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# Phenolics from the Fruits of *Maclura pomifera*

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Phenolics from the Fruits of *Maclura pomifera*Zushang Su<sup>a</sup>, Ping Wang<sup>a</sup>, Wei Yuan<sup>a</sup>, Greg Grant<sup>b</sup> and Shiyu Li<sup>a,\*</sup><sup>a</sup> National Center for Pharmaceutical Crops, Arthur Temple College of Forestry and Agriculture, Stephen F. Austin State University, Nacogdoches, TX 75962-6109, USA<sup>b</sup> Pineywoods Native Plant Center, Arthur Temple College of Forestry and Agriculture, Stephen F. Austin State University, Nacogdoches, TX 75962-6109, USA

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Two new compounds, maclurin A (**1**) and maclurin B (**2**), and six known ones, ononin (**3**), pterofuran (**4**), osajin (**5**), pomiferin (**6**), 3,4-dihydroxybenzoic acid (**7**), and 2,3,4-trihydroxybenzoic acid (**8**) were isolated from the fruit of *Maclura pomifera*. Compounds **3** and **4** were isolated from the genus for the first time. Structure elucidation was achieved by spectroscopic measurements and by comparison with literature data. Compounds **2–4** exhibited activities against the cancer cell lines A549 and Panc-28 with GI<sub>50</sub> values from 18.1 to 32.2, and 20.6 to 43.5  $\mu$ M, respectively. Compounds **2** and **4** also showed cytotoxicity against HCT 116 with GI<sub>50</sub> values of 47.2 and 24.4  $\mu$ M, respectively.

**Keywords:** *Maclura pomifera*, Osage orange, Moraceae, Bishomoflavone, 2-Arylbenzofuran, Cytotoxicity.

*Maclura pomifera* (Raf.) C.K. Schneid, known as Osage orange, is a small deciduous tree of the mulberry family (Moraceae). It is native to eastern Texas, southeastern Oklahoma, southwestern Arkansas, and the extreme northwest corner of Louisiana in the United States [1-2]. The wood is hard, durable, resistant to decay, and has been primarily used for tool handles and fence posts [1]. Seed oil of Osage orange was investigated as a low-cost, non-food, high-oil-producing feedstock source for production of biodiesel [3]. Elemol, a sesquiterpene extracted from the essential oil of Osage orange fruit, was found to be repellent to German cockroaches [4]. Previous chemical studies led to the isolation and characterization of flavonoids [5a-5f], xanthenes [5e] and triperpenoids [5g, 5h]. Among these, prenylated isoflavones are the major bioactive components. Osajin and pomiferin and their linear isomers, scandenone and auriculasin showed anticancer, antibacterial, antidiabetic, anti-inflammatory and antinociceptive properties [5b,6,7]. In particular, pomiferin has strong activity against the superoxide anion in a photochemiluminescence (PCL) assay system [6]. Scandenone was reported to have the potential to interact with PDE5 and could be investigated as a novel inhibitor [5f]. Here we report two new and six known phenolic compounds isolated from the fruit of Osage orange and their cytotoxicities.

Compound **1** has the molecular formula C<sub>17</sub>H<sub>14</sub>O<sub>6</sub> as established by the molecular ion peak at  $m/z$  315.0860 [M + H]<sup>+</sup> (Calcd. for C<sub>17</sub>H<sub>15</sub>O<sub>6</sub>, 315.0869) in the HR ESIMS. The <sup>1</sup>H-NMR spectral data (Table 1) of **1** indicated the presence of one singlet aromatic proton at  $\delta_H$  6.16, and two pairs of an ABX spin system, one oxygenated proton (CH) at  $\delta_H$  5.74 and two sp<sup>3</sup> protons (CH<sub>2</sub>) at  $\delta_H$  2.47 and 3.02. The <sup>13</sup>C-NMR spectrum displayed 17 signals including 15 aromatic carbons. In the HMBC spectrum, the singlet proton  $\delta_H$  6.16 coupling to the typical carbon signal (C-4) must be assigned to H-3. Observation of the HMBCs (Figure 2), H-3/C-7', H-7'/C-2, C-3, C-8' and C-1', and the H<sub>2</sub>-8'/C-1', C-2' and C-6', indicated that the fragment -CH(OH)-CH<sub>2</sub>- was inserted between C-2 and C-1'. This information indicated that **1** had a bishomoflavone skeleton [8]. The NMR spectra of **1** were similar to those of **3** except for an additional hydroxyl group in aromatic ring B, which was further deduced by the extra 16 mass units [8]. The OH group was determined to be at C-3' by the three proton ABX spin system at H-2', H-5' and H-6' of

