15α-Hydroxylation of a bile acid analogue, sodium 3α,7α-dihydroxy-25,26-bishomo-5β-cholane-26-sulfonate in the hamster

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Abstract The metabolism of 3α,7α-dihydroxy-25,26-bishomo-5β-cholane-26-sulfonate (bishomoCDC-sul), the sulfonate analogue of bishomochenodeoxycholic acid, and its effect on biliary bile acid composition were studied during chronic administration in the hamster. After oral administration of radiolabeled bishomoCDC-sul, more than 80% of the radioactivity was excreted into the feces within 7 days, both as the unchanged sulfonate (38.5%) and two more polar metabolites (50.0% and 11.5%). The half time of the fecal excretion was 1.6 days. In gallbladder bile, the unchanged sulfonate and its major metabolite accounted for 19.1% and 19.8% of total bile acids, respectively. In another experiment, hamsters were fed bishomoCDC-sul with antibiotics to evaluate the site of biotransformation. Even when the number of intestinal microorganisms was greatly reduced, the same three metabolites were found in the feces: bishomoCDC-sul (44.0%) and the two polar metabolites (30.8% and 25.1%). The major metabolite was isolated from feces of the hamsters fed bishomoCDC-sul without antibiotics. Its chemical structure was identified by mass spectrometry and nuclear magnetic resonance spectroscopy as the 15α-hydroxylated derivative, namely sodium 3α,7α,15α-trihydroxy-25,26-bishomo-5β-cholane-26-sulfonate. These results indicate that after oral administration, the sulfonate analogue of bishomochenodeoxycholic acid underwent enterohepatic circulation like a natural bile acid and was transformed, in part, into the 15α-hydroxylated derivative and another more polar metabolite in the liver of hamsters. There was no evidence that bishomoCDC-sul was dehydroxylated to a lithocholic acid analogue during enterohepatic cycling.—**Mikami, T., A. Ohshima, E. H. Mosbach, B. I. Cohen, N. Ayyad, M. Yoshii, K. Ohtani, K. Kihira, C. D. Schteingart, and T. Hoshita.** 15α-Hydroxylation of a bile acid analogue, sodium 3α,7α-dihydroxy-25,26-bishomo-5β-cholane-26-sulfonate in the hamster. *J. Lipid Res.* 1996. **37:** 1189–1197.

Supplementary key words *Mesocricetus auratus* • bile acid analogue • hydrophobicity • 15α-hydroxylation • 7-dehydroxylation • nuclear magnetic resonance

Chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA) are currently used as therapeutic agents for cholesterol gallstone disease (1, 2). After oral administration, CDCA and UDCA undergo enterohepatic circulation. They are conjugated with glycine or taurine by the liver, and after deconjugation, are 7-dehydroxylated by the intestinal microorganisms. As a result of 7-dehydroxylation, CDCA and UDCA are transformed into lithocholic acid (LCA) (3), which is known to be a hepatotoxic agent (4, 5) and a promoter of colon cancer (6, 7). In addition, repeated conjugation and deconjugation of the administered bile acids can induce a decline of the hepatic taurine pool (8). Conjugated bile acids resist bacterial 7-dehydroxylation (9); a free carboxylic acid group is needed for this reaction (10). For this reason, we have synthesized analogues of conjugated bile acids that possess a sulfonic acid moiety at the end

Abbreviations: CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid; CDC-sul, sodium 3α,7α-dihydroxy-5β-cholane-24-sulfonate; UDC-sul, 3α,7β-dihydroxy-5β-cholane-24-sulfonate; norUDC-sul, sodium 3α,7β-dihydroxy-24-nor-5β-cholane-23-sulfonate; LCA, lithocholic acid; bishomoCDC-sul, sodium 3α,7α-dihydroxy-25,26-bishomo-5β-cholane-26-sulfonate; ^{1}H-NMR, proton nuclear magnetic resonance; ^{13}C-NMR, carbon-13 nuclear magnetic resonance; DEPT, distortionless enhancement by polarization transfer; COSY, correlated spectroscopy; HSQC, heteronuclear single quantum correlation; GARP, globally optimized alternating-phase rectangular pulses; PS-NOESY, phase sensitive- nuclear Overhauser enhancement and exchange spectroscopy; TLC, thin-layer chromatography; HPLC, high pressure liquid chromatography

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of the side chain similar to taurine-conjugated bile acids (11–13). Oral administration of the sulfonate analogues of CDCA (CDC-sul), UDCA (UDC-sul), and norursodeoxycholic acid (norUDC-sul) in the hamster revealed that these analogues were apparently resistant to biotransformation by the liver and the intestinal microorganisms (8, 11–15).

