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## Three new bibenzyl derivatives from *Dendrobium nobile*

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Three new bibenzyl derivatives have been isolated from the stems of *Dendrobium nobile* Lindl. (Orchidaceae). Their structures were established as 4,5-dihydroxy-3,3', $\alpha$ -trimethoxybibenzyl (**1**), 4,4'-dihydroxy-3,3',5, $\alpha$ -tetramethoxybibenzyl (**2**) and 4-hydroxy-3,3',4',5, $\alpha$ -pentamethoxybibenzyl (**3**) on the basis of spectroscopic analysis.

**Keywords:** *Dendrobium nobile*; Orchidaceae; Bibenzyl derivatives; *Dendrobium nobile*

### 1. Introduction

The traditional Chinese medicine “Shi Hu”, derived from the dried or fresh stems of several *Dendrobium* species (Orchidaceae), are widely used as a Yin tonic to nourish the stomach and promote the production of body fluid [1]. Of these, *Dendrobium nobile* Lindl. is one of the most famous and has been recorded in the Chinese Pharmacopoeia (2005 Edition) as one of the original materials of “Shi Hu”. Several chemical components of this species have been identified before [2–6], and some of which exhibited antitumor, antimutagenic and immunomodulatory activities [3,6,7]. In order to discover more bioactive components, a systematical study on the chemical constituents of *Dendrobium nobile* has been undertaken. Herein, we report the isolation and structure elucidation of three new bibenzyl derivatives from the 60% ethanol extract of the stems of *Dendrobium nobile* Lindl.

### 2. Results and discussion

Compound **1** was obtained as a colorless oil,  $[\alpha]_D^{27} + 1.1$  (*c* 0.7, MeOH). The molecular formula of C<sub>17</sub>H<sub>20</sub>O<sub>5</sub> was determined by HREIMS (*m/z* 304.1300, [M]<sup>+</sup>), which was compatible with the

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results of ESIMS ( $m/z$  327.1,  $[M + Na]^+$ ;  $m/z$  303.1,  $[M - H]^-$ ) and NMR analysis. UV absorptions  $\lambda_{\max}$  (MeOH) at 273 and 280 nm ( $\log \epsilon$  2.51 and 2.48) showed characteristic of bibenzyls. The  $^1H$  NMR spectrum of **1** displayed signals for two phenolic hydroxyl protons at  $\delta$  5.34 (2H, disappearing on deuterium exchange), three methoxyl groups at  $\delta$  3.19 (3H, s), 3.75 (3H, s) and 3.83 (3H, s), one methine proton at  $\delta$  4.19 (1H, dd,  $J = 7.5, 5.7$  Hz), two methylene protons at  $\delta$  2.83 (1H, dd,  $J = 13.8, 5.7$  Hz) and 3.04 (1H, dd,  $J = 13.8, 7.5$  Hz) and six aromatic protons at  $\delta$  6.34 (1H, d,  $J = 1.7$  Hz), 6.51 (1H, d,  $J = 1.7$  Hz), 6.66 (1H, m), 6.71 (1H, m), 6.73 (1H, m), 7.15 (1H, t,  $J = 7.9$  Hz). In the  $^{13}C$  NMR and DEPT spectra of **1**, seventeen carbon signals belonging to three methyls (oxygenated), one methylene, one methine (oxygenated), six aromatic methines and six aromatic quaternary carbons (four oxygenated) were observed. According to the  $^1H$  and  $^{13}C$  NMR data, a bibenzyl skeleton with two hydroxyl and three methoxyl groups was deduced to the structure of **1**. But the significant difference between **1** and the other bibenzyls reported [5,8,9] was observed at benzylic protons. The four equivalent benzylic proton signals in upfield region in the other known bibenzyls were replaced by the signals for one oxygenated methine and two methylene protons in **1**. In combination with the  $^{13}C$ - $^1H$  long-range correlation between  $\delta$  3.19 (H- $\alpha$ -OCH<sub>3</sub>) and  $\delta$  84.9 (C- $\alpha$ ) in the HMBC spectrum, the fragment of one benzylic proton substituted by a methoxyl group was determined. A pair of *meta*-coupled doublets at  $\delta$  6.34 (1H, d,  $J = 1.7$  Hz) and 6.51 (1H, d,  $J = 1.7$  Hz) in  $^1H$  NMR spectrum of **1** revealed a 1,3,4,5-substituted benzene ring, then the remaining aromatic protons should be in another benzene ring, which consisted of a 1,3-substituted one. In the HMBC spectrum of **1**,  $^{13}C$ - $^1H$  long-range correlations were found for H-2/C-3,4,6, $\alpha$ ; H-6/C-2,4,5, $\alpha$ ; H-2'/C-3',4',6', $\alpha'$ ; H-4'/C-3',6'; H-5'/C-1',3'; H-6'/C-2',4', $\alpha'$ ; H-4,5-OH/C-4,5; H-3-OCH<sub>3</sub>/C-3 and H-3'-OCH<sub>3</sub>/C-3', which resulted in the positions of hydroxyl and methoxyl groups and the assignment of all proton and carbon signals of **1**. Thus, the structure of **1** was established as 4,5-dihydroxy-3,3', $\alpha$ -trimethoxybibenzyl (figure 1), and designated as nobilin A.

