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Flavonoids Derivatives from *Arundina graminifolia* and Their Cytotoxicity

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A new flavonoid, 3(S),4(S)-3',4'-dihydroxyl-7,8,-methylenedioxylpterocarpan (**1**), together with ten known flavonoids derivatives (**2-11**), were isolated from the whole plant of *Arundina gramnifolia*. The structure of compounds **1-11** were elucidated by spectroscopic methods including extensive 1D and 2D NMR techniques. Compound **1** was also evaluated for its cytotoxicity against five human tumor cell lines. The results revealed that compound **1** showed high cytotoxicity against HSY5Y cell with IC_{50} values of 2.2 μM and moderate cytotoxicities with IC_{50} valves 5-10 μM for other four tested cell lines.

Key Words: *Arundina gramnifolia*, Flavonoids, Cytotoxicity.

INTRODUCTION

Arundina gramnifolia (bamboo orchid) is a terrestrial plant belongs to species of orchid and the sole of the genus *Arundina*. This plant is considered to possess activities of detoxification, antiarthritis and abirritation and it is used as antidote and demulcent in traditional Chinese medicine[1]. The previous phytochemical researches on *A. gramnifolia* had revealed that stilbenoids[2-4], sterols[5,6], triterpenes[7,8] and phenols[5,9] are major components isolated from this plant.

In our previous studies, two new phenols were isolated from the *A. gramnifolia* originated in Xishuangbanna Prefecture and these compounds were found to be shown antitobacco mosaic virus activity[9]. Motivated by a search for more new bioactive metabolites from this plant, our group had reinvestigated the chemical constituents of the whole plant of *A. gramnifolia*, which led to the isolation and characterization of a new flavonoid (**1**) and ten known flavonoid derivatives (**2-11**). The structures of the isolated compounds were established by means of spectroscopic methods including extensive 1D and 2D NMR techniques. This article deals with the isolation, structural elucidation and anti tobacco mosaic virus activity of the isolated flavonoids.

EXPERIMENTAL

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. CD spectra were measured on a JASCO J-810 spectropolarimeter. 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 an 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), Sephadex LH-20 (Sigma-Aldrich, Inc, USA) and MCI gel (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan) 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.

The whole plant of *A. gramnifolia* was collected in Dehong 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-10-09-28) has been deposited in our Laboratory.

Extraction and isolation: The air-dried and powdered whole plant of *A. gramnifolia* (3.5 kg) was extracted four times with 70 % aqueous methanol (4 × 3 L) at room temperature and filtered. The crude extract (226 g) was applied to silica

gel (200-300 mesh) column chromatography, eluting with a chloroform-acetone gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A-F. The separation of fraction C (8:2, 20.2 g) by silica gel column chromatography, eluted with chloroform-methanol and preparative HPLC (38 % methanol, flow rate 12 mL/min) to give **1** (16.3 mg), **2** (23.2 mg) and **3** (25.5 mg). The further separation of fraction D (7:3, 18.4 g) by silica gel column chromatography, eluted with chloroform-methanol and preparative HPLC (34 % methanol, flow rate 12 mL/min) to give **4** (12.6 mg), **5** (28.2 mg), **6** (128.5 mg), **8** (119.2 mg) and **10** (85.4 mg). On the other hand, separation of fraction E (6:4, 34.5 g) by silica gel column chromatography and preparative HPLC (23 % methanol, flow rate 12 mL/min) led to the purification of **7** (46.5 mg), **9** (55.4 mg) and **11** (32.8 mg).

