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Two New Diphenylethylenes from *Arundina graminifolia* and Their Cytotoxicity

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Two new diphenylethylenes, gramniphenols H and I (**1** and **2**), together with six known diphenylethylenes (**3**-**8**), were isolated from A*rundina graminifolia*. The structures of **1**-**8** were elucidated by spectroscopic methods including extensive 1D- and 2D-NMR techniques. Compounds **1** and **2** were evaluated for their cytotoxicity against five human tumor cell lines. Compound **1** showed cytotoxicity against PC3 cells with IC_{50} value of 3.5 μM. Compound **2** showed cytotoxicity against NB4 and PC3 cells with IC_{50} values of 3.6 and 3.8 μM, respectively.

Key Words : A*rundina graminifolia*, Diphenylethylenes, Structure elucidation, Cytotoxicity

Introduction

Arundina graminifolia (bamboo orchid) is a terrestrial multiperennial orchid.[1] It has been widely used for clearing heat, detoxicating, and dissipating blood stasis by Dai people lived in Xishuangbanna, Yunnan province.[2] Previous phytochemical studies of *A. graminifolia* have shown the presence of stilbenoids,[3] bibenzyls,[4] phenanthrenes,[5,6] and other phenolic compounds.[7,8] In our previous studies, some new phenolic compounds possessing anti-tobacco mosaic virus (anti-TMV) and anti-HIV-1 properties were isolated from *A. gramnifolia* grown in the Xishuangbanna and Honghe Prefecture.[7,8] Motivated by a search for new bioactive metabolites from local plants, our group has investigated the chemical constituents of the whole plant of *A. graminifolia* growing in the Wuzhishan Prefecture, Hainan province, which led to the isolation and characterization of two new diphenylethylenes (**1** and **2**), and six known diphenylethylenes (**3**-**8**). This paper deals with the isolation, structural characterization of the new compounds, and their cytotoxicity against five human tumor cell lines.

Results and Discussion

The whole plant of *A. graminifolia* was extracted with 70% aqueous acetone. The extract was subjected repeatedly to column chromatography on silica gel, RP-18, and semi-preparative RP-HPLC separation to afford compounds **1**-**8**. Their structures were shown in Figure 1. The ^{1}H- and ^{13}C NMR data of the compounds **1** and **2** were listed in Table 1. By compared with the literature, the known compounds were identified as pinosylvin (**3**),[9] 3,5-dihydroxy-stilbene-3-*O*-*β*-D-glucoside (**4**),[10] rhapontigen (**5**),[11] bauhiniastatin D (**6**),[12] 3-hydroxy-4,3,5-trimethoxy-*trans*-stilbene (**7**).[13] 2,3-dihydroxy-3,5-dimethoxystilbene (**8**).[14]

Compound **1** was obtained as a yellow gum. Its HRESIMS in the positive mode revealed a peak at *m/z* 353.1008 $[M+Na]^+$ indicative of the molecular formula of $C_{18}H_{18}O_6$, corresponding to 10 degrees of unsaturation. Its UV spectrum showed the maximum absorption at 315, 236 and 210 nm, and its IR spectrum also exhibited the presence of hydroxy group (3412 cm^{-1}) and aromatic ring (1612, 1586, 1524, 1458 cm^{-1}). Its ^{1}H, ^{13}C, and DEPT NMR spectra

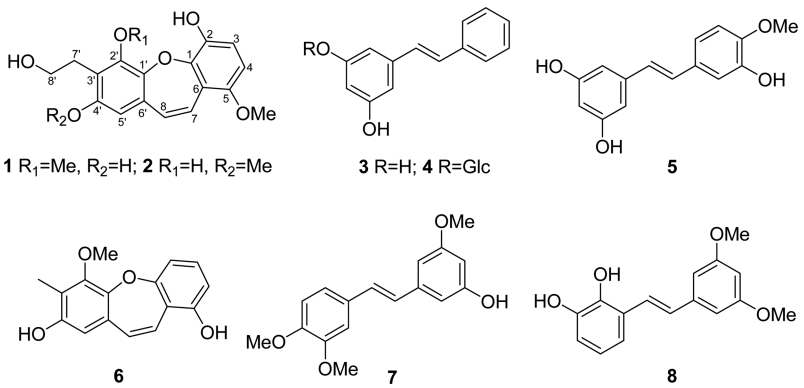

Figure 1. The structures of diphenylethylenes from *A. graminifolia*.

