Polar steroidal compounds from the Far-Eastern starfish *Lethasterias fusca*

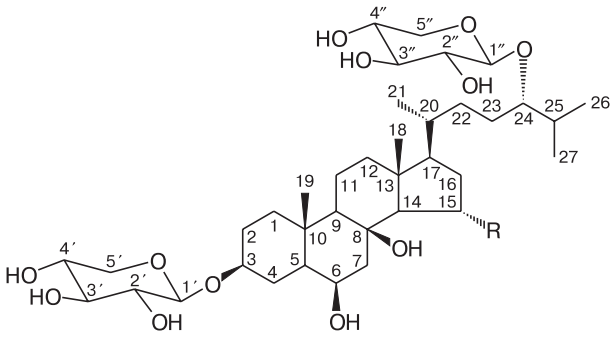
N. V. Ivanchina, T. V. Malyarenko, A. A. Kicha, A. I. Kalinovskii, and P. S. Dmitrenok*

Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch of the Russian Academy of Sciences, 159 prosp. 100 let Vladivostoku, 690022 Vladivostok, Russian Federation. Fax: +7 (423 2) 31 4050. E-mail: ivanchina@piboc.dvo.ru

Nine steroidal compounds including three new steroidal glycosides, *viz.*, sodium (24*S*)-3,24-di-*O*-(β-D-xylopyranosyl)-5α-cholestane-3β,6β,8,15α,24-pentol 15-sulfate (fuscaside A), (24*S*)-3,24-di-*O*-(β-D-xylopyranosyl)-5α-cholestane-3β,6β,8,15α,24-pentol (fuscaside B), and (22*E*,24*R*)-24-*O*-(β-D-xylopyranosyl)-5α-cholest-22-ene-3β,6α,8,15β,24-pentol (desulfated minutoside A); three previously known glycosides, *viz.*, distolasterosides D_1 and D_2 and pycnopodioside A; two previously known polyhydroxysteroids, *viz.*, 5α-cholestane-3β,6α,8,15β,16β,26-hexaol and 5α-cholestan-3β,4β,6α,7α,8,15β,16β,26-octol; and the known sodium 24,25-dihydromarthasterone 3-sulfate were isolated from the Far-Eastern starfish *Lethasterias fusca*. The structures of these compounds were elucidated by NMR spectroscopy and mass spectrometry.

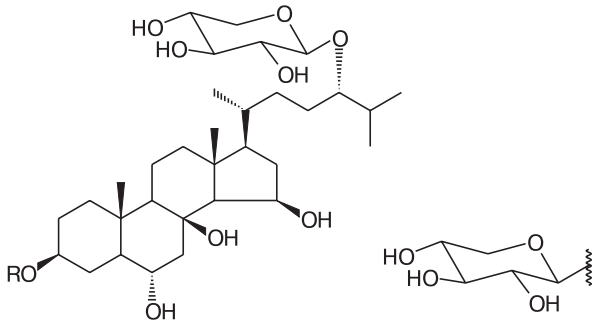
Key words: starfish, *Lethasterias fusca*, steroidal glycosides, polyhydroxylated steroids, NMR spectra.

Unlike other echinoderms, starfishes contain a diversity of highly oxidized steroidal compounds. As a rule, these include polyhydroxysteroids, polyhydroxysteroid mono- and biosides, and toxic steroidal oligoglycosides called asterosaponins.[1,2] The oxidized steroids are encountered in both free and sulfated forms. In continuation of our studies of physiologically active steroidal metabolites from Far-Eastern starfishes,[3] we studied the steroid composition of the starfish *Lethasterias fusca* (order Forcipulatida, family Asteriidae) collected at the experimental Marine station of the Pacific Institute of Bioorganic Chemistry (Possiet Bay, Japanese Sea). As a result, nine steroidal compounds (**1**—**9**) were isolated, in particular, three new steroidal glycosides, fuscasides A (**1**)



