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A new triterpenoid and other constituents from the stem bark of *Juglans mandshurica*

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1. **Subject and source**

*Juglans mandshurica* Maxim. (Juglandaceae), characterized as a deciduous tree, is widely distributed in the northeast of China, as well as some areas of Korea and the Russian Far East (Lu, 1982; Wu and Raven, 1999). Its fruits, roots and stem bark have been used as a folk medicine for the treatment of cancer in Asia and Europe (Kim et al., 1998; Fukuda et al., 2003). The stem bark of *J. mandshurica* Maxim. was collected at the mountainous area of Jian (Jilin Province, P. R. of China) and was identified by Prof. Shaobo Fan (Jilin Agricultural University). A voucher specimen (No. 2007007) was deposited in Research Center of Agriculture and Medicine Gene Engineering of Ministry of Education, Northeast Normal University.

2. **Previous work**

Previous phytochemical research on *J. mandshurica* has led to the isolation of nearly a hundred secondary metabolites. The main constituents of *J. mandshurica* could be categorized as naphthoquinones and their glycosides, which were reviewed by Guan et al. (2009), diarylheptanoids (Kim et al., 1998; Lee et al., 2002; Li et al., 2003, 2004, 2005, 2008b; Liu et al., 2007), flavonoids (Liu et al., 2004b; Si et al., 2008), as well as some phenolic compounds and their glycosides (Min et al., 2000, 2003;
Previously, we reported one new anthracene derivative and two new anthraquinones from the stem bark of *J. mandshurica* (Lin et al., 2011).

### 3. Present study

In continuation of our earlier investigation, the air-dried and powdered stem bark of *J. mandshurica* (8 kg) was extracted with 70% EtOH (3 × 10 l, 3 × 2.0 h, 80°C) under reflux conditions to give a crude extract, which was suspended in H2O and extracted with petroleum ether, CHCl3, EtOAc and n-BuOH, successively. A part of the CHCl3 extract (80 g) was subjected to CC (SiO2, gradient of petroleum ether/EtOAc) to get 8 fractions (Fr.1–Fr.8). Fr.1 (8.3 g) was rechromatographed on silica gel eluted with gradient of petroleum ether–EtOAc, to give 5 subfractions (SFr.1–SFr.5), SFr.3 (1.2 g) was purified by PTLC [CHCl3–MeOH–H2O (20:1:0.12), Rf 0.51] to obtain 1 (6 mg). Fr.2 (5.5 g) was resubjected to CC (SiO2, gradient of petroleum ether/EtOAc) to get 7 subfractions (SFr.1–SFr.7), SFr.2 (0.8 g) was then purified by PTLC [petroleum ether–EtOAc (2:3), Rf 0.45] to yield juglanin B (5 mg, 9) (Liu et al., 2007), while SFr.4 (1.5 g) was rechromatographed on CC (Sephadex LH-20, CHCl3/MeOH, 6:4) and recrystallized using CHCl3 to afford myricatomentogenin (11 mg, 8) (Morihara et al., 1997). A part of the EtOAc extract (100 g) was subjected to CC (SiO2, gradient of CHCl3/MeOH) to obtain 12 fractions (Fr.A–Fr.L). Fr.E (13.1 g) was rechromatographed on silica gel with gradient of CHCl3–MeOH to give 6 subfractions (SFr.E1–SFr.E6), SFr.E3 (2.0 g) was subjected to CC (Polyamide, 100–140 mesh, gradient of MeOH/H2O) to afford 4-[6-O-(syringyl)-β-glucopyranosyl]-3-methoxybenzoic acid (54 mg, 6) (Yokosuka and Mimaki, 2007). Fr.F (11.2 g) was resubjected to CC (Sephadex LH-20, CHCl3/MeOH, 4:6) to obtain 5 main subfractions (SFr.F1–SFr.F5), SFr.F3 (2.4 g) was then rechromatographed on polyamide (100–140 mesh) with gradient of MeOH/H2O to yield quercetin-7-O-β-glucopyranoside (17 mg, 3) and kaempferol-7-O-β-glucopyranoside (8 mg, 4). Fr.G (14.8 g) was resubjected to CC (Polyamide, 40–80 mesh, gradient of MeOH/H2O) to get 6 main subfractions (SFr.G1–SFr.G6), SFr.G3 (1.7 g) was then chromatographed on polyamide (100–140 mesh) eluted with MeOH–H2O (6:4) to yield myricetin-7-O-β-glucopyranoside (22 mg, 2) (Liu et al., 2010), while SFr.G4 (5.3 g) was purified by Sephadex LH-20 (CHCl3/MeOH, 3:7) to afford 1,6-di-O-galloyl glucose (1.75 g, 5) (Nonaka and Nishioka, 1983). Fr.H (2.1 g) was resubjected to CC (Sephadex LH-20, CHCl3/MeOH, 1:1) to obtain 3 main subfractions (SFr.H1–SFr.H3), SFr.H2 (0.9 g) was then chromatographed on ODS with gradient of MeOH/H2O to yield ellagic acid-4-O-β-xylidine-3,3′-dimethyl ether (13 mg, 7) (Sinha et al., 1999). The structures of the known isolates were established on the basis of spectroscopic evidence and by comparison with literature data.

