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# Synthesis of Monosulfates of Cholic Acid Derivatives[1]

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The 3-, 7- and 12-monosulfates of bile acids having an oxo or acetoxyl group on the steroid nucleus have been synthesized. Cholic acid derivatives appropriately protected were sulfated with chlorosulfonic acid and pyridine in the usual manner. The utilization of partial acetylation and hydrolysis together with chromium trioxide oxidation and sodium borohydride reduction provided the desired sulfates of cholic acid derivatives in satisfactory yields. The nuclear magnetic resonance spectral properties of the bile acid derivatives are briefly discussed.

**Keywords**——sulfation; bile acid monosulfate; partial acetylation; cholic acid monoacetate; 3-dehydrocholate; 7-dehydrocholate; 12-dehydrocholate

In recent years, considerable attention has been focused on the metabolic significance of sulfation of bile acids in hepato-biliary diseases.[3-6] For the purpose of clarifying these problems, the monosulfates of unconjugated and conjugated bile acids have been previously synthesized as authentic specimens.[7] It seems likely that sulfated bile acids having an oxo group may be potential intermediates of bile acids in living animals.[8] In addition, a novel method has been developed for the separation of sulfated bile acids by high-performance liquid chromatography (HPLC).[9,10] In these studies it was recognized that the chromatographic behavior of the 12-sulfates were somewhat different from those of other sulfates. An explanation for this interesting finding will require more detailed investigation using the acetates of sulfated cholic acid. The present paper deals with the preparation of the 3-, 7- and 12-monosulfates of dehydrocholic acids and cholic acid monoacetates.

Our initial efforts were directed to the synthesis of the 3-sulfates of bile acids having a 7- or 12-oxo group. For this purpose methyl 7-dehydrocholate diacetate (**2b**),[7] derivable from cholic acid (**1a**), was chosen as a suitable starting material. On exposure to 1.8% methanolic sodium hydroxide for a brief period, **2b** underwent partial hydrolysis to yield 7-dehydrocholic acid 12-monoacetate (**2c**), which, on treatment with chlorosulfonic acid and pyridine, was led to the 3-sulfate (**3a**). Elimination of the protecting group at C-12 by alkaline hydrolysis gave the desired 7-dehydrocholic acid 3-sulfate (**3b**) in satisfactory yield. Reduction of **3a** with sodium borohydride under mild conditions afforded cholic acid 12-acetate 3-sulfate (**4a**). The preparation of 12-dehydrocholic acid 3-sulfate (**6b**) was then undertaken.

