Study on the Bile Salt, Sodium Scymnol Sulfate, from *Rhizoprionodon acutus*. IV. Structures of Chimaerol and Sodium Chimaerol Sulfate in the Bile of *Lamna ditropis* and *Rhizoprionodon acutus*[1)]

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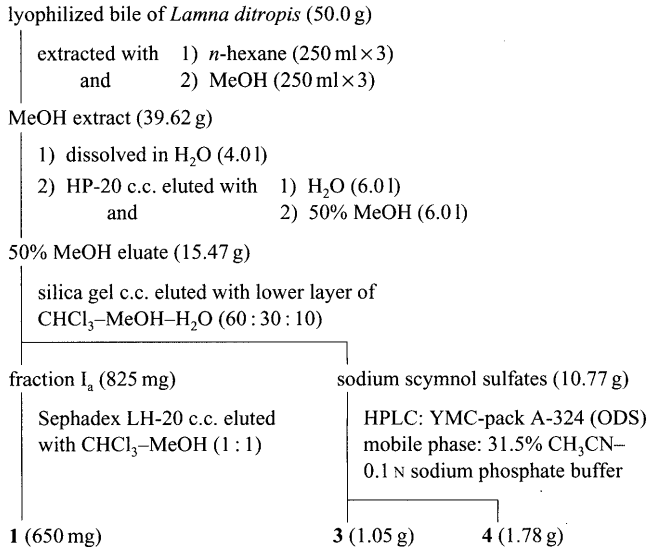
Sodium chimaerol sulfate (1) was isolated from the bile of *Lamna ditropis* and *Rhizoprionodon acutus* by chromatography on silica gel and Sephadex LH-20, together with sodium scymnol sulfates, (24*R*,25*S*)- and (24*R*,25*R*)-(+)-3α,7α,12α,24,26-pentahydroxy-5β-cholestan-27-yl sodium sulfates (3 and 4) and 3, respectively. On acid hydrolysis, compound 1 afforded chimaerol (2), which was identified as (24*R*,25*R*)-(+)-5β-cholestane-3α,7α,12α,24,26-pentol by direct comparison with an authentic sample, prepared by reduction of (24*R*,25*S*)-(+)-24,26-epoxy-5β-cholestane-3α,7α,12α,27-tetrol (5) with $LiAlH_4$. The structure of 1 was concluded to be (24*R*,25*R*)-(+)-3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-yl sodium sulfate, based on the chemical transformation and spectral data.

Keywords bile salt; sodium chimaerol sulfate; sodium scymnol sulfate; *Rhizoprionodon acutus*; *Lamna ditropis*; chimaerol

We have reported the presence of two sodium scymnol sulfates, (24*R*,25*S*)- and (24*R*,25*R*)-(+)-3α,7α,12α,24,26-pentahydroxy-5β-cholestan-27-yl sodium sulfates (**3** and **4**), in the bile of sharks, *Lamna ditropis*, *Chamydoselachus anguineus* GARMAN and *Glyphis glaucus*, whereas only **3** is present as a major component in *Rhizoprionodon acutus*.[1)] We also found that besides the above main bile salt, sodium scymnol sulfate, there is a cofactor bile salt, sodium 5β-chimerol sulfate, in the biles of *Lamna ditropis* and *Rhizoprionodon acutus*.

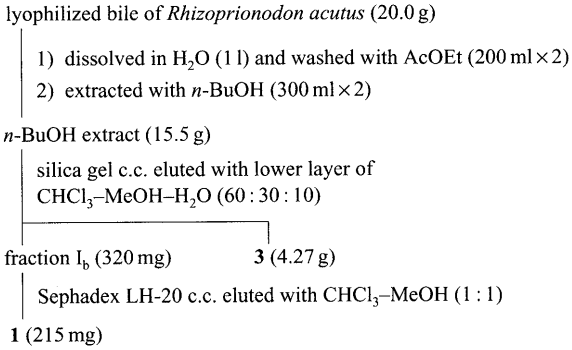
Sodium 5β-chimaerol sulfate has been isolated as a major bile salt from the biles of shark, *Chimaera monstrosa*,[2)] and sting-ray, *Dasyatis akajei*,[3)] and as a cofactor of sodium scymnol sulfate from three sturgeon species, *Acipenser guldenstaedti* BRANDT, *A. stellatus* PALL and *Huso fuso* L.[4)] The structure of the bile alcohol, chimaerol, prepared from the native salt was confirmed synthetically to be 5β-cholestane-3α,7α,12α,24,26-pentol (**2**),[5)] and the native salt was concluded to be 3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-yl sodium sulfate (**1**) on the basis of chemical data.[2,4)] It is regarded as a typical component of the bile in all Elasmobranch fishes, like sodium scymnol sulfate. However, to our knowledge, there has been no report describing in detail the isolation of the native salt and no stereochemical study to determine its absolute configuration. Here, we would like to report a novel isolation procedure and the absolute configuration of the bile salt, sodium 5β-chimaerol sulfate, from *Lamna ditropis* and *Rhizoprionodon acutus*.

