SUPPLEMENTAL MATERIAL

Characterization of alkaloids in Radix *Sophora tonkinensis* by UPLC-Q-TOF-MS/MS and its application in the comparison of two different habitats

Xing-Xing Zong[a*], Yu Wang[a], Jia-Liang Xiong[a], Yu-Hui Ping[b*], Qiong-Lin Liang[a*]

[a]*Department of Chemistry, Tsinghua University, Beijing, 100084, PR China;* [b]*Department of Pharmacy, Jiangxi University of Traditional Chinese Medicine, Nanchang 330004, China*

[*]Corresponding author. Xing-Xing Zong, Email: jxjjzong@163.com; Yu-Hui Ping, Email: Pingyh@163.com or Qiong-Lin Liang, Email: liangql@tsinghua.edu.cn

ABSTRACT

Sophora tonkinensis is widely used as traditional Chinese medicine for treating the swelling of the gums and tongue and mouth sores due to flame stomach fire. It is mainly origin from Guangxi, Sichuan provinces of China. Alkaloids are considered as the major bioactive components. A method was established for identifying alkaloids in *S. tonkinensis* root by UPLC-Q-TOF-MS/MS and was applied in characterizing alkaloids in *S. tonkinensis* root of two different habitats. Consequently, twenty-four alkaloids including six new compounds were identified in *S. tonkinensis* root. Additionally, the difference of alkaloids in *S. tonkinensis* from Guozhou, Sichuan province was investigated. In the present study, we firstly characterize total alkaloids in *S. tonkinensis* root by UPLC-Q-TOF-MS/MS and firstly established the characteristic fragmentation pathway of alkaloids with hydroxy in *S. tonkinensis* root.

KEYWORDS: UPLC-Q-TOF-MS/MS; *Sophora tonkinensis*; Alkaloid; Characterization

1. Experimental

1.1. *Chemicals, reagents, and materials*

UHPLC grade acetonitrile and HPLC grade ammonium acetate were purchased from Sigma-Aldrich Laboratories, Inc. (St. Louis, MO, USA). Deionized water was prepared using a Millipore Milli Q-Plus system (Bedford, MA, USA). The ammonia solution (25% NH_3) for UHPLC was obtained from Merck (Darmstadt, Germany). The chloroform, methanol and ammonia solution (25% NH_3) used was of AR grade supplied by Beijing Chemical Reagent Factory (Beijing, People's Republic of China).

The reference compounds (matrine, matrine *N*-oxide, sophoranol, 14β-hydroxymatrine, 9α-hydroxymatrine, cytisine) were separated and purified in our lab. The purity of all compounds is more than 95% (determined by HPLC). The structures are confirmed by their UV, MS, ^{1}H NMR and ^{13}C NMR data compared with the data from literatures. Two herbal materials were respectively collected from Guozhou, Sichuan province and Yizhou, Guangxi province, then dried in the sun. Two plant species were identified by the authors, and a voucher specimen (No. SL20160908, No. SL2016011021) were deposited in the author's laboratory.

1.2. *Sample preparation*

An amount of 1.0 g of *S. tonkinensis* root powder (sieved through 40 mesh) were added 25 ml of chloroform containing 1% ammonia water (25% NH_3), and soaked it overnight. Then it was ultrasonicated for 60 min, and filtrated extracting solution. After cleaning the residue 3 times, combine the filtrate. The solvent was recovered and the extract was concentrated to dry. The residue was dissolved with methanol transferring to a 50 mL volumetric flask and fixed volume with methanol. The solution was filtered using 0.22 μm microporous membrane discarding the primary filtrate and taking the continuous filtrate to obtain the test sample. Finally, the samples were stored in a refrigerator at 4°C.

1.3. *UHPLC-Q TOF MS analysis*

All analyses were performed on an Agilent 1290 Infinity UHPLC Coupled to an Agilent 6550 iFunnel Q-TOF LC/MS System (Agilent Technologies, Santa Clara, CA, USA). An Agilent Eclipse Plus C18 column (100×2.1 mm, 1.8 μm, Agilent Technologies, Santa Clara, CA, USA) was used for separation with gradient elution of aqueous solution (A) and acetonitrile (B). The aqueous solution (A) was added 5 mmol/L ammonium acetate, and then adjust the aqueous solution (A) pH to 8. The detailed gradient conditions are: 0–5 min, 5–12% B; 5–8 min, 12–20% B; 8–12 min, 20–30% B; 12–20 min, 30–60% B; then cleaning the column with 100% B for 5 min and finally, reconditioning the column with 5% B isocratic for 5 min. The flow rate was 0.2 mL/min and the injection volume was 1 μL. The column temperature was maintained at 25 °C.

An Agilent 6550 iFunnel Q-TOF LC/MS System was used in positive ion modes coupled with electrospray ionization source (ESI). The mass spectrometer was operating at the following parameters: dry gas (N_2) flow rate, 10 L/min; dry gas temperature, 205 °C; spray voltage, 200 V; capillary voltage, 3.5 kV. Auto MS/MS mode was used to obtain MS^2 data. The most intensive three ions from each TOF-MS scan were selected as AUTO MS/MS fragmentation precursor ions. Collision

Energy was set to 30 V. Data was acquired on the profile mode. The LC-MS/MS data was controlled and analyzed by Agilent MassHunter workstation Software B.06.00 Build 633.0 software.

