ORIGINAL ARTICLE

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Rec. Nat. Prod. 16:4 (2022) 353-357

records of natural products

Two New Bibenzyls from *Dendrobium hercoglossum*

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(Received September 3, 2021; Revised October 12, 2021; Accepted October 16, 2021)

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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The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products July-August 2022 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.291.2109.2198
Available online: November 02, 2021

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].

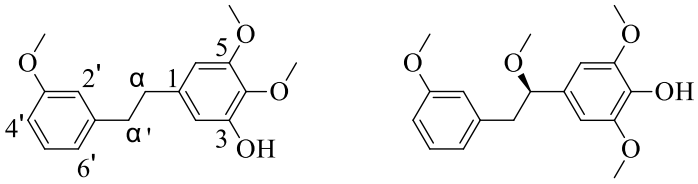

Figure 1. Chemical structures of compounds **1** and **2**

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).

Acknowledgments

This work was financially supported by the National Natural Sciences Foundation of China (No. 82060706 and 31960204), the State Key Laboratory of Functions and Applications of Medicinal Plants, Guizhou Medicial University (No. FAMP201902K); Science and Technology Department of Guizhou Province (No.QKHPTRC[2017]5733-052, No.QKHRCPT [2017]5101; No.QKHJC[2020] 1Y402, No.QKHZC[2020]4Y211, No.QKHPTRC[2017]5733-049); Talent Team Project of Zunyi City (No.ZSKRC[2020]10), Honghuagang District Science and Technology Bureau of Zunyi (No.ZHKHSZ[2019]27).

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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