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

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[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-C_{18} 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

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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

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△ These authors contributed equally to this work.
These authors have no conflict of interest to declare.

discriminant analysis (OPLS-DA) [19] as an extension *of* partial least squares discriminant analysis (PLS-DA) [20] can solve the multi-collinearity and information redundancy issues, and improve the rationality of constructed models [21]. Finally, 31 metabolites of Angelol B were detected in rat plasma, feces and urine samples., among them 20 were phase I metabolites and 11 were phase II metabolites. Based on the search of SCI-Finder database, the 31 metabolites are new compounds.

Experimental

Chemicals and reagents

Angelol B was obtained from our lab [22] 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-aci d in water (B). A programmed in 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-C_{18} 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 (N_2), 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 8 mg·mL^{-1} for oral administration. The appropriate of Angelol B was dissolved in acetonitrile (ACN) at a concentration of 100 ng·mL^{-1} as standard for LC-MS analysis.

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 $C_{20}H_{24}O_7$. 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 (Fig. 2). According to the abundance ranks (more than 75%) and correlations after primary screening, the difference ions that caused by drug treatment were separated and selected. Several potential metabolites of angelol B (**M1**−**M31**) were then identified based on the molecular ions, retention time, and mass fragment ions data (Table 1).

Table 1 UPLC-Q-TOF-MS analysis of metabolites

No.	t_R/min	Formula	Measured	Error (ppm)	Metabolic process	Product ion (MS/MS) (*m/z*)	Location
	The phase I metabolites						
M0	3.55	$C_{20}H_{24}O_7$	377.1607[M + H]$^+$	1.86		359.1495, 319.1171, 317.1080, 301.1071, 279.1230, 277.1068, 219.0630, 205.0509, 203.0702, 191.0342, 177.0536, 175.0379, 163.0401, 149.0608, 147.0439, 135.0444	p, f, u
	3.54	$C_{20}H_{24}O_7$	375.1444[M − H]$^-$	0		317.1003, 315.0891, 275.0883, 217.0511, 203.0344, 189.0191, 173.0219, 145.0307	
M1	2.64	$C_{20}H_{24}O_8$	393.1562[M + H]$^+$	4.07	Mono-oxidized	319.1187, 317.1029, 301.1066, 279.1239, 219.0654, 205.0497, 203.0704, 191.0358, 177.0568, 175.0394, 149.0598, 147.0436, 135.0439	p, f, u
M2	2.52	$C_{20}H_{24}O_9$	407.1340[M−H]$^-$	−0.49	Di-oxidized	387.0732, 359.0709, 297.0746, 269.0866, 220.0382, 187.0384	f
M3	3.85	$C_{20}H_{24}O_{10}$	425.1445[M + H]$^+$	0.47	Tri-oxidized	407.1333, 389.1237, 371.1144, 339.1276, 335.1163, 317.1028, 259.0977, 219.0655, 149.0603	f
M4	1.90	$C_{20}H_{26}O_9$	411.1617[M + H]$^+$	−9.24	Mono-oxidized and hydration	393.1550, 333.1301, 289.1429, 265.1096, 217.1241, 215.1035, 209.0795, 177.0548, 133.0620	u
M5	4.26	$C_{20}H_{26}O_{10}$	427.1627[M + H]$^+$	5.38	Di-oxidized and hydration	373.1272, 355.1152, 245.0813, 213.0912, 147.0437	f
M6	2.64	$C_{21}H_{26}O_{10}$	437.1464[M − H]$^-$	3.66	Tri-oxidized and methylation	391.1391, 387.1049, 341.1044, 275.0935, 259.0949, 203.0341, 201.0569, 175.0378	p, f, u
M7	2.16	$C_{20}H_{26}O_8$	395.1707[M + H]$^+$	0.25	Hydration	353.1644, 337.1307, 311.1254, 265.0703, 235.0600, 205.0513, 177.0563, 149.0590, 147.0441	u
M8	0.44	$C_{21}H_{28}O_8$	409.1845[M + H]$^+$	−4.15	Hydration and methylation	365.1599, 349.1264, 305.1010, 233.0811, 219.0659, 177.0540, 147.0456, 133.0636	f, u
M9	0.49	$C_{19}H_{24}O_7$	365.1589[M + H]$^+$	−3.01	Reduction and demethylation	349.1638, 305.1775, 203.0732, 177.0562, 163.0770, 149.0619, 133.0636	f, u
M10	0.42	$C_{22}H_{30}O_{10}$	455.1395[M + H]$^+$	3.95	Reduction, alkenes to dihydrodiol and acetylation	409.1861, 367.1729, 349.1304, 305.1722, 207.0636, 205.0501, 165.0554,149.0602, 147.0444, 133.0642	p
M11	6.53	$C_{20}H_{22}O_6$	359.1508[M + H]$^+$	3.90	Dehydration	341.1367, 319.1027, 297.1456, 279.0864, 265.0730, 245.0838, 205.0502, 199.1172, 177.0577, 149.0590	f
M12	2.94	$C_{21}H_{24}O_6$	373.1659[M + H]$^+$	2.14	Dehydration and methylation	344.1246, 327.1233, 279.0819, 257.0816, 205.0508, 177.0542, 149.0613	f
M13	4.88	$C_{21}H_{26}O_7$	391.1752[M + H]$^+$	−1.28	Methylation	373.1565, 355.1531, 337.1317, 309.1317, 277.1058, 215.1083, 201.0928, 187.0754, 149.0611	f
M14	2.94	$C_{22}H_{26}O_8$	417.1567[M − H]$^-$	4.08	Acetylation	389.1254, 377.1244, 371.1163, 313.1094, 283.0609, 259.0976, 255.0654	f
M15	4.97	$C_{22}H_{22}O_8$	415.1429[M + H]$^+$	8.67	Dehydration, di-oxidized and acetylation	397.1266, 339.1275, 317.1017, 205.0867, 174.0678	p, u
M16	3.91	$C_{20}H_{22}O_9$	407.1360[M + H]$^+$	4.42	Carboxylation Carboxylation and dehydration	391.1377, 373.1303, 355.1163, 337.1274, 319.1189, 307.1153, 243.1022, 213.0909, 177.0543, 147.0446	f

Continued

No.	t_R/min	Formula	Measured	Error (ppm)	Metabolic process	Product ion (MS/MS) (*m/z*)	Location
The phase I metabolites							
M17	2.76	$C_{18}H_{16}O_7$	345.0999[M + H]$^+$	7.24	Carboxylation, demethylation and desaturation Carbonlation	305.0665, 301.1099, 287.0915, 247.0602, 219.0663, 177.0560, 147.0434, 133.0679	u
M18	2.54	$C_{19}H_{18}O_9$	389.0898[M − H]$^-$	6.42		373.0723, 359.0801, 305.0676, 301.0724, 245.0837, 243.0659, 229.0490, 187.0393	u
M19	3.26	$C_{20}H_{22}O_7$	375.1441[M + H]$^+$	−0.80	Carbonlation and demethylation	317.1079, 301.1071, 279.1245, 203.0740, 201.1298, 177.0551, 149.0624, 147.0445, 133.0623	f, u
M20	2.91	$C_{19}H_{18}O_7$	357.0993[M − H]$^-$	5.04		343.0827, 317.0678, 315.0862, 273.0342, 255.0665	u
The phase II metabolites							
M21	2.22	$C_{20}H_{24}O_{10}S$	455.1014[M−H]$^-$	0.44	Sulfation	355.1204, 339.1215, 309.1105, 293.1155, 257.0804, 175.0406, 129.0352	f
M22	2.46	$C_{20}H_{24}O_{11}S$	471.0961[M−H]$^-$	0	Mono-oxidized and sulfation	413.0548, 357.1363, 311.0946, 303.0875, 275.0931, 271.0592, 201.0541, 175.0409	u
M23	2.09	$C_{20}H_{26}O_{11}S$	473.1121[M−H]$^-$	0.63	Hydration and sulfation	357.1367, 329.1365, 299.0910, 271.0960, 147.0426	f
M24	2.52	$C_{20}H_{26}O_{10}S$	457.1162[M−H]$^-$	−3.28	Reduction and Sulfation	443.1041, 387.0402, 359.1459, 297.0457, 269.1544, 219.1001	f
M25	2.54	$C_{19}H_{26}O_{12}S$	477.1108[M−H]$^-$	8.59	Di- hydration, decarbonylation, hydroxylation and sulfation	443.1041, 407.0802, 359.1114, 291.0858	f
M26	5.31	$C_{17}H_{18}O_{11}S$	429.0470[M−H]$^-$	−5.12	Carbonylation, demethylation and sulfation	385.0602, 341.0665, 313.0377, 281.0457, 254.9983	p
M27	2.48	$C_{26}H_{32}O_{13}$	553.1940[M + H]$^+$	3.43	Glucuronidation	377.1566, 301.1067, 213.0935, 177.0561, 149.0593	p, u
M28	2.68	$C_{32}H_{40}O_{19}$	729.2206[M + H]$^+$	−4.93	2x Glucuronidation	553.1929, 463.1217, 441.1347, 317.0994, 287.0928, 247.0968, 219.0662, 205.0498, 177.0545, 175.0383,149.0621	f
M29	2.06	$C_{26}H_{32}O_{14}$	569.1913[M + H]$^+$	7.55	Mono-oxidized and Glucuronidation	453.1356, 393.1549, 317.0994, 205.0513, 177.0569, 149.0597	p, f, u
M30	2.08	$C_{26}H_{32}O_{15}$	585.1815[M + H]$^+$	−0.68	Di-oxidized and Glucuronidation	569.1825, 525.1984, 487.1735, 305.1392, 295.1194, 239.0902, 233.1192, 177.0531, 149.0614, 133.0633	p, f, u
M31	1.43	$C_{24}H_{26}O_{13}$	521.1287[M−H]$^-$	−1.53	Double bond rupture, hydroxylation, dehydration and Glucuronidation	507.1151, 395.0975, 367.0651, 219.0678, 175.0376	u

