Haterumaimides J and K, Potent Cytotoxic Diterpene Alkaloids from the Ascidian *Lissoclinum* Species

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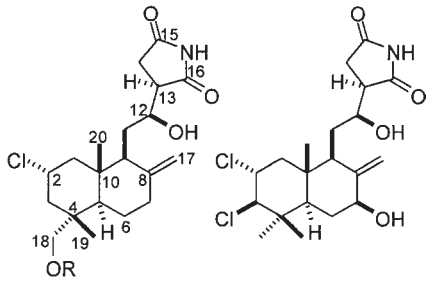
(Received December 13, 2001; CL-011256)

Two unique cytotoxic diterpene alkaloids, haterumaimides J and K, were isolated from the ascidian *Lissoclinum* sp. Their structures were elucidated by an extensive analysis of spectral data. Haterumaimides J and K showed potent cytotoxicity against P388 cells, with IC_{50} values of 0.23 ng/mL and 0.45 ng/mL, respectively.

As part of our continuing search for bioactive secondary metabolites from Okinawan marine organisms,[1,2] we investigated the ascidian *Lissoclinum* sp. collected off the coast of Hateruma Island, Okinawa, and previously reported the isolation, structure elucidation and absolute stereochemistries of haterumaimides A–E,[3] and F–I,[4] from the lipophilic extract of the ascidian. Further bioassay-guided fractionation of a cytotoxic extract yielded haterumaimides J (**1**) and K (**2**).[5] This unique class of diterpene alkaloid, dichlorolissoclimide (**3**),[6] was first discovered from the New Caledonia ascidian *Lissoclinum voeltzkowi* by Verbist and his co-workers in 1996. In this report, we describe the isolation, structure elucidation, relative stereochemistries and cytotoxicities of haterumaimides J (**1**) and K (**2**).

The *Lissoclinum* sp. (1.0 kg, wet weight) was first extracted with acetone. The acetone extract was partitioned between H_2O and EtOAc. The EtOAc extract (3.4 g) was suspended in aqueous MeOH (1 : 1) and then successively partitioned between aqueous MeOH and hexane, $CHCl_3$ and 1-BuOH. Bioassay-directed fractionation of the $CHCl_3$ extract (2.5 g) by a series of chromatographic processes, including column chromatography (SiO_2 and ODS) and HPLC on SiO_2 and ODS, gave haterumaimides J (**1**, 0.00075% of wet ascidian) and K (**2**, 0.00032%).



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Haterumaimide J (**1**) was isolated as a colorless oil. Its molecular formula was deduced to be $C_{20}H_{30}ClNO_4$ based on HRFABMS [m/z 384.1925 $(M+H)^+$, Δ-1.7 mmu and m/z 386.1920 $(M+H)^+ + 2$]. The IR spectrum showed absorption bands at ν_{max} 3400, 1720 and 1710 cm^{-1} that were assigned to hydroxyl and two imide carbonyl groups, respectively.[7] The ^1H and ^{13}C NMR data of **1** and **2** are presented in Table 1. The ^1H NMR spectrum of haterumaimide J (**1**) contained two methyl singlets at δ_H 0.67 and 0.68, a singlet at δ_H 11.01 for NH, a triplet at δ_H 4.74 ($J = 5.5$ Hz) for a primary hydroxyl proton at C-18, a doublet at δ_H 4.94 ($J = 5.0$ Hz) for a secondary hydroxyl proton at C-12, two doublets of doublets at δ_H 3.20 ($J = 11.0, 5.5$ Hz)

Table 1. NMR data for Haterumaimides J (**1**) and K (**2**)

