The Journal of Antibiotics (2015) 68, 139–141

www.nature.com/ja

NOTE

Barceloneic acid C, a new polyketide from an endophytic fungus *Phoma* sp. JS752 and its antibacterial activities

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The Journal of Antibiotics (2015) **68,** 139–141; doi:10.1038/ja.2014.116; published online 3 September 2014

Phoma is a genus of common coelomycetous fungi that includes many pathogenic species.[1,2] About 140 *Phoma* taxa have been defined and recognized.[1,2] *Phoma* sp. has been reported to produce many bioactive metabolites including phomapyrrolidones A–C with anti-tubercular activity,[3] phomazines A–C with cytotoxic properties;[4] barceloneic acids A, B and barceloneic lactone that inhibit farnesyl protein transferase,[5] a novel compound related to apiosporamide and fischerin with antifungal effects,[6] and α-tetralone derivative along with cercosporamide that are cytotoxic.[7] *Phoma* sp. is a promising source of natural novel bioactive metabolites, and has therefore attracted much attention.[8] We isolated a new barceloneic acid C (compound **1**) together with two known compounds, barceloneic acid A (compound **2**) and questin (compound **3**), from cultures of *Phoma* sp. JS752, and evaluated their antibacterial activities. Here we describe the cultures, isolation, structure determination and antibacterial activities of compounds **1**–**3**.

A strain (JS752) of *Phoma* sp. was isolated from reed plants (*Phragmites communis* Trinius) collected from a swamp at Seochun, South Korea. Stem tissues were cut into small pieces (0.5 × 0.5 cm) and surfaces were sterilized with 2% sodium hypochlorite for 1 min, 70% ethanol for 1 min, followed by being washed with sterilized distilled water. Fungal strains were grown out from plant tissues after about 1 week incubation on malt extract agar (Difco, Sparks, MD, USA) supplemented with 50 p.p.m. kanamycin, 50 p.p.m. chloramphenicol and 50 p.p.m. Rose bengal at 22 °C. Fungal strains were purely cultured by transferring actively growing edges onto a new potato dextrose agar (Difco). Pure cultured strains were stored as 20% glycerol stocks at liquid nitrogen tank of Wildlife Genetic Resources Bank at National Institute of Biological Resources (Incheon, Korea) before use. Fungal strains were identified by sequencing internal transcribed spacer (ITS) regions with ITS1 and ITS4 primers.[9] After homology search over NCBI nt DB with BlastN algorithm and phylogenetic analysis with ITS sequences from NCBI, JS752 was identified to belong to genus *Phoma*. The fungus was cultured at 28 °C for 28 days in four 500 ml Erlenmeyer flasks containing 80 g of rice with 120 ml of water.

The culture media underwent extraction three times with ethyl acetate (1:1, v-v), and the extracts were evaporated in a rotary evaporator at 40 °C. The resulting crude extract (4.2 g) was subjected to vacuum liquid chromatography over silica gel (60, < 0.663 mm; Merck, Billerica, MA, USA). The column was sequentially eluted with 500 ml of each of the following solvents: hexane/ethyl acetate (9:1, 4:1, 7:3, 1:1, 1:4; v:v) and ethyl acetate/MeOH (4:1, 7:3, 1:1, 1:4; v-v). Ten fractions were recovered and all fractions underwent thin liquid chromatography with a UV detector (254 and 365 nm). Fraction 4 (20.6 mg) was subjected to Sephadex LH-20 with the elution of CH_2Cl_2/MeOH (1:1, v-v) to isolate compound **3** (1.1 mg; Figure 1). Fr. 7 (21.0 mg) was purified using column chromatography over Sephadex LH-20 in CH_2Cl_2/MeOH (1:1, v-v) lead to compound **1** (4.5 mg; Figure 1). Fr. 8 was further purified with Sephadex LH-20 and CH_2Cl_2/MeOH (1:1, v-v) to obtain compound **2** (14.0 mg; Figure 1).

