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Noreudesmane sesquiterpenoids from the leaves of *Nicotiana tabacum*

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ABSTRACT

Six new 14-noreudesmane sesquiterpenoids, nicotabacosides A–F (**1**–**6**), along with five known sesquiterpenoids (**7**–**11**), were isolated from the leaves of *Nicotiana tabacum*. The structures of compounds **1**–**6** were elucidated as isorishitin 3-*O*-*β*-D-glucopyranoside (**1**), rishitin 3-*O*-*β*-D-glucopyranoside (**2**), rishitin 2-*O*-*β*-D-glucopyranoside (**3**), 1, 6-dehydro-rishitin 3-*O*-*β*-D-glucopyranoside (**4**), 2-hydroxyl-ligudentatol 3-*O*-*β*-D-glucopyranoside (**5**) and oxyglutinosone 3-*O*-*β*-D-glucopyranoside (**6**) based on extensive spectroscopic analyses (HRESIMS, UV, IR, 1D and 2D NMR). Their absolute configurations were determined by X-ray single-crystal diffraction and comparison of their electronic circular dichroism (ECD) spectra.

1. Introduction

Nicotiana tabacum belonging to *Nicotiana* genus of the Solanaceace family, is an important economic crop whose leaves are well-known in tobacco production. In addition, its aerial part is also used for sedative, diaphoretic, anesthetic and emetic purposes [1]. Phytochemical investigation revealed that *Nicotiana* plants were rich in terpenoids, alkaloids and flavonoids [2–7]. Most of the sesquiterpenoids in *Nicotiana* plants are structurally categorized as monocyclofarnesane, vatispirane and eudesmane-types including 3 cases of unusual noreudesmane-type. Currently, about 20 noreudesmane-type sesquiterpenoids were obtained from natural sources and classified into 13-noreudesmane, 14-noreudesmane and 11, 12, 13-tri-noreudesmane sesquiterpenoids according to the positions of carbon decreasing [8–13]. Pharmacological studies showed that nicotine, the most important alkaloid in *Nicotiana* plants, was closely related to smoking addiction, and possessed neuroprotective effect against the toxicity of amyloid-β (Aβ) oligomers [14]. As a continuous search for active compounds from natural sources, our investigation on *N. tabacum* afforded six new 14-noreudesmane sesquiterpenoids, nicotabacosides A–F (**1**–**6**), as well as five known sesquiterpenoids, actinidioionoside (**7**), byzantionoside B (**8**), (*Z*)-4-[3′-(*β*-D-glucopyranosyloxy) butylidene]-3, 5, 5-trimethyl-2-cyclohexen-l-one (**9**), (*6R*, *9R*)-3-oxo-*α*-ionol *β*-D-glucopyranoside (**10**) and (*6R*, *9S*)-3-oxo-*α*-ionol *β*-D-glucopyranoside (**11**) based on extensive spectroscopic analyses (HRESIMS, UV, IR, 1D and 2D NMR). The absolute configurations of compounds **1** and **4** were determined by X-ray single-crystal diffraction, and compounds **2**, **3**, **5** and **6** were determined by comparing their electronic circular dichroism (ECD) spectra. Herein, we described their isolation and structural elucidation.

2. Experimental

2.1. General apparatus and chemicals

Melting points (mp) were measured by a SGW®X-4B melting point apparatus (Shanghai Precision & Scientific Instrument Co., Ltd. Shanghai, China). Optical rotations were obtained on a Jasco model 1020 polarimeter (Horiba, Tokyo, Japan). HRESIMS data were recorded on a LCMS–IT–TOF mass

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spectrometer (Shimadzu, Kyoto, Japan). UV spectra were conducted on a UV-2401A spectrophotometer (Shimadzu, Kyoto, Japan). Electronic circular dichroism (ECD) spectra were performed on an Applied Photophysics Chirascan instrument (Agilent, America). IR spectra were collected on a Bio-Rad FTS-135 spectrometer (Bio-Rad, Hercules, CA). 1D and 2D NMR spectra were acquired using AM-400, DRX-500 or Advance III-600 NMR spectrometers (Bruker, Bremerhaven, Germany) with TMS as internal standard. Semi-preparative HPLC was performed on a Waters Alliance 2695 (pump: Waters 600, detector: Waters 2996) with a reversed-phase (RP) C_{18} column (9.4 × 250 mm, 5 μm, Agilent). Silica gel (200–300 mesh, Qingdao Makall group Co., Ltd; Qingdao, China), C_{18} (Merck, Darmstadt, Germany) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography.

