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Comprehensive separation and analysis of alkaloids from *Stephania yunnanensis* by counter-current chromatography coupled with liquid chromatography tandem mass spectrometry analysis

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ABSTRACT

The polar compounds such as alkaloid compounds are important bioactive components in traditional Chinese medicines. In present study, a comprehensive method for separation and analysis of polar compounds from the polar fraction of traditional Chinese medicine *Stephania yunnanensis* was established. Both the major components and minor components were analyzed by counter-current chromatography combined with liquid chromatography tandem mass spectrometry (LC–MS^n). From 50 mg polar fraction of crude extract, 15.2 mg corydine and 4.8 mg stepharine with purities over 90% were successfully separated via a polar solvent system *n*-butanol: methanol: water (4:1:5, v/v) with 10 mM NaOH as an additive in the lower phase, in one step operation. Their structures were further identified by ^{1}H NMR and FTICR–MS. Besides, three minor components were identified by HPLC–MS^n based on the fragmentation behavior of the purified compounds.

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1. Introduction

The genus *Stephania*, a member of the Menispermaceae family, is an important part of traditional Chinese medicines. There are about 39 species of genus *Stephania* grown in China. The plants of genus *Stephania* have been used in folk medicine for the treatment of various ailments such as tuberculosis, asthma, hyperglycemia, dysentery, malaria and fever for a long time [1]. *Stephania yunnanensis* is one species of genus *Stephania* and widely distributed in Yunnan Province, China. The root of this plant contains several kinds of alkaloids, like aporphine, protoberberine and morphine alkaloids [1–3]. Previous studies have shown that corydine, stepharine, *N*-methylcorydalmine and corydalmine [2–6] existed in this genus have antiprotazoal, antihypertensive, acetylcholinesterase inhibitory, cholinesterase and pseudocholinesterase inhibitory activities [3,7–10]. Therefore, it is of great importance to develop an effective method for separation and analysis of these polar compounds. While traditional separation methods are tedious and complicated, a more efficient method is demanded.

Counter-current chromatography (CCC) is an efficient method for separation of complex samples. As a continuous liquid–liquid partition chromatography, CCC eliminates the complications resulting from the solid support matrix, such as irreversible adsorptive sample loss and deactivation, tailing of solute peaks, and contamination. Since CCC was developed by Ito in 1970s [11], it has been chosen for separating many active components, especially in separations of natural products [12–16]. The key step of CCC separation is the selection of optimum solvent system. Solvent systems which established for the CCC usually have an organic phase and an aqueous phase. The polarities of the two phases are quite different. This difference makes it difficult to separate polar compounds. Currently, several organic acids and bases have been separated by polar solvent systems via CCC [17–20], but the researches for polar solvent systems were not enough. Therefore, to establish a polar solvent system for polar compounds is of great importance to the development of CCC.

Some analytes are hard to be separated because they are thermally labile, highly polar or present in a trace amount in crude samples. These difficulties can be overcome by using high-performance liquid chromatography and tandem mass spectrometry (HPLC–MS^n) because of its superior sensitivity and selectivity. Consequently, HPLC–MS^n has been widely utilized in pharmaceutical research [21–23].

In this study, the polar fraction from the crude sample of *S. yunnanensis* was chosen as our research object. A biphasic solvent system with NaOH as additive was developed as the polar separation environment for the alkaloid compounds. Two alkaloids, corydine and stepharine were successfully separated by counter-current chromatography. FTICR–MS^2 and HPLC–MS^n experiments

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were introduced to analyze the polar fraction. Three other alkaloids were given promising structures by the analysis of the fragmentation. By this method, both the major polar components and minor polar components from the plant extract were analyzed.

2. Experimental

2.1. Reagents and materials

Methanol, *n*-butanol and NaOH used for counter-current chromatography were of analytical grade and purchased from Huadong Chemicals, Hangzhou, China. Reverse osmosis Milli-Q water (18 MΩ) (Millipore, Bedford, MA, USA) was used for all solutions and dilutions. Acetonitrile used for HPLC analysis was of chromatographic grade and purchased from Merck, Darmstadt, Germany. The dried roots of *S. yunnanensis* were purchased from a drugstore in Hangzhou.