The metabolism of bile acids is affected by the structure of both the nucleus and the side chain. The hepatic metabolism of unusual bile acids, such as nor- or homobile acids differed from that of the natural bile acids (11–17). Recently, Schteingart et al. (18) demonstrated that in the hamster, norursodeoxycholic acid (norUDC) and norchenodeoxycholic acid (norCDC) were 5β-hydroxylated by the liver. Similarly, Yoshii et al. (19) identified a 5β-hydroxy metabolite of 7β-methyl-norCDCA in the same species. In addition, unusual hydroxylated bile acids were identified in patients with liver disease (20, 21). The hydroxylated metabolites might represent hepatic detoxification mechanisms. Sulfonate analogues of bile acids with side chain lengths different from those of CDC-sul or norCDC-sul could conceivably be biotransformed by the liver or the intestinal microorganisms by virtue of their differences in hydrophobicity. In the present study, we investigated the metabolism of the sulfonate analogue of bishomochenodeoxycholic acid, namely sodium 3α,7α-dihydroxy-25,26-bishomo-5β-cholane-26-sulfonate (bishomoCDC-sul) in the hamster. This sulfonate has a longer side chain and is more hydrophobic than CDC-sul and norCDC-sul which were not biotransformed by the liver or intestinal microorganisms (11–15).

MATERIALS AND METHODS

Chromatography

Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F_{254} plates (0.2 mm thickness, EM Science, Darmstadt, Germany). A 10% ethanolic solution of phosphomolybdic acid was used to render the spots visible. High pressure liquid chromatography (HPLC) of bile salts 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). A NOVA C_{18} 4-μ column (Waters Associates, Milford, MA) was used in a Waters Z-module (solvent system: 0.01 M potassium phosphate in methanol–water 75:25, pH adjusted to 5.37 with phosphoric acid, flow rate 1.0 mL/min). Radioactivity was measured on a Beckman LS 3801 liquid scintillation system (Beckman Instruments, Fullerton, CA) with automatic quench correction, using Aquasol 2 (NEN Research Products, Boston, MA) as the scintillation fluid.

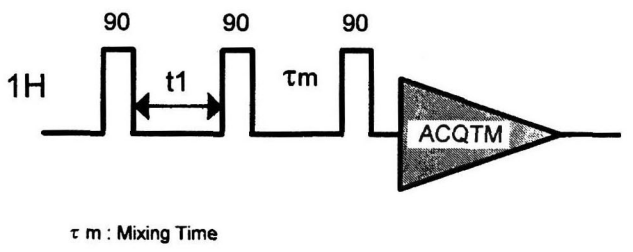

Fig. 1. Pulse sequence for PS-NOESY experiment.

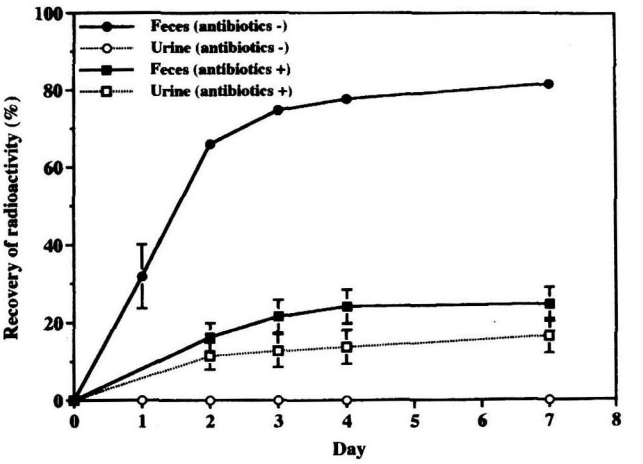

Fig. 2. Cumulative recovery of radioactivity in feces and urine. Feces (●) and urine (○) of group 1 (antibiotics -), and feces (■) and urine (□) of group 2 (antibiotics +) were collected for 7 days after intragastric administration of [^{3}H]bishomoCDC-sul.

Fast atom bombardment positive ion mass spectrometry (FABMS)

FABMS was performed on a JEOL D-300 mass spectrometer using polyethylene glycol 400 as internal standard. Typical experimental conditions were: xenon atom beam, 10 kV accelerating potential and 10 mA emission current. The underivatized bile salts were dissolved in glycerol, and the solution was inserted into the ion source.

