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Two New Bibenzyl Glucosides from *Dendrobium chrysotoxum*

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Two new bibenzyl glucosides, 3,3',4',5-tetramethoxybibenzyl-4-*O*-*β*-D-glucopyranoside (**1**) and 3,4,4',5-tetramethoxybibenzyl-3'-*O*-*β*-D-glucopyranoside (**2**), together with five known ones, chrysotobibenzyl (**3**), erianin (**4**), chrysotoxine (**5**), gigantol (**6**) and tristin (**7**) were isolated from the stems of *Dendrobium chrysotoxum*. The structures of those compounds were elucidated by extensive spectroscopic analysis. Moreover, compounds **1**-**7** were assessed for inhibitory activity of two enzymes-AChE (acetylcholine esterase) and BChE (butyrylcholine esterase).

Key Words : *Dendrobium chrysotoxum*, Orchidaceae, Bibenzyl glucosides, AChE and BChE

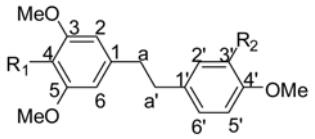
Introduction

"Shi-hu", an important traditional Chinese and folk medicine, which were prepared from the stems of *Dendrobium* species (Orchidaceae) and sometimes used as a health-food.[1,2] *D. chrysotoxum* Lindl. is used for treatment of loss of appetite with nausea, fever in deficiency condition after a severe disease and is good for the health of elder.[3,4] Previous investigations on chemical constituents of *D. chrysotoxum* have been resulted in the isolation of some aromatic compounds, such as bibenzyls, phenanthrenes, phenanthrene-quinones, and fluorenones *etc*.[5-8] To further investigate the chemical constituents of this new species involved by Pharmacopoeia of China (2010), two new bibenzyl glucosides and five known bibenzyls were isolated. ACh (acetylcholine) and BCh (butyrylcholine) are required for cholinergic neurotransmission in the central and peripheral nervous systems, AChE and BChE activity have been used as a marker for cholinergic activity, which plays a crucial role in the learning and memory.[11,12] Controlled inhibition of brain AChE and BChE may slow neurodegeneration in Alzheimer's and kinson's disease.[13] Respecting the traditional usage of "Shi-Hu" and bioactivities of bibenzyl glucosides,[9,10] those 7 compounds isolated from *D. chrysotoxum* this time were evaluated for the inhibitory activity of AChE and BChE. Herein, we describe the isolation and structure elucidation of compounds **1** and **2** and evaluation of bioactivities of **1**-**7**.

Results and Discussion

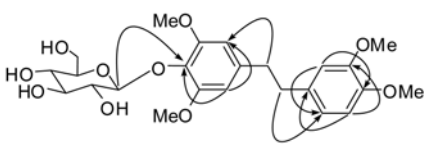
After repeated column chromatographic of the BuOH-soluble portion of the ethanolic extract from the stems of *D. chrysotoxum* on silica gel and Sephadex LH-20, compounds **1** and **2** were isolated.

Compound **1** was obtained as a white amorphous powder. Its molecular formula was determined as $C_{24}H_{32}O_{10}$, which was further confirmed by negative HR-ESI-MS (*m/z* 515.1698 $[M+Cl]^-$). UV absorbing λ_{max} (MeOH) at 208 and 278 nm revealed the presence of benzyl moieties.[14] ^{13}C NMR and DEPT spectra of **1** combined with HSQC experiment exhibited the signals for 24 carbons including 3 methylenes, 4 methoxyls, 5 oxygenated methylenes, along with 12 aromatic carbons (2 quaternary carbons, 5 protonated carbons, and 5 oxygenated carbons). The proton signals at δ 6.70 (1H, d, *J* = 8.8 Hz), δ 6.84 (1H, d, *J* = 8.8 Hz) and δ 6.73 (1H, br s)] in the ^1H NMR spectrum displayed one 1,3,4-trisubstituted benzene, and two *meta*-coupling protons [δ 6.47 (2H, br s)]



1 R_1 = OGlc, R_2 = OMe
2 R_1 = OMe, R_2 = OGlc

Figure 1. Structures of **1** and **2**.



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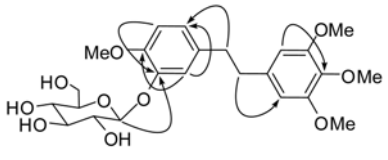

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Figure 2. Key HMBC correlations of **1** and **2**.

