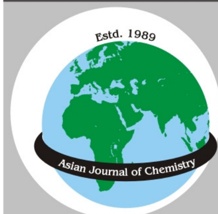

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Isolation of Isoaurone from the Tobacco Stem and its Anti-tobacco Mosaic Virus Activity

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A new isoaurone, 4,4'-dimethoxy-6-*O*-β-D-glucopyranosyl-isoaurone (**1**), were isolated from the tobacco stem. The structure of **1** was elucidated by spectroscopic methods including [1]D- and [2]D-NMR techniques. Compound **1** was also evaluated for its anti-tobacco mosaic virus (anti-TMV) activity and it showed high anti-tobacco mosaic virus activity with inhibition rate of 31.5 %. This valve is higher than that of positive control, ningnanmycin (28.4 %).

Key Words: 4,4'-Dimethoxy-6-*O*-β-D-glucopyranosyl-isoaurone, Tobacco stem, Anti-tobacco mosaic virus activity.

INTRODUCTION

Tobacco is one of the most important sources of income to farmers who live in over 100 countries and it has become one of the most commercially valued agricultural crops in the international markets[1,2]. In addition to its commercial value, tobacco leaf and stem also contain many useful chemical compounds, such as sesquiterpenes, diterpenoids, alkaloids, lignans, flavonoid, phenylpropanoids, chromanones, *etc.*[3-11]. The utilizations of these active compounds in tobacco leaf and its stem were received more and more attentions[12,13]. In order to investigate the components of the tobacco stem and search for potential leads for drug development, the phytochemical investigation on tobacco stem was carried out. This study led to the isolation of a new isoaurone, 4, 4'-dimethoxy-6-*O*-β-D-glucopyranosyl-isoaurone (**1**). Its structure (Fig. 1) was established by means of HRESIMS and extensive NMR spectra. Compound **1** was also evaluated for their anti-tobacco mosaic virus (anti-TMV) activity. It showed high anti-TMV activity with inhibition rate of 31.5 %, which is higher than that of positive control.

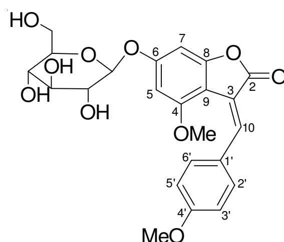

Fig. 1. Structures of compound **1**

EXPERIMENTAL

UV spectra were obtained using 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 [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). Preparative HPLC was used an Agilent 1100 HPLC equipped with ZORBAX-C_{18} (21.2 mm × 250 mm, 7.0 mm) column and DAD detector.

The stem of *Nicotiana tabacum* L. (tobacco stem) was collected from Dali County, Yunnan Province, P.R. China, in September 2010. The identification of the plant material was verified by Dr. Yuan. N, of Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (YNNU 10-9-20) has been deposited in our Laboratory.

Extraction and isolation: The air-dried and powdered tobacco stem (3.5 kg) were extracted four times with 90 % aq. ethanol (4 × 5.0 L) at room temperature and filtered. The crude extract (460 g) was applied to silica gel (200-300 mesh) column chromatography, eluting with a $CHCl_3$-$(CH_3)_2CO$ gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A-F. Further separation of fraction E (6:4, 18.6 g) by silica gel column chromatography and preparative HPLC (30 % methanol, flow rate 12 mL/min) to give **1** (32.6 mg).

4,4'-Dimethoxy-6-*O*-β-D-glucopyranosyl-isoaurone (1): Obtained as pale yellow gum; UV (MeOH) max (log ε)

210 (4.15), 260 (3.82), 385 (3.54) nm; IR (KBr, ν_{max}, cm^{-1}): 3422, 2905, 2846, 1687, 1620, 1542, 1433, 1358, 1132, 918, 843; ^1H NMR and ^{13}C NMR data (CD_3OD, 400 and 100 MHz), see Table-1; positive ESIMS *m/z* 483 $[M+H]^+$; negative HRESIMS m/z 483.1260 $[M+H]^+$ (calcd. for $C_{23}H_{24}O_{10}Na$, 483.1267).

TABLE-1
^1H AND ^{13}C NMR DATA OF COMPOUND **1** (CD_3OD)

No.	δ_C (mult.)	δ_H (mult, *J*, Hz)
2	169.9 s	
3	119.2 s	
4	158.9 s	
5	96.5 d	6.24 d, *J* = 1.8
6	159.8 s	
7	93.0 d	6.15 d, *J* = 1.8
8	155.0 s	
9	105.2 s	
10	142.2 d	7.80 s
1'	127.9 s	
2', 6'	135.9 d	8.10 d, *J* = 8.9
3', 5'	115.4 d	6.88 d, *J* = 8.9
4'	163.0 s	
OMe-4	56.1 q	3.88 s
OMe-4'	56.0 q	3.82 s
1"	103.5 d	5.30, d, *J* = 7.3
2"	75.8 d	3.44 m
3"	78.6 d	3.42 m
4"	71.2 d	3.29 m
5"	78.0 d	3.21 m
6"	62.5 t	3.50 m
		3.69 m

Anti-TMV Assays: TMV (U1 strain) was obtained from the Key Laboratory of Tobacco Chemistry of Yunnan Province, Yunnan Academy of Tobacco Science, P.R. China. The virus was multiplied in *Nicotiana tabacum cv*. K326 and purified as described in literature[14]. The concentration of TMV was determined as 20 mg/mL with an ultraviolet spectrophotometer [virus concentration = (A260 × dilution ratio)/ $E_{1cm}^{0.1\%,260\,nm}$]. The purified virus was kept at -20 ºC and was diluted to 32 μg/mL with 0.01 M PBS before use.

