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A New Isobenzofuranone Derivative from Arctic Fungus *Gyoerffyella* sp. CPCC 401434

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Abstract: A new isobenzofuranone derivative, (*R*)-3-acetyl-[5,7]-dimethoxy-3*H*-isobenzofuran-l-one (**1**), together with six known compounds, (*R*)-3-acetyl-7-hydroxy-5-methoxy-3*H*-isobenzofuran-l-one (**2**), banksialactone D (**3**), (3*R*,4*S*)-3,8-dihydroxy-3-hydroxymethyl-6-methoxy-4,5-dimethyl-isochroman-1-one (**4**), 1-*O*-[*α*-*L*-rhamnopyranosyl]-2,5-dimethyl-3-phenol (**5**), *p*-hydroxybenzaldehyde (**6**), 2-(4-hydroxyphenyl) ethanol (**7**), were isolated from Arctic fungus *Gyoerffyella* sp. CPCC 401434. Their structures and absolute configurations were elucidated by the analysis of spectroscopic data, electronic circular dichroism, and comparison with reported literature data. Herein, all compounds were reported for the first time from the genus *Gyoerffyella* and their cytotoxic and antibacterial activities.

Keywords: Arctic fungus; *Gyoerffyella*; isobenzofuranone; cytotoxicity.

1. Fungal Source

The strain of *Gyoerffyella* sp. CPCC 401434 was isolated from an Arctic plant which was collected from Ny-Ålesund Region, Svalbard of the Arctic. The strain was identified as a *Gyoerffyella* species by analyses of the ITS region of rDNA sequence. The ITS sequence of the strain was recorded at the GenBank database (http://www.ncbi.nlm.nih.gov) with the accession number of OQ535030. The strain was deposited at the China Pharmaceutical Culture Collection, Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College.

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2. Previous Studies

Fungi have been demonstrated to be rich source of structurally diverse natural products [1-2]. Polar fungi inhabiting special and extreme environments may have unique physiological and biochemical metabolic pathways. Due to their extreme environmental conditions of their living media, they might be a source of structurally novel natural products with various biological activities [3]. Recently, several bioactive secondary metabolites have been isolated from polar fungi, such as eutypellacytosporins A-D [4], eutypellenones A-B [5], pseudogymnoascins A-C [6], svalbardines A-B [7], and palitantines B-C [8]. However, the chemical constituents of the members of genus *Gyoerffyella* have not been reported till now. Thus, chemical investigation of the genus might be important and be likely to discover new natural products.

3. Present Study

As a part of our ongoing work on bioactive natural products from Arctic fungi, *Gyoerffyella* sp. CPCC 401434 was chemically investigated. As a result of this study, we isolated a new isobenzofuranone derivative, (*R*)-3-acetyl-[5,7]-dimethoxy-3*H*-isobenzofuran-l-one (**1**) together with six known compounds (**2**-**7**) (Figure 1). In this paper, we report the isolation, structure elucidation, and biological activities of these compounds.

