HETEROCYCLES, Vol. 90, No. 1, 2015, pp. 173 - 185. © 2015 The Japan Institute of Heterocyclic Chemistry
Received, 24th January, 2014, Accepted, 24th February, 2014, Published online, 4th March, 2014
DOI: 10.3987/COM-14-S(K)2

HALICHONADINS M–Q, SESQUITERPENES FROM AN OKINAWAN MARINE SPONGE *HALICHONDRIA* SP.

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Abstract – Four new dimeric sesquiterpenes, halichonadins M–P (**1**–**4**), and one new sesquiterpene, halichonadin Q (**5**), were isolated from an Okinawan marine sponge *Halichondria* sp. The sesquiterpenes have eudesmane skeleton in common. Halichonadin M (**1**) is a symmetrical dimer linked to a nitrilotriacetic acid fragment through amide bonds. Halichonadin N (**2**) is a structurally unique dimeric sesquiterpene connected via a pyrrolidine unit, while halichonadins O (**3**) and P (**4**) have linker moieties consisting of a piperidine unit. Halichonadin Q (**5**) is a sesquiterpene possessing a pyrrolidine unit. The structures of **1**–**5** were elucidated by spectroscopic analysis. Halichonadin O (**3**) showed antimicrobial activity against *Staphylococcus aureus*, *Micrococcus luteus*, and *Trichophyton mentagrophytes*.

INTRODUCTION

Marine sponges have been recognized as a rich source of interesting bioactive metabolites with fascinating chemical structures.[1] Among them, sponges belonging to the genus *Halichondria* are known to contain sesquiterpenes with various functionalities (e.g. isothiocyanate, isonitrile, and formamide).[2] These sesquiterpenes are thought to have a role in maintaining ecological systems, such as an allomon in the browser-prey relationship.[3] During our search for structurally unique metabolites from marine organisms, we have recently reported the isolation of dimeric sesquiterpenes connected through a variety of linker moieties, halichonadins G–I, K, and L, from the extracts of an Okinawan marine sponge *Halichondria* sp. (NSS-2).[4,5] Further investigation of the extracts resulted in the isolation of five new

sesquiterpenes, halichonadins M–Q (**1**–**5**). In this paper, we describe the isolation and structure elucidation of **1**–**5**.

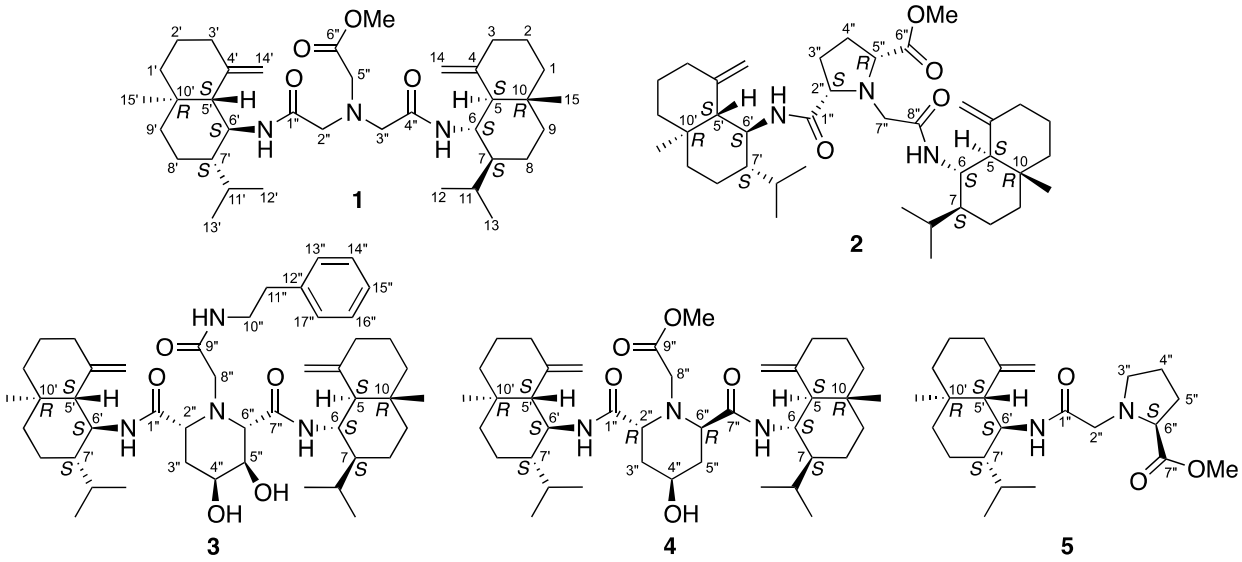


Chart 1. Structures of halichonadins M–Q (**1**–**5**)

RESULTS AND DISCUSSION

The sponge *Halichondria* sp. (NSS-2, 1.0 kg wet weight) collected at Unten Port, Okinawa, was extracted with MeOH, and the extract was partitioned between $CHCl_3$ and water. The $CHCl_3$-soluble materials were subjected to a silica gel column and a C_{18} column to give fractions containing sesquiterpenes. The fractions were purified using C_{18} HPLC to afford halichonadins M (**1**, 0.00007%, wet weight), N (**2**, 0.00005%), O (**3**, 0.00018%), P (**4**, 0.00003%), and Q (**5**, 0.00005%). In the purification process, five know sesquiterpenes, halichonadins A, C (**6**), and E and acanthenes B and C were isolated and identified by comparison of their physicochemical data with the reported data.[6-8]

Halichonadin M (**1**) was isolated as an optically active colorless amorphous solid {$[\alpha]^{20}_D$ –27.4 (*c* 0.24, MeOH)}. The HRESIMS revealed the molecular formula of **1** to be $C_{37}H_{61}N_3O_4$ (*m/z* 634.45630 $[M+Na]^+$, Δ+0.87 mmu). The ^{13}C NMR spectrum (Table 1) displayed 20 signals due to two carbonyl groups, two nitrogen bearing sp^3 methylenes, one methoxy group, and one sesquiterpene moiety. Since only 20 of 37 resonances were observed in the ^{13}C NMR spectrum, **1** was deduced to be a symmetrical dimeric sesquiterpene possessing a linker moiety (unit C). The structure including the relative configurations of the sesquiterpene moieties (units A and B) of **1** were elucidated to be identical to those of halichonadin K,[5] a dimer of eudesmane sesquiterpene, by resemblance of the 1D NMR data. In the HMBC spectrum of **1** (Figure 1), correlations for H_2-5" to C-6" and C-3" (C-2"), H_2-3" (H_2-2") to C-4" (C-1") were observed, indicating unit C (C-1"–C-6") to be a nitrilotriacetic acid. The presence of a methoxy group at C-6" was revealed by an HMBC correlation for 6"-OMe to C-6", while the connectivity of C-6 (C-6') to C-4"

(C-1") through an amide bond(s) was disclosed by an HMBC cross-peak of 6-NH (6'-NH) to C-4" (C-1").

