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Antiviral sesquiterpenes from leaves of *Nicotiana tabacum*

Shan-Zhai Shang [a,b], Wei Zhao [a,b], Jian-Guo Tang [a], Xing-Meng Xu [a], Han-Dong Sun [b], Jian-Xin Pu [b], Zhi-Hua Liu [a], Ming-Ming Miao [a], Yong-Kuan Chen [a,*], Guang-Yu Yang [a,*]

[a] Key Laboratory of Tobacco Chemistry of Yunnan Province, China Tobacco Yunnan Industrial Co., Ltd, Kunming 650231, PR China
[b] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, PR China

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ABSTRACT

Three unreported sesquiterpenes possessing two new skeletons, tabasesquiterpenes A–C (**1**–**3**), together with three known sesquiterpenes (**3**–**6**) were isolated from the leaves of *Nicotiana tabacum*. Their structures were determined mainly by spectroscopic methods, including extensive 1D- and 2D-NMR techniques. Compounds **1**–**6** were evaluated for their anti-tobacco mosaic virus (anti-TMV) activities. The results showed that compound **2** exhibited high anti-TMV activity with inhibition rate of 35.2%, which were higher than that of positive control (ningnanmycin). The other compounds also showed potential anti-TMV activity with inhibition rates in the range of 20.5–28.6%.

1. Introduction

Nicotiana tabacum, tobacco, is a stout herbaceous plant in the Solanaceae (nightshade family) that originated in the tropical Americas (South America, Mexico, and the West Indies) and now 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 a kind of plant containing most complex secondary metabolites in nature. It was reported that more than 2549 kinds of chemical compositions have been identified in *N. tabacum*, while up to 8700 kinds of chemical compositions were found in tobacco and tobacco substitutes and cigarette smoke (including combustion products) [3]. Our previous investigation of this species led to the discovery of a number of new compounds that showed various bioactivities, such as anti-HIV-1, anti-TMV, and cytotoxicity by our groups [4–9]. 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 three new (**1**–**3**) and three known (**4**–**6**) sesquiterpenes, of which possessed two unprecedented skeletons (one skeleton for compounds **1** and **2** while the other for compound **3**). This paper deals with the isolation, structural elucidation, and anti-TMV activity of these compounds.

2. Experimental

2.1. General experimental procedures

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. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. HRESIMS was performed on an API QSTAR time-of-flight spectrometer, or 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.

2.2. Plant material

The leaves of tobacco (Yunyan 201, a variety of *N. tabacum* L widely planted in Yunnan) were collected from Yuxi County, Yunnan Province, P. R. China, in September 2014.

2.3. Extraction and isolation

The air-dried and powdered leaves of *N. tabacum* (6.0 kg) were extracted four times with 70% aqueous acetone (3 × 12 L) at room

* Corresponding authors.
E-mail addresses: cyk1966@163.com (Y.-K. Chen), ygy1110@163.com (G.-Y. Yang).

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Table 1
^{1}H and ^{13}C NMR data for compounds **1–3** (500 and 125 MHz, in CD_3OD).

No.	1		2		3	
	δ_C	δ_H (m, *J*, Hz)	δ_C	δ_H (m, *J*, Hz)	δ_C	δ_H (m, *J*, Hz)
1	38.3 t	2.90 s	38.7 t	2.99 s	38.4 t	2.90 s
2	40.1 s		41.0 s		41.3 s	
3	43.7 t	2.82 s	43.9 t	2.80 s	44.7 t	2.86 s
4	152.6 s		159.0 s		126.0 s	
5	124.8 s		117.6 s		132.8 s	
6	134.4 s		136.2 s		119.7 d	6.61 s
7	122.3 d	6.80 s	121.7 d	6.69 s	152.0 s	
8	144.2 s		150.8 s		133.4 s	
9	132.7 s		130.9 s		147.5 s	
10	27.4 t	2.64 t (7.8)	198.1 s		32.9 t	2.54 t (7.8)
11	32.2 t	1.83 m	41.8 t	3.40 t (6.6)	35.8 t	1.91 m
12	62.6 t	3.54 t (6.5)	58.6 t	4.34 t (6.6)	62.7 t	3.55 t (6.5)
13,14	28.5 q	1.17 s	28.2 q	1.21 s	28.2 q	1.1723 s
15	23.5 q	2.20 s	23.1 q	2.19 s	21.1 q	2.12 s

temperature and filtered. The solvent was evaporated in vacuo, and the crude extract was dissolved in H_2O and partitioned with EtOAc. The EtOAc partition (220 g) was applied to silica gel (200–300 mesh) column chromatography, eluting with a $CHCl_3$–$(CH_3)_2CO$ gradient system (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 0:10), to give six fractions A–F. Further separation of fraction B (9:1, 25.4 g) by silica gel column chromatography, eluted with $CHCl_3$–$(CH_3)_2CO$ (14:1–1:1), yielded mixtures B1–B6. Fraction B2 (11:1, 4.1 g) was subjected to silica gel column chromatography using petroleum ether–acetone and semi-preparative HPLC (40% Acetonitrile-H_2O, flow rate 12 mL/min) to give **1** (11.2 mg), **2** (8.5 mg), and **3** (9.7 mg). Fraction B2 (9:1, 5.1 g) was subjected to silica gel column chromatography using petroleum ether–acetone and semi-preparative HPLC (37% Acetonitrile-H_2O, flow rate 12 mL/min) to give **4** (10.8 mg), **5** (12.4), and **6** (14.4).

