Nakadomarin A, a Novel Hexacyclic Manzamine-Related Alkaloid from *Amphimedon* Sponge

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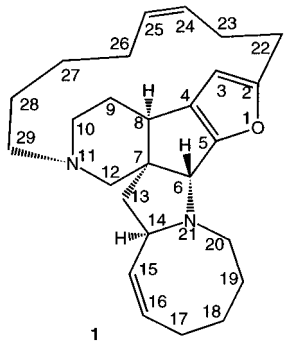
A novel manzamine-related alkaloid consisting of unprecedented 8/5/5/5/15/6 ring system, nakadomarin A (**1**), has been isolated from an Okinawan marine sponge *Amphimedon* sp., and the unique structure containing a furan ring was elucidated on the basis of the spectroscopic data. The relative stereochemistry was deduced from the NOE data and proton couplings, and a plausible biogenetic path of **1** through ircinal A was proposed.

Recently a series of unique polycyclic alkaloids with intricate skeletons have been isolated from marine Haplosclerid sponges of genera *Haliclona*, *Xestospongia*, *Amphimedon*, and *Reniera*,[1] among which the representative alkaloids, manzamines A[2,3] and B,[4] are characterized by a penta- or tetracyclic nitrogen-containing ring system bound to a β-carboline, respectively. These unusual ring systems have attracted great interest as one of the most challenging targets for total synthesis. Our continuing search for biogenetic precursors of manzamines A−C resulted in the isolation of several novel alkaloids, ircinals A and B,[5] keramaphidins B[6] and C,[7] ircinols A and B,[8] and keramamine C[7] from an *Amphimedon* sponge. Ircinals and keramaphidin B correspond to tetra- and pentacyclic biogenetic precursors of manzamines A and B, respectively, proposed by Baldwin and Whitehead.[9] Further investigation of biogenetically related compounds to manzamines from another *Amphimedon* sponge led to the isolation of nakadomarin A (**1**), a novel furan-containing hexacyclic alkaloid consisting of an unprecedented 8/5/5/5/15/6 ring system. In this paper we describe the isolation and structure elucidation of **1** and propose a biogenesis of **1** through ircinal A.

The sponge *Amphimedon* sp. (SS-264) collected off Kerama Islands, Okinawa, was extracted with MeOH. EtOAc-soluble materials of the MeOH extract were purified by silica gel chromatographies ($CHCl_3$/MeOH and then cyclohexane/acetone/Et_2NH) to afford nakadomarin A (**1**, 1.8×10^{-3} %, wet weight) as a free base, together with known manzamine alkaloids.

Nakadomarin A (**1**) {$[\alpha]^{25}_D$ −16° (*c* 0.12, MeOH)} was obtained as a colorless amorphous solid and the molecular formula was established as $C_{26}H_{36}N_2O$ by HREIMS (*m*/*z* 392.2826, M^+, Δ −0.2 mmu). The ^1H and ^{13}C NMR



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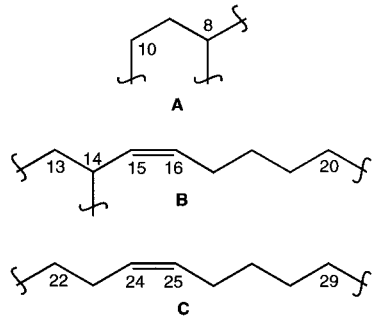
data (Table 1) revealed the presence of eight sp^2 carbons, which were attributed to two di-, one tri-, and one tetrasubstituted double bonds, and eighteen sp^3 carbons containing three methines, fourteen methylenes, and one quaternary one. Since 4 out of 10 elements of unsaturation implied by the molecular formula were accounted for, **1** was inferred to possess six rings. Three partial structures **A**−**C**, one isolated methylene (C-12), and two