	1		**2**	
C No.	^{13}C[a] (mult.)	^1H[b] (mult., *J*/Hz)	^{13}C[c] (mult.)	^1H[d] (mult., *J*/Hz)
1	48.4 (t)	2.12 (ddd, 12.5, 4.0, 1.5) 1.27 (t, 12.5)	49.0 (t)	2.22 (ddd, 13.0, 4.0, 2.0) 1.23 (dd, 13.0, 12.0)
2	57.3 (d)	4.38 (tt, 12.5, 4.0)	54.7 (d)	4.17 (tt, 12.0, 4.0)
3	45.9 (t)	1.67 (ddd, 12.5, 4.0, 1.5) 1.78 (t, 12.5)	46.2 (t)	1.97 (ddd, 12.0, 4.0, 2.0) 1.68 (m)
4	40.3 (s)	—	39.1 (s)	—
5	46.0 (d)	1.52 (dd, 12.5, 2.5)	48.4 (d)	1.39 (dd, 12.5, 6.0)
6	22.9 (t)	1.57 (dddd, 12.5, 5.0, 3.5, 2.5) 1.15 (dq, 12.5, 4.0)	23.6 (t)	1.60 (m) 1.30 (dddd, 13.0, 12.5, 12.0, 6.5)
7	37.0 (t)	2.30 (ddd, 13.0, 4.0, 3.5) 1.96 (dt, 13.0, 5.0)	37.5 (t)	2.41 (ddd, 13.0, 6.5, 2.5) 1.92 (ddd, 13.0, 12.0, 6.4)
8	147.6 (s)	—	147.1 (s)	—
9	51.4 (d)	1.65 (m)	53.5 (d)	1.70 (m)
10	41.3 (s)	—	41.7 (s)	—
11α	30.0 (t)	1.58 (m)	29.3 (t)	1.75 (m)
11β		1.40 (ddd, 13.0, 9.5, 5.0)		1.55 (m)
12	66.9 (d)	3.99 (m)	69.2 (d)	4.33 (dt, 7.5, 2.0)
13	45.5 (d)	2.89 (ddd, 9.0, 5.0, 1.5)	46.8 (d)	2.89 (ddd, 8.5, 5.0, 2.0)
14	29.0 (t)	2.54 (dd, 17.5, 5.0) 2.46 (dd, 17.5, 9.5)	29.4 (t)	2.85 (dd, 17.5, 5.0) 2.68 (dd, 17.4, 8.5)
15	181.1[e] (s)	—	178.8[e] (s)	—
16	178.8[e] (s)	—	176.4[e] (s)	—
17	107.7 (t)	4.87 (brs) 4.69 (brs)	109.0 (t)	4.96 (brs) 4.73 (brs)
18	69.3 (t)	3.20 (dd, 11.0, 5.5) 2.84 (dd, 11.0, 5.5)	71.7 (t)	3.85 (d, 11.0) 3.63 (d, 11.0)
19	17.9 (q)	0.68 (s)	18.0 (q)	0.85 (s)
20	15.0 (q)	0.67 (s)	15.4 (q)	0.67 (s)
21			171.0 (s)	—
22			21.0 (q)	2.1 (s)
		11.01 (s, NH) 4.94 (d, 5.0, OH-12) 4.74 (t, 5.5, OH-18)		8.05 (s, NH)

[a]Recorded at 125 MHz (δ_{DMSO-d_6} 39.5). [b]Recorded at 500 MHz (δ_{DMSO-d_6} 2.49). [c]Recorded at 125 MHz (δ_{CDCl3} 77.2). [d]Recorded at 500 MHz (δ_{CDCl3} 7.24).

and 2.84 ($J = 11.0$, 5.5 Hz) for two *gem* methylene protons and two broad singlets at δ_H 4.87 and 4.69 for exomethylene protons. Resonances at δ_C 181.1 (s), 178.8 (s), 147.6 (s) and 107.7 (t) in the ^{13}C NMR spectrum were assigned to two imide carbonyl carbons at C-15 and C-16, a tetrasubstituted olefinic carbon at C-8 and an exomethylene carbon at C-17, which accounted for three sites of unsaturation. Thus, haterumaimide J (**1**) must be tricyclic, to account for the six sites of unsaturation required by the molecular formula.

