Structures of new polar steroids from the Far-Eastern starfish *Ctenodiscus crispatus**

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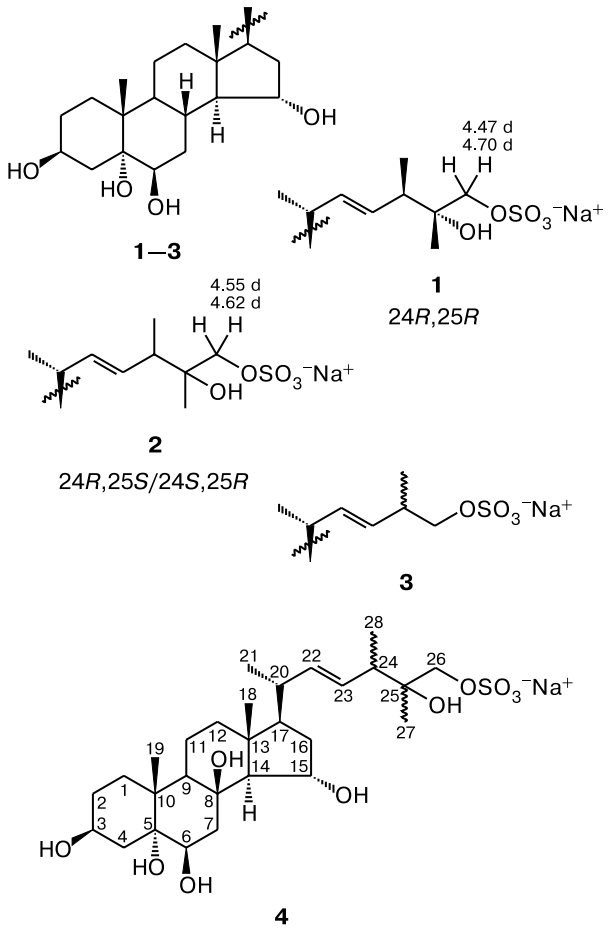
A fraction of sulfated polyhydroxylated steroids from the Far-Eastern starfish *Ctenodiscus crispatus* was investigated. The main component of this fraction was identified as (22*E*,24*R*,25*R*)-24-methyl-5α-cholest-22-en-3β,5,6β,15α,25,26-hexol 26-*O*-sulfate. For the compound stereoisomeric with respect to the side chain, the (24*R*,25*S*) or (24*S*,25*R*) relative configurations were assigned to the C(24) and C(25) chiral centers. The structures of two other compounds isolated from the fraction were identified as (22*E*,24ξ)-26,27-bisnor-24-methyl-5α-cholest-22-en-3β,5,6β,15α,25-pentol 25-*O*-sulfate and (22*E*,24ξ,25ξ)-24-methyl-5α-cholest-22-en-3β,5,6β,8,15α,25,26-heptol 26-*O*-sulfate.

Key words: starfish, *Ctenodiscus crispatus*, polyhydroxylated steroidal, steroidal sulfate, side chain, NMR spectra, α-methoxy-α-trifluoromethylphenylacetic acid ester, absolute configuration.

Polyhydroxysteroids and their glycosides are the most widespread secondary metabolites of starfishes. These compounds are of interest not only because of their unusual chemical structures but also due to their properties, including embriotoxic, fungicidal, bactericidal, antifouling, neuritogenic, and other types of physiological activities.[1] Earlier,[2] we have isolated four new polyhydroxylated steroids, two of which are sulfates, from the starfish *Ctenodiscus crispatus* (family Ctenodiscidae, the order Paxillosida) collected in the Japan Sea (the Posiet Bay). As part of continuing investigations of steroid metabolites from Far-Eastern starfishes,[2,3] we studied the composition of a fraction of sulfated polyhydroxysteroids from this starfish, isolated several new steroids, and established the absolute configuration of the side chain of steroid **1**, whose structure has been determined earlier without elucidation of the stereochemical features of the side chain.



Results and Discussion

A fraction of sulfated polyhydroxylated steroids was obtained from an aqueous-ethanolic extract of the starfish *Ctenodiscus crispatus* by reversed-phase, gel-permeation, and adsorption chromatography on Amberlite XAD-2, Sephadex LH-20, and silica gel. The following four compounds were isolated from this fraction by HPLC: previously known (22*E*)-24-methyl-5α-cholest-22-en-

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3β,5,6β,15α,25,26-hexol 26-*O*-sulfate (**1**),[2] its new stereoisomer with respect to the side chain (**2**), and new steroids, *viz.*, (22*E*,24ξ)-26,27-bisnor-24-methyl-5α-cholest-22-en-3β,5,6β,15α,25-pentol 25-*O*-sulfate (**3**) and (22*E*,24ξ,25ξ)-24-methyl-5α-cholest-22-en-3β,5,6β,8,15α,25,26-heptol 26-*O*-sulfate (**4**).

