Acanthosterol Sulfates A−J: Ten New Antifungal Steroidal Sulfates from a Marine Sponge *Acanthodendrilla* sp.

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Ten new steroidal sulfates, acanthosterol sulfates A−J (**1**−**10**), have been isolated from a marine sponge, *Acanthodendrilla* sp., collected in western Japan. Acanthosterol sulfates I and J (**9** and **10**) showed antifungal activity against the yeast *Saccharomyces cerevisiae* A364A and its mutants at 0.1 mg/disk.

Sulfated metabolites are often found in marine organisms.[1] Steroidal sulfates, an emerging group of sponge metabolites, show a variety of biological activities including antimicrobial,[2] antithrombin,[3] HIV-inhibitory,[4] guanosine diphosphate/G-protein RAS exchange inhibitory,[5] tyrosine kinase inhibitory,[6] and antifouling action.[7] During our new approach to discovery of potential biomedicines from marine invertebrates by a mechanism-based bioassay using genetic-deficient mutants of the budding yeast *Saccharomyces cerevisiae*, we found growth inhibitory activity against the strain A364A[8] and its mutants in the MeOH extract of a marine sponge of the genus *Acanthodendrilla* (family Irciniidae) collected in Gokasho Bay on the Kii Peninsula, 600 km west of Tokyo. Bioassay-monitored isolation afforded 10 new steroidal sulfates, acanthosterol sulfates A−J (**1**−**10**). This paper describes the isolation and structure elucidation of these steroid sulfates.

The frozen sponge (90 g, wet wt) was extracted with MeOH. The concentrated aqueous residue was successively extracted with Et_2O and *n*-BuOH. The active organic extracts were separated by Si gel and ODS column chromatographies followed by reversed-phase HPLC to afford 10 steroidal sulfates, acanthosterol sulfates A (**1**, 0.5 mg), B (**2**, 1.1 mg), C (**3**, 0.7 mg), D (**4**, 0.8 mg), E (**5**, 0.9 mg), F (**6**, 1.0 mg), G (**7**, 1.9 mg), H (**8**, 0.7 mg), I (**9**, 2.1 mg), and J (**10**, 1.2 mg).

Acanthosterol sulfate B (**2**) showed a molecular ion peak at *m*/*z* 553 $(M - Na)^-$ in the FABMS, which matched a formula of $C_{29}H_{45}O_8S$. The ^{1}H NMR spectrum measured in CD_3OD (Table 1) revealed five methyl signals [δ 0.55 (3H, s), 0.90 (3H, s), 0.97 (6H, d, J = 6.6 H), and 1.01 (3H, d, J = 6.6 Hz)], an acetyl methyl group [δ 2.09 (3H, s)], methylene and methine protons (δ 1.2−2.7), three oxymethine signals [δ 3.73 (1H, ddd, J = 11.4, 9.6, 4.8 Hz), 4.33 (1H, t, J = 9.6 Hz), and 4.80 (1H, t, J = 9.6 Hz)], and an olefinic proton at δ 5.26 (1H, br d, J = 4.8 Hz) (Table 1). The steroidal nature of **2** was readily inferred from ^{13}C NMR, COSY, HMQC, and HMBC data (Table 1) and supported by the molecular formula. Oxygenation at C-2, C-3, and C-4 in ring A was straightforward by interpretation of 2D NMR data; a sulfate group could be accommodated on C-4 as judged by the lowfield resonance of H-4 (δ 4.33),[9] whereas the acetyl group could be placed on C-3, which was substantiated by the lowfield resonance of H-3 (δ 4.80) and an HMBC cross peak between δ 4.80 and 173.5. The $\Delta^{7(8)}$ double bond (δ_H 5.26; δ_C 118.7 d and 139.2 s) was consistent with HMBC cross peaks δ 5.26/139.2 (C-8), 50.9 (C-9), and 55.9 (C-14) with optimization for J_{CH} = 12.5 Hz. The relative stereochemistry of 2α-hydroxy, 3β-acetoxy, and 4α-sulfate groups on ring A was deduced by the coupling constants, $J_{2,3} = J_{3,4} = 9.6$ Hz, and by NOESY correlations. The A/B trans junction was confirmed by a

Table 1. ^{1}H and ^{13}C NMR Data for Acanthosterol Sulfate B (**2**) in CD_3OD

	^{1}H		^{13}C	HMBC (J = 8.3 Hz)
1α	1.22 dd	12.6, 11.4	46.1 t	2, 3, 9, 10, 19
β	2.10 dd	12.6, 4.8		2, 5, 10, 19
2	3.73 ddd	11.4, 9.6, 4.8	68.8 d	3
3	4.80 t	9.6	80.8 d	2, 4, CH_3CO
4	4.33 t	9.6	81.8 d	3, 5, 6
5	1.52 m		47.4 d	4, 6, 9, 10, 19
6α	2.38 br d	16.2	26.6 t	
β	1.88 m			
7	5.26 br d	4.8	118.7 d	
8			139.2 s	
9	1.83 m		50.9 d	
10			36.2 s	
11α	1.63 m		22.5 t	
β	1.52 m			9, 10
12α	1.32 m		40.5 t	13
β	2.02 br d	13.2		
13			44.7 t	
14	1.88 m		55.6 d	
15α	1.59 m		24.1 t	
β	1.46 m			13, 14
16α	1.96 m		27.6[a] t	
β	1.59 m			
17	1.48 m		57.3 d	13, 14, 18, 20, 21, 22
18	0.55 s		12.4 q	12, 13, 14, 17
19	0.90 s		15.8 q	1, 5, 9, 10
20	1.29 m		40.0 d	22
21	1.01 d	6.6	16.5 q	17, 20, 22
22	2.52 dd	7.2, 2.4	65.2 d	21
23	2.69 dt	2.4, 5.4	56.8 d	24
24	1.37 m		42.5 t	22, 23, 26, 27
25	1.77 septet	6.6	27.7[a] d	23, 24, 26, 27
26	0.97 d	6.6	23.0[b] q	24, 25, 27
27	0.97 d	6.6	23.3[b] q	24, 25, 26
CH_3CO	2.09 s		21.7 q	CH_3CO
CH_3CO			173.5 s	