1) Part CLXIII of "Studies on Steroids" by T. Nambara; Part CLXII: H. Hosoda, S. Miyairi, N. Kobayashi, and T. Nambara, *Chem. Pharm. Bull.*, **28**, 3369 (1980). The following trivial names are used in this paper: cholic acid=3α,7α,12α-trihydroxy-5β-cholan-24-oic acid; 3-dehydrocholic acid=7α,12α-dihydroxy-3-oxo-5β-cholan-24-oic acid; 7-dehydrocholic acid=3α,12α-dihydroxy-7-oxo-5β-cholan-24-oic acid; 12-dehydrocholic acid=3α,7α-dihydroxy-12-oxo-5β-cholan-24-oic acid.
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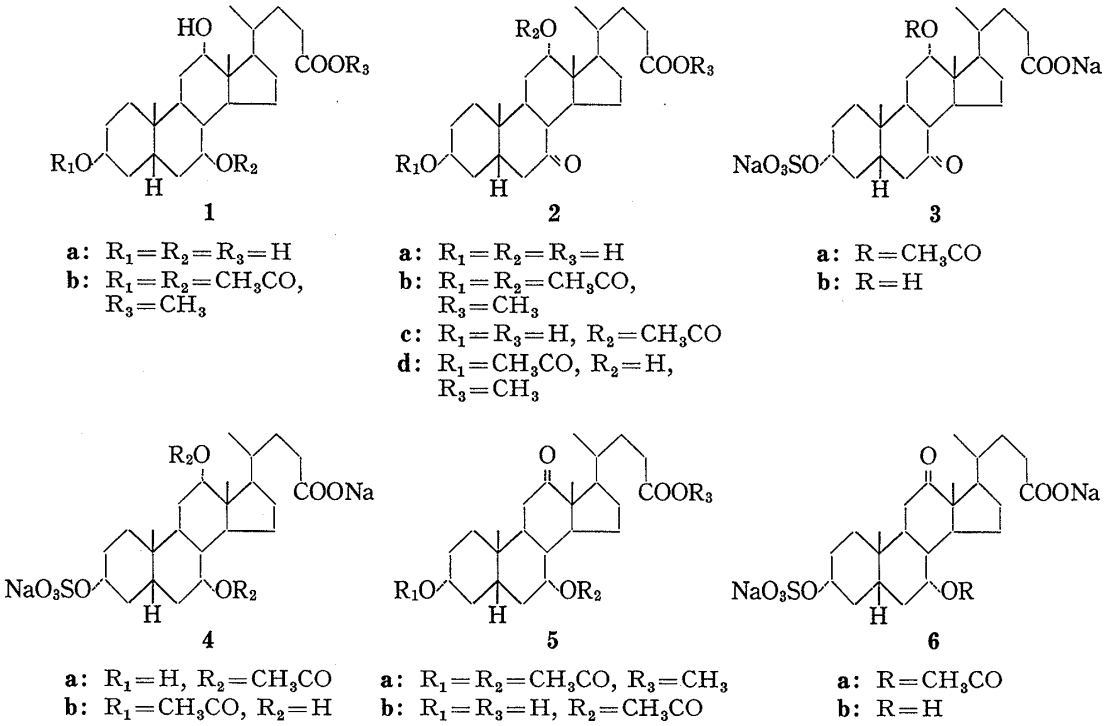



Chart 1. Bile Acid 3-Sulfates and Related Compounds

Partial hydrolysis of methyl 12-dehydrocholate diacetate (**5a**), readily obtainable from methyl cholate 3,7-diacetate (**1b**),[7] provided 12-dehydrocholic acid 7-acetate (**5b**), which, in turn, was converted into the 3-sulfate (**6a**). The desired 12-dehydrocholic acid 3-sulfate (**6b**) was obtained from **6a** by hydrolytic cleavage of the 7-acetoxyl group with alkali, while cholic acid 7-acetate 3-sulfate (**4b**) was obtained by reduction with metal hydride.

Next, the preparation of the 7-sulfates of 3-dehydrocholic acid and related compounds was carried out. Usual sulfation of methyl cholate 3,12-diacetate (**7**), which was formed

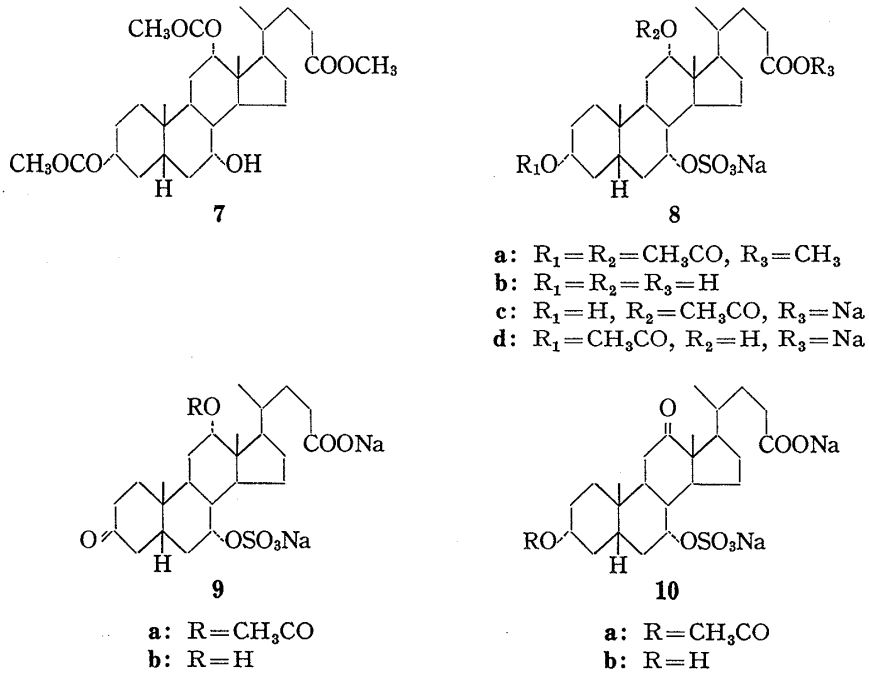
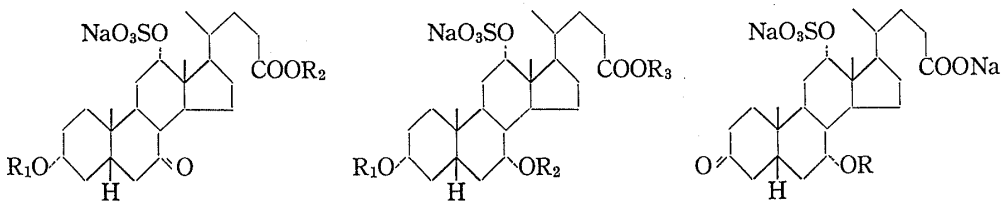



