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Janthinolide A-B, two new 2,5-piperazinedione derivatives from the endophytic *Penicillium janthinellum* isolated from the soft coral *Dendronephthya* sp.

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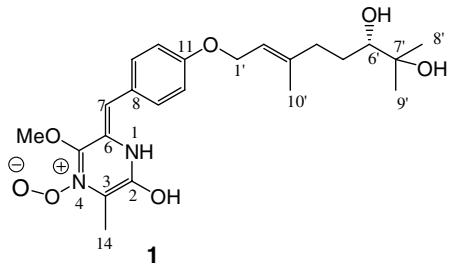
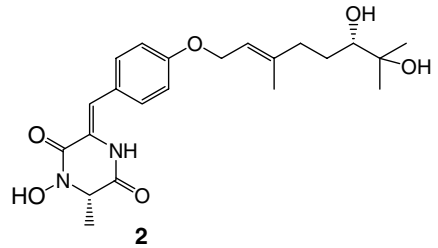
Two new 2,5-piperazinedione derivatives, janthinolide A and B (**1**–**2**), along with deoxymycelianamide, griseofulvin and dechlorogriseofulvin were isolated from the fermentation broths of the endophytic fungus *Penicillium janthinellum* isolated from a soft coral, *Dendronephthya* sp. Their structures were established by extensive spectroscopic data analysis.

1. Introduction

The fungus *Penicillium janthinellum* was reported to be associated with ryegrass staggers, a neuromuscular disease in New Zealand affecting cattle and sheep grazing in pastures dominated by perennial ryegrass (Jesus et al. 1984). Previous chemical investigation on this fungus resulted in the isolation of a member of tremorgenic mycotoxins, janthitrems A–G, belonging to the indole-diterpenoid family (Penn et al. 1993; Wilkins et al. 1992; Gallagher et al. 1980), along with the antimicrobial principals curvulic acid (Nakakita et al. 1984a) and phenolic compounds (Nakakita et al. 1984b). In our continuous investigation on chemical diversity from marine endophytic microorganisms, the fungus *P. janthinellum* was isolated from *Dendronephthya* sp., a soft coral growing off the coral reef in Hainan Island. Previous chemical investigation of this soft coral by our group resulted in the isolation and characterization of a group of unique A-nor steroids (Li et al. 2005). The primary bioassay of the EtOAc extract from the fermentation broth showed moderate inhibition against *Alternaria solani*. A repeated column chromatography on the EtOAc extract afforded three 2,5-piperazinedione derivatives (**1**–**3**) together with griseofulvin and dechlorogriseofulvin (Jarvis et al. 1996). This paper reports the structure elucidation of the two new compounds **1** and **2**.



1



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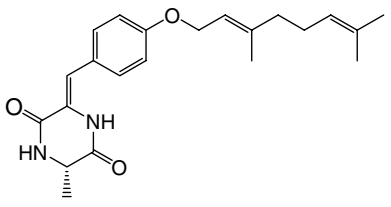


3

2. Investigations, results and discussion

Compound **3** was found to be identical to deoxymycelianamide by spectroscopic data analysis and by comparison of its chemical and physic property with those reported in the literature (Gallina et al. 1968, 1966). This is the first time to report the isolation of **3** from nature and to fully assign its ^{1}H and ^{13}C NMR data (Table).

The molecular formula of janthinolide A (**1**) was determined to be $C_{23}H_{32}N_2O_7$ on the basis of HRFABMS (m/z 449.2281, calcd 449.2282), indicating 9 degrees of unsaturation. The UV bands at 227, 312 nm and IR absorptions at 3376, 1713, 1677, 1602, 1511, and 1465 cm^{-1} suggested the presence of hydroxyl, amide or enol, and aromatic groups. The NMR data of **1** (Table) were characteristic of a mycelianamide-type, similar with those of deoxymycelianamide (**3**). The ^{1}H NMR displayed a vinyl singlet at δ_H 7.34 (s, H-7) and two aromatic doublets at δ_H 6.89 (2 H, d, J = 8.5 Hz) and 7.46 (2 H, d, J = 8.5 Hz) representing four protons on the 1,4-disubstituted benzene ring, which were attributable to the benzylidene unit. A

