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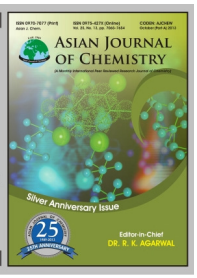

2-Arylbenzofurans from the Leaves of Oriental Tobacco and Their Cytotoxicity

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Four types of 2-arylbenzofurans (**1-4**), including a new compound, tobarylbenzofuran A (**1**), were isolated from the leaves of oriental tobacco (a variety of *Nicotiana tabacum* L). Their structures were elucidated by spectroscopic methods, including extensive 1D and 2D NMR techniques. The two compounds were tested for their cytotoxicity against five human tumors (NB4, A549, SHSY5Y, PC3, and MCF7) cell lines. The results showed that compound **1** showed notable inhibitory effect against SHSY5Y and MCF7 cell lines, with IC_{50} values of 3.5 and 1.8 μM, respectively.

Key Words: 2-Arylbenzofuran, Oriental tobacco, Cytotoxicity, *Nicotiana tabacum* L.

INTRODUCTION

Nicotiana tabacum L, a perennial herbaceous plant, is one of the most commercially valued agricultural crops in the world[1,2]. The leaves of *N. tabacum* are the most important raw material for cigarette industry. In addition to being used in cigarette industry, *N. tabacum* was found to be rich in many useful chemical compounds, such as sesquiterpenes[3,4], diterpenoids[5-7], alkaloids[8-10], lignans[11,12], flavonoid[13], phenylpropanoids[14,15] and the like. Motivated by search for bioactive metabolites from this plant, an investigation on the chemical constituents of the leaves of oriental tobacco (a variant of *N. tabacum*) was carried out. As a result, Four 2-arylbenzofurans (**1-4**), including a new compound, tobarylbenzofuran A (**1**), were isolated from this plant. In addition, the cytotoxicities of the compounds **1-4** were evaluated. This article deals with the isolation, structural elucidation and biological activities of the compounds.

EXPERIMENTAL

General methods: UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on DRX-500 spectrometers with TMS as internal standard. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. HRESIMS was performed on an API QSTAR time-of-flight spectrometer and a VG Autospec-3000 spectrometer, respectively. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a ZORBAX PrepHT GF (21.2 mm × 25 cm, 7 μm) column or a Venusil MP C_{18} (20 mm × 25 cm, 5 μm) column. Column chromatography was performed with Si gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China), Lichroprep RP-18 gel (40-63 μm, Merck, Darmstadt, Germany), Sephadex LH-20 (Sigma-Aldrich, Inc, USA) and MCI gel (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan) and MCI gel (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). The fractions were monitored by TLC and spots were visualized by heating Si gel plates sprayed with 5 % H_2SO_4 in EtOH.

The leaves of oriental tobacco were collected in Baoshan Prefecture, Yunnan Province, People's Republic of China, in September 2010. 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 of oriental tobacco (5.8 kg) was extracted with 70 % aq. MeOH (3 × 5 L) at room temperature and filtered. The filtrate was evaporated under reduced pressure and the crude extract (480 g) was applied to silica gel (150-200 mesh) column chromatography, eluting with a $CHCl_3$-MeOH gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to afford fractions A-F. Further separation of fraction B (32.2 g) on by silica gel (300-400 mesh), eluted with $CHCl_3$-$CO(CH_3)_2$ (9:1-1:2), yielded fractions B1-B7. Fraction B2 (6.28 g) was subjected to silica gel column chromatography using petroleum ether - $CH_3COOC_2H_5$ followed by semi-preparative HPLC (62 % MeOH-H_2O, flow

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

No.	δ_C (mult.)	$\delta\delta_H$ (mult, *J* = Hz)	No.	δ_C (mult.)	$\delta\delta_H$ (mult, *J* = Hz)
2	155.2 s	–	2', 6'	130.2 d	7.92 d, *J* = 8.6
3	105.9 d	7.04 s	3', 5'	115.8 d	6.85 d, *J* = 8.6
4	106.8 d	6.96 s	4'	158.2 s	–
5	152.7 s	–	1"	116.7 d	6.62 d, *J* = 10.0
6	113.5 s	–	2"	129.1 d	5.64 d, *J* = 10.0
7	110.4 d	7.35 s	3"	78.5 s	–
3a	123.2 s	–	4", 5"	29.3 q	1.53 s
7a	150.8 s	–	-OMe-4'	–	–
1'	124.0 s	–	Ar-OH-4'	–	10.88 s

rate 12 mL/min) to give **1** (22.8 mg). On the other hand, separation of fraction C (19.5 g) by silica gel (300-400 mesh) column chromatography, eluted with $CHCl_3$-$(CH_3)_2CO$ and followed by semi-preparative HPLC (38 % MeOH-H_2O, flow rate 12 mL/min) offered **2** (10.6 mg). Further separation of fraction D (38.8 g) by silica gel (300-400 mesh) column chromatography, eluted with $CHCl_3$-$(CH_3)_2CO$ and followed by semi-preparative HPLC (28 % MeOH-H_2O, flow rate 12 mL/min) led to the purification of **3** (10.6 mg) and **4** (22.5 mg).

