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LIGNAN DERIVATIVES FROM THE LEAVES *NICOTIANA TABACUM* AND THEIR ACTIVITIES

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Abstract – Two new lignan derivatives, nicotnorlignan A and nicotlactone A (**1**, **2**), together with two known lignan derivatives (**3**, **4**) were isolated from the leaves of *Nicotiana tabacum*. Their structures were elucidated by spectroscopic methods, including extensive 1D- and 2D-NMR techniques. Compounds **1-4** were tested for their anti-HIV-1 activity and anti-tobacco mosaic virus activities. The results showed compound **2** has high anti-tobacco mosaic virus activities, and all other compounds have modest anti-HIV-1 activity and anti-tobacco mosaic virus activities.

Nicotiana tabacum L. is one of the most commercially valued agricultural crops in the world.[1,2] Its leaves are the most important row material for cigarette industry. However, *N. tabacum* is also used as insecticides, anesthetics, diaphoretics, sedatives, and emetic agents in Chinese folklore medicine due to its containing many useful chemical compounds.[1,3-5] Therefore, the multipurpose utilization of *N. tabacum* is an interesting topical, and receives more and more attentions.[6-8]

In previous work, a number of bioactive compounds, such as alkaloids,[8-10] sesquiterpenes,[11,12] diterpenoids,[13-15] phenols,[16,17] and their homologous, were isolated from the *N. tabacum*. With the aim of continuing efforts to multipurpose utilization of *N. tabacum* and identify bioactive natural products from this plants, the phytochemical investigation on *N. tabacum* was carried out. As a result, four lignan derivatives (with two new one) were isolated from this plant. All of the compounds were evaluated in

anti-HIV-1 activity and anti-tobacco mosaic virus (Anti-TMV) activity, and the results are described herein.

A 95% aq. methanol extract prepared from the leaves of *N. tabacum* was subjected repeatedly to column chromatography and preparative HPLC to afford compounds **1-4**, including two new lignan derivatives, named nicotnorlignan A and nicotlacone A (**1-2**), together with two known compounds, sequirin C (**3**),[18] and benzodioxane (**4**).[19] The structures of **1-4** were shown in **Figure 1**, and their ^{1}H and ^{13}C-NMR spectroscopic data of **1-2** were listed in **Table 1**.

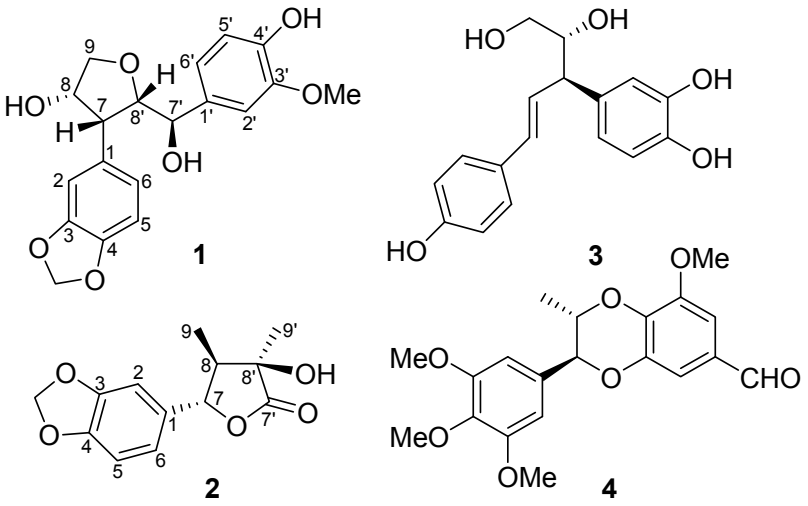

Figure 1. The structure of lignan derivatives from *N. tabacum*.

Compound **1** was obtained as pale yellow gum. Its molecular formula was determined as $C_{19}H_{20}O_7$ by HR-ESI-MS m/z 359.1138 $[M-H]^-$ (calcd 359.1131). Its ^{1}H and ^{13}C NMR spectral data (**Table 1**) showed signals to two 1,3,4-trisubstituted aromatic rings (δ_H 6.41, 6.65, 6.52, and δ_H 7.02, 6.76, 6.86), one methoxyl group (δ_C 55.9), one methylenedioxy group (δ_C 101.4), one oxidated methylene group (δ_C 76.2), one methine group (δ_C 53.5), and three oxidated methine groups (δ_C 74.5, 79.5, 91.2). Strong absorption bands accounting for hydroxyl (3432 cm^{-1}) and aromatic group (1615, 1515, 1458 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of **1** showed absorption maxima at 280, 230 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. These evidences suggested **1** was structurally similar to metasequirin E.[20] Comparison of their ^{1}H and ^{13}C NMR indicated the differences could be rationalized to the substituted patterns on aromatic rings. The HMBC correlations (**Figure 2**) of methylenedioxy group proton signal (δ_H 5.97, 6.00) with C-3 (δ_C 145.7) and C-4 (δ_C 145.0) indicated that the methylenedioxy group should be located at C-3 and C-4; the correlation of methoxyl proton signal (δ_H 3.79) with C-3′ (δ_C 148.1) indicated that the methoxyl group should be located at C-3′. The HMBC correlations observed from H-9α (δ_H 4.04) and H-9β (δ_H 3.83) to C-8′ (δ_C 91.2), C-8 (δ_C 79.5) and C-7

