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Zamamidines A and B, New Manzamine Alkaloids from the Sponge *Amphimedon* Species

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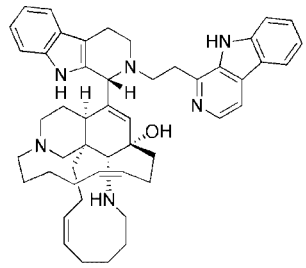
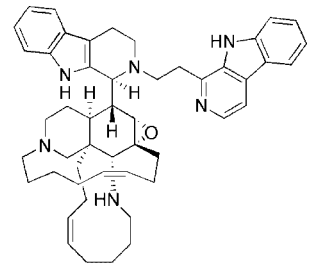
ABSTRACT



zamamidine A (1)



zamamidine B (2)

New manzamine alkaloids, zamamidines A (1) and B (2), have been isolated from an Okinawan marine sponge *Amphimedon* species. The structures and stereochemistry of 1 and 2 were elucidated from the spectroscopic data and conformational analysis. Zamamidines A (1) and B (2) are the first manzamine alkaloids possessing a second *β*-carboline ring via an ethylene unit at N-2 of manzamine H and 1,2,3,4-tetrahydromanzamine B, respectively.

The manzamine alkaloids have been reported from several marine sponge genera and are well-known to have unique polycyclic ring systems.[1] During our search for bioactive metabolites from marine organisms,[2] we have investigated extracts of an Okinawan marine sponge *Amphimedon* sp. (SS-975) and isolated new manzamine alkaloids, zamamidines A (**1**) and B (**2**).[3,4] Zamamidines A (**1**) and B (**2**) are the first manzamine alkaloids possessing a second *β*-carboline ring linked via an ethylene unit at N-2 of manzamine H and 1,2,3,4-tetrahydromanzamine B, respectively. Here we describe the isolation and structure elucidation of **1** and **2**.

The sponge *Amphimedon* sp. (SS-975) collected off Seragaki beach, Okinawa, was extracted with MeOH. EtOAc-soluble materials of the extract were subjected to silica gel column chromatographies to yield zamamidines A (**1**, 0.0011% wet weight) and B (**2**, 0.0006%) together with known manzamine alkaloids.

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(2) Takahashi, Y.; Kubota, T.; Ito, J.; Mikami, Y.; Fromont, J.; Kobayashi, J. *Bioorg. Med. Chem.* **2008**, *16*, 7561–7564.

(3) Zamamidine A (**1**): pale yellow amorphous solid; $[\alpha]_D^{21}$ −74 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} 352 (*ε* 4900), 339 (5000), 290 (22000), 283 (sh 18000), 251 (sh 24000), and 229 nm (58000); CD (MeOH) λ_{ext} 293 (Δ*ε* −3.6), 269 (−2.9), and 223 nm (+16.4); IR (film) ν_{max} 3200, 2850, 1630, and 1460 cm^{-1}; ESIMS (pos.) *m*/*z* 749.5 $(M + H)^+$; HRESIMS (pos.) *m*/*z* 749.4893 $[(M + H)^+$, calcd for $C_{49}H_{61}N_6O$, 749.4907].

(4) Zamamidine B (**2**): pale yellow amorphous solid; $[\alpha]_D^{21}$ +51 (*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} 352 (*ε* 6000), 339 (6000), 290 (27000), 283 (sh 22000), 253 (sh 31000), and 230 nm (76000); CD (MeOH) λ_{ext} 295 (Δ*ε* +5.8), 268 (+6.1), and 223 nm (−17.7); IR (film) ν_{max} 3200, 2850, 1630, and 1460 cm^{-1}; ESIMS (pos.) *m*/*z* 749.5 $(M + H)^+$; HRESIMS (pos.) *m*/*z* 749.4894 $[(M + H)^+$, calcd for $C_{49}H_{61}N_6O$, 749.4907].