2. Fragment rules of reference compounds

The ESI-MS^2 behaviours of reference standards including matrine, *N*-oxidematrine, sophoranol, 9α-hydroxymatrine, 14β-hydroxymatrine and cytisine were investigated. Matrine showed ion peaks at *m/z* 247 ($[M+H-2H]^+$), 176 ($[M+H-C_2H_7N-C_2H_4]^+$), 148 ($[M+H-C_2H_7N-C_2H_4-CO]^+$), 134 ($[M+H-C_2H_7N-C_2H_4-CO-CH_2]^+$), 120 ($[M+H-C_2H_7N-C_2H_4-CO-C_2H_4]^+$), *m/z* 150 ($[M+H-C_4H_6O-CH_3N]^+$), 136 ($[M+H-C_4H_6O-C_2H_5N]^+$), 122 ($[M+H-C_4H_6O-C_3H_7N]^+$) and the ion peaks of matrine at *m/z* 176, 150, 148 were as main ion peaks in MS^2 spectrum (Figure S2). The *N*-oxidematrine has intense ion peaks at *m/z* 247 ($[M+H-H_2O]^+$), 205 ($[M+H-H_2O-C_3H_6]^+$), 150 ($[M+H-H_2O-C_4H_6O-CHN]^+$), 148 ($[M+H-H_2O-C_2H_5N-C_2H_4-CO]^+$), 136 ($[M+H-H_2O-C_4H_6O-C_2H_3N]^+$) and other small ion peak at *m/z* 176 ($[M+H-H_2O-C_2H_5N-C_2H_4]^+$), 122 ($[M+H-H_2O-C_4H_6O-C_3H_5N]^+$) in MS^2 spectrum (Figure S3). Intense neutral loss of C_3H_6 and H_2O was characteristic for *N*-oxidematrine. Meanwhile, *N*-oxidematrine could not showed ion peaks at *m/z* 263 $[M+H-2H]^+$. Matrine-type alkaloids have hydroxyl substitution, which is at positions 5, 9, and 14. These compounds all exhibited $[M+H-2H]^+$ fragment ions in MS^2 spectra. Sophoranol exhibited intense ion peaks at *m/z* 150 ($[M+H-H_2O-C_4H_6O-CHN]^+$), 148 ($[M+H-H_2O-C_2H_5N-C_2H_4-CO]^+$), 122 ($[M+H-H_2O-C_4H_6O-C_3H_5N]^+$), 112 ($[C_6H_{10}NO]^+$) and other small ion peak at *m/z* 247 ($[M+H-H_2O]^+$), 176 ($[M+H-H_2O-C_2H_5N-C_2H_4]^+$) (Figure S4). Therefore, a peak at *m/z* 112 ($[C_6H_{10}NO]^+$) and neutral loss of H_2O was characteristic fragmentation behaviors for a hydroxyl substitution at C_5. 9α-hydroxymatrine exhibited intense ion peaks at *m/z* 247 ($[M+H-H_2O]^+$), 219 ($[M+H-H_2O-CO]^+$), 150 ($[M+H-H_2O-C_4H_6O-CHN]^+$), 148 ($[M+H-H_2O-CO-C_2H_5N-C_2H_4]^+$), and other small ion peak at *m/z* 136 ($[M+H-H_2O-C_4H_6O-C_2H_3N]^+$), 122 ($[M+H-H_2O-C_4H_6O-C_3H_5N]^+$) on so on (Figure S5). It was obvious that neutral loss of CO was characteristic fragmentation behaviors for a hydroxyl substitution at C_9. However, 14β-hydroxymatrine was observed fragmentation ions at *m/z* 150.1272 ($[M+H-C_2H_5NO-C_2H_4-CO]^+$), 152.1427 ($[M+H-C_4H_6O-CHNO]^+$), which was characteristic fragmentation behaviors for a hydroxyl substitution at C_{14} (Figure S6). Cytisine have intense ion peaks at *m/z* 148 ($[M+H-C_2H_5N]^+$), and weak ion peaks *m/z* 174 ($[M+H-H_3N]^+$), 162 ($[M+H-CH_3N]^+$), 160 ($[M+H-CH_5N]^+$), 146 ($[M+H-C_2H_7N]^+$) in MS^2 spectrum. It was indicated that these fragmentation ions come from *N* ring split (Figure S7). The possible fragmentation pathways of reference compounds were showed in Figure S8-S13.

According to reference reported (Liu et al. 2011; Wu et al. 2013), sophocarpine displayed ion peaks at *m/z* 245, 179, 176, 150, 148, 136, 134, 122, 120 which were considered as fragment ions $[M+H-2H]^+$, $[M+H-C_4H_4O]^+$, $[M+H-C_2H_5N-C_2H_4]^+$, $[M+H-C_4H_4O-CH_3N]^+$, $[M+H-C_2H_5N-C_2H_4-CO]^+$, $[M+H-C_4H_4O-C_2H_5N]^+$, $[M+H-C_2H_5N-C_2H_4-CO-CH_2]^+$, $[M+H-C_4H_4O-C_3H_7N]^+$, $[M+H-C_2H_5N-C_2H_4-CO-C_2H_4]^+$ respectively. The peaks of *m/z* 176, 148, 122 were puniness, but *m/z* 179, 150, 136, 122 were intense. Therefore, the main fragmentation behaviors were the undergoes RDA reaction and then neutral losses of C_2H_5N and CH_3N for sophocarpine.

3. Characterization of compounds

Compounds **12**, **21**, **26**, **29**, and **31** was designated as *N*-oxidematrine, sophoranol, 9α-hydroxymatrine, 14β-hydroxymatrine, and matrine by comparing with reference compound. Compound **7** gave the molecular as $C_{17}H_{26}N_2O_3$ and showed main fragment ion peaks at m/z 247

($[M+H-C_2H_4O_2]^+$), 176, 150, and 148. These fragment ions were similar with matrine-type alkaloid except $[M+H-C_2H_4O_2]^+$ fragment ion. Combining with reporting alkaloids, compound **7** was proposed as 14β-acetomatrine. Compound **30** gave the molecular as $C_{16}H_{23}N_3O$ and fragment ions were similar with 9α-hydroxymatrine except $[M+H-CHN]^+$ fragment ion, but not $[M+H-H_2O]^+$ fragment ion. Compound **30** was proposed as 9α-cyanomatrine. Compound **28** was determined as sophoridine on base of reference reported data (Wu et al. 2013).

