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Densanins A and B, New Macrocyclic Pyrrole Alkaloids Isolated from the Marine Sponge *Haliclona densaspicula*

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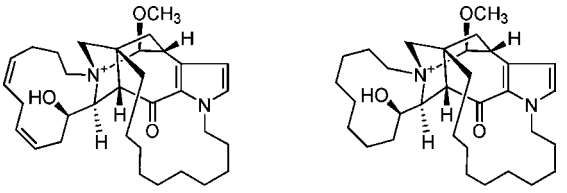
ABSTRACT



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Densanins A (1) and B (2) were isolated from the sponge *Haliclona densaspicula*. On the basis of spectral data and the Mosher ester method, the complete structures were characterized as hexacyclic diamine alkaloids, which were probably derived from 3-alkylpyridine. Compounds 1 and 2 showed relatively potent inhibitory effects on lipopolysaccharide-induced nitric oxide production in BV2 microglial cells with IC_{50} values of 1.05 and 2.14 μM, respectively.

Since the discovery of manzamine A in 1986, numerous macrocyclic diamine alkaloids have been isolated from marine sponges.[1] Most of these alkaloids, which are biogenetically derived from 3-alkylpyridine or reduced 3-alkylpyridine, are structurally diverse and biologically active. Much attention has been paid to the total synthesis and biosynthesis of these alkaloids because of their unprecedented structural scaffolds and bioactivities.[2] Recently, we carried out studies on the extract from the sponge *Haliclona densaspicula*, which initially showed moderate cytotoxicity, to search for new bioactive compounds from Korean marine organisms. From the fractionation guided by the brine shrimp lethality test, two interesting compounds, densanins A and B, were isolated but showed no apparent cytotoxic effect. The structure of densanins was characterized as fused hexacyclic diamine alkaloids, with a pyrrole ring joined to the tricyclic core. In addition, they have the tertiary amine group as saraine A, a peculiar diamine alkaloid.[3] In this study, we focus on the isolation and complete structural characterization of densanins A and B and discuss their biological activity, i.e., their potent inhibitory effect on nitric oxide (NO) production in BV2 microglial cells. Furthermore, we proposed a hypothetical biogenetic pathway for the densanins through reduced 3-alkylpyridine, similar to the manzamine biosynthesis suggested by Baldwin and Whitehead.[4]

The sponge *H. densaspicula* was collected offshore at Keomun Island, Korea, in 2008. The mixture of densanins

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(1) (a) Sakai, R.; Higa, T.; Jefford, C. W.; Benardinelli, G. *J. Am. Chem. Soc.* **1986**, *108*, 6404–6405. (b) Rodriguez, J. *Studies in Natural Products Chemistry*; Elsevier: New York, 2000; Vol. 24, pp 573−681 and references cited therein. (c) Jiménez, J. I.; Goetz, G.; Mau, C. M. S.; Yoshida, W. Y.; Scheuer, P. J.; Williamson, R. T.; Kelly, M. *J. Org. Chem.* **2000**, *65*, 8465–8469. (d) Jang, K. H.; Kang, G. W.; Jeon, J.-E.; Lim, C.; Lee, H.-S.; Sim, C.-J.; Oh, K.-B.; Shin, J. *Org. Lett.* **2009**, *11*, 1713–1716.

(2) (a) Toma, T.; Kita, Y.; Fukuyama, T. *J. Am. Chem. Soc.* **2010**, *132*, 10233–10235. (b) Garg, N. K.; Hiebert, S.; Overman, L. E. *Angew. Chem., Int. Ed.* **2006**, *45*, 2912–2915. (c) Defant, A.; Mancini, I.; Raspor, L.; Guella, G.; Turk, T.; Sepčic, K. *Eur. J. Org. Chem.* **2011**, 3761–3767.

(3) (a) Cimino, G.; Scognamiglio, G.; Spinella, A.; Trivellone, E., J. *Nat. Prod.* **1990**, *53*, 1519–1525.

(4) Baldwin, J. E.; Whitehead, R. C. *Tetrahedron Lett.* **1992**, *33*, 2059–2062.

