

A New Diphenyl Ether Derivative from an Endolichenic Fungus *Preussia africana*

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Abstract: A new diphenyl ether derivative, namely barceloneic lactone D (**1**), along with five known compounds (**2-6**) were isolated from the solid cultures of an endolichenic fungus, *Preussia africana*. The structure of new compound was elucidated by HR-ESI-MS, 1D and 2D NMR spectroscopy. All the isolates were evaluated for their antifungal activities. All compounds showed definite inhibitory activities with inhibition rate ranging from 9.2% to 79.2%.

Keywords: *Preussia africana*; diphenyl ether; antifungal. © 2024 ACG Publications. All rights reserved.

1. Introduction

The *preussia* genus belongs to the family Sporormiaceae, and have been found as endophytic and endolichenic fungi [1, 2]. Previous studies on the natural products of some species have obtained many new bioactive secondary metabolites, such as six new chromones, preussochromone A–F, with cytotoxic activity from *Preussia africana* [3], two novel polyketides, minimoidiones A and B, as α -glucosidase inhibitors from *Preussia minimoides* [4], two new dibenzofurans, preussiafurans A and B, obtaining from *Preussia* sp. with cytotoxic and antiplasmodial activities [5], six aromatic bisketals, preussomerins A–F, possessing antifungal and antibacterial activities from *Preussia isomera* [6, 7], a pair of enantiomeric norsesquiterpenoids isolating from secondary metabolites of *Preussia isomera*, (+)- and (–)-preusolactone A, with antibacterial activity against *Micrococcus luteus* [8], etc. Accordingly, its diverse secondary metabolites by fungi of the *Preussia* genus and extensive biological activity have attracted us to mine the novel natural products.

In this study, the fungus *Ppreussia africana* was isolated from lichen *Ramalina calicaris* (L.) Fr. (Ramalinaceae), and an ethyl acetate extract prepared from solid-substrate fermentation products of the *P. africana* was found to exhibit antifungal activities against four phytopathogenic fungi (*S. ginseng*, *R. solani*, *C. destructans*, *E. turcicum*) with inhibition rate of 90.1%, 83.1%, 80.5% and 60.6%, respectively, at the concentration of 0.1 mg/mL. Thus, chemical investigation of *P. africana* led to the identification of a new diphenyl ether derivative, barceloneic lactone D (**1**), and five known compounds (**2-6**). Herein, the details of isolation, structure elucidation and antifungal activities of these compounds are described.

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2. Materials and Methods

2.1. General Experimental Procedures

UV spectra were recorded on a TU-1810DPC spectrophotometer (Persee, Beijing, China). IR were recorded on a Bruker TENSOR 27 spectrophotometer (Bruker, Germany). All NMR spectra were obtained on Bruker DRX-600 spectrometer (Bruker, Germany). HR-ESI-MS were collected on a Waters Xevo G2 Q-TOF spectrometer (Waters, Milford, MA, USA). Semi-preparative HPLC was performed on a Waters 2535 chromatography system (Waters, Milford, MA, USA) equipped with Waters 515 HPLC pump and Waters 2489 UV/visible detector. HPLC grade solvents: methanol, water, and acetonitrile were purchased from Thermo Fisher Scientific Korea Ltd (Seoul, Korea). Analytical grade solvents: dichloromethane, ethyl acetate and n-hexane were purchased from Beijing Chemical Works (Beijing, China). Separation was achieved using a YMC-Pack ODS-A (250 × 10 mm, 5 μm) column (YMC Co., Ltd., Kyoto, Japan).

2.2. Microorganism Material

The fungus *P. africana* was isolated from the lichen *Ramalina calicaris* (L.) Fr. (Ramalinaceae), collected from Suncheon, Korea, in 2006. The isolate was identified as *P. africana* by analysis of its morphological characteristics and ITS sequence in GenBank. The sequenced data have been deposited in GenBank (<http://www.ncbi.nlm.nih.gov>) with the accession number PP130126.

