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6-Hydroxydiscodermindole, A New Discodermindole from the Marine Sponge *Discodermia polydiscus*

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Abstract

6-Hydroxydiscodermindole (**2**), a new discodermindole analogue, has been isolated from the deep-water marine sponge *Discodermia polydiscus*. It showed marginal *in vitro* cytotoxicity against cultured murine P388 leukemia cells. The isolation, structure elucidation and cytotoxicity of 6-hydroxydiscodermindole (**2**) is described.

Keywords: Cytotoxicity, *Discodermia polydiscus* de Bocage, Porifera, Demospongia, Lithistida, marine sponge, NMR data, 6-hydroxydiscodermindole.

Introduction

Marine sponges belonging to the genus *Discodermia* are a rich source of many biologically active compounds (Faulkner, 2002). Sun and Sakemi (1991) reported the isolation and structure determination of a brominated aminoimidazolinyl indole, discodermindole (**1**) from the deep water sponge *Discodermia polydiscus* collected at a depth of 185 m off Chub Cay, Berry Islands, Bahamas. From collections of the same species made at the north east point of Acklins Island, Bahamas at a depth of 170 m in June 1993, and off Grand Bahama Island at a depth of 170–181 m in December, 1999, we have isolated discodermindole (**1**) and a new discodermindole (**2**) in trace amount. Here, we report the isolation and structure determination of the new discodermindole analogue, 6-hydroxydiscodermindole (**2**).

Materials and methods

General experiment procedures

UV spectra were taken with a Hitachi U-3010 spectrophotometer. 1D and 2D NMR spectra were measured on a Bruker AMX-500 instrument. The ^{1}H NMR chemical shifts (referenced to CD_3OD observed at 3.30 ppm) were assigned using a combination of data from COSY and HMQC experiments. Similarly, ^{13}C NMR chemical shifts (referenced to CD_3OD observed at 49.0 ppm) were assigned on the basis of DEPT and HMQC experiments. The HRMS were obtained on a Finnigan MAT95Q mass spectrometer at the Spectroscopic Services Group, University of Florida, Gainesville, FL.

Sponge material and collection

Four samples (HBOI sample numbers: 1-XII-99-1-1, 1-XII-99-2-2, 2-XII-99-1-1, and 2-XII-99-2-1) of *Discodermia polydiscus* of the family Theonellidea, were collected at depths of 181, 181, 175 and 170 m, respectively off Lucaya, Grand Bahama Island in December, 1999 using the Johnson-Sea-Link II manned submersible operated by Harbor Branch Oceanographic Institution.

Extraction and isolation

The samples were immediately frozen and maintained below –20 °C until extraction. Freshly thawed sponge samples were

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combined (wet wt 678.9 g) and extracted with EtOH (2 × 1 L) and concentrated to give a brown extract (22.1 g). The crude EtOH extract was suspended in water (500 mL) and partitioned three times with EtOAc (3 × 1 L) and then with *n*-butanol (1 × 750 mL). The concentrated *n*-BuOH soluble fraction 1.2 g was separated by reversed-phase C_{18} column chromatography using a H_2O/MeOH gradient. The fraction that eluted with 10% MeOH/H_2O furnished pure discodermindole (151.5 mg). The subsequent faction that eluted with 20% MeOH/H_2O on further purification by HPLC (Phenomenex 5μ, NH_2, 10 × 250 mm, 15% MeOH/CH_2Cl_2, UV: 254 nm) gave discodermindole (2.0 mg) and 6-hydroxydiscodermindole (2.4 mg, 0.00035% of wet sponge) as a colorless gummy oil.

6-Hydroxydiscodermindole (2): $[\alpha]^{21}_D = -41.6°$ (c 0.1, MeOH); UV (MeOH) λ_{max} 310 (log ε 4.79), 260 (3.94), 215 (4.34) nm; IR (NaCl) ν_{max} 3179 (br), 2328, 1679, 1584, 1428, 1342, 1288, 1100, 994 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1. HRFABMS (3-nitrobenzyl alcohol) *m/z* 372.9299, 374.9280, 376.9261 $[M+H]^+$ (calculated for $C_{11}H_{11}OBr_2N_4$, 372.9306, 374.9350, 376.9331).

Hydrogenolysis of 2: 6-hydroxydiscodermindole (1.0 mg, 2.67 μM) in absolute EtOH (5.0 mL) and a catalytic amount of 10% palladium deposited on activated carbon was allowed to shake under a hydrogen atmosphere at 50 psi in a Parr apparatus overnight at room temparature. The reaction mixture was filtered and the filtrate was evaporated under a stream of N_2 to give a pink gum (1.2 mg) which was further purified by HPLC on an NH_2 column with 12% MeOH/CH_2Cl_2, to yield debromo-6-hydroxydiscodermindole (**3**, 0.8 mg) as a colorless gum. UV (MeOH) λ_{max} 298 (log ε 3.51), 270 (3.59), 215 (3.81) nm; IR (NaCl) ν_{max} 3413, 2929, 1692, 1679, 1644, 1205, 1136, 952, 844 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1.^1H and ^{13}C NMR data, see Table 2. HRFABMS (glycerol) *m/z* 217.1092 $[M+H]^+$ (calculated for $C_{11}H_{13}ON_4$, 217.1093).

Biological activity

6-Hydroxydiscodermindole inhibited the *in vitro* proliferation of cultured murine P388 leukemia and cultured human lung adenocarcinoma A549 cells with IC_{50} values of 4.6 and >5 μg/mL, respectively.