The (+)-MALDI TOF mass spectrum of steroid **1** has a pseudomolecular ion peak at *m/z* 605 $[M_{Na} + Na]^+$. The (−)-MALDI TOF mass spectrum of this steroid has a pseudomolecular ion peak at *m/z* 559 $[M - cation]^-$. A comparison of the data from mass spectrometry and ^1H NMR spectroscopy for compound **1** (Tables 1 and 2) with the corresponding data for the steroidal hexol that we have isolated earlier[2] from *C. crispatus* allowed us to identify steroid **1** as sodium (22*E*)-24-methyl-5α-cholest-22-en-3β,5,6β,15α,25,26-hexol 26-*O*-sulfate. Since compound **1** has been isolated earlier in a small amount, here we recorded its ^{13}C NMR spectrum for the first time (see Tables 1 and 2). The structure of compound **1** was confirmed by $^1H—^1H$ COSY, HSQC, and DEPT NMR experiments.

Earlier, the (22*E*)-24-methyl-25,26-dihydroxy fragment of the side chain has been found only in two polyhydroxylated steroids isolated from the starfishes *Archaster typicus*[4] and *Styracaster caroli*.[5] The absolute stereochemistry of one of these compounds has been established as follows. Initially, the relative configurations of the C(24) and C(25) atoms were determined by comparing the NMR spectra of this compound with the spectra of model synthetic compounds, *viz.*, enantiomeric pairs of (2*R*,3*R*)/(2*S*,3*S*)- and (2*S*,3*R*)/(2*R*,3*S*)-2,3-dimethylpentane-1,2-diols.[4,5] Then esters of *R*-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA, Mosher's reagent) for individual (2*R*,3*R*)- and (2*S*,3*S*)-2,3-dimethylpentane-1,2-diols were prepared. A comparison of their spectra with the spectra of the corresponding MTPA ester prepared from polyhydroxysteroid from *A. typicus* allowed the identification of the latter as (22*E*,24*R*,25*R*)-24-methyl-5α-cholest-22-en-3β,4β,5,6α,8,14,15α,25,26-nonol.[4]

We used an analogous approach to determine the absolute configuration of steroid **1**. Several derivatives of

Table 1. Chemical shifts of the carbon atoms and the chemical shifts and multiplicities of the protons of the polycyclic moiety of steroids **1**, **2**, and **4** (δ, *J*/Hz, C_5D_5N)*

Atom	**1**, **2**		**4**	
	δ_C (DEPT)	δ_H	δ_C	δ_H
1	33.2 (CH_2)	1.60, 2.20 (both m)	31.6	2.32 (m)
2	32.3 (CH_2)	2.11, 2.28 (both m)	34.1	2.35, 1.71 (both m)
3	67.2 (CH)	4.90 (m)	67.0	4.94 (m)
4	42.3 (CH_2)	2.99 (dd, H_{ax}(4), J = 11.5, J = 12.7); 2.36 (dd, H_{eq}(4), J = 5.0, J = 12.7)	42.4	2.97 (dd, H_{ax}(4), J = 11.3, J = 12.3); 2.36 (m, H_{eq}(4))
5	75.6 (C)	—	75.5	—
6	76.1 (CH)	4.24 (m)	77.7	4.33 (m)
7	35.9 (CH_2)	2.78 (m)	40.6	3.16 (dd, J = 2.8, J = 14.0); 2.36 (dd, J = 2.3, J = 14.0)
8	31.1 (CH)	2.46 (m)	76.5	—
9	45.9 (CH)	2.11 (m)	48.4	2.18 (m)
10	39.0 (C)	—	39.0	—
11	21.5 (CH_2)	1.57 (m)	19.2	2.16, 1.65 (both m)
12	40.6 (CH_2)	1.28, 1.96 (both m)	42.2	2.18 (m)
13	43.8 (C)	—	44.6	—
14	63.3 (CH)	1.62 (m)	66.1	1.75 (d, J = 9.7)
15	73.1 (CH)	4.18 (td, J = 4.0, J = 9.0)	68.9	4.88 (m)
16	42.1 (CH_2)	1.96, 2.12 (both m)	42.0	2.05, 1.25 (both m)
17	53.8 (CH)	1.49 (m)	54.8	1.45 (m)
18	15.3 (CH_3)	0.76 (s)	15.5	1.30 (s)
19	17.1 (CH_3)	1.70 (s)	17.9	1.86 (s)

* The ^1H and ^{13}C NMR spectra were recorded at 500 and 125.8 MHz, respectively; the assignment of the signals was made using two-dimensional $^1H—^1H$ COSY and HSQC spectroscopy.