[a,b] May be interchangeable. NOE cross-peaks: H-1α/H-3, H-1α/H-5, H-1α/H-9, H-1β/H-19, H-2/H-4, H-2/H-19, H-3/H-5, H-4/H-6β, H-4/H-19, H-5/H-9, H-6β/H-19, H-7/H-15β, H-11β/H-18, H-11β/H-19, H-12α/H-14, H-12β/H-18, H-12β/H-21, H-14/H-17, H-15β/H-18, H-16β/H-18, H-16β/H-20, H-17/H-21, H-18/H-19, H-18/H-20, H-18/H-21, H-20/H-23, H-21/H-22, H-21/H-23, H-22/H-24, H-23/H-26, H-23/H-27, H-24/H-26, and H-24/H-27.

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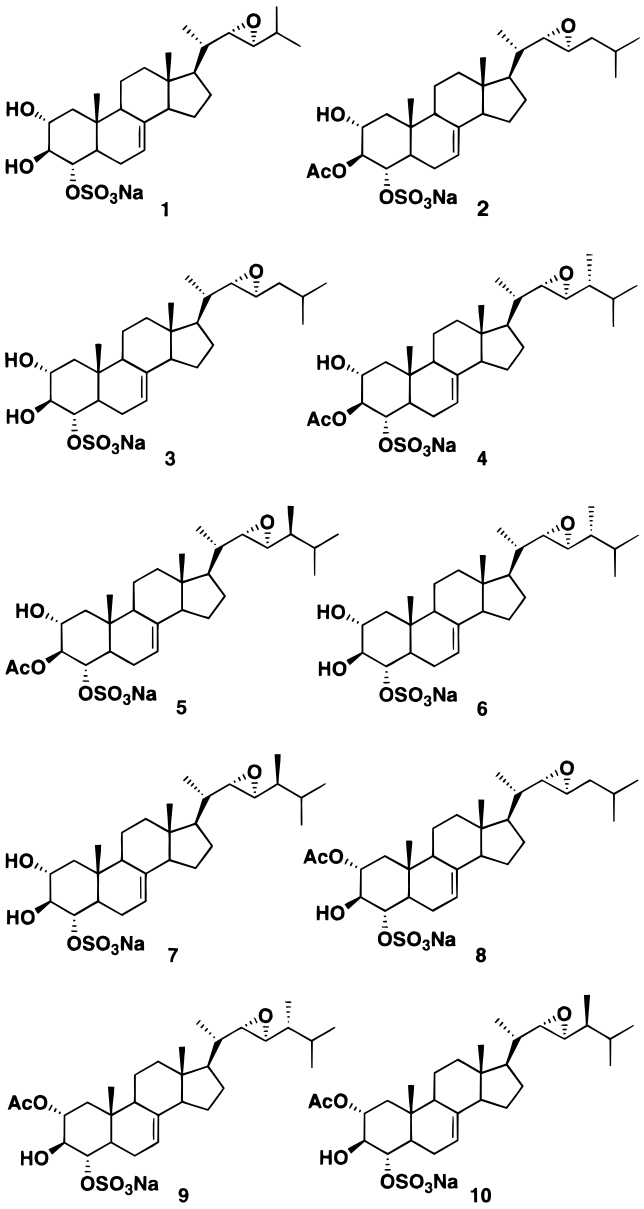

NOESY cross peak, H-3/H-5. The C/D trans junction, 17β-orientation of the side chain, and 20*S*-stereochemistry were disclosed by NOESY cross peaks, H-7/H-15β, H-12α/H-14, H-14/H-17, H-15β/Me-18, H-17/Me-21, and H-16β/H-20 (Figure 1).

The presence of a 22,23-epoxy functionality was implied by NMR signals at δ 2.52 (dd, J = 7.2, 2.4 Hz, H-22)/65.2 (d, C-22) and 2.69 (dt, J = 2.4, 5.4 Hz, H-23)/56.8 (d, C-23), and its trans-geometry was consistent with the coupling constant of J = 2.4 Hz (e.g., J_{cis} = 4.5 Hz).[10] ^{13}C NMR data for the side-chain portion, C-17 (δ 57.3), C-20 (δ 40.0), and C-21 (δ 16.5), were superimposable on those reported for downeyoside C,[9] thereby suggesting 22*S*,23*S*-stereochemistry.[11] Thus, the structure of acanthosterol sulfate A (**2**) was established.

