



A New Dihydrobenzofuran Neolignan from Stem of Flue-Cured Tobacco and its Anti-tobacco Mosaic Virus Activity

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Received: 16 June 2014;

Accepted: 22 August 2014;

Published online: 27 April 2015;

AJC-17172

A new dihydrobenzofuran neolignan, tobdihydrofuran A (**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 also tested for its anti-tobacco mosaic virus (anti-TMV) activity and it shows potential anti-tobacco mosaic virus activity with inhibition rates of 28.6 %.

Keywords: Dihydrobenzofuran neolignan, Tobdihydrofuran, Tobacco, Anti-tobacco mosaic virus.

INTRODUCTION

Nicotiana tabacum L. 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, phenylpropanoids¹⁴, and the homologous, were isolated from this plant. 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 multi-purpose utilization of the roots and stems of flue-cured tobacco is an interesting topical and receives more and more attentions^{15,16}. In this study, we report the isolation of a new dihydrobenzofuran neolignan, tobdihydrofuran A (**1**). Its structure was evaluated by spectroscopic methods, including HRMS and ¹D and ²D NMR. In addition, the anti-tobacco mosaic virus (anti-TMV) activity of compound **1** was also evaluated.

EXPERIMENTAL

Optical rotations were obtained on a Perkin-Elmer 341 digital polarimeter; UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. CD spectra were measured on a JASCO J-810 spectropolarimeter. 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 mm) column and DAD detector.

Stems of flue-cured tobacco were collected in Lijiang Prefecture, Yunnan Province, People's Republic of China, in September 2012. 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 tobacco stems (2 kg) were extracted four times with 90 % methanol (4 × 5 L) at room temperature and filtered to yield a filtrate. The crude extract (65.4 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. Further purification of the fraction C (8:2, 12.0 g) by silica gel column chromatography, eluted with petroleum ether-acetone (9:1, 8:2, 7:3, 6:4, 5:5), yielded mixtures C-1-C-5. Fraction C-3 (7:3, 1.67 g) was subjected to preparative HPLC (55 % MeOH-H₂O, flow rate 12 mL/min) to yield compound **1** (12.8 mg).

Tobadihydrofuran A (1): C₂₁H₂₄O₈, pale yellow gum; [α]_D^{24.6} -8.5 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 290 (3.58), 230 (3.64), 210 (4.12) nm; CD (c = 0.2, MeOH) λ_{max} (nm, Δε): 240 (+ 4.27); IR (KBr, ν_{max}, cm⁻¹) 3358, 2942, 2861,

TABLE-1
¹H AND ¹³C NMR DATA OF COMPOUND 1 (δ IN ppm, IN C₅D₅N, 500 AND 125 MHz)

Position	δ _c (m)	δ _H (m, J, Hz)	Position	δ _c (m)	δ _H (m, J, Hz)
1	126.9 s	-	3'	145.8 s	-
2	145.3 s	-	4'	150.5 s	-
3	152.4 s	-	5'	132.8 s	-
4	102.5 d	6.57 (d) 1.8	6'	116.9 d	7.62 s
5	153.2 s	-	7'	196.5 s	-
6	108.7 d	6.62 (d) 1.8	8'	42.6 t	3.08 t (6.5)
7	82.5 d	5.46 (d) 6.8	9'	58.9 t	3.62 t (6.5)
8	54.2 d	3.48 m	2-OMe	61.5 q	3.85 s
9	63.1 t	3.54 m	3-OMe	56.2 q	3.92 s
1'	130.1 s	-	3'-OMe	55.9 q	3.79 s
2'	113.2 d	7.48 s	5-OH	-	11.82 s

1839, 1664, 1605, 1527, 1438, 1317, 1038, 898, 635; ¹H and ¹³C NMR data (500 and 125 MHz) (Table-1); ESIMS *m/z* 427; HRESIMS *m/z* 427.1365 [M + Na]⁺ (calcd C₂₁H₂₄NaO₈ for 427.1369).

RESULTS AND DISCUSSION

A 90 % methanol extract prepared from the stems flue-cured tobacco was subjected repeatedly to column chromatography on Silic gel, Sephadex LH-20, RP-18 and Preparative HPLC to afford compound **1**. The structure of **1** was shown in Fig. 1 and its ¹H and ¹³C NMR data were listed in Table-1.

