Notes

Halichonadin E, a Dimeric Sesquiterpenoid from the Sponge *Halichondria* sp.[⊥]

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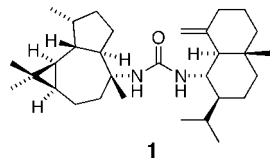
A new dimeric sesquiterpenoid with eudesmane and aromadendrane skeletons linked through a urea fragment, halichonadin E (**1**), was isolated from a marine sponge *Halichondria* sp., and the gross structure and relative configuration of **1** were elucidated on the basis of spectroscopic data. Halichonadin E (**1**) is the first hetero-dimeric sesquiterpenoid with eudesmane and aromadendrane skeletons linked through a urea fragment.

A number of sesquiterpene isothiocyanates, isonitriles, and formamides have been isolated from marine sponges of the genus *Halichondria*,[1] and these compounds are thought to have a role in maintaining ecological systems, such as an allomon in the browser−prey relationship.[2,3] During a search for structurally unique metabolites from Okinawan marine sponges,[4,5] a new dimeric sesquiterpenoid, halichonadin E (**1**), was isolated from a sponge, *Halichondria* sp. Herein, we describe the isolation and structure elucidation of **1**.



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The sponge *Halichondria* sp. (SS-1163) collected off Unten Port, Okinawa, was extracted with MeOH, and the extract was partitioned between EtOAc and water. The EtOAc-soluble materials were subjected to passage over a silica gel column (*n*-hexane/EtOAc, 95:5) followed by C_{18} HPLC (MeOH/H_2O, 95:5) to afford halichonadin E (**1**) (**1**, 0.0001%, wet wt) together with four known related terpenoids, halichonadins A and C[5] and acanthenes B and C.[6] The molecular formula, $C_{31}H_{52}N_2O$, of halichonadin E (**1**) was established by HREIMS [*m/z* 468.4066 (M^+), Δ −1.3 mmu]. IR (1638 cm^{-1}) and ^{13}C NMR (δ_C 157.1) data suggested the presence of a urea functionality. The gross structure of **1** was deduced from detailed analysis of the ^1H and ^{13}C NMR data (Table 1) aided by 2D NMR experiments ($^1H-^1H$ COSY, HOHAHA, HMQC, and HMBC). The ^{13}C NMR data indicated that **1** possesses two sp^2 and three sp^3 quaternary carbons, nine sp^3 methines, one sp^2 and nine sp^3 methylenes, and seven methyl groups. Among them, one sp^3 methine (δ_C 47.4; δ_H 3.65) and one sp^3 quaternary carbon (δ_C 58.1) were ascribed to those bearing a nitrogen atom, while one sp^2 quaternary carbon (δ_C 157.1) was attributed to that bearing an oxygen atom. The $^1H-^1H$ COSY and HOHAHA spectra revealed connectivities of C-1 to C-3, C-5 to C-9, and C-11 to C-12 and C-13 (Figure 1). HMBC correlations of H_3-15 to C-1, C-5, C-9, and C-10, H_3-12 to C-7, H-14a to C-3 and C-5, and H_2-2 to C-4 revealed the presence of a eudesmane skeleton (Figure 1). Connectivities of C-1′ to C-6′, C-1′ to C-5′, C-4′ to C-14′, and C-7′ to C-9′ were deduced from $^1H-^1H$ COSY and HOHAHA correlations (Figure 1). HMBC correlations of H-1′ to C-10′, H_3-15′ to C-9′ and C-10′, H-6′ to C-12′, H-7′ to C-13′, H_3-12′ and H_3-13′ to C-11′, and H-8′a to C-6′ suggested the presence of an aromadendrane skeleton (Figure 1). NOESY correlations of H-6 to H_3-15′ and H-14a to H-3′a in **1** indicated the connection of the eudesmane and aromadendrane skeletons though a urea linkage.

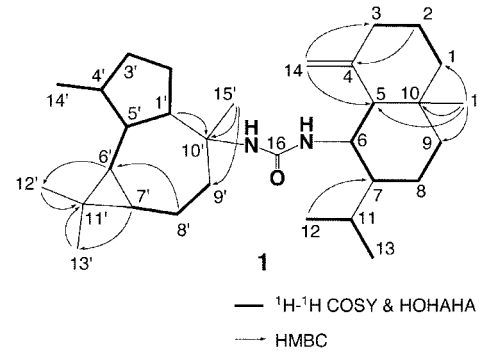

Figure 1. Selected 2D NMR correlations for halichonadin E (**1**).