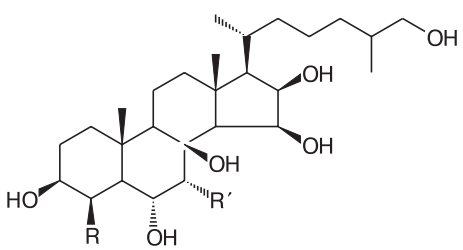
1, 2

R = OSO_3Na (**1**), OH (**2**)



3—6 **A**

3: Δ^{22}, R = H; **4:** R = **A**; **5:** Δ^{22}, R = **A**; **6:** R = H



7, 8

R = R′ = H (**7**), OH (**8**)

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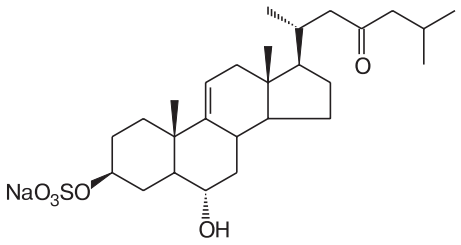
and B (**2**) and desulfated minutoside A (**3**), three known glycosides, *viz.*, distolasterosides D_1 (**4**) and D_2 (**5**) and pycnopodioside A (**6**), two known polyhydroxysteroids, *viz.*, 5α-cholestan-3β,6α,8,15β,16β,26-hexol (**7**) and 5α-cholestan-3β,4β,6α,7α,8,15β,16β,26-octol (**8**), and known native aglycon of the asterosaponin series, *viz.*, sodium 24,25-dihydromarthasterone 3-sulfate (**9**).



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Results and Discussion

Pure steroidal compounds **1—9** were isolated from a water—ethanolic extract of the starfish *L. fusca* by repeated column chromatography on Polychrom 1, Sephadex LH-20, silica gel, and florisil and HPLC on Zorbax ODS, Diaspher-110-C18, Kromasil 100A-C18, and Cosmosil 5C18-AR-2. The structures of new compounds were determined in $^1H—^1H$ COSY, HSQC, HMBC, and DEPT NMR experiments and data from mass spectrometry. Known compounds were identified by comparison of the NMR spectra, mass spectra, and physical parameters with published data.

The high-resolution (+)-MALDI-TOF mass spectrum of compound **1** contained a pseudomolecular ion peak with *m/z* 841.3692 $[M + Na]^+$, which corresponded to the molecular formula $C_{37}H_{63}O_{16}Na_2S$, and the (−)-MALDI-TOF mass spectrum of the same compound exhibited a pseudomolecular ion peak with *m/z* 795 [M − cation]$^-$. The (+)-LSI mass spectrum contained, apart from the pseudomolecular ion peak with *m/z* 841 $[M_{Na} + Na]^+$, also peaks with *m/z* 721, 691, and 571 corresponding to elimination of the sulfate group $[M_{Na} + Na - NaHSO_4]^+$, a pentose residue $[M_{Na} + Na - C_5H_{10}O_5]^+$, and simultaneous loss of the sulfate group and the pentose residue $[M_{Na} + Na - NaHSO_4 - C_5H_{10}O_5]^+$. The (−)-LSI mass spectrum of compound **1** contained a pseudomolecular ion peak with *m/z* 795 [M − cation]$^-$, a peak with *m/z* 645 corresponding to the loss of the pentose residue $[M - cation - C_5H_{10}O_5]^-$, and a peak for the sulfate group with *m/z* 97 $[HSO_4]^-$. These data indicated that **1** is a sulfated bioside. The ^{13}C NMR and DEPT spectra of compound **1** contained signals for 37 carbon atoms including signals for five methyl, eleven methylene, and eighteen methine groups and three carbon atoms bearing no hydrogen. The chemical shifts of the anomeric protons at δ 4.22 and 4.36 (J = 7.6 Hz), anomeric carbon atoms at δ 103.2 and 105.1, carbon atoms bound to oxygen at δ 66.7, 66.8, 71.3 (×2), 74.2, 75.0, 75.3, 76.9, 77.9 (×2), 78.0, 80.0, and 86.2 also attested to the presence of two pentoside fragments and a pentasubstituted steroidal aglycon in the molecule. Using 2D NMR experiments, the signals of all protons and carbon atoms were assigned (Table 1). Analysis of NMR data of glycoside **1** demonstrated that the proton and carbon chemical shifts and the corresponding spin-spin coupling constants of the protons of rings A and B in the steroidal core and one monosaccharide residue almost coincided with the corresponding signals of asteriidoside L from the Antarctic starfish of the family Asteriidae[4] containing 3β,6β-hydroxy groups and the β-D-xylopyranose residue attached to O(3) of the aglycon. The proton chemical shifts and spin-spin coupling constants and the carbon chemical shifts of rings C and D for the steroidal core, side chain, and the second monosaccharide residue were in good agreement with the data for coscinasteroside B from the starfish *Coscinasterias tenuispina*.[5] Thus it was demonstrated that compound **1** also has 8,15α,24-hydroxy groups, C(15) bears a sulfate group, and the O(24) atom of the aglycon bears a β-D-xylopyranose residue. In the HMBC spectrum, the attachment sites of monosaccharide residues were confirmed by correlations of the anomeric protons HC(1′) and HC(1″) with C(3) and C(24), respectively. We ascribed the *S*-configuration to the asymmetric center C(24) by analogy with coscinasteroside B (see Ref. 5) and other 24-glycosylated steroids found in starfishes.[1] Thus, new fuscaside A (**1**) was identified as sodium (24*S*)-3,24-di-*O*-(β-D-xylopyranosyl)-5α-cholestan-3β,6β,8,15α,24-pentol 15-sulfate.

