A New Phenylpropanoid from the Leaves of Flue-Cured Tobacco and Its Anti-Tobacco Mosaic Virus Activity

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Received: 7 May 2013;

Accepted: 17 August 2013;

Published online: 28 April 2014;

AJC-15072

A new phenylpropanoid, nicotpanoid C (**1**), was isolated from the leaves 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 modest anti-tobacco mosaic virus activity with inhibition rates of 22.4 %.

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

INTRODUCTION

Nicotiana tabacum L. is one of the most commercially valued agricultural crops in the world^{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, phenylpropanoids¹⁴⁻¹⁶, and the homologous, were isolated from this plant. Motivated by search for bioactive metabolites from this plant, the investigation on the chemical constituents of the leaves of flue-cured tobacco (a variety of *Nicotiana tabacum* L) was carried out. As a result, a new phenylpropanoid, nicotpanoid C (**1**), was isolated from this plant. 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.

Structures of compound **1**

EXPERIMENTAL

Optical rotation was measured in Horiba SEPA-300 high sensitive polarimeter. 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 × 250 mm, 7.0 μm) column and DAD detector.

The leaves 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 leaves of oriental tobacco (2.8 kg) were extracted four times with 90 % methanol (4 × 2.5 L) at room temperature and filtered to yield a filtrate. The crude extract (151 g) was applied to silica gel (200-300 mesh) column chromatography, eluting with a CHCl₃-(CH₃)₂CO 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, 18.3 g) by silica gel column chromatography, eluted with CHCl₃-MeOH (9:1, 8:2, 7:3, 6:4, 5:5), yielded mixtures D1 - D5. Fraction D2 (8:2, 1.82 g) was subjected to preparative HPLC (28 % MeOH-H₂O, flow rate 12 mL/min) to yield compound **1** (12.6 mg).

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

No.	δ _c (mult.)	δ _H (mult, J, Hz)	No.	δ _c (mult.)	δ _H (mult, J, Hz)
1	132.4 s		2'	38.3 t	2.75-2.84, overlap
2	106.8 d	6.52, d, J = 1.8			2.94-2.98, overlap
3	148.6 s		3'	71.6 d	6.20 m
4	139.5 s		4'	73.5 d	4.38 m
5	152.2 s		5'	70.8 d	4.82 m
6	104.9 d	6.65, d, J = 1.8	6'	39.0 t	2.75-2.84, overlap
7	145.2 d	7.76, d, J = 15.5	7'	176.8 s	10.8 brs
8	116.8 d	6.82, d, J = 15.5	4-OMe	55.8 q	3.79 s
9	167.8 s		5-OMe	61.2 q	3.81, s
1'	76.6 s		Ar-OH		11.85 brs

Nicotpanoid C (1): Obtained as a pale yellow gum; $[\alpha]_D^{25}$ -46.8 (*c* 0.20, MeOH); UV (MeOH), $\lambda_{\max}$ (log ϵ) 326 (3.92), 300 (3.68), 242 (4.08), 210 (4.36) nm; IR (KBr, $\nu_{\max}$, cm⁻¹) 3382, 2955, 2873, 1718, 1705, 1618, 1536, 1469, 1421, 1375, 1250, 1185, 1055, 958, 864; ¹H NMR and ¹³C NMR data (C₅D₅N, 500 MHz and 125 MHz, respectively), see Table-1; ESIMS (positive ion mode), *m/z* 421 [M+Na]⁺; HRESIMS (positive ion mode), *m/z* 421.1118 [M+Na]⁺ (calcd. 421.1111 for C₁₈H₂₂NaO₁₀).

RESULTS AND DISCUSSION

Compound **1** was obtained as pale yellow gum. Its molecular formula was determined as C₁₈H₂₂O₁₀ by HRESIMS, *m/z* 421.1118 [M+Na]⁺ (calcd. 421.1111), corresponding to eight degrees of unsaturation. Its ¹H and ¹³C NMR spectral data (Table-1) showed signals to 22 hydrogens and 18 carbons, respectively, corresponding to one aromatic ring (δ_c 132.4 s, 106.8 d, 148.6 s, 139.5 s, 152.2 s, 104.9 d) with two aromatic protons (δ_H 6.52 (d) *J* = 1.8 and 6.65 (d) *J* = 1.8), one acryl group (δ_c 145.2, 116.8, 167.8; δ_H 7.76 (d) *J* = 15.5, 6.82 (d) *J* = 15.5), one 3-*O*-quinic acid group (δ_c 76.6 s, 38.3 t, 71.6 d, 73.5 d, 70.8 d, 39.0 t, 176.8 s; δ_H 2.75-2.84 overlap, 2.94-2.98 overlap, 6.20 m, 4.38 m, 4.82 m, 2.75-2.84 overlap, 10.8 brs), two methoxy groups (δ_c 55.8 q, 61.2 q; δ_H 3.79 s, 3.81 s) and one phenolic hydroxy group (δ_H 11.85). Strong absorption bands accounting for hydroxy (3382 cm⁻¹), carbonyl (1718, 1705 cm⁻¹) and aromatic group (1618, 1536, 1469, 1421 cm⁻¹) could be observed in its IR spectrum. The UV spectrum of **1** showed absorption maxima at 326, 300, 242 and 210 nm also confirmed the existence of the aromatic function. The HMBC correlations (Fig. 1) of H-8 (δ_H 6.82) with C-1 (δ_c 132.4), of H-7 (δ_H 7.76) with C-1 (δ_c 132.4), C-2 (δ_c 106.8) and C-6 (δ_c 104.9), of H-2 (δ_H 6.52) with C-7 (δ_c 145.2) and of H-6 (δ_H 6.65) with C-7 (δ_c 145.2) suggested the acryl (-CH=CH-COO-) unit was attached to C-1. The long-range correlations of two methoxy proton signals (δ_H 3.79 and 3.81) with C-4 (δ_c 139.5) and C-5 (δ_c 152.2) clearly indicated that two methoxy groups located at C-4 and C-5. The HMBC correlations of phenolic hydroxy proton signal (δ_H 11.85) with C-2 (δ_c 106.8), C-3 (δ_c 148.6) and C-4 (δ_c 139.5) indicated that the hydroxy group should be located at C-2. The HMBC correlation of H-3' (δ_H 6.20) with C-9 (δ_c 167.8) indicated that the 3-*O*-quinic acid group should be located at C-9. Thus, the structure of **1** was established and it was named as nicotpanoid C.

Fig. 1. The key HMBC and ¹H-¹H COSY correlations of **1**

Since certain of the phenylpropanoids exhibit potential Anti-TMV activity, compounds **1** was tested for it anti-tobacco mosaic virus activity. The anti-TMV activities were tested using the half-leaf method¹⁷. 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 22.4 %.

ACKNOWLEDGEMENTS

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

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