Thus, the gross structure of halichonadin E was elucidated to be **1**, consisting of eudesmane and aromadendrane skeletons linked through a urea fragment.

α-Orientations of H-5 and H-7 and β-orientations of H-6 and Me-15 of **1** in the eudesmane skeleton and chair conformations of two cyclohexane rings in the decalin skeleton were elucidated by NOESY correlations of H_3-15 to H_2-2, H-6, and H-8b and of H-5 to H-3b and H-7. NOESY correlations of H-1′ to H-6′, H-9′a, and H_3-14′, H-5′ to H_3-12′, H-7′ to H-9′a, H-8′a to H_3-13′, and H-8′b to H_3-15′ in the aromadendrane skeleton indicated α-orientations of H-1′, Me-14′, H-6′, and H-7′ and β-orientations of H-5′ and Me-15′ (Figure 2). Furthermore, ^1H and ^{13}C NMR data of **1** were similar to those of halichonadin C[5] and axisonitrile-2,[7] which have the same relative stereochemistry as that of **1** and have been isolated from *Halichondria* sp. and *Axinella cannabina*, respectively. The relative stereochemistry and conformation of the two ring systems through a urea linkage were elucidated by NOESY correlations of H-6 to H_3-15′ and H-14a to H-3′a, as depicted in Figure 2.

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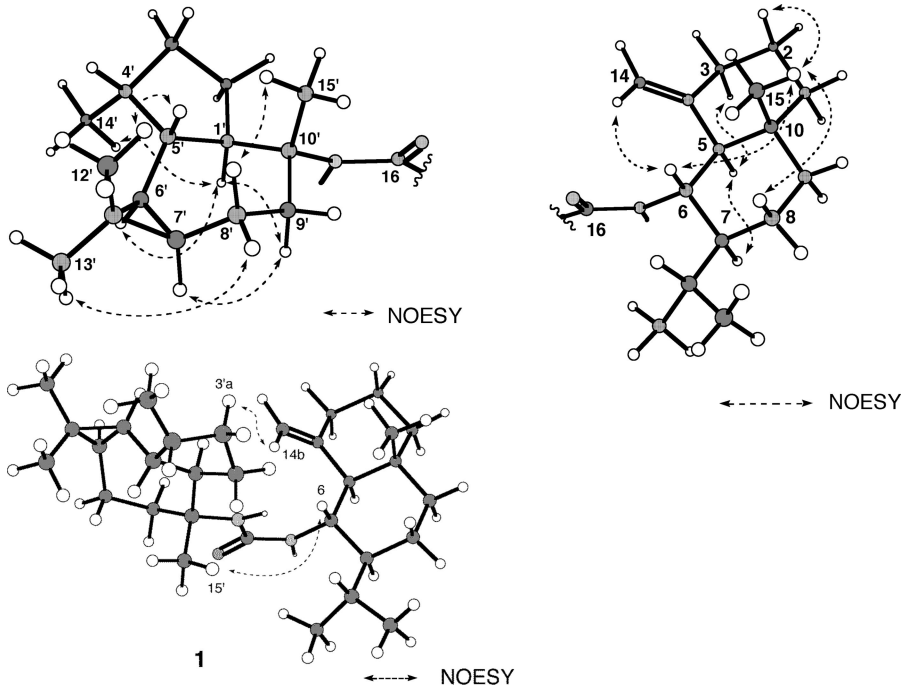

Figure 2. Selected NOESY correlations for halichonadin E (**1**) (lower) and for aromadendrane and eudesmane moieties (upper right and left, respectively) of **1**.

