A New Diphenylethylene from *Arundina graminifolia* and Its Cytotoxicity

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Received: 6 July 2013;

Accepted: 2 December 2013;

Published online: 23 June 2014;

AJC-15370

A new diphenylethylene, gramniphinol J (**1**), was isolated from *Arundina graminifolia*. Its structure was elucidated by spectroscopic methods including extensive 1D- and 2D-NMR techniques. Compound **1** was evaluated for its cytotoxicity against five human tumor cell lines and showed cytotoxicity against PC3 and SHSY5Y cells with IC₅₀ values of 8.2 and 7.6 μ M.

Keywords: *Arundina graminifolia*, Diphenylethylene, Structure elucidation, Cytotoxicity.

INTRODUCTION

Arundina graminifolia (bamboo orchid) is a terrestrial multiperennial orchid¹. It has been widely used for clearing heat, detoxicating and dissipating blood stasis by Dai people lived in Xishuangbanna, Yunnan province of China². Previous phytochemical studies of *A. graminifolia* have shown the presence of stilbenoids³, dibenzyls⁴, 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. graminifolia* 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 Dehong Prefecture, Yunnan province, which led to the isolation and characterization of a new diphenylethylene (**1**). This paper deals with the isolation and structural characterization of this new compound and its cytotoxicity against five human tumor cell lines.

EXPERIMENTAL

Ultra-violet 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 $\times$ 25 cm) or Venusil MP C₁₈ (20 mm $\times$ 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 μ m, Merck, Darmstadt, Germany) and MCI gel (75-150 μ m, Mitsubishi Chemical Corporation, Tokyo, Japan). The fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 5 % H₂SO₄ in EtOH.

The whole plant of *A. graminifolia* was collected in Dehong Prefecture, Yunnan 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 2011-9-39) has been deposited in our laboratory.

Extraction and isolation: The air-dried and powdered *A. graminifolia* (4.5 kg) were extracted four times with 70 % aqueous acetone (4 $\times$ 6 L) at room temperature and filtered. The filtrate was evaporated under reduced pressure and the crude extract (305 g) was decolorized by MCI. The 90 % methanol part (225 g) was chromatographed on a silica gel column eluting with a CHCl₃-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, 22.6 g) by silica gel column chromatography, eluted with petroleum ether-acetone (9:1-1:2), yielded mixtures C1-C7. Fraction C4 (6:4, 5.45 g) was subjected to silica gel column chromatography using petroleum

ether-acetone and semi-preparative HPLC (50 % MeOH-H₂O, flow rate 12 mL/min) to give **1** (11.8 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 cell lines 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 % CO₂. 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). In brief, 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 IC₅₀ value of each compound was calculated by Reed and Muench's method.

Gramniphénol J: Yellow gum; UV (MeOH), $\lambda_{\max}$ (log ϵ) 316 (3.87), 238 (4.05), 210 (4.35); IR (KBr, $\nu_{\max}$, cm⁻¹): 3410, 3025, 2962, 2873, 1610, 1584, 1522, 1456, 1425, 1396, 1182, 1150, 1122, 1075, 853. ¹H and ¹³C NMR data (CDCl₃, 500 MHz and 125 MHz, respectively), see Table-1. ESIMS (positive ion mode), m/z 353 [M + Na]⁺; HRESIMS (positive ion mode), m/z 353.0993 [M + Na]⁺ (Calcd. 353.1001 for C₁₈H₁₈O₆Na).

RESULTS AND DISCUSSION

The whole plants 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 compound **1**. Its structure was shown in Fig. 1. The ¹H and ¹³C NMR data of **1** were listed in Table-1.

Fig. 1. Structure of new compound

Compound **1** was obtained as a yellow gum. Its HRESIMS in the positive mode revealed a peak at m/z 353.0993 [M + Na]⁺

TABLE-1
¹H AND ¹³C NMR DATA OF COMPOUND **1**
(δ IN ppm, DATA OBTAINED IN CDCl₃)