Table 1. ^{1}H and ^{13}C NMR data of compounds **1** and **2** (δ in ppm, data obtained in C_5D_5N)

No.	Compound **1**		Compound **2**	
	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)
1	145.1 s		145.0 s	
2	141.0 s		140.0 s	
3	115.5 d	6.89 d (8.8)	115.4 d	6.84 d (8.8)
4	106.5 d	6.60 d (8.8)	106.1 d	6.56 d (8.8)
5	150.5 s		151.0 s	
6	120.9 s		121.2 s	
7	126.0 d	7.07 d (11.6)	125.7 d	7.03 d (11.6)
8	130.0 d	6.79 d (11.6)	129.7 d	6.76 d (11.6)
1′	136.4 s		137.4 s	
2′	154.0 s		147.8 s	
3′	112.9 d		112.2 d	
4′	149.4 s		152.2 s	
5′	104.1 d	6.52 s	102.4 d	6.43 s
6′	127.8 s		128.0 s	
7′	35.6 t	2.59 t (7.2)	34.4 t	2.53 t (7.2)
8′	63.6 t	3.64 t (7.2)	63.9 t	3.61 t (7.2)
5-OMe	55.9 q	3.80 s	55.9 q	3.81 s
2′-OMe			55.8 q	3.79 s
4′-OMe	61.3 q	3.87 s		
2-Ar-OH		9.43 brs		9.43 brs
2′-Ar-OH		9.62 brs		
4′-Ar-OH				9.72 brs

(Table 1) showed signals for 18 carbons and 18 hydrogen atoms, corresponding to the following functional groups: a 1,2,5,6-tetrasubstituted benzene [C-1 to C-6; δ_C 145.1, 141.0, 115.5, 106.5, 150.5, and 120.9; δ_H 6.89 d (J = 8.8) and 6.60 d (J = 8.8)], a 1',2',3',4',6'-pentasubstituted benzene (C-1' to C-6'; δ_C 136.4, 154.0, 112.9, 149.4, 104.1, 127.8; δ_H 6.52 s), a pair of double bond [CH-7 and CH-8; δ_C 126.0 and 130.0; δ_C 7.07 d (J = 11.6) and 6.79 d (J = 11.6)], a hydroxyethyl unit [CH_2-7' and CH_2-8'; δ_C 35.6, 63.6; 2.59 t (7.2), 3.64 t (7.2)], two methoxy groups (δ_C 55.9, 61.3; δ_H 3.80 s, 3.87 s), and two phenolic hydroxy groups (δ_C 9.43 brs, 9.62 brs). Detailed analysis the functional groups suggested that **1** should be an dibenz[*b*,*f*]oxepin derivatives.[15] The general features of the ^{1}H and ^{13}C NMR spectra of **1** resembled to those of bauhiniastatin C[15] except that a vinyl methyl in bauhiniastatin C was replaced by a hydroxyethyl unit in **1**. In HMBC spectrum, the long-range correlations (Figure 2) of H-7' (δ_H 2.59) to C-2' (δ_C 154.0), C-3' (δ_C 112.9) and C-4' (δ_C 149.4), of H-8' (δ_H 3.64) to C-3' (δ_C 112.9), were observed in **1**. This led us to conclude that the hydroxyethyl unit was located on C-3'. The HMBC correlations of two methoxy protons (δ_H 3.80, 3.87) with C-5 (δ_C 150.5) and C-2 (δ_C 154.0) revealed that two methoxy groups should be located at C-5 and C-2. The HMBC correlations between the phenolic hydroxy proton (δ_H 9.43) and C-1 (δ_C 145.1), C-2 (δ_C 141.0), and C-3 (δ_C 115.5), as well as those between the other hydroxy proton (δ_H 9.26) and C-3' (δ_C 112.9), C-4' (δ_C 149.4), and C-5' (δ_C 104.1), led to the assignment of two phenolic hydroxy groups at C-2 and C-4. The above evidence led to oxepin structure **1** for gramniphenol H.

