Four New Bibenzyl Derivatives from *Dendrobium candidum*

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Four new bibenzyl derivatives, namely, dendrocandins F—I (1—4), were isolated from the stems of *Dendrobium candidum*. Their structures were elucidated by the analysis of spectroscopic data. Dendrocandins F and G represent the fourth and fifth example of bisbibenzyl derivates with a dibenzopyran ring between two units, respectively. Dendrocandin H represents the first example of a bibenzyl derivative formed by a bibenzyl and a 1,4-phenanthraquinone unit *via* a dibenzopyran ring. Compounds 1—4 were examined for antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay.

Key words *Dendrobium candidum*; bibenzyl derivative; dendrocandin F; dendrocandin G; dendrocandin H; dendrocandin I

In the previous study, five new compounds and some known ones from *Dendrobium candidum* have been obtained and reported.[1,2] The further research on the phytochemical constituents of *D. candidum*, led to the isolation of four new bibenzyl derivatives, dendrocandins F—I (**1**—**4**). To the best of our knowledge, dendrocandins F (**1**) and G (**2**) represent the fourth and fifth example of bisbibenzyl derivates with a dibenzopyran ring between two units,[3,4] respectively. Dendrocandin H (**3**) represents the first example of a bibenzyl derivative formed by a bibenzyl and a 1,4-phenanthraquinone unit *via* a dibenzopyran ring. The structural elucidation and the evaluation of antioxidant activities of these compounds are reported in this contribution.

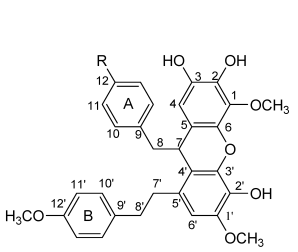
Results and Discussion

The air-dried and powdered stems of *D. candidum* were extracted with boiling EtOAc and concentrated. The EtOAc extract was chromatographed over silica gel with petroleum/EtOAc. The subsequent fractions were further purified using a varity of chromatographic techniques to yield four new bibenzyl derivatives (**1**—**4**).

Dendrocandin F (1) Compound **1** was obtained as a reddish-yellow syrup, and its molecular formula was determined as $C_{32}H_{32}O_8$ by HR-electron spray ionization (ESI)-MS (*m*/*z* 543.2026, $[M-H]^-$). The ^{1}H- and ^{13}C-NMR data of **1** were similar to those of nobilin E,[3] except for the substituted positions of methoxyl groups at A and B rings. Two symmetrical aromatic rings signals at δ 6.42 (2H, d, J=8.5 Hz), 6.56 (2H, d, J=8.5 Hz), 6.68 (2H, d, J=8.5 Hz) and 6.93 (2H, d, J=8.5 Hz) in ^{1}H-NMR spectrum, and the heteronuclear multiple bond connectivity (HMBC) correlations [δ 3.63 (12-OCH_3)/δ 160.4 (C-12) and δ 3.63 (12′-OCH_3)/δ 160.3 (C-12′)] suggested that the methoxyl groups were attached at C-12 and C-12′ of compound **1**. Thus, the structure of **1** was determined as showed in Fig. 1.

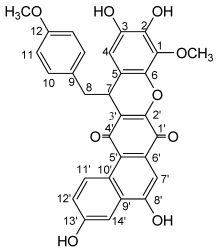
Dendrocandin G (2) Compound **2** was obtained as a reddish-yellow syrup, and its molecular formula $C_{31}H_{30}O_8$ was established by HR-ESI-MS (*m*/*z* 529.1866, $[M-H]^-$). The 1D-NMR data of compound **2** were very similar to those of compound **1**, except for the absence of a methoxyl group singnal. On the basis of 1D- and 2D-NMR data and molecular formula, it was confirmed that the methoxyl group at C-12 in compound **1** is replaced by a hydroxyl group in compound **2**.

