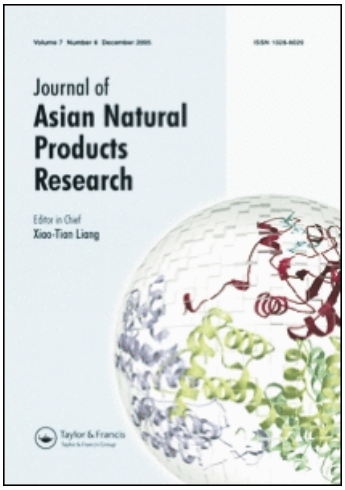

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Two diketopiperazines from marine fungus *Gliocladium* sp. YUP08

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Two new diketopiperazines, PJ147 (**1**) and PJ157 (**2**), were isolated from the mycelium of a fungus, *Gliocladium* sp. YUP08, which was separated from sea mud collected in Rushan, Shandong, China. Their structures were elucidated by spectroscopical and chemical methods.

Keywords: Marine fungus; *Gliocladium* sp; Diketopiperazines; Mycelium

1. Introduction

Gliocladium is a genus of soil-borne fungus, which occurs in diverse ecological niches including marine and freshwater environments associated with plants and animals. It has long been studied as biological control agents of phytopathogens and is characterised by its ability to produce a wide range of secondary metabolites with diverse biological actions, including antifungal activity [1,2]. In our ongoing searching for bioactive metabolites from marine microorganisms, two new compounds, PJ147 (**1**) and PJ157 (**2**), were obtained from the mycelium of *Gliocladium* sp. YUP08, which was isolated from sea mud collected in Rushan. The two compounds are a pair of geometric isomers of diketopiperazine derivatives. Here, we report the isolation and structural elucidation of compounds **1** and **2**.

2. Results and discussion

Compound **1** was obtained as white amorphous powder (MeOH), $[\alpha]_D^{20}$ −150 (*c* 0.1, CH_3OH). The molecular formula was established as $C_{22}H_{28}N_2O_3$ by HRFAB-MS at *m/z*

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Table 1. NMR data of compounds **1** and **2** in DMSO-d_6.

Position	***1***		***2***	
	δ_C	δ_H (J_{Hz})	δ_C	δ_H (J_{Hz})
1		9.96 (s)		10.31 (s)
2	166.3		162.0	
3	60.0	4.31(q, 6.8)	59.3	4.40 (q, 6.8)
4		10.22 (s)		10.49 (s)
5	157.3		157.8	
6	123.4		126.0	
7	17.1	1.45 (d, 6.8)	16.8	1.45 (d, 6.8)
8	115.2	6.74 (s)	117.9	6.92 (s)
9	125.7		125.1	
10, 10′	131.0	7.45 (d, 8.7)	132.5	7.36 (d, 8.7)
11, 11′	114.9	6.96 (d, 8.7)	113.7	6.84 (d, 8.7)
12	158.4		158.7	
13	64.5	4.57 (d, 6.6)	64.5	4.56 (d, 6.6)
14	119.6	5.43 (t, 6.6)	119.7	5.42 (t, 6.6)
15	140.5		140.5	
16	38.9	2.04 (m)	38.9	2.04 (m)
17	25.9	2.09 (m)	25.9	2.08 (m)
18	123.9	5.08 (t)	123.9	5.08 (t)
19	131.2		131.1	
20	25.6	1.64 (s)	25.6	1.63 (s)
21	17.7	1.57 (s)	17.7	1.57 (s)
22	16.5	1.71 (s)	16.5	1.71 (s)