3(S),4(S)-3',4'-dihydroxyl-7,8,-methylenedioxylptero carpan (1): Obtained as pale yellow gum; $[\alpha]^{24.2}_{D}$ + 252 (c 0.020, MeOH); CD (c 0.02, MeOH), nm (Δε) 287 (-3.26), 247 (+16.7), 232 (-202); UV (MeOH), λ_{max} (log ε) 342 (2.18), 294 (4.02), 210 (4.38) nm; IR (KBr, ν_{max}, cm^{-1}): 3380, 2964, 2882, 1614, 1538, 1480, 1422, 1359, 1257, 1150, 1036, 867; ^1H and ^{13}C NMR data (CD_3OD, 500 and 125 MHz), Table-1; ESI-MS (negative ion mode) *m/z* 259; HR-ESI-MS (negative ion mode) *m/z* $[M-H]^-$ 299.0544 (calcd. (%) 299.0556 for $C_{16}H_{11}O_6$).

RESULTS AND DISCUSSION

The air-dried and powdered whole plant of *A. gramnifolia* (3.5 kg) was extracted with 70 % aqueous methonal (3 × 3.5 L) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure to obtain a crude extract (226 g). This crude extract was subjected repeatedly to column chromatography on Si gel, Sephadex LH-20, RP-18 and preparative HPLC to afford to afford a new flavonoid, 3(S),4(S)-3',4'-dihydroxyl-7,8,-methylenedioxyl-pterocarpan (**1**), together with five known flavonoid derivatives (**2-11**). The structures of the compounds **1-11** were as shown in Fig. 1 and the ^1H and ^{13}C NMR data of the compound **1** were listed in Table-1. The known compounds, compared with literature, were identified as: medicarpin (**2**)[10], 5-hydroxy-2",2"-dimethylchromene-(3",4":6:7)-flavone (**3**)[11], butein (**4**)[12], sulfuretin (**5**)[13], quercetin (**6**)[14], quercetin-β-3-O-glycosides (**7**)[14], kaempferol (**8**)[14], kaempferol-β-3-O-glycosides (**9**)[14], (+)-catechin (**10**)[15], steppogenin-4'-O-β-D-glucosiade (**11**)[16].

