Halichonadin F and the Cu(I) Complex of Halichonadin C from the Sponge *Halichondria* sp.

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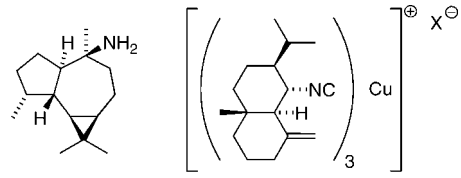
A new sesquiterpenoid with an aromadendrane skeleton, halichonadin F (**1**), and the Cu(I) complex of haliconadin C (**2**) were isolated from a marine sponge *Halichondria* sp., and the structures and relative stereochemistries of **1** and the complex of **2** were elucidated on the basis of spectroscopic data and chemical methods.

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, for example, an allomone in the browser−prey relationship.[2,3] During a search for structurally unique metabolites from Okinawan marine sponges,[4,5] a new sesquiterpenoid with the aromadendrane skeleton, halichonadin F (**1**), and the Cu(I) complex of halichonadin C (**2**) were isolated from a sponge *Halichondria* sp. In this paper, we describe the isolation and structure elucidation of **1** and **2**.



1 **2**

The sponge *Halichondria* sp. (SS-1163) collected off Unten Port, Okinawa, was extracted with MeOH, and the MeOH extract was partitioned between EtOAc and water. The EtOAc-soluble materials were subjected to passage over a silica gel column (*n*-hexane/EtOAc, 1:1 → MeOH). The fraction eluted with MeOH was separated on a silica gel column ($CHCl_3$/MeOH, 95:5 → 7:3), and then the fraction eluted with $CHCl_3$/MeOH (7:3) was separated on a silica gel column (EtOAc/MeOH, 5:1 → MeOH) to yield halichonadine F (**1**, 0.7 mg, 0.0001% wet wt) and the Cu(I) complex of halichonadin C (**2**, 2.6 mg, 0.0004%) together with six known related terpenoids, halichonadins A and C,[5] acanthenes B, C,[6] and E,[7] and epipolasin B.[8a]

The molecular formula, $C_{15}H_{27}N$, of **1** was established by HREIMS [*m*/*z* 221.2144 (M^+), Δ 0.1 mmu]. IR (3285 cm^{-1}) data suggested the presence of an amino and/or hydroxyl functionality. The gross structure of **1** was deduced from detailed analysis of the ^1H and ^{13}C NMR data (Table 1) aided with 2D NMR experiments ($^1H-^1H$ COSY, HOHAHA, HMQC, and HMBC). The ^{13}C NMR data indicated that **1** possessed two sp^3 quaternary carbons, five sp^3 methines, four sp^3 methylenes, and four methyl groups. Among them, one sp^3 quaternary carbon (δ_C 60.1) was ascribed to that bearing a nitrogen atom. The $^1H-^1H$ COSY and HOHAHA spectra revealed connectivities of C-1 to C-5 and C-9 and C-4 to C-14 (Figure 1). HMBC correlations of H-5 to C-10, H_3-15 to C-9 and C-10, H_3-12 to C-6 and C-13, and H_3-13 to C-1 suggested the presence of an aromadendrane skeleton (Figure 1). Thus, the gross structure of halichonadin F was elucidated to be **1** with an aromadendrane skeleton.

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

position	$\delta_H{}^a$	$\delta_C{}^a$	H coupled with C[b]
1	2.20 (m)	54.2	6
2a	1.88 (m)	27.1	
2b	1.88 (m)		
3a	1.53 (m)	34.6	
3b	1.73 (m)		
4	1.28 (m)	36.1	
5	2.01 (m)	39.2	10
6	1.37 (m)	28.5	7
7	0.58 (t, 10.2)	26.4	
8a	0.65 (m)	19.4	
8b	1.84 (m)		
9a	2.09 (m)	40.2	
9b	1.82 (m)		
10		60.1	
11		20.3	
12	1.00 (s)	28.5	6, 13
13	0.98 (s)	15.8	11
14	0.92 (d, 5.4)	16.0	3
15	1.30 (s)	16.9	9, 10

[a] δ in ppm. [b] HMBC correlations.

