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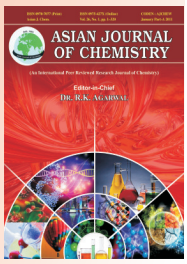

A New Sesquiterpene from *Nicotiana tabacum* and Its Cytotoxicity

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A new sesquiterpene, tabsesquiterpene A (**1**) was isolated from the leaves of *Nicotiana tabacum*. Its structure was elucidated by spectroscopic methods, including extensive 1D and 2D NMR techniques. Compound **1** was tested for its cytotoxicity. The results showed that **1** exhibited modest cytotoxicity against SHSY5Y, PC3 and MCF7 with IC_{50} values of 6.9, 4.5 and 8.8 μM, respectively.

Keywords: *Nicotiana tabacum*, Sesquiterpene, Cytotoxicity, Tabsesquiterpene A.

INTRODUCTION

Nicotiana tabacum L. 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 terpenoids[6-8], alkaloids[9,10], lignans[11,12], flavonoid[13], phenylpropanoids[14] and the 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, tabsesquiterpene A (**1**), was isolated from this plant. In addition, the cytotoxicity of **1** was evaluated. This article deals with the isolation, structural elucidation and biological activities of this new compound.

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 Wininfmred spectrophotometer. APCIMS were measured on a VG Auto Spec-3000 MS spectrometer. ^1H, ^{13}C 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 μm, Qingdao Marine Chemical Inc., China). Second separate was used an Agilent 1100 HPLC equipped with ZORBAX-C_{18} (21.2 nm × 250 nm, 7.0 μm) column and DAD detector.

The leaves of *nicotiana tabacum* L (tobacco leaves) was collected from Yuxi County, Yunnan Province, P. R. China, in September 2011.

Extraction and isolation: The air-dried and powdered leaves of *nicotiana tabacum* (2.8 kg) were extracted with 70 % aqueous ethanol (3.5 L × 3, 24 h each) at room temperature and the extract was concentrated under vacuum condition. The dried extract (98.5 g) was applied to Si gel (200-300 mesh) column chromatography eluting with a $CHCl_3$-Me_2CO gradient system (9:1, 8:2, 7:3, 6:4, 5:5, 2:1) to give six fractions A-F. Fraction A1 (9:1, 6.47 g) was subjected to silica gel column chromatography using $CHCl_3$-MeOH and preparative HPLC (75 % MeOH-H_2O, flow rate 12 mL/min) to give **1** (26.8 mg).

Cytotoxicity assays: The cytotoxicities was tested against five human tumor cell lines (NB4, A549, SHSY5Y, PC3 and MCF7) using the MTT method as reported previously[16]. Taxol was used as the positive control. The results shown that compounds **1** exhibited modest cytotoxicity against SHSY5Y, PC3 and MCF7 with IC_{50} values of 6.9, 4.5 and 8.8 μM, respectively.

Tabsesquiterpene A: Obtained as obtained as a colourless oil; $[\alpha]_D^{24.5}$ +32.1° (c = 0.1, MeOH); UV (MeOH), λ_{max} (log ε) end absorbance; IR (KBr, ν_{max}, cm^{-1}): 3415, 2926, 1762, 1736, 1639, 1547, 1476, 1136, 1075, 976; ^1H and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), Table-1; APCIMS (negative ion mode) *m/z* 339; HRAPCIMS (negative ion mode) *m/z* 339.1449 $[M-H]^-$ (calcd. (%) 339.1444 for $C_{17}H_{23}O_7$).

TABLE-1
^{1}H AND ^{13}C NMR DATA OF COMPOUND **1** IN $CDCl_3$

No.	δ_C (mult.)	δ_H (mult, *J*, Hz)
1	43.8 d	7.76 (m)
2	38.5 t	2.08 (m); 1.67 (m)
3	78.0 d	3.58 (m)
4	48.2 d	1.75 (m)
5	53.0 d	1.92 (m)
6	83.5 d	4.08 (dd, *J* = 10.2, 9.6)
7	62.3d	2.75 (dd, *J* = 10.2, 9.6)
8	70.9 d	2.74 (m)
9	48.2 t	2.75 (dd, *J* = 12.0, 4.2), 2.09 (brd, 12.0)
10	145.8 s	–
11	75.8 s	–
12	181.2 s	–
13	66.8 t	4.55 (d, *J* = 10.8)
	–	4.22 (d, *J* = 10.8)
14	115.2 t	4.99 (s); 5.08 (s)
15	19.2 q	1.28 (d, *J* = 6.5)
1'	169.5 s	–
2'	20.8 q	2.12 (s)

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 compound **1**[15]. Its structure was shown in Fig. 1 and its ^{1}H and ^{13}C NMR data was listed in Table-1.

