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Halichonadins K and L, New Dimeric Sesquiterpenoids from a Sponge *Halichondria* sp.

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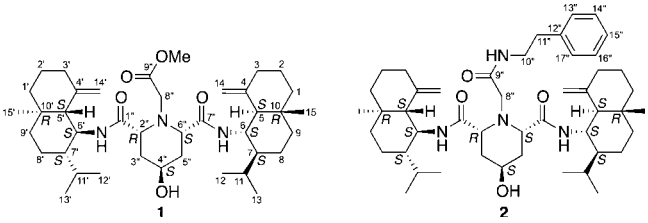
ABSTRACT



Two new structurally unique dimeric sesquiterpenoids, halichonadins K (1) and L (2), were isolated from an Okinawan marine sponge *Halichondria* sp. The structures of 1 and 2 were elucidated on the basis of spectroscopic analysis including a single crystal X-ray diffraction analysis and chemical conversion. Halichonadins K (1) and L (2) are homodimers of the eudesmane sesquiterpene linked with a piperidine ring through amide bonds. Halichonadin K (1) showed moderate cytotoxicity against KB cells.

Marine sponges belonging to the genus *Halichondria* are known to be a source of sesquiterpene isothiocyanates, isonitriles, and formamides and dimeric sesquiterpenoids with a urea linkage.[1,2] During our search for structurally unique metabolites from Okinawan marine sponges, we reported the isolation of a series of sesquiterpenoids, halichonadins A−F, from *Halichondria* spp.[3] Recently, we have also reported dimeric sesquiterpenoids, halichonadins G−I, and a sesquiterpenoid, halichonadin J, from the extracts of an Okinawan marine sponge *Halichondria* sp. (NSS-2).[4] Further investigation of the extracts resulted in the isolation of two new dimeric sesquiterpenoids, halichonadins K (**1**) and L (**2**). In this Letter, we describe the isolation and structure elucidation of **1** and **2**.

The sponge *Halichondria* sp. (NSS-2, 1.0 kg, wet weight) collected at Unten Port, Okinawa, was extracted with MeOH, and the extracts were partitioned between $CHCl_3$ and water. The $CHCl_3$-soluble materials were subjected to silica gel columns and then purified using C_{18} HPLC to afford halichonadins K (**1**, 0.00094%, wet weight) and L (**2**, 0.00093%) together with a known eudesmane-type sesquiterpenoid, halichonadin C.[3a]

Halichonadin K (**1**)[5] was obtained as an optically active colorless amorphous solid {$[\alpha]^{21}_D$ −25.2 (*c* 1.07, MeOH)}. IR absorption bands at 1738 and 1650 cm^{-1} implied the presence of ester and amide carbonyl functionalities, respectively. The molecular formula, $C_{40}H_{65}N_3O_5$, was established by the HRAPCIMS (*m*/*z* 668.49987 $[M+H]^+$, Δ+0.17 mmu). The ^1H and ^{13}C NMR spectra (Table 1) suggested that **1** has two sesquiterpene moieties {units A

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(1) Wright, A. D.; Köning, G. M. *J. Nat. Prod.* **1996**, *59*, 710–716.
(2) Zhan, Z.-J.; Ying, Y.-M.; Ma, L.-F.; Shan, W.-G. *Nat. Prod. Rep.* **2011**, *28*, 594–629.
(3) (a) Ishiyama, H.; Hashimoto, A.; Fromont, J.; Hoshino, Y.; Mikami, Y.; Kobayashi, J. *Tetrahedron* **2005**, *61*, 1101–1105. (b) Kozawa, S.; Ishiyama, H.; Fromont, J.; Kobayashi, J. *J. Nat. Prod.* **2008**, *71*, 445–447. (c) Ishiyama, H.; Kozawa, S.; Aoyama, K.; Mikami, Y.; Fromont, J.; Kobayashi, J. *J. Nat. Prod.* **2008**, *71*, 1301–1303.
(4) Suto, S.; Tanaka, N.; Fromont, J.; Kobayashi, J. *Tetrahedron Lett.* **2011**, *52*, 3470–3473.