Chart 2. Bile Acid 7-Sulfates and Related Compounds

from **2b** by metal hydride reduction, furnished methyl cholate 3,12-diacetate 7-sulfate (**8a**). Partial saponification of **8a** under mild conditions gave the desired chloic acid 12-monoacetate 7-sulfate (**8c**) in reasonable yield. Upon hydrolytic removal of the protecting groups followed by partial acetylation with acetic anhydride and pyridine in benzene, **8a** was easily converted into cholic acid 3-acetate 7-sulfate (**8d**). 3-Dehydrocholic acid 7-sulfate (**9b**) and 12-dehydrocholic acid 7-sulfate (**10b**) were prepared from **8c** and **8d** through the 12- and 3-acetates (**9a**, **10a**) by chromium trioxide oxidation and subsequent alkaline hydrolysis, respectively.




**11**

**a**: $R_1=CH_3CO$, $R_2=CH_3$
**b**: $R_1=H$, $R_2=Na$
**c**: $R_1=CH_3CO$, $R_2=Na$

**12**

**a**: $R_1=R_2=CH_3CO$, $R_3=CH_3$
**b**: $R_1=H$, $R_2=CH_3CO$, $R_3=Na$
**c**: $R_1=R_2=R_3=H$
**d**: $R_1=CH_3CO$, $R_2=H$, $R_3=Na$

