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THREE NEW ARYLBENZOFURAN LIGNANS FROM *ARCTIUM LAPPA* AND THEIR ANTI-TOBACCO MOSAIC VIRUS ACTIVITY

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Abstract – Three new (**1-3**), together with two known (**4** and **5**) arylbenzofuran lignans were isolated from the seed of *Arctium lappa*. Their structures were elucidated by spectroscopic methods, including extensive 1D- and 2D-NMR techniques. Compounds **1-3** were evaluated for their anti-tobacco mosaic virus (Anti-TMV) activity. The results showed that compounds **1-3** showed potential anti-TMV activities with inhibition rates of 33.5%, 32.8%, and 34.2%, at the concentration of 20 μ M, respectively. These rates are higher than that of positive control.

Burdock (*Arctium lappa*) is a biennial herb plant belongs to the genus of *Arctium*. It is also called niubang in Chinese and gobo in Japanese.^{1,2} This plant has been cultivated in Eastern Asia (particularly in China, Japan, and Korea) as a root vegetable or traditional medicinal plant for centuries, and remains popular now.³ Its seed (known as niubangzi) had widely been used as traditional Chinese medicine as a diuretic, diaphoretic, and a blood purifying agent.^{4,5}

The previous studies have shown the presence of lignans,⁶⁻⁸ terpenoids,^{9,10} phenolic acids,¹¹⁻¹³ flavonoids,^{14,15} and the like, in this plants. Lignans are an important kind of secondary plant metabolites composed from two (or more) phenylpropanoid units. Although their molecular backbone consists only of two phenylpropane (C6-C3) units, lignans show an enormous structural diversity. There is a growing interest in lignans and their synthetic derivatives due to their applications in various pharmacological effects.^{16,17} In our previous researches, some lignans had also found to be exhibited potential anti-TMV

activity.¹⁸⁻²⁰

In our continuing efforts to identify bioactive natural products from traditional Chinese medicine, we now investigated the chemical constituents of the seed of *A. lappa*. This leads to the isolation of three new (**1-3**) and two known (**4** and **5**) arylbenzofuran lignans. Described in this paper are the structure elucidation of compounds **1-5**, and the anti-tobacco mosaic virus (Anti-TMV) activity of compounds **1-3**.

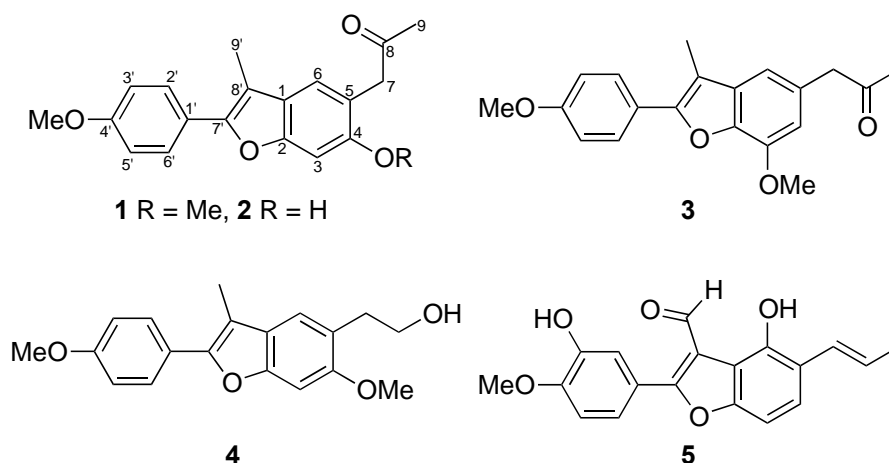
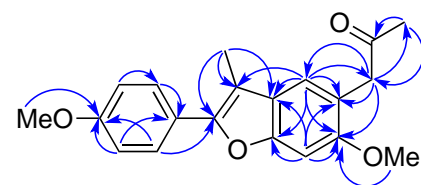


