HETEROCYCLES, Vol. 92, No. 8, 2016, pp.1462 - 1467. © 2016 The Japan Institute of Heterocyclic Chemistry
Received, 12th April, 2016, Accepted, 31st May, 2016, Published online, 20th June, 2016
DOI: 10.3987/COM-16-13482

TWO NEW 2-ARYLBENZOFURANS FROM THE STEMS OF *NICOTIANA TABACUM* AND THEIR BIOACTIVITIES

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Abstract – Two new 2-arylbenzofurans, 4-(7-methoxy-3,5-dimethyl-benzofuran-2-yl)phenol (**1**), 4-(6-methoxy-3,5-dimethylbenzofuran-2-yl)phenol (**2**), together with two known 2-arylbenzofurans (**3** and **4**) were isolated from the stems of *Nicotiana tabacum*. Their structures were determined by means of HRESIMS and extensive 1D and 2D NMR spectroscopic studies. Compounds **1**-**4** were tested for their anti-tobacoo mosaic virus (TMV) activities and cytotoxicity activities. The results showed that compound **2** exhibited high anti-TMV activities with inhibition rates of 36.4% at the concentration of 20 μM. This rate is higher than that of positive control. Compounds **1**-**4** showed moderate-to-weak cytotoxicities against some tested human tumor cell lines with IC_{50} values in the range of 2.2–8.4 μM.

Nicotiana tabacum, tobacco is an important economic crop.[1,2] Its leaves are used as a raw material for the tobacco industry, aerial plant as an insecticide, and also as anesthetic, diaphoretic, sedative, and emetic agents in Chinese folklore medicine.[1-3] In previous literatures, many new bioactive compounds, such as, sesquiterpenes,[4-6] alkaloids,[7,8] lignans,[9,10] flavonoids,[11-14] phenylpropanoids,[15,16] chromanones,[17,18] biphenyls,[19] phenolic amides,[20] isocoumarins,[21] were isolated from *N. tabacum*. The stems of *N. tabacum* are the main by-product in tobacco planting and are normally used as organic fertilizer. The multipurpose utilization of the roots and stems of *N. tabacum* is an interesting topic and has attracted more and more attentions.[16,21] In continuing efforts to utilize *N. tabacum* and identify bioactive natural products, the phytochemistry investigation of the stems of Yunyan 201 (a variety of *N. tabacum*) led to the isolation of two new (**1** and **2**) and two known 2-arylbenzofurans (**3** and **4**). This paper deals with the isolation, structural elucidation, and their bioactivities of these compounds.

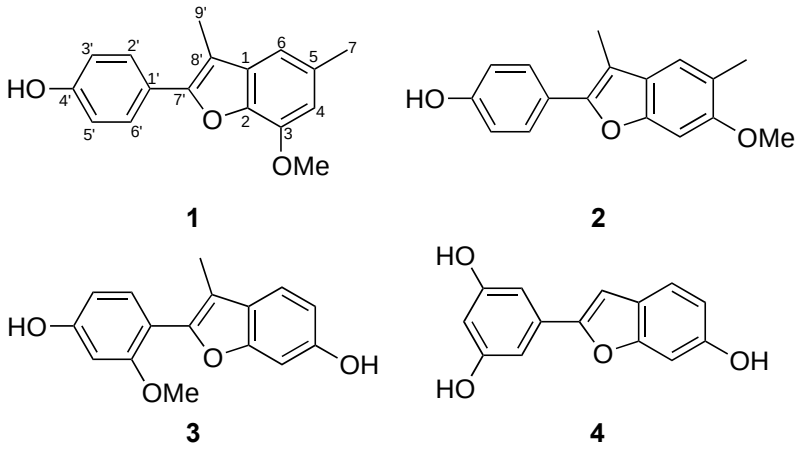

Figure 1. The structures of 2-arylbenzofurans from stems of *Nicotiana tabacum*

A 95% aq. MeOH extract prepared from the stems of tobacco was subjected repeatedly to column chromatography on silica gel and preparative HPLC to afford two new 2-arylbenzofurans, 4-(7-methoxy-3,5-dimethyl-benzofuran-2-yl)phenol (**1**) and 4-(6-methoxy-3,5-dimethylbenzofuran-2-yl)phenol (**2**), together with two known 2-arylbenzofurans (**3** and **4**). The structures of the compounds **1**-**4** were as shown in **Figure 1**, and the ^{1}H and ^{13}C NMR data of **1** and **2** were listed in Table 1. The known compounds, compared with literature, were identified as 2-[(2′-methoxy-4′-hydroxy)phenyl]-3-methy-6-hydroxybenzofuran (**3**) [22] and moracin M (**4**).[23]

Table 1. ^{1}H and ^{13}C NMR data of compounds **1** and **2** (in C_5D_5N)

