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Synthesis of Disulfates of Unconjugated and Conjugated Bile Acids[1)]

JUNICHI GOTO, YOSHIHISA SANO, TOSHIYUKI CHIKAI
and TOSHIO NAMBARA*

Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980, Japan

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The disulfates of unconjugated, and glycine- and taurine-conjugated bile acids have been synthesized. Cholic acid derivatives appropriately protected were sulfated with sulfur trioxide–triethylamine complex in pyridine in the usual manner. Subsequent hydrolysis and/or sodium borohydride reduction provided the desired disulfates of cholates in satisfactory yields. Dihydroxylated bile acid disulfates were also prepared. The nuclear magnetic resonance spectral data for bile acid disulfates and related compounds are tabulated.

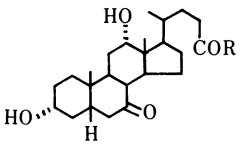
Keywords——sulfation; bile acid disulfate; glycine conjugate; taurine conjugate; *p*-nitrophenyl ester; active ester method

In recent years, considerable attention has been focused on the metabolic significance of sulfation of bile acids in hepatobiliary diseases. In order to aid research, the monosulfates of unconjugated and conjugated bile acids have been previously synthesized as standard samples.[2,3)] In addition, a novel method for simultaneous determination of the 3-sulfates in biological fluids by high-performance liquid chromatography (HPLC) has been developed.[4)] Recently, we disclosed the occurrence of bile acid 7-sulfates in urine from patients with primary biliary cirrhosis and congenital biliary atresia.[5)] Accordingly, it seems likely that disulfated bile acids are potential metabolites of bile acids in living animals. A particular interest in the relationship between bile acid metabolism and liver diseases prompted us to develop a new method for simultaneous determination of the disulfates using HPLC without prior deconjugation. For this purpose the 3,7-, 3,12- and 7,12-disulfated bile acids were required as authentic specimens.

Our initial effort was directed to the preparation of unconjugated, and glycine- and taurine-conjugated cholate 7,12-disulfates. Difficulties were encountered with selective acylation of the 3α-hydroxyl group of cholic acid since there was no marked difference in reactivity between the 3α- and 7α-hydroxyl groups, so 7-oxodeoxycholic acid methyl ester (**2**), obtainable from cholic acid by the known method,[6)] was used as a starting material. Selective acetylation of **2** proceeded with ease, providing a partially acylated product, on treatment with acetic anhydride and pyridine in benzene under mild conditions. The nuclear magnetic resonance (NMR) spectral data justified the structural assignment as the 3-monoacetate (**6**). The 3β-hydrogen appeared at *ca.* 4.5 ppm as a broad signal ($W_{1/2}=20$ Hz), while the 12β-hydrogen exhibited a signal ($W_{1/2}=7$ Hz) at *ca.* 3.9 ppm. Compound **6** was then reduced with sodium borohydride under ice-cooling to afford methyl cholate 3-acetate (**9**). Sulfation of **9** with sulfur trioxide–triethylamine complex in pyridine[7)] followed by alkaline hydrolysis furnished the desired cholic acid 7,12-disulfate (**12**).

The 7,12-disulfates of glycine- and taurine-conjugated bile acids were also synthesized in the same manner. 7-Oxodeoxycholic acid (**1**), derived from **2** by alkaline hydrolysis, was subjected to partial acetylation to give the 3-monoacetate (**5**). Condensation of **5** with *p*-nitrophenol was effected by use of *N*,*N*′-dicyclohexylcarbodiimide, yielding the *p*-nitrophenyl

ester. Further treatment with ethyl glycinate and taurine provided the glycine (**7**) and taurine (**8**) conjugates, respectively. Upon metal hydride reduction these conjugates were transformed into 7α-hydroxylated compounds (**10**, **11**), which, on sulfation followed by elimination of the protecting groups, were converted to the desired glycine- and taurine-conjugated cholate 7,12-disulfates (**13**, **14**).