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Table 1. ^{1}H and ^{13}C NMR Data for Zamamidine A (**1**) in $CDCl_3$

position	^{13}C	^{1}H	HMBC	NOESY	position	^{13}C	^{1}H	HMBC	NOESY
1	67.3 d	4.09 (brs)	4a, 9a, 10, 11, 12, 24, 11′	3b, 4b, 9, 11, 11′a, 11′b	24	42.6 d	0.98 (m)	23b	22b, 35b
3	49.6 t	3.80 (m)	1, 4, 4a	3b, 4b, 10′a, 11′a, 11′b	25	42.6 s			
		2.63 (m)		1, 3a, 4b	26	59.2 d	3.36 (m)	24, 28, 35	13a, 13b, 17a, 18b, 28a
4	21.4 t	3.20 (m)	11′	4b, 5	28	53.3 t	2.76 (m)		13b, 26, 28b, 29, 32
		2.99 (m)	4a, 9a	1, 3a, 3b, 4a, 5			2.60 (m)	26, 30	13b, 18b, 28a, 29, 30a
4a	107.2 s				29	28.8 t	1.51[a] (m)		28a, 28b
4b	127.5 s				30	28.4 t	1.81 (m)		28b, 32
5	118.1 d	7.59 (d[b])	4a, 4b, 7, 8a	4a, 4b			1.34 (m)		
6	119.4 d	7.17 (t[b])	4b, 8	5, 7	31	25.9 t	2.47 (m)		
7	121.5 d	7.18 (t[b])	5, 5a	6, 8			1.34 (m)		
8	111.0 d	7.26 (d[b])	4b, 6, 8a	7, 13a, 14	32	130.4 d	5.27 (m)		28a, 30a, 34a, 36a, 36b
8a	135.5 s				33	131.1 d	5.20 (m)		34b, 35b, 36a, 36b
9		7.57 (brs)	4a, 4b, 8a	1, 11, 13a, 14, 23a, 23b	34	27.1 t	2.41 (m)		32, 34b
9a	133.7 s						1.49 (m)		33, 34a
10	142.7 s				35	36.0 t	1.59 (m)		33, 35b
11	131.3 d	5.66 (s)	1, 13, 14, 24, 26	1, 9, 13a, 14, 11′a			−0.54 (m)		23a, 24, 33, 35a, 36b
12	69.8 s				36	64.3 t	2.69 (m)		18b, 32, 33, 35b, 36b
13	40.5 t	1.97 (m)		8, 9, 11, 13b, 26			0.73 (m)		22b, 33, 35b, 36a
		1.33 (m)	11, 12, 14, 15	13a, 15, 26, 28a, 28b	1′	145.4 s			
14	1.8 t	1.98[a] (m)		8, 9, 11, 23a	3′	137.7 d	8.36 (d, 5.3)	1′, 4′, 4a′	4′
15	128.7 d	5.24 (m)		13b	4′	113.5 d	7.86 (d, 5.4)		3′, 5′
16	129.2 d	5.12 (m)		17a, 17b, 18a, 18b	4a′	129.7 s		3′, 4a′, 4b′, 9a′	
17	29.0 t	2.52 (m)	18	16, 17b, 23a, 26	4b′	122.4 s			
		1.51 (m)		16, 17a	5′	121.8 d	8.10 (d, 7.9)	4a′, 4b′, 6′, 7′, 8′, 8a′	4′, 6′
18	28.6 t	1.44 (m)	17, 20	16, 18b, 28b	6′	120.1 d	7.24 (t, 7.6)	4b′, 8′	5′, 7′
		0.85 (m)		16, 18a, 26, 36b	7′	128.4 d	7.42 (t, 7.6)	5′, 8a′	6′, 8′
19	24.6 t	1.23 (m)		18b, 19b, 20a, 20b	8′	112.3 d	7.33 (d, 8.2)	4b′, 6′, 7′, 8a′	23b, 10′b
		1.02 (m)		19a	8a′	141.5 s			
20	58.8 t	1.98 (m)		19a, 22b	9′		10.79 (brs)		8′
		1.87 (m)		18b, 19a	9a′	136.3 s			
22	48.0 t	1.97 (m)		22b	10′	31.9 t	3.58 (m)	1′, 11	10′b, 11′b
		−0.12 (m)		22a, 23b, 24, 36b			3.32 (m)	1′, 9a′, 11	3a, 8′, 10′a
23	31.1 t	0.92 (m)		9, 14, 17a, 23b	11′	54.7 t	3.25 (m)	1	1, 3a, 11, 11′b
		0.73 (m)		9, 22b, 23a, 8′			2.86 (m)		1, 3a, 10a′, 11a′

[a] 2H. [b] *J*-values were not determined since overlapping with other signals.

