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

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A new phenolic compound, (6*S*)-6-(4-(hydroxymethyl)-2-methoxyphenyl)-6-methoxy-2-methylheptan-1-ol (**1**), was isolated from the roots 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 16.4 %.

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

INTRODUCTION

The flue-cured tobacco is a type of cigarette tobacco. It belongs to the plants of *Nicotiana* genus. Along with burley tobacco, it accounts for more than 90 % of world tobacco production[1,2]. In addition, it is also used as insecticide, anesthetic, diaphoretic, sedative and emetic agents in Chinese folklore medicine due to it 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 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 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 of flue-cured tobacco. As a result, a new phenolic compound (**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

Optical rotations were measured with a Horiba SEPA-300 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 mm) column and DAD detector.

The roots 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. Yuan N (Yunnan University of Nationalities).

Extraction and isolation: The air-dried and powdered roots of flue-cured tobacco (1.8 kg) were extracted four times with 70 % aqueous acetone (3 × 2.5 L) at room temperature and filtered to yield a filtrate. The crude extract (105 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, 8.26 g) by silica gel column chromatography, eluted with petroleum ether-acetone (9:1, 8:2, 7:3, 6:4, 5:5, 0:1), yielded mixtures A1-A5. Fraction A1 (9:1, 1.06 g) was subjected to preparative HPLC (75 % MeOH-H_2O, flow rate 12 mL/min) to yield compound **1** (11.2 mg).

(6*S*)-6-(4-(Hydroxymethyl)-2-methoxyphenyl)-6-methoxy-2-methylheptan-1-ol (1): $C_{17}H_{28}NaO_4$; obtained as yellow gum; $[\alpha]_D^{24.5}$ + 8.6 (c = 0.1, MeOH); UV (CH_3OH), λ_{max} (log ε) 285 (3.64), 226 (3.85) 210 (4.22) nm; IR (KBr, ν_{max}, cm^{-1}) 3325, 2962, 2871, 1630, 1576, 1467, 1268, 1157, 1052, 857, 762; ^{13}C NMR and ^1H NMR data ($CDCl_3$, 500 and 125 MHz) (Table-1); postive ESIMS *m/z* 319 $[M + Na]^+$; postive HRESIMS *m/z* 319.1880 $[M + Na]^+$ (calcd. for $C_{17}H_{28}O_4Na$, 319.1885).

TABLE-1
^1H NMR AND ^{13}C NMR DATA OF COMPOUND **1** ($CDCl_3$, δ, ppm, J/HZ)

No.	δ_C (m)	δ_H (m, *J* in Hz)
1	126.2 s	
2	158.2 s	
3	113.6 d	6.71, d (1.8)
4	141.8 s	
5	117.9 d	6.80, dd (7.8, 1.8)
6	127.6 s	7.12, d (7.8)
7	82.2 s	
8	41.5 t	1.80, m
9	21.5 t	1.32, m, 1.15, m
10	36.3 t	1.25, m
11	35.5 d	1.52 m
12	68.2 t	3.36 m; 3.28, dd (10.4, 6.4)
13	18.8 q	0.80, d (6.8)
14	28.0 q	1.56, s
15	65.3 t	4.60 brs
2-OMe	55.9 q	3.78 s
7-OMe	52.2 q	3.28 s

RESULTS AND DISCUSSION

The air-dried and powdered roots of flue-cured tobacco (1.8 kg) was extracted with 70 % aqueous acetone (3 × 2.5 L) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure to obtained a crude extract (105 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.

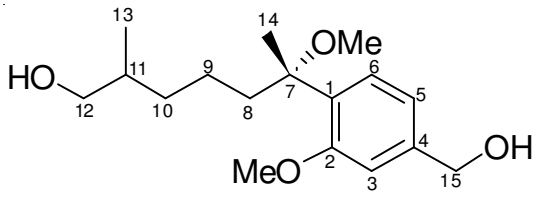

Fig.1. Structure of compound **1**

Compound **1** was obtained as a pale yellow gum with the molecular formula $C_{17}H_{28}O_4Na$ indicating four degrees of unsaturation, which was confirmed by HRESIMS at *m/z* 319.1880 $[M + Na]^+$ (calcd. for $C_{17}H_{28}NaO_4$, 319.1885). The UV spectrum exhibited absorptions at 226 and 285 nm suggesting an aromatic chromophore. The IR spectrum displayed absorption for a benzene ring (1630, 1576, 1467 cm^{-1}) and a hydroxyl group (3325 cm^{-1}), respectively. Analysis of $^{13}CNMR$ and DEPT spectra of **1** showed 17 carbons including one quaternary oxygenated aromatic carbon, two quaternary aromatic carbons, three tertiary aromatic carbons, one quaternary oxygenated carbon, two secondary oxygenated carbon, two methoxy, three methylene, one methine and two methyl groups. The ^1H NMR spectra displayed an ABX system of an aromatic ring [δ_H 7.12 (d, *J* = 7.8 Hz, H-6), 6.71 (d, *J* = 1.8 Hz, H-3), 6.80 (dd, *J* = 7.8, 1.8 Hz, H-5)], one methoxy group (δ_H 3.78 s), one hydroxymethyl group (δ_H 4.60 brs) and an (6S)-6-methoxy-2-methyl-heptan-1-ol structure unit [$CH_2OHCH(CH_3)(CH_2)_3C(CH_3)(OCH_3)$-][18]. The ^{13}NMR data of C-7-C-14 also supported the existence of (6*S*)-6-methoxy-2-methyl-heptan-1-ol structure unit. The methoxy group located at C-2 was supported by the HMBC correlation (Fig. 2) of methoxy proton signal (δ_H 3.78) with C-2 (δ_C 158.2). The HMBC correlationsof H-15 (δ_H 4.60) with C-3 (δ_C 113.6), C-4 (δ_C 141.8) and C-5 (δ_C 117.9), of H-3 (δ_H 6.71) and H-5 (δ_H 6.80) with C-15 (δ_C 65.3) suggested the hydroxymethyl group should be located at C-4. The (6*S*)-6-methoxy-2-methyl-heptan-1-ol structure unit located at C-1 was supported by the HMBC correlations of one H-8 (δ_H 1.80) with C-1 (δ_C 126.2) and of H-6 (δ_H 7.12) with C-7 (δ_C 82.2). The *S*-configuration of C-7 was also supported by its optical rotation data $[\alpha]^{24.5}{}_D$ + 8.6. Consequently, compound **1** was elucidated as (6*S*)-6-(4-(hydroxymethyl)-2-methoxyphenyl)-6-methoxy-2-methylheptan-1-ol.

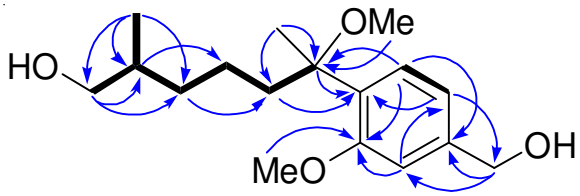

Fig.2. Key HMBC (↷) 1H-1H COSY (—) correlations of **1**

Since certain of the phenolic compounds exhibit potential Anti-TMV activity[19-21], compounds **1** was tested for it 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 16.4 %.

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