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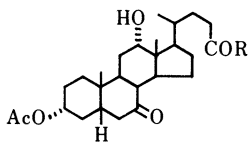
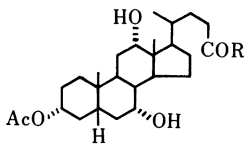
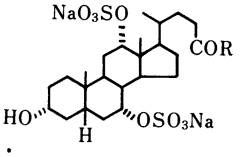
2: R=OCH_3
3: R=$NHCH_2COOC_2H_5$
4: R=$NHCH_2CH_2SO_3H$



5: R=OH
6: R=OCH_3
7: R=$NHCH_2COOC_2H_5$
8: R=$NHCH_2CH_2SO_3H$



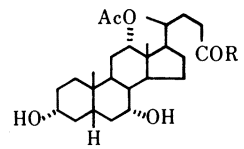
9: R=OCH_3
10: R=$NHCH_2COOC_2H_5$
11: R=$NHCH_2CH_2SO_3H$



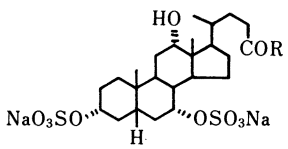
12: R=ONa
13: R=$NHCH_2COONa$
14: R=$NHCH_2CH_2SO_3Na$

Chart 1. Cholate 7,12-Disulfates and Related Compounds

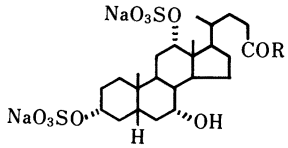
The synthesis of cholate 3,7-disulfates was then undertaken. Methyl cholate 3,12-diacetate, formed from **2** by usual acetylation with acetic anhydride in pyridine and metal hydride reduction, was converted to cholic acid 12-monoacetate (**15**) by brief exposure to 5% methanolic sodium hydroxide. In a similar fashion **15** was transformed into the *p*-nitrophenyl ester, which in turn was condensed with ethyl glycinate and taurine to yield the glycine (**17**) and taurine (**18**) conjugates. Treatment of the resulting cholate 12-monoacetates (**16**, **17**, **18**) with sulfur trioxide–triethylamine complex in pyridine and subsequent alkaline hydrolysis afforded the desired 3,7-disulfated cholates (**19**, **20**, **21**).



15: R=OH
16: R=OCH_3
17: R=$NHCH_2COOC_2H_5$
18: R=$NHCH_2CH_2SO_3H$



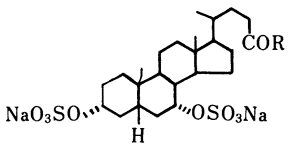
19: R=ONa
20: R=$NHCH_2COONa$
21: R=$NHCH_2CH_2SO_3Na$



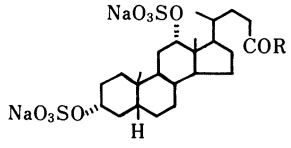
22: R=ONa
23: R=$NHCH_2COONa$
24: R=$NHCH_2CH_2SO_3Na$

Chart 2. Cholate 3,7- and 3,12-Disulfates and Related Compounds

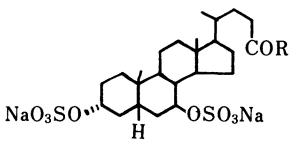
Next, preparation of cholate 3,12-disulfates was carried out. Condensation of **1** with *p*-nitrophenol yielded the *p*-nitrophenyl ester, which, on treatment with ethyl glycinate and taurine, were led to glycine (**3**) and taurine (**4**) conjugates, respectively. Compounds **2**, **3** and **4** were subjected to sulfation in the same manner as described above followed by borohydride reduction and elimination of the protecting groups, providing the desired cholate 3,12-disulfates (**22**, **23**, **24**).