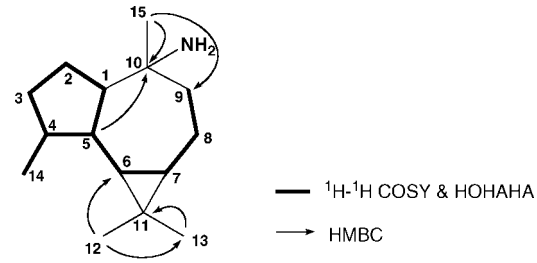


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Figure 1. Selected 2D NMR correlations for halichonadin F (**1**).

ROESY correlations of H-1 to H-6, H-2 to H-4 and H_3-15, and H-4 to H-5 in the aromadendrane skeleton indicated α-orientations of H-1, H-6, H-7, Me-14, and Me-15 and β-orientations of H-4 and H-5 (Figure 2).

To confirm the relative stereochemistry of halichonadin F (**1**), a diastereomer (**3**) of **1** was prepared from commercially available (+)-aromadendrene (**4**).[9] Treatment of **4** with thiocyanic acid (HSCN) in $CHCl_3$ provided the enantiomer (**5**) of epipolasin B,[8b] which was hydrolyzed by NaOH in glycol to give compound **3** (Scheme 1). The ^1H NMR resonances of **3** were not identical with those of **1**. Acetylation of **1** and **3** provided the acetoamides **6** and **7**, respectively (Scheme 2). The ^1H NMR resonance for Me-15 in

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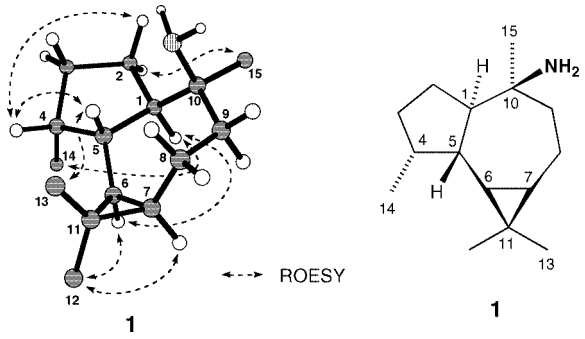
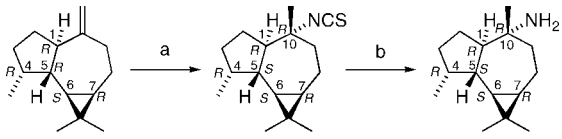


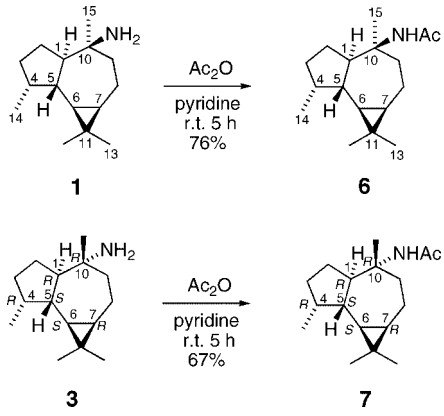
Figure 2. Selected ROESY correlations and relative stereochemistry for halichonadin F (**1**).

Scheme 1. Synthesis of the C-10 Epimer (**3**) of Halichonadin F (**1**) from (+)-Aromadendrene (**4**)[a]



[a] Reagents and conditions: (a) HSCN, $CHCl_3$, rt, 2 days (74%); (b) NaOH, glycol, 165 °C, 3 h (6%).

Scheme 2. Acetylation of Halichonadin F (**1**) and Compound **3**

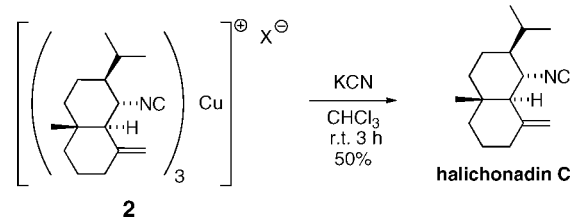


6 was δ 1.20, while that in **7** was δ 1.41, indicating that the stereochemistry of C-10 in **1** was opposite of that in **3**.

