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Phenolic amides from the leaves of *Nicotiana tabacum* and their anti-tobacco mosaic virus activities

Shan-Zhai Shang [a,b], Yuan-Xing Duan [a], Xia Zhang [a], Jian-Xin Pu [b], Han-Dong Sun [b], Zhang-Yu Chen [a], Ming-Ming Miao [a], Guang-Yu Yang [a,*], Yong-Kuan Chen [a,**]

[a] Key Laboratory of Tobacco Chemistry of Yunnan Province, Yunnan Academy of Tobacco Science, Kunming 650106, PR China
[b] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, PR China

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ABSTRACT

Three new phenolic amides, tabamides A–C (**1**–**3**), together with three known phenolic amides (**4**–**6**), were isolated from the leaves of *Nicotiana tabacum*. Their structures were elucidated by spectroscopic methods, including extensive 1D- and 2D NMR techniques. Compounds **1**–**6** were also tested for their anti-tobacco mosaic virus (anti-TMV) activity. The results showed that compound **1** exhibited high anti-TMV activity with inhibition rate of 38.6%, which is higher than that of positive control (ningnanmycin). The other compounds also showed potential anti-TMV activity with inhibition rates in the range of 15.3–26.5%, respectively.

1. Introduction

Nicotiana tabacum, tobacco, is a stout herbaceous plant in the Solanaceae (nightshade family) that originated in the tropical Americas (South America, Mexico, and the West Indies) and now cultivated worldwide as the primary commercial source of tobacco, which is smoked or chewed as a drug for its mild stimulant effects (Kuang and Lu, 2005; Hu et al., 2006). In addition, *N. tabacum* is also used as insecticides, anesthetics, diaphoretics, sedatives, and emetic agents in Chinese folklore medicines because of its containing many useful chemical compounds (Kuang and Lu, 2005; Rodgman and Perfetti, 2008). Previous investigation of this species led to the discovery of a number of new compounds that showed various bioactivities, such as anti-HIV-1, anti-TMV, and cytotoxicity by our groups (Tan et al., 2011; Chen et al., 2012a; Gao et al., 2012; Chen et al., 2012b; Chen et al., 2013; Mou et al., 2012). In continuing efforts to the phytochemistry research on the leaves of Honghua Dajinyuan (a variety of *N. tabacum*) led to the isolation of three new (**1**–**3**) and three known (**4**–**6**) phenolic amides (Fig. 1). This paper deals with the isolation, structural elucidation, and anti-TMV activity of these compounds.

2. Results and discussion

A 70% aq. acetone extract prepared from the leaves of *N. tabacum* was partitioned between EtOAc and H_2O. The EtOAc layer was subjected repeatedly to column chromatography on Si gel, Sephadex LH-20, RP-18 and preparative HPLC to afford compounds **1**–**6**, which included three new phenolic amides, named tabamides A–C (**1**–**3**), together with three known phenolic amides, N-*E*-caffeoyl tyramine (**4**) (Trabelsi et al., 2014), N-*E*-feruloyl tyramine (**5**) (Trabelsi et al., 2014), and dihydro-*N*-caffeoyltyramine (**6**) (Han et al., 2002). The structures of compounds **1**–**6** are shown in Fig. 1, and the ^1H and ^{13}C NMR data of compounds **1**–**3** are listed in Table 1.

Compound **1** was isolated as a white powder. Its molecular formula $C_{19}H_{15}NO_5$ was determined from the quasimolecular ion peak observed using electrospray ionization (ESIMS) and HRESIMS measurement at *m/z* 360.0842 $[M+Na]^+$ (calcd for 360.0848), suggesting a 13 degrees of unsaturation. Strong absorption bands accounting for hydroxy (3418 cm^{-1}), amino (3315 cm^{-1}), carbonyl (1675, 1650 cm^{-1}), and aromatic group (1608, 1529, 1456 cm^{-1}) could be observed in its IR spectrum. The UV spectrum of **1** showed absorption maxima at 215, 253, and 338 nm confirmed the

* Corresponding author. Tel.: +86 871 68319228.
** Corresponding author.
E-mail addresses: ygy1110@163.com (G.-Y. Yang), cyk1966@163.com (Y.-K. Chen).

