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PHENYLPROPANOIDS FROM THE LEAVES OF *NICOTIANA TABACUM* AND THEIR ANTI-TOBACCO MOSAIC VIRUS ACTIVITIES

Qin-Peng Shen,[1] Lan Li,[1,2] Xin-Meg Xu,[1,2] Chun-Bo Liu,[1] Neng-Jun Xiang,[1] Feng-Mei Zhang,[1] Pei He,[1] Zhi-Hua Liu,[1] Shan-Zhai Shang,[1] Ming-Ming Miao,[1,*] and Guang-Yu Yang [1,*]

[1] Key Laboratory of Tobacco Chemistry of Yunnan Province, Research and Development of Center, China Tobacco Yunnan Industrial Co., Ltd., Kunming 650231, P.R. China; [2] School of Pharmaceutical Science & Yunnan Key Laboratory of Pharmacology for Nature Products, Kunming Medical University, Kunming, Yunnan 650500, PR China; E-mail: miaomm2014@163.com ; ygy1110@163.com

Abstract – Two new phenylpropanoids, 5-hydroxy-6-(3-hydroxypropanoyl)-isobenzofuran-1(3*H*)-one (**1**), 6-(3-hydroxypropanoyl)-5-methylisobenzofuran-1(3*H*)-one (**2**), together with four known phenylpropanoids (**3-6**) were isolated from the leaves of *Nicotiana tabacum*. Their structures were determined by means of HRESIMS and extensive 1D and 2D NMR spectroscopic studies. Compounds **1-5** were tested for their anti-tobacco mosaic virus (anti-TMV) activities. The results showed that Compound **2** showed high anti-TMV activity with inhibition rate of 29.2%. This rate is close to that of positive control. The other compounds also showed potential anti-TMV activity with inhibition rates in the ranges of 20.3-24.6%, respectively.

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 are cultivated worldwide as the primary commercial source of tobacco, which is smoked or chewed as a drug for its mild stimulant effects.[1,2] In addition, *N. tabacum* is also used as insecticide, anesthetic, diaphoretic, sedative, and emetic agents in Chinese folklore medicine because of it containing many useful chemical compounds.[1-4] In previous work, a number of bioactive compounds, such as terpenoids,[5-7] alkaloids,[8,9] lignans,[10,11] flavonoid,[12,13] phenylpropanoids,[14,15] chromanones,[16,17] biphenyls,[18] phenolic amides,[19]

phenanthrene,[20] isocoumarins,[21] and the homologous, were isolated from this plant. Therefore, the multipurpose utilization of this plant is an interesting topical and attracts more and more attentions. In continuing efforts to the phytochemistry research on the tobacco leaves of Yunyan-85 (a variety of *N. tabacum*) led to the isolation of two new (**1**-**2**) and four known (**3**-**6**) phenylpropanoids. This paper deals with the isolation, structural elucidation, and anti-TMV activities of these compounds.

