Isolation of Two 13-*epi*-Neoverrucosane-Type Diterpenoids from a Marine Sponge *Axinyssa tethyoides*

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New 13-*epi*-neoverrucosane-type diterpenes **1** and **2** were isolated from the marine sponge *Axinyssa tethyoides*. Their structures were elucidated by 2D-NMR spectroscopy, the modified Mosher's method, and fluorescent X-ray analysis. **1** and **2** are the first 13-*epi*-neoverrucosane-type diterpenes that possess a sulfate group.

A number of 13-*epi*-neoverrucosane-type diterpenoids with a fused 3,6,6,5-tetracyclic carbon skeleton have been known as secondary metabolites of some species of terrestrial plants.[1,2] Recently, some verrucosane-type diterpenoides were discovered from marine sponges *Axinyssa aplysinoides*[3] and *Epipolasis kushimotoensis*.[4] In this paper, we report the isolation and characterization of two new 13-*epi*-neoverrucosane-type diterpenoides, **1** and **2**, which possess a sulfate group.

A sample (1.2 kg) of the marine sponge *Axinyssa tethyoides*[5] was extracted with methanol. The methanol extract was partitioned between H_2O and EtOAc, and the EtOAc extract was partitioned between 90% aqueous methanol and hexane. The 90% aqueous methanol layers were concentrated and separated by column chromatography (SiO_2 and ODS) to give **1** (4.3 mg) and **2** (8.9 mg).

The fluorescent X-ray analysis of **1** suggested the presence of sulfur atom (K$\alpha = 2.3$ keV). The molecular formula of **1** was determined to be $C_{20}H_{33}NaO_5S$ by HRESIMS (m/z 385.2033 $[M - Na]^-$, Δ 1.6 mmu).[6] The NMR data for **1** are summarized in Table 1. The ^1H and ^{13}C NMR spectra indicated the presence of an oxymethine group, three tertiary methyl groups, an isopropyl group, and a cyclopropane ring. The signal patterns of the ^1H NMR spectrum were very similar to those of 13-*epi*-neoverrucosan-5β-ol (**3**),[7] except for the presence of the oxygen-functional group at C-11. Considering the molecular formula and chemical shifts at H-5 and H-11 (3.98 and 4.27 ppm), we identified **1** as a 13-*epi*-neoverrucosane-type diterpenoid possessing a sulfate moiety at C-11. This assumption was confirmed by the ^1H–^1H COSY, HMQC, and HMBC spectra showing the connectivity of each carbon, as shown in Fig. 1. The location and stereochemistry of the sulfate moiety at C-11 were confirmed by the NOESY spectrum

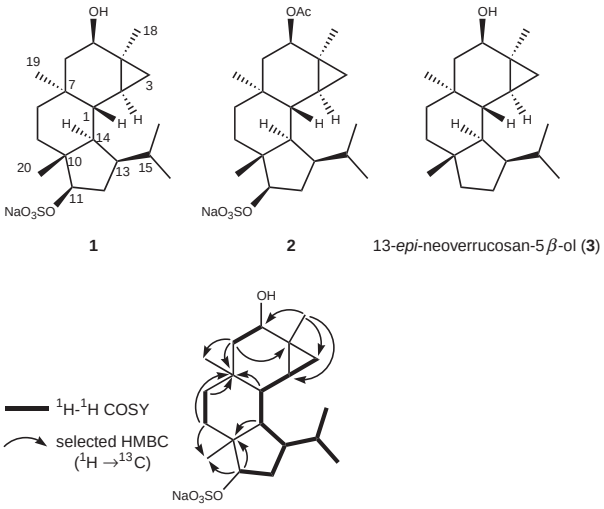

Fig. 1.