Table 1. ^{1}H and ^{13}C NMR Data of Halichonadin E (**1**) in $CDCl_3$

position	^{1}H[a]	^{13}C[a]	H coupled with C[b]
1a	1.41 (m)	42.3	
1b	1.23 (m)		
2	1.59 (m)	24.3	4, 10
3a	2.28 (brd, 12.0)	38.6	
3b	1.88 (m)		
4		146.2	
5	1.65 (m)	57.8	
6	3.65(m)	47.4	
7	1.39 (m)	37.8	
8a	1.47 (m)	18.8	7
8b	1.32 (m)		
9a	1.49 (m)	40.6	
9b	1.17 (m)		
10		29.7	
11	1.97 (m)	26.4	
12	0.90 (d, 6.6)	21.8	7
13	0.89 (d, 7.2)	16.6	
14a	4.84 (brs)	108.2	3, 5
14b	4.68 (brs)		
15	0.75 (s)	17.3	1, 5, 9, 10
16		157.1	
1′	2.49 (m)	53.6	5′, 6′, 10′
2′a	1.27 (m)	26.8	
2′b	1.22 (m)		5′
3′a	1.64 (m)	34.5	
3′b	1.20 (m)		
4′	1.98 (m)	36.6	
5′	1.34 (m)	38.7	4′, 6′, 10′
6′	0.53 (t, 10.8)	28.7	12′
7′	0.63 (dt, 6.0, 10.8)	26.9	13′
8′a	1.74 (m)	20.2	6′, 9′, 10′
8′b	0.95 (m)		
9′a	2.10 (m)	40.3	
9′b	1.89 (m)		
10′		58.1	
11′		19.8	
12′	0.99 (s)	28.7	
13′	0.95 (s)	16.2	
14′	0.91 (m)	15.9	4′, 5′
15′	1.11 (s)	18.9	9′, 10′

[a] δ in ppm. [b] HMBC correlations.

Halichonadin E (**1**) is the first hetero-dimeric sesquiterpenoid with eudesmane and aromadendrane skeletons linked through a urea fragment isolated from a sponge (*Halichondria* sp.), although some homo-dimeric sesquiterpenoids such as halichonadin A[5] and *N,N*′-bis[(1*Z*,4*Z*)-7α*H*-germacra-1(10),4-dienyl]urea[8] have been isolated from sponges of the genera *Halichondria* and *Axinyssa*, respectively. Halichonadin E (**1**) showed cytotoxicity against L1210 murine leukemia (IC_{50}, 3.0 μg/mL) and KB human epidermoid carcinoma cells (IC_{50}, 2.6 μg/mL) *in vitro*.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. The IR spectrum was taken on a JASCO FT/IR-5300 IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 NMR spectrometer. EI mass spectra were obtained on a DX-303 mass spectrometer.

Animal Material. The sponge *Halichondria* sp. (order Halichondrida, family Halichondriidae) was collected off Unten Port, Okinawa, Japan, in May 2006, and kept frozen until used. The sponge was a dense compact mound or thickly encrusted, and the surface was smooth and faintly hispid. The sponge skeleton consisted of irregular tracts of spicules in a haphazard arrangement, rare fiber development at nodes, and occasional ascending tracts, which were 80 μm wide. The sponge spicules were long, slender oxeas with long, tapering pencil points, in a large size range, 580 × 10 μm.

A voucher specimen (SS-1163) was deposited at the Graduate School of Pharmaceutical Sciences, Hokkaido University.

Extraction and Isolation. The sponge (0.55 kg, wet weight) was extracted with methanol (1 L × 3). The methanolic extract (24.5 g) was partitioned between water (300 mL) and EtOAc (300 mL × 3). The EtOAc-soluble materials (2.9 g) were subjected to passage over a silica gel column (*n*-hexane/EtOAc, 95:5) followed by C_{18} HPLC (YMC-Pack Pro C18, YMC Co., Ltd., 1.0 × 250 mm; flow rate 2.5 mL/min; MeOH/H_2O, 95:5; UV detection at 220 nm) to afford halichonadin E (**1**, t_R 21.3 min, 0.7 mg, 0.0001%, wet wt).

Halichonadin E (1): colorless, amorphous solid; $[\alpha]^{24}_D$ +7.1 (*c* 0.10, MeOH); IR (NaCl) ν_{max} 3323 and 1638 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); EIMS (positive) *m/z* 468 (M^+); HREIMS *m/z* 468.4066 (M^+), calcd for $C_{31}H_{52}N_2O$, 468.4079.

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Supporting Information Available: A photograph of *Halichondria* sp. is provided free of charge via the Internet at http://pubs.acs.org.

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