Table 2. Chemical shifts of the carbon atoms and the chemical shifts and multiplicities of the protons of the side chains of steroids **1—4** (δ, *J*/Hz)*

Atom	**1** (C_5D_5N)		**2** (C_5D_5N)		**3** (CD_3OD)		**4** (C_5D_5N)	
	δ_C (DEPT)	δ_H	δ_C (DEPT)	δ_H	δ_C	δ_H	δ_C	δ_H
20	40.3 (CH)	1.98 (m)	40.3 (CH)	1.99 (m)	41.0	2.03 (m)	40.1	2.07 (m)
21	20.6 (CH_3)	1.00 (d, J = 6.6)	20.6 (CH_3)	0.98 (d, J = 6.6)	21.1	1.01 (d, J = 6.6)	20.5	1.04 (d, J = 6.6)
22	137.4 (CH)	5.24 (dd, J = 9.0, J = 15.3)	137.1 (CH)	5.27 (dd, J = 8.8, J = 15.2)	138.2	5.35 (dd, J = 8.3, J = 15.4)	137.3	5.77 (dd, J = 8.5, J = 14.5)
23	130.1 (CH)	5.73 (dd, J = 8.5, J = 15.3)	130.6 (CH)	5.47 (dd, J = 8.7, J = 15.2)	130.6	5.26 (dd, J = 6.9, J = 15.4)	130.1	5.81 (dd, J = 8.5, J = 14.5)
24	44.6 (CH)	2.53 (m)	43.9 (CH)	2.65 (m)	37.8	2.45 (m)	44.4	2.58 (m)
25	73.2 (C)	—	73.0 (C)	—	73.6	3.74 (dd, J = 7.6, J = 9.3); 3.87 (dd, J = 6.0, J = 9.3)	73.2	—
26	74.4 (CH_2)	4.47, 4.70 (both d, J = 10.2)	74.4 (CH_2)	4.55, 4.62 (both d, J = 10.0)	—	—	74.3	4.52, 4.75 (both d, J = 10.0)
27	22.7 (CH_3)	1.44 (s)	20.7 (CH_3)	1.37 (s)	—	—	22.8	1.48 (s)
28	13.6 (CH_3)	1.22 (d, J = 7.0)	13.6 (CH_3)	1.27 (d, J = 7.0)	17.5	1.03 (m)	15.3	1.24 (d, J = 6.9)

* The ^1H and ^{13}C NMR spectra were recorded at 500 and 125.8 MHz, respectively; the assignment of the signals was made using two-dimensional $^1H—^1H$ COSY and HSQC spectroscopy.

this compound were prepared. Mild solvolytic cleavage of **1** with a 1 : 1 dioxane—pyridine mixture at 100 °C afforded desulfated derivative **1a**. Hydrogenation of **1a** over the Adams catalyst (reduced PtO_2) gave 22,23-dihydro derivative **1b**. Treatment of **1b** with the corresponding Mosher's reagent afforded 3,15,26-tri-*R*-MTPA ester **1c**. In the ^1H NMR spectrum of compound **1b**, the chemical shifts of the HC(26), H´C(26), HC(27), and HC(28) protons at δ 3.39 (d), 3.46 (d), 1.01 (s), and 0.85 (d) agree well with the chemical shifts at δ 3.42 (d), 3.49 (d), 1.04 (s), and 0.89 (d)* in the spectra of (2*R*,3*R*)/(2*S*,3*S*)-2,3-dimethylpentane-1,2-diol and differ substantially from the signals for these protons in the spectra of (2*S*,3*R*)/(2*R*,3*S*)-2,3-dimethylpentane-1,2-diol.[4] Hence, it follows that only the (24*R*,25*R*) or (24*S*,25*S*) configuration of the chiral centers of the side chain is possible in steroid **1**. In the ^1H NMR spectrum** of 3,15,26-tri-(*R*-MTPA) ester **1c**, the chemical shifts of the HC(26) and H´C(26) protons at δ 4.22 (d) and 4.26 (d) are similar to those at δ 4.21 (d) and 4.27 (d) in the ^1H NMR spectrum of 3,15,26-tri-*R*-(+)-MTPA ester of natural (22*E*,24*R*,25*R*)-24-methyl-5α-cholest-22-en-3β,4β,5,6α,8,14,15α,25,26-nonol and differ substantially from the signals for HC(1) and H´C(1) at δ 4.06 (d) and 4.30 (d) in the ^1H NMR spectrum of *R*-MTPA ester of (2*S*,3*S*)-2,3-dimethylpentane-1,2-diol.[4] Based on these data, the (24*R*,25*R*) absolute configuration was assigned to the C(24) and C(25) chiral centers in steroid **1**.

