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Synthesis of 3-Glucuronides of Unconjugated and Conjugated Bile Acids[1]

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The 3-glucuronides of unconjugated, glyco- and tauro-conjugated bile acids have been prepared by an unequivocal route. Among three synthetic routes leading to the desired compounds, a method involving glucuronidation of the *p*-nitrophenyl ester by means of the Koenigs-Knorr reaction and subsequent conversion of the activated ester into the glyco- and tauro-conjugates was found to be most suitable. The nuclear magnetic resonance spectral data for bile acid glucuronides and related compounds are tabulated.

Keywords——bile acid 3-glucuronide; glycine conjugate; taurine conjugate; glucuronidation; Koenigs-Knorr reaction; cadmium carbonate; *p*-nitrophenyl ester; activated ester method

In recent years considerable work has been done on the metabolism of bile acids in patients with hepatobiliary diseases.[3,4] Particular attention has been focused on the physiological significance of sulfation and glucuronidation of bile acids. As a part of series of studies we reported the preparation of bile acid monosulfates[5] and their separation by high-performance liquid chromatography.[6,7] Bile acid glucuronides have previously been shown to be present in the urine and plasma of patients with extra- and intrahepatic cholestasis and in trace amounts in the urine of healthy subjects.[8,9] However, the complete structures of bile acid glucuronides have not definitely been 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, glyco- and tauro-conjugated bile acids by an unequivocal route.

Our initial effort was directed to the synthesis of 3-glucuronides of unconjugated bile acids. Although direct glucuronidation of unconjugated bile acids by means of the Koenigs-Knorr reaction has been reported,[10] the structures of the products still remain unclear. Accordingly, methyl lithocholate (**2**), the 7-monoformate of methyl chenodeoxycholate (**13**), the 7-monoacetate of methyl ursodeoxycholate (**22**), the 12-monoformate of methyl deoxycholate (**32**) and the 7,12-diformate of methyl cholate (**41**), obtainable by reported methods, were taken as starting materials. Introduction of the glucuronyl residue into suitably protected bile acid methyl esters was achieved using the Koenigs-Knorr reaction with methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyranuronate in anhydrous toluene, employing cadmium carbonate as a catalyst, providing the corresponding glucuronide acetate-methyl esters (**4**, **15**, **24**,

1) Part CLIV of "Studies on Steroids" by T. Nambara; Part CLIII: M. Numazawa, Y. Kiyono, and T. Nambara, *Anal. Biochem.*, in press. The following trivial names are used in this paper: lithocholic acid=3α-hydroxy-5β-cholan-24-oic acid; chenodeoxycholic acid=3α,7α-dihydroxy-5β-cholan-24-oic acid; ursodeoxycholic acid=3α,7β-dihydroxy-5β-cholan-24-oic acid; deoxycholic acid=3α,12α-dihydroxy-5β-cholan-24-oic acid; cholic acid=3α,7α,12α-trihydroxy-5β-cholan-24-oic acid.

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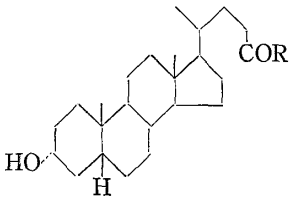
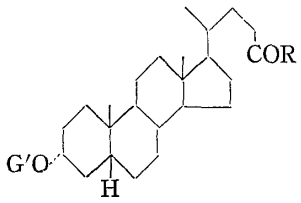
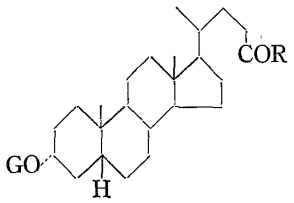


1: R=OH
2: R=OCH_3
3: R=$OC_6H_4NO_2$ (*p*)

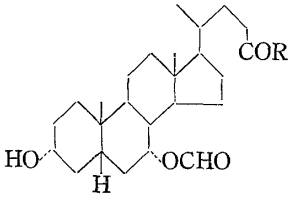


4: R=OCH_3
5: R=OH
6: R=$OC_6H_4NO_2$ (*p*)
7: R=$NHCH_2COOC_2H_5$
8: R=$NHCH_2CH_2SO_3H$

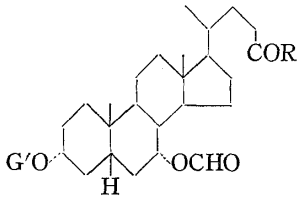


9: R=ONa
10: R=$NHCH_2COONa$
11: R=$NHCH_2CH_2SO_3Na$

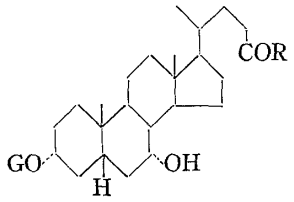
Chart 1. Lithocholate 3-Glucuronide and Related Compounds



