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TWO NEW ISOINDOLIN-1-ONES FROM THE LEAVES OF *NICOTIANA TABACUM* AND THEIR ANTI-TOBACCO MOSAIC VIRUS ACTIVITIES

Guang-Hui Kong,[1] Yu-Ping Wu,[1,2] Wei Li,[1] Zhen-Yuan Xia,[1,*] Qiang Liu,[2] Kun-Miao Wang,[2] Pei He,[2] Rui-Zhi Zhu,[2] Xiao-Xi Si,[2] and Guang-Yu Yang [2,*]

[1]Yunnan Academy of Tobacco Agricultural Sciences, Kunming, Yunnan 650031, P.R China; [2]Key Laboratory of Tobacco Chemistry of Yunnan Province, China tobacco yunnan industrial Co., Ltd., Kunming 650231, P.R. China; E-mail: xiazhenyuan@ynst.cn; ygy1110@163.com

Abstract – Two new isoindolin-1-ones, 2-(2-hydroxyethyl)-5-methyl-6-(3-methylbut-2-enyl)isoindolin-1-one (**1**) and 2,5-dimethyl-6-(3-methylbut-2-enyl)-isoindolin-1-one (**2**), were isolated from the leaves of *Nicotiana tabacum*. Their structures were determined by means of HRESIMS and extensive 1D and 2D NMR spectroscopic studies. Compounds **1** and **2** were tested for their anti-tobacco mosaic virus (anti-TMV) activities. The results revealed that compounds **1** and **2** showed potential anti-TMV activities with inhibition rates of 48.2 and 45.6%, respectively.

Nicotiana tabacum, tobacco, is a stout herbaceous plant in the Solanaceae (nightshade family) and cultivated worldwide as the primary commercial source of tobacco, which is smoked or chewed as a drug for its mild stimulant effects.[1,2] *N. tabacum* is also a kind of plant containing most complex secondary metabolites in nature, of which more than 2549 kinds of chemical compositions has been identified according to Dube and Green's reports[3] in 1982 while Perfetti and Rodgman reported[4] that compounds found in tobacco, tobacco substitutes and cigarette smoke were up to 8700 kinds totally by 2008. In previous literatures, *N. tabacum* is used as insecticides, anesthetics, diaphoretics, sedatives, and emetic agents in Chinese folklore medicines because of its containing many useful chemical compounds.[1,4-6] Previous phytochemical studies of tobacco have shown the presence of sesquiterpenes,[6-8] alkaloids,[9,10] lignans,[11,12] flavonoids,[13-15] phenylpropanoids,[16,17] chromanones,[18,19] biphenyls,[20] phenolic amides,[21] isocoumarins,[22] and the homologous.

In continuing efforts to utilize *N. tabacum* and identify bioactive natural products, the phytochemistry investigation of the leaves of Yunyan 201 (a variety of *N. tabacum*) led to the isolation of two new

isoindolin-1-ones (**1** and **2**). This paper deals with the isolation, structural elucidation, and their anti-TMV activities of these compounds.

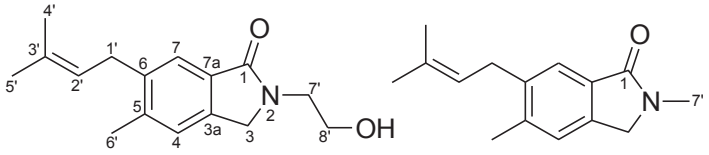

Figure 1. Isoindolin-1-ones from the leaves of *Nicotiana tabacum*

A 70% aq. acetone extract prepared from the leaves of tobacco was subjected repeatedly to column chromatography on silica gel and preparative HPLC to afford two new isoindolin-1-ones, 2-(2-hydroxyethyl)-5-methyl-6-(3-methylbut-2-enyl)isoindolin-1-one (**1**) and 2,5-dimethyl-6-(3-methylbut-2-enyl)isoindolin-1-one (**2**). The structures of the compounds **1** and **2** were shown in Figure 1, and the ^{1}H and ^{13}C NMR data of **1** and **2** were listed in Table 1.