Dendrocandin H (3) Compound **3** was obtained as a red amorphous powder, and its molecular formula $C_{30}H_{22}O_9$ was established by HR-ESI-MS (*m*/*z* 525.1190, $[M-H]^-$). The ^{1}H-NMR spectrum of compound **3** exhibited resonances for two methoxyl groups at δ 3.58 (3H, s) and 3.76 (3H, s); three protons of one methylene group and a methine one, as an ABX system at δ 2.84 (1H, dd, J=13.5, 4.5 Hz), 2.91 (1H, dd, J=13.5, 5.0 Hz) and 4.29 (1H, t, J=5.0, 4.5 Hz); and nine aromatic protons, appearing as a pair of *o*-coupled doublets at δ 6.53 (2H, d, J=9.0 Hz) and 6.55 (2H, d, J=9.0 Hz), an ABX system at δ 7.20 (1H, br d, J=9.5 Hz), 7.51 (1H, br s) and 9.37 (1H, d, J=9.5 Hz), and two singlet signals at δ 6.34 (1H, s) and 7.29 (1H, s). In combination with the heteronuclear single quantum coherence (HSQC) spectrum, the ^{13}C-NMR spectrum showed the presence of two methoxyl, one methylene, one methine, nine aromatic methine, 15 aromatic quaternary carbons, and two carbonyl carbons. On the basis



1 R=OCH_3

2 R=OH



3

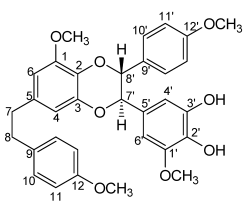


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Fig. 1. Compounds **1**—**4** Isolated from *Dendrobium candidum*

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of the ^{1}H- and ^{13}C-NMR data and molecular formula, the skeleton of **3** was identified as a compound composed of a bibenzyl unit and a 1,4-phenanthraquinone unit, and substituted by four hydroxyl and two methoxyl groups.

In the HMBC spectrum, ^{13}C–^{1}H long-range correlation peaks found at H-4/C-2, 3, 5, 6; H-7/C-4, 5, 9 and H-8/C-5, 10 deduced the bibenzyl unit. The HMBC correlations of H-7′/C-1′, 5′, 6′, 8′, 9′ and H-11′/C-5′, 9′, 13′ confirmed the structure of 1,4-phenanthraquinone unit. Compound **3** exhibited diagnostic resonances for H-7 and H-8 at δ 2.84 (1H, dd, J=13.5, 4.5 Hz), 2.91 (1H, dd, J=13.5, 5.0 Hz) and 4.29 (1H, t, J=5.0, 4.5 Hz), and the C-7 and C-8 at δ 36.1 and 43.8, respectively, which was consistent with what found in **1**. And by the molecular formula and its unsaturation degree, compound **3** was found to contain a dibenzopyran ring between two units. The fused locations were determined at the C-5 and C-3′ *via* C-7 and at the C-6 and C-2′ *via* an oxygen atom by the HMBC correlations of H-7/C-2′, 3′, 5, 6, and H-8/C-3′, 5. The carbonyl carbons of δ 180.5 and 187.6 were located at C-1′ and C-4′, respectively, which were confirmed by the correlations of H-7′/C-1′ and H-7/C-4′ in HMBC spectrum. Thus, the structure of **3** was determined as showed in Fig. 1.

Dendrocandin I (4) Compound **4** was obtained as a yellow syrup, and its molecular formula was determined as $C_{32}H_{32}O_8$ by HR-ESI-MS (m/z 543.2026, $[M-H]^-$). The ^{1}H-NMR spectrum of compound **4** exhibited resonances for four methoxyl groups at δ 3.64 (3H, s), 3.74 (3H, s), and 3.75 (6H, s); two methylene groups at δ 2.79 (2H, m) and 2.82 (2H, m); two oxygenated methine groups at δ 4.70 (1H, d, J=8.0 Hz) and 4.74 (1H, d, J=8.0 Hz); and 12 aromatic protons, appearing as two pairs of *m*-coupled signals at δ 6.13 (1H, br s), 6.30 (1H, d, J=1.5 Hz), 6.36 (1H, br s) and 6.45 (1H, br s), and two pairs of *o*-coupled doublets at δ 6.79 (2H, d, J=8.5 Hz), 6.81 (2H, d, J=8.5 Hz), 7.04 (2H, d, J=8.5 Hz) and 7.08 (2H, d, J=8.5 Hz), which indicated the presence of two 1,3,4,5-tetrasubstituted and two 1,4-disubstituted aromatic rings. The ^{13}C-NMR data showed the presence of four methoxyl, two methylene, two methine, 12 aromatic methane, and 12 aromatic quaternary carbons. On the basis of the ^{1}H- and ^{13}C-NMR data and molecular formula, the skeleton of compound **4** was identified as a bisbibenzyl derivative with two hydroxyl and four methoxyl groups.

