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NOTE

A New Homoisoflavonoid from Flue-Cured Tobacco and Its Anti-tobacco Mosaic Virus Activity

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A new homoisoflavonoid, tabeanin A (**1**), was isolated from the stems of flue-cured tobacco (a variety of *Nicotiana tabacum* L). Its structure was elucidated by spectroscopic methods, including extensive 1D and 2D NMR techniques. Compound **1** was also tested for its anti-tobacco mosaic virus activity and it shows potential anti-tobacco mosaic virus activity with inhibition rates of 36.8 %.

Keywords: Homoisoflavonoid, Flue-cured tobacco, Anti-tobacco mosaic virus activity.

Nicotiana tabacum L. is the most commonly grown of all plants in the *Nicotiana* genus and its leaves are commercially grown in many countries to be processed into tobacco[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 it 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. In this study, we report the isolation of a new homoisoflavonoid, tabeanin A (**1**). Its structure was evaluated by spectroscopic methods, including HRMS and ^{1}D and ^{2}D NMR. In addition, the anti-tobacco mosaic virus (anti-TMV) activity of compound **1** was also evaluated.

Optical rotations were obtained on a Perkin-Elmer 341 digital polarimeter; UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. 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 μm), 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 mm) column and DAD detector.

Plant material: The stems of flue-cured tobacco were collected in Honghe Prefecture, Yunnan Province, People's Republic of China, in September 2012. The identification of the plant material was verified by Prof. Y. J. Chen (Yunnan University of Nationalities).

Extraction and isolation: The air-dried and powdered tobacco leaves (3.5 kg) were extracted four times with 90 % methanol (4 × 5 L) at room temperature and filtered to yield a filtrate. The crude extract (83.4 g) was applied to silica gel (200-300 mesh) column chromatography, eluting with a chloroform-acetone system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A-F. The further purification of fraction D (7:3, 16.9 g) by silica gel column chromatography, eluted with petroleum ether-acetone (9:1, 8:2, 7:3, 6:4, 5:5), yielded mixtures D-1-D-5. Fraction D-4 (6:4, 2.53 g) was subjected to preparative HPLC (46 % MeOH-H_2O, flow rate 12 mL/min) to yield compound **1** (16.4 mg).

Tabeanin A (1): Obtained as pale yellow powder; $[\alpha]_D^{23.6}$ + 28.5 (*c* 0.20, MeOH); UV (MeOH), λ_{max} (log ε) 210 (4.15), 229 (3.02), 276 (3.63) nm; ECD (*c* 0.2, MeOH) $\Delta\varepsilon_{282}$ +11.2, $\Delta\varepsilon_{318}$-7.8; IR (KBr, ν_{max}, cm^{-1}) 3460, 2932, 2973, 1680, 1602, 1478, 1450, 1284, 1125, 1102, 994, 815; ^{1}H NMR and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz, respectively), see Table-1; ESIMS (positive ion mode), *m/z* 383 $[M + Na]^+$; HRESIMS (positive ion mode), *m/z* 383.1102 $[M + Na]^+$ (calcd. 383.1107 for $C_{19}H_{20}NaO_7$).

A 90 % methanol extract prepared from the fermentation products of the endophytic fungus *Phomopsis amygdali* was subjected repeatedly to column chromatography on Silic gel, Sephadex LH-20, RP-18 and Preparative HPLC to afford compound **1**. The structure of **1** was shown in Fig. 1 and its ^{1}H and ^{13}C NMR data were listed in Table-1.

