TWO NEW BENZALDEHYDE DERIVATIVES FROM MANGROVE ENDOPHYTIC FUNGUS (No. ZZF 32)

Changlun Shao,[1,2] Changyun Wang,[1] Meiyan Wei,[3] Zhenbin Jia,[3] Zhigang She,[2*] and Yongcheng Lin[2*]

UDC 547.571

*Two new benzaldehyde derivatives, 6-ethyl-2-hydroxy-4-methoxy-3-methylbenzaldehyde (**1**), 6-ethyl-2,4-dihydroxy-3-methylbenzaldehyde (**2**), together with 2, 4-dihydroxy-3,6-dimethylbenzaldehyde (**3**) were isolated from mangrove fungus (No. ZZF 32#) from the South China Sea. The structures of compounds **1** and **2** were established by comprehensive analysis of the spectral data, especially 2D NMR spectral results.*

Key words: benzaldehyde derivatives, cytotoxicity, mangrove fungus.

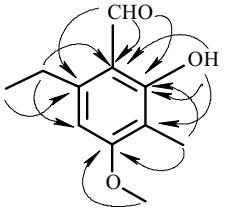
Marine-derived fungi are a rich source of bioactive secondary metabolites with diverse structures [1]. In the course of our search for bioactive metabolites, we have isolated a number of novel natural compounds from marine mangrove fungi collected from the South China Sea [2–5]. As part of an ongoing program to search for new bioactive natural compounds, we studied the constituents of the marine fungus (ZZF 32#). From the ethyl acetate extract, three compounds have been isolated, including two new benzaldehyde derivatives, 6-ethyl-2-hydroxy-4-methoxy-3-methylbenzaldehyde (**1**) and 6-ethyl-2, 4-dihydroxy-3-methylbenzaldehyde (**2**). The new compounds were elucidated on the basis of a comprehensive analysis of the detailed 1D and 2D spectroscopic data. We report herein the isolation and structural elucidation of two new benzaldehyde derivatives **1** and **2**.

Compound **1** was obtained as a colorless solid. The molecular formula of **1** was established as $C_{11}H_{14}O_3$ based on the results of EI-MS and NMR data. The molecular formula indicated five degrees of unsaturation within the molecule. The ^{13}C NMR and DEPT spectra showed that **1** had 11 carbon signals, attributable to two methyl (δ_C 7.2, 17.6), one methoxyl (δ_C 56.3), one methylene (δ_C 25.6), two methine (δ_C 104.8, 194.8), and five quaternary (δ_C 111.1, 113.2, 149.9, 163.6, 165.3) carbon atoms. The ^1H NMR spectrum and HMQC data displayed the presence of a hydrogen-bonded hydroxyl group [δ_H 12.59 (1H, s)], one aldehyde proton signal at δ_H 10.19 (s), one singlet due to an aromatic proton at δ_H 6.56, one methoxyl group at δ_H 3.95, one methylene group at δ_H 3.00 (q, J = 7.5 Hz, CH_2), and two methyl groups at δ_H 1.99 (s, CH_3), 1.29 (t, J = 7.5 Hz, CH_3). According to the above NMR data, compound **1** is a five-substituted benzene derivative. The whole structure of **1** was confirmed by the HMBC spectra. The correlations between 2-OH and C-1, C-2, C-3 and between the aldehyde proton and C-2 suggested that the hydroxyl group is located at C-2. HMBC correlations between CH_3 (δ_H 1.99) and C-2, C-3, C-4 and between OCH_3 (δ_H 3.95) and C-4 revealed that the methyl group and the methoxyl group are located at C-3 and C-4 of the benzene ring, respectively. The methylene protons of the ethyl group show the HMBC correlation to C-1, C-5, and C-6, placing the ethyl moiety at the C-6 position of the benzene ring. Thus, the structure of compound **1** was identified as 6-ethyl-2-hydroxy-4-methoxy-3-methylbenzaldehyde.

Compound **2** was also isolated as a colorless solid. The molecular formula was deduced to be $C_{10}H_{12}O_3$ based on the results of EI-MS and NMR data. Compared with the ^1H NMR data of compound **1**, compound **2** has an extra hydroxyl group at δ_H 6.35, and compound **1** lost one methoxyl group at δ_H 3.95. There is no methoxyl group signal in compound **2**. According to the above data, compound **2** is 6-ethyl-2,4-dihydroxy-3-methylbenzaldehyde.

