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Iheyamines, new cytotoxic bisindole pigments from a colonial ascidian, *Polycitorella* sp.

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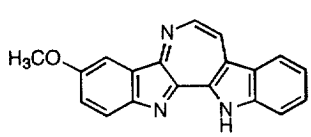
Abstract

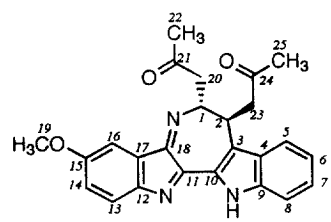
A new class of bisindole pigments, iheyamines A and B, have been isolated from an ascidian, *Polycitorella* sp. and their structures elucidated by interpretation of spectral data. Both compounds exhibited moderate cytotoxicity.

Keywords: cytotoxic; ascidian; alkaloid; deuterium-induced shifts

A number of chemically and biologically interesting compounds have been reported from ascidians [1]. The majority of them are nitrogenous compounds such as cyclic peptides and polycyclic aromatic alkaloids based on the pyrido[2,3,4-*k*,*l*]acridine nucleus. Recently we found new aromatic alkaloids, iheyamines A (**1**) and B (**2**), which exhibited moderate cytotoxicity, from a colonial ascidian, *Polycitorella* sp. collected off the island of Iheya, Okinawa. They were not pyrido-acridines but belonged to a new class of heterocycles having a bisindole skeleton. In this report we describe the isolation and structure elucidation of these compounds.



Iheyamine A (1)



Iheyamine B (2)

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A sample (1.2 kg wet weight) of the purple ascidian was extracted with acetone. The concentrated extract was partitioned between ethyl acetate and water. The ethyl acetate layer (3.6 g) was separated on Sephadex LH-20 (CH_2Cl_2-MeOH 1:1) followed by successive chromatography on silica gel and by HPLC (ODS and/or amino) to afford iheyamines A (**1**, 7.0 mg) and B (**2**, 3.3 mg) as purple, amorphous solids.

Iheyamine B (**2**)[1] had a molecular formula $C_{25}H_{23}N_3O_3$ as deduced from HR FABMS (MH^+ *m/z* 414.1824, Δ +0.6 mmu). The aromatic nature of **2** was evident from the high unsaturation requirement (16 sites) and from its ^1H NMR spectrum. Interpretation of the ^1H and ^{13}C NMR, COSY, and HOHAHA spectra led to the elucidation of partial structures **a**, **b**, and **c** (Figure 1A, solid line portions).

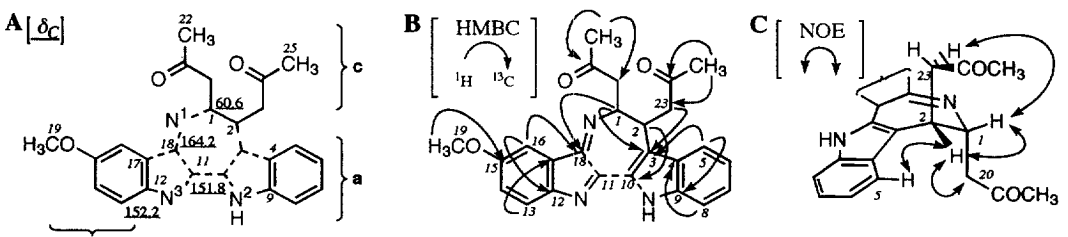

Figure 1. Partial structures (**A**) and HMBC (**B**) and NOE (**C**) correlations for iheyamine B (**2**).

