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Keramaphidin B, a Novel Pentacyclic Alkaloid from a Marine Sponge *Amphimedon* sp. : A Plausible Biogenetic Precursor of Manzamine Alkaloids

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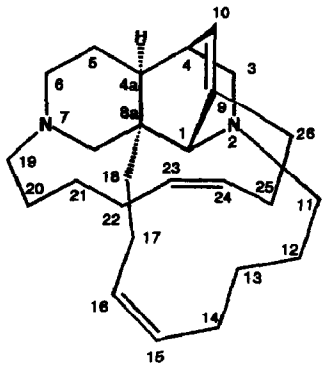
Abstract: Keramaphidin B (**1**), a novel cytotoxic alkaloid possessing a 1,4-etheno-2,7-decahydronaphthylidine core with two macrocyclic rings has been isolated from an Okinawan marine sponge *Amphimedon* sp., and the structure was elucidated by extensive 2D NMR investigation as well as a single crystal X-ray analysis. Keramaphidin B (**1**) might be a plausible biogenetic precursor of manzamine alkaloids and a very important key compound to clarify the biogenetic path.

The manzamines[1~5] are some of the most intricate β-carboline alkaloids from marine sources. This is especially true for manzamine A[1,2], owing to the unprecedented structure containing a decahydropyrrolo[2,3-i]isoquinoline core as well as biological activities, is of interest to synthetic chemists as one of the most challenging targets for total synthesis[6,7]. Manzamine-related alkaloids, ircinals A and B[8], which we isolated previously from an Okinawan marine sponge *Ircinia* sp., are considered to be precursors to generate the manzamines through coupling with tryptamine in the biosynthetic path. However, origin of the complicated ring system consisting of a decahydropyrrolo[2,3-i]isoquinoline core and two macrocycles of the ircinals has remained unknown. Further investigation of biogenetic precursors of the ircinals resulted in the isolation of keramaphidin B (**1**), a novel pentacyclic alkaloid possessing a 1,4-etheno-2,7-decahydronaphthylidine core with two macrocyclic rings, from an Okinawan marine sponge *Amphimedon* sp. This alkaloid might be a plausible biogenetic precursor of the ircinals and a very important key compound in clarifying the biogenetic path of the manzamines. Here we describe the isolation and structure elucidation of **1**. The relative stereochemistry of **1** was established by a single crystal X-ray analysis. Keramaphidin B (**1**) exhibited cytotoxicity against tumor cells in vitro.

The sponge *Amphimedon* sp. was collected off the Kerama Islands, Okinawa, and kept frozen until used. The methanolic extract of this sponge was partitioned between ethyl acetate and water. The ethyl acetate soluble material was subjected to silica gel chromatography using $CHCl_3$/MeOH (95:5) and then hexane/acetone/Et_2NH (80:20:2) to afford keramaphidin B (**1**, 0.003 % wet weight of the sponge) together



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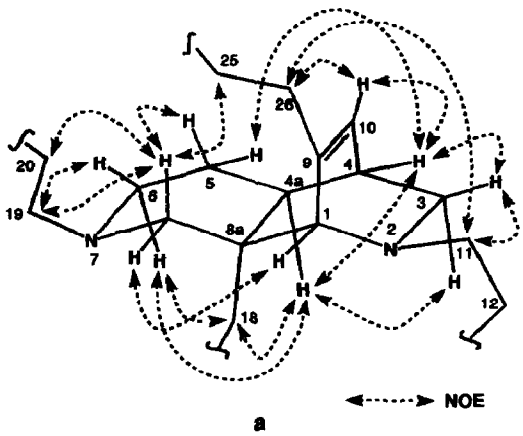

a

with known alkaloids ircinals A^8 (0.001 %) and B^8 (0.0004 %) and manzamines $A^{1,2}$ (0.03 %), B^3 (0.007 %), G^9 (0.002 %), and H^8 (0.008 %).