Isolation of sodium 5β-chimaerol sulfate from the bile of *Lamna ditropis* was achieved by three steps of column chromatography (HP-20, silica gel and Sephadex LH-20), as summarized in Chart 1. The bile was defatted with *n*-hexane, and applied to an HP-20 column, that removed most of the inorganic material and impurities. Sodium scymnol sulfates and a small amount of compound **1** were obtained in the 50% methanol eluate. Compound **1** was isolated by use of a combination of silica gel and Sephadex



( ) indicates yields c.c., column chromatography

Chart 1. Isolation Procedure for Compound **1** from *Lamna ditropis*



( ) indicates yields c.c., column chromatography

Chart 2. Isolation Procedure for Compound **1** from *Rhizoprionodon acutus*

LH-20 column chromatographies from the above eluate. Thus, 650 mg of **1** was isolated as an amorphous powder from 50 g of lyophilized bile of *Lamna ditropis*. It was similarly isolated from the bile of *Rhizoprionodon acutus*, as shown in Chart 2. The details of the isolation processes are described in the experimental section.

Compound **1** has not yet been crystallized. The structure elucidation of the compound was carried out as follows. From direct atomic absorption analysis, **1** has a sodium atom in the molecule. Its positive FAB-mass spectrum showed the ion peak at *m/z* 555, indicating that the molecular weight of **1** is 554. From these data and elemental analysis, its molecular formula was determined to be $C_{27}H_{47}NaSO_8$. The IR spectrum, which resembles that of sodium scymnol sulfate, showed absorption bands at 3426 and 1217 cm^{-1}, which are assignable to alcohol and sulfate ester functions. A detailed comparison of the ^{13}C-NMR data of compound **1** with those of sodium scymnol sulfate, (24*R*,25*S*)-(+)-3α,7α,12α,24,26-pentahydroxy-5β-cholestan-27-yl sodium sulfate (**3**),[1] indicated that **1** is the sodium sulfate salt of a bile alcohol with a 5β-cholestane skeleton. The ^{13}C-NMR signals of the side chains of **1** and **3** are shown in Table I. There are no significant differences in the chemical shifts of the corresponding carbons, except for those of the carbons at C-25 and 26 in the side chain, between **1** and **3**. From these ^{13}C-NMR data and other NMR (^{1}H, distortionless enchancement by polarization transfer (DEPT), two dimensional correlation spectroscopy (2D-COSY), and C–H-shift COSY) spectra of **1**, the compound was suggested to be 3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-yl sodium sulfate, designated as sodium 5β-chimaerol

TABLE I. 500 MHz ^{1}H- and ^{13}C-NMR Data for Side Chains of **1**, **2** and **3** (Coupling Constants in Parenthesis)

Position	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
20	1.42 m	37.8 d	1.43 m	37.9 d	1.41 m	38.6 d
21	1.02 d (6.7)	18.9 q	1.02 d (6.5)	18.9 q	1.01 (6.5)	19.9 q
22	1.42 m	34.3 t	1.42 m	34.3 t	1.53 m	34.6 t
23	1.53 m	32.7 t	1.53 m	32.9 t	1.51 m	33.1 t
	1.67 m		1.65 m		1.80 m	
24	3.45 dt (6.0, 6.0)	73.6 d	3.41 dt (6.0, 6.0)	73.6 d	4.72 ddd (5.1, 7.6, 8.0)	72.9 d
25	1.99 m	43.7 d	2.00 m	43.7 d	3.02 ddddd (5.7, 7.4, 7.7, 7.6, 8.0)	48.5 d
26	3.87 dd (6.4, 9.6 (*gem*))	72.5 t	3.44 dd (7.0, 10.8 (*gem*))	67.0 t	4.11 dd (5.7, 5.7 (*gem*))	62.3 t
	4.01 dd (7.3, 9.6 (*gem*))		3.58 dd (6.5, 10.8 (*gem*))		4.57 dd (5.7, 8.0 (*gem*))	
27	0.92 d (6.7)	11.6 q	0.91 d (6.2)	11.8 q	3.79 dd (7.6, 10.8 (*gem*))	68.9 t
					3.91 dd (7.4, 10.8 (*gem*))	

δ values in ppm and coupling constants in Hz. Multiplicities of carbon signals were determined by means of the DEPT method and are indicated as d, t and q. Solvents: methanol-d_4 for **1** and **2** and D_2O for **3**.

