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Two New Polycyclic Aromatic Alkaloids from the Okinawan Marine Sponge *Biemna* sp.

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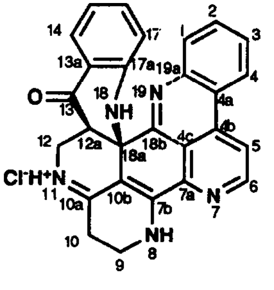
Abstract: *Two new polycyclic aromatic alkaloids, biemnadin (1) and 8,9-dihydro-11-hydroxyascididemin (2), have been isolated from the Okinawan marine sponge* Biemna *sp. The X-ray diffraction analysis of 1 has established its octacyclic structure and the structure of 2 elucidated on the basis of extensive spectroscopic and chemical studies.*

A series of polycyclic aromatic alkaloids possessing significant biological activities and unique structural features have been isolated from marine organisms in the past several years.[1] In our continuing search for bioactive compounds from Okinawan marine organisms,[2] we reported previously the isolation of several alkaloids of this series such as ascididemin[3] and cystodytins[4] from Okinawan tunicates. Here we describe the isolation and structure elucidation of two new polycyclic alkaloids, biemnadin (1) and 8,9-dihydro-11-hydroxyascididemin (2), from the Okinawan marine sponge *Biemna* sp. The structure of the octacyclic alkaloid biemnadin (1) with a unique carbon skeleton was unambiguously determined by X-ray diffraction analysis.

The sponge *Biemna* sp. was collected at Unten Harbor, Okinawa Island, and kept frozen until used. The methanol extract was partitioned between ethyl acetate and water and the aqueous phase was subsequently extracted with *n*-butanol. The ethyl acetate-soluble fraction was subjected to silica gel flash column



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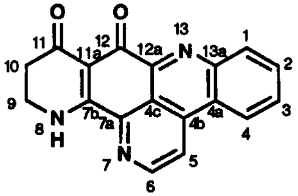

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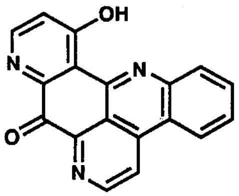

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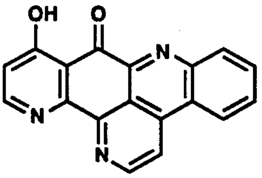

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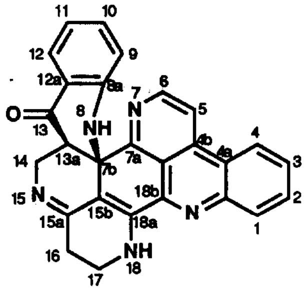

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Table 1. ^{1}H NMR Chemical Shifts of Compounds **1** and **5** (DMSO-d_6/CD$_3$OD, 2:1)

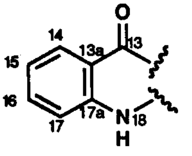
	1			**5**[a]	
position	δ_H	*J*/Hz	position	δ_H	*J*/Hz
1	7.78 d	8.1	1	8.33 br d	8.0
2	7.88 dd	8.1, 7.4	2	8.01 br t	7.5
3	7.72 dd	7.4, 7.7	3	7.88 br t	7.5
4	8.82 d	7.7	4	8.79 br d	7.5
5	8.96 d	5.5	5	8.57 s	
6	9.20 d	5.5	6	8.57 s	
14	7.75 dd	8.1, 1.5	12	7.68 dd	8.0, 1.0
15	6.65 ddd	8.1, 7.3, 1.1	11	6.62 br t	8.0
16	7.10 ddd	7.3, 8.4, 1.5	10	7.11 br t	8.0
17	6.32 dd	8.4, 1.1	9	6.32 d	8.5

[a] ref. 6.

