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A NEW ISOCOUMARIN FROM MANGROVE ENDOPHYTIC FUNGUS (No. dz17) ON THE SOUTH CHINA SEA COAST

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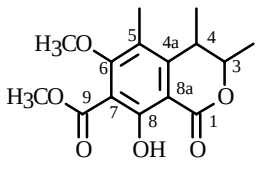
*A new isocoumarin, 3,4-dihydro-6-methoxy-8-hydroxy-3,4,5-trimethylisocoumarin-7-carboxylic acid methyl ester (**1**), together with three known compounds, 3,4-dihydro-4,8-dihydroxy-3,5-dimethylisocoumarin (**2**), 3,4-dihydro-8-hydroxy-3-methylisocoumarin-5-carboxylic acid (**3**), and Entinclole SB (4) were isolated from the mangrove endophytic fungus (No. dz17). The structure of the compound **1** was elucidated by analysis of spectroscopic data. Primary bioassays showed that **1** exhibited weak cytotoxicity against Hep-2 and HepG2 cells.*

Key words: mangrove endophytic fungus, isocoumarin, metabolites.

A diverse array of secondary metabolites has been isolated and characterized from marine-derived fungi. Endophytic fungi from marine habitats has great potential for the discovery of new pharmacologically active metabolites [1]. In our search for new metabolites from marine-derived mangrove endophytic fungi from the South China Sea, we have isolated many significant new bioactive metabolites [2–7]. Now we report one new isocoumarin, 3,4-dihydro-6-methoxy-8-hydroxy-3,4,5-trimethylisocoumarin-7-carboxylic acid methyl ester and three known compounds [8, 9] from mangrove endophytic fungus No. dz17 from the South China Sea coast.

The ethyl acetate extract of a fermentation broth of the fungus was repeatedly chromatographed on silica gel using gradient elution from petroleum to ethyl acetate to give compound **1** from the 20% ethyl acetate/petroleum fraction as a colorless solid. Compound **1** has the molecular formula $C_{15}H_{18}O_6$, as determined by HR-EIMS (*m/z* 294.1084 $[M]^+$ calcd. for $C_{15}H_{18}O_6$, 294.1098) and NMR spectra (Table 1), being indicative of seven degrees of unsaturation.

The IR spectrum of compound **1** suggested the presence of a hydroxyl group (3378 cm^{-1}), two carbonyl groups (1736, 1666 cm^{-1}) and a benzene ring (1616, 1587, 1458 cm^{-1}). The ^{13}C NMR and HMQC spectra showed 15 carbon signals, including two carbonyl signals (δ 167.9, 165.9), six olefinic carbon signals, three carbon-bearing oxygen signals [δ 80.1 (CH), 61.3 (CH_3), 52.6 (CH_3)], and three methyl carbons. The ^1H NMR spectrum displayed signals for a strongly deshielded hydroxyl hydrogen (δ_H 11.68), two methoxyls (δ_H 3.96, 3.86), one singlet methyl (δ_H 2.13), and two doublet methyls (δ_H 1.33, 1.30).



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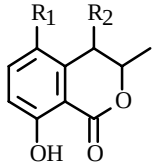

2, 3

2: $R_1 = CH_3$, $R_2 = OH$

3: $R_1 = COOH$, $R_2 = H$

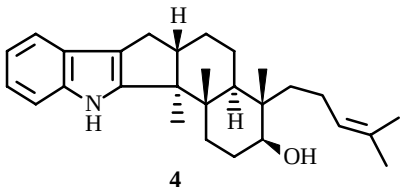

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TABLE 1. NMR Data of **1** ($CDCl_3$, δ, ppm, J/Hz)

Position	δ_C (DEPT)	δ_H	COSY	HMBC
1	167.9			H-3
3	80.1 (CH)	4.72 (dq, J = 0.8, 6.8)	H-4, 3-CH_3	3-CH_3, 4-CH_3
4	34.9 (CH)	3.00 (dq, J = 0.8, 6.8)	H-3, 4-CH_3	H-3, 3-CH_3, 4-CH_3
4a	144.3			H-3, 4, 4-CH_3, 5-CH_3
5	119.3			H-4, 5-CH_3
6	162.0			H-11, 5-CH_3
7	114.8			8-OH
8	159.2			8-OH
8a	103.4			H-4, 8-OH
9	165.9			9-OCH_3
3-CH_3	19.9 (CH_3)	1.33 (d, J = 6.8)	H-3	H-3, 4
4-CH_3	19.5 (CH_3)	1.30 (d, J = 6.8)	H-4	H-3, 4
5-CH_3	10.4 (CH_3)	2.13 (s)		
6-OCH_3	61.3 (CH_3)	3.86 (s)		
8-OH		11.68 (s)		
9-OCH_3	52.6 (CH_3)	3.96 (s)		

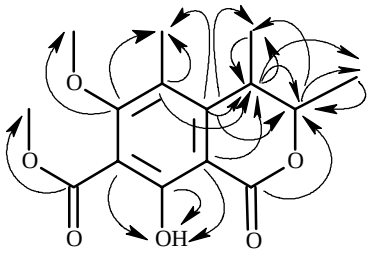

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Fig. 1. HMBC correlations for **1**.

