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Noreudesmane sesquiterpenoids from the leaves of *Nicotiana tabacum*

Cai-Yan Yang [a,b], Chang-An Geng [a], Xiao-Yan Huang [a], Hao Wang [a,b], Hong-Bo Xu [a,b], Wen-Juan Liang [a,b], Yun-Bao Ma [a], Xue-Mei Zhang [a], Jun Zhou [a], Ji-Jun Chen [a,*]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650201, PR China
[b] University of Chinese Academy of Sciences, Beijing 100049, PR China

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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

* Corresponding author. Tel.: +86 871 65223265; fax: +86 871 65227197.
E-mail address: chenjj@mail.kib.ac.cn (J.-J. Chen).

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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

Table 1
^{1}H NMR data for compounds **1**–**3** (δ in ppm, J in Hz).

No.	**1**[a]	**2**[a]	**2**[b]	**3**[a]
1a	2.10, m	2.26, m	2.04, m	2.26, m
1b		2.18, m	1.91, m	2.13, m
2	3.88, m	3.65, m	3.54, m	3.69, m
3	3.73, dd, 11.7, 5.5	3.33, m	3.08, m	3.32, m
4	2.34, m	2.06, m	2.12, m	2.07, m
6a	2.13, m	2.26, m	2.14, m	2.26, m
6b	1.73, m	1.65, m	1.65, m	1.77, m
7	2.10, m	2.06, m	2.12, m	2.26, m
8a	1.73, m	1.77, m	1.62, m	1.69, m
8b	1.43, m	1.60, m	1.47, m	1.60, m
9a	1.94, m	1.88, m	1.76, m	1.88, m
9b	2.08, m	1.98, m	1.93, m	1.98, m
12a	4.71, s	4.74, s	4.69, s	4.73, s
12b		4.64, s	4.61, s	4.62, s
13	1.73, s	1.73, s	1.69, s	1.73, s
15	1.08, d, 7.2	1.21, d, 7.2	1.13, d, 6.8	1.16, d, 6.8
1′	4.32, d, 8.0	4.39, d, 7.8	4.24, d, 7.8	4.36, d, 8.0
2′	3.21, m	3.24, m	3.07, m	3.21, m
3′	3.28, m	3.35, m	3.12, m	3.28, m
4′	3.27, m	3.26, m	2.99, m	3.21, m
5′	3.34, m	3.39, m	3.12, m	3.35, m
6′a	3.87, dd, 11.7, 2.2	3.86, dd, 11.5, 1.8	3.62, dd, 11.1, 1.9	3.85, dd, 11.5, 2.0
6′b	3.63, dd, 11.7, 5.5	3.68, dd, 11.5, 5.1	3.41, dd, 11.1, 5.1	3.66, dd, 11.5, 5.4

[a] Data were reported in CD_3OD.
[b] Data were recorded in DMSO-d_6.

R_1 values were 0.2022 ($I > 2\sigma(I)$) and 0.2407 (all data). The final wR (F^2) values were 0.4110 ($I > 2\sigma(I)$) and 0.4483 (all data). The goodness of fit on F^2 was 1.606, flack parameter was 0.5 (5), the Hooft parameter was 0.2 (2) for 5300 Bijvoet pairs.

Crystallographic data for the structures of **1** (deposition no.: CCDC 978782) and **4** (deposition no.: CCDC 978783) (Fig. 1) have been deposited in the Cambridge Crystallographic Data Centre. Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.htm (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, U.K.; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

Table 2
^{1}H NMR data for compounds **4**–**6** in CD_3OD (δ in ppm, J in Hz).