Figure 1. Arylbenzofuran lignans from the seed of *A. lappa*

The seed of *A. lappa* were extracted with 80% acetone, followed by repeated column chromatography on silica gel, Sephadex LH-20 and RP-18 silica gel. Final purification by semi-preparative RP-HPLC afforded three new arylbenzofuran lignans, 1-(6-methoxy-2-(4-methoxyphenyl)-3-methylbenzofuran-5-yl)propan-2-one (**1**), 1-(6-hydroxy-2-(4-methoxyphenyl)-3-methylbenzofuran-5-yl)propan-2-one (**2**), 1-(7-methoxy-2-(4-methoxyphenyl)-3-methylbenzofuran-5-yl)propan-2-one (**3**), together with two known arylbenzofuran lignans (**4** and **5**). The structures of the compounds **1-5** were as shown in Figure 1, and the ¹H and ¹³C NMR data of **1-3** were listed in Table 1. The known compounds, compared with literature, were identified as 2-(6-methoxy-2-(4-methoxyphenyl)-3-methylbenzofuran-5-yl)ethanol (**4**)²¹ and iteafuranal B (**5**).²²

Compound **1** was obtained as a pale yellow gum. Its molecular formula was established as C₂₀H₂₀O₄ by its HR-ESI-MS spectrum (*m/z* 347.1252, [M+Na]⁺, calcd 347.1259). The IR spectrum of **1** indicated the presence of carbonyl group (1726 cm⁻¹) and aromatic groups (1620, 1534, and 1457 cm⁻¹), and its



UV absorptions at 320 and 278 nm were characteristics for benzene chromophore. On the basis of ¹H and ¹³C NMR spectral analysis (Table 1), compound **1** showed characteristic peaks for one *p*-disubstituted benzene unit (C-1'~C-6'; H₂-2',6' and H₂-3',5'), one 1,2,4,5-tetrasubstituted benzene unit (C-1~C-6; H-3

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

and H-6), one 2-oxopropyl group (-CH₂-CO-CH₃, C-7~C-9; H₂-7 and H₃-9),²³ a pair of double bond (C-7' and C-8'), one methyl group (C-9' and H₃-9'), and two methoxy group (δ_C 56.0 and 56.4; δ_H 3.85 and 3.80 s, each 3H, s). The NMR data of two benzene unit, double bond (C-7' and C-8'), and methyl group (C-9' and H₃-9') suggested that compounds **1** should be a 3-methylbenzofurans.²¹ This deduction also supported by the HMBC correlations (Figure 2) of H₃-9' with C-1, C-7', and C-8'; of H₂-2',6' with C-1' and C-7'; and of H-6 with C-1, C-2, and C-8'. Since the methylbenzofurans skeleton was determined, one 2-oxopropyl group and two methoxy groups were accounted for the remaining substituents. The HMBC correlations from two methoxy protons (δ_H 3.85 and 3.80) to C-4 (δ_C 156.7) and C-4' (δ_C 160.6) indicated that two methoxy group was located at C-4 and C-4' respectively. The HMBC correlations of the H₂-7 (δ_H 3.56) with C-4 (δ_C 156.7), C-5 (δ_C 114.6) and C-6 (δ_C 122.2); of H-6 (δ_H 7.37) with C-7 (δ_C 46.7) confirmed that the 2-oxopropyl group was located at C-5. Thus, the structure of **1** was established, and gave the system name of 1-(6-methoxy-2-(4-methoxyphenyl)-3-methylbenzofuran-5-yl)propan-2-one.

Table 1. ¹H and ¹³C NMR data for compounds **1-3** (500 and 125 MHz, in CDCl₃)

No.	Compound 1		Compound 2		Compound 3	
	δ_C	δ_H (m, J, Hz)	δ_C	δ_H (m, J, Hz)	δ_C	δ_H (m, J, Hz)
1	118.4 s		118.0 s		126.7 s	
2	150.2 s		150.5 s		145.5 s	
3	101.3 d	7.00 s	102.9 d	6.96 s	149.2 s	
4	156.7 s		154.0 s		110.3 d	7.16 d (2.2)
5	114.6 s		115.8 s		132.3 s	
6	122.2 d	7.37 s	122.7 d	7.41 s	115.0 d	7.40 d (2.2)
7	46.7 t	3.56 s	46.0 t	3.57 s	51.7 t	3.54 s
8	206.3 s		206.5 s		206.2 s	
9	30.2 q	2.18 s	30.4 q	2.19 q	30.7 q	2.17 s
1'	120.6 s		120.8 s		122.4 s	
2',6'	130.7 d	7.79 d (8.8)	130.8 d	7.77 d (8.8)	130.6 d	7.81 d (8.8)
3',5'	115.9 d	6.79 d (8.8)	116.0 d	6.80 d (8.8)	115.9 d	6.78 d (8.8)
4'	160.6 s		160.6 s		160.5 s	
7'	152.7 s		153.0 s		152.5 s	
8'	111.4 s		111.9 s		111.7 s	
9'	8.4 q	1.93 s	8.6 q	1.88 s	8.7 q	1.91s
3-OMe					56.4 q	3.82 s
4-OMe	56.4 q	3.85 s				
4'-OMe	56.0 q	3.80 s	55.9 q	3.79 s	56.0 q	3.80 s
Ar-OH				10.27 s		