**13**

**a**: $R=CH_3CO$
**b**: $R=H$

Chart 3. Bile Acid 12-Sulfates and Related Compounds

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] | 3$\beta$-H | 7$\beta$-H | 12$\beta$-H | -$COCH_3$ |
|---|---|---|---|---|---|---|---|---|
| **1b** | C | 0.69 | 0.94 | | | | | |
| **2a** | C | 0.69 | 1.18 | 0.98 | 3.60 | | 4.03 | |
| **2b** | C | 0.76 | 1.21 | 0.83 | 4.69 | | 5.12 | 2.01, 2.12 |
| **2c** | C | 0.74 | 1.18 | 0.84 | 3.62 | | 5.06 | 2.07 |
| **2d** | C | 0.69 | 1.20 | 0.98 | 4.65 | | 4.01 | 2.01 |
| **3a** | M | 0.79 | 1.23 | 0.84 | 4.24 | | 5.04 | 2.07 |
| **3b** | M | 0.71 | 1.22 | 1.00 | 4.20 | | 3.94 | |
| **4a** | M | 0.78 | 0.93 | 0.84 | 4.09 | 3.88 | 5.03 | 2.09 |
| **4b** | M | 0.72 | 0.96 | 1.02 | 4.14 | 4.90 | 3.97 | 2.05 |
| **5a** | C | 1.05 | 1.05 | 0.83 | 4.58 | 5.00 | | 2.05, 2.05 |
| **5b** | C | 1.05 | 1.05 | 0.87 | 3.47 | 5.00 | | 2.05 |
| **6a** | M | 1.07 | 1.07 | 0.83 | 4.14 | 4.95 | | 2.01 |
| **6b** | M | 1.11 | 1.05 | 0.80 | 4.16 | 3.96 | | |
| **7** | C | 0.74 | 0.90 | 0.83 | 4.61 | 3.93 | 5.13 | 2.01, 2.12 |
| **8a** | M | 0.78 | 0.96 | 0.82 | 4.62 | 4.52 | 5.10 | 2.00, 2.12 |
| **8b** | W | 0.74 | 0.96 | 0.98 | 3.53 | 4.53 | 4.08 | |
| **8c** | M | 0.78 | 0.94 | 0.84 | 3.60 | 4.45 | 5.06 | 2.08 |
| **8d** | M | 0.72 | 0.95 | 1.00 | 4.57 | 4.42 | 3.92 | 1.97 |
| **9a** | M | 0.82 | 1.05 | 0.82 | | 4.52 | 5.09 | 2.07 |
| **9b** | M | 0.76 | 1.04 | 1.02 | | 4.48 | 3.99 | |
| **10a** | M | 1.09 | 1.09 | 0.85 | 4.57 | 4.54 | | 1.97 |
| **10b** | M | 1.09 | 1.07 | 0.84 | 3.42 | 4.52 | | |
| **11a** | M | 0.75 | 1.22 | 1.00 | 4.64 | | 4.69 | 2.04 |
| **11b** | M | 0.77 | 1.21 | 1.05 | 3.46 | | 4.64 | |
| **11c** | M | 0.78 | 1.25 | 1.08 | 4.47 | | 4.67 | 1.97 |
| **12a** | M | 0.79 | 0.98 | 1.05 | 4.54 | 4.92 | 4.69 | 2.02, 2.06 |
| **12b** | M | 0.77 | 0.94 | 1.06 | 3.30 | 4.87 | 4.67 | 2.05 |
| **12c** | W | 0.76 | 0.92 | 0.98 | 3.49 | 3.90 | 4.04 | |
| **12d** | W | 0.74 | 0.91 | 0.96 | 4.60 | 3.84 | 4.70 | 2.02 |
| **13a** | M | 0.82 | 1.06 | 1.09 | | 4.92 | 4.70 | 2.03 |
| **13b** | M | 0.82 | 1.03 | 1.06 | | 3.83 | 4.69 | |

*a*) C: $CDCl_3$, M: $CD_3OD$, W: $D_2O$.
*b*) doublet, $J=6$ Hz.

Finally, the preparation of the 12-sulfates was undertaken. For the purpose of obtaining the 7-oxo derivative, 7-dehydrocholic acid (**2a**) was taken as a starting material. Treatment of **2a** with acetic anhydride and pyridine in benzene provided the partially acetylated compound (**2d**), which, in turn, was converted into 7-dehydrocholic acid 12-sulfate (**11b**) by sulfation followed by saponification. Usual acetylation of **11b** yielded the 3-acetate (**11c**). Similarly, cholic acid 7-acetate 12-sulfate (**12b**) was prepared from **1b** *via* methyl cholate 3,7-diacetate 12-sulfate (**12a**) by sulfation and subsequent partial hydrolysis. Upon alkaline hydrolysis followed by partial acetylation in the manner described above, **12a** was transformed into cholic acid 3-acetate 12-sulfate (**12d**). 3-Dehydrocholic acid 12-sulfate (**13b**) was readily obtained from **12b** by chromium trioxide oxidation followed by saponification in the usual way.

The nuclear magnetic resonance (NMR) spectral data for bile acid derivatives having an oxo or acetoxyl function on the steroid nucleus are collected in Table I. The signal of hydrogen attached to carbon bearing a sulfate group appears as a broad singlet at lower field as compared to that of hydrogen on carbon carrying a hydroxyl group. The shift value (0.6—0.7 ppm) exerted by sulfation is useful as a diagnostic parameter for the structural elucidation of sulfated bile acids. The presence of an oxo group at C-12 causes a significant downfield shift of the 18-methyl proton signal. The availability of these NMR spectral data should be useful in characterizing bile acids and related compounds.