25: R=ONa
26: R=$NHCH_2COONa$
27: R=$NHCH_2CH_2SO_3Na$



28: R=ONa
29: R=$NHCH_2COONa$
30: R=$NHCH_2CH_2SO_3Na$



31: R=ONa
32: R=$NHCH_2COONa$
33: R=$NHCH_2CH_2SO_3Na$

Chart 3. Dihydroxylated Bile Acid Disulfates

TABLE I. NMR Spectral Data for Disulfated Bile Acids and Related Compounds

Compd.	Solv.[a]	18-CH_3	19-CH_3	21-CH_3[b]	3β-H	7β-H	7α-H	12β-H	$>NCH_2CO-$	$>NCH_2CH_2S-$[c]	-NH-
2	C	0.68	1.17	0.97	3.50			3.98			
3	C	0.67	1.15	1.01	3.56			3.92	3.93 d, 5.5 Hz		6.06
4	M	0.72	1.21	1.07	3.60			3.96		3.01 3.60	
6	C	0.69	1.20	0.97	4.63			4.00			
7	C	0.70	1.18	1.03	4.65			4.00	4.00 d, 5.5 Hz		6.02
8	M	0.73	1.23	1.06	4.60			3.96		2.98 3.59	
9	C	0.68	0.90	0.97	4.53	3.81		3.94			
10	C	0.68	0.90	1.00	4.53	3.83		3.95	3.98 d, 5.5 Hz		6.05
11	M	0.71	0.96	1.05	4.53	3.76		3.90		2.96 3.56	
12	M	0.76	0.92	1.07	3.28	4.42		4.65			
13	M	0.76	0.92	1.08	3.29	4.42		4.64	3.71 s		
14	M	0.76	0.92	1.07	3.30	4.43		4.64		2.94 3.56	
16	C	0.72	0.88	0.81	3.42	3.84		5.06			
17	C	0.73	0.87	0.83	3.40	3.83		5.02	3.96 d, 5.5 Hz		5.96
18	M	0.77	0.90	0.87	3.50	3.78		5.03		2.95 3.58	
19	M	0.71	0.94	1.01	4.13	4.43		3.93			
20	M	0.71	0.94	1.02	4.12	4.42		3.92	3.71 s		
21	M	0.71	0.93	1.01	4.12	4.42		3.91		2.94 3.57	
22	M	0.76	0.92	1.08	4.11	3.77		4.66			
23	M	0.76	0.92	1.08	4.09	3.76		4.64	3.71 s		
24	M	0.76	0.92	1.08	4.10	3.76		4.63		2.94 3.57	
25	M	0.68	0.94	0.95	4.13	4.42					
26	M	0.68	0.94	0.97	4.13	4.42			3.72 s		
27	M	0.69	0.95	0.96	4.14	4.43				2.95 3.58	
28	M	0.76	0.93	1.06	4.22			4.65			
29	M	0.76	0.94	1.08	4.23			4.65	3.73 s		
30	M	0.76	0.93	1.06	4.21			4.63		2.94 3.56	
31	M	0.69	0.97	0.95	4.20		4.20				
32	M	0.69	0.98	0.97	4.23		4.23		3.71 s		
33	M	0.69	0.97	0.97	4.23		4.23			2.94 3.56	

a) C, $CDCl_3$; M, CD_3OD. *b*) Doublet, $J=6$ Hz. *c*) Triplet, $J=7$ Hz.

Finally, the disulfates of dihydroxylated bile acids (**25**—**33**) were synthesized from unconjugated, and glycine- and taurine-conjugated chenodeoxycholates, deoxycholates and ursodeoxycholates by sulfation with sulfur trioxide–triethylamine complex in pyridine.

These disulfates were purified by use of octadecyl silyl bonded silica instead of Amberlite XAD-2 resin, which is widely employed for the polar compounds in biological fluids, because the disulfates were partially decomposed into the monosulfates during the extraction procedure with the latter resin.

The NMR spectral data for the disulfates of bile acids and their derivatives are collected in Table I. These data may be helpful for the characterization of bile acids and related compounds.

Studies on the chromatographic separation of the disulfates of unconjugated and conjugated bile acids are being conducted in these laboratories and the details will be reported elsewhere in the near future.

Experimental

All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 polarimeter in water. NMR spectra were recorded on a JEOL FX-100 spectrometer at 100 MHz with tetramethylsilane as an internal standard.

General Procedure for Sulfation——Sulfur trioxide–triethylamine complex[7] (1.5 g) was added to a solution of bile acid (1 g) in anhydrous pyridine (6 ml), and the solution was stirred at room temperature overnight. The reaction mixture was poured into ice-cooled petroleum ether (70 ml) and the solid product obtained was collected. After being washed with petroleum ether the product was dissolved in 1 N methanolic NaOH (30 ml) and the solution was refluxed for 2—10 h for deprotection. After filtration of the reaction mixture, the filtrate was concentrated *in vacuo* and diluted with ether (200 ml). The resulting precipitate was collected, redissolved in 0.5 M sodium phosphate buffer (pH 7.0, 2 ml) and passed through a column (23 × 22 mm i.d.) of PrePAK-500/C_{18} (3 g, Waters Assoc., Milford, MA). The column was washed with water (5 ml), then the disulfate in the form of the sodium salt was eluted with 30% ethanol.