The strain CPCC 401434 was inoculated to slants of PDA medium, and then cultured at 20 °C for 10 days to get spores. The spores were inoculated in PDB at 15 °C for 11 days to obtain seed culture, and then inoculated 10 mL to the solid rice (rice 80 g; distilled water 100 mL; the media were autoclaved at 121 °C for 30 min) in 500 mL flasks. The flasks were incubated at 20 °C for 40 days. The fermentation product was extracted repeatedly with ethyl acetate (EtOAc) four times and 27.0 g of crude extract were obtained. The EtOAc extract (27.0 g) was initially subjected to silica gel column chromatography (CC) eluting with dichloromethane-methanol gradient solvent system to yield six fractions (Fr.1-Fr.6). Fraction Fr.1 (20.5 g) was further subjected to silica gel CC using petroleum ether-EtOAc gradient solvent system to give five fractions (Fr.1.1-Fr.1.5). Fraction Fr.1.3 (2.8 g) was chromatographed on RP-18 CC eluting with CH_3CN-water gradient solvent system to obtain six fractions (Fr.1.3.1-Fr.1.3.6), fraction Fr.1.3.2 (10.4 mg) was isolated by semi-preparative HPLC eluting with CH_3CN-H_2O (15:85) to offer **6** (3.0 mg). Fraction Fr.1.4 (1.2 g) was chromatographed on RP-18 CC eluting with CH_3CN-water gradient solvent system to obtain five fractions (Fr.1.4.1-Fr.1.4.5), and fraction Fr.1.4.2 (18.2 mg) was further purified by semi-preparative HPLC eluting with CH_3CN-H_2O (38:62) to afford **1** (2.0 mg). Fraction Fr.3 (4.5 g) was chromatographed on RP-18 CC eluting with CH_3CN-water gradient solvent system to obtain nine fractions (Fr.3.1-Fr.3.9), fraction Fr.3.2 (40 mg) was purified by semi-preparative HPLC eluting with CH_3CN-H_2O (10:90) to produce **7** (2.0 mg). The fraction Fr.3.3 (16.4 mg) was purified by semi-preparative HPLC eluting with CH_3CN-H_2O (20:80) to yield **5** (1.5 mg), fraction Fr.3.4 (60 mg) was purified by semi-preparative HPLC eluting with CH_3CN-H_2O (40:60) to give **4** (3.3 mg), fraction Fr.3.5 (24 mg) was purified by semi-preparative HPLC eluting with CH_3CN-H_2O (38:62) to obtain **3** (6.0 mg), fraction Fr.3.6 (18 mg) was purified by semi-preparative HPLC eluting with CH_3CN-H_2O (30:70) to yield **2** (5.1 mg).

*(R)-3-acetyl-[5,7]-dimethoxy-3H-isobenzofuran-l-one (**1**)*: colorless powder; $[\alpha]_D^{25}$ +164.3 (*c* 0.014, CH_3OH); UV (MeOH) λ_{max} (log ε): 303 (0.11), 262 (0.1869), 216 (0.40) nm; HR-ESI-MS: *m/z* 287.0888 $[M+Na]^+$ (calcd for $C_{14}H_{16}O_5Na$, 287.0890); CD (MeOH) λ_{max} (Δε): 215 (−20.4), 243 (+5.4), 288 (+4.5) nm. ^1H and ^{13}C NMR data, see Table 1.

Biological Activity Assay: All isolated compounds were evaluated for cytotoxic activities against the cell lines HeLa (human cervical carcinoma), HCT116 (human colorectal carcinoma), HepG2 (human hepatocellular carcinoma), A549 and H460 (human lung carcinoma) by MTT method as previous report [9]. All compounds were also evaluated for their antibacterial activities against *Staphylococcus*

aureus (ATCC 29213) and *Escherichia coli* (ATCC 25922) using dilution turbidimetric method as the standard protocols published by the Clinical and Laboratory Standard Institute [10].

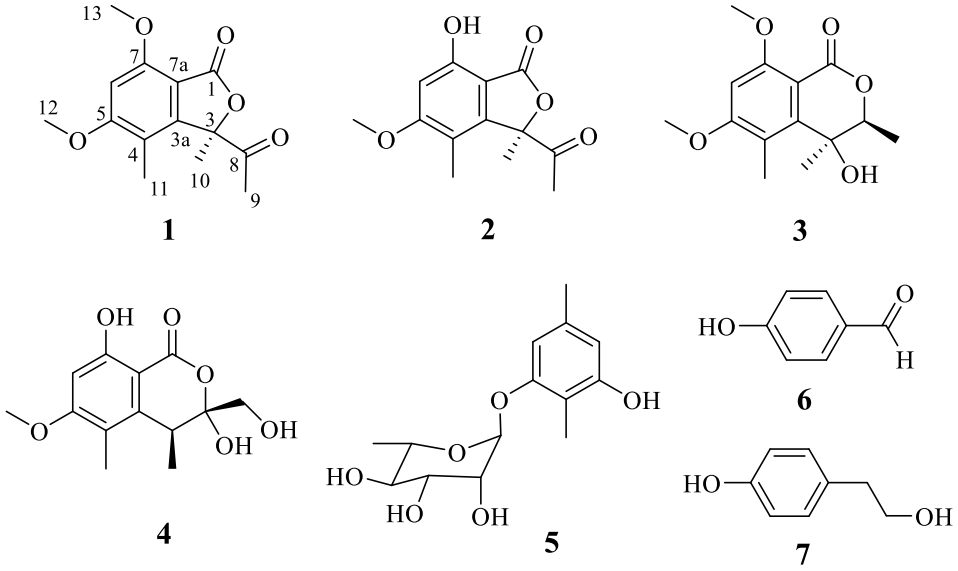
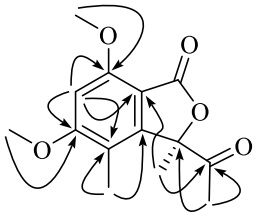


