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Synthesis of Monosulfates of Unconjugated and Conjugated Bile Acids[1]

Junichi Goto, Hiroaki Kato, Fusae Hasegawa (née Sato),[2] and Toshio Nambara[2a]

Pharmaceutical Institute, Tohoku University[2]

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The 3-, 7- and 12-monosulfates of unconjugated, and glycine- and taurine-conjugated bile acids have been prepared. Bile acid derivatives protected by appropriate groups were sulfated in the usual manner with chlorosulfonic acid in pyridine. Subsequent removal of the protecting groups provided the desired sulfates of bile acids in satisfactory yields. The nuclear magnetic resonance spectral properties of sulfated bile acids and related compounds are briefly discussed.

Keywords——sulfation; *p*-nitrophenyl ester; active ester method; glycine-conjugate; taurine-conjugate; 7-monoacetate; 3-mono-*tert*-butyldimethylsilyl ether; ursodeoxycholate

Since the first report on the occurrence of sulfated bile acids in man[3,4] there has been considerable interest in the metabolic significance of sulfation of bile acids in liver diseases.[5,6] A marked increase in the level of 3-sulfated bile acids in biological fluids is observed in patients with liver cirrhosis, hepatitis, and hepatic obstruction.[7,8] In addition, Chen *et al.* recently demonstrated that the 7α-hydroxyl group of bile acids is also conjugated with sulfuric acid in the kidney.[9] The commonly used methods for determination of sulfated bile acids involve prior hydrolysis and/or solvolysis followed by chromatographic separation of liberated bile acids. However, these procedures have disadvantages such as the lack of reliability owing to incomplete hydrolysis, artifact formation and loss of information about the conjugated forms. 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 sulfates using high-performance liquid chromatography without deconjugation and derivatization. For this purpose the 3-, 7- and 12-monosulfates of bile acids were required as standard samples.

Our initial efforts were directed to the preparation of 3-monosulfated bile acids. The 7-monoformate of chenodeoxycholic acid (**34**), 12-monoformate of deoxycholic acid (**55**), and 7,12-diformate of cholic acid (**1**), obtainable by the procedure of Tserng *et al.*,[10] were condensed with *p*-nitrophenol by the dicyclohexylcarbodiimide method, yielding the *p*-nitrophenyl esters. Reaction of the activated esters with ethyl glycinate and taurine provided the glycine- (**38**, **59**, **5**) and taurine-conjugates (**41**, **62**, **8**), respectively. Treatment of the resulting 3-monohydroxy bile acids with chlorosulfonic acid in pyridine under mild conditions and subsequent hydrolysis with alkali to remove the protecting groups afforded the desired

1) Part CXXXXIV of "Studies on Steroids" by T. Nambara; Part CXXXXIII: H. Hosoda, Y. Sakai, H. Yoshida, S. Miyairi, K. Ishii, and T. Nambara, *Chem. Pharm. Bull.* (Tokyo), **27**, 742 (1979).
2) Location: *Aobayama, Sendai, 980, Japan*; *a*) To whom inquiries should be addressed.
3) R.H. Palmer, *Proc. Nat. Acad. Sci. U.S.A.*, **58**, 1047 (1967).
4) R.H. Palmer and M.G. Bolt, *J. Lipid Res.*, **12**, 671 (1971).
5) I. Makino, H. Hashimoto, K. Shinozaki, K. Yoshino, and S. Nakagawa, *Gastroenterology*, **68**, 545 (1975).
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8) I. Makino, K. Shinozaki, and S. Nakagawa, *Lipids*, **8**, 47 (1973).
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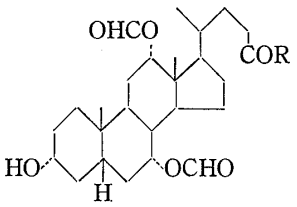

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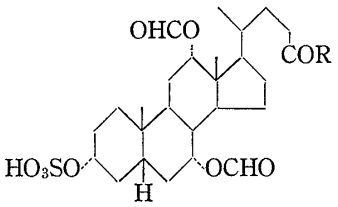
5: R=$NHCH_2COOC_2H_5$
8: R=$NHCH_2CH_2SO_3Na$



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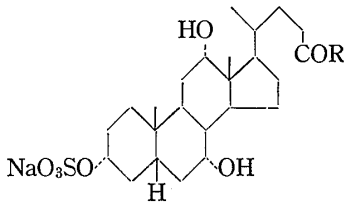

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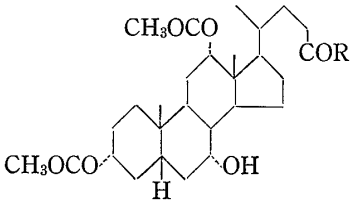

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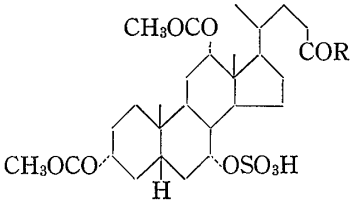
18: R=$NHCH_2CH_2SO_3H$



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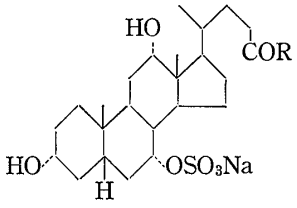

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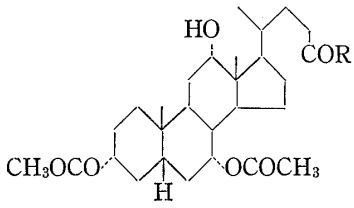
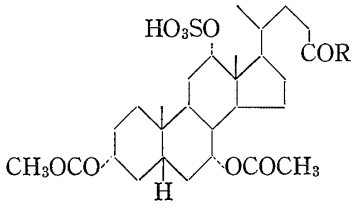


21: R=OH
22: R=OCH_3
25: R=$NHCH_2COOC_2H_5$
28: R=$NHCH_2CH_2SO_3H$



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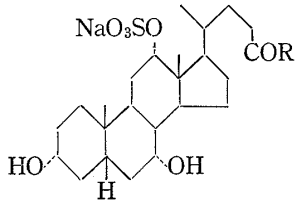

