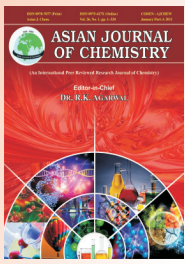
Asian Journal of Chemistry; *Vol. 26, No. 10 (2014), 2820-2822*

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.15807



A New Coumarin from Roots and Stems of Flue-Cured Tobacco and its Anti-Tobacco Mosaic Virus Activity

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Received: 21 May 2013; *Accepted*: 17 August 2013; *Published online*: 10 May 2014; AJC-15126

A new coumarin, 6-hydroxy-3-(4-hydroxyphenyl)-7-methoxy-2*H*-chromen-2-one (**1**), was isolated from the roots and 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 tested for its anti-tobacco mosaic virus (anti-TMV) activity and it shows potential anti-tobacco mosaic virus activity with inhibition rates of 23.6 %.

Keywords: Coumarin, Flue-cured tobacco, Structure elucidation, Anti-tobacco mosaic virus activity.

INTRODUCTION

The flue-cured tobacco belongs to the plants of nicotiana genus. It is a type of cigarette tobacco. Along with burley tobacco, it accounts for more than 90 % of world tobacco production[1,2]. In addition to being used in cigarette industry, the flue-cured tobacco is also used as insecticide, anesthetic, diaphoretic, sedative and emetic agents in Chinese folklore medicine due to containing many useful chemical compounds[1,3-5]. Previous phytochemical studies of flue-cured tobacco have shown the presence of terpenoids[6-8], alkaloids[9,10], lignans[11,12], flavonoid[13], phenylpropanoids[14] and the homologous. The roots and stems of flue-cured tobacco are big amount of by-product in tobacco planting and are normally used as organic fertilizer. The multipurpose utilization of the roots and stems of flue-cured tobacco is an interesting topical and receives more and more attentions[15-17]. Motivated by a search for new bioactive metabolites from this plant, our group has investigated the chemical constituents of the roots and stems of flue-cured tobacco. As a result, a new coumarin (**1**) was isolated. This article deals with the isolation, structural elucidation and the anti-tobacco mosaic virus (anti-TMV) activity of this new compound.

EXPERIMENTAL

Ultra-violet spectra were obtained using a Shimadzu UV-2401A spectrophotometer. Infrared 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-C18 (21.2 mm × 250 mm, 7.0 mm) column and DAD detector.

The roots and stems of flue-cured tobacco were collected in Honghe Prefecture, Yunnan Province, People's Republic of China, in September 2011. The identification of the plant material was verified by Prof. Chen Y. J (Yunnan University of Nationalities).

Extraction and isolation: The air-dried and powdered roots and stems of flue-cured tobacco (2.2 kg) were extracted four times with 70 % aqueous acetone (3 × 5 L) at room temperature and filtered to yield a filtrate. The crude extract (183 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 C (8:2, 14.8 g) by silica gel column chromatography, eluted with petroleum ether-acetone (8:2, 7:3, 6:4, 5:5, 0:1), yielded mixtures C1-C5. Fraction C4 (1:1, 1.81 g) was subjected to preparative HPLC (40 % MeOH-H_2O, flow rate 12 mL/min) to yield compound **1** (15.8 mg).

6-Hydroxy-3-(4-hydroxyphenyl)-7-methoxy-2*H*-chromen-2-one (1): $C_{16}H_{12}O_5$; obtained as yellow gum; UV (CH_3OH), λ_{max} (log ε) 275 (3.89), 220 (4.32) nm; IR (KBr, ν_{max}, cm^{-1}) 3435, 1717, 1589, 1511, 1486, 1438, 1266, 1174, 1076, 890; ^{13}C NMR and ^{1}H NMR data (C_5D_5N, 500 and 125

MHz) see Table-1; postive ESIMS *m/z* 307 $[M + Na]^+$; postive HRESIMS *m/z* 307.0586 $[M + Na]^+$ (calcd. for $C_{16}H_{12}NaO_5$, 307.0582).

RESULTS AND DISCUSSION

The air-dried and powdered roots and stems of flue-cured tobacco (4.5 kg) was extracted with 70 % aqueous acetone (3 × 5 L) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure to obtained a crude extract (183 g). This crude extract was subjected repeatedly to column chromatography on silica gel, Sephadex LH-20, RP-18 and preparative HPLC to afford the new compound **1**. The structures of the compound **1** were as shown in Fig. 1 and its ^1H and ^{13}C NMR data were listed in Table-1.