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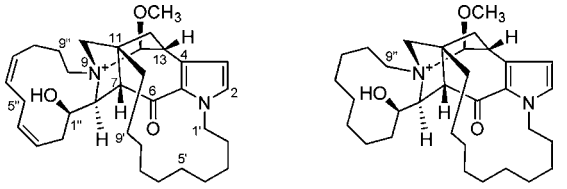
obtained after methanol extraction and solvent partition was purified by reverse-phased HPLC eluting with gradient solvents to obtain A (**1**, yellow oil, 12 mg) and B (**2**, yellow oil, 8 mg).

The molecular formula of densanin A (**1**)[5] was determined to be $C_{33}H_{49}N_2O_3^+$, which corresponded to 11 degrees of unsaturation, on the basis of positive high-resolution FABMS ($[M]^+$ peak at m/z = 521.3745, Δ = 0.2 mmu). The IR spectrum of **1** showed strong absorption bands attributed to the hydroxyl and carbonyl functionalities at 3400 and 1635 cm^{-1}, respectively. The ^{13}C NMR spectrum of **1** featured eight olefinic resonances (δ_c 112.9, 126.6, 126.8, 130.1, 130.4, 132.0, 135.1, and 138.5), one ketone resonance (δ_c 190.0), one methoxy resonance (δ_c 58.6), and a downfield-shifted methine signal (δ_c 96.1), which could be attributed to a carbon attached to two heteronuclei. Further analysis of the edited HSQC spectra of **1** revealed 17 additional methylenes and five methines but no methyl group. These data accounted for five out of the 11 degrees of unsaturation, indicating the presence of six rings in **1**.



Detailed interpretation of 1D and 2D NMR (COSY, TOCSY, HSQC, and HMBC) data indicated that **1** was composed of a tetracyclic core, substructure A, with two linear carbon chains B and C, as shown in Figure 1. The nitrogen atom and ^{13}C NMR chemical shifts with large one-bond coupling constants ($^1J_{C2H2}$ = 183 and $^1J_{C3H3}$ = 174 Hz) indicated the existence of a pyrrole ring, and this was supported by the HMBC correlations of H-2 (δ_H 7.15) and H-3 (δ_H 6.35) with the two quaternary carbons C-4 (δ_C 138.5) and C-5 (δ_C 126.8) (Figure 2). All of the carbons in the pyrrole unit could be unambiguously assigned by the HMBC correlations between the *N*-methylene protons and two pyrrole carbons C-2 (δ_C 132.0) and C-5 (δ_C 126.8). Furthermore, the conjugation of a keto group with the pyrrole through C-5 was revealed by HMBC correlations from the doublet methine proton H-7 (δ_H 3.75) to the two corresponding quaternary carbons C-5 and C-6; this was supported by the strong UV absorption at a long wavelength of 303 nm[6] and the ^{13}C chemical shifts for an α,β-unsaturated ketone. On the other hand, AB-split methylene protons on the nitrogen-bearing carbon C-10 (δ_C 68.8) showed extensive HMBC correlations with five adjacent carbons: one quaternary C-11 (δ_C 47.7), three methines C-7 (δ_C 58.7), C-8 (δ_C 76.4), and C-14 (δ_C 96.1), and one methylene C-12 (δ_C 38.5) (Table 1). Among them, the methine carbon C-7, bonded directly with H-7, was further connected with the nitrogen-bearing methine C-8 by the COSY correlation between their corresponding protons. Together with all these correlations, the HMBC correlation for H-7/C-11 led to the existence of a pyrrolidine moiety. In addition, the HMBC correlations between H-13, coupled with H-12, and the quaternary C-11 and other methine carbon C-14 allowed us to construct 1-azabiscyclo[3.2.1]octane (Figure 2). This was also supported by the correlations in the $^{15}N-H$ HMBC experiment: H-7/N-9, H-13/N-9, and H-14/N-9. In this unit, the carbon chemical shifts for C-9 and C-14 were unusually shifted to the downfield due to the ring current by the pyrrole functional group. Furthermore, HMBC correlations from H-13 to the quaternary carbons C-3 and C-4 in pyrrole established the tetracyclic frame. Thus, substructure A was completed with the attachment of the methoxy group to the carbon resonating at C-14, as justified by the HMBC correlation with the methoxy proton at δ_H 3.74.