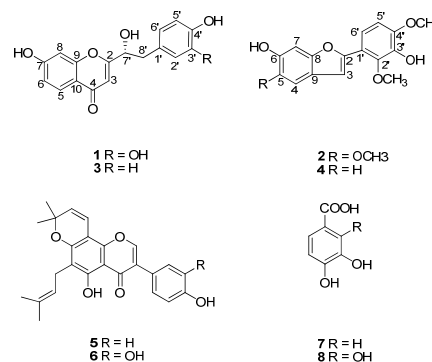


Figure 1: Structures of the isolated compounds (1–8).

ring B. This was also confirmed by the HMBC correlations of H<sub>2</sub>-8'/C-1', C-2' and C-6'. The (*R*)-configuration at position 11 of **1** was determined by comparison of optical rotation values ( $[\alpha]_D^{20} + 32.6$ ) and NMR spectra data with the known compound **3** ( $[\alpha]_D^{25} + 46$ ) [8]. Based on the above spectral evidence, the structure of **1** is shown in Figure 1, and named maclurin A.

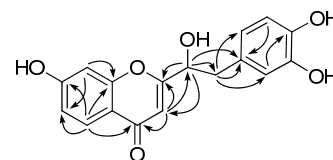


Figure 2: Selected HMBC correlations (→) of (**1**).

Compound **2** was obtained as a yellow solid with the molecular formula C<sub>17</sub>H<sub>16</sub>O<sub>6</sub>, deduced from the HR ESIMS ( $m/z$  339.0841 [M + Na]<sup>+</sup>, Calcd for 339.0845). The <sup>1</sup>H-NMR spectral data (Table 1) of **2** indicated the presence of three singlet aromatic protons at  $\delta_H$  6.94, 7.05 and 7.09, together with a pair of AB coupling system protons at  $\delta_H$  6.79 (d,  $J = 9.0$ ) and 7.21 (d,  $J = 9.0$ ). The <sup>13</sup>C-NMR spectrum displayed 14 aromatic signals and three methoxyl groups. This information indicated that **2** was a 2-arylbenzofuran derivative [9]. The two broad proton singlets at  $\delta_H$  7.05 and 6.94 were assigned to the two aromatic protons H-3 and H-7. The AB

coupling system at  $\delta_H$  6.79 (d,  $J = 9.0$ ) and 7.21 (d,  $J = 9.0$ ), similar to the  $^1H$ -NMR data of **4** [10], was assigned to the 2',4'-dimethoxy-3'-hydroxyl-2-phenyl moiety (ring C). This was confirmed by the HMBC spectrum (Figure 3). Another methoxy at  $\delta_H$  3.77 must be positioned at C-5 and one aromatic proton singlet at  $\delta_H$  7.09 assigned as H-4 was determined by the key HMBC correlations of OCH<sub>3</sub>-5/C-5, H-4/C-5, C-6 and C-8 and the ROESY correlation between H-4/5-OMe. Thus, the structure of **2** was determined and named maclurin B.

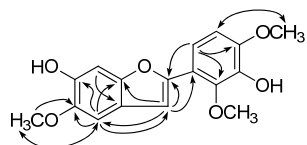


Figure 3: Selected HMBC correlations (→) and selected ROESY correlation (↔) of (**2**).

Table 1: NMR Spectroscopic Data (400 MHz, DMSO-*d*<sub>6</sub>) for **1–2** ( $\delta$  in ppm,  $J$  in Hz).

