Dendridine A, a Bis-indole Alkaloid from a Marine Sponge *Dictyodendrilla* Species

Masashi Tsuda,[†] Yohei Takahashi,[†] Jane Fromont,[‡] Yuzuru Mikami,[§] and Jun'ichi Kobayashi*,[†]

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan, Western Australian Museum, Perth, WA 6000, Australia, and Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba 260-0856, Japan

Received March 4, 2005

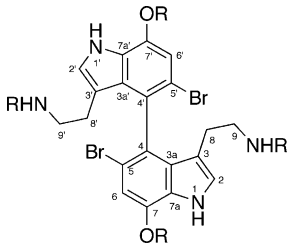
A unique C_2-symmetrical 4,4′-bis(7-hydroxy)indole alkaloid, dendridine A (**1**), was isolated from an Okinawan marine sponge *Dictyodendrilla* sp., and the structure was elucidated by spectroscopic data. Dendridine A (**1**) exhibited antibacterial and antifungal activities.

Marine sponges are a rich source of unique alkaloids possessing various chemical structures.[1] From marine sponges of the genus *Dictyodendrilla*, some pyrrolcarbazole alkaloids with interesting biological activities have been isolated so far.[2] In our continuing search for new metabolites from marine sponges,[3] a recent investigation of extracts of an Okinawan sponge *Dictyodendrilla* sp. (SS-1096) resulted in the isolation of a new bis-indole alkaloid, dendridine A (**1**). Here we describe the isolation and structure elucidation of **1**.

The sponge *Dictyodendrilla* sp. (0.2 kg, wet weight), collected off Unten-Port, Nakijin, Okinawa, was extracted with MeOH. *n*-BuOH-soluble materials of the extract were subjected to SiO_2 and C_{18} column chromatographies followed by reversed-phase HPLC to yield dendridine A (**1**, 0.00075%, wet weight) as TFA salts together with a known alkaloid, makaluvamine O (**3**).[4] On the other hand, a known terpenoid hydroquinone, aureol (**4**),[5] was isolated from EtOAc-soluble materials of the extract.

Dendridine A (**1**) was obtained as yellowish and optically inactive amorphous solid. The ESIMS spectrum of **1** showed the pseudomolecular ion peaks in the ratio of 1:2:1 at *m/z* 507, 509, and 511, respectively, indicating the presence of two bromine atoms in the molecule, and HRESIMS data of **1** revealed the molecular formula $C_{20}H_{20}N_4O_2Br_2$ [*m/z* 507.0044 $(M + H)^+$, Δ +1.2 mmu]. IR absorptions indicated the presence of OH and/or NH (3419 cm^{-1}) groups. The UV absorptions [λ_{max}303 (ϵ 4000), 292 (5800), and 282 nm (5700)] indicated the presence of other ring system(s) conjugated to the benzenoid ring(s). ^{1}H NMR data (Table 1) of **1** revealed three D_2O-exchangeable [δ 11.17 (s), 10.29 (s), and 7.51 (2H, brs)], two aromatic [δ 7.05 (s) and 6.81 (s)], and two methylene proton signals [δ 2.46 (2H, t) and 2.02 (2H, t)]. The ^{13}C NMR (Table 1) spectrum of **1** disclosed totally 10 signals due to eight sp^2 carbons [six quaternary carbons (δ 143.87, 127.98, 125.75, 122.68, 115.03, and 120.66) and two methines (δ 124.54 and 108.86)] and two sp^3 methylene carbons [δ 38.64 and 23.15], thus indicating that **1** had a symmetrically dimeric structure.