According to the HMBC correlations found at H-4/C-2, 3, 6, 7; H-8/C-7, 9, 10; H-4′/C-2′, 3′, 5′, 6′, 7′; and H-8′/C-7′, 9′, 10′ in HMBC spectrum, two bibenzyl units were determined. Based on the molecular formula and its unsaturation degree, compound **4** was found to contain a 1,4-dioxane ring. The ring linked at C-2/C-8′ *via* an oxygen atom and at C-3/C-7′ *via* another oxygen atom, which was determined definitely by HMBC correlations H-7′/C-3 and H-8′/C-2. The relative configurations of the chiral centers of dioxane ring were deduced as *trans* from the coupling constant ($J_{7',8'}$= 8.0 Hz) between H-7′ and H-8′,[5—7] and the correlations of H-7′/H-10′ and H-8′/H-4′, H-6′ in rotating frame Overhauser enhancement spectroscopy (ROESY) spectrum.

Due to the small amount of compounds **1**—**4**, the absolute configurations of the chiral centers were prevented from being determined. The small optical rotations of compound **3** and **4**, may be due to their very small $[\alpha]_D$ values. The circular dichroism (CD) spectrum of **1** ($\Delta\varepsilon_{210}$ +3.13, $\Delta\varepsilon_{235}$ +1.34, and $\Delta\varepsilon_{282}$ +0.80) which is different from **2** ($\Delta\varepsilon_{216}$ +2.31, and $\Delta\varepsilon_{275}$ +0.71), is similar to **3** ($\Delta\varepsilon_{201}$ +1.93, $\Delta\varepsilon_{225}$ +0.74, and $\Delta\varepsilon_{284}$ +0.66).

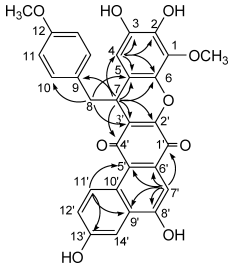

Fig. 2. Key HMBC Correlations of Compound **3**

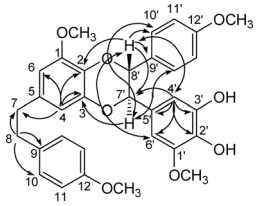

Fig. 3. Key HMBC (⟶) and ROESY (⟷) Correlations of Compound **4**

The antioxidant activity of compounds **1**—**4** was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay: Vitamin C was used as positive control with IC_{50} 23.2 μM. Among the tested compounds, **3** and **4** showed significant scavenging activity with IC_{50} values of 19.8 and 21.3 μM, while **1** and **2** exhibited moderate potent antioxidant activities with IC_{50} 55.8 and 32.4 μM, respectively.

Experimental

General ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) spectra were measured on an Inova-501 spectrometer, ^{13}C-NMR (150 MHz) and ROESY spectra were measured on a Varian Unity 600 instrument. Chemical shifts are given in δ values (ppm) relative to tetramethylsilane (TMS) as an internal standard. The HR-ESI-MS was recorded on a LTQ Orbitrap XL instrument. Optical rotations were measured using a Perkin-Elmer 341 digital polarimeter. UV spectra were measured with a Shimadzu UV-2550 UV–VIS recording spectrometer. Silica gel (300—400 mesh, Qingdao Marine Chemical Factory) and Sephadex LH-20 (Pharmazia) was used for column chromatography, and silica gel GF_{254} plates (Yantai Marine Chemical Co., Ltd.) were used for thin-layer chromatography. The analytical HPLC was performed on a Waters HPLC system equipped with a PAD-2996 detector using a Waters Symmetry C_{18} column (3.9×150 mm). The preparative HPLC was carried out on a Waters HPLC system with DAD-2487 detector using a Waters SymmetryPrep C_{18} column (7.8×300 mm).

Plant Materials The stems of *D. candidum* were collected in Zhejiang Province in 2006 and identified by Prof. Shun-Xing Guo of the Institute of Medicinal Plant Development, Peking Union Medical College. A voucher specimen (TPSH-2006) was deposited in the herbarium of the Institute of Medicinal Plant Development.

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).

Fraction 6 was further separated by column chromatography on silica gel and eluted with petroleum/acetone (9 : 1, 8.5 : 1.5, 8 : 2, 7.5 : 2.5, 7 : 3, 6 : 4) to yield 6 subfractions. The petroleum/acetone (7 : 3) subfraction was passed over a Sephadex LH-20 column with $CHCl_3$/MeOH (1 : 1) as eluent and then purified by preparative HPLC (77% MeOH) to yield compounds **1** (5.8 mg) and **4** (5.6 mg).

