Sesquiterpene Glucosides from *Nicotiana tabacum* and Their Biological Activity

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A new sesquiterpene glucoside (**1**), together with two known sesquiterpene glucosides (**2-3**) were isolated from the leaves of *Nicotiana tabacum*. Their structures were elucidated by spectroscopic methods, including extensive ¹D and ²D NMR techniques. Compounds **1-3** were tested for their anti HIV-1 activities and cytotoxicity. The results showed that compounds **1-3** have weak cytotoxic abilities and anti HIV-1 bioactivities, respectively.

Key Words: *Nicotiana tabacum*, Sesquiterpene glucosides, Anti HIV-1 activity, Cytotoxicity.

INTRODUCTION

Nicotiana tabacum L. belongs to Solanaceae family. It is one of the most commercially valued agricultural crops in the world^{1,2}. In addition to being used in cigarette industry, *N. tabacum* is also used as insecticide, anesthetic, diaphoretic, sedative and emetic agents in Chinese folklore medicine because of containing many useful chemical compounds^{1,3-5}.

In previous work, a number of bioactive compounds, such as sesquiterpenes^{6,7}, diterpenoids⁸⁻¹⁰, alkaloids^{11,12}, phenols¹³ and their homologous, were isolated from this plant. Motivated by search for bioactive metabolites from this plant, the investigation on the chemical constituents of the leaves of *N. tabacum* was carried out. As a result, a new sesquiterpene glucoside (**1**), together with two known sesquiterpene glucosides (**2-3**), were isolated from this plant. In addition, the anti HIV-1 activities and cytotoxicities of compounds **1-3** were evaluated, respectively. This work deals with the isolation, structural elucidation and biological activities of the compounds.

EXPERIMENTAL

General experimental procedures: Optical rotation was measured in Horiba SEPA-300 High Sensitive Polarimeter. IR spectra were obtained in KBr disc on a Bio-Rad Wininfrared spectrophotometer. ESI-MS were measured on a VG Auto Spec-3000 MS spectrometer. ¹H, ¹³C and ²D NMR spectra were recorded on Bruker DRX-500 instrument with TMS as internal standard. Column chromatography was performed on silica gel (200-300 mesh), or on silica gel H (10-40 mm, Qingdao

Marine Chemical Inc., China). Second separate was used an Agilent 1100 HPLC equipped with ZORBAX-C₁₈ (21.2 nm × 250 nm, 7.0 μm) column and DAD detector.

Plant material: The leaves of *nicotiana tabacum* L (tobacco leaves) was collected from Yuxi County, Yunnan Province, P.R. China, in September 2009.

Extraction and isolation: The air-dried and powdered leaves of *nicotiana tabacum* (2.5 kg) were extracted with 70 % aqueous ethanol (3.0 L × 3 L, 24 h each) at room temperature and the extract was concentrated under vacuum condition. The dried extract (68.5 g) was applied to Si gel (200-300 mesh) column chromatography eluting with a CHCl₃-Me₂CO gradient system (9:1, 8:2, 7:3, 6:4, 5:5, 2:1) to give six fractions A-F. Fraction A3 (7:3, 7.21 g) was subjected to silica gel column chromatography using CHCl₃-MeOH and preparative HPLC (30 % MeOH-H₂O, flow rate 12 mL/min) to give **1** (22.6 mg), **2** (39.8 mg) and **3** (33.5 mg).

Anti HIV-1 assay: The cytotoxicity assay against C8166 cells (CC50) was assessed using the MTT method and anti HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC₅₀)¹⁴. Compound **1** shows anti HIV-1 activity with EC₅₀ of 5.22 μg/mL, CC50 of above 200 μg/mL and TI (therapeutic index) value of above 38.3. Compound **2** shows anti HIV-1 activity with EC₅₀ of 4.73 μg/mL, CC50 of 105.6 μg/mL and TI of 20.58. Compound **3** shows anti HIV-1 activity with EC₅₀ of 6.15 μg/mL, CC50 of 88.5 μg/mL and TI value of 14.4.

Cytotoxicity assays: The cytotoxicity tests for the isolates were performed using a previously reported procedure¹⁵. All

treatments were performed in triplicate. In the MTT assay, the IC_{50} was defined as the concentration of the test compound resulting in a 50 % reduction of absorbance compared with untreated cells. The cytotoxic abilities against HL-60, Hep-G2, KB and MDA-MB-231 tumor cell lines by MTT-assay (with camptothecin as the positive control) were shown in Table-1.

TABLE-1
CYTOTOXICITIES OF COMPOUNDS 1-3

Compounds	Cell lines			
	HL-60	HepG2	KB	MDA-MB-231
1	4.42	6.05	2.22	15.50
2	5.90	13.8 3	5.96	4.21
3	5.50	7.6 8	5.41	11.29
Camptothecin	1.78	1.01	1.68	2.26

Data are IC_{50} values in $\mu\text{mol/L}$. For a compound to be deemed effective, an IC_{50} value $< 100 \mu\text{mol/L}$ is required. Camptothecin was used as a positive control. HL-60, human acute promyelocytic leukemia; Hep-G2, human hepatocellular carcinoma; KB, human oropharyngeal epidermoid carcinoma; MDA-MB-231, human breast cancer cells.

