Chemical Constituents from *Nicotiana tabacum* L. and Their Antifungal Activity

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Abstract

A phytochemical investigation of an aqueous ethyl alcohol (EtOH) extract of the flowers of *Nicotiana tabacum* L. led to the identification of one new eudesmane-type sesquiterpenoid (I) and 14 known compounds, including 7 flavones (2-8), 6 phenolic acids (9-14), and 1 coumarin (15). The chemical structure of I was established on the basis of high-resolution electrospray ionization (HRESIMS) and 1D and 2D NMR analyses, while the other structures were elucidated by 1D NMR and LC–MS data. The antiphthopathogenic activities of all the isolated compounds were tested to determine their antifungal activity against *Valsa mali* var. *mali*, *Alternaria porri*, and *Botrytis cinerea* at a concentration of 10 µg/mL. Compound 4 exhibited more potent antifungal effects against *Valsa mali* var. *mali* with a % growth inhibition of 47.9 ± 1.2%, while the others only showed insignificant activities.

Keywords

*Nicotiana tabacum*, *Valsa mali* var. *mali*, *Alternaria porri*, *Botrytis cinerea*, antifungal activity

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Introduction

*Nicotiana tabacum* L. (family Solanaceae), also known as tobacco, was first introduced from the tropical Americas.¹ As one of the most commercially valued agricultural crops worldwide, it is widely grown in China, especially the southwest provinces of Yunnan, Guizhou, and Sichuan. In addition to providing the raw materials for cigarette production, the aerial part of *N. tabacum* was used as an insecticide, sedative, diaphoretic, anesthetic, and emetic agent in traditional Chinese medicine.²⁻⁵ Previous phytochemical studies on *Nicotiana* plants led to the discovery of more than 2500 compounds, including sesquiterpenoids, diterpenoids, alkaloids, and flavonoids.⁶⁻⁹ Our research team previously found that a 95% ethanol extract of tobacco leaves possessed significant phytopathogenic fungal activities against *Valsa mali* and 10 other plant pathogens.¹⁰,¹¹ In our search for antifungal agents from *N. tabacum*, one new eudesmane-type sesquiterpenoid (I) and 14 known compounds (2 to 15) were isolated from an aqueous ethyl alcohol (EtOH) extract of the flowers using various column chromatography methods (Figure 1). Their chemical structures were elucidated on the basis of extensive spectroscopic data, while antifungal activity was evaluated on the strains *Valsa mali* var. *mali*, *Alternaria porri*, and *Botrytis cinerea* at a concentration of 10 µg/mL.

Results and Discussion

Compound 1 was isolated as a white powder. An HRESIMS adduct ion at \( m/z \) 437.2152 [M + Na]⁺ corresponds to a molecular formula of C₂₁H₃₄O₈Na, which suggests five degrees of unsaturation. With the assistance of the HSQC spectrum, the [H] NMR data (Table 1) show three methyl protons at δ_H 1.16, 1.25, and 1.78, one anomeric proton at δ_H 4.28, and a series of methylene and methine protons. The ¹³C NMR data (Table 1) exhibit 21 carbons, 6 of which are assigned to a glucosyl moiety (δ_C 104.4, 75.3, 78.2, 72.0, 77.8, and 63.1). The downfield carbon signals at δ_C 129.7, 166.3, and 201.7 can be attributed to an α,β-unsaturated carbonyl moiety. The key HMBC correlations (Figure 2) from H-14 to C-3, C-4, and C-5 indicate that the olefinic proton is substituted by a methyl group at δ_C 11.2 and δ_H 1.78. Through the HSQC and ¹H–¹H COSY (Figure 2) data, two spin systems, C(1)H₂–C(2)
H₂ and C(6)H₂–C(7)H–C(8)H₂–C(9)H₂, were established, revealing a skeleton of eudesmane-type sesquiterpenoids. Except for those of the glucosyl moiety, the obtained ¹³C NMR spectroscopic data were similar to those of a reported compound, 10β-eudesm-4-en-3-one-11,12-diol (Table 1).¹² A key HMBC correlation (Figure 2) from the proton at δ_H 4.28

Table 1. ¹H and ¹³C NMR Spectroscopic Data of 1 (600 MHz, ppm in MeOH-d₄) and the ¹³C NMR Spectroscopic Data of 10 β-Eudesm-4-en-3-one-11,12-Diol (62.5 MHz, ppm in CDCl₃).