Compound **2** was obtained as a yellow solid,  $[\alpha]_D^{27} + 1.4$  ( $c$  1.0, MeOH). The molecular formula of C<sub>18</sub>H<sub>22</sub>O<sub>6</sub> was determined by HREIMS ( $m/z$  334.1402,  $[M]^+$ ), which was compatible with the results of ESIMS ( $m/z$  357.1,  $[M + Na]^+$ ;  $m/z$  333.1,  $[M - H]^-$ ) and NMR analysis. UV absorption  $\lambda_{\max}$  (MeOH) at 280 nm ( $\log \epsilon$  2.41) was similar to other bibenzyls. The IR spectrum of **2** indicated the presence of hydroxyl group(s) (3471 cm<sup>-1</sup>) and aromatic ring(s) (1616, 1516, 1454 cm<sup>-1</sup>) in its structure. The  $^1H$  NMR spectrum showed signals for two phenolic hydroxyl protons at  $\delta$  5.46 (2H, disappearing on deuterium exchange), four methoxyl groups at  $\delta$  3.22 (3H, s), 3.78 (3H, s) and 3.84 (6H, s), one methine proton at  $\delta$  4.17 (1H, dd,  $J = 7.0, 6.2$  Hz), two methylene protons at  $\delta$  2.78 (1H, dd,  $J = 13.7, 6.2$  Hz) and 3.02 (1H, dd,  $J = 13.7, 7.0$  Hz) and five aromatic protons, appearing as a two-proton singlet at  $\delta$  6.42 (2H, s) and an ABX coupling system at  $\delta$  6.79 (1H, d,  $J = 8.0$  Hz),

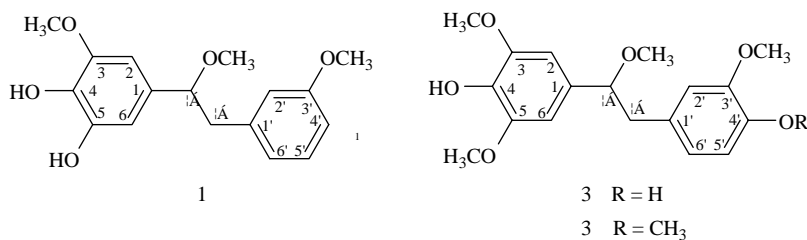


Figure 1. Structures of compounds 1–3.