12: R=OH
13: R=OCH_3
14: R=$NHCH_2COOC_2H_5$

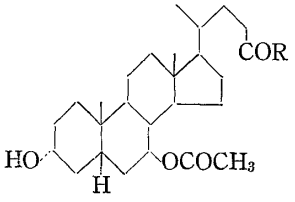


15: R=OCH_3
16: R=$OC_6H_4NO_2$ (*p*)
17: R=$NHCH_2COOC_2H_5$
18: R=$NHCH_2CH_2SO_3H$

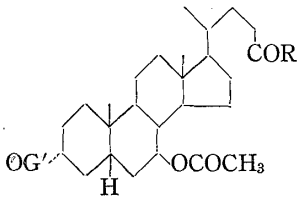


19: R=ONa
20: R=$NHCH_2COONa$
21: R=$NHCH_2CH_2SO_3K$

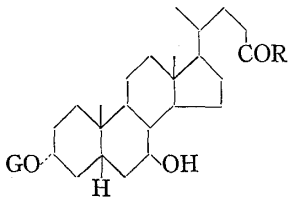
Chart 2. Chenodeoxycholate 3-Glucuronide and Related Compounds



22: R=OCH_3
23: R=OH

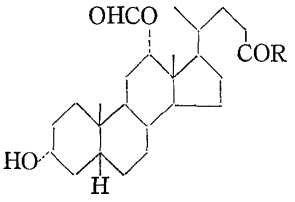


24: R=OCH_3
25: R=$OC_6H_4NO_2$ (*p*)
26: R=$NHCH_2COOC_2H_5$
27: R=$NHCH_2CH_2SO_3H$

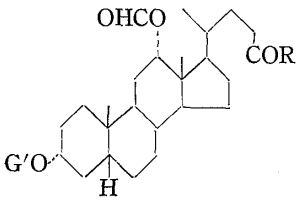


28: R=ONa
29: R=$NHCH_2COONa$
30: R=$NHCH_2CH_2SO_3K$

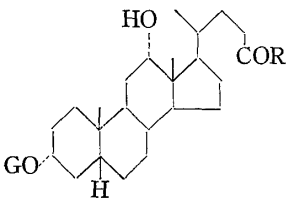
Chart 3. Ursodexycholate 3-Glucuronide and Related Compounds



31: R=OH
32: R=OCH_3



33: R=OCH_3
34: R=$OC_6H_4NO_2$ (*p*)
35: R=$NHCH_2COOC_2H_5$
36: R=$NHCH_2CH_2SO_3H$



37: R=ONa
38: R=$NHCH_2COONa$
39: R=$NHCH_2CH_2SO_3K$

Chart 4. Deoxycholate 3-Glucuronide and Related Compounds

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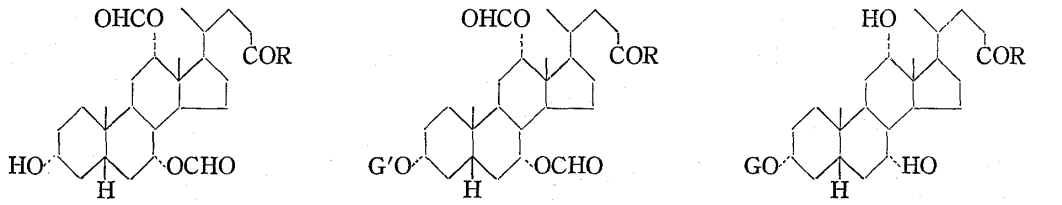

40: R=OH
41: R=OCH_3
42: R=$NHCH_2COOC_2H_5$

43: R=OCH_3
44: R=$OC_6H_4NO_2$ (*p*)
45: R=$NHCH_2COOC_2H_5$
46: R=$NHCH_2CH_2SO_3H$

47: R=ONa
48: R=$NHCH_2COONa$
49: R=$NHCH_2CH_2SO_3K$

Chart 5. Cholate 3-Glucuronide and Related Compounds

33, **43**) in satisfactory yields. Simultaneous removal of the protecting groups in both the steroid and sugar moieties was effected by treatment with methanolic sodium hydroxide to afford the desired 3-glucuronides of unconjugated bile acids (**9**, **19**, **28**, **37**, **47**).

The second project was focused on 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 ester by treatment with ethyl glycinate or taurine in pyridine.[5] Accordingly, the preparation of the desired 3-glucuronides of conjugated bile acids was attempted employing three possible procedures. These synthetic routes are different in the sequence of three reactions, glucuronidation at C-3, esterification with *p*-nitrophenol and amide formation with ethyl glycinate or taurine at C-24.