Fraction 10 was subjected to column chromatography on silica gel and eluted with $CHCl_3$/MeOH (10 : 0, 9.5 : 0.5, 9 : 1, 8.5 : 1.5, 8 : 2, 7 : 3, 6 : 4) to

Table 1. ^{1}H-NMR Data for Compounds **1**—**4** (500 MHz, CD_3OD, δ ppm, J in Hz)

No.	1	2	3	4
4	5.97 s	5.98 s	6.34 s	6.45 br s
6				6.36 br s
7	3.80 t (6.0)	3.79 t (6.0)	4.29 t (5.0, 4.5)	2.79 m
8	2.56 dd (12.5, 6.5)	2.54 dd (12.5, 7.0)	2.84 dd (13.5, 4.5)	2.82 m
	2.63 dd (12.5, 5.0)	2.60 dd (12.5, 5.5)	2.91 dd (13.5, 5.0)	
10	6.42 d (8.5)	6.34 d (8.0)	6.53 d (9.0)	7.08 d (8.5)
11	6.56 d (8.5)	6.44 d (8.0)	6.55 d (9.0)	6.81 d (8.5)
4′				6.30 d (1.5)
6′	6.47 s	6.47 s		6.13 br s
7′	2.68 m, 2.79 m	2.70 m, 2.81 m	7.29 s	4.70 d (8.0)
8′	2.73 m	2.74 m		4.74 d (8.0)
10′	6.93 d (8.5)	6.94 d (8.0)		7.04 d (8.5)
11′	6.68 d (8.5)	6.70 d (8.0)	9.37 d (9.5)	6.79 d (8.5)
12′			7.20 br d (9.5)	
14′			7.51 br s	
MeO-1	3.82 s	3.83 s	3.76 s	3.75 s
MeO-12	3.63 s		3.58 s	3.75 s
MeO-1′	3.75 s	3.75 s		3.64 s
MeO-12′	3.63 s	3.65 s		3.74 s

Table 2. ^{13}C-NMR Data for Compounds **1**—**4** (CD_3OD, δ ppm)

No.	1[a]		2[b]		3[b]		4[a]	
	δ_C	HMBC[c]	δ_C	HMBC[c]	δ_C	HMBC[c]	δ_C	HMBC[c]
1	138.1		137.3		137.8		149.9	6
2	138.9	4	138.1	4	138.8	4	133.3	4, 6, 8′
3	143.0	4	142.2	4	144.6	4	145.7	4, 7′
4	111.1	7	110.3	7	109.8	7	110.7	6, 7
5	118.7	4, 7, 8	118.0	7, 8	123.2	7, 8	135.6	7
6	141.2	4, 7	140.4	4, 7	138.5	4, 7	106.5	4, 7
7	40.8	4, 8	40.2	4, 8	36.1	4, 8	39.1	4, 6, 8
8	46.5	7, 10	45.7	7, 10	43.8	7, 10	38.2	7, 10
9	132.7	7, 11	130.8	7, 11	130.9	7, 11	135.1	8, 11
10	132.4	8	131.6	8	131.8	8	130.5	8
11	114.9		115.5		114.2		114.7	
12	160.4	10, 11	156.7	10, 11	159.8	10, 11	159.4	10, 11
1′	148.7	6′	147.9	6′	180.5	7′	149.2	6′
2′	134.7	6′	133.9	6′	151.0	7	135.3	4′, 6′
3′	143.7	7	142.9	7	114.9	7, 8	146.3	4′
4′	120.0	7, 8, 6′, 7′	119.4	7, 8, 6′, 7′	187.6	7	109.4	6′, 7′
5′	130.9	7, 6′, 7′	130.2	7′, 8′	121.3	7′, 11′	128.7	4′, 6′, 7′
6′	109.8	7′	109.0	7′	131.7	7′	104.5	4′, 7′
7′	35.7	6′, 8′	34.9	6′, 8′	104.3		82.0	4′, 6′, 8′
8′	39.0	7′, 10′	38.2	7′, 10′	158.7	7′, 14′	81.7	7′, 10′
9′	135.7	7′, 11′	134.9	7′, 8′, 11′	131.5	7′, 11′	130.3	11′
10′	131.4	8′	130.6	8′	127.9	12′, 14′	130.2	8′
11′	115.5		114.7		131.2		114.5	
12′	160.3	10′, 11′	159.5	10′, 11′	123.1	14′	161.2	10′, 11′
13′					158.3	11′		
14′					105.6	12′		
MeO-1	62.5		61.7		61.8		55.7	
MeO-12	56.3				55.5		55.7	
MeO-1′	57.6		56.8				56.5	
MeO-12′	56.4		55.6				56.6	

a) 125 MHz for ^{13}C. *b*) 150 MHz for ^{13}C. *c*) HMBC correlations are from the carbon(s) specified to the indicated proton.

yield 7 subfractions. The $CHCl_3$/MeOH (9.5 : 0.5) subfraction was purified by Sephadex LH-20 column with $CHCl_3$/MeOH (1 : 1) as eluent, then followed by preparative HPLC (65% MeOH) to yield compound **2** (1.5 mg). The $CHCl_3$/MeOH (9 : 1) subfraction was purified by preparative HPLC (65% MeOH) to yield compound **3** (2.5 mg).