Compounds **5**, **9**, **14**, **18**, and **24** all gave the protonated molecular ion at *m/z* 281.1860. Their formulae more O_2 than matrine. Compounds **5**, **9**, and **14** did not exhibited $[M+H-2H]^+$ fragment ions, meanwhile had high abundance $[M+H-H_2O]^+$ fragment ions in their MS^2 spectra. Therefore, these compounds were *N*-oxide alkaloid. Compound **5** exhibited base peak of fragment ion at *m/z* 245 ($[M+H-2H_2O]^+$) and characteristic $[C_6H_{10}NO]^+$ fragment ion in MS^2 spectrum. Therefore, compound **5** was characterized as *N*-oxidesophoranol. Neutral losses of CO were observed such as fragment ions at *m/z* 193.1663 ($[M+H-H_2O-C_3H_6-CO]^+$), 235.1737 ($[M+H-H_2O-CO]^+$) compare compound **9** with *N*-oxidematrine. Therefore, compound **9** was characterized as 9α-hydroxy-*N*-oxidematrine. Compound **14** exhibited peaks at *m/z* 152.1425, 150.1271 which were characteristic fragmentation behaviors for a hydroxyl substitution at C_{14}. Therefore, compound **14** was characterized as 14β-hydroxy-*N*-oxidematrine. The compound **18, 24** exhibited $[M+H-2H]^+$ and $[M+H-H_2O]^+$ fragment ions in their MS^2 spectra. Therefore, these compounds were dihydroxymatrine-type alkaloid. Neutral losse of CO was observed such as fragment ions at *m/z* 217.1683 ($[M+H-H_2O-CO]^+$) in MS^2 spectrum of compound **18**. It was indicated that compound **18** was present a hydroxyl substitution at C_9. Meanwhile, compound **18** exhibited characteristic $[C_6H_{10}NO]^+$ fragment ion. Thus, another hydroxyl substitution of compound **18** was at C_5. Therefore, compound **18** was characterized as 5α,9α-dihydroxymatrine. Fragmentation behaviour of compound **24** was similar with 14β-hydroxymatrine except the peaks of fragment ion at m/z 263 ($[M+H-H_2O]^+$), 235 ($[M+H-H_2O-CO]^+$) were observed. Therefore, compound **24** was characterized as 9α,14β-dihydroxymatrine.

The formulae of compound **32** was suggested as $C_{15}H_{22}N_2O$ on the basis of the protonated molecular ion. It also given main ion peaks at *m/z* 247.1809 ($[M+H]^+$), 245.1644 ($[M+H-2H]^+$), 179.1537 ($[M+H-C_4H_4O]^+$), 150 ($[M+H-C_4H_4O-CH_3N]^+$), 136 ($[M+H-C_4H_4O-C_2H_5N]^+$) in MS^2 spectrum. It was agreement with reference reported data of sophocarpine (Wu et al. 2013). The compounds **13**, **20**, **25** were suggested for formulae as $C_{15}H_{22}N_2O_2$. The compound **13** exhibited base ion peak at *m/z* 245 ($[M+H-H_2O]^+$). Thus, it is proposed that the *N* of this compound links to the O atom. Other ion peaks were at *m/z* 203 ($[M+H-H_2O-C_3H_6]^+$), 177 ($[M+H-H_2O-C_4H_4O]^+$), 150 ($[M+H-H_2O-C_4H_4O-CH_3N]^+$), 136, 177 ($[M+H-H_2O-C_4H_4O-C_2H_5N]^+$). The compound **13** was determined as *N*-oxidesophocarpine. The compound **20** exhibited intense ion peak at *m/z* 245 ($[M+H-H_2O]^+$) and other ion peak at *m/z* 195 ($[M+H-C_4H_4O]^+$), 177 ($[M+H-H_2O-C_4H_4O]^+$), 150 ($[M+H-H_2O-C_4H_4O-CH_3N]^+$), 112 ($[C_6H_{10}NO]^+$). The compound **20** was considered as 5α-hydroxysophocarpine. The compound **25** exhibited puniness ion peak at *m/z* 245 ($[M+H-H_2O]^+$), 235 ($[M+H-CO]^+$), 164 ($[M+H-CO-C_2H_5N-C_2H_4]^+$) and base ion peak at *m/z* 148 ($[M+H-H_2O-C_4H_4O-CH_3N]^+$). The compound **25** was speculated as 9α-hydroxysophocarpine. Compound **3** showed a tense peak of losing H_2O from the molecular ion. Compound **3** was suggested as 9α-hydroxy-*N*-oxidesophocarpine on base of comparison fragment ions and formula with compound **25**.

Compound **11** was identified as cytisine by comparing with reference standard. Compound **8** was considered as *N*-formylcytisine on account of it could produce one more $[M+H-CO]^+$ fragment ion compares with cytisine and molecular formulas. For the same, compound **10**, **27** produced one more $[M+H-C_6H_{10}NO]^+$, $[M+H-C_2H_2O]^+$ fragment ion respectively. Therefore, they were identified as *N*-acetylcytisine, *N*-hexanoylcytisine. Combining with reported alkaloids, compound **15** have similar fragment ion with cytisine except have more $[M+H-C_6H_{10}O_5]^+$, $[M+H-H_2O]^+$, $[M+H-2H_2O]^+$, $[M+H-3H_2O]^+$ fragment ion. Thus, it was characterized as *N*-gulcytisine. Compound **19** gave molecular formulas as $C_{12}H_{16}N_2O$. The formulae of compound **19** were more CH_3 than cytisine, and it exhibited more $[M+H-C_2H_5N]^+$, $[M+H-C_3H_9N]^+$ fragment ion compares with cytisine. Compound **19** determined as *N*-methylcytisine.

Reference

Liu G, Dong J, Wang H, Hashi Y, Chen S. 2011. Characterization of alkaloids in *Sophora flavescens* Ait. by high-performance liquid chromatography–electrospray ionization tandem mass spectrometry. J Pharm Biomed Anal. 54(5):1065-1072.

