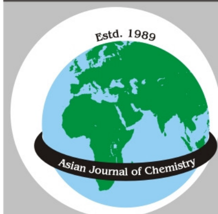
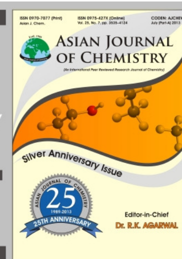
Asian Journal of Chemistry; Vol. 25, No. 11 (2013), 6133-6135



ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.14288



A New 4-Hydroxyisoflavanone from the Root of Oriental Tobacco and Their Antivirus Activities

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(*Received*: 11 July 2012; *Accepted*: 30 April 2013) AJC-13419

A new 4-hydroxyisoflavanone *i.e.*, tobflavanone D (**1**), was isolated from the roots of oriental tobacco. Its structure was elucidated by spectroscopic methods, including extensive 1D and 2D NMR techniques. Tobflavanone D (**1**) was tested for their anti HIV-1 activities and anti-tobacco mosaic virus activities. The results showed that tobflavanone D (**1**) modest anti HIV-1 activities and antitobacco mosaic virus activities, respectively.

Key Words: 4-Hydroxyisoflavanone, Oriental tobacco, Anti HIV-1 activities, Antitobacco mosaic virus.

INTRODUCTION

Nicotiana tabacum L is a perennial herbaceous plant originating from South America, and it is one of the most commercially valued agricultural crops in the world[1,2]. In addition to cigarette industry use, *N. tabacum* also contains many useful chemical compounds, such as sesquiterpenes[3,4], diterpenoids[5-7], alkaloids[8,9], phenols[10], *etc*. The stems and roots of *N. tabacum* are big amount of by-product in tobacco planting and are normally used as organic fertilizer. The multipurpose utilization of the stems and roots of *N. tabacum* is an interesting topical and receives more and more attentions[11-13]. Motivated by search for bioactive metabolites from this plant, an investigation on the chemical constituents of the roots of oriental tobacco (a variant of *N. tabacum*) was carried out. As a result, a new 4-hydroxyisoflavanone was isolated from this plant. In addition, the anti-tobacco mosaic virus (anti TMV) activity of the new 4-hydroxyisoflavanone was evaluated. This article deals with the isolation, structural elucidation and biological activities of the new compound.

EXPERIMENTAL

Optical rotation was measured in Horiba SEPA-300 high sensitive polarimeter. IR spectra were obtained in KBr disc on a Bio-Rad Wininfmred spectrophotometer. ESI-MS were measured on a VG Auto Spec-3000 MS spectrometer. ^{1}H, ^{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 mm, Qingdao Marine Chemical Inc., China). Preparative HPLC was used an Agilent 1100 HPLC equipped with ZORBAX-C_{18} (21.2 mm × 250 mm, 7.0 μm) column and DAD detector.

The roots of oriental tobacco were collected in Baoshan Prefecture, Yunnan Province, People's Republic of China, in September 2009. The identification of the plant material was verified by Prof. Chen Y.J. (Yunnan University of Nationalities). A voucher specimen (YNNI 10-9-22) has been deposited in our laboratory.

Extraction and isolation: The air-dried and powdered roots of oriental tobacco (1.5 kg) were extracted four times with 70 % methanol (4 L × 2.0 L) at room temperature and filtered. The crude extract (68 g) was applied to silica gel (200-300 mesh) column chromatography, eluting with a chloroform-acetone gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A-F. The further separation of fraction C (8:2, 13.2 g) by silica gel column chromatography, eluted with chloroform-methanol (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures C1-C5. Fraction C2 (9:1, 0.85 g) was subjected to preparative HPLC (42 % methanol, flow rate 12 mL/min) to give **1** (11.6 mg).