Further studies on the chromatographic behavior of sulfated bile acids in HPLC are being conducted in these laboratories, and the details will be reported elsewhere in the near future.

## Experimental[11]

### General Procedure for Sulfation

Chlorosulfonic acid (0.5 ml) in anhydrous pyridine (5 ml) was added to a solution of bile acid (500 mg) 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 down. The crude product was dissolved in $H_2O$ (50—100 ml), adjusted to pH 10 with 2 N NaOH, and percolated through a column of Amberlite XAD-2 resin (200 ml). After thorough washing with $H_2O$, sulfated bile acid was eluted with MeOH (200 ml).

### Preparation of 3-Sulfates

**Disodium 12α-Acetoxy-3α-hydroxy-7-oxo-5β-cholan-24-oate 3-Sulfate (3a)**——NaOH solution (9%, 15 ml) was added to a solution of methyl 3α, 12α-diacetoxy-7-oxo-5β-cholan-24-oate (**2b**) (1.5 g) in MeOH (60 ml), and the solution was stirred at room temperature for 3 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 concentrated *in vacuo* to give 12α-acetoxy-3α-hydroxy-7-oxo-5β-cholan-24-oic acid (**2c**) (1.2 g) as an oily material. **2c** was sulfated for 30 min. The oily product obtained was dissolved in $H_2O$ (100 ml), adjusted to pH 10 with 2 N NaOH, and subjected to Amberlite XAD-2 column chromatography. Recrystallization of the eluate from MeOH–ether gave **3a** (314 mg) as colorless crystals. mp 216.5—219° (dec.). $[\alpha]_D^{24}$ +48.4° ($c$=0.10). *Anal.* Calcd for $C_{26}H_{38}Na_2O_9S$: C, 54.54; H, 6.69. Found: C, 54.36; H, 7.06.

**Disodium 3α,12α-Dihydroxy-7-oxo-5β-cholan-24-oate 3-Sulfate (3b)**——NaOH solution (20%, 10 ml) was added to a solution of **3a** (100 mg) in MeOH (10 ml), and the solution was stirred at room temperature overnight. The resulting solution was neutralized with 5% HCl and evaporated down. The crude product was subjected to Amberlite XAD-2 column chromatography. Recrystallization of the eluate from MeOH–ether gave **3b** (40 mg) as colorless crystals. mp 220—223° (dec.). $[\alpha]_D^{24}$ +15.4° ($c$=0.08). *Anal.* Calcd for $C_{24}H_{36}Na_2O_8S \cdot 5/2H_2O$: C, 50.08; H, 7.18. Found: C, 50.27; H, 7.22.

**Disodium 12α-Acetoxy-3α,7α-dihydroxy-5β-cholan-24-oate 3-Sulfate (4a)**——$NaBH_4$ (30 mg) was added to a solution of **3a** (150 mg) in MeOH (9 ml) under ice-cooling, and the solution was stirred for 1 hr. The resulting solution was neutralized with 5% HCl and evaporated down. The crude product was subjected to Amberlite XAD-2 column chromatography. Recrystallization of the eluate from MeOH–ether gave

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 in $H_2O$. NMR spectra were recorded on a JEOL FX-100 spectrometer at 100 MHz using tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard.

**4a** (46 mg) as colorless crystals. mp 212—212.5° (dec.). $[\alpha]_D^{24}$ +73.8° ($c$=0.10). *Anal.* Calcd for $C_{26}H_{40}Na_2O_9S \cdot 3/2H_2O$: C, 51.90; H, 7.20. Found: C, 52.03; H, 7.45.

**Disodium 7α-Acetoxy-3α-hydroxy-12-oxo-5β-cholan-24-oate 3-Sulfate (6a)**——NaOH solution (9%, 10 ml) was added to a solution of methyl 3α,7α-diacetoxy-12-oxo-5β-cholan-24-oate (**5a**) (1 g) in MeOH (40 ml), and the solution was stirred at room temperature for 2 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. 7α-Acetoxy-3α-hydroxy-12-oxo-5β-cholan-24-oic acid (820 mg) (**5b**) thus obtained was sulfated for 40 min. The crude product was subjected to Amberlite XAD-2 column chromatography. Recrystallization of the eluate from MeOH–ether gave **6a** (280 mg) as colorless crystals. mp 187—188.5° (dec.). $[\alpha]_D^{24}$ +69.0° ($c$=0.10). *Anal.* Calcd for $C_{26}H_{33}Na_2O_9S$: C, 54.54; H, 6.69. Found: C, 54.12; H, 6.99.

