Asian Journal of Chemistry; Vol. 26, No. 13 (2014), 3903-3905

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16010

# 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 diphenylethylenes, gramniphenol 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 $IC_{50}$ values of 8.2 and 7.6 μM.

**Keywords: *Arundina graminifolia*, Diphenylethylene, 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 of China[2]. Previous phytochemical studies of *A. graminifolia* have shown the presence of stilbenoids[3], dibenzyls[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 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 × 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.

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 × 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 $CHCl_3$-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, 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-$H_2O$, 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 cellcells 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). 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 $1 \times 10^5$ 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 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 $^{13}C$ 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* 353.0993 $[M + Na]^+$ (Calcd. 353.1001 for $C_{18}H_{18}O_6Na$).

## 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 structures was shown in Fig. 1. The $^1H$ and $^{13}C$ NMR data of **1** were listed in Table-1.

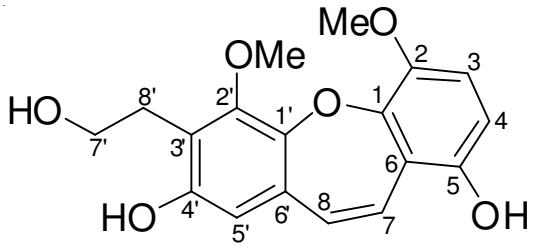


Fig. 1. Structure of new compound

Compound **1** was obtained as a yellow gum. Its HRESIMS in the positive mode revealed a peak at *m/z* 353.0993 $[M + Na]^+$ 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 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 $^1H$, $^{13}C$ 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; $\delta_C$ 145.4, 141.8, 115, 107, 152.5, 121.6; $\delta_H$ 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'; $\delta_C$ 136.8, 154.2, 112.5, 149.1, 104.1, 128; $\delta_H$ 6.53 s), a pair of double bond [CH-7 and CH-8; $\delta_C$ 125.9 and 129.9; $\delta_C$ 7.08 d ($J$ = 11.6) and 6.85 d ($J$ = 11.6)], a hydroxyethyl unit [$CH_2$-7' and $CH_2$-8'; $\delta_C$ 35.9, 63.8; 2.60 t (7.2), 3.62 t (7.2)], two methoxyl groups ($\delta_C$ 55.9 q and 61 q; $\delta_H$ 3.80 s, 3.84 s) and two phenolic hydroxyl groups ($\delta_C$ 9.58 brs, 9.75 brs). Detailed analysis the functional groups suggested that **1** should be an dibenz[b,f]oxepin derivatives[9]. The general features of the $^1H$ and $^{13}C$ NMR spectra of **1** resembled to those of bauhiniastatin C[9] except that a vinyl methyl in bauhiniastatin C was replaced by a hydroxyethyl unit in **1**. In HMBC spectrum, the long-range correlations (Fig. 2) of H-7' ($\delta_H$ 2.60) to C-2' ($\delta_C$ 154.2), C-3' ($\delta_C$ 112.5) and C-4' ($\delta_C$ 149.1), of H-8' ($\delta_H$ 3.62) to C-3' ($\delta_C$ 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 ($\delta_H$ 3.80, 3.84) with C-2 ($\delta_C$ 150.5) and C-2' ($\delta_C$ 161.8) revealed that two methoxy groups should be located at C-2 and C-2'. The HMBC correlations between the phenolic hydroxy proton ($\delta_H$ 9.58) and C-4 ($\delta_C$ 107), C-5 ($\delta_C$ 152.5) and C-6 ($\delta_C$ 121.6), as well as those between the other hydroxy proton ($\delta_H$ 9.75) and C-3' ($\delta_C$ 112.5), C-4' ($\delta_C$ 149.1) and C-5' ($\delta_C$ 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 gramniphenol J.

TABLE-1
$^1H$ AND $^{13}C$ NMR DATA OF COMPOUND **1**
(δ IN ppm, DATA OBTAINED IN $CDCl_3$)

| No. | $\delta_C$ (m) | $\delta_H$ (m, J, Hz | No. | $\delta_C$ (m) | $\delta_H$ (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 |

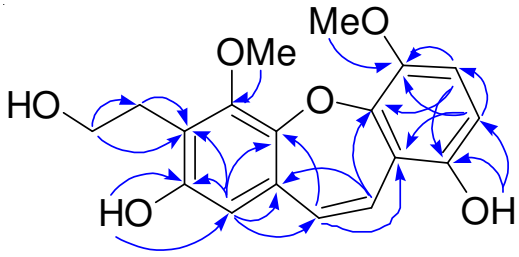


Fig. 2. Selected HMBC ( ) correlations of **1**

Since certain of the stilbenoids from Orchidaceae exhibit potential cytotoxicity[10-12]. 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 previously[13]. Paclitaxel was used as the positive control. Compound **1** showed obvious cytotoxicity against PC3 and SHSY5Y cells with $IC_{50}$ values of 8.2 and 7.6 μM.

## 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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