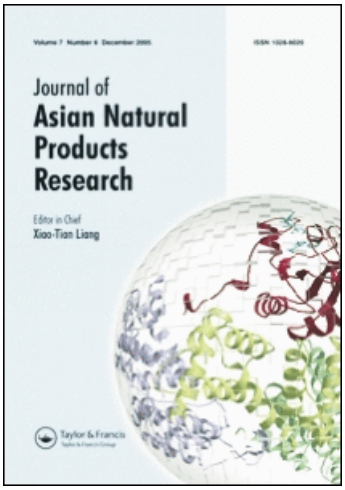

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Two new sesquiterpene glucosides from the leaves of *Nicotiana tabacum*

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Two new sesquiterpene glucosides from the leaves of *Nicotiana tabacum*

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Two new sesquiterpene glycosides, 11*R*,12-dihydroxy-6(7)-spirovetiven-8-one-9-*O*-β-D-glucopyranoside (**1**) and rishitin M1-13-*O*-β-D-glucopyranoside (**2**), have been isolated from the leaves of *Nicotiana tabacum*. Their structures were established by spectroscopic methods including ^{1}H, ^{13}C, and 2D NMR. The absolute configuration of the side chain in compound **1** was determined as *R* by NOESY and NOE difference spectra.

Keywords: *Nicotiana tabacum*; sesquiterpene glycoside; absolute configuration; spirovetiven; rishitin; Solanaceae

1. Introduction

Nicotiana tabacum is an important economic crop originating from South America [1]. Its leaves are used as a raw material for the tobacco industry, aerial plant as an insecticide, and also as anesthetic, diaphoretic, sedative, and emetic agents in Chinese folklore medicine [1]. Whereas a large number of volatile compounds and sesquiterpenoids have been found in tobacco [2], only few non-volatile compounds and sesquiterpene glycosides have been reported. Sesquiterpene glycosides were difficult to isolate, so previously these compounds were proved to be present either by hydrolysis and then the isolation of the aglycones [3] or by acetylation and then followed by GC analysis [4]. These methods were tedious and prone to breakdown the structure of sesquiterpene glycosides. In our continuing endeavor to discover new bioactive natural products, an investigation of the leaves of *N. tabacum* was undertaken. As a result, two new sesquiterpene glycosides, 11*R*,12-dihydroxy-6(7)-spirovetiven-8-one-9-*O*-β-D-glucopyranoside (**1**) and rishitin M1-13-*O*-β-D-glucopyranoside (**2**) (Figure 1), were isolated by successive chromatographic methods and final preparative HPLC purification. Their structures were determined mainly by spectroscopic methods, especially 2D NMR.

2. Results and discussion

Compound **1** was obtained as a viscous oil and gave a quasi-molecular ion $[M+Na]^+$at *m/z* 453.2087 in the HR-ESI-MS, consistent with the elemental composition $C_{21}H_{34}O_9Na$. The fragment ion at *m/z* 291 $[M+Na-162]^+$ suggested the presence of a hexose. The ^{1}H NMR spectrum of **1** revealed the presence of one doublet methyl group at δ_H 0.94 (d, $J = 6.7$ Hz), two singlet methyl groups at δ_H 1.47 (s) and 1.82 (s), one olefinic proton at δ_H 5.81 (s), and one anomeric proton at δ_H 5.13 (d, $J = 7.4$ Hz). The presence of an AB system composed of protons at δ_H 3.78

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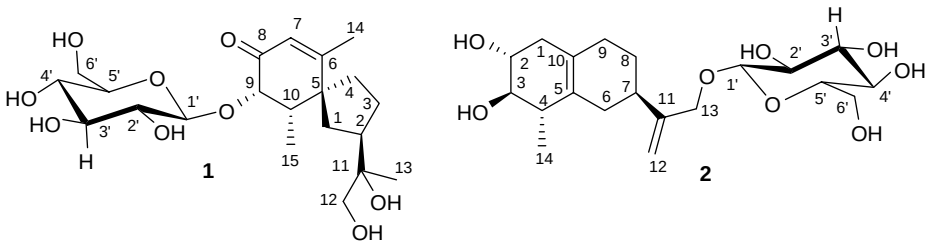

Figure 1. Structures of compounds **1** and **2**.

and 3.83 with a coupling constant of 10.5 Hz was characteristic of an oxymethene carbon attaching to a chiral carbon. Its ^{13}C NMR spectrum exhibited 21 carbons, including one anomeric carbon (δ_C 105.6) and five oxygenated carbons (δ_C 78.6, 78.5, 75.9, 71.6, and 62.8) assignable to a glucopyranosyl moiety, suggesting a sesquiterpene glucoside. The β-configuration for the glucopyranose was determined from a large coupling constant value (7.4 Hz) of the anomeric proton at δ_H 5.13. On acid hydrolysis, **1** afforded glucose, which was identified by co-TLC with standard monosaccharide.