6.61 (1H, dd,  $J = 8.0, 1.9$  Hz) and 6.50 (1H, d,  $J = 1.9$  Hz). Eighteen carbon signals due to four methyls (oxygenated), one methylene, one methine (oxygenated), five aromatic methines and seven aromatic quaternary carbons (five oxygenated) were observed in the  $^{13}\text{C}$  NMR and DEPT spectra of **2**. A bibenzyl skeleton with two hydroxyl and four methoxyl groups was proposed to the structure of **2**. Analysis of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **1** and **2** showed that the difference between these two compounds was the number and position of substituted groups in aromatic rings. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra suggested a symmetrical aromatic ring by the signals found at  $\delta$  6.42 (2H, s, H-2 and H-6), 3.84 (6H, s, H-3-OCH<sub>3</sub> and H-5-OCH<sub>3</sub>), and  $\delta$  103.4 (C-2 and C-6), 146.9 (C-3 and C-5), 56.3 (C-3-OCH<sub>3</sub> and C-5-OCH<sub>3</sub>). The remaining aromatic methoxyl and hydroxyl groups were assigned to the other benzene ring, which was a 1,3,4-substituted one.  $^{13}\text{C}$ - $^1\text{H}$  long-range correlation signals were observed between H-2,6/C-3,4,5, $\alpha$ ; H-2'/C-3',4',6', $\alpha'$ ; H-5'/C-1',3',4',6'; H-6'/C-2',4', $\alpha'$ ; H-4,4'-OH/C-4,4'; H-3,5-OCH<sub>3</sub>/C-3,5; H-3'-OCH<sub>3</sub>/C-3' and H- $\alpha$ -OCH<sub>3</sub>/C- $\alpha$  in HMBC spectrum of **2**. NOE correlation between H-2' and H-3'-OCH<sub>3</sub> was found in its NOESY spectrum. Based on the above evidence, the structure of **2** was deduced to be 4,4'-dihydroxy-3,3',5, $\alpha$ -tetramethoxybibenzyl (figure 1), and designated as nobilin B.

Compound **3** was obtained as a colorless oil,  $[\alpha]_{\text{D}}^{27} + 2.2$  ( $c$  0.8, MeOH). The molecular formula of C<sub>19</sub>H<sub>24</sub>O<sub>6</sub> was determined by HREIMS ( $m/z$  348.1554,  $[\text{M}]^+$ ), which was compatible with the results of ESIMS ( $m/z$  371.1,  $[\text{M} + \text{Na}]^+$ ;  $m/z$  347.1,  $[\text{M} - \text{H}]^-$ ) and NMR analysis. UV absorption  $\lambda_{\text{max}}$  (MeOH) at 276 nm ( $\log \epsilon$  2.53) showed characteristics of bibenzyls. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **3** were similar to those of **2**, except for an additional methoxyl signal and the disappearance of a hydroxyl group. In accordance with **2**, the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **3** showed the existence of a symmetrical aromatic ring in its structure. Thus the additional methoxyl group should be located at C-4', which was further confirmed by  $^{13}\text{C}$ - $^1\text{H}$  long-range correlation between  $\delta$  3.84 (H-4'-OCH<sub>3</sub>) and  $\delta$  147.5 (C-4') in the HMBC spectrum. On the basis of above evidence and results of HSQC and HMBC spectra, the structure of **3** was established to be 4-hydroxy-3,3',4',5, $\alpha$ -pentamethoxybibenzyl (figure 1), and designated as nobilin C.

### 3. Experimental

#### 3.1 General experimental procedures

UV spectra were obtained with a Shimadzu UV2401PC UV-Vis recording spectrophotometer. IR spectra were recorded on a Shimadzu FTIR8400 spectrophotometer. Optical rotations were measured using a JASCO P-1020 polarimeter. ESIMS spectra were performed on a Bruker esquire 2000 mass spectrometer and HREIMS spectra were obtained on a Finnigan MAT95 mass spectrometer. NMR spectra were run on a Bruker AVANCE 400 NMR spectrometer (400 MHz for  $^1\text{H}$ , 100 MHz for  $^{13}\text{C}$ ) with TMS as internal standard. The analytical and preparative HPLC were performed on a SHIMADZU Pak with RI detector using a Shim-pack VP-ODS column (4.6  $\times$  250 mm) and a Shim-pack PREP-ODS column (10  $\times$  250 mm), respectively. Column chromatography was carried out on silica gel H60 (Qingdao Haiyang Chemical Group Corporation, Qingdao, China), Sephadex LH-20 (Amersham Biosciences AB) and ODS (60-80  $\mu\text{m}$ , Merck) as packing materials. Silica gel G was used for analytical TLC.

### 3.2 Plant material

The fresh stems of *Dendrobium nobile* were collected in Yunnan province and identified by Ms. Li-ping Xiao of Hongkong Kadoorie Farm & Botanic Garden. A voucher specimen is deposited at Research Center of Traditional Chinese Medicine and Natural Products, Shenzhen, China.

Table 1.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectral data of compounds **1** and **2** (in acetone- $d_6$ ).