**Disodium 3α,7α-Dihydroxy-12-oxo-5β-cholan-24-oate 3-Sulfate (6b)**——NaOH solution (20%, 5 ml) was added to a solution of **6a** (100 mg) in MeOH (5 ml), and the solution was stirred at room temperature overnight. The resulting solution was evaporated down, and the crude product was subjected to Amberlite XAD-2 column chromatography. Recrystallization of the eluate from MeOH–ether gave **6b** (40 mg) as colorless crystals. mp 217—218° (dec.). $[\alpha]_D^{24}$ +70.4° ($c$=0.10). *Anal.* Calcd for $C_{24}H_{36}Na_2O_8S \cdot 5/2H_2O$: C, 50.08; H, 7.18. Found: C, 50.09; H, 7.23.

**Disodium 7α-Acetoxy-3α,12α-dihydroxy-5β-cholan-24-oate 3-Sulfate (4b)**——Treatment of **6a** (150 mg) as described for **4a** followed by recrystallization from MeOH–ether gave **4b** (100 mg) as colorless crystals. mp 206—216° (dec.). $[\alpha]_D^{24}$ +39.7° ($c$=0.10). *Anal.* Calcd for $C_{26}H_{40}Na_2O_9S \cdot 3H_2O$: C, 49.67; H, 7.38. Found: C, 49.50; H, 6.99.

**Preparation of 7-Sulfates**

**Disodium 12α-Acetoxy-3α,7α-dihydroxy-5β-cholan-24-oate 7-Sulfate (8c)**——Methyl cholate 3,12-diacetate (**7**) (1.38 g) was sulfated to give methyl 3α,12α-diacetoxy-7α-hydroxy-5β-cholan-24-oate 7-sulfate (**8a**) (1.4 g) as an oily material. A solution of **8a** (1.4 g) in MeOH (36 ml) and 9% NaOH (18 ml) was stirred for 2 hr. The resulting solution was poured into ice-water, neutralized with 5% HCl, and evaporated down. The residue was subjected to Amberlite XAD-2 column chromatography, and the eluate was further chromatographed on silica gel (40 g). Elution with $CHCl_3$–MeOH–AcOH–$H_2O$ (95: 24: 15: 5) and recrystallization of the eluate from MeOH–ether gave **8c** (252 mg) as colorless crystals. mp 185—187° (dec.). $[\alpha]_D^{24}$ +38.0° ($c$=0.10). *Anal.* Calcd for $C_{26}H_{40}Na_2O_9S \cdot 3/2H_2O$: C, 51.90; H, 7.20. Found: C, 52.16; H, 7.50.

**Disodium 3α-Acetoxy-7α,12α-dihydroxy-5β-cholan-24-oate 7-Sulfate (8d)**——NaOH solution (20%, 25 ml) was added to a solution of **8a** (1.8 g) in MeOH (25 ml) and the whole was stirred for 8 hr. The resulting solution was evaporated down, and the residue was subjected to Amberlite XAD-2 column chromatography. Recrystallization of the eluate from MeOH–ether gave disodium cholate 7-sulfate (**8b**) (482 mg) as colorless crystals. $Ac_2O$ (9 ml) and pyridine (18 ml) were added to a solution of **8b** (300 mg) in $H_2O$ (1.5 ml)–benzene (36 ml), and the solution was allowed to stand at room temperature for 8 hr. The reaction mixture was poured into ice-water and evaporated down. The residue was subjected to Amberlite XAD-2 column chromatography. Recrystallization of the eluate from MeOH–ether gave **8d** (254 mg) as colorless crystals. mp 182—184° (dec.). $[\alpha]_D^{24}$ +9.8° ($c$=0.10). *Anal.* Calcd for $C_{26}H_{40}Na_2O_9S \cdot 4H_2O$: C, 48.29; H, 7.48. Found: C, 48.02; H, 7.26.