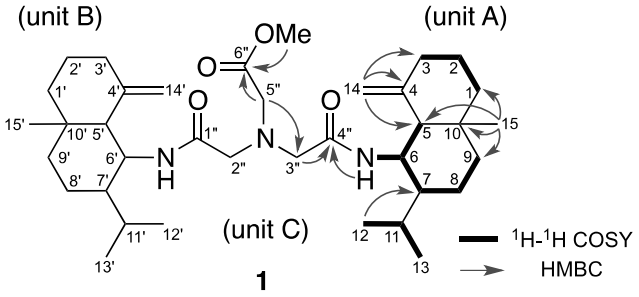
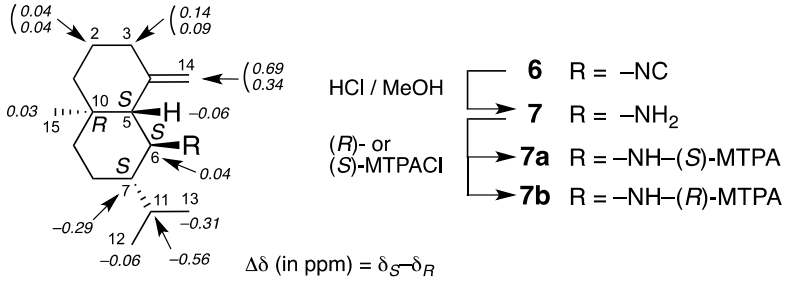


Figure 1. Selected 2D NMR correlations for halichonadin M (**1**)

Halichonadin M (**1**) was optically active, indicating that the absolute configurations of the sesquiterpene moieties (units A and B) in **1** were identical. If unit B was an enantiomer of unit A, **1** should be a *meso*-compound. In the case of halichonadin K, whose absolute stereochemistry was assigned by X-ray analysis,[5] the absolute configurations of the sesquiterpene moieties were the same. These facts might suggest that the absolute configuration of eudesmane sesquiterpenes contained in the sponge NSS-2 is identical. To confirm the assumption, a stereochemical analysis of halichonadin C (**6**),[6] a known eudesmane sesquiterpene with an isonitrile group at C-6, was carried out as follows. Halichonadin C (**6**) was treated with 5% HCl in MeOH to give an amine, halichonadin D (**7**)[6] (Scheme 1). Treatment of **7** with (*R*)-(–)- and (*S*)-(+)-2-methoxy-2-(trifluoromethyl)phenylacetyl chloride (MTPACl) gave the 6-(*S*)- and 6-(*R*)-MTPA amides (**7a** and **7b**, respectively). The Δδ values ($\Delta\delta=\delta_S-\delta_R$) obtained from the ^{1}H NMR spectra for **7a** and **7b** in C_5D_5N were shown in Scheme 1. Though an irregular distribution for H-5 (Δδ –0.06) was found,[9] the distributions of Δδ values might indicate the absolute configurations of C-5, C-6, C-7, and C-10 to be *S*, *S*, *S*, and *R*, respectively. The deduced absolute stereochemistry of **6** was identical to that of the sesquiterpene moieties of halichonadin K.[5] From these facts, eudesmane sesquiterpenes included in NSS-2 were deduced to have the 5*S*, 6*S*, 7*S*, and 10*R* configurations. Consequently, the absolute stereochemistry of halichonadin M (**1**) was concluded as shown in Chart 1.



Scheme 1. Derivatization of halichonadin C (**6**) to halichonadin D (**7**) and (*S*)- and (*R*)-MTPA amides (**7a** and **7b**, respectively) of **7**. Δδ values obtained for **7a** and **7b** in C_5D_5N are shown

Table 1. ^{1}H and ^{13}C NMR data for halichonadin M (**1**) in C_5D_5N

position	δ_C	δ_H (*J* in Hz)
1, 1’	42.2	1.30, 1.11 (1H each, m)
2, 2’	24.5	1.50 (2H, m)
3, 3’	38.6	2.23 (1H, brd, 12.2), 1.86 (1H, td, 12.2, 5.6)
4, 4’	147.8	–
5, 5’	56.5	2.00 (1H, brd, 9.5)
6, 6’	47.0	4.26 (1H, q, 9.5)
7, 7’	50.2	1.32 (1H, m)
8, 8’	18.9	1.39, 1.32 (1H each, m)
9, 9’	40.6	1.39, 1.11 (1H each, m)
10, 10’	37.7	–
11, 11’	27.1	2.14 (1H, m)
12, 12’	21.8	0.88 (3H, d, 6.8)
13, 13’	16.7	1.09 (3H, d, 6.8)
14, 14’	107.4	5.08, 4.92 (1H each, brd)
15, 15’	17.3	0.74 (3H, s)
1”, 4”	170.6	–
2”, 3”	58.6	3.77, 3.69 (1H each, d, 14.7)
5”	55.3	3.79, 3.69 (1H each, d, 16.2)
6”	172.1	–
6-NH, 6’-NH		8.29 (1H, brd, 9.5)
OMe	51.4	3.50 (3H, s)