24: R=ONa
27: R=$NHCH_2COONa$
30: R=$NHCH_2CH_2SO_3Na$

Chart 1. Cholate and Related Compounds

3-sulfates of cholate (**4**, **7**, **10**), chenodeoxycholate (**37**, **40**, **43**), and deoxycholate (**58**, **61**, **64**) in fairly good yields. An attempt to prepare ursodeoxycholic acid 7-monoformate, *i.e.*, the C-7 epimer of **34**, resulted in failure, because there was no significant difference in reactivity between the 3- and 7-hydroxyl groups. Therefore, an alternative synthetic method involving selective protection of the C-3 hydroxyl function with a bulky substituent was undertaken. When ursodeoxycholic acid was treated with *tert*-butyldimethylsilyl chloride in dimethylformamide and pyridine, silylation occurred exclusively at the sterically less hindered position to provide the 3-monosilyl ether (**75**) almost quantitatively. Usual acetylation with acetic anhydride in pyridine gave the 3-silyl ether 7-acetate (**76**), which on desilylation with 5% hydrochloric acid in aqueous acetone[11] yielded the 7-monoacetate (**77**). In a similar fashion **77** was transformed into the *p*-nitrophenyl ester, which in turn was condensed with ethyl glycinate and taurine to yield the glycine- (**80**) and taurine-conjugates (**83**). In the nuclear magnetic resonance (NMR) spectra, the 3β-hydrogen of these intermediates appeared at *ca.* 3.6 ppm as a broad signal ($W_{1/2}$=20 Hz), while the 7α-hydrogen exhibited a broad signal ($W_{1/2}$=15 Hz) at *ca.* 4.8 ppm. Sulfation with chlorosulfonic acid in pyridine followed by alkaline hydrolysis furnished the desired ursodeoxycholate 3-sulfates (**79**, **82**, **85**).

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Table I. NMR Spectral Data for Sulfated 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–[d]	–CHO	–$COCH_3$
1	C	0.78	0.95	0.87	3.57	5.12		5.32				8.12 8.18	
3	M	0.81	0.97	0.86	4.12	5.06		5.24				8.12 8.18	
4	W	0.72	0.93	0.97	4.21	3.91		4.06					
5	C	0.76	0.94	0.87	3.64	5.10		5.30	4.04 d, 5.5 Hz		6.12	8.10 8.14	
6	M	0.84	1.01	0.92	4.12	5.09		5.29	3.95 s			8.15 8.22	
7	W	0.71	0.94	0.98	4.20	3.90		4.06	3.75 s				
9	M	0.83	1.00	0.89	4.20	5.09		5.29		3.00 3.63		8.15 8.21	
10	W	0.71	0.93	0.98	4.20	3.90		4.04		3.08 3.67			
12	C	0.74	0.90	0.83	4.61	3.93		5.13					2.01 2.12
13	M	0.78	0.96	0.82	4.62	4.52		5.10					2.00 2.12
14	W	0.74	0.96	0.98	3.53	4.53		4.08					
15	C	0.75	0.91	0.85	4.62	3.86		5.07	4.00 d, 5.5 Hz		5.96		2.02 2.11
17	W	0.72	0.94	0.98	3.64	4.48		4.06	3.78 s				
18	M	0.78	0.94	0.85	4.56	3.82		5.06		2.96 3.60			2.00 2.09
19	M	0.80	0.96	0.87	4.61	4.50		5.08		3.00 3.62			2.00 2.11
20	W	0.73	0.94	0.98	3.60	4.52		4.05		3.10 3.59			
21	C	0.69	0.94	0.99	4.60	4.93		4.04					2.04 2.08
23	M	0.79	0.98	1.05	4.54	4.92		4.69					2.02 2.06
24	W	0.76	0.92	0.98	3.49	3.90		4.68					
25	C	0.70	0.94	1.00	4.64	4.95		4.04	4.07 d, 5.5 Hz		6.06		2.06 2.10
26	M	0.78	0.97	1.09	4.58	4.90		4.68	3.88 s				2.00 2.04
27	W	0.77	0.96	1.02	3.60	3.84		4.71	3.50 s				
28	M	0.72	0.97	1.03	4.56	4.88		3.98		2.98 3.60			2.01 2.05
29	M	0.78	0.97	1.08	4.59	4.91		4.71		3.00 3.65			2.01 2.05
30	W	0.78	0.95	0.98	3.60	3.85		4.70		3.07 3.54			
32	C	0.76	1.21	0.83	4.69			5.12					2.03 2.11
34	C	0.68	0.96	0.96	3.55	5.09						8.12	
35	C	0.67	0.96	0.93	3.54	5.07						8.08	
36	M	0.73	1.01	0.95	4.20	5.05						8.11	
37	W	0.70	0.97		4.28	3.94							
38	C	0.68	0.96	0.95	3.54	5.07			4.05 d, 5.5 Hz		5.96	8.08	
39	M	0.72	0.99	1.00	4.20	5.05			3.93 s			8.11	
40	W	0.70	0.97		4.28	3.95			3.79 s				
41	M	0.71	0.98	0.99	3.58	5.02				2.99 3.61		8.10	
42	M	0.69	0.97		4.23	5.08				2.98 3.64			
43	W	0.68	0.96		4.22	3.92				3.11 3.59			
45	C	0.64	0.93		3.42	4.50							
46	W	0.69	0.97		3.54	4.49							
47	C	0.70	0.95	0.97	3.52	3.90			4.07 d, 5.5 Hz		6.08		
48	T	0.65	0.89		3.40	3.80			3.96 d, 5.5 Hz		6.40		
49	M	0.67	0.92	0.95	3.44	4.42			3.87 s				

