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A NEW DIHYDROISOCOUMARIN WITH AN ISOPRENYL GROUP FROM THE ENDOPHYTIC FUNGUS *Cephalosporium* SP.

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*A new dihydroisocoumarin derivative containing an isoprenyl group, 5-(3′-methylbut-2′-enyloxy)-3,4-dihydro-8-hydroxy-3-methylisochromen-1-one (**1**), was isolated from the mangrove fungus* Cephalosporium *sp. from the South China Sea. The structure of compound **1** was established by comprehensive analysis of the spectral data, especially 2D NMR spectra.*

Keywords: dihydroisocoumarin, endophytic fungus, structure elucidation.

A diverse array of bioactive metabolites has been isolated and characterized from mangrove endophytic fungi collected from the countries around the South China Sea [1]. In the course of our study, new or bioactive secondary metabolites from marine fungi have been isolated [2–7], and the ethyl acetate extract of the fermentation broth of the mangrove endophytic fungus *Cephalosporium* sp. was separated using silica gel column and preparative thin layer chromatography (pre-TLC). A new dihydroisocoumarin derivative with an isoprenyl group, 5-(3′-methylbut-2′-enyloxy)-3,4-dihydro-8-hydroxy-3-methylisochromen-1-one (**1**), was isolated and identified. Herein, we report the isolation and structure elucidation of the new compound **1** from the culture liquid of the endophytic fungus.

Compound **1** was obtained as a white amorphous solid. The EI-MS of **1** exhibited a molecular ion at *m/z* 262. Its HR-EI-MS (*m/z* 262.1201, calcd 262.1200) implied it has a molecular formula of $C_{15}H_{18}O_4$, which indicated that the compound has 7 degrees of unsaturation. In the ^{1}H NMR spectrum, compound **1** has one hydroxyl group at δ 10.58, two *o*-coupled aromatic proton signals at δ 7.09 and 6.85 (each d, J = 9.0 Hz), one methylene group (δ 3.20, 2.64), an oxygenated methine proton at δ 4.66, and one methyl group at δ 1.53. The ^{1}H NMR spectrum also showed one methylene group attached to an oxygen atom at δ 4.46, one signal due to an olefinic proton at δ 5.43, and two methyl groups at δ 1.78, 1.71, indicating the presence of the isoprenyl group. The ^{13}C NMR and DEPT data implied that compound **1** has three methyl groups, two methylene groups, four methine carbons, and six quaternary carbons, including one carbonyl carbon at δ 169.9. Detailed assignments of the carbon and proton signals were unambiguously accomplished by an intensive analysis of 2D NMR spectral data (Table 1).

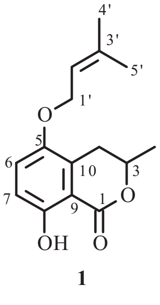

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TABLE 1. NMR Data of **1** ($CDCl_3$, δ, ppm, J/Hz)

C atom	δ_C (DEPT)	δ_H	HMBC
1	169.9 (C=O)		
3	76.1 (CH)	4.66 (dqd, J = 11.5, 6.5, 3.5)	
4	28.6 (CH_2)	3.20 (dd, J = 17.0, 3.5)	C-5, C-9, C-10
		2.64 (dd, J = 17.0, 11.5)	C-3, C-5, C-9, C-10, 3-Me
5	147.1 (C)		
6	121.9 (CH)	7.09 (d, J = 9.0)	C-5, C-8, C-10
7	115.7 (CH)	6.85 (d, J = 9.0)	C-5, C-8, C-9
8	156.0 (C)		
9	108.6 (C)		
10	127.5 (C)		
3-Me	20.9 (CH_3)	1.53 (d, J = 6.5)	C-3, C-4
8-OH		10.58 s	C-7, C-8, C-9
1′	66.4 (CH_2)	4.46 (dd, J = 12.0, 6.5)	C-5, C-2′, C-3′
		4.49 (dd, 12.0, 6.5)	
2′	119.6 (CH)	5.43 (t, sept, J = 6.5, 1.0)	C-4′, C-5′
3′	138.2 (C)		
4′	25.8 (CH_3)	1.78 (d, J = 1.0)	C-2′, C-3′
5′	18.2 (CH_3)	1.71 (d, J = 1.0)	C-2′, C-3′

In the HMBC spectrum, correlations were observed between 8-OH and C-7, C-8, and C-9. The low-field shift of C-8 signal (δ 156.0) indicated that the hydroxyl group was attached to C-8. The HMBC spectrum of compound **1** also showed correlations between H-4 and C-3, C-5, C-9, C-10, and 3-Me. Furthermore, two *o*-coupled aromatic protons at δ 7.09 (H-6) and δ 6.85 (H-7) showed correlations with C-5, C-8, C-10 and C-5, C-8, C-9, respectively. The correlation between H-1′ and C-5 at δ_C 147.1 indicated that the isoprenyl group was located at C-5 through one oxygen atom. On the basis of these results, the structure of **1** was determined to be 5-(3′-methylbut-2′-enyloxy)-3,4-dihydro-8-hydroxy-3-methylisochromen-1-one.

EXPERIMENTAL

The ^1H 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 spectrum was 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 *Cephalosporium* sp. was isolated from a leaf of a mangrove tree 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 5–7 days. The mycelium was aseptically transferred to 500 mL Erlenmeyer flasks containing culture liquid (300 mL). The flasks were then incubated at 25°C for 4 weeks.

Extraction and Separation of Metabolites. The cultures (70 L) were 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 compound **1** (6.5 mg) from 10% ethyl acetate–petroleum ether.

5-(3′-Methylbut-2′-enyloxy)-3,4-dihydro-8-hydroxy-3-methylisochromen-1-one (1). White amorphous solid. ^1H, ^{13}C NMR, see Table 1. Mass spectrum (EI-MS$^+$ m/z, I_{rel}, %): 262 (8), 194 (100), 178 (31), 176 (51), 163 (11), 160 (21), 149 (32), 69 (89), 41 (49). Mass spectrum (HR-EI$^+$ m/z, I_{rel}, %): 262.1201 (100) $[M]^+$, calcd for $C_{15}H_{18}O_4$, 262.1200.

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