Study on the Bile Salts from Sunfish, *Mola mola* L. I. The Structures of Sodium Cyprinol Sulfates, the Sodium Salt of a New Bile Acid Conjugated with Taurine, and a New Bile Alcohol and Its New Sodium Sulfates

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New sodium bile alcohol sulfates (1a and 1b), two sodium cyprinol sulfates (2a and 2b), and the sodium salt of a new bile acid conjugated with taurine (3) were isolated from the bile of sunfish, *Mola mola* L., by chromatography on silica gel and octadecylsilane (ODS), together with sodium taurocholate (4). On hydrolysis with pyridine and dioxane, the new sulfates 1a and 1b both afforded a new bile alcohol (5), whose structure was determined to be 5β-cholestane-3α,7α,11α,26,27-pentol, based on the physico-chemical data. On the basis of this result and their physicochemical data, the new sulfates were established as (25*S*)- and (25*R*)-(+)-3α,7α,11α,26-tetrahydroxy-5β-cholestan-27-yl sodium sulfate. Based on the physicochemical data and chemical transformations, the sodium cyprinol sulfates were identified as (25*S*)- and (25*R*)-(+)-3α,7α,12α,26-tetrahydroxy-5β-cholestan-27-yl sodium sulfate and compound 3 as sodium 2-[[3α,7α,11α-trihydroxy-24-oxo-5β-cholan-24-yl]amino]ethanesulfonate.

Key words sunfish; bile; 5β-cyprinol; 5β-cholestane-3α,7α,11α,26,27-pentol; 3α,7α,11α,26-tetrahydroxy-5β-cholestan-27-yl sodium sulfate; sodium 2-[[3α,7α,11α-trihydroxy-24-oxo-5β-cholan-24-yl]amino]ethanesulfonate

Sodium scymnol sulfate and/or sodium 5β-chimaerol sulfate is a typical bile salt of living elasmobranchs,[1,2] while sodium 5α-cyprinol sulfate or sodium 5α-chimerol sulfate is characteristic of freshwater fish of the family Cyprinidae.[3,4] In contrast, the bile of marine teleosts contains only C24-bile acid, such as cholic acid and chenodeoxycholic acids, conjugated with taurine as the chief bile salts, though eels, such as *Conger myriaster* and *Anguilla japonicus*, and yellowtail, *Seriola quinqueradiata*, also contain sodium 5β-cyprinol sulfate as a cofactor bile salt.[5,6]

In the course of our studies on bile salts of fish, we investigated the bile salts of marine teleost *Mola mola* L. (sunfish), in comparison with those of sharks and carp, and we found a completely new bile acid conjugated with taurine and new bile alcohol sulfates, together with sodium taurocholate and sodium 5β-cyprinol sulfate. Here we report the isolation and structural elucidation of these bile salts from *Mola mola* L.

Isolation of four sodium bile alcohol sulfates (**1a**, **1b**, **2a** and **2b**), the sodium salt of a new bile acid conjugate with taurine (**3**) and sodium taurocholate (**4**) from the gallbladder of *Mola mola* L. was achieved by three steps of column chromatography as summarized in Chart 1. These compounds have not yet been crystallized. The isolation processes are described in the experimental section.

Hydrolysis with pyridine and dioxane of the two sodium bile alcohol sulfates,[2] **2a** and **2b**, afforded a good yield of compound **6**, which was identified as 5β-cyprinol (5β-cholestane-3α,7α,12α,26,27-hexol) by direct comparison of the spectral data (IR, NMR, FAB-MS) with those of an authentic sample prepared from 5β-cholic acid by reference to a reported procedure (Charts 2, 3).[7] From the positive FAB-MS and elemental analyses of **2a** and **2b**, their molecular formulae were each determined to be $C_{27}H_{47}NaO_8S$. In the IR spectrum, each showed an absorption band assigned to the sulfate ester function (**2a**: 1250 cm^{-1}, **2b**: 1213 cm^{-1}). The ^{13}C-NMR data of **2a**, **2b** and 5β-cyprinol are given in Table 1. There are no significant differences in the chemical shifts of corresponding carbons, except for those of the carbon and proton at C_{27} in the side chain, among these compounds. The downfield shift of the carbons of C_{27} of **2a** and **2b** in comparison with that of 5β-cyprinol, as shown in Table 1, indicates that the hydroxy group at C_{27} is esterified with SO_3Na in **2a** and **2b**.[2,8] Further, there is no significant difference in the chemical shifts of the corresponding carbons and protons between **2a** and **2b**, as in the case of sodium scymnol sulfates of *Lamna ditropis*.[2b] Thus, these sulfates are concluded to be (25*R*)- and (25*S*)-(+)-3α,7α,12α,26-tetrahydroxy-5β-cholestan-27-yl sodium sulfate.

The structure elucidation of the other two new sodium bile alcohol sulfates, **1a** and **1b**, was carried out as follows: From direct atomic absorption analysis,[9] the sulfates have a sodium atom in the molecule. The positive FAB-MS showed the ion peak 555, indicating that their molecular weights were 554. From these data and elemental analysis, the molecular formulae were determined to be $C_{27}H_{47}NaO_8S$. The IR spectrum, which resembles that of sodium 5β-cyprinol sulfate, showed absorption bands (**1a**: 3426, 1246 cm^{-1}, **1b**: 3424, 1250 cm^{-1}) assignable to alcohol and sulfate ester functions. A detailed comparison of the ^{13}C-NMR data of the sulfates with those of sodium 5β-cyprinol sulfates indicated that the former sulfates are the sodium sulfate salts of a bile alcohol with a 5β-cholestane skeleton.

