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NOTE

A New Phenolic Compound from the Leaves of *Nicotiana tabacum* and its Anti-tobacco Mosaic Virus Activities

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A new phenolic compound, 5-(4-hydroxybutyl)-2,3-dimethoxyphenol (**1**), was isolated from the leaves of *Nicotiana tabacum*. Its structure was elucidated by spectroscopic methods, including extensive 1D-NMR and 2D-NMR techniques. Compound **1** was also tested for its anti-tobacco mosaic virus (anti-TMV) activity and it showed high anti-TMV activity with inhibition rate of 22.2 %.

Keywords: *Nicotiana tabacum*, Phenolic compound, Anti-tobacco mosaic virus activity.

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[1,2]. 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[1,3]. In previous investigation of this species led to the discovery of a number of new compounds by our groups and those compounds were found to be shown various bioactivities, such as anti-HIV-1, anti-TMV and cytotoxicity[4-9]. With the aim of continuing efforts to utilize *N. tabacum* and identify bioactive natural products, the phytochemical investigation of the leaves of Honghua Dajinyuan (a variety of *N. tabacum*) was carried out and led to the isolation of a new phenolic compound (**1**) (Fig. 1). This paper reports the isolation, structural elucidation and anti-TMV activity of this new compound.

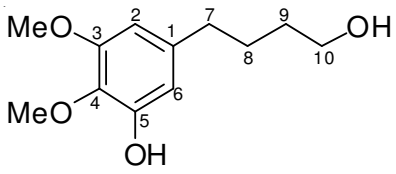

Fig. 1. Structure of compound **1**

UV spectra were obtained on a Shimadzu UV-2401A spectrophotometer. 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 mm, Qingdao Marine Chemical Inc., China). Second separate was used an Agilent 1100 HPLC equipped with ZORBAX-C_{18} (21.2 × 250 mm, 7 mm) column and DAD detector.

The leaves of Honghua Dajinyuan (a variety of *N. tabacum*) were collected in Yuxi Prefecture, Yunnan Province, People's Republic of China, in September 2012.

Extraction and Isolation: The air-dried and powdered leaves of *N. tabacum* (2 kg) were extracted four times with 90 % aq. ethanol (4 × 3 L) at room temperature and filtered. The crude extract (152 g) was applied to silica gel (200-300 mesh) column chromatography, eluting with a $CHCl_3$-MeOH (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures A-E. The further separation of fraction B (8:2, 8.22 g) by silica gel column chromatography, eluted with $CHCl_3$-$(CH_3)_2CO$ gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions B1-B6. Subfraction B4 (7:3, 1.05 g) was subjected to preparative HPLC (40 % methanol, flow rate 12 mL/min) to give **1** (9.2 mg).

5-(4-Hydroxybutyl)-2,3-dimethoxyphenol (1): obtained as pale yellow oils; UV (MeOH) λ_{max} (log ε) 315 (δ_H 2.08),

TABLE-1
^1H AND ^{13}C NMR DATA OF COMPOUND **1** (δ in ppm, in $CDCl_3$)

No.	δ_C	δ_H (J in Hz)	No.	δ_C	δ_H (J in Hz)
1	132.1 s		8	26.6 t	2.05 m
2	105.3 d	6.53 d (1.8)	9	29.7 t	1.63 m
3	153.5 s	-	10	62.1 t	3.77 t (7.2)
4	139.3 s	-	-OMe-3	55.9 s	3.80 s
5	149.7 s	-	-OMe-4	60.2 s	3.82 s
6	112.4 d	6.28 d (1.8)	Ar-OH-5	-	9.81 brs
7	35.5 t	3.15 t (6.8)	-	-	-

272 (δ_H 3.87), 210 (δ_H 4.28) nm; IR (KBr, ν_{max}, cm^{-1}): 3385, 2926, 2836, 1605, 1527, 1483, 1430, 1368, 1232, 1170, 1135, 1067, 972, 826; ^1H and ^{13}C NMR data ($CDCl_3$, 500 MHz and 125 MHz), see Table-1; positive ESIMS *m/z* 249 $[M + Na]^+$; HRESIMS *m/z* 249.1108 $[M + Na]^+$ (calc. 249.1103 for $C_{12}H_{18}NaO_4$).