The high-resolution (+)-MALDI-TOF mass spectrum of steroidal glycoside **2** contained a pseudomolecular ion peak with *m/z* 739.4297 $[M + Na]^+$, which corresponded to the molecular formula $C_{37}H_{64}O_{13}Na$. A comparison of the ^1H NMR data (see Table 1) of compound **2** with the data obtained for fuscaside A (**1**) showed that glycoside **2** differed from fuscaside A by only the lack of the sulfate group at C(15). Indeed, the H-15 signal is observed in a higher field (δ 4.26) than that for **1** (δ 4.89). In addition, the chemical shifts and the spin-spin coupling constants of the protons of the steroidal core and one monosaccharide residue were in good agreement with the corresponding data for asteriidoside L from the starfish of the family Asteriidae,[4] which also confirmed the presence of the 3β,6β,8,15α-tetrahydroxysteroidal core and the β-D-xylopyranose residue attached to the O(3) atom of the aglycon. Mild solvolysis of fuscaside A (**1**) gave a desulfated derivative the ^1H NMR spectrum of which was identical to the spectrum of glycoside **2**. In its ^{13}C NMR spectrum, the C(15) signal resonates in a higher field (δ 70.1) than in the spectrum of glycoside **1** (δ 78.0).

Table 1. ^{1}H and ^{13}C NMR spectra of compound **1** and ^{1}H NMR spectrum of compound **2** (CD_3OD, δ, *J*/Hz)[a]

