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Studies on Analysis of Bile Acids. Preparation of 3-Glucuronides of 7- and 12-Oxo Bile Acids

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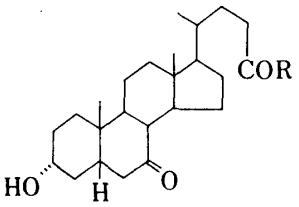
The 3-glucuronides of unconjugated and glyco- and tauro-conjugated bile acids having a 7- or 12-oxo group have been synthesized. Introduction of a glucuronyl residue at the C-3 position was achieved by the Koenigs–Knorr reaction using cadmium carbonate as a catalyst. The 3-glucuronides of conjugated bile acids were prepared by three sequential reactions: esterification with *p*-nitrophenol, glucuronidation at C-3, and amide formation with ethyl glycinate or taurine. The nuclear magnetic resonance spectral properties of 3-glucuronides of oxo bile acids and related compounds are briefly discussed.

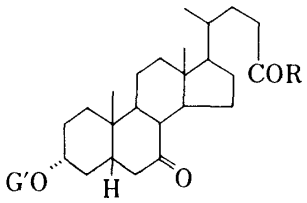
Keywords——glucuronidation of bile acid; Koenigs–Knorr reaction of bile acid; glucuronide of bile acid; 7-dehydrochenodeoxycholate; 12-dehydrodeoxycholate; 7-dehydrocholate; 12-dehydrocholate; bile acid conjugate

Considerable attention has recently been directed to the metabolism of bile acids in patients with hepatobiliary diseases.[2-4] Particular interest has been focused on the physiological significance of glucuronidation and sulfation. It has previously been demonstrated that bile acid glucuronides are present in the urine and plasma of patients with extra- and intrahepatic cholestasis and in trace amounts in the urine of healthy subjects.[5-7] However, the complete structures of bile acid glucuronides have not been definitely established. Synthetic specimens are therefore required to establish a reliable method for the analysis of bile acid glucuronides in biological fluids in connection with metabolic studies in man. The present paper deals with the preparation of 3-glucuronides of unconjugated and glyco- and tauro-conjugated bile acids having a 7- or 12-oxo group by unequivocal routes.

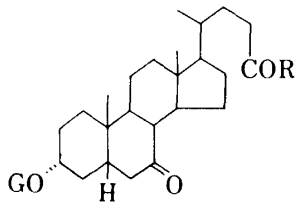
Our initial target was the synthesis of 3-glucuronides of unconjugated bile acids. For this purpose, 7-dehydrochenodeoxycholic acid (**1**),[8] 12-dehydrodeoxycholic acid (**11**),[8] 7-dehydrocholic acid 12-acetate (**20**),[9] and 12-dehydrocholic acid 7-acetate (**29**)[9] were chosen as



1: R=OH
2: R=OCH_3
3: R=$OC_6H_4NO_2(p)$



4: R=OCH_3
5: R=$OC_6H_4NO_2(p)$
6: R=$NHCH_2COOC_2H_5$
7: R=$NHCH_2CH_2SO_3H$



8: R=ONa
9: R=$NHCH_2COONa$
10: R=$NHCH_2CH_2SO_3K$

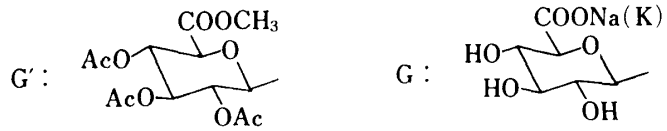
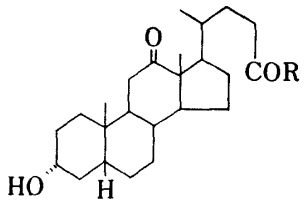
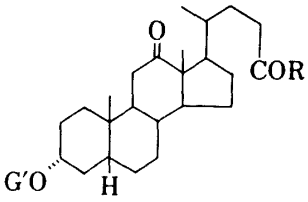


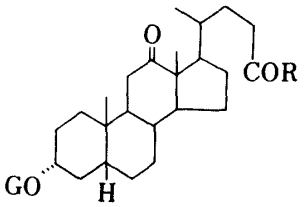
Chart 1. 7-Dehydrochenodeoxycholate 3-Glucuronides and Related Compounds