2.3. Fermentation and Isolation

The fungus *P. africana* stored in a 4°C freezer was cultured on PDA medium and incubated at a constant temperature incubator at 25 ° C for 7 d. The activated fungal clots (about 0.5 × 0.5 × 0.5 cm³) were inoculated into a liquid PDB medium, cultured at 25°C and 170 rpm for 7 d to prepare the seed culture. The seed culture was then used to inoculated 50 flasks (500 mL) each containing 80 g rice and 120 mL water. Then the flasks were incubated at a constant temperature of 25°C for 30 d.

The fermented material was extracted repeatedly with ethyl acetate (3 × 500 mL), and the organic solvent was evaporated to dryness under vacuum to afford the crude extract (32.5 g). The extract (PA) was subjected to silica gel CC (600 g, 200-300 mesh) and eluted with a gradient system of CH₂Cl₂-MeOH (100:1-1:1) to afford 12 fractions (PA1-PA12). PA5 (4.5 g) was further fractionated on ODS CC eluting with mixtures of MeOH-H₂O (20:80-80:20) to give 6 subfractions (PA5A-PA5F). PA5C (590 mg) was purified by semi-preparative RP-HPLC to give compounds **1** (5.5 mg, 35% CH₃CN *t_R* = 20.1 min), **2** (6.2 mg, 30% CH₃CN, *t_R* = 25.5 min), **4** (10.6 mg, 30% CH₃CN, *t_R* = 28.5 min). Compounds **3** (6.2 mg, *t_R* = 18.5 min) and **5** (17.1 mg, *t_R* = 16.4 min) were obtained from PA5E (230 mg) by semi-preparative RP-HPLC eluting with CH₃CN-water (22:78). PA6 (3.3 g) was further fractionated on ODS CC eluting with mixtures of MeOH-H₂O (20:80-80:20) to give 5 subfractions (PA6A-PA6E). PA6B (860 mg) was purified by semi-preparative RP-HPLC to give compound **6** (3.1 mg, 12% CH₃CN *t_R* = 20.8 min).

Barceloneic lactone D (1): White amorphous powder. UV (c 0.05, MeOH) λ_{max} (log ε) 286 (3.35) nm. IR (KBr) ν_{max} cm⁻¹: 3445 (OH), 1734 (C=O). ¹H and ¹³C NMR data see Table 1. HR-ESI-MS (*m/z* 287.0910 [M + H]⁺, calcd. 287.0914).