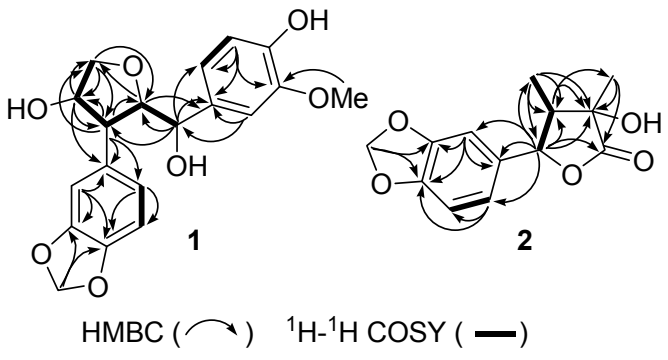

Figure 2. The Key HMBC and ^{1}H-^{1}H COSY correlations of **1** and **2**

(δ_C 53.5); from H-7 (δ_H 3.24) to C-1 (δ_C 134.6), C-6 (δ_C 119.7), C-7′ (δ_C 74.5), C-8 (δ_C 79.5), C-8′ (δ_C 91.2), and C-9 (δ_C 76.2); from H-7′ (δ_H 4.88) to C-1′ (δ_C134.0), C-6′ (δ_C 119.7), C-7 (δ_C 53.5), and C-8′ (δ_C 91.2); from H-8′ (δ_H 4.17) to C-1′ (δ_C 134.0); from H-8 (δ_H 4.23) to C-9 (δ_C 76.2), C-7 (δ_C 53.5) and C-1 (δ_C 134.6) were also supporting the structure of compound **1**. The configurations of 7*R*, 8*S*, 7′*R*, 8′*S* in **1** were deduced from the comparison of coupling constants and ROESY correlations (**Figure 3**) with these of metasequirin E, of which absolute configuration was unambiguously established by mosher method and ROESY experiments.[20] The ^{1}H and ^{13}C NMR spectral data of C-7′ (δ_H 4.88; δ_C 74.5) and C-8 (δ_H 4.23; δ_C 79.5) (**Table 1**) were almost identical to those of metasequirin E [C-7′ (δ_H 4.88; δ_C 74.8) and C-8 (δ_H 4.23; δ_C 79.2)].[20] The ROESY correlations of H-7/H-8′, H-7/H-8, H-8/H-9a, and H-6/H-9b (**Figure 3**) as well as the small coupling constants of H-7 (J = 2.9, 5.2 Hz) indicated H-7, H-8, and H-8′ were on one side.[20] Thus, the structure of **1** was determined as shown and given the name as nicotnorlignan A.