Figure 1. Chemical structures of compounds **1**-**7**

Compound **1** was isolated as colorless powder. Its molecular formula was assigned as $C_{14}H_{16}O_5$ with seven degrees of unsaturation based on the HR-ESI-MS ion peak at *m/z* 287.0888 $[M+Na]^+$ (calcd for $C_{14}H_{16}O_5Na$, 287.0890). The ^{1}H-NMR and HSQC spectra (Table 1) of **1** displayed the presence of one olefinic protons at δ_H 6.46 (1H, s), two methoxy peaks at δ_H 4.00 (3H, s) and 3.93 (3H, s), and three methyl signals at δ_H 2.04 (3H, s), 2.02 (3H, s), and 1.74 (3H, s). The ^{13}C NMR and DEPT spectra (Table 1) of **1** showed 14 carbons, including eight quaternary carbons (δ_C 203.5, 168.0, 164.9, 158.5, 149.1, 113.7, 104.7, and 89.0 including two carbonyl, two oxygenated olefinic, and one oxygenated aliphatic), one methine carbon (δ_C 95.1), and five methyl carbons (δ_C 56.2, 56.1, 23.9, 20.1, and 9.9, including two oxygenated). The HMBC spectrum (Figure 2) of **1** showed the correlations from H-6 to C-1, C-4, C-5, C-7, and C-7a, from H-9 to C-3 and C-8, from H-10 to C-3, C-3a, and C-8, from H-11 to C-3a, C-4, and C-5 established the multisubstituted isobenzofuranone unit. Furthermore, the HMBC cross-peaks of H-12/C-5 and H-13/C-7 determined the locations of two methoxy groups. The absolute configuration of **1** was determined by the electronic circular dichroism (ECD) spectrum. In the ECD spectrum (Figure 3), negative Cotton effect at 215 nm and positive Cotton effects at 243-288 nm were observed for **1**, which was similar to those of the known compound, (*R*)-3-acetyl-7-hydroxy-5-methoxy-3,4-dimethylisobenzofuran-1(3*H*)-one [11], indicating the 3*R* configuration for **1**. Therefore, the structure of **1** was established as (*R*)-3-acetyl-5,7-dimethoxy-3,4-dimethylisobenzofuran-1(3*H*)-one, as illustrated in Figure 1.

The ^{1}H and ^{13}C NMR data (Table 1) of **2** were found to be same with the known compound, (*R*)-3-acetyl-7-hydroxy-5-methoxy-3,4-dimethylisobenzofuran-1(3*H*)-one [12]. HMBC correlations (Figure S12) of **2** were observed from H-6 to C-1, C-4, C-5, C-7, and C-7a, from H-9 to C-3 and C-8, from H-10 to C-3, C-3a, and C-8, from H-11 to C-3a, C-4, and C-5, from H-12 to C-5. The experimental ECD spectrum (Figure 3) and the NMR data (Table 1) of **2** was elucidated as (*R*)-3-acetyl-7-hydroxy-5-methoxy-3,4-dimethylisobenzofuran-1(3*H*)-one [11,12].



