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Original article

Sesquiterpenes from the leaves of *Nicotiana tabacum* and their anti-tobacco mosaic virus activity

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ABSTRACT

In searching for more bioactive compounds, phytochemical investigations on the acetone extract of the leaves of *Nicotiana tabacum* resulted in the isolation of two new sesquiterpenes, nicosesquiterpene A and B (**1** and **2**), along with four known sesquiterpene derivatives (**3–6**). Structural elucidation of **1** and **2** was performed by spectral methods, such as HRMS, IR, UV, 1D and 2D NMR spectroscopy. Compounds **1** and **2** are the first naturally occurring pterosin-type sesquiterpene bearing an isopropyl moiety. Compounds **1–6** were also evaluated for their anti-tobacco mosaic virus (anti-TMV) activity. The results showed that compounds **1** and **2** exhibited high anti-TMV activity with inhibition rates of 36.7% and 45.6%, respectively, which is higher than that of positive control. The other compounds also showed potential activity with inhibition rates in the range of 22.7%–29.2%.

1. Introduction

Nicotiana tabacum tobacco is a stout herbaceous plant, and it is cultivated worldwide as the primary commercial source of tobacco [1,2]. In addition, *N. tabacum* is also used as insecticide, anesthetic, diaphoretic, sedative, and emetic agents in Chinese folklore medicine because it contains many useful chemical compounds [2–4]. Previous phytochemical studies on this plants have shown the presence of sesquiterpene [5,6], alkaloids [7,8], lignans [9,10], flavonoids [11–13], phenylpropanoids [14,15], chromanones [16,17], biphenyls [18], isocoumarins [19], and the homologous compounds. Therefore, the multipurpose utilization of this plant is an interesting topic and attracts more and more attention. Motivated by the possibility of the existence of more bioactive metabolites from this plant, an investigation on the chemical constituents of the tobacco leaves of K-326 (a variety of *N. tabacum*) was carried out. As a result, two new (**1** and **2**) and three known (**3–6**) sesquiterpenes were isolated from this plant. Compounds **1** and **2** are the first naturally occurring pterosin-type sesquiterpene bearing an isopropyl moiety. In addition, the anti-tobacco mosaic virus (anti-TMV) activity of compounds **1–6** was evaluated. This article describes the isolation, structural elucidation and the anti-TMV activity of these sesquiterpenes.

2. Experimental

Optical rotations were measured with a Horiba SEPA-300 polarimeter; UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. ECD spectra were measured on a JASCO J-810 spectropolarimeter. A Tenor 27 spectrophotometer was used for scanning IR spectra with KBr pellets. 1D and 2D NMR spectra were recorded on a DRX-500 NMR spectrometer using Trimethylsilane (TMS) as an internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. HRESIMS was performed on a VG Autospec-3000 spectrometer. Semipreparative HPLCwas performed on a Shimadzu LC-8A preparative liquid chromatography machine using Zorbax PrepHT GF (21.2 mm × 25 cm) or Venusil MP C_{18} (20 mm × 25 cm) columns. Column chromatography (CC) was performed using silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., China), Lichroprep RP-18 gel (40–63 μm, Merck, Germany), and MCI gel (75–150 μm, Mitsubishi Chemical Co., Japan). Fractions were monitored by TLC analysis, and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4 in EtOH.

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2.1. Plant material

The leaves of *N. tabacum* L (tobacco leaves) were collected from Honghe County, Yunnan Province, China, in September 2013. The tobacco is K326, which is widely cultivated in China. The identification of the plant material was verified by Prof. H. W. Yang (School of Tobacco, Yunnan Agriculture University).

2.2. Extraction and isolation

The air-dried and powdered leaves of *N. tabacum* (4.8 kg) were extracted with 70% aqueous acetone (3 h × 8 L × 4) at room temperature and filtered. The extract (242.5 g) was applied to silica gel (200–300 mesh) column chromatography, eluted with a $CHCl_3$–CH_3OH gradient system (10:0, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A–F. Further separation of fraction B (9:1, 28.4 g) by silica gel column chromatography, eluting with $CHCl_3$ –$(CH_3)_2CO$ (9:1–2:1), yielded mixtures B1–B6. Fraction B2 (8:2, 4.86 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semi-preparative HPLC (50–56% MeOH–H_2O, flow rate 12 mL/min) to give **1** (12.4 mg), **2** (10.8 mg), **3** (15.2 mg), **4** (11.7 mg), **5** (14.6 mg) and **6** (16.4 mg).

Compounds **1** and **2** were identified as new compounds. The known compounds, by comparing with the published literature, were identified as glutinosone (**3**) [20], capsidiol (**4**) [21], 1-β-hydroxy-α-cyperone (**5**) [22], and arundinol B (**6**) [23]. The structures of the compounds **1**–**6** were shown in Fig. 1, and the ^{1}H NMR and ^{13}C NMR data of **1** and **2** were listed in Table 1.

Nicosesquiterpene A (**1**): Pale yellow gum; obtained as light yellow gum, $[\alpha]_D^{25.8}$ + 14.8 (*c* 0.20, MeOH); UV (MeOH) λmax (log ε): 285 (3.20), 248 (3.68), 210 (4.18) nm; CD (MeOH, c 0.020) λ_{max} ($\Delta\varepsilon$) 326.8 (+18.2) nm; IR (KBr, cm^{-1}): ν_{max} 3368, 1685, 1600, 1536, 1472, 1358, 1203, 1070, 891, 562; ^{13}C NMR (125 MHz, $CDCl_3$, Fig. S1 in Supporting information) and ^{1}H NMR (500 MHz, $CDCl_3$, Fig. S2 in Supporting information) data, see Table 1; negative ESIMS *m/z* 231 $[M-H]^-$; negative HRESIMS *m/z* 231.1379 $[M-H]^-$ (calcd. for $C_{15}H_{19}O_2$ $[M-H]^-$, 231.1385) (Fig. S5 in Supporting information).