No.	**4**	**5**	**6**
1	5.47, br.s	6.44, s	5.79, s
2	4.20, br.d, 6.7		
3	3.19, dd, 9.4, 6.7		4.45, d, 12.4
4	2.31, m		2.07, dq, 12.4, 6.7
6a	5.60, br.s	2.67, m	2.25, dd, 9.6, 2.6
6b		2.34, m	1.28, dd, 9.6, 3.2
7	2.86, br.s	2.30, m	2.55, m
8a	1.73, m	1.93, m	1.91, m
8b	1.61, m	1.53, m	1.37, m
9a	2.34, m	2.71, m	2.44, ddd, 14.2, 3.1, 3.1
9b	2.20, m	1.65, m	2.70, ddd, 14.2, 5.1, 3.3
12a	4.81, s	4.76, s	4.74, s
12b	4.65, s		4.67, s
13	1.76, s	1.80, s	1.75, s
15	1.22, d, 6.5	2.22, s	1.23, d, 6.7
1′	4.41, d, 7.8	4.46, d, 7.8	4.37, d, 7.8
2′	3.22, m	3.52, m	3.28, m
3′	3.28, m	3.26, m	3.23, m
4′	3.27, m	3.45, m	3.38, m
5′	3.32, m	3.42, m	3.37, m
6′a	3.88, dd, 11.7, 2.2	3.85, dd, 11.9, 2.2	3.80, dd, 11.9, 2.1
6′b	3.65, dd, 11.7, 5.7	3.75, dd, 11.9, 4.9	3.67, dd, 11.9, 4.9

Table 3
^{13}C NMR data for compounds **1**–**6**.

No.	**1**[a]	**2**[a]	**2**[b]	**3**[a]	**4**[a]	**5**[a]	**6**[a]
1	36.4, t	38.7, t	37.7, t	36.6, t	122.4, d	114.7, d	123.7, d
2	66.8, d	70.6, d	68.2, d	80.3, d	83.3, d	148.2, s	201.0, s
3	85.8, d	91.6, d	89.8, d	77.9, d	76.8, d	143.3, s	80.2, d
4	40.9, d	41.9, d	39.1, d	43.2, d	39.8, d	131.5, s	47.0, d
5	129.9, s	130.2, s	128.4, s	130.1, s	138.1, s	127.8, s	72.9, s
6	33.8, t	32.5, t	30.7, t	32.3, t	127.7, d	33.4, t	44.1, t
7	43.2, d	42.4, d	40.0, d	41.7, d	44.3, d	43.6, d	40.6, d
8	28.8, t	27.8, t	26.3, t	27.6, t	28.1, t	29.1, t	32.8, t
9	31.5, t	30.4, t	29.1, t	30.3, t	29.0, t	30.9, t	33.3, t
10	126.0, s	125.8, s	124.2, s	125.7, s	136.9, s	134.7, s	166.2, s
11	151.1, s	150.1, s	148.5, s	149.8, s	149.3, s	151.1, s	150.4, s
12	109.2, t	109.7, t	109.2, t	109.6, t	111.9, t	109.5, t	110.0, t
13	20.9, q	21.5, q	20.9, q	21.4, q	21.6, q	20.9, q	21.2, q
15	18.5, q	17.1, q	16.4, q	17.2, q	14.0, q	12.8, q	10.6, q
1′	104.4, d	105.7, d	103.9, d	102.8, d	103.6, d	107.6, d	104.9, d
2′	74.7, d	75.5, d	73.7, d	74.9, d	74.9, d	75.6, d	74.7, d
3′	78.0, d	78.2, d	76.8, d	77.9, d	78.1, d	78.4, d	78.3, d
4′	71.6, d	71.5, d	69.9, d	71.5, d	71.6, d	71.2, d	71.1, d
5′	78.0, d	78.1, d	76.7, d	77.7, d	78.0, d	78.1, d	77.8, d
6′	62.6, t	62.6, t	60.9, t	62.6, t	62.6, t	62.5, t	62.5, t

[a] Data were reported in CD_3OD.
[b] Data were recorded in DMSO-d_6.