2.2. Plant material

The leaves of *N. tabacum* Linn. were collected from Luliang County of Yunnan Province of China, on September 16, 2011 and identified by Prof. Dr. Li-Gong Lei, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 2011-09-16) was deposited in the Laboratory of Antivirus and Natural Medicinal Chemistry, Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The dried leaves of *N. tabacum* (3.5 kg) were extracted with 90% EtOH at room temperature for 3 times, each for 72 h. All the extract was combined and condensed under reduced pressure (<60 °C), which was partitioned between EtOAc and H_2O.

The EtOAc part (615 g) was subjected to silica gel column chromatography (CC) (3.0 kg, 18.0 × 70 cm) using H_2O–MeOH–$CHCl_3$ (0:0:100, 0:5:95, 0:10:90, 2:20:80, v/v) as the eluent to afford Frs.1–8. Fr.4 (35.5 g) was fractionated by a MCI CHP 20P gel column (310 g, 4.0 × 40 cm) eluted with MeOH–H_2O (20:80, 40:60, 60:40, 80:20, 100:0) to get Frs.4.1–4.5. Fr.4.3 (1.8 g) was subjected to a silica gel CC (40 g, 2.0 × 50 cm) eluted with MeOH–$CHCl_3$ (10:90) to provide Frs.4.3.1–4.3.2. Fr.4.3.2 (538 mg) was separated on a sephadex LH-20 column (50 g, 1.4 × 150 cm) to yield Frs.4.3.2.1–4.3.2.2. Fr.4.3.2.1 (427 mg) was purified by HPLC on a RP C_{18} column using MeOH–H_2O (48:52) as the eluent to obtain compounds **8** (23 mg), **9** (8 mg), **10** (46 mg) and **11** (36 mg). Fr.5 (29.5 g) was fractionated by a MCI CHP 20P gel column (310 g, 4.0 × 40 cm) eluted with MeOH–H_2O (20:80, 40:60, 60:40, 80:20, 100:0) to produce Frs.5.1–5.5. Fr.5.2 (1.6 g) was chromatographed on a RP C_{18} column (124 g, 2.54 × 40 cm) eluted with MeOH–H_2O (10:90, 30:70, 50:50, 70:30, 100:0) to obtain Frs.5.2.1–5.2.7. Fr.5.2.7 (100 mg) was purified through HPLC on a RP C_{18} column with MeCN–H_2O (25:75) as mobile phase to afford compound **6** (4 mg). Fr.5.3 (3.2 g) was submitted to a silica gel CC (100 g, 4.0 × 50 cm) eluted with H_2O–MeOH–$CHCl_3$ (0:10:90, 1.0:15:85) to give Frs.5.3.1–5.3.6. Fr.5.3.3 (500 mg) was separated by HPLC on a RP C_{18} column eluted with MeOH–H_2O (72:28) to provide Fr.5.3.3.1 and Fr.5.3.3.2. Fr.5.3.3.1 (60 mg) was purified by HPLC on a RP C_{18} column using MeCN–H_2O (35:65) as the eluent to yield compounds **1** (10 mg) and **4** (10 mg). Fr.5.3.3.2 (200 mg) was purified through HPLC on a RP C_{18} column eluted with MeOH–H_2O (55:45) to generate **2** (12 mg) and **3** (30 mg). Fr.5.4 (3.7 g) was chromatographed on a silica gel column (100 g, 3.5 × 50 cm) using HCOOH–MeOH–$CHCl_3$ (1:10:90) as the eluent to give Frs.5.4.1–5.4.4. Fr.5.4.4 (2.2 g) was subjected to a silica gel CC (40 g, 2.0 × 50 cm) eluted with H_2O–MeOH–EtOAc (0.5:5:95) to generate Frs.5.4.4.1–5.4.4.3. Fr.5.4.4.1 (130 mg) was separated on a sephadex LH-20 column (50 g, 1.4 × 150 cm) to produce compound **5** (5 mg). Fr.8 (15.0 g) was fractionated by a MCI CHP 20P gel column (100 g, 2.54 × 40 cm) eluted with MeOH–H_2O (20:80, 40:60, 60:40, 80:20, 100:0) to produce Frs.8.1–8.5. Fr.8.1 (5.5 g) was chromatographed on a silica gel column (100 g, 4.0 × 50 cm) eluted with H_2O–MeOH–$CHCl_3$ (2:20:80) to generate **7** (20 mg).

