Systematic analysis of the metabolites of Angelol B by UPLC-Q-TOF-MS after oral administration to rats

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Available online 20 Nov., 2019

[ABSTRACT] Angelicae Pubescentis Radix (APR), a widely used traditional Chinese medicine (TCM), is mainly used to treat rheumatism and headache diseases. Angelol B is one of the bioactive constituents of APR with significant anti-inflammatory activity. This paper is aimed to illustrate the metabolites of angelol B in vivo. To achieve this objective, a metabolomics approach based on a rapid and accurate UPLC-Q-TOF-MS method was used to detect the metabolites of Angelol B in rat. A gradient elution system (ACN and 0.1% formic acid water) equipped with an Agilent SB-C18 column (1.8 μm, 2.1 mm × 50 mm) to complete the separation. Scanning area at m/z 100−800 operated on an electrospray ionization (ESI). The data were collected in both positive and negative ion mode and analyzed by the Masslynx 4.1 and SIMCA 13.0 software. A total of 31 metabolites including 20 phase I and 11 phase II metabolites were identified. Their structure and fragmentation process were deduced based on the MS and MS/MS data. All of thirty-one metabolites are new compounds based on the search of SCI-Finder database.

[KEY WORDS] Angelol B; UPLC-Q-TOF-MS; Metabolomics approach; Metabolism

[CLC Number] R917

Introduction

The dried roots of Angelica pubescens Maxim. f. biserrata Shan et Yuan (APR) is commonly used as an analgesic and anti-rheumatic drug for centuries and known as Duhuo in Chinese. APR shows multiple pharmacological activities such as anti-inflammatory [1], analgesic [2], anticancer [3], anti-oxidative [4] effects, and inhibitory effects on 5-lipoxygenase and cyclooxygenase [5]. In addition, it is also the main constituent of many Chinese herbal formula, such as ‘Jitong Ning Tablet’ and ‘Huo Luo Xiao Ling Dan’ which have been used for treating ankylosing spondylitis as well as acute and chronic inflammatory reactions [6] and other disorders, including arthritis [7-8]. With the further research of the pharmacological mechanism of APR, its chemical constituents and metabolism have addressed more and more attention [9-11]. Angelol B is a simple Angelol-type coumarin and one of the main bioactive constituents of Angelica pubescens Radix [12]. According to the pharmacological studies, it has a significant inhibitory activity on human platelet aggregation induced by 2 μmol·L⁻¹ ADP [13] and appears as a well-absorbed compound in Caco-2 cell monolayer absorption model [14]. While the studies on metabolism of Angelol B are few reported by now.

In this study, a high-resolution mass spectrometry based metabolomics was performed to profile the metabolism of Angelol B after oral administration to rats. UPLC combined with high accuracy mass spectrometry (Q-TOF-MS) not only can provide the accurate mass and retention time data for known compounds but also allows detecting unknown analytes, and plays an important role in identifying and structure elucidation of metabolites [15-18]. Orthogonal partial least-squares
metabolic cages with the temperature at 22–24 °C and relative
humidity at 50% and drug group (n = 5). They were housed individually in
metabolites. Comparing with SCI-Finder database, these 31 metabolites are all new
compounds.

Experimental

Chemicals and reagents

Angelol B was obtained from our lab with the purity above 98% and suitable for LC-MS analysis. Acetonitrile (ACN) and formic-acid (FA) were used of LC-MS grade and purchased from Fisher Scientific (Pittsburgh, PA, USA). Corn oil was obtained from Sigma Chemical Co. (St. Louis, MO, USA) and water was purified by Milli-Q system (Millipore, Bedford, MA, USA). Other reagents were all of analytical grade.

UPLC-Q-TOF-MS conditions

The separation and detection were performed on a Waters ACQUITY UPLC/Xevo-G2 Q-TOF mass spectrometer system with an electrospray ionization (ESI) interface (Milford, MA, USA). The control of instruments was completed on Waters Empower software (Version 2) and data processing and analysis were conducted on Masslynx 4.1 (Waters) software. The mobile phases were acetonitrile (A) and 0.1% formic-acid in water (B). A programmed gradient was carried out as follows: 0–0.1 min 5% A; 0.1–8 min 5%–95% A; 8–9 min 95%–5% A; 9–11 min 5% A. An Agilent SB-C18 column (1.8 μm, 2.1 mm × 50 mm, USWEY10227) was used for separating the metabolites. The injection volume was 5 μL. The flow rate was at 0.4 mL·min⁻¹. The column oven and auto-sampler temperature were maintained at 30 °C and room temperature, respectively. MS conditions: mass scan ranged from m/z 100 to 800 in positive and negative mode. Other parameters were set as follows: the ESI source temperature was 100 °C; de-solvation temperature, 450 °C; de-solvation gas (N₂), 800 L·h⁻¹; cone gas, 50 L·h⁻¹; the capillary voltage, 3.00 kV; sample cone voltage, 20 V, and extraction cone voltage, 4.0 V.

Animals and preparation of Angelol B

Ten male Sprague-Dawley (SD) rats (180–200 g) were obtained from the Laboratory Animal Center of Peking University Health Science Center (Beijing, China). All animal experiments were approved by the Biomedical Ethical Committee of Peking University and in line with the animals testing requirements. Angelol B was suspended in corn oil at a concentration of 100 ng·mL⁻¹ as standard for LC-MS analysis. The ion of angelol B (M0) was showed at m/z 377.1607 [M + H]+ / 375.1444 [M – H]⁻ with the molecular formula is C20H24O7. The MS/MS data and fragmentation process of angelol B were shown in Table 1 and Fig. 1, respectively.