When ethyl 7,12-diformylglycocholate (**42**) and methyl α-bromoacetoglucuronate were stirred in refluxing anhydrous benzene with cadmium carbonate, the glucuronide acetate-methyl ester derivative (**45**) was obtained in only 6% yield. This unsatisfactory result was ascribable to difficulties in the separation of the desired product. Therefore, glucuronidation at C-3 followed by conjugation with ethyl glycinate or taurine *via* the *p*-nitrophenyl ester was carried out. Condensation of 7,12-diformylcholate 3-glucuronide acetate-methyl ester with *p*-nitrophenol was effected in ethyl acetate-dioxane by the use of N,N'-dicyclohexylcarbodiimide to provide the *p*-nitrophenyl ester (**44**). The reaction of the activated ester with ethyl glycinate and taurine proceeded easily, yielding the desired conjugates (**45**, **46**). This method, however, was still unsatisfactory owing to difficulties in the purification step.

The remaining synthetic route starting from the activated ester was then undertaken. The Koenigs-Knorr reaction of *p*-nitrophenyl 7,12-diformylcholate with α-acetobromosugar occurred readily to give the glucuronide derivative (**44**) in reasonable yield. Subsequent condensation with ethyl glycinate and taurine provided the glyco- and tauro-conjugates (**45**, **46**), respectively. On brief exposure to methanolic alkali, elimination of the protecting groups in both the sugar and steroid moieties was effected to give the desired glyco- and taurocholate 3-glucuronides (**48**, **49**) in satisfactory yields.

The 3-glucuronides of glyco- and taurochenodeoxycholates (**20**, **21**) were similarly synthesized using chenodeoxycholic acid 7-formate (**12**) as a starting compound. Among the three synthetic routes described above, the method involving glucuronidation of the *p*-nitrophenyl ester and subsequent conversion into the glyco- and tauro-conjugates was found to be most favorable. The desired glucuronides of conjugated deoxycholate (**38**, **39**) and lithocholate (**10**, **11**) were prepared by one of two methods, glucuronidation of the *p*-nitrophenyl ester and conversion of the unconjugated bile acid 3-glucuronide derivative into the activated ester. The 3-glucuronides of glyco- and tauroursodeoxycholate (**29**, **30**) were synthesized by Koenigs-Knorr reaction of the *p*-nitrophenyl ester and subsequent transformation into the glyco- and tauro-conjugates.

The nuclear magnetic resonance (NMR) spectra of the glucuronides and their acetate-methyl esters were indicative of the formaton of the β-D-glucopyranuronoside structure. The anomeric proton of the sugar moiety appeared at 4.20—4.46 ppm as a doublet (J=7—9 Hz),

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

Compd.	Solv.[a]	18-CH_3	19-CH_3	21-CH_3[b]	3β-H	7β-H	7α-H	12β-H	Pyranose-[c] C_1–H	Pyranose-C_5–H	$>NCH_2CO-$	$>NCH_2CH_2S-$[d]	–NH–	–CHO	$-COCH_3$
4	C	0.61	0.88	0.88	3.60				4.66	4.04					2.04 2.06
6	C	0.64	0.89	0.97	3.60				4.65	4.03					2.02 2.05
7	C	0.64	0.90	0.92	3.60				4.65		4.05d, 6 Hz		6.00		2.00 2.06
8	M	0.69	0.95	0.95					4.28			3.02 3.64			2.01 2.03 2.06
9	W	0.71	0.99	0.99											
10	W/M/C	0.69	0.97	0.97							3.81s				
11	D	0.61	0.88	0.88					4.29						
15	C	0.64	0.92	0.92	3.48	5.04			4.62	4.02				8.06	2.04 2.07
16	C	0.64	0.91	0.97	3.50	5.02			4.64	4.02				8.04	2.01 2.04
17	C	0.64	0.92	0.95	3.50	5.04			4.64		4.05d, 5 Hz		5.95	8.02	2.04
18	M	0.70	0.97	0.97		5.04			4.30			3.02 3.65		8.18	2.02 2.03 2.07
19	M	0.68	0.91	0.95		3.80			4.41						
20	M	0.68	0.92	0.97		3.80			4.42		3.76				
21	W/M	0.68	0.94	0.95		3.82			4.47			3.00 3.56			
24	C	0.67	0.95	0.91	3.50		4.76		4.64	4.02					1.97 2.03 2.05
25	C	0.69	0.95	0.98	3.54		4.76		4.63	4.02					1.96 2.02 2.04
26	C	0.67	0.95	0.93	3.50		4.76		4.63		4.02d, 5 Hz		5.95		1.96 2.01 2.03
27	M	0.72	0.98	0.98			4.76		4.26			2.98 3.61			1.96 1.98 1.99 2.03
28	M	0.74	0.97	0.97			3.56		4.43						
29	M	0.71	0.96	0.98			3.56		4.44		3.77s				
30	M/W	0.70	0.96	0.96			3.56		4.52			3.03 3.60			
33	C	0.72	0.88	0.80	3.60			5.24	4.64	4.04				8.06	2.03
34	C	0.75	0.89	0.89	3.56			5.24	4.64	4.04				8.08	2.02 2.04
35	C	0.73	0.89	0.83	3.60			5.26	4.65		4.05d, 5 Hz		5.95	8.06	2.04 2.05
36	C/M	0.77	0.92	0.86				5.23	4.20			3.00 3.62		8.13	2.02 2.04
37	M	0.71	0.93	1.01				3.98	4.42						
38	M/W	0.72	0.96	1.00				4.07	4.57		3.80s				
39	M/W	0.70	0.92	0.97				4.00	4.50			3.01 3.56			
43	C	0.74	0.91	0.82	3.44	5.03		5.22	4.60	4.00				8.06 8.10	2.03 2.04
44	C	0.79	0.93	0.93	3.50				4.63	4.03				8.08 8.15	2.06
45	C	0.74	0.91	0.85	3.44	5.05		5.25	4.62		4.03d, 5 Hz		5.95	8.10 8.16	2.04 2.05
46	C/M	0.82	0.97	0.89		5.06		5.26	4.22			3.01 3.64		8.14 8.20	2.04 2.06
47	M	0.72	0.93	1.03		3.82		3.98	4.43						
48	M	0.72	0.92	1.04		3.84		4.00	4.47		3.80				
49	M/W	0.71	0.92	0.99		3.84		4.00	4.47			3.00 3.56			