Structural confirmation was carried out in the following way. As depicted in Chart 2, the two sulfates, **1a** and **1b**, afforded compound **5** in good yield on hydrolysis with pyridine and dioxane. From the positive FAB-MS and elemental analysis of **5**, its molecular formula was determined to be $C_{27}H_{48}O_5$. The ^{13}C-NMR data of **5** and 11α-hydroxyandrostane are given in Table 2.[10] By detailed

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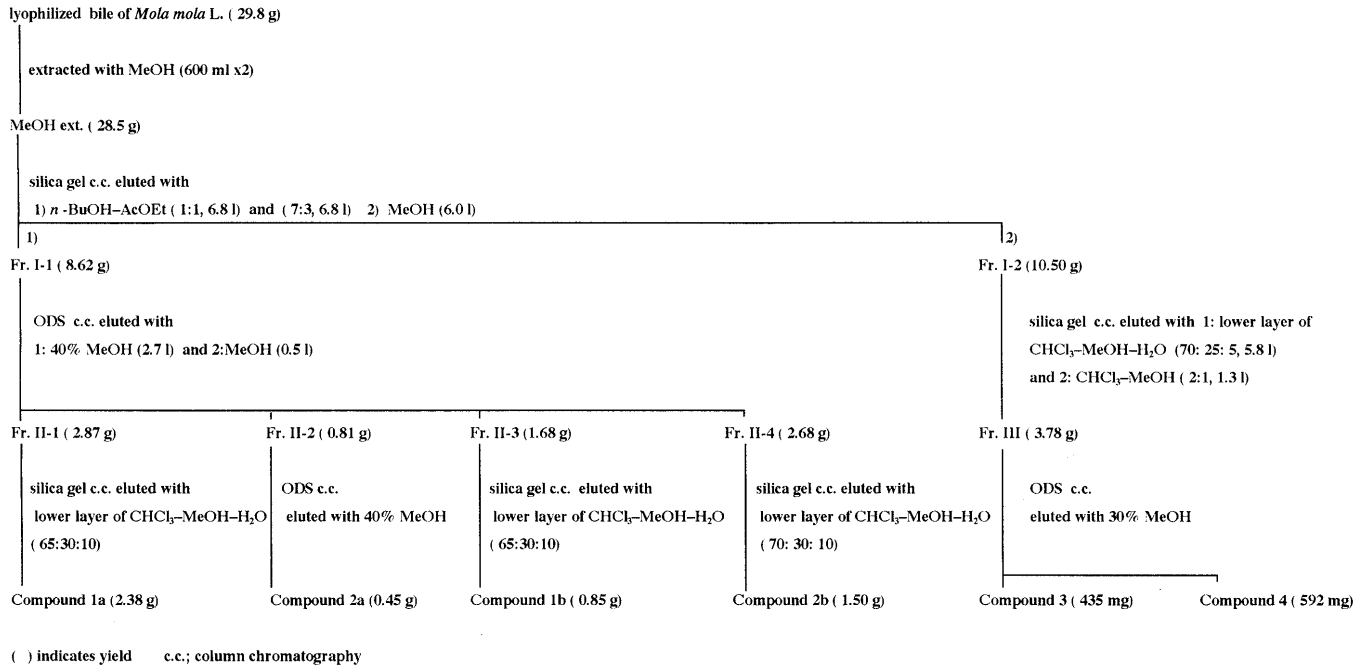


Chart 1. Isolation Procedure for Compounds **1**—**4** from *Mola mola* L.

comparison of the data of **5** with those of 5β-cyprinol, the signals for C_1 to C_8, C_{15}, C_{16} and C_{18} to C_{27} of **5** were assigned. Among the remaining 7 signals, five [38.1 (s), 70.4 (d), 52.9 (t), 44.5 (s), 51.7 (t)] are assignable to C_{10} to C_{14} on the basis of a comparison with those of 11α-hydroxyandrostane. In addition, the downfield shifts of 13 ppm for C_9 and 9 ppm for C_{17} of **5**, as compared with those of 5β-cyprinol, are well accounted for by the shift effects of the hydroxy group of both compounds on the β-carbon (+2—+9 ppm) and γ carbon (−1——8 ppm).[10] Thus, **5** is concluded to be 5β-cholestane-3α,7α,11α,26,27-pentol, designated as 5β-molanol. This was supported by analyses of the NMR (^{1}H, ^{13}C noise-decoupled, DEPT (distortionless enhancement by polarization transfer), ^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H–^{13}C COSY and HMBC (heteronuclear multiple bond connectivity)) spectra and nuclear Overhauser effects (NOEs) (Fig. 1A).

The deshielding of the C_{27} carbon in the new salts, **1a** and **1b**, compared with **5** indicated that the hydroxy group at C_{27} in these salts was indeed esterified with SO_3Na (Table 1), as in the sodium sulfates of 5β-cyprinol noted above. In addition, there is no significant difference in the chemical shifts of the corresponding carbons and protons between **1a** and **1b**. Thus, the salts were concluded to be (25*S*)- and (25*R*)-(+)-3α,7α,11α,26-tetrahydroxy-5β-cholestan-27-yl sodium sulfate, designated as sodium 5β-molanol sulfate.

