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Circumdatin I, a New Ultraviolet-A Protecting Benzodiazepine Alkaloid from a Marine Isolate of the Fungus *Exophiala*

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Abstract A new ultraviolet-A (UV-A) protecting benzodiazepine alkaloid, circumdatin I (**1**), along with the previously described circumdatin C (**2**) and circumdatin G (**3**), have been isolated from the mycelium of a marine-derived fungus of the genus *Exophiala*. The structures of the three circumdatins were assigned on the basis of physicochemical evidence. The absolute stereochemistry of **1** was determined by comparison of optical rotation and CD experiments with those of **2** and **3**.

Keywords marine-derived fungus, *Exophiala*, circumdatin, benzodiazepine alkaloid, ultraviolet-A (UV-A)

Marine-derived microorganisms such as bacteria, fungi, and cyanobacteria have proven to be a rich source of new biologically active secondary metabolites [1]. As part of our search for bioactive substances from marine-derived microorganism [2], the marine sponge-associated fungus was studied because the mycelium extract showed potent UV-A protecting activity. An assay-guided purification resulted in isolation of a new benzodiazepine alkaloid, circumdatin I (**1**), and two known circumdatins C (**2**) [3] and G (**3**) [4] from the marine isolate of fungus *Exophiala* sp. We report here on the isolation and structural elucidation of compounds **1**～**3**.

Fungal Isolation and Culture

The fungal strain was isolated from the surface of the marine sponge *Halichondria panicea* collected at Bogil Island, Jeonnam Province, Korea, in 2006 and identified as an *Exophiala* sp. (Family: Herpotrichiellaceae) on the basis of morphological evaluation and fatty acid methyl ester analysis (Korean Culture Center of Microorganism, Seoul, Korea, similarity index of 0.828). A voucher specimen is deposited at Pukyong National University with the code MFC353-1. The fungus was cultured (10 liters) for 3 weeks (static) at 29°C in SWS medium containing of soytone 0.1% and soluble starch 1.0% in seawater.

Extraction and Isolation

The cultured broth was filtered with through cheesecloth to give a mycelium cake. The freeze-dried mycelium cake was extracted with CH_2Cl_2-MeOH (1 : 1) to afford crude extract (1.8 g), which was subjected to silica gel flash chromatography. Elution was performed with *n*-hexane-EtOAc (stepwise, 0～100% EtOAc) to yield four fractions. Fractions 3 and 4, which were active in UV-A protecting assay, were separated by medium-pressure liquid chromatography (MPLC) (ODS) using a H_2O-MeOH gradient elution to afford crude compounds **1**, **2**, and **3**, respectively. These were further purified by HPLC (YMC, ODS-A, 10×250 mm, 1.0 ml/minute) utilizing a 30 minutes gradient program of 50～100% MeOH in H_2O to furnish **1** (6.5 mg), **2** (6.0 mg), and **3** (2.0 mg), respectively.

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1: a colorless solid; $[\alpha]_D^{20} = -236°$ (*c* 0.2, MeOH); CD (MeOH) nm ($\Delta\varepsilon$) 302 (−2.2), 296 (−2.4), 254 (+7.5), 236 (−16.8), 214 (+29.7); UV (MeOH) λ_{max} nm (log ε) 210 (7.4), 240 (7.5), 283 (6.9), 329 (6.9), 340 (sh) (6.8); IR (KBr) ν_{max} 3435, 1654, 1615 cm^{-1}; LR-EI-MS *m/z* 323 $[M]^+$ (100), 281 (32), 252 (20), 224 (7), 189 (10), 162 (29), 121 (69), 106 (28), 92 (19), 63 (66). HR-EI-MS *m/z* 323.0907 $[M]^+$ (calcd for $C_{17}H_{13}N_3O_4$, 323.0906). See Table 1 for NMR spectral data.

2 and **3** were isolated as a colorless solid, and showed spectral data virtually identical to those reported in the literature [3, 4].

Ultraviolet-A Protecting Assay

Samples to be tested were dissolved in MeOH, and the solution (200 μl) was dispensed into wells of a 96-well microtiter tray. The absorbance of the sample solution was measured at 340 nm with microplate reader (Packard Co., Spectra Count^{TM}). The ultraviolet-A protecting activity was expressed as ED_{50}, which is the concentration of the tested compound required to give a 50% increase of the absorbance from that of the blank solution [MeOH (200 μl)]. ED_{50}'s were determined by linear regression of data plotted on a semi-log scale.

Structural Elucidation and Bioactivity

1 was isolated as a colorless solid which yielded a molecular formula of $C_{17}H_{13}N_3O_4$ by HR-EI-MS and ^{13}C-NMR methods. The IR spectrum of **1** exhibited bands characteristic for hydroxyl (3435 cm^{-1}) and amide (1654 cm^{-1}) functionalities.

The ^{1}H- and ^{13}C-NMR data for benzodiazepine **1**, including the results from DEPT, COSY and TOCSY experiments, showed the presence of 1,2,4-trisubstituted benzene, 1,2,3-trisubstituted benzene, 1,1-disubstituted ethane, and two amides groups (Table 1, Fig. 1).