Tobarylbenzofuran A (1): $C_{19}H_{16}O_3$, orange gum; UV (CH_3OH), λ_{max} (log ε) 210 (4.05), 295 (3.86), 345 (3.48) nm; IR (KBr, ν_{max}, cm^{-1}): 3340, 2984, 2875, 1603 1538, 1440, 1122, 1061; ^{13}C and ^{1}H NMR data (C_5D_5N, 500 and 125 MHz) see Table-1; negative ESIMS m/z 291 $[M-H]^-$; negative HRESIMS m/z $[M-H]^-$ 291.1018 (calcd. (%) for $C_{19}H_{15}O_3$, 291.1021).

RESULTS AND DISCUSSION

A 70 % aq. methanol extract prepared from the leaves of oriental tobacco was subjected repeatedly to column chromatography on silica gel, sephadex LH-20, RP-18 and preparative HPLC to afford a new 2-arylbenzofuran, tobarylbenzofuran, together with three known 2-arylbenzofurans (**1-4**). The structures of the compounds **1-4** were as shown in Fig. 1 and the ^{1}H and ^{13}C NMR data of **1** were listed in Table-1. The known compounds, compared with literature, were identified as moracin M (**2**)[16], moracin M 3'-O-β-D- glucopyranoside (**3**)[16] and schoenoside (**4**)[17].

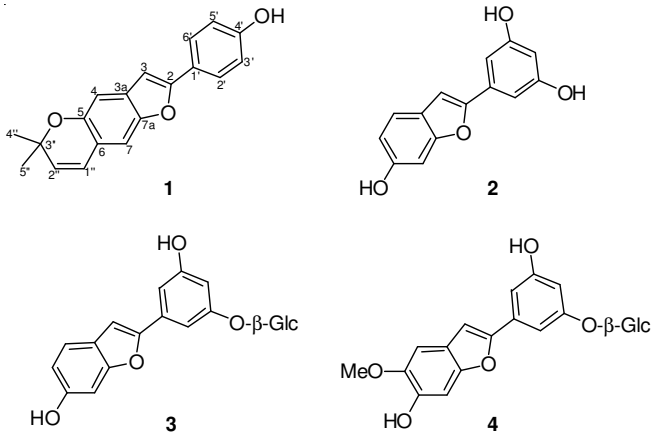

Fig. 1. Structures of arylbenzofurans from oriental tobacco

Compound **1** was obtained as an orange gum. It gave a parent ion by HRMS at m/z 291.1018 $[M-H]^-$ (calcd. for 291.1021 corresponding to a molecular formula $C_{19}H_{16}O_3$. Strong absorption bands accounting for hydroxy (3340 cm^{-1}) and aromatic groups (1603 1538, 1440 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of **1** showed absorption maxima at 295 and 345 nm, which confirmed the existence of the aromatic functions. Its ^{1}H, ^{13}C and DEPT NMR spectra (Table-1) showed signals for 19 carbons and 16 hydrogen atoms, corresponding to one 2-arylbenzofuran system[17] δ_C (155.2, 105.9, 106.8, 152.7, 113.5, 110.4, 123.2, 150.8, 124.0, 130.2 (2C) , 115.8 (2C), 158.2 s) with seven aromatic protons δ_H (7.04 s, 1H; 7.96 s, 1H; 7.35 s, 1H; 7.92 d, *J* = 8.6, 2H; and 6.85 d, *J* = 8.6, 2H), one gem-dimethylchromene moiety [δ_C (116.7, 129.1, 78.5, 29.3 (2C)) and δ_H (6.62 d, *J* = 10.0, 1H; 5.64 d, *J* = 10.0, 1H and 1.53 s, 6H)] and one phenolic hydroxy signal (δ_H 10.88). Long-range correlations (Fig. 2) of H-1" (δ_H 6.62) to C-5 (δ_C 152.7), C-6 (δ_C 113.5) and C-7 (δ_C 110.4) and of H-2" (δ_H 5.64) to C-6 (δ_C 113.5) were observed. This led us to conclude that the gem-dimethylchromene moiety was fused in an angular manner at C-5 and C-6. The position of the phenolic group at C-4' in **1** was established by the HMBC correlation (Fig. 2) of the hydroxy proton δ_H (10.88) to C-4' δ_C (158.2) and C-3',5' δ_C (115.8). On the basis of these observations, the structure of tobarylbenzofuran A (**1**) was elucidated as show.

Fig. 2. Selected HMBC (↷) correlations of **1**

Since certain of the 2-arylbenzofurans exhibit potential cytotoxicity[18,19], compounds **1-4** were tested for their cytotoxicities against five human tumor cell lines (NB4, A549, SHSY5Y, PC3 and MCF7) using the MTT method as reported previously[20]. Taxol was used as the positive control. The results were shown in Table-2. Compound **1** showed notable inhibitory effect against SHSY5Y and MCF7 cell lines, with IC_{50} values of 3.5 and 1.8 μM, respectively.

TABLE-2
CYTOTOXICITY DATA FOR
COMPOUNDS **1-4** (IC_{50} VALUES IN μM)

Compounds	NB4	A549	SHSY5Y	PC3	MCF7
1	8.0	7.4	3.5	7.4	1.8
2	8.2	>10	5.2	>10	4.8
3	6.9	8.2	4.5	>10	>10
4	>10	8.5	>10	>10	5.3
Taxol	0.03	0.02	0.2	0.2	0.1

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