The molecular formula, $C_{26}H_{40}N_2$, of keramaphidin B (**1**)[10] was established by HREIMS (*m/z* 380.3199, M^+, Δ +0.8 mmu). The ^1H and ^{13}C NMR (Table 1) spectra of **1** showed three (two di- and one trisubstituted) double bonds (δ_H 5.81, 5.69, 5.64, 5.36, and 5.24; δ_C 141.8, 132.0, 131.5, 131.2, 130.9, and 122.6), suggesting that **1** possessed five rings including two nitrogen atoms since three of eight unsaturations in the molecule were accounted for. The ^{13}C NMR data including DEPT experiments disclosed three sp^3 methines, sixteen sp^3 methylenes, and one sp^3 quaternary carbon. Detailed analyses of ^1H-^1H COSY, HOHAHA, HMQC, and HMQC-HOHAHA[11] spectra of **1** allowed assignments of four segments H_2-3 to H_2-6, H-4 to H-10, H_2-11 to H_2-18, and H_2-19 to H_2-26. The ^{13}C chemical shifts at C-1 (δ_C 64.3), C-3 (δ_C 53.6), C-6 (δ_C 47.4), C-8 (δ_C 50.8) , C-11 (δ_C 54.1), and C-19 (δ_C 56.2) indicated that each carbon was attached to a nitrogen atom. A methine proton at C-1 showed allyl couplings to H-10 and H_2-26, indicating the presence of connections between C-1, C-10, and C-26. Interpreting the HMBC[12] data allowed to assign connectivities of for segments via two nitrogens and two quaternary carbons. Detailed analysis of the NOESY spectrum (mixing time; 800 ms) of **1** allowed assignments of relative stereochemistry of the 1,4-etheno-2,7-decahydronaphthylidine ring (**a**). Two disubstituted $\Delta^{15(16)}$ and $\Delta^{23(24)}$ double bonds were assigned as both *Z*-configurations by NOESY correlations observed for H-14/H-18 and H_2-22/H_2-25 as well as the chemical shifts of allylic methylene carbons at C-14, C-17, C-22, and C-25 (δ_C 22.9, 21.0, 25.0, and 25.6, respectively). Thus the structure of keramaphidin B including the relative stereochemistry was concluded to be **1**. To verify the proposed structure, X-ray difraction analysis was undertaken using a suitable crystal of **1** grown in acetonitrile. The relative stereostructure established by the X-ray analysis (Figure 1)[13] was the same as that elucidated on the basis of NMR data. Interestingly, the X-ray study revealed that **1** is racemic though it possesses four asymmetric centers.

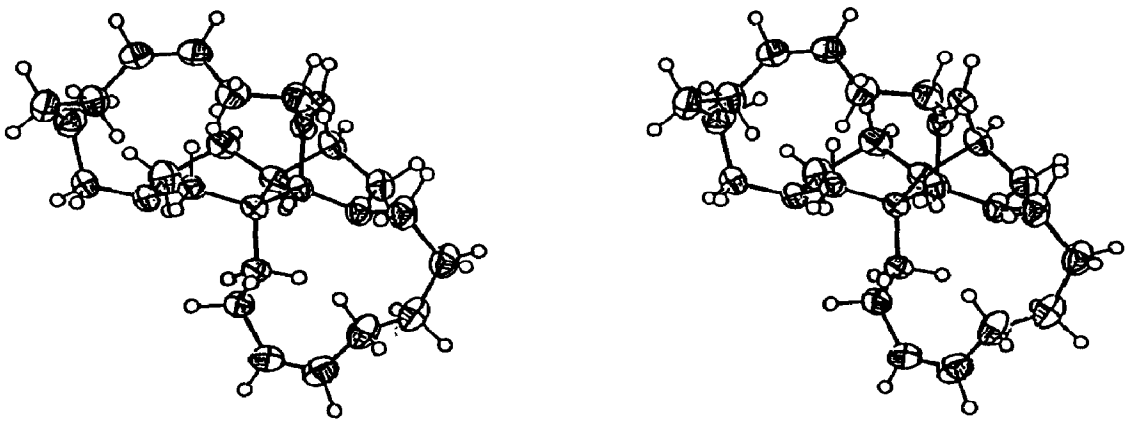

Fig. 1. A Computer Generated Perspective Drawing of the Final X-ray Model for Keramaphidin B (1)

The manzamine-related compound, keramaphidin B (1), is a unique alkaloid possessing a 1,4-etheno-2,7-decahydronaphthylidine core with two macrocyclic rings. Manzamine alkaloids may be biogenetically derived from condensation between tryptamine and ircinals A or B isolated from the *Ircinia* sponge[8]. Keramaphidin B (1) might be a plausible biogenetic precursor of the ircinals, which may be generated by hydrolysis of N2/C3 bond of 2,3-imino form of 1[14]. On the other hand, xestocyclamine A[15] isolated recently from a sponge *Xestospongia* sp. seems to be an alkaloid parallel to 1 in the biosynthesis of manzamines. Keramaphidin B was cytotoxic against P388 murine leukemia and KB human epidermoid carcinoma cells (IC_{50} 0.28 and 0.3 μg/mL, respectively).