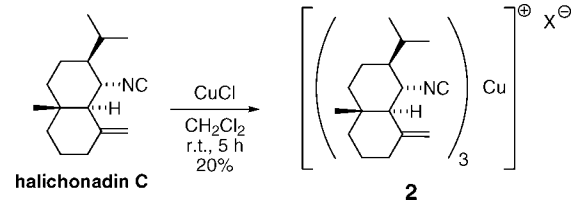
The Cu(I) complex of halichonadin C (**2**) showed a molecular ion peak at *m*/*z* 756 (M^+) in the ESIMS. HRESIMS analysis of **2** revealed the molecular formula to be $C_{48}H_{75}CuN_3$ [*m*/*z* 756. 5254 (M^+), Δ −0.3 mmu]. IR (2182 cm^{-1}) and ^{13}C NMR (δ_C 155.3 and 54.9) data suggested the presence of an isonitrile functionality. The ^1H and ^{13}C NMR data of **2** were similar to those of halichonadin C.[5] The molecular formula $C_{48}H_{75}CuN_3$ and the ^1H and ^{13}C NMR data of **2** suggested that the structure of **2** was the Cu(I)-coordinated trimer of halichonadin C. To confirm the complex structure of **2**, compound **2** was treated with KCN to give a Cu(I)-free compound (Scheme 3), whose ^1H NMR data were identical with those of halichonadin C. Furthermore, natural halichonadin C was treated with CuCl in CH_2Cl_2 to yield a Cu(I)-coordinated complex (Scheme 4),[10] whose ^1H NMR data were identical with those of the isolated Cu(I)-coordinated trimer of halichonadin C (**2**). Therefore, the structure of compound **2** was concluded to be a complex of Cu(I) coordinated to three molecules of halichonadin C, possessing a eudesmane skeleton with an isonitrile group at C-6.

In conclusion, the structures and relative stereochemistries of halichonadin F (**1**) and the Cu(I) complex of halichonadin C (**2**) were elucidated on the basis of spectroscopic data and chemical

Scheme 3. Generation of Halichonadin C from the Cu(I) Complex of Halichonadin C (**2**)



Scheme 4. Formation of the Cu(I) Complex of Halichonadin C (**2**) from Halichonadin C



methods. Halichonadin F (**1**) is a new aromadendrane sesquiterpenoid having an amino group, although aromadendrane sesquiterpenoids with a thioisocyanate group,[8a] an isonitrile group,[12] and an *N*-formylamide[13] group, and a dimeric sesquiterpenoid with eudesmane and aromadendrane skeletons linked through a urea fragment[7] have been isolated from marine sponges *Epipolasis kushimotoensis*, *Axinella cannabina*, or *Halichondria* sp. Halichonadin F (**1**) and the Cu(I) complex of halichonadin C (**2**) showed antimicrobial activity against *Micrococcus luteus* (MIC, 4 μg/mL, both), *Trichophyton mentagrophytes* (MIC, 16 and 8 μg/mL, respectively), and *Cryptococcus neoformans* (MIC, 16 μg/mL, both).

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR spectra were taken on a JASCO FT/IR-5300 IR spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 NMR spectrometer and a JEOL ECA500 NMR spectorometer. The 7.26 and 77.0 ppm resonances of residual $CDCl_3$ were used as internal references for ^1H and ^{13}C NMR spectra, respectively. EI and HREI mass spectra were obtained on a DX-303 mass spectrometer. ESI and HRESI mass spectra were obtained on a JEOL JMS-SX102A spectrometer.

Animal Material. 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 wt) was extracted with MeOH (1 L × 3). The methanolic extract (24.5 g) was partitioned between H_2O (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, 1:1 → MeOH) to yield the Cu(I) complex of halichonadin C (**2**, 2.6 mg, 0.0004%, wet wt). The fraction eluted with MeOH was separated on a silica gel column ($CHCl_3$/MeOH, 95:5 → 7:3), and then the fraction eluted with $CHCl_3$/MeOH (7:3) was separated on a silica gel column (EtOAc/MeOH, 5:1 → MeOH) to yield halichonadin F (**1**, 0.7 mg, 0.0001%).

Halichonadin F (1): colorless, amorphous solid; $[\alpha]^{24}_D$ +19 (*c* 0.10, MeOH); IR (NaCl) ν_{max} 3285 and 2925 cm^{-1}; ^1H and ^{13}C NMR (Table 1); EIMS *m*/*z* 221 $[M]^+$; HREIMS *m*/*z* 221.2144 (calcd for $C_{15}H_{27}N$, 221.2143).