Nuclear magnetic resonance spectra

Proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra were determined on a JEOL A-400 Spectrometer (400 MHz) in CD_3OD solution, using tetramethylsilane as internal standard.

The two-dimensional NMR experiments were performed on a JNM A-400 spectrometer with VAX-station

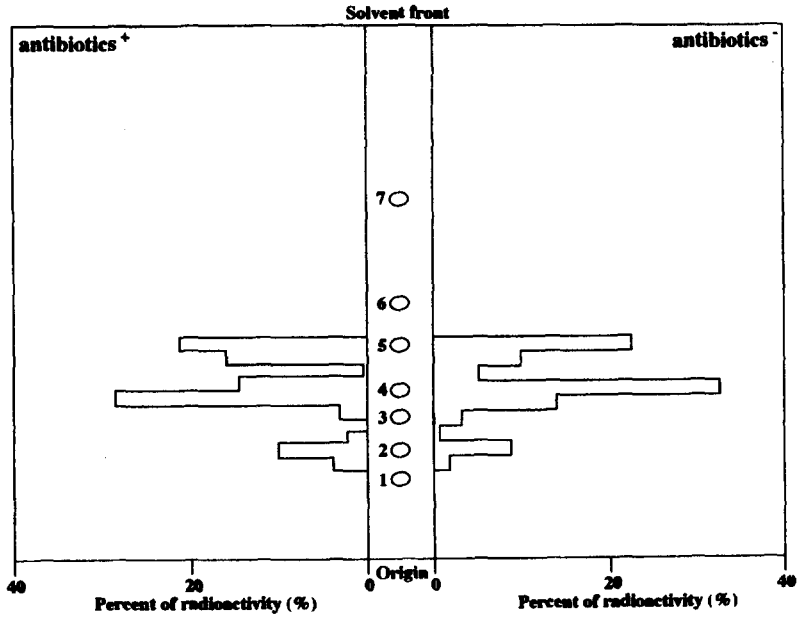

Fig. 3. Thin-layer chromatographic (TLC) analysis of radioactivity in feces for 7 days after intragastric administration of [^{3}H]bishomoCDC-sul. Right panel: group 1 (antibiotics -); left panel: group 2 (antibiotics +). The fecal bile acids were extracted and analyzed by TLC with solvent system EAW-1 (see Materials and Methods). Reference compounds are as follows: 1, cholyltaurine; 3, chenodeoxycholyltaurine; 5, bishomoCDC-sul; 6, cholylglycine; 7, chenodeoxycholylglycine; 2 and 4 were metabolites of bishomoCDC-sul.

3100, a 5 mm f reverse detection probe, and a broad-banded heteronuclear decoupling accessory. All experiments were carried out at 25°C. Heteronuclear single quantum correlation (HSQC) data were acquired without sample spinning.

The 1H–1H correlated spectroscopy (COSY) experiment was performed by applying the 90°–t1–90°–t2 pulse sequence using a 1024 (f2) × 512 (f1) data matrix with a spectral width of 2103 Hz in both dimensions. Before data processing, the t1 data were zero-filled once. Processing was done with sine squared filters in both dimensions. The final matrix had the dimensions 1024 × 1024.

The pulse sequence for the phase sensitive-nuclear Overhauser enhancement and exchange spectroscopy (PS-NOESY) experiment is shown in **Fig. 1.** For the acquisition 64 scans were made for each 512 individual experiments with a spectral width of 2310 Hz in both dimensions. Processing was done with shift sine squared filters in both dimensions. The final matrix had the dimensions 1024 × 1024.

The HSQC experiments were performed with spin-locked purge pulse using globally optimized alternating-phase rectangular pulses (GARP) decoupling. For the acquisition, 32 scans were made for each 256 individual experiments with a spectral width of 2160 Hz (f1) and 129571 Hz (f2). After zero-filling for t1 data, processing was carried out with an exponential filter coupled with apodization in both dimensions. The final data matrix had the dimensions 1024 × 512.

Bile acids

BishomoCDC-sul and [24,25-^{3}H]bishomoCDC-sul (sp. act., 2.6×10^6 dpm/μg) were prepared as described previously (22).