In this study, The difference between vehicle- and drug-treated groups were analyzed by a metabonomics approach. Among them, 20 were phase I and 11 were phase II metabolites. Based on the search of SCI-Finder database, these 31 metabolites are all new compounds.

Animal experiments and biological sample collection

The SD rats were randomly divided into vehicle group (n= 5) and drug group (n= 5). They were housed individually in metabolic cages with the temperature at 22–24 °C and relative humidity at 70%. The rats were given food and water ad libitum of three days to adapt the environment. Then, they were fasted for 4 hours with free drinking water before experiment. The rats in drug group were orally administered suspension of Angelol B at a dose of 80 mg·kg⁻¹ body weight. Corn oil was treated to vehicle group. The duration of drug administration was 24 h and fasted with free access to water. Blood samples were collected at 1.5, 4 and 8 h after oral administration and stored into heparinized 1.5 mL EP tube, respectively. Urine and feces samples were continuously collected 24 h after the administration.

Biological sample preparation

Blood samples were centrifuged at 8 000 r·min⁻¹ for 10 min at 4 °C to obtain plasma samples. 300 μL of plasma samples (including 1.5 h, 150 μL; 4 h 100 μL and 8 h, 50 μL) were mixed with 900 μL LC-MS grade acetonitrile (ACN) and vortex-mixed for 1 min. Then, the mixture was centrifuged at 15 000 r·min⁻¹ for 10 min and the supernatant was evaporated to dryness (CV2000 vacuum centrifugal concentrator). The residue was re-dissolved in 200 μL acetonitrile (LC-MS grade) and vortex-mixed for 1 min. Finally, the supernatant was filtered through a 0.22 μm filter before LC-MS analysis.

Urine samples (100 μL) were added 3-fold volume of acetonitrile (ACN) and vortex-mixed for 1 min to obtain the mixture. The mixture was centrifuged at 15 000 r·min⁻¹ for 10 min and the supernatant was evaporated to dryness. The residue was re-dissolved in 200 μL acetonitrile and filtered through a 0.22 μm filter before analysis.

Feces samples were lyophilized (SCIENTZ-10ND freezer dryer) and grounded into powder. 100 mg of feces samples were extracted with 600 μL acetonitrile, and then vortex-mixed and ultrasonic extracted for 20 min. The mixture was centrifuged at 15 000 r·min⁻¹ for 10 min and the supernatant was filtered through 0.22 μm filter before analysis.

Results

The prototype compound (Angelol B) and its 31 metabolites were identified by using UPLC-Q-TOF-MS method combined with metabolomics approaches. Among them, 20 were phase I and 11 were phase II metabolites. Comparing with SCI-Finder database, these 31 metabolites are all new compounds.

Angelol B fragments and multivariate data analysis

The ion of angelol B (M0) was showed at m/z 377.1607 [M + H]+ / 375.1444 [M – H]⁻ with the molecular formula is C20H24O7. The MS/MS data and fragmentation process of angelol B were shown in Table 1 and Fig. 1, respectively.
## Table 1  UPLC-Q-TOF-MS analysis of metabolites