Nicotabacoside E (**5**): White powder, $[\alpha]_D^{20}$: +12.4 (c 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 280 (3.49) nm; ECD (MeOH) $\Delta\varepsilon_{195}$ + 5.12, $\Delta\varepsilon_{196}$ + 6.58, $\Delta\varepsilon_{208}$ − 3.05, $\Delta\varepsilon_{219}$ + 0.31, $\Delta\varepsilon_{230}$ + 0.72; IR (KBr) ν_{max} 3422, 1642, 1483, 1451, 1294, 1070 cm^{-1}; ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data (see Tables 2 and 3); HRESIMS m/z 403.1750 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_7Na$, 403.1727, +2.3 mDa).

Nicotabacoside F (**6**): White powder, $[\alpha]_D^{20}$: +54.8 (c 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.00) nm; ECD (MeOH) $\Delta\varepsilon_{195}$ + 1.53, $\Delta\varepsilon_{198}$ − 1.62, $\Delta\varepsilon_{215}$ + 0.93, $\Delta\varepsilon_{218}$ + 0.64, $\Delta\varepsilon_{221}$ + 0.91; IR (KBr) ν_{max} 3425, 1680, 1641, 1381, 1165, 1078, 1027 cm^{-1}; ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data (see Tables 2 and 3); HRESIMS m/z 421.1786 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_8Na$, 421.1833, −4.7 mDa).

2.4. Acid hydrolysis and sugar identification

Compounds **2**, **3**, **5** and **6** (each 1 mg) were hydrolyzed with 1.0 M HCl (MeOH–H_2O, 1:1, 1 ml) under reflux for 2 h. The reaction mixture was neutralized by $NaHCO_3$, and partitioned between CH_2Cl_2 and H_2O. The H_2O part was separated on a silica gel CC (8 g, 1.0 × 40 cm) using H_2O–MeOH–$CHCl_3$ (3:30:70) as the eluent to give glucose. The glucoses from compounds **2**, **3**, **5** and **6** were determined to be D-glucoses by PC comparison with the authentic sample (H_2O–AcOEt–BuOH 5:1:4, upper layer, R_f 0.45; H_2O–PhOH 1:4, R_f 0.50) that was visualized by spraying with phthalic acid–aniline reagent (1.66 g phthalic acid and 0.93 g aniline dissolved in 100 ml H_2O–sat. BuOH), followed by heating and their $[\alpha]_D^{21}$ values [+50.4 (c 0.09, MeOH), 49.8 (c 0.07,

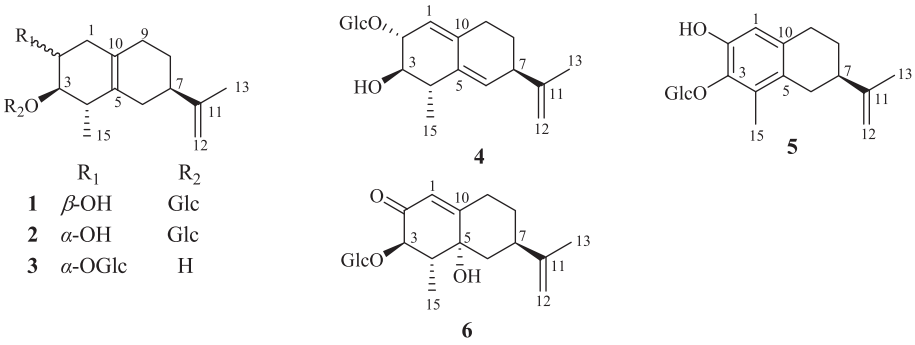

Fig. 1. The structures of compounds **1**–**6**.

MeOH), +51.0 (c 0.08, MeOH) and 49.2 (c 0.06, MeOH)], respectively [15].

