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A New Benzofuran Derivatives from Flue-Cured Tobacco and Its Anti-Tobacco Mosaic Virus Activity

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A new benzofuran derivative, (2S,3*R*,4*R*)-3,4-dihydroxy-2,7-dimethoxy-3,4-dihydro-1(2*H*)-dibenzofuranone (**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 also tested for its anti-tobacco mosaic virus activity and it shows potential anti-tobacco mosaic virus activity with inhibition rates of 42.3 %.

Keywords: Benzofuran derivative, Flue-cured tobacco, Mosaic virus activity.

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 it contains many useful chemical compounds[1,3-5]. In previous work, a number of bioactive compounds, such as terpenoids[6-8], alkaloids[9,10], lignans[11,12], flavonoid[13], phenylpropanoids[14] and the homologous, were isolated from this plant. In this study, we report the isolation of a new benzofuran derivative *i.e.*, tabofuran 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. 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, 7.0 μm) column and DAD detector.

The leaves 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 leaves (2.5 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 fraction D (7:3, 14.8 g) by silica gel column chromatography, eluted with petroleum ether-acetone (9:1, 8:2, 7:3, 6:4, 5:5) and yielded mixtures D-1-D-5. Fraction D-4 (6:5, 1.52 g) was subjected to preparative HPLC (43 % MeOH-H_2O, flow rate 12 mL/min) to yield compound **1** (11.4 mg).

(2S,3R,4*R*)-3,4-Dihydroxy-2,7-dimethoxy-3,4-dihydro-1(2*H*)-dibenzofuranone (1): Obtained as a white powder; $[\alpha]_D^{23.6}$ -29.2 (*c* 0.20, MeOH); UV (MeOH), λ_{max} (log ε) 210 (4.22), 232 (3.45), 270 (3.81) nm; CD (*c* 0.2, MeOH) $\Delta\varepsilon_{195}$ + 1.46, $\Delta\varepsilon_{208}$ -1.57; IR (KBr, ν_{max}, cm^{-1}) 3421, 2943, 2870, 1682, 1602, 1563, 1478, 1452, 1136, 1090, 860, 749; ^{1}H NMR and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz, respectively), see Table-1; ESI-MS (positive ion mode), *m/z* 301 $[M^+Na]^+$; HRESIMS (positive ion mode), *m/z* 301.0682 $[M + Na]^+$ (calcd. 301.0688 for $C_{14}H_{14}O_6Na$).

TABLE-1
^{1}H AND ^{13}C NMR DATA OF COMPOUND **1** (δ IN ppm, IN C_5D_5N, 500 AND 125 MHz)

Position	δ_C (m)	δ_H (m, *J*, Hz)	Position	δ_C (m)	δ_H (m, *J*, Hz)
1	192.5 s	-	6	97.2 d	6.93 d (1.8)
1a	118.4 s	-	7	156.8 s	-
2	86.9 d	4.08 d (9.8)	8	113.6 d	6.72 dd (1.8, 8.5)
3	78.5 d	4.02 d (9.8, 7.5)	9	122.3 d	7.46 d (8.5)
4	70.6 d	4.96 d (7.5)	9a	118.6 s	-
4a	168.4 s	-	2-OMe	60.5 q	3.68 s
5a	157.4 s	-	7-OMe	56.2 q	3.82 s

RESULTS AND DISCUSSION

A 90 % methanol extract prepared from the 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 ^{1}H and ^{13}C NMR data were listed in Table-1.