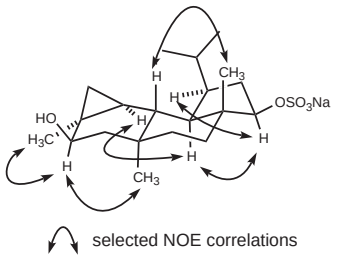

Fig. 2. Relative stereochemistry of **1**.

of **1**, in which the NOEs were observed at H-11/H-13 and H-11/H-14 (Fig. 2). For determination of the absolute stereochemistry, **1** was transformed into the corresponding MTPA esters.[8] Analysis of the chemical shifts for the (*S*)- and (*R*)-esters showed negative $\Delta\delta_{S-R}$ values for H-2, H-3, and H-18 and positive values for H-6, H-8, and H-19, indicating that the absolute configuration of the C-5 chiral center was *R*. Therefore, the chiral centers at C-1, C-2, C-7, C-10, C-11, C-13, and C-14 were assigned as *S*, *S*, *R*, *S*, *R*, *S*, and *R* on the basis of their relative configurations as determined by the NOESY spectrum (Fig. 2).

The molecular formula of **2** was determined to be $C_{22}H_{35}NaO_6S$ by HRESIMS (m/z 427.2141 $[M - Na]^-$, Δ 1.3 mmu).[6] A comparison of the ^1H and ^{13}C NMR data of **2** with those of **1** suggests that **2** possesses the same carbon framework with an acetyl group at the 5-hydroxy group (Table 1). This was confirmed by the fact that the acetylation of **1** with acetic anhydride gave **2**.

In summary, **1** and **2** were isolated from the marine sponge *Axinyssa tethyoides*. Their structures were determined to be 13-*epi*-neoverrucosane-type diterpenes by their 2D-NMR and fluorescent X-ray analysis results, and their absolute stereochemistry was determined by the modified Mosher's method. **1** and **2** are the first 13-*epi*-neoverrucosane-type diterpenes that possess a sulfate moiety.

Experimental

General Procedures. Thin-layer chromatography was carried out with glass TLC plates precoated with Merck silica-gel 60

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Table 1. NMR Spectral Data of **1** and **2** in CD_3OD

C No.	^{13}C/ppm[a)]	^{1}H/ppm[b)] mult. (J/Hz)	HMBC	^{13}C/ppm[a)]	^{1}H/ppm[b)] mult. (J/Hz)	HMBC
	Compound 1			**Compound 2**		
1	44.9	1.37 dd (4.4, 13.1)	C2, 3, 7, 10, 14, 19	44.8	1.44 dd (4.4, 13.1)	C2, 3, 7, 10, 14, 19
2	26.3	0.71 ddd (4.4, 4.5, 8.1)	C3, 4, 14	26.4	0.76 ddd (4.4, 4.5, 8.1)	C3, 14
3	20.1	0.41 t (4.5)	C1, 2, 4, 5, 18	21.1	0.53 t (4.5)	C1, 2, 4, 5, 18
		0.58 dd (4.5, 8.1)	C1, 2, 4, 5, 18		0.68 dd (4.5, 8.1)	C1, 2, 4, 5, 18
4	22.1			19.7[c)]		
5	70.5	3.98 dd (7.4, 10.8)	C4, 6, 8	74.9	5.27 dd (7.6, 10.6)	C3, 4, 6, 8, 21
6	46.4	0.76 dd (10.8, 12.2)	C4, 5, 7, 19	42.4	0.81 dd (10.6, 12.6)	C5, 7
		1.60 dd (7.4, 12.2)	C4, 5, 7		1.72 dd (7.6, 12.6)	C4, 5, 7
7	37.1			37.2		
8	34.1[c)]	1.28 td (13.5, 3.8)	C1, 7, 9, 19	34.1[c)]	1.29 td (14.0, 3.8)	C7
		1.60 td (3.8, 13.5)	C19		1.61 td (3.8, 14.0)	C10, 19
9	34.4[c)]	1.08 td (3.8, 13.5)	C7, 10	34.2[c)]	1.09 td (3.8, 14.0)	C10, 14
		1.50 td (13.5, 3.8)	C11, 20		1.51 td (14.0, 3.8)	
10	44.0			44.0		
11	86.7	4.27 dd (8.0, 9.3)	C9, 10, 12, 20	86.7	4.29 dd (8.2, 9.0)	C9, 10, 12, 20
12	31.4	1.65 m	C11, 13	31.4	1.65 ddd (6.8, 9.0, 13.4)	C11, 13, 15
		2.27 m	C11, 13		2.28 m	C10, 11
13	42.0	2.12 m	C12, 16, 17	41.9	2.14 m	C16
14	46.2	1.72 dd (9.7, 13.1)	C7, 10, 13, 15, 20	46.2	1.75 dd (9.7, 13.0)	C1, 11, 13
15	29.3	2.22 m	C13, 16, 17	29.3	2.24 m	C16, 17
16	20.1	0.91 d (6.6)	C13, 15, 17	20.1	0.91 d (6.6)	C13, 15, 17
17	23.1	0.94 d (6.8)	C13, 15, 16	23.1	0.94 d (6.7)	C13, 15, 16
18	24.8	1.18 s	C2, 3, 4, 5	24.5	1.12 s	C2, 3, 4, 5
19	15.9	0.82 s	C1, 7, 8	15.9	0.90 s	C1, 6, 7
20	13.8	0.90 s	C9, 10, 11, 14	13.8	0.91 s	C10, 11, 14
21				171.8		
22				19.8[c)]	2.02 s	C21