50	W	0.67	0.94	0.96	3.52	4.48			3.72 s						
52	M	0.69	0.93	0.95	3.50	3.82					2.99	3.62			
53	M	0.69	0.93	0.95	3.50	4.42					2.99	3.63			
54	W	0.70	0.97		3.55	4.51					3.08	3.57			
55	C	0.77	0.94	0.85	3.68			5.26						8.12	
56	C	0.76	0.92	0.84	3.64			5.25						8.13	
57	M	0.79	0.95	0.84	4.26			5.22						8.16	
58	W	0.72	0.94		4.38			4.07							
59	C	0.75	0.92	0.85	3.64			5.25	4.02 d, 5.5 Hz				6.03	8.13	
60	M	0.79	0.95	0.88	4.24			5.24	3.88 s					8.17	
61	W	0.73	0.96	0.99	4.42			4.12	3.81 s						
62	C	0.80	0.95	0.87	3.60			5.22			2.98	3.60		8.16	
63	M	0.80	0.97	0.88	4.31			5.27			2.98	3.62		8.20	
64	W	0.71	0.95		4.39			4.08			3.11	3.62			
65	C	0.70	0.94	0.99	4.72			4.00							2.04
66	C	0.71	0.95	1.00	4.74			4.01							2.05
67	M	0.75	0.95	1.04	4.58			4.64							1.99
68	W	0.74	0.91		3.55			4.65							
69	C	0.68	0.92	0.98	4.70			3.97	4.02 d, 5.5 Hz				6.04		2.02
70	M	0.77	0.95	1.08	4.60			4.66	3.88 s						2.00
71	W	0.77	0.95	1.01	3.60			4.65	3.74 s						
72	M	0.72	0.96	1.01	4.70			3.98			2.98	3.62			2.02
73	M	0.79	0.97	1.09	4.67			4.72			3.04	3.66			2.02
74	W	0.77	0.95		3.60			4.70			3.12	3.63			
75	C	0.61	0.88		3.54		3.54								
76	C	0.67	0.95		3.54		4.80								2.00
78	M	0.73	1.02	0.96	4.26		4.78								2.00
79	W	0.74	0.99		4.26		3.64								
80	C	0.68	0.96	0.93	3.60		4.80		4.05 d, 5.5 Hz				6.05		2.00
81	M	0.73	1.01	0.97	4.26		4.78		3.75 s						2.00
82	W	0.74	1.00		4.34		3.64		3.78 s						
83	M	0.72	0.98	0.93	3.54		4.78				2.99	3.62			1.99
84	M	0.68	0.96	0.94	4.24		4.75				2.94	3.57			1.95
85	W	0.72	0.99		4.32		3.68				3.10	3.60			
86	M	0.69	0.98		3.52		4.32								
87	W	0.71	1.00		3.65		4.35								
88	C	0.67	0.93	0.93	3.58		3.58		4.05 d, 5.5 Hz				6.00		
89	M	0.70	0.98	0.98	3.50		4.30		3.80 s						
90	W	0.71	1.00		3.65		4.35		3.78 s						
91	M	0.69	0.95	0.95	3.56		3.56				2.98	3.60			
92	M	0.67	0.95		3.50		4.32				3.00	3.62			
93	W	0.71	1.00		3.66		4.37				3.11	3.60			

a) C: $CDCl_3$, M: CD_3OD, T: CCl_4, W: D_2O. *b*) doublet, $J=6$ Hz. *c*) triplet, $J=7$ Hz. *d*) triplet, $J=5.5$Hz.

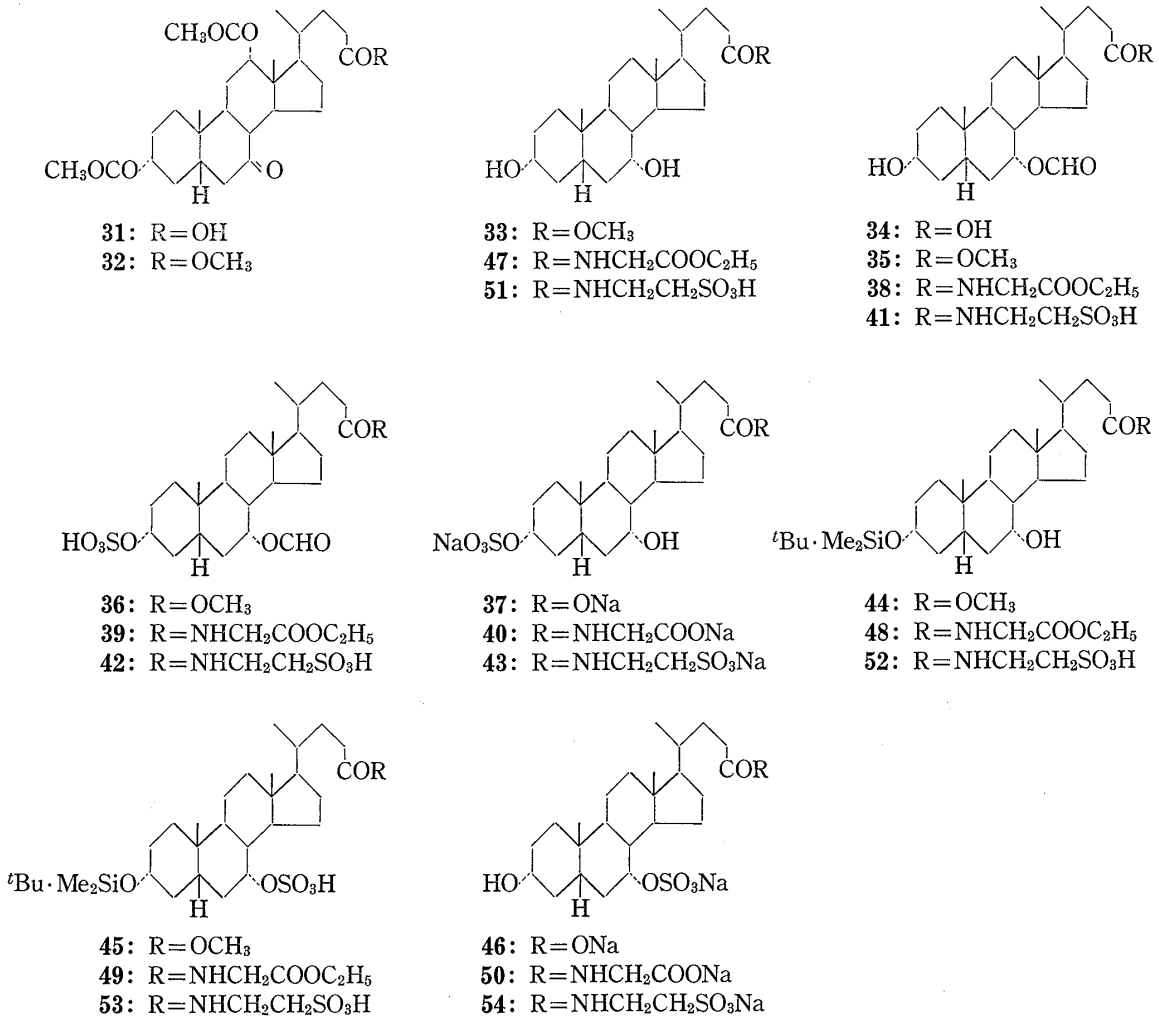
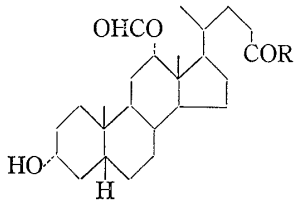