**Disodium 12α-Acetoxy-7α-hydroxy-3-oxo-5β-cholan-24-oate 7-Sulfate (9a)**——$CrO_3$ (0.74 g) in AcOH (4.4 ml)-$H_2O$ (0.4 ml) was added to a solution of **8c** (500 mg) in AcOH (5 ml) and the whole was stirred at 10° for 30 min. The resulting solution was poured into ice-water, neutralized with 2 N NaOH, and evaporated down. The residue was subjected to Amberlite XAD-2 column chromatography, and the eluate obtained was further chromatographed on silica gel (15 g). Elution with $CHCl_3$–MeOH (3: 2) and recrystallization of the eluate from MeOH–ether gave **9a** (48 mg) as colorless crystals. mp 196—198° (dec.). $[\alpha]_D^{24}$ +53.0° ($c$=0.10). *Anal.* Calcd for $C_{26}H_{38}Na_2O_9S \cdot 5/2H_2O$: C, 50.56; H, 7.02. Found: C, 50.33; H, 6.76.

**Disodium 7α,12α-Dihydroxy-3-oxo-5β-cholan-24-oate 7-Sulfate (9b)**——NaOH solution (20%, 5 ml) was added to a solution of **9a** (80 mg) in MeOH (5 ml) and the mixture was stirred at room temperature overnight. The resulting solution was evaporated down, and the residue was subjected to Amberlite XAD-2 column chromatography. Recrystallization of the eluate from MeOH-ether gave **9b** (45 mg) as colorless crystals. mp 197—199° (dec.). $[\alpha]_D^{24}$ +6.8° ($c$=0.10). *Anal.* Calcd for $C_{24}H_{36}Na_2O_8S \cdot 2H_2O$: C, 50.87; H, 7.12. Found: C, 51.07; H, 6.89.

**Disodium 3α-Acetoxy-7α-hydroxy-12-oxo-5β-cholan-24-oate 7-Sulfate (10a)**——Treatment of **8d** (200 mg) as described for **9a** followed by recrystallization from MeOH–ether gave **10a** (42 mg) as colorless crystals. mp 207—210° (dec.). $[\alpha]_D^{24}$ −7.3° ($c$=0.10). *Anal.* Calcd for $C_{26}H_{38}Na_2O_9S \cdot H_2O$: C, 52.87; H, 6.83. Found: C, 53.21; H, 7.32.

**Disodium 3α,7α-Dihydroxy-12-oxo-5β-cholan-24-oate 7-Sulfate (10b)**——Treatment of **10a** (30 mg) as described for **9b** followed by recrystallization from MeOH–ether gave **10b** (16 mg) as colorless crystals. mp 198—200° (dec.). $[\alpha]_D^{24}$ +38.9° ($c$=0.10). *Anal.* Calcd for $C_{24}H_{36}Na_2O_8S \cdot H_2O$: C, 52.55; H, 6.98. Found: C, 52.59; H, 7.17.

**Preparation of 12-Sulfates**

**Disodium 3α,12α-Dihydroxy-7-oxo-5β-cholan-24-oate 12-Sulfate (11b)**——Methyl 3α-acetoxy-12α-hy-

droxy-7-oxo-5β-cholan-24-oate (**2d**) (330 mg), obtainable from **2a** by the procedure described for **8d**, was sulfated for 1 hr. Methyl 3α-acetoxy-12α-hydroxy-7-oxo-5β-cholan-24-oate 12-sulfate (**11a**) (260 mg) thus obtained was hydrolyzed with NaOH solution as described above. Recrystallization from MeOH–ether gave **11b** (218 mg) as colorless crystals. mp 217—219° (dec.). $[\alpha]_D^{24}$ +2.9° ($c$=0.10). *Anal.* Calcd for $C_{24}H_{36}Na_2O_8S \cdot 7/2H_2O$: C, 48.56; H, 7.30. Found: C, 48.69; H, 7.01.