Zamamidine A (**1**) was obtained as an optically active pale yellow amorphous solid. The ESIMS spectrum of **1** showed the pseudomolecular ion peak at *m*/*z* 749.5, and the molecular formula of **1** was revealed to be $C_{49}H_{60}N_6O$ by HRESIMS data [*m*/*z* 749.4894 $(M + H)^+$, Δ −1.4 mmu]. UV absorptions [λ_{max} 352 (ε 4900) and 339 nm (5000)] were attributed to a β-carboline chromophore,[5] while the IR absorption indicated the existence of NH and/or OH (3200 cm^{-1}) functionality. The ^{1}H (Table 1) spectrum showed two NH proton signals (δ 10.79 and 7.57) and ten aromatic proton signals (δ 8.36, 8.10, 7.86, 7.59, 7.42, 7.33, 7.26, 7.24, 7.18, and 7.17), suggesting that **1** had two β-carboline rings. It was noted that two proton signals (δ −0.12 and −0.54) were observed at higher field. The ^{13}C NMR, DEPT, and HMQC spectra disclosed 49 signals due to 10 sp^2 quaternary carbons, 15 sp^2 methines, 2 sp^3 quaternary carbons, 3 sp^3 methines, and 19 sp^3 methylenes.

Detailed analyses of the 2D NMR spectra of **1** including $^{1}H-^{1}H$ COSY, TOCSY, NOESY, HMQC, HMQC-TOCSY, and HMBC revealed the existence of a characteristic 6/6/11/13 ring system of manzamine alkaloids such as manzamines H and L[5,6] (C-10∼C-36) in addition to 1,2,3,4-tetrahydro-β-carboline (C-1∼C-9a) and β-carboline (C-1′∼C-9′a) moieties (Figure 1).HMBC cross-peaks of H-1/C-11 and H-1/C-24 indicated that C-1 of the 1,2,3,4-tetrahydro-β-carboline ring was attached to C-10, while HMBC correlations for H-1/C-11′, H-10′/C-1′, and H-10′/C-9′a suggested that N-2 of tetrahydro-β-carboline was connected to C-1′ of a second β-carboline ring via an ethylene unit. Thus, the gross structure of zamamidine A was elucidated to be **1**.

The relative stereochemistry of zamamidine A (**1**) was deduced from analysis of the NOESY spectrum. A chair conformation of a piperidine ring (N-21, C-22∼C-25, and C-36) and a boat conformation of a cyclohexene ring (C-

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(6) Tsuda, M.; Inaba, K.; Kawasaki, N.; Honma, K.; Kobayashi, J. *Tetrahedron* **1996**, *52*, 2319–2324.

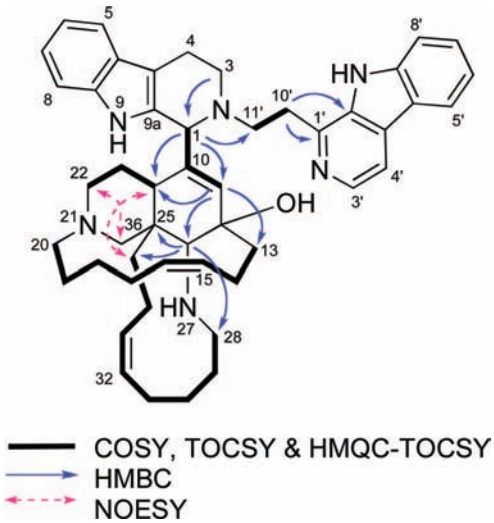

Figure 1. Selected 2D correlations for zamamidine A (**1**).