No.	**1**		**2**	
	δ_C	δ_H (m, *J*, Hz)	δ_C	
1	126.7 s		118.1 s	
2	144.7 s		148.6 s	
3	151.4 s		101.3 d	7.02 s
4	112.3 d	7.37 (d) 1.8	155.6 s	
5	134.5 s		120.2 s	
6	116.8 d	7.73 (d) 1.8	121.5 d	7.33 s
7	24.9 q	2.31 s	18.6 q	2.40 s
1′	122.5 s		122.7 s	
2′,6′	129.8 d	8.00 (d) 8.6	130.0 d	7.98 (d) 8.6
3′,5′	116.3 d	6.78 (d) 8.6	116.5 d	6.79 (d) 8.6
4′	157.9 s		157.6 s	
7′	152.7 s		152.1 s	
8′	111.2 s		111.1 s	
9′	9.1 q	2.14 s	9.3 q	2.17 s
3-OMe	56.1 q	3.86 s		
4-OMe			56.4 q	3.84 s
Ar-OH		10.81 s		10.90 s

Compound **1** was obtained as a yellow gum. Its molecular formula was determined as $C_{17}H_{16}O_3$ by HRESIMS (*m/z* 291.0990 $[M+Na]^+$; calcd $C_{17}H_{16}NaO_3$ for 291.0997). It UV spectrum showed maximum absorption at 210, 270 and 309 nm. The IR absorptions at 3415, 1620, 1487, 1436 cm^{-1} indicated the presence of hydroxy and aromatic rings. The ^{1}H, ^{13}C NMR and DEPT spectra of **1** (**Table 1**) displayed 17 carbon and 16 proton signals, respectively, corresponding to a 1,2,3,5-tetrasubstituted phenyl ring (C-1~C-6, H-4 and H-6), a 1,4-substituted phenyl ring (C-1'~C-6', H_2-2',6', and H_2-3',5'), two methyl group (C-7 and C-9'; H_3-7 and and H_3-9'), two olefin carbons (C-7' and C-8'), one methoxy group (δ_C 56.1 q; δ_H 3.86 s), and one phenolic hydroxy group (δ_H 10.81). Its ^{1}H and ^{13}C NMR spectroscopic data [two phenyl rings, two olefin carbons (C-7' and C-8'), and methyl group (C-9' and H_3-9')] were similar to those of known compound (**3**), which suggested that **1** should be a

3-methyl-2-arylbenzofuran.[22] In addition, the 3-methyl-2-arylbenzofuran skeleton of **1** was also assigned by the HMBC correlations (**Figure 2**) of H_3-9′ with C-1, C-7′, and C-8′; of H_2-2′,6′ with C-7′; and of H-6 with C-8′. The position of the methyl group (C-7) at C-5 of the arylbenzofuran was elucidated by the HMBC correlations from H_3-7 to C-4, C-5, and C-6; from H-4 to C-7; and from H-6 to C-7. The HMBC correlations from the methoxy proton (δ_H 3.86) to C-3 (δ_C 151.4) supported the methoxy group located at C-3. The phenolic hydroxy group located at C-4′ was supported by the HMBC correlation of phenolic hydroxy proton (δ_H 10.81) with C-4′ and C-3′,5′. Thus, the structure of **1** was established as 4-(7-methoxy-3,5-dimethylbenzofuran-2-yl)phenol.

Compound **2** was also obtained as yellow gum and it gave an $[M+Na]^+$ peak at *m/z* 291.0998 in HRESIMS, consistent with a molecular formula of $C_{17}H_{16}O_3$. The ^1H and ^{13}C spectra data of **2** was very similar to these of **1** (see **Table 1**). The major differences were due to the substituent position variations on the tetrasubstituted phenyl ring (C-1~C-6). The methyl group (C-7) located at C-5 of was supported by the HMBC correlations from H_3-7 to C-4, C-5, and C-6; from H-4 to C-7; and from H-6 to C-7. The HMBC correlations from the methoxy proton (δ_H 3.84) to C-4 (δ_C 155.6) supported the methoxy group located at C-4. The phenolic hydroxy group located at C-4′ was supported by the HMBC correlation of phenolic hydroxy proton (δ_H 10.90) with C-4′ and C-3′,5′. Thus, the structure of 4-(6-methoxy-3,5-dimethylbenzofuran-2-yl)phenol (**2**) was established.

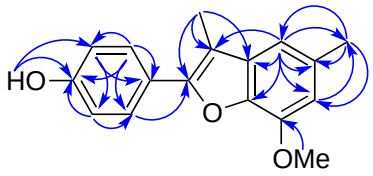

Figure 2. key HMBC (↷) correlations of **1**.

Table 2. TMV Infection inhibition activities of compounds **1-4**

Compounds	Inhibition rate (%)	IC_{50} (μM)	Compounds	Inhibition rates (%)	IC_{50} (μM)
1	25.3 ± 3.5	62.5	**4**	21.2 ± 3.0	78.3
2	36.4± 3.2	31.6	ningnanmycin	31.5 ± 3.1	36.3
3	20.6 ± 2.8	71.6			

All results are expressed as mean ± SD; n = 3 for all groups.

Compounds **1-4** were tested for their anti-tobacoo mosaic virus (TMV) activities. The anti-TMV activities were tested by half-leaf method, using ningnanmycin (a commercial product for plant disease in China, with inhibition rate of 32.2%) as a positive control.[24,25] The results showed that compounds **2** exhibited high anti-TMV activities with inhibition rates of 36.4% at the concentration of 20 μM. This rate is higher than that of positive control. The other compounds also showed potential activities with inhibition rates in the range of 20.6%~25.3 % at the concentration of 20 μM, respectively.