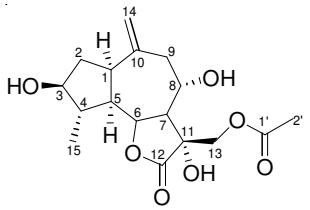

Fig. 1. Structure of new sesquiterpene

Compound **1** was obtained as colourless oil. The negative-ion APCIMS showed a quasi-molecular ion peak $[M-H]^-$ at *m/z* 339. The molecular formula, $C_{17}H_{24}O_7$, was established by HRAPCIMS (*m/z* 339.1449 $[M-H]^-$; calcd. (%) 339.1444), indicating six degrees of unsaturation. The IR spectrum of **1** exhibited absorption at 3415, 1762, 1736 and 1639 cm^{-1} ascribable to hydroxyl, γ-lactone and C=C functional groups . The ^{1}H NMR spectrum (Table-1) displayed one secondary methyl at δ_H 1.28 (3H, d, *J* = 6.5 Hz, H-15) and one acetyl methyl at δ_H 2.12 (3H, s, H-2'). The ^{13}C NMR and DEPT spectra (Table-1) revealed 17 carbon resonances including two methyl, four methylenes, seven methines, four quaternary carbons. The lactone carbonyl resonances were located at δ_C 83.5 (d, C-6), 181.2 (s, C-12), four oxygenated carbon resonances were observed at δ_C 78.0 (d, C-3), 70.9 (d, C-8), 75.8 (s, C-11) and 66.8 (t, C-13), exocyclic methylene resonances at δ_C 145.8 (s, C-10) and 115.2 (t, C-14), an acetoxy group at δ_C 169.5 (s, C-1') and 20.8 (q, C-2'), respectively.

The ^{1}H and ^{13}C spectra data of **1** was very similar to these of 3β,8α,11α,13-tetrahydroxy-10 (14)-guaien-1α,4β,5α, 6β*H*-6α,12-olide,[15] except for the additional acetoxy group signal [δ_C 169.5 (s, C-1') and 20.8 (q, C-2'); δ_H at 2.12 (s, H-2')] of compound **1**. The partial structural unit C-1 to C-9 was deduced from the analysis of the HSQC and ^{1}H ^{1}H COSY spectra of **1**. The HMBC correlations (Fig. 2) between the AB system signals at δ_H 4.22 (1H, d, *J* = 10.8 Hz, Hα-13) and δ_H 4.55 (1H, d, *J* = 10.4 Hz, Hβ-13) and C-12 and the oxygenated quaternary carbon signal at δ_C 75.8 (s, C-11) confirmed two hydroxyl groups attached to C-11 and C-13, respectively. The acetoxy group attached at C-13 was supported by the HMBC correlation of H-13 [δ_H 4.55 (d, *J*=12.0 Hz), 4.22 (d, *J* = 12.0 Hz)] with the acetoxyl carbonyl carbon δ_C 169.5 (s, C-1'). The HMBC showed the cross-peaks of H-15 [δ_H 1.28 (d, *J* = 6.5 Hz)] to C-3, C-4 and C-5, H-6 [δ_H 4.08 (dd, *J* = 10.4, 10.1 Hz)] to C-12 (δ_C 181.2), H-14 [δ_H 4.99 (s), 5.08 (s)] to C-1, C-9 and C-10 and H-9 [δ_H 2.75 (dd, *J* = 12.0, 4.2 Hz), 2.09 (brd, *J* = 12.0 Hz)] to C-1, C-10 and C-14, respectively. These informations confirmed the planar structure of **1**.

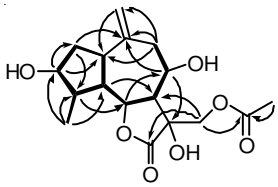

Fig. 2. ^{1}H ^{1}H COSY (–) and selected HMBC (⌒) correlations of **1**

The relative configuration of **1** was determined by comparison with 3β,8α,11α,13-tetrahydroxy-10 (14)-guaien-1α,4β,5α,6βH-6α,12-olide[15] and confirmed by a ROESY experiment. The ROESY correlations (Fig. 3) of Hα-1 with H-3 and Hα-5 with H-3, H-7 and Me-15 indicated that H-3, H-7 and Me-15 possessed α-orientations, respectively. The ROESY correlations of H-6 with H β-4 and H-8 suggested that H-6 and H-8 possessed β-orientations, respectively. On the basis of the evidence mentioned above, the structure of **1** was elucidated as shown and gives the trivial name of tabsesquiterpene A.

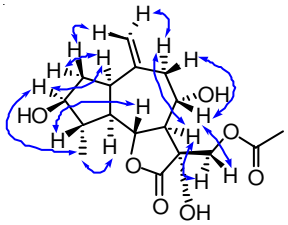

Fig. 3. Key ROESY (⌒) correlations of **1**

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