

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

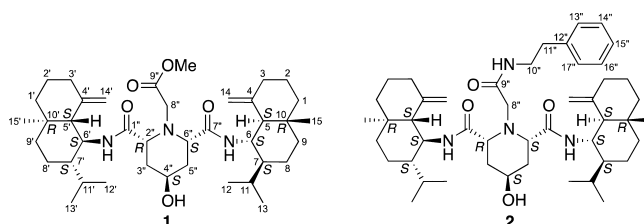
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## ABSTRACT



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.<sup>1,2</sup> 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.<sup>3</sup> 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).<sup>4</sup> 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<sub>3</sub> and water. The CHCl<sub>3</sub>-soluble materials were subjected to silica gel columns and then purified using C<sub>18</sub> HPLC to afford halichonadins K (**1**, 0.00094%, wet weight) and L (**2**, 0.00093%) together with a known eudesmane-type sesquiterpenoid, halichonadin C.<sup>3a</sup>

Halichonadin K (**1**)<sup>5</sup> was obtained as an optically active colorless amorphous solid {[ $\alpha$ ]<sub>D</sub><sup>21</sup> –25.2 (*c* 1.07, MeOH)}. IR absorption bands at 1738 and 1650 cm<sup>–1</sup> implied the presence of ester and amide carbonyl functionalities, respectively. The molecular formula, C<sub>40</sub>H<sub>65</sub>N<sub>3</sub>O<sub>5</sub>, was established by the HRAPCIMS (*m/z* 668.49987 [M+H]<sup>+</sup>,  $\Delta$ +0.17 mmu). The <sup>1</sup>H and <sup>13</sup>C NMR spectra (Table 1) suggested that **1** has two sesquiterpene moieties {units A

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<sup>§</sup> Western Australian Museum.

(1) Wright, A. D.; Köning, G. M. *J. Nat. Prod.* **1996**, *59*, 710–716.

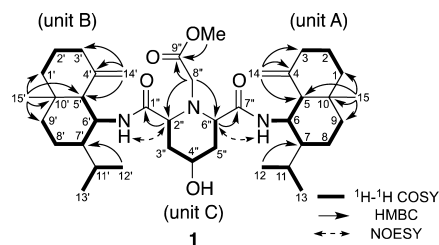
(2) Zhan, Z.-J.; Ying, Y.-M.; Ma, L.-F.; Shan, W.-G. *Nat. Prod. Rep.* **2011**, *28*, 594–629.

(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; [ $\alpha$ ]<sub>D</sub><sup>21</sup> –25.2 (*c* 1.07, MeOH); IR (film)  $\nu_{\text{max}}$  3284, 1738, and 1650 cm<sup>–1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR (Table 1); HRAPCIMS: *m/z* 668.49987 [M+H]<sup>+</sup> (calcd for C<sub>40</sub>H<sub>66</sub>N<sub>3</sub>O<sub>5</sub>, 668.49970).

(C-1–C-15) and B (C-1'–C-15')). They were identical to those of halichonadin H,<sup>4</sup> 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).

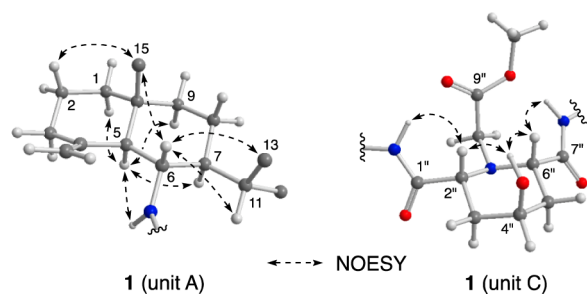


**Figure 1.** Selected 2D NMR correlations for halichonadin K (**1**).

Furthermore, the <sup>1</sup>H and <sup>13</sup>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<sup>3</sup> methines, and three sp<sup>3</sup> methylenes (Table 1). Among them, two sp<sup>3</sup> methines {C-2'' (δ<sub>C</sub> 60.3) and C-6'' (δ<sub>C</sub> 60.5)} and one sp<sup>3</sup> methylene {C-8'' (δ<sub>C</sub> 53.4)} were ascribed to those bearing a nitrogen atom. The gross structure of unit C was assigned as follows. The <sup>1</sup>H–<sup>1</sup>H COSY spectrum demonstrated the connectivities of C-2'' to C-6'' (Figure 1). HMBC correlations for protons of nitrogen bearing sp<sup>3</sup> methylene (H<sub>2</sub>-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<sub>C</sub> 62.3). This was supported by the downfield shift for H-4'' (Δδ 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.

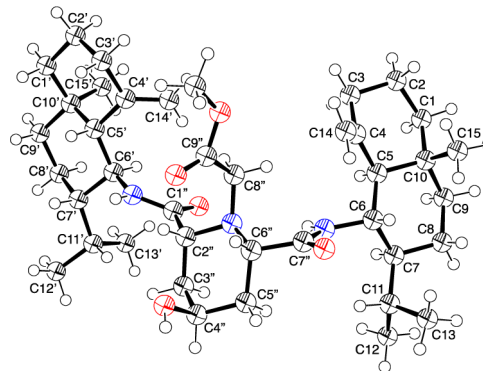
The relative configuration of unit A was deduced to be the same as that of halichonadin H<sup>4</sup> based on the NOESY analysis (Figure 2). Resemblance of the <sup>13</sup>C chemical shifts for units A and B of **1** implied that both units have the same relative configuration (Table 1). In unit C, 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 be assigned by the NOESY analysis.