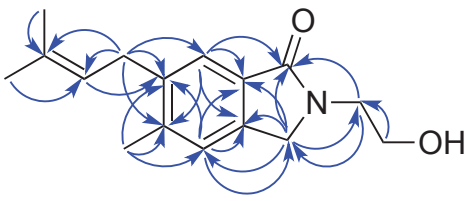

Figure 2. Key HMBC (↷) correlations of **1**

Compound **1** was isolated as a yellow gum. The molecular formula of **1** was determined to be $C_{16}H_{21}NO_2$ by the pseudomolecular ion peak at *m/z* 260.1658 $[M+H]^+$ in its HRESIMS, suggesting 7 degrees of unsaturation. The UV spectrum showed absorption maxima at 210, 260 and 298 nm, and the IR spectrum showed absorption bands at 3312, 2930, 1665, 1610, 1547, 1460 cm^{-1}, indicating the presence of hydroxy group, carbonyl group, and aromatic ring. The ^{1}H, ^{13}C NMR data (Table-1), and HSQC correlations of **1** showed resonances due to a isoindolin-1-one nucleus[23] (C-1 to C-7a; H_2-3, H-4, and H-7), a prenyl group[22] (C-1' to C-5'; H_2-1', H-2', H_3-4', and H_3-5'), a 2-hydroxyethyl group[24] (C-7' and C-8'; H_2-7' and H_2-8'), and a methyl group (C-6' and H_3-6'). The HMBC correlations (Figure 2) of H_2-3 with C-1, C-3a, C-4, C-7a, and C-7', of H-4 with C-3, of H-7 with C-1, and of H_2-7' with C-1 and C-3 also suggested that compound **1** should be an isoindolin-1-one. The HMBC correlations of H_2-1′ (δ_H 3.36) with C-5 (δ_C 138.0), C-6 (δ_C 136.5), and C-7 (δ_C 126.5), and of H-2′ (δ_H 5.35) with C-6 (δ_C 136.5) indicated that the prenyl group was attached to C-6. The location of the methyl group was assigned to C-5 position on the basis of HMBC correlations of the methyl proton signal (δ_H 1.72) with C-4 (δ_C 128.2), C-5 (δ_C 138.0) and C-6 (δ_C 136.5). Finaly, the 2-hydroxyethyl group linked to nitrogen-atom (N-2) was confirmed by the HMBC correlation of H-7′ (δ_H 3.59) with C-1 (δ_C 166.5) and C-3 (δ_C 42.3). Thus, the structure of **1** was established as 2-(2-hydroxyethyl)-5-methyl-6-(3-methylbut-2-enyl)isoindolin-1-one.

Compound **2** was also obtained as yellow gum. A molecular formula $C_{15}H_{19}NO$ was assigned from HRESIMS (*m/z*: 230.1540 $[M+H]^+$, calcd 230.1545). The ^{1}H and ^{13}C NMR data of **2** (Table 1) displayed 15 carbon and 19 proton signals, corresponding to a isoindolin-1-one nucleus[24] (C-1 to C-7a; H_2-3, H-4,

and H-7), a prenyl group (C-1' to C-5'; H_2-1', H-2', H_3-4', and H_3-5'), a methyl group linked to aromatic ring (C-6 position), and a methyl group linked to nitrogen-atom (N-2 position). The ^{1}H and ^{13}C NMR spectral data of **2** were similar to those of **1**. The obvious chemical shift differences resulted from the substituent group variation on the nitrogen-atom. The appearance of one methyl signals and the disappearance of a 2-hydroxyethyl signal were observed in compound **2**. These changes indicated that the 2-hydroxyethyl group in **1** was replaced by a methyl group in **2**. The detailed structures of **2** were also confirmed by further analysis of its HMBC correlations. Accordingly, the structure of 2,5-dimethyl-6-(3-methylbut- 2-enyl) isoindolin-1-one (**2**) was established.