Chart 2. Chenodeoxycholate and Related Compounds

Next, preparation of the 7-monosulfates was carried out. 3-Mono-*tert*-butyldimethylsilyl ethers of chenodeoxycholate (**44**, **48**, **52**) and ursodeoxycholate (**75**, **88**, **91**), readily prepared in the manner described above, were converted into the sulfates by treatment with chlorosulfonic acid in pyridine. Upon removal of the protecting groups with acid under mild conditions[11] the sulfated 3-silyl ethers (**45**, **49**, **53**, **86**, **89**, **92**) yielded the 7-sulfates of chenodeoxycholate (**46**, **50**, **54**) and ursodeoxycholate (**87**, **90**, **93**). In the case of cholic acid, difficulties were encountered with partial deacylation and silylation because there appeared to be no marked difference in reactivity between the 7α- and 12α-hydroxyl groups. Fieser *et al.* showed that selective oxidation of the 7α-hydroxyl group of cholic acid can be effected by treatment with N-bromosuccinimide.[12] Indeed, oxidation of cholic acid with this reagent under weakly basic conditions followed by usual acetylation afforded solely 7-oxodeoxycholic acid 3,12-diacetate (**31**). The presence of the 19-methyl proton signal at 1.21 ppm and the absence of the 7β-hydrogen signal in the NMR spectra confirmed the structural assignment. Reduction of **31** with sodium borohydride under ice-cooling yielded the 7α-hydroxyl intermediate (**11**) as a main product. Condensation of the *p*-nitrophenyl ester with ethyl glycinate and taurine furnished the corresponding conjugates (**15**, **18**). After usual sulfation and elimination of the protecting groups, **11**, **15** and **18** were transformed into the desired cholate 7-sulfates (**14**, **17**, **20**), respectively.

12) L.F. Fieser and S. Rajagopalan, *J. Am. Chem. Soc.*, **71**, 3935 (1949).



55: R=OH
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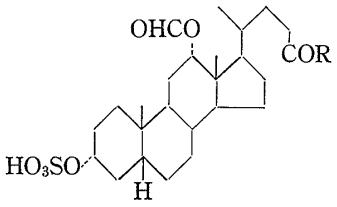

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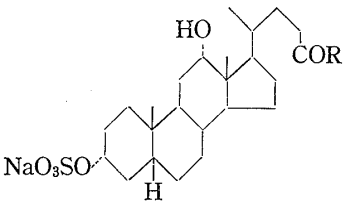
60: R=$NHCH_2COOC_2H_5$
63: R=$NHCH_2CH_2SO_3H$



58: R=ONa
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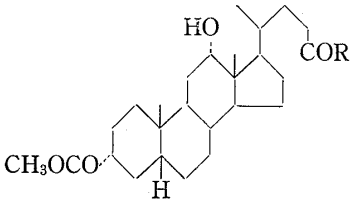

65: R=OH
66: R=OCH_3
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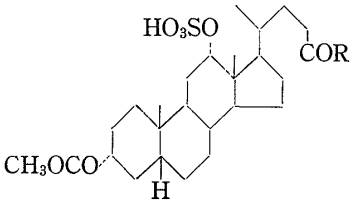
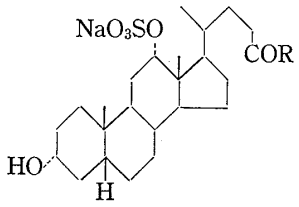


67: R=OCH_3
70: R=$NHCH_2COOC_2H_5$
73: R=$NHCH_2CH_2SO_3H$



68: R=ONa
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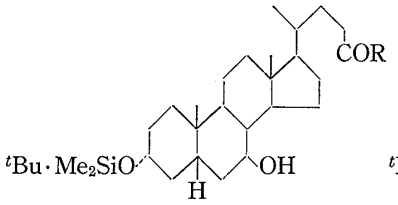
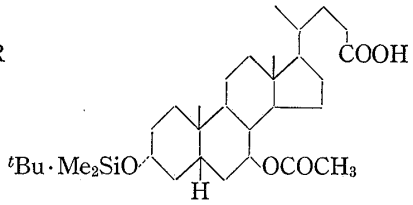
74: R=$NHCH_2CH_2SO_3Na$

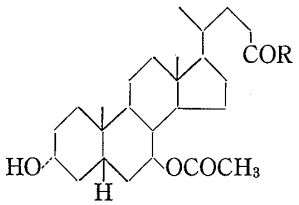
Chart 3. Deoxycholate and Related Compounds



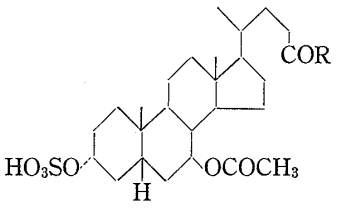
75: R=OH
88: R=$NHCH_2COOC_2H_5$
91: R=$NHCH_2CH_2SO_3H$



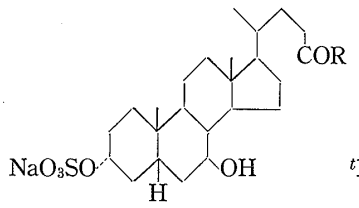
76



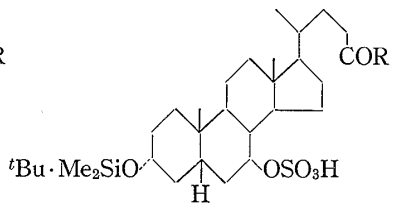
77: R=OH
80: R=$NHCH_2COOC_2H_5$
83: R=$NHCH_2CH_2SO_3H$



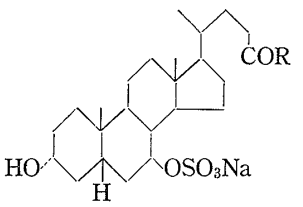
78: R=OH
81: R=$NHCH_2COOC_2H_5$
84: R=$NHCH_2CH_2SO_3H$