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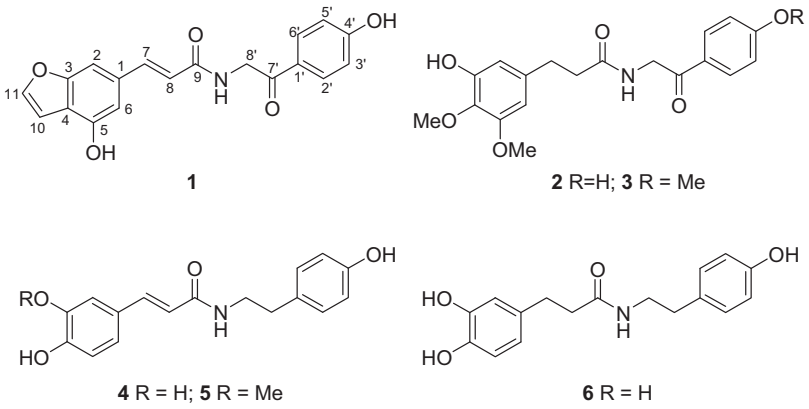

Fig. 1. Structures of phenolic amides from *Nicotiana tabacum*.

Table 1
^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data of compounds **1**–**3** in C_5D_5N.

Position	**1**		**2**		**3**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	130.3 s		131.8 s		132.0 s	
2	105.1 d	7.03 (d) 1.8	107.2 d	6.70 (d) 1.8	107.5 d	6.73 (d) 1.8
3	154.2 s		147.5 s		147.8 s	
4	118.5 s		138.7 s		139.0 s	
5	148.3 s		151.0 s		151.1 s	
6	109.3 d	6.56 (d) 1.8	105.8 d	6.49 (d) 1.8	105.4 d	6.47 (d) 1.8
7	145.4 d	7.66 (d) 16.1	31.0 t	2.77 (t) 7.2	31.1 t	2.81 (t) 7.2
8	116.0 d	6.68 (d) 16.1	36.6 t	2.49 (t) 7.2	36.8 t	2.46 (t) 7.2
9	166.8 s		173.3 s		173.1 s	
10	107.1 d	6.70 (d) 2.6				
11	146.5 d	7.40 (d) 2.6				
1′	127.1 s		127.7 s		127.9 s	
2′,6′	131.4 d	7.85 (d) 8.6	131.0 d	7.84 (d) 8.6	130.1 d	7.89 (d) 8.6
3′,5′	115.3 d	6.88 (d) 8.6	115.8 d	6.87 (d) 8.6	115.0 d	6.90 (d) 8.6
4′	160.5 s		160.7 s		164.0 s	
7′	191.5 s		190.8 s		190.2 s	
8′	45.0 t	4.52 (d) 5.6	45.4 t	4.50 (d) 5.6	45.2 t	4.51 (d) 5.6
NH		8.32 (t) 5.6		8.33 (t) 5.6		8.34 (t) 5.6
–OMe-4			60.9 q	3.79 s	61.0 q	3.78 s
–OMe-5			56.0 q	3.81 s	56.0 q	3.81 s
–OMe-4′					55.8 q	3.80 s
Ar-OH-3				10.87 s		10.90 s
Ar-OH-5		10.13 s				
Ar-OH-4′		10.45 s		10.41 s		

