

Monodictyquinone A: a New Antimicrobial Anthraquinone from a Sea Urchin-Derived Fungus *Monodictys* sp.

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A new antimicrobial anthraquinone, 1,8-dihydroxy-2-methoxy-6-methylanthraquinone, monodictyquinone A (1), was isolated from a culture of a marine-derived fungus of the genus *Monodictys* which was isolated from the sea urchin, *Anthocardia crassispina*, along with three known compounds, pachybasin (2), chrysophanol (3), and emodin (4).

Key words monodictyquinone A; fungus; *Monodictys* sp.; antimicrobial; *Anthocardia crassispina*

Marine microorganisms such as bacteria and fungi have been proven to be a rich source of new biologically active secondary metabolites.^{1–3)} In our search for new natural products from marine-derived organisms,^{4–6)} here we report the isolation and structure elucidation of a new antimicrobial anthraquinone, monodictyquinone A (1), along with three known compounds, pachybasin (2),⁷⁾ chrysophanol (3),⁷⁾ and emodin (4).⁸⁾

The marine-derived fungus *Monodictys* sp. was isolated from the sea urchin *Anthocardia crassispina*, collected in Toyama Bay in the Sea of Japan. The fungus was grown on agar plates composed of 50% seawater with nutrients. The culture plates were extracted with EtOH. The extract was partitioned between EtOAc and H₂O. The EtOAc-soluble fraction (15 g) was further partitioned between hexane and 90% MeOH–H₂O. The aqueous MeOH layer was subjected to normal and reverse-phase silica gel column chromatography to furnish 1 (0.67 mg) as a yellow solid.

Compound 1 has a molecular formula of C₁₆H₁₂O₅ as established by HR-EI-MS, and the molecular formula requires eleven degrees of unsaturation. The ¹H-NMR spectrum in CDCl₃ (Table 1) revealed two methyl groups at δ 2.45 (s, 6-Me) and 4.00 (s, 2-OMe), two doublet [δ 7.15 (1H, d, *J*=8.4 Hz, H-3) and 7.85 (1H, d, *J*=8.4 Hz, H-4)] and two singlet resonances [δ 7.06 (1H, s, H-7) and 7.64 (1H, s, H-5)] in the aromatic region, and two D-exchangeable protons at δ 11.97 (s, 8-OH) and 12.51 (s, 1-OH). The ¹³C-NMR spectrum in CDCl₃ (Table 1) showed two methyl, four methine, and ten quaternary carbons including two carbonyl carbons assignable to C-9 (δ 192.9) and C-10 (δ 181.1); these data strongly suggested the characteristic feature of anthraquinone derivative. The positions of substituted groups were determined by heteronuclear multiple-bond correlation (HMBC) experiments using the optimized *J* value of 8.3 or

5.0 Hz (Fig. 1); plain arrows showed correlations with the optimized *J* value of 8.3 Hz, while dashed arrows revealed correlations with the optimized *J* value of 5.0 Hz. The experiments indicated that two hydroxy (δ 11.97, 12.51), methyl (δ 2.45), and methoxy (δ 4.00) groups are attached to C-8, C-1, C-6, and C-2, respectively; thereby the structure of 1 was established (Fig. 1).

Several biological activities have been hitherto reported for anthraquinones isolated from the marine environment; protein kinase inhibitory activity for 1,3,6,8-tetrahydroxyanthraquinone congeners isolated from the sponge-associated fungus *Microsphaeropsis* sp.,⁹⁾ antibacterial activity for 1,8-dihydroxy-4-methylanthraquinone isolated from the cyano-

Table 1. NMR Data (CDCl₃) of 1

	δ _H	<i>J</i> (Hz)	δ _C	HMBC	
				<i>J</i> =8.3 Hz	<i>J</i> =5.0 Hz
1			152.6 C		
2			154.1 C		
3	7.15 d	8.4	116.0 CH	C-1, C-4a	
4	7.85 d	8.4	121.4 CH	C-2, C-3	C-10
4a			125.5 C		
5	7.64 s		121.3 CH	C-7, C-8a	C-10
6			149.5 C		
7	7.06 s		123.9 CH	C-5, 6-Me	
8			162.8 C		
8a			114.1 C		
9			192.9 C		
9a			115.9 C		
10			181.1 C		
10a			133.8 C		
6-Me	2.45 s		22.3 CH ₃	C-5, C-6, C-7	
8-OH	11.97 s			C-7, C-8	C-8a
1-OH	12.51 s			C-1	C-2, C-9a
2-OMe	4.00 s		56.4 CH ₃	C-2	

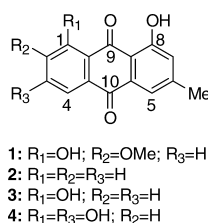


Chart 1

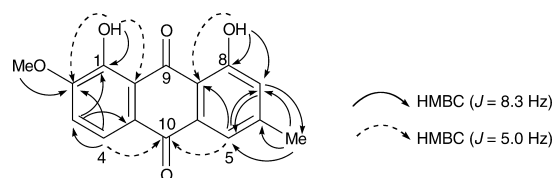


Fig. 1. HMBC Correlations with Optimized *J* Values of 8.3 Hz or 5.0 Hz Observed for 1