a) Recorded at 100 MHz. b) Recorded at 400 MHz. c) Interchangeable.

F254. Column chromatography was accomplished with Fuji Silysia Chemical silica-gel BW820MH. Proton nuclear magnetic resonance spectra were recorded in CD_3OD at 400 MHz. High-resolution mass spectra were obtained with a Q-star spectrometer (Applied Biosystems) on an ESI mode. Energy-dispersive X-ray fluorescence analyses were effected with a Seiko instruments SEA2010.

Isolation and Purification. A sample (1.2 kg) of the sponge *Axinyssa tethyoides* was collected at Bise in Okinawa, and extracted with methanol for a week. The extract (3.5 g) was diluted with water (500 mL), and partitioned with EtOAc (500 mL × 3). The material (0.9 g) obtained from the EtOAc layers was partitioned between 90% aqueous methanol (200 mL) and hexane (200 mL × 3). The material (150 mg) obtained from the 90% aqueous methanol layers was separated by silica-gel column chromatography using $CHCl_3$:MeOH (1:0/40:1/20:1/10:1/5:1/0:1) and purified with reversed-phase column chromatography using ODS (60% MeOH) to give **1** (4.3 mg) and **2** (8.9 mg).

5β-Hydroxy-13-*epi*-11β-neoverrucosanyl Sulfate (1): $[\alpha]^{22}_{D}$ +18.4 (*c* 0.19, CH_3OH). IR (thin film) 3362, 2926, 1724, 1245, 1222, 1001 cm^{-1}. ^{1}H NMR and ^{13}C NMR see Table 1. HRMS (ESI) Exact mass calcd for $C_{20}H_{33}NaO_5S$ $[M - Na]^-$ requires m/z 385.2049. Found m/z 385.2033.

5β-Acetoxy-13-*epi*-11β-neoverrucosanyl Sulfate (2): $[\alpha]^{22}_{D}$ +14.7 (*c* 0.23, CH_3OH). IR (thin film) 3393, 2955, 1730, 1244, 1222, 1000 cm^{-1}. ^{1}H NMR and ^{13}C NMR see Table 1. HRMS (ESI) Exact mass calcd for $C_{22}H_{35}NaO_6S$ $[M - Na]^-$ requires m/z 427.2154. Found m/z 427.2141.

Preparation of (*S*)- and (*R*)-MTPA Ester Derivatives of 1. To a solution of **1** (0.5 mg, 1.3 μmol) in pyridine (0.5 mL) was added (*R*)-MTPA chloride (26 μmol). The mixture was stirred at room temperature for 2 h under N_2 and then concentrated in vacuum. The residue was purified by silica-gel column chromatography eluting with $CHCl_3$/MeOH (5:1) to give the (*S*)-ester **4** (0.7 mg, quant) as a colorless oil. On the other hand, treatment of **1** (0.5 mg, 1.3 μmol) with (*S*)-MTPA chloride furnished the (*R*)-ester **5** (0.7 mg, quant) as a colorless oil.