a) C: $CDCl_3$, M: CD_3OD, W: D_2O, D: d_6-DMSO.
b) doublet, J=5—6 Hz.
c) doublet, J=7—10 Hz.
d) triplet, J=6—7 Hz.

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indicating a *trans*-diaxial relationship to the vicinal 2′-proton. Further evidence for the β-glucoside linkage in these monoglucuronides was obtained by characterizing the bile acid liberated after incubation with the β-glucuronidase preparation.

The NMR spectral data for 3-glucuronides of various bile acids and their derivatives are collected in Table I. The availability of these data may be helpful for the characterization of bile acids and related compounds.

Studies on the chromatographic separation of 3-glucuronides of unconjugated and conjugated bile acids are being conducted in these laboratories and the details will be reported elsewhere in the near future.

Experimental[11]

General Procedure for the Preparation of 3-β-D-Glucopyranuronoside (Glucuronide)——Freshly prepared $CdCO_3$ (800 mg) and methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyranuronate (500 mg) were added to a solution of bile acid (0.001 mol) in anhydrous toluene or benzene (24 ml) and the suspension was azeotropically distilled with stirring over a period of 1.5 hr. Additional portion of the acetobromosugar (300 mg) and $CdCO_3$ (500 mg) were added and the solution was refluxed for another 1.5 hr. The precipitate was removed by filtration and washed with toluene or benzene. The filtrate and washings were combined and evaporated down *in vacuo*. The oily residue was subjected to column chromatography on silica gel (20 g). Elution with hexane–AcOEt and recrystallization of the eluate gave the glucuronide acetate–methyl ester.

Methyl Lithocholate 3-Glucuronide Acetate-Methyl Ester (4)——Methyl lithocholate (2) (490 mg) was subjected to glucuronidation by means of the Koenigs-Knorr reaction in toluene. Recrystallization of the crude product from acetone–hexane gave **4** (121 mg) as colorless needles. mp 175—176°. $[\alpha]_D^{25}$ −26.9° (c=0.1, $CHCl_3$). *Anal.* Calcd for $C_{38}H_{58}O_{12}\cdot 1/2H_2O$: C, 63.73; H, 8.31. Found: C, 64.01; H, 8.38.

Lithocholic Acid 3-Glucuronide Disodium Salt (9)——NaOH solution (7%, 30 ml) was added to a solution of **4** (85 mg) in MeOH (70 ml) and the mixture was stirred at 40° for 32 hr. The resulting solution was poured into ice-water, neutralized with conc. HCl and evaporated down *in vacuo*. The residue obtained was dissolved in 2% NaOH (55 ml) and subjected to column chromatography on Amberlite XAD-2. 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 H_2O–MeOH–acetone gave **9** (43 mg) as a colorless amorphous substance. mp 257—259° (dec.). $[\alpha]_D^{25}$ −10.3° (c=0.1, H_2O). *Anal.* Calcd for $C_{30}H_{34}Na_2O_9\cdot 2H_2O$: C, 56.95; H, 7.96. Found: C, 56.60; H, 7.55.