The (+)- and (−)-MALDI TOF mass spectra of steroid **2** have the same pseudomolecular ion peaks as those observed in the mass spectra of **1**. The chemical shifts, the coupling constants of the protons, and the chemical shifts of the carbon atoms in the NMR spectra of steroid **2** are virtually identical to those observed in the NMR spectra of **1**, except for certain signals belonging to the side chain (see Tables 1 and 2). The signals for HC(23), HC(24), HC(26), and H´C(26) in the ^1H NMR spectrum of steroid **2** are observed at δ 5.47, 2.65, 4.55, and 4.62, whereas the corresponding signals in the spectrum of compound **1** are observed at δ 5.73, 2.53, 4.47, and 4.70, respectively. The signals for C(24) and C(27) in the ^{13}C NMR spectrum of steroid **2** are observed at δ 43.9 and 20.7, whereas the corresponding signals in the spectrum of compound **1** are observed at δ 44.6 and 22.7, respectively. These differ-

* In the study,[4] the chemical shifts of the protons in the ^1H NMR spectra were determined relative to residual CH_3OH in CD_3OD as the internal standard and they differ from the chemical shifts of the protons in the spectra measured with the use of $SiMe_4$ as the internal standard by +0.04 ppm.

** The spectrum was recorded in $CDCl_3$.

ences in the NMR spectra suggest that compound **2** differs from steroid **1** in the configuration of the chiral centers at the C(24) or C(25) atoms.

Derivatives **2a—c** were prepared analogously to the above-described synthesis of derivatives **1**. In the ^{1}H NMR spectrum of 22,23-dihydro derivative **2b**, the HC(26) and H′C(26) protons give a broad singlet at δ 3.44, and the signals for the HC(27) and HC(28) protons are observed at δ 1.05 (d) and 0.91 (s). These values are similar to the corresponding signals at δ 3.45 (AB system), 1.07 (s), and 0.96 (d) in the spectrum of the enantiomeric pair of (2*S*,3*R*)/(2*R*,3*S*)-2,3-dimethylpentane-1,2-diols.[4] In the ^{1}H NMR spectrum of 3,15,26-tri-(*R*-MTPA) ester **2c**, the signals for the HC(26) and H′C(26) protons at δ 4.18 (d) and 4.34 (d), respectively, differ substantially from the signals for these protons at δ 4.21 (d) and 4.27 (d) in the ^{1}H NMR spectrum of 3,15,26-tri-(*R*-MTPA) ester of natural (22*E*,24*R*,25*R*)-24-methyl-5α-cholest-22-en-3β,4β,5,6α,8,14,15α,25,26-nonol and the corresponding signals for the HC(1) and H′C(1) protons at δ 4.06 (d) and 4.30 (d) in the ^{1}H NMR spectrum of *R*-MTPA ester of (2*S*,3*S*)-2,3-dimethylpentane-1,2-diol.[4] These data suggest that the chiral centers in steroid **2**, unlike those in steroid **1**, have the (24*S*,25*R*) or (24*R*,25*S*) configuration.

The (+)-MALDI TOF mass spectrum of steroid **3** shows the pseudomolecular ion peak $[M_{Na} + Na]^+$ at *m*/*z* 561. The (−)-MALDI TOF mass spectrum contains the pseudomolecular ion peak $[M - cation]^-$ at *m*/*z* 515. The mass-spectrometric data and the data from ^{1}H and ^{13}C NMR spectroscopy suggest that steroid **3** has the molecular formula $C_{26}H_{43}O_8SNa$. The ^{13}C NMR spectrum of steroid **3** showed the presence of 26 carbon atoms in the molecule. The spectrum had signals at δ 68.4, 73.6, 74.3, 76.4, and 76.6 corresponding to five carbon atoms bound to oxygen and signals at δ 130.6 and 138.2 belonging to two carbon atoms at the double bond. In the ^{1}H and ^{13}C NMR spectra of steroid **3**, the signals belonging to the steroid nucleus moiety (see the Experimental) are identical to the corresponding signals in the NMR spectra of compounds **1** and **2** and in the NMR spectra of other steroids from the starfish *Luidia clathrata*[6] containing an analogous 3β,5α,6,15α-tetrahydroxy-substituted tetracyclic system. Analysis of the ^{13}C NMR spectrum demonstrated that the side chain in steroid **3** consists of seven carbon atoms, two of which belong to the double bond and one carbon atom is bound to oxygen (see Table 2). The ^{1}H NMR spectrum of steroid **3** has the following characteristic signals for the protons of the side chain: two doublets of doublets for the olefinic protons at δ 5.26 and 5.35, two doublets of the methyl groups at δ 1.01 and 1.03, and two doublets of doublets for the methylene protons at δ 3.74 and 3.87 (see Table 2). The coupling constant for the protons of the 22,23-double bond (J = 15.4 Hz) is indicative of its *trans* configuration.[4] A comparison of the NMR spectra of compound **3** with those of the steroid from the starfish *Acodontaster conspicuus* containing the (22*E*)-26,27-bisnor-24-methyl-25-hydroxycholestane side chain showed that the signals for the C(25) atom and the HC(25) and H′C(25) protons at δ 73.6, 3.74, and 3.87, respectively, in the spectra of **3** are shifted downfield compared to the corresponding signals of this steroid at δ 68.3, 3.30, and 3.43.[7] Based on these results and the data from mass spectrometry and ^{1}H—^{1}H COSY spectroscopy, we concluded that there is a sulfate group at C(25) in compound **3**. Therefore, new steroid **3** is sodium (22*E*,24ξ)-26,27-bisnor-24-methyl-5α-cholest-22-en-3β,5α,6β,15α,25-pentol 25-*O*-sulfate. We found the same compound in the Far-Eastern starfish *Henricia leviuscula*.