Table 1. ^{1}H and ^{13}C NMR assignments and 2D NMR correlations of compound **1** in CD_3OD

Position	δ_C (mult)	δ_H (mult, *J* in Hz)	HMBC (H→C)
1	140.0		
2	107.6	6.47 (1H, br s)	C-1, 4, 6, a
3	153.9		
4	134.5		
5	153.9		
6	107.6	6.47 (1H, br s)	C-1, 2, 4, a
1'	135.9		
2'	113.8	6.73 (1H, br s)	C-4', 6', a'
3'	148.7		
4'	150.2		
5'	113.0	6.84 (1H, d, *J* = 8.8)	C-1', 3'
6'	121.9	6.70 (1H, d, *J* = 8.8)	C-2', 4'
a	39.4	2.84 (2H, s)	C-2, 6, 1', a'
a'	38.5	2.84 (2H, s)	C-1, 2', 6', a
3-OMe	56.9	3.78 (3H, s)	C-3
5-OMe	56.9	3.78 (3H, s)	C-5
3'-OMe	56.5	3.76 (3H, s)	C-3'
4'-OMe	56.3	3.76 (3H, s)	C-4'
Glucose moiety			
1"	105.6	4.79 (1H, d, *J* = 7.35)	C-4, 3"
2"	75.7	3.46 (1H, m)	
3"	77.8	3.41 (1H, m)	C-5"
4"	71.3	3.40 (1H, m)	C-2"
5"	78.3	3.19 (1H, m)	
6"	62.5	3.67 (1H, m)	C-4"
		3.77 (1H, m)	

Table 2. ^{1}H and ^{13}C NMR assignments and 2D NMR correlations of compound **2** in CD_3OD

Position	δ_C (mult)	δ_H (mult, *J* in Hz)	HMBC (H→C)
1	139.4		
2	106.9	6.42 (1H, br s)	C-1, 3, 4, 6, a
3	154.2		
4	137.0		
5	154.2		
6	106.9	6.42 (1H, br s)	C-1, 2, 3, 4, a
1'	136.2		
2'	118.5	6.97 (1H, d, *J* = 1.6)	C-4', 6', a'
3'	147.8		
4'	148.8		
5'	113.6	6.88 (1H, d, *J* = 8.4)	C-1', 3'
6'	123.9	6.80 (1H, d, *J* = 8.4, 1.6)	C-2', 4'
a	38.0	2.80 (2H, m)	C-2, 6, 1', a'
a'	37.7	2.80 (2H, m)	C-1, 2', 6', a
3-OMe	56.5	3.76 (3H, s)	C-3
4-OMe	61.2	3.72 (3H, s)	C-4
5-OMe	56.5	3.76 (3H, s)	C-5
4'-OMe	56.9	3.82 (3H, s)	C-4'
Glucose moiety			
1"	103.0	4.78 (1H, d, *J* = 7.31)	C-3', 3"
2"	74.9	3.47 (1H, m)	C- 4"
3"	77.8	3.46 (1H, m)	C- 5"
4"	71.4	3.32 (1H, m)	C- 2"
5"	78.2	3.31 (1H, m)	
6"	62.6	3.66 (1H, m)	C-4"
		3.82 (1H, m)	

revealed that the other benzene ring was 1,3,4,5-tetrasubstituted. Two benzene rings together with two methylenes [δ 2.84 (4H, br s)] indicated a pentasubstituted bibenzyl skeleton,[9,10,15,16] which was confirmed by the correlations between H-6' (δ 6.70) and H-2', H-5'; H-2' (δ 6.73) and H-6'; H-5' (δ 6.84) and H-6'; and between H-2 and H-6 in the COSY spectrum, along with the correlations of H-6' (δ 6.70) to C-a', C-2', C-4'; H-5' (δ 6.84) to C-1', C-3'; H-2' (δ 6.73) to C-a', C-4', C-6'; H-2 (δ 6.47) to C-a, C-4, C-6; and H-6 (δ 6.47) to C-a, C-2, C-4 in the HMBC spectrum. The ^{1}H -^{13}C NMR spectrum (Table 1) exhibited 12 proton signals at δ 3.76-3.78 and 4 carbons at δ 56.3-56.9 consisted of four methoxyls, the other six protons signals at δ 3.19-3.68 and an anomeric proton at δ 4.79, together with 6 carbon signals [δ 105.6 (d, C-1"), 75.7 (d, C-2"), 77.8 (d, C-3"), 71.3 (d, C-4"), 78.3 (d, C-5"), 62.5(t, C-6")] indicated the presence of a sugar moiety,[10,16] and the coupling constant value at 7.35 Hz (δ 4.79) of anomeric proton indicated that the glucosyl moiety was connected to the aglycone by a *β*-linkage.[10,15,16] Long-range correlations in the HMBC spectrum between H-1" and C-4 indicated the glucosyl was linked to C-4. Thus, the structure of **1** was elucidated as 3,3',4',5-teramethoxybibenzyl-4-*O*-*β*-D-glucopyranoside.