368.4689 [M^+] and NMR data. The IR spectrum displayed bands at 3310, 1688 and 1606 cm^{-1}, suggesting the presence of amide functions. Three methyl singlets in the ^1H NMR spectrum (table 1) showed HMBC correlations (figure 1) characteristic of geranyl side chains. For example, proton at δ 1.71 (3H, s, H-22) showed correlations with C-16 (δ 38.9), C-15 (δ 140.5) and C-14 (δ 119.6), and proton at δ 1.64 (3H, s, H-20) showed correlations with C-21 (δ 17.7), C-19 (δ 131.2) and C-18 (δ 123.9). Signals at δ 6.96 (2H, d, $J = 8.7$ Hz, H-11′, 11), 7.45 (2H, d, $J = 8.7$ Hz, H-10′, 10), and 6.74 (1H, s, H-8) were assigned as a *para*-oxygenated benzylidene by analysis of HMQC and HMBC data. Proton signals at δ 9.96 (1H, s, H-1), 10.23 (1H, s, H-4) and carbon signals at δ 166.3 (C-2), 157.3 (C-5) suggested the presence of the diketopiperazine group. Methyl proton at δ 1.45 (3H, d, $J = 6.8$ Hz, H-7) showed HMBC correlations with C-3 (δ 60.0) and C-2 (δ 166.3), indicating an Ala residue present in the diketopiperazine. Further structural assignments were completed by HMBC analysis. Methene protons at δ 4.57 (2H, d, $J = 6.6$ Hz, H-13) displayed correlation with

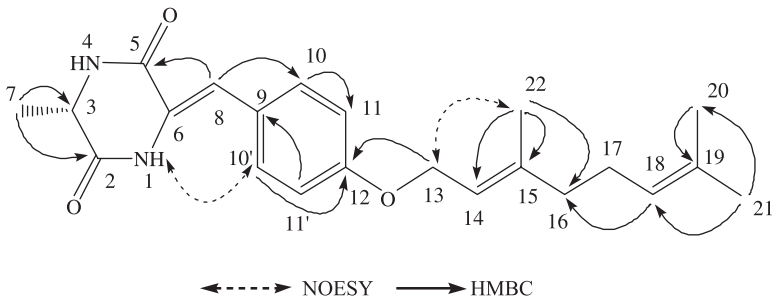

Figure 1. Selective HMBC and NOESY correlations of compound **1**.

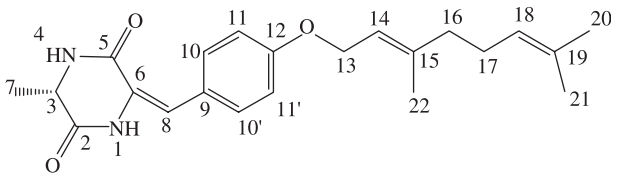

Figure 2. Structure of compound **2**.

C-12 (δ 158.4), so the geranyl group was obviously linked to the benzylidene at the 12-position through an oxygen atom. Vinyl proton at δ 6.74 (1H, s, H-8) showed correlation with C-5 (δ 157.3), suggesting the benzylidene was attached to the diketopiperazine ring at the 6-position.

A NOESY experiment was conducted to confirm the geometry of **1**. NOE between the NH proton at δ 9.96 (1H, s, H-1) and phenyl proton at δ 7.45 (1H, d, $J = 8.7$ Hz, H-10′) was obviously observed, which confirmed the *Z*-configuration of the $\Delta^{6,8}$ olefin [3]. NOE between methyl proton at δ 1.71 (3H, s, H-22) and methene proton at δ 4.57 (2H, d, $J = 6.6$ Hz, H-13) confirmed the *E*-configuration of $\Delta^{14,15}$ double bond in the geranyl group. In the ^{13}C NMR of **1**, C-22 (δ 16.5) observed in high field also confirmed this conclusion [4]. To determine the absolute stereochemistry, compound **1** was hydrolysed [5]. The acid hydrolysate was separated to get Ala in compound **1**, which was determined to possess an L-configuration by comparing its NMR, EI-MS and $[\alpha]_D^{20}$ data with those reported values. Thus, the structure of **1** was determined to be as shown in figure 1.