Atom	**1**[b]			**2**
	δ_H	HMBC	δ_C[c]	δ_H
1	1.71 (m)		41.4 CH_2	
	0.98 (m)			
2	1.81 (m)		30.1 CH_2	
	1.61 (m)			
3	3.70 (m)		80.0 CH	3.70 (m)
4	1.82 (m)		33.0 CH_2	
	1.75 (m)			
5	1.19 (m)		48.7 CH	
6	3.86 (m)		74.2 CH	3.86 (m)
7	2.54 (dd, 3.1, 15.0)	C(5),C(6), C(8), C(9)	44.7 CH_2	2.38 (dd, *J* = 3.0, *J* = 14.7)
	1.55 (dd, 6.0, 15.0)			1.60 (m)
8			76.9 C	
9	0.95 (m)		56.9 CH	
10			36.8 C	
11	1.84 (m)		19.9 CH_2	
	1.53 (m)			
12	1.96 (m)		42.7 CH_2	
	1.25 (m)			
13			44.7 C	
14	1.37 (d, 9.5)	C(13), C(15), C(18)	64.6 CH	
15	4.89 (dt, 2.8, 9.5)		78.0 CH	4.26 (dt, *J* = 3.6, *J* = 10.0)
16	2.20 (dd.d., 3.0, 8.5, 15.0)	C(13)	38.6 CH_2	
	1.90 (dt, 15.0, 9.5)			
17	1.31 (m)		56.0 CH	
18	0.97 (s)	C(12), C(13), C(14), C(17)	15.3 CH_3	0.95 (s)
19	1.16 (s)	C(1), C(5), C(9), C(10)	15.8 CH_3	1.16 (s)
20	1.39 (m)		36.1 CH	
21	0.89 (d, 6.5)	C(17), C(20), C(22)	18.8 CH_3	0.90 (d, *J* = 6.6)
22	1.58 (m)		32.4 CH_2	
	1.01 (m)			
23	1.59 (m)		28.8 CH_2	
	1.37 (m)			
24	3.34 (m)		86.2 CH	
25	1.84 (m)		31.9 CH	
26	0.91 (d, 6.5)	C(24), C(25), C(27)	18.3 CH_3	0.93 (d, *J* = 6.9)
27	0.92 (d, 6.5)	C(24), C(25), C(26)	18.5 CH_3	0.92 (d, *J* = 6.9)
1′	4.36 (d, 7.6)	C(3)	103.2 CH	4.35 (d, *J* = 7.6)
2′	3.13 (dd, 7.7, 9.4)	C(1′), C(3′), C(4′)	75.0 CH	3.13 (dd, *J* = 7.7, *J* = 9.4)
3′	3.30[d]		77.9 CH	3.32[d]
4′	3.47 (m)		71.3 CH	3.47 (m)
5′	3.82 (dd, 5.3, 11.4)	C(1′), C(3′), C(4′)	66.8 CH_2	3.82 (dd, 5.3, *J* = 11.4)
	3.18 (dd, 9.4, 11.3)			3.18 (dd, 9.4, *J* = 11.4)
1″	4.22 (d, 7.6)	C(24)	105.1 CH	4.22 (d, *J* = 7.6)
2″	3.19 (dd, 7.4, 8.9)	C(1″), C(3″), C(4″)	75.3 CH	3.18 (dd, *J* = 7.5, *J* = 8.9)
3″	3.27 (t, 8.8)	C(2″), C(4″)	77.9 CH	3.32[d]
4″	3.52 (m)		71.3 CH	3.52 (m)
5″	3.87 (dd, 5.3, 11.3)	C(1″), C(3″), C(4″)	66.7 CH_2	3.87 (dd, 5.3, *J* = 11.4)
	3.13 (dd, 9.4, 11.3)			3.13 (dd, 9.4, *J* = 11.4)

[a] The ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125.8 MHz, respectively.
[b] The signals were assigned by ^{1}H—^{1}H COSY and HSQC 2D NMR techniques.
[c] The signal multiplicity was determined using the DEPT spectrum.
[d] The signals overlap with the solvent signals.

Conversely, the signals for the C(14) and C(16) atoms carbon occur in a lower field (d 66.5 and 41.6) than in the case of compound **1** (d 64.6 and 38.6, respectively). These data were also indicative of the fact that fuscaside B (**2**) is

a native desulfated derivative of fuscaside A (**1**) having the structure of (24*S*)-3,24-di-*O*-(β-D-xylopyranosyl)-5a-cholestane-3b,6b,8,15a,24-pentol.