Halichonadin N (**2**) was obtained as an optically active colorless amorphous solid {$[\alpha]^{20}_D$ −21.1 (*c* 0.13, MeOH)}. The molecular formula, $C_{39}H_{63}N_3O_4$, was established by the HRAPCIMS (*m/z* 638.48927 $[M+H]^+$, Δ+0.14 mmu). The ^{1}H and ^{13}C NMR spectra (Table 2) showed the resonances of three carbonyl groups, one methoxy group, two sp^3 methines, and three sp^3 methylenes, as well as a couple of the signals due to sesquiterpene moieties (units A and B). From these data, **2** was presumed to be a dimer of sesquiterpene with a linker moiety. The structures of units A and B of **2** including the relative configurations were deduced to be the same as those of **1** by resemblance of the ^{1}H and ^{13}C NMR data. The gross structure of the linker moiety {unit C (C-1”–C-8”)} and the connectivities among units A–C were assigned as follows. The ^{1}H-^{1}H COSY spectrum of **2** indicated the connectivites of C-2” to C-5” (Figure 2). HMBC correlations for protons of a nitrogen bearing sp^3 methylene (H_2-7”) to C-2” and C-5” suggested the existence of a pyrrolidine ring (C-2”–C-5” and N-2”) and the connectivity of N-2” to C-7”. The connectivities of C-7” to one amide carbonyl carbon (C-8”) and of C-2” to the other amide carbonyl carbon (C-1”) were assigned by HMBC cross-peaks of H_2-7” to C-8” and H_2-3” to C-1”. In addition, HMBC correlations for H-6 to C-8” and H-6’ to C-1” indicated that C-6 and C-6’ were connected to C-8” and C-1”, respectively, through amide linkages. These connectivities were supported by NOESY correlations for 6-NH/H_2-7” and 6’-NH/H-2”. The presence of a methoxycarbonyl group at C-5” was deduced by an HMBC cross-peak of 6”-OMe to C-6”, taking the molecular formula of **2** into consideration. Thus, the gross structure of **2** was assigned as shown in Figure 2.

Table 2. ^{1}H and ^{13}C NMR data for halichonadin N (**2**) in C_5D_5N

position	δ_C	δ_H (*J* in Hz)	position	δ_C	δ_H (*J* in Hz)
1	42.3[a]	1.30, 1.07 (1H each, m)	7’	50.5	1.23 (1H, m)
2	24.6[b]	1.46 (2H, m)	8’	18.9	1.36, 1.27 (1H each, m)
3	38.7	2.21 (1H, m), 1.77 (1H, td, 12.3, 5.4)	9’	40.6	1.38, 1.15 (1H each, m)
4	147.8	–	10’	37.6	–
5	56.6	1.86 (1H, brd, 10.3)	11’	27.0	2.02 (1H, m)
6	46.5	4.23 (1H, q, 10.3)	12’	21.6	0.78 (3H, d, 6.9)
7	50.4	1.23 (1H, m)	13’	16.7	0.98 (3H, d, 6.9)
8	18.9	1.36, 1.27 (1H each, m)	14’	106.9	4.96, 4.83 (1H each, brs)
9	40.6	1.38, 1.03 (1H each, m)	15’	17.3[c]	0.74 (3H, s)
10	37.6	–	1”	173.3	–
11	27.1	2.13 (1H, m)	2”	69.0	4.01 (1H, dd, 8.5, 3.9)
12	21.8	0.91 (3H, d, 7.0)	3”	31.2	2.46, 2.14 (1H each, m)
13	16.7	1.08 (3H, d, 7.0)	4”	31.0	2.20, 1.96 (1H each, m)
14	107.6	5.08, 4.93 (1H each, brs)	5”	66.3	4.39 (1H, t, 7.5)
15	17.5[c]	0.72 (3H, s)	6”	176.5	–
1’	42.2[a]	1.30, 1.07 (1H each, m)	7”	55.5	3.82, 3.73 (1H each, d, 16.3)
2’	24.5[b]	1.52 (2H, m)	8”	170.4	–
3’	38.5	2.25 (1H, brd, 13.1), 2.02 (1H, m)	NH-6		8.09 (1H, brd, 10.3)
4’	147.3	–	NH-6’		8.14 (1H, brd, 10.4)
5’	56.8	2.04 (1H, m)	OMe	52.1	3.66 (3H, s)
6’	46.5	4.13 (1H, q, 10.4)			

[a-c] Signals may be interchangeable.

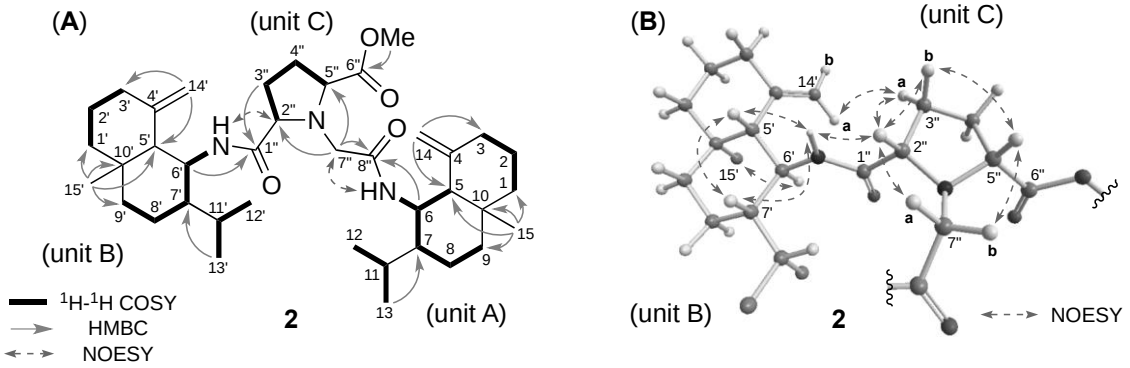


Figure 2. (**A**) Selected 2D NMR correlations and (**B**) relative stereochemistry for unit B (C-1’–C-15’) and unit C (C-1”–C-8”) of halichonadin N (**2**)

In unit C (C-1”–C-8”) of **2**, the following NOESY correlations were observed: H-2”/H-7”a, H-2”/H-3”b, H-2”/H-3”a (weak), H-5”/H-7”b, and H-5”/H-3”b (Figure 2). These observations implied the *syn* relationship for H-2”/H-5”. The relative relationship between units B and C was assigned as shown in Figure 2 by NOESY cross-peaks of H-5’/6’-NH, H-7’/6’-NH, H-2”/6’-NH, and H-14’a/H-3”a. Though the relative relationship between units A and C could not been assigned by NOESY analysis, the absolute configurations of units A and B in **2** were deduced to be identical as those in **1**. Therefore, the absolute stereochemistry of halichonadin N (**2**) was concluded as shown in Chart 1.

