Chemistry of Natural Compounds, Vol. 47, No. 6, January, 2012 [Russian original No. 6, November–December, 2011]

PORRIC ACID D FROM MARINE-DERIVED FUNGUS *Alternaria* sp. ISOLATED FROM BOHAI SEA

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UDC 547.565.2

*Porric acid D (**1**), a new dibenzofuran derivative, was isolated from the methanol extract of a marine-derived fungus,* Alternaria *sp., isolated from the Bohai Sea, together with a known compound, altenusin. Their structures were elucidated by spectroscopic analysis, including high-resolution mass spectroscopy, and 1 and 2-dimensional NMR techniques. Compounds **1** and **2** showed inhibitory activity against* Staphyloccocus aureus *with MIC 100 and 25 μg/mL, respectively.*

Keywords: *Alternaria* sp., dibenzofuran, *Staphyloccocus aureus.*

Alternaria is a genus of ascomycete fungi which are known to be major plant pathogens in economically important crops grown worldwide [1, 2]. Chemical investigations have led to identification of anthraquinones [3], phthalides [4], porric acids A–C [5], phytotoxins [6], alterporriol F [7], and perylene derivatives [8]. In the course of screening for bioactive compounds against *Staphyloccocus aureus*, a strain of *Alternaria* was isolated from seawater of the Bohai Sea. Its EtOAc extract showed bioactivity. Continued studies on the chemical constituents of the EtOAc extract yielded a new dibenzofuran derivative, named porric acid D, together with a known compound, altenusin [9]. Herein, we report the isolation, structure elucidation, and antimicrobial activity of these compounds.

Compound **1** was obtained as a yellow amorphous powder. High-resolution ESI-MS revealed a pseudomolecular ion peak at *m/z* 289.0708 for $[M + H]^+$ (calcd 289.0712) corresponding to the molecular formula $C_{15}H_{12}O_6$ and ten degrees of unsaturation. The UV spectrum (bands at 338, 260, 238 nm) was suggestive of a dibenzofuran skeleton [10], which was confirmed by the presence of 12 sp^2 hybridized carbons, other than that of the carboxylic group, in the ^{13}C NMR spectrum (Table 1). A sharp singlet at δ_H 11.91 in the ^1H NMR spectrum, as well as a carbon signal at δ_C 164.1 (C-11), interrelated in the HMBC correlations from H-COOH to C-6 and C-11, revealed the carboxylic group. The signals in the ^1H NMR (Table 1) spectrum showed a methyl group at δ 2.66 (3H, s, H_3-10), a methoxy group at δ 3.91 (3H, s, H_3-12), an aromatic singlet at δ 6.73 (1H, s, H-2), and a *meta* relationship of aromatic protons at δ 6.63 (1H, d, J = 2.0 Hz, H-7) and 7.24 (1H, d, J = 2.0 Hz, H-9). The protonated carbons and their corresponding protons were unambiguously assigned by the HSQC experiment. The substitution pattern of the aromatic rings was elucidated by the analysis of HMBC correlations. In the HMBC spectrum, the correlations from H_3-10 to C-1, C-2, and C-9b and from H-2 to C-3, C-4, C-9b, and C-10 established that the methyl group was attached to C-1. The *meta* relationship of H-7 and H-9 was confirmed by the HMBC correlations from H-7 to C-9 and from H-9 to C-7. The HMBC correlations from H-7, H-9, and H_3-12 to C-8 established that the methoxyl group was assigned to C-8. The HMBC correlations from the carboxyl proton to C-6 and C-12 revealed that the carboxyl group was attached to C-6. Compound **1** was established as porric acid D, in which the methoxyl group was located at C-8 in contrast to that of porric acid B at C-4 [5].

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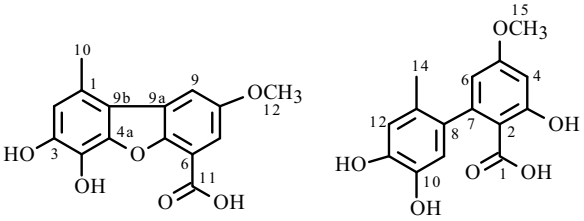
0009-3130/12/4706-0893 ©2012 Springer Science+Business Media, Inc.

TABLE 1. ^{1}H and ^{13}C NMR Spectral Data of Compound **1**, J/Hz*

C atom	δ_C	δ_H	HMBC	C atom	δ_C	δ_H	HMBC
1	126.4 s			8	166.1 s		
2	116.8 d	6.73 (1H, s)	3, 4, 9b, 10	9	103.4 d	7.24 (1H, d, J = 2.0)	7, 8, 9b
3	147.0 s			9a	138.4 s		
4	131.1 s			9b	109.1 s		
4a	141.5 s			10	24.5 q	2.66	1, 2, 9b
5a	164.6 s			11	164.1 s		
6	98.3 s			12	55.8 q	3.91	8
7	99.2 d	6.63 (1H, d, J = 2.0)	5a, 6, 8, 9, 11	COOH		11.91	6, 11

*Mutiplicities and coupling constants are in parentheses. Assignments are based on HMBC and HMQC experiments.