79: R=ONa
82: R=$NHCH_2COONa$
85: R=$NHCH_2CH_2SO_3Na$



86: R=OH
89: R=$NHCH_2COOC_2H_5$
92: R=$NHCH_2CH_2SO_3H$



87: R=ONa
90: R=$NHCH_2COONa$
93: R=$NHCH_2CH_2SO_3Na$

Chart 4. Ursodeoxycholate and Related Compounds

Finally, synthesis of the 12-sulfates was undertaken. Partial acetylation of cholic acid and deoxycholic acid was accomplished by treatment with acetic anhydride in pyridine and benzene[13] to provide 12-monohydroxy bile acids (**21**, **65**). In the NMR spectra of these compounds the 3β- and 7β-hydrogen signals appeared at *ca.* 4.6 ppm ($W_{1/2}$=20 Hz) and 4.9 ppm ($W_{1/2}$=7 Hz), each as a broad signal, while the 12β-hydrogen signal of the 12α-hydroxyl compound appeared at *ca.* 4.0 ppm ($W_{1/2}$=6 Hz) and that of the 12-acetate at *ca.* 5.1 ppm. The methyl esters (**22**, **66**), and glycine- (**25**, **69**) and taurine-conjugates (**28**, **72**), obtained from **21** and **65**, respectively, were subjected to sulfation with chlorosulfonic acid in pyridine followed by alkaline hydrolysis as described above to provide the desired 12-sulfates of cholate (**24**, **27**, **30**) and deoxycholate (**68**, **71**, **74**).

The NMR spectral data for various sulfated bile acids and their derivatives are collected in Table I. It should be noted that the signal of hydrogen attached to carbon bearing a sulfate group appears at lower field as a broad singlet compared to that of hydrogen on carbon carrying a hydroxyl function. The shift value (0.6—0.7 ppm) on sulfation is useful as a diagnostic for the structural elucidation of sulfated bile acids. The 18-methyl proton of the 12-hydroxyl compounds resonated at *ca.* 0.70 ppm whereas that of the corresponding sulfates appeared at *ca.* 0.77 ppm. The availability of these NMR spectral data may be helpful for the characterization of bile acids and related compounds.

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

Experimental[14]

General Procedure for Sulfation

To a solution of bile acid (500 mg) in anhydrous pyridine (5 ml) was added chlorosulfonic acid (0.5 ml) in anhydrous pyridine (5 ml) under ice-cooling and the solution was heated at 50° for 30—60 min. The resulting solution was poured into ice-water, acidified with conc. HCl, and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product was dissolved in H_2O (50—100 ml), adjusted to pH 10 with NaOH, and percolated through a column of Amberlite XAD-2 resin (200 ml). After thorough washing with H_2O the sulfated bile acid was eluted with MeOH (200 ml).

General Procedure for the Preparation of Glycine-Conjugate

To a solution of bile acid (3 g) in anhydrous dioxane (30 ml) were added *p*-nitrophenol (1.5 g) in anhydrous AcOEt (70 ml) and dicyclohexylcarbodiimide (2.7 g), 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 (80 g). Elution with hexane–AcOEt (7: 1—2: 1) gave *p*-nitrophenyl ester (2 g). To a solution of *p*-nitrophenyl ester (2 g) in pyridine (8 ml) was added ethyl glycinate (1 g) in pyridine (2 ml) and the solution was stirred at room temperature for 3 hr. The reaction mixture was poured into ice-water, acidified with conc. HCl, and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated to give the glycine-conjugate.

General Procedure for the Preparation of Taurine-Conjugate

To a solution of bile acid *p*-nitrophenyl ester (2 g) in pyridine (50 ml) was added taurine (600 mg) in H_2O (5 ml) and the solution was stirred at room temperature overnight. The resulting solution was concentrated and the oily residue obtained was chromatographed on silica gel (60 g). Elution with $CHCl_3$–MeOH (6: 1—3: 1) gave the taurine-conjugate.

Preparation of 7-,12-Monoformate and 7,12-Diformate of Bile Acids

To a solution of cholic acid, chenodeoxycholic acid or deoxycholic acid (500 mg) in benzene (20 ml) were added 85% formic acid (4 ml) and *p*-toluenesulfonic acid monohydrate (200 mg) and the solution was azeotropically distilled over a period of 3 hr. The resulting solution was diluted with AcOEt, washed with

13) L.F. Fieser and S. Rajagopalan, *J. Am. Chem. Soc.*, **72**, 5530 (1950).

14) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured by a JASCO Model DIP-4 polarimeter in H_2O unless otherwise specified. NMR spectra were recorded on a JEOL Model PS-100 spectrometer at 100 MHz using tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard.

H_2O, dried over anhydrous Na_2SO_4, and evaporated to give performylated bile acid in almost quantitative yield. To a solution of performylated bile acid (1 g) in acetone (11 ml) was added 0.2 N NaOH (22 ml) dropwise over a period of 30 min and the solution was stirred at room temperature for 2 hr. The reaction mixture was poured into ice-water, neutralized with 5% HCl, and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The 3-monohydroxy compounds (**1**, **34**, **55**) thus obtained were used for further elaboration.

Preparation of 3-Sulfates

Disodium Cholate 3-Sulfate (**4**)——Methyl cholate 7,12-diformate (**2**) (1.4 g), obtainable from cholic acid 7,12-diformate (**1**) by methylation with diazomethane, was sulfated for 30 min to give methyl cholate 7,12-diformate 3-sulfate (**3**) (2.2 g) as an oily product. To a solution of **3** (2 g) in MeOH (30 ml) was added 20% NaOH (30 ml) and the solution was stirred at room temperature overnight. The resulting solution was evaporated *in vacuo* and the residue obtained was dissolved in H_2O (100 ml) and submitted to Amberlite XAD-2 column chromatography. Recrystallization of the eluate from MeOH–ether gave **4** (450 mg) as colorless crystals. mp 198.5—199.5° (dec.). $[\alpha]_D^{15}$ +37.0° (c=0.08). *Anal.* Calcd. for $C_{24}H_{38}Na_2O_8S \cdot 4H_2O$: C, 47.67; H, 7.67. Found: C, 47.67; H, 7.32.