11: R=OH
12: R=OCH_3



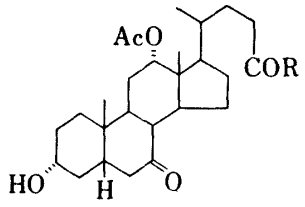
13: R=OCH_3
14: R=$OC_6H_4NO_2(p)$
15: R=$NHCH_2COOC_2H_5$
16: R=$NHCH_2CH_2SO_3H$



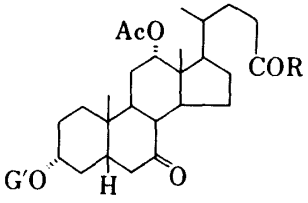
17: R=ONa
18: R=$NHCH_2COONa$
19: R=$NHCH_2CH_2SO_3Na$

Chart 2. 12-Dehydrodeoxycholate 3-Glucuronides and Related Compounds

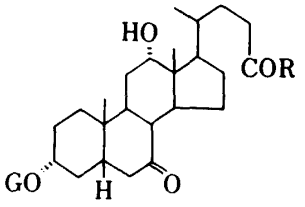
starting materials. Introduction of a glucuronyl residue into suitably protected bile acid methyl esters (**2**, **12**, **21**, **30**) was achieved by use of the Koenigs–Knorr reaction with methyl 1-bromo-1-deoxy-2,3,4-tri-*O*-acetyl-α-D-glucopyranuronate in anhydrous toluene, employing cadmium carbonate as a catalyst, to provide the corresponding 3-glucuronide acetate-methyl esters (**4**, **13**, **22**, **31**) in satisfactory yields. Simultaneous removal of the protecting groups in both the sugar and the steroid moieties was effected by treatment with methanolic sodium hydroxide to afford the desired 3-glucuronides of unconjugated bile acids having a C-7 or C-12 oxo group (**8**, **17**, **26**, **35**).



20: R=OH
21: R=OCH_3

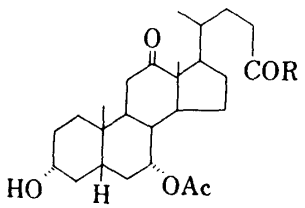


22: R=OCH_3
23: R=$OC_6H_4NO_2(p)$
24: R=$NHCH_2COOC_2H_5$
25: R=$NHCH_2CH_2SO_3H$

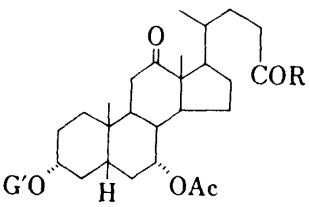


26: R=ONa
27: R=$NHCH_2COONa$
28: R=$NHCH_2CH_2SO_3K$

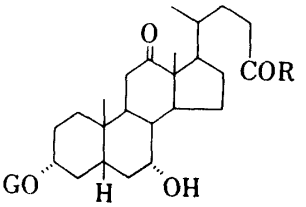
Chart 3. 7-Dehydrocholate 3-Glucuronides and Related Compounds



29: R=OH
30: R=OCH_3



31: R=OCH_3
32: R=$OC_6H_4NO_2(p)$
33: R=$NHCH_2COOC_2H_5$
34: R=$NHCH_2CH_2SO_3H$



35: R=ONa
36: R=$NHCH_2COONa$
37: R=$NHCH_2CH_2SO_3K$

Chart 4. 12-Dehydrocholate 3-Glucuronides and Related Compounds

Our next target was the synthesis of 3-glucuronides of glyco- and tauro-conjugated bile acids. It has previously been established that the glyco- and tauro-conjugates can be readily obtained from the corresponding *p*-nitrophenyl esters by treatment wih ethyl glycinate or taurine in pyridine.[8] The preparation of 3-glucuronides of conjugated bile acids was under-