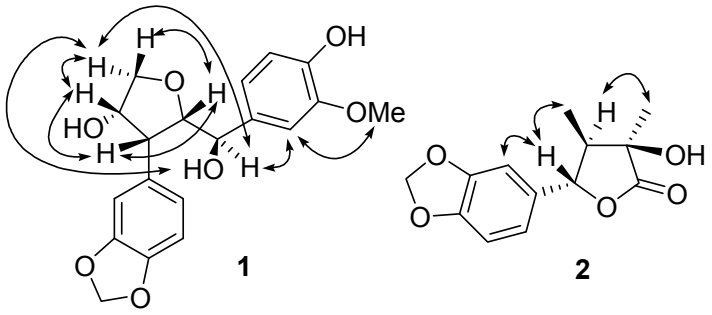

Figure 3. The Key ROESY (↷) correlations of **1** and **2**

Compound **2** was obtained as pale yellow gum. Its molecular formula was determined as $C_{13}H_{14}O_5$ by HR-ESI-MS m/z 249.0756 $[M-H]^-$ (calcd 249.0763). Its ^{1}H and ^{13}C NMR spectral data (**Table 1**) showed signals to one 1,3,4-trisubstituted aromatic rings (δ_H 6.73, 6.84, 6.61), one methylenedioxy group (δ_C 101.1), two methyl groups (δ_C 7.9, 22.3), one methine group (δ_C 49.9), one oxidated methine group (δ_C 85.6), one oxidated quaternary carbon (δ_C 74.1), and one carbonyl group (δ_C 176.3). Strong absorption bands accounting for hydroxyl (3435 cm^{-1}), carbonyl group (1762 cm^{-1}) and aromatic group (1614, 1512, 1436 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of **1** showed absorption maxima at 282, 235 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 ^{1}H and ^{13}C NMR spectra of **2** were very similar to these of (+)-(7*S*,8*R*,8′*R*)-4,8′-dihydroxy-3-methoxy-1′,2′,3′,4′,5′,6′-hexanorligna-7′,7-lactone.[21] The obvious differences are the substitutents on aromatic ring**.** The phenolic hydroxyl group and methoxyl group were replaced by a methylenedioxy group in **2**. The HMBC correlations (**Figure 2**) of methylenedioxy group proton signal (δ_H 5.97 s, 5.99 s) with C-3 (δ_C 145.8) and C-4 (δ_C 145.0) indicated that the methylenedioxy group should be located at C-3 and C-4. The HMBC correlations observed from H-7 (δ_H 5.10) to C-1 (δ_C 128.4), C-2 (δ_C 107.5), C-6 (δ_C 119.6), C-7′ (δ_C 176.3), C-8 (δ_C 49.9), C-8′ (δ_C 74.1), and C-9 (δ_C 7.91); from H-8 (δ_H 2.01) to C-8′ (δ_C 74.1), C-9′ (δ_C 22.3); from CH_3-9 (δ_H 1.06) to C-7 (δ_C 85.6), C-8 (δ_C 49.9),

and C-8′ (δ_C 74.1); from CH_3-9′ (δ_H 1.49) to C-7′ (δ_C 176.3), and C-8′ (δ_C 74.1) were also supporting the structure of compound **2**. The ROESY correlations of H-7/H-2, H-7/H_3-9, H-8/H_3-9′ (**Figure 3**) suggested the configurations of 7*S*, 8*R*, 8′*R* in **2**. The configurations of 7*S*, 8*R*, 8′*R* were further confirmed by the comparison of NMR spectral data and ROESY correlations with these of (+)-(7*S*,8*R*,8′*R*)-4,8′-dihydroxy-3-methoxy-1′,2′,3′,4′,5′,6′-hexanorligna-7′,7-lactone.[21] Thus, the structure of **2** was determined as shown and given the name as nicotlacone A.

Table 1. ^{1}H NMR and ^{13}C NMR data of compounds **1** - **2** in CD_3COCD_3 (125 and 500 MHz)

No.	Compound **1**		Compound **2**	
	δ_C (mult.)	δ_H (mult, *J*, Hz)	δ_C (mult.)	δ_H (mult, *J*, Hz)
1	134.6 s		128.4 s	
2	111.7 d	6.41, d, *J* = 1.8	107.5 d	6.73, d, *J* = 1.8
3	145.7 s		145.8 s	
4	145.0 s		145.0 s	
5	114.2 d	6.65, d, *J* = 8.2	113.3 d	6.84, d, *J* = 8.0
6	119.7 d	6.52, dd, *J* = 1.8, 8.2	119.6 d	6.61, dd, *J* = 1.8, 8.0
7	53.5 d	3.24, dd, *J* = 2.9, 5.2	85.6 d	5.10, d, *J* = 9.4
8	79.5 d	4.23 brs	49.9 d	2.01 m
9α	76.2 t	4.04, dd, *J* = 4.2, 9.2	7.9 q	1.06, d, *J* = 6.6
9β		3.83, dd, *J* = 2.8, 9.2		
1′	134.0 s			
2′	110.7 d	7.02, d, *J* = 1.8		
3′	148.1 s			
4′	146.3 s			
5′	115.3 d	6.76, d, *J* = 8.2		
6′	119.9 d	6.86, dd, *J* = 1.8, 8.2		
7′	74.5 d	4.88, brs	176.3 s	
8′	91.2 d	4.17, dd, *J* = 3.2, 4.8	74.1 s	
9′			22.3 q	1.49 s
-OMe	55.9 q	3.79, s		
-OCH_2O-	101.4 t	5.97, 6.00, s	101.1 t	5.97, 5.99

Since some of the lignans exhibited anti virus activities,[22,23] compounds **1-4** were tested for the Anti-TMV activity using the half-leaf method,[24] and anti-HIV activity according to literature.[25]

In Anti-TMV activity test, the anti-viral inhibition rates of the compounds at the concentration of 20 μM were tested by the half-leaf method. The results showed that the compounds **1-4** exhibited inhibition rates of 15.2, 58.4, 22.6, and 16.1%, respectively. The results showed that compound **2** exhibited high Anti-TMV activity; its inhibition rate is higher than that of a positive control. Other compounds also have modest Anti-TMV activity.

