

## A NEW BENZOPHENONE FROM *Dobinea delavayi*

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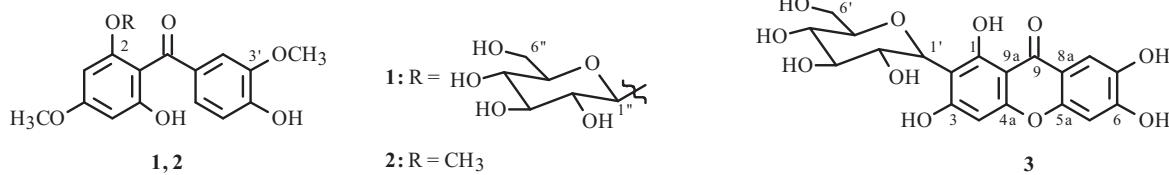
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*A new benzophenone sesquiterpene dobiniside A (1), together with two known compounds, 4',6-dihydroxy-2,3',4-trimethoxybenzophenone (2) and mangiferin (3), were isolated from the root of Dobinea delavayi. The structure of the new compound was determined on the basis of spectroscopic analysis, including 1D, 2D NMR, and FAB-MS techniques as well as by comparison of the spectral data with those of related compounds reported in the literature. Compounds 1–3 were screened for antitumor activity in vitro, and compound 3 was shown to possess antitumor activity with  $IC_{50}$  value of  $7.4 \times 10^{-5} \mu\text{M}$  for A-549.*

**Keywords:** *Dobinea delavayi*, benzophenone, dobiniside A.

*Dobinea* Buch.-Ham. ex D. Don is a special genus with only two species endemic to east Asia, and *Dobinea delavayi* is one of this genus, placed in three different families, the Podoaceae, tribe Acerineae of the Sapindaceae, and tribe Dobineae of the Anacardiaceae [1]. The root of *D. delavayi* has been used as an antitumor agent in Chinese folk medicine. Only one publication reported on the chemical constituents of this plant [2]. In the search for new antitumor compounds from *D. delavayi*, we carried out a chemical investigation of the root, collected from Kunming, Yunnan Province, People's Republic of China, which led to the isolation of three benzophenones, including a new one. This paper describes the isolation and structural elucidation of the new benzophenone 1 and the screening for antitumor activity *in vitro*, whereby it was shown that compound 3 possessed antitumor activity.

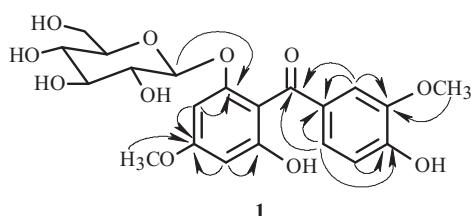
Dobiniside A (1) was obtained as yellow needle crystals with the molecular formula  $C_{21}H_{24}O_{11}$  as evidenced by the HR-ESI-MS ( $m/z$  451.1230 [ $M - H^-$ ]) and  $^{13}\text{C}$  NMR spectra, indicating ten unsaturation degrees. The IR absorption bands at  $3442 \text{ cm}^{-1}$ , 1618, 1513, and  $1463 \text{ cm}^{-1}$  showed the presence of OH and aromatic ring, respectively. The  $^1\text{H}$  NMR spectrum of compound 1 clearly showed protons of two  $\text{OCH}_3$  at  $\delta$  3.80 and 3.87 and five aromatic protons at 6.18, 6.40 (each d,  $J = 2.0 \text{ Hz}$ ), 6.82 (d,  $J = 8.8 \text{ Hz}$ ), 7.30 (dd,  $J = 1.8, 8.8 \text{ Hz}$ ), and 7.45 (d,  $J = 1.8 \text{ Hz}$ ). The  $^{13}\text{C}$  NMR (DEPT) spectroscopic data (Table 1) revealed the presence of two methyls, one methylene, 10 methines, and eight quaternary carbons.



