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Phenolic Compounds from *Arundina graminifolia* and Their Anti-Tobacco Mosaic Virus Activity

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Arundina gramnifolia belongs to Orchidaceae family. It is a terrestrial plant extends from India, Nepal, Thailand, Malaysia, Singapore, South China to Indonesia and across the Pacific Islands. The genus is considered to possess activities of detoxification, antiarthritis and abirritation and is used as antidote and demulcent.[1] In recent years, several papers have described phytochemistry investigations of *A. gramnifolia*, and it was found to be rich in stilbenoids and triterpenes.[2-5] Motivated by a search for bioactive compounds from this plant, further chemical investigation were carried out. As a result, two new phenolic compounds (**1-2**), together with four known phenols (**3-6**), were isolated from this plant. In addition, the anti-tobacco mosaic virus activity of compounds **1-6** were evaluated for the first time. This article deals with the isolation, structural elucidation and biological activities of the isolated compounds.

Results and Discussion

The air-dried and powdered whole plant of *A. gramnifolia* (2.0 kg) was extracted with 70% aqueous methanol (3 × 3.5 L) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure to obtained a crude extract (152 g). This crude extract was subjected repeatedly to column chromatography on Si gel, Sephadex LH-20, RP-18 and preparative HPLC to afford compounds **1-6** (Figure 1), including two new phenolic compounds, gramniphenol A and B (**1-2**), together with four known phenols, 9'-dehydroxy-vladinol F (**3**),[6] vladinol F (**4**),[7] 9-*O*-*β*-D-xylopyranoside-vladinol F (**5**),[8] and 4,9-dihydroxy-4',7-epoxy-8',9'-dinor-8,5'-neolignan-7'-oic acid (**6**).[9] The structures of the compounds **1-6** were as shown in Figure 1, and the NMR data of **1** and **2** were listed in Table 1.

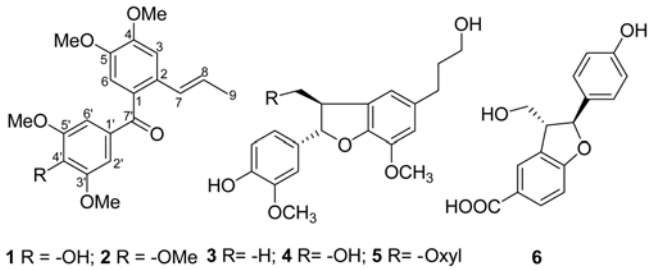


Figure 1. The structures of compounds **1-6**.

Compound **1** was obtained as white amorphous powder, and was assigned the molecular formula $C_{20}H_{22}O_6$ by HRESIMS *m*/z 381.1318 $[M + Na]^+$ (calcd 381.1314). Its ^1H and ^{13}C NMR spectra showed signals to 22 hydrogens and 20 carbons, respectively, corresponding to two aromatic rings with four aromatic protons (δ_H 6.48, 6.48, 6.82, 7.08), one carbonyl carbon (δ_C 196.1), one allyl group (δ_C 131.4, 123.9, 18.5; δ_H 6.48 d, J = 15.8, 6.24 m, 1.95 d, J = 6.5), four methoxy groups (δ_C 55.9, 55.9, 56.0, 56.1), which were in accordance with the molecular formula, $C_{20}H_{22}O_6$. Strong

Table 1. ^1H and ^{13}C NMR Data of compounds **1** and **2** (δ in ppm, data obtained in $CDCl_3$)

No.	Compound **1**		Compound **2**	
	δ_C (mult.)	δ_H (mult, *J*, Hz)	δ_C (mult.)	δ_H (mult, *J*, Hz)
1	127.3 s		127.1 s	
2	136.7 s		136.5 s	
3	110.7 d	6.82 s	109.9 d	6.82 s
4	152.5 s		152.7 s	
5	147.4 s		147.4 s	
6	117.8 d	7.08 s	117.5 d	7.07 s
7	131.4 d	6.48 d, *J* = 15.8	131.1 d	6.48 d, *J* = 15.5
8	123.9 d	6.24 m	123.6 d	6.20 m
9	18.5 q	1.95 d, *J* = 6.5	18.6 q	1.86 d, *J* = 6.6
1′	139.6 s		137.6 s	
2′	109.1 d	6.50 s	107.5 d	6.49 s
3′	151.9 s		150.0 s	
4′	134.9 s		143.6 s	
5′	151.9 s		150.0 s	
6′	109.1 d	6.50 s	107.5 d	6.49 s
7′	196.1 s		196.4 s	
OMe-4	56.0 q	3.88 s	55.9 s	3.86 s
OMe-5	56.1 q	3.96 s	56.0 s	3.92 s
OMe-3′	55.9 q	3.76 s	55.8 s	3.72 s
OMe-4′			60.8 s	3.81 s
OMe-5′	55.9 q	3.76 s	55.8 s	3.72, s
4′-Ar-OH		9.64 brs		

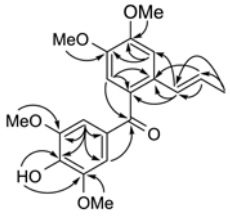


Figure 2. Selected HMBC (⌒) correlations of compound **1**.