The sulfated (22*E*)-26,27-bisnor-24-methyl-25-hydroxycholestane side chain has been found in steroid metabolites of starfishes for the first time. Up to now, only three similar polyhydroxylated steroids from the starfishes *Acodontaster conspicuus*[7] and *Hacelia attenuata*[8] have been characterized. These steroids contain an analogous, but sulfate-free, side chain.

The (+)-MALDI TOF mass spectrum of steroid **4** shows the pseudomolecular peak $[M_{Na} + Na]^+$ at *m*/*z* 621. The (−)-MALDI TOF mass spectrum of **4** has the pseudomolecular peak $[M - cation]^-$ at *m*/*z* 575. Based on the data from ^{13}C and ^{1}H NMR spectroscopy, we assigned the formula $C_{28}H_{47}O_{10}SNa$ to compound **4**. The ^{13}C NMR spectrum of **4** contains signals for 28 carbon atoms. Seven of these signals, at δ 67.0, 75.5, 77.7, 76.5, 68.9, 73.2, and 74.3, belong to the carbon atoms bound to the oxygen atoms, and two signals at δ 130.1 and 137.3 correspond to the double bond. The ^{1}H—^{1}H COSY and HSQC experiments allowed us to assign all signals for the protons and carbon atoms in the NMR spectra (see Tables 1 and 2). A comparison of the ^{1}H and ^{13}C NMR spectra of compound **4** and 24-propyl-5α-cholestane-3β,5,6β,8,15α,28,29-heptol from *C. crispatus* described earlier[2] demonstrated that they have the structurally identical steroid moiety containing hydroxy groups at the 3β,5α,6β,8 and 15α positions and differ only in the structure of the side chains. The chemical shifts of the carbon atoms and protons and the coupling constants of the protons of the side chain in the NMR spectra of compound **4** are similar to the corresponding characteristics in the NMR spectra of steroid **1**. Hence, we concluded that compound **4** contains the (22*E*)-26-*O*-sulfo-24-methyl-22-en-25,26-dihydroxycholestane side chain. Since compound **4** was isolated in a small amount (1.5 mg), unfortunately we could not prepare derivatives necessary for the determiation of the configurations of the C(24) and C(25) chiral centers. Based on the available data, we assigned the structure of sodium (22*E*,24ξ,25ξ)-24-methyl-5α-cholest-22-en-3β,5,6β,8,15α,25,26-heptol 26-*O*-sulfate to new steroid **4**.

To summarize, the fraction under study contains one previously known (the major component of the fraction)

and three new polar steroids. For the major component, the absolute configuration of the side chain was determined. For other components, the chemical structures, except for the absolute configurations of the chiral centers in the side chains, were established. These data will be used to reveal the biological activity—structure relationship in a series of polar steroids isolated from starfishes.

Experimental

The ^{1}H and ^{13}C NMR spectra were recorded on Bruker DPX 300 (300 and 75.5 MHz, respectively) and Bruker DPX 500 (500 and 125.8 MHz, respectively) spectrometers with $SiMe_4$ as the internal standard. The optical rotation was measured on a Perkin—Elmer 141 polarimeter.

The MALDI TOF mass spectra were obtained on a Biflex III mass spectrometer (Bruker, Germany, N_2 laser, 337 nm). A sample was dissolved in MeOH (1 mg mL^{-1}), and an aliquot (1 μL) was analyzed with the use of 2,5-dihydroxybenzoic acid as the matrix. The HPLC analysis was carried out on a DuPont Model 8800 chromatograph equipped with a refractometer as the detector using YMC-Pack ODS-A (5 μm, 12 nm, 10×250 mm) and Diasfer-110-C18 (5 μm, 4×250 mm) columns.

Column chromatography was performed on Amberlite XAD-2 (20—80 mesh, Sigma Chemical Co.), Sephadex LH-20 (Sigma Chemical Co.), silica gel L (40/100 μm, Chemapol, Czech Republic), and Florisil (100—200 mesh, Aldrich Chemical Co.). The TLC analysis was carried out on Sorbfil plates (4.5×6.0 cm, Krasnodar, Russia) with a layer of silica gel STKh-1A (5—17 μm) fixed on a foil.