2.2. Apparatus

2.2.1. Counter-current chromatography

The separations were performed on a semi-preparative apparatus (Ito scheme IV) with one 140 mL coil and a counterweight. This instrument was manufactured by the Zhejiang University machine shop (Hangzhou, China). The multilayer coil was prepared by winding a 26.4 m × 2.6 mm i.d. PTFE tube. The β-value varied from 0.33 at the internal terminal to 0.60 at the external terminal ($\beta = r/R$ where r is the distance from the coil to the holder shaft, and R is the revolution radius or the distance between the holder axis and central axis of the centrifuge). The revolution speed of the apparatus can be regulated with a speed controller in the range of 0 and 1000 rpm and the sample injection was accomplished by an injection valve with a 10 mL sample loop, producing about 100 G gravitational field at most. Furthermore, a Model 2W-2B constant-flow pump (Beijing Xingda Equipment, Beijing, China) was used to fill the CCC apparatus with the stationary phase and to elute the mobile phase. The effluent was continuously monitored by an HD-9704 UV spectrometer (Jingke Equipment, Shanghai, China) operating at 254 nm. Eluent was collected by a BSZ-100 fraction collector and an N2000 data analysis system (Institute of Automation Engineering, Zhejiang University, Hangzhou, China) was used to record the CCC chromatogram. A PHS-3B pH Meter (Shanghai Precision & Scientific instrument, Shanghai, China) was used for pH measurement.

2.2.2. HPLC analysis

An Agilent 1100 analytical HPLC system with a G1312 Binpump, G1314A variable-wavelength detector (VWD), model 7725 injector fitted with a 20 μL sample loop, along with an Agilent ChemStation data system, was used. A Hypersil reverse phase C18 column, (250 mm × 4.6 mm i.d., 5 μm, Yilite) was used for separation; the column was maintained at room temperature.

2.2.3. Mass spectrometry

HPLC/ESI–MS^n analyses were performed using the Agilent HPLC system described above combined with a Bruker Esquire 3000 plus ion trap mass spectrometer (Bruker-Franzen Analytik GmbH, Bremen, Germany) equipped with electrospray ionization (ESI) source. Instrument control and data acquisition were performed using Esquire 5.0 software. The ion source temperature was 250 °C, and needle voltage was always set at −4.0 kV. Nitrogen was used as the drying and nebulizer gases at a flow rate of 10 L/min and a backpressure of 30 psi. Helium was introduced into the trap with an estimated pressure 6×10^{-6} mbar to improve trapping efficiency and to act as the collision gas for the MS^n experiment; the mass spectrometer was optimized in the collision energy range of 0.5–1.0 V to maximize the ion current in the spectra.

The offline FTICR–MS experiments were performed using an Apex III Fourier transform ion cyclotron resonance mass spectrometer with 7.0 T actively shielded superconducting magnet (Bruker Daltonics, Billerica, MA, USA) combined with an Apollo electrospray ionization source operated in the positive ion mode. The solutions were infused at a rate of 3.0 μL/min by using a Cole–Parmer syringe pump. Accurate mass measurements were performed using NaI as an external calibration compound. Each spectrum was an average of eight transients, each composed of 512 K points, acquired using a workstation operating XMASS version 6.1.1.

2.3. Preparation of crude extract

The roots of *S. yunnanensis* were dried to constant mass at 55 °C in a vacuum oven and then pulverized. 1 kg of *S. yunnanensis* powder was extracted by 5 L of 95% ethanol for 2 h under reflux. The procedure was repeated for three times. The combined 15 L ethanol solution was concentrated to dryness under reduced pressure at 40 °C producing about 95 g of ethanol extract, of which 5 g ethanol extract was separated by silica gel column chromatography. After the column chromatography procedure, the different ratio of the elution was collected separately and concentrated by rotary evaporator. And then the fractions were air-dried in vacuum oven, 0.5 g polar fraction compounds (elute solvent ratio: MeOH: EA 1:1) was obtained and stored in a refrigerator (4 °C) for CCC separation and HPLC/ESI–MS^n analysis.