Tobflavanone D: Obtained as pale yellow gum; $[\alpha]^{D}_{22.5}$ -89.2 (c 0.20, MeOH); UV (MeOH), λ_{max} (log ε) 348 (2.18), 298 (4.05), 210 (4.74) nm; IR (KBr, ν_{max}, cm^{-1}): 3398, 2965, 2874, 1738, 1628, 1542, 1475, 1420, 1378, 1228, 1168, 1046, 879; ^{1}H and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz), Table-1;

ESI-MS (positive ion mode) m/z 383; HR-ESI-MS (positive ion mode) m/z 383.1100 $[M + Na]^+$ (calcd. (%) 383.1107 for $C_{19}H_{20}NaO_7$).

TABLE-1
^{1}H AND ^{13}C NMR DATA OF TOBFLAVANONE D (**1**) IN C_5D_5N

No.	Compound **1**	
	δ_C (mult.)	δ_H (mult, *J*, Hz)
2α	66.8 (t)	3.61 (dd, *J* = 10.2, 11.2)
2β	–	4.33 (dd, *J* = 5.2, 10.8)
3	38.8 (d)	3.53 (m)
4	79.0 (d)	5.31 (d, *J* = 6.9)
5	115.0 (d)	7.72 (s)
6	138.8 (s)	–
7	149.3 (s)	–
8	104.0 (d)	7.27 (s)
9	148.9 (s)	–
10	134.1 (s)	–
1′	120.3 (s)	–
2′	160.1 (s)	–
3′	103.3 (d)	6.72 (d, *J* = 2.2)
4′	155.2 (s)	
5′	109.1 (d)	6.61 (dd, *J* = 2.2, 8.6)
6′	125.0 (d)	7.03 (d, *J* = 8.6)
7-OMe	55.8 (q)	3.81 (s)
2′-OMe	55.9 (q)	3.78 (s)
4′-OAc	21.2 q, 170.0 s	1.91(s)
6-OH	–	11.21 (brs)

RESULTS AND DISCUSSION

A 70 % aq. methanol extract prepared from the roots of oriental tobacco was subjected repeatedly to column chromatography and preparative HPLC to afford compound **1**. Its structure was shown in Fig. 1 and its ^{1}H and ^{13}C NMR spectroscopic data were listed in Table-1.

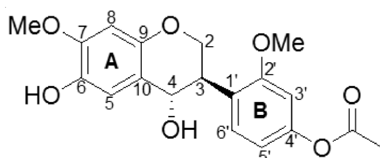

Fig. 1. Structure of tobflavanone D (**1**)

Tobflavanone D (**1**) was obtained as pale yellow gum. Its molecular formula was determined as $C_{19}H_{20}O_7$ by HR-ESI-MS m/z 383.1100 $[M + Na]^+$ (calcd. (%) 383.1107). Its ^{1}H and ^{13}C NMR spectra data (Table-1) showed signals to 20 hydrogens and 19 carbons, respectively, corresponding to two aromatic rings with five aromatic protons, one oxidated methine group (δ_C 79.0), one methine groups (δ_C 38.8) one oxidated methylene group (δ_C 66.8), two methoxy group (δ_C 55.8, 55.9), an acetoxy group (δ_C 21.2, 170.0) and a phenolic hydroxyl group (δ_H 11.21). Strong absorption bands accounting for hydroxyl (3398 cm^{-1}), acetoxy (1738 cm^{-1}) and aromatic groups (1628, 1542, 1475, 1420 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of tobflavanone D (**1**) showed absorption maxima at 298, 210 nm confirmed the existence of the aromatic functions. The ^{1}H NMR spectrum showed a pair of doublets at δ_H 3.61 (*J* = 10.2, 11.2 Hz), δ_H 4.33 (*J* = 5.2, 10.8 Hz), a multiplet at δ_H 3.54 (m) and a doublet at δ_H 5.31 (*J* = 6.9 Hz). These signals were assignable to H-2, H-3 and H-4 protons of a 4-hydroxyisoflavanone skeleton[14], respectively. The typical proton signals at (δ_H 7.72 s 1H) and (7.27 s 1H) revealed that the ring A should be a 6,7-substituted moiety[15]. Proton signals at δ_H 6.72 (*J* = 2.2 Hz), δ_H 6.61 (*J* = 2.2, 8.6 Hz) and δ_H 7.03 (*J* = 8.6 Hz) also revealed that the ring B should be 2',4'-disubstituted[16]. The HMBC correlations (Fig. 2) of phenolic hydroxy proton signal (δ_H 11.21) with C-5 (δ_C 115.0), C-6 (δ_C 138.8) and C-7 (δ_C 149.3) indicated that the phenolic hydroxy group should be located at C-6. Two methoxy groups located at C-7 and C-2' was supported by the HMBC correlations of the methoxy proton signals (δ_H 3.81, 3.77) with C-7 (δ_C 149.3) and C-2' (δ_C 160.1), respectively. This was further confirmed through the NOESY correlations (Fig. 3) between OMe-7 (δ_H 3.81) with H-8 (δ_H 7.27) and OMe-2' (δ_H 3.78) with H-3' (δ_H 6.72 *J* = 2.2 Hz), respectively. The position of methoxy and hydroxy groups was be determined, the acetoxy group should be located at C-4' accordingly.

