2017

Phenolics from the Fruits of Maclura pomifera

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Su, Zushang; Wang, Ping; Yuan, Wei; Grant, Greg; and Li, Shiyou, "Phenolics from the Fruits of Maclura pomifera" (2017). *NCPC Publications and Patents*. 51.  
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Two new compounds, maclurin A (I) 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 aururalins 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 C₁₇H₁₄O₆ as established by the molecular ion peak at m/z 315.0860 [M + H]⁺ (Calcd. for C₁₇H₁₄O₆, 315.0869) in the HR ESIMS. The ¹H-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 oxygennated proton (CH) at δH 5.74 and two sp³ protons (CH₂) at δH 2.47 and 3.02. The ¹³C-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 H-2', H-5' and H-6' 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]D° + 32.6) and NMR spectra data with the known compound 3 ([α]D° + 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 C₁₇H₁₃O₆, deduced from the HR ESIMS (m/z 339.0841 [M + Na]⁺, Calcd for 339.0845). The ¹H-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 ¹³C-NMR spectrum displayed 14 aromatic signals and three methoxyl groups. This information indicated that 2 was a 2-arylbenzo[fluran 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 $\delta_{H} 6.79$ (d, $J = 9.0$) and 7.21 (d, $J = 9.0$), similar to the $^1H$-NMR data of 4 [10], was assigned to the 2,4,6-trimethoxy-3-hydroxy-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$_2$/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).

<table>
<thead>
<tr>
<th>No.</th>
<th>$\delta_1$</th>
<th>$\delta_2$</th>
<th>$\delta_3$</th>
<th>$\delta_4$</th>
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</thead>
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<tr>
<td>1</td>
<td>6.16, s</td>
<td>120.6</td>
<td>7.05, s</td>
<td>104.5</td>
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<tr>
<td>2</td>
<td>173.7</td>
<td>131.6</td>
<td>145.8</td>
<td></td>
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<tr>
<td>3</td>
<td>6.31, d (8.4)</td>
<td>111.7</td>
<td>146.0</td>
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<tr>
<td>4</td>
<td>158.8</td>
<td>103.4</td>
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<tr>
<td>5</td>
<td>6.40, s</td>
<td>161.9</td>
<td>120.9</td>
<td></td>
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<tr>
<td>6</td>
<td>109.3</td>
<td>117.5</td>
<td>143.5</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>127.6</td>
<td>117.4</td>
<td>143.5</td>
<td></td>
</tr>
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<td>8</td>
<td>145.2</td>
<td>144.3</td>
<td>149.3</td>
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<tr>
<td>9</td>
<td>6.52, d (8.8)</td>
<td>115.6</td>
<td>108.2</td>
<td></td>
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<td>10</td>
<td>6.28, d (8.8)</td>
<td>108.4</td>
<td>115.9</td>
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<td>1'</td>
<td>2.47, d (14.4), 3.02, d (14.4)</td>
<td>39.9</td>
<td></td>
<td></td>
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<tr>
<td>2'</td>
<td>3.77, s</td>
<td>56.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3'</td>
<td>3.75, s</td>
<td>59.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5'</td>
<td>3.79, s</td>
<td>56.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Phytochemical study of this plant also resulted in the isolation of six known compounds, ononin (3) [8], petroruran (4) [10], osajin (5) [Sc], pomiferin (6) [Sc], 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. Octadeyl-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-20111020-8001) 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$_2$O 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$_2$O 0.1%: 7:1 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 get three subfractions (Fr.B4a–Fr.B4c). Fr.B4b (120 mg) was further purified by HPLC (CH$_3$CN/0.1% HOAc in H$_2$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/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% HOAc in H$_2$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.

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

UV (MeOH) $\lambda_{max}$ (log $e$): 211 (3.8), 286 (2.8), 325 (3.3).

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

EIMS: $m/z$ 314.3, HR ESIIMS: $m/z$ 315.0869 [M + H]$^+$ (calcd. for C$_{17}$H$_{15}$O$_{6}$Na, 315.0869).

Maclurin B (2)

Pale brown powder.

UV (MeOH) $\lambda_{max}$ (log $e$): 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 ESIIMS: $m/z$ 339.0841 [M + Na]$^+$ (calcd. for C$_{17}$H$_{16}$O$_{6}$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$_{50}$ 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.

<table>
<thead>
<tr>
<th>Compound</th>
<th>A549</th>
<th>Panc-28</th>
<th>HCT-116</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt;50 μM</td>
<td>&gt;50 μM</td>
<td>&gt;50 μM</td>
</tr>
<tr>
<td>2</td>
<td>26.1 ± 5.57 μM</td>
<td>43.5 ± 3.68 μM</td>
<td>47.2 ± 3.15 μM</td>
</tr>
<tr>
<td>3</td>
<td>32.2 ± 5.19 μM</td>
<td>22.7 ± 2.86 μM</td>
<td>&gt;50 μM</td>
</tr>
<tr>
<td>4</td>
<td>18.1 ± 6.35 μM</td>
<td>20.6 ± 3.08 μM</td>
<td>24.4 ± 1.05 μM</td>
</tr>
<tr>
<td>5</td>
<td>&gt;50 μM</td>
<td>&gt;50 μM</td>
<td>&gt;50 μM</td>
</tr>
<tr>
<td>6</td>
<td>&gt;50 μM</td>
<td>&gt;50 μM</td>
<td>&gt;50 μM</td>
</tr>
</tbody>
</table>

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

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

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