The high-resolution (+)-MALDI-TOF mass spectrum of compound **3** contained a pseudomolecular ion peak with *m/z* 605.3614 $[M + Na]^+$ corresponding to the molecular formula $C_{32}H_{54}O_9Na$. The chemical shifts and the spin-spin coupling constants for the protons and carbon atoms of the steroidal core observed in the ^1H and ^{13}C NMR и DEPT spectra of glycoside **3** were in good agreement with those known for pycnopodioside A from the starfish *Pycnopodia helianthoides*.[6] One could suggest that steroid **3** contains a 3β,6α,8,15β-tetrahydroxysteroidal core. The NMR chemical shifts of the side chain and the monosaccharide residue in **3** were similar to those for minutoside A isolated from the starfish *Anasterias minuta*,[7] which might be indicative of the presence of (24*R*)-24-*O*-(β-D-xylopyranosyl)-cholest-22-ene side chain in glycoside **3**. The spin-spin coupling constant $J_{22,23}$ = 15.4 Hz corresponded to the *trans*-configuration of the 22(23)-double bond. The chemical shifts of all protons and carbon atoms in **3** were determined by NMR spectroscopy. Data of 2D NMR experiments confirmed the structure of glycoside **3** as (22*E*,24*R*)-24-*O*-(β-D-xylopyranosyl)-5α-cholest-22-ene-3β,6α,8,15β,24-pentol. Glycoside **3** is desulfated minutoside A being found for the first time as a natural compound. Simultaneously, we isolated this compound in a repeated study of the Far-Eastern starfish *Distolasterias nipon*.[8]

The NMR and mass spectra of compounds **4** and **5** were found to be almost identical to those reported for distolasterosides D_1 and D_2, respectively, which were found previously in the starfish *Distolasterias nipon*.[9,10] Similarly, glycoside **6** was identified as pycnopodioside A detected previously in the starfish *Pycnopodia helianthoides*.[6] In addition, we isolated two known polyhydroxysteroids, *viz.*, 5α-cholestan-3β,6α,8,15β,16β,26-hexol (**7**) found previously in the starfish *Halityle regularis*[11] and 5α-cholestan-3β,4β,6α,7α,8,15β,16β,26-octol (**8**) first found in the starfish *Asterina pectinifera*,[12] and sodium 24,25-dihydromarthasterone 3-sulfate (**9**) known as native sulfated aglycon of marthasteroside C from the starfish *Marthasterias glacialis*[13] and isolated first in a pure state from the starfish *Aphelasterias japonica*.[14]

Glycosides **1** and **2** refer to a rather scarce structural group of natural glycosylated steroidal polyols containing monosaccharide residues attached to both O(3) and O(24) atoms of the aglycon.

Experimental

^1H and ^{13}C NMR spectra were recorded on Bruker DPX 300 (300.13 and 75.5 MHz) and Bruker DRX 500 (500.13 and 125.8 MHz, respectively) spectrometers using CD_3OD as the internal standard (δ_H 3.30, δ_C 49.0). Optical rotation was measured on a Perkin—Elmer 343 polarimeter.

MALDI-TOF mass spectra were run on a Bruker Biflex III mass spectrometer (Germany, N_2 laser, 337 nm). The sample was dissolved in MeOH (1 mg mL^{-1}) and a 1 μL aliquot was analyzed using 2,5-dihydroxybenzoic acid as the matrix. The LSI mass spectra were recorded on an Intectra AMD-604S mass spectrometer (Germany), Cs^+ ion energy 10 keV, accelerating voltage 8 kV. Glycerol (Sigma) was used as the matrix. HPLC was carried out on an Agilent 1100 Series chromatograph (refractometer as the detector) with Zorbax ODS (5 μ, 250×9.4 mm), Diaspher-110-C18 (S-5 μ, 250×4 mm), Kromasil 100A-C18 (5 μ, 250×4.6 mm), and Cosmosil 5C18-AR-2 columns (5 μ, 250×4.6 mm).

Column chromatography was perfomed using Polychrom 1 (Teflon powder, Biolar, Latvia), KSK silica gel (50—160 μm, Sorbpolimer, Krasnodar, Russia), and Florisil (200—300 mesh, Aldrich). Thin layer chromatography was carried out on Sorbfil plates (4.5×6.0 cm, Sorbpolimer, Krasnodar, Russia) with a foil-supported STKh-1A silica gel layer (5—17 μm) in a butanol : ethanol : water system (4 : 1 : 2). The substances were visualized by spraying the chromatograms with concentrated H_2SO_4 followed by heating at 110 °C for 10 min.