Halichonadin O (**3**) was obtained as an optically active colorless amorphous solid {$[\alpha]^{20}_{D}$ −34.2 (*c* 0.24, MeOH)}. The molecular formula, $C_{47}H_{72}N_4O_5$, was established by the HRAPCIMS (*m/z* 773.55762 $[M+H]^+$, Δ+0.07 mmu). The ^{1}H and ^{13}C NMR spectra of **3** (Table 3) were reminiscent of halichonadin L,[5]

a dimer of eudesmane sesquiterpene with a linker unit consisting of a piperidine and phenethylamide moieties. Resemblance of the ^{1}H and ^{13}C chemical shifts for the sesquiterpene moieties in **3** and those for halichonadin L implied that their gross structures and relative configurations are identical. Comparison of ^{1}H and ^{13}C NMR data for the linker moiety (unit C, C-1”–C-17”) of **3** with those for halichonadin L indicated that **3** has two hydroxy groups on the piperidine ring, whereas halichonadin L has one hydroxy group at C-4”. The positions of the hydroxy groups in **3** were assigned as C-4” and C-5” on the basis of ^{1}H-^{1}H COSY cross-peaks of H-2”/H$_2$-3”, H$_2$-3”/H-4”, H-4”/H-5”, H-5”/H-6”, H-4”/4”-OH, and H-5”/5”-OH (Figure 3). NOESY correlations for H-3”a/H-5”, H-2”/H-8”a, and H-6”/H-8”b and large values of $^{3}J_{\text{H-2”/H-3”a}}$ (9.3 Hz) and $^{3}J_{\text{H-5”/H-6”}}$ (7.4 Hz) suggested the chair conformation of the piperidine ring in unit C as well as the equatorial orientations for the substituents at C-2”, C-5”, and C-6” (Figure 3). The equatorial orientation of H-4” was assigned by NOESY cross-peaks of H-4”/H-3”a, H-4”/H-3”b, and H-4”/H-5”. The relative relationships among units A–C of **3** were not assigned, since an effective correlation was not observed in the NOESY spectrum.

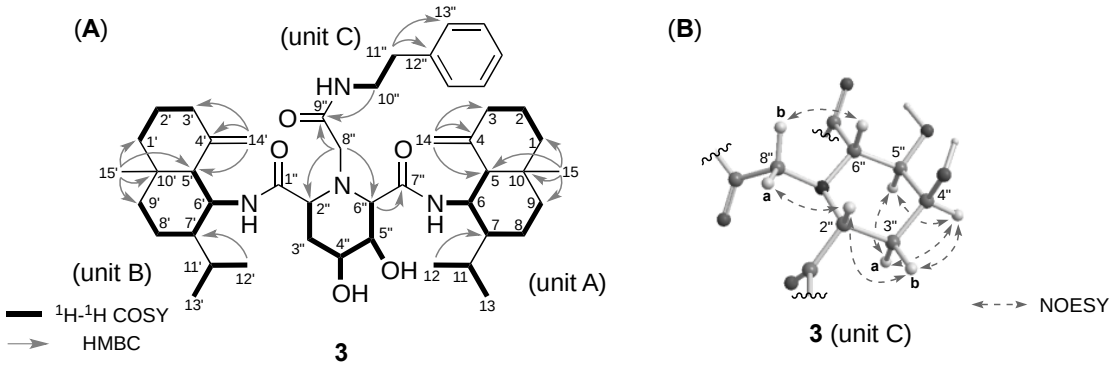


Figure 3. (**A**) Selected 2D NMR correlations and (**B**) relative stereochemistry for unit C (C-1”–C-9”) of halichonadin O (**3**)

Halichonadin P (**4**) was isolated as an optically active colorless amorphous solid {[α]$^{20}_{D}$ −26.2 (*c* 0.12, MeOH)}. The HRAPCIMS spectrum gave a pseudomolecular ion peak [M+H]$^{+}$ at *m/z* 668.49976, which was consistent with the molecular formula of $C_{40}H_{65}N_3O_5$. The ^{1}H and ^{13}C NMR spectra of **4** (Table 3) were similar to those of halichonadin K.[5] Subtle differences between the ^{1}H and ^{13}C NMR data for unit C (C-1”–C-9”) in **4** and those in halichonadin K implied that **4** is a stereoisomer of halichonadin K in unit C. The gross structure of **4** was confirmed to be the same as that of halichonadin K by analysis of the 2D NMR spectra (Figure 4). NOESY analysis supported that the relative configurations of the sesquiterpene moieties in **4** are the same as those of halichonadin K. In unit C of **4**, large ^{3}J values of H-4”/H-5”b and H-5”b/H-6” (10.3 Hz each) and NOESY cross-peaks of H-4”/H-5”a, H-5”a/H-6”, H-3”b/H-8”a disclosed the axial orientations for H-3”b, H-4”, H-5”b, and H-6” (Figure 4). Thus, the piperidine ring adopts the chair conformation. The equatorial orientation of H-2” was implied by small ^{3}J values of H-2”/H$_2$-3” (2.9 and 4.9 Hz) and NOESY correlations for H-2”/H-3”a and H-2”/H-3”b. The relative relationship between

the sesquiterpene moiety (unit B) and unit C was deduced as shown in Figure 4 on the basis of NOESY correlations for H-11'/H-3"a, 6'-NH/H-2", 6'-NH/H-5', and 6'-NH/H-7'.

