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A NEW XANTHONE *O*-GLYCOSIDE FROM THE MANGROVE ENDOPHYTIC FUNGUS *Phomopsis* sp.

Zhongjing Huang,[1] Jianxiang Yang,[2] Fuhou Lei,[1*] Zhigang She,[3] and Yongcheng Lin[3]

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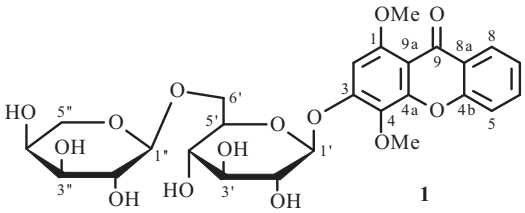
A new xanthone O*-glycoside, 3-*O*-(6-*O*-α-L-arabinopyranosyl)-β-D-glucopyranosyl-1,4-dimethoxyxanthone (**1**), together with three known compounds, phomapyrone D (**2**), 2-methoxy-3,4-methylenedioxybenzophenone (**3**), and cyclo(D-6-Hyp-L-Phe) (**4**), was isolated from the mangrove endophytic fungus,* Phomopsis *sp. (ZH76). Their structures were determined by analysis of spectroscopic data. Compound **1** inhibited HEp-2 and HepG2 cells with IC_{50} values of 9 and 16 μmol/mL, respectively.*

Keywords: xanthone *O*-glycoside, mangrove endophytic fungus, structure elucidation, cytotoxic activity.

Mangrove endophytic fungi have received some attention, and some promising compounds with unique chemical structures and potent activities have been isolated [1–5]. This paper reports the isolation, structural elucidation, and biological activities of a new xanthone *O*-glycoside, 3-*O*-(6-*O*-α-L-arabinopyranosyl)-β-D-glucopyranosyl-1,4-dimethoxyxanthone (**1**), together with three known compounds, phomapyrone D (**2**), 2-methoxy-3,4-methylenedioxybenzophenone (**3**), and *cyclo*(D-6-Hyp-L-Phe) (**4**), from the mangrove endophytic fungus *Phomopsis* sp. ZH76 that was isolated from the stem of the mangrove tree *Excoecaria agallocha* from Dong Zai, Hainan, China.

Compound **1** was obtained as a yellow amorphous solid. The molecular formula of $C_{26}H_{30}O_{14}$ was established by HR-FAB-MS (*m/z* 565.1552, calcd for $[M - H]^-$ 565.1557) and the NMR spectral data (Table 1). The UV absorption bands at 232, 258, 299, and 376 nm and IR (KBr) absorption bands at 3468, 1658, 1605, 1589, and 1478 cm^{-1} suggested the presence of the xanthone skeleton [6]. On acid hydrolysis, **1** gave glucose and arabinose, suggesting that **1** was a xanthone glycoside.

The ^{1}H NMR spectrum exhibited four aromatic protons at δ 7.64 (d, J = 8.3 Hz), 7.78 (t, J = 8.3 Hz), 7.42 (t, J = 7.5 Hz), and 8.09 (d, J = 7.8 Hz), and one aromatic proton at 6.87 (s), indicating that one aromatic ring was a 1,2-disubstituted benzene ring and the other was a pentasubstituted benzene ring. The ^{1}H NMR spectrum also showed the signals of one anomeric proton of Glc at δ 4.97 (d, J = 7.5 Hz), one anomeric proton of Ara at 4.17 (d, J = 6.5 Hz), and two aromatic methoxyl groups at 3.85 and 3.81. The remaining proton signals upfield were attributable to two sugar moieties.



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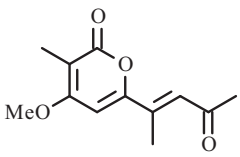

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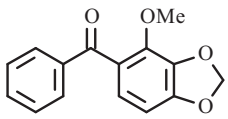

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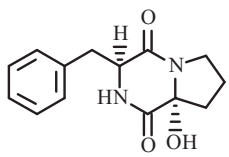

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1) College of Chemistry and Ecology Engineering, Guangxi University for Nationalities, 530006, Nanning, P. R. China, e-mail: leifuhou@163.com; 2) Department of Chemistry and Engineering, Guilin Medical University, 541004, Guilin, P. R. China; 3) College of Chemistry and Chemical Engineering, Sun Yat-Sen (Zhongshan) University, 510275, Guangzhou, P. R. China. Published in *Khimiya Prirodnykh Soedinenii*, No. 1, January–February, 2013, pp. 27–29. Original article submitted September 24, 2011.

