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A New Antibacterial Dioxopiperazine Alkaloid Related to Gliotoxin from a Marine Isolate of the Fungus *Pseudallescheria*

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Abstract A new antibacterial dioxopiperazine, dehydroxy*bis*dethio*bis*(methylthio)gliotoxin (**1**), and the previously described *bis*dethio*bis*(methylthio)gliotoxin (**2**) and gliotoxin (**3**), have been isolated from the broth of a marine-derived fungus of the genus *Pseudallescheria*. The structure and absolute stereochemistry of the new compound was assigned on the basis of NMR and CD experiments. Compounds **1**∼**3** exhibit potent antibacterial activity against the methicillin-resistant and multidrug-resistant *Staphylococcus aureus* with MIC values of 31.2, 31.2, and 1.0 μg/ml, respectively. Compound **3** also exhibited a significant radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) with IC_{50} value of 5.2 μM.

Keywords marine-derived fungus *Pseudallescheria*, dehydroxy*bis*dethio*bis*(methylthio)gliotoxin, *bis*dethio*bis*-(methylthio)gliotoxin, gliotoxin, antibacterial activity, radical scavenging activity

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Marine microorganisms such as bacteria and fungi inhabit virtually any environment in the sea, and they are the source of greatest diversity in the sea [1, 2]. These microbes have been shown to produce novel substances with utilities as potential new drug leads [3].

In our screening aimed at identifying antimicrobial compounds of microbial origin [4], we investigated antibacterial activity against the methicillin-resistant and multidrug-resistant *Staphylococcus aureus* (MRSA and MDRSA) from the fungal extracts, and a significant activity was observed in the marine-derived algicolous fungus *Pseudallescheria* sp. (MFB165).

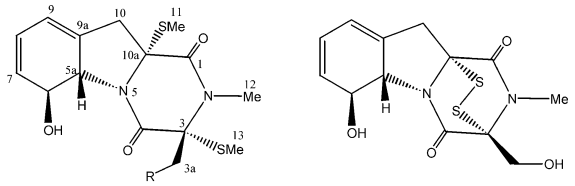
Fungal Isolation and Culture

The fungal strain, *Pseudallescheria* sp., was isolated from the surface of the marine brown alga *Agarum cribrosum* collected in the Uljin, Gyeongbuk Province, Korea in 2002 and identified based on the morphological evaluation and fatty acid methyl ester analysis (Korean Culture Center of Microorganism, Seoul, Korea, a similarity index of 0.65). A voucher specimen is deposited at Pukyong National University with the code MFB165.

The isolate was cultured in ten × one liter volumes using SWS medium consisting of soytone 0.1%, soluble starch 1.0%, and seawater 100% for 30 days (static) at 29°C.

Extraction and Isolation

The mycelium and broth were separated by filtration, and the whole broth was extracted with EtOAc (10 liters) to afford crude extract of 0.8 g. The broth extract showed strong antibacterial activity against MRSA and MDRSA,



1: R = H
2: R = OH

3

Fig. 1 Chemical structures of dehydroxy*bis*dethio*bis*-(methylthio)gliotoxin (**1**), *bis*dethio*bis*(methylthio)gliotoxin (**2**), and gliotoxin (**3**).

and the active components were purified by assay-guided isolation.

Broth extract (0.8 g) was subjected to silica gel flash chromatography and eluted with *n*-hexane/EtOAc (1 : 1), *n*-hexane/EtOAc (1 : 5), *n*-hexane/EtOAc (1 : 10), and finally with EtOAc. Each collections (30 ml each) were combined on the basis of their TLC profiles to yield four major fractions. MPLC of antibacterial active fractions 2, 3, and 4 on ODS (YMC Gel, ODS-A, 12 nm, S-150 μm) by elution with MeOH afforded compounds **1**∼**3**, respectively. The isolated compounds were further purified by HPLC (YMC ODS-A, 10×250 mm, 1 ml/minute) utilizing a 30 minutes gradient program of 50% to 100% MeOH in H_2O to furnish dehydroxy*bis*dethio*bis*(methylthio)gliotoxin (**1**, 5.5 mg), *bis*dethio*bis*(methylthio)gliotoxin (**2**, 9.0 mg) and gliotoxin (**3**, 7.0 mg), respectively.

Dehydroxy*bis*dethio*bis*(methylthio)gliotoxin (**1**): a colorless oil; $[\alpha]_D^{20} = -48°$ (*c* 0.3, MeOH); CD (MeOH) nm ($\Delta\varepsilon$) 287 (+2.8), 225 (−7.5); UV λ_{max}^{MeOH} nm (log ε) 271 (4.25); IR (KBr) ν_{max} 3360, 1661, 1641, 1423, 1383, 1255, 1189, 1062, 960 cm^{-1}; LR-EI-MS *m*/*z* 340 $[M]^+$ (0.3), 323 (1), 309 (2), 293 (74), 265 (16), 246 (55), 245 (67), 218 (58), 56 (100). HR-FAB-MS *m*/*z* 363.0816 $[M+Na]^+$ (calcd for $C_{15}H_{20}N_2O_3S_2Na$, 363.0813). See Table 1 for NMR spectral data.