Nicosesquiterpene B (**2**): Light yellow gum, $[\alpha]_D^{25.2}$ + 16.5 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε): 283 (3.25), 248 (3.59), 210 (4.07) nm; CD (MeOH, c 0.020) λ_{max} ($\Delta\varepsilon$) 327.6 (+19.6) nm; IR (KBr) ν_{max} 3360, 1683, 1600, 1542, 1465, 1347, 1215, 1058, 890, 575 cm^{-1}; ^{13}C NMR (125 MHz, $CDCl_3$, Fig. S7 in Supporting information) and ^{1}H NMR (500 MHz, $CDCl_3$, Fig. S8 in Supporting information) data, see Table 1; negative ESIMS *m/z* 231 $[M-H]^-$; negative HRESIMS *m/z* 231.1393 $[M-H]^-$ (calcd. for $C_{15}H_{19}O_2$ $[M-H]^-$, 231.1385) (Fig. S10 in Supporting information).

2.3. Anti-TMV assays

TMV (U1 strain) was obtained from the Key Laboratory of Tobacco Chemistry of Yunnan Province, Yunnan Academy of Tobacco Science, China. The virus was multiplied in *Nicotiana tabacum* cv. K326 and purified as described. [24] The concentration of TMV was determined as 20 mg/mL with a UV spectrophotometer [virus concentration = (A_{260} × dilution ratio)/$E_{1\ cm}^{0.1\%,\ 260\ nm}$]. The purified virus was kept at −20 °C and was diluted to 32 μg/mL with 0.01 mol/L PBS before use.

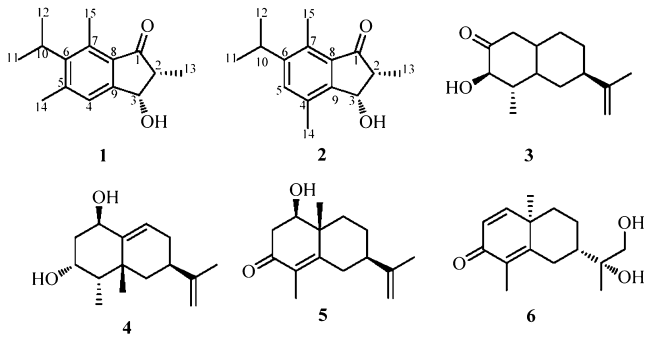

Fig. 1. The structures of sesquiterpenes from *Nicotiana tabacum*.

Table 1
^{1}H NMR and ^{13}C NMR data for compounds **1** and **2** (500 and 125 MHz, in CD_3OD).

No.	1		2	
	δ_C	δ_H (m, *J*, Hz)	δ_C	δ_H (m, *J*, Hz)
1	207.5 s		207.8 s	
2	49.7 d	2.94 m	49.0 d	2.95 m
3	70.1 d	4.59 d (6.4)	71.2 d	4.58 d (6.4)
4	128.4 d	7.12 s	132.2 s	
5	143.8 s		131.5 d	7.38 s
6	147.8 s		143.4 s	
7	135.4 s		133.9 s	
8	130.5 s		134.9 s	
9	152.0 s		148.2 s	
10	27.2 d	2.54 m	31.4 d	2.73 m
11	22.6 q	1.10 d (6.8)	23.4 q	1.14 d (6.9)
12	22.6 q	1.10 d (6.8)	23.4 q	1.14 d (6.9)
13	10.9 q	1.32 d (7.6)	10.1 q	1.27 d (7.6)
14	20.9 q	2.35 s	20.1 q	2.26 s
15	15.0 q	2.43 s	13.2 q	2.49 s

Nicotiana glutinosa plants were cultivated in an insect-free greenhouse. *N. glutinosa* was used as a local lesion host. The experiments were conducted when the plants grew to the 5/6-leaf stage. The tested compounds were dissolved in DMSO and diluted with distilled H_2O to the required concentrations. A solution of equal concentration of DMSO was used as a negative control. The commercial antiviral agent ningnanmycin (purity >98%) was used as a positive control.

For the half-leaf method [25], the virus was inhibited by mixing with the solution of the compounds. 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 a 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:

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

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

3. Results and discussion

3.1. Structure elucidation

Compound **1** was obtained as a light yellow gum. The negative mode HRESIMS data showed a quasimolecular ion peak at *m/z* 231.1379 $[M-H]^-$, suggesting a molecular formula of $C_{15}H_{20}O_2$, and indicating the presence of 6 degrees of unsaturation in the molecule. The IR spectrum showed major absorption bands at 3368 cm^{-1} (–OH), 1685 cm^{-1} (–C=O), and 1600 cm^{-1} (aromatic –C=C–). The NMR data in Table 1 and spectra of HSQC (Fig. S3 in Supporting information) and HMBC (Fig. S4 in Supporting information) indicated that **1** has a pentasubstituted benzene ring, a methine, an oxidated methine, a carbonyl group, a isopropyl group, and three methyl group. The HMBC correlations (Fig. 2) of H-2 with C-1, C-8, C-13, C-9, and C-3; H-3 with C-1, C-2, C-4, C-8, C-9, and C-13; H-4 with C-3; H-13 with C-1, C-2, and C-3, indicated