The starfish samples were collected in August, 2002, in the Possiet Bay of the Japanese Sea at the Marine Experimental Station of the Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch of the Russian Academy of Sciences, and identified by C. Sh. Dautov (Institute of Marine Biology, Far-Eastern Branch of the Russian Academy of Sciences, Vladivostok, Russia).

Isolation of compounds 1—9. Crushed starfishes (1.14 kg) were extracted twice with 70% ethanol (3 L kg^{-1}) with heating on a water bath and the precipitate was separated by filtration through cotton. Nonpolar compounds (sterols and lipids) were removed by extracting the filtrate twice with hexane (1 mL per 3 mL of the filtrate). The water—ethanol layer was concentrated *in vacuo*, the dry residue was divided into two parts, and each part was dissolved in water (500 mL) and passed separately through a Polychrom 1 column (7.5×18 cm). The column was washed with water until the eluate contained no chloride ions and then with ethanol, and the ethanolic eluate was concentrated. The resulting overall fraction of steroidal compounds (8.2 g) was successively chromatographed on a Sephadex LH-20 column (3.5×30 cm) in an ethanol : water system (2 : 1) and a silica gel column (6×19 cm) in a chloroform : ethanol system (stepwise gradient, 8 : 1 → 1 : 1). The fractions thus formed were subjected to HPLC on a Zorbax ODS column using 65% ethanol as the eluent and rechromatographed on Diaspher-110-C18, Kromasil 100A-C18, and Cosmosil 5C18-AR-2. This gave 13 mg of compound **1** (R_f = 0.59), 0.5 mg of compound **2** (R_f = 0.69), 6 mg of compound **3** (R_f = 0.62), 1 mg of compound **4** (R_f = 0.76), 4 mg of compound **5** (R_f = 0.71), 2.5 mg of compound **6** (R_f = 0.86), 6 mg of compound **7** (R_f = 0.91), 3.5 mg of compound **8** (R_f = 0.83), and 0.5 mg of compound **9** (R_f = 0.80).

Fuscaside A (1). Amorphous compound, $[\alpha]_D$ +0.4 (*c* 0.3, MeOH). ^1H and ^{13}C NMR spectra are presented in Table 1. HR (+)-MALDI-TOF MS: found *m/z* 841.3692 $[M_{Na} + Na]^+$. $C_{37}H_{63}O_{16}Na_2S$. Calculated: M = 841.3632. MS (–)-MALDI-TOF, *m/z*: 795 [M – cation]$^-$. MS (+)-LSI, *m/z*: 841 $[M_{Na} + Na]^+$, 721 $[M_{Na} + Na - NaHSO_4]^+$, 691 $[M_{Na} + Na - C_5H_{10}O_5]^+$ and 571 $[M_{Na} + Na - NaHSO_4 - C_5H_{10}O_5]^+$. MS (–)-LSI, *m/z*: 795 [M – cation]$^-$, 645 [M – cation – $C_5H_{10}O_5$]$^-$ and 97 $[HSO_4]^-$.

Fuscaside B (2). Amorphous compound, $[\alpha]_D$ −9.3 (*c* 0.05, MeOH). The ^{1}H NMR spectra are presented in Table 1. HR (+)-MALDI-TOF MS: found *m/z* 739.4297 $[M_{Na} + Na]^+$. $C_{37}H_{64}O_{13}Na$. Calculated: M = 739.4247.