3. Results and discussion

Nicotabacoside A (**1**) had a quasi-molecular ion peak at *m/z* 407.2040 $[M + Na]^+$ in the positive HRESIMS, indicating the molecular formula of $C_{20}H_{32}O_7$ with five degrees of unsaturation. Its IR spectrum exhibited hydroxyl (3423 cm^{-1}), double-bond (1642 cm^{-1}) and ether-bond (1077, 1031 cm^{-1}) groups. Two methyls (δ_H 1.73, s and 1.08, d, $J = 7.2$ Hz), two protons of exocyclic double-bond (4.71, s) as well as one anomeric proton (4.32, d, $J = 8.0$ Hz) were observed by its ^1H NMR spectrum. Besides a set of signals due to β-D-glucosyl group, its ^{13}C NMR (DEPT) spectrum showed 2 methyls, 5 methylenes, 4 methines and 3 quaternary carbons. The NMR data of its aglycone part (Tables 1 and 3) were similar to those of rishitin except for the obvious down-fielded shift of C-3 from 79.2 in rishitin to 85.8 in **1**, indicating that the glucosyl group was linked to C-3 [16–18]. This deduction was verified by the HMBC correlation from H-1′ (δ_H 4.32) to C-3 (δ_C 85.8). ROESY correlations of H-2 (3.88)/H-15 (1.08), H-3 (3.73)/H-15 (1.08) and H-4 (2.34)/H-6b (1.73)/H-12a (4.71) suggested that H-2, C-15 and H-7 were situated at the same face (Fig. 3). Finally, its absolute configuration was determined by an X-ray single-crystal diffraction to be 2*S*, 3*R*, 4*S* and 7*R* (Fig. 4).

Nicotabacoside B (**2**) showed a quasi-molecular ion peak at *m/z* 407.2053 $[M + Na]^+$ in the positive HRESIMS, suggesting the molecular formula of $C_{20}H_{32}O_7$. Hydroxyl (3473, 3406 cm^{-1}), double-bond (1643 cm^{-1}) and ether-bond (1079, 1031 cm^{-1}) groups were deduced by its IR spectrum. Acid hydrolysis of compound **2** provided a D-glucose moiety which was deduced by its $[\alpha]_D$ value [+50.4 (c 0.09, MeOH)]. In addition to a β-D-glucosyl group, the NMR data of its aglycone part were similar to those of rishitin except for the down-fielded shift of C-3 from 79.2 in rishitin to 89.8 in **2** [16–18], revealing that the glucosyl group was connected to C-3. This deduction was affirmed by the HMBC correlation from H-1′ (δ_H 4.24) to C-3 (δ_C 89.8). ROESY correlations (Fig. 3) of H-3 (δ_H 3.08)/H-15 (1.13), H-4 (2.12)/H-6b (1.65)/H-12a (4.69) indicated that its relative configuration of C-3, C-4, and C-7 was identical with those of compound **1**. Different from compound **1**, the ROESY correlations of H-2/Me-4 in compound **2** were not detected, and the correlations of H-2/H-1a and H-2/H-1b were observed, which suggested that OH-2 was α-oriented. In the ECD spectrum of **2**, the first cotton effect (CE) is positive and the second CE is negative, which is identical to those of compound **1**. Therefore, its absolute configuration was determined to be 2*R*, 3*R*, 4*S* and 7*R* (see Fig. S1 in Supporting Information).

Nicotabacoside C (**3**) had a molecular formula of $C_{20}H_{32}O_7$ by the quasi-molecular ion peak at *m/z* 407.2038 $[M + Na]^+$ in its positive HRESIMS. Its IR spectrum showed hydroxyl (3408 cm^{-1}), double-bond (1644 cm^{-1}) and ether-bond (1076, 1042, 1023 cm^{-1}) groups. The D-glucose from acid hydrolysis of compound **3** was revealed by its $[\alpha]_D$ value [49.8 (c 0.07, MeOH)]. The ^1H and ^{13}C NMR spectral data of **3** (Tables 1 and 3) were similar to those of **2** except for the obvious down-fielded shift of C-2 from 70.6 in **2** to 80.3 in **3** and up-fielded shift of C-3 from 91.6 in **2** to 77.9 in **3**, suggesting the glucosyl group at C-2 which was confirmed by