Ethyl Glycolithocholate 3-Glucuronide Acetate-Methyl Ester (7)——i) *p*-Nitrophenyl lithocholate (3)[5] (700 mg) was subjected to glucuronidation by means of the Koenigs-Knorr reaction in toluene. Recrystallization of the crude product from acetone–hexane gave *p*-nitrophenyl lithocholate 3-glucuronide acetate-methyl ester (**6**) (262 mg) as colorless needles. mp 187—187.5°. Ethyl glycinate (50 mg) in pyridine (1 ml) was added to a solution of **6** (120 mg) in pyridine (3 ml) and the solution was stirred at room temperature for 1 hr. 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 *in vacuo*. The oily residue was subjected to column chromatography on silica gel (6 g). Elution with hexane–AcOEt and recrystallization of the eluate from acetone–hexane gave **7** (99 mg) as colorless needles. mp 170—171°. $[\alpha]_D^{25}$ −21.8° (c=0.1, $CHCl_3$). *Anal.* Calcd for $C_{41}H_{63}NO_{13}$: C, 63.30; H, 8.16; N, 1.80. Found: C, 63.42; H, 8.31; N, 2.15.

ii) Lithocholic acid (**1**) (500 mg) was subjected to glucuronidation by means of the Koenigs-Knorr reaction in toluene. Recrystallization of the crude product from acetone–hexane gave lithocholic acid 3-glucuronide acetate–methyl ester (**5**) (492 mg) as colorless needles. mp 170°. *p*-Nitrophenol (110 mg) and N,N′-dicyclohexylcarbodiimide (190 mg) in anhydrous AcOEt (7 ml) were added to a solution of **5** (235 mg) in anhydrous AcOEt (7 ml)–dioxane (6 ml) and the solution was stirred at room temperature for 7.5 hr. After removal of the precipitate by filtration, the filtrate was evaporated down *in vacuo*. The oily residue was chromatographed on silica gel (15 g). Elution with hexane–AcOEt (5: 1—3: 1) gave **6** (139 mg) as colorless needles. Treatment of **6** with ethyl glycinate as described in i) gave **7**. mp 170—171°. Mixed melting point determination after admixture with the sample obtained in i) showed no depression.

Glycolithocholic Acid 3-Glucuronide Disodium Salt (10)——NaOH solution (20%, 3 ml) was added to a solution of **7** (100 mg) in H_2O (300 ml) and the solution was stirred at 40° for 15 hr. The reaction mixture was subjected to column chromatography on Amberlite XAD-2 and then to ion-exchange chromatography on Dowex-50W-X8 (Na^+ form). Recrystallization of the eluate from H_2O–MeOH–acetone gave **10** (55 mg)

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

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as a colorless amorphous substance. mp 253—256° (dec.). $[\alpha]_D^{25}$ −19.2° (c=0.1, H_2O). *Anal.* Calcd for $C_{32}H_{49}NNa_2O_{10}\cdot 21/2H_2O$: C, 55.00; H, 7.79; N, 2.00. Found: C, 55.07; H, 7.86; N, 1.88.

Taurolithocholic Acid 3-Glucuronide Disodium Salt (11)——Taurine (63 mg) in H_2O (5.5 ml) was added to a solution of **6** (250 mg) in pyridine (22 ml) and the mixture was stirred at room temperature for 6 hr. The resulting solution was concentrated and the oily residue was chromatographed on silica gel (10 g). Elution with $CHCl_3$–MeOH (5: 1—2: 1) gave taurolithocholate 3-glucuronide acetate-methyl ester (**8**) (136 mg) as an oily product. Aq. NH_4OH (28%, 12 ml) was added to a solution of **8** (40 mg) in MeOH (16 ml) and the mixture was stirred at 37° for 2 days. The resulting solution was evaporated down *in vacuo* and the residue was dissolved in MeOH (40 ml). After addition of 20% NaOH (2 ml) the solution was stirred at room temperature overnight. The resulting solution was concentrated and the precipitate was collected by filtration. The precipitate was dissolved in 72% EtOH (15 ml) and subjected to ion-exchange chromatography on Amberlyst A-15 (H^+ form). The eluate was rechromatographed on Amberlyst A-15 (Na^+ form). Recrystallization of the eluate from H_2O–MeOH–ether gave **11** (10 mg) as a pale brown amorphous substance. mp 263—266° (dec.). $[\alpha]_D^{25}$ −32.6° (c=0.1, 80% EtOH). *Anal.* Calcd for $C_{32}H_{51}NNa_2O_{11}S\cdot 2H_2O$: C, 51.95; H, 7.88; N, 1.89. Found: C, 51.64; H, 7.82; N, 2.32.