methines (C-3 and C-6) were assigned by detailed analyses of $^1H-^1H$ COSY, HOHAHA, and HMQC spectra. The ^{13}C chemical shifts of C-6 (δ 75.9), C-10 (δ 46.6), C-12 (δ 60.6), C-14 (δ 60.4), C-20 (δ 51.7), and C-29 (δ 59.6) suggested that these carbons were adjacent to a nitrogen atom. Geometries of two disubstituted $\Delta^{15(16)}$ and $\Delta^{24(25)}$ double bonds were elucidated to be both *Z*-configuration by NOESY correlations for H-15/H-16, H-14/H-17a, H-24/H-25, and H_2-23/H_2-26 as well as $^1H-^1H$ coupling constants ($J_{15,16}$ = 10.1 Hz and $J_{24,25}$ = 10.8 Hz). Connection among C-6, C-8, C-12, and C-13 via C-7 was implied by HMBC cross-peaks for H-6/C-12, H-8/C-12, H-8/C-13, H_2-12/C-6, H-13β/C-6, H-12β/C-7, and H_2-13/C-7. Long-range C−H couplings for H-10β/C-12, H-10α/C-29, H_2-12/C-10, H-12β/C-29, H_2-29/C-10 revealed connection among the partial structures **A** and **C** and C-12 via N-11.

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

position		δ_H[a]	m	J (Hz)	δ_C[b]	m	HMBC[c] (H)
2					164.6	s	3, 22, 23
3		5.99	s		105.6	d	22a[d]
4					137.9	s	3, 6, 8, 9
5					156.2	s	3, 6
6		4.25	brs		75.9	d	12, 13α
7					64.2	s	9, 12β, 13
8		2.95	dd	2.7, 4.3	43.2	d	9β, 10α, 12α, 13
9	α	2.04	dddd	2.4, 4.3, 12.1, 14.2	23.1	t	8, 10
	β	1.85	ddd	2.7, 4.5, 14.2			
10	α	2.22	dd	2.4, 12.1	46.6	t	8, 9α, 12, 29
	β	2.66	dt	4.5, 12.1			
12	α	3.13	d	12.2	60.6	t	6, 8, 10β, 13β,
	β	2.39	d	12.2			29b[e]
13	α	2.17	dd	5.0, 12.7	43.0	t	8, 12, 14, 15[e]
	β	1.65	dd	10.9, 12.7			
14		4.05	m		60.4	d	13β, 15, 16
15		5.61	dd	8.3, 10.1	129.5	d	17α
16		5.97	dt	10.1, 7.2	136.4	d	17α, 18α[e]
17	α	2.42	m		26.4	t	15, 16
	β	2.19	m				
18	α	1.76	m		28.9	t	17α, 19[e]
	β	1.51	tt	4.3, 13.0			
19		1.77[b]	m		25.3	t	17,[e] 18α[e]
20	α	2.93	m		51.7	t	19[e]
	β	3.20	m				
22		2.77	ddd	2.9, 7.4, 14.6	30.0	t	3,[e] 24
		2.76	ddd	3.0, 10.1, 14.6			
23		2.56	m		29.2	t	22, 24,[e] 25
		2.22	m				
24		5.28	ddd	7.0, 8.8, 10.8	129.8	d	22, 23b,[e] 26b
25		5.50	dt	10.8, 7.8	132.7	d	23b,[e] 26, 27a[e]
26		1.97	m		27.6	t	24, 25, 27,[e] 28[e]
		1.70	m				
27		1.13	m		29.7	t	25, 26, 28,[e] 29
		0.96	m				
28		1.39	m		27.4	t	26, 27, 29b
		1.17	m				
29		2.47	dt	11.9, 3.7	59.6	t	10α, 12β, 28[e]
		2.36	dt	11.9, 3.6			

[a] Recorded at 600 MHz. [b] Recorded at 125 MHz. [c] Delay time (Δ) for C−H long-range coupling was set to 50 ms. [d] a and b denote upfield and downfield resonances, respectively, of a geminal pair for C-22, C-23, C-26, C-27, C-28, and C-29. [e] These correlations were observed in D-HMBC[10] spectrum (Δ = 80 ms).