General Procedure for Preparation of Glycine Conjugates——*N,N'*-Dicyclohexylcarbodiimide (0.9 g) and *p*-nitrophenol (0.5 g) were added to a solution of bile acid (1 g) in anhydrous dioxane (10 ml)–AcOEt (20 ml), and the solution was stirred at room temperature overnight. After removal of the precipitate by filtration the filtrate was evaporated *in vacuo* and the oily residue obtained was chromatographed on silica gel (20 g). Elution with hexane–AcOEt (2 : 1—1 : 1) gave the *p*-nitrophenyl ester. Ethyl glycinate (500 mg) in pyridine (1 ml) was added to a solution of the *p*-nitrophenyl ester (1 g) in pyridine (4 ml) and the solution was stirred at room temperature for 3 h. The reaction mixture was poured into ice-water, acidified with 5% HCl and extracted with AcOEt. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated down *in vacuo*. The oily residue was subjected to column chromatography on silica gel (20 g). Elution with hexane–AcOEt (1 : 2—1 : 10) gave the glycine conjugate.

General Procedure for Preparation of Taurine Conjugates——Taurine (400 mg) in water (4 ml) was added to a solution of bile acid *p*-nitrophenyl ester (1 g) in pyridine (20 ml) and the solution was stirred at room temperature overnight. The resulting solution was concentrated and the oily residue was subjected to column chromatography on silica gel (20 g). Elution with $CHCl_3$–MeOH (4 : 1—2 : 1) gave the taurine conjugate.

Trisodium Cholate 7,12-Disulfate (12)——Acetic anhydride (3 ml) in benzene (20 ml) was added to a solution of methyl 7-oxodeoxycholate[6] (**2**) (4 g) in pyridine (3 ml) and the solution was stirred at room temperature for 6.5 h. The reaction mixture was poured into ice-water, acidified with 5% HCl and extracted with AcOEt. The organic layer was washed successively with water, 5% $NaHCO_3$ and water, dried over anhydrous Na_2SO_4 and evaporated down *in vacuo*. Recrystallization of the crude product from acetone–hexane gave methyl 7-oxodeoxycholate 3-acetate (**6**) (2.9 g) as colorless needles. mp 176—177°C. A solution of **6** (1.9 g) in MeOH (50 ml) was treated with $NaBH_4$ (150 mg) under ice-cooling and the solution was stirred for 25 min. The reaction mixture was poured into ice-water, acidified with 5% HCl and extracted with AcOEt. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated down *in vacuo*. Recrystallization of the crude product from acetone–hexane gave methyl cholate 3-acetate (**9**) (1.3 g) as colorless needles. mp 151—152 °C. Compound **9** (1.2 g) was subjected to sulfation and the crude product was recrystallized from MeOH–ether to give **12** as colorless crystals. mp 164—166 °C (dec.). $[\alpha]_D^{15}$ +33.0° (c=0.11). *Anal.* Calcd for $C_{24}H_{37}Na_3O_{11}S_2 \cdot 2H_2O$: C, 42.98; H, 6.16. Found: C, 43.01; H, 6.23.

Trisodium Glycocholate 7,12-Disulfate (13)——7-Oxodeoxycholic acid (**1**) (2 g) was subjected to partial acetylation in the same manner as described for **6** to give 7-oxodeoxycholic acid 3-acetate (**5**) (1.5 g). Compound **5** was condensed with ethyl glycinate to give ethyl glyco-7-oxodeoxycholate 3-acetate (**7**). Treatment of **7** in the same manner as described for **12** followed by recrystallization from MeOH–ether gave **13** (300 mg) as colorless crystals. mp 163—164 °C (dec.). $[\alpha]_D^{17}$ +23.6° (c=0.11). *Anal.* Calcd for $C_{26}H_{40}NNa_3O_{12}S_2 \cdot 2H_2O$: C, 42.91; H, 6.09; N, 1.92. Found: C, 43.02; H, 6.25; N, 1.70.

Trisodium Taurocholate 7,12-Disulfate (14)——Compound **5** (600 mg) was condensed with taurine to give tauro-7-oxodeoxycholate 3-acetate (**8**). Treatment of **8** in the same manner as described for **12** followed by recrystallization from MeOH–ether gave **14** (150 mg) as colorless crystals. mp 150—152 °C (dec.). $[\alpha]_D^{18}$ +17.5° (c=0.09). *Anal.* Calcd for $C_{26}H_{42}NNa_3O_{13}S_3 \cdot 2H_2O$: C, 40.15; H, 5.96; N, 1.80. Found: C, 40.22; H, 5.85; N, 1.85.