2.4. Preparation of the two-phase solvent systems and sample solution

The two-phase solvent system used for CCC separation was thoroughly equilibrated in a separatory funnel at room temperature and the two phases were separated. NaOH was added to the lower phase at 10 mM as an eluter. The sample solution was prepared by dissolving 50 mg fraction sample in 1.5 mL of the upper phase and 1.5 mL of the lower phase without NaOH.

2.5. Determination of solute partition coefficient

The partition coefficient (K) is the solute physicochemical parameter responsible for retention in CCC. The measurement of K values was performed as follows: a small amount of crude sample was added into a test tube to which about 2 mL of each phase of the pre-equilibrated two-phase solvent system were added. After shaking vigorously for 10 min, the mixture was separated by centrifugation at 3000 rpm for 3 min. Then, an aliquot of each phase (100 mL) was introduced into two test tubes. Each tube was diluted with an equal volume (1 mL) of methanol and analyzed by HPLC. The K value was expressed as the ratio of the peak area of a given compound in the upper phase divided by that in the lower phase.

2.6. Separation procedure

For each separation, the CCC column was first entirely filled with the upper organic phase, then the aqueous mobile phase was pumped through the column at a flow rate of 1.5 mL/min in the head to tail direction (reversed mode), while the column was rotated at 600 rpm. When the system was equilibrium, the crude sample which dissolved in equal volume of upper phase and lower phase was injected through the sample port. The effluent was monitored continuously at 254 nm and automatically collected in test tube per 5 min using a BSZ-100 fraction collector. After the separation was

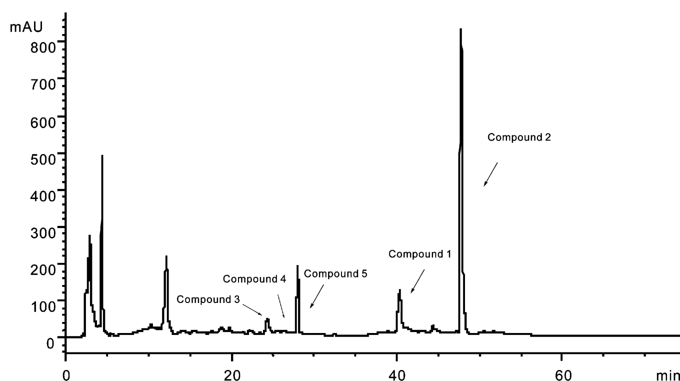

Fig. 1. HPLC chromatogram of the polar fraction obtained from *S. yunnanensis*. Solvent A: acetonitrile; solvent B: 0.1% triethylamine (TEA). The percentage of solvent A was changed linearly as follows: 0 min 5% A; 10 min 12% A; 20 min 18% A; 30 min 18% A; 35 min 25% A; 40 min 40% A; 45 min 55% A; 50 min 95% A. The flow rate was 0.8 mL/min and was monitored at 280 nm.

completed, retention of the stationary phase was measured by collecting the column contents into a graduated cylinder by forcing them out of the column with pressurized nitrogen gas. Peak fractions were analyzed by HPLC. The retention of stationary phase was 10%.

2.7. HPLC analysis and identification of CCC peak fractions

HPLC analyses of the crude sample and CCC peak fractions were carried out in gradient mode with acetonitrile (solvent A) and 0.1% triethylamine (TEA) (solvent B). The percentage of solvent A was changed linearly as follows: 0 min 5% A; 10 min 12% A; 20 min 18% A; 30 min 18% A; 35 min 25% A; 40 min 40% A; 45 min 55% A; 50 min 95% A. The flow rate was 0.8 mL/min and the effluent was monitored at 280 nm.

Identification of the CCC peak fraction was performed by ESI–MS and ^{1}H NMR. ESI-MS analyses were performed using Bruker Esquire 3000 plus mass spectrometer with an electrospray ionization (ESI) interface in the positive mode. NMR experiments were carried out using a Bruker Avance DMX 500 NMR spectroscopy with chloroform ($CDCl_3$) as solvent and TMS as internal standard.

3. Results and discussion

3.1. CCC separation

The polar fraction obtained from *S. yunnanensis* was first analyzed by HPLC. The chromatogram is shown in Fig. 1, two major compounds, compound **1** and compound **2** are the target compounds in the CCC separation.

