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TWO NEW BENZOFURANYLPROPANOIDS FROM *NICOTIANA TABACUM* AND THEIR ANTI-TOBACCO MOSAIC VIRUS ACTIVITIES

Jianlin Tan,[1,3] Zhangyu Chen,[1,2] Guangyu Yang,[1] Mingming Miao,[1] Yongkuan Chen,[1*] and Tianfei Li[1,2*]

[1] Key Laboratory of Tobacco Chemistry of Yunnan Province, Yunnan Academy of Tobacco Science, Kunming 650106, P. R. China. [2] China Tobacco Yunnan Industry Company(Ltd.) Kunming 650000, P. R. China. [3] Laboratory for Conservation and Utilization of Bio-Resources & Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan University, Kunming 650091, P. R. China. E-mail:cyk1966@163.com, ygy1110@163.com

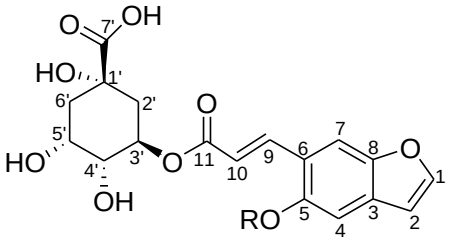
Abstract – Two new benzofuranylpropanoids, nicotfurans A and B (**1 - 2**), were isolated from the roots and stems of *Nicotiana tabacum*. Their structures were elucidated by spectroscopic methods, including extensive 1D- and 2D- NMR techniques. Compounds **1 - 2** were tested for their anti-tobacco mosaic virus (anti-TMV) activities. The results showed that compounds **1 - 2** have high anti-TMV activities.

Nicotiana tabacum L. belongs to Solanaceae family. It is a perennial herbaceous plant originating from South America, and it is one of the most commercially valued agricultural crops in the world.[1,2] In addition to being used in cigarette industry, *N. tabacum* is also used as insecticides, anesthetics, diaphoretics, sedatives, and emetic agents in Chinese folklore medicine because of its containing many useful chemical compounds.[1,3] The stems and roots of *N. tabacum* are big amount of by-product in tobacco planting, and are normally used as organic fertilizer. The multipurpose utilization of the stems and roots of *N. tabacum* is an interesting topical, and receives more and more attentions.[4,5]

In previous work, a number of bioactive compounds, such as alkaloids,[5,6] sesquiterpenes,[7,8] diterpenoids,[9] phenols,[10,11] and their homologous, were isolated from the *N. tabacum*. Motivated by search for bioactive metabolites from this plant, an investigation on the chemical constituents of the stems and roots of *N. tabacum* was carried out. As a result, two new benzofuranylpropanoids were isolated from this plant. In

addition, the anti-tobacco mosaic virus (Anti-TMV) activities of compounds **1** and **2** were evaluated. This article deals with the isolation, structural elucidation and biological activities of the two new compounds.

A 95% aq. methanol extract prepared from the stems and roots of *N. tabacum* was subjected repeatedly to column chromatography on silica gel, Sephadex LH-20, RP-18 and preparative HPLC to afford compounds **1** and **2**. The structures of **1** and **2** were shown in **Figure 1**, and their ^{1}H and ^{13}C-NMR spectroscopic data were listed in **Table 1**.



