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Griseusin C, a Novel Quinone Derivative from a Marine-Derived Fungus *Penicillium* sp.

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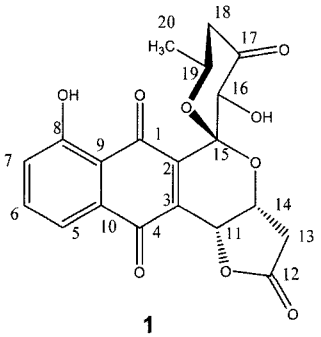
A novel quinone derivative, Griseusin **C** (**1**), along with a known quinone, Naphthoquinone **C** (**2**), was isolated from the lyophilized culture broth of the marine-derived fungus *Penicillium sp.* The structures were elucidated on the basis of extensive 1D- and 2D-NMR, as well as HRESI-MS, spectroscopic analysis. The relative stereochemistries of the compounds were assessed by NOESY analysis.

Key words: Quinone; Griseusin **C**; Naphthoquinone **C**; Marine-derived fungus; *Penicillium* sp.

INTRODUCTION

Mangrove endophytes, including actinomycetes and fungi, have been recognized as rich sources of structurally unique and biologically active secondary metabolites[1-3]. During the course of our screening for antimicrobial and cytotoxic constituents from marine-derived fungi[4], a novel quinone derivative, namely griseusin ***C*** (**1**), was isolated from the culture broth of marine-derived fungus *Penicillium* sp. along with a known quinone naphthoquinone ***C*** (**2**). Their structures were elucidated on the basis of HRESI-MS, ^{1}H- and ^{13}C-NMR together with 2D-NMR spectroscopic analysis. The relative stereochemistries of **1** and **2** were assessed mainly by NOESY analysis. Previous papers (Naoki *et al.*, 1976, Masayuki *et al.*, 1995) have reported the isolation and characterization of griseusin ***A***, ***B*** and their derivatives from actinomycete strains; these compounds exhibited broad, strong antimicrobial activities against Gram-positive bacteria, including MRSA, and some Gram-negative bacteria[5-7]. In this paper, we report the isolation and structure elucidation of griseusin ***C*** from fungus *Penicillium* sp. for the first time.



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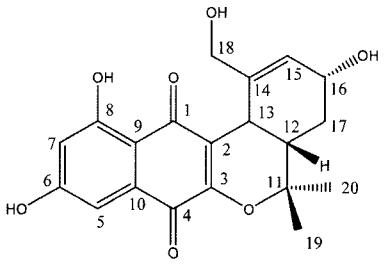

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MATERIALS AND METHODS

General experiments

^{1}H- and ^{13}C-NMR spectra were measured on a Bruker Avance DRX 500 NMR spectrometer using TMS as an internal standard. Chemical shifts (δ) are expressed in parts per million (ppm), and coupling constants (J) are reported in Hertz (*Hz*). ESI-MS spectra were recorded on

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a triple quadrupole mass spectrometer Quattro (VG Biotech, Altrincham, England) and HRESI-MS spectra were obtained on a Bruker FT-ICRMS spectrometer. Column chromatography was carried out with Silica gel 60M (200-300 mesh), Lichrospher RP-8 (20 μm) and Sephadex LH-20 (Pharmacia); TLC was performed with silica gel plates (Macherey-Nagel, SilG/UV254, 20x20, 0.20 mm); spots were detected by UV_{254} and anisaldehyde/H_2SO_4 (10%); Fermentation was carried out in a 300-L fermenter (Braun Diessel).

Fungal strain

The fungal strain (GT20026105) was collected from mangrove plants, *Kandelia Candel*, collected from Hainan Island, South China, in October 2002, and the mangrove was identified by Prof. P. Lin from Xiamen University. The mangrove was deposited in State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing, P. R. China. The fungal strain was stored in the Hans-Knöll-Institute for Natural Products Research, Jena, Germany. The strain was cultured at 21°C in a 300-L fermenter with pH controlled at 5.5 for 7 days.