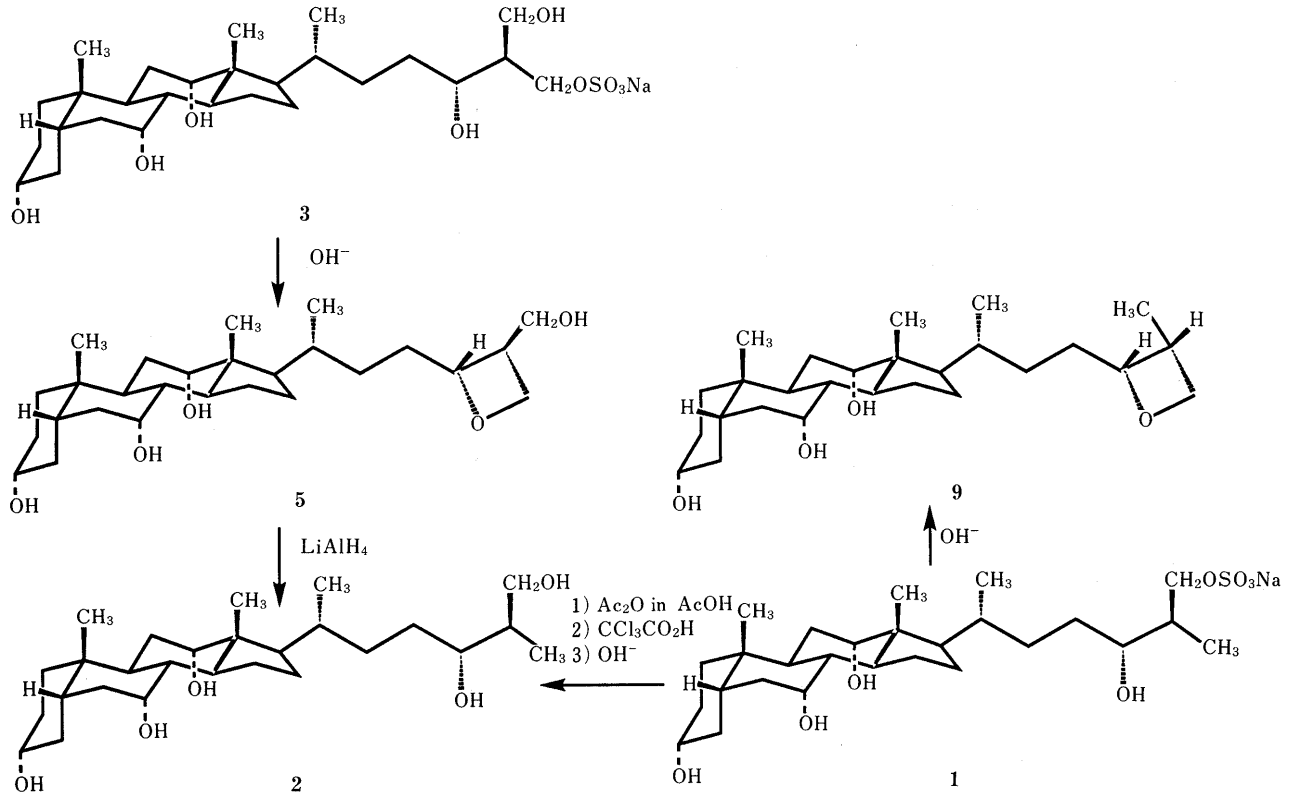


Chart 3. Preparation of Chimaerol **2** from Sodium Chimaerol Sulfate and Sodium Scymnol Sulfate, **1** and **3**, and Anhydrochimaerol **9** from **1**

sulfate by Haslewood *et al.*[2]