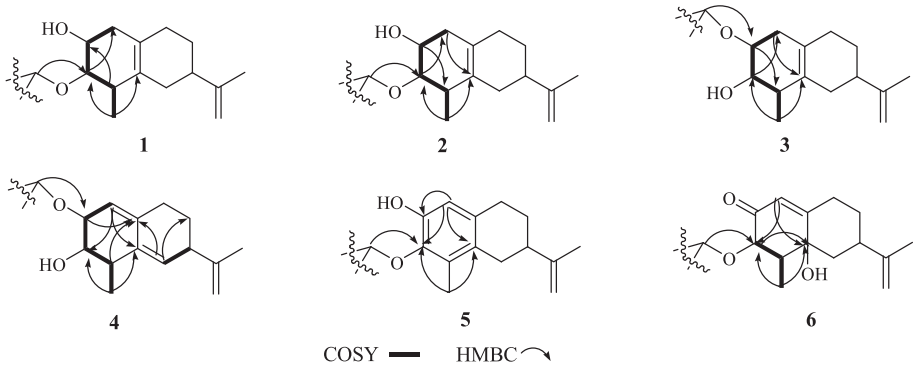

Fig. 2. The key ^1H–^1H COSY and HMBC correlations of compounds **1**–**6**.

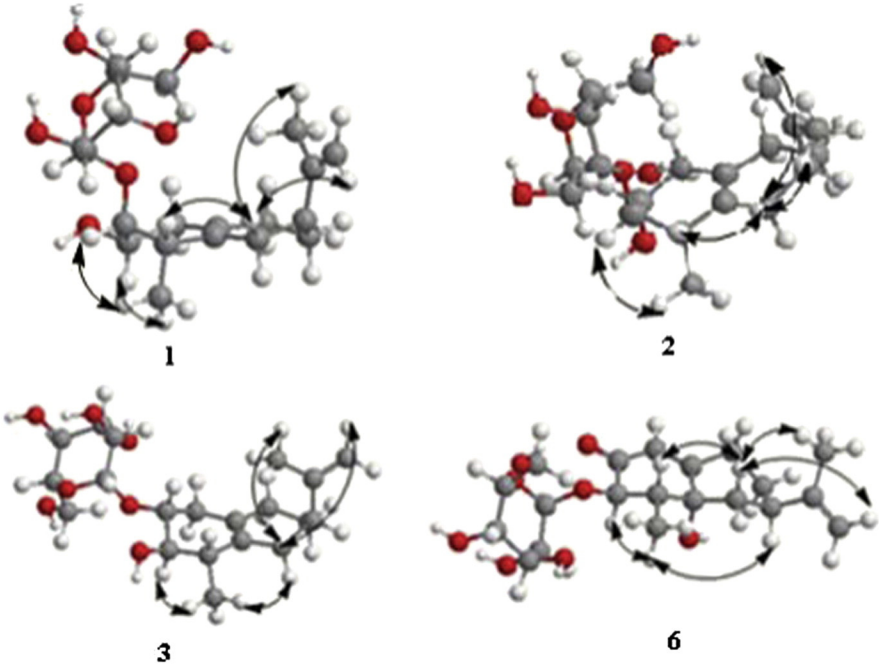

Fig. 3. The key ROESY correlations of compounds **1**–**3** and **6**.

HMBC correlation from H-1′ (δ_H 4.36) to C-2 (80.3). ROESY correlations of H-3 (δ_H 3.32)/H-15 (1.16)/H-6a (2.26) and H-6b (1.77)/H-12a (4.73) suggested that the C-2, C-3, C-4 and C-7 of **3** had the same relative configuration with those of **2**. Thus, the stereochemistry of **3** was determined to be 2*R*, 3*R*, 4*S* and 7*R* by comparing the ECD spectrum with **2** (see Fig. S1 in Supporting Information).