Acanthosterol sulfate H (**8**) has the same molecular formula as **2** and revealed ^{1}H and ^{13}C NMR spectra almost superimposable on those of **2**, except for the downfield shift of H-2 and upfield shift of H-3. The COSY spectrum was consistent with the presence of 2α-acetoxyl and 3β-hydroxyl groups. Thus, the structure of **8** was as shown.

A molecular formula of $C_{27}H_{43}O_7SNa$ was established for acanthosterol sulfate C (**3**) by HRFABMS, a C_2H_2O unit

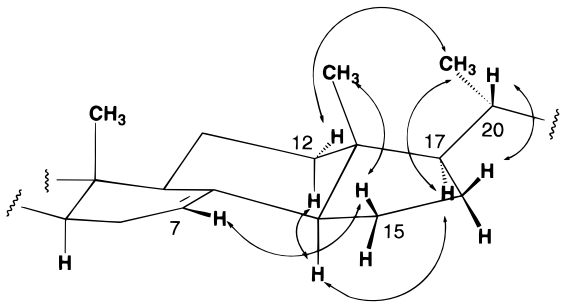

Figure 1. Stereochemistry of acanthosterol sulfate B (**2**) established by NOE.

less than acanthosterol sulfate B (**2**). The ^{1}H NMR spectrum readily revealed that the acetoxy methyl signal was missing and that H-3 was shifted upfield (δ 3.39), thereby suggesting that **3** was deacetylacanthosterol sulfate B (**2**).

Acanthosterol sulfate A (**1**) has a molecular formula of $C_{26}H_{41}O_7SNa$ as established by HRFABMS, one CH_2 unit less than acanthosterol sulfate C (**3**). The COSY spectrum revealed that **1** had the same steroidal nucleus as **3**, although their side chains were different (Table 2). A methine proton at δ 1.40 (1H, sextet, J = 6.6 Hz, H-24) was coupled not only to two doublet methyl signals at δ 0.95 and 1.00 (each 3H, d, J = 6.6 Hz, Me-25 and Me-26) but also to an epoxide methine proton at δ 2.41 (1H, dd, J = 6.6, 2.4 Hz, H-23), thus indicating that a C-24 methylene unit was missing from acanthosterol sulfate B (**2**). The structure of this side chain was identical to that of a saponin isolated from a starfish.[11] ^{13}C NMR signals [δ 57.3 (d, C17), 39.3 (d, C20), 16.5 (q, C21), 64.5 (d, C22), 63.4 (d, C23), 32.0 (d, C24), 18.8 (q, C25), and 19.4 (q, C16)] indicated the same stereochemistry.

Acanthosterol sulfates I (**9**) and J (**10**) possessed identical molecular formulas of $C_{30}H_{47}O_8SNa$. Their NMR data (Table 2) revealed that the two steroids were quite similar to **8**, except for the presence of an additional methyl group at C24 [δ 0.96 (3H, d, J = 6.6 Hz) in **9** and 0.90 (1H, d, J = 6.6 Hz) in **10**], which was also implied by the COSY spectra. The configuration of C22, C23, and C24 for **9** and **10** was established by comparison of NMR data with those of Minale's synthetic models.[12] The C17 signals (δ 57.1 for **9**; 57.2 for **10**) were incompatible with the 22*R*,23*R*-configuration for **9** and **10**; the models having 22*S*,23*S*-configuration showed δ 57.5 for C17.[12] It is known that the stereochemistry of C24 influences the chemical shifts of the 22,23-epoxide methine protons;[12] thus, two well-separated doublets at δ 2.50 (1H, dd, J = 8.4, 2.4 Hz, H-23) and 2.63 (1H, dd, J = 7.2, 2.4 Hz, H-22) of **9** indicated the 24*R*-configuration for **9**, while two partially overlapping doublets at δ 2.50 (1H, dd, J = 7.2, 2.4 Hz, H-22) and 2.51 (1H, dd, J = 7.2, 2.4 Hz, H-23) pointed to the 24*S*-configuration for **10**. Thus, the 22*S*,23*S*,24*R*- and 22*S*,23*S*,24*S*-configurations were established for **9** and **10**, respectively.

Similarly, the structures of the remaining compounds, acanthosterol sulfates D (**4**), E (**5**), F (**6**), and G (**7**), were elucidated on the basis of FABMS and COSY data. Compounds **4** and **5** had the same steroidal nucleus as **2**, while **6** and **7** matched **1**. Acanthosterol sulfates D (**4**) and F (**6**) had the same side chain as **9**; **5** and **7** matched **10**.

Steroidal sulfates have been isolated from marine sponges of the families Halichondriidae, Irciniidae, Adociidae, and Axinellidae.[1] This is the first report of the isolation of steroidal sulfates from sponges of the genus *Acanthodendrilla*.