Position	<b>1</b>		<b>2</b>	
	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$
1	0.74, dt (13.0, 3.5)	36.1, t	0.70, m	36.1, t
2	2.24, m		2.10, m	
3	1.40, m	19.1, t	1.39, m	19.1, t
4	1.62, m		1.57, m	
5	1.21, d (3.5)	42.4, t	1.18, m	42.4, t
6	1.46, m		1.43, m	
7		42.4, s		42.4, s
8	1.06, d (11.0)	57.6, d	1.04, d (10.5)	57.5, d
9	1.46, m	21.1, t	1.43, m	21.1, t
10	1.60, m		1.57, m	
11	1.64, m	38.9, t	1.64, m	38.8, t
12	1.83, m		1.79, m	
13		45.3, s		45.3, s
14	1.26, d (8.5)	56.9, d	1.22, m	56.9, d
15		42.4, s		42.4, s
16	1.62, m	19.3, t	1.61, m	19.2, t
17	1.97, m		1.90, m	
18	1.54, m	26.2, t	1.49, m	26.1, t
19	1.74, m		1.71, m	
20	2.12, m	46.9, d	2.10, m	46.9, d
1'	1.62, m	42.9, t	1.57, m	42.9, t
2'	1.69, m		1.67, m	
3'	1.57, m	54.6, t	1.53, m	54.6, t
4'	1.72, m		1.68, m	
5'		80.0, s		80.0, s
6'	4.32, d (12.5), 2H	68.9, t	4.28, d (10.0), 2H	68.9, t
7'	0.91, s, 3H	34.5, q	0.87, s, 3H	34.5, q
8'	0.92, s, 3H	22.6, q	0.88, s, 3H	22.6, q
9'	4.51, d (12.5)	63.9, t	4.47, d (12.6)	63.9, t
10'	4.77, d (12.5)		4.73, d (12.6)	
11'		127.6, s		127.6, s
12'	6.86, d (3.0)	116.4, d	6.82, d (3.0)	116.3, d
13'		146.2, s		146.2, s
14'		148.8, s		148.8, s
15'	7.15, d (8.0)	115.7, d	7.12, d (9.0)	115.7, d
16'	7.03, dd (8.0, 3.0)	122.6, d	7.01, dd (9.0, 3.0)	122.6, d
17'	7.59, d (16.0)	145.7, d	7.57, d (12.0)	145.8, d
18'	6.34, d (16.0)	115.7, d	6.30, d (12.0)	115.7, d
19'		167.7, s		167.7, s
20'		127.5, s		127.4, s
1''		116.3, d		116.3, d
2''	6.84, d (3.0)	146.2, s	6.80, d (3.0)	146.2, s
3''		148.7, s		148.6, s
4''		148.7, s		148.6, s
5''	7.13, d (8.0)	115.1, d	7.09, d (9.0)	115.1, d
6''	7.01, dd (8.0, 3.0)	122.5, d	7.01, dd (9.0, 3.0)	122.5, d
7''	7.54, d (16.0)	145.6, d	7.50, d (12.0)	145.7, d
8''	6.28, d (16.0)	115.7, d	6.24, d (12.0)	115.1, d
9''		167.7, s		167.7, s

### 3.3 Extraction and isolation

The powdered air-dried stems of *Dendrobium nobile* (5 kg) were refluxed with 60% EtOH for three times. After evaporation of EtOH *in vacuo*, the aqueous residue was extracted with EtOAc and *n*-BuOH successively. The EtOAc extract (63 g) was first subjected to column chromatography on silica gel eluted with CHCl<sub>3</sub>-MeOH (100:0 → 0:100) to afford 12 fractions. Fraction 5 (11 g) was further chromatographed on silica gel MPLC by gradient elution with cyclohexane-EtOAc (95:5 → 0:1) to give 13 fractions. Subfraction 8 (276 mg) was passed over Sephadex LH-20 column with CHCl<sub>3</sub>-MeOH (1:1) as eluent and then applied to ODS column eluted with MeOH-H<sub>2</sub>O (4:6 → 8:2). The eluent of 60% MeOH was purified by preparative HPLC (55% MeOH) to yield compound **1** (1.6 mg). Subfractions 10 (1.4 g) and 11 (238 mg) were passed over Sephadex LH-20 column with CHCl<sub>3</sub>-MeOH (1:1) as eluent and then applied to ODS column eluted with MeOH-H<sub>2</sub>O (4:6 → 7:3), respectively. Compounds **2** and **3** (8.2 mg and 4.8 mg) were finally obtained from respective eluent of 50% MeOH by purification with preparative HPLC (50% MeOH).

