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Two New Bibenzyls from *Dendrobium hercoglossum*

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Abstract: Two new bibenzyl compounds 3-hydroxy-4, 5, 3′-trimethoxybibenzyl (**1**) and (*R*)-4-hydroxy-3, 5, 3′, *α*-tetramethoxybibenzyl (**2**), along with twelve known compounds (**3**−**14**), were isolated from the stems of *Dendrobium hercoglossum* Reichb. f. The structures of the new compounds were elucidated on the basis of detailed spectroscopic analysis. The cytotoxic effects of the isolated compounds on two human tumors cell lines (MDA-MB-231 and Hela) were evaluated by the MTT assay.

Keywords: Orchidaceae; *dendrobium hercoglossum*; bibenzyl; cytotoxicity.

1. Introduction

Dendrobium species (Orchidaceae), were known as "Shihu" or "Huangcao" in China, it is widely distributed throughout Asia, Europe, and Australia [1]. Previous phytochemical investigations on "Shihu" showed that alkaloids, bibenzyls, phenanthrenes, phenolic acids, and sesquiterpenoids, were the main secondary metabolites. *Dendrobium* species have the activities of antioxidant, anti-tumor, anti-angiogenesis, anti-inflammatory, anti-aggregation, *etc* [2-6]. In our continuing endeavor to discover new structures from *Dendrobium* species [7-11], two new bibenzyl compounds 3-hydroxy-4, 5, 3′-trimethoxybibenzyl (**1**) and (*R*)-4-hydroxy-3, 5, 3′, *α*- tetramethoxybibenzyl (**2**) (Figure 1), along with twelve known compounds were isolated from the stems of *Dendrobium hercoglossum* Reichb. f. The structures of the new compounds were elucidated on the basis of detailed spectroscopic analysis. The cytotoxic effects of the isolated compounds on two human tumors cell lines (MDA-MB-231 and Hela) were evaluated by the MTT assay. We herein present the isolation, structural elucidation, and biological evaluation of these new bibenzyl compounds

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2. Materials and Methods

2.1. Instruments and Materials

Agilent DD2400-MR nuclear magnetic resonance instrument (Agilent company, USA) and Bruker AVANCE III-600 instruments (Bruker, Bremen, Germany), TMS is internal standard; LTQ Orbitrap XL mass spectrometer (Thermo Fisher, USA); J-1500 Circular Dichroism Chiroptical Spectrometer (JASCO company, Japan), LC3000 high performance liquid chromatograph (Chuang Xing Tong Heng Science And Technology Co., Ltd., Beijing, China); ODS column (Daisogel C_{18} 10 μm 30 mm×250 mm; YMC C_{18} 5 μm 10 mm×250 mm); GF_{254} and 300-400 mesh silica gel (Marine Chemical Industry Factory, Qingdao, China); Sephadex LH-20 gel (Merck company, Germany). The plant was collected from Baoshan city in Yunnan Province, the People's Republic of China, in December 2018, and identified as *Dendrobium hercoglossum* Rchb. f. by Professor Faming Wu at Zunyi Medical University. A voucher specimen with the catalogue No.20181029 was deposited in the Herbarium of the the School of Pharmacy, Zunyi Medical Universty.

