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Zamamiphidin A, a New Manzamine Related Alkaloid from an Okinawan Marine Sponge *Amphimedon* sp.

Takaaki Kubota,[†] Yuki Kamijyo,[†] Azusa Takahashi-Nakaguchi,[‡] Jane Fromont,[§] Tohru Gonoi,[‡] and Jun'ichi Kobayashi*[,†]

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan, Medical Mycology Research Center, Chiba University, Chiba 260-0856 Japan, and Western Australian Museum, Locked Bag 49, Welshpool DC, WA 6986, Australia

jkobay@pharm.hokudai.ac.jp

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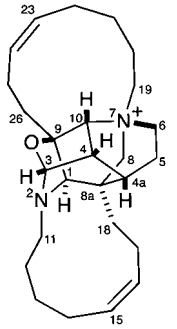
ABSTRACT



zamamiphidin A (**1**)

A manzamine related alkaloid, zamamiphidin A (1), consisting of a new heptacyclic ring system has been isolated from an Okinawan marine sponge *Amphimedon* sp. The structure of 1 including the relative stereochemistry was elucidated on the basis of the spectroscopic data. Compound 1 showed antibacterial activity against *Staphylococcus aureus* (MIC, 32 μg/mL).

The manzamine alkaloids have been reported from several marine sponge genera and are attractive as biosynthetically intriguing bioactive natural products.[1,2] During our search for bioactive metabolites from marine organisms, we have investigated extracts of an Okinawan marine sponge *Amphimedon* sp. (SS-1231) and isolated a new manzamine related alkaloid, zamamiphidin A (**1**), and an analog of ircinal A,[3] ircinic acid A (**2**). Here we describe the isolation and structure elucidation of **1** and **2** (Figure 1).

The sponge *Amphimedon* sp. (SS-1231) collected at Zamami, Okinawa, was extracted with MeOH. EtOAc-soluble materials of the extract were purified by silica gel column chromatography to yield zamamiphidin A (**1**, 0.00015% wet weight) and ircinic acid A (**2**, 0.00011% wet weight) together with known manzamine alkaloids, manzamines A,[4] B,[5] C,[5] H,[3] and L,[6] 3,4-dihydromanzamine A,[7] ircinals A[3] and B,[3] ircinol A,[8] keramaphidin B,[9,10] and ma'eganedin A.[11]

[†] Graduate School of Pharmaceutical Sciences, Hokkaido University.
[‡] Medical Mycology Research Center, Chiba University.
[§] Western Australian Museum.

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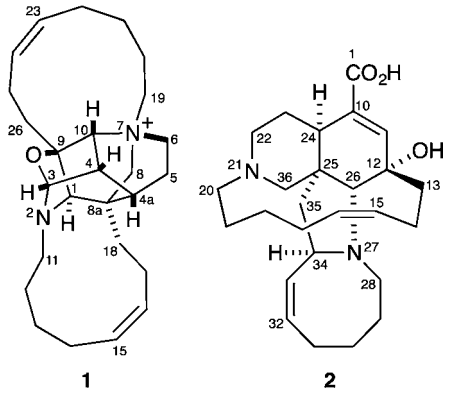

Figure 1. Structures of zamamiphidin A (**1**) and ircinic acid A (**2**).

Table 1. ^{1}H and ^{13}C NMR Data of Zamamiphidin A (**1**) in CD_3OD

position	δ_H[a]	multi (J in Hz)	δ_C[b]
1	2.64	br s	73.4
3	5.12	dd (5.1, 1.9)	94.0
4	2.61	br dd (7.6, 5.3)	41.8
4a	2.53	nd[c]	29.6
5*l*	2.14	nd[c]	19.8
5*h*	1.88	nd[c]	
6*l*	3.35	nd[c]	56.4
6*h*	3.26	br dd (10.4, 10.0)	
8*l*	3.11	nd[c]	63.1
8*h*	3.00	br d (12.5)	
8a			40.9
9			86.5
10	4.05	d (7.6)	62.9
11*l*	2.95	ddd (12.6, 4.1, 4.1)	49.2
11*h*	2.67	ddd (12.6, 9.7, 4.1)	
12*l*	1.68	m	27.5
12*h*	1.58	m	
13	1.64[d]	nd[c]	26.1
14*l*	2.21	ddd (14.0, 9.3, 4.7)	26.1
14*h*	2.13	nd[c]	
15	5.47	m	132.2
16	5.33	ddd (10.8, 7.0, 7.0)	131.4
17	2.41[d]	nd[c]	24.0
18*l*	1.82	nd[c]	33.6
18*h*	1.45	ddd (14.2, 6.9, 3.5)	
19*l*	3.64	br dd (12.7, 12.7)	61.1
19*h*	3.14	nd[c]	
20*l*	2.05	m	25.8
20*h*	1.96	nd[c]	
21*l*	1.91	nd[c]	24.4
21*h*	1.33	m	
22*l*	2.39	nd[c]	28.5
22*h*	2.33	nd[c]	
23	5.60	nd[c]	132.6
24	5.60	nd[c]	131.6
25*l*	2.51	nd[c]	22.0
25*h*	2.28	nd[c]	
26*l*	2.50	nd[c]	33.8
26*h*	1.99	nd[c]	

