Flavonoid Compounds from *Arundina graminifolia*

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A new flavonoid compounds, gramflavonoid A (**1**), together with four flavonoid compounds (**2-5**), were isolated from the whole plant of *Arundina graminifolia*. 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 graminifolia*, Flavonoid compounds, Anti HIV-1 activity.

INTRODUCTION

Arundina graminifolia 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. graminifolia* had used as antidote and demulcent in traditional Chinese herb medicine. This plant is considered to possess activities of detoxification, antiarthritis and abirritation¹. In recent years, several papers have described phytochemistry investigations of *A. graminifolia* and it was found to be rich in stilbenoids, triterpenes, flavonoids and lignans²⁻⁶. With the aim of continuing efforts to multipurpose utilization of *A. graminifolia* and identify bioactive natural products from this plants, the phytochemical investigation on *A. graminifolia* 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 $\times$ 25 cm, 7 μ m) column or a Venusil MP C₁₈ (20 mm $\times$ 25 cm, 5 μ m) column. Column chromatography was performed with Si gel (200-300 mesh, Qingdao Marine Chemical, Inc., Qingdao, 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 Si gel plates sprayed with 5 % H₂SO₄ in EtOH.

Plant material: The whole plant of *A. graminifolia* 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. graminifolia* (2.5 kg) were extracted three times with 70 % aqueous MeOH (3 L $\times$ 4 L) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure to obtain a crude extract (95.5 g). This crude extract was applied to Si gel (200-300 mesh) column chromatography 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 separation of fraction B (15.65 g) by Si gel column chromatography eluted with $\text{CHCl}_3\text{-CH}_3\text{COCH}_3$ (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 % $\text{MeOH-H}_2\text{O}$, 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 % $\text{MeOH-H}_2\text{O}$, 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 (EC_{50})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 $[\text{M} + \text{Na}]^+$; HRESIMS (positive ion mode) m/z 335.0889 $[\text{M} + \text{Na}]^+$, calcd. (%) 335.0895 for $\text{C}_{18}\text{H}_{16}\text{O}_5\text{Na}$.

TABLE-1
 ^1H AND ^{13}C NMR DATA OF COMPOUND 1
(δ IN ppm, DATA OBTAINED IN $\text{C}_6\text{D}_6\text{N}$)

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. graminifolia* (2.5 kg) was extracted with 70 % aqueous methanol ($3 \times 4\text{ L}$) 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, lonchocarpin⁹. 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 $\text{C}_{18}\text{H}_{16}\text{O}_5$ by HR-ESI-MS m/z 335.0889 $[\text{M} + \text{Na}]^+$ (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,

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

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, $J = 8.6\text{ Hz}$, 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 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-trimethoxy-aurone and gives the trivial name of gramflavonoid A.

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 ($\text{EC}_{50} = 0.045\text{ }\mu\text{g/mL}$ and $\text{CC}_{50} = 200\text{ }\mu\text{g/mL}$)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

^aTI (therapeutic index) = CC₅₀/EC₅₀.

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