The selection of the biphasic solvent system is of great importance for CCC separation. The partition coefficient (K) values of two target compounds were determined for evaluating the separating capability of the biphasic solvent system (Table 1). Because of the high polarity of the target compounds, the relative polar solvent system *n*-hexane: ethyl acetate: methanol: water (1:6:1:6, v/v) was selected first. But the K values were too small. Two target compounds were mainly distributed in aqueous phase, so they would be eluted quickly with the impurities. Then a more polar solvent system *n*-butanol:ethyl acetate:water (4:1:5, v/v) was selected, the K values were still not large enough to separate the two target compounds. In order to increase the solubility of target compounds in the upper phase, ethyl acetate was replaced by methanol because of the larger polarity of methanol compared with that of ethyl acetate. The K values and α value ($\alpha = K_2/K_1$) of this solvent system matched the requirements of normal solvent systems. However, the settling time of this solvent system is much longer than the two solvent systems. So the retention of stationary phase of the solvent system *n*-butanol:methanol:water (4:1:5, v/v) was low, only 10%, the low retention and the peak broadening resulted in a bad peak resolution, the two target compounds could not be successfully separated. According to the previous research [24], for a similar solvent system composed of 1-butanol/acetic acid/water (4:1:5, v/v), using the lower mobile phase eluted from the tail toward the head can improve the retention of the stationary phase. Thus the experiment was repeated by using the lower mobile phase eluted from the tail toward the head. The retention of the stationary phase was 40% at first, however, the stationary phase would lose in the separation procedure. When the separation was finished, the liquid in the column was pushed out by the nitrogen. The volume of the rest upper phase was measured to calculate the retention of the stationary phase. The value was still about 10%, showed that the retention of the stationary phase did not get a significant improvement. So the K values needed to be larger to fit the requirement of this separation. Adding NaOH to the solvent system can deprotonate the alkaloid molecules, the compounds become hydrophobic and distribute more in the upper hydrophobic phase [25].

Table 1
The K value of the solvent systems.

	K_1	K_2
n-hexane:ethyl acetate:methanol:water (1:6:1:6, v/v)	≈0	≈0
n-butanol:ethyl acetate:water (4:1:5, v/v)	0.47	0.75
n-butanol:methanol:water (4:1:5, v/v)	0.95	1.86
n-butanol:methanol:water (4:1:5, v/v, lower phase added NaOH 10 mM)	3.03	11.7

Therefore, NaOH was added into the lower phase as an additive to enlarge the K values. The new solvent system was attempted, the two K values became larger ($K_1 = 3.03$, $K_2 = 11.7$), so did the α value ($\alpha = 3.86$). The solvent system *n*-butanol:methanol:water (4:1:5, v/v) with 10 mM NaOH in the lower phase was chosen as the solvent system for the separation.

Fig. 2 shows the preparative CCC separation of 50 mg polar fraction sample using the solvent system composed of *n*-butanol:methanol:water (4:1:5, v/v), with10 mM NaOH as additive in lower phase. Each CCC fraction was analyzed by HPLC. The chromatograms of two pure fractions are also shown in Fig. 2. Fractions were concentrated dryness under reduced pressure at 40 °C. NaOH was removed by extraction with chloroform and water. As a result, 4.8 mg compounds 1 and 15.2 mg compound **2** were obtained from area I and area II, respectively, with purities more than 90%, in a single step separation.

3.2. Identification

The structure identification of CCC fractions in Fig. 2 was carried out by positive FTICR–MS, ^{1}H NMR.

3.2.1. Compound *1*

^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.04 (1H, dd), 6.90(1 H, dd), 6.65 (1H, s), 6.42 (1H, dd), 6.30 (1H, dd), 4.30 (1H, dd), 3.79 and 3.61 (6H, 2s), 3.46 (1H, m), 3.17 (1H, m), 2.78 (2H, m), 2.41 (1H, m), 2.21 (1H, m); positive FTICR–MS m/z: 298.1438 $[M+H]^+$, refer to previous literature, compound **1** is stepharine [26].