Starfish samples were collected from a depth of 4—20 m in the Japan Sea (Posiet Bay) in June 1998 and identified by S. Sh. Dautov (Institute of Marine Biology, Far-Eastern Branch of Russian Academy of Sciences, Vladivostok).

Isolation of compounds 1—4. The ground starfish (650 g) was twice extracted with 70% ethanol (3 mL g^{-1}) with heating on a water bath. An ethanolic extract was concentrated *in vacuo*, and the residue was dissolved in water (0.5 L) and passed through a column (7×25 cm) with Amberlite XAD-2. Water was passed through the column until Cl^- ions disappeared from the eluate and then compounds were eluted with ethanol. The ethanolic eluate was concentrated. The resulting total fraction of steroidal compounds was successively chromatographed on a column with Sephadex LH-20 (3×60 cm) using a 2 : 1 ethanol—water mixture and on a column with silica gel (4×18 cm) using a chloroform—ethanol system (stepwise gradient, 8 : 1 → 1 : 2) to isolate a fraction containing (TLC data) sulfated polyhydroxysteroids (130 mg, R_f 0.86—0.72 using a 4 : 1 : 2 butanol—ethanol—water mixture). Then this faction was separated by HPLC on a YMC-Pack ODS-A column in 50% aqueous ethanol. The resulting subfractions were rechromatographed on a Diasfer-110-C18 column in 60% aqueous methanol (0.5 mL min^{-1}). Compounds **1** (R_t = 12.7 min), **2** (R_t = 14.8 min), **3** (R_t = 13.3 min), and **4** (R_t = 10.9 min) were isolated in yields of 10, 7, 1.5, and 1.5 mg, respectively.

Sodium (22*E*,24*R*,25*R*)-24-methyl-5α-cholest-22-en-3β,5,6β,15α,25,26-hexol 26-*O*-sulfate (1). Amorphous compound, $[\alpha]_D$ +11.6 (*c* 0.6, MeOH). Data from the ^{1}H and ^{13}C NMR spectra are given in Tables 1 and 2. (+)-MALDI TOF-MS, *m/z*: 605 $[M_{Na} + Na]^+$. (–)-MALDI TOF-MS, *m/z*: 559 $[M - cation]^-$.

Sodium (22*E*,24ξ,25ξ)-24-methyl-5α-cholest-22-en-3β,5,6β,15α,25,26-hexol 26-*O*-sulfate (2). Amorphous compound, $[\alpha]_D$ +12.4 (*c* 0.5, MeOH). Data from the ^{1}H and ^{13}C NMR spectra are given in Tables 1 and 2. (+)-MALDI TOF-MS, *m/z*: 605 $[M_{Na} + Na]^+$. (–)-MALDI TOF-MS, *m/z*: 559 $[M - cation]^-$.

Sodium (22*E*,24ξ)-26,27-bisnor-24-methyl-5α-cholest-22-en-3β,5,6β,15α,25-pentol 25-*O*-sulfate (3). Amorphous compound, $[\alpha]_D$ +7.0 (*c* 0.1, MeOH). Data from the ^{1}H NMR spectrum of the polycyclic moiety of compound **3** (CD_3OD), δ: 0.73 (s, 3 H, Me(18)C); 1.12 (m, 1 H, H(14)); 1.16 (s, 3 H, Me(19)C); 1.17 (m, 1 H, H(9)); 1.23 (m, 1 H, H(12)); 1.28 (m, 1 H, H(8)); 1.35 (m, 1 H, H(11)); 1.41 (m, 1 H, H(17)); 1.48 (m, 1 H, H(1)); 1.50 (m, 1 H, H_{eq}(2)); 1.54 (m, 1 H, H_{eq}(4)); 1.75 (m, 1 H, H_{ax}(2)); 1.76 (m, 1 H, H(16)); 1.77 (m, 1 H, H′(1)); 1.85 (m, 1 H, H(7)); 1.90 (m, 1 H, H′(16)); 2.05 (dd, 1 H, H(4), *J* = 11.2 Hz, *J* = 13.0 Hz); 3.50 (br.s, 1 H, H(6)); 3.85 (dt, 1 H, H(15), *J* = 3.0 Hz, *J* = 9.4 Hz); 4.00 (m, 1 H, H(3)). The ^{13}C NMR spectrum of the polycyclic moiety of compound **3** (CD_3OD), δ: 31.7 (C(1)); 33.6 (C(2)); 68.4 (C(3)); 41.5 (C(4)); 76.6 (C(5)); 76.4 (C(6)); 35.3 (C(7)); 30.8 (C(8)); 46.6 (C(9)); 39.3 (C(10)); 22.2 (C(11)); 41.7 (C(12)); 45.0 (C(13)); 63.6 (C(14)); 74.3 (C(15)); 41.8 (C(16)); 54.9 (C(17)); 13.8 (C(18)); 17.4 (C(19)). Data from the ^{1}H and ^{13}C NMR spectra of the side chain of compound **3** are given in Table 2. (+)-MALDI TOF-MS, *m/z*: 561 $[M_{Na} + Na]^+$. (–)-MALDI TOF-MS, *m/z*: 515 $[M - cation]^-$.

