Notes

Hyrtinadine A, a Bis-indole Alkaloid from a Marine Sponge[⊥]

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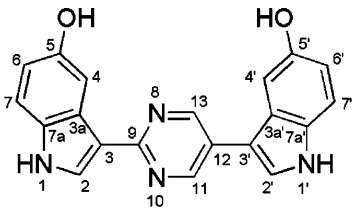
A new cytotoxic bis-indole alkaloid, hyrtinadine A (**1**), with a pyrimidine moiety has been isolated from an Okinawan marine sponge *Hyrtios* sp., and the structure was elucidated on the basis of spectroscopic data.

Marine sponges of the genus *Hyrtios* have been found to contain a variety of structurally unique metabolites with interesting biological activities,[1] and a number of indole alkaloids have been isolated from sponges of this genus so far.[2] In our search for bioactive substances from marine sponges,[3] we have investigated extracts of an Okinawan marine sponge *Hyrtios* sp. and isolated a new cytotoxic bis-indole alkaloid, hyrtinadine A (**1**), with a pyrimidine moiety. Here we describe the isolation and structure elucidation of **1**.



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The sponge *Hyrtios* sp. (SS-1127) (order Dictyoceratida; family Thorectidae) collected off Unten-Port, Okinawa, was extracted with MeOH. The EtOAc-soluble materials of the extract were subjected to silica gel chromatography ($CHCl_3$/MeOH) and then C_{18} columns (MeOH/H_2O) followed by C_{18} HPLC (MeOH/H_2O) to yield hyrtinadine A (**1**, 1.0 mg, 0.0046%, wet weight) as a colorless, amorphous solid, together with hyrtiosins A and B,[4] 5-hydroxyindole-3-aldehyde,[4] and hyrtiosulawesine.[5] The ESIMS spectrum of hyrtinadine A (**1**) showed the pseudomolecular ion peak at *m*/*z* 343 $(M + H)^+$, and HRESIMS data of **1** revealed the molecular formula, $C_{20}H_{14}N_4O_2$ [*m*/*z* 343.1191 $(M + H)^+$, Δ −0.4 mmu]. The UV absorption [λ_{max} 277 nm (ϵ 7600)] was attributable to substituted benzenoid chromophore(s), while the absorption at 339 nm (ϵ 3100) indicated the presence of the other ring system(s) conjugated to the benzenoid ring(s). IR absorptions indicated the presence of OH and/or NH (3390 cm^{-1}) group(s). In the ^1H NMR (Table 1) spectrum of **1**, four D_2O-exchangeable protons were observed, two (δ_H 8.87 and 8.85, both brs) of which were suggestive of phenolic OH signals, while the remaining two (δ_H 11.4 and 11.3, both brs) were due to aromatic NH signals. The ^{13}C NMR data (Table 1) of **1** disclosed signals due to 10 sp^2 quaternary and 10 sp^2 methine carbons. The existence of two 1,2,4-trisubstituted benzene rings was deduced from the following proton signals: δ 7.97 (brs), 7.29 (d, J = 8.6 Hz), 7.26 (d, J = 8.6 Hz), 7.20 (brs), 6.71 (brd, J = 8.6 Hz), and 6.69 (brs, J = 8.6 Hz). The presence of two 5-hydroxy-3-indolyl moieties was concluded from HMBC correlations as shown in Figure 1.

Table 1. ^1H and ^{13}C NMR Data of Hytinadine A (**1**) in DMSO-d_6

position	δ_C		δ_H	
1			11.4	brs
2	128.7	CH	8.11	s
3	114.2	C		
3a	126.4	C		
4	106.2	CH	7.97	brs
5	151.8	C		
5-OH[a]			8.87	brs
6	112.0	CH	6.71	brd, 8.6
7	112.6	CH	7.29	d, 8.6
7a	131.5	C		
9	160.7	C		
11, 13	153.5	CH	9.00[b]	s
12	125.0	C		
1′			11.3	brs
2′	124.2	CH	7.79	s
3′	108.6	C		
3a′	125.4	C		
4′	102.7	CH	7.20	brs
5′	151.7	C		
5′-OH[a]			8.85	brs
6′	112.2	CH	6.69	brd, 8.6
7′	112.6	CH	7.26	d, 8.6
7a′	131.3	C		

[a] Interchangeable. [b] 2H.