The ^{13}C NMR spectrum also showed signals belonging to the aglycone: one α,β-unsaturated carbonyl group (δ_C 199.3, 166.6, and 124.5), one quaternary carbon (δ_C 52.8), and three methyl carbons at δ_C 9.5, 20.1, and 23.8, showing HMQC correlations with three methyl groups at δ_H 0.94, 1.82, and 1.47, respectively. The NMR spectral data of the aglycone of **1** (Table 1) were consistent with those of 9,11,12-trihydroxy-6(7)-spirovetiven-8-one [5], except that the signal of C-9 was shifted downfield by 7.3 ppm and C-15 upfield by 2.8 ppm [6], suggesting the site of glycosidation to be C-9. This conclusion was further confirmed by the HMBC correlation (Figure 2) from the anomeric proton (δ_H 5.13) of glucose with C-9 of aglycone at δ_C 81.4.

In the NOESY spectrum (Figure 3) of **1**, the cross-peaks distinguished H-1β, H-3β, and H-4β on the C-11 side of the cyclopentane ring from H-2, H-1α, H-3α, and H-4α on the opposite side. The NOESY cross-peak from H-9 to H-10 suggested the homonymy of the two protons, and the coupling constant ($J = 4.0$ Hz) between H-10 and H-9 showed that the cyclohexenone adopted a half-chair conformation with Me-15 predominantly in a pseudoaxial position, for the bulky group of glucose at the equatorial position is energy preferred (Figure 3). Significant correlations were observed between H-1α and H-10, and between H-4β and Me-14.

The configuration of C-11 was determined according to the published method [7] which was authenticated by CD exciton chirality, using the NOESY spectrum. The relative intensities (Table 2) of the cross-peaks between Me-13 and the neighboring protons (Figure 4) in the NOESY spectrum suggested that Me-13 is closer to C-3 than to C-1, and thus concluded that the chiral C-11 of the aglycone is in the *R* configuration. The conclusion was further supported by the NOE difference spectra (Table 2) as NOEs observed in it were not interfered by overlapped protons and thus helped to give more reliable evidence than the NOESY spectrum. Therefore, compound **1** was determined as 11*R*,12-dihydroxy-6(7)-spirovetiven-8-one-9-*O*-β-D-glucopyranoside.

Compound **2** was obtained as a viscous oil. The molecular formula, $C_{20}H_{32}O_8$, was inferred from the positive HR-ESI-MS at *m/z* 423.1997 $[M+Na]^+$. Its ^{13}C

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data of **1** and **2** in C_5D_5N.

	1		**2**	
No.	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)
1	36.6	H_α 1.98 (dd, 11.1, 12.4), H_β 2.43–2.47 (m)	39.5	2.36–2.41 (m)
2	46.1	2.33–2.40 (m)	71.6	4.23–4.25 (m)
3	27.6	H_α 1.68–1.72 (m), H_β 1.82–1.88 (m)	79.2	3.60 (dd, 8.5, 9.1)
4	35.5	H_β 1.37–1.41 (m), H_α 1.88–1.92 (m)	42.6	2.36–2.41 (m)
5	52.8	–	129.6	–
6	166.6	–	31.7	1.79–1.88 (m), 2.36–2.41 (m)
7	124.5	5.81 (s)	36.2	2.63 (m)
8	199.3	–	26.8	1.52–1.55 (m), 1.62–1.68 (m)
9	81.4	5.12 (d, 4.0)	29.7	1.79–1.88 (m)
10	46.3	2.47–2.51 (m)	125.4	–
11	73.2	–	149.2	–
12	69.9	3.78 (d, 10.5), 3.83 (d, 10.5)	110.6	4.97 (s), 5.43 (brs)
13	23.8	1.47 (s)	71.0	4.36 (d, 12.0), 4.65 (d, 12.0)
14	20.1	1.82 (s)	16.8	1.35 (d, 6.6)
15	9.5	0.94 (d, 6.7)		
1′	105.6	5.13 (d, 7.4)	103.6	4.95 (d, 7.5)
2′	75.9	4.14 (dd, 8.3, 7.4)	75.2	4.06 (dd, 7.5, 8.7)
3′	78.5	3.87 (dd, 8.3, 9.5)	78.5	3.96 (dd, 8.7, 9.4)
4′	71.6	4.26 (dd, 9.5, 9.1)	71.7	4.24 (dd, 9.4, 8.1)
5′	78.6	4.21 (m)	78.6	4.24 (m)
6′	62.8	4.51 (dd, 1.8, 11.5), 4.29 (dd, 5.6, 11.5)	62.8	4.37 (dd, 12.3, 6.8), 4.55 (dd, 12.3, 2.1)

