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Sesquiterpenoids and Diterpenoids from the Flowers of *Nicotiana tabacum* L. and Their Antifungal Activity

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Abstract: Two new guaiane-type sesquiterpenoids, nicotiasesquiterpenes A (**1**) and B (**2**), and seven known cembrane-type diterpenoids (**3**–**9**), were isolated from glandular trichome secretions of fresh flowers of *Nicotiana tabacum* L. These new chemical structures were established by extensive analyses of HRESIMS and 1D and 2D NMR data, while the other structures were elucidated by comparison of 1D NMR data with those reported in the literature. All of the isolated compounds were tested for their antiphytopathogenic fungal activity against *Valsa mali* var. *mali*, *Alternaria porri*, and *Botrytis cinerea* at a concentration of 10 μg/mL. Compounds **3**, **4**, and **5** exhibited medium antifungal effects against *Valsa mali* var. *mali*, with inhibitory rates of 45.3±16.1, 53.4± 4.6, and 40.1±4.5%, respectively, while the other compounds showed only insignificant activities.

Keywords: *Nicotiana tabacum*; *Valsa mali var. mali*; *Alternaria porri*; *Botrytis cinerea*; antifungal activity.

1. Plant Source

The flowers of *Nicotiana tabacum* L. (Solanaceae, NYBG Herbarium number was 656253) were cultivated in Zhucheng City, Shandong Province, China, in July 2020 and identified by Yong-Mei Du (Tobacco Research Institute). A voucher specimen (NT-XK-2020-07) was deposited in the laboratory of Tobacco Research Institute of Chinese Academy of Agricultural Sciences, Qingdao 266101, China.

2. Previous Studies

To date, more than 400 compounds including alkaloids, aromatic compounds, flavonoids, volatiles, sesquiterpenoids, diterpene alcohols, and sugar esters have been identified from *N. tabacum* L. in bioassay-guided purification approaches or other ways [1,2]. In particular, nicotine alkaloids and cembrane-type diterpenoids represent the two most characteristic classes from this plant. In contrast to the alkaloids stored in plant cells, terpenes are present as complex mixtures in the cuticular wax of the

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tobacco leaf and flower, where they co-occur with aliphatic hydrocarbons, fatty alcohols, wax esters and sucrose esters [3]. A literature survey disclosed that a total of 105 cembrane-type diterpenoids were isolated from *N. tabacum* L. by the end of 2020 [4].

3. Present Study

In our search for antiphytopathogenic fungal agents from *N. tabacum* L., two new guaiane-type sesquiterpenoids, nicotiasesquiterpenes A (**1**) and B (**2**), and seven known cembrane-type diterpenoids (**3**–**9**) were isolated from an aqueous EtOH extract (glandular trichome secretions) of fresh flowers using various column chromatographic methods. Herein, we report the structural elucidation and antifungal test of these natural compounds (Figure 1).

The fresh flowers of *N. tabacum* L. plants were macerated and washed three times in 95% ethanol (solid-liquid ratio of 1:7) at room temperature and then filtered. The filtrate was concentrated under vacuum to give 200 g of residue. The crude extract was separated by medium-pressure liquid chromatography (Suzhou Huitong Chromatography Co., Ltd., Jiangsu, China) and eluted through an HT-10002 SP-120-30/50-ODS-B column (100×460 mm, 30–50 μm) with a gradient mobile phase of CH_2CN-H_2O at a flow rate of 200 mL/min. The eluate was collected for 3 min per bottle and then merged into 20 fractions (Fr.1–Fr.20) by UPLC analyses at a wavelength of 200 nm. The subfractions were subjected to reversed-phase preparative HPLC with a gradient of MeOH-H_2O solution to yield the final compounds. The two new compounds **1** (4.3 mg) and **2** (5.6 mg), as well as the known **7** (66.3 mg), were isolated from Fr.18–Fr.20, while **8** (26.2 mg) and **9** (7.1 mg) were isolated from Fr.15–Fr.17. Further purification of Fr.11–Fr.14 yielded **3** (350.7 mg) and **4** (135.1 mg), while a similar procedure for Fr.6–Fr.8 gave **5** (98.2 mg) and **6** (101.9 g).