Cu(I) complex of halichonadin C (2): colorless solid; mp 170−172 °C; $[\alpha]^{25}_D$ −6 (*c* 1.0, $CHCl_3$); IR (NaCl) ν_{max} 3270, and 1740 cm^{-1}; ^1H and ^{13}C NMR (Tables 1 and 2); ESIMS *m*/*z* 756 $[H]^+$; HRESIMS *m*/*z* 756.5254 (calcd for $C_{48}H_{75}CuN_3$, 756.5257).

Diastereomer (3) of Halichonadin F (1). To (+)-aromadendrene **4** (Wako Pure Chemical Co., Ltd., 182.4 mg, 0.893 mmol) was added a HSCN chloroform solution[8b] (2 mL), and the reaction mixture was stirred at room temperature for 2 days. The reaction mixture was filtered and concentrated in vacuo. Purification of the residue by column chromatography on silica gel (pentane) gave **5** (174.9 mg) as a colorless oil. Isothiocyanate **5** (59.2 mg, 0.225 mmol) was treated with NaOH

Table 2. ^{1}H and ^{13}C NMR Data of the Cu(I) Complex of Halichonadin C (**2**) in $CDCl_3$

position	δ_H[a]	δ_C[a]	H coupled with C[b]
1a	1.45 (m)	41.5	
1b	1.28 (m)		
2a	1.64 (m)	23.5	
2b	1.59 (m)		
3a	2.34 (m)	37.5	
3b	1.96 (m)		
4		144.9	
5	2.07 (m)	55	
6	3.50 (t, 10.8)	54.9	
7	1.56 (m)	48.2	
8	1.54 (m)	18.1	
9a	1.54 (m)	39.3	
9b	1.22 (m)		
10		37.2	
11	2.08 (m)	27.4	
12	0.98 (d, 7.2)	20.9	7, 11, 13
13	0.86 (d, 7.2)	15.7	7, 11, 12
14a	4.95 (s)	107.8	3, 5
14b	4.53 (s)		5
15	0.68 (s)	16.6	1, 5, 9, 10
16		152.7	

[a] δ in ppm. [b] HMBC correlations.

(16 mg) in glycol (0.6 mL) at 165 °C for 3 h. The reaction mixture was diluted with saturated aqueous K_2CO_3 (1 mL), extracted with $CHCl_3$ (3 × 0.5 mL), dried with K_2CO_3, and concentrated in vacuo. Purification with silica gel column chromatography ($CHCl_3$/MeOH, 4:1 → 0:1) provided **3** (3.1 mg, 0.014 mmol) in 6% yield as a colorless, amorphous solid: $[\alpha]^{20}_D$ −16 (*c* 0.10, MeOH); IR (NaCl) ν_{max} 3340 and 2925 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.51 (1H, dd, J = 10.9, 9.2 Hz), 0.57 (1H, m), 0.91 (1H, br s), 0.99 (6H, s), 1.00 (3H, s), 1.21−1.32 (4H, m), 1.44 (1H, m), 1.59−1.74 (5H, m), 1.99 (1H, m); EIMS *m*/*z* 221 $[M]^+$; HREIMS *m*/*z* 221.2129 (calcd for $C_{15}H_{27}N$, 221.2143).

Acetylation of Halichonadin F (1). To a solution of halichonadin F (**1**; 0.1 mg, 0.45 μmol) in pyridine (0.1 mL) was added Ac_2O (0.1 mL), and the mixture was reacted at room temperature for 5 h. The mixture was concentrated and purified with silica gel column chromatography (*n*-hexane/EtOAc, 1:1 → 0:1) to provide **6** (0.09 mg, 0.34 μmol) in 76% yield.