The structure of dendridine A (**1**) was elucidated by spectroscopic data including 2D NMR data such as the ^{1}H−^{1}H COSY, ROESY, and HMBC spectra (Figure 1). The



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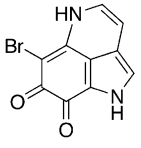
2 R = Ac



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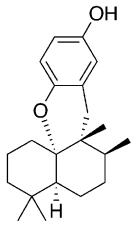

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presence of an ethylamine unit (H_2-8 to 9-NH_2) was suggested by analysis of the ^{1}H−^{1}H COSY spectrum. The existence of a primary amino group at C-9 was supported by conversion into the acetamide group by acetylation [tetraacetate **2**: CH_3CO; δ 1.67 (6H, s), 9-NH; δ 7.20 (2H, s)]. The lower-field one [δ 11.17 (H-1)] of D_2O-exchangeable protons showed HMBC correlations for C-3 and C-3a, while one [δ 7.05 (H-2)] of two sp^2 methine protons correlated to C-3a and C-7a. These suggested the presence of a 2,3,4-trisubstituted pyrrole ring. Connection of the 2-ethylamine unit at C-3 was deduced from ROESY correlations for H-1/H-2 and H-2/H_2-8. The other [δ 10.29 (7-OH)] D_2O-exchangeable proton was assigned as a phenolic proton from the two-bond HMBC correlation for a lower-field sp^2 quaternary carbon [δ 143.9 (C-7)]. In the ^{1}H NMR spectrum of tetraacetate **2**, an acetylmethyl signal was observed at δ 2.38 in place of the phenol proton for **1**. The presence of a pentasubstituted benzene ring was elucidated on the basis of the following HMBC and ROESY correlations: H-6/C-4, H-6/C-5, H-6/C-7, H-6/C-7′a, and 7-OH/C-7a, and H-6/7-OH, respectively. The chemical shift of C-5 (δ 115.0)

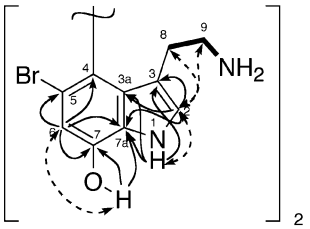
* To whom correspondence should be addressed. Phone & Fax: +81 11 706 4985. Fax: +81 11 706 4989. E-mail: jkobay@pharm.hokudai.ac.jp.
[†]Hokkaido University.
[‡]Western Australian Museum.
[§]Chiba University.

10.1021/np050076e CCC: $30.25
Published on Web 06/28/2005

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

positn	δ_C		δ_H	m, Hz	HMBC
1			11.17	s	
2	124.5	CH	7.05	s	
3	110.7	C			H-1, H-2
3a	128.0	C			H-1, H-2
4	122.7	C			H-6
5	115.0	C			H-6
6	108.9	CH	6.81	s	
7	143.9	C			H-6, 7-OH
7-OH			10.29	s	
7a	125.8	C			H-1, H-2, H-6, 7-OH
8	23.2	CH_2	2.02[a]	t, 7.0	
9	38.6	CH_2	2.46[a]	m	
9-NH_2			7.51[a]	brs	

[a] 2H.



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^{1}H-^{1}H COSY HMBC ROESY

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

corresponded to those of carbons bearing a bromine atom, indicating that a bromine atom was attached to C-5. These results suggested the presence of a dimeric structure possessing a 3-(2′-aminoethyl)-5-bromo-7-hydroxyindol-4-yl moiety at both C-4 and C-4′.[6] Therefore, the structure of dendridine A was concluded to be **1**.

Dendridine A (**1**) is a new C_2-symmetrical 4,4′-bis(7-hydroxy)indole alkaloid. Isolation of 7-hydroxyindole alkaloids such as **1** is rare,[7] although many hydroxyindole alkaloids have been reported as natural products.[8] Dendridine A (**1**) exhibited inhibitory activities against Gram-positive bacteria *Bacillus subtilis* and *Micrococcus* luteus (MIC, 8.3 and 4.2 μg/mL, respectively) and the fungus *Cryptococcus neoformans* (MIC, 8.3 μg/mL), while **1** showed a weak cytotoxicity against murine leukemia L1210 cells (IC_{50}, 32.5 μg/mL).

Experimental Section

General Experimental Procedures. The IR and UV spectra were taken on JASCO FT/IR-5300 and Shimadzu UV1600PC spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a 600 MHz spectrometer using 2.5 mm microcells for DMSO-d_6 (Shigemi Co., Ltd.). ESIMS mass spectra were obtained on a JEOL 700TZ spectrometer.