The structure of compound **3** was elucidated as follows: From the positive FAB-MS and elemental analysis, the molecular formula of **3** was determined to be $C_{26}H_{44}NNaO_7S$. From a detailed comparison of its ^{13}C-NMR data with those of 5β-molanol (**5**), the signals for C_1 to C_{21} of **3** were assigned (Table 2). The remaining 5 signals, [35.0 (t), 33.8 (t), 177.2 (s), 37.4 (t), 52.3 (t)] were assigned to C_{22} to C_{24}, $C_{1'}$ and $C_{2'}$ on the basis of comparison with those of sodium taurocholate (**4**). These results suggest

Table 1. ^{13}C-NMR Data for **1a**, **1b**, **2a**, **2b**, **5**, and 5β-Cyprinol (**6**) in C_5D_5N

Position	**1a**	**1b**	**5**	**2a**	**2b**	5β-Cyprinol (**6**)
20	36.5 d	35.9 d	36.3 d	36.2 d	36.2 d	36.3 d
21	19.0 q	18.7 q	18.8 q	17.8 q	17.8 q	17.9 q
22	36.6 t	36.3 t	36.6 t	36.6 t	36.6 t	36.9 t
23	24.3 t	24.1 t	24.1 t	23.9 t	23.9 t	24.2 t
24	28.8 t	28.6 t	28.6 t	28.8 t	28.9 t	29.2 t
25	42.1 d	41.7 d	42.9 d	41.7 d	41.8 d	42.5 d
26	62.5 t	62.2 t	63.8 t	62.3 t	62.3 t	63.8 t
27	68.3 t	68.6 t	64.0 t	68.6 t	68.4 t	64.1 t

δ_C values in ppm. Multiplicities of carbon signals were determined by means of the DEPT method and are indicated as d, t and q. Assignments were based on ^{1}H–^{1}H and ^{1}H–^{13}C COSY, DEPT, and HMBC experiments.

that this compound is sodium 2-[[3α,7α,11α-trihydroxy-24-oxo-5β-cholan-24-yl]amino]ethanesulfonate. This was supported by analyses of the NMR (^{1}H, ^{13}C noise-decoupled, DEPT, ^{1}H–^{1}H and ^{1}H–^{13}C COSY and HMBC) spectra and NOEs (Fig. 1B), and by the finding that one equivalent of taurine was obtained by HCl hydrolysis of **3**.

As noted above, the bile of *Mola mola* L. contains mainly sodium bile alcohol sulfates, sodium 5β-cyprinol and 5β-molanol sulfates, together with two sodium salts of bile acid conjugated with taurine, sodium taurocholate and sodium 2-[[3α,7α,11α-trihydroxy-24-oxo-5β-cholan-24-yl]amino]ethanesulfonate. Previously, it has been shown that among marine teleosts, the eels, *Conger myriaster*, *Anguila japonicus* and *Muraenesox cinereus*, and yellowtail, *Seriola quinqueradiata*, have only cholic and chenodeoxycholic acids conjugated with taurine as their chief bile salts and sodium 5β-cyprinol sulfate as a cofactor in their biles.[5,6] In contrast, other marine teleostean fishes have only taurine conjugates of these C-24 bile acids.[11]

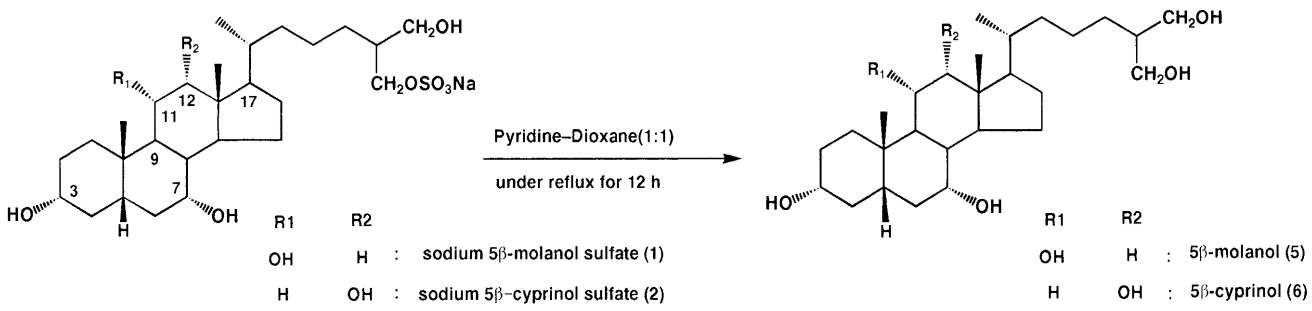


Chart 2. Preparation of **5** from Sodium 5β-Molanol Sulfate (**1**) and **6** from Sodium 5β-Cyprinol Sulfate (**2**)

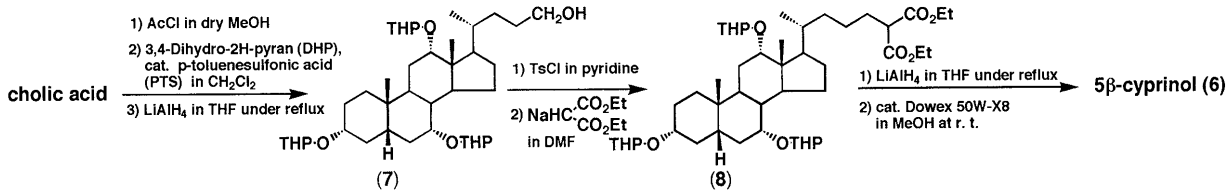


Chart 3. Synthesis of 5β-Cyprinol (**6**) from Cholic Acid

Table 2. ^{13}C-NMR Data for Sodium Taurocholate (ST) (**4**), **3**, **5**, **6** and 11α-Hydroxyandrostane (11HA) in CD_3OD