Considering the molecular formula, $C_{20}H_{14}N_4O_2$, of **1** and the presence of two 5-hydroxy-3-indolyl moieties, it was suggested that the remaining part consisted of $C_4H_2N_2$ with four double-bond equivalents. Chemical shifts and intensities of signals for δ_H 9.00 (2H, s, H-11 and H-13) and δ_C 153.5 (2C, C-11 and C-13) suggested that **1** has a symmetrical heteroaromatic ring, while signals due to two 5-hydroxy-3-indolyl moieties were not equivalent, thus indicating that the residual $C_4H_2N_2$ part was a C_2-symmetrical heteroaromatic structure, namely, a 2,5-substituted pyrimidine ring, which was supported by HMBC correlations for H-11 (or H-13) to C-9 (δ_C 160.7), C-11 (or C-13) (δ_C 153.5), and C-12 (δ_C 125.0). The HMBC correlation for H-11 (or H-13)/C-3′ and NOESY correlations for H-11 (or H-13)/H-2′ and H-11 (or H-13)/H-4′ suggested that C-3′ is attached to C-12 in the pyrimidine ring, and the other

[⊥] Dedicated to the late Dr. Kenneth L. Rinehart of the University of Illinois at Urbana−Champaign for his pioneering work on bioactive natural products.

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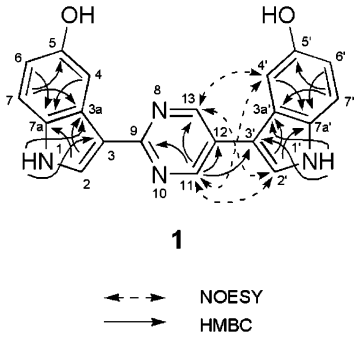

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Figure 1. Selected 2D NMR correlations for hyrtinadine A (**1**).

quaternary carbon (C-9) was connected to C-3. Thus, the structure of hyrtinadine A was concluded to be **1**.

Hyrtinadine A (**1**) is the first example of a bis-indole alkaloid with a 2,5-disubstituted pyrimidine ring between two indole rings, although natural bis-indole alkaloids with imidazole, piperidine, or pyrrolidone rings such as topsentins,[6−9] rhopaladins,[10] dragmacidins,[11−14] and violacein,[15] and monoindole alkaloids with a pyrimidine ring such as psammopenins[16] and meridianins,[17] have been isolated from natural sources so far. Hyrtinadine A (**1**) exhibited cytotoxicity against murine leukemia L1210 cells (IC_{50} 1 μg/mL) and human epidermoid carcinoma KB cells (IC_{50} 3 μg/mL) in vitro.

Experimental Section

General Experimental Procedures. IR and UV spectra were recorded on a Shimadzu UV-1600PC and a JASCO FT/IR-5300 spectrophotometer, respectively. ^{1}H, ^{13}C, and 2D NMR spectra were measured on a Bruker AMX-600 spectrometer using 2.5 mm microcells for DMSO-d_6 (Shigemi Co., Ltd.). Positive-mode ESIMS were obtained on a JEOL JMS 700-TZ spectrometer at −80 V as a focus voltage using a sample dissolved in MeOH with flow rate of 200 μL/min.

Sponge Description. The sponge *Hyrtios* sp. (order Dictyoceratida; family Thorectidae) was collected off Unten-Port, Okinawa, Japan, March 2005, and kept frozen until used. The sponge was firm but compressible, and the surface was finely conulose from underlying primary fibers, which look sand impregnated macroscopically. The color of its porous surface was blackish-brown, but the interior mesohyl was slightly lighter in color than the exterior surface. Its undersurface was not conulose with oscules, and its interior was somewhat compact and porous with small canals. All fibers were cored with sand grains.

Its primary fibers tended to fasciculation and were approximately 250 μm wide; the secondary fibers were fairly regular square or oval meshes, 30−50 μm wide, and were centrally cored with sand grains. The voucher specimen (SS-1127) was deposited at Graduate School of Pharmaceutical Sciences, Hokkaido University.

Extraction and Isolation. The sponge (1.13 kg, wet weight) was extracted with MeOH (500 mL × 2), and the extract was partitioned between EtOAc (700 mL × 3) and H_2O (500 mL). An aliquot of the EtOAc-soluble material (5 g) was subjected to a SiO_2 gel column ($CHCl_3$/MeOH) and then a C_{18} column (Cosmosil 140 C_{18} PREP, Nakarai Tesque Inc., Japan; eluent, MeOH/H_2O, 45:55) to give a crude fraction of alkaloids. The crude fraction was purified by C_{18} HPLC [YMC Pak Pro C_{18}, YMC Co., Inc., Japan, 10 × 250 mm; eluent, MeOH/H_2O, 40:60; flow rate, 2.0 mL/min; UV detection at 210 nm] to afford hyrtinadine A (**1**, 0.0046%, wet weight, t_R 33 min).

Hyrtinadine A (1): colorless, amorphous solid; UV (MeOH) λ_{max} 339 (ϵ 3100) and 277 nm (7600); IR (KBr) ν_{max} 3390 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); ESIMS (pos) *m*/*z* 343 $(M + H)^+$; HRESIMS *m*/*z* 343.1191 [calcd for $C_{20}H_{14}N_4O_2$, $(M + H)^+$; 343.1195].

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