*Nicotiasesquiterpene A (**1**):* White amorphous powder, $[\alpha]_D^{25}$ +23.5 (*c* 0.02, MeOH); ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 249.1487 $[M + H]^+$ (calcd for $C_{15}H_{21}O_3$, 249.1491).

*Nicotiasesquiterpene B (**2**)*: White amorphous powder, $[\alpha]_D^{25}$ +43.7 (*c* 0.07, MeOH); ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 251.1641 $[M + Na]^+$ (calcd for $C_{15}H_{23}O_3$, 251.1647).

Table 1. ^{1}H and ^{13}C NMR spectroscopic data of **1** and **2** (600 MHz, ppm in DMSO-d_6)

Position	Compound **1**		Compound **2**	
	δ_H (*J* in Hz)	δ_C (m)	δ_H (*J* in Hz)	δ_C (m)
1	3.11, brs	42.9, CH	4.19, brs	40.2, CH
2a	2.54, overlap	39.1, CH_2	2.42, dd (7.1, 18.6)	40.4, CH_2
2b	2.13, dd (2.1, 18.8)		2.01, dd (2.0, 18.6)	
3		207.3, C		208.2, C
4		139.9, C		135.9, C
5		162.4, C		166.2, C
6	7.21, s	122.8, CH	6.79, s	118.7, CH
7		157.3, C		161.5, C
8		205.8, C	4.44, d (3.0)	65.6, CH
9a	2.91, dd (7.5, 13.1)	52.2, CH_2	2.30, m	40.8, CH_2
9b	2.54, overlap		1.29, overlap	
10	2.27, m	30.0, CH	2.19, m	30.3, CH
11		71.6, C		72.5, C
12	1.37, s	29.8, CH_3	1.26, s	29.0, CH_3
13	1.30, s	29.3, CH_3	1.32, s	28.4, CH_3
14	1.75, d (1.7)	8.3, CH_3	1.68, d (1.6)	8.0, CH_3
15	0.72, d (7.0)	13.9, CH_3	0.59, d (7.0)	15.4, CH_3
11-OH	5.25, s		4.88, s	
8-OH			5.04, d (4.2)	

Antifungal activity assay: The antifungal activity against three phytopathogenic fungi (*Valsa mali* var. *mali*, *Alternaria porri*, and *Botrytis cinerea*) were tested using a modified method previously described in the literature [5]. All plant pathogens were purchased from Qingdao Agricultural University (Qingdao, China). The isolated compounds were separately dissolved in 95% ethanol at a concentration of 1 mg/mL. After steam sterilization, culture dishes (90 mm) filled with liquid potato dextrose agar (PDA) medium were immediately added to 1 mL of the aforementioned solution and mixed thoroughly as the experimental group (EG). The final concentration of each compound was 100 μg/mL (the dilution ratio was 1:100). Moreover, PDA medium containing 1 mL of 95% ethanol was used as a control group (CG). After the medium was naturally cooled and solidified, the fungal strains cultured in another PDA culture dish (φ = 9 mm) were inoculated into the center of each dish and repeated three times. The treated fungus was fermented under static conditions at 25 °C for 7 days. The final growth inhibition ratio of the samples was calculated by the cross patch method using the formula [(φCG–9 mm) – (φEG–9 mm)]/(φCG–9 mm) ×100%.