Trisodium Cholate 3,7-Disulfate (19)——Methyl cholate 3,12-diacetate (3 g) was dissolved in 5% methanolic KOH and the solution was stirred at room temperature for 30 min. The reaction mixture was poured into ice-water, acidified with 5% HCl and extracted with AcOEt. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated down *in vacuo* to give cholic acid 12-acetate (**15**) in almost quantitative yield. Treatment of **15** with diazomethane followed by recrystallization from MeOH gave methyl cholate 12-acetate (**16**) (2.7 g) as colorless needles. mp 100—102 °C. Compound **16** (1.5 g) was subjected to sulfation and the crude product was

recrystallized from MeOH–ether to give **19** as colorless crystals. mp 163.5—167 °C (dec.). $[\alpha]_D^{17}$ +4.8° (c=0.10). *Anal.* Calcd for $C_{24}H_{37}Na_3O_{11}S_2 \cdot 2H_2O$: C, 42.98; H, 6.16. Found: C, 43.12; H, 6.18.

Trisodium Glycocholate 3,7-Disulfate (20)——Compound **15** (1 g) was condensed with ethyl glycinate to give ethyl glycocholate 12-acetate (**17**) (850 mg). Compound **17** (800 mg) was subjected to sulfation and the crude product was recrystallized from MeOH–ether to give **20** (450 mg) as colorless crystals. mp 165—167 °C (dec.). $[\alpha]_D^{18}$ +23.6° (c=0.11). *Anal.* Calcd for $C_{26}H_{40}NNa_3O_{12}S_2 \cdot H_2O$: C, 45.15; H, 5.82; N, 2.02. Found: C, 45.02; H, 5.86; N, 2.05.

Trisodium Taurocholate 3,7-Disulfate (21)——Taurocholate 12-acetate (**18**) (1.5 g), obtainable from **15** by condensation with taurine, was subjected to sulfation and the crude product was recrystallized from MeOH–ether to give **21** (700 mg) as colorless crystals. mp 156—160 °C (dec.). $[\alpha]_D^{20}$ +19.1° (c=0.10). *Anal.* Calcd for $C_{26}H_{42}NNa_3O_{13}S_3$: C, 42.10; H, 5.71; N, 1.89. Found: C, 42.35; H, 5.81; N, 1.90.

Trisodium Cholate 3,12-Disulfate (22)——Compound **2** (1.8 g) was subjected to sulfation and the crude product obtained was reduced with $NaBH_4$ in the same manner as described for **9**. Recrystallization of the crude product from MeOH–ether gave **22** (700 mg) as colorless crystals. mp 163—164.5 °C (dec.). $[\alpha]_D^{20}$ +46.7° (c=0.11). *Anal.* Calcd for $C_{24}H_{37}Na_3O_{11}S_2 \cdot H_2O$: C, 44.17; H, 6.02. Found: C, 44.29; H, 6.12.

Trisodium Glycocholate 3,12-Disulfate (23)——Treatment of ethyl glyco-7-oxodeoxycholate (**3**), obtainable from **1** (1.5 g) by condensation with ethyl glycinate, in the same manner as described for **22** followed by recrystallization from MeOH–ether gave **23** (750 mg) as colorless crystals. mp 167—168 °C (dec.). $[\alpha]_D^{20}$ +65.4° (c=0.10). *Anal.* Calcd for $C_{26}H_{40}NNa_3O_{12}S_2 \cdot 2H_2O$: C, 42.91; H, 6.09; N, 1.92. Found: C, 43.10; H, 6.03; N, 1.95.

Trisodium Taurocholate 3,12-Disulfate (24)——Treatment of tauro-7-oxodeoxycholate (**4**), obtainable from **1** (2 g) by condensation with taurine, in the same manner as described for **22** followed by recrystallization from MeOH–ether gave **24** (700 mg) as colorless crystals. mp 156—158 °C (dec.). $[\alpha]_D^{17}$ +72.7° (c=0.10). *Anal.* Calcd for $C_{26}H_{42}NNa_3O_{13}S_3 \cdot H_2O$: C, 41.10; H, 5.84; N, 1.84. Found: C, 41.02; H, 5.95; N, 1.80.

Trisodium Chenodeoxycholate 3,7-Disulfate (25)——Methyl chenodeoxycholate (2 g) was subjected to sulfation and the crude product was recrystallized from MeOH–ether to give **25** (1.3 g) as colorless crystals. mp 174.5—177 °C (dec.). $[\alpha]_D^{19}$ +0.2° (c=0.11). *Anal.* Calcd for $C_{24}H_{37}Na_3O_{10}S_2$: C, 46.60; H, 6.03. Found: C, 46.36; H, 6.01.