existence of the aromatic function. The ^{1}H and ^{13}C NMR spectrum of **1** revealed an (*E*)-3-(4-hydroxybenzofuran-6-yl)acryl moiety (Leng et al., 2014) (C-1–C-11; H-2, H-6–H-8, H-10, H-11, and Ar-OH-5) and an 2-amino-1-(4-hydroxyphenyl) ethanone moiety (Hong et al., 2013) (C-1′–C-8′; H-2′,6′, H-3′,5′, H-8′, Ar-OH-4′ and NH) (Table 1). The HMBC correlations (Fig. 2) of the amino proton (δ_H 8.32) with C-8 (δ_C 116.0), C-9 (δ_C 166.8), C-7′ (δ_C 191.5), and C-8′ (δ_C 45.0) indicated that **1** should be a amide derivative, and the (*E*)-3-(4-hydroxybenzofuran-6-yl)acryl moiety connected to 2-amino-1-(4-hydroxyphenyl) ethanone moiety by nitrogen atom. The HMBC correlations of the hydroxy proton (δ_H 10.13) with C-4 (δ_C 118.5), C-5 (δ_C 148.3), and C-6 (δ_C 109.3); as well as those of the other hydroxy proton (δ_H 10.45) with C-3′,5′ (δ_C 115.3) and C-4′ (δ_C 160.5), led to the assignment of two phenolic hydroxy groups at C-5 and C-4′. Additionally, H-10 (δ_H 6.70) showed correlations with the carbon signal of C-3 (δ_C 154.2), C-4 (δ_C 118.5), and C-5 (δ_C 148.3); and H-11 (δ_H 7.40) showed correlations with the C-3 (δ_C 154.2) and C-4 (δ_C 118.5) clearly indicated that the furan ring should be located between C-3 and C-4. On the basis of the above evidences, the structure of **1** was established as shown, and gives the trivail name of tabamide A.

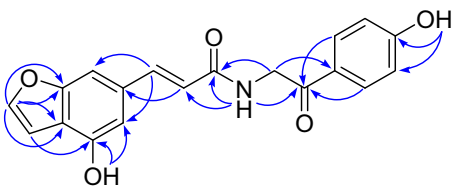

Fig. 2. Key HMBC correlations (↷) of **1**.

Compound **2** was isolated as a white powder. Its molecular formula $C_{19}H_{21}NO_6$ was determined from its HRESIMS measurement at m/z 382.1272 $[M+Na]^+$. The ^{1}H and ^{13}C NMR spectrum of **2** revealed a 3-(3-hydroxy-4,5-dimethoxyphenyl)propan moiety (Leng et al., 2014) (C-1–C-9, -OMe-3, and -OMe-5; H-2, H-6–H-8, and Ar-OH-3) and an 2-amino-1-(4-hydroxyphenyl) ethanone moiety (Hong et al., 2013) (C-1′–C-8′; H-2′,6′, H-3′,5′, H-8′, Ar-OH-4′ and NH) (Table 1). The HMBC correlations of the amino proton (δ_H 8.33) with C-8 (δ_C 36.6), C-9 (δ_C 173.3), C-7′ (δ_C 190.8), and C-8′ (δ_C 45.4) indicated that **2** should be a amide derivative composed of a 3-(3-hydroxy-4,5-dimethoxyphenyl)propan moiety and a

2-amino-1-(4-hydroxyphenyl) ethanone moiety. The HMBC correlations of two methoxy protons (δ_H 3.79 and 3.81) with C-4 (δ_C 138.7) and C-5 (δ_C 151.0) led to the assignment of two methoxy groups at C-4 and C-5, respectively. Additionally, two phenolic hydroxy groups located at C-3 and C-4′ were also supported by the HMBC correlations of one hydroxy proton (δ_H 10.87) with C-2 (δ_C 107.2), C-3 (δ_C 147.5), and C-4 (δ_C 138.7); and the other hydroxy proton (δ_H 10.41) with C-3′,5′ (δ_C 115.8) and C-4′ (δ_C 160.7). Thus, the structure of tabamide B (**2**) was established as shown.

Tabamides C (**3**) was also obtained as white powder. It was assigned the molecular formula $C_{20}H_{23}NO_6$ by HRESIMS at *m/z* 396.1426 $[M+Na]^+$. The ^1H and ^{13}C NMR spectra of **3** (Table 1) were similar to those of **2**, the major difference being the replacement of a hydroxy proton signal in **2** (δ_H 10.41) by a methoxy signal (δ_C 55.8, δ_H 3.80) in **3**. The HMBC correlation of the methoxy proton (δ_H 3.80) with C-4′ (δ_C 164.0) indicated that the methoxy group was located at C-4′. Compound **3** is therefore the 4′-*O*-methyl derivative of **2**.