TABLE I. NMR Spectral Data for the 3-Glucuronides of Oxo Bile Acids

Compd.	Solv.[a]	18-CH_3	19-CH_3	21-CH_3	3β-H	7β-H	12β-H	Pyranose C_1–H[b]	Pyranose C_5–H	>NCH_2CO–	>NCH_2CH_2S–[c]		–NH–	–$COCH_3$	
4	C	0.63	1.15	0.93	3.50			4.55	3.90					1.95	1.97
5	C	0.65	1.17	1.02	3.50			4.56	3.90					1.97	
6	C	0.65	1.17	0.95	3.50			4.55		3.90 d, 5 Hz			6.38	1.95	
7	C/M	0.69	1.26	0.98				4.30			2.98	3.56		2.02	2.04
8	W	0.87	1.40	1.13											
9	W	0.85	1.37	1.15						3.84s					
10	W	0.84	1.38	1.16											
13	C	1.00	1.00	0.88	3.50			4.56	3.94					1.96	2.00
14	C	1.02	1.02	0.90	3.50			4.57	4.02					2.00	
15	C	1.00	1.00	0.90	3.50			4.57		3.92 d, 5 Hz			6.30	1.96	2.00
16	C/M	1.06	1.08	0.88				4.34			3.00	3.56		2.03	2.06
17	W	1.23	1.23	1.00											
18	W	1.20	1.20	1.00						3.82s					
19	W	1.18	1.18	0.95							3.10	3.54			
22	C	0.72	1.16	0.80	3.50		4.93	4.55	3.95					1.93	1.99
23	C	0.75	1.17	0.88	3.50		4.93	4.56	4.00					1.93	2.02
24	C	0.74	1.17	0.83	3.50		4.95	4.54		3.91 d, 5 Hz			6.20	1.96	2.00
25	C/M	0.73	1.18	0.83	3.55		5.06	4.66			2.98	3.60		2.01	2.07
26	W	0.90	1.37	1.18			4.16								
27	W	0.90	1.37	1.20			4.16			3.85s					
28	W	0.90	1.40	1.24			4.17				3.10	3.54			
31	C	1.01	1.01	0.87	3.50	4.82		4.55	3.94					1.97	1.99
32	C	1.02	1.02	0.90	3.40	4.82		4.54	3.95					1.97	1.99
33	C	1.00	1.00	0.90	3.50	4.83		4.56		3.90 d, 5 Hz			6.20	1.96	
34	C/M	1.05	1.05	0.86	3.55	4.90		4.60			2.96	3.56		2.01	2.05
35	W	1.20	1.26	1.00		4.08									
36	W	1.22	1.26	1.02		4.08				3.85s					
37	W	1.20	1.25	1.01		4.08									

a) C: $CDCl_3$, M: CD_3OD, W: D_2O.
b) Doublet, J=7—10 Hz.
c) Triplet, J=6 Hz.

taken by employing three sequential reactions involving esterification with *p*-nitrophenol, glucuronidation at C-3, and amide formation with ethyl glycinate or taurine. This method was recognized to be the most favorable among three possible routes leading to the desired compounds.[10]

Condensation of bile acids having a C-7 or C-12 oxo group (**1**, **11**, **20**, **29**) with *p*-nitrophenol was carried out in ethyl acetate–dioxane by the use of *N*,*N*′-dicyclohexylcarbodiimide to afford the *p*-nitrophenyl esters. The Koenigs–Knorr reaction of the *p*-nitrophenyl esters with α-acetobromosugar occurred readily to provide the 3-glucuronide acetate-methyl esters (**5**, **14**, **23**, **32**) in reasonable yields. Subsequent condensation with ethyl glycinate and taurine furnished the glyco- (**6**, **15**, **24**, **33**) and tauro-conjugates (**7**, **16**, **25**, **34**), respectively. Upon exposure to methanolic alkali, simultaneous elimination of the protecting groups in both the sugar and the steroid moieties was attained to give the desired glyco-conjugate 3-glucuronides (**9**, **18**, **27**, **36**) and tauro-conjugate 3-glucuronides (**10**, **19**, **28**, **37**) in satisfactory yields.

The nuclear magnetic resonance (NMR) spectral properties of the glucuronides, acetate-methyl esters, and related compounds are listed in Table I. The data are indicative of the formation of the β-D-glucopyranuronoside structure. The anomeric proton signal of the glucuronyl moiety appeared at 4.30—4.66 ppm as a doublet (J=7—10 Hz), indicating a *trans*-diaxial relationship to the vicinal 2′-proton. The β-glucuronoside linkage in these monoglucuronides was confirmed by characterizing the bile acids liberated by incubation with a β-glucuronidase preparation.