Metabolism of bishomoCDC-sul

In the first experiment, four male hamsters (Sasco, Omaha, NE) weighing 105 ± 6 g (group 1) were fed Purina chow containing 0.1% unlabeled bishomoCDC-sul for 13 days. On the 6th day, a solution of [^{3}H]bishomoCDC-sul (1 mg in saline) was administered by stomach tube. Feces and urine were collected for 7 days. Fecal bile acids were extracted with ethanol for 16 h in a Soxhlet apparatus. Radioactivity of feces and urine was determined by liquid scintillation counting. Fecal bile acids were analyzed by TLC to detect the metabolites of the administered sulfonate using solvent system EAW-1 (ethyl acetate–acetic acid–water, 7:2:1, by vol), as described previously (13). On the 13th day, the hamsters were anesthetized by intra-abdominal injection of 20 mg/100 g body weight of ketamine (Ketaset, Bristol Labs, Syracuse, NY) and bile was removed from the

TABLE 1. Effect of bishomoCDC-sul administration on biliary bile acid composition compared to chow fed controls

Bile Acids	Chow	BishomoCDC-sul
	%	%
Cholyltaurine	46.1 ± 5.3	13.7 ± 1.4
Cholylglycine	20.6 ± 4.0	5.9 ± 0.8
Chenodeoxycholyltaurine	18.6 ± 3.3	20.2 ± 1.2
Chenodeoxycholylglycine	7.0 ± 2.5	10.0 ± 2.3
Deoxycholyltaurine	4.7 ± 2.4	3.6 ± 0.4
Deoxycholylglycine	2.1 ± 0.2	1.0 ± 0.1
Lithocholyltaurine	0.4 ± 0.1	0.4 ± 0.1
Lithocholylglycine	0.5 ± 0.1	1.3 ± 0.8
bishomoCDC-sul	–	19.1 ± 0.8
Metabolite	–	19.8 ± 4.3
Others	–	5.0 ± 0.6

Hamsters were fed chow containing 0.1% sodium 3α,7α-dihydroxy-25,26-bishomo-5β-cholane-26-sulfonate (bishomoCDC-sul) for 13 days. Chow-fed controls were fed for comparison.

gallbladder with a 100 μL Hamilton syringe (Hamilton Co., Reno, NV). The biliary bile acid composition was determined by HPLC. In a second experiment, after a 3-day induction period on chow with antibiotics [0.25% neomycin sulfate (Sigma, St. Louis, MO) and 2,500 units polymyxin B sulfate (Sigma, St. Louis, MO)/g of food], four male hamsters weighing 123 ± 12 g (group 2) were fed chow with bishomoCDC-sul and these antibiotics for 13 days. In a preliminary experiment, the administration of neomycin and polymyxin B abolished deoxycholic acid and lithocholic acid in bile (data not shown). Administration of radiolabeled bishomoCDC-sul and manipulation of feces and urine were performed as with group 1.

Isolation and purification of the major metabolite

The fecal ethanolic extract of group 1 was evaporated and the residue was dissolved in methanol. This solution was applied as a band on a silica gel H plate and developed with solvent system EAW-1. The compounds were made visible with iodine vapor, and the band containing the metabolite was scraped into a glass column. The column was eluted with benzene (20 mL), ethyl acetate (40 mL), acetone (40 mL), and methanol (40 mL). The methanol fraction was evaporated and 29 mg of a crude residue was obtained. This residue was further purified by preparative TLC as described above. The material obtained was crystallized from methanol/ethyl acetate; 9.1 mg of the metabolite was obtained.

Calculations

The numerical data are expressed as mean ± SEM.

RESULTS

A feeding experiment was carried out to study the in vivo metabolism of bishomoCDC-sul. Eight hamsters were fed a diet containing 0.1% bishomoCDC-sul with or without antibiotics for 13 days and ingested, on the average, 9.1 ± 0.5 g of food per day. All animals remained healthy during the feeding period. On day 7, ^{3}H-labeled bishomoCDC-sul was administered intragastrically. **Figure 2** shows the cumulative recovery of radioactivity in feces and urine. In group 1 (without antibiotics), more than 80% of the ^{3}H was recovered in the feces within 7 days; no label was detected in the urine. The half-time ($t_{1/2}$) of fecal excretion was 1.6 days. In group 2 (with antibiotics), fecal and urinary recovery of radioactivity were 25% and 17%, respectively.

