 5, it can be calculated that the half-times ($t_{1/2}$) of biliary recovery of CDC-tau, norCDC-sul, and homoCDC-sul were 194 min, 221 min, and 252 min (intraduodenal injection) and 13 min, 12 min, and 19 min (intra-ileal injection), respectively.

Metabolism of the sulfonates

Chromatographic analysis, shown in **Fig. 6,** of the material appearing in the bile after intravenous or intraduodenal administration revealed that there was little biotransformation of homoCDC-sul and norCDC-sul (over 90% of the radioactivity was accounted for by the starting material). Less than 5% of the radioactivity was found in a spot somewhat less polar than the administered material. This spot was not stained by the phosphomolybdate spray.

Bile acid composition

The biliary bile acid composition of bile fistula hamsters infused intravenously with the labeled sulfonates is shown in **Table 1.** The biliary bile acid composition did not change during the control period (saline infusion for 2 h). During the infusion of each bile acid analogue, bile composition was shifted in the direction of the administered bile acid sulfonate. The proportion of the infused compounds in bile increased to more than 50% of total bile acid, and exceeded that of the total taurocholate and glycocholate during infusion of the compound (20–40 min) as well as during the subsequent 20-min period (40–60 min).

Effects of the sulfonates on bile flow

Infusion experiments were carried out using unlabeled compounds in order to investigate the effect of the sulfonate analogues on bile flow. **Fig. 7** summarizes the results obtained following the infusion of homoCDC-sul, norCDC-sul, and CDC-tau at the rate of 3 μmol/min per kg for 2 h. When CDC-tau was infused, bile flow increased as long as the bile acid was administered. However, homoCDC-sul caused a severe cholestasis. After infusion of this analogue, bile flow decreased immediately and ceased at the end of about 1.5 h. NorCDC-sul induced a milder cholestatic effect which was noticeable after about 0.5 h. When this compound was infused, bile flow increased, but then began to fall and continued at a low level throughout the experimental period. After the

TABLE 1. Biliary bile acid composition of bile fistula hamsters infused intravenously with labeled norCDC-sul or homoCDC-sul

