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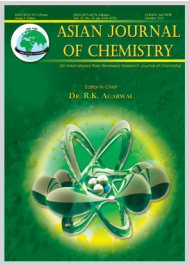

Norlignans from the Roots and Stems of *Nicotiana tabacum* and Their Antitobacco Mosaic Virus Activity

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A new norlignan, nicotnorlignan C (**1**), together with four known norlignans (**2-5**) were isolated from the roots and stems of *Nicotiana tabacum*. The structures of compounds **1-5** were elucidated on the basis of extensive NMR and MS means. The anti-tobacco mosaic virus activity of **1-5** was evaluated. All the compounds showed modest anti-tobacco mosaic virus activity.

Key Words: Norlignan, *Nicotiana tabacum*, Antitobacco mosaic virus activity.

INTRODUCTION

Nicotiana tabacum L. belongs to solanaceae family. It is one of the most commercially valued agricultural crops in the world[1,2]. In addition to used as raw material for cigarette industry, *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 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[11,12].

Motivated by search for bioactive metabolites from this plant and multipurpose utilization of the stems and roots of *N. tabacum*, an investigation on the chemical constituents of the roots and stems of *N. tabacum* was carried out. As a result, a new norlignan, together with four known norlignans was isolated. In addition, the anti-tobacco mosaic virus (Anti-TMV) activity of compounds **1-5** was evaluated. This article deals with the isolation, structural elucidation and biological activities of the compounds.

EXPERIMENTAL

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 ^{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 mm × 250 mm, 7.0 μm) column and DAD detector.

The roots and stems of *Nicotiana tabacum* L. was collected from Dali County, Yunnan Province, P.R. China, in September 2009.

Extraction and isolation: The air-dried and powdered roots and stems of *Nicotiana tabacum* (4.5 kg) were extracted with 70 % aqueous methanol (4.0 L × 3, 24 h each) at room temperature and the extract was concentrated under *vacuum* condition. The dried extract (53.8 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 and 2:1) to give six fractions A-F. Fraction B (8:2, 13.8 g) was subjected to silica gel column chromatography using $CHCl_3$-MeOH and preparative HPLC (40 % MeOH-H_2O, flow rate 12 mL/min) to give **1** (15.4 mg) and **5** (13.8 mg). Fraction C (7:3, 16.4 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 **2** (22.6 mg), **3** (228.7 mg) and **4** (17.6 mg).

Anti-TMV assays: The Anti-TMV activity was tested using the half-leaf method[13]. The inhibitory activities of the 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 compounds in the systemic infection host *N. tabacum cv*. K326. Ningnanmycin (20 μM), a commercial product for plant disease in China, was used as a positive control.

Nicotnorlignan C: Obtained as a pale yellow gum; $[\alpha]_D^{24.0}$ + 18.2 (c 0.22, MeOH); UV (MeOH), λ_{max} (log ε) 280 (2.86), 238 (3.16), 210 (4.41) nm; IR (KBr, ν_{max}, cm^{-1}): 3436, 2920, 1765, 1618, 1515, 1437, 1273, 1036, 950, 845, 759; ^1H NMR and ^{13}C NMR data (CD_3COCD_3, 500 MHz and 150 MHz, respectively), Table-1; ESIMS (positive ion mode) *m/z* 303 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 303.0840 $[M-H]^-$ (calcd. 303.0845 for $C_{14}H_{16}O_6Na$).

RESULTS AND DISCUSSION

A 70 % aq. ethanol extract prepared from the roots and stems of *Nicotiana tabacum* was subjected repeatedly to column chromatography on Si gel, Sephadex LH-20, RP-18 and Preparative HPLC to afford, nicotnorlignan C(**1**), recurphenol C(**2**)[14], recurphenol D(**3**)[14], sequirin C(**4**)[15] and benzodioxane (5)[16]. The structure of the compounds **1-5** was shown in Fig. 1 and the ^1H and ^{13}C NMR spectroscopic data of **1** were listed in Table-1.

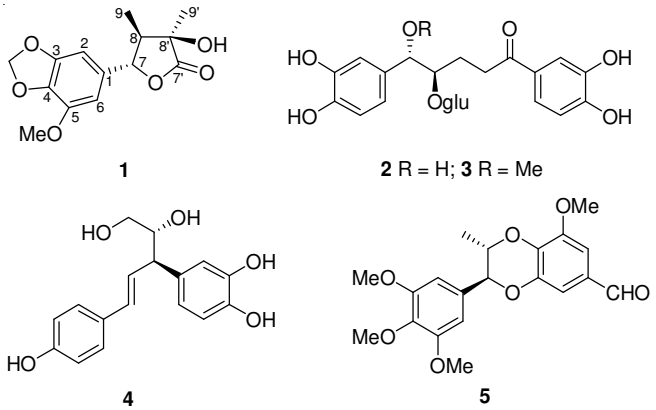

Fig. 1. Structure of norlignans from *N. tabacum*

TABLE-1
^1H NMR AND ^{13}C NMR DATA OF COMPOUND **1** IN CD_3COCD_3 (125 AND 500 MHz)