Nicotabacoside D (**4**) had a quasi-molecular ion at *m/z* 405.1879 $[M + Na]^+$ in the positive HRESIMS, indicating the molecular formula of $C_{20}H_{30}O_7$ with six degrees of unsaturation. The presence of conjugated double-bonds was speculated by its UV spectrum [λ_{max} (log ε) 241 (4.45) nm] while hydroxyl (3419 cm^{-1}), double-bond (1643 cm^{-1}) as well as ether-bond (1079, 1030 cm^{-1}) groups were revealed by its IR spectrum. The ^1H and ^{13}C NMR data of **4** were similar with **3** except that two methylenes (δ_C 36.6, C-1; 32.3, C-6) in **3** were replaced by two olefinic carbons (δ_C 122.4, C-1; 127.7, C-6) in **4**, suggesting that $\Delta^{5,10}$ double-bond in **3** was changed to be $\Delta^{1,10}$ and $\Delta^{5,6}$ double-bonds in **4**. This speculation was reinforced by the key ^1H–^1H COSY correlations of H-1 (δ_H 5.47)/H-2 (4.20) and H-6 (5.60)/H-7 (2.86) together with the key HMBC correlations of H-1 (5.47)/C-3 (δ_C 76.8), C-5 (138.1) and C-9 (29.0); H-6 (5.60)/C-4 (39.8), C-8 (28.1) and C-10 (136.9) (Fig. 2). In addition, the glucosyl group attaching to C-2 was confirmed by the HMBC correlation from H-1′ (4.41) to C-2 (83.3). The absolute configuration of compound **4** was elucidated as 2*R*, 3*R*, 4*S* and 7*R* by an X-ray single-crystal diffraction (Fig. 4).

Nicotabacoside E (**5**) had a molecular formula of $C_{20}H_{28}O_7$ with seven degrees of unsaturation by its positive HRESIMS which gave a quasi-molecular ion at *m/z* 403.1750 $[M + Na]^+$. The presence of hydroxyl (3422 cm^{-1}), aromatic ring (1642 cm^{-1}) and ether-bond (1294, 1070 cm^{-1}) groups was revealed by its IR spectrum. Acid hydrolysis of compound **5** produced the D-glucose by its $[\alpha]_D$ value [+51.0 (c 0.08, MeOH)]. Its ^1H NMR spectrum indicated two methyls (δ_H 1.80, s and 2.22, s), a penta-substituted phenyl ring (6.44, s) and one anomeric proton (4.46, d, J = 7.8 Hz). The ^{13}C NMR data showed the presence of one set of β-D-glucosyl signals

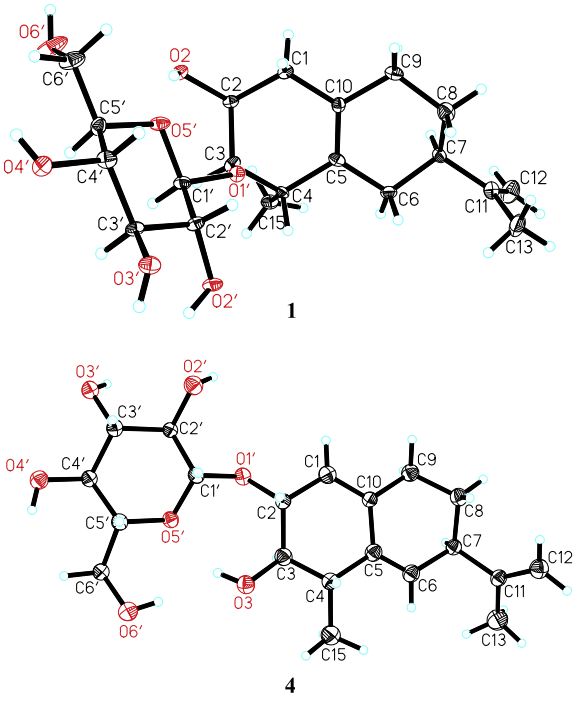

Fig. 4. X-ray crystal structures of compounds **1** and **4**.