The chromatographic separation of 3-glucuronides of unconjugated and glyco- and tauro-conjugated bile acids having an oxo group is being studied 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 Model DIP-180 polarimeter. NMR spectra were recorded on a NEVA Model NV-14 spectrometer at 60 MHz using tetramethylsilane as an internal or external standard.

General Procedure for the Preparation of 3-β-D-Glucopyranuronoside——Freshly prepared $CdCO_3$ (800 mg) and methyl 1-bromo-1-deoxy-2,3,4-tri-*O*-acetyl-α-D-glucopyranuronate (500 mg) were added to a solution of oxo bile acid (1.23 mmol) in anhydrous toluene (30 ml), and the suspension was azeotropically distilled with stirring over a period of 3 h. Additional portions of the α-acetobromosugar (300 mg) and $CdCO_3$ (500 mg) were added, and the suspension was refluxed for a further 2 h. The precipitate was removed by filtration and washed with toluene. The filtrate and washings were combined and evaporated down. The oily residue was subjected to column chromatography on silica gel (30 g). Elution with hexane–AcOEt and recrystallization of the product from the appropriate solvent gave the glucuronide acetate-methyl ester.

Methyl 7-Dehydrochenodeoxycholate 3-Glucuronide Acetate-methyl Ester (**4**)——Methyl 7-dehydrochenodeoxycholate (**2**) (500 mg) was subjected to glucuronidation by means of the Koenigs–Knorr reaction. Recrystallization of the crude product from hexane–AcOEt gave **4** (570 mg) as colorless needles. mp 170—173°C. $[\alpha]_D^{26}$ −57.9° (c=0.19, $CHCl_3$). *Anal.* Calcd for $C_{38}H_{56}O_{13}$: C, 63.22; H, 7.96. Found: C, 62.96; H, 8.11.

7-Dehydrochenodeoxycholate 3-Glucuronide Disodium Salt (**8**)——NaOH solution (20%, 10 ml) was added to a solution of **4** (130 mg) in MeOH (30 ml), and the mixture was stirred at room temperature for 2 d. The resulting solution was poured into ice-water, neutralized with conc. HCl, and evaporated down. The residue obtained was dissolved in 2% NaOH (60 ml) and subjected to column chromatography on Amberlite XAD-2 resin. The eluate was redissolved in H_2O (20 ml) and subjected to ion-exchange chromatography on Dowex-50W-X8 (Na^+ form). Recrystallization of the eluate from aq. MeOH–acetone gave **8** (63 mg) as a colorless amorphous substance. mp 265—268°C (dec.). $[\alpha]_D^{26}$ −38.6° (c=0.44, H_2O). *Anal.* Calcd for $C_{30}H_{44}Na_2O_{10}\cdot 2H_2O$: C, 57.13; H, 7.67. Found: C, 57.21; H, 7.58.

***p*-Nitrophenyl 7-Dehydrochenodeoxycholate 3-Glucuronide Acetate-methyl Ester** (**5**)——*p*-Nitrophenol (0.81 g) and *N*,*N*′-dicyclohexylcarbodiimide (1.46 g) were added to a solution of **1** (1.46 g) in anhydrous AcOEt (35 ml)–dioxane (20 ml), and the solution was stirred at room temperature overnight. After removal of the precipitate by filtration, the filtrate was evaporated down. The oily residue was chromatographed on silica gel (100 g). Elution with hexane–AcOEt (2: 1—3: 2) and recrystallization of the product from hexane–AcOEt gave *p*-nitrophenyl 7-dehydrochenodeoxycholate (**3**) (1.18 g) as colorless needles. mp

183—187°C. $[\alpha]_D^{26}$ −32.1° (c=0.28, $CHCl_3$). *Anal.* Calcd for $C_{30}H_{41}NO_6$: C, 70.42; H, 8.08; N, 2.74. Found: C, 70.32; H, 8.16; N, 2.60. **3** (1.1 g) was subjected to glucuronidation by means of the Koenigs–Knorr reaction carried out in toluene. Recrystallization of the crude product from hexane–AcOEt gave **5** (255 mg) as colorless needles. mp 233—234.5°C. $[\alpha]_D^{26}$ −31.3° (c=0.32, $CHCl_3$). *Anal.* Calcd for $C_{43}H_{57}NO_{15}$: C, 62.38; H, 6.94; N, 1.69. Found: C, 62.12; H, 6.83; N, 1.62.