2.2. Separation and Purification

Dried and powdered stems of *D. hercoglossum* Rchb. f. (3.15 kg) were extracted with MeOH refluxed. The extracts were concentrated to give a residue (300 g), then subjected to 300~400 mesh silica gel, extracted successively with EtOAc (3×2 L) and n-BuOH (3×2 L). The EtOAc extract (60 g) was subjected to silica gel column chromatography (80 mm×600 mm, 400 g, 300~400 mesh), eluted with a gradient of petroleum ether−EtOAc (*v/v* 100:0→10:1→5:1→3:1→2:1→1:1→1:2 →0:100) to yield 5 fractions (Fr.1−Fr.5). Fr.3 was subjected to column chromatography (CC) over MCI gel (85 × 100 mm), eluting with MeOH–H_2O (*v/v*, 30:70→50:50→70:30→90:10→100:0), to yield four fractions (Fr.3.1−Fr.3.4) based on TLC analysis results. Fr.3.4 was purified by semipreparative HPLC (MeOH−H_2O *v/v*, 65:35, 6.0 mL/min) to yield three fractions (Fr.3.4.1−Fr.3.4.3), Fr.3.4.3 was purified by semipreparative HPLC (MeOH−H_2O *v/v*, 69:31, 3.0 mL/min) to give **1** (t_R=19.1 min, 2.3 mg), 4′-hydroxy-3, 3′, 5-trimethoxybibenzyl (t_R=25.5 min, 2.9 mg) [12], and 4-hydroxy-3, 3′, 5-trimethoxybibenzyl (t_R=22.1 min, 1.5 mg) [13]. Fr.3.1 was purified by semipreparative HPLC (MeOH−H_2O *v/v*, 22:78, 6.0 mL/min) to give 4-hydroxybenzaldehyde (t_R=17.2 min, 3.1 mg) [14] and vanillin (t_R=23.6 min, 13.1 mg) [15]. Fr.3.3 was purified by semipreparative HPLC (MeOH−H_2O *v/v*, 69:31, 6.0 mL/min) to give **2** (t_R=14.7 min, 10.2 mg). Fr.4 to yield three fractions (Fr.4.1−Fr.4.3) based on TLC analysis results. Fr.4.2 was purified by Sephadex LH-20 (30×1200 mm, MeOH) to afford seven fractions (Fr.4.2.1−Fr.4.2.7), Fr.4.2.3 was purified by semipreparative HPLC (MeOH−H_2O *v/v*, 60:40, 6.0 mL/min) to give 4, 5-dihydroxy-3, 3′-dimethoxybibenzyl (t_R=14.8 min, 49.1 mg) [16], Fr.4.2.4 was purified by semipreparative HPLC (MeOH−H_2O *v/v*, 60:40, 6.0 mL/min) to give 4′, 5-dihydroxy-3, 3′-dimethoxybibenzyl (t_R=10.0 min, 8.1 mg) [17], Fr.4.2.6 was purified by semipreparative HPLC (MeOH−H_2O *v/v*, 62:38, 6.0 mL/min) to give batatasin III (t_R=8.9 min, 20.1 mg) [18]. Fr.4.3 was purified by Sephadex LH-20 (30×1200 mm, MeOH) to afford four fractions (Fr.4.3.1−Fr.4.3.4), Fr.4.3.2 was purified by Sephadex LH-20 (30×1200 mm, MeOH) to afford four fractions (Fr.4.3.2.1−Fr.4.3.2.4), Fr.4.3.2.4 was purified by semipreparative HPLC (MeOH−H_2O *v/v*, 49:51, 6.0 mL/min) to yield two fractions (Fr.4.3.2.4.1 −Fr.4.3.2.4.2), Fr.4.3.2.4.2 was purified by semipreparative HPLC (n-hexane−isopropanol *v/v*, 87:13, 5.0 mL/min) to give dendrosinens B (t_R=5.7 min, 9.5 mg) [13], Fr.4.3.2.3 was purified by semipreparative HPLC (MeOH−H_2O *v/v*, 50:50, 6.0 mL/min) to give (*E*)-*p*-hydroxycinnamic acid (t_R=5.8 min, 12.5 mg) [19]. Fr.5 to yield three fractions (Fr.5.1−Fr.5.3) based on TLC analysis results. Fr.5.1 was purified by Sephadex LH-20 (30×1200 mm, MeOH) to afford seven fractions (Fr.5.1.1−Fr.5.1.7), Fr.5.1.5 was purified by semipreparative HPLC (MeOH−H_2O *v/v*, 70:30, 6.0 mL/min) to give nobilin E (t_R=24.8 min, 28.5 mg) [20]. Fr.5.3 by MCI sample, and further purifified by semi-preparative HPLC (MeOH−H_2O *v/v*, 40:60→100:0, 30.0 mL/min) to yield seven fractions (Fr.5.3.1−Fr.5.3.7), Fr.5.3.1 was purified by semipreparative HPLC (MeOH−H_2O *v/v*, 30:70, 6.0 mL/min) to yield seven fractions (Fr.5.3.1.1−Fr.5.3.1.7), Fr.5.3.1.7 was

purified by semipreparative HPLC (acetonitrile−H_2O *v/v*, 25:75→100:0→25:75, 4.0 mL/min) to give *N-trans*-feruloyltyramine (t_R=21.5 min, 37.1 mg) [21] and *N-trans*-coumaroyltyranine (t_R=19.0 min, 17.1 mg) [22].