1 R = -H; **2** R = -Me

Figure 1. The structure of compounds **1-2**

Compound **1** was obtained as pale yellow gum. Its molecular formula was determined as $C_{18}H_{18}O_9$ by HR-ESI-MS *m/z* 401.0856 $[M+Na]^+$ (calcd 401.0849). Its ^{1}H and ^{13}C NMR spectral data (**Table 1**) showed signals to 18 hydrogens and 18 carbons, respectively, corresponding to a benzofuran nucleus (δ_C 147.0, 106.4, 129.0, 104.7, 154.9, 110.0, 108.9, 149.0) with four aromatic protons (δ_H 6.99 s, 7.35 s, 6.72 d J = 2.5, 7.67 d J = 2.5), one acryl group (δ_C 145.8, 115.9, 167.3; δ_H 7.98 d J = 15.9, 6.57 d J = 15.9), one 3-*O*-quinic acid group (δ_C 76.1, 38.9, 71.2, 73.6, 72.4, 39.2, 177.3; δ_H 2.72-2.81 overlap, 2.94 m, 4.81 m, 4.34 m, 6.22 m, 2.72-2.81overlap). One phenolic hydroxy group (δ_H 10.12). Strong absorption bands accounting for hydroxy (3368 cm^{-1}), carbony (1721, 1704 cm^{-1}) and aromatic group (1643, 1514, 1458 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of **1** showed absorption maxima at 310, 260 nm also confirmed the existence of the aromatic function. The ^{1}H NMR data of **1** (δ_H 6.99 s, 7.35 s, 6.72 d J = 2.5, 7.67 d J = 2.5) revealed the benzofuran nucleus is 5,6-substituted.[12] The ^{1}H-^{1}H COSY of H-9/H-10; together with HMBC correlations (**Figure 2**) of H-7 (δ_H 7.35) with C-9 (δ_C 145.8), of H-9 (δ_H 7.98) with C-5 (δ_C 154.9), C-6 (δ_C 110.0), C-7 (δ_C 108.9), C-10 (δ_C 115.9), C-11 (δ_C 167.3), of H-10 (δ_H 6.59) with C-9 (δ_C 145.8), C-6 (δ_C 110.0) and C-11 (δ_C 167.3) suggested the presence of a acryl (-CH=CH-COO-) structure unit, and this structure unit was attached to C-6. The HMBC correlations of phenolic hydroxy proton signal (δ_H 10.12) with C-4 (δ_C 104.7), C-5 (δ_C 154.9) and C-6 (δ_C 110.0) indicated that the hydroxy group should be located at C-5. The HMBC correlation of H-3' (δ_H 4.81) with C-11 (δ_C 167.3) indicated that the 3-*O*-quinic acid group should be located at C-11. Thus, the structure of **1** was established and named as nicotfuran A.

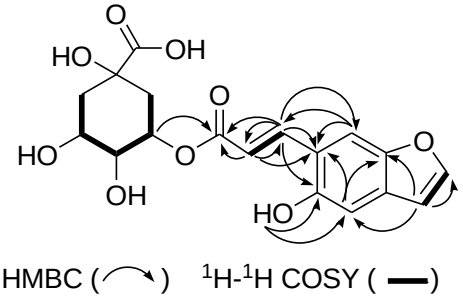


Figure. 2 The Key HMBC and ^{1}H-^{1}H COSY correlations of **1**

Compounds **2** was also obtained as pale yellow gum, and should sodiated molecular ions at *m/z* 415.1001 $[M+Na]^+$ in the HRESIMS (calcd *m/z* 415.1005), corresponding to the molecular formula of $C_{19}H_{20}O_9$.

The ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **1**. The only difference was a hydroxy group in **1** was substituted by a methoxy group in **2** on the aromatic rings, which was supported by the disappearance of phenolic hydroxy proton signal (δ_H 10.12 brs) and appearance of methoxy group signals (δ_C 55.9 q, δ_H 3.78 s) in **2**. Thus, the structure of **2** was established, and it has been accorded the trivial name of nicotfuran B.

Table 1. ^{1}H NMR and ^{13}C NMR data of compounds **1 - 2** in C_5D_5N

No.	Compound **1**		Compound **2**	
	δ_C (mult.)	δ_H (mult, *J*, Hz)	δ_C (mult.)	δ_H (mult, *J*, Hz)
1	147.0 d	6.72 d, *J* = 2.5	146.8 d	6.76 d, *J* = 2.5
2	106.4 d	7.67 d, *J* = 2.5	106.2 d	7.68 d, *J* = 2.5
3	129.0 s		129.2 s	
4	104.7 d	6.99 s	104.4 d	7.03 s
5	154.9 s		152.9 s	
6	110.0 s		109.8 s	
7	108.9 d	7.35 s	108.4 d	7.36 s
8	149.0 s		148.9 s	
9	145.8 d	7.98, d, *J* = 15.9	145.4 d	7.96, d, *J* = 15.9
10	115.9 d	6.59, d, *J* = 15.9	115.5 d	6.56, d, *J* = 15.9
11	167.3 s		167.1 s	
1′	76.1 s		76.0 s	
2′	38.9 t	2.72-2.81, overlap 2.94, m	38.9 t	2.71-2.80, overlap 2.93, m
3′	71.2 d	4.81 m	71.1 d	4.80 m
4′	73.6 d	4.34 m	73.4 d	4.33 m
5′	72.4 d	6.22 m	72.2 d	6.21 m
6′	39.2 t	2.72-2.81, overlap 2.72-2.81, overlap	39.2 t	2.71-2.80, overlap 2.71-2.80, overlap
7′	177.3 s		177.2 s	
5-OMe			55.9 q	3.78 s
ArOH		10.12 brs		