Position	ST (4)	3	5	5β-Cyprinol (6)	11HA
1	37.3 t	40.2 t	40.0 t	37.3 t	40.2 t
2	32.0 t	33.2 t	33.0 t	32.2 t	22.6 t
3	73.7 d	74.1 d	73.8 d	73.7 d	26.7 t
4	41.2 t	41.9 t	41.7 t	41.3 t	29.7 t
5	43.7 d	45.4 d	45.2 d	43.8 d	47.0 d
6	36.6 t	37.0 t	37.0 t	36.7 t	29.7 t
7	69.8 d	69.8 d	69.6 d	69.9 d	32.8 t
8	41.8 d	41.0 d	40.9 d	41.8 d	35.4 d
9	28.7 d	42.0 d	41.8 d	28.7 d	61.2 d
10	36.7 s	38.3 s	38.1 s	36.7 s	38.4 s
11	30.4 t	70.5 d	70.4 d	29.6 t	69.2 d
12	74.8 d	53.0 t	52.9 t	74.9 d	50.5 t
13	48.3 s	44.7 s	44.0 s	48.2 s	41.2 s
14	44.0 d	51.8 d	51.7 d	44.0 d	53.7 d
15	25.0 t	25.4 t	25.4 t	25.0 t	25.6 t
16	29.4 t	30.0 t	30.0 t	30.4 t	20.6 t
17	48.8 d	58.1 d	58.2 d	49.1 d	40.8 t
18	13.8 q	13.8 q	13.9 q	13.8 q	18.4 q
19	24.0 q	24.9 q	24.9 q	24.0 q	12.8 q
20	37.7 d	37.6 d	37.8 d	38.0 d	
21	18.5 q	19.6 q	20.0 q	18.9 q	
22	35.0 t	35.0 t	38.1 t	38.3 t	
23	33.9 t	33.8 t	25.4 t	25.6 t	
24	177.5 s	177.2 s	30.0 t	30.2 t	
25			44.9 d	45.2 d	
26			64.5 t	64.6 t	
27			64.7 t	64.8 t	
1′	37.4 t	37.4 t			
2′	52.3 t	52.3 t			

δ_C values in ppm. Multiplicities of carbon signals were determined by means of the DEPT method and are indicated as d, t and q. Assignments were based on ^{1}H–^{1}H and ^{1}H–^{13}C COSY, DEPT, and HMBC experiments.

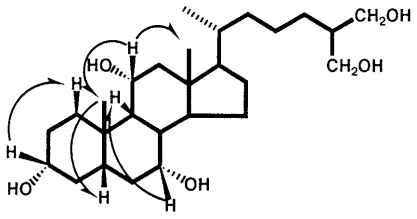
Thus, the bile salts pattern of *Mola mola* L. partially resembles those of eels and yellowtail. In higher vertebrates, including teleosts, bile alcohol sulfate has been completely replaced by taurine and/or glycine conjugates of C-24 bile acids, such as cholic acid and chenodeoxycholic acid, so it may be concluded that the bile salts of *Mola mola* L., as well as those of eels and yellowtail, are those to be expected of fishes largely advanced in an evolutionary sense, but still possessing primitive features.

Sodium 5β-cyprinol sulfate is widely distributed in different species. The biles of sturgeons, Acipenseridae and *Huso huso* L., and paddlefish, *Plyodon spathula*, contain this sulfate.[12,13] It was also isolated from the bile of a frog, *Rana nigromaculate*.[14] Structural determination of this sulfate in bile should be helpful for clarifying evolutionary relationships, as in the case of bile salt of the shark.[2]

As noted above, two sodium 5β-molanol sulfates, (25*S*)- and (25*R*)-(+)-3α,7α,11α,26-tetrahydroxy-5β-cholestan-27-yl sodium sulfate (**1a** and **1b**), and sodium 2-[[3α,7α,11α-trihydroxy-24-oxo-5β-cholan-24-yl]amino]-ethanesulfonate (**3**) were isolated from the bile of *Mola mola* L. To our knowledge, this is the first isolation of an 11α-hydroxylated steroidal constituent from the bile of a vertebrate, including secondary metabolic products formed from primary bile salts. It is noteworthy, because this result implies that during its evolution, *Mola mola* L. has gained an enzyme which can introduce an α-hydroxy group directly at C_{11} of the 5β-cholestane skeleton to produce **5**. Another possibility is that sunfish can catalyze 11β-hydroxylation of the 5β-cholestane skeleton and then the configuration of the product is converted from β to α with the help of intestinal flora, as in the case of formation of ursodeoxycholic acid from chenodeoxycholic acid. Thus, sunfish may contain a steroidogenic 11α or 11β-hydroxylase, like P450c11 in human adrenocortical cells,[15] for its bile component. It is noteworthy that the new C-27 bile alcohol, **5**, is metabolized to the corresponding new C-24 bile acid and then to its taurine conjugate, **3**: this seems to resemble the conversion of 5β-cyprinol, **6**, to taurocholate, **4**.

In summary, we have established an isolation procedure for new sodium bile alcohol sulfates, two sodium 5β-cyprinol sulfates and a new bile acid conjugated with taurine from the bile of *Mola mola* L. and prepared a new bile alcohol from the new sulfates. The new bile alcohol

A) 5β-molanol (5β-cholestane-3α,7α,11α,26,27-pentol) (5)



B) sodium 2-[[3α,7α,11α-trihydroxy-24-oxo-5β-cholan-24-yl]amino]ethanesulfonate (3)

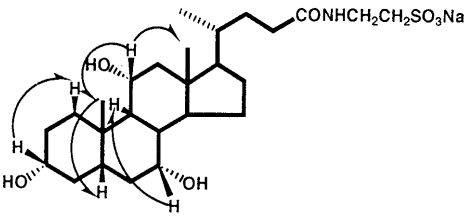


Fig. 1. Results of Two Dimensional Correlations and NOE Experiments on **3** and **5**

Heavy lines indicate the connectivities assigned on the basis of ^{1}H–^{1}H COSY and HMBC. Arrows denote irradiated protons (tail)/observed protons (head) in NOE difference experiments.

was identified as 5β-cholestane-3α,7α,11α,26,27-pentol, and the new sulfates as (25*S*)- and (25*R*)-(+)-3α,7α,11α,26-tetrahydroxy-5β-cholestan-27-yl sodium sulfate. Sodium 5β-cyprinol sulfates were identified as (25*S*)- and (25*R*)-(+)-3α,7α,12α,26-tetrahydroxy-5β-cholestan-27-yl sodium sulfate and the salt of the new bile acid as sodium 2-[[3α,7α,11α-trihydroxy-24-oxo-5β-cholan-24-yl]amino]-ethanesulfonate. Stereochemical and pharmacological studies on the new bile alcohol, its sodium sulfate and the new bile acid are in progress.