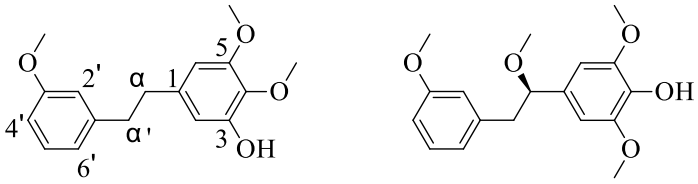

Figure 1. Chemical structures of compounds **1** and **2**

Table 1. NMR data of compounds **1** and **2** in $CDCl_3$ (*J* in Hz)

No.	**1**[a]		**2**[b]	
	δ_H	δ_C	δ_H	δ_C
1		138.1		132.7
2	6.45 (1H, d, 1.6)	107.8	6.41 (1H, s)	103.2
3		149.1		146.9
4		133.7		133.9
5		152.1		146.9
6	6.23 (1H, d, 1.6)	104.5	6.41 (1H, s)	103.2
α	2.84 (2H, m)	37.9	4.20 (1H, t, 6.6)	85.2
1′		143.3		140.0
2′	6.75 (1H, br.s)	114.2	6.62 (1H, br.s)	115.1
3′		159.6		159.3
4′	6.77 (1H, d, 7.8)	111.3	6.71 (1H, d, 7.9)	111.6
5′	7.23 (1H, t, 7.8)	129.3	7.13 (1H, t, 7.9)	129.0
6′	6.81 (1H, d, 7.8)	120.9	6.66 (1H, d, 7.9)	121.9
α′	2.89 (2H, m)	37.9	3.05 (1H, dd, 13.6, 7.2) 2.81 (1H, dd, 13.6, 6.0)	44.9
3-OCH_3			3.81 (3H, s)	56.2
4-OCH_3	3.85 (3H, s)	55.2		
5-OCH_3	3.80(3H, s)	55.8	3.81 (3H, s)	56.2
3′-OCH_3	3.77 (3H, s)	61.0	3.72 (3H, s)	55.1
α-OCH_3			3.19 (3H, s)	56.7

[a] 600/150 MHz; [b] 400/100 MH

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was isolated as yellow oil. Its molecular formula was determined as $C_{17}H_{20}O_4$ by the HRESIMS (*m/z* 289.1428 $[M+H]^+$, calcd for 289.1440), demonstrated 8 degrees of unsaturation. The ^{1}H-NMR (Table 1) and HSQC spectra of compound **1** showed the existence of six aromatic protons signals at δ_H 7.23 (1H, t, *J* = 7.8 Hz), 6.81 (1H, d, *J* = 7.8 Hz), 6.77 (1H, d, *J* = 7.8 Hz), 6.75 (1H, br.s), 6.45 (1H, d, *J* = 1.6 Hz), 6.23 (1H, d, *J* = 1.6 Hz); three methoxyl singlet peaks at δ_H 3.85, 3.80, 3.77 (each 3H, s); two methylene groups at δ_H 2.89 (2H, m) and 2.84 (2H, m). The ^{13}C NMR (Table 1) and HSQC spectra showed 17 carbon signals, including two methylene carbon signals at δ_C 37.9 and 37.9; three methoxy signals at δ_C 61.0, 55.8, 55.2; twelve aromatic carbon signals at δ_C 104.5, 107.8, 111.3, 114.2, 120.9, 129.3, 133.7, 138.1, 143.3, 149.1, 152.1, and 159.6. Analyze of the NMR data of compound **1** showed that it is a typical bibenzyl compound [10]. The HMBC correlations (Figure S1) of H-2 (δ_H 6.45) with C-4, H-6 (δ_H 6.23) with C-4, -OCH_3 (δ_H 3.85) with C-4, indicated that one methoxyl was located at C-4; The HMBC correlations of H-6 (δ_H 6.23) with C-5, -OCH_3 (δ_H 3.80) with C-5, H-2′ (δ_H 6.75) with C-3′, H-5′ (δ_H 7.23) with C-3′, -OCH_3 (δ_H 3.77) with C-3′, positioned