Table 2. ^{1}H NMR Data for Acanthosterol Sulfates A (1), I (9), and J (10) in CD_3OD

	1		**9**		**10**	
1α	1.13 t	12.6	1.18 t	12.6	1.18 t	12.6
β	2.01 dd	12.6, 3.2	2.07 dd	12.6, 4.2	2.07 dd	12.6, 4.2
2	3.62 ddd	12.6, 9.6, 3.2	4.88 ddd	12.6, 9.6, 4.2	4.88 ddd	12.6, 9.0, 4.2
3	3.39 t	9.6	3.65 t	9.6	3.65 t	9.0
4	4.18 dd	10.2, 9.6	4.24 dd	10.8, 9.6	4.25 dd	9.6, 9.0
5	1.47 m		1.50 m		1.52 m	
6α	2.33 br d	15.6	2.35 br d	13.2	2.36 br d	15.6
β	1.85 m		1.87 m		1.87 m	
7	5.25 br d	4.8	5.26 br d	4.2	5.26 br d	4.2
8						
9	1.86 m		1.87 m		1.87 m	
10						
11α	1.60 m		1.57 m		1.57 m	
β	1.49 m		1.50 m		1.48 m	
12α	1.32 m		1.29 m		1.31 m	
β	2.02 m		2.03 br.d	13.2	2.02 br.d	13.2
13						
14	1.80 m		1.82 m		1.82 m	
15α	1.62 m		1.57 m		1.59 m	
β	1.51 m		1.49 m		1.50 m	
16α	1.96 m		1.97 m		1.96 m	
β	1.60 m		1.62 m		1.61 m	
17	1.47 m		1.47 m		1.46 m	
18	0.55 s		0.55 s		0.55 s	
19	0.88 s		0.94 s		0.94 s	
20	1.26 m		1.30 m		1.26 m	
21	0.99 d	6.6	1.01 d	6.6	1.00 d	6.6
22	2.59 dd	7.2, 2.4	2.63 dd	7.2, 2.4	2.50 dd	7.2, 2.4
23	2.41 dd	6.6, 2.4	2.50 dd	8.4, 2.4	2.51 dd	7.2, 2.4
24	1.40 sextet	6.6	1.02 m		1.04 m	
25	0.95[a] d	6.6	1.67 sextet	6.6	1.71 sextet	6.6
26	1.00[a] d	6.6	0.94[b] d	6.6	0.93[c] d	6.6
27			0.97[b] d	6.6	0.97[c] d	6.6
28			0.96 d	6.6	0.90 d	6.6
AcO			2.04 s		2.04 s	

[a–c] May be interchangeable.

Among 10 acanthosterol sulfates, three types of steroidal nuclei and four types of side chains are represented. Polyhydroxysteroids with a 2α,3*β*,4α-oxygenated system have been isolated from the ophiuroid *Astrotoma agassizii*;[13] however, acanthosterol sulfates B (**2**), D (**4**), E (**5**), H (**8**), I (**9**), and J (**10**) are the first polyhydroxysteroids with an acetoxyl group.

The antifungal activities of acanthosterol sulfates I (**9**) and J (**10**)[14] have been determined using the yeast *Saccharomyces cerevisiae* (Table 3). They inhibited the growth of the parent strain A364A[8] and their mutants, STX338-2C[15] and 14028g,[16] but were inactive against other parent strains, such as GT160-45C[15] and RAY-3Aα.[17] The reason for this selectivity is not known.

Interestingly, acanthosterol sulfate E (**5**) was desulfated during storage at 4 °C in CD_3OD; the half-life was 10 days, with 100% conversion in five months. In the case of acanthosterol sulfate G (**7**), one-fourth was desulfated within five months. No such phenomenon, however, was observed for the other compounds. At present, it is unknown why these compounds were desulfated in MeOH.

Experimental Section

General Experimental Procedures. Optical rotations were determined with a JASCO DIP-1000 digital polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL A600 NMR spectrometer in CD_3OD. MS were measured on a JEOL SX-102 mass spectrometer.

Antifungal Assay Against the Yeast *S. cerevisiae*. The yeast strains A364A, STX338-2C, 14028g, and GT160-45C were obtained from the yeast genetic stock center at the University of California, Berkeley, and the strain RAY-3Aα from Prof. A. Toh-e of the University of Tokyo. Growth-inhibitory activity was determined by the paper disk method.

Table 3. Growth-Inhibitory Activity of Acanthosterol Sulfates I (9) and J (10) against the Strains of the Yeast *S. serevisiae*

	inhibitory zone (mm)[a]				
	A364A	STX338-2C	14028g	GT160-45C	RAY-3Aa
9	7	8	9	inactive	inactive
10	11	10	11	inactive	inactive

[a] Paper disks (*ϕ*6 mm), impregnated with 0.1 mg of each sample, were incubated on agar plates containing yeast at 26.5 °C.

Paper disk (*ϕ*6 mm, Toyo Roshi Kaisha, Ltd., Tokyo) with sample (0.1 mg) was incubated on an agar plate containing yeast at 26.5 °C.

Sponge Sample. The marine sponge was collected by snorkeling at a depth of 2 m in Gokasho Bay on the Kii Peninsula, 600 km west of Tokyo, frozen immediately, and kept frozen until processed. The sponge was identified as *Acanthodendrilla* sp. (family Dictyodendrillidae). It has small fingerlike lobes, diameter 1−3 cm, with a coarsely conulose surface and dark-brown color. Consistency is compressible, spongy. Surface is lightly encrusted by *Haliclona* and other foreign objects. Skeleton has an irregular reticulation of spongin fibers, cored by foreign material (mostly broken sponge spicules), with strongly laminated bark and pith often visible. Interconnecting fibers are mostly lighter cored, occasionally uncored. Diameter of fibers is 40−150 *μ*m. Comparatively tightly meshed, mesh size 200−1000 *μ*m. A voucher specimen (ZMA POR. 13012) was deposited at the Institute for Systematics and Population Biology, University of Amsterdam, The Netherlands.