Compound 1 is new, and was isolated as colorless needles, $[\alpha]_{D}^{25} = +37^\circ$ (c 0.12, MeOH). Its molecular formula was determined as C30H49O2 on the basis of HR-TOF-MS ($m/z = 441.3723 [M + H]^+$, calcd. for C30H48O2, 441.3727). It was considered to be a triterpenoid due to the positive Lieberman–Burchard reaction, as well as its NMR spectral data. The IR spectrum of 1 indicated the presence of hydroxyl (3484 cm$^{-1}$) and double bond (1462 cm$^{-1}$) functional groups. The $^1$H NMR spectrum showed eight methyl singlets at $\delta$ 0.83, 0.88, 0.89, 0.91, 1.02, 1.07, 1.15 and 1.22 (each 3H, s), two coupled olefinic proton signals at $\delta$ 5.72 (1H, d, $J = 5.7$ Hz, H-11) and 5.57 (1H, d, $J = 5.7$ Hz, H-12), as well as two oxygen-bearing methine protons at $\delta$ 4.15 (1H, t, $J = 3.2$ Hz, H-1) and 3.80 (1H, dd, $J = 12.0, 5.7$ Hz, H-3). The $^{13}$C NMR spectrum displayed 30 carbon signals that were sorted by a DEPT experiment as eight methyls, eight methylenes, six methines and eight quaternary carbons, including two oxygen-bearing methine carbons at $\delta$ 73.0 and 72.8 and two pairs of double bond carbons at $\delta$ 150.3, 149.0, 119.9 and 117.0. Based on the above data, compound 1 was determined as an oleanane-type triterpenoid (Shasi and Asish, 1994). The structure of 1 was subsequently established by comprehensive analysis of 2D NMR spectroscopic data and comparison with data for related compounds. In the HMBC spectrum (Fig. 1), long-range correlations from H-12, H-25, and H-26 to C-9,

![Fig. 1. Key HMBC and NOESY correlations of 1.](image-url)
and from H-11 and H-26 to C-9 were observed, indicating the olean-9(11),12-diene skeleton of 1, which was further confirmed by comparison of the $^{13}$C NMR spectrum of 1 with that of 3α,21β-dihydroxy-olean-9(11),12-diene (Cáceres-Castillo et al., 2008) and saikogenin B (Mahato et al., 1988). Moreover, splitting pattern of H-1 (t, $J = 3.2$ Hz) and H-3 (dd, $J = 12.0, 5.7$ Hz) revealed that the hydroxyl groups at C-1 and C-3 were α- and β-oriented (Rogers and Subramony, 1988), respectively, which was further confirmed by the correlations between H-1/H-25, H-1/H-11, H-3/H-23 and H-24/H-25 in the NOESY spectrum (Fig. 1). It is noteworthy that, 1α,3β-dihydroxy-olean-9(11),12-dietyl-3-palmitate, whose splitting pattern of H-1 is different from that of 1, was isolated from Saussurea ussuriensis (Asteraceae) (Li et al., 2008a) and Celastrus rosthornianus (Celastraceae) (Wang, 2007).

![Fig. 2. X-Ray crystal structure of 1.](image)

**Table 1**

$^1$H (400 MHz) and $^{13}$C (100 MHz) NMR data of compound 1 in CDCl$_3$ ($\delta$ in ppm, $J$ in Hz).