Wu ZJ, Sun DM, Fang DM, Chen JZ, Cheng P, Zhang GL. 2013. Analysis of matrine-type alkaloids using ESI-QTOF. Int J Mass Spectrom. 341:28-33.

Legends for figures and table:

Figure S1. The reported alkaloids from *S. tonkinensis* root ……P8

Figure S2. The MS^2 spectrum of matrine ……P9

Figure S3. The MS^2 spectrum of *N*-oxidematrine ……P9

Figure S4. The MS^2 spectrum of sophoranol ……P10

Figure S5. The MS^2 spectrum of 9α-hydroxymatrine ……P10

Figure S6. The MS^2 spectrum of 14β-hydroxymatrine ……P11

Figure S7. The MS^2 spectrum of cytisine ……P11

Figure S8. Possible fragmentation pathway of matrine ……P12

Figure S9. Possible fragmentation pathway of *N*-oxidematrine ……P13

Figure S10. Possible fragmentation pathway of sophoranol ……P14

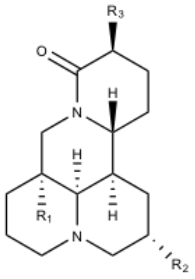
Figure S11. Possible fragmentation pathway of 9α-hydroxymatrine ……P15

Figure S12. Possible fragmentation pathway of 14α-hydroxymatrine ……P16

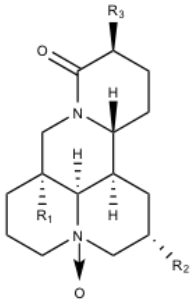
Figure S13. Possible fragmentation pathway of cytisine ……P17

Figure S14. The total ion chromatogram in positive mode for Radix Sophora Tonkinensis: (red) Guozhou, Sichuan province; (black) Yizhou, Guangxi province ……P18

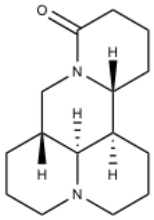
Table S1. Accurate *m/z*, fragment ions of analytes using UHPLC-QTOF-MS from *S. tonkinensis* root …… P19



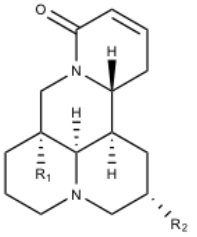
matine R_1=H, R_2=H, R_3=H
14β-hydroxymatrine R_1=H, R_2=H, R_3=OH
5α-hydroxymatrine R_1=OH, R_2=H, R_3=H
9α-hydroxymatrine P_1=H, P_2=OH, P_3=H
14β-acetomatrine R_1=H, R_2=H, R_3=$OCOCH_3$



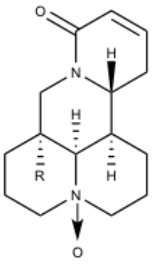
N-oxidematine R_1=H, R_2=H, R_3=H
14β-hydroxy-*N*-oxidematine R_1=H, R_2=H, R_3=OH
5α-hydroxy-*N*-oxidematine R_1=OH, R_2=H, R_3=H



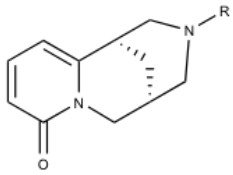
sophoridine



sophocarpine R_1=H, R_2=H
5α-hydroxysophocarpine R_1=OH, R_2=H



N-oxidesophocarpine R=H
5α-hydroxy-*N*-oxidesophocarpine R=OH



cytisine R=H
N-methylcytisine R=CH_3
N-formylcytisine R=CHO
N-hexanoylcytisine R=$C_6H_{11}O$