Desulfated minutoside A (3). Amorphous compound, $[\alpha]_D$ +3.3 (*c* 0.2, MeOH). ^{1}H NMR (CD_3OD), δ: 0.84 (m, 1 H, H(9)); 0.86 (d, 3 H, Me(26)C, *J* = 6.6 Hz); 0.93 (d, 3 H, Me(27)C, *J* = 6.6 Hz); 0.97 (m, 1 H, H(1)); 0.98 (s, 3 H, Me(19)C); 1.01 (d, 3 H, Me(21)C, *J* = 6.6 Hz); 1.02 (d, 1 H, H(14), *J* = 5.5 Hz); 1.04 (m, 1 H, H(17)); 1.05 (m, 1 H, H(5)); 1.19 (both m, 1 H each, H_{eq}(4), H(12)); 1.27 (m, 1 H, H_{ax}(7)); 1.29 (s, 3 H, Me(18)C); 1.39 (m, 1 H, H(16)); 1.48 (m, 1 H, H_{eq}(2)); 1.51 (m, 1 H, H(11)); 1.71 (m, 1 H, H´(1)); 1.73 (m, 1 H, H_{ax}(2)); 1.81 (m, 1 H, H´(11)); 1.82 (m, 1 H, H(25)); 1.97 (m, 1 H, H´(12)); 2.18 (m, 1 H, H_{ax}(4)); 2.23 (m, 1 H, H(20)); 2.24 (m, 1 H, H´(16)); 2.36 (dd, 1 H, H_{eq}(7), *J* = 12.2 Hz, *J* = 4.0 Hz); 3.13 (dd, 1 H, H(5´), *J* = 11.5 Hz, *J* = 10.4 Hz); 3.16 (dd, 1 H, H(2´), *J* = 7.4 Hz, *J* = 8.5 Hz); 3.26 (t, 1 H, H(3´), *J* = 8.7 Hz); 3.44 (m, 1 H, H(4´)); 3.48 (m, 1 H, H(3)); 3.69 (both m, 1 H each, H(6), H(24)); 3.79 (dd, 1 H, H(5″), *J* = 11.5 Hz, *J* = 5.3 Hz); 4.21 (d, 1 H, H(1´), *J* = 7.4 Hz); 4.39 (br.t, 1 H, H(15), *J* = 5.6 Hz); 5.35 (dd, 1 H, H(23), *J* = 15.4 Hz, *J* = 7.7 Hz); 5.39 (dd, 1 H, H(22), *J* = 15.4 Hz, *J* = 7.2 Hz). ^{13}C NMR (CD_3OD), δ: 39.4 (C(1)); 31.4 (C(2)); 72.2 (C(3)); 32.3 (C(4)); 53.8 (C(5)); 67.6 (C(6)); 49.4 (C(7)); 77.5 (C(8)); 57.4 (C(9)); 38.0 (C(10)); 19.7 (C(11)); 43.2 (C(12)); 44.2 (C(13)); 62.6 (C(14)); 71.1 (C(15)); 43.4 (C(16)); 57.6 (C(17)); 16.7 (C(18)); 14.1 (C(19)); 40.8 (C(20)); 20.9 (C(21)); 141.2 (C(22)); 128.1 (C(23)); 89.2 (C(24)); 33.8 (C(25)); 18.3 (C(26)); 19.3 (C(27)); 104.3 (C(1´)); 75.4 (C(2´)); 78.1 (C(3´)); 71.2 (C(4´)); 67.0 (C(5´)). HMBC (CD_3OD): H(18)/C(12), C(13), C(14), C(17); H(21)/C(17), C(20), C(22); H(26)/C(24), C(25), C(27); H(27)/C(24), C(25), C(26); H(1´)/C(24); H(2´)/C(1´), C(3´); H(3´)/C(2´), C(4´); H(5´)/C(3´), C(4´). HR (+)-MALDI-TOF MS: found *m/z* 605.3614 $[M + Na]^+$. $C_{32}H_{54}O_9Na$. Calculated: M = 605.3660.

Distolasteroside D_1 (4). Amorphous compound, $[\alpha]_D$ −5.3 (*c* 0.1, MeOH), *cf.* Ref. 9: $[\alpha]_D$ −17.9 (*c* 0.24, MeOH). The ^{1}H and ^{13}C NMR spectra were identical to the spectra described previously.[9,10] (+)-MALDI-TOF MS, *m/z*: 739 $[M + Na]^+$.