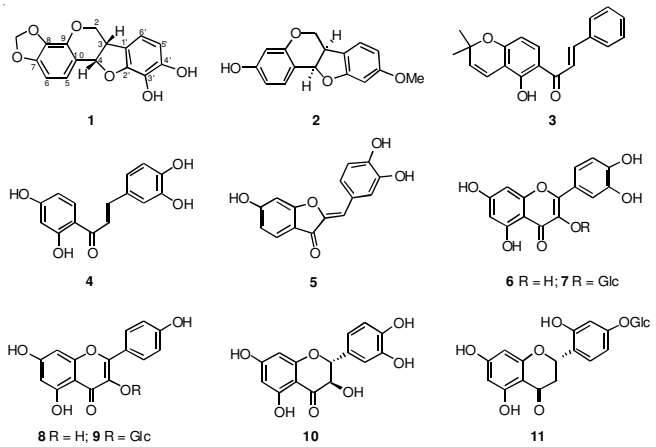

Fig. 1. Flavonoids derivatives from *Arundina graminifolia*

Compound **1** was obtained as a pale yellow gum with $[\alpha]^{22.5}_{D}$ + 252 (c 0.020, MeOH). The absorption bands accounting for hydroxyl (3380 cm^{-1}) and aromatic groups (1614, 1538, 1480, 1422 cm^{-1}) could be observed in its IR spectrum. Its molecular formula was established by the negative mode HRESIMS (high-resolution electron spray ionization mass spectra) peak at *m/z* 99.0544 $[M-H]^-$ (calcd. (%) 337.0688 for $C_{16}H_{11}O_6$). The ^1H NMR spectrum suggested a pterocarpan structure due to the splitting pattern of the protons at δ_H 4.24 (dd, *J* = 4.6, 10.5 Hz, H-4α), δ_H 3.56 (t, *J* = 10.5 Hz, H-2β), δ_H 3.44 (m, H-3) and δ_H 5.43 (d, *J* = 6.7 Hz, H-4), related to the protons of the heterocyclic ring B. This spectrum also allowed the identification of two pairs of *ortho* situated aromatic protons at δ_H 6.96 (d, *J* = 8.5 Hz, H-5), δ_H 6.53 (d, *J* = 8.5 Hz, H-6), δ_H 6.40 (d, *J* = 8.1 Hz, H-5) and δ_H 6.74 (d, *J* = 8.1 Hz, H-6) and gave a clear evidence of the 7,8,3',4'-substitution pattern of the pterocarpan moiety[17]. In addition, one methylenedioxy group signals (δ_H 5.86, 5.91 s) was also observed. All these data were supported by the ^{13}C and DEPT spectrum that revealed 16 carbon atoms corresponding to two methylene, six methines and eight non-hydrogenated carbons. However, the confirmation of the above suggestion for **1** was supported by the HSQC and HMBC (Fig. 2) experiments, which allowed the unequivocal assignments of its ^{13}C and ^1H NMR data. The methylenedioxy group located at C-7 and C-8 was supported by the HMBC correlations of methylenedioxyl proton at δ_H 5.88, 5.91 (-OCH_2O-) with the carbon at δ_C 146.3 (C-7) and δ_C 134.1 (C-8). Since the position of the methylenedioxy group was determined, two hydroxyl group should be located at C-3' and C-4' to support the tetra substituted aromatic B-ring.

TABLE-1
^1H NMR AND ^{13}C NMR DATA OF COMPOUNDS **1** (OBTAINED IN CD_3OD)

No.	δ_C (mult.)	δ_H (mult, *J*, Hz)	No.	δ_C (mult.)	δ_H (mult, *J*, Hz)
2α	67.5 t	4.24, dd, *J* = 4.6, 10.5	10	113.2 s	–
2β	67.5 t	3.56, t, *J* = 10.5	1'	122.8 s	–
3	41.3 d	3.44 m	2'	148.8 s	–
4	80.0 d	5.43, d, *J* = 6.7	3'	133.0 s	–
5	122.1 d	6.96, d, *J* = 8.5	4'	148.2 s	–
6	110.0 d	6.53, d, *J* = 8.5	5'	106.6 d	6.40, d, *J* = 8.1
7	146.3 s	–	6'	115.9 d	6.74, d, *J* = 8.1
8	134.1 s	–	-OCH_2O-	101.2 t	5.88, 5.91 s
9	145.5 s	–	–	–	–

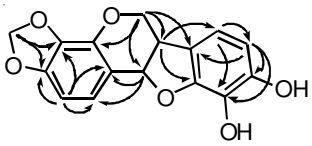

Fig. 2. Selected HMBC (↷) correlations of **1**

It is well known from the literature that, according to biogenetical regulations, the hydrogens (H-3 and H-4) at the B/C rings junction of all natural pterocarpans are always *cis*, either α, α or β, β, thus leading to only two enantiomeric forms. It is also known, through CD (circular dichroism) and/or ORD (optical rotatory dispersion) analyses, that (-) optical rotation can be associated with α,α positioning (3R, 4R), while the (+) optical rotation can be associated with the β, β positioning (3S, 4S) of both series[18,19]. From the (+) optical rotation of compound **1**, it could be assumed an (3S, 4S) absolute configuration for it. As expected, the CD spectrum of **1** should a similar profile of that from (+)-pterocarpin and almost a mirror image of (-)-maackiain, what is in agreement with the suggested (3S, 4S) absolute stereochemistry for compound **1**. Thus, the structure of **1** was determined as 3(S),4(S)-3',4'-dihydroxyl-7,8,-methylenedioxylpterocarpan.

Since certain of the flavonoids derivatives exhibit potential cytotoxicity[20,21], the compounds **1** was tested for their cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3 and MCF7) using the MTT method as reported previously[22]. Taxol was used as the positive control. The results revealed that compound **1** showed high cytotoxicity against HSY5Y cell with IC_{50} values of 2.2 μM and moderate cytotoxicities with IC_{50} valves 5-10 μM for other four tested cell lines.

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