Figure S1. The reported alkaloids from *S. tonkinensis* root

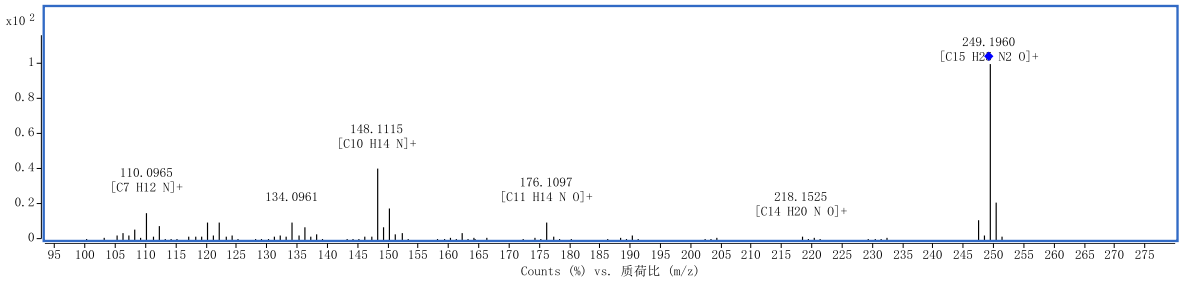


Figure S2. The MS^2 spectrum of matrine

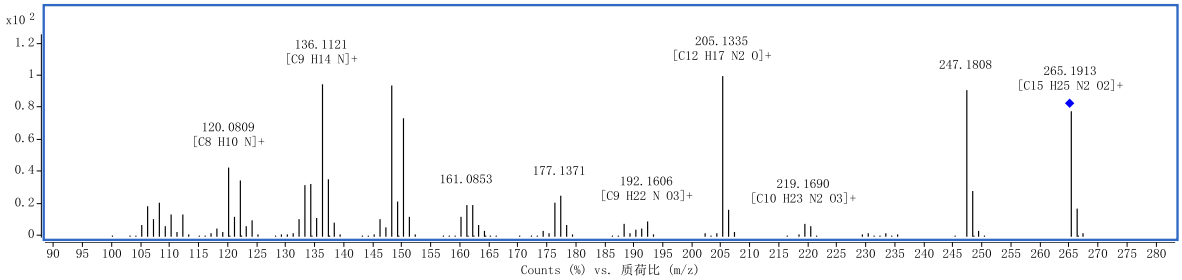


Figure S3. The MS^2 spectrum of *N*-oxidematrine

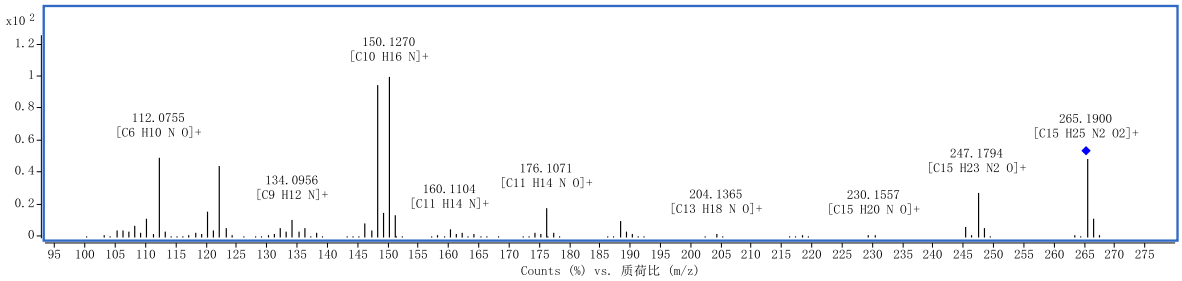


Figure S4. The MS^2 spectrum of sophoranol

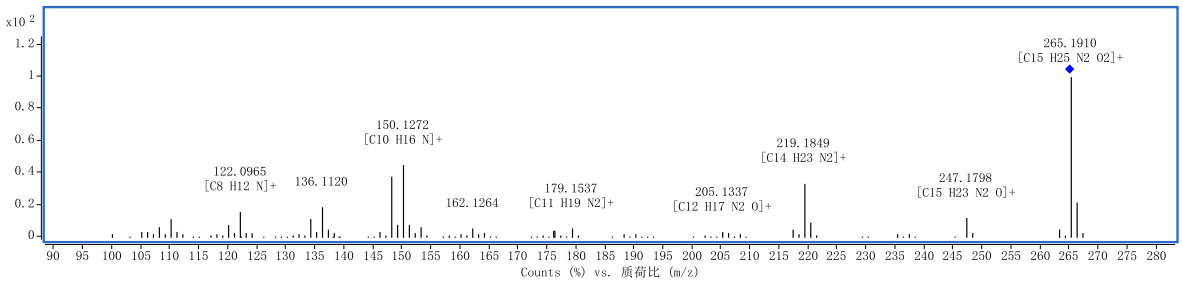


Figure S5. The MS^2 spectrum of 9α-hydroxymatrine

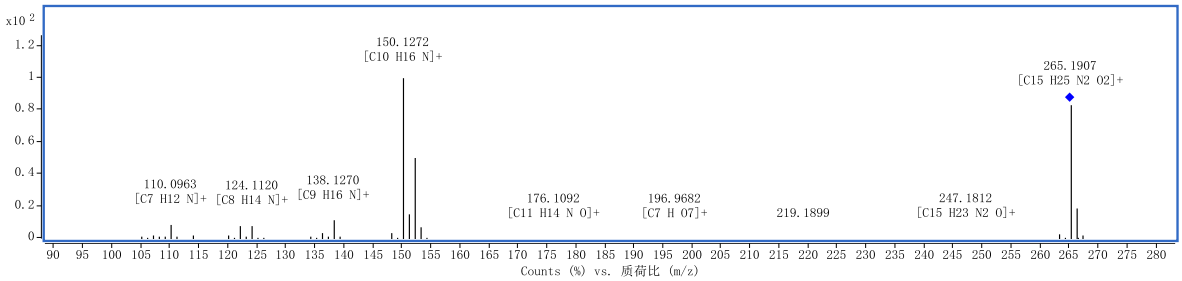


Figure S6. The MS^2 spectrum of 14β-hydroxymatrine