Table 1. ^{1}H and ^{13}C NMR Data of compounds **1** and **2** ($CDCl_3$, δ, ppm, J/Hz)

No.	Compound **1**		Compound **2**	
	δ_C	δ_H (m, J, Hz)	δ_C	δ_H (m, J, Hz)
1	166.5 s		167.8 s	
3	42.3 t	4.25 s	44.9 t	4.23 s
3a	135.5 s		135.8 s	
4	128.2 d	6.75 s	127.0 d	6.78 s
5	138.0 s		137.5 s	
6	136.5 s		136.4 s	
7	126.5 d	7.43 s	126.3 d	7.50 s
7a	128.5 s		128.6 s	
1′	28.2 t	3.36 (d) 6.9	28.6 t	3.35 (d) 6.9
2′	123.2 d	5.35 (t) 6.9	123.5 d	5.36 (t) 6.9
3′	133.6 s		133.3 s	
4′	16.3 q	1.57 s	16.5 q	1.55 s
5′	25.8 q	1.77 s	25.5 q	1.78 s
6′	18.8 q	1.72 s	16.9 q	1.75 s
7′	46.3 t	3.59 (t) 5.6	33.3 q	4.23 s
8′	59.2 t	3.82 (t) 5.6		

Compounds **1** and **2** were tested for their anti-TMV activities. The anti-TMV activities were tested by half-leaf method, using ningnanmycin (a commercial product for plant disease in China, with inhibition rate of 31.6%) as a positive control.[25,26] The results revealed that compounds **1** and **2** showed high anti-TMV activities with inhibition rates of 48.2 and 45.6% at the concentration of 20 μM, respectively.

EXPERIMENTAL

General. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27

spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on DRX-500 spectrometers with TMS as internal standard, and the chemical shifts (δ) were expressed in ppm. HRESIMS was performed on an API QSTAR time-of-flight spectrometer and a VG Autospec-3000 spectrometer, respectively. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a ZORBAX PrepHT GF (21.2 mm × 25 cm, 7 μm) column or a Venusil MP C_{18} (20 mm × 25 cm, 5 μm) column. Column chromatography was performed with Si gel (200–300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China). The fractions were monitored by TLC, and spots were visualized by heating Si gel plates sprayed with 5% H_2SO_4 in EtOH.

Plant material. The leaves of *N. tabacum L* (tobacco leaves) was collected from Yuxi County, Yunnan Province, P.R. China, in September 2014. The tobacco variety is Yunyan-201, which had widely cultivated in China. The identification of the plant material was verified by Prof. H. W. Yang (School of Tobacco, Yunnan Agriculture University).

Extraction and Isolation. The air-dried and powdered tobacco leaves (6.5 kg) were extracted three times with 70% aqueous acetone (3 × 8.0 L) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure to obtain a crude extract (426 g). This crude extract was applied to Si gel (200-300 mesh) column chromatography eluting with a $CHCl_3$-MeOH gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5) to give six fractions A–F. The separation of fraction C (8:2, 20.5 g) was subjected to Si gel column chromatography eluting with $CHCl_3$-$(Me)_2CO$ and then run on preparative HPLC (40% MeOH-H_2O, flow rate 12 mL/min) to yield compounds **1** (15.2 mg) and **2** (10.8 mg).

2-(2-Hydroxyethyl)-5-methyl-6-(3-methylbut-2-enyl)isoindolin-1-one (**1**): Obtained as yellow gum; UV (MeOH) λ_{max} nm (log ε) 210 (4.32), 260 (3.86), and 298 (3.05); IR (KBr) ν_{max} 3312, 2930, 1665, 1610, 1547, 1460, 1354, 1213, 1152, 1068, 838, and 746 cm^{-1}; positive ESIMS *m/z* 260 $[M+H]^+$, positive HRESIMS *m/z* 260.1658 (calcd for $C_{16}H_{22}NO_2$, 260.1651).

2,5-Dimethyl-6-(3-methylbut-2-enyl)isoindolin-1-one (**2**): Obtained as yellow gum; UV (MeOH) λ_{max} nm (log ε) 210 (4.18), 258 (3.80), and 295 (3.11); IR (KBr) ν_{max} 2935, 1672, 1612, 1536, 1465, 1357, 1215, 1168, 1060, 826, and 740 cm^{-1}; positive ESIMS *m/z* 230 $[M+H]^+$, positive HRESIMS *m/z* 230.1540 (calcd for $C_{15}H_{20}NO$, 230.1545).

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