p, plasma samples; f, feces samples and u, urine samples

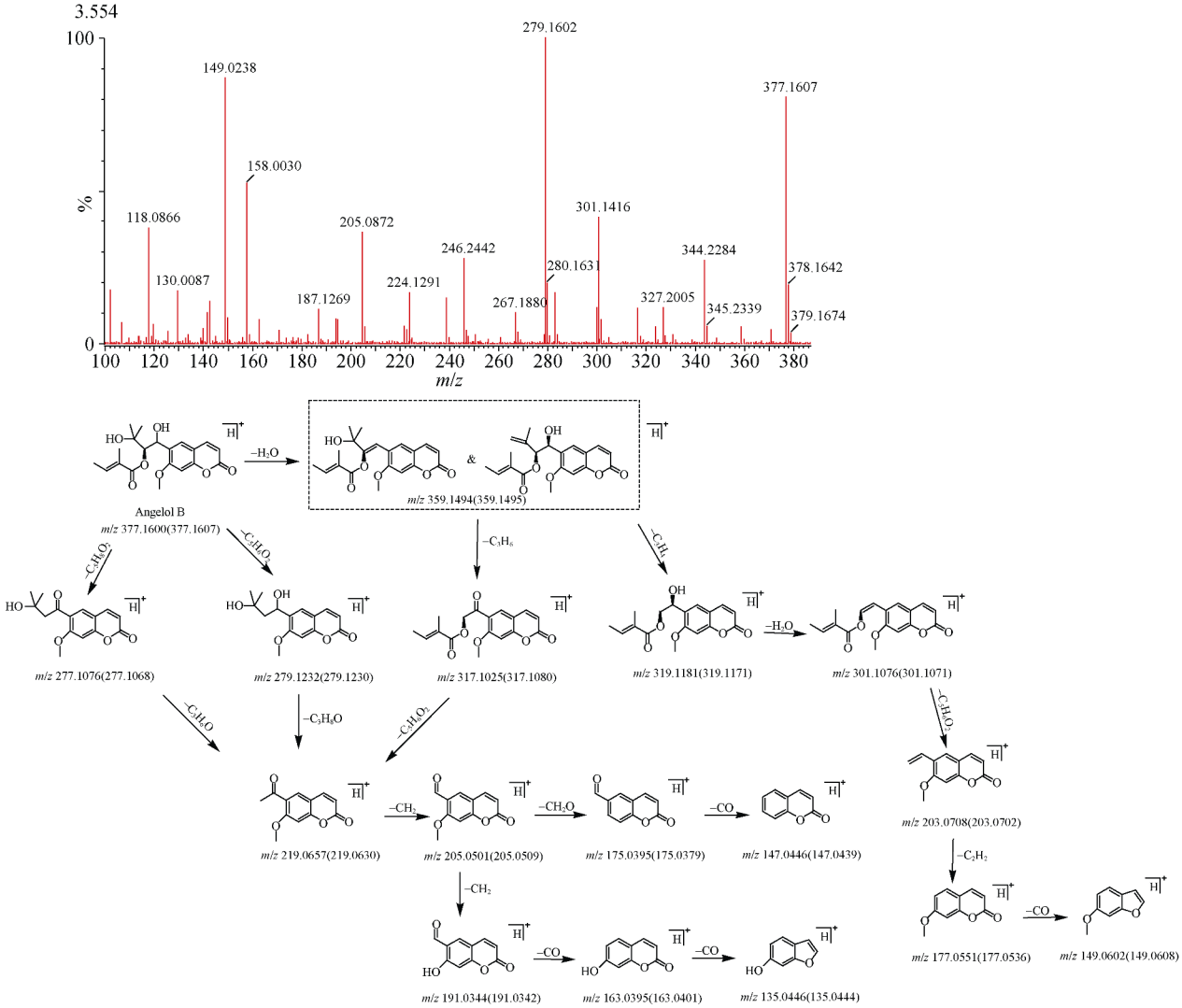
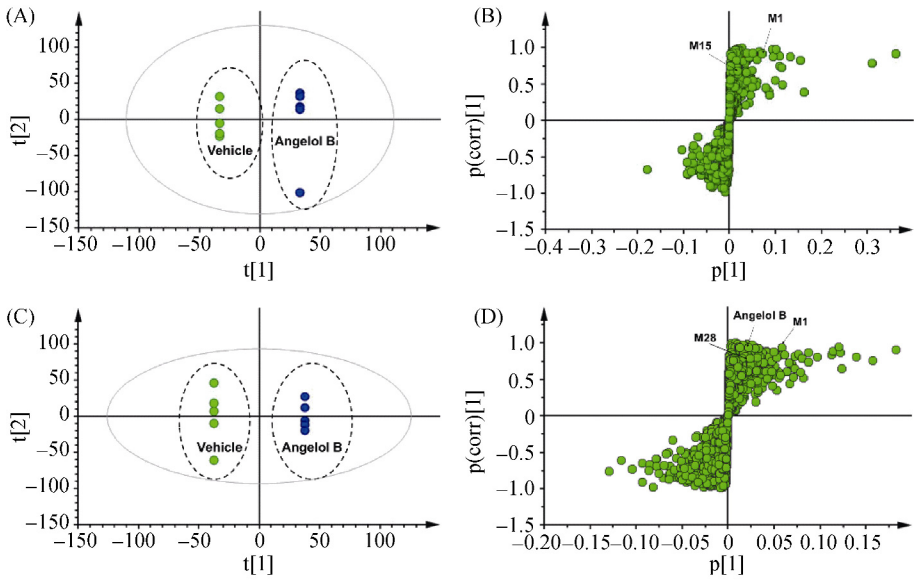


Fig. 1 The MS/MS spectrum and fragmentation process of angelol B



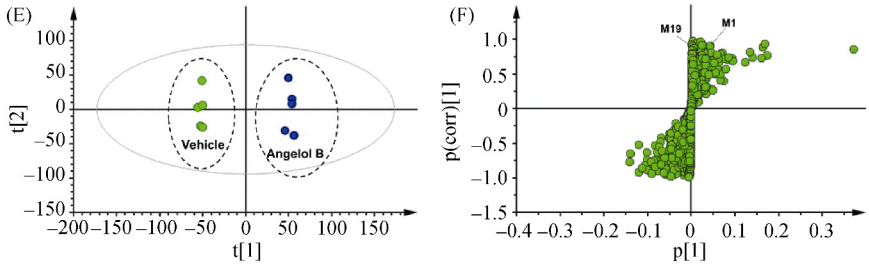


Fig. 2 OPLS analysis and S-plot of global metabolomes of rat plasma, feces and urine; each point represents an individual rat biological sample. (A and B) OPLS analysis and S-plot of global metabolomes in rat plasma treated with blank (corn oil) and Angelol B (80 mg·kg^{-1}), respectively. (C and D) 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 I metabolites