Isolation. The frozen sponge (90 g, wet wt) was extracted with MeOH. The concentrated aqueous residue was extracted with ether and then *n*-BuOH. The *n*-BuOH fraction (0.41 g) was subjected to Si gel (Wakogel C-200, Wako Pure Chemical Industries, Ltd., Osaka, *ϕ*4 × 7.5 cm) column chromatography with 15% MeOH−$CHCl_3$ to afford a fraction (90.5 mg), which

was combined with the ether layer (0.37 g) and fractionated by Si gel column chromatography (ϕ3.5 × 9.0 cm) with 10 and 20% MeOH−$CHCl_3$. The fraction (114.0 mg) eluted with 20% MeOH−H_2O was purified by ODS column chromatography (YMC-GEL ODS-A 60−400/230, Yamamura Chem. Co., Ltd., Kyoto, ϕ2.5 × 4.5 cm) with MeOH and reversed-phase HPLC (COSMOSIL 5C18-AR−II, ϕ10 × 250 mm, 80% MeOH-1N $NaClO_4$, Nacalai Tesque Inc, Kyoto) followed by desalting with a short ODS column to afford 10 steroidal sulfates, acanthosterol sulfates A (**1**, 0.5 mg, 5.5 × 10^{-4} %, wet wt), B (**2**, 1.1 mg, 1.2 × 10^{-3} %), C (**3**, 0.7 mg, 7.8 × 10^{-4} %), D (**4**, 0.8 mg, 8.9 × 10^{-4} %), E (**5**, 0.9 mg, 1.0 × 10^{-3} %), F (**6**, 1.0 mg, 1.1 × 10^{-3} %), G (**7**, 1.9 mg, 2.1 × 10^{-3} %), H (**8**, 0.7 mg, 7.8 × 10^{-4} %), I (**9**, 2.1 mg, 2.3 × 10^{-3} %), and J (**10**, 1.2 mg, 1.3 × 10^{-3} %), in this elution order.

Acanthosterol sulfate A (1): $[\alpha]^{25}_D$ −18° (*c* 0.038, MeOH); ^{1}H NMR (CD_3OD) in Table 2; ^{13}C NMR (CD_3OD) δ 16.5 (C21), 18.8 (C25), 19.4 (C26), 26.9 (C6), 32.0 (C24), 39.3 (C20), 40.6 (C12), 45.4 (C1), 46.5 (C5), 51.0 (C9), 55.6 (C14), 57.3 (C17), 63.4 (C24), 64.5 (C22), 71.6 (C2), 80.6 (C3), 84.7 (C4), 118.4 (C7); FABMS (negative, TEA) *m/z* 497 (M − Na)$^-$; HRFABMS (negative, PEG sulfate) *m/z* 497.2599 (calcd for $C_{26}H_{41}O_7S$, 497.2573).

Acanthosterol sulfate B (2): $[\alpha]^{25}_D$ +7.1° (*c* 0.085, MeOH); ^{1}H and ^{13}C NMR (CD_3OD) in Table 1; FABMS (negative, TEA) *m/z* 553 (M − Na)$^-$; HRFABMS (negative, PEG sulfate) *m/z* 553.2821 (calcd for $C_{29}H_{45}O_8S$, 553.2835).

Acanthosterol sulfate C (3): $[\alpha]^{25}_D$ +23° (*c* 0.054, MeOH); ^{1}H NMR (CD_3OD) δ 0.55 (3H, s, Me-18), 0.88 (3H, s, Me-19), 0.97 (6H, d, *J* = 6.6 Hz, Me-26 and Me-27), 1.01 (3H, d, *J* = 6.6 Hz, H-21), 1.14 (1H, t, *J* = 12.6 Hz, H-1α), 1.28 (1H, m, H-20), 1.31 (1H, m, H-12α), 1.37 (2H, m, H_2-24), 1.47 (1H, m, H-5), 1.47 (1H, m, H-17), 1.50 (1H, m, H-15*β*), 1.51 (1H, m, H-11*β*), 1.60 (1H, m, H-15α), 1.60 (1H, m, H-16*β*), 1.62 (1H, m, H-11α), 1.76 (1H, septet, *J* = 6.6 Hz, H-25), 1.80 (1H, m, H-9), 1.86 (1H, m, H-6*β*), 1.86 (1H, m, H-14), 1.96 (1H, m, H-16α), 2.01 (1H, dd, *J* = 12.6, 3.2 Hz, H-1*β*), 2.03 (1H, m, H-12*β*), 2.33 (1H, br d, 16.8 Hz, H-6α), 2.52 (1H, dd, *J* = 7.2, 2.4 Hz, H-22), 2.69 (1H, dt, *J* = 2.4, 6.0 Hz, H-23), 3.39 (1H, t, *J* = 9.0 Hz, H-3), 3.62 (1H, ddd, *J* = 12.6, 9.0, 4.3 Hz, H-2), 4.18 (1H, dd, *J* = 9.6, 9.0 Hz, H-4), and 5.25 (1H, br d, *J* = 5.4 Hz, H-7); FABMS (negative, TEA) *m/z* 511 (M − Na)$^-$; HRFABMS (negative, PEG sulfate) *m/z* 511.2718 (calcd for $C_{27}H_{43}O_7S$, 511.2730).