NMR spectrum showed 20 carbons, including four olefinic ones (δ_C 149.2, 129.6, 125.4, and 110.6). The ^{1}H NMR spectrum of **2** revealed the presence of one methyl group at δ_H 1.35 (d, $J = 6.6$ Hz) and two olefinic protons at δ_H 5.43 (brs) and 4.97 (s) which correlated with one olefinic carbon at δ_C 110.6 in HMQC, revealing the presence of one exo-olefinic carbon. The ^{1}H and ^{13}C NMR spectra (Table 1) showed signals of a glucopyranosyl moiety (Table 1), indicating that **2** is a norsesquiterpene glucoside. A large coupling constant ($J = 7.5$ Hz) for the anomeric proton (δ_H 4.95) of the glucose in the ^{1}H NMR spectrum suggested a β-configuration for the glucose unit. The ^{13}C NMR spectrum of the aglycone of **2**

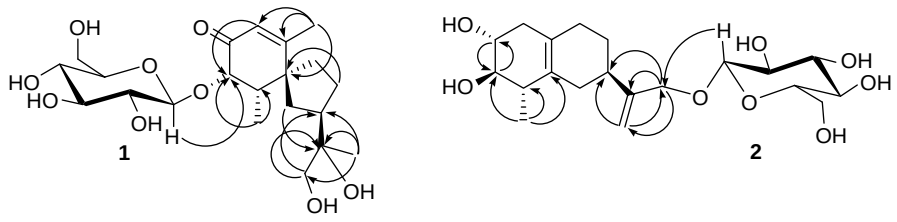

Figure 2. Key HMBC correlations of compounds **1** and **2**.

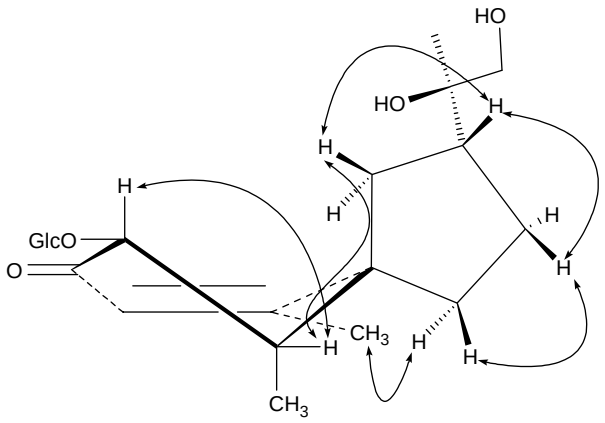

Figure 3. Key NOESY correlations for compound **1**.

(Table 1) was similar to those of rishitin M1 [8,9], except for the signal of C-13 (δ_C 71.0), which was downfield shifted severely from that at δ_C 66.2 of rishitin M1. In the HMBC spectrum (Figure 2), the carbon at δ_C 71.0 correlated with the anomeric proton at δ_H 4.95 (d, $J = 7.5$ Hz), which demonstrated that the glucose was attached to C-13. Thus, compound **2** was elucidated as rishitin M1-13-*O*-β-D-glucopyranoside.

3. Experimental

3.1 General experimental procedures

Optical rotations were recorded on a JASCOP-1020 polarimeter. The IR spectra were measured on a Bruker Tensor-27 spectrometer with KBr disk. The UV spectra were obtained on a Shimadzu UV-2450 spectrophotometer. Mass spectra were obtained on an MS Agilent 1100 series LC/MSD ion trap mass spectrometer (ESI-MS), and the positive-ion HR-ESI-MS was performed on a Mariner ESI-TOF spectrometer. The NMR spectra were obtained on Bruker DRX-500 spectrometers (500 and 125 MHz, respectively). HPLC separations were performed on an Agilent 1100 series instrument with a Shim-park RP-C18 column (200 × 20 mm i.d.) and a UV detector at 210 and 254 nm. Column chromatography was performed on silica gel (Qingdao Haiyang Chemical Co. Ltd, Qingdao, China), ODS-C18 (Fuji Silysia Chemical

Table 2. NOE correlations between the CH_3-13 and neighboring protons for compound **1** in C_5D_5N.