Table 3. ^{1}H and ^{13}C NMR data for halichonadins O (**3**) and P (**4**) in C_5D_5N

	3[a]		**4**	
position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	42.3	1.31, 1.16 (1H each, m)	42.1	1.30, 1.16 (1H each, m)
2	24.6	1.54 (2H, m)	24.5[b]	1.53, 1.46 (1H each, m)
3	38.6	2.27, 1.92 (1H each, m)	38.5	2.19 (1H, brd, 12.2), 1.77 (1H, td, 12.2, 6.0)
4	147.8	–	147.3[c]	–
5	56.8	2.07 (1H, m)	56.6	1.84 (1H, d, 9.5)
6	47.0	4.25 (1H, m)	46.7	4.26 (1H, q, 9.5)
7	50.3	1.44 (1H, m)	50.3	1.29 (1H, m)
8	18.8	1.42, 1.33 (1H each, m)	18.7	1.36, 1.27 (1H each, m)
9	40.6	1.40, 1.12 (1H each, m)	40.4[d]	1.38, 1.06 (1H each, m)
10	37.7	–	37.8	–
11	27.1	2.29 (1H, m)	27.2	2.08 (1H, sept, 7.0)
12	22.0	1.02 (3H, d, 6.9)	22.0	0.94 (3H, d, 7.0)
13	16.7	1.16 (3H, d, 6.9)	16.5	1.06 (3H, d, 7.0)
14	107.5	5.08, 4.94 (1H each, brs)	107.5[e]	5.04, 4.89 (1H each, brs)
15	17.4	0.74 (3H, s)	17.3[f]	0.70 (3H, s)
1'	42.3	1.31, 1.16 (1H each, m)	42.3	1.30, 1.16 (1H each, m)
2'	24.6	1.54 (2H, m)	24.6[b]	1.53, 1.46 (1H each, m)
3'	38.6	2.32, 1.99 (1H each, m)	38.6	2.31 (1H, brd, 11.8), 1.91 (1H, td, 11.8, 6.6)
4'	147.6	–	147.5[c]	–
5'	56.5	2.14 (1H, d, 10.4)	56.9	1.96 (1H, d, 9.5)
6'	47.4	4.35 (1H, m)	46.7	4.26 (1H, q, 9.5)
7'	50.5	1.44 (1H, m)	50.5	1.25 (1H, m)
8'	18.9	1.42, 1.33 (1H each, m)	18.9	1.36, 1.27 (1H each, m)
9'	40.7	1.40, 1.12 (1H each, m)	40.6[d]	1.38, 1.06 (1H each, m)
10'	37.7	–	37.7	–
11'	26.4	2.54 (1H, m)	27.1	2.16 (1H, sept, 7.0)
12'	22.0	0.93 (3H, d, 6.9)	21.8	0.88 (3H, d, 7.0)
13'	16.7	1.08 (3H, d, 6.9)	16.6	1.05 (1H, d, 7.0)
14'	107.5	5.26, 5.08 (1H each, brs)	107.7[e]	5.17, 4.98[g] (1H each, brs)
15'	17.4	0.75 (3H, s)	17.4[f]	0.74 (3H, s)
1"	174.9	–	172.9	–
2"	60.3	4.15 (1H, dd, 9.3, 5.7)	61.4	4.17 (1H, dd, 4.9, 2.9)
3"	34.5	2.63, 2.23 (1H each, m)	35.2	2.67 (1H, brd, 13.1), 2.09 (1H, m)
4"	66.4	4.37 (1H, brs)	64.3	4.43 (1H, m)
5"	71.8	4.53 (1H, m)	37.0	2.64 (1H, brd, 12.4), 2.01 (1H, dt, 12.4, 10.3)
6"	68.0	4.13 (1H, d, 7.4)	62.6	4.50 (1H, brd, 10.3)
7"	173.7	–	172.6	–
8"	57.6	3.77, 3.65 (1H each, d, 17.7)	53.3	3.90, 3.76 (1H each, d, 18.0)
9"	171.0	–	172.7	–
10"	41.4	3.61 (2H, m)		
11"	36.2	2.88 (2H, t, 7.7)		
12"	140.1	–		
13", 17"	129.1	7.21 (2H, d, 7.4)		
14", 16"	128.8	7.26 (2H, t, 7.4)		
15"	126.6	7.18 (1H, t, 7.4)		
NH-6		8.56 (1H, brs)		7.31 (1H, d, 9.5)
NH-6'		8.80 (1H, brs)		7.89 (1H, d, 9.5)
NH-9"		8.91 (1H, brs)		
OH-4"		6.99 (1H, brs)		6.55 (1H, d, 4.9)
OH-5"		6.99 (1H, brs)		
9"-OMe			51.8	3.69 (3H, s)

[a] Assignment for the sesquiterpene moieties (C-1–C-15 and C-1'–C-15') may be interchangeable.
[b-f] Signals may be interchangeable. [g] Signal was overlapped with that of HOD.

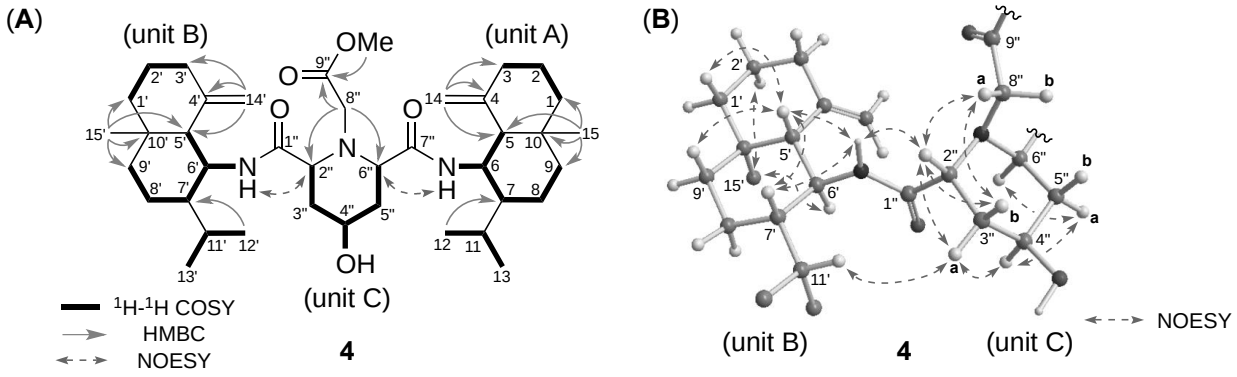


Figure 4. (**A**) Selected 2D NMR correlations and (**B**) relative stereochemistry for units B (C-1'–C-15') and C (C-1"–C-9") of halichonadin P (**4**)

