Hamacanthins A and B, New Antifungal Bis Indole Alkaloids from the Deep-Water Marine Sponge, Hamacantha Sp.

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HAMACANTHINS A AND B, NEW ANTIFUNGAL BIS INDOLE ALKALOIDS FROM THE DEEP-WATER MARINE SPONGE, *HAMACANTHA* SP.

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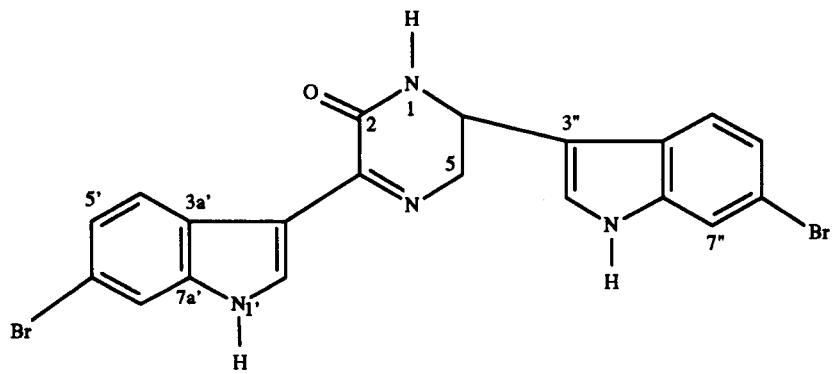
ABSTRACT.—Hamacanthin A [**1**] and hamacanthin B [**2**] are two bioactive dihydropyrazinonediylbis(indole) alkaloids isolated from a new species of deep-water marine sponge, *Hamacantha* sp. The hamacanthins are growth inhibitors of *Candida albicans* and *Cryptococcus neoformans*. Isolation and structure elucidation of **1** and **2** by nmr spectroscopy are described.

Several interesting biologically active bis indole alkaloids have been reported from marine organisms over the past few years. The examples reported from sponges include the antitumor and antiviral active topsentins from *Topsentia genitrix* (1), *Spongosorites* spp. (2,3), and *Hexadella* sp. (4), which have a ketone and imidazole moiety between the two indole rings; the cytotoxic dragmacidins reported from both *Dragmacidon* sp. (5) and *Hexadella* sp. (4) which have a piperazine moiety; the cytotoxic and antifungal nortopsentins reported from *Spongosorites ruetzleri* (6) which lack the ketone observed in the topsentins; the biologically active dragmacidin-d, which has a 2(1H)-pyrazinone moiety, reported from a *Spongosorites* sp. (7), and a fully aromatized antimicrobial pigment, fascaplysin, reported from *Fascaplysinopsis* sp. (8). Bis indole alkaloids have also been reported from the ascidians *Dendrodoa grossularia* (9) and *Didemnum candidum* (10). In this paper, we report the isolation and structure elucidation of two new isomeric bis indole alkaloids which we have named hamacanthins A [**1**] and B [**2**] from a deep-water sponge of the genus *Hamacantha*. Hamacanthins A and B both inhibited the growth of the opportunistic fungal pathogens *Candida albicans* and *Cryptococcus neoformans*.

In our search for biologically active substances from marine organisms, an EtOH extract from a deep-water marine sponge *Hamacantha* sp. was found to inhibit the growth of *C. albicans*. This new species of *Hamacantha* was collected by a



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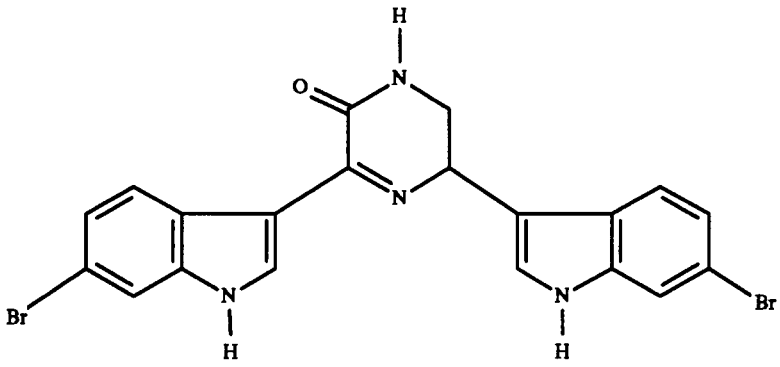

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manned submersible vessel off the southeast coast of Madeira at a depth of 548 m. An EtOH extract of the frozen sponge yielded an extract that was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction on bioassay-guided cc over Si gel followed by hplc furnished the two new antifungal constituents, hamacanthin A [**1**] and hamacanthin B [**2**]. The hamacanthins are related in structure to the reported dragmacidins (5) and nortopsentins (6). The structural charac-

TABLE 1. ^{13}C- and ^{1}H-Nmr Data[a] for Hamacanthins A [**1**] and B [**2**] in DMSO-d_6.