11∼C-12 and C-24∼C-26) in the *cis*-decahydroisoquinoline ring in addition to α-orientation of 12-OH and *β*-orientation of H-26 were derived from NOESY correlations for H-14/H-23a, H-22b/H-24, H-22b/H-36b, H-23a/H-26, H-24/H-26b, H-24/H-35b, and H-24/H-36b (Figure 2). NOESY correlations for H-1/H-11, H-9/H-11, and H-8′/H-22b suggested that the planes of a 1,2,3,4-tetrahydro-*β*-carboline ring and a cyclohexene ring were perpendicular to each other and that a second *β*-carboline ring was located behind a piperidine ring. Consequently, an orientation of H-1 was assigned as *β*.

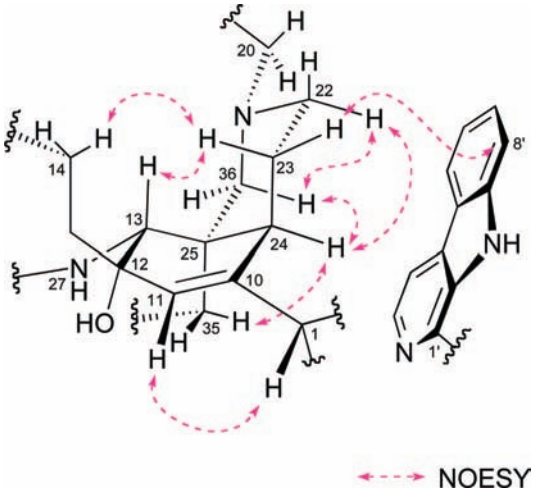

Figure 2. Selected NOESY correlations for partial structure of zamamidine A (**1**).

Though the structure of the C-1∼C-37 part of zamamidine A (**1**) was the same as those of manzamine H[5,6] including relative stereochemistry, proton signals for H-22b and H-35b were shifted to higher field (*δ* −0.12 and −0.54, respectively) as compared with those of manzamine H. These phenomena may be explained by the anisotropic effect of the *β*-carboline plane. To confirm this speculation, the comformational analysis was carried out by the MacroModel program (MMFF94s force-field). On the basis of NOESY correlation for H-23/H-8′, the distance between these protons was restricted within 1.0∼4.0 Å. Six stable conformers for **1** obtained from 1000 conformers by the calculation were superimposable, and each conformer was consistent with a conformation expected from NOESY correlations. The distances for the center of the benzene ring (C-4′b, C-5′∼C-8′, and C-8′a) to H-22 and the center of the pyridine ring (C-1′, N-2′, C-3′, C-4′, and C-9′) to H-35b in the most stable confomer were 3.1 and 3.0 Å, respectively (Figure 3).

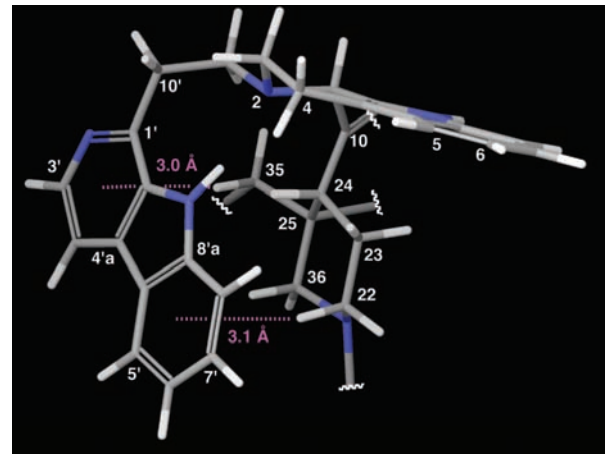

Figure 3. Most stable conformer for zamamidine A (**1**). C-11∼C-20, C-26, N-27, and C-28∼C-34 are not shown.

Absolute stereochemistry of zamamidine A (**1**) was elucidated on the basis of the CD spectrum.[7] 1*R*-configuration at C-1 was assigned on the basis of a positive Cotton effect (Δ*ε* + 16.4) at 221 nm. Thus, the structure of zamamidine A was elucidated to be **1**.

