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

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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 ^1H and ^{13}C -NMR spectroscopic data were listed in **Table 1**.

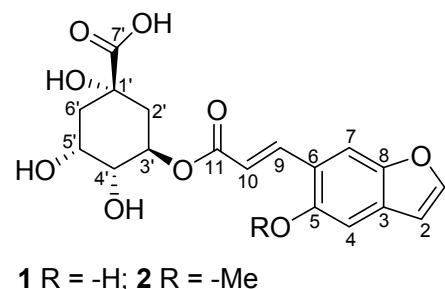


Figure 1. The structure of compounds **1-2**

Compound **1** was obtained as pale yellow gum. Its molecular formula was determined as $\text{C}_{18}\text{H}_{18}\text{O}_9$ by HR-ESI-MS m/z 401.0856 $[\text{M}+\text{Na}]^+$ (calcd 401.0849). Its ^1H 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, 2.72-2.81 overlap). One phenolic hydroxy group (δ_{H} 10.12). Strong absorption bands accounting for hydroxy (3368 cm^{-1}), carbonyl (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 ^1H 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 ^1H - ^1H 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}

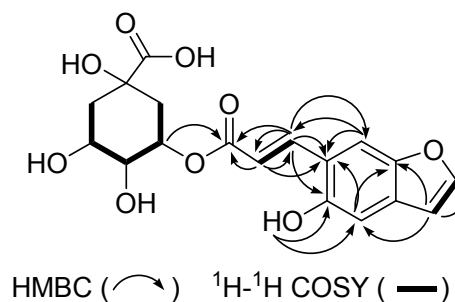


Figure 2 The Key HMBC and ^1H - ^1H COSY correlations of **1**

110.0) and C-11 (δ_{C} 167.3) suggested the presence of a acryl ($-\text{CH}=\text{CH}-\text{COO}-$) 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.

Compounds **2** was also obtained as pale yellow gum, and should sodiated molecular ions at m/z 415.1001 $[\text{M}+\text{Na}]^+$ in the HRESIMS (calcd m/z 415.1005), corresponding to the molecular formula of $\text{C}_{19}\text{H}_{20}\text{O}_9$.

The ^1H 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. ^1H NMR and ^{13}C NMR data of compounds **1** - **2** in $\text{C}_5\text{D}_5\text{N}$

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 Wininfrared spectrophotometer. ESI-MS were measured on a VG Auto Spec-3000 MS spectrometer. ^1H , ^{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 \times 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 \times 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 \times 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; $[\alpha]_{\text{D}}^{24.2}$ -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} ; ^1H NMR and ^{13}C NMR data ($\text{C}_5\text{D}_5\text{N}$, 500 MHz and 150 MHz, respectively), **Table 1**; ESIMS (positive ion mode) *m/z* 401; HRESIMS (positive ion mode) *m/z* 401.0856 $[\text{M} + \text{Na}]^+$ (calcd 401.0849 for $\text{C}_{18}\text{H}_{18}\text{NaO}_9$).

Nicotfuran B. Obtained as a pale yellow gum; $[\alpha]_{\text{D}}^{24.5}$ -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} ; ^1H NMR and ^{13}C NMR data ($\text{C}_5\text{D}_5\text{N}$, 500 MHz and 150 MHz, respectively), **Table 1**; ESIMS (positive ion mode) *m/z* 415; HRESIMS (positive ion mode) *m/z* 415.1001 $[\text{M} + \text{Na}]^+$ (calcd 415.1005 for $\text{C}_{19}\text{H}_{20}\text{NaO}_9$).

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REFERENCES

1. The Editorial Committee of the Administration Bureau of Flora of China, *Flora of China*, 67 vols. Beijing Science and Technology Press, Beijing, 2005.
2. T. W. Hu and Z. Mao, *Tob. Control*, 2006, **15**, i37.
3. A. Rodgman and T. A. Perfetti, *The Chemical Components of Tobacco and Tobacco Smoke*, CRC Press, Taylor and Francis Group, Boca Raton, Florida, 2008; A. P. Cavender and M. Alban, *J. Ethnobot. Ethnomed*, 2009, **5**, 3; I. Angkhana, S. J. Pei, B. Henrik, and T. Chusie, *J. Ethnopharm.*, 2008, **116**, 134.
4. W. Li, L. B. Zhang, J. H. Peng, N. Li, and X. Y. Zhu, *Ind. Crop. Prod.*, 2008, **27**, 341; B. B. Zhang, C. J. Xie, and X. Y. Yang, *Peptides*, 2008, **29**, 350.
5. W. H. Zhong, C. J. Zhu, M. Shu, K. D. Sun, L. Zhao, C. Wang, Z. J. Ye, and J. M. Chen, *Bioresour. Tech.*, 2010, **101**, 6935.
6. X. C. Wei, S. C. Sumithran, A. G. Deaciuc, H. R. Burton, L. P. Bush, L. P. Dwoskin, and P. A. Crooks, *Life. Sci.*, 2005, **78**, 495; T. Braumann, G. Nicolaus, W. Hahn, and H. Elmenhorst, *Phytochemistry*, 1990, **29**, 3693.
7. F. Xin, J. S. Wang, J. Luo, and L. Y. Kong, *J. Asian Nat. Prod. Res.*, 2010, **12**, 252.
8. F. Xin, J. S. Wang, J. Luo, and L. Y. Kong, *J. Asian Nat. Prod. Res.*, 2009, **11**, 675.
9. I. Wahlberg, I. Wallin, K. Nordfors, T. Nishida, C. R. Enzell, and W. W. Reid, *Acta. Chem. Scand.*, 1979, **33b**, 541; Y. Shinozaki, T. Tobita, M. Mizutani, and T. Matsuzaki, *Biosci. Biotechnol. Biochem.*, 1996, **60**, 903; T. Petterson, A. M. Eklund, and I. Wahlberg, *J. Agric. Food. Chem.*, 1993, **41**, 2097.
10. D. Vereecke, E. Messens, K. Klarskov, A. Bruyn, M. Montagu, and K. Goethals. *Planta*, 1997, **201**, 342.
11. X. G. Gu, J. B. Cai, X. L. Zhu, and Q. D. Su, *J. Sep. Sci.*, 2005, **28**, 2477.
12. M. L. Hammond, I. E. Kopka, R. A. Zambias, C. G. Caldwell, J. Boger, F. Baker, T. Bach, S. Luell, and D. E. MacIntyre, *J. Med. Chem.*, 1989, **32**, 1006.
13. J. H. Westwood and M. Stevens, *Adv. Virus. Res.*, 2010, **76**, 179.
14. G. L. Shadle, S. Wesley, K. Varsha, L. Kenneth, F. Chen, C. Lamb, and R. A. Dixon, *Phytochemistry*, 2003, **64**, 153.
15. X. H. Yan, J. Chen, Y. T. Di, X. Fang, J. H. Dong, P. Sang, Y. H. Wang, H. P. He, Z. K. Zhang, and X. J. Hao, *J. Agric. Food Chem.*, 2010, **58**, 1572.