Acanthosterol sulfate D (4): $[\alpha]^{25}_D$ +20° (*c* 0.062, MeOH); ^{1}H NMR (CD_3OD) *δ*: 0.56 (3H, s, Me-18), 0.90 (3H, s, Me-19), 0.94 (3H, d, *J* = 6.6 Hz, Me-26[a]), 0.96 (3H, d, *J* = 6.6 Hz, Me-28), 0.97 (3H, d, *J* = 6.6 Hz, Me-27[a]), 1.02 (1H, d, *J* = 6.6 Hz, H-21), 1.03 (1H, m, H-24), 1.22 (1H, t, *J* = 12.6 Hz, H-1α), 1.30 (1H, m, H-20), 1.32 (1H, m, H-12α), 1.47 (1H, m, H-17), 1.50 (1H, m, H-15*β*), 1.53 (1H, m, H-5), 1.53 (1H, m, H-11*β*), 1.60 (1H, m, H-15α), 1.62 (1H, m, H-16*β*), 1.63 (1H, m, H-11α), 1.67 (1H, sextet *J* = 6.6 Hz, H-25), 1.83 (1H, m, H-9), 1.88 (1H, m, H-14), 1.89 (1H, m, H-6*β*), 1.97 (1H, m, H-16α), 2.03 (1H, br d, *J* = 13.2 Hz, H-12*β*), 2.09 (1H, dd, *J* = 12.6, 4.8 Hz, H-1*β*), 2.09 (3H, s, AcO), 2.38 (1H, br d, *J* = 16.2 Hz, H-6α), 2.50 (1H, dd, *J* = 7.8, 2.4 Hz, H-23), 2.63 (1H, dd, *J* = 7.2, 2.4 Hz, H-22), 3.73 (1H, dddd, *J* = 12.6, 9.6, 4.8 Hz, H-2), 4.33 (1H, dd, *J* = 10.2, 9.6 Hz, H-4), 4.80 (1H, t, *J* = 9.6 Hz, H-3), and 5.26 (1H, br d, *J* = 4.8 Hz, H-7) ([a] May be interchangeable); FABMS (negative, TEA) *m/z* 567 (M − Na)$^-$; HRFABMS (negative, PEG sulfate) *m/z* 567.2966 (calcd for $C_{30}H_{47}O_8S$, 567.2992).

Acanthosterol sulfate E (5): ^{1}H NMR (CD_3OD, MeOH) δ 0.55 (3H, s, Me-18), 0.90 (3H, s, Me-19), 0.90 (3H, d, *J* = 6.6 Hz, Me-28), 0.93 (3H, d, *J* = 6.6 Hz, Me-26[a]), 0.97 (3H, d, *J* = 6.6 Hz, Me-27[a]), 1.00 (3H, d, *J* = 6.6 Hz, H-21), 1.04 (1H, m, H-24), 1.22 (1H, t, *J* = 12.6 Hz, H-1α), 1.25 (1H, m, H-20), 1.32 (1H, dt, *J* = 4.8 and 12.6 Hz, H-12α), 1.47 (1H, m, H-15*β*), 1.47 (1H, m, H-17), 1.51 (1H, m, H-5),1.51 (1H, qd, *J* = 12.0, 4.8 Hz, H-11*β*), 1.59 (1H, m, H-15α), 1.61 (1H, m, H-16*β*), 1.63 (1H, m, H-11α), 1.71 (1H, sextet, *J* = 6.6 Hz, H-25), 1.82 (1H, m, H-9), 1.88 (1H, m, H-14), 1.89 (1H, m, H-6*β*), 1.97 (1H, m, H-16α), 2.03 (1H, br d, *J* = 12.6 Hz, H-12b), 2.09 (1H, dd, *J* = 12.6, 4.8 Hz, H-1*β*), 2.38 (1H, br d, *J* = 16.2 Hz, H-6α), 2.50 (1H, dd, *J* = 6.6, 2.4 Hz, H-22), 2.52 (1H, dd, *J* = 7.2, 2.4 Hz, H-23), 3.73 (1H, ddd, *J* = 12.6, 9.6, 4.8 Hz, H-2), 4.33 (1H, dd, *J* = 10.2, 9.6 Hz, H-4), 4.80 (1H, t, *J* = 9.6 Hz, H-3), and 5.26 (1H, br d, *J* = 3.6 Hz, H-7) ([a] May be interchangeable); FABMS (negative, TEA) *m/z* 567 (M − Na)$^-$; HRFABMS (negative, PEG sulfate) *m/z* 567.2986 (calcd for $C_{30}H_{47}O_8S$, 567.2992). Optical rotation was not measured due to the desulfation of the sample.