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Table 2. Antimicrobial Activities of **1**

Compound (μg)	Inhibitory zones (mm) ^{a)}		
	<i>B. subtilis</i>	<i>E. coli</i>	<i>C. albicans</i>
2.5	7	8	7
5.0	10	12	8
10	15	15	11

a) Paper disks (ϕ 6 mm), impregnated with **1**, were incubated on agar plates containing microorganisms.

bacterium *Nostoc commune*¹⁰⁾ and for lunatin (1,3,8-trihydroxy-6-methoxyanthraquinone) isolated from the sponge-derived fungus *Curvularia lunata*,¹¹⁾ and cytotoxicity for evariquinone (1,2,3-trihydroxy-6-methyl-8-methoxyanthraquinone) isolated from a sponge-derived fungus, *Emerella variegata*.¹²⁾ Compound **1** showed antibacterial activities against *Bacillus subtilis*, *Escherichia coli*, and *Candida albicans* with 2.5 $\mu\text{g}/\text{disk}$ (Table 2); but showed no cytotoxicity against HeLa cells at the concentration of 50 $\mu\text{g}/\text{ml}$.

Although isolation of *Monodictys* fungi from the marine environment was reported, only two papers with respect to the fungal metabolites have so far been reported; a perylene derivative, stemphytriol, from *M. fluctuata*¹³⁾ and four monomeric xanthenes, monodictysins A–C and monodictyxanthone, and a benzophenone, monodictyphenone, from *M. putredinis*.¹⁴⁾

Experimental

General Experimental Procedures UV spectra were measured on a Shimadzu UV-1600 UV–visible spectrometer. IR spectra were recorded on a Shimadzu IR-460 infrared spectrophotometer. NMR spectra were recorded on a JEOL GSX500 in CDCl_3 . Mass spectra were measured on a JEOL SX-102 mass spectrometer.

Fungal Strain A strain of the fungus *Monodictys* sp. was isolated from the sea urchin *Anthocidaris crassispina*, collected in Toyama Bay in the Sea of Japan. The identification of the fungus was evaluated by TechonoSuruga Co., Ltd. (Shizuoka, Japan). A voucher specimen is deposited in Kanazawa University with the code MB576.

Extraction and Isolation The fungus was cultivated on agar medium composed of 1.0% peptone, 0.5% yeast extract, 0.25% beef extract, and 2.0% glucose in 50% natural seawater for 14 d at 25 °C. The culture was extracted with EtOH three times. The extract (98 g) was evaporated under reduced pressure and partitioned between EtOAc and H_2O . The EtOAc soluble fraction (15 g) was partitioned between hexane and 90% MeOH– H_2O , and the aqueous MeOH fraction (13 g) was subjected to silica gel column chromatography using a step-wise gradient from CHCl_3 to MeOH. The $\text{CHCl}_3/\text{MeOH}$ (10:1) fraction was further purified by ODS column chromatography with MeOH/ H_2O (9:1) and ODS HPLC with MeOH/ H_2O (8:2) to afford **3** (5.8 mg). The $\text{CHCl}_3/\text{MeOH}$ (4:1) fraction from the first silica gel column chromatography was further purified by ODS column chromatography with MeOH/ H_2O (9:1) to afford a fraction (37.8 mg) containing **1**, **2**, and **4**. The fraction was purified by silica gel column chromatography with hexane/EtOAc. The hexane/EtOAc (10:1) fraction afforded **2** (14.0 mg). The hexane/EtOAc (3:1) fraction was further purified by silica

gel column chromatography with $\text{CHCl}_3/\text{MeOH}$. The CHCl_3 fraction afforded **1** (0.67 mg), and the $\text{CHCl}_3/\text{MeOH}$ (1:1) fraction afforded **4** (3.3 mg). The known compounds (**2**–**4**) were identified on the basis of their spectroscopic data.^{7,8)}

1: UV λ_{max} (EtOH) nm (log ϵ): 230 (3.52), 261 (3.27), 300 (2.90), 436 (2.85). IR (film) cm^{-1} : 3500; ^1H - and ^{13}C -NMR, see Table 1; EI-MS m/z : 284 $[\text{M}]^+$; HR-EI-MS m/z : 284.0686 (Calcd for $\text{C}_{16}\text{H}_{12}\text{O}_5$, 284.0685).

Antimicrobial Assay Antimicrobial activity was determined by the paper disk method. A paper disk (ϕ 6 mm, Toyo Roshi Kaisha, Ltd., Tokyo), with the sample was incubated on an agar plate containing *Bacillus subtilis*, *Escherichia coli*, or *Candida albicans* at 25 °C.

Cytotoxicity Test Cytotoxicity test was carried out with HeLa cells. Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, penicillin (50 units/ml), and streptomycin (50 $\mu\text{g}/\text{ml}$) under a humidified atmosphere of 5% CO_2 at 37 °C. The cells were seeded into 96-well microplates (3×10^3 cells/well) and pre-cultured for a day. The medium was replaced with that containing test compounds at various concentrations and the cells were further cultured at 37 °C for 3 d. The medium was then replaced with 50 ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (0.2 mg/ml in medium) and the cells were incubated under the same conditions for 4 h. After the addition of 200 μl of DMSO, optical density at 570 nm was measured with a microplate reader.

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