	NOESY data		NOE difference spectra	
H	δ	Area[a]	δ	Area[b]
1α	1.98	26	1.98	34
1β	2.43–2.47	12	2.43–2.47	–
2	2.33–2.40	162	2.33–2.40	85
12a	3.78	50	3.78	60
12b	3.83	50	3.83	60
3α	1.68–1.72	217	1.68–1.72	53
3β	1.82–1.88	593	1.82–1.88	100

[a] Relative areas of the cross-peaks.
[b] Enhancement of the NOE interaction.

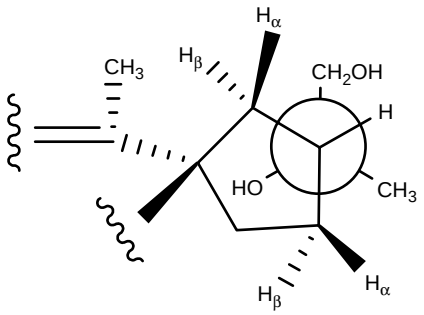

Figure 4. Staggered conformer of compound **1**.

Ltd, Aichi, Japan), and MCI (Mitsubishi Chemical Corporation, Tokyo, Japan).

3.2 Plant material

The leaves of *N. tabacum* were collected in Kunming City, Yunnan Province, China, in September, 2006. The plant material was identified by Prof. Min-jian Qin, Department of Medicinal Plants, China Pharmaceutical University and a voucher specimen (No. 060916) is deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

3.3 Extraction and isolation

The leaves of *N. tabacum* (20 kg) were extracted thrice with MeOH at 60°C for 6 h. The MeOH extract was concentrated under reduced pressure to give a residue (500 g), which was suspended in MeOH–H_2O (1:1), and then partitioned with petroleum ether, ethyl acetate, and *n*-butanol, respectively. The *n*-butanol extract (65 g) was chromatographed over a silica gel column (100–200 mesh), and eluted with $CHCl_3$–MeOH (100:0, 100:2, 100:5, 100:10, 100:20, 100:30, 100:50, and 0:100) to give fractions 1–9. Fraction 6 (10 g) was subjected to an MCI column (5 × 20 cm), and eluted successively with H_2O, 10% MeOH, 30% MeOH, 50% MeOH, 70% MeOH, and 100% MeOH. The 50% MeOH eluate (120 mg) was subjected to an RP-18 column and eluted by MeOH–H_2O from 20 to 50% to give four subfractions, and the third subfraction (14 mg) was purified by prep-HPLC (column: 10 × 250 mm, RP-18, flow rate: 10 ml/min) eluted with MeCN–H_2O (20:80) to afford **2** (4 mg). Compound **1** (8 mg) was obtained likewise from the fourth subfraction (40 mg) by prep-HPLC eluted with MeCN–H_2O (25:75).

3.3.1 Compound *1*

A colorless oil; $[\alpha]_D^{25}$ − 73.2 ($c = 0.2$, MeOH); UV (MeOH) λ_{max} (log ε): 244 (4.50), 202 (4.31) nm; IR (KBr): 3422, 2967, 2927, 2881, 1675 cm^{-1}; ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N) spectral data, see Table 1; HR-ESI-MS *m/z*: 453.2087 $[M+Na]^+$ (calcd for $C_{21}H_{34}O_9Na$, 453.2095).

3.3.2 Compound *2*

A colorless oil; $[\alpha]_D^{25}$ − 22.1 ($c = 0.15$, MeOH); IR (KBr): 3417, 2924, 1078, 1035 cm^{-1}; ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N) spectral data, see Table 1; HR-ESI-MS *m/z*: 423.1997 $[M+Na]^+$ (calcd for $C_{20}H_{32}O_8Na$, 423.1989).

3.4 Hydrolysis experiments

Each of the compounds (2 mg) was hydrolyzed using 1% HCl at 95°C for 3 h. The reaction mixture was neutralized with Na_2CO_3, and then the liberated sugar moiety was detected with standard D-glucose using silica gel TLC plate with *n*-butanol–acetone–H_2O (5:4:1) as a developing solvent. Phenylamine/*o*-phthalic acid was used as a spraying reagent for color (yellow) detection of glucose.

Acknowledgements

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