Compounds **1**–**6** were tested for their anti-TMV activity. The inhibitory activity of compounds **1**–**6** against TMV replication was tested using the half-leaf method (Hu et al., 2013). Ningnanmycin, a commercial product for plant disease in China, was used as a positive control. The antiviral inhibition rates of compounds **1**–**6** at the concentration of 20 μM were listed in Table 2. The results showed that compound **1** exhibited high anti-TMV activity with inhibition rate of 38.6%. The inhibition rate is higher than that of positive control. The other compounds also showed potential anti-TMV activities with inhibition rates in the range of 15.3–26.5%, respectively.

3. Experimental

3.1. General

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, or 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.

Table 2
TMV infection inhibition activity of compounds **1**–**6**.

Compounds	Inhibition rates (%)
1	38.6 ± 3.8
2	26.5 ± 2.6
3	22.4 ± 2.8
4	15.3 ± 2.0
5	18.2 ± 2.5
6	20.1 ± 2.2
Ningnanmycin	31.2 ± 3.4

All results are expressed as mean ± SD; $n = 3$ for all groups.

3.2. Plant material

The leaves of *N. tabacum* L. (tobacco leaves) were collected from Yuxi County, Yunnan Province, PR China, in September 2011.

3.3. Extraction and isolation

The air-dried and powdered leaves of *N. tabacum* (4.8 kg) were extracted four times with 70% aqueous acetone (4× 5 L) at room temperature and filtered, with the filtrate evaporated under reduced pressure and partitioned with EtOAc (4× 3 L). The EtOAc partition (205 g) was applied to silica 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. Further separation of fraction B (9:1, 22.8 g) by silica gel column chromatography, eluted with $CHCl_3$–$(CH_3)_2CO$ (9:1–2:1), yielded mixtures B1–B6. Fraction B2 (8:2, 5.4 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semi-preparative HPLC (40% MeOH–H_2O, flow rate 12 mL/min) to give **1** (8.6 mg), **2** (14.6 mg), and **3** (11.9 mg). Fraction B3 (7:3, 6.75 g) was subjected to silica gel column chromatography using $CHCl_3$–$(CH_3)_2CO$ and semi-preparative HPLC (36% MeOH–H_2O, flow rate 12 mL/min) to give **4** (10.2 mg), **5** (14.5 mg), and **6** (13.8 mg).

3.3.1. Tabamide A (*1*)

Obtained as white powder; UV (MeOH) λ_{max} (log ε): 215 (4.26), 253 (3.86), 338 (3.75); IR (KBr) ν_{max}: 3418, 3315, 2913, 2576, 1675, 1650, 1608, 1529, 1456, 1232, 1165, 1121; ^1H and ^{13}C (500 and 125 MHz) NMR spectroscopic data, see Table 1; positive ESI-MS *m/z* 360 $[M+Na]^+$; positive HRESIMS *m/z* 360.0842 $[M+Na]^+$ (calcd for $C_{19}H_{15}NNaO_5$, 360.0848).

3.3.2. Tabamide B (*2*)

Obtained as white powder; UV (MeOH) λ_{max} (log ε): 220 (4.38), 287 (3.22); IR (KBr) ν_{max}: 3318, 2970, 1682, 1654, 1610, 1536, 1445, 1368, 1246, 1160, 1124 cm^{-1}; ^1H and ^{13}C (500 and 125 MHz) NMR spectroscopic data, see Table 1; positive ESI-MS *m/z* 382 $[M+Na]^+$; positive HRESIMS *m/z* 382.1272 $[M+Na]^+$ (calcd for $C_{19}H_{21}NNaO_6$, 382.1267).

3.3.3. Tabamide C (*3*)

Obtained as white powder; UV (MeOH) λ_{max} (log ε): 220 (4.26), 285 (3.34); IR (KBr) ν_{max}: 3316, 2968, 1685, 1657, 1610, 1532, 1442, 1367, 1249, 1158, 1120 cm^{-1}; ^1H and ^{13}C (500 and 125 MHz) NMR spectroscopic data, see Table 1; positive ESI-MS *m/z* 396 $[M+Na]^+$; positive HRESIMS *m/z* 396.1426 $[M+Na]^+$ (calcd for $C_{20}H_{23}NNaO_6$, 396.1423).

3.4. Anti-HIV-1 assay

The anti-TMV activities were tested using the half-leaf method, and ningnanmycin (Hu et al., 2013), a commercial product for plant disease in China, was used as a positive control.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2014.06.012.

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