Tabasesquiterpene A (**1**): obtained as pale-yellow gum; UV (MeOH), λ_{max} (log ε) 210 (4.17), 252 (3.56) nm; IR (KBr) ν_{max} 3386, 2926, 1605, 1538, 1446, 1142, 1057, 857 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz, respectively), Table 1; ESIMS (negative ion mode) *m/z* 233 $[M - H]^-$; HRESIMS (negative ion mode) *m/z* 233.1549 $[M - H]^-$ (calcd 233.1542 for $C_{15}H_{21}O_2$).

Tabasesquiterpene B (**2**): obtained as pale-yellow gum; UV (MeOH), λ_{max} (log ε) 210 (4.22), 257 (3.63) nm; IR (KBr) ν_{max} 3386, 2928, 1657, 1600, 1546, 1433, 1158, 1064, 936 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz, respectively), Table 1; ESIMS (negative ion mode) *m/z* 247 $[M - H]^-$; HRESIMS (negative ion mode) *m/z* 247.1327 $[M - H]^-$ (calcd 247.1334 for $C_{15}H_{20}O_3$).

Tabasesquiterpene (**3**): obtained as pale-yellow gum; UV (MeOH), λ_{max} (log ε) 210 (4.09), 250 (3.50) nm; IR (KBr) ν_{max} 3386, 2,926,130, 1602, 1535, 1450, 1136, 1063, 865 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz, respectively), Table 1; ESIMS (negative ion mode) *m/z* 233 $[M - H]^-$; HRESIMS (negative ion mode) *m/z* 233.1546 $[M - H]^-$ (calcd 233.1542 for $C_{15}H_{21}O_2$).

2.4. Anti-MTV assay

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 *N. tabacum* cv. K326 and purified as described [10]. The concentration of TMV was determined as 20 mg/mL with a UV spectrophotometer [virus concentration = $(A_{260} \times \text{dilution ratio}) / E_{1\,m}^{0.1\%,260nm}$]. The purified virus was kept at −20 °C 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 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 was used as a positive control.

For the half-leaf method [11], 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 or 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.

3. Results and discussion

A 70% aq. acetone extract prepared from the leaves of *N. tabacum* was partitioned between EtOAc and H_2O. The EtOAc layer was subjected repeatedly to column chromatography on Si gel and preparative HPLC to afford compounds **1–6**. Compounds **1–3** were identified as new compounds, and being named as tabasesquiterpenes A–C (**1–3**). Their structures are shown in Fig. 1, and the ^{1}H and ^{13}C NMR data of compounds **1–3** are listed in Table 1. The known compounds, comparing with the published literatures, were identified as balsamiferine B (**4**) [12], samboginone (**5**) [13], and *ent*-4(15)-eudesmen-1α,11-diol (**6**) [14].

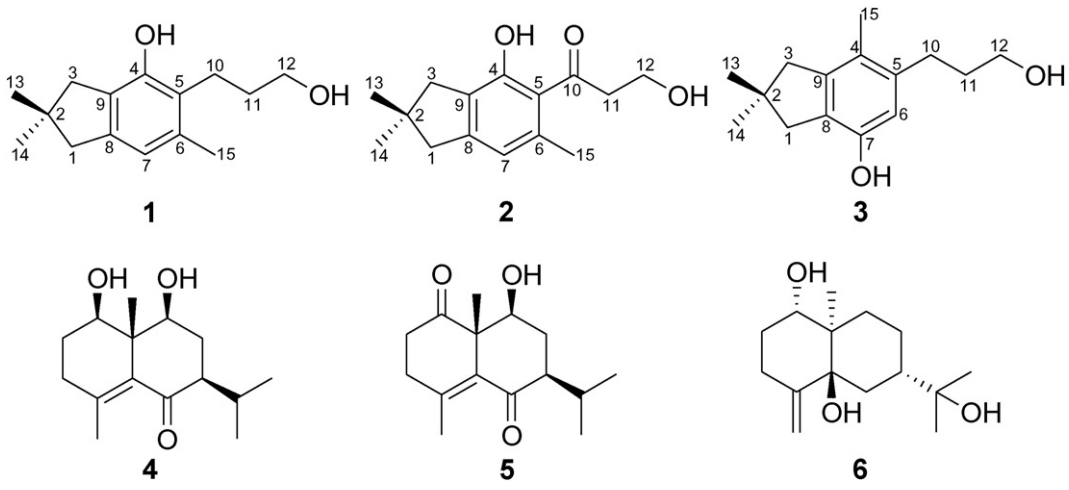

Fig. 1. The structures of sesquiterpenes from *Nicotiana tabacum*.