Nicotiana glutinosa plants were cultivated in an insect-free greenhouse. *N. glutinosa* was used as a local lesion host. The experiments could be conducted when the plants grow to the 5-6-leaf stage. The tested compounds were dissolved in DMSO and diluted with distilled H_2O to the required concentrations. The solution of equal concentration of DMSO was used as negative control. The commercial antiviral agent ningnanmycin was used as a positive control.

For half-leaf method[15], the virus was inhibited by mixing with the solution of compound. After 30 min, the mixture was inoculated on the left side of the leaves of *N. glutinosa*, whereas the right side of the leaves was inoculated with the mixture of DMSO solution and the virus as control. The local lesion numbers were recorded 3-4 days after inoculation. Three repetitions were conducted for each compound. The inhibition rates were calculated according to the formula:

$$\text{Inhibition rate (\%)} = [(C-T)/C] \times 100\ \%$$

where C is the average number of local lesions of the control and T is the average number of local lesions of the treatment.

RESULTS AND DISCUSSION

Compound **1** was obtained as a yellow solid. It gives a parent ion by HR-ESIMS at *m/z* 483.1260 $[M+H]^+$ (calcd. for 483.1267) corresponding to a molecular formula of $C_{23}H_{24}O_{10}$, requiring twelve degrees of unsaturation. The ^1H NMR spectrum of **1** showed the presence of an AA'BB' aromatic system at δ_H 8.10 (2H, d, *J* = 8.9 Hz, H-2',6') and 6.88 (2H, d, *J* = 8.8 Hz, H-3',5'), two *meta*-coupled aromatic protons at δ_H 6.15 (1H, d, *J* = 1.8 Hz, H-7) and 6.24 (1H, d, *J* = 1.8 Hz, H-5), an isolated olefinic proton at δ_H 7.80 (1H, s, H-10), two methoxy group proton (δ_H 3.88 and 3.82 s) and a glucosyl moiety [δ_H 5.30 (1H, d, *J* = 7.3, H-1"); δ_H 3.21-3.69 (6H, m, H-2", H-3", H-4", H-5", H-6")]. The ^{13}C NMR spectrum of **1** revealed the presence of a isoaurone nucleus[16] (δ_C 69.9 s, 119.2 s, 158.9 s, 96.5 d, 159.8 s, 93.0 d, 155.0 s, 105.2 s, 142.2 d, 127.9 s, 135.9 d (2C), 115.4 d (2C), 163.0 s), a glucosyl moiety (δ_C 103.5 d, 75.8 d, 78.6 d, 71.2 d, 78.0 d, 62.5 t) and a methoxy group (δ_C 56.0 and 56.1 q). The HMBC correlations of **1** (Fig. 2) showing the deshielded H-10 (δ_H 7.80) coupled to C-2 (δ_C 169.9) suggested that the structure of **1** could be a isoaurone

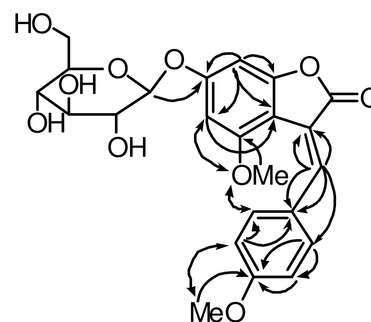

Fig. 2. Key HMBC (⌒↘) and NOESY (↗↘) correlations of **1**

too[17,18]. The AA'BB' spin system in the ^1H NMR spectrum indicated one substitutent should be attached to C-4' (δ_C 163.0). In the class of C6-C3-C6 compounds possessing substituents at C-5 and C-7, the chemical shift of C-6 (or C-5 in the case of **1**) appears at lower field than C-8 (or C-7 in the case of **1**)[16]; therefore, the signals at δ_C 96.5 and 93.0 were assigned to C-5 and C-7, respectively. The HSQC spectrum indicated that the *meta*-coupled aromatic protons at δ_H 6.24 and 6.15 associated with C-5 and C-7, respectively. The HMBC spectrum revealed the coupling from the methoxy group (δ_H 3.88, 3.82) to the carbon atom at δ_C 158.9 and δ_C 163.0, which further showed the NOESY correlation with H-5 (δ_H 6.24) and H-3' (δ_H 6.88), but not with H-7 (δ_H 6.15). This indicated that the methoxy group was located at C-4 and C-4'. The long-range correlations in the HMBC spectrum between H-1" (δ_H 5.30 d) and C-6 (δ_C 159.8 s) indicated the glucosyl was linked to C-6 and the coupling constant value of H-1" (*J* = 7.3 Hz) indicated that the glucosyl moiety was connected to the aglycone by a β-linkage[19,20]. The configuration of E- and Z-isoaurones is determined on the basis of the chemical shift of H-10, which is anisotropically and diamagnetically affected by the C-2 carbonyl group. It is known that the chemical shift of H-10 in Z-isoaurone resonates at higher field (7.4-7.5 ppm) than in the E-isomer (7.8-8.0 ppm)[18,21]. Therefore, the H-10 chemical shift at δ_H 7.80 suggested that **1** is E-isoaurone. This conclusion

was supported by the cross-peak between the methoxy group (δ_C 3.88) and H-2',6' (δ_H 8.10) in a NOESY experiment. Thus, compound **1** was established as 4,4'-dimethoxy-6-O-β-D-glucopyranosyl-isoaurone.

Since certain of the phenolic derivatives exhibit potential anti-virus activity[22-24], compound **1** was tested for their anti-TMV activity. The inhibitory activity of compound **1** against TMV replication were tested using the half-leaf method[15,25]. Ningnanmycin, a commercial product for plant disease in China, was used as a positive control. The antiviral inhibition rate of compound **1** at the concentration of 20 μM was tested for 31.5 %, which is higher than that of ningnanmycin (28.4 %).

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