Trisodium Glycochenodeoxycholate 3,7-Disulfate (26)——Ethyl glycochenodeoxycholate (1.2 g) was subjected to sulfation and the crude product was recrystallized from MeOH–ether to give **26** (700 mg) as colorless crystals. mp 174.5—176 °C (dec.). $[\alpha]_D^{20}$ +18.4° (c=0.11). *Anal.* Calcd for $C_{26}H_{40}NNa_3O_{11}S_2 \cdot H_2O$: C, 45.01; H, 6.10; N, 2.02. Found: C, 44.92: H, 6.18; N, 2.05.

Trisodium Taurochenodeoxycholate 3,7-Disulfate (27)——Taurochenodeoxycholate (1.2 g) was subjected to sulfation and the crude product was recrystallized from MeOH–ether to give **27** (650 mg) as colorless crystals. mp 160—164 °C (dec.). $[\alpha]_D^{18}$ +9.3° (c=0.10). *Anal.* Calcd for $C_{26}H_{42}NNa_3O_{12}S_3 \cdot H_2O$: C, 41.99; H, 5.96; N, 1.88. Found: C, 42.05; H, 5.85; N, 1.80.

Trisodium Deoxycholate 3,12-Disulfate (28)——Methyl deoxycholate (1.8 g) was subjected to sulfation and the crude product was recrystallized from MeOH–ether to give **28** (1 g) as colorless crystals. mp 176—177 °C (dec.). $[\alpha]_D^{17}$ +67.0° (c=0.10). *Anal.* Calcd for $C_{24}H_{37}Na_3O_{10}S_2 \cdot H_2O$: C, 45.28; H, 6.17. Found: C, 45.04; H, 5.96.

Trisodium Glycodeoxycholate 3,12-Disulfate (29)——Ethyl glycodeoxycholate (800 mg) was subjected to sulfation and the crude product was recrystallized from MeOH–ether to give **29** (350 mg) as colorless crystals. mp 177—179 °C (dec.). $[\alpha]_D^{20}$ +40.0° (c=0.10). *Anal.* Calcd for $C_{26}H_{40}NNa_3O_{11}S_2 \cdot 3H_2O$: C, 42.79; H, 6.35; N, 1.92. Found: C, 42.51; H, 6.10; N, 1.84.

Trisodium Taurodeoxycholate 3,12-Disulfate (30)——Taurodeoxycholate (500 mg) was subjected to sulfation and the crude product was recrystallized from MeOH–ether to give **30** (200 mg) as colorless crystals. mp 165—168 °C (dec.). $[\alpha]_D^{18}$ +53.3° (c=0.10). *Anal.* Calcd for $C_{26}H_{42}NNa_3O_{12}S_3 \cdot 3H_2O$: C, 40.05; H, 6.20; N, 1.80. Found: C, 39.77; H, 6.18; N, 1.66.

Trisodium Ursodeoxycholate 3,7-Disulfate (31)——Methyl ursodeoxycholate (1.6 g) was subjected to sulfation and the crude product was recrystallized from MeOH–ether to give **31** (1.3 g) as colorless crystals. mp 182—184 °C (dec.). $[\alpha]_D^{17}$ +22.9° (c=0.11). *Anal.* Calcd for $C_{24}H_{37}Na_3O_{10}S_2 \cdot H_2O$: C, 45.28; H, 6.17. Found: C, 45.45; H, 6.55.

Trisodium Glycoursodeoxycholate 3,7-Disulfate (32)——Ethyl glycoursodeoxycholate (1 g) was subjected to sulfation and the crude product was recrystallized from MeOH–ether to give **32** (500 mg) as colorless crystals. mp 184—186 °C (dec.). $[\alpha]_D^{18}$ +43.9° (c=0.10). *Anal.* Calcd for $C_{26}H_{40}NNa_3O_{11}S_2 \cdot 3H_2O$: C, 42.79; H, 6.35; N, 1.92. Found: C, 42.61; H, 6.22; N, 1.87.

Trisodium Tauroursodeoxycholate 3,7-Disulfate (33)——Tauroursodeoxycholate (600 mg) was subjected to sulfation and the crude product was recrystallized from MeOH–ether to give **33** (230 mg) as colorless crystals. mp 166—168 °C (dec.). $[\alpha]_D^{19}$ +27.4° (c=0.11). *Anal.* Calcd for $C_{26}H_{42}NNa_3O_{12}S_2 \cdot 2H_2O$: C, 40.99; H, 6.09; N, 1.84. Found: C, 40.97; H, 6.10; N, 1.92.

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References and Notes

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