In anti-HIV-1 activity test, the cytotoxicity assay against C8166 cells (CC_{50}), and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}), using azidothymidine (AZT) as a positive control (EC_{50} = 0.034 μg/mL and CC_{50} > 200 μg/mL).[25] Compounds **1-4** showed modest anti-HIV-1 activities with EC_{50} values of 3.16, 1.28, 9.56, and 7.62 μg/mL, respectively, and the all exerted minimal cytotoxicity against C8166 cells (CC_{50} > 200 μg /mL). The therapeutic index (TI) values (CC_{50}/EC_{50}) of **1-4** were more than 63.3, 109.9, 20.9, and 26.2, respectively.

EXPERIMENTAL

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

Plant material. The leaves of *Nicotiana tabacum* L (tobacco leaves) was collected from Yuxi County, Yunnan Province, P. R. China, in September 2009.

Extraction and isolation. The air-dried and powdered leaves of *Nicotiana tabacum* (4.5 kg) were extracted with 95% aqueous MeOH (4.0 L × 3, 24 h each) at room temperature and the extract was concentrated under vacuum condition. The dried extract (96.7 g) was applied to Si gel (200–300 mesh) column chromatography (10 × 100 cm column, with Si gel 2.8 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 (8.0 L of eluant was used for each fractions). Fraction B (5:5, 12.5 g) was subjected to silica gel column chromatography using $CHCl_3$–MeOH and preparative HPLC (30% – 45% MeOH-H_2O, flow rate 12 mL/min) to give **1** (11.2 mg), **2** (14.8 mg), **3** (13.5 mg), and **4** (22.1 mg).

Anti-TMV Assays. The Anti TMV activity were tested using the half-leaf method.[24] 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 (20 μM), a commercial product for plant disease in China, was used as a positive control.

Anti-HIV-1 Assays. The cytotoxicity assay against C8166 cells (CC_{50}) was assessed using the MTT method and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1

(EC_{50}).[25]

Nicotnorlignan A (1). Obtained as a pale yellow gum; $[\alpha]_D^{24.8}$ -25.6 (*c* 0.25, MeOH); UV (MeOH), λ_{max} (log ε) 280 (3.62), 230 (3.85), 205 (4.46) nm; IR (KBr) ν_{max} 3432, 1615, 1515, 1458, 1271, 1034, 958, 862 cm^{-1}; ^1H and ^{13}C NMR data (CD_3COCD_3, 500 and 150 MHz, respectively), **Table 1**; ESIMS (negative ion mode) *m/z* 359 $[M-H]^-$; HRESIMS (negative ion mode) *m/z* 359.1138 $[M-H]^-$ (calcd 359.1131 for $C_{19}H_{19}O_7$).

Nicotlacone A (2). Obtained as a pale yellow gum; $[\alpha]_D^{24.0}$ +15.8 (*c* 0.25, MeOH); UV (MeOH), λ_{max} (log ε) 282 (2.22), 235 (3.54), 205 (4.35) nm; IR (KBr) ν_{max} 3435, 2923, 1762, 1614, 1512, 1436, 1270, 1032, 953, 847, 756 cm^{-1}; ^1H and ^{13}C NMR data (CD_3COCD_3, 500 and 150 MHz, respectively), **Table 1**; ESIMS (negative ion mode) *m/z* 249 $[M-H]^-$; HRESIMS (negative ion mode) *m/z* 249.0756 $[M-H]^-$ (calcd 249.0763 for $C_{13}H_{13}O_5$).

Sequirin C (3). ^{13}C NMR data (CD_3COCD_3, 150 MHz). δ: 134.2 (s, C-1), 122.8 (d, C-2), 146.5 (s, C-3), 145.2 (s, C-4), 114.2 (d, C-5), 118.8 (d, C-6), 54.7 (d, C-7), 75.6 (d, C-8), 65.2 (t, C-9), 131.2 (s, C-1′), 127.8 (d, C-2′), 117.0 (d, C-3′), 155.9 (s, C-4′), 117.0 (d, C-5′), 127.8 (d, C-6′), 131.6 (d, C-7′), 128.5 (d, C-8′).

Benzodioxane (4). ^{13}C NMR data (CD_3COCD_3, 150 MHz). δ: 139.8 (s, C-1), 151.6 (s, C-2), 107.5 (d, C-3), 130.8 (s, C-4), 108.7 (d, C-5), 146.9 (s, C-6), 192.6 (d, C-7), 132.4 (s, C-1′), 103.8 (d, C-2′), 151.6 (s, C-3′), 138.2 (s, C-4′), 151.6 (d, C-5′), 103.8 (d, C-6′), 81.5 (d, C-7′), 73.6 (d, C-8′), 16.8 (q, C-9′), 55.8, 55.9, 55.8, 60.7 (q, 4 × -OMe).

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