The following differential NOE experiments justified linking of C-6, C-14, and C-20 via N-21: irradiation of H-6 (δ 4.25) yielded NOE's for H-20α (δ 2.93, 1.2%) and H-20β (δ 3.20, 2.3%), while irradiation of H-20α afforded 2.8% NOE for H-14 (δ 4.05). The presence of an 8/5-fused azabicyclic system was supported by the ^{13}C chemical shifts at C-6 (δ 75.9), C-14 (δ 60.4), and C-20 (δ 51.7) of **1**, which were similar to those of the corresponding carbons (C-26; δ 77.6, C-34; δ 57.7, C-28; δ 53.3) of manzamine A[2,3] in CD_3OD. HMBC correlations for H-3/C-2, H-22/C-2, H-3/C-4, H-6/C-4, H-8/C-4, H-9/C-4, H-3/C-5, and H-6/C-5 and a long-range ^{1}H−^{1}H coupling for H-6/H-8 indicated that the three sp^2 quaternary carbons at δ 164.6, 137.9, and 156.2 were assignable to C-2, C-4, and C-5, respectively. The low-field ^{13}C chemical shifts at C-2 (δ 164.6) and C-5 (δ 156.2) and the unsaturation degree of **1** indicated the presence of an ether linkage between C-2 and C-5, which was supported by the UV absorption at 228 nm (ϵ 10000).[11] Thus the structure of nakadomarin A was elucidated to be **1**.

The relative configurations of all chiral centers of nakadomarin A (**1**) as well as conformation of each ring were elucidated on the basis of NOESY data, differential NOE experiments, and ^{1}H−^{1}H coupling constants (Figure 1). NOESY cross-peaks for H-9α/H-12α and H-10α/H-12α and ^{1}H−^{1}H coupling constants of H-8/H-9α (J = 4.3 Hz), H-8/H-9β (2.7 Hz), H-9α/H-10α (2.4 Hz), H-9α/H-10β (12.1 Hz), H-9β/H-10α (<1 Hz), and H-9β/H-10β (4.5 Hz) showed that the piperidine ring (C-7−C-12) had a boat conformation with H-9α and H-12α occupying flagpole orientations.[12] β-Axial orientation of the lone pair at N-11 was deduced from NOESY correlations for H-10α/H_2-29 and H-12α/H_2-29. The boat conformation of the piperidine ring elucidated by the NOESY data and ^{1}H−^{1}H coupling constants corresponded well to the most stable conformation of a piperidine ring, which was afforded by conformational search[13] of the 5/5/15/6-tetracyclic moiety using MacroModel version 5.0[14] (Figure 2). The 7,8-cis configuration was indicated by NOESY correlations observed for H-6/H-12β, H-8/H-13α, and H-8/H-14. NOESY data for H-6/H-13β, H-6/H-15, H-6/H-20β, H-8/H-14, and H-13β/H-14 suggested that H-6, H-14, and the lone pair at N-21 were oriented β, α, and α, respectively. Considering the NOE's for H-14/H-17α, H-14/H-19α, H-14/H-20α, H-16H-17β, H-16/H-18β, and H-16/H-19β, the eight-membered ring (C-14−N-21) seemed to be an envelope-boat conformation,[15] similar to the N-27 to C-34 ring in manzamine A.[1a,5] The conformation of the eight-membered ring in **1** deduced from NOE's was close to the most stable conformation of the 8/5-fused azabicyclic system calculated by MacroModel version 5.0 (Figure 3). Thus the relative stereostructure and ring conformation of nakadomarin A were concluded to be **1**.

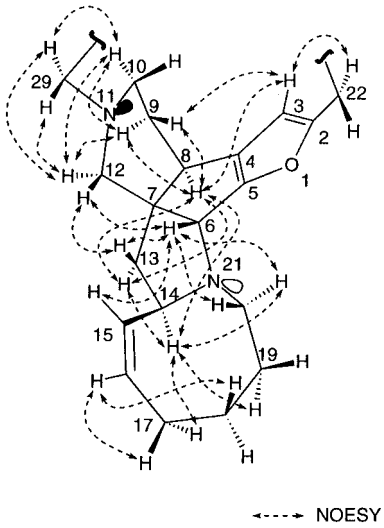

Figure 1. Relative stereochemistry for pentacyclic core of nakadomarin A (**1**) based on the NOESY correlation and proton couplings. The coupling constants for this moiety (H/H in Hz) are as follows: 8/9α = 4.3, 8/9β = 2.7, 9α/10α = 2.4, 9α/10β = 12.1, 9b/10α = <1, 9β/10β = 4.5, 13α/14 = 5.0, 13β/14 = 10.9, 14/15 = 8.3, and 15/16 = 10.1.

