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Phenolics from the Fruits of *Maclura pomifera*

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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_{50} values from 18.1 to 32.2, and 20.6 to 43.5 μM, respectively. Compounds **2** and **4** also showed cytotoxicity against HCT 116 with GI_{50} values of 47.2 and 24.4 μM, 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], xanthones [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 $C_{17}H_{14}O_6$ as established by the molecular ion peak at *m/z* 315.0860 $[M + H]^+$ (Calcd. for $C_{17}H_{15}O_6$, 315.0869) in the HR ESIMS. The ^{1}H-NMR spectral data (Table 1) of **1** indicated the presence of one singlet aromatic proton at δ_H 6.16, and two pairs of an ABX spin system, one oxygenated proton (CH) at δ_H 5.74 and two sp^3 protons (CH_2) at δ_H 2.47 and 3.02. The ^{13}C-NMR spectrum displayed 17 signals including 15 aromatic carbons. In the HMBC spectrum, the singlet proton δ_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_2-8'/C-1', C-2' and C-6', indicated that the fragment –CH(OH)–CH_2– 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 ring B. This was also confirmed by the HMBC correlations of H_2-8'/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.

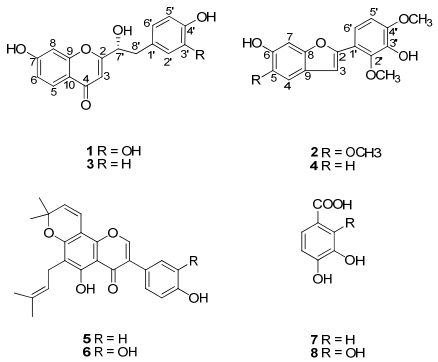

Figure 1: Structures of the isolated compounds (**1**–**8**).

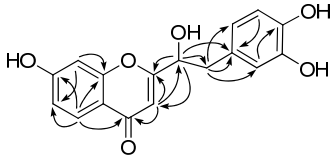

Figure 2: Selected HMBC correlations (→) of (**1**).

Compound **2** was obtained as a yellow solid with the molecular formula $C_{17}H_{16}O_6$, deduced from the HR ESIMS (*m/z* 339.0841 [M + Na]$^+$, Calcd for 339.0845). The ^{1}H-NMR spectral data (Table 1) of **2** indicated the presence of three singlet aromatic protons at δ_H 6.94, 7.05 and 7.09, together with a pair of AB coupling system protons at δ_H 6.79 (d, *J* = 9.0) and 7.21 (d, *J* = 9.0). The ^{13}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 δ_H 7.05 and 6.94 were assigned to the two aromatic protons H-3 and H-7. The AB

coupling system at δ_H 6.79 (d, J = 9.0) and 7.21 (d, J = 9.0), similar to the ^{1}H-NMR data of **4** [10], was assigned to the 2',4'-dimethoxyl-3'-hydroxyl-2-phenyl moiety (ring C). This was confirmed by the HMBC spectrum (Figure 3). Another methoxy at δ_H 3.77 must be positioned at C-5 and one aromatic proton singlet at δ_H 7.09 assigned as H-4 was determined by the key HMBC correlations of OCH_3-5/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.

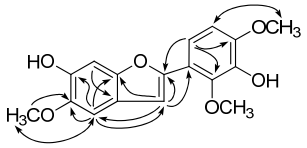

Figure 3: Selected HMBC correlations (→) and selected ROESY correlation (↔) of (**2**).

Table 1: NMR Spectroscopic Data (400 MHz, DMSO-d_6) for **1**–**2** (δ in ppm, J in Hz).

No.	**1**		**2**	
	δ_H	δ_C	δ_H	δ_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_3			3.77, s	56.6
2' OCH_3			3.75, s	59.6
4' OCH_3			3.79, s	56.5

Phytochemical study of this plant also resulted in the isolation of six known compounds, ononin (**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, 3.5μm). 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_2O and EtOAc, to yield an EtOAc-soluble residue (36g). The residue showing potent cytotoxicity against A549 cancer cell line (GI_{50} 19.7 μg/mL) was chromatographed on HP-20 resin (MeOH/H_2O 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 $CHCl_2$/MeOH (20:1, 10:1 and 5:1, v/v). Fr.B (7 g) was separated on Si gel with a CH_2Cl_2/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 $CHCl_3$/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_3CN/0.1% HOAc in H_2O: 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_3CN/0.1% HOAc in H_2O: 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) λ_{max} (log ε): 218 (3.8), 286 (2.8), 325 (3.3).
^{1}H and ^{13}C NMR: Table 1.
EIMS: *m/z* 314.3, HR ESIMS: *m/z* 315.0860 $[M + H]^+$ (calcd. for $C_{17}H_{15}O_6$, 315.0869).

Maclurin B (2)
Pale brown powder.
UV (MeOH) λ_{max} (log ε): 211 (4.3), 284 (3.1), 291 (3.6), 325 (4.4), 340 (4.1).
^{1}H and ^{13}C NMR: Table 1.
EIMS: *m/z* 316.3, HR ESIMS: *m/z* 339.0841 $[M + Na]^+$ (calcd. for $C_{17}H_{16}O_6Na$, 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_{50} values from 18.1 to 32.2, and 20.6 to 43.5 μM, respectively. Compounds **2** and **4** showed cytotoxicity against HCT116 with GI_{50} values of 47.2 and 24.4 μM, respectively. The other compounds showed no activity against the three tested cell lines with GI_{50} values > 50 μM.

Table 2: Cytotoxicity Evaluation of compounds **1**–**6**[a].

Compound	A549	Panc-28	HTC-116
1	>50 μM	>50 μM	>50 μM
2	26.1 ± 5.57μM	43.5 ± 3.68 μM	47.2 ± 3.15 μM
3	32.2 ± 5.19 μM	22.7 ± 2.86 μM	>50 μM
4	18.1 ± 6.35 μM	20.6 ± 3.08 μM	24.4 ± 1.05 μM
5	>50 μM	>50 μM	>50 μM
6	>50 μM	>50 μM	>50 μM
Doxorubicin	0.44 ± 0.03 μM	0.72 ± 0.09 μM	1.00 ± 0.01 μM

[a]Each GI_{50} 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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