Methyl 7-Formylchenodeoxycholate 3-Glucuronide Acetate-Methyl Ester (15)——Treatment of methyl 7-formylchenodeoxycholate (**13**) (700 mg) as described for **4** followed by recrystallization from acetone–hexane gave **15** (273 mg) as colorless needles. mp 209.5—210.5°. $[\alpha]_D^{25}$ −12.4° (c=0.1, $CHCl_3$). *Anal.* Calcd for $C_{39}H_{58}O_{14}$: C, 62.38; H, 7.79. Found: C, 62.59; H, 7.74.

Chenodeoxycholic Acid 3-Glucuronide Disodium Salt (19)——Treatment of **15** (100 mg) as described for **9** followed by recrystallization from MeOH–ether gave **19** (50 mg) as a colorless amorphous substance. mp 261—263° (dec.). $[\alpha]_D^{25}$ −10.4° (c=0.1, MeOH). *Anal.* Calcd for $C_{30}H_{34}Na_2O_{10}\cdot H_2O$: C, 57.13; H, 7.67. Found: C, 57.25; H, 8.00.

Ethyl 7-Formylglycochenodeoxycholate 3-Glucuronide Acetate-Methyl Ester (17)——i) Treatment of 7-formylchenodeoxycholic acid[5,12] (**12**) (223 mg) as described for **7**-i) followed by recrystallization from acetone–hexane gave **17** (32 mg) as colorless needles. mp 217—219°. $[\alpha]_D^{25}$ −9.6° (c=0.1, $CHCl_3$). *Anal.* Calcd for $C_{42}H_{63}NO_{15}$: C, 61.37; H, 7.73; N, 1.70. Found: C, 61.23; H, 7.76; N, 2.00.

ii) Treatment of **12** (1.49 g) as described for **7**-ii) followed by recrystallization from acetone-hexane gave **17** (178 mg) as colorless crystals. mp 217—219°. Mixed melting point determination after admixture with the sample obtained in i) showed no depression.

iii) Ethyl 7-formylglycochenodeoxycholate (**14**) (500 mg) was subjected to glucuronidation by means of the Koenigs-Knorr reaction in benzene to give **17** (128 mg) as colorless needles. mp 216—219°. Mixed melting point determination after admixture with the sample obtained in i) showed no depression.

Glycochenodeoxycholic Acid 3-Glucuronide Disodium Salt (20)——Treatment of **17** (100 mg) as described for **19** followed by recrystallization from MeOH–ether gave **20** (50 mg) as a colorless amorphous substance. mp 255—259° (dec.). $[\alpha]_D^{25}$ −8.2° (c=0.1, MeOH). *Anal.* Calcd for $C_{32}H_{49}NNa_2O_{11}\cdot 11/2H_2O$: C, 55.16; H, 7.52; N, 2.01. Found: C, 55.26; H, 7.25; N, 1.98.

Taurochenodeoxycholic Acid 3-Glucuronide Dipotassium Salt (21)——*p*-Nitrophenyl 7-formylchenodeoxycholate 3-glucuronide acetate-methyl ester (**16**) (70 mg) was condensed with taurine as described above to give 7-formyltaurochenodeoxycholate 3-glucuronide acetate-methyl ester (**18**) (36 mg) as an oily product. KOH solution (10%, 20 ml) was added to a solution of **18** (50 mg) in MeOH (30 ml) and the mixture was stirred at 40° for 2 days. The resulting solution was poured into ice-water, neutralized with conc. HCl and evaporated down *in vacuo*. The residue was dissolved in 3% KOH (70 ml) and subjected to column chromatography on Amberlite XAD-2. The eluate was redissolved in H_2O (10 ml) and subjected to ion-exchange chromatography on Dowex-50W-X8 (K^+ form). Recrystallization of the eluate from aq. acetone gave **21** (29 mg) as colorless needles. mp 281—284° (dec.). $[\alpha]_D^{25}$ +11.0° (c=0.1, H_2O). *Anal.* Calcd for $C_{32}H_{51}K_2NO_{12}S\cdot H_2O$: C, 49.91; H, 6.94; N, 1.82. Found: C, 49.59; H, 7.24; N, 1.78.

Methyl 7-Acetylursodeoxycholate 3-Glucuronide Acetate-Methyl Ester (24)——Methyl 7-acetylursodeoxycholate (**22**)[5] (400 mg) was subjected to glucuronidation by means of the Koenigs-Knorr reaction in toluene. The oily residue was subjected to column chromatography on silica gel (25 g). Elution with benzene–ether (10: 1—6: 1) and recrystallization of the eluate from hexane–ether gave **24** (182 mg) as colorless needles. mp 161—162°. $[\alpha]_D^{25}$ +3.8° (c=0.1, $CHCl_3$). *Anal.* Calcd for $C_{40}H_{60}O_{14}$: C, 62.81; H, 7.91. Found: C, 62.72; H, 7.63.