Although the relative relationship between units A and C was not assigned by NOESY analysis, the absolute configurations of sesquiterpene units (units A and B) were presumed to be identical, like the case of **1**. Accordingly, the structure of halichonadin P was concluded to be **4** (Chart 1), where the absolute configuration of C-6" was *R* in contrast with the 6"*S* configuration in related sesquiterpene dimers, halichonadins K and L.[5]

Halichonadin Q (**5**) was obtained as an optically active colorless amorphous solid {$[\alpha]^{20}_D$ −19.8 (*c* 0.12, MeOH)}, and the HRAPCIMS revealed the molecular formula to be $C_{23}H_{38}N_2O_3$ (*m/z* 391.29590 $[M+H]^+$, Δ+0.38 mmu). The ^{1}H and ^{13}C NMR spectra (Table 4) showed the resonances of a sesquiterpene moiety, which was identical to that of **1**, as well as two carbonyl carbons, one nitrogen bearing sp^3 methine, four sp^3 methylenes, two of which were attached to a nitrogen atom, and one methoxy group. Inspection of the ^{1}H-^{1}H COSY and HMBC spectra of **5** (Figure 5) suggested the existence of a pyrrolidine ring (C-3'–C-6') and the connectivity of C-1' to N-3' through C-2'. The connectivity of C-1' to the sesquiterpene moiety (C-6) via an amide linkage was implied by a NOESY cross-peak of 6-NH/H-2'b. A methoxy carbonyl group at C-6' was disclosed by HMBC correlations for 7'-OMe to C-7' and H_2-5' to C-7'. Thus, the gross structure of **5** was elucidated as shown in Figure 5.

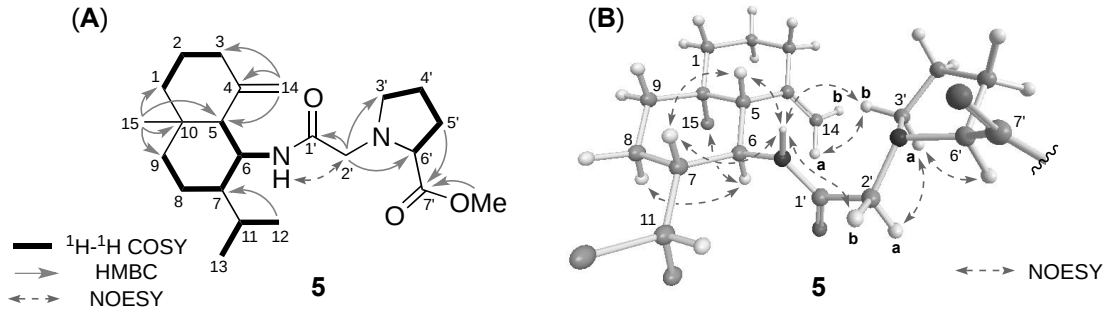


Figure 5. (**A**) Selected 2D NMR correlations and (**B**) relative stereochemistry for halichonadin Q (**5**)

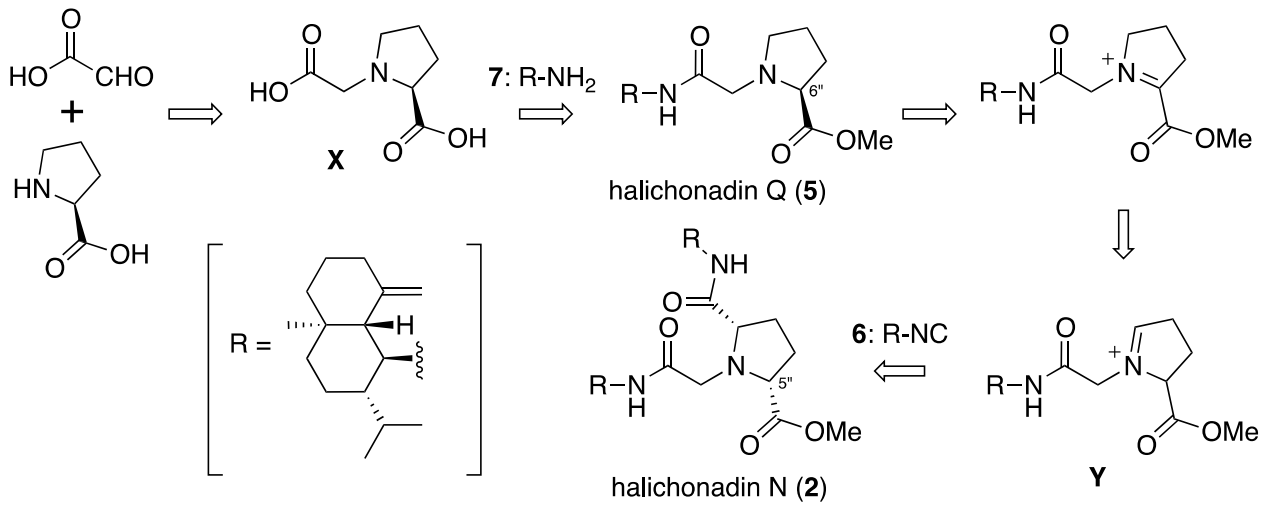
Table 4. ^{1}H and ^{13}C NMR data for halichonadin Q (**5**) in C_5D_5N

position	δ_C	δ_H (*J* in Hz)
1	42.2	1.44, 1.28 (1H each, m)
2	24.3	1.81, 1.61 (1H each, m)
3	38.3	2.28, 1.89 (1H each, m)
4	146.2	–
5	57.0	1.82 (1H, m)
6	46.5	3.49 (1H, q, 10.2)
7	50.3	1.17 (1H, m)
8	18.6	1.48, 1.36 (1H each, m)
9	40.5	1.51, 1.25 (1H each, m)
10	37.6	–
11	26.7	1.79 (1H, m)
12	21.6	0.90 (3H, d, 6.8)
13	16.3	0.90 (3H, d, 6.8)
14	107.5	4.78, 4.62 (1H each, brs)
15	17.4	0.78 (3H, s)
1’	169.9	–
2’	58.5	3.37, 3.04 (1H each, d, 16.4)
3’	54.7	3.07 (1H, m), 2.39 (1H, q, 8.3)
4’	24.3	1.80, 1.59 (1H each, m)
5’	29.8	2.14, 1.93 (1H each, m)
6’	65.8	3.39 (1H, dd, 9.4, 5.5)
7’	174.5	–
NH-6		6.06 (1H, brd, 10.2)
OMe	51.9	3.69 (3H, s)

NOESY correlations for H-2’a/H-3’a and H-3’a/H-6’ suggested that these protons were oriented to the same side of the pyrrolidine ring (Figure 5). Given NOESY cross-peaks of H-3’b/H-14a, H-3’b/6-NH, 6-NH/H-2’b, 6-NH/H-5, and 6-NH/H-7, the relative relationship between the sesquiterpene moiety and the pyrrolidine unit in **5** was assigned as shown in Figure 5. Since the absolute configuration of the sesquiterpene moiety of **5** was presumed to be the same as that of **1**, the absolute stereochemistry of **5** was concluded as shown in Chart 1.