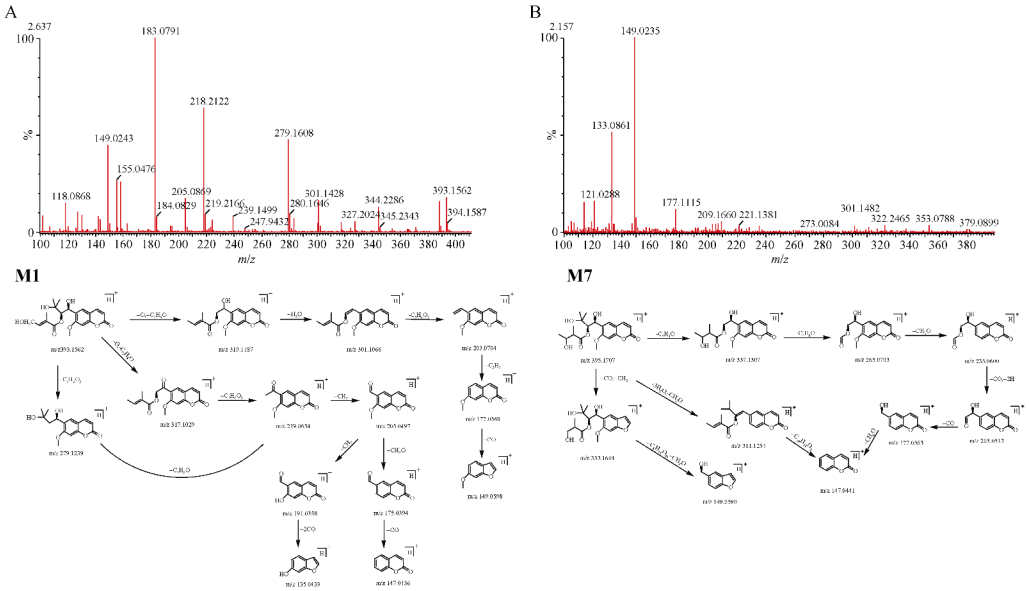
A total of 20 phase I metabolites were identified in plasma, feces and urine samples of rats. According to the MS^2 information, the metabolic pathways include oxidization, reduction, hydration, methylation, demethylation, dehydration, hydroxylation, acetylation, carboxylation, and desaturation.

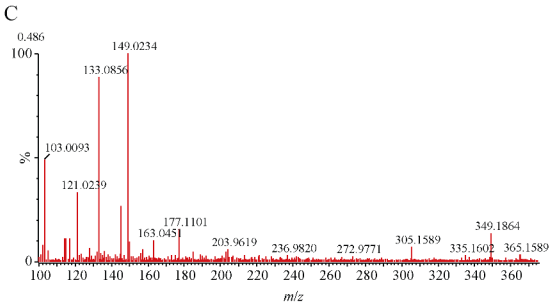
The oxidization and oxidization- metabolites

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 $[M + H - O - C_3H_6O]^+$, 317.1029 $[M + H - O - C_3H_8O]^+$, 301.1066 $[M + H - O - C_3H_6O - H_2O]^+$, 279.1239 $[M + H - C_5H_6O_3]^+$, 219.0630 $[M + H - C_5H_6O_3 - C_3H_8O]^+$/ $[M + H - O - C_3H_8O - C_5H_6O_2]^+$, 205.0509 $[M + H - C_5H_6O_3 - C_3H_8O - CH_2]^+$, 203.0702 $[M + H - O - C_3H_6O - H_2O - C_5H_6O_2]^+$, 191.0342 $[M + H - C_5H_6O_3 - C_3H_8O - 2CH_2]^+$, 177.0536 $[M + H - O - C_3H_6O - H_2O - C_5H_6O_2 - C_2H_2]^+$, 175.0379 $[M + H - C_5H_6O_3 - C_3H_8O - CH_2 - CH_2O]^+$, 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 oxidization 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 $[M - H - CH_2 - 3H_2 - CO]^-$, 297.0746 $[M - H - CH_2 - 3H_2 - CO_2 - O_2 - CH_2]^-$, 269.0866 $[M - H - CH_2 - 3H_2 - CO_2 - O_2 - CH_2 - CO]^-$, 220.0382 $[M - H - CH_2 - 3H_2 - CO - C_7H_7O_3]^-$, and 187.0384 $[M - H - CH_2 - 3H_2 - CO - C_7H_7O_3 - O - OH]^-$. **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 $[M + H - 2H_2O]^+$, 371.1144 $[M + H - 3H_2O]^+$, 339.1276 $[M + H - 3H_2O - 2O]^+$, 335.1163 $[M + H - 2O - C_3H_6O]^+$ 317.1028 $[M + H - 2O - C_3H_6O - H_2O]^+$, 259.0977 $[M + H - 3H_2O - 2O - C_5H_4O]^+$, 219.0655 $[M + H - 2O - C_3H_6O - H_2O - C_5H_6O_2]^+$ and 149.0603 $[M + H - 2O - C_3H_6O - H_2O - C_5H_6O_2 - C_2H_2O - CO]^+$. **M3** was obtained by the oxidization (16 Da) of **M2**. **M1**, **M2** and **M3** were generated by the continuous oxidization of **M0** according to their MS^2 data and possible fragmentation process (Fig. 3A).