Extraction and isolation

The lyophilized culture broth was extracted with MeOH at room temperature. The extract was concentrated to give the dark brown oily residue (27 g), which was chromatographed on the Lichrospher RP-8 column and eluted with H_2O, aqueous MeOH (30%, 60%, 90%) and MeOH to yield five fractions. The 60% MeOH fraction was chromatographed on a Silica gel column ($CHCl_3$: MeOH=9:1) to produce five fractions. Fr.3-2 was further separated by Sephadex LH-20 chromatography (90% MeOH) to afford compound **1** (18 mg, yield: 0.66×10^{-3}); Fr.3-3 was chromatographed on RP-8 (75% aqueous MeOH) to yield compound **2** (6.2 mg, yield: 0.23×10^{-3}).

Griseusin C (1)

Compound **1** was obtained as an orange powder; ESI-MS[+]: *m/z*=823.70[2M+Na]$^+$, 400.80 [M+H]$^+$; 418.65 [M +NH_4]$^+$; HRESI-MS[+]: *m/z*=423.0698 [M+Na]$^+$; $[\alpha]_D^{25}$-132.1 (*c* 0.950, MeOH); ^{1}H- and ^{13}C-NMR data: see Table I.

Naphthoquinone C (2)

Compound **2** was obtained as an orange powder; ESI-MS[+]: *m/z*=395.9[M+Na]$^+$, 767.6[2M+Na]$^+$; ESI-MS[-]: *m/z*=371.6[M-H]$^-$; 743.3[2M-H]$^-$; ^{1}H- and ^{13}C-NMR data: see Table I.

Bioactivity results

Compounds **1** and **2** were tested in bioassays with Xanthin-Oxidase (XOD), 3α-Hydroxysteroid dehydrogenase (3α-HSD) and Horseradish peroxidase (HRP). Compound **1** was effective on 3α-HSD at concentrations under 290 μM, and compound **2** was effective on HRP at concentrations under 250 μM. Compound **1** had no effect on XOD or HRP at concentrations under 250 μM, and compound **2** had no effect on XOD and 3α-HSD at concentrations under 250 μM and 290 μM, respectively.

RESULTS AND DISCUSSION

Structure elucidation of 1

Compound **1** was isolated as an amorphous orange powder that gave a [M+Na]$^+$ ion at *m/z* 423.0698 in the HRESI-MS, which corresponds to a molecular formula of $C_{20}H_{16}O_9$ (Calcd 423.0692), requiring 13 degrees of unsaturation. The ^{1}H-NMR spectrum (Table I) indicated the presence of one methyl signal at δ1.36 (d, *J*=6.0), three aromatic protons at δ7.77 (t, *J*= 7.5), 7.60 (dd, *J*=7.5,1.3), 7.37 (dd, *J*=7.5,1.3), four oxymethine protons at δ5.50 (s), 3.14 (d, *J*= 5.1), 4.7 ,4.27 (m), two methylene protons at δ2.59-2.69 (m). The ^{13}C-NMR spectrum (Table I), together with the HMBC data of **1**, showed signals for 20 carbons with the multiplicities of the carbon signals determined

Table I. ^{1}H- and ^{13}C-NMR data of Griseusin **C** and Naphthoquinone **C** (in CD_3OD)