Structure confirmation and determination of the configurations at C-24 and -25 were carried out in the following way. As depicted in the scheme, compound **1** afforded **2** in moderate yield, on nonaqueous hydrolysis of its *O*-acetylated derivative with trichloroacetic acid, followed by alkaline hydrolysis with aqueous potassium hydroxide. The physicochemical data of **2** suggested it to be chimaerol, 5β-cholestane-3α,7α,12α,24,26-pentol. By analogy with the structures of the bile alcohols, ranol and scymnol, the absolute configuration of **2** at C-24 is supposed to be *R*.[6,7] So, the two (24*R*)-chimaerols, (24*R*,25*R*)- and (24*R*,25*S*)-(+)-5β-cholestane-3α,7α,-12α,24,26-pentol (**2** and **8**), were synthesized by $LiAlH_4$ reduction of the anhydroscymnols, (24*R*,25*S*)- and (24*R*,25*R*)-(+)-24,26-epoxy-5β-cholestane-3α,7α,12α,27-tetrol (**5** and **6**),[8] which were prepared from **3** and **4**,[1] respectively. Compound **2** was identified as (24*R*,25*R*)-(+)-5β-cholestane-3α,7α,12α,24,26-pentol by direct comparisons. The deshielded shifts of carbinyl carbon and the proton at C-27 of **1** with comparison of those of **2** indicated that the hydroxyl group at C-27 in **1** is indeed esterified with SO_3Na (Table I).[9] Alkaline degradation of **1** with aqueous potassium hydroxide gave anhydrochimaerol, (24*R*,25*R*)-(+)-24,26-epoxy-5β-cholestane-3α,7α,12α-triol (**9**) (Chart 3). Thus, **1** is determined to be (24*R*,25*R*)-(+)-3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-yl sodium sulfate. This is noteworthy, because chimaerol is supposed to be a metabolic precursor of scymnol: C-terminal hydroxylation would lead to this compound directly and secondary *O*-sulfonation at C-27 of scymnol would afford sodium scymnol sulfate(s), **3** and/or **4**, in both sharks, *Rhizoprionodon acutus* and *Lamina ditropis*, respectively, depending on the sulfated position.[1,2,6]

In summary, we have established an isolation procedure for sodium 5β-chimaerol sulfate and confirmed the structure as (24*R*,25*R*)-(+)-3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-yl sodium sulfate, based on chemical transformations and physicochemical data.

Experimental

Melting points were determined on a Yanaco micro melting point apparatus and are uncorrected. IR spectra were taken on a JASCO IRA-2 grating infrared spectrometer. Optical rotation was measured with a Jasco DIP-140. Mass spectra (MS) were recorded on a JMS-AX505W instrument and high-resolution mass spectra (MS) and FAB-MS were obtained on a JMS-SX102 machine, using ethylene glycol as the matrix. NMR spectra were recorded on JEOL GX-500 and GX-270 spectrometers using tetramethylsilane (TMS) as an internal standard. Chemical shifts are recorded in δ values (ppm) and coupling constants in hertz (Hz). Multiplicities of ^{13}C-NMR signals were determined by means of the DEPT method. ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY spectra were obtained with the JEOL standard pulse sequences and data processing was performed with the standard software.

Isolation of Sodium Chimaerol Sulfate Material: Gallbladders, obtained from sharks, *Lamna ditropis* (*ca.* 60 kg × 5) caught in July 1992 off the coast of Sanriku, Japan, and *Rhizoprionodon acutus* (*ca.* 8 kg × 5) caught in December 1991 near Suruga Bay, Japan, were homogenized and each homogenate was freeze-dried.