(5) Halichonadin K (**1**): colorless amorphous solid; $[\alpha]^{21}_D$ −25.2 (*c* 1.07, MeOH); IR (film) ν_{max} 3284, 1738, and 1650 cm^{-1}; ^1H and ^{13}C NMR (Table 1); HRAPCIMS: *m*/*z* 668.49987 $[M+H]^+$ (calcd for $C_{40}H_{66}N_3O_5$, 668.49970).

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(C-1−C-15) and B (C-1′−C-15′)}. They were identical to those of halichonadin H,[4] a homodimer of sesquiterpene with the eudesmane skeleton. The gross structure of units A and B were confirmed by detailed analysis of the 2D NMR spectra (Figure 1).

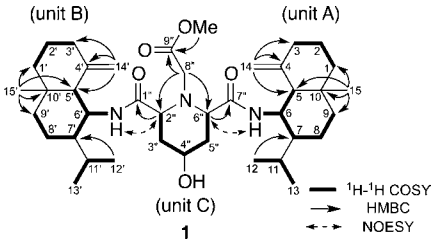


Figure 1. Selected 2D NMR correlations for halichonadin K (**1**).

Furthermore, the ^{1}H and ^{13}C NMR spectra showed the signals of the linker moiety {unit C (C-1″−C-9″)}, namely one ester and two amide carbonyl groups, one methoxy group, three sp^3 methines, and three sp^3 methylenes (Table 1). Among them, two sp^3 methines {C-2″ (δ_C 60.3) and C-6″ (δ_C 60.5)} and one sp^3 methylene {C-8″ (δ_C 53.4)} were ascribed to those bearing a nitrogen atom. The gross structure of unit C was assigned as follows. The ^{1}H−^{1}H COSY spectrum demonstrated the connectivities of C-2″ to C-6″ (Figure 1). HMBC correlations for protons of nitrogen bearing sp^3 methylene (H_2-8″) to C-2″, C-6″, and C-9″ and 9″-OMe to C-9″ revealed the presence of a piperidine ring (C-2″−C-6″ and 2″-N) and the connectivity of 2″-N to a methyl acetate moiety (C-8″ and C-9″). The existence of a hydroxy group at C-4″ was implied by the chemical shift of C-4″ (δ_C 62.3). This was supported by the downfield shift for H-4″ ($\Delta\delta$ 1.12 ppm) of the 4″-*p*-bromobenzoate (**1a**) of halichonadin K (**1**) (Supporting Information). The connectivities among units A−C through amide bonds were disclosed by HMBC correlations for H-2″/C-1″ and H-6″/C-7″ and NOESY cross-peaks of H-2″/6′-NH and H-6″/6-NH. Therefore, the gross structure of **1** was assigned as shown in Figure 1.

The relative configuration of unit A was deduced to be the same as that of halichonadin H[4] based on the NOESY analysis (Figure 2). Resemblance of the ^{13}C chemical shifts for units A and B of **1** implied that both units have the same relative configuration (Table 1). In unit C, NOESY correlations for 4″-OH/H-2″ and 4″-OH/H-6″ were observed, suggesting that the piperidine ring adopts the chair conformation and the axial orientations for H-2″, H-6″, and 4″-OH (Figure 2). Thus, the relative configurations for each unit of **1** were elucidated. However, their relative relationship could not been assigned by the NOESY analysis.

Although a crystal of the 4″-*p*-bromobenzoate (**1a**) was not obtained, crystallization of halichonadin K (**1**) from

(6) 0.01% of hydroquinone is added as a stabilizer to diisopropyl ether used for the crystallization.

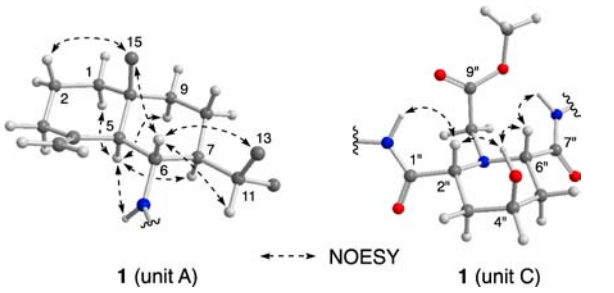


Figure 2. Selected NOESY correlations and relative configurations for units A (C-1−C-15) and C (C-1″−C-9″) of halichonadin K (**1**) (protons of methyl groups in unit A were not shown).