No.	δ_c (m)	δ_H (m, J, Hz)	No.	δ_c (m)	δ_H (m, J, Hz)
1	145.4 s		3'	112.5 s	
2	141.8 s		4'	149.1 s	
3	115.0 d	6.85 d (8.8)	5'	104.1 d	6.53 s
4	107.0 d	6.62 d (8.8)	6'	128.0 s	
5	152.5 s		7'	35.9 t	2.60 t (7.2)
6	121.6 s		8'	63.8 t	3.62 t (7.2)
7	125.9 d	7.08 d (11.6)	2-OMe	55.9 q	3.80 s
8	129.9 d	6.81 d (11.6)	2'-OMe	61.0 q	3.84 s
1'	136.8 s		5-Ar-OH		9.58 brs
2'	154.2 s		4'-Ar-OH		9.75 brs

indicative of the molecular formula of C₁₈H₁₈O₆, corresponding to 10 degrees of unsaturation. Its UV spectrum showed the maximum absorption at 316, 238 and 210 nm and its IR spectrum also exhibited the presence of hydroxy group (3410 cm⁻¹) and aromatic ring (1610, 1584, 1522, 1456 cm⁻¹). Its ¹H, ¹³C and DEPT NMR spectra (Table-1) showed signals for 18 carbons and 18 hydrogen atoms, corresponding to a the following functional groups: a 1,2,5,6-tetrasubstituted benzene [C-1 to C-6; δ_c 145.4, 141.8, 115, 107, 152.5, 121.6; δ_H 6.85 d (J = 8.8) and 6.62 d (J = 8.8)], a 1',2',3',4',6'-pentsubstituted benzene (C-1' to C-6'; δ_c 136.8, 154.2, 112.5, 149.1, 104.1, 128; δ_H 6.53 s), a pair of double bond [CH-7 and CH-8; δ_c 125.9 and 129.9; δ_H 7.08 d (J = 11.6) and 6.85 d (J = 11.6)], a hydroxyethyl unit [CH₂-7' and CH₂-8'; δ_c 35.9, 63.8; 2.60 t (7.2), 3.62 t (7.2)], two methoxyl groups (δ_c 55.9 q and 61 q; δ_H 3.80 s, 3.84 s) and two phenolic hydroxyl groups (δ_c 9.58 brs, 9.75 brs). Detailed analysis the functional groups suggested that **1** should be an dibenz[b,f]oxepin derivatives⁹. The general features of the ¹H and ¹³C NMR spectra of **1** resembled to those of bauginiastatin C⁹ except that a vinyl methyl in bauginiastatin C was replaced by a hydroxyethyl unit in **1**. In HMBC spectrum, the long-range correlations (Fig. 2) of H-7' (δ_H 2.60) to C-2' (δ_c 154.2), C-3' (δ_c 112.5) and C-4' (δ_c 149.1), of H-8' (δ_H 3.62) to C-3' (δ_c 112.5), were observed in **1**. This led us to conclude that the hydroxyethyl unit moiety was located on C-3'. The HMBC correlations of two methoxy protons (δ_H 3.80, 3.84) with C-2 (δ_c 150.5) and C-2' (δ_c 161.8) revealed that two methoxy groups should be located at C-2 and C-2'. The HMBC correlations between the phenolic hydroxy proton (δ_H 9.58) and C-4 (δ_c 107), C-5 (δ_c 152.5) and C-6 (δ_c 121.6), as well as those between the other hydroxy proton (δ_H 9.75) and C-3' (δ_c 112.5), C-4' (δ_c 149.1) and C-5' (δ_c 104.1), led to the assignment of two phenolic hydroxyl groups at C-5 and C-4'. The above evidence led to oxepin structure **1** for gramniphénol J.

Fig. 2. Selected HMBC (→) correlations of **1**

Since certain of the stilbenoids from Orchidaceae exhibit potential cytotoxicity¹⁰⁻¹². Compound **1** were tested for their cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3 and MCF7) using the MTT method as reported previously¹³. Paclitaxel was used as the positive control. Compound **1** showed obvious cytotoxicity against PC3 and SHSY5Y cells with IC₅₀ values of 8.2 and 7.6 μ M.

ACKNOWLEDGEMENTS

This research was supported by the National Natural Science Foundation of China (No. 21032085), the excellent Scientific and Technological Team of Yunnan High School (2010CI08), the Yunnan University of Nationalities Green Chemistry and Functional Materials Research for Provincial Innovation Team (2011HC008) and Open Research Fund Program of Key Laboratory of Ethnic Medicine Resource Chemistry (Yunnan University of Nationalities) (2010XY08).

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