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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 GI50 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 GI50 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 auricalusin showed anticancer, antibacterial, antidiabetic, anti-inflammatory and antimicrobial 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 $C_{17}H_{13}O_{6}$, 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 δH 6.16, and two pairs of an ABX spin system, one oxygenated proton (CH) at δH 5.74 and two sp3 protons (CH2) at δH 2.47 and 3.02. The $^{13}$C-NMR spectrum displayed 17 signals including 15 aromatic carbons. In the HMBC spectrum, the singlet proton δH 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'-C-1', C-2' and C-6', indicated that the fragment -CH(OH)-CH2 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 δH 2.11, 5.53 and 6.47 of ring B. This was also confirmed by the HMBC correlations of H2-8'/C-1', C-2' and C-6'. The (R)-configuration at position 11 of 1 was determined by comparison of optical rotation values ([α]D20 + 32.6) and NMR spectra with the known compound 3 ([α]D25 + 46) [8]. Based on the above spectral evidence, the structure of 1 is shown in Figure 1, and named maclurin A.

Compound 2 was obtained as a yellow solid with the molecular formula $C_{17}H_{14}O_{6}$, 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 δH 6.94, 7.05 and 7.09, together with a pair of AB coupling system protons at δH 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-arylbenezofuran derivative [9]. The two broad proton singlets at δH 7.05 and 6.94 were assigned to the two aromatic protons H-3 and H-7. The AB
coupling system at $\delta_4 6.79$ (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'-dimethoxy-3'-hydroxy-2-phenyl moiety (ring C). This was confirmed by the HMBC spectrum (Figure 3). Another methoxy at $\delta_1 3.77$ must be positioned at C-5 and one aromatic proton singlet at $\delta_1 7.09$ 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 and H-5-OH. Thus, the structure of 2 was determined and named maclurin B.

**Extraction and isolation:** Air-dried fruit of *M. pomifera* (630 g) were powdered and extracted three times with 95% EtOH at room temperature ($3 \text{ L} \times 3$). After removal of the solvent, the crude extract (93 g) was partitioned between H$_2$O and EtOAc, to yield an EtOAc-soluble residue (36 g). The residue showing potent cytotoxicity against A549 cancer cell line (GI$_{50}$ 19.7 $\mu$g/mL) was chromatographed on HP-20 resin (MeOH/H$_2$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$_3$/MeOH (20:1, 10:1 and 5:1, v/v). Fr. B (7 g) was separated on Si gel with a CHCl$_3$/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 1:2 v/v) to give three subfractions (Fr.B4a–Fr.B4c). Fr.B4b (120 mg) was further purified by HPLC (CH$_3$CN/0.1% HCO$_3$ in H$_2$O: 17/83, v/v, 0.6 mL/min) to afford compounds 1 (10 mg, $t_f$ 34.9 min) and 3 (6 mg, $t_f$ 38 min). Fr. C (2.5 g) was first chromatographed on Si gel with n-hexane/EtOAc 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$_3$CN/0.1% HCO$_3$ in H$_2$O: 25/75, v/v, 0.6 mL/min) to give compounds 2 (5 mg, $t_f$ 72.1 min) and 4 (7 mg, $t_f$ 121.2 min).

**Phytochemical study:** Fruit from *M. pomifera* was collected in Nacogdoches, Texas, USA, and identified by Dr. Shiyou Li. The voucher specimen (TX-Nac-20111020-8001) was deposited at the National Center for Pharmaceutical Crops at Stephen F. Austin State University.

**Plant material:** Fruit from *M. pomifera* was collected in Nacogdoches, Texas, USA, and identified by Dr. Shiyou Li. The voucher specimen (TX-Nac-20111020-8001) was deposited at the National Center for Pharmaceutical Crops at Stephen F. Austin State University, Nacogdoches, USA.

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

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

**Acknowledgment:** This project was funded by Stephen F. Austin State University.

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

<table>
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Each GI$_{50}$ was determined as the mean ± SD in triplicate determinations for each concentration.
References