dichloromethane/diisopropyl ether gave a cocrystal of **1** and hydroquinone which is an additive of diisopropyl ether.[6] The single crystal X-ray diffraction analysis of the crystal revealed the relative stereochemistry of **1**.[7] The ORTEP drawing of **1** without hydroquinone was shown in Figure 3. In addition, the analysis disclosed the absolute stereochemistry of **1** {Flack parameter, 0.26(20), calculated using 3893 Friedel pairs}.[8] Therefore, the absolute configurations at 11 chiral centers of halichonadin K (**1**) were assigned as 5*S*, 6*S*, 7*S*, 10*R*, 5′*S*, 6′*S*, 7′*S*, 10′*R*, 2″*R*, 4″*S*, and 6″*S*.

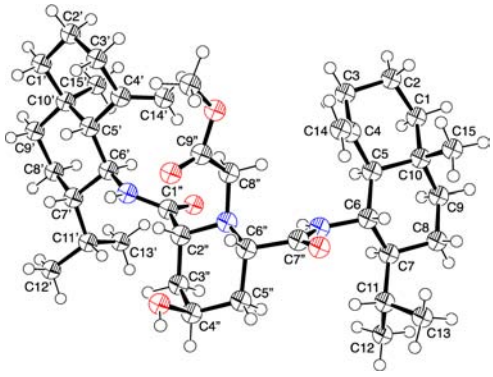


Figure 3. ORTEP drawing of halichonadin K (**1**).

Halichonadin L (**2**)[9] was obtained as an optically active colorless amorphous solid {$[\alpha]^{21}_D$ −14.3 (*c* 0.73, MeOH)}. An IR absorption band at 1647 cm^{-1} implied the presence of amide carbonyl functionality. The molecular formula, $C_{47}H_{72}N_4O_4$, was established by the

(7) Crystallographic data for halichonadin K (**1**) have been deposited at the Cambridge Crystallographic Data Center (deposition number CCDC 882486).

(8) (a) Flack, H. D. *Acta Crystallogr., Sect. A* **1983**, *A39*, 876–881. (b) Flack, H. D.; Bernardinelli, G. *J. Appl. Crystallogr.* **2000**, *33*, 1143–1148. (c) Flack, H. D.; Bernardinelli, G. J. *Chirality* **2008**, *20*, 681–690.

(9) Halichonadin L (**2**): colorless amorphous solid; $[\alpha]^{21}_D$ −14.3 (*c* 0.73, MeOH); IR (film) ν_{max} 3274 and 1647 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); HRAPCIMS: *m*/*z* 757.56233 $[M+H]^+$ (calcd for $C_{47}H_{73}N_4O_4$, 757.56263).

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data for Halichonadins K (**1**) and L (**2**) in C_5D_5N