**Disodium 3α-Acetoxy-12α-hydroxy-7-oxo-5β-cholan-24-oate 12-Sulfate (11c)**——A solution of **11b** (150 mg) in $Ac_2O$ (1.0 ml) and pyridine (1.5 ml) was allowed to stand at room temperature for 4 hr. The resulting solution was evaporated to dryness *in vacuo*, and the residue was subjected to Amberlite XAD-2 column chromatography. Recrystallization of the eluate from MeOH–ether gave **11c** (23 mg) as colorless crystals. mp 197—199° (dec.). $[\alpha]_D^{24}$ +24.3° ($c$=0.10). *Anal.* Calcd for $C_{26}H_{38}Na_2O_9S \cdot 4H_2O$: C, 48.44; H, 7.19. Found: C, 48.95; H, 7.03.

**Disodium 7α-Acetoxy-3α,12α-dihydroxy-5β-cholan-24-oate 12-Sulfate (12b)**——Sulfation of methyl 3α,7α-diacetoxy-12α-hydroxy-5β-cholan-24-oate (**1b**) for 1 hr gave methyl cholate 3,7-diacetate 12-sulfate (**12a**). **12a** (2.55 g) was hydrolyzed with 9% NaOH as described above. The resulting solution was neutralized with 5% HCl and evaporated down. The residue was subjected to Amberlite XAD-2 column chromatography. Recrystallization of the eluate from MeOH–ether gave **12b** (298 mg) as colorless crystals. mp 209.5—228° (dec.). $[\alpha]_D^{24}$ +33.0° ($c$=0.10). *Anal.* Calcd for $C_{26}H_{40}Na_2O_9S \cdot 7/2H_2O$: C, 48.97; H, 7.43. Found: C, 48.88; H, 7.38.

**Disodium 3α-Acetoxy-7α,12α-dihydroxy-5β-cholan-24-oate 12-Sulfate (12d)**——**12a** was hydrolyzed with 20% NaOH as described above to give cholate 12-sulfate (**12c**). $Ac_2O$ (0.72 ml) and pyridine (2.9 ml) were added to a solution of **12c** (40 mg) in $H_2O$ (0.4 ml)–benzene (9.6 ml), and the solution was allowed to stand at room temperature for 6 hr. Additional portions of $Ac_2O$ (0.72 ml) and pyridine (2.9 ml) were added, and the mixture was allowed to stand overnight. The resulting solution was evaporated down, and the residue was subjected to Amberlite XAD-2 column chromatography. The eluate was chromatographed on silica gel (20 g). Elution with $CHCl_3$–MeOH (3 : 1) and recrystallization of the eluate from MeOH–ether gave **12d** (26 mg) as colorless crystals. mp 206—210° (dec.). $[\alpha]_D^{24}$ +32.5° ($c$=0.10). *Anal.* Calcd for $C_{26}H_{40}Na_2O_9S \cdot 3H_2O$: C, 49.67; H, 7.38. Found: C, 49.88; H, 7.11.

**Disodium 7α-Acetoxy-12α-hydroxy-3-oxo-5β-cholan-24-oate 12-Sulfate (13a)**——Treatment of **12b** (500 mg) as described for **9a** followed by recrystallization from MeOH–ether gave **13a** (120 mg) as colorless crystals. mp 214—216° (dec.). $[\alpha]_D^{24}$ +54.4° ($c$=0.10). *Anal.* Calcd for $C_{26}H_{38}Na_2O_9S \cdot 7/2H_2O$: C, 48.44; H, 7.19. Found: C, 48.72; H, 6.68.

**Disodium 7α,12α-Dihydroxy-3-oxo-5β-cholan-24-oate 12-Sulfate (13b)**——Treatment of **13a** (200 mg) as described for **9b** followed by recrystallization from MeOH–ether gave **13b** (60 mg) as colorless crystals. mp 167—168° (dec.). $[\alpha]_D^{24}$ +32.5° ($c$=0.10). *Anal.* Calcd for $C_{24}H_{36}Na_2O_8S \cdot 2H_2O$: C, 50.87; H, 7.12. Found: C, 50.85; H, 7.16.

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