No.	$\delta_H$	<b>1</b>	$\delta_C$	<b>2</b>	$\delta_C$
2			165.9		150.9
3	6.16, s		120.6	7.05, s	104.5
4			173.7	7.09, s	103.5
5	7.29, d (8.4)		131.6		145.8
6	6.31, d (8.4)		111.7		146.0
7			158.8	6.94, s	98.3
8	6.40, s		103.4		148.7
9			161.9		120.9
10			109.3		
1'			127.6		117.5
2'	6.47, s		117.4		145.3
3'			145.2		140.4
4'			144.3		149.3
5'	6.52, d (8.8)		115.6	6.79, d (9.0)	108.2
6'	6.28, d (8.8)		108.4	7.21, d (9.0)	115.9
7'	5.74, brs		83.5		
8'	2.47, d (14.4), 3.02, d (14.4)		39.9		
5 OCH <sub>3</sub>				3.77, s	56.6
2' OCH <sub>3</sub>				3.75, s	59.6
4' OCH <sub>3</sub>				3.79, s	56.5

Phytochemical study of this plant also resulted in the isolation of six known compounds, nononin (**3**) [8], pterofuran (**4**) [10], osajin (**5**) [5c], pomiferin (**6**) [5c], 3,4-dihydroxybenzoic acid (**7**) [11] and 2,3,4-trihydroxybenzoic acid (**8**) [12], which were determined by comparison of their NMR and MS spectroscopic data with the reported values in the literature. Among them, compounds **3** and **4** were isolated from the genus *Maclura* Nutt for the first time.

## Experimental

**General:** NMR experiments were performed using a JEOL ECS 400 spectrometer, with spectroscopic data referenced to the solvent used. HR-mass spectra were acquired using a MDS Sciex API QStar Pulsar mass spectrometer. UV spectra were recorded on a UV 210A spectrophotometer. Optical rotation values were measured on a JASCO P-1010 polarimeter. Octadecyl-functionalized silica gel, silica gel, Diaion® HP-20 absorbent resin, and TLC plates were purchased from Aldrich Chemical Co. HPLC analysis was performed on an Agilent 1260 HPLC system using Agilent ODS columns (Zorbax SB-C18, 4.6 × 250 mm, 3.5 $\mu$ m). Doxorubicin (98%) was purchased from Sigma-Aldrich Chemical Co.

**Plant material:** Fruit from *M. pomifera* was collected in Nacogdoches, Texas, USA, and identified by Dr. Shiyou Li. The voucher specimen (TX-Nac-20111020-#001) was deposited at the National Center for Pharmaceutical Crops at Stephen F. Austin State University, Nacogdoches, USA.

**Extraction and isolation:** Air-dried fruit of *M. pomifera* (630 g) were powdered and extracted three times with 95% EtOH at room temperature (3 L × 3). After removal of the solvent, the crude extract (93 g) was partitioned between H<sub>2</sub>O and EtOAc, to yield an EtOAc-soluble residue (36g). The residue showing potent cytotoxicity against A549 cancer cell line (GI<sub>50</sub> 19.7  $\mu$ g/mL) was chromatographed on HP-20 resin (MeOH/H<sub>2</sub>O 0:1, 7:3 and 1:0, each 6 L) to give three fractions, Fr. A–C. Compounds **7** (55 mg) and **8** (82 mg) were isolated from Fr. A (15g) by chromatography on Si gel CC by CHCl<sub>2</sub>/MeOH (20:1, 10:1 and 5:1, v/v). Fr.B (7 g) was separated on Si gel with a CH<sub>2</sub>Cl<sub>2</sub>/MeOH gradient system to give five subfractions (Fr. B1–Fr.B5). Compounds **5** (22 mg) and **6** (16 mg) were isolated from Fr.B3 (980 mg) by Si gel CC with a CHCl<sub>3</sub>/MeOH gradient system (0:1 to 10:1 v/v). Fr.B4 (2.9 g) was applied on Si gel CC with *n*-hexane/EtOAc gradient system (0:1 to 1:2 v/v) to give three subfractions (Fr.B4a–Fr.B4c). Fr.B4b (120 mg) was further purified by HPLC (CH<sub>3</sub>CN/0.1% HOAc in H<sub>2</sub>O: 17/83, v/v, 0.6 mL/min) to afford compounds **1** (10 mg,  $t_R$  34.9 min) and **3** (6 mg,  $t_R$  38 min). Fr. C (2.5 g) was first chromatographed on Si gel with *n*-hexane/acetone gradient system (0:1 to 2:1 v/v) to get three subfractions (Fr. C1–Fr.C2), then Fr. C2 was purified by analytical HPLC (CH<sub>3</sub>CN/0.1% HOAc in H<sub>2</sub>O: 25/75, v/v, 0.6 mL/min) to give compounds **2** (5 mg,  $t_R$  72.1 min) and **4** (7 mg,  $t_R$  121.2 min).