<table>
<thead>
<tr>
<th>No.</th>
<th>% retention (min)</th>
<th>Formula</th>
<th>Measured m/z [M + H]+ (ppm)</th>
<th>Metabolic process</th>
<th>Product ion (MS/MS) (m/z)</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>2.64</td>
<td>C₇₀H₁₂₆₀₈</td>
<td>393.1562 [M + H]+</td>
<td>4.07 Mono-oxidized</td>
<td>387.0732, 359.0709, 297.0746, 269.0866, 220.0382, 187.0384</td>
<td>f</td>
</tr>
<tr>
<td>M2</td>
<td>2.52</td>
<td>C₇₀H₁₂₀₉</td>
<td>407.1340 [M−H]−</td>
<td>−0.49 Di-oxidized</td>
<td>407.1333, 389.1237, 371.1144, 339.1276, 335.1163, 317.1028, 259.0977, 219.0655, 149.0603</td>
<td>f</td>
</tr>
<tr>
<td>M3</td>
<td>3.85</td>
<td>C₇₀H₁₂₅₁₀</td>
<td>425.1445 [M + H]+</td>
<td>0.47 Tri-oxidized</td>
<td>391.1391, 387.1049, 341.1044, 275.0935, 259.0949, 203.0341, 201.0569, 175.0378</td>
<td>f</td>
</tr>
<tr>
<td>M5</td>
<td>4.26</td>
<td>C₇₀H₁₂₅₁₀</td>
<td>427.1627 [M + H]+</td>
<td>5.38 Di-oxidized and hydration</td>
<td>373.1272, 355.1152, 245.0813, 213.0912, 147.0437</td>
<td>f</td>
</tr>
<tr>
<td>M6</td>
<td>2.64</td>
<td>C₇₀H₁₂₅₁₀</td>
<td>437.1464 [M−H]−</td>
<td>3.66 Tri-oxidized and methylation</td>
<td>391.1391, 387.1049, 341.1044, 275.0935, 259.0949, 203.0341, 201.0569, 175.0378</td>
<td>u</td>
</tr>
<tr>
<td>M7</td>
<td>2.16</td>
<td>C₇₀H₁₂₅₁₀</td>
<td>395.1707 [M + H]+</td>
<td>0.25 Hydration</td>
<td>353.1644, 337.1307, 311.1254, 265.0703, 235.0600, 205.0513, 177.0563, 149.0590, 147.0441</td>
<td>u</td>
</tr>
<tr>
<td>M8</td>
<td>0.44</td>
<td>C₇₀H₁₂₅₁₀</td>
<td>409.1845 [M + H]+</td>
<td>−4.15 Hydration and methylation</td>
<td>365.1599, 349.1264, 305.1010, 233.0811, 219.0659, 177.0540, 147.0456, 133.0636</td>
<td>f, u</td>
</tr>
<tr>
<td>M9</td>
<td>0.49</td>
<td>C₇₀H₁₂₅₁₀</td>
<td>365.1589 [M + H]+</td>
<td>−3.01 Reduction and demethylation</td>
<td>349.1638, 305.1775, 203.0722, 177.0562, 163.0770, 149.0619, 133.0636</td>
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<tr>
<td>M10</td>
<td>0.42</td>
<td>C₇₀H₁₂₅₁₀</td>
<td>455.1395 [M + H]+</td>
<td>3.95 Reduction, alkenes to dihydrodiol and acetylation</td>
<td>409.1861, 367.1729, 349.1304, 305.1722, 207.0636, 205.0501, 165.0554, 149.0602, 147.0441, 133.0642</td>
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<tr>
<td>M11</td>
<td>6.53</td>
<td>C₇₀H₁₂₅₁₀</td>
<td>359.1508 [M + H]+</td>
<td>3.90 Dehydration</td>
<td>341.1367, 319.1027, 297.1456, 279.0864, 265.0730, 245.0838, 205.0502, 199.1172, 177.0577, 149.0990</td>
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<td>M12</td>
<td>2.94</td>
<td>C₇₀H₁₂₅₁₀</td>
<td>373.1659 [M + H]+</td>
<td>2.14 Dehydration and methylation</td>
<td>344.1246, 327.1233, 279.0819, 257.0816, 205.0508, 177.0542, 149.0613</td>
<td>f</td>
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<tr>
<td>M14</td>
<td>2.94</td>
<td>C₇₀H₁₂₅₁₀</td>
<td>417.1567 [M−H]−</td>
<td>4.08 Acetylation</td>
<td>389.1254, 377.1244, 371.1163, 313.1094, 283.0609, 259.0976, 255.0654</td>
<td>f</td>
</tr>
<tr>
<td>No.</td>
<td>$t_{R}$ (min)</td>
<td>Formula</td>
<td>Measured</td>
<td>Error (ppm)</td>
<td>Metabolic process</td>
<td>Product ion (MS/MS) (m/z)</td>
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<tr>
<td><strong>The phase I metabolites</strong></td>
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</tr>
<tr>
<td>M17</td>
<td>2.76</td>
<td>$C_{16}H_{20}O_{12}$</td>
<td>345.0999[M + H]$^+$</td>
<td>7.24</td>
<td>Carboxylation, demethylation and desaturation Carbonation</td>
<td>305.0665, 301.1099, 287.0915, 247.0602, 219.0663, 177.0560, 147.0434, 133.0679, 373.0723, 359.0801, 305.0676, 301.0724, 225.0857, 243.0659, 229.0940, 187.0393, 317.1079, 301.1071, 279.1245, 203.0740, 201.1298, 177.0551, 149.0624, 147.0445, 133.0623, 343.0827, 317.0678, 315.0862, 273.0342, 255.0665</td>
</tr>
<tr>
<td>M18</td>
<td>2.54</td>
<td>$C_{16}H_{18}O_{8}$</td>
<td>389.0898[M − H]$^-$</td>
<td>6.42</td>
<td></td>
<td>237.0687, 215.0536, 173.0367, 149.0490</td>
</tr>
<tr>
<td>M19</td>
<td>3.26</td>
<td>$C_{20}H_{22}O_{12}$</td>
<td>375.1441[M + H]$^+$</td>
<td>−0.80</td>
<td>Carbonation and demethylation</td>
<td>317.1079, 301.1071, 279.1245, 203.0740, 201.1298, 177.0551, 149.0624, 147.0445, 133.0623, 343.0827, 317.0678, 315.0862, 273.0342, 255.0665</td>
</tr>
<tr>
<td>M20</td>
<td>2.91</td>
<td>$C_{16}H_{16}O_{10}$</td>
<td>357.0993[M − H]$^-$</td>
<td>5.04</td>
<td></td>
<td>235.0687, 215.0536, 173.0367, 149.0490</td>
</tr>
<tr>
<td><strong>The phase II metabolites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M21</td>
<td>2.22</td>
<td>$C_{16}H_{22}O_{10}S$</td>
<td>455.1014[M − H]$^−$</td>
<td>0.44</td>
<td>Sulfation</td>
<td>355.1204, 339.1215, 309.1105, 293.1155, 257.0804, 175.0496, 129.0352</td>
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<tr>
<td>M22</td>
<td>2.46</td>
<td>$C_{20}H_{22}O_{10}S$</td>
<td>471.0961[M − H]$^−$</td>
<td>0</td>
<td>Mono-oxidized and sulfation</td>
<td>413.0548, 357.1363, 311.0946, 303.0875, 275.0931, 271.0592, 201.0541, 175.0409</td>
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<tr>
<td>M23</td>
<td>2.09</td>
<td>$C_{20}H_{22}O_{10}S$</td>
<td>473.1121[M − H]$^−$</td>
<td>0.63</td>
<td>Hydration and sulfation</td>
<td>357.1367, 329.1365, 299.0910, 271.0960, 147.0426</td>
</tr>
<tr>
<td>M24</td>
<td>2.52</td>
<td>$C_{16}H_{22}O_{10}S$</td>
<td>457.1162[M − H]$^−$</td>
<td>−3.28</td>
<td>Reduction and Sulfation</td>
<td>443.1041, 387.0402, 359.1459, 297.0457, 269.1544, 219.1001</td>
</tr>
<tr>
<td>M25</td>
<td>2.54</td>
<td>$C_{16}H_{22}O_{10}S$</td>
<td>477.1108[M − H]$^−$</td>
<td>8.59</td>
<td>Di- hydration, decarbonylation, hydroxylation and sulfation</td>
<td>443.1041, 407.0802, 359.1114, 291.0858</td>
</tr>
<tr>
<td>M26</td>
<td>5.31</td>
<td>$C_{16}H_{22}O_{10}S$</td>
<td>429.0470[M − H]$^−$</td>
<td>−5.12</td>
<td>Carbonylation, demethylation and sulfation Glucuronidation</td>
<td>385.0602, 341.0665, 313.0377, 281.0457, 254.9983, 377.1566, 301.1067, 213.0935, 177.0561, 149.0593</td>
</tr>
<tr>
<td>M27</td>
<td>2.48</td>
<td>$C_{16}H_{22}O_{10}S$</td>
<td>553.1940[M + H]$^+$</td>
<td>3.43</td>
<td>2x Glucuronidation</td>
<td>553.1929, 463.1217, 441.1347, 317.0994, 287.0928, 247.0968, 219.0662, 205.0498, 177.0545, 175.0383, 149.0621</td>
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<tr>
<td>M28</td>
<td>2.68</td>
<td>$C_{16}H_{22}O_{10}S$</td>
<td>729.2206[M + H]$^+$</td>
<td>−4.93</td>
<td></td>
<td>453.1356, 393.1549, 317.0994, 205.0513, 177.0569, 149.0597</td>
</tr>
<tr>
<td>M29</td>
<td>2.06</td>
<td>$C_{16}H_{22}O_{10}$</td>
<td>569.1913[M + H]$^+$</td>
<td>7.55</td>
<td>Mono-oxidized and Glucuronidation</td>
<td>569.1825, 525.1984, 487.1735, 305.1392, 295.1194, 239.0902, 233.1192, 177.0531, 149.0614, 133.0633</td>
</tr>
<tr>
<td>M30</td>
<td>2.08</td>
<td>$C_{20}H_{22}O_{10}$</td>
<td>585.1815[M + H]$^+$</td>
<td>−0.68</td>
<td>Di-oxidized and Glucuronidation</td>
<td>507.1151, 395.0975, 367.0651, 219.0678, 175.0376</td>
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<tr>
<td>M31</td>
<td>1.43</td>
<td>$C_{16}H_{22}O_{10}S$</td>
<td>521.1287[M − H]$^−$</td>
<td>−1.53</td>
<td>Double bond rupture, hydroxylation, dehydration and Glucuronidation</td>
<td></td>
</tr>
</tbody>
</table>