One of the three rings was clearly deduced to be a succinimide moiety from the HMBC correlations of the NH signal at δ_H 11.01 (s) to δ_C 181.1 (s), 178.8 (s), 29.0 (t) and 45.5 (d). The remaining two carbocyclic rings were assigned to be a substituted decalin structure with a chlorine atom at C-2, and an exocyclic double bond between C-8 and C-17 based on the analysis of HOHAHA and COSY spectra together with the HMBC correlations, as shown in Figure 1.

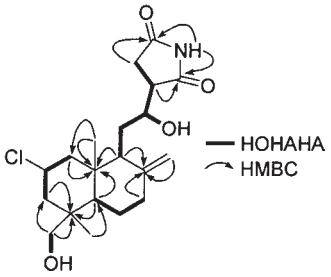

Figure 1. Partial structures of haterumaimide J (**1**) based on HOHAHA (bold line) and some important HMBC correlations (arrows).

A detailed analysis of the HOHAHA, ^1H–^1H COSY and HMBC spectra finally led to the entire planar carbon framework of haterumaimide J as a monochlorinated labdane alkaloid with a rare succinimide moiety, as shown in **1**.

The relative stereochemistry of the decalin part of the haterumaimide J (**1**) was determined to be 2*S**, 4*S**, 5*S**, 9*R** and 10*R** from the NOESY correlations of H-2/H_3-20, H-2/H_3-19, H_3-19/H_3-20, H_3-20/H-6β, H-1β/H_3-20, H-1α/H-3α, H-1α/H-5, H-1α/H-9, H-3α/H_2-18, H-3α/H-5, H-5/H-7α, H-5/H-9, H-5/H-6α and H-9/H-7α (Figure 2) together with an analysis of the vicinal coupling constants of H-2 (tt, $J = 12.5$, 4.0 Hz) and H-5 (dd, $J = 12.5$, 2.5 Hz). The relative stereochemistries, 12*S** and 13*R** were tentatively proposed by a careful examination of the NOESY correlations of H-12/H-13, H-12/H-9, H-11α/H-12, H-13/H-14α, and H-11β/H-14β (Figure 2) together with the coupling constants of H-13 (ddd, $J = 9.0$, 5.0 and 1.5 Hz) and comparison with the related compounds isolated from the same organism.[3,4]

The ^1H and ^{13}C NMR spectra (Table 1) of haterumaimide K (**2**) resembled to those of **1** except that there were two more carbon signals at δ_C 171.0 (s) and 21.0 (q) together with a proton signal at δ_H 2.1 (s). The strong HMBC correlation of δ_H 2.1 (s) to δ_C 171.0 (s) clearly indicated that **2** is a monoacetylated derivative of **1** and the acetyl group was assigned to be located at C-18 based on the HMBC correlation of δ_H 3.85 (d, $J = 11.0$ Hz) and 3.63 (d, $J = 11.0$ Hz) to δ_C 171.0 (s). Extensive analysis of the HOHAHA, ^1H–^1H COSY and HMBC spectra led to the planar structure of haterumaimide K (**2**) as a monoacetylated derivative of haterumaimide J (**1**). The structure of **2** was further clarified by

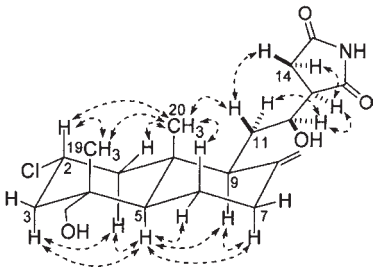

Figure 2. Some selected NOESY correlations of haterumaimide J (**1**).

the base-catalyzed hydrolysis (cat. NaOMe, MeOH, rt, 90%) of **2** into its congener, **1**. The ^1H, ^{13}C NMR and $[\alpha]_D$ data for **1** and the hydrolysate of **2** were identical,[8] thus confirming the structure of haterumaimide K, as shown in **2**.

Haterumaimides J (**1**) and K (**2**) completely inhibited the first cleavage of the cell division of fertilized sea urchin eggs at 3 ppm, and showed potent cytotoxicity against P388 cells with IC_{50} values of 0.23 ng/mL and 0.45 ng/mL, respectively. Chlorinated labdane alkaloids with a succinimide moiety are extremely rare in nature. These labdane alkaloids are extremely important for drug leads.[9] Further chemical and biological studies are underway in our laboratory.