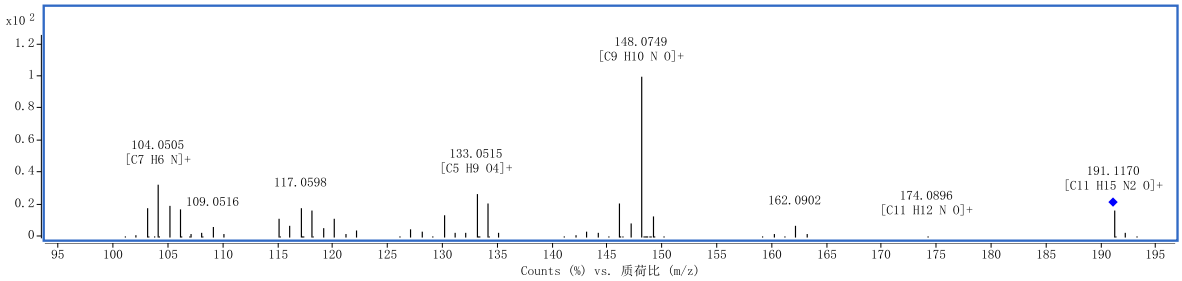


Figure S7. The MS^2 spectrum of cytisine

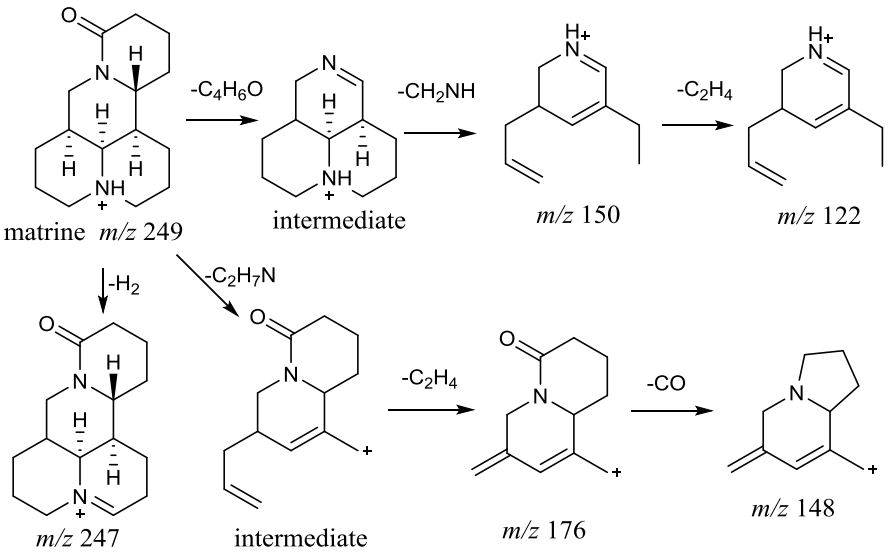


Figure S8. Possible fragmentation pathway of matrine

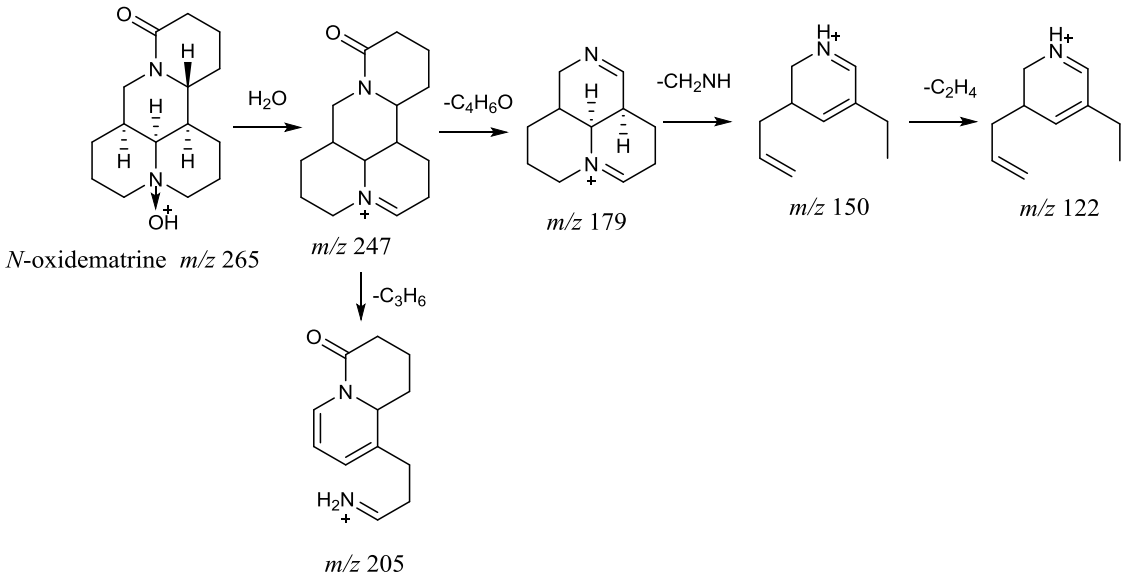


Figure S9. Possible fragmentation pathway of *N*-oxidematrine

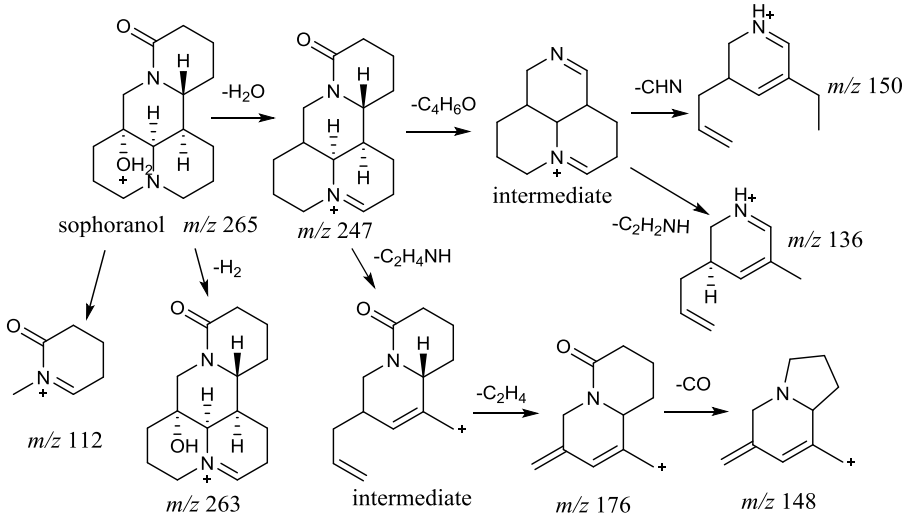


Figure S10. Possible fragmentation pathway of sophoranol

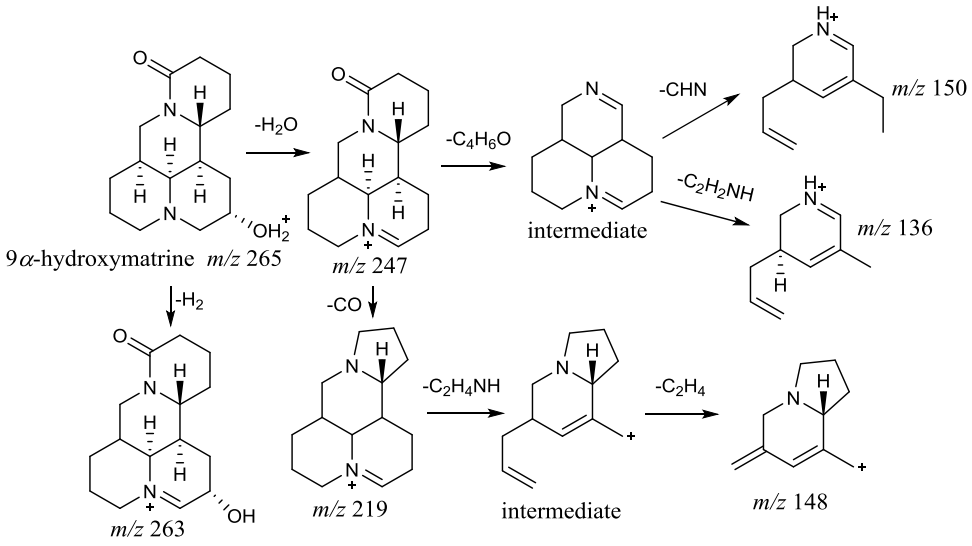


Figure S11. Possible fragmentation pathway of 9α-hydroxymatrine