3.2.2. Compound *2*

^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 8.79 (1H, s), 7.11 (1H, d), 6.90 (1H, d), 6.70 (1H, s), 3.90 (6H, s), 3.71 (3H, s), 2.90 (3H, s); positive

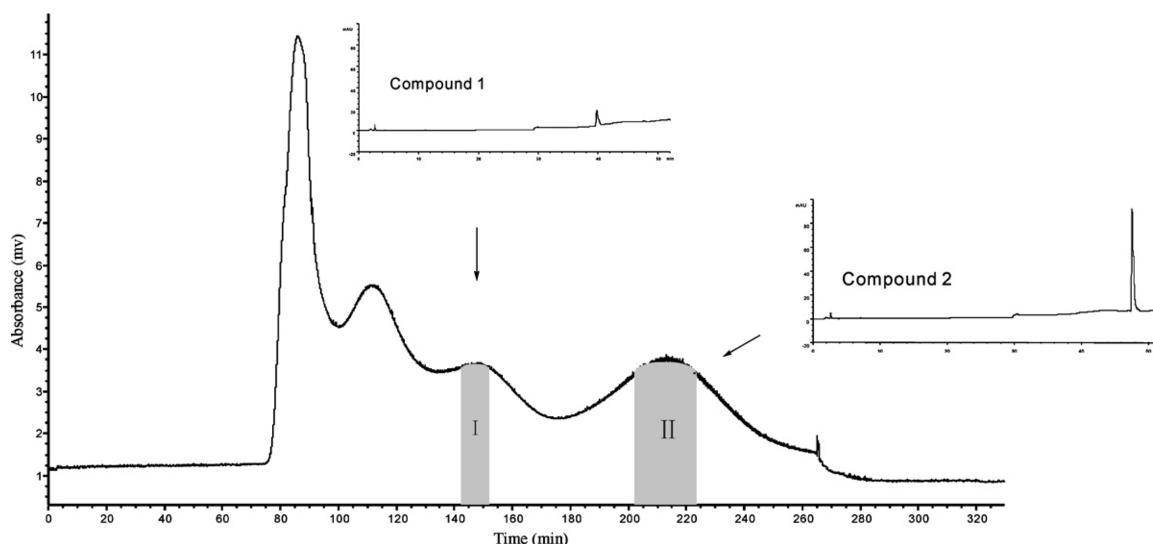

Fig. 2. CCC chromatogram of the typical preparative CCC separation of 50 mg polar fraction sample using the solvent system composed of *n*-butanol:methanol:water (4:1:5, v/v), lower phase was added 10 mM NaOH. The aqueous mobile phase was pumped through the column at a flow rate of 1.5 mL/min in the head to tail direction and the column was rotated at 600 rpm. The HPLC chromatograms of the two alkaloids were also displayed.

FTICR–MS *m/z*: 342.1694 $[M+H]^+$, refer to previous literature, compound **2** is corydine [27].

3.3. FTICR–MS^2 and HPLC/ESI–MS^n analyses

The compounds obtained by CCC separation were analyzed by FTICR–MS^2. And a standard alkaloid compound called tetrahydropalmatine which had been separated from the same plant [5] was also been analyzed. Major fragment ions (*m/z*) observed in MS^2 spectra were listed in Table 2. The fragmentation pathways proposed [28,29] for the $[M+H]^+$ ion of the compounds are shown in Fig. 3. The polar fraction of the crude sample obtained from the root of *S. yunnanensis* was analyzed by HPLC/ESI–MS^n. The total ion chromatography (TIC) is shown in Fig. 4. The fragmentation product ions of several other components are listed in Table 3.

The positive ion ESI mass spectrum of compound **3** gave the major ion at *m/z* 448, the fragmentation of this precursor ion yielded a product ion at *m/z* 286, which was attributed to the elimination of a fragment of 162 Da. It is the feature loss of glucoside. The fragment ion at *m/z* 269 was generated by the loss of 17 Da from the ion at *m/z* 286. It is assigned to a neutral loss of ammonia. Two product ions at *m/z* 237, 175 were generated by the losses of