Nicotabacoside A (**1**): Colorless acicular crystal [MeOH–H_2O (1:1, v/v)], mp 107.2–108.2 °C; $[\alpha]_D^{16}$: +37.1 (c 0.20, MeOH); ECD (MeOH) $\Delta\varepsilon_{195}$ + 15.66, $\Delta\varepsilon_{205}$ + 6.29, $\Delta\varepsilon_{212}$ + 7.56, $\Delta\varepsilon_{231}$ − 0.01; IR (KBr) ν_{max} 3423, 1642, 1439, 1374, 1167, 1077, 1031 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data (see Tables 1 and 3); HRESIMS *m/z* 407.2040 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_7Na$, 407.2040, 0 mDa).

Crystal data of compound **1**: $C_{20}H_{32}O_7 \cdot 2H_2O$, M = 420.49, monoclinic, a = 21.4086 (7) Å, b = 7.3541 (3) Å, c = 28.0134 (9) Å, α = 90.00°, β = 95.0100 (10)°, γ = 90.00°, V = 4393.6(3) $Å^3$, T = 100 (2) K, space group *C*2, Z = 8, μ (CuKα) = 0.830 mm^{-1}, 15,414 reflections measured, 5780 independent reflections (R_{int} = 0.0565). The final R_1 values were 0.1023 ($I > 2\sigma(I)$) and 0.1054 (all data). The final wR (F^2) values were 0.2790 ($I > 2\sigma(I)$) and 0.2875 (all data). The goodness of fit on F^2 was 1.368, flack parameter was 0.2 (3), the Hooft parameter was 0.24 (13) for 1820 Bijvoet pairs.

Nicotabacoside B (**2**): White powder; $[\alpha]_D^{17}$: −49.2 (c 0.09, MeOH); ECD (MeOH) $\Delta\varepsilon_{195}$ − 11.91, $\Delta\varepsilon_{200}$ − 15.42, $\Delta\varepsilon_{218}$ + 0.98, $\Delta\varepsilon_{228}$ − 0.33; IR (KBr) ν_{max} 3473, 3406, 1643, 1443, 1373, 1163, 1079, 1031 cm^{-1}; ^1H (400 MHz) and ^{13}C (100 MHz) NMR data (see Tables 1 and 3); HRESIMS *m/z* 407.2053 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_7Na$, 407.2040, + 1.3 mDa).

Nicotabacoside C (**3**): White powder; $[\alpha]_D^{16}$: −42.5 (c 0.60, MeOH); ECD (MeOH) $\Delta\varepsilon_{195}$ − 11.91, $\Delta\varepsilon_{196}$ − 8.95, $\Delta\varepsilon_{200}$ − 13.30, $\Delta\varepsilon_{216}$ + 3.72, $\Delta\varepsilon_{239}$ − 0.55; IR (KBr) ν_{max} 3408, 1644, 1602, 1450, 1375, 1284, 1164, 1076, 1042, 1023 cm^{-1}; ^1H (400 MHz) and ^{13}C (100 MHz) NMR data (see Tables 1 and 3); HRESIMS *m/z* 407.2038 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_7Na$, 407.2040, −0.2 mDa).

Nicotabacoside D (**4**): Colorless acicular crystals [MeOH–H_2O (6:4)], mp 195.0–196.0 ºC; $[\alpha]_D^{16}$: +5.8 (c 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 241 (4.45) nm; ECD (MeOH) $\Delta\varepsilon_{195}$ + 8.40, $\Delta\varepsilon_{210}$ − 4.44, $\Delta\varepsilon_{239}$ + 14.88, $\Delta\varepsilon_{259}$ − 0.49; IR (KBr) ν_{max} 3419, 1643, 1452, 1372, 1165, 1079, 1030 cm^{-1}; ^1H (400 MHz) and ^{13}C (100 MHz) NMR data are seen in Tables 2 and 3; HRESIMS *m/z* 405.1879 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_7Na$, 405.1884, −0.5 mDa).

Crystal data of compound **4**: $C_{20}H_{30}O_7 \cdot H_2O$, M = 400.46, monoclinic, a = 8.0041 (6) Å, b = 63.282 (5) Å, c = 8.9989 (7) Å, α = 90.00°, β = 116.076 (4)°, γ = 90.00°, V = 4094.1 (5) $Å^3$, T = 100 (2) K, space group *P*21, Z = 8, μ (CuKα) = 0.831 mm^{-1}, 27,133 reflections measured, 12,552 independent reflections (R_{int} = 0.1413). The final