Nakadomarin A (**1**) is a novel hexacyclic alkaloid consisting of an unprecedented 8/5/5/5/15/6 ring system. Since nakadomarin A (**1**) possesses a piperidine ring with

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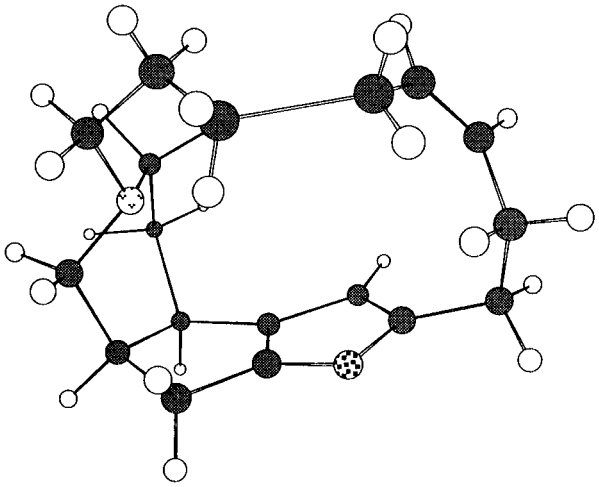


Figure 2. Most stable conformation (total energy, 47.6 kcal/mol) of 5/5/15/6-tetracyclic system in nakadomarin A (**1**) calculated by Macromodel ver. 5.0.

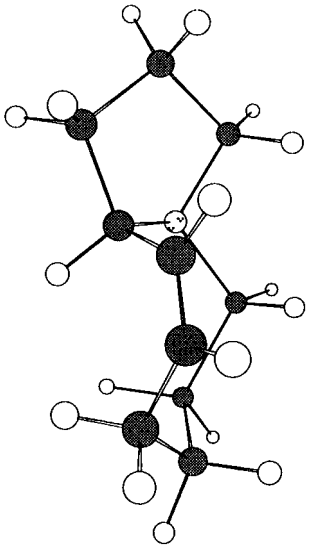


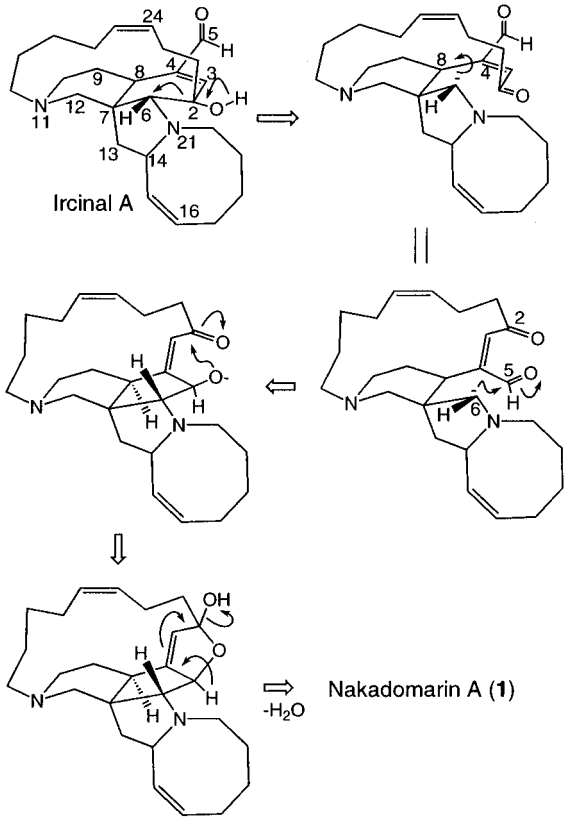
Figure 3. Most stable conformation (total energy, 28.7 kcal/mol) of 8/5-bicyclic system in nakadomarin A (**1**) calculated by Macromodel ver. 5.0.