Compound **2** was also isolated as a white amorphous powder, possessing the same molecular formula $C_{24}H_{32}O_{10}$ as **1** based on its negative HR-ESI-MS (*m/z* 515.1671 $[M+Cl]^-$). According to ESI-MS and NMR spectra of **2**, it could be predicted that **2** was an isomer of **1**. The ^{13}C NMR and HSQC spectral data of **2** were almost the same as that of **1**, except for the little difference in chemical shift value between **1** and **2**. The ^{1}H NMR spectrum of **2** also showed one benzene was 1,3,4-trisubstituted [δ_H 6.77 (1H, dd, *J* = 8.4, 1.6 Hz), 6.87 (1H, d, *J* = 8.4 Hz) and 6.97 (1H, d, *J* = 1.6 Hz)], the other one was 1,3,4,5-tetrasubstituted with two similar *meta*-coupling protons [δ_H 6.42 (2H, br s)]. The ^{1}H and ^{13}C NMR spectral data displayed four methoxy group [δ_H 3.66-3.88 (12H, m); δ_C 56.5-61.2], two methylenes [δ_H 2.80 (4H, m); δ_C 38.4, 39.3] and one sugar moiety.[10, 16] The long-range correlation signal in the HMBC spectrum of **2** between H-1" [δ_H 4.77 (1H, d, *J* = 7.08 Hz)] and C-3' [δ_C 147.7] indicated that the glucosyl was attached to C-3'. Cross-peaks in the HMBC and COSY spectrum further confirmed the substituted patterns. Therefore, the structure of **2** was determined as 3,4,4',5-teramethoxybibenzyl-3'-*O*-*β*-D-glucopyranoside.

By comparison with the previously published data, five known bibenzyls were identified as chrysotobibenzyl (**3**),[6] erianin (**4**),[6] chrysotoxine (**5**),[7] gigantol (**6**),[17] tristin (**7**).[18] The AChE and BChE inhibition rates of isolated compounds are compared in Table 3 respectively, tacrine was used as positive control. As shown in Table 3, it could be deduced that compounds **3**, **4** and **5** have a certain degree of inhibition

Table 3. The AchE and BchE inhibition rates of compounds **1-7**

Compound	Concentration (μmol\L)	AchE (%)	BchE (%)
Tacrine	0.333	55.08	86.93
1	50	3.75	4.85
2	50	0.47	0.57
3	50	2.43	30.68
4	50	14.48	41.66
5	50	9.87	19.35
6	50	4.98	−14.44
7	50	3.72	−2.80

ratios against BChE and compounds **4** and **5** have weak inhibition ratios against AChE, and those bibenzyls from this kind of "Shi-hu" may be active compounds for its health care function.

Experimental

General Procedures. Optical rotations were determined on a JASCO model 1020 polarimeter (Horiba, Tokyo, Japan). UV spectra were measured on a Shimadzu UV-2401A spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were obtained on a Bio-Rad FTS-135 infrared spectrometer (Bio-Rad, Hercules, CA, USA) using KBr pellets. MS and HR-MS were run on a VG Auto Spec-3000 mass spectrometer (VG, Manchester, England). 1D and 2D NMR spectra were recorded on a Bruker AM-400 or DRX-500 spectrometer (Bruker, Bremerhaven, Germany) with TMS as the internal standard. Silica gel (200-300 mesh) for column chromatography (CC) and TLC was obtained from Qindao Marine Chemical Factory, Qingdao, China. Sephadex LH-20 was purchased from Amersham Biosciences.

Plant Material. The stems of *D. chrysotoxum* were collected in January 2010 from Puer (Yunnan province) and identified by Professor Hong Yu. A voucher specimen (No. ZDC-002) has been deposited in State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, the Chinese Academy of Sciences, Kunming, China.