The HMBC correlations of the hydroxyl proton at δ_H 11.68 with C-7, C-8, C-8a confirmed the location of the hydroxyl group at C-8 (δ_C 159.2). The methoxy group (δ_H 3.86, δ_C 61.3) is located at C-6 (δ_C 162.0) as determined by its HMBC correlations with C-6. The methyl group (δ_H 2.13, δ_C 10.4) is located at C-5 as revealed by the HMBC correlations with C-4a, C-5, and C-6. The positions of two methyl groups (δ_H 1.30, δ_C 19.4, and δ_H 1.33, δ_C 19.9) were respectively determined to be at C-4, C-3, as indicated by the HMBC spectrum. COSY correlations observed between 4-CH_3 and H-4 and 3-CH_3 and H-3 supported these assignments. The HMBC data established the overall structure shown in Fig. 1.

Primary bioassays showed that compound **1** exhibited weak cytotoxicity against Hep-2 and HepG2 cells (IC_{50}>50 μg/mL).

EXPERIMENTAL

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. EIMS spectrum was obtained on a VG-ZABHS mass spectrometer, and HREIMS spectrum was obtained on a VG Autospec-500 mass spectrometer. 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 and was uncorrected.

Fungus Material and Culture Conditions. A strain of the fungus dz17 was isolated from the South China Sea coast. It is apospory and its general species have not been identified. Starter cultures were maintained on cornmeal seawater agar. 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 cultures (100 L) were filtered through cheesecloth. The filtrate was concentrated to 2 L below 60°C and extracted several 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 to give compounds **1** (2.5 mg), **2** (3 mg), **3** (4.3 mg), and **4** (5.3 mg) from the 20%, 30, 50, and 20% ethyl acetate/petroleum respectively.

3,4-Dihydro-6-methoxy-8-hydroxy-3,4,5-trimethyl-isocoumarin-7-carboxylic acid methyl ester (1): colorless solid, mp 235–238°C; $[\alpha]_D^{22}$ –167.2° (*c* 0.15, $CHCl_3$).

UV spectrum (CH_3OH, λ_{max}, nm) (log ε): 214 (5.19), 257 (3.73), 317 (3.53).

IR spectrum (KBr, ν, cm^{-1}): 3378 (OH), 2923, 1736 (C=O), 1666 (C=O), 1616, 1587, 1458 (Ph), 1414, 1308, 1217, 1159, 1116, 1019, 985, 816, 775.

Mass spectrum (EI^+, *m/z*, I_{rel}, %): 294 (100) $[M]^+$, 279 (12), 262 (91), 218 (42), 189 (15), 57 (19), 43 (18). Mass spectrum (HR-EI^+, *m/z*, I_{rel}, %): 294.1084 (25.3) $[M]^+$, (calc. 294.1098). ^1H, ^{13}C NMR see Table 1.

3,4-Dihydro-4,8-dihydroxy-3,5-dimethylisocoumarin (2): colorless solid, mp 243–245°C.

^1H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 10.97 (1H, s), 7.38 (1H, d, J = 9), 6.94 (1H, d, J = 9), 4.64 (1H, d, J = 2), 2.36 (3H, s), 1.81 (1H, br.s), 1.63 (3H, d, J = 7).

^{13}C NMR (125 MHz, acetone-d_6, δ, ppm): 169.8 (C), 160.4 (C), 138.9 (CH), 137.6 (C), 126.2 (C), 118.1 (CH), 106.7 (C), 78.0 (CH), 64.4 (CH), 17.1 (CH_3), 16.3 (CH_3).

3,4-Dihydro-8-hydroxy-3-methylisocoumarin-5-carboxylic acid (3): white solid, mp 190–220°C (sublimation).

^1H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 12.95 (1H, br.s), 11.67 (1H, s), 8.07 (1H, d, J = 9), 6.96 (1H, d, J = 9), 4.76 (1H, m), 3.80 (1H, dd, J = 18, 3.5), 3.01 (1H, dd, J = 18, 12), 2.10 (1H, dd, J = 18, 12), 1.44 (3H, d, J = 6.5).

^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 169.2 (C), 166.7 (C), 163.8 (C), 143.2 (C), 138.1 (CH), 119.6 (C), 115.3 (CH), 108.9 (C), 75.1 (CH), 32.0 (CH_2), 20.1 (CH_3).

Entinclole SB (4): amorphous power. Mass spectrum (EI^+, *m*/z, I_{rel}, %): 405 (100) $[M]^+$, 390 (75), 182 (81), 130 (37).

^1H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.75 (1H, br.s, NH), 7.41 (1H, m), 7.27 (1H, m), 7.06 (2H, m), 5.13 (1H, br.t, J = 7.0, CH_2CH=), 3.59 [1H, dd, J = 8.3, 6.7, $CH_2CH(OH)$], 2.72 (1H, m), 2.68 (1H, dd, J = 12.9, 6.4), 2.34 (1H, dd, J = 12.9, 10.3), 1.72 (3H, br.s, =CMe_2), 1.66 (3H, br.s, =CMe_2), 1.24–1.50 (14H, m), 1.12 (3H, s, Me), 1.04 (3H, s, Me), 0.84 (3H, s, Me).

^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 151.1, 140.1, 131.5, 125.3, 124.8, 120.6, 119.7, 118.6, 118.5, 111.6, 73.6, 53.4, 49.1, 41.5, 40.2, 39.6, 37.8, 33.8, 27.8, 27.8, 26.1, 25.5, 23.1, 21.7, 19.5, 18.0, 16.8, 15.0.

Bioassays. The cytotoxic assays were performed using the MTT assay method [10]. Compound **1** inhibited the growth of Hep-2 and HepG2 cells with IC_{50} values of 52 and 55 μg/mL, respectively.

ACKNOWLEDGMENT

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