A possible biogenetic pathway of halichonadins N (**2**) and Q (**5**), which have a pyrrolidine moiety in common, is proposed as shown in Scheme 2. It is noteworthy that the deduced absolute configuration of C-5” in **2** is different from that of C-6” in **5**. A plausible biogenetic intermediate (**X**) seems to be derived by condensation of glyoxylic acid and L-proline. Halichonadin Q (**5**) might be generated from **X** and halichonadin D (**7**).[6] Then, intermediate **Y** might be derived from **5**. In the process, epimerization of the methine carbon in pyrrolidine ring seems to have occurred. Addition of halichonadin C (**6**) to **Y** might give halichonadin N (**2**).

Antimicrobial activities of halichonadins M (**1**) and O (**3**) as well as halichonadins G–L[4,5] against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *Aspergillus niger*, *Trichophyton mentagrophytes*, *Candida albicans*, and *Cryptococcus neoformans* were evaluated. As a result, halichonadin O (**3**) showed antimicrobial activity against *Staphylococcus aureus* (MIC 8 μg/mL), *Micrococcus luteus* (MIC 8 μg/mL), and *Trichophyton mentagrophytes* (IC_{50} 16 μg/mL). Other tested sesquiterpenes did not show such activity against any strains.



Scheme 2. Possible biogenetic pathway of halichonadins N (**2**) and Q (**5**)

CONCLUSION

Recently, we have reported the isolation and structure elucidation of sesquiterpenes, halichonadins G–L, from an Okinawan marine sponge *Halichondria* sp. (NSS-2).[4,5] Further study for constituents of the sponge resulted in the isolation of four new dimeric sesquiterpenes, halichonadins M–P (**1**–**4**) and one new sesquiterpene, halichonadin Q (**5**). These new sesquiterpenes have eudesmane skeleton in common. Halichonadin M (**1**) is a symmetrical dimer linked to a nitrilotriacetic acid fragment through amide linkages. Halichonadin N (**2**) is an unique dimeric sesquiterpene connected via a pyrrolidine unit. Halichonadins O (**3**) and P (**4**) have linker moieties consisting of a piperidine unit, and are structurally related to halichonadins K and L, respectively. Halichonadin Q (**5**) is a sesquiterpene possessing a pyrrolidine unit. The absolute stereochemistry for **1**, **2**, **4**, and **5** was deduced on the basis of spectroscopic analysis taking the absolute stereochemistry for known compounds isolated from the same origin into consideration. The absolute configuration of the piperidine unit in **3** remains unsolved.

EXPERIMENTAL

General procedures: Optical rotations were recorded on a JASCO P-1030 digital polarimeter. NMR spectra were recorded on a JASCO FT/IR-230, spectrophotometer. NMR spectra were measured by a Bruker AMX-600 NMR spectrometer and a JEOL ECA 500 spectrometer. The 7.19 and 123.5 ppm resonances of residual pyridine were used as internal references for ^{1}H and ^{13}C NMR spectra, respectively. Mass spectra were recorded on a Thermo Scientific Exactive spectrometer. HPLC column: column A, YMC-Pack Pro C18, YMC Co., Ltd., 10 x 250 mm; column B, Mightysil RP-18 GP, Kanto Chemical Co., Inc., 20 x 250 mm; column C, Mightysil RP-18 GP, 10 x 250 mm).

Sponge descriptioin: The light-brown sponge *Halichondria* sp. (Order Halichondria, Family Halichondriidae) (NSS-2) collected at Unten Port, Okinawa, was kept frozen until used. The sponge is firm, slightly compressible, springy texture with an irregular surface. The sponge has an irregular isotropic skeleton. Spicules are oxeas long, slender, occasionally with stepped ends, 780 x 10 μm. The voucher specimen was deposited at the Graduate School of Pharmaceutical Sciences, Hokkaido University.

Isolation of halichonadins M–Q (1–5): The sponge *Halichondria* sp. (1.0 kg, wet weight) was extracted with MeOH (1.5 L x 3) to give the extract (70.3 g). A part of the extract (22.3 g) was partitioned between $CHCl_3$ (400 mL x 3) and water (400 mL). The $CHCl_3$-soluble materials (2.37 g) were subjected to a silica gel column (*n*-hexane/EtOAc, 95:5→60:40 and then $CHCl_3$/MeOH, 99:1→0:100) to give 23 fractions (frs. 1–23). Fr. 9 was purified by a silica gel column (*n*-hexane/$CHCl_3$/MeOH, 5:5:0→5:5:2) and C_{18} HPLC (column A, flow rate 2.0 mL/min; UV detection at 220 nm; eluent MeCN/H_2O, 95:5) to yield halichonadin Q (**5**, 0.5 mg). Fr. 14 was applied to a C_{18} column (MeOH/H_2O, 9:1) and then subjected to C_{18} HPLC (column A, 2.0 mL/min; 220 nm; MeOH/H_2O, 95:5) to give seven fractions (frs. 14.1–7) including halichonadin M (**1**, 0.7 mg, fr. 14.6). Purification of fr. 14.5 using C_{18} HPLC (column A, 2.0 mL/min; 220 nm; MeCN) gave halichonadin N (**2**, 0.5 mg). Fr. 15 was loaded on a C_{18} column (MeOH/H_2O, 9:1) and C_{18} HPLC (column A, 2.0 mL/min; 220 nm; MeOH/H_2O, 95:5) to isolate halichonadin P (**4**, 0.3 mg). Fr. 19 was subjected to C_{18} HPLC (column B, 6.0 mL/min; 220 nm; MeOH/H_2O, 95:5), and then purified by C_{18} HPLC (column C, 2.0 mL/min; 220 nm; MeCN) to afford halichonadin O (**3**, 1.8 mg).