The molecular formula of zamamidine B (**2**) was established as $C_{49}H_{60}N_6O$ by HRESIMS data [*m*/*z* 749.4894 (M + H)$^+$, Δ −1.3 mmu]. The ^{1}H and ^{13}C NMR spectra suggested that **2** was also a new manzamine alkaloid possessing a second *β*-carboline ring as in **1**. Lack of a proton signal due to a double bond at H-11 of **1** in the ^{1}H spectrum for **2** implied that the polycyclic ring system in **2** (C-10∼C-36) was the same as manzamine B.[8] Inspection of 2D NMR spectra revealed that the structure of **2** was 1,2,3,4-tetrahydromanzamine B[9] possessing a second *β*-carboline ring via an ethylene unit at N-2. The relative stereochemistry of the polycyclic ring system in **2** (C-10∼C-36) was elucidated to be the same as manzamine B by comparison of ^{13}C NMR data of **2** with those of manzamine B[8] and its congeners[9,10] (Tables 2 and 3, Supporting Information). The absolute

(7) Stöckigt, J.; Zenk, M. H. *J. Chem. Soc., Chem. Commun.* **1977**, *n/a*, 646–648.

configuration at C-1 in **2** was assigned as *S* from negative Cotton effect of the CD spetrum at 223 nm ($\Delta\varepsilon$ −17.7). Thus, the structure of zamamidine B was elucidated to be **2**.

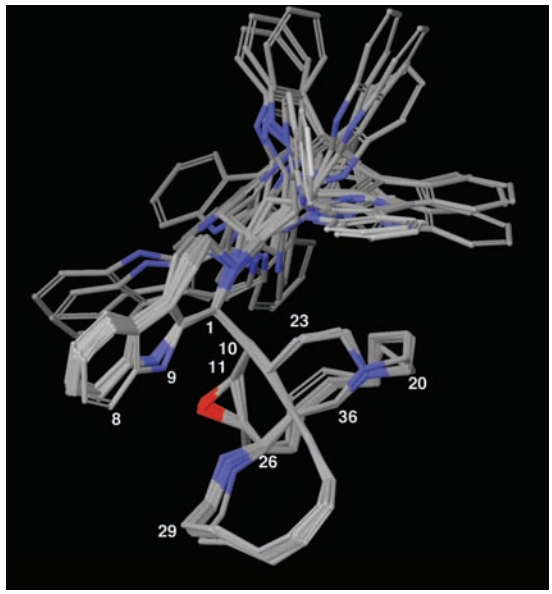

Figure 4. Superimposed view of stable conformations for zamamidine B (**2**). Hydrogens are not shown.

Despite the existence of a second β-carboline ring in **2**, remarkable higher-field shifts of any protons were not observed as compared with those of 1,2,3,4-tetrahydromanzamine B. The superimposed view for the most stable 20 conformers of **2** (total energy: 32.53−32.75 kcal/mol) obtained from 1000 conformers by the calculation was shown in Figure 4. The planes of a 1,2,3,4-tetrahydro-β-carboline moiety and a cyclohexene ring of the stable conformers were perpendicular to each other, while the direction of the 1,2,3,4-tetrahydro-β-carboline moiety was opposite to that of **1**. Thus, the position of the additional β-carboline ring was elucidated to be relatively far from the polycyclic ring system in **2** (C-10∼C-36), and its anisotropic effects might not be effective to any protons.

Zamamidines A (**1**) and B (**2**) are the first manzamine alkaloids possessing a second β-carboline ring via an ethylene unit at N-2 of manzamine H and 1,2,3,4-tetrahydromanzamine B, respectively. Zamamidines A (**1**) and B (**2**) showed cytotoxicity against P388 murine leukemia (IC_{50}, 13.8 and 14.8 μg/mL, respectively), while **1** and **2** did not show cytotoxicity against KB human epidermoid carcinoma cells (IC_{50} > 20 μg/mL) in vitro. Antimalarial activities of **1** and **2** are currently investigated.

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Supporting Information Available: Experimental details, Tables 2 and 3, and one- and two-dimensional NMR spectra for zamamidines A and B. This material is available free of charge via the Internet at http://pubs.acs.org.

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