Isolation of Sodium Chimaerol Sulfate **1** from the Bile of *Lamna ditropis*: The lyophilized bile (50.0 g) of *Lamna ditropis* was extracted with *n*-hexane (250 ml × 2) and then MeOH (250 ml × 2). The MeOH extract (39.62 g) was dissolved in H_2O (4.0 l) and the solution was applied to an HP-20 (5.02 l) column. The column was eluted with H_2O (6.0 l), 50% MeOH (6.0 l) and then MeOH (4.0 l). The 50% MeOH eluate (15.47 g) was chromatographed on silica gel with the lower layer of $CHCl_3$–MeOH–H_2O (65 : 35 : 10) to afford fraction Ia (825 mg) and sodium scymnol sulfates (10.77 g). Fraction Ia (825 mg) was applied to a Sephadex LH-20 column and elution with $CHCl_3$ and MeOH (1 : 1) afforded sodium chimaerol sulfate (**1**) (650 mg). Purification of sodium scymnol sulfates (380 mg) by HPLC yielded 63 mg of (24*R*,25*R*)-(+)-3α,7α,12α,24,26-pentahydroxy-5β-cholestan-27-yl sodium sulfate (**4**) and 37 mg of (24*R*,25*S*)-(+)-3α,7α,12α,24,26-pentahydroxy-5β-cholestan-27-yl sodium sulfate (**3**), as reported previously.[1] The conditions for HPLC were as follows: column, YMC-Pack A-324 (ODS) 10 × 300 mm; flow rate, 3 ml/min; mobile phase, 31.5% CH_3CN–0.1 N sodium phosphate buffer (pH 6.70); detector, refractive index (RI). Compounds **1** gave the following physical data. Compound **1**: White amorphous powder, $[\alpha]_D^{25}$ 23.9° (c = 0.5, methanol). *Anal.* Calcd for $C_{27}H_{47}NaO_8S \cdot 7/2H_2O$: C, 52.51; O, 8.75. Found: C, 52.66; O, 8.53. FAB-MS *m/z*: 555 $(C_{27}H_{47}NaO_8S+H)^+$. IR ν_{max}^{KBr} cm^{-1}: 3426, 2992, 1467, 1380, 1217, 1072, 979. ^{1}H-NMR (in methanol-d_4) δ: 4.01 (1H, dd, J = 7.3, 9.6 Hz, 27-H_A), 3.96 (1H, s, 12-H), 3.87 (1H, dd, J = 6.4, 9.6 Hz, 27-H_B), 3.79 (1H, s, 7-H), 3.64 (1H, dddd, J = 6.0, 6.0, 7.5, 8.2 Hz, 3-H), 3.45 (1H, dt, J = 8.6, 6.9 Hz, 24-H), 2.32—2.20 (2H, m, 4 and 9-H), 2.03—0.87 (23H, m), 1.03 (3H, d, J = 6.7 Hz, 21-H), 0.92 (3H, d, J = 6.7 Hz, 27-H), 0.91 (3H, s, 19-H), 0.72 (3H, s, 18-H). ^{13}C-NMR (in methanol-d_4) δ: 74.8 (C-12, d), 73.6 (C-24, d), 73.1 (C-3, d), 72.5 (C-26, d), 69.8 (C-7, t), 49.2 (C-17, d), 48.2 (C-13, s), 43.9 (C-14, d), 43.7 (C-25, d), 41.7 (C-5, d), 41.2 (C-8, d), 40.4 (C-4, t), 37.8 (C-20, d), 37.3 (C-1, t), 36.6 (C-10, s), 36.6 (C-6, t), 34.3 (C22, t), 32.7 (C-23, t), 31.9 (C-2, t), 30.3 (C-11, t), 29.6 (C-16, t), 28.6 (C-9, d), 25.0 (C-15, t), 24.0 (C-19, q), 18.9 (C-21, q), 13.8 (C-18, q), 11.6 (C-27, q).

Isolation of Sodium Chimaerol Sulfate **1** from the Bile of *Rhizoprionodon acutus*: The lyophilized bile (20.0 g) of *Rhizoprionodon acutus* was dissolved in H_2O (1000 ml). The solution was washed with AcOEt (200 ml × 2), saturated with NaCl, and then extracted with *n*-BuOH (400 ml × 2). The *n*-BuOH extract (15.5 g) was applied to a silica gel column. Elution with the lower layer of $CHCl_3$–MeOH–H_2O (65 : 35 : 10) gave fraction Ib (320 mg) and sodium scymnol sulfate, (24*R*,25*S*)-(+)-3α,7α,12α,24,26-pentahydroxy-5β-cholestan-27-yl sodium sulfate, (**3**) (4.27 g), as reported previously.[1,6] Fraction I_b (320 mg) was applied to a Sephadex LH-20 column and eluted with $CHCl_3$ and MeOH (1 : 1) to obtain **1** (215 mg).