Semi-quantitative analysis of the radioactive compounds in feces by TLC is illustrated in **Fig. 3.** The data of group 1 showed that 38.5% of the tritium-labeled bishomoCDC-sul remained unchanged and that the remainder was transformed into two, more polar compounds (50.0% and 11.5%, respectively). The same compounds were found in group 2; bishomoCDC-sul and the two polar products amounted to 44.0%, 30.8% and 25.1%, respectively. There was no evidence of any less polar material (a "litho-derivative") suggesting that bishomoCDC-sul was completely resistant to bacterial 7-dehydroxylation.

The bile acid composition of gallbladder bile of group 1 was analyzed by HPLC at the end of the 13-day feeding period. The data for these animals and hamsters fed only a chow diet are summarized in **Table 1.** Significant proportions of the biliary bile acids were present as the administered compound (19.1%) and the major polar metabolite (19.8%), with concomitant reductions of cholyl bile acid conjugates compared to chow-fed controls.

The major metabolite from feces of group 1 was isolated and purified by preparative TLC, as described above, then analyzed by FABMS and NMR. The positive FAB spectrum of the major metabolite showed an ion

TABLE 2. Proton NMR of bishomoCDC-sul and its metabolite[a]

Proton	BishomoCDC-sul[b]	Metabolite
3β-H	3.36, m	3.39, m
7β-H	3.79, m	4.01, m
18-Me	0.67, s	0.72, s
19-Me	0.92, s	0.93, s
21-Me	0.95, d, J = 6.4 Hz	0.94, d, J = 6.6 Hz
26-CH_2	2.78, m	2.78, m
Hydroxylated		3.97, m

[a]The spectra were measured in CD_3OD solution.
[b]BishomoCDC-sul, sodium 3α,7α-dihydroxy-25,26-bishomo-5β-cholane-26-sulfonate.

at m/z 509, $(M+H)^+$, which was increased by 16 mass units in comparison to the parent bishomoCDC-sul (m/z 493). The ^{1}H-NMR spectrum of the metabolite (**Table 2**) showed a new triplet of doublets at 3.97 ppm corresponding to a proton geminal to the new hydroxyl group, which was coupled to three other hydrogens. The H,H-COSY spectrum located these at 1.49, 1.76, and 1.87 ppm (**Fig. 4**). The only positions for the new hydroxyl group compatible with these data were on C-11, 15, 16, or 22. The quartet corresponding to H-4α at 2.26 ppm in the parent compound was still present in the ^{1}H-NMR spectrum of the metabolite, suggesting that in the metabolite the A/B junction was still *cis* and that the hydroxyl groups on 3α and 7α were unchanged (23). The signals corresponding to the methyl groups had not been shifted appreciably; H-7 was deshielded from 3.79 to 4.01 ppm, indicating that the new hydroxyl group was located in its proximity. A model compound survey