Disodium Glycocholate 3-Sulfate (**7**)——Ethyl glycocholate 7,12-diformate (**5**) (1.5 g) was sulfated for 30 min to give ethyl glycocholate 7,12-diformate 3-sulfate (**6**) (2.2 g) as an oily product. To a solution of **6** (2 g) in MeOH (30 ml) was added 20% NaOH (30 ml) and the solution was stirred at room temperature overnight. The resulting solution was evaporated *in vacuo* and the residue obtained was submitted to Amberlite XAD-2 column chromatography. Recrystallization of the eluate from MeOH–ether gave **7** (520 mg) as colorless crystals. mp 194.5—195.5° (dec.). $[\alpha]_D^{15}$ +19.6° (c=0.10). *Anal.* Calcd. for $C_{26}H_{41}NNa_2O_9S \cdot 2H_2O$: C, 49.91; H, 7.25; N, 2.24. Found: C, 50.21; H, 7.13; N, 2.15.

Disodium Taurocholate 3-Sulfate (**10**)——Taurocholic acid 7,12-diformate (**8**) (700 mg) was sulfated for 35 min. The oily product obtained was dissolved in H_2O (50 ml), adjusted to pH 10 with 2 N NaOH, and submitted to Amberlite XAD-2 column chromatography. Taurocholic acid 7,12-diformate 3-sulfate (**9**) (650 mg) was obtained as an oily product. To a solution of **9** (600 mg) in MeOH (15 ml) was added 20% NaOH (21 ml) and the solution was stirred at room temperature overnight. The resulting solution was evaporated *in vacuo* and the residue obtained was submitted to Amberlite XAD-2 column chromatography. Recrystallization of the eluate from MeOH–ether gave **10** (380 mg) as colorless crystals. mp 175.5—176.5° (dec.). $[\alpha]_D^{15}$ +29.1° (c=0.10). *Anal.* Calcd. for $C_{26}H_{43}NNa_2O_{10}S_2 \cdot 21/2H_2O$: C, 45.60; H, 7.07; N, 2.05. Found: C, 45.61; H, 6.86; N, 1.81.

Disodium Chenodeoxycholate 3-Sulfate (**37**)——Treatment of methyl chenodeoxycholate 7-formate (**35**) (380 mg) as described for **4** followed by recrystallization from MeOH–ether gave **37** (230 mg) as colorless crystals. mp 201—202°, $[\alpha]_D^{15}$ +31.7° (c=0.10). *Anal.* Calcd. for $C_{24}H_{38}Na_2O_7S \cdot 2H_2O$: C, 52.16; H, 7.66. Found: C, 52.56; H, 7.27.

Disodium Glycochenodeoxycholate 3-Sulfate (**40**)——Treatment of ethyl glycochenodeoxycholate 7-formate (**38**) (900 mg) as described for **7** followed by recrystallization from MeOH–ether gave **40** (260 mg) as colorless crystals. mp 199—200° (dec.). $[\alpha]_D^{15}$ +27.5° (c=0.11). *Anal.* Calcd. for $C_{26}H_{41}NNa_2O_8S \cdot H_2O$: C, 52.78; H, 7.33; N, 2.37. Found: C, 53.01; H, 7.42; N, 2.50.

Disodium Taurochenodeoxycholate 3-Sulfate (**43**)——Treatment of **34** (800 mg) as described for **10** followed by recrystallization from MeOH–ether gave **43** (255 mg) as colorless crystals. mp 183—184° (dec.). $[\alpha]_D^{15}$ +29.9° (c=0.10). *Anal.* Calcd. for $C_{26}H_{43}NNa_2O_9S_2 \cdot 2H_2O$: C, 47.04; H, 7.14; N, 2.11. Found: C, 47.36; H, 7.16; N, 2.56.

Disodium Deoxycholate 3-Sulfate (**58**)——Treatment of methyl deoxycholate 12-formate (**56**) (1 g) as described for **4** followed by recrystallization from MeOH–ether gave **58** (876 mg) as colorless crystals. mp 202—203° (dec.). $[\alpha]_D^{15}$ +19.8° (c=0.10). *Anal.* Calcd. for $C_{24}H_{38}Na_2O_7S \cdot 3H_2O$: C, 50.51; H, 7.77. Found: C, 50.83; H, 7.82.

Disodium Glycodeoxycholate 3-Sulfate (**61**)——Treatment of ethyl glycodeoxycholate 12-formate (**59**) (1.55 g) as described for **7** followed by recrystallization from MeOH–ether gave **61** (835 mg) as colorless crystals. mp 198—199° (dec.). $[\alpha]_D^{15}$ +40.0° (c=0.10). *Anal.* Calcd. for $C_{26}H_{41}NNa_2O_8S \cdot 2H_2O$: C, 51.22; H, 7.44; N, 2.30. Found: C, 51.17; H, 7.44; N, 1.94.

Disodium Taurodeoxycholate 3-Sulfate (**64**)——Treatment of **55** (1.6 g) as described for **10** followed by recrystallization from MeOH–ether gave **64** (410 mg) as colorless crystals. mp 183—184 (dec.). $[\alpha]_D^{15}$ +20.0° (c=0.10). *Anal.* Calcd. for $C_{26}H_{43}NNa_2O_9S_2 \cdot H_2O$: C, 48.36; H, 7.02; N, 2.17. Found: C, 48.05; H, 7.43; N, 2.26.

Disodium Ursodeoxycholate 3-Sulfate (**79**)——To a solution of ursodeoxycholic acid (2 g) in anhydrous dimethylformamide (1 ml)–pyridine (0.5 ml) were added imidazole (3.5 g) and *tert*-butyldimethylsilyl chloride (1.6 g) under ice-cooling and the solution was stirred for 1 hr. The reaction mixture was poured into ice-water and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The oily residue obtained was chromatographed on silica gel (40 g) with hexane–AcOEt (3 : 1). Recrystallization of the eluate from aq. EtOH gave ursodeoxycholic acid 3-*tert*-butyldimethylsilyl ether (**75**) (2.2 g). mp 206—207°. $[\alpha]_D^{15}$ +19.1° (c=0.10, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{54}O_4Si$: C, 71.09; H, 10.74. Found: C, 70.83; H, 10.85. Treatment of **75** (1.3 g) with Ac_2O (10 ml) and pyridine (10 ml) in the usual

manner gave ursodeoxycholic acid 7-acetate 3-*tert*-butyldimethylsilyl ether (**76**) (1.3 g) as an oily product. To a solution of **76** (1.2 g) in acetone (17 ml) was added 21% HCl (1.7 ml) and the solution was stirred at room temperature for 30 min. The resulting solution was neutralized with 2 N NaOH, concentrated *in vacuo*, acidified with 5% HCl, and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated to give ursodeoxycholic acid 7-acetate (**77**) (990 mg) as an oily product. Sulfation of **77** (1 g) followed by deacetylation as described above and recrystallization from MeOH–ether gave **79** (550 mg) as colorless crystals. mp 209—210° (dec.). $[\alpha]_D^{15}$ +19.9° (c=0.09). *Anal.* Calcd. for $C_{24}H_{38}Na_2O_7S \cdot H_2O$: C, 53.91; H, 7.54. Found: C, 53.69; H, 7.24.

