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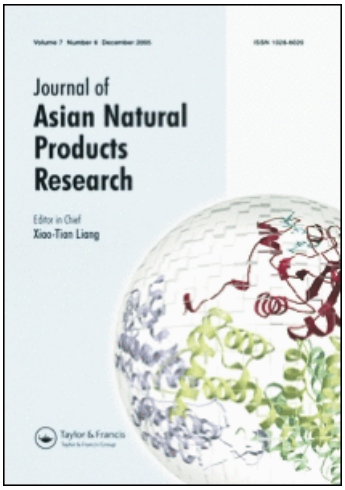

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A pair of sesquiterpene glucosides from the leaves of *Nicotiana tabacum*

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NOTE

A pair of sesquiterpene glucosides from the leaves of *Nicotiana tabacum*

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A pair of sesquiterpene glucosides, 3-hydroxysolavetivone-β-D-glucoside A (**1**) and 3-hydroxysolavetivone-β-D-glucoside B (**2**), have been isolated from the leaves of *Nicotiana tabacum*. The former is a new compound, while the latter is a known one. Their structures were established by spectroscopic methods including ^{1}H, ^{13}C, and 2D NMR. The relative configuration of C-3 in compound **2** was revised by NOESY experiment.

Keywords: *Nicotiana tabacum*; sesquiterpene glucoside; 3-hydroxysolavetivone; Solanaceae

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1. Introduction

Nicotiana tabacum L. belongs to Solanaceae family and is an important economic crop originating from South America [1]. Its leaves are used as raw material for the tobacco industry, aerial plant as insecticide, and also as anesthetic, diaphoretic, sedative, and emetic agents in Chinese folklore medicine [1]. The Solanaceae family is one of the richest sources of sesquiterpenoids and their glycosides [2,3]. Previous phytochemical studies on *N. tabacum* are mainly focused on aglycones because of the difficulty in the isolation of the sesquiterpene glycosides, the presence of which were mostly proved indirectly by hydrolysis and isolation of the aglycones [4]. In this work, an investigation is undertaken to research genuine sesquiterpene glycosides originating from the leaves of *N. tabacum* by chromatographic process without derivatization. As a result, a pair of sesquiterpene glucosides, 3-hydroxysolavetivone-β-D-glucoside A (**1**), a new compound, and 3-hydroxysolavetivone-β-D-glucoside B (**2**), a known one, were isolated by successive chromatographic methods and final preparative HPLC purification (Figure 1). Their structures were determined mainly by spectroscopic methods, especially 2D NMR. The two compounds are epimers at C-3 and the relative configuration of C-3 in **2** reported in the literature [5] should be revised.

2. Results and discussion

Compound **1** was obtained as a viscous oil and gave a quasi-molecular ion $[M+Na]^+$ at *m/z* 419.2036 in the HR-ESI-MS, consistent with the elemental composition $C_{21}H_{32}O_7Na$. The ^{1}H NMR spectrum of **1** revealed the presence of one doublet methyl group at δ_H 1.15 (d, $J = 6.9$ Hz), two singlet methyl groups at δ_H 1.72 (s) and 1.76 (s), one olefinic proton

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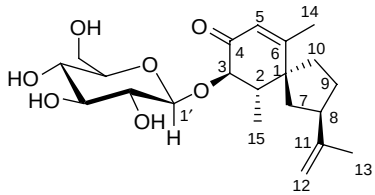

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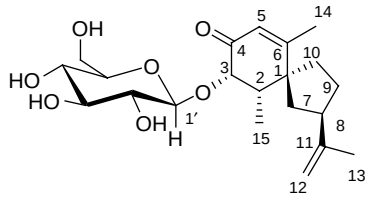

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Figure 1. Structures of compounds **1** and **2**.