Table 2. ^{13}C NMR Chemical Shifts of Compounds **1** and **5** (DMSO-d_6/CD$_3$OD, 2:1)

1		**5**[a]		**1**		**5**[a]	
position	δ_C	position	δ_C	position	δ_C	position	δ_C
1	128.6 d	1	131.0	10b	117.8 s	15b	114.9
2	132.2 d	2	132.4	12	40.6 t	14	41.4
3	129.4 d	3	130.5	12a	44.9 d	13a	45.7
4	124.1 d	4	124.3	13	191.3 s	13	191.8
4a	121.5 s	4a	123.6	13a	117.8 s	12a	117.9
4b	137.7 s	4b	137.8	14	125.7 d	12	126.3
4c	114.7 s	4c	113.7	15	117.8 d	11	117.9
5	120.2 d	5	116.7	16	135.0 d	10	135.4
6	148.8 d	6	148.0	17	115.9 d	9	116.0
7a	144.5 s	7a	157.9	17a	145.5 s	8a	146.4
7b	155.1 s[b]	18a	149.9	18a	54.7 s	7b	55.0
9	38.3 t	17	38.2	18b	154.1 s[b]	18b	144.6
10	29.1 t	16	27.4	19a	143.5 s	19a	144.4
10a	162.9 s	15a	163.8				

[a] ref. 6. [b] interchangeable signals.



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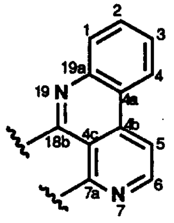

b

chromatographies (hexane/ethyl acetate and chloroform/methanol), followed by gel filtration on a Sephadex LH-20 column to yield biemnadin (**1**, 0.0003% yield, wet weight) together with a known alkaloid, meridine[5] (**3**, 0.0002%). From the butanol-soluble fraction, 8,9-dihydro-11-hydroxyascididemin (**2**, 0.0001%) and a known related compound, 11-hydroxyascididemin[5] (**4**, 0.0001%), were obtained by the similar separation procedures.

Biemnadin (**1**) was shown to have the molecular formula of $C_{27}H_{19}N_5O$ by HRFABMS data [*m/z* 430.1657, $(M+H)^+$, Δ -1.1 mmu], containing 21 degrees of unsaturation. The ^1H NMR spectrum of **1** revealed signals due to 10 aromatic protons and 7 aliphatic protons. Interpreting the ^1H-^1H COSY spectrum suggested that the aromatic proton signals consisted of three isolated spin systems, two of which were ascribable to 1,2-substituted benzene rings (H-1 ~ H-4 and H-14 ~ H-17). The signals for third aromatic spin system at δ_H 8.80 (1H, d, *J*=5.5 Hz; H-5) and 9.19 (1H, d, *J*=5.5 Hz; H-6) were indicative of the presence of a pyridine ring according to their chemical shifts and coupling constant.[3-5] Seven aliphatic protons were attributed to two isolated spin systems ($-CH_2CH_2-$ and $-CH_2CH-$). In the HMBC spectrum of **1** the ^1H-^{13}C long-range correlations were observed for NH-18/C-13a, NH-18/C-17; H-14/C-16, H-14/C-17a, and H-14/C-13; H-6/C-7a, H-6/C-4b, H-6/C-5; H-5/C-4c, H-5/C-4a, and H-5/C-6, which indicated the presence of the partial structures **a** and **b**. These partial structures were also contained in eudistone A (**5**),[6] an octacyclic alkaloid previously isolated from a Seychelles tunicate *Eudistoma* sp., and the molecular formula of compound **1** is the same as that of **5**. Detailed comparison of the ^1H and ^{13}C NMR data of **1** and **5** suggested that their ^1H and ^{13}C chemical shifts were slightly different from each other (Tables 1 and 2). From these results biemnadin (**1**) was deduced to possess an isomeric structure closely-related to eudistone A (**5**). Although it appeared considerably difficult to obtain further information on the connectivities of the partial structures from the NMR studies, crystals of hydrochlolide salt of biemnadin (**1**) were fortunately obtained and unambiguous proof for the structure of **1** was provided by X-ray diffraction analysis. A computer-generated perspective drawing of the final X-ray model is given in Figure 1, which represented that the N-11 atom is protonated and a chloride ion is attached on it. Thus, biemnadin (**1**) proved to be a new octacyclic aromatic alkaloid; the position of one benzene ring is different from that of eudistone A (**5**). Interestingly, the X-ray study revealed that the compound **1** is recemic though it possessess two asymmetric centers at C-12a and C-18a, whereas eudistone A (**5**) was reported to be optically active.[7,8]