TABLE 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data for **1** (DMSO-d_6, δ, ppm, J/Hz)

C atom	δ_C (DEPT)	δ_H	HMBC
1	156.8 (C)		H-2, 1-OCH_3
2	96.3 (CH)	6.87 (s)	
3	157.0 (C)		H-2, 1′
4	138.5 (C)		H-2, 4-OCH_3
4a	148.2 (C)		
4b	154.4 (C)		H-5, 6, 8
5	117.8 (CH)	7.64 (d, J = 8.3)	H-6, 7
6	134.6 (CH)	7.78 (t, J = 8.3)	H-5, 7, 8
7	124.1 (CH)	7.42 (t, J= 7.5)	H-5, 6, 8
8	125.6 (CH)	8.09 (d, J = 7.8)	H-6, 7
8a	121.6 (C)		H-5, 7, 8
9	179.8 (C)		H-8
9a	110.9 (C)		H-2
1-OCH_3	56.3 (CH_3)	3.81 (s)	
4-OCH_3	61.7 (CH_3)	3.85 (s)	
Glc-1′	101.5 (CH)	4.97 (d, J = 7.5)	H-2′, 3′, 5′
2′	73.4 (CH)	3.35 (m)	H-1′, 3′
3′	76.1 (CH)	3.37 (m)	H-1′, 2′, 4′
4′	69.9 (CH)	3.23 (m)	H-5′
5′	75.6 (CH)	3.62 (m)	H-1′, 4′, 6′
6′	68.3 (CH_2)	3.96 (m), 3.55 (dd, J = 6.5, 11.0)	H-5′, 1″
Ara-1″	103.4 (CH)	4.17 (d, J = 6.5)	H-6′, 2″, 3″
2″	70.5 (CH)	3.46 (t, J = 8.0)	H-1″, 3′, 4″
3″	72.7 (CH)	3.35 (m)	H-2″, 4″
4″	67.3 (CH)	3.58 (m)	H-2″, 3″, 5″
5″	64.8 (CH_2)	3.65 (dd, J = 4.0, 11.6), 3.30 (m)	H-1″, 4″

In the HMBC spectrum cross-peaks from H-2 (δ_H 6.87) to C-1, C-3, C-4, and C-9a revealed the presence of a 1,3,4-trisubstituted xanthone. Two methoxyl groups (δ_H 3.81 and 3.85) were placed at C-1 and C-4, respectively, as determined by the HMBC cross-peaks of the former proton with C-1, and of the latter proton with C-4. The HMBC cross-peaks from H-1′ (δ_H 4.97) of Glc to C-3 (δ_C 157.0) of the aglycon and from H-1″ (δ_H 4.17) of Ara to C-6′ (δ_C 68.3) of Glc showed that the glucosyl residue was linked to C-3 of the aglycon, and the arabinosyl residue was linked to the glucosyl moiety by a (1″→6′) linkage. The anomeric configuration of the glucosyl residue was deduced to be β from the J value (J = 7.5 Hz) of anomeric proton, and that of the arabinosyl residue to be α by comparison of the ^{13}C NMR data [7]. So, compound **1** was identified as 3-*O*-(6-*O*-α-L-arabinopyranosyl)-β-D-glucopyranosyl-1,4-dimethoxyxanthone.

In addition, three known compounds, **2**, **3**, and **4**, were identified as phomapyrone D, 2-methoxy-3,4-methylenedioxybenzophenone, and *cyclo*(D-6-Hyp-L-Phe), respectively, through the comparison of spectroscopic data with those in the literature [8–10].

Primary bioassays showed that **1** exhibited cytotoxicity against HEp-2 and HepG2 cells with IC_{50} values of 9 and 16 μg/mL, respectively.

EXPERIMENTAL

General Methods. NMR data were recorded in DMSO-d_6 or $CDCl_3$, using TMS as internal reference, on a Varian INOVA 500 NB nuclear magnetic resonance spectrometer (^{1}H, 500 MHz; ^{13}C, 125 MHz). Mass spectra were recorded on a Thermo VG ZAB-HS double-focussing mass spectrometer or a Thermo MAT95XP high-resolution mass spectrometer. IR spectra were measured on a Bruker Equinox 55 spectrophotometer. UV spectra were obtained on a Shimadzu UV-2501PC spectrophotometer. Optical rotations were measured on a Schmidt + Haensch Polartronic HH W5 polarimeter and were uncorrected. Column chromatography was performed on silica gel (200–300 mesh, Qingdao, China), and TLC on precoated GF_{254} plates (Qiangdao, China). β-D-glucose (Sigma) and α-L-arabinose (Sigma) were used as reference compounds.

Fungus Material and Culture Conditions. The fungus *Phomopsis* sp. (ZH76) was isolated from the stem of *Excoecaria agallocha* of the mangrove tree Euphorbiaceae from Dong Sai of the South China Sea coast. Starter cultures were maintained on cornmeal seawater agar. *Phomopsis* sp. (ZH76) was stored at Sun Yat-Sen (Zhongshan) University, Guangzhou, P. R. China. Plugs of agar supporting mycelium growth were cut and transferred aseptically into 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 25 g/L). The flask was incubated at 30°C on a rotary shaker for 6 days. The mycelium was aseptically transferred to 1000 mL Erlenmeyer flasks containing culture liquid (600 mL) and incubated at room temperature for 30 days.