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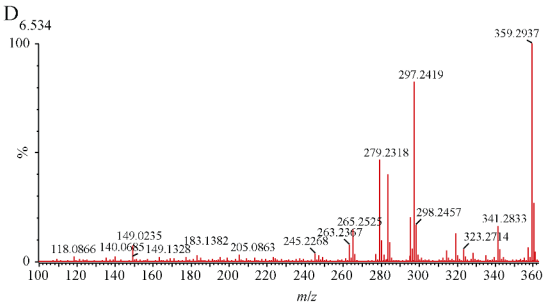
m/z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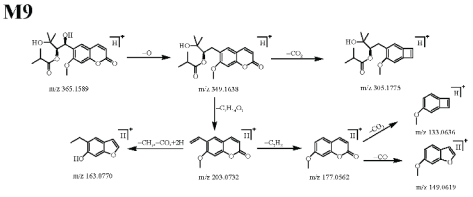
M9
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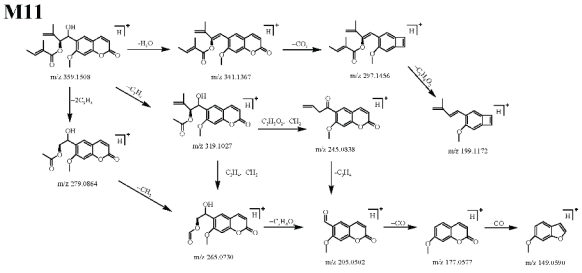
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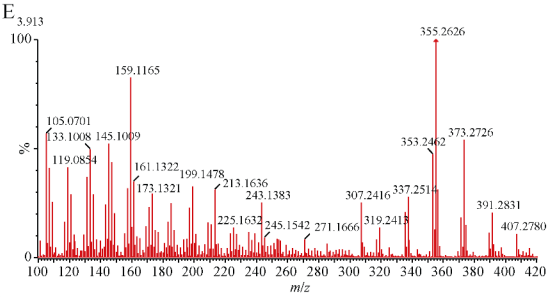
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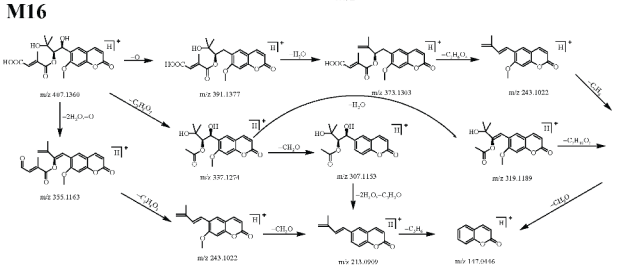
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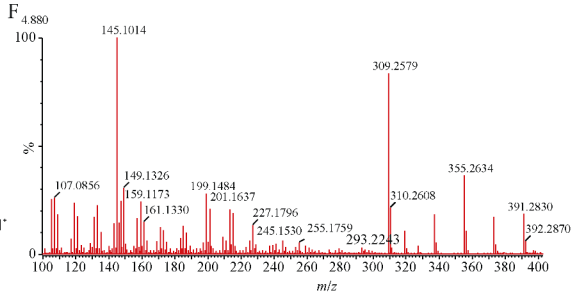
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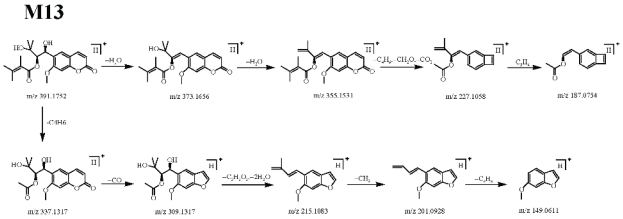
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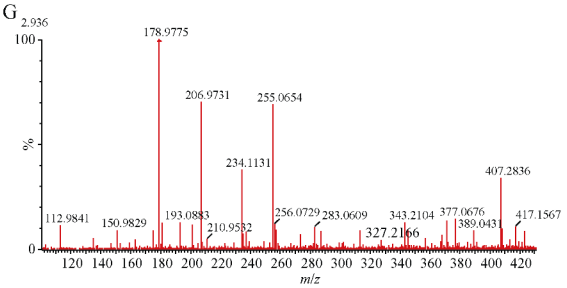
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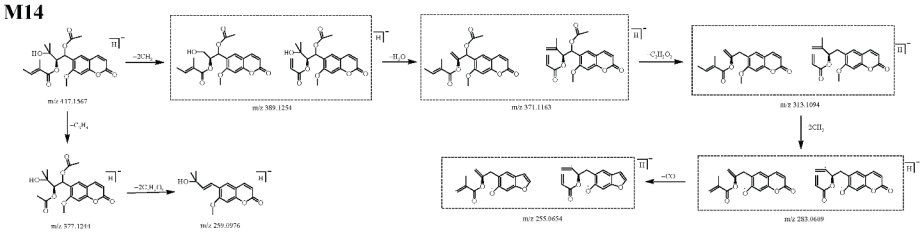
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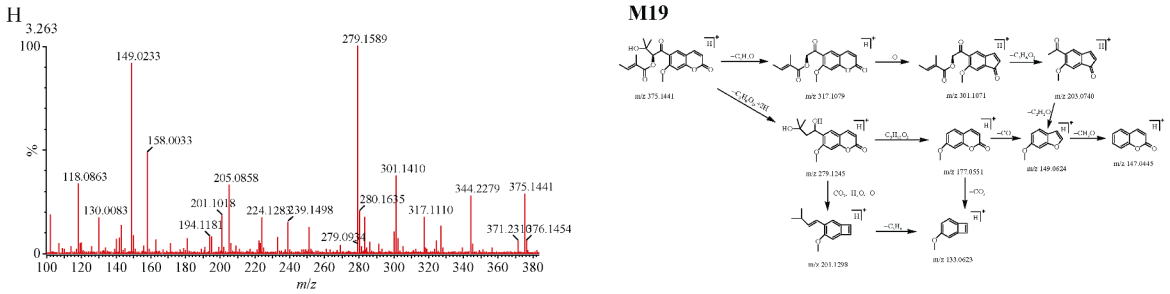


Fig. 3 The MS/MS spectra and possible fragmentation process of 8 phase I metabolites. (A) M1; (B) M7; (C) M9; (D) M11; (E) M16; (F) M13; (G) M14; (H) M19

The $[M + H]^+$ ion of **M4** (t_R = 1.90 min) was at *m/z* 411.1617 and only found in rat urine. Its molecular formula is deduced as $C_{20}H_{26}O_9$. The main fragment ions were at *m/z* 393.1550 $[M + H - H_2O]^+$, 333.1301 $[M + H - H_2O - C_2H_4O_2]^+$, 289.1429 $[M + H - H_2O - C_2H_4O_2 - CO_2]^+$, 265.1096 $[M + H - C_5H_8O_4 - CH_2]^+$, 209.0795 $[M + H - C_5H_8O_4 - 3CH_2 - CO]^+$, and 177.0548 $[M + H - C_5H_8O_4 - 3CH_2 - CO - CH_4O]^+$. **M4** was 18 Da more than that of **M1** which indicated that **M4** was gennerated by the hydration of **M1**.

M5 was only detected in rat feces. It gave molecular ion at *m/z* 427.1627 $[M + H]^+$ (t_R = 4.26 min) and its molecular formula is speculated as $C_{20}H_{26}O_{10}$. **M5** yielded major MS^2 ions at *m/z* 373.1272 $[M + H - 3H_2O]^+$, 355.1152 $[M + H - 4H_2O]^+$, 245.0813 $[M + H - 4H_2O - C_5H_4O_2 - CH_2]^+$, 213.0912 $[M + H - 4H_2O - C_5H_4O_2 - CH_2 - CO]^+$ and 147.0437 $[M + H - 4H_2O - C_5H_4O_2 - CH_2 - CO - C_5H_6]^+$. 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 infromation and possible fragmentation prcess (Fig. C and D), **M4** and **M5** were the oxidized- hydration metabolites of angelol B.

M6 (t_R = 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 $C_{21}H_{26}O_{10}$ and yielded characteristic product ions at *m/z* 391.1391 $[M - H - 2O - CH_2]^-$, 387.1049 $[M - H - CH_3OH - H_2O]^-$, 341.1044 $[M - H - CH_3OH - H_2O - 2O - CH_2]^-$, 275.0935 $[M - H - 2O - CH_2 - C_5H_8O_3]^-$, 259.0949 $[M - H - CH_3OH - H_2O - 2O - CH_2 - C_4H_2O_2]^-$, 203.0341 $[M - H - 2O - CH_2 - C_5H_8O_3 - C_4H_8O]^-$, 201.0569 $[M - H - CH_3OH - H_2O - 2O - CH_2 - C_4H_2O_2 - C_3H_6O]^-$ and 175.0378 $[M - H - CH_3OH - H_2O - 2O - CH_2 - C_4H_2O_2 - C_3H_6O - C_2H_2]^-$ / $[M - H - 2O - CH_2 - C_5H_8O_3 - C_4H_8O - CO]^-$. According to the MS/MS data, **M6** was generated by tri-oxidization and methylation of Angelol B.

The hydration and hydration- metabolites

Metabolite **M7** (t_R = 2.16 min) was only detected in urine. Its molecular formula is deduced as $C_{20}H_{26}O_8$ by *m/z* 395.1707 $[M + H]^+$. The main fragments at *m/z* 353.1644 $[M + H - CO - CH_2]^+$, 337.1307 $[M + H - C_3H_6O]^+$, 311.1254 $[M + H - 3H_2O - CH_2O]^+$, 265.0703 $[M + H - C_4H_8O]^+$, 235.0600 $[M + H - C_4H_8O - CH_2O]^+$, 205.0513 $[M + H - C_4H_8O - CH_2O - CO - 2H]^+$, and 177.0563 $[M + H - C_4H_8O - CH_2O - CO - 2H - CO]^+$. **M7** was 18 Da more than that of **M0**, which indicated that **M7** was producted by hydration 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 $C_{21}H_{28}O_8$. **M8** was found in rat feces and urine, which had the typical fragments at *m/z* 365.1599 $[M + H - CH_2 - CH_2O]^+$, 349.1264 $[M + H - CH_2 - CH_2O - CH_4]^+$, 305.1010 $[M + H - CH_2 - CH_2O - CH_4 - CH_2O - CH_2]^+$, 233.0811 $[M + H - CH_2 - CH_2O - CH_4 - CH_2O - CH_2 - C_3H_4O_2]^+$, and 219.0659 $[M + H - CH_2 - CH_2O - CH_4 - CH_2O - CH_2 - C_4H_6O_2]^+$. **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 $C_{19}H_{24}O_7$, which was 12 Da less than that of **M0** and had the product ions at *m/z* 349.1638 $[M + H - O]^+$, 305.1775 $[M + H - O - CO_2]^+$, 203.0732 $[M + H - O - C_7H_{14}O_3]^+$, 177.0562 $[M + H - O - C_7H_{14}O_3 - C_2H_2]^+$, 163.0770 $[M + H - O - C_7H_{14}O_3 - CH_2 - CO + 2H]^+$, 149.0619 $[M + H - O - C_7H_{14}O_3 - C_2H_2 - CO]^+$, and 133.0636 $[M + H - O - C_7H_{14}O_3 - C_2H_2 - CO_2]^+$. According to the fragments information, **M9** was speculated as the reduction and demethylation metabolite of Angelol B (Fig. 3C).