Table 1: ^{1}H and ^{13}C NMR data of compounds 1 to 3

No.	**1**[b]		**2**[a]		**3**[b]	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	161.7		166.7		168.0	
3	152.0		60.3	4.32 1 H, q, 7.0	50.7	4.12 1 H, q, 7.0
5	166.0		157.6		161.1	
6	122.2		123.7		125.7	
7	132.1	7.34 1 H, s	115.5	6.75 1 H, s	114.8	6.65 1 H, s
8	126.1		126.0		126.1	
9	131.5	7.46 1 H, d, 8.5	131.2	7.46 1 H, d, 8.0	131.2	7.45 1 H, d, 8.5
10	115.1	6.89 1 H, d, 8.5	115.3	6.97 1 H, d, 8.0	115.3	6.96 1 H, d, 8.5
11	159.6		158.8		158.7	
12	115.1	6.89 1 H, d, 8.5	115.3	6.97 1 H, d, 8.0	115.3	6.96 1 H, d, 8.5
13	131.5	7.46 1 H, d, 8.5	131.2	7.46 1 H, d, 8.0	131.2	7.45 1 H, d, 8.5
14	9.1	2.13 3 H, s	17.4	1.46 3 H, d, 7.0	19.7	1.34 3 H, d, 7.0
1′	64.8	4.64 2 H, d, 6.0	65.0	4.58 2 H, d, 6.0	64.9	4.58 2 H, d, 6.0
2′	120.2	5.50 1 H, t, 6.0	119.5	5.44 1 H, t, 6.0	120.0	5.43 1 H, t, 6.0
3′	141.0		141.8		140.9	
4′	36.2	2.13 1 H, m[c] 2.33 1 H, m[c]	36.8	1.98 1 H, m[c] 2.25 1 H, m[c]	39.4	2.05 2 H, m[c]
5′	29.2	1.48 1 H, m[c] 1.65 1 H, m[c]	29.5	1.23 1 H, m[c] 1.65 1 H, m[c]	26.3	2.08 2 H, m[c]
6′	77.8	3.32 1 H, brd, 9.0	77.4	3.06 1 H, brd, 10	124.2	5.08 1 H, m[c]
7′	73.3		72.0		131.5	
8′	23.1	1.18 3 H, s	24.9	0.99 3 H, s	18.0	1.58 3 H, s
9′	26.3	1.19 3 H, s	29.9	1.06 3 H, s	25.9	1.64 3 H, s
10′	16.8	1.78 3 H, s	17.0	1.72 3 H, s	16.8	1.72 3 H, s
NH-1		8.29 1 H, s		9.99 1 H, s		9.80 1 H, s
NH-4						8.37 1 H, s
N−OH				10.24 1 H, s		
2-OH		8.77 1 H, s				
5-OCH_3	52.6	3.86 3 H, s				