An exchangeable proton at δ 10.77 was located on the nitrogen (N^2) in the partial structure **a** by a long range coupling (J = 0.9 Hz) with the signal (δ 7.65) of H-5. The partial structures and the remaining nitrogen atom (N^1) could be assembled as shown in Figure 1B by observing HMBC cross peaks and considering the chemical shift values of δ 60.6 (C-1) and 164.2 (C-18). At this point only one carbon (δ 151.8) remained to be connected. The connectivity of this carbon (C-11) to C-10 was provided by deuterium-induced shift experiment [2]. When ^{13}C NMR spectrum (acetone-d_6) was taken in the presence of CD_3OD, some signals were observed to split into two peaks due to the isotopic effect (Table 1). The shifts for C-9 (Δδ 0.141) and C-10 (Δδ 0.122) indicated that these carbons were α to the nitrogen (N^2) bearing an exchangeable proton. A small but distinct shift (Δδ 0.032) observed with the signal at δ 151.8 indicated that this carbon was located β to N^2, thus establishing the connectivity of C-10 to C-11 and completing the planar structure for **2**. The same magnitude of the shifts were observed with C-3, C-4, and C-8 (Table 1). All of the NMR signals were assigned as shown in Table 1. The relative stereochemistry was elucidated by NOESY and NOEDS observation as shown in Figure 1C and supported by an H-H coupling constant (3.2 Hz) between H-1 and H-2. Although a CD spectrum was recorded, we were unable to determine the absolute configuration

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[1] Iheyamine B (**2**): $[\alpha]_D^{25}$ -16° (*c* 2x10^{-4}, $CHCl_3$); UV / visible ($CHCl_3$) λ_{max} 276 sh (ε 18,000), 300 (ε 36,000), 307 (ε 36,000), 330 sh (ε 14,000), 526 nm (ε 7,400); IR ($CHCl_3$) ν_{max} 3500-2500, 1710, 1590, 860, 800 cm^{-1}; CD ($CHCl_3$) 265 nm (Δε 4.35), 298.5 (Δε -4.57), 295.5 (Δε -5.33), 305.5 (Δε -6.83).

Table 1

NMR Data (in Acetone-d_6) for Iheyamine B (**2**)

No.	^{13}C NMR[a]	^{1}H NMR [mult. *J* (Hz)][b]	NOE[c]	HMBC[d]	isotope shift ($\Delta\delta$)[e]
1	δ 60.6 d	δ 5.33 td 7.0, 3.2	H-2, H_2-23, H_2-20	H_2-23,23', H_2-20	0
2	34.7 d	4.14 td 7.0, 3.2	H-5, H-1, H_2-20	H_2-23,23', H_2-20	0
3	122.1 s			H-5, H-1, H_2-23,23', H-2	0.046
4	128.2 s			H-5, H-6, H-8	0.042
5	121.0 d	7.65 ddt 8.1, 1.2, 0.9	H-6, H-2	H-7	0
6	120.8 d	7.09 ddd 8.1, 7.0, 0.9	H-5, H-7	H-8	0
7	126.1 d	7.273 ddd 8.3, 7.0, 1.2	H-8, H-6	H-5, H-6	0
8	112.7 d	7.51 dt 8.3, 0.9	H-13, NH	H-6	0.051
9	139.9 s			H-5, H-7	0.141
10	130.0 s			H-2	0.122
11	151.8 s				0.032
12	152.2 s			H-16, H-14	0
13	121.9 d	7.270 dd 8.1, 0.4	H-8, H-14		0
14	117.8 d	6.97 dd 8.1, 2.8	H_3-19, H-13	H-16	0
15	160.2 s			H_3-19, H-16, H-14, H-13	0
16	108.6 d	7.15 dd 2.8, 0.4	H_3-19	H-14	0
17	132.0 s			H-13	0
18	164.2 s			H-1, H-16	0
19	56.2 q	3.87 s	H-16, H-14		0
20	47.0 t	2.71 d 7.0	H-1, H-2, H_3-22	H-1, H_3-22	0
21	205.6 s			H_3-22, H_2-20	0
22	30.3 q	1.99 s	H_2-20		0
23	48.9 t	2.96 dd 17.1, 7.0	H-1	H-2, H_3-25	0
		2.87 dd 17.1, 7.0			
24	206.4 s			H_3-25, H_2-23,23'	0
25	29.3 q	2.01 s			0
NH		10.77 brs	H-8		

[a] Taken at 125 MHz [b] Taken at 500 MHz [c] Obtained by NOESY and NOE difference spectra [d] Optimized for 8 Hz
[e] δ_C (acetone-d_6) - δ_C (acetone-d_6/CD_3OD)

of **2**. The presence of optical activity rules out a question that **2** may be an artifact of the extraction with acetone.