The 90 % aqueous ethanol extract prepared from the leaves of *N. tabacum* was subjected repeatedly to column chromatography on Si gel, Sephadex LH-20, RP-18 and preparative HPLC to afford 5-(4-hydroxybutyl)-2,3-dimethoxyphenol (**1**).

Compound **1** was obtained as pale yellow oils. Its molecular formula was determined as $C_{12}H_{18}O_4$ by HR-ESI-MS *m/z* 249.1108 $[M+Na]^+$ (calc. 249.1103). Its ^1H and ^{13}C NMR spectra (Table-1) showed signals to 18 hydrogens and 12 carbons, respectively, corresponding to one aromatic ring (δ_C 132.1 s, 105.3 d, 153.5 s, 139.3 s, 149.7 s, 112.4 d) with two aromatic protons (δ_H 6.53 d, J = 1.8 Hz; 6.28 d, J = 1.8 Hz), three methylene groups [δ_C 35.5 t, 26.6 t, 29.7 t; δ_H 3.15 t (6.8), 2.05 m, 1.63 m], one oxidated methylene group [δ_C 60.2 s, δ_C 3.77 t (7.2)], two methoxy groups (δ_C 55.9 s, 60.2 s; δ_H 3.80 s, 3.82 s) and a phenolic hydroxy group (δ_H 9.81). Strong absorption bands accounting for hydroxy (3385 cm^{-1}) and aromatic group (1605, 1527, 1483 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of **1** showed absorption maxima at 272 nm confirmed the existence of the aromatic function. The ^1H-^1H COSY of H-7/H-8/H-9/H-10; together with HMBC correlations (Fig. 2) of aromatic protons, H-2 (δ_H 6.53), H-6 (δ_H 6.28) with C-7 (δ_C 35.5); of H-8 (δ_H 2.05) with C-1 (δ_C 132.1); and of H-7 (δ_H 3.15) with C-1 (δ_C 132.1), C-2 (δ_C 105.3) and C-6 (δ_C 112.4) suggested that **1** is a 4-hydroxybutylphenol (Ar(OH)-CH_2-CH_2-CH_2-CH_2OH) possessing two methoxy groups on the aromatic ring. The HMBC correlations of aromatic hydroxy proton signal (δ_H 9.81) with C-4 (δ_C 139.3), C-5 (δ_C 149.7) and C-6 (δ_C 112.4) indicated that the phenolic hydroxy group should be located at C-5. The HMBC correlations of two methoxy proton signals (δ_H 3.80, 3.82) with C-3 (δ_C 153.5) and C-4 (δ_C 139.3) indicated two methoxy groups should be located at C-3 and C-4, respectively. Thus, the structure of **1** was established as 5-(4-hydroxybutyl)-2,3-dimethoxyphenol.

Since certain of the phenolic compounds exhibit potential anti-TMV activities[6,7,10], compound **1** was tested for its anti-TMV activity. The inhibitory activities of compound **1** against

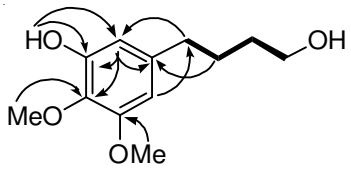

Fig. 2. Key HMBC (⌒) and ^1H-^1H COSY (–) correlations of **1**

TMV replication were tested using the half-leaf method[10]. Ningnanmycin, a commercial product for plant disease in China, was used as a positive control. Compound **2** showed high anti-TMV activity with inhibition rate of 22.2 %. It inhibition rate is close that of positive control (28.8 %).

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