p, plasma samples; f, feces samples and u, urine samples
Fig. 1  The MS/MS spectrum and fragmentation process of angelol B
Fig. 2  OPLS analysis and S-plot of global metabolomes of rat plasma, feces and urine; each point represents an individual rat biological sample. (A and B) OPLS analysis and S-plot of global metabolomes in rat plasma treated with blank (corn oil) and Angelol B (80 mg·kg$^{-1}$), respectively. (C and D) OPLS analysis and S-plot of global metabolomes in rat feces treated with blank (corn oil) and Angelol B (80 mg·kg$^{-1}$), respectively. (E and F) OPLS analysis and S-plot of global metabolomes in rat urine treated with blank (corn oil) and Angelol B (80 mg·kg$^{-1}$), respectively.

**Analysis of phase І metabolites**

A total of 20 phase І metabolites were identified in plasma, feces and urine samples of rats. According to the MS$^2$ information, the metabolic pathways include oxidation, reduction, hydration, methylation, demethylation, dehydration, hydroxylation, acetylation, carboxylation, and desaturation.

**The oxidation and oxidation- metabolites**

M1 (t$_R$ = 2.64 min) was detected in rat plasma, feces, and urine. It had the [M + H]$^+$ ion at m/z 393.1562 and its molecular formula is speculated as C$_{20}$H$_{24}$O$_8$. The fragment ions of M1 were at m/z 319.1187 [M + H – O – C$_4$H$_8$O$_3$]$^+$, 317.1029 [M + H – O – C$_4$H$_8$O$_3$]$^+$, 301.1066 [M + H – O – C$_4$H$_8$O$_3$]$^+$, 279.1239 [M + H – C$_8$H$_6$O$_3$]$^+$, 219.0630 [M + H – C$_8$H$_6$O$_3$]$^+$, 205.0509 [M + H – C$_8$H$_6$O$_3$]$^+$, 203.0702 [M + H – O – C$_8$H$_6$O$_3$]$^+$, 191.0342 [M + H – C$_8$H$_6$O$_3$]$^+$, 177.0536 [M + H – O – C$_8$H$_6$O$_3$]$^+$, 175.0379 [M + H – C$_8$H$_6$O$_3$]$^+$, and ions at m/z 149.0608, 147.0439, and 135.0444 were obtained by continuous loss of CO. M1 was 16 Da more than that of M0, which indicated that M1 was generated by oxidation of Angelol B.