Acanthosterol sulfate F (6): $[\alpha]^{25}_D$ −11° (*c* 0.077, MeOH); ^{1}H NMR (CD_3OD) δ 0.55 (3H, s, Me-18), 0.88 (3H, s, Me-19), 0.94 (3H, d, *J* = 6.6 Hz, Me-26[a]), 0.96 (3H, d, *J* = 6.6 Hz, Me-28), 0.97 (3H, d, *J* = 6.6 Hz, Me-27[a]), 1.02 (3H, d, *J* = 6.6 Hz, H-21), 1.03 (1H, m, H-24), 1.13 (1H, t, *J* = 12.6 Hz, H-1α), 1.31 (1H, m, H-12α), 1.31 (1H, m, H-20), 1.46 (1H, m, H-5), 1.46 (1H, m, H-17), 1.48 (1H, m, H-15*β*), 1.51 (1H, m, H-11*β*), 1.59 (1H, m, H-15α), 1.61 (1H, m H-16*β*), 1.63 (1H, 1H, m, H-11α), 1.66 (1H, sextet, *J* = 6.6 Hz, H-25), 1.81 (1H, m, H-9), 1.85 (1H, m, H-6*β*), 1.87 (1H, m, H-14), 1.96 (1H, m, H-16α), 2.01 (1H, dd, *J* = 12.6, 4.2 Hz, H-1*β*), 2.03 (1H, m, H-12*β*), 2.33 (1H, br d, *J* = 16.2 Hz, H-6α), 2.50 (1H, dd, *J* = 7.8, 2.4 Hz, H-23), 2.63 (1H, dd, *J* = 7.8, 2.4 Hz, H-22), 3.39 (1H, t, *J* = 9.6 Hz, H-3), 3.62 (1H, ddd, *J* = 12.6, 9.6, 4.2 Hz, H-2), 4.18 (1H, dd, *J* = 10.2, 9.6 Hz, H-4), and 5.25 (1H, br d, *J* = 4.2 Hz, H-7) ([a] May be interchangeable); FABMS (negative, TEA) *m/z* 525 (M − Na)$^-$; HRFABMS (negative, PEG sulfate) *m/z* 525.2909 (calcd for $C_{28}H_{45}O_7S$, 525.2886).

Acanthosterol sulfate G (7): $[\alpha]^{25}_D$ +4.9° (*c* 0.15, MeOH); ^{1}H NMR (CD_3OD) δ 0.55 (3H, s, Me-18), 0.88 (3H, s, Me-19), 0.90 (3H, d, *J* = 6.6 Hz, Me-28), 0.93 (3H, d, *J* = 6.6 Hz, Me-26[a]), 0.97 (3H, d, *J* = 6.6 Hz, Me-27[a]), 1.00 (3H, d, *J* = 6.6 Hz, Me-21), 1.04 (1H, m, H-24), 1.13 (1H, t, *J* = 12.6 Hz, H-1α), 1.25 (1H, m, H-20), 1.32 (1H, m, H-12α), 1.46 (1H, m, H-17), 1.47 (1H, m, H-5), 1.51 (1H, m, H-11b), 1.51 (1H, m, H-15b), 1.61 (1H, m, H-11α), 1.61 (1H, m, H-16*β*), 1.62 (1H, m, H-15α), 1.71 (1H, sextet, *J* = 6.6 Hz, H-25), 1.80 (1H, m, H-14), 1.85 (1H, m, H-1*β*), 1.88 (1H, m, H-9), 1.96 (1H, m, H-16α), 2.01 (1H, dd, *J* = 12.6, 2.4 Hz, H-1*β*), 2.03 (1H, br d, *J* = 11.4 Hz, H-12*β*), 2.33 (1H, br d, *J* = 18.4 Hz, H-6α), 2.51 (1H, dd, *J* = 7.2, 2.4 Hz, H-22), 2.52 (1H, dd, *J* = 7.2, 2.4 Hz, H-23), 3.39 (1H, t, *J* = 9.0 Hz, H-3), 3.63 (1H, ddd, *J* = 12.6, 9.0, 2.4 Hz, H-2), 4.18 (1H, dd, *J* = 10.8, 9.0 Hz, H-4), and 5.25 (1H, br d, *J* = 4.2 Hz, H-7) ([a] May be interchangeable); FABMS (negative, TEA) *m/z* 525 (M − Na)$^-$; HRFABMS (negative, PEG sulfate) *m/z* 525.2909 (calcd for $C_{28}H_{45}O_7S$, 525.2886).

Acanthosterol sulfate H (8): $[\alpha]^{25}_D$ −6.9° (*c* 0.054, MeOH); ^{1}H NMR (CD_3OD) δ 0.55 (3H, s, Me-18), 0.94 (3H, s, Me-19), 0.97 (6H, d, *J* = 6.6 Hz, Me-26 and Me-27), 1.00 (3H, d, *J* = 6.6 Hz, H-21), 1.18 (1H, t, *J* = 12.6 Hz, H-1α), 1.28 (1H, m, H-20), 1.31 (1H, m, H-12α), 1.38 (2H, m, H_2-24), 1.46 (1H, m, H-17), 1.47 (1H, m, H-15*β*), 1.49 (1H, m, H-5), 1.49 (1H, m, H-11*β*), 1.58 (1H, m, H-15α), 1.61 (1H, m, H-16*β*), 1.62 (1H, m, H-11α), 1.77 (1H, septet, *J* = 6.6 Hz, H-25), 1.81 (1H, m, H-9), 1.87 (1H, m, H-6*β*), 1.87 (1H, m, H-14), 1.96 (1H, m, H-16α), 2.02 (1H, dt, *J* = 12.6, 4.2 Hz, H-12*β*), 2.02 (3H, s, AcO), 2.07 (1H, dd, *J* = 12.6, 4.2 Hz, H-1*β*), 2.35 (1H, br d, *J* = 15.0 Hz, H-6α), 2.52 (1H, dd, *J* = 7.2, 2.4 Hz, H-22), 2.69 (1H, dt, *J* = 2.4, 6.0 Hz, H-23), 3.65 (1H, t, *J* = 9.0 Hz, H-3), 4.24 (1H, dd, *J* = 10.8, 9.0 Hz, H-4), 4.88 (1H, ddd, *J* = 12.6, 9.0, 4.2 Hz, H-2), and 5.26 (1H, br d, *J* = 3.6 Hz, H-7) ([a] May be interchangeable); FABMS (negative, TEA) *m/z* 553 (M − Na)$^-$; HRFABMS (negative, PEG sulfate) *m/z* 553.2821 (calcd for $C_{29}H_{45}O_8S$, 553.2835).