(*S*)-MTPA Ester 4: IR (thin film) 3445, 2952, 1740, 1245, 1232, 1001 cm^{-1}. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.51 (m, 2H), 7.43 (m, 3H), 5.54 (dd, $J = 7.7$, 10.8 Hz, 1H), 4.29 (dd, $J = 8.0$, 9.2 Hz, 1H), 3.53 (s, 3H), 2.27 (m, 1H), 2.15 (m, 1H), 2.14 (m, 1H), 1.87 (dd, $J = 7.7$, 12.4 Hz, 1H), 1.76 (dd, $J = 9.5$, 13.0 Hz, 1H), 1.67 (ddd, $J = 6.8$, 9.0, 13.4 Hz, 1H), 1.61 (dt, $J = 4.0$, 13.2 Hz, 1H), 1.51 (td, $J = 13.2$, 4.0 Hz, 1H), 1.45 (dd, $J = 4.6$, 13.0 Hz, 1H), 1.33 (td, $J = 13.2$, 4.0 Hz, 1H), 1.14 (dt, $J = 13.2$, 4.0 Hz, 1H), 1.11 (s, 3H), 0.95 (dd, $J = 10.8$, 12.4 Hz, 1H), 0.95 (s, 3H), 0.93 (s, 3H), 0.92 (d, $J = 6.5$ Hz, 3H), 0.89 (d, $J = 6.6$ Hz, 3H), 0.81 (ddd, $J = 4.6$, 4.8, 8.1 Hz, 1H), 0.62 (dd, $J = 4.8$, 8.1 Hz, 1H), 0.45 (t, $J = 4.8$ Hz, 1H). HRMS (ESI) Exact mass calcd for $C_{30}H_{40}F_3NaO_7S$ $[M - Na]^-$ requires m/z 601.2447. Found m/z 601.2441.

(*R*)-MTPA Ester 5: IR (thin film) 3445, 2924, 1741, 1246, 1232, 1002 cm^{-1}. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.54 (m, 2H), 7.41 (m, 3H), 5.57 (dd, $J = 7.8$, 10.5 Hz, 1H), 4.28 (dd, $J = 8.1$, 9.2 Hz, 1H), 3.56 (s, 3H), 2.27 (m, 1H), 2.17 (m, 1H), 2.15 (m, 1H), 1.77 (dd, $J = 7.7$, 12.6 Hz, 1H), 1.76 (dd,

$J = 9.3$, 13.0 Hz, 1H), 1.67 (ddd, $J = 6.8$, 9.0, 13.4 Hz, 1H), 1.61 (dt, $J = 3.7$, 13.2 Hz, 1H), 1.51 (td, $J = 13.2$, 3.7 Hz, 1H), 1.43 (dd, $J = 4.5$, 13.0 Hz, 1H), 1.28 (td, $J = 13.2$, 3.7 Hz, 1H), 1.12 (dt, $J = 13.2$, 3.7 Hz, 1H), 1.21 (s, 3H), 0.93 (s, 3H), 0.93 (d, $J = 6.5$ Hz, 3H), 0.89 (d, $J = 6.6$ Hz, 3H), 0.89 (s, 3H), 0.84 (m, 1H), 0.80 (dd, $J = 10.5$, 12.6 Hz, 1H), 0.74 (dd, $J = 4.7$, 8.2 Hz, 1H), 0.51 (t, $J = 4.7$ Hz, 1H). HRMS (ESI) Exact mass calcd for $C_{30}H_{40}F_3NaO_7S$ $[M - Na]^-$ requires m/z 601.2447. Found m/z 601.2458.

Acetylation of 1. **1** (1.1 mg, 2.8 μmol) was treated with acetic anhydride (0.2 mL) in pyridine (0.5 mL) for 1 h at room temperature and concentrated in vacuum. The residue was purified by silica-gel column chromatography with $CHCl_3$/MeOH (5:1) to give **2** (0.9 mg, 74%) as a colorless oil.

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