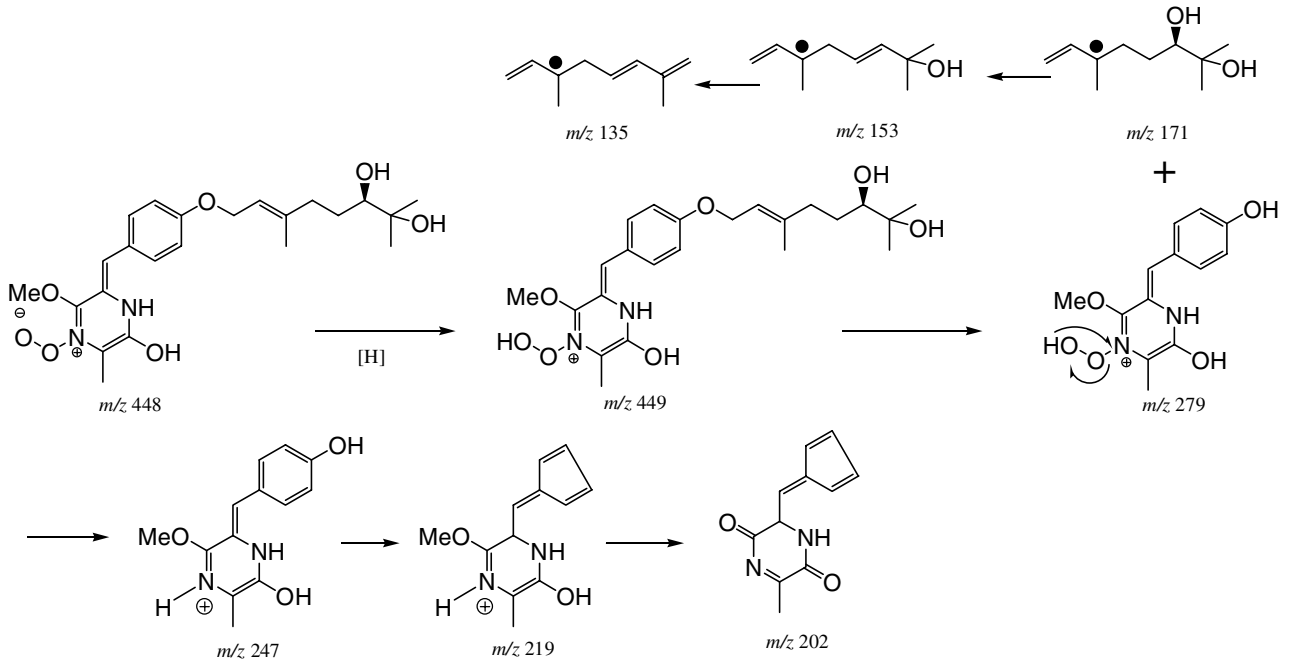
[a] Measured in DMSO; [b] Measured in $CDCl_3$; [c] Overlapping signals

vinyl triplet at δ_H 5.50 (t, J = 6.0 Hz) and its coupling oxymethylene protons at δ_H 4.64 (2 H, d, J = 6.0 Hz) were in consistent with H-2′ and H-1′ of geranyloxy side chain. Of the three methyl resonances in side chain, a vinyl methyl signal at δ_H 1.78 (3 H, s, H-10′) was positioned at C-3′ on the basis of HMBC correlation between H-10′ and the vinyl carbons at δ_C 120.2 (d, C-2′) and 141.0 (s, C-3′), while the signals at δ_H 1.18 (3 H, s, H-8′) and 1.19 (3 H, s, H-9′) indicated the two methyl groups in a saturated environment. The HMBC correlations from H-8′ and H-9′ to two hydroxylated carbons at δ_C 77.8 (d, C-6′) and 73.3 (s, C-7′) and to the respective carbon supposed the presence of a dihydroxy geranyloxy moiety. The remaining NMR data consisted of a methoxyl group [δ_H 3.86 (3 H, s), δ_C 52.6 (q)], an vinyl methyl group [δ_H 2.13 (3 H, s), δ_C 9.1 (q)], and four quaternary carbons at δ_C 122.2 (s), 152.0 (s), 161.7 (s), and 166.0 (s), which distinguished to those of piperazine-2,5-dione while comparison of the NMR data with those of **3**. The HMBC correlations observed from the methoxyl protons and H-7 to δ_C 166.0 (s, C-5) and from the methyl protons at δ_H 2.13 (3 H, s) to carbons at δ_C 152.0 (s, C-3) and 161.7 (s, C-2) suggested the presence of a pyrazine-like core, in which a methoxyl group was substituted at C-5. Furthermore, a D_2O exchangeable proton at δ_H 8.77 (s) showing HMBC correlation with C-2 and C-3 was due to a hydroxyl group to be substituted at C-2. The other D_2O exchangeable proton at δ_H 8.29 (s) was assigned to NH-1 on the basis of its long range COSY correlation with H-7 and HMBC correlations with C-2, C-3, C-5, C-6, and C-7. Thus, the remaining two oxygen atoms accounted from the molecular composition and from its degrees of unsaturation suggested a peroxide group to be located at N-4, the sole position allowed for substitution. The gross structure was also supported by ESIMS fragmentation as shown in the Scheme.