No.	Compound **1**	
	δ_C (mult.)	δ_H (mult, *J*, Hz)
1	130.5 s	
2	113.4 d	6.48, s
3	148.5 s	
4	138.4 s	
5	150.3 s	
6	122.7 d	6.62, s
7	84.7 d	5.12, d, *J* = 9.4
8	50.2 d	2.06 m
9	8.12 q	1.04, d, *J* = 6.5
7′	175.9 s	
8′	74.8 s	
9′	22.5 q	1.51 s
-OMe	55.8 q	3.82 s
$-OCH_2O-$	101.6 t	5.96, 5.98

Compound **1** was obtained as pale yellow gum. Its molecular formula was determined as $C_{14}H_{16}O_6$ by HR-ESI-MS *m/z* 303.0840 $[M + Na]^+$ (calcd. 303.0845). Its ^1H and ^{13}C NMR spectral data (Table-1) showed signals to one 1,3,4,5-tetrasubstituted aromatic rings (δ_H 6.48, 6.62), one methylenedioxy group (δ_C 101.6), one methoxyl group (δ_C 55.8), two methyl groups (δ_C 8.12, 22.5), one methine group (δ_C 50.2), one oxidated methine group (δ_C 84.7), one oxidated quaternary carbon (δ_C 74.8) and one carbonyl group (δ_C 175.9). Strong absorption bands accounting for hydroxyl (3436 cm^{-1}), carbonyl group (1765 cm^{-1}) and aromatic group (1618, 1515, 1437 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of **1** showed absorption maxima at 280, 238 nm also confirmed the existence of the aromatic function. On the basis of the molecular formula, one ring was needed to meet the required degrees of unsaturation. The ^1H and ^{13}C NMR spectra of **1** were very similar to these of (+)-(7S,8R,8'R)-4,8'-dihydroxy-3-methoxy-1',2',3',4',5',6'-hexanorligna-7',7-lactone[17]. The obvious differences are the substitutents on aromatic rings; a phenolic hydroxyl group replaced was by a methylenedioxy group. The HMBC correlations (Fig. 2) of methylenedioxy group proton signal (δ_H 5.96 s, 5.98 s) with C-3 (δ_C 148.5) and C-4 (δ_C 138.4) indicated that the methylenedioxy group should be located at C-3 and C-4; of methoxyl group proton signal (δ_H 3.82 s) with C-5 (δ_C 150.3) indicated that the methoxyl group should be located at C-5. The HMBC correlations observed from H-7 (δ_H 5.12) to C-1 (δ_C 130.5), C-2 (δ_C 113.4), C-6 (δ_C 122.7), C-7' (δ_C 175.9), C-8 (δ_C 50.2), C-8' (δ_C 74.8) and C-9 (δ_C 8.12); from H-8 (δ_H 2.06) to C-1 (δ_C 130.5), C-7' (δ_C 175.9), C-8' (δ_C 74.8), C-9 (δ_C 8.12), C-9' (δ_C 22.5); from CH_3-9 (δ_H 1.04) to C-7 (δ_C 84.7), C-8 (δ_C 50.2) and C-8' (δ_C 74.8); from CH_3-9' (δ_H 1.51) to C-7' (δ_C 175.9), C-8 (δ_C 50.2) and C-8' (δ_C 74.8) were also supporting the structure of compound **1**. The configurations of 7S, 8R, 8'R in **1** were deduced from the comparison of coupling constants and ROESY correlations (Fig. 3) with these of (+)-(7S,8R,8'R)-4,8'-dihydroxy-3-methoxy-1',2',3',4',5',6'-hexanorligna-7',7-lactone[17]. Thus, the structure of **1** was determined as shown and given the name as nicotnorlignan C.

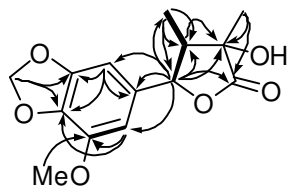

Fig. 2. Key HMBC and ^1H-^1H COSY correlations of **1**

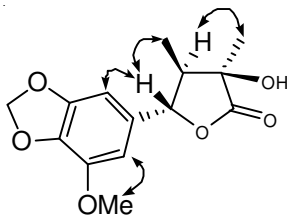

Fig. 3. Key ROESY ( ) correlations of **1**

Since some of the lignans exhibited anti virus activities[18,19], compounds **1-5** were tested for the Anti-TMV activity using the half-leaf method according to literature[13].

In Anti-TMV activity test, the antiviral inhibition rates of the compounds at the concentration of 20 μM were tested by

the half-leaf method. The results showed that the compounds **1-5** exhibited inhibition rates of 14.7, 22.5, 23.4, 17.6 and 21.4 %, respectively. The results showed that all compounds exhibited modest Anti-TMV activity. Its inhibition rate is close to that of positive control.

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