Ethyl Glyco-7-dehydrochenodeoxycholate 3-Glucuronide Acetate-methyl Ester (6)——Ethyl glycinate (83 mg) in pyridine (2 ml) was added to a solution of **5** (250 mg) in pyridine (4 ml), and the solution was stirred at room temperature for 2 h. The reaction mixture was poured into ice-water, acidified with 5% HCl, and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. The oily residue was subjected to column chromatography on silica gel (20 g). Elution with hexane–AcOEt and recrystallization of the product from hexane–AcOEt gave **6** (215 mg) as colorless needles. mp 192—194°C. $[\alpha]_D^{26}$ −19.5° (c=0.41, $CHCl_3$). *Anal.* Calcd for $C_{41}H_{61}NO_{14}\cdot 1/2H_2O$: C, 61.52; H, 7.81; N, 1.75. Found: C, 61.72; H, 7.88; N, 1.84.

Glyco-7-dehydrochenodeoxycholate 3-Glucuronide Disodium Salt (9)——NaOH solution (20%, 10 ml) was added to a solution of **6** (130 mg) in MeOH (30 ml), and the solution was stirred at room temperature for 24 h. The reaction mixture was subjected to column chromatography on Amberlite XAD-2 resin and then to ion-exchange chromatography on Dowex-50W-X8 (Na^+ form). Recrystallization of the product from aq. MeOH–acetone gave **9** (64 mg) as a colorless amorphous substance. mp 265—268°C (dec.). $[\alpha]_D^{26}$ −38.9° (c=0.36, H_2O). *Anal.* Calcd for $C_{32}H_{47}NNa_2O_{11}\cdot 2H_2O$: C, 54.61; H, 7.31; N, 1.99. Found: C, 54.83; H, 7.25; N, 1.79.

Tauro-7-dehydrochenodeoxycholic Acid 3-Glucuronide Acetate-methyl Ester (7)——Taurine (93 mg) in H_2O (5 ml) was added to a solution of **5** (350 mg) in pyridine (20 ml), and the mixture was stirred at room temperature for 7 h. The resulting solution was concentrated and the oily residue was chromatographed on silica gel (20 g). Elution with $CHCl_3$–MeOH (10: 1—3: 1) and recrystallization of the product from hexane–$CHCl_3$ gave **7** (112 mg) as a colorless amorphous substance. mp 280—285°C. $[\alpha]_D^{26}$ −24.0° (c=0.50, $CHCl_3$). *Anal.* Calcd for $C_{39}H_{59}NO_{15}S\cdot 2H_2O$: C, 55.06; H, 7.41; N, 1.65. Found: C, 55.35; H, 7.30; N, 1.64.

Tauro-7-dehydrochenodeoxycholate 3-Glucuronide Dipotassium Salt (10)——KOH solution (20%, 10 ml) was added to a solution of **7** (53 mg) in MeOH (10 ml), and the mixture was stirred at room temperature for 3 d. The reaction mixture was subjected to column chromatography on Amberlite XAD-2 resin and then to ion-exchange chromatography on Dowex-50W-X8 (K^+ form). Recrystallization of the product from aq. EtOH gave **10** (24 mg) as colorless needles. mp 287—288.5°C (dec.). $[\alpha]_D^{26}$ −39.5° (c=0.76, H_2O). *Anal.* Calcd for $C_{32}H_{49}K_2NO_{12}S\cdot 2H_2O$: C, 48.90; H, 6.80; N, 1.78. Found: C, 49.12; H, 6.74; N, 1.98.