The molecular formula of **M10** (t_R = 0.42 min) is deduced as $C_{22}H_{30}O_{10}$ 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 $[M + H - CO - H_2O]^+$, 367.1729 $[M + H - CO - H_2O - CH_2CO]^+$, 349.1304 $[M + H - CO - H_2O - C_3H_8O]^+$, 305.1722 $[M + H - O - CH_2O - CH_2COO - H_2O - CO]^+$. 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 $C_{20}H_{22}O_6$ The characteristic product ions of **M11** were at *m/z* 341.1367 $[M + H - H_2O]^+$, 319.1027 $[M + H - C_3H_4]^+$, 297.1456 $[M + H - H_2O - CO_2]^+$,

279.0864 $[M + H - 2C_3H_4]^+$, 265.0730 $[M + H - 2C_3H_4 - CH_2]^+$, 245.0838 $[M + H - C_3H_4 - C_2H_3O_2 - CH_2]^+$, 205.0502 $[M + H - 2C_3H_4 - CH_2 - C_2H_4O_2]^+$, and 199.1172 $[M + H - H_2O - CO_2 - C_5H_6O_2]^+$, and 177.0577 and 149.0590 were produced by remove 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 $[M + H - CH_2 - CH_3\cdot]^+$, 327.1233 $[M + H - CH_2 - CH_3\cdot - OH]^+$ and 279.0819 $[M + H - C_4H_6 - C_3H_4]^+$. **M12** was 14 Da more than that of **M11** which infered that **M12** was generated by methylation of **M11**.

The molecular formula of **M15** is speculated as $C_{22}H_{22}O_8$ 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 $[M + H - H_2O]^+$, 339.1275 $[M + H - H_2O - C_2H_2O_2]^+$, 317.1017 $[M + H - C_2H_2O_2 - C_3H_4]^+$, 205.0867 $[M + H - C_2H_2O_2 - C_3H_4 - C_5H_6O_3 + 2H]^+$ and 174.0678 $[M + H - C_2H_2O_2 - C_3H_4 - C_5H_6O_3 + 2H - C_3HO]^+$. According to the MS and MS/MS information, **M15** was generated by loss of two H_2O (32 Da) and acetylation after di-oxidization of **M0**. **M12** and **M15** were dehydration- metabolites and their possible structure and fragmentation pathway were shown in Fig. S1, H and I.

The carboxylation and carboxylation-metabolites

The molecular ion of **M16** was at *m/z* 407.1360 $[M + H]^+$ (t_R = 3.91 min) and was only found in rat feces. Its molecular formula is deduced as $C_{20}H_{22}O_9$, which was 30 Da more than that of **M0** and yielded major fragment ions at *m/z* 391.1377 $[M + H - O]^+$, 373.1303 $[M + H - O - H_2O]^+$, 355.1163 $[M + H - 2H_2O - O]^+$, 337.1274 $[M + H - C_3H_2O_2]^+$, 319.1189 $[M + H - C_3H_2O_2 - H_2O]^+$ and 307.1153 $[M + H - C_3H_2O_2 - CH_2O]^+$. **M16** was obtained by carboxylation of **M0** based on the MS and MS/MS data (Fig. 3E).

M17 had the $[M + H]^+$ ion at *m/z* 345.0999 and was found in rat urine with retention time at 2.76 min. Its possessed typical product ions at *m/z* 305.0665 $[M + H - C_3H_4]^+$, 301.1099 $[M + H - CO_2]^+$, 287.0915 $[M + H - CO_2 - CH_2]^+$, 247.0602 $[M + H - C_3H_4 - C_2H_2O_2]^+$, and 219.0663 $[M + H - C_3H_4 - C_2H_2O_2 - CO]^+$. According to the MS^2 data, **M17** was produced by loss of C_2H_2 group and 2 H_2O after carboxylation of **M0**.

M18 was detected in rat urine with retention time at 2.54 min. It showed the molecular ion at *m/z* 389.0898 $[M - H]^-$ which indicated that the molecular formula is $C_{19}H_{18}O_9$. Its fragment ions were at *m/z* 373.0723 $[M - H - H_2O - C_2H_2O_2]^-$, 359.0801 $[M - H - CH_2O]^-$, 305.0676 $[M - H - C_4H_4O_2]^-$, 301.0724 $[M - H - C_2H_4O - CO_2]^-$, 245.0837 $[M - H - C_4H_4O_2 - CO_2 - O]^-$, 243.0659 $[M - H - C_4H_4O_2 - CO_2 - H_2O]^-$, 229.0490 $[M - H - C_4H_4O_2 - CO_2 - H_2O - CH_2]^-$, and 187.0393 $[M - H - C_4H_4O_2 - CO_2 - H_2O - CH_2 - CO - CH_2]^-$ suggesting that **M18** was generated by the carboxylation, demethylation and desaturation of prototype compound. **M17** and **M18** were identified as the carboxylation and carboxylation-metabolites based on the MS and MS/MS data.

The other metabolites

Metabolite **M13** (t_R = 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 $C_{21}H_{26}O_7$ and further fragmentation yielded characteristic ions at *m/z* 373.1565 $[M + H - H_2O]^+$, 355.1531 $[M + H - 2H_2O]^+$, 337.1317 $[M + H - C_4H_6]^+$, and 309.1317 $[M + H - C_4H_6 - CO]^+$. **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 $C_{22}H_{26}O_8$ by UPLC-QTOF-MS (*m/z* 417.1567 $[M - H]^-$). It had fragment ions at *m/z* 389.1254 $[M - H - 2CH_2]^-$, 377.1244 $[M - H - C_3H_4]^-$, 371.1163 $[M - H - 2CH_2 - H_2O]^-$, 313.1094 $[M - H - 2CH_2 - H_2O - C_2H_2O_2]^-$, 283.0609 $[M - H - 2CH_2 - H_2O - C_2H_2O_2 - 2CH_3\cdot]^-$, 259.0976 $[M - H - C_3H_4 - 2C_3H_2O_2]^-$ and 255.0654 $[M - H - 2CH_2 - H_2O - C_2H_2O_2 - 2CH_3\cdot - CO]^-$. **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).

M19 (t_R = 3.26 min) had the $[M + H]^+$ ion at *m/z* 375.1441 ($C_{20}H_{22}O_7$) and was detected in rat feces and urine. Further fragmentation yielded product ions at *m/z* 317.1079 $[M + H - C_3H_6O]^+$, 301.1071 $[M + H - C_3H_6O - O]^+$, 279.1245 $[M + H - C_5H_6O_2 + 2H]^+$, 203.0740 $[M + H - C_3H_6O - O - C_5H_6O_2]^+$, 201.1298 $[M + H - C_5H_6O_2 + 2H - CO_2 - H_2O - O]^+$, 177.0551 $[M + H - C_5H_6O_2 + 2H - C_5H_{10}O_2]^+$, 149.0624 $[M + H - C_5H_6O_2 + 2H - C_5H_{10}O_2 - CO]^+$, 147.0445 $[M + H - C_5H_6O_2 + 2H - C_5H_{10}O_2 - CH_2O]^+$, and 133.0623 $[M + H - C_5H_6O_2 + 2H - C_5H_{10}O_2 - CO_2]^+$. **M19** was 2 Da less than that of **M0** which speculated that it was a carbonlation metabolite of Angelol B (Fig. 3H).

M20 was only detected in rat urine. Its $[M - H]^-$ ion was at *m/z* 357.0993 ($C_{19}H_{18}O_7$) with retention time at 2.91 min. It had fragment ions at *m/z* 343.0827 $[M - H - CH_2]^-$, 317.0678 $[M - H - CH_2 - C_2H_2]^-$, 315.0862 $[M - H - CH_2 - CO]^-$, 273.0342 $[M - H - C_5H_8O]^-$, and 255.0665 $[M - H - CO_2 - C_2H_4 - CH_2O]^-$. It was carbonlation and demethylation metabolite of Angelol B based on the MS and MS/MS information (Fig. S1 L).