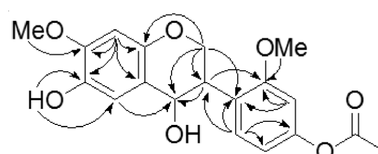

Fig. 2. Selected HMBC (↷) correlations of **1**

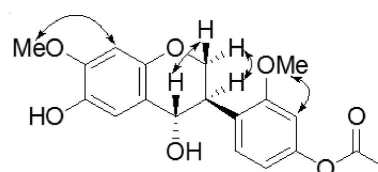

Fig. 3. the NOESY correlations (↶↷) of compound **1**

The larger magnitude of coupling constants between H-2β and H-3 inferred α and pseudoaxial orientation of H-3. Moreover, the larger coupling constant between H-3 and H-4 revealed their *trans* relationship and indicating β orientation of H-4. The relative stereochemistry at C-3 and C-4 were established as shown, which was confirmed by strong NOESY correlations between H-4 and H-2β and between H-3 and H-2α and also by ^{1}H and ^{13}C NMR chemical shifts of C-2, C-3 and C-4, which showed complete agreement with those of conferol A[14] and bolusanthol A[17]. Thus, structure of tobflavanone D (**1**) was determined as 4,6-dihydroxy-7,2'-trimethoxy-4'-acetoxyl-isoflavanone and named as cordifoliflavanone A.

Since some of the flavonoids exhibited anti virus activities[18-20], compounds **1** was tested for their anti HIV-1 activities and anti tobaco mosaic virus activities.

The anti HIV-1 activities were tested for their potencies in preventing the cytopathic effects of HIV-1 in C8166 and cytotoxicity measured in parallel with the determination of antiviral activity, using AZT as a positive control (EC_{50} = 0.045 μg/mL and CC_{50} > 200 μg/mL)[21]. Compound **1** showed modest anti HIV-1 activities with EC_{50} value of 2.68 μg/mL and minimal cytotoxicity against C8166 cells (CC_{50} > 200 μg/mL). The therapeutic index (TI) values (CC_{50}/EC_{50}) of **1** were more than 69.9.

The anti tobacco mosaic virus activities were tested using the half-leaf method[22]. The inhibitory activities of the new

compounds (at the concentration of 20 μM) against tobaco mosaic virus replication were tested using two approaches. First, the half-leaf method was used to test the antiviral activity in the local lesion host *N. glutinosa in vivo*. Then, the leaf-disk method was used to evaluate the antiviral activity of the compound in the systemic infection host *N. tabacum* cv. K326. Ningnanmycin (2 % water solution), a commercial product for plant disease in China, was used as a positive control. The results showed that the compound **1** exhibited inhibition rates of 8.26 %.

ACKNOWLEDGEMENTS

This project was supported financially by the Basic Research Foundation of Yunnan Tobacco Industry Co. Ltd. (2012JC01), the Excellent Scientific and Technological Team of Yunnan High School (2010CI08), and Open Research Fund Program of Key Laboratory of Ethnic Medicine Resource Chemistry (Yunnan University Nationalities) (2010XY08.

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