1-(6-Hydroxy-2-(4-methoxyphenyl)-3-methylbenzofuran-5-yl)propan-2-one (**2**) was obtained as a pale yellow gum and showed a quasi-molecular ion at m/z 333.1108 [M+Na]⁺ in the HRESIMS (calcd m/z 333.1103), corresponding to the molecular formula C₁₉H₁₈O₄. The ¹H and ¹³C NMR spectra of **2** were

similar to those of **1**. The chemical shift differences resulted from the downfield shift of C-4 from δ_C 156.7 ppm to δ_C 154.0 ppm, and the disappearance of a methoxy resonance and appearance of a phenolic hydroxy resonance (δ_H 10.27 s) in **2**. These changes indicated that a methoxy group at C-4 in **1** was converted into a phenolic hydroxy group in **2**. The HMBC correlation of the phenolic hydroxy proton (δ_H 10.27) with C-3 (δ_C 102.9), C-4 (δ_C 154.0) and C-5 (δ_C 115.8) also indicated that the phenolic hydroxy group was located at C-4. The structure of **2** was therefore defined.

Compound **3** was also obtained as a pale yellow gum, and its molecular formula was determined to be $C_{20}H_{20}O_4$, by HREIMS experiment (m/z 347.1263 $[M+Na]^+$), requiring eleven degrees of unsaturation. The 1H and ^{13}C spectral data of **3** also depict similar structure to compound **1**. The obvious chemical shift differences resulted from the substituents positions variation on the aromatic rings. The 2-oxopropyl group attached to C-5 was deduced from the HMBC cross peaks between H₂-7 with C-4, C-5 and C-6; of H-6 with C-7. The HMBC correlations of two methoxy protons (δ_H 3.82 and 3.80) with C-3 and C-4' suggested two methoxy groups at C-3 and C-4', respectively. Thus, the structure of 1-(7-methoxy-2-(4-methoxyphenyl)-3-methylbenzofuran-5-yl)propan-2-one (**3**) was determined.

Since certain lignans exhibit potential anti-TMV activities,¹⁸⁻²⁰ compounds **1-3** were tested for their anti-TMV activity. The anti-TMV activity was tested using the half-leaf method. Ningnanmycin (a commercial product for plant disease in China) with inhibition rate of 31.2%, was used as a positive control.²⁴ The results revealed that compounds **1-3** showed high anti-TMV activity with inhibition rates of 33.5%, 32.8%, and 34.2%, at the concentration of 20 μM , respectively. These rates are higher than that of positive control.

General Experimental Procedures. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectra. 1D- and 2D-NMR spectroscopic data were recorded on a DRX-500 NMR spectrometer with TMS as internal standard. Chemical shifts (δ) are expressed in ppm with reference to the TMS signal. HRESIMS was performed on a VG Autospec-3000 spectrometer. Semi-preparative HPLC was performed on an Agilent 1260 preparative liquid chromatograph with Zorbax PrepHT GF (2.12 mm \times 25 cm) or Venusil MP C₁₈ (2.0 mm \times 25 cm) columns. Column chromatography was performed using silica 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), or MCI gel (75-150 μm , Mitsubishi Chemical Corporation, Tokyo, Japan). Column fractions were monitored by TLC visualized by spraying with 5% H₂SO₄ in EtOH and heating.

Plant Material. The seed of *Arctium lappa* L. (niubangzi) was purchased from Juhuaacun Chinese Traditional Medicine Market, Kunming, Yunnan Province, P. R. China, in February 2016, and identified by Prof. Xiao Cheng, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen

(No. KM06-158) has been deposited in Yunnan Key Laboratory of Pharmacology for Natural Products, Kunming Medical University, P. R. China.

