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A NEW ISOPRENYL PHENYL ETHER COMPOUND FROM MANGROVE FUNGUS

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*A new isoprenyl phenyl ether, 3-hydroxy-4-(3-methylbut-2-enyloxy)benzoic acid methyl ester (**1**), together with 4-hydroxybenzoic acid (**2**), 2-hydroxy-6-methylbenzoic acid (**3**), and 4-hydroxy-3-methoxybenzoic acid (**4**) were isolated from Mangrove fungus (No. B60) from the South China Sea. The structures of the compounds were established on the basis of NMR spectroscopic and mass spectrometric data. In the preliminary bioassay, compound **1** exhibited antibacterial and antifungal activities. Compound **1** also inhibited cytotoxicity to the hepG2 cell line with an IC_{50} value of 10.0 μg/mL.*

Key words: marine fungi, biological activity, isoprenyl phenyl ether.

In the search for new bioactive natural products from marine organisms, increasing attention is being given to microorganisms such as bacteria and fungi, especially the mangrove fungi. Eight years ago, we embarked on the study of the metabolites of marine fungi, including those from mangroves from the South China Sea, and this has yielded a lot of novel and bioactive secondary metabolites [1–6]. In this paper, we describe the isolation, structure elucidation, and biological activities of a new isoprenyl phenyl ether, 3-hydroxy-4-(3-methyl-but-2-enyloxy)benzoic acid methyl ester (**1**). Compound **1** exhibited antibacterial, antifungal, and anticancer activities.

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 obtain compound **1** from the 20% ethyl acetate/petroleum fraction as colorless crystals. EIMS, ^{13}C NMR, ^{1}H NMR, COSY, HMQC, HMBC and DEPT data unambiguously confirmed the structure of **1**. Compound **1** has the molecular formula $C_{13}H_{16}O_4$ as determined by HR-EIMS (*m/z* 236.1077 $[M]^+$, calcd 236.1043) and NMR spectra.

The IR spectrum of **1** showed absorptions for a hydroxyl (3397 cm^{-1}), a carbonyl group (1700 cm^{-1}), and a benzene ring (1599, 1513 cm^{-1}). In the ^{13}C NMR spectrum, there were one carbonyl signal (δ 166.91) and eight aromatic signals. There were two signals assigned to carbons bearing oxygen at δ 65.92 (CH_2), 51.92(CH_3) and two methyl carbons. The ^{1}H NMR spectra showed one exchangeable proton at 6.06 (1H, s) and one ABX spin system assignable to a 1,3,4-trisubstitued benzene rings at 7.62 (1H, dd, J = 8.5, 2.0 Hz, H-6), 6.92 (1H, d, J = 8.5 Hz, H-5), and 7.55 (1H, d, J = 2.0 Hz, H-2). The HMQC spectrum exhibited correlations between protons and carbons, which verified that the signal at δ 5.48 belongs to an olefinic proton correlating with the carbon signal at δ 118.74.

Analysis of the HMBC data established the overall structure of **1** (Fig. 1), especially the multiple correlations from H-1 to C-2 and C-3 and H-2 to C-4 and C-5, which were used to define the isoprenyl moiety. The H-1 proton shows an HMBC correlation to the C-3′, placing the isoprenyl moiety at the C-3′ position of the benzene ring. The correlation between C-4′ and OH, and the multiple correlations from OH to C-3′ and C-5′, located OH at C-4′. Proton H-2′ and H-6′ correlate the ester carbonyl carbon, placing the ester at the C-1′ position of the benzene ring.

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

C atom	δ_C (DEPT)	δ_H	COSY
1	65.92 CH_2	4.63 (d, 7.0)	H-2
2	118.74 CH	5.48 (tqq, 7.0, 1.5, 1.5)	H-1
3	139.49		
4	25.79 CH_3	1.81 (d, 1.5)	H-1
5	18.23 CH3	1.76 (d, 1.5)	
1′	122.15		
2′	112.99 CH	7.55 (d, 2.0)	H-6′
3′	145.40		
4′	150.31		
5′	114.00 CH	6.92 (d, 8.5)	H-6′
6′	124.08 CH	7.62 (dd, 2.0, 8.5)	H-2′,5′
7′	166.91		
8′	51.92 OCH_3	3.88 (s)	
4′-OH		6.06 (s)	

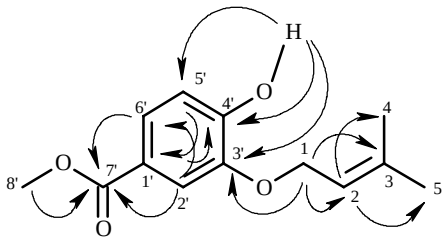

Fig. 1. The key HMBC correlations of **1**.

In the LC-MS spectrum, the molecular ion peak was observed at *m/z* 235 $[M-H]^-$. As the informative fragment, an ion at *m/z* 166 generated by the loss of $[CH_2\text{-}CH{=}C\text{-}(CH_3)_2]$ as well as a peak at *m/z* 151 arising from the loss $[CH_2CH{=}C(CH_3)_2{+}CH_3]$ from the molecular ion provided additional structural information.

In the preliminary bioassay, compound **1** exhibited strong inhibitory activities against Gram-positive bacteria *Staphylococcus aureus* (ATCC27154) and Gram-negative bacteria *Escherichia coli* (ATCC 25922) (MIC value 6.25 and 25 μg/mL, respectively) and the fungus *Fusarium oxysporum* (MIC value, 12.5 μg/mL), while **1** showed no activity against the fungus *Candida albicans* (ATCC 10231). Compound **1** also exhibited the growth of hepG2 cell line with an IC_{50} value of 10 μg/mL.

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. 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 micro-melting point apparatus and was uncorrected.

Fungus Material and Culture Conditions. A strain of the fungus (No. B60) was isolated from the South China Sea coast and was stored at the Department of Applied Chemistry, Zhongshan University, Guangzhou, China. The culture conditions have been reported previously [1–6].

Extraction and Separation of Metabolites. The cultures (120 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 obtain compound **1** (2.1 mg) from 20% ethyl acetate/petroleum: 3-hydroxy-4-(3-methylbut-2-enyloxy)benzoic acid methyl ester,

$C_{13}H_{16}O_4$, M^+ 236.1043, colorless solid; mp 49–50°C. IR spectrum (KBr, ν, cm^{-1}): 3397.6 (OH), 2925.6, 1700.0 (C=O), 1599.3, 1513.1 (Ph), 1438.8, 1378.6, 1283.2, 1211.5, 1100.6, 1004.4, 765.6, 700.5.

UV (MeOH), λ_{max}, nm (*c* 0.015, log e): 261 (4.11), 292 (3.42).

^1H, ^{13}C NMR see Table 1.

Mass spectrum (LC-MS^- *m/z*, I_{rel}, %): 235 (100) $[M-H]^-$, 166 (8). Mass spectrum (EI^+ *m/z*, I_{rel}, %): 236 (3) $[M]^+$, 205 (5), 168 (99), 137 (85), 69 (100), 41 (68). Mass spectrum (HR-EI^+: *m/z*, I_{rel}, %): 236.1077 (25.3) $[M]^+$, calcd for $C_{13}H_{16}O_4$, 236.1043.

The antibacterial activities of compound **1** were tested by the usual procedure [7]. The cytotoxic assay was performed using the MTT assay method [8].

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