HR FABMS of iheyamine A (**1**)[2] gave a formula of $C_{19}H_{13}N_3O$, which differed from that of **2** by $C_6H_{10}O_2$, equivalent to two units of 2-oxopropyl. The ^{1}H and ^{13}C NMR spectra revealed that **1** was closely related to **2** except for the absence of two 2-oxopropyl side chains. Instead, **1** exhibited two highly deshielded signals (δ 9.04 and 8.55) which could be assigned to H-1 and H-2, respectively. 2D NMR study (COSY, HOHAHA, HMQC, HMBC) allowed us to depict the structure **1** and to assign all the signals as shown in Table 2.

The skeleton (**3**) of iheyamine A is a new polycyclic heteroaromatic system composed of an azocine unit fused onto a bisindole unit. Alternatively, it can be viewed as a diaza-azulene derivative. Bisindole pigments from marine sources include the well-known Tyrian purple [4] from snails and its derivatives from an acorn worm [5], caulerpin from green algae [6], and

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[2] Iheyamine A (**1**): isolated as a salt form by addition of TFA; IR ($CHCl_3$) ν_{max} 3700-2500, 1620, 1600, 1390, 1340, 1290, 970, 810, 800 cm^{-1}; HR FABMS (glycerol) MH^+ *m/z* 300.1161, Δ +2.4 mmu.

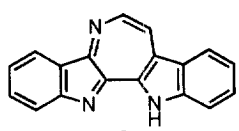
11-hydroxystaurosporine from an ascidian [7]. Iheyamines A (1) and B (2) showed cytotoxicity with IC_{50} in the level of 1 μg/mL against P388, A549, and HT29 cell lines.

Table 2

NMR (in $CDCl_3$) and UV/visible Data for Iheyamine A (1)

No.	^{13}C NMR[a] Salt form	^{1}H NMR [mult. J (Hz)][b] Salt form			^{1}H NMR [mult. J (Hz)][b] Free base form[c]			UV/visible Salt form	UV/visible Free base form
1	δ 143.2 d	δ 9.38	d	6.4	δ 9.04	d	6.4	λ_{max} (MeOH)	λ_{max} (MeOH + NaOH)
2	124.5 d	8.85	d	6.4	8.55	d	6.4	284 nm (ε 16,000)	338 nm (ε 42,000)
3	128.3 s							325 sh (ε 33,000)	370 sh (ε 20,000)
4	123.8 s							341 (ε 36,000)	480 (ε 4,400)
5	121.4 d	8.42	brd	7.9	8.37	brd	7.9	360 sh (ε 30,000)	~500 sh (ε 4,200)
6	124.4 d	7.61	brdd	7.9, 7.0	7.52	brdd	7.9, 7.0	388 (ε 19,000)	
7	132.8 d	7.84	brdd	7.9, 7.0	7.74	brdd	7.9, 7.0	499 (ε 5,200)	
8	115.1 d	8.05	brd	7.9	7.86	brd	7.9		
9	144.0 s							λ_{max}($CHCl_3$)	
10	138.1 s							287 nm	
11	148.9 s							330 sh	
12	138.2 s							346	
13	115.7 d	7.99	d	8.9	7.70	d	8.9	365 sh	
14	124.1 d	7.52	dd	8.9, 2.4	7.37	dd	8.9, 2.4	395	
15	157.5 s							515	
16	102.8 d	7.96	d	2.4	8.01	d	2.4		
17	126.1 s								
18	135.7 s								
19	56.0 q	4.02	s		4.02	s			

[a] Taken at 125 MHz [b] Taken at 500 MHz [c] Recorded after washing with 0.1 N NaOH solution



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