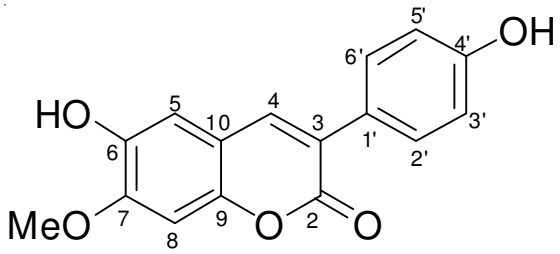

Fig.1. Structure of new coumarin

TABLE 1
^1H NMR AND ^{13}C NMR DATA OF COMPOUND **1**
(C_5D_5N, δ, ppm, J/Hz)

No.	δ_C (mult.)	δ_H (mult, J, Hz)	No.	δ_C (mult.)	δ_H (mult, J, Hz)
2	162.5 s		10	112.6 s	
3	121.4 s		1′	128.3 s	
4	139.7 d	7.89 s	2′,6′	129.3 d	7.75 d (8.6
5	112.8 d	7.08 s	3′,5′	114.2 d	6.92 d (8.6
6	142.5 s		4′	158.8 s	
7	152.2 s		7-OMe	55.9 q	3.83 s
8	103.4 d	6.65 s	Ar-OH-6		11.26 brs
9	148.3 s		Ar-OH-4′		11.02 brs

Compound **1** was isolated as a yellow gum. High-resolution ESIMS analysis gave an quasi-molecular ion at *m/z* 307.0586 $[M + Na]^+$ (calcd. 307.0582), consistent with a molecular formula of $C_{16}H_{12}O_5$, which indicated 11 degrees of unsaturation. Its UV spectrum showed the maximum absorption at 275 and 220 nm. Strong absorption bands accounting for hydroxy (3435 cm^{-1}), carbonyl (1717 cm^{-1}) and aromatic groups (1589, 1511, 1486 cm^{-1}) could also be observed in its IR spectrum. The ^1H and ^{13}C NMR spectra of **1** (Table-1) displayed signals for all 16 carbons and 12 protons, including a 3,6,7-subsititued-coumarin system (δ_C 162.5 s, 121.4 s, 139.7 d, 112.8 d, 142.5 s, 152.2 s, 103.4 d, 148.3 s, 112.6 s; δ_H 7.89 s, 7.08 s, 6.65 s[18], a *para*-subsititued phenyl moiety [δ_C 128.3 s, 129.3 d (2C), 114.2 d (2C), 158.8 s; δ_H 7.75 (d) *J* = 8.6 (2H), 6.92 (d) *J* = 8.6 (2H)][19], one methoxy group (δ_C 55.9 q; δ_H 3.83 s) and two phenolic hydroxy group (δ_H 11.26 and 11.02 brs). The HMBC correlation (Fig. 2) of one phenolic hydroxyl proton (δ_H 11.02) with C-3',5' (δ_C 114.2), C-4' (δ_C 158.8) showed a phenolic hydroxyl group was located at C-4'. The HMBC correlations between H-2',6' (δ_H 7.75) and C-3 (δ_C 121.4), H-4 (δ_H 7.89) and C-1' (δ_C 128.3) concluded the linkage of the *p*-hydoroxyphenyl was located at the C-3 position of the coumarin system. The location of another phenolic hydroxy group was assigned to C-6 position on the basis of HMBC correlation between the hydroxy proton signal (δ_H 11.26) and C-5 (δ_C 112.8), C-6 (δ_C 142.5) and C-7 (δ_C 152.2). Finally, a methoxy group at C-7 position was supported by the HMBC correlation observed between methoxy proton (δ_H 3.83) and C-7 (δ_C 152.2). On the basis of the above evidence, the structure of **1** was established as 6-hydroxy-3-(4-hydroxyphenyl)-7-methoxy-2*H*-chromen-2-one.

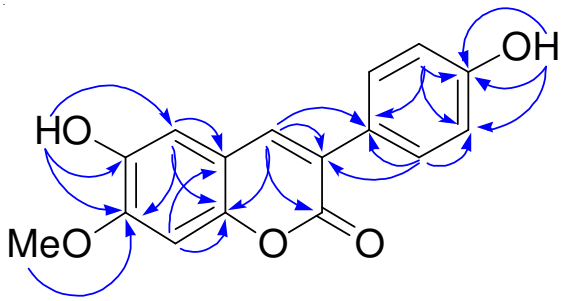

Fig. 2. Key HMBC (↷) correlations of **1**

Since certain of the phenolic compounds exhibit potential anti-TMV activity[19-21], compounds **1** was tested for its anti-tobacco mosaic virus activity. The anti-TMV activities were tested using the half-leaf method[21]. 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 rates of 23.6 %.

ACKNOWLEDGEMENTS

This project was supported financially by the National Natural Science Foundation of China (No. 31360081) the Basic Research Foundation of Yunnan Tobacco Industry Co. Ltd (2012JC01), the National Natural Science Foundation of China (No. 21002085), the Excellent Scientific and Technological Team of Yunnan High School (2010CI08).

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