Sodium salt of (22*E*,24ξ,25ξ)-24-methyl-5α-cholest-22-en-3β,5,6β,8,15α,25,26-heptol 26-*O*-sulfate (4). Amorphous compound, $[\alpha]_D$ +11.8 (*c* 0.1, MeOH). Data from the ^{1}H and ^{13}C NMR spectra are given in Tables 1 and 2. (+)-MALDI TOF-MS, *m/z*: 621 $[M_{Na} + Na]^+$. (–)-MALDI TOF-MS, *m/z*: 575 $[M - cation]^-$.

Desulfation of compounds 1 and 2. Compound **1** or **2** (3 mg) in a 1 : 1 dioxane—pyridine mixture (1 mL) was heated at 100 °C for 4 h. The reaction mixture was concentrated *in vacuo*, and the dry residue was chromatographed on a column with Florisil (1.5×3 cm) in a 3 : 2 chloroform—ethanol system. Compound **1a** or **2a**, respectively, was obtained in a yield of 2 mg.

Desulfated steroid 1a. Amorphous compound, $[\alpha]_D$ +10.5 (*c* 0.1, MeOH). ^{1}H NMR (CD_3OD), δ: 0.75 (s, 3 H, Me(18)C); 0.98 (d, 3 H, Me(28)C, *J* = 6.8 Hz); 1.02 (d, 3 H, Me(21)C, *J* = 6.8 Hz); 1.09 (s, 3 H, Me(27)C); 1.17 (s, 3 H, Me(19)C); 3.37 (d, 1 H, H(26), *J* = 11.0 Hz); 3.44 (d, 1 H, H′(26), *J* = 11.0 Hz); 3.46 (br.s, 1 H, H(6)); 3.85 (dt, 1 H, H(15), *J* = 3.0 Hz, *J* = 9.5 Hz); 4.01 (m, 1 H, H(3)); 5.27 (dd, 1 H, H(22), *J* = 8.5 Hz, *J* = 15.0 Hz); 5.42 (dd, 1 H, H(23), *J* = 8.0 Hz, *J* = 15.0 Hz).

Desulfated steroid 2a. Amorphous compound, $[\alpha]_D$ +9.3 (*c* 0.1, MeOH). ^{1}H NMR (CD_3OD), δ: 0.75 (s, 3 H, Me(18)C); 1.00 (d, 3 H, Me(28)C, *J* = 6.8 Hz); 1.01 (d, 3 H, Me(21)C, *J* = 6.8 Hz); 1.04 (s, 3 H, Me(27)C); 1.17 (s, 3 H, Me(19)C); 3.38 (br.s, 2 H, H(26), H′(26)); 3.47 (br.s, 1 H, H(6)); 3.85 (dt, 1 H, H(15), *J* = 3.0 Hz, *J* = 9.5 Hz); 4.01 (m, 1 H, H(3)); 5.28 (m, 2 H, H(22), H(23)).

Hydrogenation of compounds 1a and 2a. A solution of compound **1** or **2** (2 mg) in methanol (2 mL) was hydrogenated with hydrogen over a platinum catalyst (PtO_2) for 24 h. The catalyst was removed by filtration, the solution was concentrated, and

the dry residue was chromatographed on a column with Florisil (1.5×3 cm) using a 3 : 2 chloroform—ethanol mixture. Compound **1b** or **2b**, respectively, was obtained in a yield of 1.5 mg.

Hydrogenated steroid 1b. Amorphous compound, $[\alpha]_D$ +11.5 (*c* 0.15, MeOH). ^{1}H NMR (CD_3OD), δ: 0.74 (s, 3 H, Me(18)C); 0.85 (d, 3 H, Me(28)C, *J* = 6.8); 0.89 (d, 3 H, Me(21)C, *J* = 6.8 Hz); 1.01 (s, 3 H, Me(27)C); 1.17 (s, 3 H, Me(19)C); 3.39 (d, 1 H, H(26), *J* = 11.2 Hz); 3.46 (d, 1 H, H´(26), *J* = 11.2 Hz); 3.47 (br.s, 1 H, H(6)); 3.85 (dt, 1 H, H(15), *J* = 3.3 Hz, *J* = 9.4 Hz); 4.01 (m, 1 H, H(3)).