Dendrocandin F (**1**): Reddish-yellow syrup; $[\alpha]_D^{20}$ +2.5 (c=0.08, MeOH); CD (MeOH): nm ($\Delta\varepsilon$): 210 (+3.13), 235 (+1.34), and 282 (+0.80); UV λ_{max} (MeOH): nm (log ε): 200 (4.7), 278 (3.8); ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz), see Table 1 and Table 2; HR-ESI-MS m/z: 543.2026, $[M-H]^-$ (Calcd for $C_{32}H_{31}O_8$: 543.2019).

Dendrocandin G (**2**): Reddish-yellow syrup; $[\alpha]_D^{20}$ −7.9 (c=0.07, MeOH); CD (MeOH): nm ($\Delta\varepsilon$): 216 (+2.31), and 275 (+0.71); UV λ_{max} (MeOH): nm (log ε): 201 (4.7), 279 (3.8); ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 150 MHz), see Table 1 and Table 2; HR-ESI-MS m/z: 529.1866, $[M-H]^-$ (Calcd for $C_{31}H_{29}O_8$: 529.1862).

Dendrocandin H (**3**): Red amorphous powder; $[\alpha]_D^{20}$ ±0 (c=0.09, MeOH); CD (MeOH): nm ($\Delta\varepsilon$): 201 (+1.93), 225 (+0.74), and 284 (+0.66); IR (KBr) cm^{-1}: 3392, 1649, 1616, 1585, 1510, 1473, 1373, 1326, 1228, 1130, 1029; UV λ_{max} (MeOH): nm (log ε): 220 (4.6), 254 (4.4), 316 (4.4), 389 (3.8); ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 150 MHz), see Table 1 and Table 2; HR-ESI-MS m/z: 525.1190, $[M-H]^-$ (Calcd for $C_{30}H_{21}O_9$: 525.1186).

Dendrocandin I (**4**): Yellow syrup; $[\alpha]_D^{20}$ ±0 (c=0.08, MeOH), CD (MeOH): nm ($\Delta\varepsilon$): 201 (+5.89), 224 (−2.49), 245 (+0.78), and 277.5 (+0.96); UV λ_{max} (MeOH): nm (log ε): 215 (4.7), 274 (3.0); ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz), see Table 1 and Table 2; HR-ESI-MS m/z: 543.2026, $[M-H]^-$ (Calcd for $C_{32}H_{31}O_8$: 543.2019).

Measurement of DPPH Free Radical Scavenging Capacity The DPPH free radical scavenging assay was performed as in our previous report.[2)]

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References

1) Li Y., Wang C. L., Guo S. X., Yang J. S., Xiao P. G., *Chem. Pharm. Bull.*, **56**, 1477—1479 (2008).
2) Li Y., Wang C. L., Wang Y. J., Guo S. X., Yang J. S., Chen X. M., Xiao P. G., *Chem. Pharm. Bull.*, **57**, 218—219 (2009).
3) Zhang X., Xu J. K., Wang J., Wang N. L., Kurihara H., Kitanaka S., Yao X. L., *J. Nat. Prod.*, **70**, 24—28 (2007).
4) Zhang C. F., Wang M., Wang L., Iinuma M., Zhang M., Xu L. S., Wang Z. T., *Indian J. Chem.*, **47B**, 952—956 (2008).
5) Ma C. Y., Zhang H. J., Tan G. T., Hung N. V., Cuong N. M., Soejarto D. D., Fong H. H. S., *J. Nat. Prod.*, **69**, 346—350 (2006).
6) Kim T. H., Ito H., Hayashi K., Hasegawa T., Machiguchi T., Yoshida T., *Chem. Pharm. Bull.*, **53**, 641—644 (2005).
7) Banwell M. G., Bezos A., Chand S., Dannhardt G., Kiefer W., Nowe U., Parish C. R., Savage G. P., Ulbrich H., *Org. Biomol. Chem.*, **1**, 2427—2429 (2003).