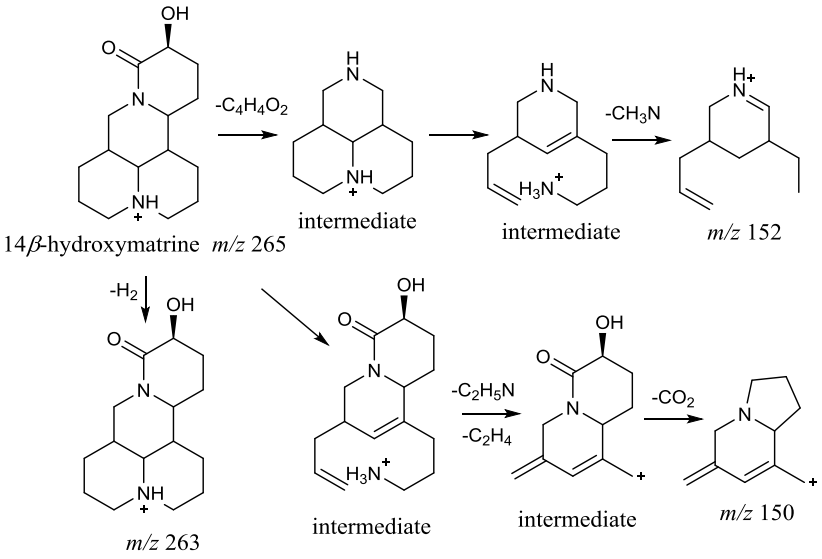


Figure S12. Possible fragmentation pathway of 14α-hydroxymatrine

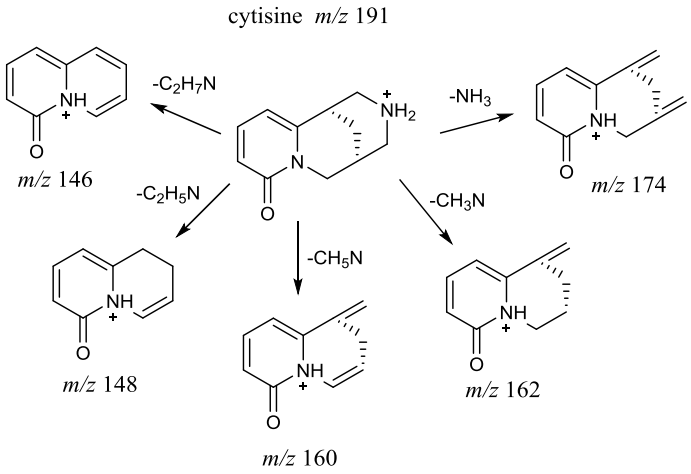


Figure S13. Possible fragmentation pathway of cytisine

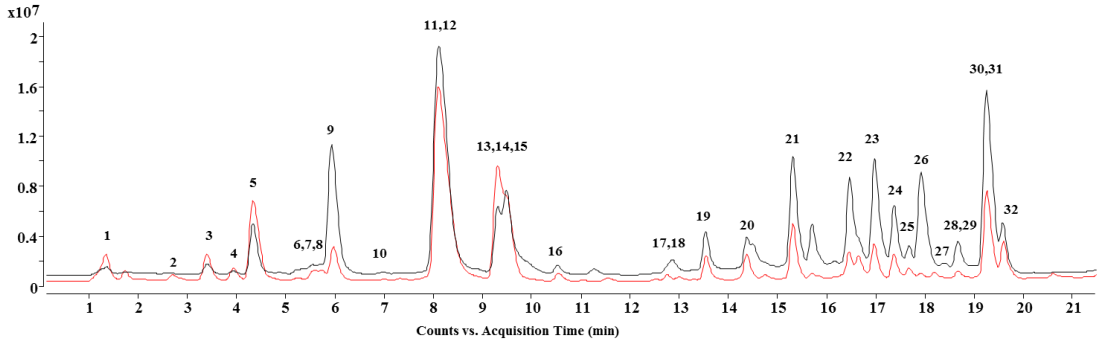


Figure S14. The total ion chromatogram in positive mode for Radix Sophora Tonkinensis: (red) Guozhou, Sichuan province; (black) Yizhou, Guangxi province

Table S1. Accurate *m/z*, fragment ions of analytes using UHPLC-QTOF-MS from *S. tonkinensis* root