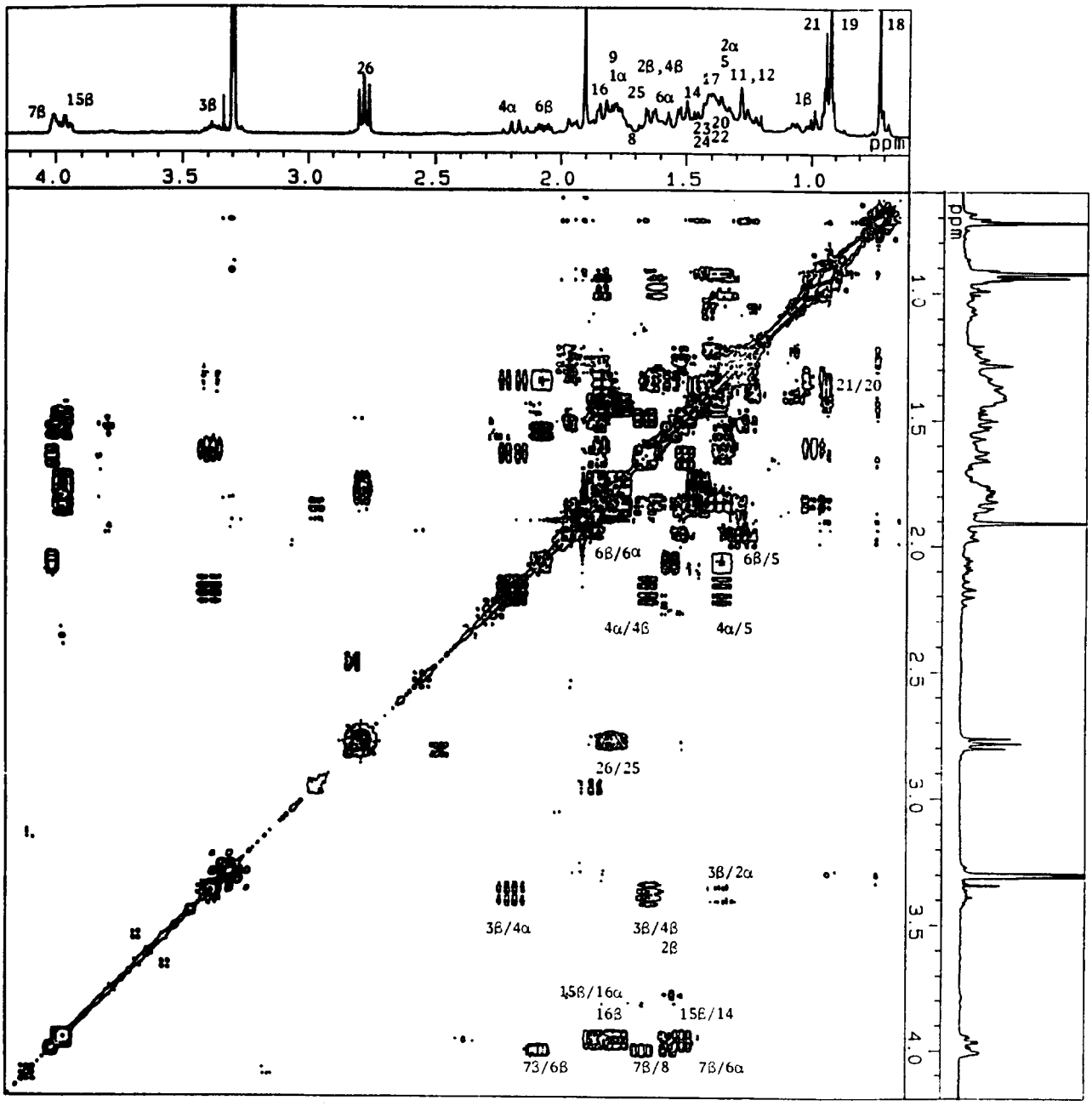

Fig. 4. H,H-COSY spectrum of the major metabolite in CD_3OD. The signal at 1.9 ppm is a solvent impurity (acetic acid or sodium acetate).

TABLE 3. NMR data of bishomoCDC-sul and its metabolite

Carbon No.	BishomoCDC-sul [^{13}C]	Metabolite [^{13}C]	Metabolite H_α	Metabolite H_β
1	36.6–	36.5–	1.85	~1.0
2	31.4–	31.3–	1.34	1.63
3	73.0+	73.0+	–	3.39
4	40.5–	40.1–	2.18	1.64
5	43.3+	43.0+	–	1.36
6	35.9–	34.1–	1.57	2.07
7	69.1+	69.0+	–	4.01
8	40.9+	40.5+	–	1.62
9	34.1+	35.4+	1.86	–
10	36.3	35.9	–	–
11	21.8–	21.9–	1.53	1.27
12	41.1–	41.7–	1.28	1.96
13	43.7	44.9	–	–
14	51.6+	59.5+	1.49	–
15	24.7–	72.7+	–	3.97
16	29.4–	41.3–	1.87[a]	1.76[a]
17	57.7+	55.9+	1.43	–
18	12.2+	13.5+	0.72	
19	23.4+	23.4+	0.93	
20	37.2+	36.6+	1.35-1.45	
21	19.3+	19.0+	0.94	
22	37.0–	36.8–	135-1.45	
23	27.4–	26.9–	1.24, 1.43	
24	30.4–	30.3–	1.36-1.50	
25	26.1–	26.0–	1.70-1.90	
26	52.8–	52.8–	2.78, 2.78	

The spectra were measured as CD_3OD solution. BishomoCDC-sul, sodium 3α,7α-dihydroxy-25,26-bishomo-5β-cholane-26-sulfonate; + or – indicates DEPT sign. ^{1}H chemical shifts are from the HSQC spectrum. α and β do not apply to the side chain; the chemical shifts for the two protons on the side chain are reported as a range if the cross peaks are not resolved.

[a]Assignments can be exchanged.

showed that only a hydroxyl group at 15α or 15β was likely to affect the signal of H-7β without shifting appreciably the signals for Me-18 and Me-19 (24).