*Bis*dethio*bis*(methylthio)gliotoxin (**2**), $[\alpha]_D^{20} = -51°$ (*c* 1.0, MeOH), and gliotoxin (**3**) were isolated as a colorless oil and white solid, respectively, and showed spectral data virtually identical to those reported in the literature [5∼7].

Table 1 NMR spectral data for dehydroxy*bis*dethio*bis*-(methylthio)gliotoxin (**1**)[a]

C no.	δ_H (mult., *J*)	δ_C (mult)	HMBC (H to C)
1		164.3 (s)	
3		67.3 (s)	
3a	1.81 (s)	23.2 (q)	3, 4
4		167.4 (s)	
5a	4.79 (d, 13.5)	68.9 (d)	6
6	4.68 (d, 13.5)	73.6 (d)	5a
6-OH	5.46 (br.s)		6, 7
7	5.62 (d, 9.7)	130.4 (d)	5a, 9
8	5.89 (m)	123.4 (d)	
9	5.96 (br.s)	118.8 (d)	
9a		133.5 (s)	
10	3.03 (s)	37.9 (t)	9, 9a, 10a
10a		71.6 (s)	
11	2.16 (s)	14.1 (q)	10a
12	2.98 (s)	28.7 (q)	1, 3
13	2.21 (s)	14.5 (q)	3

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

Structural Elucidation and Bioactivity

Compounds **2** and **3** were identified as the known compounds, *bis*dethio*bis*(methylthio)gliotoxin and gliotoxin, respectively, by comparison of their spectroscopic data to the published data [5∼7].

Dehydroxy*bis*dethio*bis*(methylthio)gliotoxin (**1**), $[\alpha]_D$ −48° (*c* 0.3, MeOH), was isolated as a colorless oil which yielded a molecular formula of $C_{15}H_{20}N_2O_3S_2Na$ by HR-FAB-MS $[(M+Na)^+$ *m*/*z* 363.0813 (dev +0.3 ppm)] and ^{13}C NMR methods. The IR spectrum of **1** exhibited bands characteristic for hydroxyl (3360 cm^{-1}) and amide (1661 cm^{-1}) functionalities, while the UV spectrum revealed the presence of a dioxopiperazine [271 nm (log ε 4.25)] chromophore. The EI-MS spectrum of dioxopiperazine **1** showed two prominent fragment ions corresponding to the parent molecule with losses of one and two CH_3S groups [*m*/*z* 293 (M^+-CH_3S), 246 (M^+-2CH_3S)], respectively.

The ^{1}H and ^{13}C NMR data for dioxopiperazine **1**, including results from COSY, DEPT, HMQC and HMBC experiments, showed the presence of a *N*,*N*-disubstituted dioxopiperazine ring possessing one methyl and two thiomethyl groups, 2,3-disubstituted-3,5-cyclohexadien-1-ol, and one methylene (Table 1). The connectivities and assignments of the carbon and proton resonances for **1**, which led to the planar structure for this metabolite, were made by interpretation of HMBC NMR data and by the comparison of the NMR data with those of *bis*dethio*bis*(methylthio)gliotoxin (**2**). The general features of its UV, IR, CD and NMR spectra closely resembled those of compound **2**, except that the NMR signals assigned to the hydroxymethyl [δ 3.73, 4.05 (each 1H, dd, *J*=11.3, 6.0, 3a-H_2), 5.50 (1H, t, *J*=6.0, 3a-OH), δ_C 63.0 (t, C-3a)] for compound **2** were changed to the methyl group [δ 1.81 (3H, s, 3a-H_3), δ_C 23.2 (q, C-3a)] for

compound **1**.

The relative configurations at the stereocenters were deduced from NOE spectral data which showed correlations between 3-SCH_3 and 10a-SCH_3 and between 5a-H and 6-OH and 10-H_a indicative of their *cis* orientations, respectively.

The absolute stereochemistry of **1** was investigated using CD. The CD spectrum of **1** showed the following Cotton effects at 287 nm ($\Delta\varepsilon$, +2.8) and 225 nm ($\Delta\varepsilon$, −7.5), which were very similar to those of **2** [278 nm ($\Delta\varepsilon$, +3.6) and 226 nm ($\Delta\varepsilon$, −7.5)]. Thus, the absolute configuration of asymmetric centers for **1** was determined to be 3(*R*), 5a(*S*), 6(*S*), and 10a(*R*). This conclusion was further supported by the comparison of the optical rotation between compounds **1** and **2**. The value of specific rotation of **1** ($[\alpha]_D$ −48°) was in negative, the same phase as that of **2** ($[\alpha]_D$ −51°], implying that both compounds shared the same configuration.

Compounds **1**∼**3** were evaluated for antibacterial activity against MRSA and MDRSA [8], and they exhibited an antibacterial activity with MIC values of 31.2, 31.2, and 1.0 μg/ml, respectively. Compounds **1**∼**3** were also screened for radical scavenging activity against DPPH [9]. Only **3** showed a significant radical scavenging activity with IC_{50} value of 5.2 μM, which is more potent than the positive control, ascorbic acid (IC_{50}, 20 μM), and the **1** and **2** were virtually inactive.

Diketopiperazines are widespread microbial products commonly found in nutrient rich cultures of both terrestrial and marine fungi [10]. Diketopiperazines are of interest because of their activity in various pharmacological assay systems [6].

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