Halichonadin M (1). Colorless amorphous solid; $[\alpha]^{20}_D$ –27.4 (*c* 0.24, MeOH); ^{1}H and ^{13}C NMR (Table 1); HRESIMS: *m/z* 634.45630 $[M+Na]^+$ (calcd for $C_{37}H_{61}N_3O_4Na$, 634.45543).

Halichonadin N (2). Colorless amorphous solid; $[\alpha]^{20}_D$ –21.1 (*c* 0.13, MeOH); ^{1}H and ^{13}C NMR (Table 2); HRAPCIMS: *m/z* 638.48927 $[M+H]^+$ (calcd for $C_{39}H_{64}N_3O_4$, 638.48913).

Halichonadin O (3). Colorless amorphous solid; $[\alpha]^{20}_D$ –34.2 (*c* 0.24, MeOH); ^{1}H and ^{13}C NMR (Table 3); HRAPCIMS: *m/z* 773.55762 $[M+H]^+$ (calcd for $C_{47}H_{73}N_4O_5$, 773.55755).

Halichonadin P (4). Colorless amorphous solid; $[\alpha]^{20}_D$ –26.2 (*c* 0.12, MeOH); ^{1}H and ^{13}C NMR (Table 3); HRAPCIMS: *m/z* 668.49976 $[M+H]^+$ (calcd for $C_{40}H_{66}N_3O_5$, 668.49970).

Halichonadin Q (5). Colorless amorphous solid; $[\alpha]^{20}_D$ –19.8 (*c* 0.12, MeOH); ^{1}H and ^{13}C NMR (Table 4); HRAPCIMS: *m/z* 391.29590 $[M+H]^+$ (calcd for $C_{23}H_{39}N_2O_3$, 391.29552).

Derivatization of halichonadin C (6) to halichonadin D (7) and (*S*)- and (*R*)-MTPA amides (7a and 7b, respectively) of 7: Halichonadin C (**6**, 25 mg) was treated with 5% HCl (500 μL) in MeOH at 60 °C for 3 h. After evaporation, the residue was purified using a SiO_2 column ($CHCl_3$/MeOH) to give halichonadin D (**7**, 8.9 mg). To a CH_2Cl_2 solution (300 mL) of **7** (1.0 mg) were added 4-(dimethylamino)pyridine (5.6 mg), triethylamine (18.4 μL), (*R*)-(–)-MTPACl (18 μL) at room temperature, and stirring was continued for 3 h. After addition of MeOH and evaporation of solvent, the residue was applied to a silica gel column ($CHCl_3$/MeOH) and reverse phase HPLC (Luna 5u Phenyl-Hexyl, Phenomenex Inc., 3.0 mL/min; 230 nm; MeCN/H_2O, 90:10) to yield the 6-(*S*)-MTPA amide (**7a**) of halihchonadin D (**7**). The 6-(*R*)-MTPA amide (**7b**) of **7** was prepared according to the same procedure as described above.

Halichonadin D (7): $[\alpha]^{20}_D$ +19.4 (*c* 0.38, MeOH); ^{13}C NMR (C_5D_5N) δ_C 147.2 (C-4), 108.2 (C-14), 55.8 (C-5), 48.6 (C-6), 47.8 (C-7), 41.6 (C-1), 40.0 (C-9), 37.9 (C-3), 37.4 (C-10), 26.5 (C-11), 24.2 (C-2), 21.3 (C-13), 18.2 (C-8), 17.2 (C-15), 15.9 (C-12); HRAPCIMS: *m/z* 222.22156 $[M+H]^+$ (calcd for $C_{15}H_{28}N$, 222.22163).

6-(*S*)-MTPA amide (7a) of halichonadin D: ^{1}H NMR (C_5D_5N) δ_H 8.186 (1H, d, *J* = 9.8 Hz, 6-NH), 7.908 (2H, m, Ph), 7.398 (3H, m, Ph), 5.225 (1H, s, H-14a), 5.003 (1H, s, H-14b, overlapped with signal of HOD), 4.296 (1H, m, H-6), 3.572 (3H, OMe), 2.329 (1H, brd, *J* = 12.0 Hz, H-3eq), 2.116 (1H, d, *J* = 10.4 Hz, H-5), 1.951 (1H, m, H-3ax), 1.732 (1H, m, H-11), 1.526 (2H, m, H_2-2), 1.188 (1H, m, H-7), 0.991 (3H, d, *J* = 6.9 Hz, H_3-12), 0.730 (3H, s, H_3-15), and 0.650 (3H, d, *J* = 6.9 Hz, H_3-13); HRAPCIMS: *m/z* 438.26101 $[M+H]^+$ (calcd for $C_{25}H_{35}NO_2F$, 438.26144).

6-(*R*)-MTPA amide (7b) of halichonadin D: ^{1}H NMR (C_5D_5N) δ_H 8.315 (1H, d, *J* = 9.8 Hz, NH-6), 7.835 (2H, m, Ph), 7.340 (3H, m, Ph), 4.881 (1H, s, H-14a), 4.397 (1H, s, H-14b), 4.252 (1H, m, H-6), 2.291 (1H, m, H-11), 2.238 (1H, brd, *J* = 12.6 Hz, H-3eq), 2.171 (1H, d, *J* = 9.7 Hz, H-5), 1.808 (1H, m, H-3ax), 1.490 (2H, m, H_2-2), 1.476 (1H, m, H-7), 1.05 (3H, d, *J* = 6.3 Hz, H_3-12), 0.961 (3H, d, *J* = 6.3 Hz, H_3-13), and 0.701 (3H, s, H_3-15); HRAPCIMS: *m/z* 438.26110 $[M+H]^+$ (calcd for $C_{25}H_{35}NO_2F$, 438.26144).

ACKNOWLEDGMENTS

We thank Z. Nagahama for his help with sponge collection, S. Oka and A. Tokumitsu, Equipment Management Center, Hokkaido University, for measurements of mass spectra. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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