1) School of Medicine and Pharmacy, Ocean University of China, Qingdao, 266003, P. R. China; 2) School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Guangzhou, 510275, P. R. China, fax: +86 20 8403 4096, e-mail: cesshzhg@mail.sysu.edu.cn, fax: +86 20 8403 9623, e-mail: ceslyc@mail.sysu.edu.cn; 3) School of Pharmacy, Guangdong Medical College, Dongguan, 523808, P. R. China. Published in Khimiya Prirodnykh Soedinenii, No. 6, pp. 656–657, November–December, 2009. Original article submitted May 7, 2008.

0009-3130/09/4506-0779



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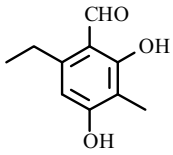

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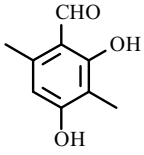

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Compound **3** was isolated from the fungus as colorless solids. Its structure was identified as 2,4-dihydroxy-3,6-dimethylbenzaldehyde [6] by comparison of the spectroscopic data with those in the related literature.

EXPERIMENTAL

The ^{1}H and ^{13}C NMR data were recorded on an INOVA-500 (499.77 and 125.68 MHz) or INOVA-300 (300.00 and 75.15 MHz) NMR spectrometer with Me_4Si as the internal standard. Mass spectrum was obtained on a VG-ZABHS 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 (No. ZZF 32) was isolated from the South China Sea coast and was stored at the Department of Applied Chemistry, Zhongshan University, Guangzhou, China. Starter cultures were maintained on cornmeal seawater agar. Plugs of agar supporting mycelium 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 0.3%). The flask was incubated at 30°C on a rotary shaker for 5–7 days. The mycelium was aseptically transferred to a 500 mL Erlenmeyer flasks containing culture liquid (300 mL). The flasks were then incubated at 30°C with shaking for 15 days.

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 to obtain compounds **1** (4.2 mg), **2** (2.6 mg), and **3** (8.1 mg) from 10% ethyl acetate–petroleum.

6-Ethyl-2-hydroxy-4-methoxy-3-methylbenzaldehyde (1). Colorless solid. Mass spectrum (EI-MS^+ *m*/*z*, I_{rel}, %): 194 (100), 179 (6), 176 (9), 161 (7), 158 (18), 146 (21), 131 (9), 121 (5), 115 (5), 91 (9), 77 (8). ^{1}H NMR (500 MHz, acetone-d_6, δ, ppm, J/Hz): 12.59 (1H, s, OH), 10.19 (1H, s, CHO), 6.56 (1H, s, CH), 3.95 (3H, s, OCH_3), 3.00 (1H, q, J = 7.5, CH_2), 1.99 (3H, s, CH_3), 1.29 (3H, t, J = 7.5, CH_3). ^{13}C NMR (125 MHz, acetone-d_6, δ, ppm): 194.8 (CHO), 165.3 (C), 163.6 (C), 149.9 (C), 113.2 (C), 111.1 (C), 104.8 (CH), 56.3 (OCH_3), 25.6 (CH_2), 17.6 (CH_3), 7.2 (CH_3).

6-Ethyl-2,4-dihydroxy-3-methylbenzaldehyde (2). Colorless solid. Mass spectrum (EI-MS^+ *m*/*z*, I_{rel}, %): 180 (100), 162 (36), 151 (8), 147 (21), 145 (6), 133 (15), 119 (4), 105 (5), 91 (9), 77 (12). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 12.66 (1H, s, OH), 10.04 (1H, s, CHO), 6.35 (1H, s, OH), 6.25 (1H, s, CH), 2.86 (2H, q, J = 7.5, CH_2), 2.10 (3H, s, CH_3), 1.27 (3H, t, J = 7.5, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 192.61 (CHO), 164.11 (C), 161.54 (C), 147.91 (C), 111.97 (C), 108.92 (C), 108.34 (CH), 24.75 (CH_2), 16.79 (CH_3), 6.99 (CH_3).

2,4-Dihydroxy-3,6-dimethylbenzaldehyde (3). Colorless solid. Mass spectrum (EI-MS^+ *m*/*z*, I_{rel}, %): 166 (70), 165 (100), 148 (3), 137 (8), 120 (6). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 12.77 (1H, s, OH), 10.07 (1H, s, CHO), 6.34 (1H, s, CH), 2.50 (3H, s, CH_3), 2.01(3H, s, CH_3).

ACKNOWLEDGMENT

We wish to acknowledge financial support from the National Natural Science Foundation of China (40776073, 20072058), the 863 Foundation of China (2006AA09Z422), and the Doctoral Startup Fund of Ocean University of China (1404-82421036).

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