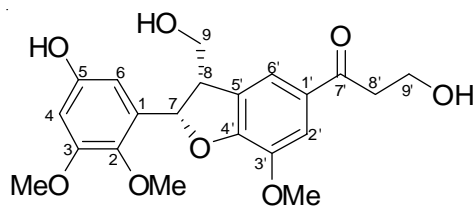


Fig. 1. Structure of compound **1**

Compound **1** was obtained as pale yellow gum. The molecular formula of **1** was determined as C₂₁H₂₄O₈ from its positive HRESIMS ion at *m/z* 427.1365 [M + Na]⁺ (calcd for C₂₁H₂₄NaO₈, 427.1369). The ¹H NMR spectrum of **1** showed the presence of a 1,2,3,5-tetrasubstituted benzene ring (δ_H 6.57, d, *J* = 1.8 and 6.62, d, *J* = 1.8), a 1,3,4,5-tetrasubstituted benzene ring (δ_H 7.48 s and 7.62 s), three methoxy group protons attached to the aromatic ring [δ_H 3.85 (3H, s), 3.92 (3H, s) and 3.79 (3H, s)], a oxidited methane proton (δ_H 5.46, 1H, d, *J* = 6.8 Hz), a methine proton (δ_H 3.48, 1H, m), two oxidited methylene protons signals (δ_H 3.54, 2H, m; 3.62, 2H, t, *J* = 6.5), a methylene protons (δ_H 3.08, 2H, t, *J* = 6.5) and a phenolic hydroxy proton (δ_H 11.82, 1H, s). The ¹H-¹H COSY correlations of H-7/H-8/H-9, in combination with its ¹³C NMR spectrum data indicated that **1** had a dihydrobenzofuran neolignan skeleton¹⁷. The ¹³C NMR spectrum of **1** (Table-1) showed 21 carbon signals. Aside from the carbon signals from the three methoxy groups (δ_c 61.5 q, 56.2 q and 55.9 q), the remaining eighteen carbon signals supported the presence of a 1,2,3,5-tetrasubstituted benzene ring (δ_c 126.9 s, 145.3 s, 152.4 s, 102.5 d, 153.2 s, 108.7 d), a 1,3,4,5-tetrasubstituted benzene ring (δ_c 130.1 s, 113.2 d, 145.8 s, 150.5 s, 132.8 s, 116.9 d), a carbonyl (δ_c 196.5), a oxidited methane (δ_c 82.5 d), a methine carbon (δ_c 54.2 d), two oxidited methylene

carbons (δ_c 63.1 t and 58.9 t) and a methylene carbon (δ_c 42.6 t). The ¹H-¹H COSY correlations of H-8'/H-9', together with the HMBC correlations (Fig. 2) of H-8' (δ_H 3.08) with C-7' (δ_c 196.5), C-9' (δ_c 58.9) and of H-9' (δ_H 3.62) with C-7' (δ_c 196.5) and C-8' (δ_c 42.6) confirmed that compound **1** possessed a 3-hydroxy-1-phenylpropan-1-one unit¹⁸, and the position of this unit at C-1' was confirmed by the HMBC correlation of H-8' (δ_H 3.62) with C-1' (δ_c 130.1), of H-2' (δ_H 7.48) and H-6' (δ_H 7.62) with C-7' (δ_c 196.5). The positions of the three methoxy groups at C-2, C-3 and C-3' were confirmed by the HMBC correlations of three methoxy protons (δ_H 3.85, 3.92 and 3.79) with C-2 (δ_c 145.3), C-3 (δ_c 152.4) and C-3' (δ_c 145.8), respectively. Meanwhile, the HMBC correlations of phenolic hydroxy proton (δ_c 11.82) with C-4 (δ_c 102.5), C-5 (δ_c 153.2) and C-6 (δ_c 108.7) confirmed the phenolic hydroxy group was located at C-4. A relative-trans configuration was determined by the coupling constant (*J*_{7,8} = 6.8 Hz) in accordance with literature reports^{19,20}. The CD spectrum showed a positive Cotton effect (Δε₂₄₀ + 4.27), so it was found that compound **1** had the 7*S*,8*R*-configuration²⁰. The structure of **1** was determined and named as tobdihydrofuran A.

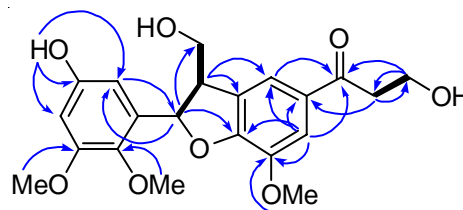


Fig. 2. ¹H-¹H COSY (---) and Key HMBC (—) correlation of compound **1**

Compounds **1** was tested for its anti-tobacco mosaic virus activity. The anti-TMV activities were tested using the half-leaf method^{18,21}. 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 an inhibition rate of 28.6 %.

ACKNOWLEDGEMENTS

This project was supported financially by the National Natural Science Foundation of China (No. 31360081), the Basic Research Foundation of Yunnan Province (2013FB097) and the Basic Research Foundation of Yunnan Tobacco Industry Co. Ltd (2012JC01).

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