Three New Bibenzyl Derivatives from *Dendrobium candidum*

Yan LI, Chun-Lan WANG, Ya-Jun WANG, Shun-Xing GUO,* Jun-Shan YANG, Xiao-Mei CHEN, and Pei-Gen XIAO

Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College; Beijing 100193, P. R. China.

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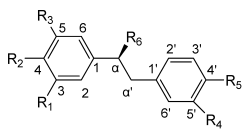
Three new compounds were isolated from the stems of *Dendrobium candidum*: (*S*)-3,4,4′-trihydroxy-5,α-dimethoxybibenzyl (1), named dendrocandin C; (*S*)-3,4,4′-trihydroxy-5-methoxy-α-ethoxybibenzyl (2), named dendrocandin D; and 3,3′,4,4′-tetrahydroxy-5-methoxybibenzyl (3), named dendrocandin E. Their structures were elucidated by 1D- and 2D-NMR spectroscopy and mass spectroscopy. The isolated compounds exhibited potent antioxidant activity in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging test, with IC_{50} values of 34.2, 34.5, and 15.6 μM for compounds 1, 2, and 3, respectively. Vitamin C was used as positive control with IC_{50} 23.2 μM.

Key words *Dendrobium candidum*; dendrocandin C; dendrocandin D; dendrocandin E; antioxidant activity

Dendrobium candidum (Orchidaceae) is one of the original materials of "Shi Hu", used as both traditional Chinese and folk remedies for antipyretic, eyes-benefiting, and tonic purposes.[1] In the previous study, we have reported the isolation of seven compounds from *D. candidum*.[2] Our continuing research on the phytochemical constituents of *D. candidum*, led to the isolation of three new bibenzyl derivatives (**1**—**3**). Because of the reported antioxidant activity of some bibenzyl derivates,[3] we evaluated the antioxidant activity of compounds **1**—**3** by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging test. The present paper deals with the structure elucidation and the antioxidant evaluation of compounds **1**—**3**.

Dendrocandin C (1) Compound **1** was obtained as a yellow syrup, $[\alpha]_D^{20}$ +1.5° (c=0.13, MeOH). Its molecular formula was deduced as $C_{16}H_{18}O_5$ on the basis of its HR-ESI-MS (m/z 289.1086, $[M-H]^-$) in tandem with the NMR data. The ^{1}H-NMR data of **1** indicated the presence of two methoxyl groups at δ_H 3.16 (3H, s) and 3.76 (3H, s); one methylene group at δ_H 2.73 (1H, dd, J=14.0, 7.0 Hz) and 2.95 (1H, dd, J=14.0, 7.0 Hz); one oxygenated methine proton at δ_H 4.12 (1H, t, J=7.0 Hz); and six aromatic protons, appearing as a pair of *m*-coupled signals at δ_H 6.28 (1H, d, J=1.5 Hz) and 6.35 (1H, d, J=1.5 Hz), and a pair of *o*-coupled signals at δ_H 6.62 (2H, d, J=8.5 Hz) and 6.87 (2H, d, J=8.5 Hz). In the ^{13}C-NMR spectrum, sixteen carbon signals belonging to two methoxyl, one methylene, one oxygenated methine, six aromatic methine, and six aromatic quaternary carbons (four oxygenated) were observed. From the above analysis we found that the ^{1}H- and ^{13}C-NMR data of **1** were similar to those of dendrocandin A,[2] except for the disappearance of a methoxyl group at C-4′ of dendrocandin A. According to the 1D- and 2D-NMR data and the molecular formula of compound **1** showed that a hydroxyl group substituted at C-4′ of **1**. The key heteronuclear multiple bond correlation (HMBC) correlations are shown in Fig. 2. The absolute configuration of **1** was established as (*S*) on the basis of the positive Cotton effect at 226 nm ($\Delta\varepsilon$ +2.64) in its circular dichroism (CD) spectrum, which was opposite to those of dendrocandin A.[2] Based on the above evidence, the structure of **1** was deduced to be (*S*)-3,4,4′-trihydroxy-5,α-dimethoxybibenzyl, named dendrocandin C.