Preparation of Chimaerol 2 from Sodium Chimaerol Sulfate 1 Acid Hydrolysis of **1** with Trichloroacetic Acid: A mixture of 25 mg of **1** and 0.3 ml of acetic acid and acetic anhydride (1 : 1) was refluxed for 3 h. The solvent was removed *in vacuo*, and the residue was dissolved in 0.2 ml of dry dioxane. To this solution, 0.1 ml of freshly distilled trichloroacetic acid was added. The mixture was left for 7 d at room temperature, then diluted with 0.12 ml of aqueous 1 M $BaCl_2$ to yield 9 mg of $BaSO_4$. After removal of the precipitates by filtration, the filtrate was concentrated under reduced pressure. A solution of 50 mg of potassium hydroxide in 0.5 ml of methanol was added to the concentrate and the mixture was refluxed for 3 h. The excess methanol was removed and the residue was diluted with 5 ml of H_2O, then neutralized with diluted hydrochloric acid, and extracted twice with 5 ml of AcOEt and *n*-BuOH (1 : 1). The organic phase was washed with saturated aqueous NaCl and dried over $MgSO_4$. The concentrate was chromatographed with $CHCl_3$–MeOH–H_2O (60 : 25 : 5) as an eluent to afford 13 mg of **2**. Upon crystallization of this product from AcOEt and MeOH, colorless plates (10 mg) were obtained. The physical properties of **2** are as follows. Compound **2**: colorless plates. mp 172—176 °C (lit. 180—182 °C).[2] $[\alpha]_D^{25}$ 32.74° (c = 0.5, methanol). *Anal.* Calcd for $C_{27}H_{48}O_5 \cdot H_2O$: C, 68.94; H, 10.64. Found: C, 68.89; H, 10.80. 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 $(C_{27}H_{48}O_5-3H_2O+H)^+$, 381 $(C_{27}H_{48}O_5-4H_2O+H)^+$, 363 $(C_{27}H_{48}O_5-5H_2O+H)^+$. IR ν_{max}^{KBr} cm^{-1}: 3388, 2928, 1453, 1374, 1034. ^{1}H-NMR (in methanol-d_4) δ: 3.96 (1H, s, 12-H), 3.79 (1H, s, 7-H), 3.58 (1H, dd, J = 7.0, 10.8 Hz, 26-H_A), 3.56 (1H, dddd, J = 6.0, 6.0, 8.0, 8.2 Hz, 3-H), 3.44 (1H, dd, J = 6.5, 10.8 Hz, 26-H_B), 3.41 (1H, dt, J = 6.0, 6.0 Hz, 24-H), 2.33—2.18 (2H, m, 4 and 9-H), 2.07—0.85 (23H, m), 1.02 (3H, d, J = 6.5 Hz, 21-H), 0.91 (3H, d, J = 6.2 Hz, 27-H), 0.88 (3H, s, 19-H), 0.72 (3H, s, 18-H). ^{13}C-NMR (in methanol-d_4) δ: 74.8 (C-12, d), 74.2 (C-3, d), 73.6 (C-24, d), 69.8 (C-7, d), 67.0 (C-26, t), 49.0 (C-17, d), 48.2 (C-13, s), 43.9 (C-14, d), 43.7 (C-25, d), 42.6 (C-5, d), 41.7 (C-8, d), 41.2 (C-4, t), 37.9 (C-20, d), 37.3 (C1, t), 36.7 (C-10, s), 36.6 (C-6, t), 34.3 (C-22, t), 32.9 (C-23, t), 31.9 (C-2, t), 30.3 (C-11, t), 29.6 (C-16, t), 28.6 (C-9, d), 25.0 (C15, t), 24.0 (C-19, q), 18.9 (C-21, q), 13.9 (C-18, q),

11.8 (C-27, q). The above data were identical with those of authentic (24*R*,25*R*)-(+)-5β-cholestane-3α,7α,12α,24,26-pentol, which was prepared by reduction of anhydroscymnol **5** with $LiAlH_4$, as described below.

Hydrolysis of **1** with Dowex 50W-X8 in MeOH: Dowex 50W-X8 (20 mg, 100—200 mesh) was added to a solution of 25 mg of sodium chimaerol sulfate (**1**) (0.044 mmol) in 0.5 ml of MeOH, and the mixture was stirred for 12 h. After removal of the resin by filtration, the filtrate was concentrated under reduced pressure. The residue was recrystallized from AcOEt and MeOH to afford 19 mg of **2** as colorless plates.

Preparation of Chimaerols 2 and 8 from Sodium Scymnol Sulfate 3 and 4 Alkaline Degradation of **3** and **4** with Potassium Hydroxide: Vigorous alkaline hydrolysis of **3** (30 mg), in the reported manner,[1,6] furnished (24*R*,25*S*)-(+)-24,26-epoxy-5β-cholestane-3α,7α,12α,27-tetrol (**5**) (22 mg). (24*R*,25*R*)-(+)-24,26-Epoxy-5β-cholestane-3α,7α,12α,27-tetrol (**6**) (18 mg) and (24*R*)-(+)-26,27-epoxy-5β-cholestane-3α,7α,-12α,24-tetrol (**7**) (14 mg) were similarly obtained from **4** (60 mg). Recrystallization of compound **5** (22 mg) from aqueous EtOH gave 17 mg of colorless needles (mp 228—230 °C), and recrystallization of **6** and **7** from aqueous MeOH and AcOEt–MeOH gave 14 mg of colorless needles (mp 194—195 °C) and 10 mg of colorless needles (mp 199—200 °C), respectively.