Experimental

Melting points were determined on a Yanaco micro melting point apparatus and are uncorrected. IR spectra were taken on a JASCO IR-700 IR spectrophotometer. Optical rotation was measured with a JASCO DIP-140. FAB-MS were obtained on a JMS-SX102 machine, using glycerin as the matrix. NMR spectra were recorded on JEOL GX-500 and α-400 spectrometers using tetramethylsilane (TMS) as an internal standard. Chemical shifts are recorded in δ values (ppm) and coupling constants (*J*) in hertz (Hz). Multiplicities of ^{13}C-NMR signals were determined by the DEPT method. ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY, HMBC and NOE difference spectra were obtained with the JEOL standard pulse sequences and data processing was performed with the standard software.

Material Gall-bladders, obtained from sunfishes, *Mola mola* L. (*ca.* 30—40 kg × 4), caught in 1992—1994 near Suruga Bay, Shizuoka Prefecture, Japan, were homogenized and the mixture was freeze-dried. This material (29.8 g) was used as the starting material for isolation.

Isolation of Bile Salts from the Bile of *Mola mola* L. The lyophilized bile (29.8 g) of *Mola mola* L. was extracted with MeOH (600 ml × 2). The MeOH extract (28.5 g) was applied to a silica gel column and elution with *n*-BuOH and AcOEt (1 : 1, 6.8 l and 7 : 3, 6.8 l) and then MeOH (6.0 l) gave fractions I-1 (8.62 g) and I-2 (10.50 g), respectively.

Fraction I-1 (8.62 g) was chromatographed on an octadecyl silica (ODS) (YMC-gel ODS-60A) column with 40% MeOH to afford four fractions, II-1 (2.87 g), -2 (0.81 g), -3 (1.68 g) and -4 (2.68 g).

Fraction II-1 (2.87 g) was applied to a silica gel column and elution with the lower layer of $CHCl_3$–MeOH–H_2O (65 : 30 : 10) afforded compound **1a** (2.38 g). In the same manner, compound **1b** (0.85 g) was obtained from fraction II-3 (1.68 g). Fraction II-2 (0.81 g) was subjected to ODS (Sep-Pak^R, Millipore) with 40% MeOH to afford compound **2a** (0.45 g). Fraction II-4 (2.68 g) was chromatographed on a silica gel column with the lower layer of $CHCl_3$–MeOH–H_2O (70 : 30 : 10) to afford compound **2b** (1.50 g).

Fraction I-2 (10.50 g) was subjected to silica gel column chromatography and elution with the lower layer of $CHCl_3$–MeOH–H_2O (70 : 25 : 5) afforded fraction III (3.78 g). This was chromatographed on ODS (YMC-gel ODS-60A) with 30% MeOH to afford compounds **3** (435 mg) and **4** (592 mg). Compound **4** was identified as sodium taurocholate by direct comparison of the spectral data (IR, NMR and FAB-MS) with those of an authentic sample (Sigma Chem. Co.).

The physical properties of **1a, b**, **2a, b** and **3** are as follows. These compounds give the same physical properties after being chromatographed on Dowex-1 with H_2O and dilute NaOH.

Compound **1a**: White amorphous powder. $[\alpha]_D^{25}$ +17.6° (*c* = 1.0, MeOH). *Anal.* Calcd for $C_{27}H_{47}NaO_8S \cdot 2H_2O$: C, 54.92; H, 8.31. Found: C, 55.05; H, 8.29. FAB-Ms *m/z*: 555 $(C_{27}H_{47}NaO_8S+H)^+$, 537 $(C_{27}H_{47}NaO_8S-H_2O+H)^+$, 519 $(C_{27}H_{47}NaO_8S-2H_2O+H)^+$, 457 $(C_{27}H_{47}NaO_8S-SO_3Na+H)^+$. IR ν_{max} (KBr) cm^{-1}: 3426, 2930, 2866, 1465, 1375, 1246, 1074, 1013, 987. ^{1}H-NMR (in C_5D_5N, 500 MHz) δ: 4.67 (1H, dd, *J* = 10.0, 4.5, 27-H_A), 4.58 (1H, dd, *J* = 10.0, 7.0, 27-H_B), 4.23—4.17 (1H, m, 11-H_β), 4.11—4.06 (1H, m, 7-H_β), 3.99 (1H, dd, *J* = 11.0, 4.5, 26-H_A), 3.95 (1H, dd, *J* = 11.0, 6.5, 26-H_B), 3.93—3.87 (1H, m, 3-H_β), 3.25 (1H, ddd, *J* = 13.2, 2.6, 2.6), 3.16 (1H, ddd, *J* = 13.5, 13.5, 13.5), 2.62—2.53 (2H, m), 2.47—2.36 (1H, m), 2.22—2.02 (4H, m), 1.96—1.88 (2H, m), 1.83—1.71 (2H, m), 1.67—1.62 (1H, m), 1.57—1.35 (5H, m), 1.37—1.23 (4H, m), 1.12 (3H, s, 19-H), 1.20—1.06 (3H, m), 0.99—0.89 (1H, m), 0.84 (3H, d, *J* = 6.0, 21-H), 0.73 (3H, s, 18-H). ^{13}C-NMR (in C_5D_5N, 125.7 MHz) δ: 72.4 (C-3), 68.8 (C-11), 68.3 (C-27), 67.9 (C-7), 62.5 (C-26), 56.7 (C-17), 52.4 (C-12), 50.8 (C-14), 44.3 (C-5), 43.2 (C-13), 42.1 (C-25), 41.9 (C-9), 41.1 (C-4), 39.9 (C-8), 39.5 (C-1), 37.3 (C-10), 36.6 (C-22), 36.5 (C-20), 36.2 (C-6), 33.5 (C-2), 29.2 (C-16), 28.8 (C-24), 24.3 (C-15, 23), 24.0 (C-19), 19.0 (C-21), 13.3 (C-18).