**3.3.1 Nobilin A (1).** obtained as a colorless oil; 1.6 mg;  $[\alpha]_D^{27} + 1.1$  (*c* 0.7, MeOH); UV (MeOH)  $\lambda_{\max}$  (nm) (log  $\epsilon$ ): 280 (2.48), 273 (2.51); <sup>1</sup>H NMR data, see table 1; <sup>13</sup>C NMR data, see table 2; ESIMS *m/z* 327.1 [M + Na]<sup>+</sup>; *m/z* 303.1 [M - H]<sup>-</sup>; HREIMS *m/z* 304.1300 [M]<sup>+</sup> (calcd for C<sub>17</sub>H<sub>20</sub>O<sub>5</sub>, 304.1311).

**3.3.2 Nobilin B (2).** obtained as a yellow solid; 8.2 mg;  $[\alpha]_D^{27} + 1.4$  (*c* 1.0, MeOH); UV (MeOH)  $\lambda_{\max}$  (nm) (log  $\epsilon$ ): 280 (2.41); IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>) 3471 (OH), 1616, 1516, 1454; <sup>1</sup>H NMR data, see table 1; <sup>13</sup>C NMR data, see table 2; ESIMS *m/z* 357.1 [M + Na]<sup>+</sup>; *m/z* 333.1 [M - H]<sup>-</sup>; HR-EIMS *m/z* 334.1402 [M]<sup>+</sup> (calcd for C<sub>18</sub>H<sub>22</sub>O<sub>6</sub>, 334.1416).

**3.3.3 Nobilin C (3).** obtained as a colorless oil; 4.8 mg;  $[\alpha]_D^{27} + 2.2$  (*c* 0.8, MeOH); UV (MeOH)  $\lambda_{\max}$  (nm) (log  $\epsilon$ ): 276 (2.53); <sup>1</sup>H NMR data, see table 1; <sup>13</sup>C NMR data, see table 2;

Table 2. <sup>13</sup>C NMR (100 MHz) data for compounds **1–3** in CDCl<sub>3</sub> ( $\delta$  ppm).

No	<b>1</b>	<b>2</b>	<b>3</b>
1	133.7 (s)	132.8 (s)	132.8 (s)
2	101.4 (d)	103.4 (d)	103.4 (d)
3	146.9 (s)	146.9 (s)	147.0 (s)
4	131.7 (s)	134.0 (s)	134.0 (s)
5	143.7 (s)	146.9 (s)	147.0 (s)
6	107.4 (d)	103.4 (d)	103.4 (d)
1'	140.2 (s)	130.3 (s)	131.0 (s)
2'	115.1 (d)	112.3 (d)	113.0 (d)
3'	159.4 (s)	146.0 (s)	148.5 (s)
4'	111.7 (d)	144.0 (s)	147.5 (s)
5'	129.0 (d)	113.9 (d)	111.0 (d)
6'	121.9 (d)	122.1 (d)	121.5 (d)
$\alpha$	84.9 (d)	85.6 (d)	85.5 (d)
$\alpha'$	44.8 (t)	44.6 (t)	44.5 (t)
3-OCH <sub>3</sub>	56.2 (q)	56.3 (q)	56.3 (q)
5-OCH <sub>3</sub>		56.3 (q)	56.3 (q)
3'-OCH <sub>3</sub>	55.1 (q)	55.9 (q)	55.8 (q)
4'-OCH <sub>3</sub>			55.9 (q)
$\alpha$ -OCH <sub>3</sub>	56.7 (q)	56.7 (q)	56.7 (q)

ESIMS  $m/z$  371.1  $[M + Na]^+$ ;  $m/z$  347.1  $[M - H]^-$ ; HR-EIMS  $m/z$  348.1554  $[M]^+$  (calcd for  $C_{19}H_{24}O_6$ , 348.1573).

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