Detailed analyses of the HMQC and HMBC spectra of **1** suggest the presence of 1,2,2-trisubstituted 3-methyl-7-hydroxy-1,4-benzodiazepine and 2,3-disubstituted 8-hydroxyquinazolin-4-one. The presence of 2,3-disubstituted 8-hydroxyquinazolin-4-one was further supported by UV spectral data [329 nm (log ε 6.9), 340 (sh) (6.8)].

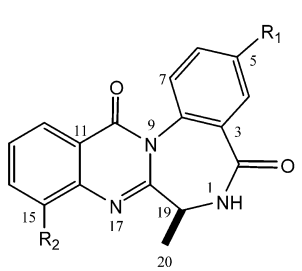
The connectivities and assignments of the functional groups for **1**, which led to the planar structure for this metabolite, were made by interpretation of HMBC NMR data (Fig. 1) and by the comparison of the NMR data with those of **2** [3] and **3** [4].

The NMR data of **1**∼**3** showed similar patterns, except

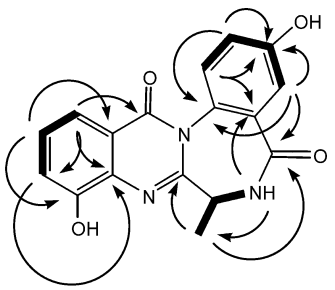
Table 1 NMR spectral data for circumdatin I (**1**)[a]

Position	δ_H (mult., *J*)	δ_C (mult.)
1	8.69 (d, 6.0)	
2		166.6 (s)
3		132.4 (s)
4	7.10 (d, 3.2)	114.1 (d)
5		157.2 (s)
6	7.00 (dd, 9.0, 3.2)	117.8 (d)
7	7.38 (d, 9.0)	130.0 (d)
8		124.5 (s)
10		161.2 (s)
11		121.8 (s)
12	7.58 (dd, 8.0, 1.0)	116.5 (d)
13	7.37 (dd, 8.0, 8.0)	127.8 (d)
14	7.27 (dd, 8.0, 1.0)	119.1 (d)
15		152.8 (s)
16		134.8 (s)
18		155.1 (s)
19	4.31 (dq, 6.5, 6.0)	49.6 (d)
20	1.56 (d, 6.5)	14.9 (q)

[a] Recorded in DMSO-d_6 at 400 MHz (^{1}H) and 100 MHz (^{13}C).



Circumdatin I (**1**): $R_1 = R_2 = OH$
Circumdatin C (**2**): $R_1 = OH$, $R_2 = H$
Circumdatin G (**3**): $R_1 = H$, $R_2 = OH$



1

Fig. 1 Chemical structures of circumdatins I (**1**), C (**2**), and G (**3**).
Structure of **1** elucidated by ^{1}H-^{1}H COSY (—) and HMBC (→) correlations.

for the appearance of a new oxygenated sp^2-quaternary carbon in place of one sp^2-methine of **2** [δ 7.76 (H-15), 128.8 (C-15)] and **3** [δ 7.57 (H-5), 128.6 (C-5)], respectively (Table 1).

Thus, **1** was characterized as 5,15-dihydroxycircumdatin F [5] on the basis of direct comparison of NMR data of **1** with those of **2** and **3**. The absolute stereochemistry of **1** was investigated using CD. The CD spectrum (MeOH) of **1** showed the following Cotton effects at 302 nm ($\Delta\varepsilon$, −2.2), 254 (+7.5), 236 (−16.8), 214 (+29.7), which were very similar to those of **2** [CD (MeOH): 307 nm ($\Delta\varepsilon$, −4.3), 263 (+8), 233 (−22) and 213 (+29)] [3]. Thus, the absolute configuration of asymmetric center for **1** was determined to be 19(*S*). This conclusion was further supported by the comparison of the optical rotations among **1**∼**3**. The value of specific rotation of **1** ($[\alpha]_D^{20}$ −236°) was in negative, the same phase as those of **2** ($[\alpha]_D^{20}$ −75°) [3] and **3** ($[\alpha]_D^{20}$ −221°) [4], implying that both compounds shared the same configuration.

2 and **3** were identified as the known compounds, circumdatins C and G, respectively, by comparison of their spectroscopic data to the published data [3, 4].

1∼**3** were evaluated for UV-A protecting activity, and they exhibited an UV-A protecting activity with ED_{50} values of 98, 101, and 105 μM, respectively, which are more potent than the positive control, oxybenzone (ED_{50}, 350 μM), a currently used sunscreen agent.

Benzodiazepine alkaloids, for example, circumdatins A∼H [3∼6], *epi*-aszonalenins A∼C [7], benzodiazepinedione [8], and asperlicins [9], were widespread microbial products commonly found in nutrient rich cultures of both terrestrial and marine fungi, and exhibited interesting biological activities, treatment of gastrointestinal and CNS disorder [5, 9], inhibition of mitochondrial NADH oxidase [6], and psychoactive properties [7]. Circumdatins A∼H have been previously isolated from *Aspergillus ochraceus* and have been suggested as good chemotaxonomic markers for this species [3∼6].

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