Asian Journal of Chemistry; Vol. 25, No. 9 (2013), 4922-4924

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.14143

Flavonoid Compounds from *Arundina graminifolia*

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(*Received*: 4 June 2012; *Accepted*: 8 March 2013) AJC-13103

A new flavonoid compounds, gramflavonoid A (**1**), together with four flavonoid compounds (**2-5**), were isolated from the whole plant of *Arundina gramnifolia*. The structure of **1-5** was elucidated by spectroscopic methods including extensive 1D and 2D NMR techniques. The anti HIV-1 activity was evaluated for compounds **1-5**. The results reveal that compound **1** showed moderate anti HIV-1 activity and other compounds also showed weak anti HIV-1 activity.

Key Words: *Arundina gramnifolia*, Flavonoid compounds, Anti HIV-1 activity.

INTRODUCTION

Arundina gramnifolia belongs to Orchidaceae genus. This species widely distributed from temperate regions, from India, Nepal, Thailand, Malaysia, Singapore, South China to Indonesia and across the Pacific Islands. *A. gramnifolia* had used as antidote and demulcent in traditional Chinese herb medicine. This plant is considered to possess activities of detoxification, antiarthritis and abirritation[1]. In recent years, several papers have described phytochemistry investigations of *A. gramnifolia* and it was found to be rich in stilbenoids, triterpenes, flavonoids and lignans[2-6]. With the aim of continuing efforts to multipurpose utilization of *A. gramnifolia* and identify bioactive natural products from this plants, the phytochemical investigation on *A. gramnifolia* was carried out, five flavonoid compounds (**1-5**), including a new compound (**1**), were isolated from this plant. In addition, the anti HIV-1 active of compounds **1-5** were evaluated. This article deals with the isolation, structural elucidation and biological activities of the isolated compounds.

EXPERIMENTAL

General procedures: Optical rotations were measured with a Horiba SEPA-300 polarimeter. 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 DRX-500 spectrometers with TMS as internal standard. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. HRESIMS was performed on an API QSTAR time-of-flight spectrometer and a VG Autospec-3000 spectrometer, respectively. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a ZORBAX PrepHT GF (21.2 mm × 25 cm, 7 μm) column or a Venusil MP C_{18} (20 mm × 25 cm, 5 μm) column. Column chromatography was performed with Si gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, 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 Si gel plates sprayed with 5 % H_2SO_4 in EtOH.

Plant material: The whole plant of *A. gramnifolia* was collected in Xishuangbanna prefecture of Yunnan Province, People s Republic of China, in September 2010. The identification of plant material was verified by Prof. Ning Yuan. A voucher specimen (Ynni-10-09-29) has been deposited in our Laboratory.

Extraction and isolation: The air-dried and powdered of whole plant of *A. gramnifolia* (2.5 kg) were extracted three times with 70 % aqueous MeOH (3 L × 4 L) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure to obtained a crude extract (95.5 g). This crude extract was applied to Si gel (200-300 mesh) column chromatography 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 separation of fraction B (15.65 g) by Si gel column chromatography eluted with $CHCl_3$-CH_3COCH_3 (1:0-1:2) yielded mixtures B1-B6. Fraction B3 (8:2, 5.16 g) was subjected to Si gel column chromatography using petroleum ether-acetone and preparative HPLC (55 % MeOH-H_2O, flow rate 12 mL/min) to give compounds **1** (11.5 mg), **2** (12.6 mg), **3** (22.4 mg). Fraction B4 (6:4, 2.94 g) was subjected to Si gel column chromatography eluting with petroleum ether-acetone and then run on preparative HPLC (45 % MeOH-H_2O, flow rate 12 mL/min) to yield compounds **4** (11.8 mg) and **5** (32.2 mg).

Anti HIV1 assays: The cytotoxicity assay against C8166 cells (CC_{50}) was assessed using the MTT method and anti HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC50)[14].

Gramflavonoid A (1): Obtained as a pale yellow gum; UV (MeOH), λ_{max} (log ε) 338 (3.72), 258 (3.92), 210 (4.22) nm; IR (KBr, ν_{max}, cm^{-1}) max 3423, 2912, 1665, 1613, 1495, 1443, 1269, 1145, 1075, 854, 828, 778; ^1H and ^{13}C NMR data (C_5ND_5, 500 and 150 MHz, respectively), Table-1; ESIMS (positive ion mode) m/z 335 $[M + Na]^+$; HRESIMS (positive ion mode) m/z 335.0889 $[M + Na]^+$, calcd. (%) 335.0895 for $C_{18}H_{16}O_5Na$.

TABLE-1
^1H AND ^{13}C NMR DATA OF COMPOUND **1**
(δ IN ppm, DATA OBTAINED IN C_5D_5N)

No.	δ_C (m)	δ_H (m, *J*, Hz)	No.	δ_C (m)	δ_H (m, *J*, Hz)
2	146.2 s	–	1'	122.9 s	
3	181.3 s	–	2'	136.2 d	7.89, d, *J* = 8.6
4	114.1 d	7.18, s	3'	115.8 d	7.04, d, *J* = 8.6
5	145.2 s	–	4'	161.2 s	–
6	154.0 s	–	5'	115.8 d	7.04, d, *J* = 8.6
7	103.5 d	6.65, s	6'	136.2 d	7.89, d, *J* = 8.6
8	158.2 s	–	OMe-5	55.9 q	3.78, s
9	117.6 s	–	OMe-6	56.0 q	3.80, s
10	111.7 d	6.72, s	OMe-4'	55.8 q	3.82, s

RESULTS AND DISCUSSION

The air-dried and powdered whole plant of *A. gramnifolia* (2.5 kg) was extracted with 70 % aqueous methanol (3 × 4 L) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure to obtained a crude extract (96.5 g). This crude extract was subjected repeatedly to column chromatography on Si gel, Sephadex LH-20, RP-18 and preparative HPLC to afford compounds **1-5** (Fig. 1), including a new flavonoid compound, gramflavonoid A (**1**), together with four known flavonoids, derriobtusone A (**2**)[7], derriobtusone B (**3**)[7], obovatin (**4**)[8], lonchocarpin[9]. The structures of the compounds **1-5** were as shown in Fig. 1 and the NMR data of **1** were listed in Table-1.