Since some of the phenylpropanoids exhibited anti virus activities,[13,14] compounds **1** and **2** were tested for their potencies in preventing Anti-TMV activity using the half-leaf method.[15]

The antiviral inhibition rates of the compound at the concentration of 20 μM were tested by the half-leaf method. The results showed that the compounds **1** and **2** exhibited inhibition rates of 74.8% and 68.4%, respectively. The rates are higher than that of positive control (56.4%).

EXPERIMENTAL

General. Optical rotation was measured in Horiba SEPA-300 high sensitive polarimeter. IR spectra were obtained in KBr disc on a Bio-Rad Wininfmred spectrophotometer. ESI-MS were measured on a VG Auto Spec-3000 MS spectrometer. ^{1}H, ^{13}C and 2D NMR spectra were recorded on Bruker DRX-500 instrument with TMS as internal standard. Column chromatography was performed on silica gel (200-300 mesh), or on silica gel H (10~40 μm, Qingdao Marine Chemical Inc., China). Second separate was used an Agilent 1100 HPLC equipped with ZORBAX-C_{18} (21.2 mm × 250 mm, 7.0 μm) column and DAD detector.

Plant material. The stems and roots of *Nicotiana tabacum* L (tobacco stems and roots) was collected from Yuxi County, Yunnan Province, P. R. China, in September 2009.

Extraction and isolation. The air-dried and powdered stems and roots of *Nicotiana tabacum* (2.5 kg) were extracted with 95% aqueous methanol (3.0 L × 3, 24 h each) at room temperature and the extract was concentrated under vacuum condition. The dried extract (68.5 g) was applied to silica gel (200–300 mesh) column chromatography (8 × 100 cm column, with Si gel 1.86 kg) eluting with a $CHCl_3$ − Me_2CO gradient system (9:1, 8:2, 7:3, 6:4, 5:5 and 2:1) to give six fractions A–F (5.2 L of eluant was used for each fractions). Fraction E (5:5, 12.5 g) was subjected to silica gel column chromatography using $CHCl_3$-MeOH and preparative HPLC (30% MeOH-H_2O, flow rate 12 mL/min) to give **1** (34.2 mg) and **2** (42.5 mg).

Anti-TMV Assays. The Anti TMV activities were tested using the half-leaf method.[15] The inhibitory activities of the new 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 *N. glutinosa* in vivo. Then, the leaf-disk method was used to evaluate the antiviral activity of the compound in the systemic infection host *N. tabacum cv*. K326. Ningnanmycin (2% water solution), a commercial product for plant disease in China, was used as a positive control.

Nicotfuran A. Obtained as a pale yellow gum; $[\alpha]_D^{24.2}$ -42.3 (*c* 0.20, MeOH); UV (MeOH), λ_{max} (log ε) 310 (3.84), 260 (4.18), 215 (4.94) nm; IR (KBr) ν_{max} 3368, 2957, 2874, 1721, 1704, 1643, 1514, 1458, 1425, 1262, 1174, 1043, 956, 874 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (C_5D_5N, 500 MHz and 150 MHz, respectively), **Table 1**; ESIMS (positive ion mode) *m/z* 401; HRESIMS (positive ion mode) *m/z* 401.0856 $[M + Na]^+$ (calcd 401.0849 for $C_{18}H_{18}NaO_9$).

Nicotfuran B. Obtained as a pale yellow gum; $[\alpha]_D^{24.5}$ -39.4 (*c* 0.22, MeOH); UV (MeOH), λ_{max} (log ε) 310 (3.80), 262 (4.22), 215 (4.98) nm; IR (KBr) ν_{max} 3365, 2954, 2876, 1725, 1707, 1640, 1517, 1456, 1428, 1264, 1177, 1062, 1045, 953, 871 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (C_5D_5N, 500 MHz and 150 MHz, respectively), **Table 1**; ESIMS (positive ion mode) *m/z* 415; HRESIMS (positive ion mode) *m/z* 415.1001 $[M + Na]^+$ (calcd 415.1005 for $C_{19}H_{20}NaO_9$).

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