Disodium Glycoursodeoxycholate 3-Sulfate (82)——Ethyl glycoursodeoxycholate 7-acetate (**80**) (1.4 g), obtainable from **77** (2 g) and ethyl glycinate, was sulfated for 30 min to give ethyl glycoursodeoxycholate 7-acetate 3-sulfate (**81**) (1.6 g) as an oily product. To a solution of **81** in MeOH (14 ml) was added 20% NaOH (14 ml) and the solution was stirred at room temperature overnight. The resulting solution was evaporated *in vacuo* and the residue obtained was submitted to Amberlite XAD-2 column chromatography. Recrystallization of the eluate from MeOH–ether gave **82** (500 mg) as colorless crystals. mp 206—209° (dec.). $[\alpha]_D^{15}$ +18.4° (c=0.11). *Anal.* Calcd. for $C_{26}H_{41}NNa_2O_8S$: C, 54.43; H, 7.20; N, 2.44. Found: C, 54.10; H, 7.45; N, 2.49.

Disodium Tauroursodeoxycholate 3-Sulfate (85)——Tauroursodeoxycholic acid 7-acetate (**83**) (1.1 g), obtainable from **77** (2 g) and taurine, was sulfated and then deacetylated as described above. Recrystallization from MeOH–ether gave **85** (300 mg) as colorless crystals. mp 192—194° (dec.). $[\alpha]_D^{15}$ +19.1° (c=0.10). *Anal.* Calcd. for $C_{26}H_{43}NNa_2O_9S_2$: C, 50.06; H, 6.95; N, 2.25. Found: C, 49.75; H, 7.05; N, 2.26.

Preparation of 7-Sulfates

Disodium Cholate 7-Sulfate (14)——To a solution of methyl 3α, 12α-diacetoxy-7-oxo-5β-cholan-24-oate (**32**)[12] (1 g) in MeOH (8 ml) was added $NaBH_4$ (900 mg) under ice-cooling and the solution was stirred for 1 hr. The resulting solution was diluted with AcOEt (50 ml), washed with 5% HCl and H_2O, dried over anhydrous Na_2SO_4, and evaporated. Methyl cholate 3,12-diacetate (**12**) thus obtained was sulfated for 1 hr to give methyl cholate 3,12-diacetate 7-sulfate (**13**) as an oily product. To a solution of **13** in MeOH (12 ml) was added 20% NaOH (12 ml) and the solution was stirred for 3 days at room temperature. The resulting solution was evaporated *in vacuo* and the residue obtained was submitted to Amberlite XAD-2 column chromatography. Recrystallization of the eluate from MeOH–ether gave **14** (450 mg) as colorless crystals. mp 198.5—200° (dec.). $[\alpha]_D^{15}$ +9.3° (c=0.11). *Anal.* Calcd. for $C_{24}H_{38}Na_2O_8S \cdot H_2O$: C, 52.35; H, 7.32. Found: C, 52.34; H, 7.21.

Disodium Glycocholate 7-Sulfate (17)——3α,12α-Diacetoxy-7-oxo-5β-cholan-24-oic acid (**31**) (2 g) was reduced with $NaBH_4$ as described above. Cholic acid 3,12-diacetate (**11**) thus obtained was condensed with ethyl glycinate to give the glycine-conjugate (**15**). Sulfation of **15** for 1 hr gave the 7-sulfate (**16**) which in turn was hydrolyzed with NaOH. Recrystallization from MeOH–ether gave **17** (620 mg) as colorless crystals. mp 184—185°. $[\alpha]_D^{15}$ +19.6° (c=0.10). *Anal.* Calcd. for $C_{26}H_{41}NNa_2O_9S \cdot H_2O$: C, 51.39; H, 7.13; N, 2.31. Found: C, 51.47; H, 7.26; N, 2.30.

Disodium Taurocholate 7-Sulfate (20)——Treatment of **11** (2.4 g) as described for **10** followed by recrystallization from MeOH–ether gave **20** (590 mg) as colorless crystals. mp 167—168° (dec.). $[\alpha]_D^{15}$ +9.6° (c=0.10). *Anal.* Calcd. for $C_{26}H_{43}NNa_2O_{10}S_2 \cdot H_2O$: C, 47.47; H, 6.90; N, 2.13. Found: C, 47.66; H, 6.65; N, 1.93.

Disodium Chenodeoxycholate 7-Sulfate (46)——Methyl chenodeoxycholate (**33**) (1 g) was transformed into the 3-*tert*-butyldimethylsilyl ether (**44**) (1.1 g) as described above. Sulfation of **44** for 30 min gave the 7-sulfate (**45**) which in turn was hydrolyzed with HCl and then with NaOH. Recrystallization from MeOH–ether gave **46** (650 mg) as colorless crystals. mp 192—194° (dec.). $[\alpha]_D^{15}$ −29.6° (c=0.10). *Anal.* Calcd. for $C_{24}H_{38}Na_2O_7S \cdot 11/2H_2O$: C, 53.02; H, 7.60. Found: C, 53.25; H, 7.43.

Disodium Glycochenodeoxycholate 7-Sulfate (50)——Treatment of ethyl glycochenodeoxycholate (**47**) (1.5 g) as described for **17** followed by recrystallization from MeOH–ether gave **50** (720 mg) as colorless crystals. mp 186—187°. $[\alpha]_D^{15}$ −8.8° (c=0.11). *Anal.* Calcd. for $C_{26}H_{41}NNa_2O_8S \cdot 2H_2O$: C, 51.22; H, 7.44; N, 2.30. Found: C, 51.79; H, 7.46; N, 2.39.