Extraction and Separation of Metabolites. All cultures (90 L) were separated into mycelium and filtrate. The mycelium was dried in room temperature and then immersed in methanol (6 L) for 20 days. The methanol extract was evaporated under reduced pressure to yield a brown gum (48 g). The gum was chromatographed repeatedly on silica gel using gradient elution from petroleum ether–ethyl acetate (9:1, 8:2, 7:3, 5:5, and 3:7), providing fractions A, B, C, D, and E. Fraction B was repeatedly purified by column chromatography on silica gel using petroleum ether–ethyl acetate (9:1, 8:2, 7:3, and 6:4), giving fractions B_1, B_2, B_3, and B_4. Fraction B_3 was subjected to column chromatography on silica gel eluting with petroleum ether–ethyl acetate (8:2) to give compound **1** as a yellow amorphous solid (7 mg). Fraction C was purified by column chromatography on silica gel with petroleum ether–ethyl acetate (8:2, 7:3) to afford compound **2** as an amorphous white solid (8 mg) and compound **3** as a colorless oil (5 mg), respectively. Fraction D was purified by column chromatography on silica gel with petroleum ether–ethyl acetate (6:4) to give compound **4** as a white solid (10 mg).

3-*O*-(6-*O*-α-L-Arabinopyranosyl)-β-D-glucopyranosyl-1,4-dimethoxyxanthone (1). Yellow amorphous solid. $[\alpha]_D^{25}$ –62.0° (*c* 0.41, CH_3OH). UV spectrum (CH_3OH, λ_{max}, nm) (log ε): 341 (4.35), 298 (4.68), 277 (4.14), 243 (3.90). IR spectrum (KBr, ν, cm^{-1}): 3434, 2942, 2886, 1615, 1469, 1422, 1316.

Mass spectrum (FAB, *m/z*, I_{rel}, %): 565 $[M - H]^-$ (25), 433 $[M - H - 132]^-$ (10), 271 $[M - H - 132 - 162]^-$ (100). Mass spectrum (HR-FAB, *m/z*, I_{rel}, %): 565.1557 $[M - H]^-$, (calcd for $C_{26}H_{29}O_{14}$, 565.1557). ^1H, ^{13}C NMR see Table 1.

Phomapyrone D (2). Amorphous white solid. ^1H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.14 (1H, s), 6.53 (1H, s), 3.95 (3H, s), 2.35 (6H, s), 2.05 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 199.7 (C), 165.2 (C), 164.5 (C), 158.2 (C), 139.8 (C), 126.5 (CH), 97.5 (CH), 56.7 (CH_3), 33.0 (CH_3), 13.9 (CH_3), 9.4 (CH_3).

2-Methoxy-3,4-methylenedioxybenzophenone (3). Colorless oil. ^1H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.77 (2H, br.dd, J = 8.0, 1.6), 7.52 (1H, tt, J = 8.0, 1.6), 7.42 (2H, br.t, J = 8.0), 6.94 (1H, d, J = 8.0), 6.58 (1H, d, J = 8.0), 5.59 (2H, br.s), 3.81 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 195.0 (C), 151.2 (C), 141.8 (C), 138.3 (C), 136.8 (C), 132.6 (CH), 129.5 (2 × CH), 128.0 (2 × CH), 126.1 (C), 124.3 (CH), 102.7 (CH), 101.5 (CH_2), 60.0 (CH_3).

***Cyclo*(D-6-Hyp-L-Phe) (4)**. White solid. ^1H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.36 (2H, t, J = 7.3), 7.29 (1H, t, J = 7.3), 7.23 (2H, d, J = 7.3), 5.79 (s), 4.25 (1H, dd, J = 9.9, 3.6), 3.65 (2H, m), 3.62 (1H, dd, J = 14.3, 3.6), 2.73 (1H, dd, J = 14.3, 9.9), 2.32 (1H, m), 1.93 (1H, m), 1.86 (2H, m). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 168.1 (C), 166.1 (C), 135.7 (C), 129.3 (2 × CH), 129.2 (2 × CH), 127.6 (CH), 87.6 (C), 55.8 (CH), 45.4 (CH_2), 41.0 (CH_2), 36.8 (CH_2), 19.9 (CH_2).

Acidic Hydrolysis of Compound 1. Compound **1** (3 mg) in 50% MeOH (3 mL) containing 5% HCl was refluxed in a boiling H_2O bath for 5 h. After cooling, the reaction mixture was poured into 10 mL of H_2O. The mixture was extracted with ethyl acetate, and the aqueous residue was then checked by co-TLC together with authentic sugar β-D-glucose and α-L-arabinose (EtOAc–MeOH–H_2O–AcOH, 13:4:4:5). Acid hydrolysis of **1** gave β-D-glucose (R_f 0.46) and α-L-arabinose (R_f 0.53).

Bioasays. The cytotoxic assays were performed using the MTT assay method [11]. In the preliminary bioassay, compound **1** inhibited cytotoxicity against HEp-2 and HepG2 cells with IC_{50} values of 9 and 16 μg/mL, respectively.

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