M2 (t$_R$ = 2.52 min) was only found in rat feces. Its molecular formula is deduced as C$_{20}$H$_{24}$O$_9$ by the [M – H]$^–$ ion at m/z 407.1340. Its characteristic ions were at m/z 359.0709 [M – H – CH$_3$ – 3H$_2$CO – O], 397.0746 [M – H – CH$_3$ – 3H$_2$CO – O$_2$ – CH$_2$], 269.0866 [M – H – CH$_3$ – 3H$_2$CO – O$_2$ – CH$_2$ – CO$^–$], 220.0382 [M – H – CH$_3$ – 3H$_2$CO – CO – C$_6$H$_7$O$_3$]$^–$, and 187.0384 [M – H – CH$_3$ – 3H$_2$CO – CO – C$_6$H$_7$O$_3$ – O – OH]$^–$. M2 was 32 Da more than that of the parent ion (m/z 375.1444 [M – H]$^–$), which speculated that M2 was a di-oxidized metabolite of Angelol B.

M3 was only detected in rat feces, it showed the molecular ion at m/z 425.1445 [M + H]$^+$ with the retention time at 3.85 min. Its molecular formula is deduced as C$_{20}$H$_{24}$O$_{10}$ and the typical ions were at m/z 389.1237 [M + H – 2H$_2$O]$^+$, 371.1144 [M + H – 3H$_2$O]$^+$, 339.1276 [M + H – 3H$_2$O – 2O$^–$], 335.1163 [M + H – 2O – C$_6$H$_7$O$_3$]$^+$, 317.1028 [M + H – 2O – C$_6$H$_7$O$_3$ – O – CH$_2$O]$^–$, 259.0977 [M + H – 3H$_2$O – 2O – C$_6$H$_7$O$_3$]$^–$, 219.0655 [M + H – 2O – C$_6$H$_7$O$_3$ – O – CH$_2$O]$^–$, and ions at m/z 149.0603, 147.0439, and 135.0444 were obtained by continuous loss of CO. M3 was obtained by the oxidation (16 Da) of M2. M1, M2 and M3 were generated by the continuous oxidation of M0 according to their MS$^2$ data and possible fragmentation process (Fig. 3A).
The [M + H]⁺ ion of M4 (tᵣ = 1.90 min) was at m/z 411.1617 and only found in rat urine. Its molecular formula is deduced as C₂₀H₂₆O₉. The main fragment ions were at m/z 393.1550 [M + H – H₂O – C₄H₈O], 333.1301 [M + H – H₂O – C₂H₆O₂ – CO₂], 265.1096 [M + H – C₂H₆O₂ – CH₂ – CO₂], 209.0795 [M + H – C₂H₆O₂ – 3CH₂ – CO₂], and 177.0548 [M + H – C₂H₆O₂ – 3CH₂ – CO – CH₂O]. M4 was 18 Da more than that of M1 which indicated that M4 was generated by the hydration of M1.

M5 was only detected in rat feces. It gave molecular ion at m/z 427.1627 [M + H]⁺ (tᵣ = 4.26 min) and its molecular formula is speculated as C₂₀H₂₆O₁₀. M5 yielded major MS² ions at m/z 373.1272 [M + H – 3H₂O]⁺, 355.1152 [M + H – 4H₂O]⁺, 245.0813 [M + H – 4H₂O – C₂H₆O₂ – CH₂], 213.0912 [M + H – 4H₂O – C₂H₆O₂ – CH₂ – CO₂]⁺ and 147.0437 [M + H – 4H₂O – C₂H₆O₂ – CH₂ – CO – CH₂O]⁻. According to the MS and MS/MS data, M5 was 50 Da more than that of M0 which indicated that M5 was di-oxidized and hydrated from M0. Based on the fragment information and possible fragmentation process (Fig. C and D), M4 and M5 were the oxidized-hydration metabolites of angelol B.

M6 (tᵣ = 2.64 min) was found in rat plasma, feces, and urine, and exhibited a [M – H]⁻ ion at m/z 437.1464. Its molecular formula is speculated as C₂₁H₂₈O₈ and yielded characteristic product ions at m/z 391.1391 [M – H – 2O – CH₂], 387.1049 [M – H – CH₂OH – H₂O]⁻, 341.1044 [M – H – CH₂OH – H₂O – 2O – CH₂], 275.0935 [M – H – 2O – CH₂ – C₂H₄O₂], 259.0949 [M – H – CH₂OH – H₂O – 2O – CH₂ – C₂H₄O₂], 203.0341 [M – H – 2O – CH₂ – C₂H₄O₂], 201.0569 [M – H – CH₂OH – H₂O – 2O – CH₂ – C₂H₄O₂ – C₂H₂O], and 175.0378 [M – H – CH₂OH – H₂O – 2O – CH₂ – C₂H₄O₂ – C₂H₂O₂]⁻ / [M – H – 2O – CH₂ – C₂H₄O₂ – C₂H₂O₂ – C₂H₂O – CO₂]⁻. According to the MS/MS data, M6 was generated by tri-oxidation and methylation of Angelol B.

