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TWO NEW DIHYDROISOCOUMARINS FROM THE ENDOPHYTIC FUNGUS *Aspergillus* sp. COLLECTED FROM THE SOUTH CHINA SEA

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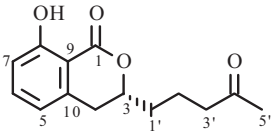
*Two new dihydroisocoumarin derivatives, aspergillumarins A and B (**1** and **2**), were isolated from the culture broth of a marine-derived fungus* Aspergillus *sp., which was isolated from the fresh leaf of the mangrove tree* Bruguiera gymnorrhiza *collected from the South China Sea. The structures of compounds **1** and **2** were established by comprehensive analysis of the spectral data, especially 2D NMR spectra results. Compounds **1** and **2** showed weak antibacterial activity against* Staphylococcus aureus *and* Bacillus subtilis *at a concentration of 50 μg/mL.*

Keywords: dihydroisocoumarin, endophytic fungus, structure elucidation, antibacterial activity.

Marine-derived fungi have been recognized as a potential source of structurally novel and biologically potent metabolites, and a growing number of marine fungi have been reported to produce novel bioactive secondary metabolites [1–3]. As part of a program to discover new bioactive secondary metabolites from marine-derived fungi collected from the South China Sea [4–8], the EtOAc extract of the culture broth of a marine-derived fungus, *Aspergillus* sp., which was isolated from the fresh leaf of the mangrove tree *Bruguiera gymnorrhiza*, was studied, and two new dihydroisocoumarin derivatives, aspergillumarins A and B (**1** and **2**), were isolated. Herein we report the isolation, structure elucidation, and biological activity of these new compounds.

Compound **1** was obtained as a colorless oil. The EI-MS of **1** exhibited a molecular ion at *m/z* 248. Its HR-EI-MS (*m/z* 248.1036, calcd 248.1043) implied it has a molecular formula $C_{14}H_{16}O_4$, which indicated that the compound has seven degrees of unsaturation. In the ^{1}H NMR spectrum (Table 1), the proton signals and the coupling constants at δ 6.69 (d, J = 7.2 Hz), 7.40 (dd, J = 8.4, 7.2 Hz), 6.87 (d, J = 8.4 Hz) indicated the presence of a 1,2,3-trisubstituted benzene system. One hydrogen-bonded hydroxyl group (δ 10.9, 1H, s), one oxygenated methine proton signal (δ 4.57, m), four methylene groups (δ 2.92, br.s; 2.53, t, J = 6.8 Hz; 1.83, m; 1.76, m), and one methyl group at δ 2.16 (s) were also observed. The ^{13}C NMR and DEPT data implied that compound **1** has one methyl group, four methylene groups, four methine carbons, and five quaternary carbons, including one lactone carbonyl carbon at δ 169.7 and one ketone carbonyl carbon at δ 208.2. Detailed assignments of the carbon and proton signals were unambiguously accomplished by an intensive analysis of 2D NMR spectral data (Table 1).



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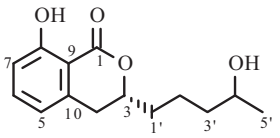

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TABLE 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data of **1** and **2** ($CDCl_3$, δ, ppm, J/Hz)

C atom	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1		169.7 (C)		169.6 (C)
3	4.57 (m)	79.3 (CH)	4.50 (m)	79.5 (CH)
4	2.92 (br.s)	33.9 (CH_2)	2.87 (br.s)	32.6 (CH_2)
5	6.69 (d, J = 7.2)	117.9 (CH)	6.62 (d, J = 7.2)	117.8 (CH)
6	7.40 (dd, J = 8.4, 7.2)	136.1 (CH)	7.32 (dd, J = 8.4, 7.2)	135.8 (CH)
7	6.87 (d, J = 8.4)	116.0 (CH)	6.78 (d, J = 8.4)	115.7 (CH)
8		162.0 (C)		161.6 (C)
9		108.2 (C)		108.1 (C)
10		139.2 (C)		139.2 (C)
1′	1.83 (m)	33.9 (CH_2)	1.69 (m)	21.0 (CH_2)
2′	1.76 (m)	18.9 (CH_2)	1.82 (m)	34.6 (CH_2)
3′	2.53 (t, J = 6.8)	42.7 (CH_2)	1.55 (m)	38.6 (CH_2)
4′		208.2 (C)	3.76 (m)	67.3 (CH)
5′	2.16 (s)	29.8 (CH_3)	2.34 (d, J = 6.0)	23.4 (CH_3)
8-OH	10.97 (s)		10.91 (s)	

In the COSY spectrum, the contiguous sequence of correlations from H-4 to H-3, H-1′, H-2′, and H-3′ confirmed the presence of an aliphatic subunit in **1**. In the HMBC spectrum, the correlations between H_3-5′ and C-3′, C-4′ confirmed that one acetoxyl group was linked at C-3′. The correlations from 8-OH to C-7, C-8, and C-9 as well as the low field shift of C-8 signal (δ_C 162.0) indicated that the hydrogen-bonded hydroxyl group was attached to C-8. The HMBC spectrum of compound **1** also showed correlations between H-4 and C-5, C-9, and C-10. Furthermore, the aromatic proton at δ 6.69 (H-5) showed correlations with C-4, C-6, C-9, and C-10. On the basis of these results, the structure of **1** was determined, and the compound was named aspergillumarin A, but its absolute stereochemistry at C-3 was not determined. However, the optical value of 3*R*-7-hydroxymellein in the literature has a negative value at –97° [9]. Therefore, the absolute configuration of compound **1** was defined tentatively as 3*R*.