Analysis of phase II metabolites

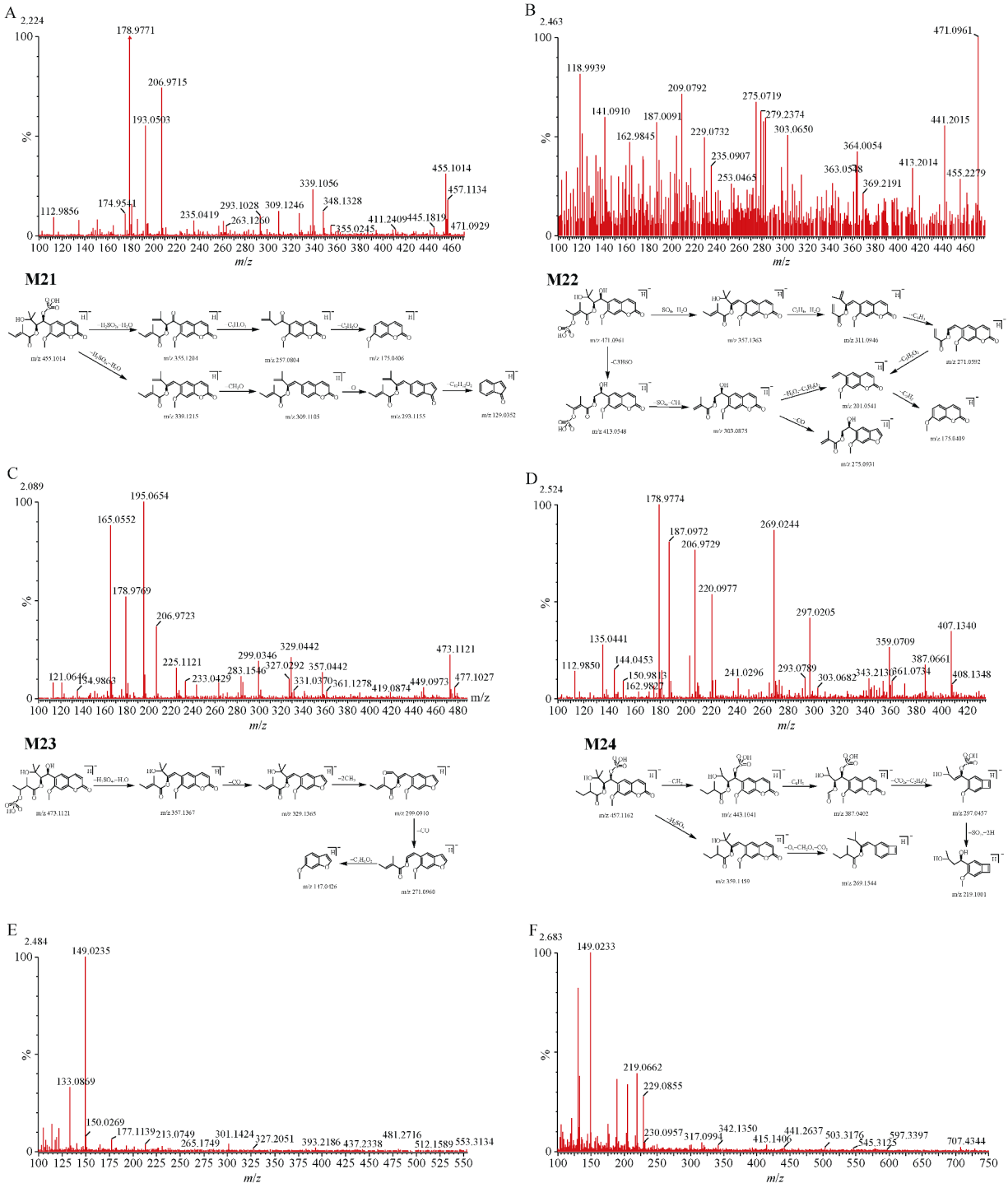
A total of 11 phase II metabolites were identified in rat plasma, feces, and urine. According to the MS and MS/MS information, the main metabolic pathways are sulfation and glucuronidation.

The sulfation metabolites

M21 was only detected in feces with retention time at 2.22 min. Its molecular formula is deduced as $C_{20}H_{24}O_{10}S$ by UPLC-QTOF-MS (*m/z* 455.1014 $[M - H]^-$). Its fragment ions

were at *m/z* 355.1204 [M − H − H_2SO_3 − H_2O]$^-$, 339.1215 [M − H − H_2SO_4 − H_2O]$^-$, 309.1105 [M − H − H_2SO_4 − H_2O − CH_2O]$^-$, 293.1155 [M − H − H_2SO_4 − H_2O − CH_2O − O]$^-$, 257.0804 [M − H − H_2SO_3 − H_2O − $C_5H_6O_2$]$^-$, 175.0406 [M − H − H_2SO_3 − H_2O − $C_5H_6O_2$ − C_5H_6O]$^-$, and 129.0352 [M − H − H_2SO_4 − H_2O − CH_2O − O − $C_{10}H_{12}O_2$]$^-$. 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_3H_6O]$^-$, 357.1363 [M − H − SO_4 − H_2O]$^-$, 311.0946 [M − H − SO_4 − H_2O − C_2H_4 − H_2O]$^-$, 303.0875 [M − H − C_3H_6O − SO_4 − CH_2]$^-$, 275.0931 [M − H − C_3H_6O − SO_4 − CH_2 − CO]$^-$, 271.0592 [M − H − SO_4 − H_2O − C_2H_4 − H_2O − C_3H_4]$^-$, 201.0541 [M − H − SO_4 − H_2O − C_2H_4 − H_2O − C_3H_4 − $C_3H_2O_2$]$^-$, and 175.0409 [M − H − SO_4 − H_2O − C_2H_4 − H_2O − C_3H_4 − $C_3H_2O_2$ − C_2H_2]$^-$, which indicated that **M22** was oxidized and sulfated by **M0** (Fig. 4B).



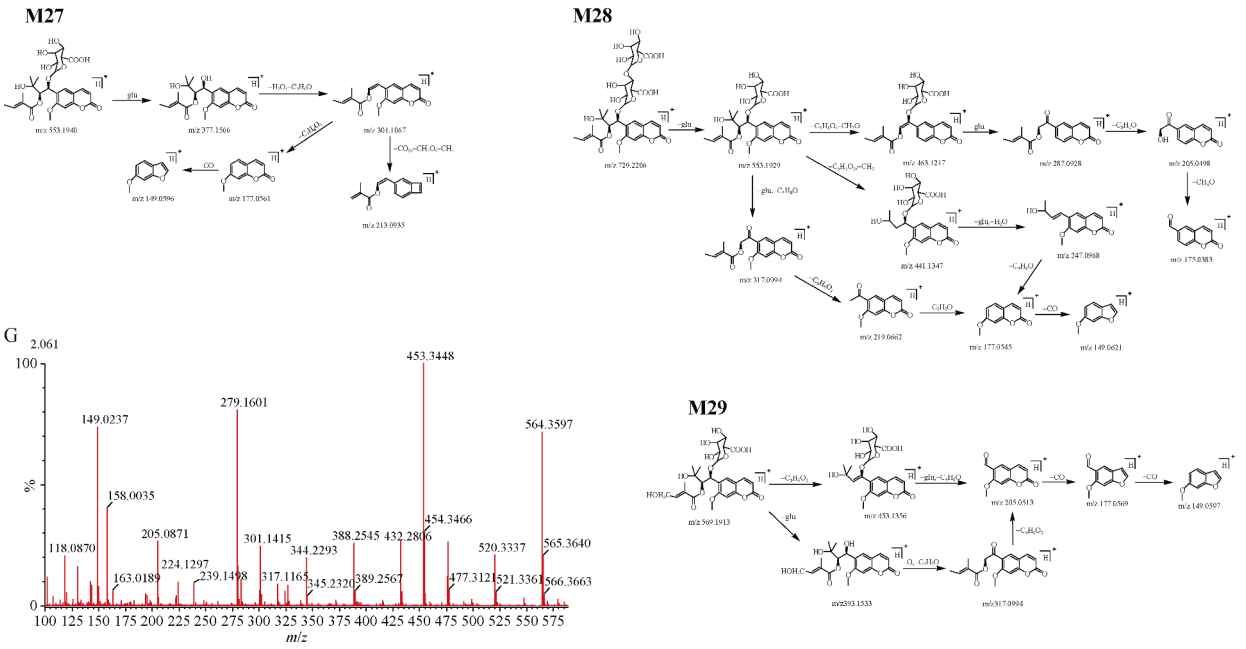


Fig. 4 The MS/MS spectra and possible fragmentation process of 7 phase II metabolites. (A) M21; (B) M22; (C) M23; (D) M24; (E) M27; (F) M28; (G) M29

Metabolite **M23** was detected in rat feces with retention time at 2.09 min, and gave molecular ion at *m/z* 473.1121[M – H]$^-$ ($C_{20}H_{26}O_{11}S$). It had fragment ions at *m/z* 357.1367 [M – H – H_2SO_4 – H_2O]$^-$, 329.1365 [M – H – H_2SO_4 – H_2O – CO]$^-$, 299.0910 [M – H – H_2SO_4 – H_2O – CO – $2CH_3$]$^-$, 271.0960 [M – H – H_2SO_4 – H_2O – CO – $2CH_3$ – CO]$^-$ and 147.0426 [M – H – H_2SO_4 – H_2O – CO – $2CH_3$ – CO – $C_7H_8O_2$]$^-$. **M23** was 98 mass units (SO_3 and H_2O) more than that of **M0** and inferred that **M23** was the hydration and sulfation metabolite of **M0** (Fig. 4C).