| Position | ¹H (J in Hz) | ¹³C (m) | ¹³C (m) |
|----------|--------------|---------|---------|
| 1        | 1.77, overlap| 38.4, CH₂| 36.31   |
| 2a       | 2.55, m      | 34.6, CH₂| 33.96   |
| 2b       | 2.33, m      |         |         |
| 3        | 201.7, C     | 199.04  | 164.8   |
| 4        | 129.7, C     | 129.7   |         |
| 5        | 166.3, C     |         |         |
| 6a       | 2.89, brd (13.5)| 30.3, CH₂| 27.38   |
| 6b       | 1.96, t (13.5)|         |         |
| 7        | 1.77, overlap| 46.4, CH  | 40.23   |
| 8a       | 1.77, overlap| 22.5, CH₂| 24.52   |
| 8b       | 1.67, m      |         |         |
| 9a       | 1.73, m      | 43.2, CH₂| 37.36   |
| 9b       | 1.42, m      |         |         |
| 10       |              | 37.3, C | 35.86   |
| 11       |              | 74.9, C | 75.20   |
| 11a      |              | 74.9, C | 75.20   |
| 12a      | 4.11, d (10.0)| 76.2, CH₂| 67.97   |
| 12b      | 3.38, overlap|         |         |
| 13       | 1.16, s      | 20.5, CH₃| 20.28   |
| 14       | 1.78, s      | 11.2, CH₃| 11.23   |
| 15       | 1.25, s      | 22.6, CH₃| 21.91   |
| Glc-1'   | 4.28, d (7.8)| 104.4, CH |         |
| 2'       | 3.21, overlap| 75.3, CH |         |
| 3'       | 3.30, overlap| 78.2, CH |         |
| 4'       | 3.22, overlap| 72.0, CH |         |
| 5'       | 3.38, overlap| 77.8, CH |         |
| 6'a      | 3.91, dd (1.8, 12.0)| 63.1, CH₂|         |
| 6'b      | 3.63, dd (6.6, 12.0)|         |         |
to the carbon at δC 76.2 suggests that the glucosyl moiety is located at C-12, whereas the β-configurations were deduced based on the coupling constant (7.5 Hz) of the anomeric proton. Therefore, the structure of compound 1 is established as 10β-eudesm-4-en-3-one-11,12-diol-12-O-β-glucopyranoside. The NOESY correlations (Figure 3) of H-15 with H-6a and H-9b and that of H-7 with H-6b and H-9a indicate that H-15 and H-7 are on opposite sides of the ring. Unfortunately, the determination of the absolute configuration could not be performed with the limited amount of isolated sample.

In addition, another fourteen known compounds were isolated in this study. They were identified as luteolin (2),13 quercetin (3),13 kaempferol (4),13 genistein (5),14 taxifolin (6),13 catechin (7),15 epicatechin (8),15 chlorogenic acid (9),16 caffeic acid (10),16 ferulic acid (11),16 vanillic acid (12),16 methyl vanillate (13),15 vanillin (14),18 and 6,7-dihydroxy-4-methylcoumarin (15),19 by comparison of their NMR spectroscopic (Supplemental Material, Figures S8–S49) and LC–MS data with literature data.

All these compounds were evaluated for 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 (CG), compound 4 exhibited more potent antifungal effects against Valsa mali var. mali with a % growth inhibition of 47.9 ± 1.2%. Compounds 2, 3, 5, and 8 to 15 showed weak activities against one or more fungal strains, while the other compounds (1, 6, and 7) showed only ineffective activities.

| Compounds | V. mali var. mali | A. porri | B. cinerea |
|-----------|------------------|----------|-----------|
| 1         | –                | –        | 5.8 ± 10.9 |
| 2         | –                | –        | 11.2 ± 13.7 |
| 3         | –                | –        | 1.0 ± 2.1  |
| 4         | 47.9 ± 1.2       | –        | 7.5 ± 11.4 |
| 5         | 13.5 ± 11.8      | –        | –         |
| 6         | –                | –        | –         |
| 7         | –                | –        | –         |
| 8         | 4.7 ± 1.6        | 6.9 ± 8.3| –         |
| 9         | 2.1 ± 3.3        | 11.9 ± 12.8| –       |
| 10        | –                | 7.3 ± 10.9| –         |
| 11        | 14.8 ± 8.6       | 10.7 ± 8.7| –         |
| 12        | 3.1 ± 5.8        | 1.1 ± 3.4| 9.9 ± 8.8 |
| 13        | –                | 7.9 ± 5.7| –         |
| 14        | –                | 3.8 ± 7.9| –         |
| 15        | –                | 0.9 ± 1.9| 4.8 ± 9.6 |

*a-CBT-diol was used as the positive control.

Table 2. Antifungal Activities of Compounds 1 to 15 at 10 μg/mL (n = 3).

Material and Methods

General Experimental Procedures

Specific rotation was measured on an Autopol IV-T spectrometer (Rudolph Technologies). NMR spectra were obtained on a Bruker Advance III-600 MHz spectrometer (Bruker Biospin); chemical shifts are given in δ (ppm) with the known residual solvent proton peak as the reference. High-resolution electrospray ionization (HRESIMS) was performed on a Thermo Orbitrap Fusion Lumos instrument (Thermo Fisher Scientific), while the LC–MS data were measured on a Shimadzu LCMS-8050 mass spectrometer (Shimadzu Corp.). Semipreparative HPLC was performed on a C18 (SunFire®, 10 μm, 19 × 250 mm) column using a Waters e2695 separation module equipped with a 2998 detector (Waters Corp.). Commercially available silica gel of 200 to 300 mesh (Qingdao Marine Chemical Co.), Diaion HP-20 macroporous resin (Mitsubishi Chemical Corp.), and Sephadex LH-20 (Pharmacia Fine Chemicals) were used for open column chromatography.