8,9-Dihydro-11-hydroxyascididemin (**2**) was obtained as yellow amorphous powder and its molecular formula, $C_{18}H_{11}N_3O_2$, suggested by HRFABMS data [*m/z* 302.0928, $(M+H)^+$, Δ -0.2 mmu] contained two more hydrogens than that of 11-hydroxyascididemin (**4**).[5] The ^1H NMR [δ_H 3.82 (2H, t, *J*=7.8 Hz) and 2.59 (2H, t, *J*=7.8 Hz)] and ^{13}C NMR data [δ_C 41.0 (t) and 37.0 (t)] corresponded to two new sp^3

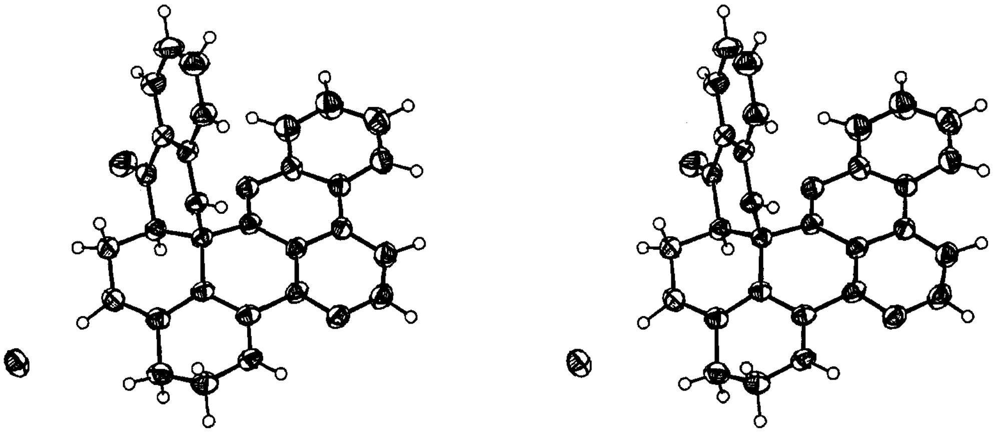

Figure 1. A computer generated perspective drawing of the final X-ray model for compound **1** (hydrochloride salt).

Table 3. ^{1}H and ^{13}C NMR Chemical Shifts of Compounds **2** and **4**

	2[a]				**4**[b]			
position	δ_H		*J*/Hz	δ_C	δ_H		*J*/Hz	δ_C
1	8.16	dd	8.0, 1.5	132.0 d	8.64	dd	8.1, 1.8	133.3
2	7.81	ddd	8.0, 7.4, 1.1	132.5 d	8.06	dt	8.1, 1.2	132.1
3	7.74	ddd	8.0, 7.4, 1.5	131.5 d	8.00	dt	8.1, 1.8	131.5
4	8.50	dd	8.0, 1.1	124.5 d	8.73	dd	8.1, 1.2	123.1
5	8.41	d	5.7	119.0 d	8.58	d	5.7	117.2
6	8.94	d	5.7	150.5 d	9.31	d	5.7	150.1
9	3.82 (2H)	t	7.8	41.0 t	7.15	d	5.7	156.7
10	2.59 (2H)	t	7.8	37.0 t	8.89	d	5.7	114.8

a) in $CDCl_3/CD_3OD$ (1:1). Assignments were based on the HSQC spectrum of **2**. b) in $CDCl_3$, ref. 5.

methylenes in place of the aromatic signals of 11-hydroxyascididemin (**4**). The two sp^3 methylenes were deduced to be located at C-9 and C-10 on the basis of comparing the ^{1}H and ^{13}C NMR data (Table 3). Compound **2** was treated with DDQ to afford 11-hydroxyascididemin (**4**), thus confirming the sturcuture of **2**.