Compound **1** was isolated as a pale red powder. The molecular formula was found to be $C_{16}H_{14}O_6$ based on HREIMS data (*m/z* 302.0795, calculated for 302.0790) corresponding to10 degrees of unsaturation. The ^{1}H-NMR spectrum (Table 1) clearly exhibited a singlet aromatic methine signal at δ_H 6.49, two *meta*-coupled aromatic methine signals at δ_H 6.34 and 6.27 (each doublet, $J = 2.4$ Hz), a methoxyl group at δ_H 3.67, a methyl group at δ_H 2.21 and a singlet sp^3 methylene group at δ_H 2.21. Sixteen carbon signals were

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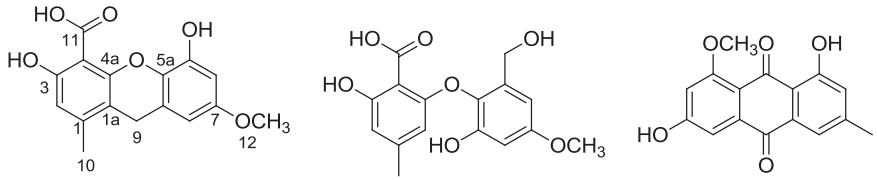
Received 19 March 2014; revised 15 July 2014; accepted 23 July 2014; published online 3 September 2014



1 2 3

Figure 1 Structures of compounds **1–3** isolated from *Phoma* sp.

Table 1 ^{1}H-NMR and ^{13}C-NMR spectroscopic data for compound 1 (DMSO-d_6)

Position	*^{13}C-NMR*	*^{1}H-NMR*	*HMBC (H→C)*
1	141.3	—	—
2	112.1	6.49 (s)	C-1, 3, 4, 10, 11
3	156.4	—	—
4	105.0	—	
5	146.2	—	—
6	101.3	6.34 (d, 2.4)	C-5, 7, 8, 5a
7	155.3	—	—
8	103.0	6.27 (d, 2.4)	C-6, 7, 9, 5a, 8a
9	25.1	3.78 (s)	C-1, 8, 1a, 4a, 5a
10	19.5	2.21 (s)	C-1, 2, 1a
11	169.1	—	—
12	55.4	3.67 (s)	C-7
1a	109.9	—	—
4a	149.6	—	—
5a	133.8	—	—
8a	121.6	—	—

observed in the ^{13}C-NMR spectrum (Table 1). A detailed analysis of the ^{13}C-NMR spectrum revealed the presence of one characteristic carbonyl group at δ_C 169.1. Furthermore, 12 aromatic ring signals for two benzene moieties at δ_C 156.4, 155.3, 149.6, 146.2, 141.3, 133.8, 121.6, 112.1, 109.9, 105.0, 103.0 and 101.3 were identified, indicating that compound **1** is a tricyclic compound to meet the requirement of the unsaturation. In addition, a methyl group at δ_C 19.5, a methoxyl group at δ_C 55.4 and a methylene group at δ_C 25.1 were observed. Based on the molecular formula combined with carbon chemical shifts, the presence of three exchangeable protons was identified.

The detailed structure of compound **1** was mainly elucidated based on the analysis of 2D NMR data (Table 1). HMBC correlations of the singlet aromatic proton at δ_H 6.49 (H-2) with δ_C 141.3 (C-1), 156.4 (C-3), 105.0 (C-4), 19.5 (C-10) and 169.1 (C-11) together with HMBC correlations of the methyl protons at δ_H 2.21 (H-10) with δ_C 141.3 (C-1), 112.1 (C-2) and 109.9 (C-1a) indicated that the methine carbon C-2 (δ_C 112.1) was adjacent to the oxygenated sp^2 quaternary carbon C-3 (δ_C 156.4). This was in turn next to the carboxylic acid-bearing carbon C-4 (δ_C 105.0), and the methine carbon C-2 was adjacent to the methyl group-bearing carbon C-1 (δ_C 141.3) as shown in Figure 2. In addition, HMBC correlations of the aromatic doublet proton at δ_H 6.34 (H-6) with aromatic carbons at δ_C 146.2 (C-5), 155.3 (C-7), 103.0 (C-8) and 133.8 (C-5a) as well as correlations between the methoxyl protons at δ_H 3.67 (H_3-12) and the oxygenated aromatic carbon at δ_C 155.3 (C-7) suggested that the methoxyl group-bearing carbon C-7 was located between two aromatic methine carbons (C-6 and C-8). Additionally, one of the oxygenated sp^2 quaternary carbons at δ_C 146.2 (C-5) or 133.8 (C-5a) was next to

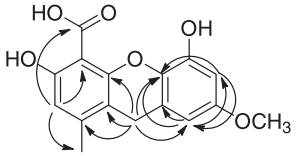


Figure 2 Key HMBCs for compound **1**.

the methine carbon at δ_C 101.3 (C-6). In this way, we were able to account for the presence of two rings in the molecule.