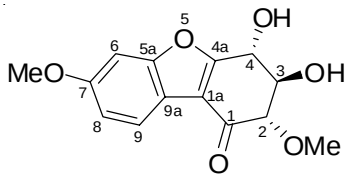

Fig. 1. Structure of compound **1**

Compound **1** was isolated as a white powder. Its molecular formula was determined to be $C_{14}H_{14}O_6$ by HREIMS. Its IR spectrum displayed absorption bands at 3421, 1682 and 1602 cm^{-1} due to hydroxy, carbonyl and aromatic groups, respectively. Analysis of its ^{1}H NMR revealed the presence of a 1,2,4-trisubstituted benzene ring at δ_H 6.93 (1H, d, *J* = 1.8 Hz), 6.72 (1H, dd, *J* = 1.8, 8.5 Hz), 7.46 (1H, d, *J* = 7.5 Hz); three contiguous oxymethines at δ_H 4.08 (1H, d, *J* = 7.5 Hz), 4.02, (1H, d, *J* = 9.8, 7.5), 4.96 (1H, d, *J* = 7.5) and two methoxy groups at δ_H 3.68 and 3.82 (3H, s, each). On the basis of DEPT and HMQC spectra, the ^{13}C NMR spectrum of **1** showed 14 carbon signals, which were assigned to a ketone carbonyl carbon at 192.5 (C-1); a benzofuran ring at δ_C 97.2 (C-6), 156.8 (C-7), 113.6 (C-8), 122.3 (C-9), 157.4 (C-5a), 118.6 (C-9a), 118.4 (C-1a) and 168.4 (C-4a); three oxygen-bearing methines at δ_C 86.9 (C-2), 78.5 (C-3) and 70.6 (C-4); and two methoxy carbons at δ_C 60.5 and 56.2. The HMBC correlations (Fig. 2) from H-2 (δ_H 4.08) to C-1, C-3 and C-4 and from H-3 (δ_H 4.02) to C-1, C-2 and C-4 were indicative of a connection between C-1 and C-2. The HMBC correlations from H-4 (δ_H 4.96) to C-4a and C-1a indicated that the structural unit (C1-C2-C3-C4) was linked to the C-4a of the benzofuran ring. Taking into account the eight degrees of unsaturation of **1**, we considered that the ketone carbonyl carbon C-1 was connected to the quaternary carbon C-1a to form a hydrogenated dibenzofuranone skeleton. Finally, the cross-peak demonstrated by the HMBC between the methoxy protons and C-2 and C-7 indicated that two methoxy groups were located at C-2 and C-7, respectively. In addition, the HMBC correlations from H-6 to C-8, C-5a and C-9a and from H-9 to C-7, C-5a and C-1a (Table-1) suggested the planar structure for compound **1**[15]. The relative configuration of **1** was established from ^{1}H-^{1}H coupling constants and NOESY experiments. First, the NOE correlation from H-2 to H-4 suggested that a 1,3-diaxial relationship existed between H-2 and H-4. In addition, the large coupling constants between H-2 and H-3 (*J* = 9.8 Hz) and between H-3 and H-4 (*J* = 7.5 Hz) indicated that H-3 was also in an axial position. Thus, 2-OCH_3, 3-OH and 4-OH were considered to occupy equatorial positions in a half-chair conformation. Its absolute configuration was determined by CD exciton chirality method[16,17]. The resultant CD spectrum showed a positive Cotton effect at 195 nm and a negative Cotton effect at 208 nm. This result suggested that **1** contains groups with 3 and 4*R* configurations and thus, the absolute configuration of C-2 was concluded to be *S*. On the basis of the above evidence, the structure of **1** was elucidated to be (2*S*,3*R*,4*R*)-3,4-dihydroxy-2,7-dimethoxy-3,4-dihydro-1(2*H*)-dibenzofuranone.

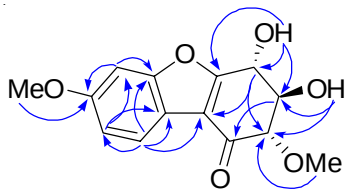

Fig. 2. Structure of HMBC correlations

Compound **1** was tested for its anti-tobacco mosaic virus activity. The anti-TMV activities were tested using the half-leaf method[17,18]. 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 rate of 42.3 %.

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