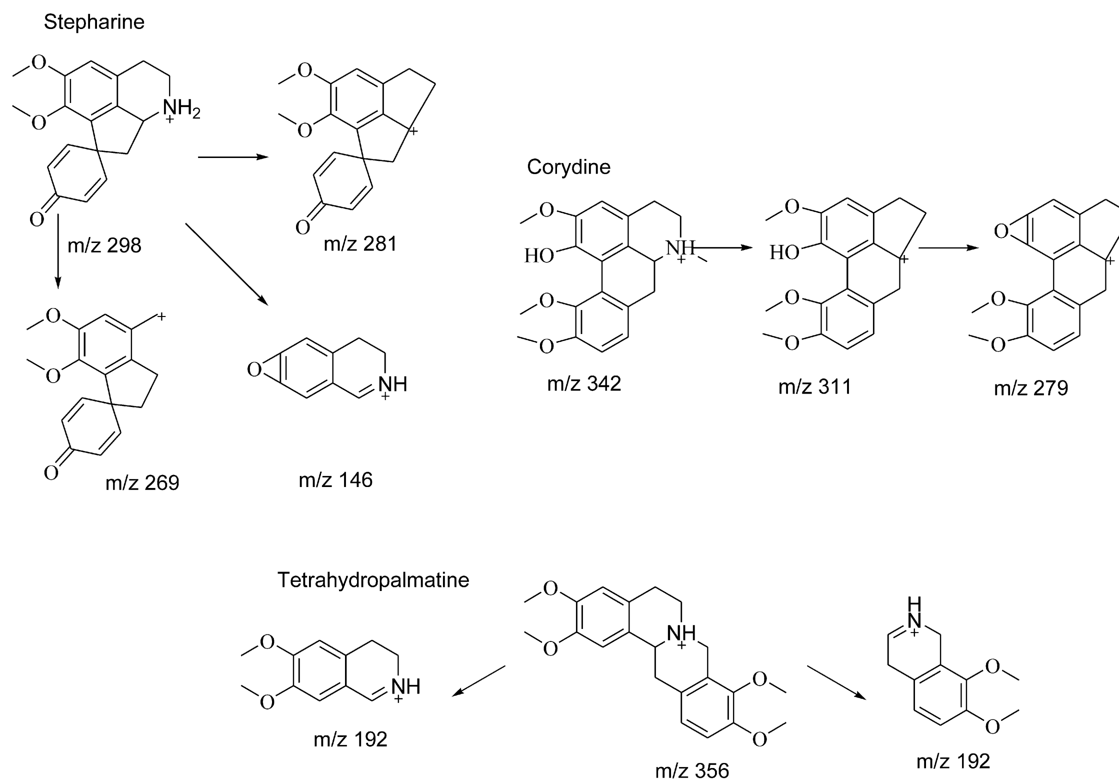

Fig. 3. The fragmentation pathways proposed for the $[M+H]^+$ ions of the three compounds.

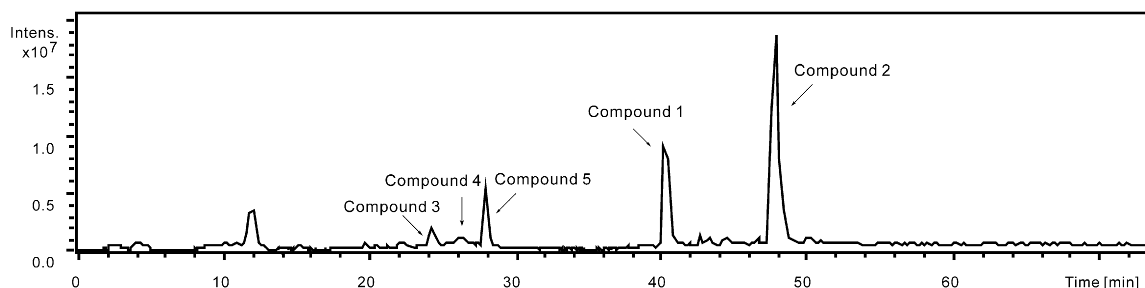

Fig. 4. The total ion chromatography (TIC) of the fraction of the crude sample obtained from the root of *Stephania yunnanensis*.