The stereochemistry of **1** was mainly determined on the basis of NOESY spectrum and optical rotation. The presence of NOE correlations between NH-1/H-9 (or H-13), H_2-1′ (δ_H 4.64, d, J = 6.0 Hz)/H_3-10′, and H-7/OMe, and absence of NOE correlation between H-7 (δ_H 7.34, s)/NH-1 were assignable to 6*Z* and 2′*E*. The configuration of C-6′ was supposed to be *S* due to the close identical NMR data of the dihydroxygeranyoxyl system in **1** with those of 6′*S*,7′-dihydroxybergamottins (Ohta et al. 2002). The optical rotation of **1** ($[\alpha]_D$ −6.2°) in contrast to that of 6′*R*-7′-hihydroxybergamottin ($[\alpha]_D$ +12.7°) (Dreyer et al. 1973; Ohta et al. 2002) also supported the stereochemistry assignment.

Janthinolide B (**2**) was isolated as a pale red oil, and its molecular formula was determined to be $C_{22}H_{30}N_2O_6$ by HRFABMS (m/z 419.2175, calcd. 419.2176) and NMR data. The UV bands (228 and 318 nm) and the IR absorptions (3422, 1688, 1630, 1605, and 1512 cm^{-1}) showed similarity with those of **1**. Comparison of the ^{1}H and ^{13}C NMR data of **2** (Table) with those of **1** revealed that **2** shared the same substructure of a 11-O-(6′,7′-dihydroxygeranyl)benzylidene. The ^{13}C NMR resonances at δ_C 166.7 (s, C-2), 60.3 (d, C-3), 157.6 (s, C-5), 123.7 (s, C-6), and 17.4 (q) were attributable to a 3-methyl-2,5-dioxopiperazine unit, which was supported by the HMBC correlations between the methyl proton at δ_H 1.46 (d, J = 7.0 Hz, H_3-14) to C-2 and C-3, between the vinyl proton at δ_H 6.75 (s, H-7) and C-5, and between a D_2O exchangeable proton at δ_H 9.99 (s, NH-1) and C-5 and C-6. Thus, the remaining OH unit accounted from molecular

Scheme: Proposed ESIMS/MS fragmentation pathway of **1**



formula was supposed to link at N-4, which induced the chemical shift of C-3 to downfield at δ_C 60.3 (d) in contrast with δ_C 50.7 (d, C-3) of **3**. The weak HMBC correlation from a hydroxyl proton at δ_H 10.24 (s) to C-3 and C-5 further confirmed the OH substitution. The geometries of the double bonds at C-6 and C-2′ of **2** were determined to be the same as those of **1** due to the similar NOE and well comparable NMR data of both compounds. The configuration of C-3 was assumed to be *S* through its optical rotation ($[\alpha]_D$ −92.5°) which showed the same sign as that of (3*S*)-mycelianamide ($[\alpha]_D$ −217°) (Brich et al. 1956).

The anti-fungi test of **2** and griseofulvin revealed that griseofulvin present the minimum inhibitory concentration (MIC) at 2.75 and 20 μg/ml against *Alternaria solani* and *Pyricularia oryzae* respectively, while **2** showed no activity.