	1		**2**	
position	^{13}C	^{1}H	^{13}C	^{1}H
1	42.2	1.31, 1.14 (1H each, m)	42.3	1.32, 1.19 (1H each, m)
2	24.5	1.49 (2H, m)	24.6	1.52 (2H, m)
3	38.5	2.25, 1.86 (1H each, m)	38.6	2.29, 1.95 (1H each, m)
4	147.3	–	147.4[b]	–
5	56.6	1.96 (1H, m)	56.6	2.09 (1H, m)
6	46.7	4.24 (1H, m)	46.9	4.28 (1H, m)
7	50.1	1.26 (1H, m)	50.3	1.42 (1H, m)
8	18.7	1.38, 1.27 (1H each, m)	18.8[b]	1.43, 1.32 (1H each, m)
9	40.6[b]	1.39, 1.10 (1H each, m)	40.6	1.42, 1.16 (1H each, m)
10	37.7	–	37.7	–
11	27.0	2.12 (1H, m)	27.0	2.15 (1H, m)
12	21.8	0.90 (3H, d, J = 7.0 Hz)	21.9	0.98 (3H, d, J = 6.8 Hz)
13	16.6	1.05 (3H, d, J = 7.0 Hz)	16.6	1.09 (3H, d, J = 6.8 Hz)
14	107.3	5.08 (1H, brs), 4.91[a] (1H, m)	107.4[b]	5.03, 4.91 (1H each, brs)
15	17.3	0.73 (3H, s)	17.4	0.73 (3H, s)
1′	42.2	1.31, 1.14 (1H each, m)	42.3	1.32, 1.19 (1H each, m)
2′	24.5	1.49 (2H, m)	24.6	1.52 (2H, m)
3′	37.5	2.25, 1.86 (1H each, m)	38.6	2.29, 1.95 (1H each, m)
4′	147.5	–	147.6[b]	–
5′	56.2	1.96 (1H, m)	56.6	2.09 (1H, m)
6′	46.7	4.24 (1H, m)	46.9	4.28 (1H, m)
7′	50.2	1.33 (1H, m)	50.3	1.42 (1H, m)
8′	18.7	1.38, 1.27 (1H each, m)	18.9[b]	1.43, 1.32 (1H each, m)
9′	40.5[b]	1.39, 1.10 (1H each, m)	40.6	1.42, 1.16 (1H each, m)
10′	37.6	–	37.7	–
11′	26.9	2.18 (1H, m)	27.1	2.24 (1H, m)
12′	21.7	0.95 (3H, d, J = 7.0 Hz)	22.0	1.03 (3H, d, J = 6.7 Hz)
13′	16.5	1.07 (3H, d, J = 7.0 Hz)	16.7	1.15 (3H, d, J = 6.7 Hz)
14′	107.5	5.05 (1H, brs), 4.91[a] (1H, m)	107.6[b]	5.15, 5.02 (1H each, brs)
15′	17.3	0.73 (3H, s)	17.4	0.73 (3H, s)
1″	173.6	–	174.3	–
2″	60.3	4.48 (1H, dd, J = 10.9, 3.2 Hz)	61.8[b]	4.04 (1H, m)
3″	37.5	2.25 (2H, m)	36.4[b]	2.26 (2H, m)
4″	62.3	4.35 (1H, brs)	61.9	4.38 (1H, brs)
5″	37.8	2.35 (2H, m)	36.6[b]	2.38 (2H, m)
6″	60.5	4.49 (1H, dd, J = 9.2, 4.1 Hz)	61.6[b]	4.04 (1H, m)
7″	173.5	–	174.3	–
8″	53.4	3.98, 3.85 (1H each, d, J = 18.1 Hz)	58.9	3.59 (2H, m)
9″	172.0	–	171.2	–
10″	–	–	41.6	3.71, 3.68 (1H each, m)
11″	–	–	36.3	2.99 (2H, t, J = 7.8)
12″	–	–	140.1	–
13″,17″	–	–	129.1	7.29 (2H, m)
14″,16″	–	–	128.9	7.29 (2H, m)
15″	–	–	126.6	7.21 (1H, m)
NH-6	–	8.06 (1H, brd, J = 8.9 Hz)	–	8.50 (1H, brd, J = 7.0 Hz)
NH-6′	–	8.12 (1H, brd, J = 9.0 Hz)	–	8.32 (1H, brd, J = 9.3 Hz)
NH-9″	–	–	–	8.84 (1H, brt, J = 5.1 Hz)
OMe	51.2	3.58 (3H, s)	–	–
OH	–	6.28 (1H, brs)	–	6.68 (1H, brs)

[a] Signals were overlapped with that of HOD. [b] Signals may be interchangeable.

HRAPCIMS (m/z 757.56233 $[M+H]^+$, Δ−0.30 mmu). The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1**, and the signals for a phenethylamine moiety in **2** were discerned in place of the resonances of a methoxy group in **1** (Table 1). These data implied that **2** is a dimer of the eudesmane-type sesequiterpene having a piperidine ring and a phenethylamine moiety. Analysis of the $^{1}H-^{1}H$ COSY and HMBC spectra suggested the gross structrue of **2** as shown in Figure 4.

(10) Kunishima, M.; Kawachi, C.; Hioki, K.; Terao, K.; Tani, S. *Tetrahedron* **2001**, *57*, 1551–1558.