Acanthosterol sulfate I (9): $[\alpha]^{25}_D$ −11° (*c* 0.15, MeOH); ^{1}H NMR (CD_3OD) in Table 2; ^{13}C NMR δ 12.4 (C18), 13.6 (C28), 15.6 (C19), 16.6 (C21), 19.8 (C26), 20.1 (C27), 21.1 (AcO), 26.4 (C6), 32.5 (C25), 40.0 (C20), 40.4 (C12), 42.2 (C1), 43.5 (C24), 46.4 (C5), 50.6 (C9), 55.6 (C14), 57.0 (C17), 61.6 (C23), 65.5 (C22), 74.1 (C2), 77.4 (C3), 84.2 (C4), and 118.4 (C7); FABMS (negative, TEA) *m/z* 567 (M − Na)$^-$; HRFABMS (negative, PEG sulfate) *m/z* 567.2986 (calcd for $C_{30}H_{47}O_8S$, 567.2992).

Acanthosterol sulfate J (10): $[\alpha]^{25}_D$ −25° (*c* 0.092, MeOH); ^{1}H NMR (CD_3OD), in Table 2; ^{13}C NMR δ 12.2 (C18), 13.4 (C28), 15.4 (C19), 16.7 (C21), 19.3 (C26), 20.7 (C27), 21.1 (AcO), 26.4 (C6), 32.6 (C25), 40.0 (C20), 40.4 (C12), 42.2 (C1), 43.3

(C24), 46.3 (C5), 50.6 (C9), 55.6 (C14), 57.2 (C17), 61.4 (C23), 64.2 (C22), 74.2 (C2), 78.1 (C3), 84.2 (C4), 118.7 (C7); FABMS (negative, TEA) *m*/*z* 567 $(M - Na)^-$; HRFABMS (negative, PEG sulfate) *m*/*z* 567.3016 (calcd for $C_{30}H_{47}O_8S$567.2992).

Desulfated derivative of acanthosterol sulfate E (11): $[\alpha]^{25}_D$ +14° (*c* 0.069, MeOH); ^{1}H NMR (CD_3OD) δ 0.55 (3H, s, Me-18), 0.86 (3H, s, Me-19), 0.90 (3H, d, J = 6.6 Hz, Me-28), 0.93 (3H, d, J = 6.6 Hz, Me-26[a]), 0.97 (3H, d, J = 6.6 Hz, Me-27[a]), 1.01 (3H, d, J = 6.6 Hz, Me-21), 1.04 (1H, m, H-24), 1.22 (1H, t, J = 12.6 Hz, H-1α), 1.25 (1H, m, H-20), 1.32 (1H, dt, J = 4.8, 12.6 Hz, H-12α), 1.39 (1H, dt, J = 4.8, 10.8 Hz, H-5), 1.47 (1H, m, H-15β), 1.47 (1H, m, H-17), 1.51 (1H, qd, J = 12.0, 4.8 Hz, H-11β), 1.59 (1H, m, H-15α), 1.61 (1H, m, H-16β), 1.63 (1H, m, H-11α), 1.71 (1H, sextet, J = 6.6 Hz, H-25), 1.73 (1H, m, H-6β), 1.82 (1H, m, H-9), 1.88 (1H, m, H-14), 1.97 (1H, m, H-16α), 2.03 (1H, br d, J = 12.6 Hz, H-12β), 2.08 (1H, dd, J = 12.6, 4.8 Hz, H-1β), 2.10 (3H, s, AcO), 2.32 (1H, br d, J = 16.2 Hz, H-6α), 2.50 (1H, dd, J = 6.6, 2.4 Hz, H-22), 2.52 (1H, dd, J = 7.2, 2.4 Hz, H-23), 3.39 (1H, dd, J = 10.8, 9.6 Hz, H-4), 3.66 (1H, ddd, J = 12.6, 9.0, 4.8 Hz, H-2), 4.67 (1H, t, 9.6 Hz, H-3), and 5.26 (1H, br d, J = 4.2 Hz, H-7) ([a] May be interchangeable); FABMS (positive, glycerol) *m*/*z* 489 $(M + H)^+$; HRFABMS (positive, PEG) *m*/*z* 489.3590 (calcd for $C_{30}H_{49}O_5$, 289.3580).

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