The [M – H]$^-$ ion of **M24** was at *m/z* 457.1162 ($C_{20}H_{26}O_{10}S$) 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 – CH_2]$^-$, 387.0402 [M – H – CH_2 – C_4H_8]$^-$, 359.1459 [M – H – H_2SO_4]$^-$, 297.0457 [M – H – CH_2 – C_4H_8 – CO_2 – C_2H_6O]$^-$, 269.1544 [M – H – H_2SO_4 – O – CH_2O – CO_2]$^-$, and 219.1001 [M – H – CH_2 – C_4H_8 – CO_2 – C_2H_6O – SO_3 + 2H]$^-$. The above data indicated that **M24** was generated by reduction of **M21** (Fig. 4D).

M25 (t_R = 2.54 min) was only detected in rat feces. Its molecular formula is deduced as $C_{19}H_{26}O_{12}S$ 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 – $2H_2O$]$^-$, 359.1114 [M – H – 2OH – H_2SO_3 – 2H]$^-$, and 291.0858 [M – H – 2OH – H_2SO_3 – 2H – C_4H_4O]$^-$. 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 min) was only detected in rat plasma and exhibited [M – H]$^-$ ion at *m/z* 429.0470. Its molecular formula is speculated as $C_{17}H_{18}O_{11}S$. **M26** had fragment ions at *m/z* 385.0602 [M – H – CO – O]$^-$, 341.0665 [M – H – CO – O – CO_2]$^-$, 313.0377 [M – H – CO – O – CO_2 – C_2H_4]$^-$, 281.0457 [M – H – CO – O – CO_2 – CH_3COOH]$^-$, and 254.9983 [M – H – $C_6H_{10}O_4$ –CO]$^-$. **M26** was generated by carbonylation, demethylation, and sulfation of Angelol B.

The glucuronidation metabolites

M27 was found in rat plasma and urine. Its [M + H]$^+$ ion (t_R = 2.48 min) was at *m/z* 553.1940 and molecular formula is deduced as $C_{26}H_{32}O_{13}$. It was 176 mass units (a glucuronide group) more than that of **M0**, which indicate that **M27** was the glucuronidation metabolite of **M0**. and Its major product ions were at *m/z* 377.1566 [M + H – glu]$^+$, 301.1067 [M + H – glu – H_2O – C_3H_6O]$^+$, 213.0935 [M + H – glu – H_2O – C_3H_6O – CO_2 – CH_2O – CH_2]$^+$, 177.0561 [M + H – glu – H_2O – C_3H_6O – $C_7H_8O_2$]$^+$ and 149.0593 [M + H – glu – H_2O – C_3H_6O – $C_7H_8O_2$ – 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]$^+$ ($C_{32}H_{40}O_{19}$) 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]$^+$, 463.1217 [M + H – glu – C_3H_8O – CH_2O]$^+$, 441.1347 [M + H – glu – $C_5H_6O_2$ – CH_2]$^+$, 317.0994 [M + H – 2glu – C_3H_8O]$^+$, 287.0928 [M + H – glu – C_3H_8O – CH_2O – glu]$^+$, 247.0968 [M + H – glu – $C_5H_6O_2$ – CH_2 – glu – H_2O]$^+$, 219.0662 [M + H – 2glu – C_3H_8O – $C_5H_6O_2$]$^+$, 205.0498 [M + H – glu – C_3H_8O – CH_2O – glu – C_5H_6O]$^+$, 177.0545 [M + H – 2glu – C_3H_8O – $C_5H_6O_2$ – C_2H_2O]$^+$, 175.0383 [M + H – glu – C_3H_8O – CH_2O – glu – C_5H_6O – CH_2O]$^+$, and 149.0621 [M + H – 2glu – C_3H_8O – $C_5H_6O_2$ – C_2H_2O – CO]$^+$. 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 $[M + H - C_5H_8O_3]^+$, 393.1549 $[M + H - glu]^+$, 317.0994 $[M + H - glu - O - C_3H_8O]^+$, 205.0513 $[M + H - C_5H_8O_3 - glu - C_4H_8O]^+$, 177.0569 $[M + H - C_5H_8O_3 - glu - C_4H_8O - CO]^+$, and 149.0597 $[M + H - C_5H_8O_3 - glu - C_4H_8O - 2CO]^+$ which inferred that **M29** was oxidized by **M27**. The possible structure and fragmentation process was shown in Fig. 4G.

M30 (t_R = 2.08 min) possessed the $[M + H]^+$ ion at *m/z* 585.1815 and was found in rat plasma, feces and urine. Its molecular formula is speculated as $C_{26}H_{32}O_{15}$ which was 32 Da (2O) more than that of **M27**, indicating that **M30** was obtained by di-oxidization of **M27**. Further fragmentation yielded MS/MS ions at *m/z* 569.1825 $[M + H - O]^+$, 525.1984 $[M + H - O - CO_2]^+$, 487.1735 $[M + H - O - CO_2 - C_2H_4O]^+$, 305.1392 $[M + H - O - CO_2 - C_2H_4O - glu]^+$, 295.1194 $[M + H - O - glu - C_5H_6O_3]^+$, 239.0902 $[M + H - O - glu - C_5H_6O_3 - 2CH_2 - CO]^+$, 233.1192 $[M + H - O - CO_2 - C_2H_4O - glu - C_3H_4O_2]^+$, 177.0531 $[M + H - O - glu - C_5H_6O_3 - 2CH_2 - CO - C_2H_6O - O]^+$, 149.0614 $[M + H - O - glu - C_5H_6O_3 - 2CH_2 - CO - C_2H_6O - O - CO]^+$, and 133.0633 $[M + H - O - CO_2 - C_2H_4O - glu - C_3H_4O_2 - C_5H_8O_2]^+$.

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 $[M - H - CH_2 - C_5H_4O_3]^-$, 367.0651 $[M - H - CH_2 - C_5H_4O_3 - 2CH_2]^-$, 219.0678 $[M - H - CH_2 - C_5H_4O_3 - glu]^-$ and 175.0376 $[M - H - CH_2 - C_5H_4O_3 - glu - C_2H_4O]^-$. 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