Compound **1b**: White amorphous powder. $[\alpha]_D^{25}$ +11.0° (*c* = 1.0, MeOH). *Anal.* Calcd for $C_{27}H_{47}NaO_8S \cdot 2H_2O$: C, 54.92; H, 8.31. Found: C, 55.03; H, 8.27. FAB-MS *m/z*: 555 $(C_{27}H_{47}NaO_8S+H)^+$, 537 $(C_{27}H_{47}NaO_8S-H_2O+H)^+$, 519 $(C_{27}H_{47}NaO_8S-2H_2O+H)^+$, 457 $(C_{27}H_{47}NaO_8S-SO_3Na+H)^+$. IR ν_{max} (KBr) cm^{-1}: 3424, 2930, 2864, 1463, 1374, 1250, 1074, 1013, 983. ^{1}H-NMR (in C_5D_5N, 500 MHz) δ: 4.72 (1H, dd, *J* = 9.6, 4.2, 27-H_A), 4.59 (1H, dd, *J* = 9.6, 6.5, 27-H_B), 4.23—4.17 (1H, m, 11-H_β), 4.11—4.06 (1H, m, 7-H_β), 3.96 (1H, dd, *J* = 11.0, 4.5, 26-H_A), 3.92 (1H, dd, *J* = 11.0, 7.0, 26-H_B), 3.93—3.88 (1H, m, 3-H_β), 3.24 (1H, ddd, *J* = 13.2, 2.6, 2.6), 3.15 (1H, ddd, *J* = 13.5, 13.5, 13.5), 2.62—2.52 (2H, m), 2.47—2.38 (1H, m), 2.21—2.02 (4H, m), 1.96—1.87 (2H, m), 1.82—1.69 (2H, m), 1.69—1.62 (1H, m), 1.57—1.49 (5H, m), 1.46—1.23 (4H, m), 1.12 (3H, s, 19-H), 1.20—1.08 (3H, m), 0.98—0.88 (1H, m), 0.86 (3H, d, *J* = 6.0, 21-H), 0.72 (3H, s, 18-H). ^{13}C-NMR (in C_5D_5N, 125.7 MHz) δ: 72.2 (C-3), 68.6 (C-11, 27), 67.6 (C-7), 62.2 (C-26), 56.5 (C-17), 52.2 (C-12), 50.6 (C-14), 44.1 (C-5), 42.9 (C-13), 41.7 (C-25, 9), 40.9 (C-4), 39.6 (C-8), 39.2 (C-1), 37.0 (C-10, 22), 36.3 (C-6), 35.9 (C-20), 33.3 (C-2), 28.7 (C-16), 28.6 (C-24), 24.1 (C-15, 23), 23.7 (C-19), 18.7 (C-21), 13.1 (C-18).

Compound **2a**: White amorphous powder; $[\alpha]_D^{25}$ +21.2°(*c* = 1.0, MeOH). *Anal.* Calcd for $C_{27}H_{47}NaO_8S \cdot 2H_2O$: C, 54.92; H, 8.31. Found: C, 50.13; H, 8.34. FAB-MS *m/z*: 555 $(C_{27}H_{47}NaO_8S+H)^+$, 537 $(C_{27}H_{47}NaO_8S-H_2O+H)^+$, 457 $(C_{27}H_{47}NaO_8S-SO_3Na+H)^+$. IR ν_{max} (KBr) cm^{-1}: 3432, 2936, 2866, 1464, 1375, 1209, 1074, 1039, 979. ^{1}H-NMR (in C_5D_5N, 500 MHz) δ: 4.71 (1H, dd, *J* = 9.7, 4.3, 27-H_A), 4.58 (1H, dd, *J* = 9.7, 6.3, 27-H_B), 4.25—4.21 (1H, m, 12-H_β), 4.11—4.07 (1H, m, 7-H_β), 3.96 (1H, dd, *J* = 11.0, 4.8, 26-H_A), 3.91 (1H, dd, *J* = 11.0, 7.0, 26-H_B), 3.76—3.70 (1H, m, 3-H $_\beta$), 3.08 (1H, dd, *J* = 12.5, 12.5, 12.5), 2.89 (1H, ddd, *J* = 11.8, 11.8, 7.0), 2.75—2.67 (1H, m), 2.27—2.20 (1H, m), 2.12—1.71 (10H, m), 1.70—1.58 (2H, m), 1.54—1.16 (10H, m), 1.12 (3H, d, *J* = 6.5, 21-H), 1.10—1.03 (1H, m), 1.00 (3H, s, 19-H), 0.79 (3H, s, 18-H). ^{13}C-NMR (in C_5D_5N, 125.7 MHz) δ: 72.4 (C-12), 71.8 (C-3), 68.6 (C-27), 67.7 (C-7), 62.3 (C-26), 47.3 (C-17), 46.8 (C-13), 42.7 (C-14), 42.5 (C-5), 41.7 (C-25), 41.0 (C-8), 40.7 (C-4), 36.6 (C-22), 36.2 (C-20), 35.8 (C-1), 35.3 (C-10, 6), 31.6 (C-2), 29.6 (C-16), 28.8 (C-24), 28.2 (C-11), 27.4 (C-9), 23.9 (C-23), 23.8 (C-15), 23.2 (C-19), 17.8 (C-21), 13.0 (C-18).

