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NOTE

A New Isoflavonoid from the Stems of *Nicotiana tabacum* and its Anti-tobacco Mosaic Virus Activity

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A new isoflavonoid (3',4',5'-trihydroxyl-5,7-dimethoxyl-isoflavonoid) was isolated from the stems of *Nicotiana tabacum*. Its structure was determined by means of HRESIMS, extensive ^{1}D and ^{2}D NMR spectroscopic studies and chemical evidences. The anti-tobacco mosaic virus activity of the new isoflavonoid was also evaluated and it shows moderate anti-tobacco mosaic virus activity.

Key Words: Isoflavonoid, *Nicotiana tabacum*, Anti-tobacco mosaic virus activity.

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 cigarette industry use, *N. tabacum* also contains many useful chemical compounds, such as sesquiterpenes[3,4], diterpenoids[5-7], alkaloids[8,9], phenols[10] and the like. The utilizations of these active compounds in the leaves and stems of *N. tabacum* were received more and more attentions.

Motivated by search for bioactive metabolites from this plant, an investigation on the chemical constituents of the stems of *N. tabacum* was carried out. As a result, a new isoflavonoid was isolated from this plant. In addition, the anti-tobacco mosaic virus (Anti-TMV) activity of the new isoflavonoid was evaluated. This article deals with the isolation, structural elucidation and biological activities of the new isoflavonoid.

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 ^{2}D 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 nm, 7.0 μm) column and DAD detector.

The stems of *Nicotiana tabacum* L (tobacco stems) was collected from Yuxi County, Yunnan Province, P.R. China, in September, 2008.

Extraction and Isolation: The air-dried and powdered stems *N. tabacum* (2 kg) were extracted with 70 % aqueous ethanol (3 L × 3, 24 h each) at room temperature and the extract was concentrated under vacuum condition. The dried extract (46.8 g) was applied to Si gel (200-300 mesh) column chromatography eluting with a $CHCl_3$-Me_2CO gradient system (9:1, 8:2, 7:3, 6:4, 5:5, 2:1) to give six fractions A-F. The separation of fraction C($CHCl_3$- Me_2CO 7:3, 18.6 g) by Si gel column chromatography eluted with $CHCl_3$-Me_2OH (9:1 - 1:2) and preparative HPLC (35 % MeOH-H_2O, flow rate 12 mL/min) can obtained the new isoflavonoid. The structure of the compound was shown in Fig. 1 and its ^{1}H and ^{13}C NMR spectroscopic data were listed in Table-1.

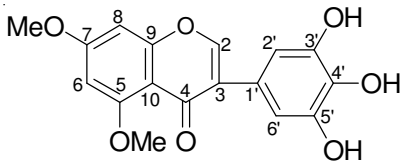

Fig. 1. Structure of the new isoflavonoid

Anti-tobacco mosaic virus assays: The anti-tobacco mosaic virus actives were tested using the half-leaf method[11]. The inhibitory activities of new isoflavonoid against tobacco mosaic virus 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, a commercial product for plant disease in China, was used as a positive control.

3',4',5'-Trihydroxyl-5,7-dimethoxyl-isoflavonoid was obtained as orange-yellow gum. The ESIMS spectrum

presented a molecular ion peak at *m/z* 329.0667 $[M-H]^-$ (calcd. 329.0661). The ^1H NMR spectrum at δ_H 7.94 (s, 1H), 6.58 (d, *J* = 2.2, 1H), 6.49 (d, *J* = 2.2, 1H) and 7.02 (s, 2H) showed the presence of 3',4',5',5,7-substituted isoflavonoid nucleus[12] and this was supported by the ^{13}H NMR data (Table-1). In addition to isoflavonoid nucleus, two methoxyl signals (δ_H 3.75, s, 3.73 s; δ_C 56.7 q, 56.0 q) were also observed in it ^1H and ^{13}C NMR spectrum. In the HMBC spectrum, the presence of cross peaks at δ_H 3.75 with δ_C 160.1 (C-5) and δ_H 3.73 with δ_C 162.0 (C-7) evidenced the attachment position of the two methoxyl groups at C-5 and C-7. Since no further aromatic carbon signals were evident, the substituents on a aromatic ring B should be three hydroxyl with 3,4,5-substituted[12] and the ^{13}C NMR spectrum of ring B (δ_C 122.7 s, 105.3 d, 146.8 s, 137.5 s) also supported this. Thus, the structure of the compound was established as shown (Fig. 2).

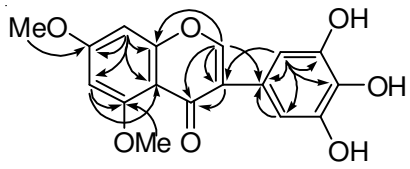

Fig. 2. Selected HMBC (⌒) correlations of new isoflavonoid

TABLE-1
^1H NMR AND ^{13}C NMR DATA OF THE COMPOUND IN $CDCl_3$

No.	δ_C (mult)	δ_H (mult, *J*, Hz)
2	154.1 d	7.94 s
3	123.1 s	
4	181.7 s	
5	160.1 s	
6	97.5 d	6.58 d, *J* = 2.2 Hz
7	162.0 s	
8	96.0 d	6.49 d, *J* = 2.2 Hz
9	158.2 s	
10	106.2 s	
1'	122.7 s	
2',6'	105.3 d	7.02, s
3',5'	146.8 s	
4'	137.5 s	
OMe-5	56.7 q	3.75, s
OMe-7	56.0 q	3.73, s

Since some of the flavonoids exhibited anti-virus activities[13,14] 3',4',5'-trihydroxyl-5,7-dimethoxyl- isoflavonoid was tested for its potencies in preventing anti-tobacco mosaic virus activity using the half-leaf method[11].

The antiviral inhibition rates of the compound at the concentration of 20 μM tested by the half-leaf method. The results showed that the compounds exhibited inhibition activities against tobacco mosaic virus replication with inhibition rates of 92.8 %. The rates higher than that of the positive control, ningnanmycin (28.9 %).

N. glutinosa were pretreated with solutions of compounds or a solution of DMSO for 6.0 h before inoculation with tobacco mosaic virus. At the concentration of 20 μM, the compound showed potent protective effects to the host plants, with the inhibition rates ranging from 74.5-86.5 %. The results indicated that pretreatment with compound increase the resistance of the host plant to tobacco mosaic virus infection.

3',4',5'-trihydroxyl-5,7-dimethoxyl-isoflavonoid: $C_{17}H_{14}O_7$, orange-yellow gum; UV (MeOH), λ_{max} (log ε) 356 (3.87), 310 (3.67), 246 (4.18), 210 (4.99) nm; IR (KBr, ν_{max}, cm^{-1}) 3384, 1658, 1615, 1548, 1508, 1426, 1125, 1047, 956, 863; ^1H NMR and ^{13}C NMR data ($CDCl_3$, 500 MHz), Table-1; ESIMS *m/z* 329; HRESIMS (positive ion mode) m/z 329.0667 $[M-H]^-$ (calcd. 329.0661 for $C_{17}H_{13}O_7$).

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