Position	1			2	
	^{13}C[b]	^{1}H[c]	HMBC (^{1}H)	^{13}C	^{1}H
1		8.78 (d, 1.0)			8.49 (t, 1.5)
2	157.4[d] s		H-1	157.2 s	
3	157.6 s		H-5, H-2′	157.0 s	
5	53.4 t	4.05 (dd, 16.2, 5.1) 4.10 (dd, 16.2, 8.5)	H-1, H-6	53.6 d	5.25 (dd, 9.5, 4.8)
6	46.1 d	4.98 (ddd, 8.5, 5.1, 1.0)	H-1, H-5, H-2″	43.2 t	3.47 (ddd, 12.9, 9.5, 1.0) 3.61 (ddd, 12.9, 4.8, 1.5)
1′.....		11.59 (d, 2.7)		11.62 s	
2′.....	132.6 d	8.41 (d, 2.7)		132.7 d	8.41 (s)
3′.....	111.0 s		H-1′, H-2′	111.0 s	
3a′	125.0 s		H-2′, H-5′, H-7′	125.0 s	
4′.....	124.1 d	8.29 (d, 8.5)		124.1 d	8.30 (d, 8.7)
5′.....	123.2 d	7.20 (dd, 8.5, 2.0)	H-7′	123.3 d	7.17 (dd, 8.7, 1.6)
6′.....	114.7 s		H-4′	114.8 s	
7′.....	114.2 d	7.62 (d, 2.0)	H-5′	114.1 d	7.63 (d, 1.6)
7a′	137.0 s		H-2′, H-4′	136.9 s	
1″.....		11.15 (d, 2.3)			11.12 (s)
2″.....	124.5 d	7.30 (d, 2.3)	H-6	123.6 d	7.28 (s)
3″.....	113.1 s		H-5, H-6, H-1″	114.8 s	
3a″	124.6 s		H-2″, H-5″, H-7″	125.0 s	
4″.....	120.7 d	7.66 (d, 8.5)		120.8 d	7.65 (d, 8.6)
5″.....	121.5 d	7.13 (dd, 8.5, 2.0)	H-7″	121.4 d	7.13 (dd, 8.6, 1.6)
6″.....	114.7 s		H-4″	113.9 s	
7″.....	114.2 d	7.56 (d, 2.0)	H-5″	114.2 d	7.59 (d, 1.6)
7a″	137.2 s		H-1″, H-2″	137.2 s	

[a]Table entries are chemical shift, ppm from solvent (multiplicity, J in Hz).

[b]Assignments based on APT, DEPT, and HMQC experiments.

[c]Assignments based on COSY and TOCSY experiments.

[d]Assignment based on SINEPT experiments.

teristics of the hamacanthins are the substitution of a 5,6-dihydro-1(2H)-pyrazinone moiety in place of an imidazole moiety between the two bromoindole rings of the related topsentins and nortopsentins. The structures of the two hamacanthins differ from each other in the position of attachment of the second bromoindole moiety to the 5,6-dihydro-1(2H)-pyrazinone ring system.

A molecular formula of $C_{20}H_{14}Br_2N_4O$ was established for hamacanthin A [**1**] by high-resolution fabms. The ^{13}C-nmr data (Table 1) showed that the molecule contained 17 unsaturated carbons, a conjugated amide carbon at δ 157.4, a nitrogen-bearing methylene carbon at δ 53.4, and another nitrogen-bearing methine carbon at δ 46.1. The presence of two independent 6-bromoindoyl-3-yl moieties was evident from analysis of ^1H- and ^{13}C-nmr data (Table 1) including ^1H-^1H COSY, TOCSY, DEPT, HMQC, and HMBC, and from the comparison of chemical shift values with those of known bromoindoles (2,6). The remaining subunit $C_4H_4N_2O$ as required by the molecular formula had to accommodate the remaining tetrasubstituted olefinic carbon at δ 157.6, the conjugated amide group, and the two nitrogen-bearing aliphatic groups described earlier. The partial structure of 3,6-disubstituted-5,16-dihydro-1(2H)-pyrazinone was established by interpretation of ^1H- and ^{13}C-nmr data (Table 1), including ^1H-^1H COSY and TOCSY spectral data, and was confirmed by long-range C-H correlations. In the COSY spectrum of **1**, the NH proton observed at δ 8.78 (d, J=1 Hz) revealed coupling to the methine proton observed at δ 4.98, which in turn was coupled to the methylene group observed at δ 4.10 and 4.05. The absence of any other ^1H-^1H couplings combined with the chemical shift value of the methylene carbon at δ 53.4 suggested that the methylene group was attached to the remaining nitrogen atom. The long-range C-H correlations (Table 1) observed from H-1 (NH, δ 8.78) to C-2 (C=O, δ 157.4), and from H-5 (δ 4.05) to C-3 (C=N-, δ 157.6), unambiguously established the presence of the disubstituted dihydropyrazinone moiety in the molecule. Furthermore, long-range correlations were also observed from H-1 and H-5 to C-3″ (δ 113.1), from H-6 (δ 4.98) to C-3″ and C-2″ (δ 124.5), and from H-2′ (δ 8.41) to C-3 (δ 157.4), thereby connecting the dihydropyrazinone ring with two 6-bromoindol-3-yl moieties at C-3 and C-6 to yield the structure of hamacanthin A as **1**.

