A New Phenolic Compound from the Stems of Flue-Cured Tobacco and Its Anti-Tobacco Mosaic Virus Activity

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A new phenolic compound, (*E*)-7-(3,7-dimethylocta-2,6-dienyl)-4-methoxybenzofuran-2(3*H*)-one (**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 tested for its anti-tobacco mosaic virus (anti-TMV) activity and it shows potential anti-tobacco mosaic virus activity with inhibition rates of 12.5 %.

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

INTRODUCTION

Nicotiana tabacum L. is a perennial herbaceous plant. It is found only in cultivation, where it 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 terpenoids⁶⁻⁸, alkaloids^{9,10}, lignans^{11,12}, flavonoid¹³, phenyl-propanoids¹⁴ and the homologous, were isolated from this plant. 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 attentions¹⁵⁻¹⁷. With the aims of search for bioactive metabolites from this plant, the investigation on the chemical constituents of the steams of flue-cured tobacco (a variety of *Nicotiana tabacum* L) was carried out. As a result, a new phenolic compound (**1**) (Fig. 1) was isolated. In addition, the anti-tobacco mosaic virus (anti-TMV) activity of **1** was evaluated. This article deals with the isolation, structural elucidation and biological activities of this new compound.

EXPERIMENTAL

UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. IR 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 μm) column and DAD detector.

The stems of flue-cured tobacco were collected in Yuxi 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 stems of tobacco stems (2.2 kg) were extracted 4 times with 90 % methanol (4 × 5 L) at room temperature and filtered to

Fig. 1. Structure of new phenolic compound

yield a filtrate. The crude extract (85.2 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 A (20:1, 22.4 g) by silica gel column chromatography, eluted with petroleum ether-acetone (9:1, 8:2, 7:3, 6:4, 5:5), yielded mixtures A1-A5. Fraction A2 (8:2, 1.65 g) was subjected to preparative HPLC (75 % MeOH-H₂O, flow rate 12 mL/min) to yield compound **1** (14.3 mg).

(E)-7-(3,7-dimethylocta-2,6-dienyl)-4-methoxybenzofuran-2(3H)-one: Obtained as a white amorphous powder; UV (MeOH), $\lambda_{\max}$ (log ϵ) 278 (3.98), 210 (4.30) nm; IR (KBr, $\lambda_{\max}$, cm⁻¹) 3028, 2965, 2918, 1768, 1602, 1536, 1449, 1150, 1105, 894; ¹H NMR and ¹³C NMR data (C₅D₅N, 500 MHz and 125 MHz, respectively), (Table-1); ESIMS (positive ion mode), m/z 323 [M + Na]⁺; HRESIMS (positive ion mode), m/z 323.1628 [M + Na]⁺ (calcd. 323.1623 for C₁₉H₂₄O₃Na).

RESULTS AND DISCUSSION

Compound **1** was obtained as white amorphous powder. Its molecular formula was determined as C₁₉H₂₄O₃Na by HRESIMS, m/z 323.1628 [M + Na]⁺ (calcd. 323.1623), corresponding to 8 degrees of unsaturation. Its ¹H and ¹³C NMR spectral data (Table-1) showed signals to 24 hydrogens and 19 carbons, respectively, corresponding to one aromatic ring (δ_c 115.6 s, 155.4 s, 112.7 d, 129.0 d, 128.6 s, 135.8 s) with two aromatic protons (6.82 (d) J = 8.8, 7.26 (d) J = 8.8), one geranyl moiety¹⁸ (δ_c 28.2 t, 121.6 d, 138.2 s, 33.6 t, 26.0 t, 124.6 d, 131.2 s, 25.2 q, 18.2 q, 16.8 q; δ_H 3.22 (d) J = 7.2, 5.26 (t) J = 7.2, 2.22 m, 2.18 m, 5.06 m, 1.62 s, 1.50 s, 1.68 s), a furan-2(3H)-one¹⁸ (39.2 t, 175.0 s; 3.58 s) and a methoxy group (δ_c 56.2 q; δ_H 3.81 s). Strong absorption bands accounting for carbonyl (1768 cm⁻¹) and aromatic group (1602, 1536, 1449 cm⁻¹) could be observed in its IR spectrum. The UV spectrum of **1** showed absorption maxima at 278 and 210 nm also confirmed the existence of the aromatic function. The HMBC correlations (Fig. 2) of H-7 (δ_H 3.22) with C-4 (δ_c 129.0), C-5 (δ_c 128.6) and C-6 (δ_c 135.8), of H-4 (δ_H 7.26) with C-7 (δ_c 28.2) suggested the geranyl moiety was attached to C-1. The long-range correlation of the methoxy proton signal (δ_H 3.81) with C-2 (δ_c 155.4) clearly indicated that the methoxy group located at C-2. The HMBC correlations of H-1' (δ_H 3.58)

with C-1 (δ_c 115.6), C-2 (δ_c 155.4), C-6 (δ_c 135.8) and the carbonyl carbon (δ_c 175.0) indicated that the furan-2(3H)-one should be fused at C-1 and C-6. The protons signals (δ_H 6.82 (d) J = 8.8, 7.26 (d) J = 8.8) also supported the substituents position on the aromatic ring. Thus, the structure of **1** was established as (E)-7-(3,7-dimethylocta-2,6-dienyl)-4-methoxybenzofuran-2(3H)-one.

HMBC ( ) ¹H-¹H COSY ( )

Fig. 2. Key HMBC and ¹H-¹H COSY 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 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 12.5 %.

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REFERENCES

1. The Editorial Committee of the Administration Bureau of Flora of China, Flora of China, Vol. 67, 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**, 508 (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).

TABLE-1
¹H NMR AND ¹³C NMR DATA OF COMPOUND **1**
(C₅D₅N, δ , ppm, J (Hz))

No.	δ_c (mult.)	δ_H (mult, J , Hz)	No.	δ_c (mult.)	δ_H (mult, J , Hz)
1	115.6 s		11	26.0 t	2.18, m
2	155.4 s		12	124.6 d	5.06, m
3	112.7 d	6.82, d, J = 8.8	13	131.2 s	
4	129.0 d	7.26, d, J = 8.8	14	25.2 q	1.62, s
5	128.6 s		15	18.2 q	1.50, s
6	135.8 s		16	16.8 q	1.68, s
7	28.2 t	3.22, d, J = 7.2	1'	39.2 t	3.58, s
8	121.6 d	5.26, t, J = 7.2	2'	175.0 s	10.8 brs
9	138.2 s		2-OMe	56.2 q	3.81 s
10	33.6 t	2.22, m			

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. T. Noshita, H. Kiyota, Y. Kidachi, K. Ryoyama, S. Funayama, K. Hanada and T. Murayama, *Biosci. Biotechnol. Biochem.*, **73**, 726 (2009).
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).