Reduction of **5** with $LiAlH_4$: A solution of 15 mg of **5** in dry tetrahydrofuran (THF, 3 ml) was added dropwise to a suspension of $LiAlH_4$ (11 mg) in dry THF (1 ml). The mixture was heated for 2 h at 60 °C, and then carefully poured into ice-water (5 ml). This solution was saturated with NaCl, and then extracted with AcOEt and *n*-BuOH (1 : 1) (5 ml × 2). The extract was chromatographed on silica gel with the lower layer of $CHCl_3$–MeOH–H_2O (65 : 25 : 10) to afford (24*R*,25*R*)-(+)-5β-cholestane-3α,7α,12α,24,26-pentol (**2**) (12 mg), which was recrystallized from acetone to obtain colorless plates (10 mg). Synthetic **2** has the following physicochemical properties. Synthetic **2**: mp 181—184 °C (lit. 168—172 and 165—173 °C).[5,8] $[\alpha]_D^{25}$ 33.46° (c=0.5, methanol). *Anal.* Calcd for $C_{27}H_{48}O_5 \cdot 1/2H_2O$: C, 70.28; H, 10.62. Found: C, 70.09; H, 10.49. 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 $(C_{27}H_{48}O_5-3H_2O+H)^+$, 381 $(C_{27}H_{48}O_5-4H_2O+H)^+$, 363 $(C_{27}H_{48}O_5-5H_2O+H)^+$. IR ν_{max}^{KBr} cm^{-1}: 3340, 2936, 1461, 1376, 1076, 1033. ^{1}H-NMR (in methanol-d_4) δ: 3.95 (1H, s, 12-H), 3.79 (1H, s, 7-H), 3.59 (1H, dd, J=7.0, 10.8 Hz, 26-H_A), 3.56 (1H, dddd, J=6.0, 6.0, 8.0, 8.2 Hz, 3-H), 3.44 (1H, dd, J=6.5, 10.8 Hz, 26-H_B), 3.40 (1H, td, J=6.0, 6.0 Hz, 24-H), 2.32—2.20 (2H, m, 4 and 9-H), 2.05—0.84 (23H, m), 1.01 (3H, d, J=6.5 Hz, 21-H), 0.90 (3H, d, J=3.0 Hz, 27-H), 0.87 (3H, s, 19-H), 0.71 (3H, s, 18-H). ^{13}C-NMR (in methanol-d_4) δ: 74.8 (C-12, d), 74.2 (C-3, d), 73.5 (C-24, d), 69.8 (C-7, d), 67.0 (C-26, t), 49.0 (C-17, d), 48.1 (C-13, s), 43.8 (C-14, d), 43.6 (C-25, d), 42.5 (C-5, d), 41.7 (C-8, d), 41.1 (C-4, t), 37.8 (C-20, d), 37.2 (C-1, t), 36.6 (C-10, s), 36.5 (C-6, t), 34.3 (C-22, t), 32.8 (C-23, t), 31.8 (C-2, t), 30.3 (C-11,t), 29.5 (C-16, t), 28.5 (C-9, d), 25.0 (C-15, t), 24.0 (C-19, q), 18.9 (C-21, q), 13.9 (C-18, q), 11.8 (C-27, q).