1 **2**

Compound 2. The ESI-MS of **2** exhibited a pseudomolecular ion peak at *m/z* 291. The ^{13}C NMR spectrum showed 15 carbons signals, which included one carboxyl group at δ 171.6 (C-1), four oxygenated quaternary carbons at δ 161.9 (C-5), 161.5 (C-3), 143.9 (C-11), and 142.1 (C-10), four quaternary carbons at δ 144.9 (C-7) 132.4 (C-8), 124.9 (C-13), and 108.9 (C-2), four sp^2 methines at δ 116.6 (C-12), 115.9 (C-9), 108.8 (C-6), and 99.6 (C-4), one methyl group at δ 18.9, and one methoxyl group at δ 55.3. The ^{1}H NMR spectrum showed a pair of *meta* protons for the benzene ring at δ 6.43 (H-4, d, J = 2.0) and 6.10 (H-6, d, J = 2.0), a pair of *para* protons for benzene at δ 6.53 (H-12, s) and 6.41 (H-9, s), a methyl group at δ 1.85 (H_3-14, 3H, s), and a methoxyl group at δ 3.76 (H_3-15, 3H, s). These data agreed with those of altenusin [9].

EXPERIMENTAL

IR spectra were recorded by the FT-IR microscope transmission method on a Nicolet 5700 FT-IR spectrophotometer. NMR spectra were recorded on a Varian Inova 500 MHz spectrometer at 500.103 MHz for ^{1}H and 125.762 MHz for ^{13}C in DMSO-d_6 using solvent signals (DMSO, δ_H 2.50/δ_C 39.5) as reference, and the coupling constants are in Hz. HR-ESI-MS data were measured with an APEX II FT-ICR-MS spectrometer (Bruker Daltonics, Inc. USA). Column chromatography was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). HPLC separation was performed on an Agilent 1100 series and an Alltima 250 cm × 2.2 cm i.d. preparative column packed with C18 (10 μm).

Microorganism. The marine fungus *Alternaria* sp. was isolated from seawater collected from the Bohai Sea, Tianjin in October 2007. The fungus was cultured and kept on PDA prepared in natural seawater. It was identified according to its morphological characteristic.

Fermentation. A small spoon of spores growing on potato dextrose agar slant was inoculated into a 250 mL conical flask containing 40 mL of liquid medium composed of glucose (1.0%), maltose (1.0%), and yeast extract (0.3%), mannitol (2.0%), monosodium glutamate (1.0%), KH_2PO_4 (0.05%), $MgSO_4$ (0.03%), corn plasm (0.1%), and natural seawater, its pH adjusted to 7.2, and cultured at 28°C for three days on a rotary shaker at 160 rpm. Then 5 mL of the resultant seed culture was inoculated into 500 mL conical flasks each containing 50 g rice and 30 mL natural seawater, and incubated stationary for 15 days.

Extraction and Isolation. The culture whole broth was extracted with EtOAc exhaustively and the solvent removed under reduced pressure at < 50°C to yield a dark residue. The EtOAc extract (2.8 g) was subjected to a normal-phase silica gel (200–300 mesh) column and eluted with a gradient of increasing Me_2CO (0–100%) in petroleum ether. The fraction eluted by 12% Me_2CO in petroleum ether was rechromatographed over Sephadex LH-20 using petroleum ether–$CHCl_3$–MeOH (5:5:1) to afford six subfractions. The fourth subfraction was further separated by reversed-phase preparative HPLC using MeOH–H_2O (65:35) as mobile phase to yield compounds **1** (3.2 mg) and **2** (2.8 mg).

Porric acid D (1), yellow amorphous powder. IR (ν_{max}, cm^{-1}): 3443, 3261, 1661, 1610, 1571, 1465, 1408, 1378, 1354, 1321, 1301, 1278, 1232, 1195, 1155, 1123, 1092, 1063, 1023, 990, 982, 923, 836, 813, 785, 767, 751, 721, 689. ESI-MS: *m/z* 289 $[M + H]^+$; HR-ESI-MS: *m/z* 289.0708 (calcd for $C_{15}H_{13}O_6$, 289.0712); for ^1H NMR and ^{13}C NMR data, see Table 1.

Assay for Antimicrobial Activity. Antimicrobial activities were determined using agar diffusion against *Staphyloccocus aureus*. The plates were inoculated and incubated at 37°C for 48 h. The MIC (minimum inhibitory concentration) was determined from plates with the lowest concentration of compounds on which the strain would not grow. Compounds **1** and **2** showed bioactivity against *Staphyloccocus aureus* with MIC of 100 and 25 μg/mL, respectively.

ACKNOWLEDGMENT

This work was supported by the Fundamental Research Funds for the Central Universities (2010ZY18, 2011YXL025), Research Fund for the Doctoral Program of Higher Education of China (200804911002), Scientific Fund of China University of Geosciences (51900961135), National Natural Science Foundation of China (30901849), and Science and Technology Planning Project of Zhejiang Province, China (2009C33004).

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