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

Ahmed Atef EL-BEIH, Tetsuro KAWABATA, Keiichiro KOIMARU, Tomihisa OHTA, and Sachiko TSUKAMOTO*

Graduate School of Natural Science and Technology, Kanazawa University; Kakuma-machi, Kanazawa 920–1192, Japan.
Received March 12, 2007; accepted May 7, 2007; published online May 8, 2007

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, *Anthocidaris crassispina*, along with three known compounds, pachybasin (2), chrysophanol (3), and emodin (4).

Key words monodictyquinone A; fungus; *Monodictys* sp.; antimicrobial; *Anthocidaris 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]

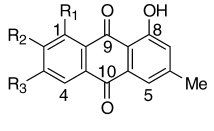
The marine-derived fungus *Monodictys* sp. was isolated from the sea urchin *Anthocidaris crassispina*, collected in Toyama Bay in the Sea of Japan. The fungus was grown on agar plates composed of 50% seawater with nutrients. The culture plates were extracted with EtOH. The extract was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction (15 g) was further partitioned between hexane and 90% MeOH–H_2O. 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 $C_{16}H_{12}O_5$ as established by HR-EI-MS, and the molecular formula requires eleven degrees of unsaturation. The ^{1}H-NMR spectrum in $CDCl_3$ (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 ^{13}C-NMR spectrum in $CDCl_3$ (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 ($CDCl_3$) of **1**

	δ_H	J (Hz)	δ_C	HMBC	
				J=8.3 Hz	J=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	



1: R_1=OH; R_2=OMe; R_3=H
2: R_1=R_2=R_3=H
3: R_1=OH; R_2=R_3=H
4: R_1=R_3=OH; R_2=H

Chart 1

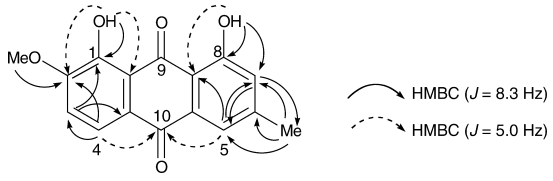


Fig. 1. HMBC Correlations with Optimized J Values of 8.3 Hz or 5.0 Hz Observed for **1**

* To whom correspondence should be addressed. e-mail: sachiko@p.kanazawa-u.ac.jp

Table 2. Antimicrobial Activities of **1**

Compound (μg)	Inhibitory zones (mm) [a]		
	B. subtilis	*E. coli*	*C. albicans*
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 form a sponge-derived fungus, *Emericella variecolor*.[12] Compound **1** showed antibacterial activities against *Bacillus subtilis*, *Escherichia coli*, and *Candida albicans* with 2.5 μg/disk (Table 2); but showed no cytotoxicity against HeLa cells at the concentration of 50 μg/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 xanthones, 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 $CHCl_3$/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 $CHCl_3$/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 $CHCl_3$/MeOH. The $CHCl_3$ fraction afforded **1** (0.67 mg), and the $CHCl_3$/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; ^{1}H- and ^{13}C-NMR, see Table 1; EI-MS *m*/*z*: 284 $[M]^+$; HR-EI-MS *m*/*z*: 284.0686 (Calcd for $C_{16}H_{12}O_5$ 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 μg/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.

Acknowledgments This work was supported by Grants-in-Aid for Scientific Research on Priority Areas (No. 18032033) from the Ministry of Education, Culture, Sports, Science and Technology of Japan and also by grants from the Hokkoku Foundation for Cancer Research and from the Naito Foundation.

References

1) Feling R. H., Buchanan G. O., Mincer T. J., Kauffman C. A., Jensen P. R., Fenical W., *Angew. Chem., Int. Ed.*, **42**, 355—357 (2003).
2) Oh D.-C., Williams P. G., Kauffman C. A., Jensen P. R., Fenical W., *Org. Lett.*, **8**, 1021—1024 (2006).
3) Kwon H. C., Kauffman C. A., Jensen P. R., Fenical W., *J. Am. Chem. Soc.*, **128**, 1622—1632 (2006).
4) Kato H., Yoshida T., Tokue T., Nojiri Y., Hirota H., Ohta T., Williams R. M., Tsukamoto S., *Angew. Chem. Int. Ed.*, **46**, 2254—2256 (2007).
5) Tsukamoto S., Yoshida T., Hosono H., Ohta T., Yokosawa H., *Bioorg. Med. Chem. Lett.*, **16**, 69—71 (2006).
6) Tsukamoto S., Hirota H., Imachi M., Fujimuro M., Onuki H., Ohta T., Yokosawa H., *Bioorg. Med. Chem. Lett.*, **15**, 191—194 (2005).
7) Jung M. E., Lowe J. A., Lyster M. A., Node M., Pfluger R. W., Brown R. W., *Tetrahedron*, **40**, 4751—4766 (1984).
8) Cohen P. A., Towers G. H. N., *J. Nat. Prod.*, **58**, 520—526 (1995).
9) Brauers G., Edrada R. A., Ebel R., Proksch P., Wray V., Berg A., Grafe U., Schachtele C., Totzke F., Finkenzeller G., Marme D., Kraus J., Munchbach M., Michek M., Bringmann G., Schaumann K., *J. Nat. Prod.*, **63**, 740—745 (2000).
10) Jaki B., Heilmann J., Sticher O., *J. Nat. Prod.*, **63**, 1283—1285 (2000).
11) Jadulco R., Brauers G., Edrada R. A., Ebel R., Wray V., Sudarsono, Proksch P., *J. Nat. Prod.*, **65**, 730—733 (2002).
12) Bringmann G., Lang G., Steffens S., Gunther E., Schaumann K., *Phytochemistry*, **63**, 437—443 (2003).
13) Krohn K., John M., Aust H.-J., Draeger S., Schulz B., *Nat. Prod. Lett.*, **14**, 31—34 (1999).
14) Krick A., Kehraus S., Gerhaeuser C., Klimo K., Nieger M., Maier A., Fiebig H.-H., Atodiresei I., Raabe G., Fleischhauer J., König G. M., *J. Nat. Prod.*, **70**, 353—360 (2007).