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10. **1**: colorless needle; mp 131-132 °C; $[\alpha]_D^{25}$ 0° (*c* 0.2, $CHCl_3$); IR (film) λ_{max} 2940 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.93 (1H, ddd, *J*=1.9, 5.6, and 11.6 Hz; H-4a), 1.17 (1H, ddd, *J*=4.4, 8.7, and 13.0 Hz; H-5), 1.24 (1H, m; H-12), 1.32 (1H, m; H-21), 1.34 (1H, m; H-20), 1.36 (1H, m; H-5), 1.45 (1H, m; H-12), 1.46 (1H, m; H-13), 1.48 (1H, m; H-21), 1.55 (1H, m; H-20), 1.57 (1H, m; H-14), 1.58 (1H, m; H-13), 1.61 (1H, m; H-18), 1.64 (1H, dd, *J*=2.3 and 9.0 Hz; H-3), 1.78 (1H, m; H-17), 1.88 (1H, dt, *J*=12.3 and 7.6 Hz; H-18), 1.96 (1H, br.d, *J*=15.2 Hz; H-22), 2.08 (1H, d, *J*=10.7 Hz; H-8), 2.12 (1H, m; H-25), 2.14 (1H, m; H-22), 2.22 (1H, m; H-4), 2.23 (1H, m; H-11), 2.23 (1H, d, *J*=12.3 Hz; H-8), 2.24 (1H, m; H-19), 2.25 (1H, m; H-26), 2.27 (1H, m; H-17), 2.29 (1H, m; H-25), 2.33 (1H, m; H-26), 2.35 (1H, m; H-14), 2.63 (1H, dt, *J*=12.3 and 3.6 Hz; H-6), 2.75 (1H, m; H-6), 2.86 (1H, dd, *J*=1.5 and 8.5 Hz; H-3), 2.91 (1H, dd, *J*=9.7 and 20.7 Hz; H-3), 3.01 (1H, s; H-1), 3.07 (1H, m; H-19), 5.24 (1H, br.d, *J*=10.8 Hz; H-23), 5.36 (1H, br.d, *J*=10.8 Hz; H-24), 5.64 (1H, ddd, *J*=5.2, 10.1, and 13.6 Hz; H-15), 5.69 (1H, ddd, *J*=6.3, 10.1, and 13.6 Hz; H-16), and 5.81 (1H, br.d, *J*=6.3 Hz; H-10); ^{13}C NMR ($CDCl_3$) δ 21.0 (t, C-17), 21.1 (t, C-20), 22.9 (t, C-14), 25.0 (t, C-22), 25.6 (t, C-25), 26.1 (t, C-12), 25.6 (t, C-13), 27.2 (t, C-21), 27.6 (t, C-5), 37.0 (t, C-26), 38.0 (d, C-4), 41.6 (t, C-18), 43.3 (d, C-4a), 45.1 (s, C-8a), 47.4 (t, C-6), 50.8 (t, C-8), 53.6 (t, C-3), 54.1 (t, C-11), 56.2 (t, C-19), 64.3 (d, C-1), 122.6 (d, C-10), 130.9 (d, C-16), 131.2 (d, C-15), 131.5 (d, C-23), 132.0 (d, C-24), and 141.8 (s, C-9); EIMS *m/z* 380 (M^+) and 283.
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13. Crystallographic data for **1**: $C_{26}H_{40}N_2$, mp 131-132 °C, crystal size 0.30 x 0.20 x 0.15 mm, orthorhombic, *P*bca, a = 16.168 (4) Å, b = 21.497 (5) Å, c = 12.631 (4) Å, V = 4390 (2) $Å^3$, Z = 8, D_{obs} = 1.15 gcm^{-3}, D_{calc} = 1.15 gcm^{-3}, $F(000)$ = 1680, μ(CuKα) = 4.25 cm^{-1}, R = 0.052, $\bar{\omega}R$ = 0.063, for 2947 observed reflections ($F > 3\sigma F$). Intensity data were collected on a Mac Science MXC18 diffractometer using CuKα radiation (λ = 1.54178). The final atomic coordinates as well as bond lengths and bond angles are deposited at the Cambridge Crystallographic Data Centre.
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