Chemistry of Natural Compounds, Vol. 44, No. 4, 2008

TWO NEW ACIDS FROM MANGROVE ENDOPHYTIC FUNGUS (No. ZZF13)

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UDC 665.37

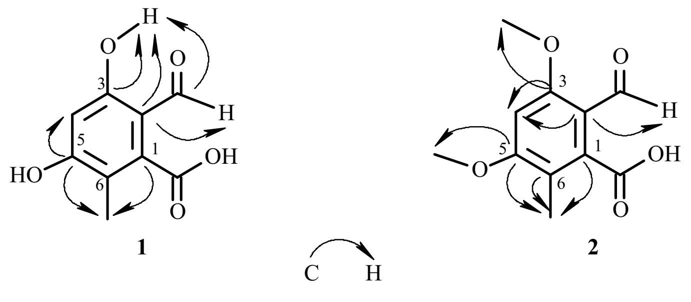
*Two new acids **1** and **2**, together with a known compound, purpactin A (**3**), were isolated from endophytic fungus No. ZZF13. Their structures were elucidated on the basis of 1D, 2D NMR, and HREIMS spectra.*

Key words: acids, endophytic fungus, cytotoxicity.

Endophytic fungi are a well-established source for structurally diverse and biologically active secondary metabolites [1]. In a continuing search for new antitumor and antifugal natural products [2, 3], the metabolites were studied and two new ones were obtained, 2-formyl-3,5-dihydroxy-6-methylbenzoic acid and 2-formyl-3,5-dimethoxy-6-methylbenzoic acid, as shown in Fig. 1. No. ZZF13 was an unidentified endophytic fungus, which was isolated from the Zhanjiang sea area, China.

Compound **1** was obtained as brown needles. A molecular formula of $C_9H_8O_5$, which gave six degrees of unsaturation, was established by HREIMS and ^{13}C NMR methods. The IR absorption spectrum of **1** showed bands that were characteristic of hydroxyl (3335 cm^{-1}) and enone (1716 cm^{-1}) functionalities. The $FeCl_3$ positive reaction confirmed the phenolic hydroxyl groups. In the ^1H NMR, the signals of one benzene protons at δ 7.18, one methyl proton at δ 2.11, a chelated hydroxyl at δ 12.98, and one aldehyde at δ 10.52 were observed. ^{13}C NMR (Table 1) and DEPT of **1** suggested one methyl, one methine, and seven quaternary carbon atoms, including two carbonyl carbon (δ 198.0, 168.3).

Diagnostic HMBC correlations of **1**, from OH-3 to C-2, 3, CHO, revealed that OH-3 was chelated by an aldehyde. Correlations from H-4 to C-5 and from CH_3-6 to C-1 and C-5 showed the positions of the other hydroxy, methyl, and carboxyl. On the basis of all the foregoing evidence, we proposed that the structure of this compound is 2-formyl-3, 5-dihydroxy-6-methylbenzoic acid.



1

2

Fig. 1. Structures of compounds **1** and **2**.

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Published in Khimiya Prirodnykh Soedinenii, No. 4, pp. 336-337, July-August, 2008. Original article submitted April 24, 2007.

TABLE 1. NMR Data of Compound **1** (acetone, δ, ppm)

C atom	^{1}H NMR	^{13}C NMR	HMBC (C to H)
1		117.1	CH_3-6
2		114.1	OH-2, H-CHO
3		163.3	OH-3
4	7.18	112.9	
5		165.9	H-4, CH_3-6
6		134.9	CH_3-6
6-CH_3	2.11	8.83	
COOH		168.3	
CHO		198.0	H-4, 3-OH
3-OH	12.98		
5-OH	10.52		

TABLE 2. NMR Data of Compound **2** (acetone, δ, ppm)

C atom	^{1}H NMR	^{13}C NMR	HMBC (C to H)
1		117.0	CH_3-6
2		114.3	H-4, H-CHO
3		167.5	H-4, OCH_3-3
4	7.16	113.0	
5		163.1	OCH_3-5, CH_3-6
6		134.8	CH_3-6
6-CH_3	2.08	8.15	
COOH		168.0	
CHO	10.4	198.0	
OCH_3-3	3.94	57.3	
OCH_3-5	4.00	53.4	

Compound **2** was isolated as a green amorphous powder with spectroscopic properties similar to **1**. EIMS delivered a mass of 224 and HREIMS determined the formula to be $C_{11}H_{12}O_5$. The $FeCl_3$ did not show a positive reaction. The ^{1}H and ^{13}C NMR (Table 2) spectra, including DEPT, showed one methyl, two oxymethyls, one carboxyl and one aldehyde. The ^{1}H NMR data of **2** had two more oxymethyl signals (3.94, 4.00) than **1** and one less signal of the chelated hydroxy (12.98). The correlation in the HMBC experiment from CH_3-6 to C-1, C-5, and C-6 indicated the positions of methyl. The correlation of OCH_3-3 and H-4 to C-3, CH_3-6 and OCH_3-5 to C-5 revealed the position of the two oxymethyls. The structure of **2** (Fig. 1) was thus defined on the basis of the analysis of 1D and 2D NMR spectroscopic experiments as 2-formyl-3,5-dimethoxy-6-methylbezoic acid.

Furthermore, the known compound **3** was identified by comparison of its spectroscopic data with that of literature as purpactin A [4], which is an inhibitor of acyl-CoA [5].

EXPERIMENTAL

General Experimental Procedures. Melting points were detected on a Fisher-Johns hot-stage apparatus and were uncorrected. NMR data were recorded on a Varian Inova-500 NB spectrometer, using acetone as solvent and TMS as internal standard. Mass spectra were acquired on a VG-ZAB mass spectrometer. IR spectra were obtained on a Nicolet 5DX-FTIR spectrophotometer. Column chromatography was carried out on silica gel (200-300 mesh; Qingdao Haiyang chemicals).

Fungus Strain. A strain of the fungus No. ZZF13 was isolated from the Zhanjiang sea area, China; the species was unidentified. This fungus was stored in the Department of Biology and Chemistry, City University of Hong Kong, Hong Kong, and the Department of Applied Chemistry, Zhongshan University, Guangzhou, PR China.

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 a twofold volume 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** (10.2 mg) and **2** (5.6 mg)

Compound 1: mp 280-281°C. HREIMS: *m/z* 196.0364 $[M]^+$ (calc. for C_9H_8O5, 196.0366). IR (KBr, ν_{max}): 3378, 3111, 2956, 2597, 1697, 1594, 1479, 1410, 1382, 1328, 1227, 1195, 1169, 1121, 1036 cm^{-1}. For ^1H NMR (500 MHz, acetone) and ^{13}C NMR (125 MHz, acetone-d_6) spectra, see Table 1.

Compound 2: mp 75–76°C. HREIMS: *m/z* 224.0679 $[M]^+$ (calc. for $C_{16}H_{20}O_8$, 224.0679). IR (KBr, ν_{max}): 2926, 2856, 1719, 1634, 1579, 1496, 1405, 1360, 1311, 1245, 1207, 1182, 1144, 1070, 1045, 1007 cm^{-1}. For ^1H NMR (500 MHz, acetone) and ^{13}C NMR (125 MHz, acetone-d_6) spectra, see Table 2.

ACKNOWLEDGMENT

This work was supported by the 863 Foundation of China (2006AA09Z422, 2006AA09Z419), the National Natural Science Foundation of China (20572136), and the Natural Science Foundation of Guangdong Provincial (05003268), Guangzhou Project of Science & Technology Planning (No. 2004 J1-C0061).

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