The hydration and hydration - metabolites

Metabolite M7 (tᵣ = 2.16 min) was only detected in urine. Its molecular formula is deduced as C₂₀H₂₆O₇ by m/z 395.1707 [M + H]⁺. The main fragments at m/z 353.1644 [M + H – CO – CH₂]⁺, 337.1307 [M + H – C₂H₆O], 311.1254 [M + H – 3H₂O – CH₂O], 265.0703 [M + H – C₂H₄O], 235.0600 [M + H – C₂H₆O – CH₂O]⁺, 205.0513 [M + H – C₄H₈O – CH₂O – CO – 2H]⁻, and 177.0563 [M + H – C₄H₈O – CH₂O – CO – 2H – CO₂]⁻. M7 was 18 Da more than that of M0, which indicated that M7 was produced by dehydration of Angelol B (Fig. 3B).

The [M + H]⁺ ion of M8 showed at m/z 409.1845 with retention time at 0.44 min, and the molecular formula is speculated as C₂₁H₂₆O₉. M8 was found in rat urine, which had the typical fragments at m/z 365.1599 [M + H – CH₂ – C₂H₂O]⁺, 349.1264 [M + H – CH₂ – C₂H₂O – CH₂], 305.1010 [M + H – CH₂ – C₂H₂O – CH₂ – C₂H₂O₂ – CH₂], 233.0811 [M + H – CH₂ – C₂H₂O – CH₂ – C₂H₂O₂ – CH₂ – C₂H₂O₂], and 219.0659 [M + H – CH₂ – C₂H₂O – CH₂ – C₂H₂O₂ – CH₂ – C₂H₂O₂]. M8 was 14 Da more than that of M7 inferring that M8 was generated by methylation of M7.

The reduction-metabolites

M9 was detected in rat feces and urine. It had the molecular ion at m/z 365.1589 [M + H]⁺ with retention time at 0.49 min. The molecular formula is deduced as C₁₉H₂₄O₇, which was 12 Da less than that of M0 and had the product ions at m/z 349.1638 [M + H – O], 305.1775 [M + H – O – CO₂], 203.0732 [M + H – O – C₂H₄O₂], 177.0562 [M + H – O – C₂H₄O₂ – C₂H₂], 163.0770 [M + H – O – C₂H₄O₂ – CH₂ – CO₂], 149.0619 [M + H – O – C₂H₄O₂ – C₂H₂ – CO₂], 133.0636 [M + H – O – C₂H₄O₂ – C₂H₂ – CO₂]⁻. According to the fragments information, M9 was speculated as the reduction and demethylation metabolite of Angelol B (Fig. 3C).

The molecular formula of M10 (tᵣ = 0.42 min) is deduced as C₂₂H₂₈O₁₄ by UPLC-QTOF-MS (m/z 455.1395 [M + H]⁺). M10 was found in rat plasma and yielded major product ions at m/z 409.1861 [M + H – CO – H₂O]⁺, 367.1729 [M + H – CO – H₂O – CH₂CO], 349.1304 [M + H – CO – H₂O – CH₂CO], 305.1722 [M + H – O – CH₂O – CH₂COO – H₂O – CO₂]⁻. Based on the fragments information, M10 was generated by the reduction, alkenes to dihydrodiol and acetylation of prototype compound.

The dehydration and dehydration - metabolites

M11 was only detected in rat feces. It had the [M + H]⁺ ion at m/z 359.1508 and was 18 Da less than that of M0. Its molecular formula is deduced as C₂₀H₂₆O₈. The characteristic product ions of M11 were at m/z 341.1367 [M + H – H₂O]⁺, 319.1027 [M + H – C₂H₄]⁺, 297.1456 [M + H – H₂O – CO₂]⁻.
The carboxylation and carboxylation-metabolites were shown in metabolites and their possible structure and fragmentation pathways produced by removal of CO. The data indicated that M11 was a dehydrated metabolite of M0 (Fig. 3D).

M12 was found in rat feces and gave the molecular ion at m/z 373.1659 [M + H]+. Its retention time was at 2.94 min. It had the main fragment ions at m/z 344.1246 [M + H – C2H2 – CH3]+, 327.1233 [M + H – CH2 – CH2 – OH]+ and 279.0819 [M + H – C4H6 – C3H4]+. M12 was 14 Da more than that of M11 which inferred that M12 was generated by methylation of M11.

The molecular formula of M15 is speculated as C23H23O8 by the [M + H]+ ion at m/z 415.1429. It was detected in rat plasma and urine with retention time at 4.97 min. Its product ions were at m/z 397.1266 [M + H – H2O]+, 339.1275 [M + H – H2O – C2H2O2]+, 317.1017 [M + H – C2H2O2 – C3H4]+, 205.0867 [M + H – C3H4 – C2H2O2 – 2H]+ and 174.0678 [M + H – C3H4O2 – C2H2 – C3H4O2 – 2H – C3H6O]+. According to the MS and MS/MS information, M15 was generated by loss of two H2O (32 Da) and acetylation after the [M + H]+ ion at m/z 397.1266 [M + H – C4H6 – C3H4]+.

Metabolite M13 (tR = 4.88 min) was found in rat feces and exhibited molecular ion at m/z 391.1752 [M + H]+. Its molecular formula is deduced as C23H22O9, and further fragmentation yielded characteristic ions at m/z 373.1565 [M + H – 2H2O]+, 355.1531 [M + H – H2O – C2H2O2]+, 337.1317 [M + H – C2H2O2]+, and 309.1317 [M + H – C4H6 – CO]+. M13 was 14 Da more than that of M0 which indicated that M13 was obtained by the methylation of M0 based on the MS and MS/MS data and the possible fragmentation process was shown in Fig. 3F.