3. Experimental

3.1. General

Optical rotations were measured on a Perkin-Elmer 243B polarimeter. IR spectra were recorded on a Thermo Nicolet Nexus 470 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance-500 FT NMR spectrometer (500 MHz for ^{1}H, 125 MHz for ^{13}C) using TMS as an internal standard. EIMS were performed with a Bruker APEX II mass spectrometer, and HRFABMS were measured on GCT-MS mass spectrometer, and EISMS were performed on MDS-SCIEX-QSTAR mass spectrometer. Column chromatography was carried with silica gel (200–300 mesh) and H silica gel, and GF_{254} silica gel for TLC was obtained from Qingdao Marine Chemistry Co. Ltd., Qingdao, People's Republic of China, Sephadex LH-20 (18–110 μm) was obtained from Pharmacia Co. ODS (chromatorex, 100–200 mesh) was from Fuji Silysia Co.

3.2. Material

The strain *Penicillium janthinellum* was isolated from a soft coral *Dendronephthya* sp. collected in South China Sea, China in 2001. The strain was identified by Professor Li Tian of the Qingdao Institute of Marine Bioactive Substances, and deposited at the State Key laboratory of Natural and Biomimetic Drugs, Peking University. The strain was cultured in a seawater-based medium (40L) (100 × 1 L Fermbach flasks) containing an extract of potato in seawater, peptone, yeast, glucose, 1.5% NaCl, 0.13% $MgCl_2 6H_2O$, 0.01% KCl, 0.001% $FePO_4$ and the pH of the medium was adjusted to 6.5. The flasks were cultured on a rotary shaker (150 rpm) for 12 days at 20 °C.

3.3. Extraction and isolation

The fermentation broth (40 L) of *Penicillium janthinellum* was separated by centrifugation, and the mycelium was extracted three times with MeOH. The combined MeOH extracts were concentrated in vacuo to afford a brown residue (56.0 g). The residue was partitioned in water against EtOAc, and *n*-BuOH, respectively. The EtOAc extract (4.0 g) was subjected to a silica gel column with petroleum ether-acetone (2 : 1) as eluent to afford 10 portions (P1 to P10). P2 (120 mg) was chromatographed on a silica gel column with petroleum ether-EtOAc (1 : 1) as an eluent to obtain griseofulvin (80.0 mg) and dechlorogriseofulvin (2.0 mg). P4 and P5 showing similar spots on TLC under UV (254 nm) absorption were combined, and this portion (50 mg) was subjected to a Saphadex LH-20 column eluting with MeOH to afford compounds **1** (2.0 mg), and **3** (3.0 mg), which were further purified on silica gel column eluting with petroleum ether-EtOAc (2 : 1). P7 (15 mg) was purified on a ODS (C_{18}) column eluting with MeOH-H_2O (70 : 30) to yield **2** (5.0 mg).

3.3.1. Janthinolide A (**1**)

Corlorless oil; $[\alpha]_D^{25}$ −6.2° (c 0.2, $CHCl_3$); UV λ_{max} (MeOH, log ε) nm 227 (3.76), 312 (3.85); IR (KBr) ν_{max} cm^{-1} 3376, 3061, 1713, 1677, 1602, 1511, 1465, 1511, 1256, 1175, 1023; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS m/z 449 $[M + 1]^+$ (22), 431 (11), 413 (8), 411 (3), 399 (4), 381 (6), 370 (3), 351 (6), 279 (14), 247 (35), 233 (6), 219 (6), 171 (11), 153 (44), 135 (13); HRFAB m/z 449.2282 (calcd for $C_{23}H_{33}N_2O_7$ 449.2281).

3.3.2. Janthinolide B (**2**)

Pale red oil; $[\alpha]_D^{25}$ −92.5° (c 0.3, MeOH); UV λ_{max} (MeOH, log ε) nm 228 (3.96), 318 (4.11); IR (KBr) ν_{max} cm^{-1} 3422, 1688, 1630, 1605, 1512, 1443, 1386, 1301, 1249, 1177, 1025; ^{1}H and ^{13}C NMR data, see Table 1; HRFABMS m/z 419.2176 $[M + 1]^+$ (calcd for $C_{22}H_{31}N_2O_6$ 419.2175).

3.4. Bioassay

The bioassay of **2** and griseofulvin against *Alternaria solani* and *Pyricularia oryzae* was performed as described in the literature (Lee et al. 2004).

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