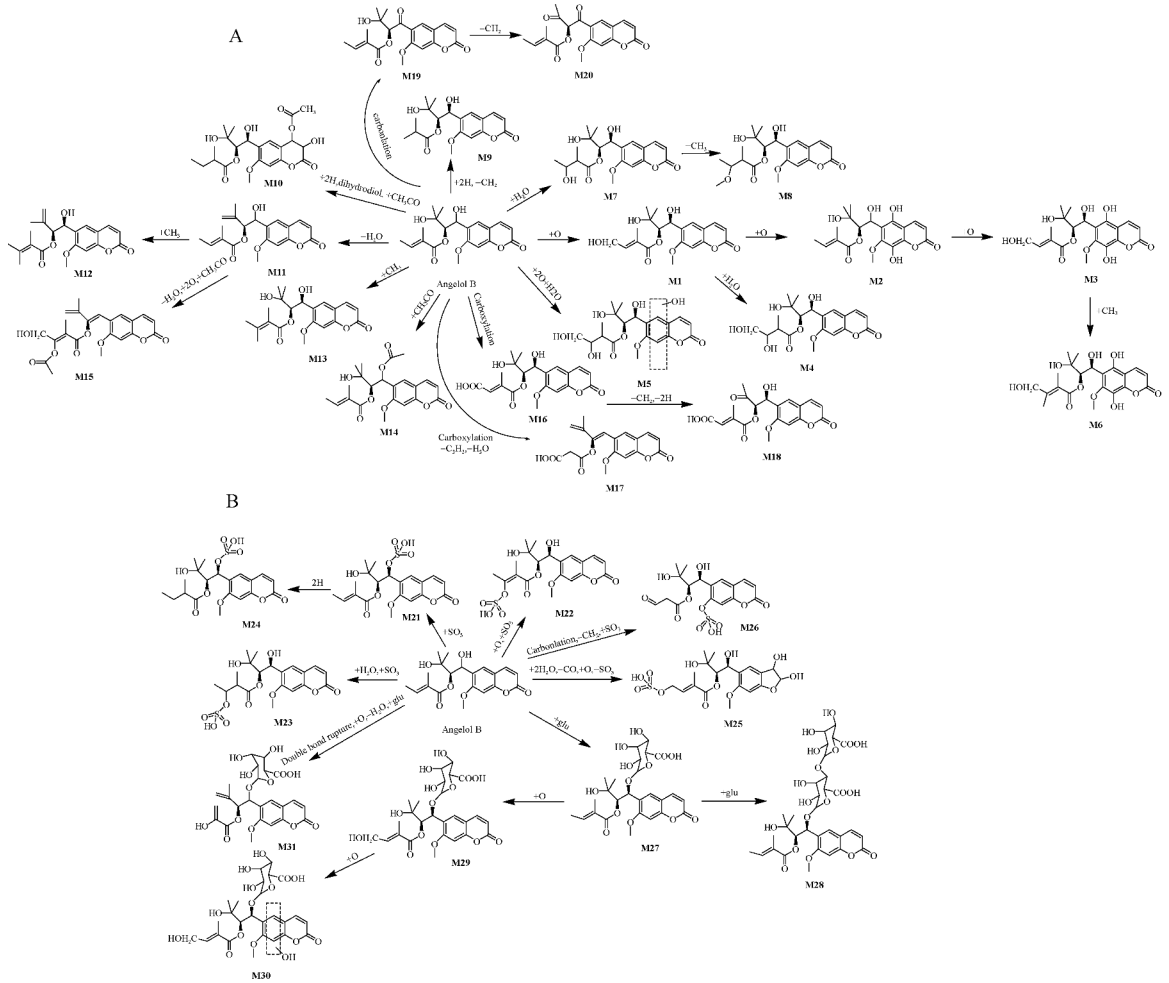


Fig. 5 The metabolic pathway of Angelol B. (A) the phase I metabolic pathway, (B) the phase II metabolic pathway

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 oxidization, reduction, hydration, methylation, demethylation, dehydration, 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

[1] Li XR, Wang JN, Gao L. Anti-inflammatory and analgesic activity of R.A.P. (Radix Angelicae Pubescentis) ethanol extracts [J]. *Afr J Tradit Complement Altern Med*, 2013, **10**(3): 422-426.

[2] Li RL, Zhao C, Yao MN, *et al*. Analgesic effect of coumarins from Radix Angelicae Pubescentis is mediated by inflammatory factors and TRPV1 in a spared nerve injury model of neuropathic pain [J]. *J Ethnopharmacol*, 2017, **195**: 81-88.

[3] Sun J, Liu BR, Hu WJ, *et al*. *In vitro* anticancer activity of aqueous extracts and ethanol extracts of fifteen traditional Chinese medicines on human digestive tumor cell lines [J]. *Phytother Res*, 2007, **21**(11): 1102-1104.

[4] Pei Y, Ma X, Yi J, *et al*. Effects of *Angelica pubescens* coumarins on anti-oxidation function and excitatory amino acid glutamic acid content of Parkinson's rat model [J]. *Chin J Gerontol*, 2014, **34**(5): 1272-1274.

[5] Liu JH, Zschocke S, Reininger E, *et al*. Inhibitory effects of *Angelica pubescens* on 5-lipoxygenase and cyclooxygenase [J]. *Planta Med*, 1998, **64**(6): 525-529.

[6] Gao D, Wang BJ, Huo ZP, *et al*. Analysis of chemical constituents in an herbal formula Jitong Ning Tablet [J]. *J Pharm Biomed Anal*, 2017, **140**: 301-312.

[7] Zhang RX, Fan AY, Zhou AN, *et al*. Extract of the Chinese herbal formula Huo Luo Xiao Ling Dan inhibited adjuvant arthritis in rats [J]. *J Ethnopharmacol* , 2009, **121**(3): 366-371.

[8] Lao L, Fan A Y, Zhang RX, *et al*. Anti-hyperalgesic and anti-inflammatory effects of the modified Chinese herbalformula Huo Luo Xiao Ling Dan (HLXL) in rats [J]. *Am J Chin Med*, 2006, **34**(5): 833-844.

[9] Zhang YB, Li W, Yang XW. Biotransformation of columbianadin by rat hepatic microsomes and inhibition of biotransformation products on NO production in RAW 264.7 cells *in vitro* [J]. *Phytochemistry*, 2012, **81**: 109-116.

[10] Chen TL, Zhang YB, Xu W, *et al*. Biotransformation of isoimperatorin by rat liver microsomes and its quantification by LC-MS/MS method [J]. *Fitoterapia*, 2014, **93**: 88-97.

[11] Zhang P, Yang XW. A new metabolite of nodakenetin by rat liver microsomes and its quantification by RP-HPLC method [J]. Biomed Chromatogr, 2010, **24** (2): 216-221.

[12] Yang XW, Guo QM, Wang Y. Absorption and transport of 6 coumarins isolated from the roots of *Angelica pubescens* in human Caco-2 cell monolayer model [J]. *Chin J Integr Med*, 2008, **6**(4): 392-398.

[13] Liu JH, Xu SX, Yao XS, *et al*. Angelol-type coumarins from *Angelica pubescence* and their inhibitory effect on platelet aggregation [J]. *Phytochemistry*, 1995, **39**(5): 1099- 1101.

[14] Yang YF, Zhang L, Zhang YB, *et al*. Simultaneous assessment of absorption characteristics of coumarins from Angelicae Pubescentis Radix: *In vitro* transport across Caco-2 cell and *in vivo* pharmacokinetics in rats after oral administration [J]. *J Chromatogr B Analyt Technol Biomed Life Sci*, 2017, **1060**: 308-315.

[15] Zhang HM, Li SL, Zhang H, *et al*. Holistic quality evaluation of commercial white and red ginseng using a UPLC-QTOF-MS/MS-based metabolomics approach [J]. *J Pharm Biomed Anal*, 2012, **62**: 258-273.

[16] Ni SM, Qian DE, Duan JA. *et al*. UPLC-QTOF/MS-based screening and identification of the constituents and their metabolites in rat plasma and urine after oral administration of *Glechoma longituba* extract [J]. *J Chromatogr B Analyt Technol Biomed Life Sci*, 2010, **878** (28): 2741-2750.

[17] Shan CX, Li W, Wen HM, *et al*. Identification of liguzinediol metabolites in rats by ultra performance liquid chromatography/quadrupole-time-of-flight mass spectrometry [J]. *J Pharm Biomed Anal*, 2012, **62**: 187-190.

[18] Li J, Chan W. Investigation of the biotransformation of osthole by liquid chromatography/tandem mass spectrometry [J]. *J Pharm Biomed Anal*, 2013, **74**: 156-161.

[19] Trygg J, Wold S. Orthogonal projections to latent structures (O-PLS) [J]. *J Chemom*, 2002, **16** (3): 119-128.

[20] Jonsson P, Gullberg J, Nordstrom A, *et al*. A strategy for identifying differences in large series of metabolomic samples analyzed by GC/MS [J]. *Anal Chem*, 2004, **76** (6): 1738-1745.

[21] Fonville JM, Richards SE, Barton RH, *et al*. The evolution of partial least squares models and related chemometric approaches in metabonomics and metabolic phenotyping [J]. *J Chemom*, 2010, **24** (11-12): 636-649.

[22] Yang YF, Zhang L, Yang XW. Distribution assessments of coumarins from Angelicae Pubescentis Radix in rat cerebrospinal fluid and brain by liquid chromatography tandem mass spectrometry analysis [J]. *Molecules*, 2018, **23** (1): 225-236.

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