Disodium Taurochenodeoxycholate 7-Sulfate (54)——Treatment of taurochenodeoxycholic acid (**51**) (1.0 g) as described for **20** followed by recrystallization from MeOH–ether gave **54** (380 mg) as colorless crystals. mp 173—175° (dec.). $[\alpha]_D^{15}$ −20.4° (c=0.10). *Anal.* Calcd. for $C_{26}H_{43}NNa_2O_9S_2 \cdot 2H_2O$: C, 47.04; H, 7.14; N, 2.11. Found: C, 47.23; H, 6.73; N, 2.53.

Disodium Ursodeoxycholate 7-Sulfate (87)——**75** (1 g) was sulfated for 30 min and hydrolyzed with HCl. Purification of the crude product by Amberlite XAD-2 column chromatography and recrystallization of the eluate from MeOH–ether gave **87** (650 mg) as colorless crystals. mp 219—221° (dec.). $[\alpha]_D^{15}$ +19.9° (c=0.10). *Anal.* Calcd. for $C_{24}H_{38}Na_2O_7S \cdot H_2O$: C, 53.91; H, 7.54. Found: C, 53.39; H, 7.24.

Disodium Glycoursodeoxycholate 7-Sulfate (90)——Treatment of **75** (1.5 g) as described for **82** followed by recrystallization from MeOH–ether gave **90** (920 mg) as colorless crystals. mp 196—198° (dec.). $[\alpha]_D^{15}$ +19.1° (c=0.10). *Anal.* Calcd. for $C_{26}H_{41}NNa_2O_8S \cdot H_2O$: C, 52.78; H, 7.33; N, 2.37. Found: C, 52.58; H, 7.39; N, 1.89.

Disodium Tauroursodeoxycholate 7-Sulfate (93)——Treatment of **75** (1.8 g) as described for **85** followed by recrystallization from MeOH–ether gave **93** (980 mg) as colorless crystals. mp 196—198° (dec.). $[\alpha]_D^{15}$ +20.4° (c=0.10). *Anal.* Calcd. for $C_{26}H_{43}NNa_2O_9S_2 \cdot 1/2H_2O$: C, 49.04; H, 6.97; N, 2.20. Found: C, 48.97; H, 7.13; N, 2.23.

Preparation of 12-Sulfates

Disodium Cholate 12-Sulfate (24)——To a solution of cholic acid (2 g) in benzene (19.2 ml)-pyridine (4.8 ml) was added Ac_2O (4.8 ml) and the solution was allowed to stand at room temperature for 48 hr. The reaction mixture was poured into ice-water and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. Cholic acid 3,7-diacetate (**21**) (1.8 g) obtained was converted to methyl cholate 3,7-diacetate (**22**) by methylation with diazomethane. **22** (1 g) was sulfated for 40 min and methyl cholate 3,7-diacetate 12-sulfate (**23**) obtained was hydrolyzed with NaOH as described above. Recrystallization from MeOH–ether gave **24** (510 mg) as colorless crystals. mp 206—209° (dec.). $[\alpha]_D^{15}$ +29.5° (c=0.10). *Anal.* Calcd. for $C_{24}H_{38}Na_2O_8S \cdot 2H_2O$: C, 50.69; H, 7.45. Found: C, 51.04; H, 7.87.

Disodium Glycocholate 12-Sulfate (27)——**21** (1 g) was condensed with ethyl glycinate to give ethyl glycocholate 3,7-diacetate (**25**). Treatment of **25** as described for **24** followed by recrystallization from MeOH–ether gave **27** (520 mg) as colorless crystals. mp 205—207° (dec.). $[\alpha]_D^{15}$ +19.0° (c=0.11). *Anal.* Calcd. for $C_{26}H_{41}NNa_2O_9S \cdot 1/2H_2O$: C, 52.16; H, 7.07; N, 2.34. Found: C, 52.44; H, 7.49; N, 2.05.

Disodium Taurocholate 12-Sulfate (30)——**21** (1.5 g) was condensed with taurine to give taurocholic acid 3,7-diacetate (**28**). Treatment of **28** as described for **24** followed by recrystallization from MeOH–ether gave **30** (380 mg) as colorless crystals. mp 192—193.5° (dec.). $[\alpha]_D^{15}$ +39.9° (c=0.10). *Anal.* Calcd. for $C_{26}H_{43}NNa_2O_{10}S_2 \cdot H_2O$: C, 47.47; H, 6.90; N, 2.13. Found: C, 47.68; H, 6.63; N, 1.95.

Disodium Deoxycholate 12-Sulfate (68)——Treatment of deoxycholic acid 3-acetate (**65**) (1.5 g) as described for **24** followed by recrystallization from MeOH–ether gave **68** (500 mg) as colorless crystals. mp 201—202° (dec.). $[\alpha]_D^{15}$ +37.2° (c=0.11). *Anal.* Calcd. for $C_{24}H_{38}Na_2O_7S \cdot 3H_2O$: C, 50.51; H, 7.77. Found: C, 50.74; H, 7.47.

Disodium Glycodeoxycholate 12-Sulfate (71)——**65** (2.2 g) was condensed with ethyl glycinate to give ethyl glycodeoxycholate 3-acetate (**69**). Treatment of **69** as described for **24** followed by recrystallization from MeOH–ether gave **71** (600 mg) as colorless crystals. mp 204—205° (dec.). $[\alpha]_D^{15}$ +38.4° (c=0.10). *Anal.* Calcd. for $C_{26}H_{41}NNa_2O_8S \cdot 1/2H_2O$: C, 53.29; H, 7.27; N, 2.40. Found: C, 53.26; H, 7.58; N, 2.33.

Disodium Taurodeoxycholate 12-Sulfate (74)——**65** (2.3 g) was condensed with taurine to give taurodeoxycholic acid 3-acetate (**72**). Treatment of **72** as described for **24** followed by recrystallization from MeOH–ether gave **74** (280 mg) as colorless crystals. mp 179—181° (dec.). $[\alpha]_D^{15}$ +38.9° (c=0.10). *Anal.* Calcd. for $C_{26}H_{43}NNa_2O_9S_2 \cdot 2H_2O$: C, 47.04; H, 7.14; N, 2.11. Found: C, 47.51; H, 7.22; N, 2.28.

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