Dendrocandin D (2) Compound **2** was obtained as a red syrup, $[\alpha]_D^{20}$ +3.6° (c=0.22, MeOH). The ^{1}H- and ^{13}C-NMR data of **2** were similar to those of compound **1**, except for an additional ethoxyl signal and the disappearance of a methoxyl group at C-α of **1**. The additional ethoxyl group located at C-α, which was confirmed by ^{13}C–^{1}H long-range correlation peaks at δ_H 3.26, 3.38/δ_C 85.1 and δ_H 4.22/δ_C 65.0 in the HMBC spectrum. Other key HMBC correlations are shown in Fig. 2. The absolute configuration of compound

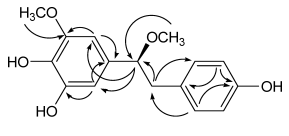


1 $R_1 = R_2 = R_5 = OH$, $R_3 = R_6 = OCH_3$, $R_4 = H$

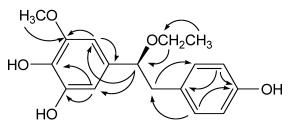
2 $R_1 = R_2 = R_5 = OH$, $R_3 = OCH_3$, $R_6 = OCH_2CH_3$, $R_4 = H$

3 $R_1 = R_2 = R_4 = R_5 = OH$, $R_3 = OCH_3$, $R_6 = H$

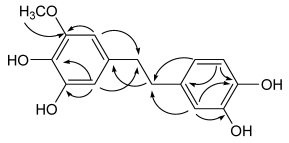
Fig. 1. Structures of Compounds **1**—**3**



1



2



3

Fig. 2. Key HBMC Correlations of Compounds **1**—**3**

* To whom correspondence should be addressed. e-mail: sxguo2006@yohoo.com.cn

2 was established as (*S*) on the basis of the positive Cotton effect at 229 ($\Delta\varepsilon$ +0.74) nm. Thus, the structure of compound **2** was determined as (*S*)-3,4,4′-trihydroxy-5-methoxy-α-ethoxybibenzyl, named dendrocandin D.

Dendrocandin E (3) Compound **3** was obtained as a red syrup. The HR-ESI-MS (*m/z* 275.0912, $[M-H]^-$) and NMR analyses revealed the molecular formula $C_{15}H_{16}O_5$. The ^{1}H-NMR spectrum of **3** exhibited 4H signal at δ_H 2.62 (4H, s) for the characteristic bibenzylic protons[4]; one methoxyl group at δ_H 3.70 (3H, s); and five aromatic protons, appearing as a pair of *m*-coupled signals at δ_H 6.17 (1H, d, J=1.5 Hz) and 6.22 (1H, d, J=1.5 Hz), and an ABX system at δ_H 6.41 (1H, dd, J=8.0, 2.0 Hz), 6.52 (1H, d, J=2.0 Hz) and 6.58 (1H, d, J=8.0 Hz). The ^{13}C-NMR spectrum showed the presence of one methoxyl, two methylene, five aromatic methine, and seven aromatic quaternary carbons (five oxygenated). According to the ^{1}H- and ^{13}C-NMR data and molecular formula, a bibenzyl skeleton with one methoxyl and four hydroxyl groups were deduced to the structure of compound **3**. The methoxyl group located at C-5, which was confirmed by ^{13}C–^{1}H long-range correlation peaks between δ_H 3.70 (MeO-5-H$_3$) and δ_C 149.4 (C-5) in the HMBC spectrum. Based on the above evidence, the structure of compound **3** was deduced to be 3,3′,4,4′-tetrahydroxy-5-methoxybibenzyl, named dendrocandin E.

The antioxidant activity of compounds **1**—**3** was evaluated by DPPH free radical scavenging assay: Vitamin C was used as positive control with IC_{50} 23.2 μM. Among the tested compounds, **3** showed significant scavenging activity with IC_{50} value of 15.6 μM, while **1** and **2** exhibited moderate potent antioxidant activities with IC_{50} 34.2 and 34.5 μM, respectively.

Table 1. ^{1}H- and ^{13}C-NMR Data of Compounds **1** and **2** (CD_3OD, 500 MHz) (δ in ppm, J in Hz)

No.	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1		134.6		134.2
2	6.35 (1H, d, 1.5)	108.6	6.36 (1H, d, 1.5)	108.4
3		146.4		146.4
4		133.5		134.4
5		149.5		149.4
6	6.28 (1H, d, 1.5)	103.4	6.28 (1H, d, 1.5)	103.4
1′		130.6		130.8
2′, 6′	6.87 (2H, d, 8.5)	131.5	6.87 (2H, d, 8.5)	131.5
3′, 5′	6.62 (2H, d, 8.5)	115.7	6.61 (2H, d, 8.5)	115.7
4′		156.6		156.6
α	4.12 (1H, t, 7.0)	86.9	4.22 (1H, t, 6.5)	85.1
α'	2.73 (1H, dd, 14.0, 7.0), 2.95 (1H, dd, 14.0, 7.0)	44.7	2.72 (1H, dd, 13.5, 6.5), 2.94 (1H, dd, 13.5, 6.5)	44.9
MeO-5	3.76 (3H, s)	56.7	3.75 (3H, s)	56.6
MeO-α	3.16 (3H, s)	56.5		
EtO-α			1.10 (3H, t, 7.0)	15.5
			3.26 (1H, m), 3.38 (1H, m)	65.0