Hydrogenated steroid 2b. Amorphous compound, $[\alpha]_D$ +9.4 (*c* 0.15, MeOH). ^{1}H NMR (CD_3OD), δ: 0.74 (s, 3 H, Me(18)C); 0.91 (d, 3 H, Me(28)C, *J* = 6.6 Hz); 0.93 (d, 3 H, Me(21)C, *J* = 6.6 Hz); 1.05 (s, 3 H, Me(27)C); 1.17 (s, 3 H, Me(19)C); 3.44 (br.s, 2 H, H(26), H´(26)); 3.47 (br.s, 1 H, H(6)); 3.86 (dt, 1 H, H(15), *J* = 3.3 Hz, *J* = 9.4 Hz); 4.01 (m, 1 H, H(3)).

Synthesis of MTPA esters of compounds 1b and 2b. Compound **1** or **2** (1.5 mg) was dissolved in dry pyridine (200 μL), after which *R*-MTPA acid chloride (10 μL) was added. The reaction mixture was kept at room temperature for 2 h and then concentrated *in vacuo*. The dry residue was purified on a column with silica gel (1×2 cm) using a 1 : 1 hexane—chloroform mixture and then chloroform as eluents. Compound **1c** or **2c**, respectively, was obtained in a yield of 1 mg.

(24*R*,25*R*)-24-Methyl-5α-cholestane-3β,5,6β,15α,25,26-hexol 3,15,26-tris[*R*-(methoxy)(trifluoromethyl)phenylacetate] (1c). Amorphous compound, $[\alpha]_D$ +10.0 (*c* 0.1, MeOH). ^{1}H NMR (CD_3OD), δ: 0.77 (s, 3 H, Me(18)C); 0.83 (d, 3 H, Me(28)C, *J* = 6.6 Hz); 0.87 (d, 3 H, Me(21)C, *J* = 6.6 Hz); 1.09 (s, 3 H, Me(27)C); 1.12 (s, 3 H, Me(19)C); 3.09 (br.s, 1 H, H(6)); 4.21 (d, 1 H, H(26), *J* = 11.2 Hz); 4.24 (d, 1 H, H´(26), *J* = 11.2 Hz); 4.95* (dt, 1 H, H(15), *J* = 3.3 Hz, *J* = 9.4 Hz); 5.40 (m, 1 H, H(3)). ^{1}H NMR ($CDCl_3$), δ: 0.72 (s, 3 H, Me(18)C); 0.83 (d, 3 H, Me(28)C, *J* = 6.6 Hz); 0.89 (d, 3 H, Me(21)C, *J* = 6.6 Hz); 1.08 (s, 3 H, Me(27)C); 1.10 (s, 3 H, Me(19)C); 3.05** (br.s, 1 H, H(6)); 4.22 (d, 1 H, H(26), *J* = 11.2 Hz); 4.26 (d, 1 H, H´(26), *J* = 11.2 Hz); 4.84 (dt, 1 H, H(15), *J* = 3.3 Hz, *J* = 9.4 Hz); 5.40 (m, 1 H, H(3)).

(24ξ,25ξ)-24-Methyl-5α-cholestane-3β,5,6β,15α,25,26-hexol 3,15,26-tris[*R*-(methoxy)(trifluoromethyl)phenylacetate] (2c). Amorphous compound, $[\alpha]_D$ +41.0 (*c* 0.1, MeOH). ^{1}H NMR (CD_3OD), δ: 0.76 (s, 3 H, Me(18)C); 0.87 (d, 3 H, Me(28)C, *J* = 6.6 Hz); 0.88 (d, 3 H, Me(21)C, *J* = 6.6 Hz); 1.07 (s, 3 H, Me(27)C); 1.13 (s, 3 H, Me(19)C); 3.09 (br.s, 1 H, H(6)); 4.24 (br.s, 2 H, H(26), H´(26)); 4.92 (dt, 1 H, H(15), *J* = 3.3 Hz, *J* = 9.4 Hz); 5.41 (m, 1 H, H(3)). ^{1}H NMR ($CDCl_3$), δ: 0.72 (s, 3 H, Me(18)C); 0.87 (d, 3 H, Me(21)C, *J* = 6.6 Hz); 0.89 (d, 3 H, Me(28)C, *J* = 6.6 Hz); 1.10 (s, 3 H, Me(19)C); 1.11 (s, 3 H, Me(27)C); 3.02 (br.s, 1 H, H(6)); 4.18 (d, 1 H, H(26), *J* = 11.2 Hz); 4.34 (d, 1 H, H´(26), *J* = 11.2 Hz); 4.85 (dt, 1 H, H(15), *J* = 3.3 Hz, *J* = 9.4 Hz); 5.41 (m, 1 H, H(3)).

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* The position of the signal in the spectrum was refined by heating a solution of the sample to 45 °C, which led to the upfield shift of the signal of H_2O.

** The position of the signal in the spectrum was refined by heating a solution of the sample to 45 °C, which led to the downfield shift of the signal of the OMe group.