The ^{13}C-NMR spectrum and distortionless enhancement by polarization transfer (DEPT) editing of the metabolite (**Table 3**) confirmed the presence of a third secondary hydroxyl group (carbon resonating at 73.0 ppm). The signals corresponding to carbons on the side chain, C-1 to C-13, and Me-19 were essentially unchanged. The easily identifiable resonances for C-15 and C-16 at 24.7 and 29.4 ppm had disappeared while a new methine at 59.5 was present. The latter could only be due to a carbon β to the new hydroxyl group. As these are typically deprotected by 6–10 ppm relative to the parent compound, it could only come from deshielding of C-14, originally at 51.6 ppm. The third hydroxyl group was therefore located on C-15. The assignment was confirmed by COSY and HSQC spectra. In the COSY spectrum of the metabolite the signal corresponding to the new CHOH group exhibited a cross peak with a proton at 1.49 ppm (H-14), which in turn connected with C-14 at 59.5 ppm in HSQC (Table 3). In addition, H-15β also showed COSY cross peaks with two protons at 1.76 and 1.87 ppm, which correlated with the signal at 41.3 ppm in HSQC, assigned to C-16. A full assignment of proton and carbon resonances of the metabolite is presented in Tables 2 and 3.

The stereochemistry of the 15-OH group was established as α by the -1.8 ppm shift exhibited by C-17 in the metabolite (relative to the parent compound). Replacement of the 15α-H in the parent compound by a hydroxyl group eliminates the 1,3-diaxial interaction as defined by Beierbeck, Saunders, and ApSimon (25) with H-17, resulting in protection of C-17. A 15β-OH group

would have caused protection of C-8 by the same mechanism, as can be found in model compounds (26). Direct evidence for the 15α-configuration was obtained by the presence of a cross peak between H-15β and Me-18 in the PS-NOESY spectrum of the metabolite (data not shown).

The observed ^{13}C-NMR spectrum of the metabolite was also in full agreement with that predicted by applying known 15α-OH shift values to the spectrum of bishomoCDC-sul (26). The structure of the metabolite is then sodium 3α,7α,15α-trihydroxy-25,26-bishomo-5β-cholane-26-sulfonate (II, **Fig. 5**). It is interesting to note that C-6 and C-9 appear at slightly different chemical shifts in I and II. These two carbons carry axial hydrogens and may reflect subtle deformations of the steroid nucleus brought about by the introduction of the 15α-OH group and/or solvation differences.

DISCUSSION

We previously demonstrated that after acute or chronic administration, the sulfonate analogues of CDCA, UDCA, and norUDCA underwent enterohepatic circulation in the hamster, without biotransformation by the liver or the intestinal microorganisms (8, 14). Similarly, in acute experiments with bile fistula animals, bishomoCDC-sul, a bile acid analogue with a longer side chain, was excreted in the bile unchanged (22). It is known that the metabolism of bile acids and bile acid analogues is affected by the structure of the nucleus and the side chain (15–18). In the present study, we investigated the metabolism of bishomoCDC-sul during chronic administration and found polar metabolites in bile and feces of the hamsters. To determine the site of biotransformation, we fed the sulfonate and antibiotics simultaneously, and the same metabolites were obtained.

When bishomoCDC-sul was administered intragastrically, more than 80% of the sulfonate radioactivity was excreted into the feces of group 1 within 7 days (Fig. 2). The $t_{1/2}$ of fecal excretion was 1.6 days. Previously, it was reported that in the hamster the $t_{1/2}$ for the fecal excretion of CDCA was 1.8 days (27). Thus, the absorption and excretion rate of bishomoCDC-sul were similar to those of CDCA conjugates. In group 2, the administration of antibiotics reduced the excretion of radioactivity in the feces (25%) and increased urinary radioactivity (17%). The exact mechanism of the effect of antibiotics on the rate and route of excretion of bishomoCDC-sul and metabolites is unclear. Similar effects of antibiotics on bile acid excretion have been reported previously; the much longer transit time of intestinal contents in germ free animals increased absorption and reduced excretion of bile acid (28, 29).