Results and Discussion

Sponge sample (wet wt 678.9 g) collected in December 1999 was soaked in EtOH for 2 h and ground in a blender. The concentrated EtOH extract was partitioned between EtOAc and H_2O. The H_2O-soluble fraction was further partitioned with *n*-BuOH. The *n*-BuOH-soluble fraction after concentration was chromatographed over a C_{18} column using MeOH/H_2O gradient. The 10% MeOH/H_2O eluant yielded pure discodermindole (**1**, 151.5 mg, yield, 0.023% wet weight). The 20% MeOH/H_2O eluant on further purification by HPLC yielded 6-hydroxydiscodermindole (**2**, 2.5 mg, yield, 0.0004% wet wt) and more discodermindole (**1**, 2.0 mg). The June 1993 collection (4-VI-93-3-005, 100g wet wt, Harbor Branch Oceanographic Museum, catalog number 003:00982) on purification under similar isolation procedures yielded 0.3 mg of 6-hydroxydiscodermindole (**2**, yield, 0.0003% wet weight). The identity of discodermindole was confirmed by comparison with an authentic sample from the HBOI depository.

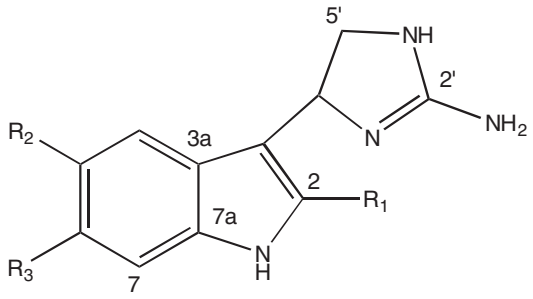
HRFABMS of **2** supported the molecular formula $C_{11}H_{10}Br_2N_4O$ [$(M+H^+)$ *m/z* 372.9299, 374.9280, 376.9261]. The UV spectrum showed absorptions at λ_{max} 215 (log ε 4.79), 260 (3.94), 310 nm (4.34), characteristic of an indole chromophore (Scott, 1964). The presence of two aromatic singlets at δ 7.58 and 6.90 in the ^1H NMR spectrum along with the occurrence of two protonated and six non-protonated sp^2 aromatic carbons in the ^{13}C spectrum suggested the existence of a tetrasubstituted indole. The

Table 1. ^1H (500 MHz) and ^{13}C (125.7 MHz) NMR data of **2** in CD_3OD.

position	δ(^1H) (m, *J*, Hz)	δ(^{13}C)	HMBC
2		109.5 (s)	H4′
3		112.3 (s)	H4′, H5′, H4
3a		121.2 (s)	H4′, H7
4	7.58 (s)	122.3 (d)	–
5		105.7 (s)	H4, H7
6		151.3 (s)	H4, H7
7	6.90 (s)	98.8 (d)	–
7a		138.6 (s)	H4, H7
2′		161.0 (s)	H5′, H4′
4′	5.36 (dd, 9.8, 10.3)	53.6 (d)	H5′
5′	4.08 (dd, 10.3, 10.0)	49.8 (t)	H4′
	3.72 (dd, 9.8, 10.0)		

Table 2. ^1H (500 MHz) and ^{13}C (125.7 MHz) NMR data of **3** in CD_3OD.

position	δ (^1H) (m, *J*, Hz)	δ (^{13}C)	HMBC
2	7.12 (s)	122.9 (d)	–
3	–	114.4 (s)	H2, H5′
3a	–	120.0 (s)	H2, H5, H4′
4	7.32 (d, 8.6)	119.8 (d)	–
5	6.62 (dd, 8.6, 1.3)	110.9 (d)	H7
6	–	154.5 (s)	H4
7	6.80 (d, 1.3)	98.2 (d)	H5
7a	–	139.8 (s)	H2, H4
2′	–	160.9 (s)	–
4′	5.35 (dd, 9.7, 9.8)	55.4 (d)	H5′
5′	4.08 (dd, 9.7, 10.0)	49.5 (t)	–
	3.72 (dd, 9.8, 10.0)		



	R_1	R_2	R_3
1	Br	Br	H
2	Br	Br	OH
3	H	H	OH

Figure 1. Structures of compounds **1**, **2** and **3**.

additional 16 mass units in the mass spectrum of **2** compared to the mass spectrum of discodermindole and the presence of a singlet at δ 151.3 in the ^{13}C spectrum established the presence of a phenolic group. The presence of an ABX system [δ 3.72 (dd, 10.0, 9.8 Hz), 4.08 (dd, 10.3, 10.0 Hz), 5.36 (dd, 10.3, 10.0 Hz)] together with the characteristic guanidine carbon singlet at δ 161.0 in **2** indicated the presence of a mono substituted dihydroimidazole functionality similar to that found in discodermindole (**1**). The three-bond long-range C-H correlations observed in the HMBC experiment (Table 1) established the tentative structure of hydroxydiscodermindole. However, these data were not sufficient to confirm the positions of the two bromines and the hydroxyl group. Hydrogenolysis of **2** in EtOH in the presence of 10% Pd/C catalyst gave debromohydroxy-discodermindole **3** in quantitative yield. The ^1H NMR spectrum (Table 2) showed a singlet at δ 7.12, a *meta*- coupled doublet at δ 6.80, an *ortho*-coupled doublet at δ 7.32 and *ortho*- and *meta*-coupled double doublet at δ 6.62. The COSY spectrum confirmed the coupling pattern. These data restricted the hydroxyl group to be at the C-5 or C-6 position. The long-range C-H correlations observed in the HMBC experiment (Table 2) established the position of the phenol group to C-6 in **3** and therefore confirmed the structure of 6-hydroxy-discodermindole (**2**).

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