<table>
<thead>
<tr>
<th>No.</th>
<th>$\delta$ (H)</th>
<th>$\delta$ (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.15 (1H, t, $J = 3.2$)</td>
<td>72.8</td>
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<tr>
<td>2</td>
<td>1.70 (1H, m), 1.38 (1H, m)</td>
<td>31.7</td>
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<tr>
<td>3</td>
<td>3.80 (1H, dd, $J = 12.0, 4.8$)</td>
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<tr>
<td>4</td>
<td>39.0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1.47 (1H, m)</td>
<td>44.3</td>
</tr>
<tr>
<td>6</td>
<td>1.60 (1H, m), 1.62 (1H, m)</td>
<td>17.4</td>
</tr>
<tr>
<td>7</td>
<td>2.02 (1H, m), 1.93 (1H, m)</td>
<td>32.4</td>
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<tr>
<td>8</td>
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<td>150.3</td>
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<tr>
<td>10</td>
<td></td>
<td>45.0</td>
</tr>
<tr>
<td>11</td>
<td>5.72 (1H, d, $J = 5.7$)</td>
<td>117.0</td>
</tr>
<tr>
<td>12</td>
<td>5.57 (1H, d, $J = 5.7$)</td>
<td>119.9</td>
</tr>
<tr>
<td>13</td>
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<td>149.0</td>
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<td>14</td>
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<td>15</td>
<td>1.89 (1H, m), 1.38 (1H, m)</td>
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<td>2.20 (1H, dd, $J = 12.8, 5.2$)</td>
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<td>1.67 (1H, m), 1.07 (1H, m)</td>
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<td>23</td>
<td>1.07 (3H, s)</td>
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<td>24</td>
<td>0.83 (3H, s)</td>
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<td>25</td>
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<tr>
<td>30</td>
<td>0.88 (3H, s)</td>
<td>33.2</td>
</tr>
</tbody>
</table>
We unambiguously confirmed the structure of 1 by X-ray analysis of a single crystal obtained from chloroform (Fig. 2). The detailed crystallographic data have been deposited with the Cambridge Crystallographic Data Centre (CCDC). Copies of the data can be obtained free of charge on application to CCDC with the number of 830567. Thus, the structure of 1 was established as 1α,3β-dihydroxy-olean-9(11),12-diene, named juglangenin A. The complete 1H and 13C NMR assignments (Table 1) of 1 were determined from a combination of DEPT, HMQC, HMBC and NOESY spectra.

**Juglangenin A (1):** colorless needles; [α]D25 +37 (c 0.12, MeOH); UV (MeOH) λmax (log ε): 210 (3.06), 277 (2.24) nm; IR (KBr) νmax: 3484, 2947, 2868, 1462, 1376, 1260, 1200, 1110, 1086, 1065, 990, 818 and 678 cm⁻¹; 1H and 13C NMR data (see Table 1); HR-TOF-MS: m/z = 441.3723 [M + H]+ (calcd. for C30H49O2, 441.3727).

4. Chemotaxonomic significance

The present study reported the first isolation of nine secondary metabolites (Fig. 3), including an oleanane-type triterpenoid (1), three flavonoids (2–4), three phenolic glycosides (5–7) and two diarylheptanoids (8–9), from the stem bark of *J. mandshurica*.

Juglangenin A (1) was isolated as a new and unusual structure, which possesses an interesting olean-9(11),12-diene skeleton and an uncommon 1α,3β-dihydroxy substitution mode. It is worthy to note that, oleanolic acid, ursolic acid and some of their derivatives have been obtained from *J. mandshurica* (Zhou et al., 2010), while no triterpenoid was reported from other species of *Juglans* as far as we know. Thus, the present isolation of 1 might have chemotaxonomic importance for *J. mandshurica*.

Surprisingly we found that, flavonoids bearing a 7-O-β-glucopyranosyl unit (2–4) were isolated for the first time from the family Juglandaceae. Notably, several C-3 glycosides of flavonoids having the same aglycones of 2–4, such as afzelin, astragalin, juglanin, quercitrin, hyperin, isoquercitrin and myricitrin, have been reported from *J. mandshurica* and *Juglans regia* L. (Min et al., 2000; Liu et al., 2004b; Si et al., 2008; Alkhawajah, 1997).

Similarly, a series of tri-, tetra- and penta-O-galloyl glucosides have been obtained from *Juglans* species, and were summarized by Si et al. (2011). However, we report herein the first isolation of 1,6-di-O-galloyl glucose (5) from the Juglandaceae family. On the other hand, this is also the first report of 4-[6-O-(syringyl)-β-glucopyranosyloxy]-3-methoxybenzoic acid (6) from the family Juglandaceae. It is worthy to note that, 4-[6-O-(galloyl)-β-glucopyranosyloxy]-3-methoxybenzoic acid, whose structure is very close to that of 6, was formerly obtained from the stem bark of *J. mandshurica*.
(Shi, 2006). Moreover, this is the first isolation of compound 7, from the genus *Juglans*, which was previously obtained form the genus *Platycarya* (Tanaka et al., 1998) of the same family.

Interestingly, the present study revealed the presence of myricatomentogenin (8) from the genus *Juglans*, which was formerly isolated from the genera *Pterocarya* (Liu et al., 2005) of the same family. It is noteworthy that, compound 8 has only ever been obtained from two other species outside Juglandaceae: *Myrica nana* (Myricaceae) (Wang et al., 2008) and *Alnus hirsuta* (Betulaceae) (Jin et al., 2007). In addition, juglalin B (9) has been exclusively isolated from *J. regia* L (Liu et al., 2007) as far as we know.

The present phytochemical research furthers our knowledge about the diversity of compounds in *J. mandshurica*. Meanwhile, we suggest that, the three phenolic glycosides (5–7) and the two diarylheptanoids (8–9) might serve as potential chemotaxonomic markers within *Juglans* species, which still need further investigation.

**Acknowledgments**

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**References**