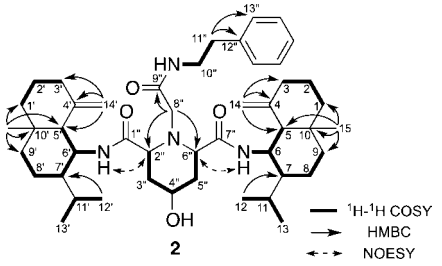
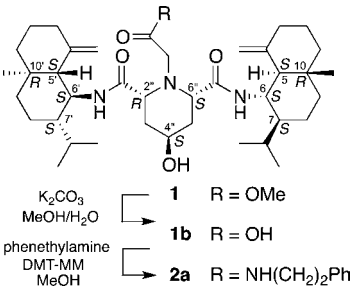


Figure 4. Selected 2D NMR correlations for halichonadin L (**2**).

Scheme 1. Derivatization of Halichonadin K (**1**) to Halichonadin L (**2a**)

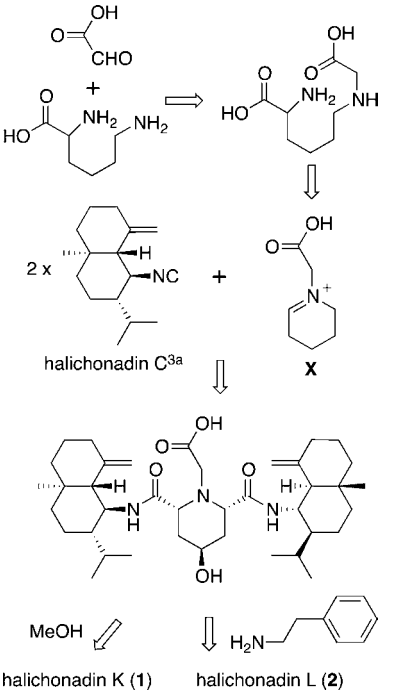


To elucidate the stereochemistry of halichonadin L (**2**), derivatization of halichonadin K (**1**) to **2a** was carried out (Scheme 1). Halichonadin K (**1**) was treated with K_2CO_3 in MeOH/water (1:1) to give the demethyl derivative (**1b**). Condensation of **1b** and phenethylamine by DMT-MM[10] in MeOH furnished halichonadin L (**2a**). Since ^{1}H NMR data (Supporting Information) and optical rotation {$[\alpha]^{18}_D$ −10.3 (c 0.20, MeOH)} of derived halichonadin L (**2a**) were in agreement with those of natural halichonadin L (**2**), the absolute stereochemistry of **2** was concluded as shown in Scheme 1.

Halichonadins K (**1**) and L (**2**) are structurally unique homodimers of the eudesmane sesquiterpene. To the best of our knowledge, they are the first example of the isolation of dimeric sesquiterpenoids linked with a piperidine ring from a natural source, while some homo- and hetero sesquiterpene dimers such as halichonadins A,[3a] E,[3b] and G−I,[4] and *N*,*N*′-bis{(1*Z*,4*Z*)-7αH-germacra-1(10),4-dienyl}urea[11] were reported from sponges of the genera *Halichondria* and *Axinyssa*. Furthermore, halichonadins K (**1**) and L (**2**) have a methyl acetate and

(11) Satitpatipan, V.; Suwanborirux, K. *J. Nat. Prod.* **2004**, *67*, 503–505.

Scheme 2. Possible Biogenetic Path of Halichonadins K (**1**) and L (**2**)



N-phenethylacetamide moieties, respectively, connected with a nitrogen atom of the piperidine ring.

A possible biogenetic path of halichonadins K (**1**) and L (**2**) is proposed as shown in Scheme 2. A plausible biogenetic intermediate (**X**) seems to be derived by condensation of glyoxylic acid and lysine. Halichonadins K (**1**) and L (**2**) might be generated from **X** and two molecules of halichonadin C.[3a]

Halichonadin K (**1**) showed cytotoxicity against human epidermoid carcinoma KB cells (IC_{50} 10.6 μg/mL) *in vitro*.

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Supporting Information Available. Experimental section, 1D and 2D NMR spectra, and X-ray crystallographic data (CIF) for halichonadins K and L and their derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.