Metabolite M14 was only detected in rat feces with retention time at 2.94 min. Its molecular formula is speculated as C22H22O12 by UPLC-QTOF-MS (m/z 417.1567 [M + H]+). It had fragment ions at m/z 389.1254 [M + H – 2H2]+, 377.1244 [M + H – C4H6]+, 371.1163 [M + H – 2CH2 – H2O]+, 313.1094 [M + H – 2CH2 – H2O – C2H2O2]+, 283.0690 [M + H – 2CH2 – H2O – C2H2O2 – 2CH3]+, 259.0976 [M + H – C4H6 – C2H2O2]+ and 255.0654 [M + H – 2CH2 – H2O – C2H2O2 – 2CH2– CO]+. M14 was 42 mass units more than that of Angelol B (m/z 375.1444 [M + H]+) which inferred that M14 was the acetylation metabolite of Angelol B (Fig. 3G).


M20 was only detected in rat urine. Its [M − H]+ ion was at m/z 357.0993 (C19H16O8S) with retention time at 2.91 min. It had fragmentation ions at m/z 343.0827 [M − H – C3H4]+, 317.0678 [M − H – CH2 – C3H4]+, 315.0862 [M − H – CH2 – CO]+, 273.0342 [M − H – C2H2O]+, and 255.0665 [M − H – CO2 – H3O – CH2O2]+. It was carboxylation and demethylation metabolite of Angelol B based on the MS and MS/MS information (Fig. S1 L).
were at $m/z$ 355.1204 [M – H – H$_2$SO$_3$ – H$_2$O]$^–$, 339.1215 [M – H – H$_2$SO$_4$ – H$_2$O]$^–$, 309.1105 [M – H – H$_2$SO$_4$ – H$_2$O – CH$_2$O]$^–$, 293.1155 [M – H – H$_2$SO$_4$ – H$_2$O – CH$_2$O – O]$^–$, 257.0804 [M – H – H$_2$SO$_3$ – H$_2$O – C$_6$H$_5$O$_2$]$^–$, and 129.0352 [M – H – H$_2$SO$_4$ – H$_2$O – CH$_2$O – O – C$_9$H$_2$O$_2$]$^–$. It was 80 Da more than that of Angelol B ($m/z$ 375.1444 [M – H]$^–$) and was deduced as a sulfation metabolite of Angelol B (Fig. 4A).

**M22** ($t_R$ = 2.46 min) showed the [M – H]$^–$ ion at $m/z$ 471.0961 (C$_{20}$H$_{24}$O$_{11}$S) and was only found in rat urine. It was 16 Da (O) more than that of **M21**. Further fragmentation yielded product ions at $m/z$ 413.0548 [M – H – C$_9$H$_6$O]$^–$, 357.1363 [M – H – SO$_4$ – H$_2$O]$^–$, 311.0946 [M – H – SO$_4$ – H$_2$O – C$_6$H$_5$O$_2$]$^–$, 303.0875 [M – H – C$_6$H$_5$O – SO$_4$ – CH$_2$]$^–$, 275.0931 [M – H – C$_6$H$_5$O – SO$_4$ – CH$_2$ – CO]$^–$, 271.0592 [M – H – SO$_4$ – H$_2$O – C$_6$H$_5$O$_2$ – C$_3$H$_4$]$^–$, 201.0451 [M – H – SO$_4$ – H$_2$O – C$_6$H$_5$O$_2$ – C$_3$H$_4$ – CH$_2$O]$^–$, and 175.0409 [M – H – SO$_4$ – H$_2$O – CH$_2$ – C$_3$H$_4$ – C$_3$H$_2$O$_2$]$^–$, which indicated that **M22** was oxidized and sulfated by **M0** (Fig. 4B).

M23 was 98 mass units (SO3 and H2O) more than that of M0 and inferred that M23 was the hydration and sulfation metabolite of M0 (Fig. 4C).

The \([\text{M} - \text{H}]^-\) ion of M24 was at \( m/z \) 457.1162 (C20H26O10S) which was 2 Da more than that of M21 and only found in rat feces with retention time at 2.52 min. It showed the product ions at \( m/z \) 443.1041 [M – H – CH2], 387.0402 [M – H – CH2 – C6H8], 359.1459 [M – H – H2SO4], 297.0457 [M – H – H2SO4 – CO – 2CH3], 269.1544 [M – H – H2SO4 – O – CH2O – CO2], and 219.1001 [M – H – CH2 – C6H8O2 – CO2 – H2O].

The above data indicated that M24 was generated by reduction of M21 (Fig. 4D).

M25 (\( t_R = 2.54 \text{ min} \)) was only detected in rat feces. Its molecular formula is deduced as C19H26O12S according to the molecular ion at \( m/z \) 477.1108 [M – H]. The MS/MS ions were at \( m/z \) 443.1041 [M – H – 2OH], 407.0802 [M – H – 2OH – 2H2O], 359.1114 [M – H – 2OH – H2SO4 – 2H2], and 291.0838 [M – H – 2OH – H2SO4 – 2H – C6H4]. According to the MS and MS/MS data, M25 was obtained by di-hydration, decarbonylation, hydroxylation and sulfation of Angelol B (Data not shown).