(δ_C 107.6, 75.6, 78.4, 71.2, 78.1, 62.5). The ^{1}H and ^{13}C NMR data of its aglycone part were similar to those of ligudentatol [19] except that one methine (C-2) in ligudentatol was changed to be quaternary carbon in **5**, as well as the up-fielded shift of C-3 from 151.3 in ligudentatol to 143.3 in **5**. The above analyses suggested that one glucosyl group was connected to C-3 and one additional hydroxyl was located at C-2 in **5**, which was also confirmed by the HMBC correlations H-1′ (δ_H 4.46)/C-3 (143.3) and H-1 (6.44)/C-2 (δ_C 148.2), C-3 (143.3) and C-5 (127.8). Therefore, its absolute configuration was determined to be 7*R* by comparing its ECD spectrum with that of compound **4** (see Fig. S1 in Supporting Information).

Nicotabacoside F (**6**) had a molecular formula of $C_{20}H_{30}O_8$ with six degrees of unsaturation by means of positive HRESIMS which exhibited the quasi-molecular ion peak at *m/z* 421.1786 $[M + Na]^+$. Its IR spectrum exhibited hydroxyl (3425 cm^{-1}), carbonyl (1680 cm^{-1}) and ether-bond (1078, 1027 cm^{-1}) groups. Acid hydrolysis of compound **6** yielded the D-glucose which was revealed by its $[\alpha]_D$ value [+49.2 (c 0.06, MeOH)]. Its ^{1}H NMR spectrum showed signals of two methyls (δ_H 1.75, s and 1.23, d, *J*-6.7 Hz), one double-bond (5.79, s) and one anomeric proton (4.37, d, $J = 7.8$ Hz). Besides a set of β-D-glucosyl signals, its ^{13}C NMR (DEPT) data were similar with those of (+)-oxyglutinosone [20,21] except for the down-fielded shift of C-3 from 75.1 in (+)-oxyglutinosone to 80.2 in **6**, which suggested that one additional glucosyl group was assigned at C-3. The above inference was verified by HMBC correlation from H-1′ (δ_H 4.37) to C-3 (δ_C 80.2). The ROESY correlations of H-3 (δ_H 4.45)/H-15 (1.23)/H-6a (2.25) and H-4 (2.07)/H-6b (1.28)/H-12a (4.74) indicated that H-3, H-15, OH-5 and H-7 were situated on the same side, consistent with the relative configuration of (+)-oxyglutinosone (Fig. 3). Consequently, its absolute configuration was determined to be 3*R*, 4*R*, 5*R* and 7*R* by comparing its ECD spectrum with that of compound **4** (see Fig. S1 in Supporting Information).

The known compounds **7**–**11** were identified to be actinidioionoside (**7**) [22,23], byzantionoside B (**8**) [24], (*Z*)-4-[3′-(β-D-glucopyranosyloxyl) butylidene]-3, 5, 5-trimethyl-2-cyclohexen-l-one (**9**) [25], (*6R*, *9R*)-3-oxo-α-ionol β-D-glucopyranoside (**10**) and (*6R*, *9S*)-3-oxo-α-ionol β-D-glucopyranoside (**11**) [26] by comparing their spectral data with literatures.

Compounds **1**–**6** are six new 14-noreudesmane sesquiterpenoid glycosides, which enrich the constituents of nor-sesquiterpenoids in *N. tabacum*. Compounds **3** and **7**–**11** were evaluated for the agonistic effects on human melatonin receptor 1 (MT_1) on HEK/293/MT_1 cell lines and human melatonin receptor 2 (MT_2) on HEK/293/MT_2 cell lines in vitro. However no obviously agonistic activity was observed at the tested concentrations (see Table S1 in Supporting Information).

Conflict of interest statement

There are no conflicts of interests among all authors in this manuscript.

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Appendix A. Supplementary data

HRESIMS, UV, IR, ^{1}H, ^{13}C (DEPT), 2D NMR (HSQC, HMBC, ^{1}H–^{1}H COSY and ROESY) and ECD spectra for nicotabacosides A–F, X-ray crystal structures of nicotabacosides A and D, bioassay of compounds **3** and **7**–**11** on MT_1 and MT_2 as well as the eluent for each fraction are available as Supporting Information. Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2014.04.010.

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