Nicotterpene A: Obtained as a viscous oil; $[\alpha]_{24.5}^{D_{24.5}} -15.8$ (c 0.22, MeOH); UV (MeOH), λ_{max} (log ϵ) 248 (3.86), 210 (4.38) nm; IR (KBr, ν_{max} , cm^{-1}): 3418, 2968, 2870, 1682, 1634, 1550, 1462, 1435, 972, 875; ^1H and ^{13}C NMR data ($\text{C}_5\text{D}_5\text{N}$, 500 and 125 MHz), Table-2; ESIMS (positive ion mode) m/z 435; HRESIMS (positive ion mode) m/z 435.1900 $[\text{M} + \text{Na}]^+$ (calcd. (%) 435.1995 for $\text{C}_{20}\text{H}_{24}\text{O}_8\text{Na}$).

TABLE-2
 ^1H AND ^{13}C NMR DATA OF COMPOUNDS 1 IN $\text{C}_5\text{D}_5\text{N}$

No.	δ_{C} (mult.)	δ_{H} (mult., J , Hz)
1	50.2 s	—
2	46.3 d	2.58, m
3	81.2 d	4.47, d, $J = 8.1$
4	198.3 s	—
5	124.2 d	5.93 s
6	168.5 s	—
7	41.8 t	1.72, m, 2.43, m
8	38.9 d	2.36 m
9	31.6 t	1.90 m, 1.48 m
10	32.7 t	1.26 m, 1.69 m
11	152.3 s	—
12	106.5 t	5.06 brs, 4.98 brs
13	62.4 t	4.35 s
14	21.4 q	1.72 s
15	13.2 q	1.08, d, $J = 7.0$
1'	104.2 d	5.22, d, $J = 8.1$
2'	74.2 d	4.15, m
3'	78.5 d	3.92, m
4'	71.6 d	4.34, m
5'	78.8 d	4.22, m
6'	63.5 t	4.30, m, 4.56, m

RESULTS AND DISCUSSION

A 70 % aq. methanol extract prepared from the leaves of *N. tabacum* was subjected repeatedly to column chromatography on silica gel, sephadex LH-20, RP-18 and preparative HPLC to afford compounds **1-3** (Fig. 1), including a new sesquiterpene, nicotterpene A (**1**), together with two known

Fig. 1. Structure of compounds **1-3**

sesquiterpenes, 3-hydroxysolavetivone- β -D-glucoside A (**2**)⁶, 11R,12-dihydroxy-6(7)-spirovetiven-8-one-9-O- β -D-glucopyranoside (**3**)⁷.

Compound **1** was obtained as a viscous oil and gave a quasi-molecular ion $[\text{M} + \text{Na}]^+$ at m/z 435.1990 (calcd. (%) 435.1995) in the HRESI-MS, consistent with the elemental composition $\text{C}_{21}\text{H}_{32}\text{O}_8\text{Na}$. The ^1H NMR spectrum of **1** revealed the presence of one doublet methyl group at δ_{H} 1.08 (d, $J = 7.0$ Hz), one singlet methyl group at δ_{H} 1.72 (s), one olefinic proton at δ_{H} 5.93 (s) and two exo-olefinic protons at δ_{H} 4.98 (brs) and 5.06 (brs). Analysis of the ^{13}C NMR spectrum, which has 21 signals, allowed the identification of one α,β -unsaturated carbonyl group at δ_{C} 198.3, 168.5, 124.2, one terminal double bond at δ_{C} 147.8, 109.1, one quaternary carbon at δ_{C} 50.2, two methyl carbons at δ_{C} 13.2, 21.4 and an oxidated methylene carbon at δ_{C} 63.5. The presence of one sugar was confirmed from one anomeric proton at δ_{H} 5.22 (d, $J = 8.1$ Hz), one anomeric carbon at δ_{C} 104.2 and five oxygenated carbons at δ_{C} 74.2, 78.5, 71.6, 78.8, 63.5. All the spectral data suggested that **1** was a spirovetiven-type sesquiterpene glycoside⁶. The location of the sugar moiety at C-3 was established according to the correlation observed between H-1' (at δ_{H} 5.22) and C-3 (at δ_{C} 80.4) in the HMBC experiment of **1** (Fig. 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 ($J = 8.1$ Hz) of the anomeric proton at δ_{H} 5.17. The NMR spectral data of **1** were similar to those of the previously reported 3-hydroxysolavetivone- β -D-glucoside A (**1**), a sesquiterpene glucoside isolated from *N. tabacum*⁶. The main differences between the two compounds were that a signal of the methyl carbon in **2** was changed to an oxidated methylene carbon in **1**. This variation resulted from a methyl group (C-13) was oxidated to a methylene group and this was supported by the HMBC correlations of H-13 (δ_{H} 4.35 s) with C-8 (δ_{C} 38.9), C-11 (δ_{C} 152.3), C-12 (δ_{C} 106.5).

Fig. 2. Key HMBC ($\text{H} \rightarrow \text{C}$) correlations of **1**

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** 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, the structure of **1** was determined and named nicotterpene A.

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