Distolasteroside D_2 (4). Amorphous compound, $[\alpha]_D$ −0.7 (*c* 0.2, MeOH), *cf.* Ref. 9: $[\alpha]_D$ −10.8 (*c* 0.27, MeOH). The ^{1}H and ^{13}C NMR spectra were identical to the spectra described previously.[9,10] (+)-MALDI-TOF MS, *m/z*: 737 $[M + Na]^+$.

Pycnopodioside A (6). Amorphous compound, $[\alpha]_D$ −1.1 (*c* 0.25, MeOH), *cf.* Ref. 6: $[\alpha]_D$ −2.0 (MeOH). The ^{1}H and ^{13}C NMR spectra were identical to the spectra described previously.[6] (+)-MALDI-TOF MS, *m/z*: 607 $[M + Na]^+$.

5α-Cholestan-3β,6α,8,15β,16β,26-hexol (7). Amorphous compound, $[\alpha]_D$ +15.7 (*c* 0.1, MeOH), *cf.* Ref. 11: $[\alpha]_D$ 0 (MeOH). The ^{1}H and ^{13}C NMR spectra were identical to the spectra described previously.[11] (+)-MALDI-TOF MS, *m/z*: 491 $[M + Na]^+$. MS (−)-MALDI-TOF, *m/z*: 467 $[M - H]^-$.

5α-Cholestane-3β,4β,6α,7α,8,15β,16β,26-octol (8). Amorphous compound, $[\alpha]_D$ +2.3 (*c* 0.05, MeOH), *cf.* Ref. 12: $[\alpha]_D$ +26.4 (*c* 1.04, MeOH). The ^{1}H and ^{13}C NMR spectra were identical to the spectra described previously.[12] (+)-MALDI-TOF MS, *m/z*: 523 $[M + Na]^+$.

24,25-Dihydromarthasteron 3-sulfate, sodium salt (9). Amorphous compound, $[\alpha]_D$ +5.6 (*c* 0.06, MeOH), *cf.* Ref. 14: $[\alpha]_D$ +4.0 (*c* 0.2, MeOH). The ^{1}H and ^{13}C NMR spectra were identical to the spectra described previously.[13,14] (−)-MALDI-TOF MS, *m/z*: 495 $[M - H]^-$.

Desulfation of compound 1. Compound **1** (3 mg) was heated for 4 h with 1 mL of a dioxane—pyridine mixture (1 : 1) at 100 °C. The reaction mixture was concentrated *in vacuo* and the dry residue was chromatographed on a column with silica gel (1×4 cm) in a chloroform—ethanol system (3 : 2) to give 1.5 mg of desulfated derivative. The ^{1}H NMR spectrum was identical to the spectrum of compound **2** (see Table 1). ^{13}C NMR (CD_3OD), δ: 41.4 (C(1)); 30.1 (C(2)); 80.0 (C(3)); 33.0 (C(4)); 48.7 (C(5)); 74.2 (C(6)); 45.4 (C(7)); 77.1 (C(8)); 57.1 (C(9)); 36.8 (C(10)); 19.8 (C(11)); 42.8 (C(12)); 45.5 (C(13)); 66.5 (C(14)); 70.1 (C(15)); 41.6 (C(16)); 56.0 (C(17)); 15.3 (C(18)); 15.8 (C(19)); 36.2 (C(20)); 19.0 (C(21)); 32.8 (C(22)); 28.6 (C(23)); 86.1 (C(24)); 31.9 (C(25)); 18.3 (C(26)); 18.4 (C(27)); 103.2 (C(1´)); 75.0 (C(2´)); 77.9 (C(3´)); 71.3 (C(4´)); 66.9 (C(5´)); 104.9 (C(1″)); 75.3 (C(2″)); 78.1 (C(3″)); 71.3 (C(4″)); 66.5 (C(5″)).

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