[a] 600 MHz. [b] 150 MHz. [c] nd: J-values were not determined because of overlapping with other signals. [d] 2H.

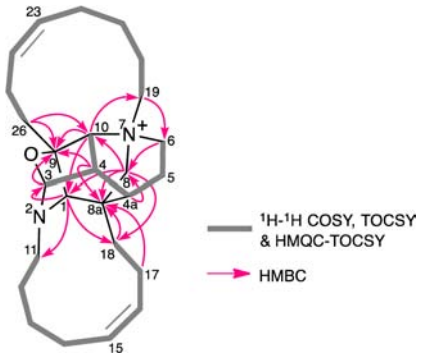

Figure 2. Selected 2D NMR correlations for zamamiphidin A (**1**) in CD_3OD.

Zamamiphidin A (**1**) was obtained as an optically active pale yellow amorphous solid. The molecular formula of **1** was established to be $C_{26}H_{39}O_1N_2$ by HRESIMS data in MeOH [m/z 395.30606 $(M)^+$ (calcd for $C_{26}H_{39}O_1N_2$, 395.30569)]. HRESIMS data of **1** in CD_3OD [m/z 395.30600 $(M)^+$ (calcd for $C_{26}H_{39}O_1N_2$, 395.30569)] disclosed that **1** has no exchangeable proton. The analysis of the HMQC spectrum with ^{1}H and ^{13}C NMR data (Table 1) indicated that **1** consists of 4 sp^2 methine, 2 sp^3 quaternary carbons, 5 sp^3 methines, and 15 sp^3 methylenes.

The inspection of the $^{1}H-^{1}H$ COSY, TOCSY, and HMQC-TOCSY spectra of **1** revealed connections of C3 to C4, C4 to C4a and C10, C4a to C5, C5 to C6, C11 to C18, and C19 to C26 (Figure 2). HMBC cross-peaks of H1/C3 and H1/C11 indicated the connections of three *N*-bearing carbons C1(δ_C 73.4), C3(δ_C 94.0), and C11(δ_C 49.2) to N2, while HMBC cross-peaks of H6/C8, H8/C10, H10/C19, and H19/C6 implied the linkings of four *N*-bearing carbons C6(δ_C 56.4), C8(δ_C 63.1), C10(δ_C 62.9), and C19(δ_C 61.1) to N7. Connectivities of C1, C4a, C8, and C18 with C8a were deduced from HMBC correlations for H1/C18, H4/C8a, H4a/C8, H8/C8a, H8/C18, and H18/C8a. HMBC cross-peaks of H4/C9, H10/C9, H26/C9, and H26/C10 suggested that C1, C10, and C26 were attached to C9. A connection of C3(δ_C 94.0) and C9(δ_C 86.5) by an ether linkage forming a hemiaminal ether was disclosed by an HMBC correlation for H3/C9. The configurations of both two double bonds (C15−C16 and C23−C24) were assigned as *E* from $^{3}J_{H/H}$ values of olefinic protons ($^{3}J_{H15/H16}$ 10.8 Hz, $^{3}J_{H23/H24}$ 10.8 Hz[12]). Thus, the gross structure of zamamiphidin A was elucidated to be **1**.