a C_8 alkyl chain attached at N-11 and an 8/5-fused azabicyclic system, which are common structures in manzamine A and ircinal A,[5] nakadomarin A (**1**) is considered to be a novel class of manzamine-related alkaloid containing a furan ring. A plausible biogenetic path of nakadomarin A (**1**) through ircinal A is shown in Scheme 1, in which cleavage of the C-2−C-6 bond of ircinal A followed by rotation of the C-4−C-8 bond, and then formation of the C-5−C-6 bond gives a pentacyclic intermediate, which finally yields nakadomarin A (**1**) after formation of an ether ring and dehydroxylation. Nakadomarin A (**1**) showed cytotoxicity against murine lymphoma L1210 cells (IC_{50} 1.3 μg/mL) and inhibitory activity against cyclin dependent kinase 4 (IC_{50} 9.9 μg/mL). Compound **1** exhibited antimicrobial activity against a fungus (*Trichophyton mentagrophytes*, MIC 23 μg/mL) and a Gram-positive bacterium (*Corynebacterium xerosis*, MIC 11 μg/mL).

Experimental Section[16]

Sponge Materials. The medium brown color sponge *Amphimedon* sp. (order Haplosclerida; family Niphatidae) was collected off Kerama Islands, Okinawa, and kept frozen until

Scheme 1. Plausible Biogenetic Path of Nakadomarin A (1) through Ircinal A



used. The piece of sponge is a small mound with irregular meandering ridged surface. Texture is firm and compressible. Mesohyl consists of a fibrous reticulation with more dense plumoreticulate fiber centrally. Spicules possess few interstitial. Spicule fans extend slightly beyond the surface at right angles. Primary fibers possessing 80−100 mm thick are centrally cored by 5−12 spicules. Secondary fibers (80−100 mm) are cored by 1−3 spicules. Megascleres are small oxeas (mean size; 187 × 9 mm), which are generally straight or slightly curved. There is no microsclere. The voucher specimen (SS-264) was deposited at the Faculty of Pharmaceutical Sciences, Hokkaido University.

Extraction and Isolation. The sponge (1.0 kg, wet weight) was extracted with MeOH (1 L × 2). The methanolic extract (71 g) was partitioned between ethyl acetate (400 mL × 3) and 1 N NaCl aq. Part (15 g) of the EtOAc soluble material (51.1 g) was subjected to SiO_2 columns three times (solvent system; $CHCl_3$/MeOH, 90:10, cyclohexane/acetone/Et_2-NH, 90:10:2, $CHCl_3$/MeOH, 90:10) to yield nakadomarin A (**1**, 6.0 mg, 1.8×10^{-3} % wet weight).

Nakadomarin A (1): a colorless amorphous solid; $[\alpha]^{25}_D$ −16° (*c* 0.12, MeOH); UV (MeOH) λ_{max} 206 (ϵ 11000) and 228 nm (10000); IR (KBr) ν_{max} 2920, 2850, 1455, and 1080 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); EIMS *m/z* 392 (M^+) and 296; HREIMS *m/z* 392.2826 (M^+), calcd for $C_{26}H_{36}N_2O$, 392.2828.

Computational Methods. Conformational searching was carried out using Pseudo Monte Carlo simulation in Macromodel program. The closure bonds for the 5/5/15/6-tetracyclic system were chosen at C-9−C-10 and C-27−C-28 with the closure limit from 1 to 4 Å, while the closure bond for 8/5-bicyclic system was chosen at C-18−C-19. Five thousand Monte Carlo steps were performed and produced 19 and 2 conformers for 5/5/15/6-tetracylcic and 8/5-bicyclic systems, respectively, which were obtained within 3 kcal/mol of the lowest energy confomers. Each conformer was finally minimized by molecular mechanics calculation of MM2* force field in H_2O.

(16) Analytical instruments and general procedures in this work were described in the previous report: Kobayashi, J.; Tsuda, M.; Fuse, H.; Sasaki, T.; Mikami, Y. *J. Nat. Prod.* **1997**, *60*, 150−154.

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Supporting Information Available: NMR spectra and PDB files of **1** (11 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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