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NOTE

A New Phenanthrene from Flue-Cured Tobacco and Its Anti-tobacco Mosaic Virus Activity

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A new phenanthrene, 9,10-dihydro-5-hydroxy-2,8-dimethoxyphenanthrene-1,4-dione (**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 ^{1}D and ^{2}D 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 18.9 %.

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

Nicotiana tabacum L. is the most commonly grown of all plants in the Nicotiana genus. 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 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 phenanthrene, 9,10-dihydro-5-hydroxy-2,8-dimethoxyphenanthrene-1,4-dione (**1**). Its structure was evaluated by spectroscopic methods, including HRESIMS and ^{1}D and ^{2}D NMR. In addition, the anti-tobacco mosaic virus (anti-TMV) activity of compound **1** was also evaluated.

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 Puer Prefecture, Yunnan Province, People's Republic of China, in September 2012. The identification of the plant material was verified by Prof. Y. J. Chen (Yunnan University of Nationalities).

Extraction and isolation: The air-dried and powdered tobacco leaves (2.2 kg) were extracted four times with 90 % methanol (4 × 3.5 L) at room temperature and filtered to yield a filtrate. The crude extract (43.8 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 C (8:2, 10.4 g) by silica gel column chromatography, eluted with petroleum ether-acetone (9:1, 8:2, 7:3, 6:4, 5:5) and yielded mixtures C-1-C-5. Fraction C-3 (7:3, 1.28 g) was subjected to preparative HPLC (48 % MeOH-H_2O, flow rate 12 mL/min) to yield compound **1** (14.6 mg).

9,10-Dihydro-5-hydroxy-2,8-dimethoxyphenanthrene-1,4-dione (1): Obtained as an orange powder; UV (MeOH), λ_{max} (log ε) 215 (3.67), 226 (3.48), 278 (3.57), 325 (3.42) nm; IR (KBr, ν_{max}, cm^{-1}) 3384, 2990, 1672, 1635, 1598, 1450, 1357, 1246, 1139, 1094, 876, 791; ^{1}H NMR and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz, respectively) (Table-1); ESIMS (positive ion mode), *m/z* 309 $[M + Na]^+$; HRESIMS (positive ion mode), *m/z* 309.0732 $[M + Na]^+$ (calcd. 309.0739 for $C_{16}H_{14}NaO_5$).

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.

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	181.2 s	-	8	148.9 s	-
2	160.3 s	-	8a	130.2 s	-
3	108.4 d	6.12 s	9	19.4 t	2.82 m
4	190.8 s	-	10	28.5 t	2.68 m
4a	138.9 s	-	10a	145.3 s	-
4b	132.4 s	-	2-OMe	56.8 q	3.89 s
5	151.7 s	-	8-OMe	55.9 q	3.78 s
6	115.2 d	6.76 d (8.2)	5-OH	-	11.84 s
7	118.7 d	6.89 d (8.2)	-	-	-

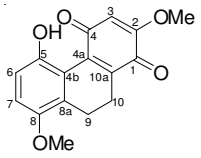

Fig. 1. Structure of compound **1**

Compound **1** was obtained as orange powder. The HRESIMS indicated a molecular ion at *m/z* 309.0732 [M + Na]$^+$ and the molecular formula was determined as $C_{16}H_{14}O_5$ by HREIMS in conjunction with NMR data analysis. The IR spectrum of **1** revealed strong absorption bands due to an aromatic ring (1598, 1450 cm^{-1}), conjugated carbonyl groups (1672, 1635 cm^{-1}) and hydroxy groups (3384 cm^{-1}). The UV spectrum also supported the presence of the aromatic ring (λ_{max} 215, 226, 242, 278, 325 nm). In the ^{1}H NMR spectrum, three aromatic proton signals at δ_H 6.12 (s), 6.76 (d, *J* = 8.2 Hz) and 6.89 (d, *J* = 8.2 Hz) were observed. The last two signals indicated the presence of a 1,2,3,4-tetrasubstituted phenyl group. Two methylene signals at δ_H 2.82 and 2.68, two methoxy signals at δ_H 3.89 and 3.78 and one phenolic hydroxy signal at δ_H 11.84 were also observed. The ^{13}C NMR spectrum also showed the presence of two carbonyl carbons (δ_C 190.8, 181.2), two methylene carbons (δ_C 28.5, 19.4) and 10 sp^2 hybridized carbons (δ_C 160.3, 108.4, 138.9, 132.4, 151.7, 115.2, 118.7, 148.9, 130.2, 145.3). These data indicated that **1** should be a phenanthrene derivative[15]. The ^{1}H-^{1}H COSY spectrum indicated two partial structures indicated by thick lines in Fig. 2. The long-range ^{1}H - ^{13}C correlations were analyzed *via* an HMBC spectrum that showed correlations of H-3 to C-1, C-2, C-4 and C-4a; H-6 to C-4b, C-5 and C-8; H-7 to C-5 and C-8a; H-9 to C-4b, C-8, C-8a and C-10a; H-10 to C-1, C-4a, C-8a and C-10a; 2-OMe to C-2; 8-OMe to C-8 and 5-OH to C-5, C-6, C-4b (Fig. 2). Furthermore, NOE correlations were observed between H-3 (δ_H 6.12) and 2-OMe (δ_H 3.89), between H-7 (δ_H 6.89) and 8-OMe (δ_H 3.78) and between H-6 (δ_H 6.76) and H-7 (δ_H 6.89). On the basis of these results, the structure of **1** was determined as 9,10-dihydro-5-hydroxy-2,8-dimethoxy-phenanthrene-1,4-dione.

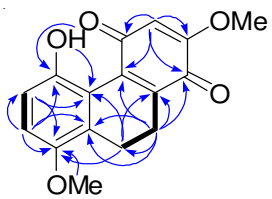

Fig. 2. ^{1}H-^{1}H COSY (—) are key HMBC ( ) correlation of **1**

Compound **1** was tested for its anti-tobacco mosaic virus activity. The anti-TMV activities were tested using the half-leaf method[16]. 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 18.9 %.

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