absorption bands accounting for hydroxy group (3438 cm^{-1}), carbonyl group (1736 cm^{-1}) and aromatic groups (1638, 1565, 1531, 1460 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of **1** showed absorption maxima at 268, 210 nm which confirmed the existence of the aromatic functions. The HMBC correlations (Figure 2) of aromatic proton H-6 (δ_H 7.08, s), H-2' (δ_H 6.50, s), H-6' (δ_H 6.50) with C-7' (δ_C 196.1) indicated that the two aromatic rings were linked to the same carbon, C-7'; the HMBC correlations of H-7 (6.48 d J = 15.8) and H-8 (δ_H 6.24 m) with C-2 (δ_C 136.7) indicated that allyl group should be attached to C-2. The four methoxy groups located at C-4, C-5, C-3', C5' were supported by the HMBC correlations of the four methoxyl proton signal (δ_H 3.76, 3.76, 3.88, 3.96) with C-4 (δ_C 56.0), C-5 (δ_C 56.1), C-3' (δ_C 55.9), C5' (δ_C 55.9), respectively. A hydroxy group located at C-4' was supported by the HMBC correlations of the phenolic hydroxyl proton signal (δ_H 9.46) with C-3' (δ_C 151.9), C-4' (δ_C 134.9), and C-5' (δ_C 151.9). The ROESY correlations of OMe-4 with H-3, OMe-5 with H-6, OMe-3' with H-2', OMe-5' with H-6' also support the substituents position of four methoxy groups. Thus, the structure of compound **1** was unambiguously determined as shown, and this compound was given the name as gramniphenol A.

Compound **2** (gramniphenol B) was also obtained as white amorphous powder. By comparison of ^1H, and ^{13}C NMR spectra of **2** with those of **1**, compounds **2** was found to be very close to those of **1**. The only difference is an aromatic hydroxyl group in **1** was substituted by a methoxyl group in **2**, which was supported by the disappearance of a hydroxyl proton signal (δ_H 9.64) in **1** and appearance a methoxyl group signal (δ_C 60.7) in **2**. Thus, the structure of **2** was established as shown.

Since certain of phenolic compounds exhibit potential anti-tobacco mosaic virus activity,[10-12] and this activity for compounds **1-6** was not evaluated in previous literature. Compounds **1-6** were tested for the Anti-TMV activity using the half-leaf method according to literature.[13]

In Anti-TMV activity test, the anti-viral inhibition rates of the compounds at the concentration of 20 μM were tested by the half-leaf method. Ningnanmycin (20 μM), a commercial product for plant disease in China with inhibition rate of 33% was used as positive control. The results showed that compounds **1-6** exhibit inhibition rates of 6.27%, 22.2%, 1.46%, 2.15%, 4.76%, and 38.6%, respectively. Compound **6** exhibited high Anti-TMV activity; its inhibition rate is higher that of positive control. Compound **2** also exhibited modest Anti-TMV activity; its inhibition rate is close to that of positive control.

Experimental Section

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 Bruker 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 mm) column or a Venusil MP C_{18} (20 mm × 25 cm, 5 mm) 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-10-09-22) has been deposited in our Laboratory.

Extraction and Isolation. The air-dried and powdered of whole plant of *A. gramnifolia* (2.0 kg) were extracted three times with 70% aqueous MeOH (3 × 3.5 L) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure to obtained a crude extract (152 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 (22.6 g) by Si gel column chromatography eluted with $CHCl_3$-acetone (20:1-1:2) yielded mixtures B1-B6. Fraction B2 (3.58 g) was subjected to Si gel column chromatography using petroleum ether-acetone and preparative HPLC (60% MeOH-H_2O, flow rate 12 mL/min) to give compounds **1** (19.2 mg), **2** (22.5 mg), and **3** (31.4 mg). Fraction B3 (2.94 g) was subjected to Si gel column chromatography eluting with petroleum ether-acetone and then run on preparative HPLC (55% MeOH-H_2O, flow rate 12 mL/min) to yield compounds **4** (58.3 mg), **5** (29.7 mg). Fraction B4 (3.57 g) was subjected to Si gel column chromatography eluting with petroleum ether-acetone and then run on preparative HPLC (35% MeOH-H_2O, flow rate 12 mL/min) to yield compound **6** (38.4 mg).

Anti-TMV Assays. The Anti TMV activity was tested using the half-leaf method.[13] The inhibitory activity of the

compounds against TMV replication were tested using two approaches. First, the half-leaf method was used to test the antiviral activity in the local lesion host *Nicotiana glutinosa* in vivo. Then, the leaf-disk method was used to evaluate the antiviral activity of the compounds in the systemic infection host *Nicotiana tabacum cv.* K326. Ningnanmycin (20 μM), a commercial product for plant disease in China, was used as a positive control.

Gramniphenol A (1): Obtain as white amorphous powder; UV (MeOH) λ_{max} (log ε): 210 (4.29), 268 (3.82), 345 (2.97) nm; IR (KBr) ν_{max}: 3438, 3026, 2934, 2869, 1736, 1638, 1565, 1531, 1460, 1358, 1262, 1145, 1046, 1025, 962, 878 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; positive ESIMS *m/z* 381 $[M+Na]^+$; HRESIMS: *m/z* 381.1318 $[M+Na]^+$, (calcd $C_{20}H_{22}NaO_6$ for 381.1314).

Gramniphenol B (2): White amorphous powder; UV (MeOH) λ_{max} (log ε): 210 (4.22), 268 (3.77), 345 (2.99) nm; IR (KBr) ν_{max}: 3031, 2940, 2865, 1734, 1635, 1564, 1538, 1462, 1355, 1260, 1143, 1040, 1028, 960, 874 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; positive ESIMS *m/z* 395 $[M+Na]^+$; HRESIMS: *m/z* 395.1465 $[M+Na]^+$, (calcd $C_{21}H_{24}NaO_6$ for 395.1471).

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