Methyl 12-Dehydrodeoxycholate 3-Glucuronide Acetate-methyl Ester (13)——Treatment of methyl 12-dehydrodeoxycholate (**12**) (500 mg) in the manner described for **4** followed by recrystallization of the product from MeOH gave **13** (490 mg) as colorless needles. mp 175—177°C. $[\alpha]_D^{26}$ +47.8° (c=0.23, $CHCl_3$). *Anal.* Calcd for $C_{38}H_{56}O_{13}$: C, 63.22; H, 7.96. Found: C, 62.94; H, 7.93.

12-Dehydrodeoxycholate 3-Glucuronide Disodium Salt (17)——Treatment of **13** (220 mg) in the manner described for **8** followed by recrystallization of the product from aq. acetone gave **17** (80 mg) as a colorless amorphous substance. mp 276—279°C (dec.). $[\alpha]_D^{26}$ +40.2° (c=0.82, H_2O). *Anal.* Calcd for $C_{30}H_{44}Na_2O_{10}\cdot 2H_2O$: C, 57.13; H, 7.67. Found: C, 57.01; H, 7.79.

***p*-Nitrophenyl 12-Dehydrodeoxycholate 3-Glucuronide Acetate-methyl Ester (14)**——Treatment of 12-dehydrodeoxycholic acid (**11**) (628 mg) in the manner described for **5** followed by recrystallization of the product from hexane–AcOEt gave **14** (350 mg) as colorless needles. mp 223.5—225°C. $[\alpha]_D^{26}$ +41.4° (c=0.29, $CHCl_3$). *Anal.* Calcd for $C_{43}H_{57}NO_{15}$: C, 62.38; H, 6.94; N, 1.69. Found: C, 62.09; H, 6.92; N, 1.67.

Ethyl Glyco-12-dehydrodeoxycholate 3-Glucuronide Acetate-methyl Ester (15)——Treatment of **14** (350 mg) in the manner described for **6** followed by recrystallization of the product from aq. MeOH gave **15** (257 mg) as colorless needles. mp 180—184°C. $[\alpha]_D^{26}$ +27.1° (c=0.48, $CHCl_3$). *Anal.* Calcd for $C_{41}H_{61}NO_{14}$: C, 62.18; H, 7.76; N, 1.77. Found: C, 62.00; H, 7.99; N, 1.97.

Glyco-12-dehydrodeoxycholate 3-Glucuronide Disodium Salt (18)——Treatment of **15** (130 mg) in the manner described for **9** followed by recrystallization of the product from aq. MeOH–acetone gave **18** (64 mg) as a colorless amorphous substance. mp 265—268°C (dec.). $[\alpha]_D^{26}$ +36.7° (c=0.30, H_2O). *Anal.* Calcd for $C_{32}H_{47}NNa_2O_{11}\cdot 2H_2O$: C, 54.61; H, 7.31; N, 1.99. Found: C, 54.88; H, 7.40; N, 1.88.

Tauro-12-dehydrodeoxycholic Acid 3-Glucuronide Acetate-methyl Ester (16)——Treatment of **14** (370 mg) in the manner described for **7** followed by recrystallization of the product from hexane–$CHCl_3$ gave **16** (215 mg) as a colorless amorphous substance. mp 280—285°C. $[\alpha]_D^{26}$ +18.2° (c=0.22, $CHCl_3$). *Anal.* Calcd for $C_{39}H_{59}NO_{15}S\cdot 2H_2O$: C, 55.06; H, 7.41; N, 1.65. Found: C, 55.16; H, 7.41; N, 1.65.

Tauro-12-dehydrodeoxycholate 3-Glucuronide Disodium Salt (19)——NaOH solution (20%, 10 ml) was added to a solution of **16** (80 mg) in MeOH (30 ml), and the solution was stirred at room temperature for 4 d. The reaction mixture was subjected to column chromatography on Amberlite XAD-2 resin and then to ion-exchange chromatography on Dowex-50W-X8 (Na^+ form). Recrystallization of the product from aq. acetone gave **19** (34 mg) as a colorless amorphous substance. mp 276—279°C (dec.). $[\alpha]_D^{26}$ +40.6° (c=0.64, H_2O). *Anal.* Calcd for $C_{32}H_{49}NNa_2O_{12}S\cdot 2H_2O$: C, 50.98; H, 7.09; N, 1.86. Found: C, 51.15; H, 7.17; N, 2.09.