	Griseusin C		Naphthoquinone C	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1	-	182.6(s)	-	183.1(s)
2	-	140.7(s)	-	122.8(s)
3	-	132.3(s)	-	155.0(s)
4	-	186.4(s)	-	184.3(s)
5	7.37 (dd, *J*=7.5,1.3)	125.1(d)	6.39 (d, *J*=1.3)	107.1(d)
6	7.77 (t, *J*=7.5)	137.2(d)	-	167.9(s)
7	7.60 (dd, *J*=7.5,1.3)	119.0(d)	6.94 (d, *J*=1.3)	110.3(d)
8	-	162.1(s)	-	165.8(s)
9	-	132.5(s)	-	108.3(s)
10	-	115.8(s)	-	136.1(s)
11	3.14 (d, *J*=5.1)	69.0(d)	-	81.7(s)
12	-	175.8(s)	2.22(m)	35.9(d)
13	2.69(m)	35.7(t)	3.57(m)	32.5(d)
14	4.7(Ol) *	67.2(d)	-	140.9(s)
15	-	99.5(s)	6.56 (d, *J*=5.4)	126.3(d)
16	5.50(s)	76.2(d)	4.20(m)	63.8(d)
17a	-	204.1(s)	2.07(m)	30.4(t)
17b	-	-	1.40(m)	-
18	2.57(m)	48.2(t)	4.13(d)	64.7(t)
19	4.27(m)	69.2(d)	1.39(s)	25.1(q)
20	1.36 (d, *J*=6.0)	20.5(q)	1.55(s)	25.8(q)

*Overlap under H_2O signal.

from DEPT spectrum as: ten quaternary, seven methine, two methylene and one methyl carbon atoms.

The ^{1}H-^{1}H COSY spectrum of 1 (Fig. 2) revealed the following correlations: H5-H6-H7; H11-H14-H13; H18-H19-H20. The substructure of unit A (Fig. 1) was established by the following HMBC correlations: H6-C8, H6-C10, H7-C9, H7-C5, H5-C4 and H5-C7. The partial structure of unit B (Fig. 1) was indicated by the following HMBC correlations: H11-C12, H11-C13, H14-C12, H11-C2, H14-C15, H16-C15, H19-C15, H19-C17, H16-C18, H20-C18 and H18-C16. There are no indications of HMBC correlations between unit A and B. After carefully studying the molecular formula of $C_{20}H_{16}O_9$, as revealed by HRESI-MS, we established the planar structure of **1**.

The relative configuration was established from the following NOE correlations (Fig. 3): H16-H11, H20-H18 and H13-H14. The optical rotation values were then compared with griseusin **A** and **B**: Compound **1**, $[\alpha]_D^{25}$-132.1 (*c* 0.950, MeOH), griseusin **A**, $[\alpha]_D^{23}$-147.8(*c* 0.997, $CHCl_3$), griseusin **B**, $[\alpha]_D^{23}$-190.2(*c* 0.5,DMF). According to the results above, the structure of **1** was elucidated as 17-one-deacetyl-griseusin **A**, which we named griseusin **C**.

Structure elucidation of 2

Compound **2** was obtained as an amorphous orange powder; ESI-MS[+]: *m/z*=395.9[M+ Na]$^+$, 767.6[2M+ Na]$^+$. The ^{13}C-NMR spectrum (Table I) of **2** showed signals for 20 carbons with the multiplicities of the carbon signals determined from DEPT spectrum as: ten quaternary carbon atoms at δ183.1(s), 184.3(s), 167.9(s), 165.8(s), 155.0(s), 140.9(s), 136.1(s), 122.8(s), 108.3(s) and 81.7(s); six methine carbon atoms at δ126.3(d), 110.3(d), 107.1(d), 63.8(d), 35.9(d) and 32.5(d); two methylene carbon atoms at δ64.7(t) and 30.4(t); and two methyl carbon atoms at δ25.8(q) and 25.1(q).

The ^{1}H-^{1}H COSY spectrum of **2** (Fig. 4) showed the following correlations: H6-H8 and H13-H12-H17-H16-H15. The HMBC correlations (Fig. 5) were: H5-C9, H5-C7, H7-C9 and H7-C5. Together with the coupling constant, $H_{5\text{-}7}$ =1.3, these correlations led us to first establish a benzo-group. HMBC correlations H5-C4 and H7-C1 then allowed us to establish the quinone skeleton. The left partial structure was indicated by the following HMBC correlations: H19, H20-C11, H12-C14, H13-C15, H15-C18, H15- C17, H16-C14, H17-C13, H12-C2, H13-C3, H13-C11 and H18-C13.