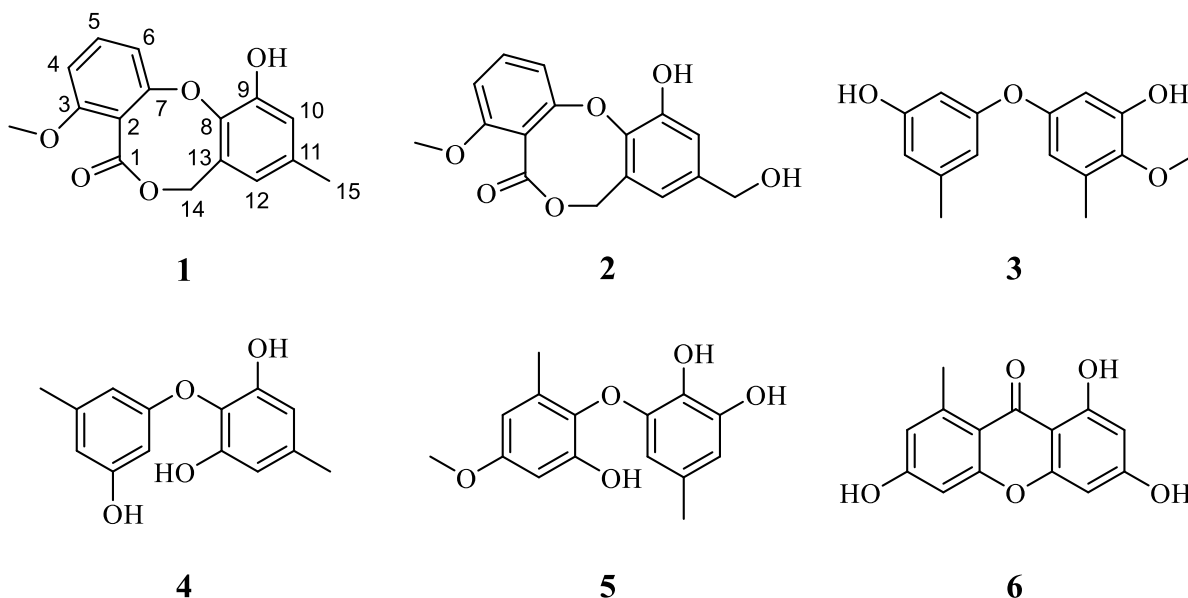


Figure 1. Structures of compounds 1-6

2.4. Antifungal Bioassay

All isolated compounds were evaluated for the antifungal activity against *Sclerotinia ginseng*, *Rhizoctonia solani*, *Cylindrocarpon destructans* and *Exserohilum turcicum* using the mycelium growth inhibition method to the previous report [9]. The compounds were dissolved in DMSO and mixed with PDA culture media to a final concentration 0.2 mg/mL. The media (6 mL) containing pure compounds were then poured into sterile Petri plates (6 cm). A 5 mm mycelial disk of the test pathogens was cut from 4 days culture and it was placed at the center of the plate. The mycelial disks on PDA without any test compound served as control, and each treatment was repeated three times and incubated in the dark at 28°C. Colony diameters were measured after 72 hours of incubation. The mycelial growth inhibition rate was calculated according to the following formula:

$$\text{Growth inhibition rate (\%)} = [(D_c - D_t)/(D_c - D_i)] \times 100$$

The D_c and D_t are the colony diameter of the control group and treated group, respectively, and D_i is the initial colony diameter.

3. Results and Discussion

3.1. Structure Elucidation

Compound **1**, obtained as white amorphous powder, possessed a molecular formula of $C_{16}H_{14}O_5$ by the negative HR-ESI-MS (m/z 287.0910 $[M + H]^+$, calcd. 287.0914), requiring 10 indices of hydrogen deficiency. The IR spectrum displayed absorption bands at 3445 and 1734 cm^{-1} , which were in agreement with hydroxyl and ester carbonyl groups. The 1H NMR spectrum (Table 1) in $DMSO-d_6$ of **1** showed a hydroxyl proton signal at δ_H 9.65 (1H, s, OH-9), a set of 1,2,3-trisubstituted aromatic protons at δ_H 7.54 (1H, t, $J = 8.4$ Hz, H-5), 7.07 (1H, d, $J = 8.4$ Hz, H-4), and 6.77 (1H, d, $J = 8.4$ Hz, H-6, overlapped), two meta-coupling aromatic protons at δ_H 6.77 (1H, br s, H-10, overlapped) and 6.41 (1H, br s, H-12), an oxygenated methylene singlet at δ_H 5.01 (2H, s, H-14), a methoxy signal at δ_H 3.85 (3H, s, 3-OCH₃), as well as a methyl proton signal at δ_H 2.15 (3H, s, H-15). The ^{13}C NMR spectrum (Table 1) showed a total of 16 carbon signals, including an ester carbonyl carbon at δ_C 166.3 (s, C-1), 12 aromatic carbon signals due to two benzene rings, a methoxy carbon at δ_C 56.2 (q, 3-OMe), an oxygenated methylene carbon at δ_C 68.4 (t, C-14), and a methyl carbon at δ_C 20.4 (q, C-15). The

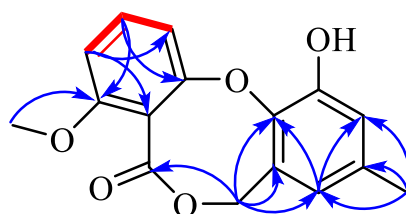
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above NMR features were similar to those of barceloneic lactone B [14]. The main difference was that a newly arisen methyl [δ_{H} 2.15 (3H, s, H-15); δ_{C} 20.4 (q, C-15)] replaced the hydroxymethyl in barceloneic lactone B [10]. The inference was clearly supported by the HMBC correlations (Figure. 1) from H-15 to C-10 [118.2 (d)], C-11 [134.0 (s)] and C-12 [119.9 (d)]. Therefore, the structure of **1** was established as 4-methoxy-9-methyl-11-hydroxy-5H,7H-dibenzo[b,g][1,5]dioxocin-5-one, shown in Figure 1 and named as barceloneic lactone D.