Collection Time	Bile Acid Composition[a]						
	TC	GC	TCDC	GCDC	TDC	GDC	NorCDC-sul HomoCDC-sul
min				%			
Saline infusion							
0–20	43.9 ± 3.8	27.0 ± 3.2	13.2 ± 1.0	8.3 ± 1.2	4.4 ± 1.2	3.2 ± 0.9	
20–40	48.7 ± 0.8	25.5 ± 2.2	13.0 ± 2.1	7.2 ± 0.5	4.3 ± 1.0	1.3 ± 0.9	
40–60	45.5 ± 6.0	24.3 ± 1.3	12.9 ± 0.5	8.2 ± 1.2	5.3 ± 1.4	3.8 ± 0.2	
60–80	47.3 ± 4.1	26.0 ± 2.7	12.8 ± 1.2	7.1 ± 0.7	5.0 ± 2.0	1.8 ± 0.9	
80–100	44.3 ± 5.1	25.8 ± 2.0	12.6 ± 0.7	8.0 ± 0.8	5.5 ± 1.5	3.8 ± 0.8	
100–120	44.4 ± 4.3	25.6 ± 2.6	15.9 ± 0.8	7.1 ± 1.4	4.3 ± 1.1	2.7 ± 2.5	
NorCDC-sul infusion							
0–20	47.7 ± 0.8	26.8 ± 5.2	11.6 ± 2.3	7.7 ± 5.6	5.6 ± 0.1	0.6 ± 0.6	
20–40	23.3 ± 8.1	7.7 ± 2.4	9.5 ± 3.4	2.7 ± 0.7	2.3 ± 0.6	0.2 ± 0.2	54.3 ± 11.0
40–60	28.3 ± 5.7	11.4 ± 3.4	11.2 ± 3.7	3.9 ± 2.3	3.9 ± 2.4	0.2 ± 0.2	41.1 ± 7.5
60–80	44.7 ± 5.9	19.6 ± 4.3	16.2 ± 6.3	7.1 ± 3.1	5.4 ± 1.6	0.7 ± 0.9	6.3 ± 3.1
80–100	42.7 ± 9.4	19.7 ± 7.4	17.6 ± 3.3	9.9 ± 3.1	6.6 ± 3.1	0.1 ± 0.1	3.4 ± 1.8
100–120	46.5 ± 8.2	18.9 ± 6.6	17.0 ± 3.3	8.4 ± 2.5	6.0 ± 2.5	1.1 ± 1.1	2.1 ± 1.0
HomoCDC-sul infusion							
0–20	47.3 ± 3.0	28.6 ± 1.2	13.8 ± 1.5	6.2 ± 1.9	3.2 ± 0.3	0.9 ± 1.0	
20–40	19.2 ± 3.6	13.0 ± 4.3	7.5 ± 0.1	4.8 ± 1.6	2.5 ± 0.3	1.5 ± 1.6	51.5 ± 9.3
40–60	19.6 ± 0.2	11.4 ± 2.5	6.8 ± 0.5	3.4 ± 0.7	2.0 ± 0.2	0.5 ± 0.5	56.3 ± 3.6
60–80	38.8 ± 3.5	22.7 ± 1.2	13.7 ± 2.3	7.2 ± 0.5	3.6 ± 0.2	1.3 ± 1.5	12.7 ± 2.4
80–100	41.5 ± 3.6	22.6 ± 1.7	14.6 ± 2.1	8.4 ± 0.1	4.1 ± 0.1	1.3 ± 1.4	7.5 ± 2.5
100–120	45.4 ± 3.2	24.8 ± 1.5	14.0 ± 2.3	6.4 ± 0.4	3.5 ± 0.2	0.8 ± 0.9	5.1 ± 2.5

After a 20-min period (time 0–20 min), the ^{3}H-labeled bile acid analogues were infused at a dose of 50 μg/min for 20 min. In the control animals, saline was infused throughout the experiment. The bile acids were analyzed by HPLC after removal of protein. TC, taurocholic acid; GC, glycocholic acid; TCDC, taurochenodeoxycholic acid; GCDC, glycochenodeoxycholic acid; TDC, taurodeoxycholic acid; GDC, glycodeoxycholic acid; norCDC-sul, 3α,7α-dihydroxy-24-nor-5β-cholane-23-sulfonate; homoCDC-sul, 3α,7α-dihydroxy-25-homo-5β-cholane-25-sulfonate.

[a]The values are expressed as mean ± SEM.

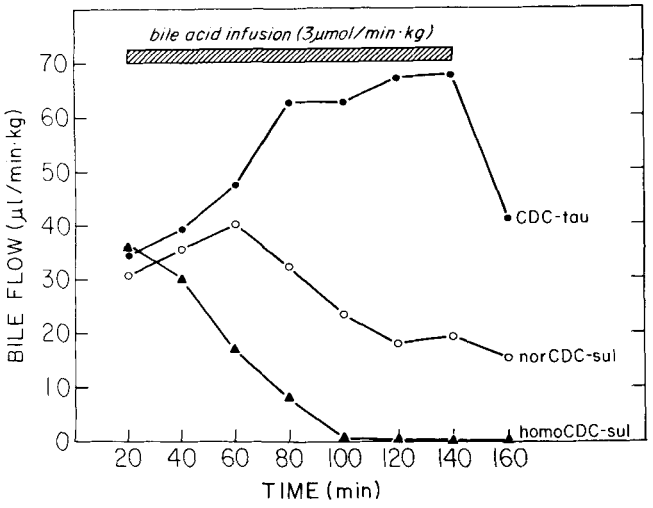

Fig. 7. Bile flow in bile fistula hamsters after intravenous infusion of homoCDC-sul, norCDC-sul, and CDC-tau. After a 20-min control period, the bile acids were infused at a dose of 3 μmol/min per kg for 2 h. Each value represents the average of three male hamsters.

infusion of these bile acid analogues was stopped, bile flow continued to decrease. The results of a dose-response experiment with homoCDC-sul and norCDC-sul are shown in **Fig. 8.** Both compounds exerted cholestatic effects, homoCDC-sul at an infusion rate of 0.75 μmol/min per kg and norCDC-sul at 2.0 μmol/min per kg.