Extraction and Isolation. The air-dried stems of the plant (55.0 kg) were powdered and extracted with 95% aqueous EtOH under reflux successively (each time 2 h, 250 liters × 3 times). The combined extracts were concentrated under reduced pressure and fractionated successively into EtOAc-soluble (2.3 kg) and *n*-BuOH-soluble (635 g) fractions. The ethyl acetate part was chromatographed on a silica gel column [silica gel (200-300 mesh, 12 kg), petroleum ether/acetone (100:0-0:100, v/v)] to give 11 fractions. Fraction 4 (220 g) was subjected to repeated CC [silica gel (200-300 mesh, 1.8 kg), petroleum ether/acetone (15:1-1:3, v/v)] to yield compound **3** (9.1 g) and **4** (18.5 g). Similarly, fraction 5 (143 g) was further separated by CC [silica gel, petroleum ether/acetone (8:1-1:1)], and then passed over ODS column [MeOH/H_2O (1:1-1:0, v/v)] and purified by Sephadex LH-20 column further [$CHCl_3$/MeOH (1:1, v/v)] to afford compound **5** (3.0 g) and **6** (7.2 g). Fraction 9 (180 g) was treated as fraction 5 to produce compound **7** (5.3 g). The *n*-BuOH extract (635 g) was subjected to CC [silica gel, 200-300 mesh, 4.8 kg, $CHCl_3$/MeOH (20:1-0:1)] to give 8 fractions. Compound **1** (13.5 mg) and **2** (22.6 mg) were obtained from fraction 1 (240 g) by repeated CC [silica gel., 200-300 mesh, 2.5 kg, $CHCl_3$/MeOH (30:1)], and further refined by ODS column [MeOH/H_2O (7:3)].

3,3',4',5-Tetramethoxybibenzyl-4-*O*-*β*-D-glucopyranoside (1): White amorphous powder (CH_3OH), $C_{24}H_{32}O_{10}$, mp 158-160 °C; $[\alpha]_D^{23}$ = −17.6 (*c* 0.34, MeOH); UV (MeOH) λ_{max} (log ε_{max}): 208 nm (4.6) and 278 nm (3.5); IR (KBr) ν_{max} (cm^{-1}): 3441, 2919, 1726, 1593, 1515, 1463, 1420, 1240, 1123, 1065, 1026, 817; ^{1}H NMR [CD_3OD, 400 MHz] and ^{13}C NMR [CD_3OD, 100 MHz] spectral data, see Table 1; ESI-MS (negative ion) *m/z* 515 $[M+Cl]^-$ (100); HR-ESI-MS *m/z* 515.1698 $[M+Cl]^-$ (calcd. 515.1684 for $C_{24}H_{32}O_{10}Cl$).

3,4,4',5-Tetramethoxybibenzyl-3'-*O*-*β*-D-glucopyranoside (2): White amorphous powder (CH_3OH), $C_{24}H_{32}O_{10}$, mp 170-172 °C; $[\alpha]_D^{23}$ = −23.81 (*c* 0.39, MeOH). UV (MeOH) λ_{max} (log ε_{max}): 208 nm (4.6) and 277 nm (3.5); IR (KBr) ν_{max} (cm^{-1}): 3397, 2934, 2839, 1722, 1591, 1511, 1460, 1421, 1329, 1261, 1233, 1126, 1073, 1043, 1013, 896, 812, 778, 634, 582, 528; ^{1}H NMR [CD_3OD, 400 MHz] and ^{13}C NMR [CD_3OD, 100 MHz] spectral data, see Table 2; ESI-MS (negative ion) *m/z* 515 $[M+Cl]^-$ (100); HR-ESI-MS *m/z* 515.1671 $[M+Cl]^-$ (calcd. 515.1684 for $C_{24}H_{32}O_{10}Cl$).

AChE and BchE Inhibitory Activity. The AchE and BchE inhibitory activity of the isolated compounds were assayed by the spectrophotometric method developed by *Ellman et al*.[19] AchE and BchE (Sigma) were used as substrate in the assay. Compounds were dissolved in DMSO. The mixture contained 110 μL phosphate buffer (pH 8.0), 10 μL of test compound soln. (50 μM), 40 μL AChE Soln. (0.002 U/μL). And the mixture was incubated for 20 min (30 °C). The reaction was initiated by the Addition of 20 μL of DTNB (5,5-dithio-bis-nitrobenzoic acid), (6.25 mM), and 20 μL of S-Butyrylthiocholine iodide (6.25 mM). The hydrolysis of acetylthiocholine was monitored at 405 nm after 30 min. Tacrine was used as positive control. All reactions were performed in triplicate. The anti-BchE activity assay was carried out under the same conditions as the AchE, except for the difference at concentration (0.04 U/100 μL) and volume (80 μL) of BchE used in the test.

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