Reduction of **6** with $LiAlH_4$: A yield of 7 mg of (24*R*,25*S*)-(+)-5β-cholestane-3α,7α,12α,24,26-pentol (**8**) was obtained by reduction of 10 mg of **6** with $LiAlH_4$ in the same manner as described above. It has the following physicochemical properties. Compound **8**: mp 131—133 °C, $[\alpha]_D^{25}$ 26.0° (c=0.5, methanol). *Anal.* Calcd for $C_{27}H_{48}O_5 \cdot H_2O$: C, 68.94; H, 10.64. Found: C, 69.26; H, 10.55. 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 $(C_{27}H_{48}O_5-3H_2O+H)^+$, 381 $(C_{27}H_{48}O_5-4H_2O+H)^+$, 363 $(C_{27}H_{48}O_5-5H_2O+H)^+$. IR ν_{max}^{KBr} cm^{-1}: 3380, 2936, 1457, 1376, 1078, 1043, 1020. ^{1}H-NMR (in methanol-d_4) δ: 3.96 (1H, s, 12-H), 3.80 (1H, s, 7-H), 3.63 (1H, dd, J=6.0, 10.8 Hz, 26-H_A), 3.49 (1H, dd, J=6.5, 10.8 Hz, 26-H_B), 3.43 (1H, td, J=6.0, 7.0 Hz, 24-H), 3.41 (1H, dddd, J=6.0, 6.0, 8.0, 8.2 Hz, 3-H), 2.33—2.20 (2H, m, 4 and 9-H), 2.01—0.82 (23H, m), 1.01 (3H, d, J=6.7 Hz, 21-H), 0.91 (3H, d, J=5.8 Hz, 27-H), 0.90 (3H, s, 19-H), 0.72 (3H, s, 18-H). ^{13}C-NMR (in methanol-d_4) δ: 76.3 (C-12, d), 74.8 (C-3, d), 73.6 (C-24, d), 69.8 (C-7, d), 67.0 (C-26, t), 49.1 (C-17, d), 48.2 (C-13, s), 43.9 (C-14, d), 43.7 (C-25, d), 43.0 (C-5, d), 41.7 (C-8, d), 41.2 (C-4, t), 37.9 (C-20, d), 37.3 (C-1, t), 36.7 (C-10, s), 36.6 (C-6, t), 33.6 (C-22, t), 32.5 (C-23, t), 31.9 (C-2, t), 30.3 (C-11, t), 29.6 (C-16, t), 28.6 (C-9, d), 25.0 (C-15, t), 24.0 (C-19, q), 18.8 (C-21, q), 14.7 (C-18, q), 13.9 (C-27, q).

Alkaline Degradation of **1** with Potassium Hydroxide: Vigorous alkaline hydrolysis of **1** (56 mg), in the manner described above, furnished **9** (31 mg). Recrystallization of compound **9** from ether gave 27 mg of colorless needles. Compound **9**: mp 143—144 °C (mp 141—144 °C).[2] $[\alpha]_D^{25}$ 26.9° (c=0.7, methanol). *Anal.* Calcd for $C_{27}H_{46}O_4 \cdot 1/2\ H_2O$: C, 73.13; H, 10.60. Found: C, 73.10; H, 10.59. FAB-MS *m/z*: 435 $(C_{27}H_{46}O_4+H)^+$, 417 $(C_{27}H_{46}O_4-H_2O+H)^+$, 399 $(C_{27}H_{46}O_4-2H_2O+H)^+$. IR ν_{max}^{KBr} cm^{-1}: 3394, 2930, 1462, 1377, 1076, 1043, 978. ^{1}H-NMR (in methanol-d_4) δ: 4.80 (1H, td, J=8.0, 5.6 Hz, 24-H), 4.75 (1H, dd, J=5.6, 7.5 Hz, 26-H_A), 4.05 (1H, dd, J=5.6, 5.6 Hz, 26-H_B), 3.95 (1H, s, 12-H), 3.80 (1H, d, J=2.5 Hz, 7-H), 3.36 (1H, dddd, J= 6.0, 6.0, 7.5, 8.2 Hz, 3-H), 3.02 (1H, qddd, J=7.5, 7.5, 5.5, 5.5 Hz, 25-H), 2.30—2.20 (2H, m, 4 and 9-H), 2.01—1.00 (23H, m), 1.18 (3H, d, J=7.5 Hz, 27-H), 1.07 (3H, d, J=6.7 Hz, 21-H), 0.96 (3H, s, 19-H), 0.71 (3H, s, 18-H). ^{13}C-NMR (in methanol-d_4) δ: 88.0 (C-24, d), 77.6 (C-26, t), 74.8 (C-12, d), 73.6 (C-3, d), 69.8 (C-7, d), 48.8 (C-17, d), 48.2 (C-13, s), 43.9 (C-14, d), 43.7 (C-5, d), 41.8 (C-8, d), 41.2 (C-4, t), 37.8 (C-20, d), 37.3 (C-1, t), 36.7 (C-6, t), 36.6 (C-10, s), 34.0 (C-25, d), 32.4 (C-23, t), 31.9 (C-22, t), 30.3 (C-2, t), 30.0 (C-11, t), 29.6 (C-16, t), 28.6 (C-9, d), 25.0 (C-15, t), 24.0 (C-19, q), 18.8 (C-21, q), 14.5 (C-27, q), 13.9 (C-18, q).

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