By comparing their NMR spectroscopic data with the literature, five known compounds (**2-6**) were identified as barceloneic lactone B (**2**) [10], quercilolin (**3**) [11], 3-hydroxy-5-methylphenyl ether (**4**) [12], 2,2',3,4'-tetrahydroxy-5,6'-dimethyldiphenyl ether (**5**) [13] and 1,3,6-trihydroxy-8-methyl-9H-xanthen-9-one (**6**) [14].

Table 1. ^1H -NMR (600 MHz) and ^{13}C -NMR (150 MHz) data of **1** in DMSO- d_6

Position	^1H NMR (integral, multiplicity, J in Hz)	^{13}C NMR (DEPT)
1	-	166.3 (s)
2	-	115.1 (s)
3	-	156.7 (s)
4	7.07 (1H, d, $J = 8.4$ Hz)	109.5 (d)
5	7.54 (1H, t, $J = 8.4$ Hz)	133.8 (d)
6	6.77 (1H, d, $J = 8.4$ Hz, overlapped)	114.3(d)
7	-	152.3 (s)
8	-	141.6 (s)
9	-	148.5 (s)
10	6.77 (1H, br s)	118.2(d)
11	-	134.0 (s)
12	6.41 (1H, br s)	119.9(d)
13	-	127.3 (s)
14	5.01 (2H, s)	68.4 (t)
15	2.15 (3H, s)	20.4 (q)
3-OMe	3.85 (3H, s)	56.2 (q)
9-OH	9.65 (1H, s, OH)	-



1

Figure 2. Key HMBC (arrows) and ^1H - ^1H COSY (bold lines) correlations of **1**

3.2. Antifungal Activity

Compound **1** exhibited week antifungal activities against four phytopathogenic fungi with inhibition rate ranging from 8.3% to 22.2% at the concentration of 0.1 mg/mL, but compound **5** showed significant inhibitory effects against *S. ginseng* and *C. destructans* with inhibition rate of 78.8% and 74.2%, respectively, compared to positive control carbendazim (80.1% and 77.2%). These compounds may be used as precursors for discovery of lead antifungal compounds by structural modification. In summary, the results of the abovementioned biological assays indicated that the heptacyclic moiety of compound **1** may play a significant role in the bioactivity of diphenyl ether derivatives.

3.3. Chemotaxonomic Evaluation

Previous phytochemical studies revealed that species of *Preussia* genus are rich in aromatic heterocycle as well as several polyketides [3–7]. In this work, five diphenyl ethers (**1-5**) and one polyketide (**6**) were isolated from *P. africana*. According to literature review, it was known that the diphenyl ether has been obtained from this genus for the first time in 2012 and was isolated in an extract of *Preussia typharum* from the Oliver Wildlife Preserve [15]. This study is the first report of five diphenyl ethers (**1-5**) including one new compound (**1**) from *P. africana*.

Table 2. Antifungal activity of compounds **1-6** at 0.1 mg/mL

Compound	Inhibition rate (%) ⁰			
	<i>S. ginseng</i>	<i>R. solani</i>	<i>C. destructans</i>	<i>E. turcicum</i>
1	22.2 ± 1.1	9.8 ± 1.4	15.4 ± 1.6	8.3 ± 1.7
2	11.2 ± 1.6	9.2 ± 2.3	—	—
3	35.3 ± 2.1	39.6 ± 1.2	21.7 ± 2.1	47.6 ± 2.0
4	46.3 ± 2.1	77.8 ± 2.7	41.2 ± 1.2	24.5 ± 1.3
5	78.8 ± 3.2	69.8 ± 2.3	74.2 ± 1.8	47.2 ± 1.3
6	9.6 ± 0.9	—	9.9 ± 1.2	—
carbendazim	80.1 ± 1.3	92.1 ± 2.3	77.2 ± 2.1	73.3 ± 1.9

*Values are shown as mean ± standard deviation of mean (n = 3).

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Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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