The high-resolution fab mass measurements established the formula $C_{20}H_{14}Br_2N_4O$ for hamacanthin B [**2**]. Based on nmr comparison (Table 1) this compound appeared to have the same dihydropyrazinonediylbis(indole) unit as in **1**. However, significant differences were observed in the ^1H-nmr chemical shift values and the coupling patterns of H-5 and H-6. In the COSY spectrum of **2** the NH (H-1) proton observed at δ 8.49 (t, J=1.5 Hz) indicated couplings to the methylene protons observed at δ 3.47 and 3.61, which in turn were coupled to the methine proton observed at δ 5.25 (dd, J=9.5 and 4.8 Hz). A set of selective INEPT nmr experiments (11) conducted in $CDCl_3$/10% CD_3OD as the solvent gave useful results. Irradiation of the methine H-5 (δ 5.27) produced enhancement in the signals of the carbons (δ 157.01, 123.18, and 124.73), corresponding to C-3, C-2″ and C-3a″, respectively. Similarly, irradiation of one of the methylene protons (δ 3.71) produced enhancement in the signals of the carbons (δ 158.55 and 114.65), corresponding to C-2 and C-3″, respectively. These observations confirmed the presence of the dihydropyrazinone ring system, and that the second bromoindole group is attached to C-5 to give the structure of hamacanthin B as **2**. The absolute stereochemistries at C-6 in **1** and C-5 in **2** were not determined.

Both hamacanthins A and B showed significant antimicrobial activity against

C. albicans, *C. neoformans*, and *Bacillus subtilis*. A number of 2(1H)-pyrazinones have been reported from microbial sources but the substitution of the second indole at C-5 in **2** is fairly unusual (12).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were taken with a Perkin-Elmer Lambda 3 B uv/visible spectrophotometer. Ir spectra were obtained on a Midac Ft-ir M 1200 spectrophotometer. ^{13}C-Nmr spectra were measured on a Bruker AM-360 instrument. ^{1}H- and all 2D nmr spectra were measured on a Bruker AMX-500 instrument. All nmr spectra were measured in DMSO-d_6. Chemical shifts were referenced to solvent DMSO-d_6 signal at 2.49 ppm for ^{1}H and 39.5 ppm for ^{13}C. All 2D spectra were run non-spinning. We used 5 Hz as $^{3}J_{CH}$ in the selective INEPT experiments. The high-resolution mass spectra were obtained on a Kratos MS-80RFA mass spectrometer at the Chemical Instrumentation Center, Yale University. Optical rotations were measured with a Jasco DIP 360 digital polarimeter.

ANIMAL MATERIAL.—The sponge is a new species of *Hamacantha* (phylum Porifera, class Demospongiae, order Poecilosclerida, family Biemnidae) which was collected by the Johnson-Sea-Link I manned submersible vessel from a sand slope at a depth of 548 m off the southeast coast of Madeira (latitude 32°42.41′ N, longitude 60°40.25′ W). In life, the sponge was amorphous and yellow-white in color. It is most closely related to *Hamacantha lundbecki* described by Vacelet (13), but differs in spicule complement. There are no sigmas present and there are two distinct size categories of oxeas. A taxomonic voucher specimen is deposited in the Harbor Branch Oceanographic Museum (catalog no. 003:00920, DBMR no. 27-V-91-3-003).