Methyl 7-Dehydrocholate 12-Acetate 3-Glucuronide Acetate-methyl Ester (**22**)——Treatment of methyl 7-dehydrocholate 12-acetate (**21**) (500 mg) in the manner described for **4** followed by recrystallization of the product from hexane–AcOEt gave **22** (430 mg) as colorless needles. mp 237—238°C. $[\alpha]_D^{26}$ −12.5° (c=0.24, $CHCl_3$). *Anal.* Calcd for $C_{40}H_{58}O_{15}$: C, 61.68; H, 7.51. Found: C, 61.40; H, 7.54.

7-Dehydrocholate 3-Glucuronide Disodium Salt (**26**)——Treatment of **22** (130 mg) in the manner described for **8** followed by recrystallization of the product from MeOH–ether gave **26** (47 mg) as a colorless amorphous substance. mp 268—272°C (dec.). $[\alpha]_D^{26}$ −25.9° (c=0.27, H_2O). *Anal.* Calcd for $C_{30}H_{44}Na_2O_{11}\cdot 2H_2O$: C, 55.72; H, 7.48. Found: C, 55.58; H, 7.54.

***p*-Nitrophenyl 7-Dehydrocholate 12-Acetate 3-Glucuronide Acetate-methyl Ester** (**23**)——Treatment of 7-dehydrocholic acid 12-acetate (**20**) (976 mg) in the manner described for **5** followed by recrystallization of the product from acetone–ether gave **23** (207 mg) as colorless needles. mp 222—225°C. $[\alpha]_D^{26}$ +15.6° (c=0.32, $CHCl_3$). *Anal.* Calcd for $C_{45}H_{59}NO_{17}$: C, 61.00; H, 6.71; N, 1.58. Found: C, 60.91; H, 6.62; N, 1.65.

Ethyl Glyco-7-dehydrocholate 12-Acetate 3-Glucuronide Acetate-methyl Ester (**24**)——Treatment of **23** (160 mg) in the manner described for **6** followed by recrystallization of the product from hexane–acetone gave **24** (150 mg) as colorless needles. mp 170—172°C. $[\alpha]_D^{26}$ +13.2° (c=0.38, $CHCl_3$). *Anal.* Calcd for $C_{43}H_{63}NO_{16}\cdot 3/2H_2O$: C, 58.89; H, 7.59; N, 1.60. Found: C, 58.93; H, 7.47; N, 1.70.

Glyco-7-dehydrocholate 3-Glucuronide Disodium Salt (**27**)——Treatment of **24** (85 mg) in the manner described for **9** followed by recrystallization of the product from aq. EtOH gave **27** (42 mg) as colorless needles. mp 267—270°C (dec.). $[\alpha]_D^{26}$ −21.1° (c=0.90, H_2O). *Anal.* Calcd for $C_{32}H_{47}NNa_2O_{12}\cdot 2H_2O$: C, 54.61; H, 7.31; N, 1.99. Found: C, 54.48; H, 7.52; N, 1.87.

Tauro-7-dehydrocholic Acid 12-Acetate 3-Glucuronide Acetate-methyl Ester (**25**)——Treatment of **23** (340 mg) in the manner described for **7** followed by recrystallization of the product from hexane–$CHCl_3$ gave **25** (240 mg) as a colorless amorphous substance. mp 238—240°C. $[\alpha]_D^{26}$ +7.4° (c=1.62, $CHCl_3$). *Anal.* Calcd for $C_{41}H_{61}NO_{17}S\cdot 2H_2O$: C, 54.18; H, 7.16; N, 1.54. Found: C, 53.90; H, 7.07; N, 1.47.

Tauro-7-dehydrocholate 3-Glucuronide Dipotassium Salt (**28**)——Treatment of **25** (80 mg) in the manner described for **10** followed by recrystallization of the product from aq. EtOH gave **28** (18 mg) as colorless needles. mp 282—285°C (dec.). $[\alpha]_D^{26}$ −23.2° (c=0.82, H_2O). *Anal.* Calcd for $C_{32}H_{49}K_2NO_{13}S\cdot 2H_2O$: C, 47.92; H, 6.66; N, 1.75. Found: C, 48.20; H, 6.52; N, 1.75.