at δ_H 5.90 (s), and two exo-olefinic protons at δ_H 4.81 (br s) and 4.78 (br s). Analysis of the ^{13}C NMR spectrum, which has 21 signals, allowed the identification of one α,β-unsaturated carbonyl group at δ_C 197.4, 168.7, 124.5, one terminal double bond at δ_C 147.8, 109.1, one quaternary carbon at δ_C 51.2 [5], and three methyl carbons at δ_C 13.6, 21.4, 21.4, showing HMQC correlations with three methyl groups at δ_H 1.15, 1.72, and 1.76, respectively. The presence of one sugar was confirmed from one anomeric proton at δ_H 5.17 (d, $J = 8.1$ Hz), one anomeric carbon at δ_C 103.5, and five oxygenated carbons at δ_C 74.8, 78.4, 71.7, 78.8, 63.0. All the spectral data suggested that **1** was a spirovetiven-type sesquiterpene glycoside [6]. The location of the sugar moiety at C-3 was established according to the correlation observed between H-1′ (at δ 5.17) and C-3 (at δ 80.4) in the HMBC experiment of **1** (Figure 2). On acid hydrolysis, **1** afforded glucose, which was identified by co-TLC with standard monosaccharide. The β-configuration for the glucose was determined from a large coupling constant value (8.1 Hz) of the anomeric proton at δ_H 5.17.

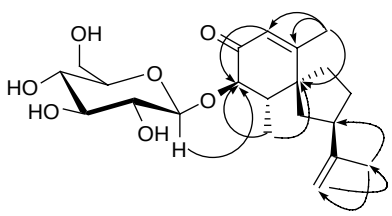

Figure 2. Key HMBC correlations of compound **1**.

The NMR spectral data of **1** were similar to those of the previously reported 3-hydroxysolavetivone-β-D-glucoside B (**2**), a sesquiterpene glucoside isolated from *N. tabacum* [5]. The main differences between the two compounds were that the two signals of the exo-olefinic proton in **1** were changed to a singlet in **2**, the H-3 was downfield shifted from δ_H 4.50 (d, $J = 8.1$ Hz) in **1** to δ_H 5.10 (d, $J = 4.5$ Hz) in **2**, and one of the methyl carbon signal at δ_C 13.6 in **1** was upfield shifted to δ_C 9.5 in **2**. These variations resulted from the opposite configuration of C-3 in the two compounds, which was proved by the NOESY spectra (Figure 3) of **1** and **2**.

In compound **1**, the NOESY cross-peak from H-3 to Me-15 suggested that H-3 and Me-15 are on the same side and the coupling constant ($J = 8.1$ Hz) between H-2 and H-3 showed that the cyclohexenone of **1** (Figure 3) adopted a half-chair conformation with H-2 and H-3 in a pseudoaxial position, since the bulky groups of glucose and methyl preferred an equatorial position. Consequently, **1** was named 3-hydroxysolavetivone-β-D-glucoside A. In compound **2**, H-3 and H-2 have the NOESY cross-peak while H-3 and Me-15 do not correlate in the NOESY spectrum, which suggested that H-3 and Me-15 are on the opposite side, and so the relative configuration of C-3 in **2** [5] should be revised to α and renamed as 3-hydroxysolavetivone-β-D-glucoside B.

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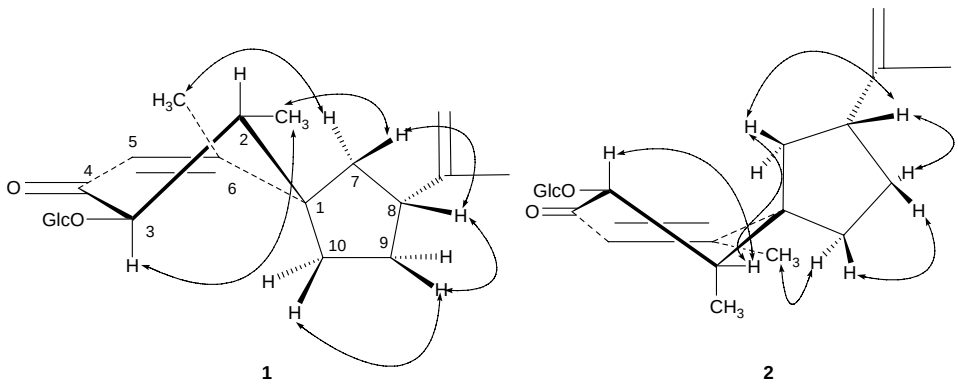

Figure 3. Key NOESY correlations of compounds **1** and **2**.

Previously, researchers proposed that only one of the 3-epimers of spirovetiven-type sesquiterpene glycoside, 3-β form, was presented in *N. tabacum* [6]. The two compounds were the first example of the co-existence of spirovetiven epimers at C-3.