It is noteworthy that two new polycyclic aromatic alkaloids (**1** and **2**) as well as known alkaloids (**3** and **4**) were now isolated from a sponge of the genus *Biemna*, while compounds **3**, **4**,[5] and eudistone A[6] (**5**), closely related to compounds **2** and **1**, respectively, were previously obtained from extracts of tunicates, *Amphicarpa meridiana*, *Leptoclinides* sp., and *Eudistoma* sp. Compounds **1** and **2** exhibited cytotoxicity against human epidermoid carcinoma KB (IC$_{50}$ values: 1.73 and 0.209 μg/mL, respectively) and murine lymphoma L1210 (IC$_{50}$ values: 4.29 and 0.675 μg/mL, respectively) cells in vitro.

Experimental Section

General Methods. Melting points were determined on a Yanaco MP-J3 melting point apparatus and uncorrected. UV, IR and CD spectra were taken on JASCO Ubest-35, JASCO Report-100, and JASCO J-500A spectrometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded on JEOL JMN GX-270 and EX-400 spectrometers. EIMS and FABMS were obtained on a JEOL JMS DX-303 and a JEOL HX-110 spectrometers, respectively. Wako C-300 silica gel (Wako Pure Chemical) was used for glass column chromatography and TLC was carried out on Merck silica gel GF$_{254}$.

Sponge Material. The sponge *Biemna* sp. (Order Poecilosclerida; Family Desmacellidae; Gray, 1867) collected by netting at Unten Harbor, Okinawa Island, was kept frozen until used. The specimen was a very dark brown to purple black sponge when preserved with some foreign material in the mesohyl. Skeleton a loose unispicular or bispicular reticulation of styles without fibre development. Numerous large sigmas throughout the mesohyl. Styles 552-612 x 12-13 µm; sigmas 96 µm, rephides 210 µm long. The voucher specimen (SS-857) was deposited at the Faculty of Pharmaceutical Sciences, Hokkaido University.

Extraction and Isolation Procedures. The MeOH extract of the sponge *Biemna* sp. (1.7 kg, wet weight) was evaporated under reduced pressure and the residue (36 g) was partitioned between EtOAc (400 mL x 3) and 1M NaCl (400 mL) and the aqueous layer was further extracted with *n*-BuOH (400 mL x 3). The EtOAc soluble material (877 mg) was partially (432 mg) subjected to silica gel flash column chromatography chromatography (50 x 3 cm) with gradient elution of EtOAc in hexane (0~100%) and MeOH in CHCl$_3$ (50~100%). The fraction (15.4 mg) eluted by 50% MeOH in CHCl$_3$ was further purified by a Sephadex LH-20 column chromatography (CHCl$_3$/MeOH, 1:1; 120 x 2.5 cm) to give biemnadin (**1**, 1.3 mg). The other fraction (71 mg) eluted with 50% MeOH in CHCl$_3$ was separated by the second silica gel column chromatography (30 x 1.5 cm) eluted with MeOH in CHCl$_3$ (0~100%). The fraction (4.7 mg) eluted with 6% MeOH in CHCl$_3$ was purified by a Sephadex LH-20 column chromatography (CHCl$_3$/MeOH, 1:1; 120 x 2.5 cm) to yield meridine (**3**, 2.0 mg). The *n*-BuOH soluble material (2.4 g) was separated by a silica gel column chromatography (50 x 3 cm) eluted with CHCl$_3$/*n*-BuOH/AcOH/H$_2$O (1.5:6:1:1). The fraction (30 mg) eluted from 815 mL to 920 mL was further purified by repeated Sephadex LH-20 column chromatographies (CHCl$_3$/MeOH, 1:1; 120 x 2.5 cm) to give 11-hydroxyascididemin (**4**, 2.0 mg) and 8,9-dihydro-11-hydroxyascididemin (**2**, 1.8 mg).

