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

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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 terpenoids⁶⁻⁸, alkaloids^{9,10}, lignans^{11,12}, flavonoid¹³, phenylpropanoids¹⁴ 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 attentions¹⁵⁻¹⁷. 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 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 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-H₂O, 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**):** C₁₆H₁₂O₅; obtained as yellow gum; UV (CH₃OH), 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; ¹³C NMR and ¹H NMR data (C₅D₅N, 500 and 125

MHz) see Table-1; positive ESIMS m/z 307 $[M + Na]^+$; positive 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 obtain 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 a 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-substituted-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 s¹⁸, a *para*-substituted 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*-hydroxyphenyl 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 activity¹⁹⁻²¹, compound **1** was tested for its anti-tobacco mosaic virus activity. The anti-TMV activities were tested using the half-leaf method²¹. 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 %.

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REFERENCES

1. The Editorial Committee of the Administration Bureau of Flora of China, *Flora of China*, 67 vols., Beijing Science and Technology Press, Beijing (2005).
2. T.W. Hu and Z. Mao, *Tob. Control*, **15**(suppl 1), i37 (2006).
3. A. Rodgman and T.A. Perfetti, *The Chemical Components of Tobacco and Tobacco Smoke*. CRC Press, Taylor and Francis Group, Boca Raton, Florida (2008).
4. A.P. Cavender and M. Alban, *J. Ethnobiol. Ethnomed.*, **5**, 3 (2009).
5. A. Inta, P. Shengji, H. Balslev, P. Wangpakapattanawong and C. Trisonthi, *J. Ethnopharmacol.*, **116**, 134 (2008).
6. X. Feng, J.-S. Wang, J. Luo and L.-Y. Kong, *J. Asian Nat. Prod. Res.*, **12**, 252 (2010).
7. Y. Shinozaki, T. Tobita, M. Mizutani and T. Matsuzaki, *Biosci. Biotechnol. Biochem.*, **60**, 903 (1996).
8. T. Pettersson, A.M. Eklund and I. Wahlberg, *J. Agric. Food Chem.*, **41**, 2097 (1993).
9. X.C. Wei, S.C. Sumithran, A.G. Deaciuc, H.R. Burton, L.P. Bush, L.P. Dvoskin and P.A. Crooks, *Life Sci.*, **78**, 495 (2005).
10. T. Braumann, G. Nicolaus, W. Hahn and H. Elmenhorst, *Phytochemistry*, **29**, 3693 (1990).

11. Y.K. Chen, X.S. Li, G.Y. Yang, Z.Y. Chen, Q.F. Hu and M.M. Miao, *J. Asian Nat. Prod. Res.*, **14**, 450 (2012).
12. Q.-F. Hu, G. Yang, X. Li, X. Yang, H. Mu, Y. Chen and X.-M. Gao, *Heterocycles*, **85**, 147 (2012).
13. Z.Y. Chen, J.L. Tan, G.Y. Yang, M.M. Miao, Z.Y. Chen and T.F. Li, *Phytochem. Lett.*, **5**, 233 (2012).
14. Y. Chen, T. Li, Z.-Y. Chen, G. Yang, M. Miao and J. Tan, *Heterocycles*, **83**, 2381 (2011).
15. W. Li, L.B. Zhang, J.H. Peng, N. Li and X.Y. Zhu, *Ind. Crops Prod.*, **27**, 341 (2008).
16. Q. Hu, M. Miao, W. Zhao, T. Zhang, L. Wan, G. Yang, Y. Chen and D. Mou, *Heterocycles*, **85**, 2485 (2012).
17. W.H. Zhong, C.J. Zhu, M. Shu, K.D. Sun, L. Zhao, C. Wang, Z.J. Ye and J.M. Chen, *Bioresour. Technol.*, **101**, 6935 (2010).
18. J.F. Liu, K.P. Xu, F.S. Li, J. Shen, C.P. Hu, H. Zou, F. Yang, G.R. Liu, H.L. Xiang, Y.J. Zhou, Y.J. Li and G.S. Tan, *Chem. Pharm. Bull. (Tokyo)*, **58**, 549 (2010).
19. J.X. Chen, H.Q. Leng, Y.X. Duan, W. Zhao, G.Y. Yang, Y.D. Guo, Y.K. Chen and Q.F. Hu, *Phytochem. Lett.*, **6**, 144 (2013).
20. Q.F. Hu, B. Zhou, J.M. Huang, X.M. Gao, L.D. Shu, G.Y. Yang and C.T. Che, *J. Nat. Prod.*, **76**, 292 (2013).
21. Q.F. Hu, B. Zhou, X.M. Gao, L.Y. Yang, L.D. Shu, Y.Q. Shen, G.P. Li, C.T. Che and G.Y. Yang, *J. Nat. Prod.*, **75**, 1909 (2012).