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



**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.<sup>6</sup> The single crystal X-ray diffraction analysis of the crystal revealed the relative stereochemistry of **1**.<sup>7</sup> 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}.<sup>8</sup> 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**)<sup>9</sup> was obtained as an optically active colorless amorphous solid {[α]<sub>D</sub><sup>21</sup> –14.3 (c 0.73, MeOH)}. An IR absorption band at 1647 cm<sup>–1</sup> implied the presence of amide carbonyl functionality. The molecular formula, C<sub>47</sub>H<sub>72</sub>N<sub>4</sub>O<sub>4</sub>, 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; [α]<sub>D</sub><sup>21</sup> –14.3 (c 0.73, MeOH); IR (film) ν<sub>max</sub> 3274 and 1647 cm<sup>–1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR (Table 1); HRAPCIMS: *m/z* 757.56233 [M+H]<sup>+</sup> (calcd for C<sub>47</sub>H<sub>73</sub>N<sub>4</sub>O<sub>4</sub>, 757.56263).

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

**Table 1.**  $^1\text{H}$  (600 MHz) and  $^{13}\text{C}$  (150 MHz) NMR Data for Halichonadins **1** and **2** in  $\text{C}_5\text{D}_5\text{N}$ 

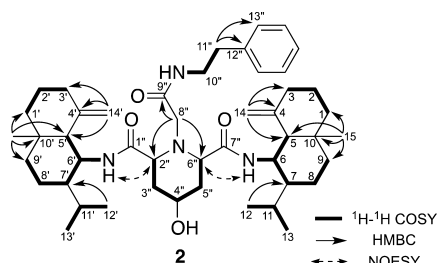
position	<b>1</b>		<b>2</b>	
	$^{13}\text{C}$	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$
1	42.2	1.31, 1.14 (1H each, m)	42.3	1.32, 1.19 (1H each, m)
2	24.5	1.49 (2H, m)	24.6	1.52 (2H, m)
3	38.5	2.25, 1.86 (1H each, m)	38.6	2.29, 1.95 (1H each, m)
4	147.3	—	147.4 <sup>b</sup>	—
5	56.6	1.96 (1H, m)	56.6	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 <sup>b</sup>	1.43, 1.32 (1H each, m)
9	40.6 <sup>b</sup>	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 <sup>a</sup> (1H, m)	107.4 <sup>b</sup>	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 <sup>b</sup>	—
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 <sup>b</sup>	1.43, 1.32 (1H each, m)
9'	40.5 <sup>b</sup>	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 <sup>a</sup> (1H, m)	107.6 <sup>b</sup>	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 <sup>b</sup>	4.04 (1H, m)
3''	37.5	2.25 (2H, m)	36.4 <sup>b</sup>	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 <sup>b</sup>	2.38 (2H, m)
6''	60.5	4.49 (1H, dd, $J = 9.2, 4.1$ Hz)	61.6 <sup>b</sup>	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)

<sup>a</sup> Signals were overlapped with that of HOD. <sup>b</sup> Signals may be interchangeable.

HRAPCIMS ( $m/z$  757.56233  $[\text{M}+\text{H}]^+$ ,  $\Delta$ –0.30 mmu). The  $^1\text{H}$  and  $^{13}\text{C}$  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 sesquiterpene having a

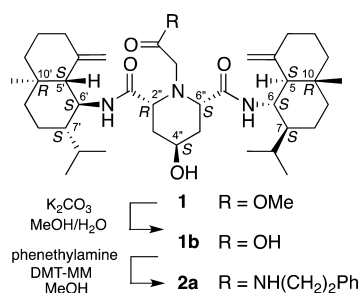
piperidine ring and a phenethylamine moiety. Analysis of the  $^1\text{H}$ – $^1\text{H}$  COSY and HMBC spectra suggested the gross structure of **2** as shown in Figure 4.

(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**)

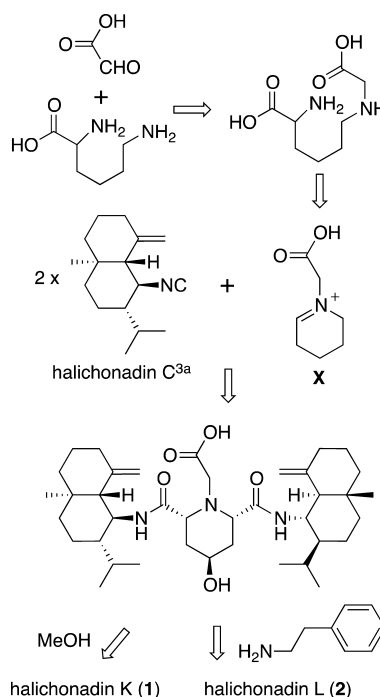


To elucidate the stereochemistry of halichonadin L (**2**), derivatization of halichonadin K (**1**) to **2a** was carried out (Scheme 1). Halichonadin K (**1**) was treated with  $K_2CO_3$  in MeOH/water (1:1) to give the demethyl derivative (**1b**). Condensation of **1b** and phenethylamine by DMT-MM<sup>10</sup> in MeOH furnished halichonadin L (**2a**). Since  $^1H$  NMR data (Supporting Information) and optical rotation  $\{[\alpha]_D^{18} -10.3$  (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,<sup>3a</sup> E,<sup>3b</sup> and G–I,<sup>4</sup> and *N,N'*-bis{(1*Z*,4*Z*)-7 $\alpha$ H-germacra-1(10),4-dienyl}urea<sup>11</sup> 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.<sup>3a</sup>

Halichonadin K (**1**) showed cytotoxicity against human epidermoid carcinoma KB cells ( $IC_{50}$  10.6  $\mu g/mL$ ) *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.