Compound **2** was obtained as white amorphous powder (MeOH), $[\alpha]_D^{20}$ −78 (*c* 0.5, CH_3OH). The HRFAB-MS of 2 indicated the molecular formula of $C_{22}H_{28}N_2O_3$, which was identical to **1**. Its UV, IR and EI-MS data were similar to those of compound **1**. In the ^{1}H NMR of **2** (table 1), H-8 (δ 6.92) was in lower field and four aromatic protons (δ 7.36, 6.84) were in higher field compared to those of **1**. In its ^{13}C NMR, C-2 (δ 162.0) shifted up-field relative to its position in the spectrum of **1**. Except for the above data, the NMR data indicated very close similarity between the two compounds. This information indicated compound **2** might be a geometric isomer of compound **1**. By analysis of NOESY data of **2**, all NOE present in those of **1** were observed, except for NOE between the NH proton and phenyl proton. Moreover, NOE between the NH proton at δ 10.31 (1H, s, H-1) and vinyl proton at δ 6.92 (1H, s, H-8), which were not present in those of **1**, were observed. Thus, $\Delta^{6,8}$ olefin in **2** was confirmed as an *E*-configuration [3]. On the basis of 1D and 2D NMR data, the structure of **2** was elucidated as shown in figure 2.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Perkin–Elmer 241 polarimeter. UV spectra were measured on a Shimadzu UV-1601. All the NMR spectra were taken on a Bruker-ARX-300 spectrometer (^{1}H at 300 MHz and ^{13}C at 75 MHz). EI-MS spectra were recorded on a DX-300 mass spectrometer. HRFAB-MS spectra were measured on a VG Atospec spectrometer. Column chromatography was performed on silica gel G (200–300 mesh,

Qingdao Haiyang Chemical Factory), Sephadex LH-20 (Pharmadex) and reversed-phase silica gel (Chromatorex C_{18}).

3.2 Fungus material

The fungus strain was isolated from sea mud collected in Rushan, Shandong province, China, in May of 2004, and identified as *Gliocladium* sp. by Professor Li Tian. A voucher specimen (No. CAAN045011) is deposited in the Key Laboratory of Marine Biology of the State Oceanography Administration, China.

3.3 Cultivation, extraction and isolation

The strain was cultured on seed medium at 24° on a rotary shaker for 9 days. The culture medium contained potato decoction 200 ml, sea mud extract 20 ml, peptone 2 g, dextrose 15 g, NaCl 12 g, $MgCl_2 \cdot 6H_2O$ 1.1 g, KCl 0.1 g, and distilled water 1000 ml. On the tenth day, the fermentation broth, including cells, was harvested and then centrifuged to separate mycelial mass from aqueous layer. The mycelial mass was exhaustively extracted with acetone to obtain a crude extract (30 g). The extract was subjected to gradient elution in petroleum ether/acetone (100:1 to 1:1) on a silica gel column to give a series of fractions. Fractions 4 and 5 were chromatographed over a Sephadex LH-20 column (Pharmadex, $CHCl_3$/MeOH 1:1) and further purified on reversed-phase silica gel (Chromatorex C_{18}, MeOH/H_2O 7:3), to give compounds **1** (70 mg) and **2** (50 mg), respectively.

3.3.1 Acid hydrolysis of compound 1. Compound **1** (60 mg) was hydrolysed by heating the sample in a seal vial at 120°C for 22 h in 6 N HCl. The hydrolysate was dried under vacuum to obtain a residue. It was subjected to a Sephadex LH-20 column (Pharmadex, $CHCl_3$/MeOH 1:1) to obtain Ala (5 mg).

3.3.2 Compound 1. White amorphous powder (MeOH); $[\alpha]_D^{20}$ −150 (*c* 0.1, CH_3OH); UV (MeOH) λ_{max} nm 315, 225, 203, IR (KBr) ν_{max} (cm^{-1}) 3310, 2921, 1688, 1606, 1521, 1442, 1251; ^1H NMR and ^{13}C NMR data: see table 1; EI-MS *m/z*: 368 $[M]^+$, 248, 232, 217, 133, 81, 69 (base), 41; HRFAB-MS *m/z*: 368.4689 (calcd for $C_{22}H_{28}N_2O_3$, 368.4693).

3.3.3 Compound 2. White amorphous powder (MeOH); $[\alpha]_D^{20}$ −78 (*c* 0.5, CH_3OH); UV (MeOH) λ_{max} nm 319, 230, 202; IR (KBr) ν_{max} (cm^{-1}) 3319, 2922, 1670, 1641, 1510, 1466, 1386, 1253; ^1H NMR and ^{13}C NMR data: see table 1; EI-MS *m/z*: 368 $[M]^+$, 248, 217, 133, 81, 69 (base), 41; HRFAB-MS *m/z*: 368.4685 (calcd for $C_{22}H_{28}N_2O_3$, 368.4693).

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