Ursodeoxycholic Acid 3-Glucuronide Disodium Salt (28)——Treatment of **24** (100 mg) as described for **9** followed by recrystallization from MeOH–ether gave **28** (50 mg) as a colorless amorphous substance. mp 264—268° (dec.). $[\alpha]_D^{25}$ +3.8° (c=0.1, MeOH). *Anal.* Calcd for $C_{30}H_{46}Na_2O_{10}\cdot 1/2H_2O$: C, 57.96; H, 7.62. Found: C, 57.86; H, 7.81.

Ethyl 7-Acetylglycoursodeoxycholate 3-Glucuronide Acetate-Methyl Ester (26)——Treatment of 7-acetylursodeoxycholic acid (**23**)[5] (360 mg) as described for **7**-i) followed by recrystallization from ether gave **26** (31 mg) as a colorless amorphous substance. mp 118—123°. $[\alpha]_D^{25}$ +6.0° (c=0.1, $CHCl_3$). *Anal.* Calcd

12) K.-Y. Tserng and P.D. Klein, *Steroids*, **29**, 635 (1977).

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for $C_{43}H_{65}NO_{15}$: C, 61.78; H, 7.84; N, 1.67. Found: C, 61.68; H, 7.63; N, 1.58.

Glycoursodeoxycholic Acid 3-Glucuronide Disodium Salt (29)——Treatment of **26** (75 mg) as described for **9** followed by recrystallization from MeOH–ether gave **29** (38 mg) as a colorless amorphous substance. mp 250—253° (dec.). $[\alpha]_D^{25}$ +6.1° (c=0.1, MeOH). *Anal.* Calcd for $C_{32}H_{49}NNa_2O_{11}\cdot 11/2H_2O$: C, 55.16; H, 7.52; N, 2.01. Found: C, 55.15; H, 7.69; N, 1.80.

Tauroursodeoxycholic Acid 3-Glucuronide Dipotassium Salt (30)——Treatment of *p*-nitrophenyl 7-acetylursodeoxycholate 3-glucuronide acetate-methyl ester (**25**) (120 mg) as described for **21** gave **30** (43 mg) as a colorless amorphous substance. mp 250—253° (dec.). $[\alpha]_{25}^{D}$ +21.1° (c=0.1, H_2O). *Anal.* Calcd for $C_{32}H_{51}K_2NO_{12}S\cdot 1/2H_2O$: C, 50.50; H, 6.76; N, 1.84. Found: C, 50.48; H, 7.22; N, 1.46.

Methyl 12-Formyldeoxycholate 3-Glucuronide Acetate-Methyl Ester (33)——Methyl 12-formyldeoxycholate (**32**)[5,12] (400 mg) was subjected to glucuronidation in toluene as described for **4** to give **33** (292 mg) as colorless needles. mp 211°. $[\alpha]_D^{25}$ +36.5° (c=0.1, $CHCl_3$). *Anal.* Calcd for $C_{39}H_{58}O_{14}$: C, 62.38; H, 7.79. Found: C, 62.47; H, 7.97.

Deoxycholic Acid 3-Glucuronide Disodium Salt (37)——Treatment of **33** (100 mg) as described for **10** followed by recrystallization from MeOH–ether gave **37** (45 mg) as a pale yellow amorphous substance. mp 264—267° (dec.). $[\alpha]_D^{25}$ +15.7° (c=0.1, MeOH). *Anal.* Calcd for $C_{30}H_{34}O_{10}Na_2\cdot 1/2H_2O$: C, 57.96; H, 7.62. Found: C, 57.84; H, 7.82.

Ethyl 12-Formylglycodeoxycholate 3-Glucuronide Acetate-Methyl Ester (35)——i) Treatment of 12-formyldeoxycholic acid (**31**) (270 mg) as described for 7-i) followed by recrystallization from hexane–AcOEt gave **35** (32 mg) as a colorless amorphous substance. mp 141—144°. $[\alpha]_D^{25}$ +20.8° (c=0.1, $CHCl_3$). *Anal.* Calcd for $C_{42}H_{63}NO_{15}\cdot H_2O$: C, 60.05; H, 7.80; N, 1.67. Found: C, 60.39; H, 7.56; N, 1.46.

ii) Treatment of **31** (1.5 g) as described for 7-ii) followed by recrystallization from hexane–AcOEt gave **35** (138 mg). mp 141—144°. Mixed melting point determination after admixture with the sample obtained in i) showed no depression.