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TABLE 1.  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data of Dobiniside A (**1**) ( $\text{CD}_3\text{OD}$ ,  $\delta$ , ppm, J/Hz)

C atom	$\delta_{\text{H}}$	$\delta_{\text{C}}$	C atom	$\delta_{\text{H}}$	$\delta_{\text{C}}$
1	—	111.6 (s)	6'	7.30 (dd, $J = 1.8, 8.8$ )	127.0 (d)
2	—	158.9 (s)	1''	4.88 (d, $J = 7.7$ )	102.4 (d)
3	6.18 (d, $J = 2.0$ )	96.7 (d)	2''	3.16 (m)	74.8 (d)
4	—	164.2 (s)	3''	3.36 (m)	78.3 (d)
5	6.40 (d, $J = 2.0$ )	94.8 (d)	4''	3.38 (m)	71.2 (d)
6	—	158.4 (s)	5''	3.28 (m)	77.8 (d)
1'	—	132.1 (s)	6''	3.66 (m); 3.84 (m)	62.6 (t)
2'	7.45 (d, $J = 1.8$ )	113.1 (d)	3'-OCH <sub>3</sub>	3.87 (s)	56.4 (q)
3'	—	148.7 (s)	4-OCH <sub>3</sub>	3.80 (s)	56.0 (q)
4'	—	153.3 (s)	7-C=O	—	197.0 (s)
5'	6.82 (d, $J = 8.8$ )	115.6 (d)			

Fig. 1. Key HMBC correlations of compound **1**.

The carbon signals at  $\delta$  102.4 (d), 78.3 (d), 77.8 (d), 74.8 (d), 71.2 (d), and 62.6 (t) suggested the presence of one glucose, which was further confirmed by its MS fragmentation peaks at  $m/z$  389 [ $\text{M} - \text{H} - \text{Glc}$ ]<sup>-</sup>. The coupling constant of the anomeric proton ( $J = 7.7$  Hz) suggested that the glucose moiety was a  $\beta$ -type sugar. Comparison of the NMR data with those reported in the literature showed that compound **1** was similar to 4',6-dihydroxy-2,3',4-trimethoxybenzophenone (**2**) [3] except for the difference that one additional glucose unit in **1** was replaced by the methoxy group at C-2 in **2**. In the HMBC (Fig. 1) spectroscopic spectrum, correlations of two OCH<sub>3</sub> ( $\delta$  3.87, s; 3.80, s) with C-3' (148.7, s) and C-4 (164.2, s) and of H-1'' with C-2 (158.9, s) were observed, which confirmed the positions of the two OCH<sub>3</sub> and glucose to the aromatic ring. Based on the above evidence, compound **1** was identified as 4',6-dihydroxy-3',4-dimethoxy-2-O- $\beta$ -D-glucopyranosyl benzophenone, named dobiniside A.

Compounds **2** and **3** were identified as 4',6-dihydroxy-2,3',4-trimethoxybenzophenone (**2**) and mangiferin (**3**) by detailed comparison of the spectral data with those reported in the literature [3, 4]. The  $^{13}\text{C}$  NMR spectroscopic data show that the carbonyl in mangiferin was at  $\delta$  179.2.

## EXPERIMENTAL

**General Comments.** Melting points were measured on an XRC-1 micro-melting point apparatus and were uncorrected. MS spectra were obtained on a VG Auto Spec-3000 mass spectrometer. 1D and 2D NMR spectra were recorded on Bruker AM-400 MHz spectrometers, with chemical shifts ( $\delta$ ) in ppm relative to TMS as internal standard and coupling constants in hertz (Hz). IR spectra were measured with a Bio-Rad FTS-135 spectrometer with KBr pellets. UV spectra were measured on a Hitachi UV-3210 spectrophotometer. Silica gel (200–300 mesh) for column chromatography was a product of the Qingdao Marine Chemical Ltd., Qingdao, P. R. China. Sephadex LH-20 for chromatography was purchased from Amersham Biosciences. Reversed-phase chromatography was with RP-18 (LiChroprep, 40–63  $\mu\text{m}$ , Merck, Darmstadt, Germany).

**Plant Materials.** The root of *D. delavayi* was collected in Kunming, Yunnan Province, People's Republic of China, in September 2009, and authenticated by Prof. Hua Peng.

**Extraction and Isolation.** The air-dried and powdered root of *D. delavayi* (10 kg) was extracted three times each with 20 L of 95% EtOH under reflux for 3 h. The extracts were evaporated and the residue was resuspended in 10 L of H<sub>2</sub>O and partitioned successively with EtOAc (5 L  $\times$  3) and *n*-BuOH (5 L  $\times$  3) to yield the EtOAc extract (500 g) and the *n*-BuOH extract (210 g), respectively. The *n*-BuOH extract was applied to a silica gel column chromatograph (200–300 mesh) eluted

with  $\text{CH}_3\text{Cl}-\text{CH}_3\text{OH}$  (10:1, v/v) to give five fractions. Fraction 1 (15 g) was purified on Sephadex LH-20 with  $\text{CH}_3\text{OH}-\text{CH}_3\text{Cl}$  1:1 and crystallized in  $\text{CH}_3\text{OH}$  to give **2** (30 mg). Subfraction 2 (10 g) was purified on Sephadex LH-20 with  $\text{CH}_3\text{OH}$ , and then subjected to RP-18 silica gel with 10%→100% aqueous  $\text{CH}_3\text{OH}$  to afford compounds **1** (15 mg) and **3** (80 mg).

