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A NEW GRISEOFULVIN DERIVATIVE FROM THE MANGROVE ENDOPHYTIC FUNGUS *Sporothrix* SP.

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*A new griseofulvin derivative, 7-chloro-2′,5,6-trimethoxy-6′-methylspiro(benzofuran-2(3H),1′-(2) cyclohexene)-3,4′-dione (**1**), together with the known 2-acetyl-7-methoxybenzofuran (**2**), was isolated from the marine-derived mangrove endophytic fungus* Sporothrix *sp. (No. 4335). The structure of new compound **1** was elucidated by analysis of spectroscopic data.*

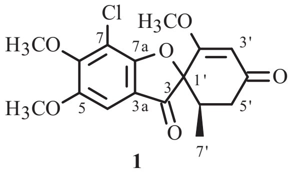
Keywords: mangrove, endophytic fungus, griseofulvin, metabolites.

Marine microorganisms have proved to be rich sources of bioactive secondary metabolites, and numerous compounds with potent biological activities and unique chemical structures have been isolated [1, 2].

In previous investigations, we have reported many natural products with potent biological activities and unique chemical structures from marine mangrove fungi from the South China Sea [3–6]. The strain (No. 4335) was collected from the bark of an estuarine mangrove from the South China Sea, whose species has been identified as *Sporothrix* sp. A new griseofulvin derivative, 7-chloro-2′,5,6-trimethoxy-6′-methylspiro(benzofuran-2(3H),1′-(2) cyclohexene)-3,4′-dione (**1**), together with the known 2-acetyl-7-methoxybenzofuran (**2**), was isolated from the fermentation broth of the fungus. Their structures were elucidated by spectroscopic methods.

Compound **1** was obtained as a colorless, amorphous powder and determined to have the molecular formula $C_{17}H_{17}ClO_6$ by HR-EI-MS data (*m/z* 352.0708).

The IR spectrum of **1** showed the presence of the carboxyl group with a band at 1707 cm^{-1}. The ^{13}C NMR and DEPT spectra displayed 17 carbon signals, including two carbonyl signals (δ 197.0, 192.5), eight olefinic carbon signals, three carbon-bearing oxygen signals [δ 57.0, 56.6, 56.4 (OCH$_3$)], and four saturated aliphatic carbon signals [δ 90.8 (C), 40.0 (CH$_2$), 36.4 (CH), 14.2 (CH$_3$)]. These data, along with the number of unsaturations, required the presence of three rings, one being aromatic. In the ^{1}H NMR spectrum, there were two singlet olefinic protons (δ 6.13, 5.54), three singlet methoxyls (δ 4.03, 3.98, 3.62), one doublet methyl (δ 0.97), one methylene (δ 3.03), and one methine (δ 2.86).



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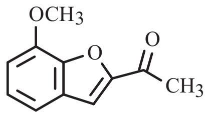

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TABLE 1. NMR Data of **1** ($CDCl_3$, δ, ppm, J/Hz)

C atom	δ_C (DEPT)	δ_H	HMBC	C atom	δ_C (DEPT)	δ_H	COSY	HMBC
3	192.5		H-4, 6′	3′	104.9 (CH)	5.54 (s)		H-5′
3a	105.2		H-4	4′	197.0			H-3′, 5′
4	89.5 (CH)	6.13 (s)		5′	40.0 (CH_2)	3.03 (dd J = 12.3, 3.0)		H-3′, 6′, 7′
5	164.6		H-4, 5-OMe			2.45 (dd, J = 12.3, 2.4)		
6	157.7		H-4, 6-OMe	6′	36.4 (CH)	2.86 (qdd, J = 5.1, 3.0, 2.4)	H-5′, 7′	H-3, 1′, 5′, 7′
7	97.1			7′	14.2 (CH_3)	0.97 (d, J = 5.1)		H-5′
7a	169.5			5-OMe	57.0 (CH_3)	4.03 (s)		
1′	90.8		H-3′, 5′, 6′, 7′	6-OMe	56.4 (CH_3)	3.98 (s)		
2′	170.8		H-3′, 2′-OMe	2′-OMe	56.6 (CH_3)	3.62 (s)		