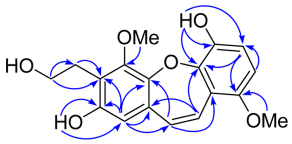

Figure 2. Selected HMBC (↷) correlations of *1*.

Table 2. The cytotoxicity data for the compounds **1-8**

Compounds	IC_{50} values (μM, the results were means of three tests)				
	NB4	A549	SHSY5Y	PC3	MCF7
1	8.2	> 10	6.5	3.5	8.6
2	3.6	8.4	6.8	3.8	8.4
3	>10	> 10	> 10	> 10	> 10
4	> 10	8.5	> 10	7.4	> 10
5	6.8	> 10	> 10	9.2	> 10
6	8.2	5.4	> 10	4.8	5.9
7	7.8	8.6	> 10	4.7	> 10
8	8.2	> 10	> 10	9.0	> 10
Paclitaxel	0.03	0.02	0.2	0.2	0.1

Compounds **2** was also obtained as yellow gum, and should sodiated molecular ion at *m/z* 353.0997 $[M+Na]^+$ in the HRESIMS. This indicated the compounds **1** and **2** have the same molecular formula. The ^{1}H- and ^{13}C NMR spectra of **2** were similar to those of **1**. The obvious chemical shift differences resulted from the down-shift of C-4 from δ_C 149.4 ppm to δ_C 152.2 ppm, and the up-shift of C-2 form δ_C 154.0 ppm to δ_C 147.8 ppm. These suggested the substituent groups at C-2 and C-4 should be varied. For compound **2**, two methoxy groups located at C-5 and C-4, two phenolic hydroxy group located at C-2 and C-2' were also be concluded by the analysis of its HMBC spectrum. Accordingly, the structure of gramniphenol I (**2**) was determined as shown. Compounds **1** and **2** are the first naturally occurring dibenz[*b*,*f*]oxepin derivatives possessing a hydroxyethyl unit.

Since certain of the stilbenoids from Orchidaceae exhibit potential cytotoxicity,[16-18] compounds **1-8** were tested for their cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3, and MCF7) using the MTT method as reported previously.[19] Paclitaxel was used as the positive control. The results were shown in Table 2. Compound **1** showed cytotoxicity against PC3 cells with IC_{50} value of 3.5 μM. Compound **2** showed cytotoxicity against NB4 and PC3 cells with IC_{50} values of 3.6 and 3.8 μM, respectively. The other compounds also showed modest cytotoxicity with IC_{50} below 10 μM for some selected cells line.

Experimental Section

General Experimental Procedures. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR

spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on a DRX-500 NMR spectrometer with TMS as internal standard. Unless otherwise specified, chemical shifts (δ) are expressed in ppm with reference to the solvent signals. HRESIMS was performed on a VG Autospec-3000 spectrometer. Semipreparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with Zorbax PrepHT GF (21.2 mm × 25 cm) or Venusil MP C_{18} (20 mm × 25 cm) columns. Column chromatography was performed using silica gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40-63 μm, Merck, Darmstadt, Germany), and MCI gel (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). The fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4 in EtOH.

Plant Material. The whole plant of *A. graminifolia* was collected in Wuzhishan Prefecture, Hainan province, People's Republic of China, in September 2011. The identification of the plant material was verified by Dr. Yuan. N, of Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (YNNU 2011-9-38) has been deposited in our laboratory.