another two -OCH_3 at C-5 and C-3′, respectively. Accordingly, the structure of compound **1** was established as 3-hydroxy-4, 5, 3′-trimethoxybibenzyl.

Compound **2** was isolated as yellow oil. Its molecular formula was determined as $C_{18}H_{22}O_5$ by the HRESIMS (*m/z* 341.1372 $[M+Na]^+$, calcd for 341.1365), which requires 8 degrees of unsaturation. The ^{1}H-NMR (Table 1) and HSQC spectra showed the existence of six aromatic protons signals at δ_H 7.13 (1H, t, *J* = 7.9 Hz), 6.71 (1H, d, *J* = 7.9 Hz), 6.66 (1H, d, *J* = 7.9 Hz), 6.62 (1H, br.s), 6.41 (2H, s); four methoxyl singlet peaks at δ_H 3.81, 3.81, 3.72, 3.19; a methylene at δ_H 3.05 (1H, dd, *J* = 13.6, 7.2 Hz) and 2.81 (1H, dd, *J* = 13.6, 6.0 Hz); an oxygenated methyne at δ_H 4.20 (1H, t, *J* = 6.6 Hz). The ^{13}C NMR (Table 1) and HSQC spectra showed 18 carbon signals, including one methylene carbon signal at δ_C 44.9; one oxygenated methyne at δ_C 85.2; four methoxy signals at δ_C 56.7, 56.2, 56.2, 55.1; twelve aromatic carbon signals at δ_C 103.2, 103.2, 111.6, 115.1, 121.9, 129.0, 132.7, 133.9, 140.0, 146.9, 146.9, 159.3. These NMR data above showed that compound **2** has a bibenzyl skeleton [10]. The HMBC correlations (Figure 2) of H-α (δ_H 4.20) with C-α', with C-2, with C-6, with C-1', and H-2, 6 (δ_H 6.41) with C-4, with C-α', -OCH_3 (δ_H 3.19) with C-α (δ_C 85.2) indicated that one methoxyl was located at C-α. The HMBC correlations of -OCH_3 (δ_H 3.81) with C-3, 5 (δ_C 146.9), H-2, 6 (δ_H 6.41) with C-3, 5, -OCH_3 (δ_H 3.72) with C-3' (δ_C 159.3), H-2' (δ_H 6.62) with C-3', H-5' (δ_H 7.13) with C-3', positioned another three -OCH_3 at C-3, C-5, and C-3', respectively. Furthermore, the negative cotton effect at 216 nm in the CD spectrum confirmed that the C-α configuration is *R* [13]. Thus, the structure of compound **2** was established as (*R*)-4-hydroxy-3, 5, 3′, *α*- tetramethoxybibenzyl.

3.2. Cell Viability Assay

MDA-MB-231 and HeLa cells were cultured in Dulbecco's modified Eagle's media (DMEM) supplemented with 10% fetal bovine serum and 2 mM L-glutamine. The cells were maintained at 37°C in a humidified atmosphere at 95% air and 5% CO_2. Antitumor activity was measured by MTT assay (9). Compounds 4, 5-dihydroxy-3, 3′-dimethoxybibenzyl (**3**), 4′, 5-dihydroxy-3, 3′-dimethoxybibenzyl (**4**), batatasin III (**5**), and nobilin E (**9)** exhibited strong *in vitro* cytotoxicity activities against MDA-MB-231 and Hela cell lines in MTT assay (Figure S14 and S15).

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Supporting Information

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