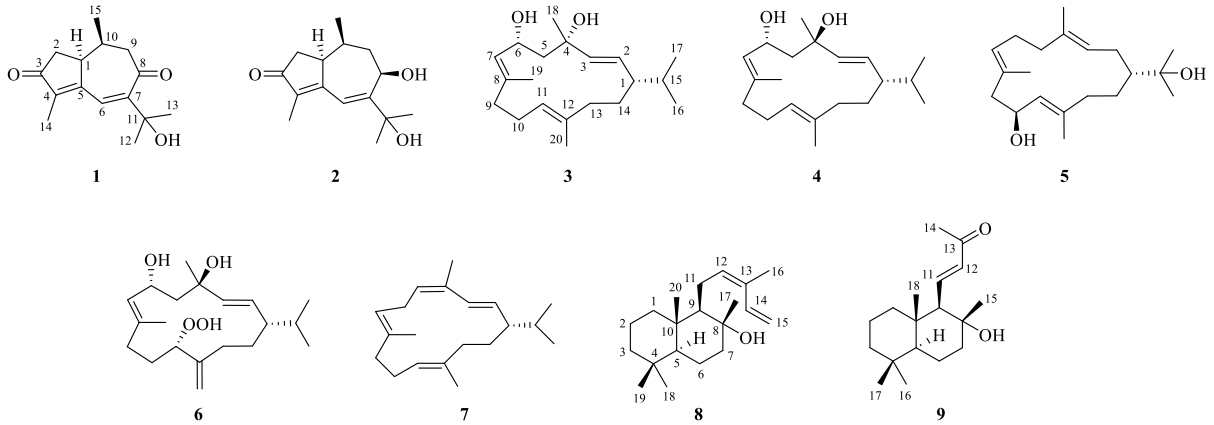

Figure 1. The chemical structures of compounds **1**–**9**

Compound **1** was isolated as a white powder. The HRESIMS adduct ion at *m*/*z* 249.1487 $[M + H]^+$ corresponds to a molecular formula of $C_{15}H_{20}O_3$, which suggests six degrees of unsaturation. This composition was in good agreement with the observation of four methyl, two methylene, three methine, and six quaternary carbon resonances in its ^{13}C NMR spectra. The six downfield quaternary carbon signals appearing at δ_C 207.3, 205.8, 162.4, 157.3, 139.9, and 122.8 are further attributed to two α,β-unsaturated carbonyl moieties. The four upfield methyl signals appearing at δ_C 29.8 (C-12), 29.3 (C-13), 13.9 (C-15), and 8.3 (C-14), along with the observation of two important groups of HMBC correlations (Figure 2) from H-13 (12) to C-6, C-7, and C-8 and from H-14 to C-3, C-4, and C-5, indicated that it was a guaiane-type sesquiterpenoid. Two spin systems, $C(15)H_3$-$C(10)H_1$ and $C(2)H_2$-$C(1)H_1$-$C(10)H_1$-$C(9)H_2$, were established by ^1H-^1H COSY (Figure 2) and HSQC analyses. Further regiochemistry of **1** was confirmed by the other detailed HMBC correlations. Finally, the structure of compound **1** was assigned as nicotiasesquiterpene A, as illustrated in Figure 1. Diagnostic NOE correlations were observed for H-1 with H-10 but not for H-1 with H-15, which indicated that H-1 and CH_3-10 were positioned on opposite sides of the seven-membered ring (Figure 2).

Compound **2** showed ^1H and ^{13}C NMR spectroscopic data that were highly similar to those of **1** (Table 1), which was characterized as a guaiane-type sesquiterpenoid. Its molecular formula $C_{15}H_{22}O_3$ with five degrees of unsaturation was deduced based on an HRESIMS adduct ion at 251.1641 $[M + Na]^+$ (calcd for $C_{15}H_{23}O_3$, 251.1647). Detailed comparison of the ^{13}C NMR data with that of **1** revealed the absence of a carbonyl group in **2**, which was supported by the reduced degree of unsaturation, and the resonance of C-8 at δ_C 65.6 in **2**, instead of the deshielded resonance at δ_C 205.8 in **1**. With the exception of this difference, the other 1D NMR spectroscopic data of **2** and **1** were well matched. Consequently, compound **2** was elucidated as nicotiasesquiterpene B. Its relative stereochemistry between H-1 and CH_3-10 was tentatively assigned to be the *trans* configuration by biosynthetic

analogy to the stereochemistry of H-1 and CH_3-10 for **1**. Unfortunately, the final absolute configuration could not be determined due to the limited amount of isolated sample.