Compound **2b**: White amorphous powder. $[\alpha]_D^{25}$ +39.6° (*c* = 1.0, MeOH). *Anal.* Calcd for $C_{27}H_{47}NaO_8S \cdot 2H_2O$: C, 54.92; H, 8.31. Found: C, 55.13; H, 8.34. FAB-MS *m/z*: 555 $(C_{27}H_{47}NaO_8S+H)^+$, 537 $(C_{27}H_{47}NaO_8S-H_2O+H)^+$, 457 $(C_{27}H_{47}NaO_8S-SO_3Na+H)^+$. IR

ν_{max} (KBr) cm^{-1}: 3418, 2936, 2864, 1466, 1374, 1213, 1073, 1039, 979. ^{1}H-NMR (in C_5D_5N, 500 MHz) δ: 4.70 (1H, dd, J=10.3, 4.6, 27-H_A), 4.58 (1H, dd, J=10.3, 6.9, 27-H_B), 4.24—4.21 (1H, m, 12-H_β), 4.12—4.09 (1H, m, 7-H_β), 3.96 (1H, dd, J=11.0, 4.3, 26-H_A), 3.91 (1H, dd, J=11.0, 6.5, 26-H_B), 3.77—3.69 (1H, m, 3-H_β), 3.09 (1H, dd, J=12.5, 12.5, 12.5), 2.90 (1H, ddd, J=11.8, 11.8, 7.0), 2.76—2.27 (1H, m), 2.28—2.21 (1H, m), 2.12—1.72 (10H, m), 1.70—1.61 (2H, m), 1.52—1.18 (10H, m), 1.13 (3H, d, J=6.0, 21-H), 1.12—1.03 (1H, m), 1.00 (3H, s, 19-H), 0.80 (3H, s, 18-H). ^{13}C-NMR (in C_5D_5N, 125.7 MHz) δ: 72.4 (C-12), 71.8 (C-3), 68.4 (C-27), 67.7 (C-7), 62.3 (C-26), 47.3 (C-17), 46.8 (C-13), 42.7 (C-14), 42.5 (C-5), 41.8 (C-25), 41.0 (C-8), 40.7 (C-4), 36.6 (C-22), 36.2 (C-20), 35.8 (C-1), 35.3 (C-10, 6), 31.6 (C-2), 29.6 (C-16), 28.9 (C-24), 28.2 (C-11), 27.4 (C-9), 23.9 (C-23, 15), 23.2 (C-19), 17.8 (C-21), 13.0 (C-18).

Compound **3**: White amorphous powder; $[\alpha]_D^{25}$ +21.8° (c=1.0, MeOH). *Anal.* Calcd for $C_{26}H_{44}NNaO_7S\cdot 2H_2O$: C, 54.45; H,8.57; N, 2.44. Found: C, 54.37; H, 8.66; N, 2.43. FAB-MS m/z: 538 $(C_{26}H_{44}NNaO_7S+H)^+$, 520 $(C_{26}H_{44}NNaO_7S-H_2O+H)^+$, 502 $(C_{26}H_{44}NNaO_7S-2H_2O+H)^+$. IR ν_{max} (KBr) cm^{-1}: 3400, 2926, 2868, 1636, 1542, 1457, 1376, 1212, 1048. ^{1}H-NMR (in CD_3OD, 500 MHz) δ: 3.83—3.80 (1H, m, 11-H_β), 3.79—3.76 (1H, m, 7-H_β), 3.58 (2H, t, J=7.0, 1′-H), 3.42—3.38 (1H, m, 3-H_β), 2.95 (2H, t, J=7.0, 2′-H), 2.58—2.54 (1H, m, 1-H_α), 2.31—2.21 (3H, m), 2.12—2.04 (1H, m), 1.99—1.90 (3H, m), 1.82—1.17 (14H, m), 1.10—1.06 (1H, m), 1.03 (3H, s, 19-H), 1.00 (3H, d, J=6.0, 21-H), 0.96—0.92 (1H, m), 0.69 (3H, s, 18-H). ^{13}C-NMR data in CD_3OD are given in Table 2.

Hydrolysis of Sodium Bile Alcohol Sulfate with Pyridine-Dioxane Preparation of Compound 5 from Sodium 5β-Molanol Sulfate 1 A solution of 250.0 mg of **1a** in 5 ml of pyridine–dioxane (1 : 1) was refluxed for 12 h and then 1 ml of aqueous 1 M $BaCl_2$ was added to it. The precipitate was removed by filtration to yield 91 mg of $BaSO_4$, and the filtrate was concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$ and MeOH (8 : 1) to afford 135 mg of 5β-molanol (**5**). Crystallization of this product from dilute MeOH afforded colorless plates (116 mg). Hydrolysis of **1b** (50 mg) in the manner described above furnished 28 mg of **5**. The physical properties of **5** are as follows. Compound **5**: Colorless plates; mp 197—198°C. $[\alpha]_D^{25}$ +24.6° (c=1.0, MeOH). *Anal.* Calcd for $C_{27}H_{48}O_5\cdot H_2O$: C, 68.88; H,10.71. Found: C, 68.95; H, 10.84. FAB-MS m/z: 453 $(C_{27}H_{48}O_5+H)^+$, 435 $(C_{27}H_{48}O_5-H_2O+H)^+$, 417 $(C_{27}H_{48}O_5-2H_2O+H)^+$, 399 $(C_{27}H_{48}O_5-3H_2O+H)^+$. IR ν_{max} (KBr) cm^{-1}: 3352, 2930, 2868, 1458, 1439, 1375, 1077, 1032, 995. ^{1}H-NMR (in CD_3OD, 500 MHz) δ: 3.82 (1H, dddd, J=10.7, 9.2, 5.3, 2.6, 11-H_β), 3.80—3.77 (1H, m, 7-H_β), 3.55 (4H, d, J=5.5, 26, 27-H), 3.36—3.43 (1H, m, 3-H_β), 2.56 (1H, ddd, J=15.8, 3.3, 3.3, 1-H_α), 2.29 (1H, dd, J=12.5, 5.3, 12-H_β), 2.25 (1H, ddd, J=13.3, 13.0, 3.3, 4-H_α), 1.96 (1H, ddd, J=14.5, 6.0, 3.3, 6-H_β), 1.95 (1H, dd, J=11.2, 9.2, 9-H_α), 1.95—1.88 (1H, m, 16-H_α), 1.73—1.68 (1H, m, 15-H_β), 1.68—1.61 (1H, m, 4-H_β), 1.61—1.56 (3H, m, 25-H, 2-H), 1.53—1.48 (3H, m, 14-H_β, 8-H_β, 6-H_α), 1.44—1.40 (3H, m, 23-H_A, 22-H_A, 20-H), 1.36—1.20 (7H, m, 5-H $_\beta$, 16-H_β, 23-HB, 12-H_α, 24-H, 17-H_α), 1.09—1.04 (2H, m, 15-H_α, 22-H_B), 1.03 (3H, s, 19-H), 0.95—0.91 (1H, m, 1-H_β), 0.94 (3H, d, J=6.5, 21-H), 0.70 (3H, s, 18-H). ^{13}C-NMR data in CD_3OD are given in Table 2.