Metabolite M26 (\( t_R = 5.31 \text{ min} \)) was only detected in rat plasma and exhibited \([\text{M} - \text{H}]^-\) ion at \( m/z \) 429.0470. Its molecular formula is speculated as C17H13O11S. M26 had fragment ions at \( m/z \) 385.0602 [M – H – CO – O], 341.0665 [M – H – CO – O – CO2 – C6H4], 281.0457 [M – H – CO – O – CO2 – C6H4O], and 254.9983 [M – H – C6H10O2 – CO]. M26 was generated by carboxylation, demethylation, and sulfation of Angelol B.

The glucuronidation metabolites

M27 was found in rat plasma and urine. Its \([\text{M} + \text{H}]^+\) ion (\( t_R = 2.48 \text{ min} \)) was at \( m/z \) 553.1940 and molecular formula is deduced as C26H32O13. It was 176 mass units (a glucuronide group) more than that of M0, which indicate that M27 was the glucuronidation metabolite of M0. Its major product ions were at \( m/z \) 377.1566 [M + H – glu], 301.1067 [M + H – glu – H2O – C3H6O], 213.0935 [M + H – glu – H2O – C3H6O – CO2], 177.0561 [M + H – glu – H2O – C3H6O – CO2 – C2H4O], and 149.0593 [M + H – glu – H2O – C3H6O – C6H10O2 – CO] (Fig. 4E).

M28 was only detected in rat feces with retention time at 2.68 min. It possessed molecular ion at \( m/z \) 729.2206 [M + H] (C32H40O19) indicating an addition of a glucuronide group (176 Da) on M27. Further fragmentation gave the product ions at \( m/z \) 553.1929 [M + H – glu]2, 463.1217 [M + H – glu – C6H10O2 – CH2O], 441.1347 [M + H – glu – C6H10O2 – CH2O], 317.0994 [M + H – 2glu – C6H10O2], 287.0928 [M + H – glu – C6H10O2 – C6H10O2], 247.0968 [M + H – glu – C6H10O2 – C6H10O2 – C2H4O], 219.0662 [M + H – glu – C6H10O2 – C6H10O2 – C2H4O], 205.0498 [M + H – glu – C6H10O2 – C6H10O2 – C2H4O – C2H4O], 177.0545 [M + H – glu – C6H10O2 – C6H10O2 – C2H4O – C2H4O], 175.0383 [M + H – glu – C6H10O2 – C6H10O2 – C2H4O – C2H4O], and 149.0621 [M + H – glu – C6H10O2 – C6H10O2 – C2H4O – C2H4O – C2H4O]. It was generated by the glucuronidation of M27 based on the MS and MS/MS information (Fig. 4F).
The molecular ion of M29 ($t_R = 2.06$ min) was at $m/z$ 569.1913 [M + H]$^+$ and the molecular formula is speculated as C$_{26}$H$_{32}$O$_{14}$ which was 16 Da (O) more than that of M27. It was detected in rat plasma, feces and urine with retention time at 2.06. Its fragment ions was at $m/z$ 453.1356 [M + H – C$_5$H$_8$O$_3$]$^+$, 393.1549 [M + H – glu]$^+$, 317.0994 [M + H – glu – O – C$_6$H$_4$O]$^+$, 205.0513 [M + H – C$_6$H$_4$O – glu – C$_6$H$_6$O]$^+$, 177.0569 [M + H – C$_6$H$_4$O – glu – C$_6$H$_6$O – CO]$^+$, and 149.0597 [M + H – C$_6$H$_4$O – glu – C$_6$H$_6$O – 2CO]$^+$ which inferred that M29 was oxidized by M27. The possible structure and fragmentation process was shown in Fig. 4G.


M31 was only detected in rat urine with retention time at 1.43 min and gave molecular ion at $m/z$ 521.1287 [M – H]$^-$. Its molecular formula is C$_{24}$H$_{26}$O$_{13}$ and 146 mass units more than that of Angelol B ($m/z$ 375.1444 [M – H]$^-$). Its product ions were at $m/z$ 507.1151 [M – H – CH$_2$]$^-$, 395.0975 [M – H – CH$_2$ – C$_6$H$_4$O]$^-$, 367.0651 [M – H – CH$_2$ – C$_6$H$_4$O – 2CH$_2$]$^-$, 219.0678 [M – H – CH$_2$ – C$_6$H$_4$O – glu]$^-$ and 175.0376 [M – H – CH$_2$ – C$_6$H$_4$O – glu – C$_2$H$_4$O]$^-$ which according to the MS and MS/MS information, M31 was generated by double bond rupture, hydroxylation, dehydration and glucuronidation of angelol B.

**Conclusions**

As one of the main bioactive constituents of Angelica pubescens Radix, metabolism of angelol B has not been reported by now. A total of thirty-one metabolites were detected with a metabolomics method and the peak area of some metabolites. Among them, 20 were phase I metabolites and 11 were phase II metabolites, and the metabolic pathway of angelol B was shown in Fig. 5. All of the 31 metabolites

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**Fig. 5** The metabolic pathway of Angelol B. (A) the phase I metabolic pathway, (B) the phase II metabolic pathway
were identified as new compounds according to the searches of SCI-Finder database. The structures of metabolites were elucidated by their accurate mass and product ions. The possible structures and predicted fragmentation process were shown in Figs. 3, 4. The results showed that the phase I metabolic pathways mainly included oxidation, reduction, hydration, methylation, demethylation, dehydrogenation, hydroxylation, acetylation, carboxylation and desaturation, and the main pathways of phase II metabolic were sulfation and glucuronidation. The study provides the information on the metabolic behavior of angelol B in vivo and contributes to further study in the future.

References