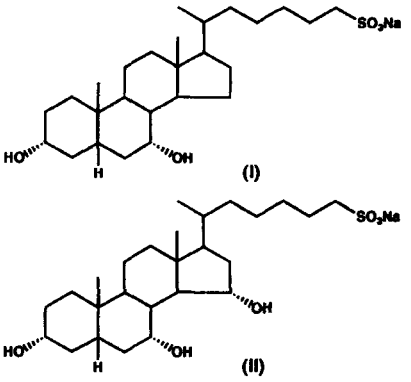

Fig. 5. I, Sodium 3α,7α-dihydroxy-25,26-bishomo-5β-cholane-26-sulfonate (bishomoCDC-sul); II, sodium 3α,7α,15α-trihydroxy-25,26-bishomo-5β-cholane-26-sulfonate.

We previously reported that CDC-sul, which is more hydrophilic than bishomoCDC-sul, was excreted without biotransformation after chronic oral administration in the hamsters used in the Hiroshima laboratory (14). When this chronic feeding experiment was repeated in the Sasco hamster in the New York laboratory, identical results (no biotransformation of CDC-sul) were obtained (A. Ohshima, E.H. Mosbach, B.I. Cohen, and N. Ayyad, unpublished observations). In the present study, bishomoCDC-sul was biotransformed to more polar compounds with or without antibiotics. Reversed phase HPLC data show that bishomoCDC-sul was more hydrophobic than CDC-sul (relative retention time was 1.0 for CDC-tau, 1.26 for CDC-sul and 2.61 for bishomoCDC-sul). The relatively high hydrophobicity of bishomoCDC-sul may explain why it was hydroxylated by the liver. The higher hydrophobicity of bishomoCDC-sul could have led to longer hepatic retention and also to different partitioning or transport into intracellular compartments (endoplasmic reticulum) where hydroxylation takes place.

There was no evidence from the TLC analyses of bile and feces that bishomoCDC-sul was 7-dehydroxylated by the intestinal bacteria. Bacterial 7-dehydroxylation occurs mainly with unconjugated bile acids and requires a free carboxylic moiety at the end of the side chain (9, 10). The side chain of bishomoCDC-sul has a terminal sulfonic acid group like a taurine-conjugated bile acid. This may explain why bishomoCDC-sul resisted bacterial 7-dehydroxylation during enterohepatic cycling so that no "litho"-type metabolites were detected.

After chronic administration (13 days), bishomoCDC-sul (19.1%) and its major metabolite (19.8%) accounted for 39% of total bile acids in gallbladder bile. When UDC-sul or norUDC-sul was administered for 14 days, 24% and 17%, respectively, of the sulfonates were present in the bile; no metabolites, either polar (hydroxylated) or non-polar (litho-derivatives), were detected. These results indicate that sulfonates with long side

chains accounted for a higher proportion of total bile acids in the bile. Administration of sulfonates with equal chain length (CDC-sul vs. UDC-sul) indicated that the relative hydrophobicity affected bile acid composition (30). The inhibitory effect of bile acids on cholesterol 7α-hydroxylase is a function of the bile acid hydrophobicity (31, 32). These findings may explain why the proportion of the more hydrophobic bishomoCDC-sul in bile was relatively elevated in comparison with CDC-sul and UDC-sul. The bile enrichment could also be due to competition among bishomoCDC-sul, its metabolites, and the endogenous conjugated bile acids for the ileal carrier.

The major metabolite obtained from the feces of group 1 hamsters was isolated and purified by preparative TLC. The chemical standard was determined by FABMS, ^{1}H-NMR, ^{13}C-NMR, H,H-COSY, HSQC, and PS-NOESY. On the basis of these experiments, the metabolite was shown to be the 15α-hydroxylated product of bishomoCDC-sul, namely 3α,7α,15α-trihydroxy-25,26-bishomo-5β-cholane-26-sulfonate. This is the first demonstration that 15α-hydroxylation occurs in the liver of the hamster. The 15α-hydroxylation of bile acids by other species has been reported previously. 15α-Hydroxy C_{21} bile acids were formed from cholesterol in the rat (33), and 3α,15α-cholan-24-oic acid was found to occur naturally in the wombat (34).

In conclusion, the sulfonate analogue of bishomoCDC, namely bishomoCDC-sul, underwent enterohepatic circulation in the hamster just like the endogenous bile acids. During enterohepatic cycling, bishomoCDC-sul was hydroxylated in the liver to form more polar metabolites and not dehydroxylated by microorganisms. The major metabolite was identified as 15α-hydroxy-bishomoCDC-sul (3α,7α,15α-trihydroxy-25,26-bishomo-5β-cholane-26-sulfonate).

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