Preparation of Compound 6 from Sodium 5β-Cyprinol Sulfate 2 Pure **6** (110 mg) and 42 mg of $BaSO_4$ were obtained from compound **2a** (125 mg) according to the same procedure as described above. Crystallization of this product from MeOH and AcOEt afforded colorless plates (54 mg). Hydrolysis of **2b** (50 mg), in the same manner as described above, furnished 38 mg of **6**. The physical properties of **6** are as follows. Compound **6**: Colorless plates; mp 180.0°C (lit. 174°C).[5] $[\alpha]_D^{25}$ +18.1° (c=1.0, MeOH). *Anal.* Calcd for $C_{27}H_{48}O_5\cdot 1/2CH_3OH$: C, 70.46; H, 10.76. Found: C, 70.34; H, 10.77. FAB-MS m/z: 453 $(C_{27}H_{48}O_5+H)^+$, 435 $(C_{27}H_{48}O_5-H_2O+H)^+$, 417 $(C_{27}H_{48}O_5-2H_2O+H)^+$, 399 $(C_{27}H_{48}O_5-3H_2O+H)^+$. IR ν_{max} (KBr) cm^{-1}: 3398, 2936, 2860, 1664, 1643, 1631, 1467, 1453, 1372, 1244, 1071, 1040, 979, 810. ^{1}H-NMR (in CD_3OD, 500 MHz) δ: 3.97—3.94 (1H, m, 12-H_β), 3.80—3.77 (1H, m, 7-H_β), 3.55 (4H, d, J=6.0, 26, 27-H), 3.39—3.30 (1H, m, 3-H_β), 2.33—2.19 (2H, m), 2.02—1.20 (22H, m), 1.16—1.05 (2H, m), 1.01 (3H, d, J=6.5, 21-H), 0.98—0.94 (1H, m), 0.91 (3H, s, 19-H), 0.71 (3H, s, 18-H). ^{13}C-NMR data in CD_3OD are given in Table 2. This compound was identified as 5β-cyprinol by direct comparison with an authentic sample prepared from 5β-cholic acid as follows.

Synthesis of 5β-Cyprinol from Cholic Acid 3,4-Dihydro-2*H*-pyran (535 mmol) was reacted with methyl cholate (23.7 mmol) in the presence of a catalytic amount of *p*-toluenesulfonic acid (43 mg), followed by reduction with $LiAlH_4$ (158 mmol) to obtain 3α,7α,12α-tri(tetrahydro-2′-pyranoxy)-5β-cholan-24-ol (**7**) (97%) (amorphous powder, *Anal.* Calcd for $C_{36}H_{66}O_7$: C, 72.63; H, 10.00. Found: C, 72.61; H, 10.28. mass m/z: 647 (M^+). IR ν_{max} (KBr) cm^{-1}: 3280). *p*-Toluenesulfonyl chloride (2.4 mmol) was then reacted with **7** (2.0 mmol) in dry pyridine (3 ml) to afford 3α,7α,12α-tri(tetrahydro-2′-pyranoxy)-24-tosyloxy-5β-cholane (89%). Reaction of the sodium salt of diethyl malonate (1.2 mmol) with this tosylate (1.2 mmol) in dry dimethylformamide (DMF) gave diethyl 3α,7α,12α-tri(tetrahydro-2′-pyranoxy)-5β-cholestane-26,27-dioate (**8**) (89%) (amorphous powder, *Anal.* Calcd for $C_{46}H_{76}O_{10}$: C, 70.00; H, 9.64. Found: C, 69.83; H, 9.51. IR ν_{max} (KBr): 1753 cm^{-1}). **8** (0.5 mmol) was reduced with $LiAlH_4$ (5 mmol) in dry tetrahydrofuran (THF) (15 ml) to yield 3α,7α,12α-tri(tetrahydro-2′-pyranoxy)-5β-cholestane-26,27-diol (88%). Finally, this product (0.4 mmol) was treated with Dowex 50W-X8 (Mitsubishi Kasei Co.) (2 ml) in MeOH (5 ml) to afford 5β-cyprinol (**6**) (97%), which was recrystallized from dilute MeOH to yield colorless needles (152 mg) (mp 197.8°C, MS m/z: 453 (M^+)) (lit. 173—174°C).[7]

References and Notes

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