The cytotoxicities of compounds **1-4** were also tested using a previously reported procedure.[26,27] The cytotoxic abilities against five human tumor cell lines (NB4, A549, SHSY5Y, PC3, and MCF7) by MTT-assay were summarized in **Table 3**. The results revealed that compounds **1-4** showed moderate-to-weak inhibitory activities against some tested human tumor cell lines with IC_{50} values in the range of 2.2–8.4 μM.

EXPERIMENTAL

General. 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, and the chemical shifts (δ) were expressed in ppm. 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). The fractions were monitored by TLC, and spots were visualized by heating Si gel plates sprayed with 5% H_2SO_4 in EtOH.

Table 3. Cytotoxic activity of compounds **1**–**4**

Compounds	Cell lines and IC_{50} (μM)				
	NB4	A549	SHSY5Y	PC3	MCF7
1	3.8	5.9	>10	6.4	>10
2	2.8	4.5	5.2	2.2	3.4
3	7.3	>10	>10	6.8	8.4
4	>10	5.5	>10	>10	6.0
Taxol	0.03	0.02	0.05	0.05	0.05

NB4, human leukemia cell; A549, carcinomic human alveolar basal epithelial cell; SHSY5Y, human neuroblastoma cell; PC3, Human prostate cancer cell; MCF7, Human breast adenocarcinoma cell.

Plant material. The stems of *N. tabacum* L (tobacco stems) was collected from Lijiang County, Yunnan Province, P. R. China, in September 2014. The tobacco variety is Yunyan-201, which had widely cultivated in China. The identification of the plant material was verified by Prof. H. W. Yang (School of Tobacco, Yunnan Agriculture University).

Extraction and Isolation. The air-dried and powdered tobacco stems (3.8 kg) were extracted with 95% MeOH, and the extract was partitioned between EtOAc. The EtOAc-soluble materials (63.8 g) were applied to silica gel (200–300 mesh) column chromatography, eluting with $CHCl_3$/MeOH gradient system (9:1, 8:2, 7:3, 6:4, 5:5, 4:6) to give six fractions A-F. Further separation of fraction A (9:1, 13.6 g) by silica gel column chromatography, eluted with $CHCl_3$/Me_2CO (8:2-2:1) yielded mixtures A1–A7. Fraction A2 (7:3, 0.67 g) was subjected to silica gel column chromatography using petroleum ether/acetone, and then semi-preparative HPLC (48% MeOH/H_2O, flow rate 12 mL/min) to give **1** (12.5 mg) and **2** (10.8 mg). Fraction A3 (6:4, 1.64 g) was subjected to silica gel column chromatography using petroleum ether/acetone, and then semi-preparative HPLC (44% MeOH/H_2O, flow rate 12 mL/min) to give **3** (14.2 mg) and **4** (13.1 mg).

Anti-TMV Assays. The anti-TMV activity was tested using the half-leaf method,[24,25] and Ningnanmycin (2% water solution), a commercial product for plant disease in China, was used as positive control.

Cytotoxicity Assay. The cytotoxicity tests for the isolates were performed by against NB4, A549, SHSY5Y, PC3, and MCF7 tumor cell lines by MTT-assay (with Taxol as the positive control).[26,27]

4-(7-Methoxy-3,5-dimethylbenzofuran-2-yl)phenol (1) : $C_{17}H_{16}O_3$, obtained as a yellow gum; UV (MeOH) λ_{max} (log ε) 210 (4.10), 270 (3.15), 309 (3.52) nm; IR (KBr) ν_{max} 3415, 1620, 1487, 1436, 1379, 1158, 1069, 865, 793 cm^{-1}; ^1H and ^{13}C NMR (500 and 125 MHz, in C_5D_5N, see Table-1; ESIMS *m/z* (positive ion mode) 291 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 291.0990 $[M+Na]^+$ (calcd $C_{17}H_{16}NaO_3$ for 291.0997).

4-(6-Methoxy-3,5-dimethylbenzofuran-2-yl)phenol (**2**)*:* $C_{17}H_{16}O_3$, obtained as a yellow gum; UV (MeOH) λ_{max} (log ε) 210 (4.06), 268 (3.26), 306 (3.57) nm; IR (KBr) ν_{max} 3420, 1618, 1482, 1440, 1382, 1153, 1062, 880, 764 cm^{-1}; ^1H and ^{13}C NMR (500 and 125 MHz, in C_5D_5N, see Table-1; ESIMS *m/z* (positive ion mode) 291 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 291.0998 $[M+Na]^+$ (calcd $C_{17}H_{16}NaO_3$ for 291.0997).

ACKNOWLEDGEMENTS

This research was supported by the National Natural Science Foundation of China (No. 21562049, No. 31360081 and No. 31400303), and the Applied Fundamental Foundation of Yunnan Province (No. 2014FB163, No. 2015FB162).

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