6: colorless, amorphous solid; $[\alpha]^{23}_D$ −88 (*c* 0.04, $CHCl_3$); IR (NaCl) ν_{max} 3300, 2925 cm^{-1};^{1}H NMR δ 0.54 (1H, t, J = 10.8 Hz, H-6), 0.62 (1H, m, H-7), 0.92 (3H, d, J = 7.2 Hz, H-14), 0.98 (3H, s, H-13), 1.00(3H, s, H-12), 1.01 (1H, m, H-8), 1.20 (3H, s, H-15), 1.26 (1H, m, H-9), 1.32 (2H, m, H-3), 1.40 (1H, m, H-5), 1.66 (2H, m, H-2), 1.76 (1H, dt, J = 14.4, 6.0 Hz, H-8), 1.90 (3H, s, NAc), 2.00 (1H, m, H-4), 2.06 (1H, dd, J = 13.2, 6.0 Hz, H-9), 2.45 (1H, q, J = 9.0 Hz, H-1); EIMS *m*/*z* 263 $[M]^+$; HREIMS *m*/*z* 263.2230 (calcd for $C_{17}H_{29}ON$, 263.2249).

Acetylation of Compound 3. To a solution of compound **3** (0.1 mg, 4.5 μmol) in pyridine (0.1 mL) was added Ac_2O (0.1 mL), and the mixture was reacted at room temperature for 5 h. The mixture was concentrated and purified with silica gel column chromatography (hexane/EtOAc, 1:1 → 0:1) to provide **7** (0.08 mg, 3.0 μmol) in 67% yield. **7**: colorless, amorphous solid; $[\alpha]^{21}_D$ +17 (*c* 0.04, $CHCl_3$); ^{1}H NMR δ 0.50 (1H, t, J = 10.2 Hz, H-6), 0.59 (1H, m, H-7), 0.94 (3H, d, J = 7.2 Hz, H-14), 1.00 (3H, s, H-13), 1.01 (3H, s, H-12), 1.05 (1H, m, H-8), 1.20 (1H, m, H-9), 1.26 (2H, m, H-2), 1.41 (3H, s, H-15), 1.51 (1H, q, J = 10.0 Hz, H-5), 1.68 (2H, m, H-3), 1.69 (1H, m, H-8), 1.78 (1H, m, H-1), 1.96 (3H, s, NAc), 2.01 (1H, m, H-4), 2.80 (1H, dd, J = 14.1, 6.9 Hz, H-9); EIMS *m*/*z* 263 $[M]^+$; HREIMS *m*/*z* 263.2266 (calcd for $C_{17}H_{29}ON$, 263.2249).

Generation of Halichonadin C from the Cu(I) Complex of Halichonadin C (2). The Cu(I) complex of halichonadin C (**2**) (0.1 mg, 1.3 μmol) was treated with KCN (1.0 mg, 154 μmol) in MeOH (50 μL) at room temperature for 3 h, and the reaction mixture was filtered and concentrated in vacuo. Purification of the residue by column chromatography on silica gel (*n*-hexane/EtOAc, 9:1) gave halichonadin C (0.05 mg, 50%): $[\alpha]^{24}_D$ −72 (*c* 0.025, $CHCl_3$). The ^{1}H NMR spectrum of the generated halichonadin C was identical with that of natural halichonadin C; ESIMS *m*/*z* 231 $[M]^+$; HRESIMS *m*/*z* 231.1987 (calcd for $C_{16}H_{25}N$, 231.1987).

Formation of the Cu(I) Complex of Halichonadin C (2) from Halichonadin C. To a CH_2Cl_2 (1 mL) solution of natural halichonadin C (36.8 mg, 0.159 mmol) was added CuCl (5.0 mg, 0.051 mmol), and the reaction mixture was stirred at room temperature for 5 h. The reaction mixture was filtered and concentrated in vacuo. Purification of the residue by column chromatography on silica gel (EtOAc/MeOH, 5:1) gave compound **2** (23.4 mg, 0.031 mmol, 20%): $[\alpha]^{23}_D$ +60 (*c* 0.64, MeOH). The ^{1}H NMR spectrum was identical with that of the natural Cu(I) complex of halichonadin C (**2**); ESIMS *m*/*z* 756 $[M]^+$; HRESIMS *m*/*z* 756.5248 (calcd for $C_{48}H_{75}CuN_3$, 756.5257).

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(10) Treatment of natural halichonadin C with CuI gave a trace of the Cu(I)-coordinated halichonadin C (**2**), although CuI rather than CuCl has been used for formation of a Cu(I)-coordinated trimer of *p*-tolyl isocyanide.[11]
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