We thank Prof. Tatsuo Higa, University of the Ryukyus, for his valuable suggestion throughout this work and Dr. Yuichi Hirose of the same university for identifying the ascidian. This work was supported in part by Wako Pure Chemical Industries Ltd. and by Grants-in-Aid (Nos. 11558079 and 12045235) from the Ministry of Education, Science, Sports, and Culture of Japan.

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7 **1**: $[\alpha]_D^{29} + 68^\circ$ (*c* 0.92); FT/IR (film) ν_{max} 3400, 2910, 1720, 1710, 1220, 1050 cm^{-1}; UV (MeOH) λ_{max} 210 nm (ε 3800); HRFABMS *m/z* $(M + H)^+$ 384.1925 (calcd for $C_{20}H_{31}ClNO_4$, 384.1942, Δ −1.7 mmu). **2**: $[\alpha]_D^{29} + 59.6^\circ$ (*c* 0.19); FT/IR (film) ν_{max} 3405, 2902, 1720, 1710, 1250, 1040 cm^{-1}; UV (MeOH) λ_{max} 210 nm (ε 3600); HRFABMS *m/z* $(M + H)^+$ 426.2032 (calcd for $C_{22}H_{33}ClNO_5$, 426.2047, Δ −1.5 mmu).
8 $[\alpha]_D^{30} + 58^\circ$ (*c* 0.04); ^1H NMR (DMSO-d_6, 500 MHz) δ 11.03 (s, NH), 4.96 (d, $J = 5.0$ Hz, OH-12), 4.88 (brs, H-17a, 1H), 4.75 (t, $J = 5.5$ Hz, OH-18), 4.70 (brs, H-17b, 1H), 4.38 (tt, $J = 12.5$, 4.0 Hz, H-2, 1H), 4.00 (m, H-12, 1H), 3.20 (dd, $J = 11.0$, 5.4 Hz, H-18a, 1H), 2.90 (ddd, $J = 9.0$, 5.0, 1.6 Hz, H-13, 1H), 2.85 (dd, $J = 11.0$, 5.5 Hz, H-18b, 1H), 2.55 (dd, $J = 17.5$, 5.0 Hz, H-14α, 1H), 2.46 (dd, $J = 17.5$, 9.0 Hz, H-14β, 1H), 2.30 (ddd, $J = 12.5$, 4.5, 3.5 Hz, H-7β, 1H), 2.12 (ddd, $J = 12.4$, 4.0, 1.5 Hz, H-1β, 1H), 1.95 (dt, $J = 12.5$, 4.5 Hz, H-7α, 1H), 1.80 (t, $J = 12.5$ Hz, H-3α, 1H), 1.68 (ddd, $J = 12.5$, 4.0, 1.5 Hz, H-3β, 1H), 1.65 (m, H-9, 1H), 1.57 (m, H-11α, 1H), 1.56 (m, H-6β, 1H), 1.50 (dd, $J = 12.5$, 3.0 Hz, H-5, 1H), 1.40 (ddd, $J = 13.0$, 9.5, 5.0, H-11β, 1H), 1.29 (t, $J = 12.5$ Hz, H-1α, 1H), 1.15 (m, H-6α, 1H), 0.69 (s, CH_3-19, 3H), 0.67 (s, CH_3-20, 3H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 181.0 (s, C-15), 178.8 (s, C-16), 147.5 (s, C-8), 107.7 (t, C-17), 69.3 (t, C-18), 66.9 (d, C-12), 57.5 (d, C-2), 51.4 (d, C-9), 48.5 (t, C-1), 46.0 (d, C-5), 45.8 (t, C-3), 45.5 (d, C-13), 41.3 (s, C-10), 40.2 (s, C-4), 37.0 (t, C-7), 30.0 (t, C-11), 29.0 (t, C-14), 22.8 (t, C-6), 18.0 (q, C-19), 15.0 (q, C-20).
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