TABLE-1
^{1}H AND ^{13}C NMR DATA OF COMPOUND **1** (δ IN PPM, IN C_5D_5N, 500 AND 125 MHZ)

Position	δ_C (m)	δ_H (m, J, Hz)	Position	δ_C (m)	δ_H (m, J, Hz)
2	72.2 t	3.93 d (11.2) 4.01 d (11.2)	11	37.8 t	2.82 d (10.6) 2.74 d (10.6)
3	73.6 s	-	1′	127.8 s	-
4	192.2 s	-	2′, 6′	131.6 d	7.28 d (8.6)
5	151.0 s	-	3′, 5′	113.8 d	6.79 d (8.6)
6	136.2 s	-	4′	158.4 s	-
7	157.2 s	-	6-OM	61.2 q	3.82 s
8	98.3 d	6.52 s	7-OM	56.3 q	3.79 s
9	156.2 s	-	4′-OM	55.9 q	3.81 s
10	108.4 s	-	Ar-OH	-	11.62 s

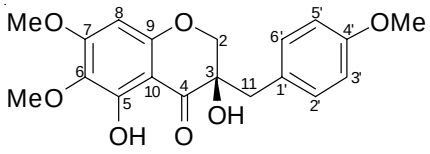

Fig. 1. Structure of compound **1**

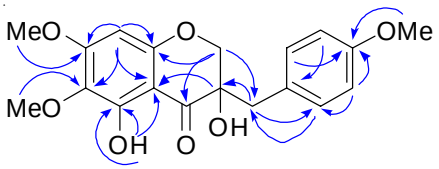

Fig. 2. Key HMBC (⌒) correlations of **1**

Compound **1** was isolated as a pale yellow powder. Its composition was established as $C_{19}H_{20}O_7Na$ by positive ion HRESIMS. Its IR spectrum exhibited absorption bands at 3460 and 1680 cm^{-1}, indicating the presence of hydroxy and conjugated carbonyl groups. Its ^{1}H NMR spectrum displayed the typical splitting pattern of a eucomoltype homoisofla-vonoid, with two pairs of geminal coupled proton signals at δ_H 3.93 and 4.01 (each a doublet, J = 11.2 Hz) and δ_H 2.74 and 2.82 (each a doublet, J = 10.6 Hz)[15]. The observation of 12 aromatic carbon signals (δ_C 151 s, 136.2 s, 157.2 s, 98.3 d, 156.2 s, 108.4 s, 127.8 s, 131.6 d (2C), 113.8 d (2C), 158.4 s), a carbonyl carbon signal (δ_C 192.2 s) and a methylene carbon signal (δ_C 37.8) in the ^{13}C NMR spectrum corroborated the homoisoflavonoid structure. The basic skeleton of 3-hydroxy-3-benzyl-4-chromanone was confirmed by the HMBC correlations (Fig. 2) of H-2 (δ_H 3.93 and 4.01) and C-3 (δ_C 73.6), C-4 (δ_C 192.2); of H-11 (δ_H 2.82 and 2.74) and C-2 (δ_C 72.2), C-3 (δ_C 73.6), C-4 (δ_C 192.2) and C-1' (δ_C 127.8). The presence of three proton singlets (each 3H) located at δ_H 3.82, 3.79 and 3.81 in the ^{1}H NMR spectrum, corresponding by HSQC to the three carbon resonances located at δ_C 61.2, 56.3 and 55.9, indicated the presence of three methoxy groups. Two doublets at δ_H 6.79 and 7.28 (2H each, J = 8.6 Hz) indicated a paradisubstituted B-ring. A singlet at δ_H 6.52 was assigned to H-8 of the A-ring, based on the HMBC correlations between H-8 (δ_H 6.52) and C-9 (δ_C 156.2), C-10 (δ_C 108.4). A phenolic hydroxyl group located at C-5 was confirmed by the HMBC correlation of phenolic hydroxyl proton signal (δ_H 11.62) with C-5 (δ_C 151.0), C-6 (δ_C 136.2) and C-10 (δ_C 108.4). The stereochemistry at C-3 was determined by comparison of its electronic circular dichroism (ECD) and spectrum and optical rotation valve with those of previously reported homoisoflavonoids[16]. Thus, the structure of **1** was established as shown and gives the trivail name of tabeanin A.

Compounds **1** was tested for it anti-tobacco mosaic virus activity. The anti-tobacco mosaic virus activities were tested using the half-leaf method[17]. Ningnanmycin (2 % water solution), a commercial product for plant disease in China, was used as a positive control. The results showed that compound **1** exhibited inhibition rate of 36.8 %.

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