Compound **1** was obtained as pale yellow gum. Its molecular formula was determined as $C_{18}H_{16}O_5$ by HR-ESI-MS m/z 335.0889 $[M + Na]^+$ (calcd. (%) 335.0895). The ^1H and ^{13}C NMR spectrum of **1** (Table-1) along with analysis of the DEPT spectra displayed 18 carbon signals and 16 proton signals, respectively, corresponding to an aurone nucleus[10,11] (δ_C 146.2 s, 181.3 s, 114.1 d, 145.2 s, 154.0 s, 103.5 d, 158.2 s, 117.6 s, 111.7 d, 122.9 s, 136.2 d, 115.8 d, 161.2 s, 115.8 d, 136.2 d), three methoxyl groups (δ_C 55.5 q, 56.0 q, 55.9 q). Strong absorption bands accounting for hydroxyl (3423 cm^{-1}), carbonyl group (1665 cm^{-1}) and aromatic groups (1613, 1495, 1443 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of **1** showed absorption maxima at 338, 258 and 210 nm, which confirmed the existence of the aromatic functions. The HMBC correlations (Fig. 2) of H-10 (δ_H 6.72) with C-2 (δ_C 146.2), C-3 (δ_C 181.3), C-1' (δ_C 122.9), C-2' (δ_C 136.2), of H-2' (δ_H 7.89) with C-10 (δ_C 111.7) and of H-4 (δ_H 7.18), with C-10 (δ_C 111.7), also supported the aurone nucleus. The signals for four coupled aromatic protons at δ_H 7.04 (d, *J* = 8.6 Hz, 2H) and 7.89 (d, *J* = 8.6, 2H), suggested a 4'-mono-substituted for C ring. The proton signals for two singlets at δ_H 7.18 (s, 1H) and δ_H 6.65 (s, 1H) also revealed that the substituents for B-ring should be located at C-4 and C-7. The HMBC correlations of of the three methoxy proton signals (δ_H 3.78, 3.80, 3.82) with C-5 (δ_C 145.2), C-6 (δ_C 154.0) and C-4' (δ_C 161.2) suggested three methoxyl groups located at C-5, C-6 and C-4', respectively. Thus, the structure of **1** was established as 4',5,6-trimethoxyl-aurone and gives the trivial name of gramflavonoid A.

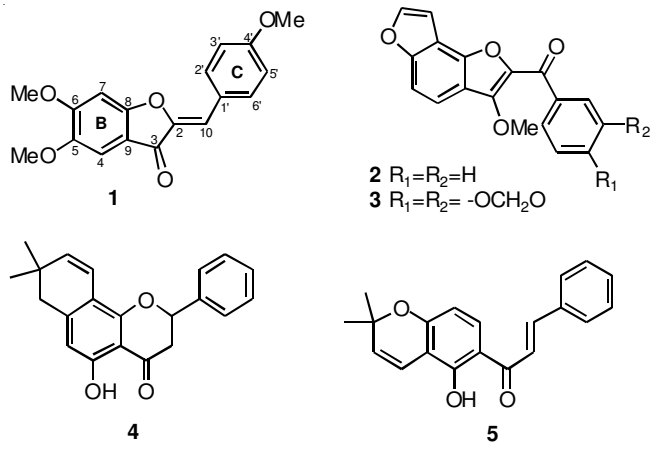

Fig. 1. Structures of compounds **1-5**

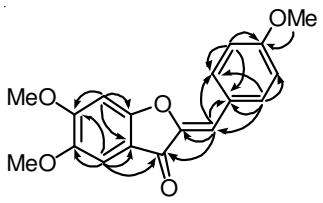

Fig. 2. Selected HMBC ( ) correlations **1**

Since some of the flavonoid compounds exhibited anti virus activities[12,13], compounds **1-5** were tested for the anti HIV-1.

For anti HIV-1 activity assay, the cytotoxicity against C8166 cells (CC_{50}) was assessed using the MTT method and anti HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}), using AZT as a positive control (EC_{50} = 0.045 μg/mL and CC_{50} 200 μg/mL)[14]. The results are shown in Table-2. The results reveal that compound **1** showed moderate anti HIV-1 activity with therapeutic index (TI) values above 30 and other compounds also showed weak anti HIV-1 activity with TI values above 10.

TABLE-2
ANTI HIV ACTIVITY OF COMPOUNDS **1-5**

Compound	CC_{50} (μg/mL)	EC_{50} (μg/mL)	TI[a]
1	176	5.27	33.4
2	124	8.28	15.0
3	155	11.9	13.0
4	125	9.48	13.2
5	186	18.2	10.2

[a]TI (therapeutic index) = CC_{50}/EC_{50}.

ACKNOWLEDGEMENTS

This project was supported financially by 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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