### Maclurin A (**1**)

Light yellow gum.

$[\alpha]_D^{20}$ : +32.6 (c 0.1, MeOH);

UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ): 218 (3.8), 286 (2.8), 325 (3.3).

$^1H$  and  $^{13}C$  NMR: Table 1.

EIMS:  $m/z$  314.3, HR ESIMS:  $m/z$  315.0860 [M + H]<sup>+</sup> (calcd. for C<sub>17</sub>H<sub>15</sub>O<sub>6</sub>, 315.0869).

### Maclurin B (**2**)

Pale brown powder.

UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ): 211 (4.3), 284 (3.1), 291 (3.6), 325 (4.4), 340 (4.1).

$^1H$  and  $^{13}C$  NMR: Table 1.

EIMS:  $m/z$  316.3, HR ESIMS:  $m/z$  339.0841 [M + Na]<sup>+</sup> (calcd. for C<sub>17</sub>H<sub>16</sub>O<sub>6</sub>Na, 339.0845).

**Cytotoxicity assay:** Compounds (**1–6**) were assayed for their cytotoxicity against three human cancer cell lines (A549, Panc-28, and HCT116) by WST-8 method, with doxorubicin as a positive control [13]. The results (Table 2) showed that compounds **2–4** exhibited cytotoxicity against A549 and Panc-28 with GI<sub>50</sub> values from 18.1 to 32.2, and 20.6 to 43.5  $\mu$ M, respectively. Compounds **2** and **4** showed cytotoxicity against HCT116 with GI<sub>50</sub> values of 47.2 and 24.4  $\mu$ M, respectively. The other compounds showed no activity against the three tested cell lines with GI<sub>50</sub> values > 50  $\mu$ M.

Table 2: Cytotoxicity Evaluation of compounds **1–6**<sup>a</sup>.

Compound	A549	Panc-28	HCT-116
<b>1</b>	>50 $\mu$ M	>50 $\mu$ M	>50 $\mu$ M
<b>2</b>	26.1 ± 5.57 $\mu$ M	43.5 ± 3.68 $\mu$ M	47.2 ± 3.15 $\mu$ M
<b>3</b>	32.2 ± 5.19 $\mu$ M	22.7 ± 2.86 $\mu$ M	>50 $\mu$ M
<b>4</b>	18.1 ± 6.35 $\mu$ M	20.6 ± 3.08 $\mu$ M	24.4 ± 1.05 $\mu$ M
<b>5</b>	>50 $\mu$ M	>50 $\mu$ M	>50 $\mu$ M
<b>6</b>	>50 $\mu$ M	>50 $\mu$ M	>50 $\mu$ M
Doxorubicin	0.44 ± 0.03 $\mu$ M	0.72 ± 0.09 $\mu$ M	1.00 ± 0.01 $\mu$ M

<sup>a</sup>Each GI<sub>50</sub> was determined as the mean ± SD in triplicate determinations for each concentration.

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**Conflict of Interest:** None of the authors has any conflicts of interest related to this study.

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