DISCUSSION

In 1968, Gustafsson, Midtvedt, and Norman (15) observed that bacterial 7-dehydroxylation took place readily with unconjugated bile acids, while the taurine and glycine amidates were unaffected. Similarly, a more recent study showed that side chain conjugation prevented 7-dehydroxylation of bile acids by *Eubacterium* sp. VP112708 (14). It is now known that the 7-dehydroxylation requires a free carboxyl group and involves an adenosine nucleotide-linked intermediate (16). Sarcosine (N-methylglycine)-conjugated UDCA was found to be resistant to bacterial hydrolysis and bacterial 7-dehydroxylation (24). Kihira et al. (18) hypothesized that this resistance was attributable to the attachment of a more polar functional group to the carboxyl group of the free (unconjugated) bile acid. To test this hypothesis they synthesized a sulfonate analogue, CDC-sul, which was unhydrolyzable and possessed a highly polar moiety at the end of the side chain. This analogue proved to be completely resistant to bacterial dehydroxylation in the hamster (19). As a result, we postulated that, in general, sulfonate analogues of 3,7-dihydroxy bile acids would be resistant to 7-dehydroxylation by the intestinal bacteria, regardless of the length of the side chain. Consequently, we synthesized sulfonate analogues from homoCDCA (C_{25}) and norCDCA (C_{23}), i.e., homoCDC-sul and norCDC-sul, which have a highly negative charge at the same position as the carboxyl groups of bishomo bile

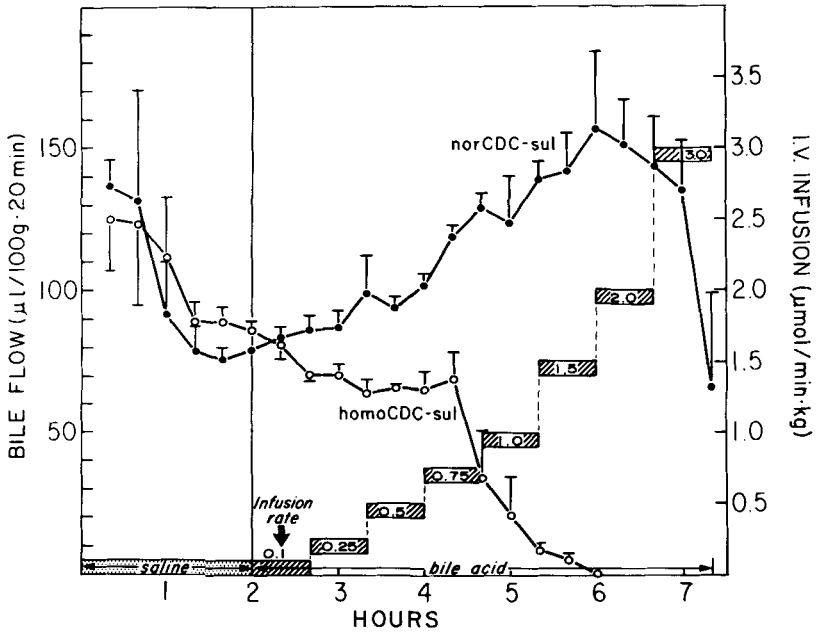

Fig. 8. Bile flow in bile fistula hamsters. After a 2-h control period (saline infusion), homoCDC-sul or norCDC-sul was infused intravenously at increasing rates (0.1, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, and 3.0 μmol/min per kg) for 5 h. Each dose was administered for consecutive periods of 40 min, and bile samples were collected during 20-min periods. The vertical bars indicate one standard deviation from the mean for three male hamsters.

acids (C_{26}) and the natural bile acids (C_{24}), respectively. Fig. 1 shows the structures of these compounds.