Interpretation of the HMQC and HMBC data of **1** revealed the substitution pattern and allowed us to assign all the ^1H and ^{13}C NMR signals. The key correlations between H-4 and C-3, 5, H-6′ and C-3, 1′, 5′, and H-3′ and C-1′, 2′, 4′, 5′ established the overall structure of **1** (Table 1). The configuration at C (6′) was not dertermined. However, it can be assumed to be (6′*R*), since all compounds containing such a residue reported to date in griseofulvin derivatives possess this configuration [7, 8].

EXPERIMENTAL

General Procedures. The ^1H and ^{13}C NMR data were recorded on a Varian Unity INOVA-500NB NMR spectrometer (500 MHz for ^1H, 125 MHz for ^{13}C) with Me_4Si as the internal standard; EI-MS spectrum was obtained on a VG-ZABHS mass spectrometer, and HR-EI-MS spectrum was obtained on a VG Autospec-500 mass spectrometer; optical rotation was measured on a Horiba SEPA-300 polarimeter; IR spectrum was measured on a Bruker VECTOR 22 spectrophotometer; UV spectrum was measured on a Shimadzu UV-2501PC spectrophotometer; melting point was determined on an X-4 micromelting point apparatus.

Fungus Material and Culture Conditions. A strain of the fungus (No. 4335) was isolated from the South China Sea coast. Starter cultures were maintained on cornmeal seawater agar, and its general species was identified as *Sporothrix* sp. Plugs of agar supporting mycelial growth were cut and transferred aseptically to a 250 mL Erlenmeyer flask containing 100 mL of liquid medium (glucose 10 g/L, peptone 2 g/L, yeast extract 1 g/L, NaCl 2 g/L). The flask was incubated at 30°C on a rotary shaker for 5–7 days. The mycelium was aseptically transferred to 500 mL Erlenmeyer flasks containing culture liquid (200 mL). The flasks were then incubated at 30°C for 25 days.

Extraction and Separation of Metabolites. The 200 L cultures were filtered through cheesecloth. The filtrate was concentrated to 3.5 L below 50°C and extracted five times by shaking with equal volumes of ethyl acetate. The combined extracts were chromatographed repeatedly on silica gel using gradient elution from petroleum ether to ethyl acetate to give compounds **1** (23 mg) and **2** (10 mg) from 30 and 20% ethyl acetate–petroleum, respectively. The structure of **1** was determined by a combination of IR, HR-EI-MS, UV, and extensive 1D and 2D NMR analysis.

7-Chloro-2′,5,6-trimethoxy-6′-methylspiro(benzofuran-2(3H),1′-(2)cyclohexene)-3,4′-dione (1): colorless, amorphous powder, mp 205–206°C, $[\alpha]_D^{20}$ +4° (*c* 0.08, MeOH). UV spectrum (CH_3OH, λ_{max}, nm) (log ε): 291 (2.75). IR spectrum (KBr, ν, cm^{-1}): 1701 (C=O), 1616, 1408, 1221, 1099, 887. Mass spectrum (EI^+, *m/z*, I_{rel}, %): 352 (100) $[M]^+$, 321 (64), 214 (49), 138 (87), 69 (45). Mass spectrum (HR-EI^+, *m/z*, I_{rel}, %): 352.0708 $[M]^+$, (calcd 352.0709). ^1H, ^{13}C NMR, see Table 1.

2-Acetyl-7-methoxybenzofuran (2): yellow amorphous solid. LC-MS: 190 $[M]^+$. ^1H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.51 (1H, dd, J = 10.8, 11.2), 6.97 (1H, d, J = 11.2), 6.77 (1H, d, J = 10.8), 6.08 (1H, s), 3.97 (3H, s), 2.32 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 178.0 (C), 163.5 (C), 159.6 (C), 158.4 (C), 133.3 (C), 112.0 (CH), 109.9 (CH), 109.7 (CH), 106.2 (CH), 56.5 (CH_3), 20.8 (CH_3).

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