Glycodeoxycholic Acid 3-Glucuronide Disodium Salt (38)——Treatment of **35** (100 mg) as described for **9** followed by recrystallization from aq. MeOH gave **38** (30 mg) as a colorless amorphous substance. mp 252—255° (dec.). $[\alpha]_D^{25}$ +18.9° (c=0.1, H_2O). *Anal.* Calcd for $C_{32}H_{49}NNa_2O_{11}\cdot 2H_2O$: C, 54.46; H, 7.57; N, 1.98. Found: C, 54.27; H, 7.57; N, 1.96.

Taurodeoxycholic Acid 3-Glucuronide Dipotassium Salt (39)——Treatment of *p*-nitrophenyl 12-formyldeoxycholate 3-glucuronide acetate-methyl ester (**34**) (160 mg) as described for **21** followed by recrystallization from aq. MeOH gave **39** (38 mg) as colorless needles. mp 282—285° (dec.). $[\alpha]_D^{25}$ +31.3° (c=0.1, H_2O). *Anal.* Calcd for $C_{32}H_{51}K_2NO_{12}S\cdot 2H_2O$: C, 48.77; H, 7.04; N, 1.78. Found: C, 49.11, H, 7.11; N, 1.25.

Methyl 7,12-Diformylcholate 3-Glucuronide Acetate-Methyl Ester (43)——Treatment of methyl 7,12-diformylcholate (**41**)[5,12] (390 mg) as described for **4** followed by recrystallization from hexane–AcOEt gave **43** (227 mg) as colorless needles. mp 232—233°. $[\alpha]_D^{25}$ +20.8° (c=0.1, $CHCl_3$). *Anal.* Calcd for $C_{40}H_{58}O_{16}\cdot 1/2H_2O$: C, 59.17; H, 7.33. Found: C, 59.46; H, 7.30.

Cholic Acid 3-Glucuronide Disodium Salt (47)——Treatment of **43** (100 mg) as described for **9** followed by recrystallization from MeOH–ether gave **47** (44 mg) as a pale yellow amorphous substance. mp 269—272° (dec.). $[\alpha]_D^{25}$ +3.8° (c=0.1, MeOH). *Anal.* Calcd for $C_{30}H_{34}Na_2O_{11}\cdot 1\,1/2\,H_2O$: C, 54.95; H, 7.53. Found: C, 54.80; H, 7.93.

Ethyl 7,12-Diformylglycocholate 3-Glucuronide Acetate-Methyl Ester (45)——i) Treatment of 7,12-diformylcholic acid (**40**) (130 mg) as described for 7-i) followed by recrystallization from hexane–AcOEt gave **45** (18 mg) as colorless needles. mp 200—203°. $[\alpha]_D^{25}$ +24.0° (c=0.1, $CHCl_3$). *Anal.* Calcd for $C_{43}H_{63}NO_{17}\cdot H_2O$: C, 58.42; H, 7.41; N, 1.58. Found: C, 58.67; H, 7.59; N, 1.85.

ii) Treatment of **40** (360 mg) as described for 7-ii) followed by recrystallization from hexane–AcOEt gave **45** (33 mg) as colorless needles. mp 199—201°. Mixed melting point determination after admixture with the sample obtained in i) showed no depression

iii) Ethyl 7,12-diformylglycocholate (**42**)[5] (730 mg) was subjected to glucuronidation by means of the Koenigs-Knorr reaction in benzene to give **45** (45 mg) as colorless needles. mp 198—200°. Mixed melting point determination after admixture with the sample obtained in i) showed no depression.

Glycocholic Acid 3-Glucuronide Disodium Salt (48)——Treatment of **45** (60 mg) as described for **9** followed by recrystallization from MeOH–ether gave **48** (20 mg) as a colorless amorphous substance. mp 257—260° (dec.). $[\alpha]_D^{25}$ −6.1° (c=0.1, MeOH). *Anal.* Calcd for $C_{32}H_{49}NNa_2O_{12}\cdot H_2O$: C, 54.61; H, 7.31; N, 1.99. Found: C, 54.79; H, 7.15; N, 2.04.

Taurocholic Acid 3-Glucuronide Dipotassium Salt (49)——Treatment of *p*-nitrophenyl 7,12-diformylcholate 3-glucuronide acetate-methyl ester (**44**) (250 mg) as described for **21** followed by recrystallization from aq. acetone gave **49** (20 mg) as colorless plates. mp 246—250° (dec.). $[\alpha]_D^{25}$ +10.0° (c=0.1, H_2O). *Anal.* Calcd for $C_{32}H_{51}K_2NO_{13}S\cdot 11/2H_2O$: C, 48.34; H, 6.85; N, 1.76. Found: C, 48.26; H, 7.06; N, 1.72.

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