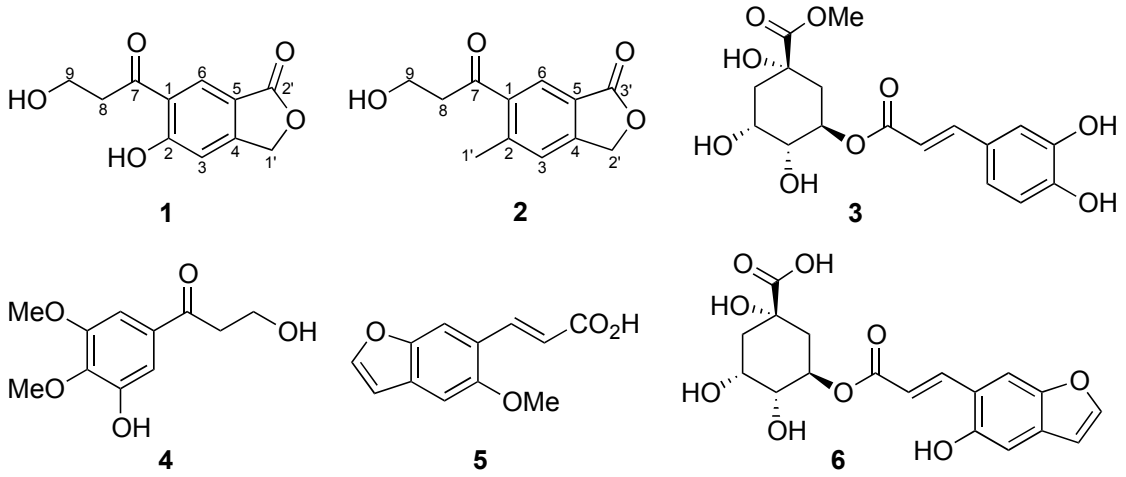

Figure 1. phenylpropanoids from the leaves of *N. tabacum*

A 70% aq. methanol extract prepared from the leaves of tobacco was subjected repeatedly to column chromatography on silica gel and preparative HPLC to afford two new phenylpropanoids, 5-hydroxy-6-(3-hydroxypropanoyl)-isobenzofuran-1(3*H*)-one (**1**), 6-(3-hydroxypropanoyl)-5-methylisobenzofuran-1(3*H*)-one (**2**), and four known phenylpropanoids (**3**-**6**). The structures of the compounds **1**-**6** were as shown in Figure 1, and the ^{1}H and ^{13}C NMR data of **1** and **2** were listed in Table 1. The known compounds, compared with literature, were identified as *trans*-chlorogenic acid methyl esters (**3**),[22] feddeiketone B (**4**),[23] lanceolune C (**5**),[24] and nicotfuran A (**6**).[15]

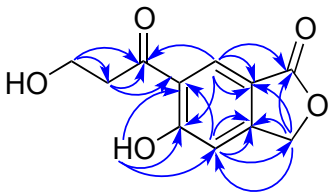

Figure 2. key HMBC (↷) correlations of **1**

Compound **1** was obtained as a pale-yellow gum. Its molecular formula was determined as $C_{11}H_{10}O_5$ by HRESIMS (*m/z* 221.0457 $[M-H]^-$; calcd for $C_{11}H_9O_5$, 221.0450), indicating the presence of seven degrees of unsaturation in the molecule. The UV spectrum showed absorption maxima at 215, 286 and 318 nm, and the IR spectrum showed absorption bands at 3460, 1730, 1652, 1608, 1536, and 1487 cm^{-1}, indicating the presence of hydroxy group, carbonyl group, and aromatic ring. The ^{1}H and ^{13}C NMR spectrum of **1** (Table 1) along with analysis of the DEPT spectra displayed 11 carbon signals and 9 proton signals, respectively, corresponding to a 1,2,4,5-tetrasubstituted phenyl ring (δ_C 120.9 s, 166.1 s, 112.0 d, 152.0 s, 116.3 s and 130.4 d; δ_H 6.94 s and 8.32 s), a 3-hydroxypropan-1-one [-C(O)-CH_2CH_2OH] moiety (δ_C 198.4 s, 42.1 t and 58.7 t; δ_H 3.45 (t) 6.8 and 4.41 (t) 6.8).[25] an ester carbonyl group (δ_C 169.1 s), an oxygenated methylene (δ_C 69.0 t; δ_H 5.40 s), and a phenolic hydroxy

group (δ_H 10.86). The HMBC correlations H-1′/C-2′, C-3, C-4, C-5; H-3/C-1′; H-6/C-2′ (Figure 2) suggested the existence of a benzolactone moiety. The HMBC correlations H-8/C-1 and H-6/C-7 suggested the 3-hydroxypropan-1-one moiety should be located at C-1. The location of the phenolic hydroxy group was assigned to C-2 on the basis of HMBC correlations of phenolic hydroxy proton (δ_H 10.86) with C-1, C-2 and C-3. Thus, the structure of 5-hydroxy-6-(3-hydroxypropanoyl)-isobenzofuran-1(3*H*)-one (**1**) was established as shown.

Compound **2** was also obtained as a pale-yellow gum. A molecular formula $C_{12}H_{12}O_4$ was assigned from HRESIMS (*m/z*: *m/z* 219.0652 [M-H]$^-$, calcd 219.0657). The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1**. The chemical shift differences resulted from the disappearance of a phenolic hydroxy proton signal and appearance of a methyl resonance (δ_C 18.3 q; δ_H 2.28 s) in **2**. This indicated that the phenolic hydroxy group in **1** was converted into a methyl group in **2**. The HMBC correlations of H-1′ to C-1, C-2 and C-3 indicated that the methyl group was located at C-2. Thus, the structure of **2** was established as 6-(3-hydroxypropanoyl)-5-methylisobenzofuran-1(3*H*)-one.