3. Experimental

3.1 General experimental procedures

Optical rotations were recorded on JASCOP-1020. The IR spectra were measured on a Bruker Tensor-27 spectrometer with a KBr disk. UV spectra were obtained on a Shimadzu UV-2450 spectrophotometer. Mass spectra were obtained on a MS Agilent 1100 series LC/MSD ion trap mass spectrometer (ESI-MS), and positive-ion HR-ESI-MS was performed on a Mariner ESI-TOF spectrometer. The NMR spectra were obtained on Bruker DRX-500 (^{13}C NMR) and DRX-300 (^{1}H NMR) spectrometers. 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) and ODS-C18 (Fuji Silysia Chemical Ltd, Aichi, 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 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 three times 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 ethyl acetate extract (200 g) was chromatographed over a silica gel column (100–200 mesh), eluted with petroleum ether–acetone (100:0, 100:2, 100:4, 100:10) and $CHCl_3$–MeOH (100:0, 100:2, 100:5, 100:10, 100:20, 100:30, 100:50, 0:100), and combined according to TLC results to give fractions 1–15. Fraction 13 (15 g) was subjected to an ODS-18 column (5 × 20 cm), and eluted successively with MeOH–H_2O from 20 to 80% to give four subfractions, and the third subfraction

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Table 1. ^{1}H NMR (300 MHz) and ^{13}C NMR (125 MHz) spectral data of **1** and **2** in C_5D_5N.

	1		**2**	
No.	δ_C	δ_H	δ_C	δ_H
1	51.2	–	52.4	–
2	47.4	2.53 (m)	46.4	2.43 (m)
3	80.4	4.50 (d, $J = 8.1$ Hz)	81.2	5.10 (d, $J = 4.5$ Hz)
4	197.4	–	198.1	–
5	124.5	5.90 (s)	124.7	5.83 (s)
6	168.7	–	165.4	–
7	42.1	1.69–1.76 (m), 2.48–2.54 (m)	40.6	1.64–1.73 (m), 2.43–2.48 (m)
8	44.4	2.33–2.38 (m)	46.4	2.32–2.39 (m)
9	32.5	1.85–1.92 (m), 1.42–1.56 (m)	32.2	1.76–1.80 (m), 1.42–1.49 (m)
10	33.9	1.26–1.35 (m), 1.67–1.72 (m)	35.7	1.30–1.38 (m), 1.60–1.67 (m)
11	147.8	–	147.3	–
12	109.1	4.81 (br s), 4.78 (br s)	109.3	4.76 (2H, s)
13	21.4	1.72 (s)	21.1	1.67 (s)
14	21.4	1.76 (s)	19.8	1.72 (s)
15	13.6	1.15 (d, $J = 6.9$ Hz)	9.5	0.97 (d, $J = 6.6$ Hz)
1′	103.5	5.17 (d, $J = 8.1$ Hz)	105.6	5.27 (d, $J = 7.8$ Hz)
2′	74.8	4.06 (m)	76.0	4.17 (m)
3′	78.4	3.96 (m)	78.7	3.94 (m)
4′	71.7	4.31 (m)	71.5	4.37 (m)
5′	78.8	4.22 (m)	78.6	4.25 (m)
6′	63.0	4.34 (m), 4.56 (m)	62.7	4.33 (m), 4.53 (m)

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(62 mg) was purified by prep-HPLC (column: 10 × 250 mm, RP-18, flow rate: 10 ml/min) eluted with MeCN–H_2O (30:70) to afford **1** (4 mg) and **2** (8 mg).

3.3.1 *Compound **1***

Colorless oil; $[\alpha]_D^{25}$ −12.2 ($c = 0.21$, MeOH); UV (MeOH) λ_{max} (log ε): 247 (4.30) nm; IR ν_{max} (KBr) cm^{-1}: 3417, 2963, 1674; ^{1}H NMR (300 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N) spectral data: see Table 1; HR-ESI-MS *m/z*: 419.2036 $[M+Na]^+$ (calcd for $C_{21}H_{32}O_7Na$, 419.2040).

3.3.2 *Compound **2***

Colorless oil; $[\alpha]_D^{25}$ −90.6 ($c = 0.17$, MeOH); UV (MeOH) λ_{max} (log ε): 247 (4.27) nm; IR ν_{max} (KBr) cm^{-1}: 3347, 2957, 1696; ^{1}H NMR (300 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N) spectral data: see Table 1; ESI-MS *m/z*: 397 $[M+H]^+$.

3.4 *Hydrolysis experiments*

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

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