**Dobiniside A (1)**. Yellow needle powder, mp 108–110°C,  $[\alpha]_D^{21}-30.4065^\circ$  (*c* 0.2.05,  $\text{CH}_3\text{OH}$ ). UV ( $\text{CH}_3\text{OH}$ ,  $\lambda_{\max}$ , nm) ( $\log \epsilon$ ): 310 (4.050), 285 (4.077), 227 (4.246), 205 (4.678), 191 (4.273). IR (KBr, *v*,  $\text{cm}^{-1}$ ): 3442, 1759, 1618, 1513, 1463, 1237, 1045.  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ ) and  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ ), see Table 1. HR-ESI-MS *m/z* 451.1230 [ $\text{M}-\text{H}$ ]<sup>−</sup> (calcd for  $\text{C}_{21}\text{H}_{23}\text{O}_{11}$ , 451.1240); EI-MS *m/z* (%) 452 (100), 389 (80), 289 (85), 227 (45).

**4',6-Dihydroxy-2,3',4-trimethoxybenzophenone (2)**.  $\text{C}_{16}\text{H}_{16}\text{O}_6$ , yellow solid, mp 161–162°.  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ ,  $\delta$ , ppm, J/Hz): 7.25 (1H, d, *J* = 2.0, H-2'), 7.15 (1H, dd, *J* = 2.0 and 7.5, H-6'), 6.88 (1H, d, *J* = 7.5, H-5'), 6.15 (1H, d, *J* = 2.0, H-5), 6.17 (1H, d, *J* = 2.0, H-3), 3.84 (3H, s, 3'-OMe), 3.75 (3H, s, 4-OMe), 3.58 (3H, s, 2-OMe).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ ,  $\delta$ , ppm): 197.5 (C-7), 166.2 (C-4), 165.1 (C-6), 158.0 (C-2), 148.5 (C-4'), 146.0 (C-3'), 134.2 (C-6'), 124.5 (C-1'), 110.8 (C-2'), 110.3 (C-5'), 105.7 (C-1), 93.5 (C-5), 92.3 (C-3), 56.0 (OCH<sub>3</sub>), 55.6 (OCH<sub>3</sub>), 55.2 (OCH<sub>3</sub>). FAB-MS (−) *m/z*: 303 [ $\text{M}-\text{H}$ ]<sup>−</sup> (100), 289 (9), 265 (10), 231 (2), 123 (20), 99 (55).

**Mangiferin (3)**. Yellow powder,  $\text{C}_{19}\text{H}_{18}\text{O}_{11}$ , mp 278–280°C. FAB-MS (+) *m/z*: 423 [ $\text{M}-\text{H}$ ]<sup>−</sup>, 397 (4), 357 (6), 282 (1), 111 (1).  $^1\text{H}$  NMR (500 MHz,  $\text{C}_5\text{D}_5\text{N}$ ,  $\delta$ , ppm, J/Hz): 8.10 (1H, s, H-5), 7.20 (1H, s, H-8), 6.36 (1H, s, H-4), 4.64 (1H, d, *J* = 8.8, H-1'), 3.98–3.12 (6H, H-2'–6').  $^{13}\text{C}$  NMR (125 MHz,  $\text{C}_5\text{D}_5\text{N}$ ,  $\delta$ , ppm): 180.4 (C-9), 165.5 (C-3), 163.4 (C-1), 157.6 (C-4a), 156.1 (C-6), 152.1 (C-5a), 145.6 (C-7), 113.2 (C-8a), 109.3 (C-8), 109.0 (C-2), 103.5 (C-5), 102.9 (C-9a), 94.3 (C-4), 83.2 (C-5'), 80.9 (C-3'), 75.6 (C-1'), 72.8 (C-4'), 72.2 (C-2'), 63.1 (C-6').

**Antitumor Activity Test.** Antitumor activities of compounds **1–3** were evaluated *in vitro* using HL-60 and A-549 cells by methods of SRB (sulforhodamine B) and MTT (methyl thiazol tetrazolium), respectively. The results suggested that compound **3** possessed remarkable antitumor activity with an  $\text{IC}_{50}$  value of  $7.4 \times 10^{-5}$   $\mu\text{M}$  for A-549.

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