Compound **2** was obtained as colorless crystals. Its molecular formula $C_{14}H_{18}O_4$ (six degrees of unsaturation) was determined by high-resolution EI mass spectrometry. This molecular formula was also corroborated by ^{1}H and ^{13}C NMR spectroscopic data (Table 1). Analysis of its ^{1}H and ^{13}C NMR spectra (Table 1) indicated that **2** was very similar to **1**. Detailed comparison of the ^{1}H NMR spectrum of **2** with that of **1** showed that the H-4′ signal in **1** was absent but in **2** was at δ_H 3.76 (H-4′), and the corresponding carbon chemical shift of C-4′ (208.2) in **1** was changed to δ_C 67.3 (s) in **2**, suggesting that the ketone carboxyl group had been replaced by an oxygenated methine group. Detailed assignments of the carbon and proton signals were unambiguously accomplished by an intensive analysis of 2D NMR spectral data (Table 1). Thus, the structure of **2** was assigned to aspergillumarin B on the basis of the structure of **1**. On the basis of the absolute configuration of aspergillumarin A (**1**) and a shared biogenesis with **1**, the configuration of aspergillumarin B (**2**) should also be assigned as 3*R*. But its absolute stereochemistry at C-4′ was not determined.

The antibacterial activities of compounds **1** and **2** were also determined against *Staphylococcus aureus* and *Bacillus subtilis* by the method of Fromtling et al. [10], and both exhibited weak antibacterial activity against *S. aureus* and *B. subtilis* at a concentration of 50 μg/mL.

EXPERIMENTAL

The ^{1}H and ^{13}C NMR data were recorded on an INOVA-500 (499.77 and 125.68 MHz) NMR spectrometer with Me_4Si as the internal standard. Mass spectra were obtained on a VG-ZABHS mass spectrometer and a MAT95XP high-resolution mass spectrometer. Column chromatography was carried out on silica gel (200–300 mesh; Qingdao Haiyang Chemicals).

Fungus Material and Culture Conditions. A strain of the fungus *Aspergillus* sp. was isolated from the leaf of the mangrove tree *Bruguiera gymnorrhiza* from the South China Sea coast and was stored at the School of Chemistry and Chemical Engineering, Sun Yat-sen University. Starter cultures were maintained on cornmeal seawater agar. Plugs of agar supporting

mycelia growth were cut and transferred aseptically to a 250 mL Erlenmeyer flask containing 100 mL of liquid medium (glucose 1%, peptone 0.2%, yeast extract 0.1%, NaCl 0.25%). The flask was incubated at 28°C on a rotary shaker for 1 week. The mycelium was aseptically transferred to 500 mL Erlenmeyer flasks containing culture liquid (200 mL). The flasks were then incubated at 28°C for 5 weeks.

Extraction and Separation of Metabolites. The culture broth (150 L) was filtered through cheesecloth. The filtrate was concentrated to 4 L below 50°C and extracted four times by shaking with twofold volumes of ethyl acetate. The combined extracts were chromatographed repeatedly on silica gel using gradient elution from petroleum ether to ethyl acetate, and by preparative thin layer chromatography to obtain compounds **1** (20.0 mg) and **2** (25.0 mg) from the 20% ethyl acetate–petroleum ether.

Aspergillumarin A (1). Colorless oil, $[\alpha]_D^{20}$ –10.3° (*c* 0.27, $CHCl_3$). IR spectrum (KBr, ν_{max}, cm^{-1}): 3344 (OH), 2954, 1714 (C=O), 1674, 1619, 1463, 1374, 1234, 1164, 1119, 808, 741, 699. Mass spectrum EI-MS^+ (*m/z*, I_{rel}, %): 248 (27), 230 (17), 212 (58), 190 (12), 172 (100). HR-EI-MS^+ (*m/z*, I_{rel}, %): 248.1036 (100) $[M]^+$, calcd for $C_{14}H_{16}O_4$, 248.1043. ^1H, ^{13}C NMR, see Table 1.

Aspergillumarin B (2). Colorless crystals, mp 40–41°C, $[\alpha]_D^{20}$ –18.6° (*c* 0.20, $CHCl_3$). IR spectrum (KBr, ν_{max}, cm^{-1}): 3410 (OH), 2950, 2868, 1658 (C=O), 1617, 1582, 1463, 1242, 1203, 1116, 1030, 982, 811, 738, 694. EI-MS^+ (*m/z*, I_{rel}, %): 250 (43), 232 (49), 217 (15), 214 (100), 199 (22), 188 (31), 172 (47), 163 (33), 160 (36), 147 (24), 135 (48), 134 (82). HR-EI-MS^+ (*m/z*, I_{rel}, %): 250.1200 (100) $[M]^+$, calcd for $C_{14}H_{18}O_4$, 250.1205. ^1H, ^{13}C NMR, see Table 1.

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