Biemnadin (1). Yellow crystal; mp > 300 °C; UV (MeOH) λ_{max} 229 (ε 29000), 256 (17000), and 370 nm (5000); IR (KBr) ν_{max} 3400, 3250, 1660, and 1560 cm^{-1}; ^{1}H NMR (DMSO-d_6/CD$_3$OD, 2:1), see Table 1; ^{1}H NMR (CD$_3$OD) δ_H 9.19 (1H, d, *J*=5.5 Hz, H-6), 8.80 (1H, d, *J*=5.5 Hz, H-5), 8.78 (1H, d, *J*=7.7 Hz, H-4), 7.87 (1H, d, *J*=8.1 Hz, H-1), 7.87 (1H, dd, *J*=1.5 and 8.1 Hz, H-14), 7.82 (1H, dd, *J*=7.4 and 7.7 Hz, H-3), 7.75 (1H, dd, *J*=7.4 and 8.1 Hz, H-2), 7.11 (1H, ddd, *J*=1.5, 7.3, and 8.4 Hz, H-16), 6.68 (1H, ddd, *J*=1.1, 7.3, and 8.1 Hz, H-15), 6.37 (1H, dd, *J*=1.1 and 8.4 Hz, H-17), 4.03 (1H, ddd, *J*=1.4, 5.1, and 15.5 Hz, H-9), 3.83 (1H, ddd, *J*=1.4, 5.1, and 15.5 Hz, H-9'), 3.79 (2H, m, H$_2$-12), 3.35 (1H, m, H-12a), 3.12 (1H, m, H-10), and 2.91 (1H, m, H-10'); ^{13}C NMR (DMSO-d_6/CD$_3$OD, 2:1) see Table 2; HRFABMS *m/z* 430.1657 (M+H; calcd for $C_{27}H_{20}N_5O$, 430.1668).

8,9-Dihydro-11-hydroxyascididemin (2). Yellow amorphous powder; mp > 300 °C; UV (MeOH) λ_{max} 218 (ε 17000), 273 (9300), 319 (6700), and 355 nm (5300); IR (KBr) ν_{max} 3250, 2900, 1660, 1600, and 1020 cm^{-1}; ^{1}H and ^{13}C NMR (CDCl$_3$/CD$_3$OD, 1:1) see Table 3; EIMS *m/z* (intensity, %) 301 (M$^+$, 85), 271 (20), and 243 (8); HRFABMS *m/z* 302.0928 (M+H; calcd for $C_{18}H_{12}N_3O_2$, 302.0930).

Crystallization of Biemnadin (1). Toluene/methanol (2:1, 1 mL) was added to a sample tube containing biemnadin (**1**, 1.6 mg) and warmed until all the solid was completely dissolved. The tube was then capped and the solvent was evaporated naturally at room temperature. After three or four days, it was observed that the crystals were forming gradually.

Crystal Data. Biemnadin hydrochloride salt, $C_{27}H_{20}N_5OCl$, crystal size 0.25 x 0.15 x 0.15 mm, monoclinic, space group $P2_1/a$, a=14.638 (2) Å, b=14.965 (4) Å, c=9.993 (2) Å, β=96.93 (1)°, V=2173.1 (8) $Å^3$, Z=4, D_{obs}=1.42 g/cm^3, D_{calc}=1.42 g/cm^3, F(000)=913, μ(CuKα)=8.62 cm^{-1}. Intensity data were collected on a Mac Science MXC18 diffractometer using CuKα radiation (λ=1.54178 Å). The final R-factor was 0.036. The final atomic coordinates as well as bond lengths and bond angles are deposited at the Cambridge Crystallographic Data Centre.

Oxidation of 8,9-Dihydro-11-hydroxyascididemin (2). DDQ (0.3 mg) was added to a solution of **2** (0.16 mg) in $CHCl_3$/MeOH (1:1, 0.5 mL). After stirring at room temperature for 14 h, the solvent was removed by evaporation under reduced pressure. The major product was concluded to be identical with 11-hydroxyascididemin (**4**) by comparison of the TLC behavior and EIMS data in which the molecular ion peak for 11-hydroxyascididemin (**4**) was clearly observed at m/z 299, while the molecular ion peak for **2** at m/z 301 disappeared.

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7. No characteristic curve was observed in the CD spectrum of biemnadin (**1**) in MeOH, while eudistone A (**5**) was reported to give a distinctive CD curve.[6]
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