The relative stereochemistry of zamamiphidin A (**1**) was deduced by analysis of the NOESY spectrum (Figure 3). NOESY correlations for H1/H8*l*, H1/H18*h*, and H1/H26*l* suggested that H8*l*, H18*h*, and H26*l* were located close to H1. Proximity of H3, H5*h*, H6*l*, and H10 to H4 was

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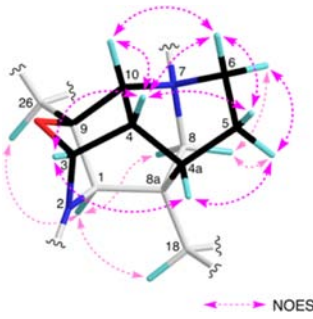

Figure 3. Selected NOESY correlations for zamamiphidin A (**1**) in CD_3OD.

indicated by NOESY correlations for H3/H4, H4/H5*h*, H4/H6*l*, and H4/H10. NOESY cross-peaks of H3/H4a and H4a//H5*l* showed proximity of H3 and H5*l* to H4a, while NOESY cross-peaks of H5*l*/H8*h* and H6*h*/H8*h* implied proximity of H5*l* and H6*h* to H8*h*. All four piperidine rings (C1−C2−C3−C4−C4a−C8a, C1−C8a−C8−N7−C10−C9, C4−C4a−C5−C6−N7−C10, and C4a−C5−C6−N7−C8−C8a) might be in the twist boat form, while both of a tetrahydrofuran ring (C3−C4−C10−C9−O) and an oxazolidine ring (C1−N2−C3−O−C9) might adopt the conformation between the envelope and half-chair.

Ircinic acid A (**2**) was obtained as an optically active pale yellow amorphous solid. The molecular formula of **2** was established to be $C_{26}H_{38}O_3N_2$ by HRESIMS data [*m*/*z* 427.29508 $(M + H)^+$ (calcd for $C_{26}H_{39}O_3N_2$, 427.29552)]. The ^1H NMR spectrum of **2** was similar to that of ircinal A,[3] except for disappearance of a signal derived from an aldehyde group. The difference of the molecular formula between **2** and ircinal A implied that **2** was the carboxy acid analog of ircinal A. To verify the hypothesis, **2** was derived to 1-*O*-methyl form by treatment with trimethylsilyldiazomethane. Since the NMR data of 1-*O*-methyl form of **2** were identical with those of synthetic 1-*O*-methyl carboxylic acid analog of ircinal A,[13] the structure of ircinic acid A was assigned as **2**.

A possible biosynthetic pathway of zamamiphidin A (**1**) was shown in Scheme 1. The biosynthetic pathway of manzamine A from dihydropyridine derivative through the hypothetical intermediate **X** has been proposed by Baldwin and Whitehead.[14] Compound **1** might be derived

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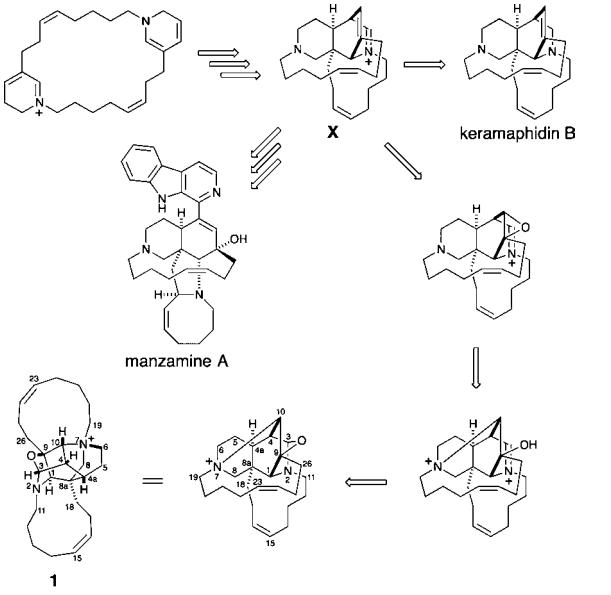
Scheme 1. Possible Biosynthetic Pathway for Zamamiphidin A (**1**)



from **X** by epoxidation of an olefin, opening of an epoxide by addition of a tertiary amine, and generation of a hemiaminal ether.

Zamamiphidin A (**1**) is a manzamine related alkaloid possessing a new heptacyclic ring system. Compound **1** showed antibacterial activity against *Staphylococcus aureus* (MIC, 32 μg/mL), but not against *Escherichia coli*, *Bacillus subtilis*, and *Micrococcus luteus* (MIC, >32 μg/mL), and antifungal activities against *Aspergillus niger*, *Trichophyton mentagrophytes*, *Candida albicans*, and *Cryptococcus neoformans* ($IC_{50} > 32$ μg/mL). Compound **1** did not show cytotoxycity against L1210 murine leukemia and KB human epidermoid carcinoma cells ($IC_{50} > 10$ μg/mL).

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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.

The authors declare no competing financial interest.