Methyl 12-Dehydrocholate 7-Acetate 3-Glucuronide Acetate-methyl Ester (**31**)——Treatment of methyl 12-dehydrocholate 7-acetate (**30**) (500 mg) in the manner described for **4** followed by recrystallization of the product from hexane–AcOEt gave **31** (510 mg) as colorless needles. mp 236—239°C. $[\alpha]_D^{26}$ +19.0° (c=0.21, $CHCl_3$). *Anal.* Calcd for $C_{40}H_{58}O_{15}$: C, 61.68; H, 7.51. Found: C, 61.73; H, 7.44.

12-Dehydrocholate 3-Glucuronide Disodium Salt (**35**)——Treatment of **31** (290 mg) in the manner described for **8** followed by recrystallization of the product from aq. MeOH–ether gave **35** (40 mg) as colorless plates. mp 272—274°C (dec.). $[\alpha]_D^{26}$ +45.8° (c=0.24, H_2O). *Anal.* Calcd for $C_{30}H_{44}Na_2O_{11}\cdot 2H_2O$: C, 55.72; H, 7.48. Found: C, 55.89; H, 7.30.

***p*-Nitrophenyl 12-Dehydrocholate 7-Acetate 3-Glucuronide Acetate-methyl Ester** (**32**)——Treatment of 12-dehydrocholic acid 7-acetate (**29**) (863 mg) in the manner described for **5** followed by recrystallization of the product from hexane–acetone gave **32** (208 mg) as colorless needles. mp 240—240.5°C. $[\alpha]_D^{26}$ +36.4° (c=0.11, $CHCl_3$). *Anal.* Calcd for $C_{45}H_{59}NO_{17}$: C, 61.00; H, 6.71; N, 1.58. Found: C, 61.20; H, 6.65; N, 1.59.

Ethyl Glyco-12-dehydrocholate 7-Acetate 3-Glucuronide Acetate-methyl Ester (**33**)——Treatment of **32** (250 mg) in the manner described for **6** followed by recrystallization of the product from hexane–acetone gave **33** (219 mg) as colorless needles. mp 200.5—202°C. $[\alpha]_D^{26}$ +10.0° (c=0.40, $CHCl_3$). *Anal.* Calcd for $C_{43}H_{63}NO_{16}$: C, 60.76; H, 7.47; N, 1.65. Found: C, 60.67; H, 7.58; N, 1.62.

Glyco-12-dehydrocholate 3-Glucuronide Disodium Salt (**36**)——Treatment of **33** (120 mg) in the manner described for **9** followed by recrystallization of the product from aq. acetone gave **36** (53 mg) as a colorless amorphous substance. mp 261—265°C (dec.). $[\alpha]_D^{26}$ +36.4° (c=0.22, H_2O). *Anal.* Calcd for $C_{32}H_{47}NNa_2O_{12}\cdot 2H_2O$: C, 54.61; H, 7.31; N, 1.99. Found: C, 54.50; H, 7.21; N, 1.80.

Tauro-12-dehydrocholic Acid 7-Acetate 3-Glucuronide Acetate-methyl Ester (**34**)——Treatment of **32** (350 mg) in the manner described for **7** followed by recrystallization of the product from hexane–$CHCl_3$ gave **34** (95 mg) as a colorless amorphous substance. mp 276—278°C. $[\alpha]_D^{26}$ +21.9° (c=0.32, $CHCl_3$). *Anal.* Calcd for $C_{41}H_{61}NO_{17}S\cdot 3H_2O$: C, 53.17; H, 7.29; N, 1.51. Found: C, 52.87; H, 7.41; N, 1.63.

Tauro-12-dehydrocholate 3-Glucuronide Dipotassium Salt (**37**)——Treatment of **34** (60 mg) in the manner described for **10** followed by recrystallization of the product from aq. EtOH gave **37** (25 mg) as colorless needles. mp 291—293°C (dec.). $[\alpha]_D^{26}$ +21.7° (c=0.46, H_2O). *Anal.* Calcd for $C_{32}H_{49}K_2NO_{13}S\cdot 2H_2O$: C, 47.92; H, 6.66; N, 1.75. Found: C, 47.69; H, 6.79; N, 1.63.

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References and Notes

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