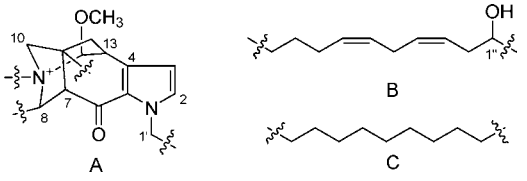


Figure 1. Substructures of densanin A (**1**).

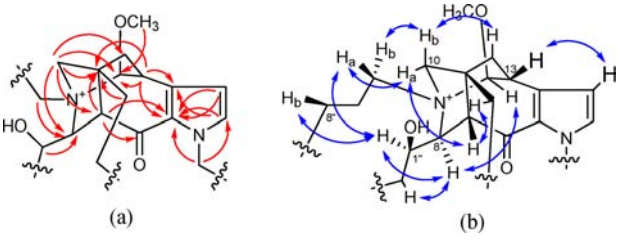


Figure 2. (a) Key HMBC correlations (H→ C) and (b) NOE correlations of **1**.

In the case of substructure B, consecutive COSY couplings starting from H-1″ (δ_H 4.45) and TOCSY correlations revealed a deca-3,6-dien-1-ol moiety, as shown in Figure 1. Substructure C was composed of nine upfield-shifted methylene groups unassigned. A combination of the COSY, TOCSY, and HMBC spectral data led substructure C to be a linear nonane chain.

Substructures A–C could be assembled on the basis of the HMBC and COSY correlations. First, the oxymethine C-1″ and methylene C-10″ at both terminals of substructure B were connected with C-8 and the nitrogen of 1-azabiscyclo[3.2.1] octane, respectively, from the HMBC correlations. Similarly, connection of the nonane with substructure A was accomplished by the COSY correlations

(5) $[\alpha]^{25}_D$ −85.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.02), 303 (4.11) nm; IR (film) ν_{max} 3397, 2927, 1635, 1413, 1200, 726 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRFAB(+)MS m/z 521.3745 $[M]^+$ (calcd for $C_{33}H_{49}O_3N_2$ 521.3743).

(6) Jiang, Z. D.; Gerwick, W. H. *J. Nat. Prod.* **1991**, *54*, 403–407.

Table 1. ^{1}H and ^{13}C NMR Data for Densanins A (**1**)[a] and B (**2**) Recorded in CD_3OD