HMBC: H ⟶ C

Figure 2. Important HMBC correlations for compound **1**

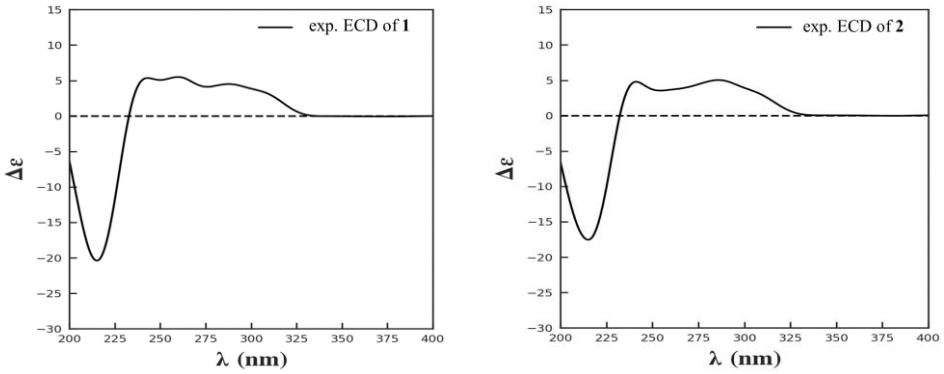


Figure 3. Experimental ECD spectra for compounds **1** and **2**

Herein, we elucidated the known compounds as banksialactone D (**3**) [13], (3*R*,4*S*)-3,8-dihydroxy-3-hydroxymethyl-6-methoxy-4,5-dimethyl-isochroman-1-one (**4**) [14], pestarhamnose C (**5**) [15], *p*-hydroxybenzaldehyde (**5**) [16], 2-(4-hydroxyphenyl)ethanol (**7**) [17] by comparison of their NMR and MS data as well as optical rotation with those reported in the literatures. There ^{1}H and ^{13}C NMR data of the known compounds were given in the Supporting Information.

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of compound **1** in $CDCl_3$

No.	**1**		**2**	
	δ_C, type	δ_H	δ_C, type	δ_H
1	168.2, C		171.6, C	
3	89.2, C		91.5, C	
3a	149.2, C		146.4, C	
4	113.9, C		114.1, C	
5	165.1, C		165.8, C	
6	95.3, CH	6.47 (1H, s)	99.2, CH	6.47 (1H, s)
7	158.7, C		157.2, C	
7a	104.8, C		102.4, C	
8	203.6, C		202.8, C	
9	24.1, CH_3	2.03 (3H, s)	24.0, CH_3	2.05 (3H, s)
10	20.3, CH_3	1.75 (3H, s)	20.1, CH_3	1.76 (3H, s)
11	10.1, CH_3	2.05 (3H, s)	10.0, CH_3	2.02 (3H, s)
12	56.4, CH_3	3.94 (3H, s)	56.3, CH_3	3.84 (3H, s)
13	56.3, CH_3	4.01 (3H, s)		

Compounds **1-7** were evaluated for cytotoxic activities against five human cancer cell lines (Hela, HCT116, HepG2, A549, and H460) by using the MTT method [9]. All compounds displayed weak cytotoxic activities (Table 2). Moreover, compounds **1-7** were tested against antibacterial activities against *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 [10]. Unfortunately, none of the compounds showed any antibacterial activity against the texted microorganisms at the concentration of 64 μg/mL.

Table 2. The cytotoxic activities of **1-7**

Compound	inhibition rate (%) in 50 μM				
	HeLa	HCT116	HepG2	A549	H460
1	13.9	20.9	-1.7	10.7	24.7
2	17.9	25.9	8.8	4.3	32.0
3	15.1	33.5	7.5	22.1	41.1
4	14.6	10.6	1.4	13.5	25.4
5	15.8	23.8	5.0	13.7	34.6
6	16.6	27.3	-4.5	17.8	37.4
7	12.5	17.9	-20.4	21.6	35.6
taxol [a]	99.9	99.3	99.7	99.4	99.1

[a] Taxol as positive control.

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Supporting Information

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