Table 1. ^{1}H NMR and ^{13}C NMR Data (in C_5D_5N) of compounds **1** and **2**

No.	Compound **1**		Compound **2**	
	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)
1	120.9 s		132.8 s	
2	166.1 s		142.0 s	
3	112.0 d	6.94 s	127.7 d	7.31 s
4	152.0 s		152.5 s	
5	116.3 s		124.7 s	
6	130.4 d	8.32 s	129.0 d	8.31 s
7	198.4 s		198.1 s	
8	42.1 t	3.45 (t) 6.8	41.9 t	3.20 (t) 6.8
9	58.7 t	4.41 (t) 6.8	58.0 t	4.32 (t) 6.8
1′	69.0 t	5.40 s	18.3 q	2.28 s
2′	169.1 s		69.2 t	5.42 s
3′			169.3 s	
Ar-OH-4′		10.86 s		

Compound **1-5** were tested for their anti-TMV activity. The anti-TMV activity were tested using the half-leaf method.[26] Ningnanmycin (a commercial product for plant disease in China), was used as a positive control. Their antiviral inhibition rates at the concentration of 20 μM were listed in Table 2. Compound **2** showed high anti-TMV activity with inhibition rate of 29.2%. This rate is close to that of positive control. The other compounds also showed potential anti-TMV activity with inhibition rates in the ranges of 20.3-24.6%, respectively.

Table 2. TMV Infection Inhibition Activities of **1-5**

Compounds	Inhibition rate (%)	Compounds	Inhibition rates (%)
1	22.5 ± 3.0	**4**	24.6 ± 2.6
2	29.2 ± 3.2	**5**	23.4 ± 3.0
3	20.3 ± 2.9	ningnanmycin	30.114 ± 3.2

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

EXPERIMENTAL

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, and the chemical shifts (δ)

were expressed in ppm. 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). 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 leaves of *N. tabacum* L (tobacco leaves) was collected from Yuxi County, Yunnan Province, P. R. China, in September 2013. The tobacco variety is Yunyan-85, which had widely cultivated in China.

Extraction and Isolation. The air-dried and powdered leaves of *N. tabacum* (4.5 kg) were extracted four times with 70% aqueous MeOH (3 × 5 L) at room temperature and filtered. The solvent was evaporated in vacuo, and the crude extract was dissolved in H_2O and partitioned with EtOAc. The EtOAc partition (152 g) was applied to silica gel (200–300 mesh) column chromatography, eluting with a $CHCl_3$-MeOH gradient system (10:0, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A–F. Further separation of fraction B (8:2, 32.6 g) by silica gel column chromatography, eluted with $CHCl_3$ –Me_2CO (8:2 - 2:1), yielded mixtures B1–B6. Fraction B3 (6:4, 3.22 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semi-preparative HPLC (35% MeOH-H_2O, flow rate 12 mL/min) to give **1** (11.3 mg), **2** (8.2 mg), and **4** (18.4 mg) Fraction B5 (4:6, 4.48 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semi-preparative HPLC (20% MeOH-H_2O, flow rate 12 mL/min) to give **3** (13.6 mg) , **5** (16.2 mg), and **6** (9.28 mg).

Anti-TMV Assays. The anti-TMV activities were tested using the half-leaf method,[26] and Ningnanmycin (2% water solution), a commercial product for plant disease in China, was used as a positive control.

5-Hydroxy-6-(3-hydroxypropanoyl)-isobenzofuran-1(3*H*)-one (1): Obtained as pale-yellow gum; UV (MeOH), λ_{max} (log ε) 318 (3.28), 286 (3.59), 215 (4.02) nm; IR (KBr) λ_{max} 3460, 2927, 1730, 1652, 1608, 1536, 1487, 1285, 1121, 1072, 927, 805 cm^{-1}; ^1H NMR and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz, respectively), Table 1; ESIMS (negative ion mode) *m/z* 221 $[M-H]^-$; HRESIMS (negative ion mode) *m/z* 221.0457 $[M-H]^-$ (calcd 221.0450 for $C_{11}H_9O_5$).

6-(3-Hydroxypropanoyl)-5-methylisobenzofuran-1(3*H*)-one (2): Obtained as pale-yellow gum; UV (MeOH), λ_{max} (log ε) 312 (3.22), 280 (3.57), 214 (4.05) nm; IR (KBr) λ_{max} 3442, 2918, 1735, 1657, 1610, 1542, 1476, 1387, 1259, 1142, 1064, 869, 768 cm^{-1}; ^1H NMR and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz, respectively), Table 1; ESIMS (negative ion mode) *m/z* 219 $[M-H]^-$; HRESIMS (negative ion mode) *m/z* 219.0652 $[M-H]^-$ (calcd 219.0657 for $C_{12}H_{11}O_4$).

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