Extraction and Isolation. The air-dried and powdered *A. graminifolia* (4.8 kg) were extracted four times with 70% aqueous acetone (4 × 6 L) at room temperature and filtered. The filtrate was evaporated under reduced pressure, and the crude extract (315 g) was decolorized by MCI. The 90% methanol part (230 g) was chromatographed on a silica gel column eluting with a $CHCl_3$-MeOH gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A–F. The further separation of fraction C (8:2, 38.2 g) by silica gel column chromatography, eluted with petroleum ether-acetone (9:1–1:2), yielded mixtures C1–C7. Fraction C4 (6:4, 5.27 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semi-preparative HPLC (50% MeOH-H_2O, flow rate 12 mL/min) to give **1** (8.33 mg), **2** (11.5 mg), **6** (13.8 mg), **7** (16.8 mg), and **8** (13.2 mg). Fraction C5 (1:1 3.85 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semi-preparative HPLC (44% MeOH-H_2O, flow rate 12 mL/min) to give **5** (13.8 mg). The further separation of fraction D (7:3, 20.8 g) by silica gel column chromatography, eluted with chloroform-acetone (8:2–1:2), yielded mixtures D1–C5. Fraction D3 (6:4, 3.18 g) was subjected to semi-preparative HPLC (30% MeOH-H_2O, flow rate 12 mL/min) to give **4** (54.8 mg).

Cytotoxicity Assay. Colorimetric assays were performed to evaluate each compound's activity. NB4 (human acute promyelocytic leukemia cells), A549 (Human lung adenocarcinoma epithelial cells), SHSY5Y (human neuroblastoma cells), PC3 (Human prostate cancer cell), and MCF7 (human breast adenocarcinoma cells) tumor cells were purchased from the American Type Culture Collection (ATCC). All cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT) supplemented with 10% fetal bovine serum (Hyclone) at 37 °C in a humidified atmosphere with 5% CO_2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO). Briefly, 100 μL of suspended adherent cells were seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition. In addition, suspended cells were seeded just before drug addition, with an initial density of 1×10^5 cells/mL in 100 μL of medium. Each tumor cell line was exposed to each test compound at various concentrations in triplicate for 48 h; paclitaxel (Sigma, purity > 95%) was used as a positive control. After the incubation, MTT (100 μg) was added to each well, and the incubation was continued for 4 h at 37 °C. The cells were lysed with 100 μL of 20% SDS-50% DMF after removal of 100 μL of the medium. The optical density of the lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680). The IC_{50} value of each compound was calculated by Reed and Muench's method.

Gramniphenol H: Yellow gum; UV (MeOH), λ_{max} (log ε) 315 (3.92), 236 (4.12), 210 (4.32); IR (KBr) cm^{-1}: 3412, 3028, 2965, 2875, 1612, 1586, 1524, 1458, 1429, 1398, 1185, 1153, 1126, 1078, 855. ^{1}H- and ^{13}C-NMR data ($CDCl_3$, 500 MHz and 125 MHz, respectively), see Table 1. ESIMS (positive ion mode), *m/z* 353 $[M+Na]^+$; HRESIMS (positive ion mode), *m/z* 353.1008 $[M+Na]^+$ (calcd. 353.1001 for $C_{18}H_{18}NaO_6$).

Gramniphenol I: Yellow gum, UV (MeOH), λ_{max} (log ε) 314 (3.88), 235 (4.10), 210 (4.38); IR (KBr) cm^{-1}: 3410, 3032, 2968, 2872, 1615, 1583, 1520, 1461, 1426, 1395, 1182, 1150, 1124, 1076, 859. ^{1}H- and ^{13}C-NMR data ($CDCl_3$, 500 MHz and 125 MHz, respectively), see Table 1. ESIMS (positive ion mode), *m/z* 353 $[M+Na]^+$; HRESIMS (positive ion mode), *m/z* 353.0997 $[M+Na]^+$ (calcd. 353.1001 for $C_{18}H_{18}NaO_6$).

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