Sponge Description. The sponge *Dictyodendrilla* sp. (order Dendroceratida; family Dictyodendrillidae) was collected off Unten-Port, Nakijin, Okinawa, and kept frozen until used. The voucher specimen (SS-1096) was deposited at the Graduate School of Pharmaceutical Sciences, Hokkaido University.

Extraction and Isolation. The sponge (0.2 kg, wet weight) was extracted with MeOH (500 mL × 2), and the extract (8.61 g) was partitioned between EtOAc (150 mL × 3) and H_2O (150 mL). Then the aqueous layer was extracted with *n*-BuOH (150 mL × 3). Parts (0.82 g) of the *n*-BuOH-soluble materials (1.42 g) were subjected to SiO_2 gel ($CHCl_3$/*n*-BuOH/AcOH/H_2O, 1.5:6:1:1, 2 L) and C_{18} column chromatography (MeOH/H_2O/CF_3CO_2H, 1:1:0.1) followed by C_{18} HPLC (YMC-Pack Pro C_{18}, YMC Co. Ltd., 10 × 250 mm; eluent, CH_3CN/H_2O/CF_3CO_2H, 25:75:0.1; flow rate, 2.5 mL/min; UV detection at 220 nm) to afford dendridine A (**1**, 1.5 mg, 0.00075%, wet weight, t_R 9.6 min) and makaluvamine O (**3**, 0.1 mg, t_R 4.2 min). Aureol (**4**) was obtained from the EtOAc-soluble materials.

Dendridine A (1): yellowish amorphous solid; UV (MeOH) λ_{max} 303 (ϵ 4000), 292 (5800), and 282 nm (5700); IR (KBr) ν_{max} 3419, 2927, and 1682 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; ESIMS (pos.) *m/z* 507, 509, and 511 [$(M + H)^+$, 1:2:1]; HRESIMS (pos.) *m/z* 507.0044 [$(M + H)^+$, calcd for $C_{20}H_{21}N_4O_2{}^{79}Br_2$, 507.0032].

Tetraacetate 2. Dendridine A (**1**, 0.1 mg) was treated with acetic anhydride (6 μL) and pyridine (50 μL) at room temperature for 14 h. After evaporation of the solvent, the tetraacetate (**2**, 0.1 mg) of **1** was afforded as a yellowish oil: ^{1}H NMR (DMSO-d_6) δ 11.41 (2H, s), 7.20 (2H, s), 7.18 (2H, s), 7.15 (2H, s), 3.3 (4H, m), 2.66 (4H, t, J = 6.5 Hz), 2.38 (6H, s), 1.67 (6H, s); ESIMS *m/z* 697, 699, and 701 [$(M + Na)^+$, 1:2:1]; HRESIMS *m/z* 697.0267 [$(M + Na)^+$, calcd for $C_{28}H_{28}N_4O_6{}^{79}Br_2$ Na, 697.0273].

Acknowledgment. We thank S. Oka, Center for Instrumental Analysis, Hokkaido University, for ESIMS measurement, and Z. Nagahama and K. Uehara for their help with collection of the sponge. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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(6) ^{13}C chemical shifts for 4,4′-bis(5-bromo-7-hydroxyl)indole [C-2: δ_C 123, C-3: δ_C 112, C-3a: δ_C 134, C-4: δ_C 125, C-5: δ_C 117, C-6: δ_C 111, C-7: δ_C 141, C-7a: δ_C 123] and 7,7′-bis(6-bromo-5-hydroxyl)indole [C-2: δ_C 123, C-3: δ_C 112, C-3a: δ_C 133, C-4: δ_C 109, C-5: δ_C 154, C-6: δ_C 100, C-7: δ_C 124, C-7a: δ_C 130] were calculated by ChemNMR Pro (CanbrigeSoft). The ^{13}C chemical shift for the phenolic carbon (δ_C 143.9) for **1** was close to that of 4,4′-bis(5-bromo-7-hydroxyl)indole rather than 7,7′-bis(6-bromo-5-hydroxyl)indole.

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NP050076E