Compound No	RT (min)	Formula	*m/z* ([M+H]$^+$)		Error (ppm)	Fragment ion	Identification
			Experimental	Theoretical			
1	1.38	$C_{15}H_{26}N_2O_4$	299.1973	299.1965	-2.42	281.1870,263.1793,253.1550,240.1458,239.1410,223.1775,168.1386,150.1274,138.1285,122.0969,110.0970	unidentifed
2	3.09	$C_{15}H_{27}N_3O_3$	298.2124	298.2125	1.03	280.2048,223.1812,205.1276,168.1385,150.1284,138.1274,112.0944	unidentified
3	3.42	$C_{15}H_{22}N_2O_3$	279.1704	279.1703	-0.35	261.1603,243.1493,215.1177,175.1228,110.0605	9α-hydroxy-*N*-oxidesophocarpine
4	3.92	$C_{15}H_{24}N_2O_3$	281.1862	281.1860	0.08	263.1755,235.1794,221.1280,193.1663,177.1386,150.1258,136.1123,122.0974	unidentified
5	4.27	$C_{15}H_{24}N_2O_3$	281.1865	281.1860	-1.40	263.1747,245.1650,247.1780,235.1756,218.1436,204.1288,180.1011,112.0755	*N*-oxidesophoranol
6	5.21	$C_{15}H_{24}N_2O_3$	281.1858	281.1860	0.6	263.1763,261.1548,245.1605,243.1454,204.1356,188.1075,166.1210,164.1052,148.1099,1120765	unidentifed
7	5.35	$C_{17}H_{26}N_2O_3$	307.2022	307.2016	-1.45	247.1788,176.1056,150.1269,148.1109,112.0746	14β-acetomatrine
8	5.62	$C_{12}H_{14}N_2O_2$	219.1130	219.1128	-0.51	160.0746,148.0743,146.0599,117.0571	*N*-formylcytisine
9	5.92	$C_{15}H_{24}N_2O_3$	281.1862	281.1860	-0.72	263.1755,235.1737,221.1280,193.1639,150.1258,148.1118,136.1121	9α-hydroxy-*N*-oxidematrine
10	6.96	$C_{13}H_{16}N_2O_2$	233.1282	233.1284	1.1	191.1156,160.0712,148.0755,133.0532,117.0590	*N*-acetylcytisine
11	7.99	$C_{11}H_{14}N_2O$	191.1182	191.1179	-2.1	162.0931,148.0755,146.0603,133.0518	cytisine
12	8.09	$C_{15}H_{24}N_2O_2$	265.1920	265.1911	-0.6	247.1795,205.1325,150.1258,148.1107,136.1110	*N*-oxidematrine
13	9.28	$C_{15}H_{22}N_2O_2$	263.1761	263.1754	-2.11	245.1639,203.1171,177.1378,160.0751,150.1264,136.1112,122.0947	*N*-oxidesophocarpine
14	9.48	$C_{15}H_{24}N_2O_3$	281.1862	281.1860	-0.36	263.1752,235.1469,222.1360,152.1425,150.1271,138.1271,124.1111,110.0966	14β-hydroxy-*N*-oxidematrine
15	9.96	$C_{17}H_{24}N_2O_6$	353.1707	253.1707	0.98	335.1611,317.1484,299.1388,256.0976,203.1178,191.1172,170.0810,148.0748	*N*-gulcytisine
16	10.51	$C_{12}H_{20}N_2O_2$	225.1604	225.1598	-0.50	166.1222,148.1109,136.1114,120.0799,110.0957	unidentified
17	12.72	$C_{15}H_{24}N_2O_2$	265.1916	265.1911	-1.32	263.1761,245.1669,205.1368,188.1105,176.1067, 166.1212,164.1069	unidentified
18	12.85	$C_{15}H_{24}N_2O_3$	281.1862	281.1860	-0.36	263.1742,245.1643,235.1805,217.1683,150.1267,148.1111,122.0959,100.0753	5α,9α-dihydroxymatrine
19	13.55	$C_{12}H_{16}N_2O$	205.1341	205.1335	-2.41	162.0911,146.0604,133.0516,117.0557,104.0473	*N*-methylcytisine
20	14.36	$C_{15}H_{22}N_2O_2$	263.1750	263.1754	0.95	245.1647,195.1486,177.1377,150.1271,134.0954,122.0959,112.0754	5α-hydroxysophocarpine
21	15.30	$C_{15}H_{24}N_2O_2$	265.1914	265.1911	-1.03	247.1797,245.1643,188.1437,176.1068,150.1264,148.1109,122.0955,112.0750	sophoranol
22	16.15	$C_{15}H_{24}N_2O_2$	265.1914	265.1911	-0.34	247.1795,179.1532,148.1108,136.1111	unidentified
23	16.85	$C_{16}H_{23}N_3O_2$	290.1862	290.1862	0.36	243.1461,217.0956,189.0996,174.0896,172.0724,160.0747,146.0604,100.0757	unidentified
24	17.20	$C_{15}H_{24}N_2O_3$	281.1859	281.1860	0.25	263.1767,235.1805,179.1509,152.1422,150.1276,138.1292	9α,14β-dihydroxymatrine
25	17.67	$C_{15}H_{22}N_2O_2$	263.1747	263.1754	2.69	235.1811,217.1670,164.1053,148.1117,146.0942	9α-hydroxysophocarpine
26	17.90	$C_{15}H_{24}N_2O_2$	265.1911	265.1911	0.16	263.1757,247.1804,219.1851,150.1276,148.1119	9α-hydroxymatrine
27	18.33	$C_{17}H_{24}N_2O_2$	289.1914	289.1911	-0.81	191.1175,160.0759,148.0756,120.0807	*N*-hexanoylcytisine
28	18.44	$C_{15}H_{24}N_2O$	249.1959	249.1961	1.28	220.1700,196.9642,176.1063,152.1422,120.0821	sophoridine

29	18.63	$C_{15}H_{24}N_2O_2$	265.1914	265.1911	-0.9	263.1738,152.1427,150.1272,138.1270,124.1120,122.0964,110.0963	14β-hydroxymatrine
30	19.05	$C_{16}H_{233}O$	274.1913	274.1914	0.68	247.1800,177.1369,150.1279,122.0958	9α-cyanomatrine
31	19.25	$C_{15}H_{24}N_2O$	249.1966	249.1961	-1.54	247.1810,190.1229,176.1102,150.1263,148.1110	matrine
32	19.58	$C_{15}H_{22}N_2O$	247.1809	247.1805	-1.31	245.1650,245.1796,179.1536,176.1078,150.1264,148.1113,136.1115,108.0799	sophocarpine