HMBCs of the sp^3 methylene protons at δ_H 3.78 (H_2-9) with the aromatic carbons at δ_C 141.3 (C-1), 103.0 (C-8), 109.9 (C-1a), 149.6 (C-4a) and 133.8 (C-5a) facilitated the connection of two rings through the methylene carbon (C-9). Finally, the ether linkage between C-4a and C-5a was identified and explained the carbon chemical shifts of C-4a and C-5a (149.6 and 133.8, respectively) while determining the molecular formula based on HRMS and fulfilling the requirement of unsaturations (tricyclic compound). Thus, we concluded that compound **1** was 3,5-dihydroxy-7-methoxy-1-methyl-9H-xanthene-4-carboxylic acid also known as barceloneic acid C (Figure 1).

Compound **2** was determined to be barceloneic acid A by comparing its spectroscopic data such as that from ^{1}H- and ^{13}C-NMR as well as MS with values from the literature.[5] Barceloneic acid A has also been isolated from *Pencilillium albocoremium*[10] and has modest FPTase inhibitory effects.[5] Barceloneic acid A forms barceloneic lactone through dehydration.[10] The hydroxyl group of the hydroxymethylene moiety in barceloneic acid A (compound **2**) was removed and attached to C-1a of the aromatic ring to form a pyran ring between two aromatic rings. This is the first report of barceloneic acids undergoing dehydration to form a xanthene ring. Based on the spectroscopic data, compound **3** was identified as 1.6-dihydroxy-8-methoxyl-3-methyl anthraquinone, which is also called questin.[11] Anthraquinones have been isolated from endophytic fungi, *Aspergillus* sp.,[12,13] as well as plants such as *Polygonum* sp. and *Cassia* sp.[14,15]

In order to see if compound **1** is an artifact during the isolation of compound **2**, the pure compound **2** was exposed to the same condition as used for its separation, and then subjected to HPLC. It showed the same retention time as the initial state, indicating that compound **1** is not an artifact of compound **2**. Compounds **1–3** can be biosynthetically related. The biosynthetic relationship between anthraquinones, benzophenones, grisanes and diphenyl ethers was reported before.[16,17] Biosynthesis of compound **2** is considered to proceed from the anthraquinone (questin, compound **3**), via the benzophenone (desmethylsulochrin), the grisane (geodin) and the diphenyl ether (asterric acid). Compound **1** is proposed to be biosynthesized from compound **2** through oxidative coupling and dehydration.

The antibacterial activities of compounds **1–3** against pathogenic Gram-positive bacteria including *Bacillus cereus* (13061), *Listeria monocytogenes* (19114) and *Staphylococcus pseuditermedius* (49444)

as well as Gram-negative bacteria including *Escherichia coli* (35150) and *Salmonella typhimurium* (43174) were assessed using the paper disc diffusion method. The bacterial strains were provided by Dr Kwang-Hyun Baek (Yeungnam University, Gyeongsan, South Korea), and cultured in potato dextrose agar medium to evaluate antibacterial activities and determine the MICs . Ampicillin was used as a positive control. Compound **1** showed moderate antibacterial activities against Gram-positive bacteria, *L. monocytogenes* and *S. pseuditermedius*, with an MIC value of $1.02\,\mu g\,ml^{-1}$ for both strains. The MIC of ampicillin was $0.89\,\mu g\,ml^{-1}$. In contrast, compounds **2** and **3** were not found to have antibacterial activity against any of the five pathogenic bacteria.

In conclusion, *Phoma* sp. JS752 was isolated from stems of reed plants *Phragmites communis* Trinius, and chemical investigation of cultures of this strain led to the isolation of three polyketides including a new compound named as barceloneic acid C. The new compound exhibited antibacterial activities against *L. monocytogenes* and *S. pseuditermedius*. To the best of our knowledge, this is the second report on isolation of the endophytic fungus from reed plants and the first report on metabolites produced by them. These results suggest that endophytic fungi could be good resources for antibacterial agents.

ACKNOWLEDGEMENTS

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0023753).

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)