Table 2. ^{1}H- and ^{13}C-NMR Data of Compound **3** (CD_3OD, 500 MHz) (δ in ppm, J in Hz)

No.	δ_H	δ_C	No.	δ_H	δ_C
1		134.3	3′		145.9
2	6.22 (1H, d, 1.5)	109.9	4′		144.2
3		146.3	5′	6.58 (1H, d, 8.0)	116.2
4		133.1	6′	6.41 (1H, dd, 8.0, 2.0)	120.8
5		149.4	α	2.62 (4H, s)	39.3
6	6.17 (1H, d, 1.5)	105.1	α'		38.7
1′		135.0	MeO-5	3.70 (3H, s)	56.5
2′	6.52 (1H, d, 2.0)	116.7			

Experimental

General Experimental Procedures and Plant Materials Details are provided in a previous paper.[2]

Extraction and Isolation The powdered air-dried stems of *D. candidum* (2.6 kg) were refluxed with EtOAc three times to get the EtOAc extract (57 g). The EtOAc extract was subjected to column chromatography on silica gel (200—300 mesh, 1000 g) and eluted with petroleum/EtOAc (100 : 0→0 : 100) to yield 13 fractions (1—13). Fr. 8 was further separated by Sephadex LH-20 column chromatography with $CHCl_3$/MeOH (1 : 1) as eluent, and then purified by prep HPLC on Waters-Symmetry Prep C_{18} column using MeOH/H_2O (45 : 55, 3.0 ml/min) as eluent to afford compounds **1** (1.9 mg) and **2** (3.1 mg). The retention time of compounds **1** and **2** were 10 min and 20 min, respectively. Fr. 10 was passed over a Sephadex LH-20 column with $CHCl_3$/MeOH (1 : 1) and then purified by prep HPLC with MeOH/H_2O (40 : 60, 3.0 ml/min) to yield compound **3** (1.7 mg). Its retention time was 11 min. The detection wavelength of prep HPLC was 203 nm.

Dendrocandin C (**1**): Yellow syrup, $[\alpha]_D^{20}$ +1.5° (c=0.13, MeOH), CD (MeOH): nm ($\Delta\varepsilon$): 226 (+2.64), UV λ_{max} (MeOH): nm (log ε): 201 (4.3), 284 (3.5), ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz), see Table 1, HR-ESI-MS *m/z* 289.1086, $[M-H]^-$ (Calcd for $C_{16}H_{17}O_5$: 289.1076).

Dendrocandin D (**2**): Red syrup, $[\alpha]_D^{20}$ +3.6° (c=0.22, MeOH), CD (MeOH): nm ($\Delta\varepsilon$): 229 (+0.74), UV λ_{max} (MeOH): nm (log ε): 215 (4.5), 278 (3.5), ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz), see Table 1, HR-ESI-MS *m/z* 303.1240, $[M-H]^-$ (Calcd for $C_{17}H_{19}O_5$: 303.1233).

Dendrocandin E (**3**): Red syrup, UV λ_{max} (MeOH): nm (log ε): 205 (4.5), 282 (3.7), ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz), see Table 2, HR-ESI-MS *m/z* 275.0912, $[M-H]^-$ (Calcd for $C_{15}H_{15}O_5$: 275.0920).

Antioxidant Activity The DPPH assay was performed by the modified method of Zhang *et al.*[3]: 100 μl of the test samples at different concentrations in EtOH and 100 μl of DPPH (Sigma) in EtOH (0.004%) were added in a 96-cell microplate. The absorbance was measured in a BT-MQX200 microplate spectrophotometer (BioTek) at 517 nm after 30 min, and the percent of inhibition activity was calculated. IC_{50} values denote the concentration of samples required to scavenge 50% DPPH free radicals. The tested samples at different concentrations without DPPH were used as controls to eliminate the influence of the samples' color. Vitamin C was used as positive control, and DPPH solution in ethanol served as negative control. All tests were performed in triplicate.

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