Certain biological properties of homoCDC-sul and norCDC-sul were studied in the biliary fistula hamster in comparison with CDC-tau. As shown in Fig. 3, homoCDC-sul and norCDC-sul were extracted effectively by the liver and rapidly secreted into the bile, in a manner similar to the naturally occurring conjugated bile acid, CDC-tau. The rate of hepatic uptake is related to the polarity of the bile acid (25); side chain conjugation is more important than modification of the steroid nucleus, and conjugation with taurine increases the affinity of the bile acid for its transport carrier (26, 27). The polarities of the sulfonate analogues of CDCA and UDCA are similar to those of the taurine-conjugated CDCA and UDCA (18), and the mobilities of homoCDC-sul and norCDC-sul on TLC resembled that of CDC-tau (Fig. 6). HomoCDC-sul and norCDC-sul appear to be sufficiently polar for efficient hepatic extraction. Presumably, because of this high polarity of the sulfonate analogues, these compounds can be secreted into bile without biotransformation regardless of the route of administration.

After intravenous infusion of 1 mg (2.1 μmol homoCDC-sul and 2.2 μmol norCDC-sul), the proportions of both of these analogues in bile increased to more than 50% of total bile acids (Table 1). These compounds were rapidly secreted into bile during the first pass through the liver. However, long-term feeding studies are required to determine how well these compounds are retained in the enterohepatic circulation.

As illustrated in Figs. 4 and 5, the intestinal absorption rates of the two sulfonates were quite similar to those of CDC-tau. After intra-ileal administration, these sulfonates were absorbed efficiently from the ileum and were processed by the liver as efficiently as CDC-tau, whereas the recoveries in bile after duodenal administration were much slower. The highly polar conjugated bile acids are absorbed poorly by passive diffusion in the upper intestine; however, they have high active transport rates across the ileum if they have a single negative charge on the side chain (25, 28). Taurine conjugates are almost totally dependent on the active transport sites in the ileum for their absorption (29). Our findings indicate that the sulfonate analogues are absorbed mainly from the ileum by an active transport system. The slight delay in the appearance of radioactivity from homoCDC-sul was apparent up to 80 min. However, after 240 min, recovery of radioactivity from all compounds studied was nearly quantitative.

For the purpose of evaluating the effects of the sulfonate analogues on bile flow, a single constant dose (3.0 μmol/min per kg) or increasing doses were infused intravenously (Figs. 3 and 8). Both sulfonate analogues produced cholestasis. In the case of homoCDC-sul, a relatively small dose (0.75 μmol/min per kg) was cholestatic. Cholestasis results from multiple disturbances in the sequence of events responsible for bile production (30). The relative hydrophilicity/hydrophobicity of bile salts might be responsible for this effect (31, 32). Since CDC-tau, which possesses both a sulfonic acid moiety and a peptide bond in the side chain, was choleretic at the dose we used, the absence of a peptide bond in the sulfonate analogues might be associated with the etiology of the cholestasis. HomoCDC-sul is hydrophobic as determined by reverse-phase HPLC. The relative retention time of this compound was 1.74 (vs. 1.00 for CDC-tau, 0.93 for norCDC-sul, and 1.80 for taurolithocholic acid). More experiments are needed to clarify the relationship between cholestasis and hydrophobicity.

In summary, we synthesized and characterized two sulfonate analogues, namely homoCDC-sul and norCDC-sul. These compounds were rapidly absorbed from the ileum, extracted efficiently by the liver, like the naturally occurring bile acid, CDC-tau. They were secreted into the bile largely without biotransformation. However, their cholestatic properties, especially in the case of homoCDC-sul, would prevent their utilization as cholelitholytic agents.

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