	densanin A (**1**)			densanin B (**2**)	
no.	δ_C	δ_H (mult, J Hz)	HMBC	δ_C	δ_H (mult, J Hz)
2	132.0, CH	7.15, d (2.5)	C-3, 4, 5, 1′	131.8, CH	7.13, d (2.5)
3	112.9, CH	6.35, d (2.5)	C-2, 4, 5, 13	112.7, CH	6.35, d (2.5)
4	138.5, C			138.9, C	
5	126.8, C			126.9, C	
6	190.0, C			189.7, C	
7	58.7, CH	3.75, d (5.9)	C-5, 6, 8, 11, 12, 10′, 1″	58.5, CH	3.68, d (5.9)
8	76.4, CH	3.87, d (5.9)	C-6, 7, 10, 14	78.5, CH	4.08, d (5.9)
10a	68.8, CH_2	3.43, dd (10.5, 1.5)	C-11, 12, 14	68.5, CH_2	3.37, dd (10.5, 2.0)
10b		3.60, dd (10.5, 1.0)	C-7, 8, 12		3.55, dd (10.5, 0.7)
11	47.7, C			47.9, C	
12a	38.5, CH_2	2.25, d (13.9)	C-4, 10, 11, 13, 14	38.8, CH_2	2.23, d (13.9)
12b		2.33, dd (13.9, 6.1),	C-7, 10, 11, 13, 14		2.31, dd (13.9, 6.1),
13	37.3, CH	3.73, d (6.1)	C-3, 4, 5, 11, 14	37.7, CH	3.73, d (6.1)
14	96.1, CH	4.85, br s	C-4, 10, OCH_3	96.2, CH	4.86, br s
OCH_3	58.6, CH_3	3.74, s	C-14	59.0, CH_3	3.75, s
1′a	47.9, CH_2	3.90, dt (13.5, 5.6)	C-2, 5, 2′, 3′	48.1, CH_2	3.87, dt (13.5, 5.6)
1′b		5.17, ddd (13.5, 9.1, 4.9)			5.14, ddd (13.5, 9.1, 4.9)
2′	30.4, CH_2	1.76, m ; 1.89, m	C-1′, 3′	30.4, CH_2	1.73, m ; 1.88, m
3′	25.3, CH_2	0.64, m ; 1.22, m	C-1′, 2′, 4′	25.4, CH_2	0.69, m ; 1.21, m
4′	26.7, CH_2	1.06, m ; 1.51, m		26.8, CH_2	1.07, m ; 1.47, m
5′	28.9, CH_2	0.99, m ; 1.29, m		28.8, CH_2	1.00, m ; 1.29, m
6′	27.2, CH_2	1.21, m		27.2, CH_2	1.20, m
7′	28.8, CH_2	1.22, m		28.9, CH_2	1.21, m
8′	29.8, CH_2	1.20, m		29.9, CH_2	1.21, m
9′	26.7, CH_2	1.51, m		26.6, CH_2	1.51, m
10′	36.7, CH_2	1.56, m ; 1.69, m	C-11, 12, 8′, 9′	36.8, CH_2	1.55, m ; 1.68, m
1″	67.9, CH	4.45, dd (9.5, 6.9)	C-7, 8, 2″	66.0, CH	3.93, d (9.8)
2″	31.8, CH_2	2.06, m ; 2.39, m	C-8, 1″	31.4, CH_2	1.26, m ; 1.51, m
3″	126.6, CH	4.93, m		21.2, CH_2	1.38, m ; 1.69, m
4″	135.1, CH	5.46, td (11.3, 4.7)		24.1, CH_2	1.52, m
5″	27.4, CH_2	2.60, m ; 3.08, m		21.1, CH_2	1.37, m
6″	130.4, CH	5.64, m		24.8, CH_2	1.47, m
7″	130.1, CH	5.61, m		25.0, CH_2	1.50, m ; 1.73, m
8″	25.5, CH_2	2.18, m ; 2.27, m		19.4, CH_2	1.63, m ; 1.84, m
9″a	26.6, CH_2	1.27, m		57.0, CH_2	2.95, dd (13.9, 8.6)
9″b		2.03, m			4.05, m
10″a	55.5, CH_2	3.06, dd (13.7, 8.6)	C-14		
10″b		3.94, m	C-8, 9″		

[a] Measured at 500 MHz (^{1}H) and 125 MHz (^{13}C).

between the terminal methylene protons (δ_H 1.76 and 1.89) of the chain and H-1′, as well as by the HMBC correlations between the other terminal protons and the quaternary carbon C-11 (Figure 2). Accordingly, the positively charged gross structure of **1** was determined, consistent with the molecular formula.

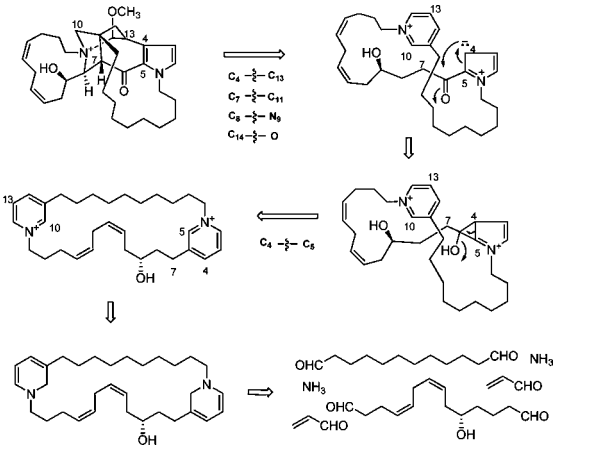
The relative stereochemistry of **1** was elucidated on the basis of the ROESY data with ^{1}H coupling constants. The ROE correlations of H-8/H-14 and H_b-10/H_b-12 established the configuration of the 1-azabiscyclo[3.2.1] octane moiety and the stereochemistry of the methoxy group at C-14. The H-8 signal was observed as a doublet because of the apparent coupling (J_{HH} = 5.9 Hz) with H-7 and the near zero coupling with H-1″. This observation revealed that H-8 is oriented in the opposite direction with respect to H-7 but at right angles with respect to H-1″. The orientation of H-7 was also confirmed by the ROE correlations of H-7 with H_a-10 and H-10′ (Figure 2). Furthermore, the ROE correlations of H-1″ with H-8, H-8″, and H_a-10″ indicated that H-1″ directed toward the interior of substructure B. Finally, the *Z*-isomerism of the two disubstituted double bonds was confirmed from the chemical shift of the allylic carbons and the ROE correlations between their protons.