In addition, another six known compounds were isolated in this study. They were identified as α-cembrenediol (**3**) [6], β-cembrenediol (**4**) [6], (3*E*,7*E*,11*E*)-3,7,11-cembratriene-10,15-diol (**5**) [7], (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*)-11-hydroperoxy-2,7,12(20)-cembratriene-4,6-diol (**6**) [8], (1*R*,2*E*,4*Z*,7*E*,11*E*)-cembra-2,4,7,11-tetrene (**7**) [9], (+)-*cis*-abienol (**8**) [10], and (11*E*)-8-hydroxy-14,15-dior-11-labden-13-one (**9**) [11] by comparison of their NMR data (Supporting Information, Figures S14–S31) with literature data.

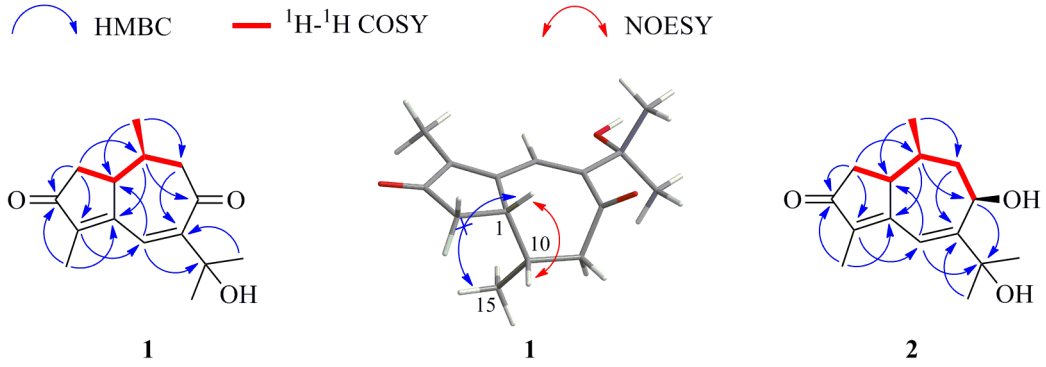

Figure 2. Important HMBC, ^{1}H-^{1}HCOSY, and 1D-NOESY correlations for compounds **1** and **2**

All of these natural compounds (**1**–**9**) were evaluated for their antifungal activity against the phytopathogenic fungal strains *Valsa mali* var. *mali*, *Alternaria porri*, and *Botrytis cinerea* at a concentration of 10 μg/mL (Table 2). Compared with the control group, compounds **3**, **4**, and **5** exhibited medium antifungal effects against *Valsa mali* var. *mali*, with inhibitory rates of 45.3±16.1, 53.4±4.6, and 40.1±4.5%, respectively, while the others showed only insignificant activities.

Table 1. Antifungal activities of compounds **1**–**9** at a concentration of 10 μg/mL (n = 3)

Compounds	**Growth inhibition ratio (%)**		
	V. mali var. mali	*A. porri*	*B. cinerea*
1	–[a]	6.3 ± 2.9	–
2	–	–	10.0 ± 7.2
3	45.3 ± 16.1	15.0 ± 8.8	19.2 ± 18.5
4	53.4 ± 4.6	21.2 ± 17.0	14.9 ± 14.7
5	40.1 ± 4.5	11.7 ± 5.3	14.1 ± 11.2
6	15.1 ± 7.9	–	11.2 ± 2.4
7	–	–	3.0 ± 4.4
8	13.1±14.3	–	9.3 ± 1.0
9	7.9 ± 4.8	–	–

[a] No significant activity was found compared to that of the control.

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Supporting Information

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