Plant Materials

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

Extraction and Isolation

Fresh flowers were collected from a total of 15 acres (about 1000 kg) of N. tabacum. At normal temperatures and pressures,
these materials were rinsed in 95% ethanol (solid–liquid ratio of 1:7), three times. The treated flowers were dried in the sun, while the solution was concentrated under reduced pressure to obtain the glandular trichome secretions (this work is reported in another study). The air-dried flowers (100 kg) were crushed and refluxed three times with 95% EtOH for 2 h. The extract (8.0 kg) was suspended in 20 L of dilute HCl solution (pH 1-2) for 12 h at room temperature and successively partitioned with 20 L of EtOAc and 20 L of n-ButOH to yield acidified crude extracts, which were named acidized EtOAc and n-ButOH, respectively. NaOH was added to the aqueous phase to adjust the pH to 12 to 13, and the solution was incubated for 12 h at room temperature. The solution was again extracted with EtOAc to obtain the alkalized crude extract, named alkalized EtOAc. The acidized EtOAc fraction (2.6 kg) was separated on a silica gel column with a step gradient of Pet (light petroleum)–EtOAc (v/v) to yield 10 fractions using TLC analyses: A (1428.5 g), B (11.1 g), C (32.3 g), D (25.5 g), E (20.4 g), F (39.3 g), G (27.2 g), H (40.9 g), I (35.5 g), and J (14.0 g). The fractions were successively eluted from an LH-20 column with a mobile phase of CHCl3–MeOH (50:50, v/v), with HPLC analyses. Further purification of these subfractions using reversed-phase preparative HPLC with a MeOH:H2O solution yielded the final compounds. The subfractions Fr.H3 to Fr.H5 yielded 2 (74.3 mg) and 5 (51.0 mg); Fr.H10 to Fr.H13 yielded 11 (18.7 mg), 12 (101.2 mg), and 14 (35.7 mg); Fr.H19 to Fr.H23 yielded 13 (63.2 mg) and 15 (14.1 mg). The subfractions Fr.I5 to Fr.I7 yielded 3 (68.0 mg) and 4 (29.9 mg); Fr.I11 to Fr.I13 yielded 6 (46.3 mg), 7 (114.9 mg), and 8 (77.5 mg); Fr.I25 to Fr.297 yielded 9 (104.3 mg). The subfractions Fr.J7 to Fr.J10 yielded 1 (4.5 mg) and 10 (35.0 mg).

**Compound 1.** White amorphous powder, [α]D20 + 41.2 (c 0.04, MeOH); 1H and 13C NMR data, see Table 1; HRESIMS m/z 437.2152 [M + Na]⁺ (c calc for C21H34O8Na, 437.2151).

**Antifungal Activity Assay.** The antifungal activity against three phytopathogenic fungi (e.g., Valsa mali var. mali, Alternaria porri, and Botrytis cinerea) were tested using a modified method previously described in the literature.10,11 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; these samples constituted the experimental group (EG). The final concentration of each compound was 10 μg/mL (the dilution ratio was 1:100). PDA medium containing 1 mL of 95% ethanol was used as the CG. After the medium was naturally cooled and solidified, the fungal strains cultured in another PDA culture dish (φ = 9 mm) were inoculated in 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 \([\{\text{α-CBT-diol}} - \{\text{φEG} - 9 \text{mm} \}/\{\text{φCG} - 9 \text{mm} \}] \times 100\%\). α-CBT-diol, which is a characteristic antifungal constituent of tobacco, was used as the positive control.20

**Conclusions**

One new eudesmane-type sesquiterpenoid glycoside (I) and 14 known phenolic compounds (2-15) were isolated from the aqueous EtOH extract of the flowers of N. tabacum L. Their chemical structures were established by extensive analysis of HRESIMS and NMR spectra and by comparison of the 1D NMR data with those reported in the literature. Compound 4 possessed potent antifungal effects against V. mali var. mali with a % growth inhibition of 47.9 ± 1.2%, while the other compounds showed insignificant antifungal activities.

**Declaration of Conflicting Interests**

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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**Trial Registration**

Not applicable because this article does not contain any clinical trials.

**Ethical Approval**

Ethical approval is not applicable for this article.

**Statement of Human and Animal Rights**

This article does not contain any studies with human or animal subjects.

**Statement of Informed Consent**

There are no human subjects in this article and informed consent is not applicable.
Supplemental Material

Supplemental material for this article is available online.

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