The relative configuration was established from the following NOE correlations (Fig. 5): H13-H18, H20-H17, H13-H12 and H19-H12. After comparing these data with the literature[6], the structure of ***2*** was elucidated as naphthoquinone ***C***.

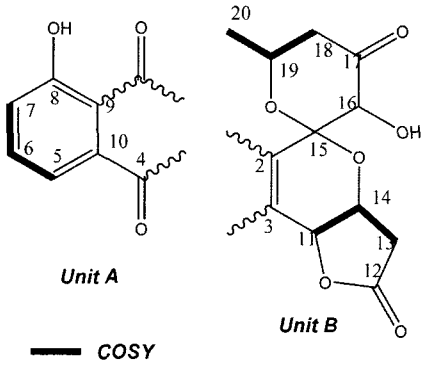

Fig. 1. Partial structure of 1

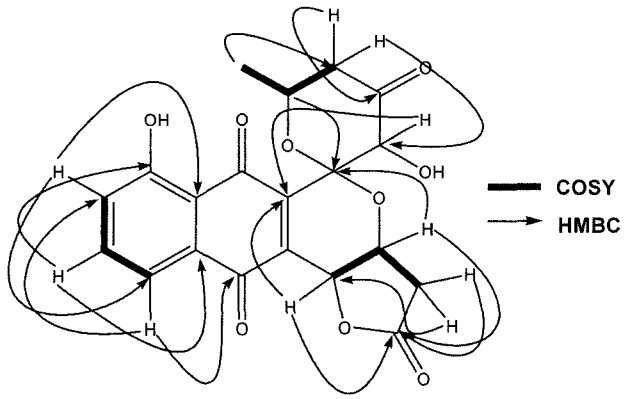

Fig. 2. Main COSY and HMBC correlations of 1

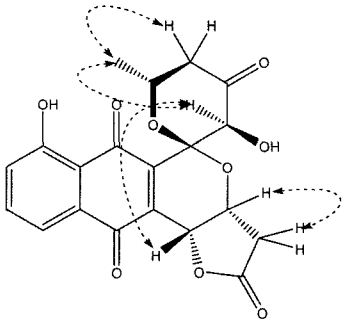

Fig. 3. Key NOESY correlations of 1

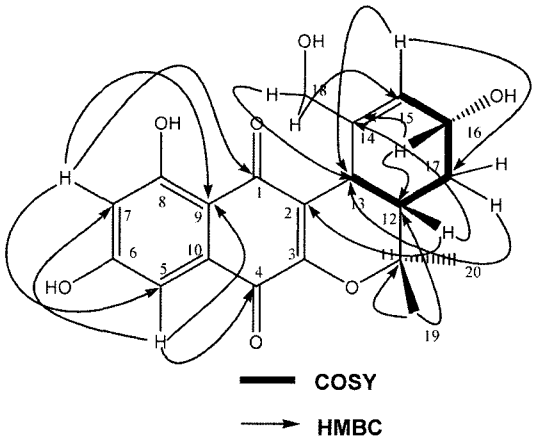

Fig. 4. Main COSY and HMBC correlations of 2

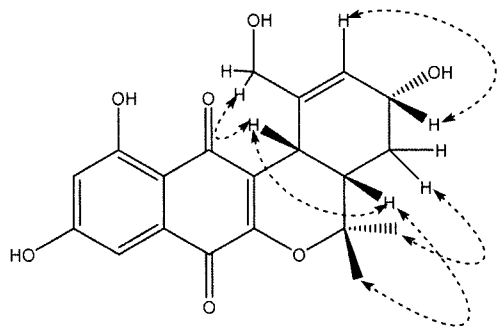

Fig. 5. Key NOESY correlations of **2**

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