EXTRACTION AND ISOLATION.—The thawed sponge (223 g, wet wt) was extracted three times with EtOH (400 ml). The concentrated extract was then partitioned between EtOAc (200 ml) and H_2O (100 ml). The EtOAc-soluble fraction (1.2 g) showed activity against *C. albicans* (MIC 3.1 μg/ml; RPMI-1640 growth medium). This active fraction was chromatographed on Si gel (Kieselgel 60 H, 25 g) using a CH_2Cl_2/MeOH step gradient. The antifungal active fraction (*C. albicans* MIC 1.6 μg/ml; RPMI-1640) that eluted with 2% MeOH/CH_2Cl_2 (70 mg) on further purification by hplc (Si gel, 5 μm, 250×10 mm) with 2.5% MeOH/CH_2Cl_2 gave hamacanthin A [**1**] and hamacanthin B [**2**] as the active components.

Hamacanthin A [**1**].—Pale yellow powder (22 mg, 0.011% from frozen sponge); $[\alpha]^{24}$D 84° (c=0.1, MeOH); ir ν max (neat) 3225 br, 2925, 1672, 1585, 1437 cm^{-1}; uv λ max (MeOH) 219 (ε 76500), 280 (20600), 325 (13300) nm; ^{1}H- and ^{13}C-nmr spectra, see Table 1; hrfabms (nitrobenzyl alcohol) m/z 486.9605, Δ 1.2 mmu for $C_{20}H_{15}{}^{79}Br^{81}BrN_4O$ (M+H); lrfabms (nitrobenzyl alcohol) m/z 489 (18), 487 (36), 485 (21), 460 (11), 307 (100), 289 (55), 245 (98).

Hamacanthin B [**2**].—Pale yellow powder (27 mg, 0.014%); $[\alpha]^{24}$D 172° (c=0.1, MeOH); ir ν max (neat) 3250 br, 2925, 1672, 1585, 1437 cm^{-1}; uv λ max (MeOH) 219 (ε 76500), 280 (20600), 325 (13300) nm; ^{1}H- and ^{13}C-nmr spectra, see Table 1; ^{1}H nmr ($CDCl_3$/10% CD_3OD) δ 3.53 (1H, dd, J=13.0 and 9.5 Hz, H-6), 3.71 (1H, dd, J=13.0 and 4.8 Hz, H-6), 5.27 (1H, dd, J=9.5 and 4.8 Hz, H-5), 7.13 (1H, dd, J=8.6 and 1.6 Hz, H-5″), 7.14 (1H, s, H-2″), 7.16 (1H, dd, J=8.6 and 1.6 Hz, H-5′), 7.49 (1H, d, J=8.6 Hz, H-4″), 7.50 (2H, m, H-7′ and H-7″), 8.28 (1H, s, H-2′), 8.31 (1H, d, J=8.6 Hz, H-4′); ^{13}C nmr ($CDCl_3$/10% CD_3OD) δ 44.03 (t, C-6), 54.08 (d, C-5), 111.48 (s, C-3′), 114.13 (d, C-7′), 114.33 (d, C-7″), 114.65 (s, C-3″), 115.22 (s, C-6″), 116.00 (s, C-6′), 120.00 (d, C-4″), 122.29 (d, C-5″), 123.18 (d, C-2″), 123.95 (d, C-5′), 124.22 (d, C-4′), 124.73 (s, C-3a″), 124.98 (s, C-3a′), 132.17 (d, C-2′), 137.17 (s, C-7a′), 137.54 (s, C-7a″), 157.01 (s, C-3), 158.55 (s, C-2); hrfabms (nitrobenzyl alcohol) m/z 486.9606, Δ 1.3 mmu for $C_{20}H_{15}{}^{79}Br^{81}BrN_4O$ (M+H); lrfabms (nitrobenzyl alcohol) m/z 489 (40), 487 (88), 485 (43), 460 (13), 429 (17), 307 (100), 289 (60), 245 (60).

ANTIMICROBIAL ASSAYS.—Minimum inhibitory concentrations (MICs) were determined by standard microdilution broth techniques (14) in a total volume of 50 μl. Growth media used were as follows: *C. albicans* (ATCC 44506), RPMI-1640; *C. neoformans* (ATCC 32045), Emmon's modification of Sabouraud dextrose broth; *B. subtilis* (ATCC 6633), Mueller-Hinton broth supplemented with Ca^{2+} and Mg^{2+}. Plates were incubated at 37° for either 24 h (*C. albicans* and bacteria) or 48 h (*C. neoformans*). RPMI-1640 plates were incubated in a humidified atmosphere of 5% (v/v) CO_2. The MIC was determined as the lowest concentration of the drug which completely inhibited growth.

BIOLOGICAL ACTIVITIES OF **1** AND **2**.—MIC against *C. albicans* RPMI (μg/ml): **1**, 1.6; **2**, 6.2. MIC against *C. neoformans* (μg/ml): **1**, 3.1; **2**, 6.2. MIC against *B. subtilis* (μg/ml): **1**, 3.1; **2**, 1.6.

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