The absolute stereochemistry of **1** could be determined by the modified Mosher ester reaction at C-1″. The shielding effect of the phenyl group in the MTPA ester of **1** resulted in different $\Delta\delta^{SR}$ signs for each proton spatially close to the MTPA group centered on C-1″, with positive and negative values for H-2″ and H_a-10, respectively

(7) $[\alpha]^{25}_D$ −98.8 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 203 (2.89), 303 (2.98) nm; IR (film) ν_{max} 3397, 2927, 1635, 1413, 1200, 726 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRFAB(+)MS *m*/*z* 511.3898 $[M]^+$ (calcd for $C_{32}H_{51}O_3N_2$ 511.3900).

(Supporting Information). Therefore, C-1″ was determined to be *R* configuration.

Scheme 1. Plausible Retrobiosynthetic Pathway of Densanin A (**1**)



Densanin B (**2**)[7] had the molecular formula $C_{32}H_{51}N_2O_3^+$, as revealed by the combination of ^{13}C NMR and positive HRFABMS (*m*/*z* 511.3898 measured for $[M]^+$) data. Comparison of the ^1H and ^{13}C NMR spectra of **1** and **2** revealed the absence of four olefinic signals and one carbon signal in the latter case. By a combination of COSY, HSQC, and HMBC experiments, **2** was identified to possess the same substructure A as **1**. Furthermore, the ^1H and ^{13}C NMR chemical shifts for nine methylenes among the 16 methylene groups resonating in the upfield region (10−40 ppm) were in good agreement with those of **1**, corresponding to substructure C. The HMBC correlations confirmed that the linear nonane chain was connected to substructure A, as in the case of **1**. In a similar way, the remaining seven methylenes and a methylene at δ_C 57.0 formed a linear octane chain, which could be proved by the COSY and TOCSY spectra. Each terminal carbon was linked to the nitrogen atom of substructure A and the oxymethine carbon at δ_C 66.0 by the HMBC and COSY spectra, thus establishing the planar structure of **2**.

Densanins A (**1**) and B (**2**) are unprecedented alkaloids including a 1-azabiscyclo[3.2.1] octane unit and a pyrrole ring. Moreover, these two compounds were characterized by a hexacyclic diamine skeleton with two long chains, reminiscent of the structures of diverse macrocyclic alkaloids derived from 3-alkylpyridine, primarily found in marine sponges. Scheme 1 depicts the retro-biosynthesis of densanin A from 3-alkylpyridine. After the cleavage of C-4/C-13, C-7/C-11, and C-8/N-9, the carbanion from the pyrrole ring attacks the neighboring ketone group to give 2-azabicyclo[3.1.0]hexa-1,3-dien-6-ol as an intermediate.[8] Subsequently, rearrangement of this intermediate can afford a pyridine ring and then form two long-chain dialdehydes with two acroleins and two ammonias, as suggested by Baldwin and Whitehead.[4]

Densanins A and B showed the inhibitory effects on NO production evaluated in lipopolysaccharide (LPS)-activated BV2 microglial cells using the Griess assay.[9] The amount of nitrite released into the medium increased 3-fold, from 14.55 ± 1.26 to 48.25 ± 2.88 μmol, after exposure to 100 ng/mL of LPS for 24 h. The NO production induced by LPS could be significantly suppressed by pretreatment with densanins A and B (IC_{50} values of 1.05 and 2.14 μM, respectively) without affecting cell viability.

In summary, we isolated new hexacyclic diamine alkaloids, densanins A and B, from the sponge *H. densaspicula*. The two compounds showed no cytotoxicity in several cells but significant potent inhibition activity for LPS-induced NO production in BV-2 microglial cells.

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Supporting Information Available. Experimental procedures and spectral data of **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

(8) Tanny, S. R.; Grossman, J.; Fowler, F. W. *J. Am. Chem. Soc.* **1972**, *94*, 6495–6501.

(9) Woo, M. S.; Jung, S. H.; Hyun, J. W.; Kim, H. S. *Neurosci. Lett.* **2004**, *356*, 187–190.

The authors declare no competing financial interest.