Extraction and Isolation. The samples (4.8 kg) were crushed to 30-50 mesh, and the powders were extracted with 80% acetone (4 × 8 L) at room temperature and filtered. The filtrate was evaporated under reduced pressure, and the crude extract (514 g) was applied to a silica gel (150-200 mesh) column eluted with chloroform-methanol (CHCl₃-MeOH) gradients (20:1, 9:1, 8:2, 7:3, 6:4, 5:5) to afford six fractions (A-F). Further separation of fraction B (9:1, 48.9 g) by silica gel column chromatography, eluted with CHCl₃-acetone (1:0-1:2), yielded subfractions B1–B7. Subfraction B2 (9:1, 12.2 g) was loaded on another silica gel column using petroleum ether-ethyl acetate (EtOAc) elution, and then separated semi-preparative HPLC (68% MeOH, flow rate 20 mL/min) to afford **1** (11.8 mg) and **3** (14.9 mg). Subfraction B3 (8:2, 8.56 g) was separated on another silica gel column eluted by petroleum ether-EtOAc, followed by semi-preparative HPLC (60% MeOH, flow rate 20 mL/min) to give **2** (15.5 mg), **4** (13.2 mg), and **5** (14.7 mg).

Anti-TMV Assays. The anti-TMV activities were tested using the half-leaf method,²⁴ and Ningnanmycin (2% water solution), a commercial product for plant disease in China, was used as a positive control. The virus was inhibited by mixing with the solution of tested compounds (20 μM in DMSO). After 30 min, the mixture was inoculated on the left side of the leaves of *N. glutinosa*, whereas the right side of the leaves was inoculated with the mixture of DMSO solution and the virus as control. The local lesion numbers were recorded 3-4 days after inoculation. Three repetitions were conducted for each compound. The inhibition rates were calculated according to the formula:

$$\text{inhibition rate (\%)} = [(C-T) / C] \times 100\%$$

where C is the average number of local lesions of the control and T is the average number of local lesions of the treatment. Ningnanmycin (20 μM in DMSO), a commercial virucide for plant disease in China, was used as a positive control.

1-(6-Methoxy-2-(4-methoxyphenyl)-3-methylbenzofuran-5-yl)propan-2-one (1), C₂₀H₂₀O₄, obtained as pale yellow gum; UV (MeOH) λ_{max} (log ε): 210 (4.15), 278 (3.27), 320 (3.62) nm; IR (KBr) ν_{max} 3081, 2968, 1726, 1620, 1534, 1457, 1380, 1162, 1071, 869, 784; ¹H and ¹³C NMR data (CDCl₃, 500 and 125 MHz), see Table 1. Positive ESIMS *m/z* 347 [M+Na]⁺; Positive HRESIMS *m/z* 347.1252 [M+Na]⁺ (calcd for C₂₀H₂₀NaO₄, 347.1259).

1-(6-Hydroxy-2-(4-methoxyphenyl)-3-methylbenzofuran-5-yl)propan-2-one (2), C₁₉H₁₈O₄, obtained as pale yellow gum; UV (MeOH) λ_{max} (log ε): 210 (4.20), 275 (3.47), 316 (3.68) nm; IR (KBr) ν_{max} 3429, 3064, 2960, 1723, 1616, 1542, 1455, 1384, 1159, 1067, 885, 793; ¹H and ¹³C NMR data (CDCl₃, 500 and 125 MHz), see Table 1. Positive ESIMS *m/z* 333 [M+Na]⁺; Positive HRESIMS *m/z* 333.1108 [M+Na]⁺ (calcd for C₁₉H₁₈NaO₄, 333.1103).

1-(7-Methoxy-2-(4-methoxyphenyl)-3-methylbenzofuran-5-yl)propan-2-one (3), C₂₀H₂₀O₄, obtained as pale yellow gum; UV (MeOH) λ_{\max} (log ϵ): 210 (4.26), 280 (3.48), 323 (3.65) nm; IR (KBr) ν_{\max} 3054, 2962, 1720, 1615, 1538, 1449, 1378, 1157, 1059, 854, 786; ¹H and ¹³C NMR data (CDCl₃, 500 and 125 MHz), see Table 1. Positive ESIMS m/z 321 [M+Na]⁺; Positive ESIMS m/z 347 [M+Na]⁺; Positive HRESIMS m/z 347.1263 [M+Na]⁺ (calcd for C₂₀H₂₀NaO₄, 347.1259).

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