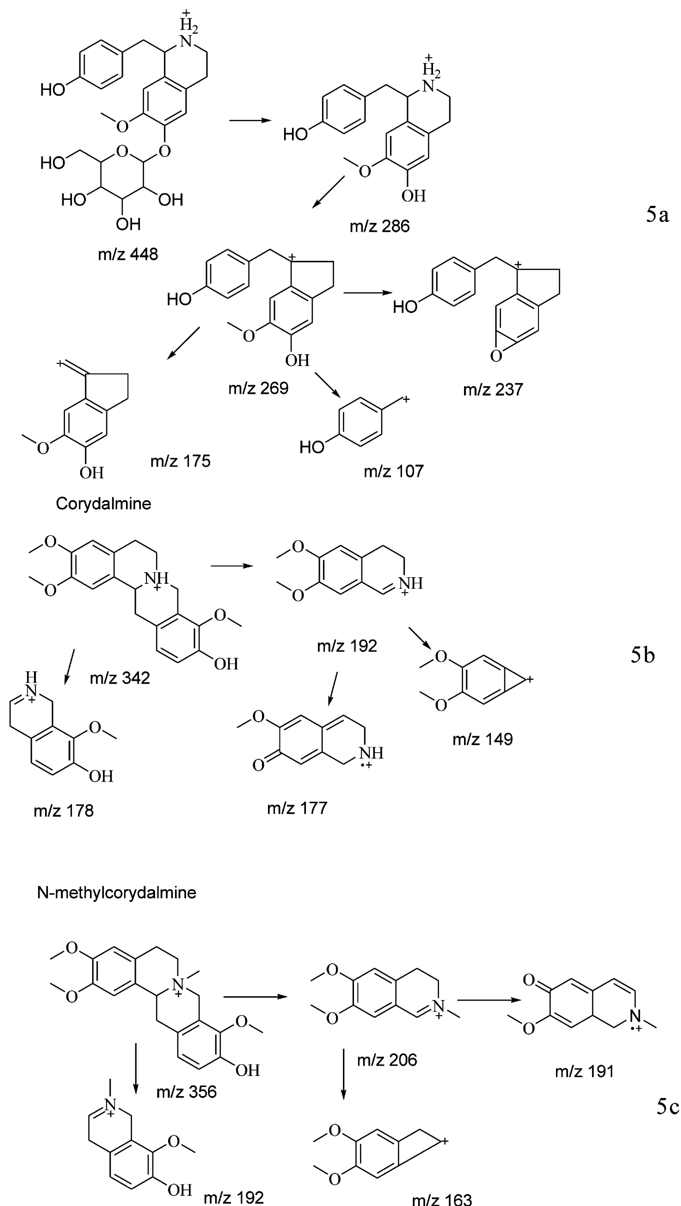

Fig. 5. The fragmentation pathways proposed for the ions of (a) compound **3**; (b) compound **4**; and (c) compound **5**.

Table 2
Major fragment ions (m/z) of the three alkaloids observed in FTICR-MS^2 spectra.

Compounds	Precursor ions (m/z)	Product ions (m/z)
Stepharine	298.1441	281.1148, 269.1149, 146.0594
Corydine	342.1694	311.1275, 279.1014
Tetrahydropalmatine	356.1856	192.1019

Table 3
The fragmentation product ions of several other components.

Compounds	MS^n	Precursor ions (m/z)	Product ions (m/z)
3	MS2	448	286
	MS3	286	269
	MS4	269	237,175,107
4	MS2	342	192,178
	MS3	192	177,149
5	MS2	356	206,192
	MS3	206	191,163

32 Da and 94 Da. The loss of 32 Da is assigned to a neutral loss of CH_3OH, and the loss of 94 Da is assigned to a neutral loss of phenol. The fragmentation product at m/z 107 is assigned to a 4-hydroxybenzyl cation. Thus compound **3** is presumed to be the compound O^6-methylhigenamine-7-*O*-β-D-glucopyranoside. The fragmentation pathways proposed for the ions of compound **3** are shown in Fig. 5a.

The positive ion ESI mass spectrum of compound **4** gave the major ion at m/z 342. The major fragmentation product ions at m/z 178 and 192 are similar with the two fragmentation product ions at m/z 192 generated from tetrahydropalmatine. The mass difference between compound **4** and tetrahydropalmatine is 14 Da, presuming that tetrahydropalmatine has one more methylene group. Refer to previous research of the alkaloids separated from the genus *Stephania* [1], compound **4** is presumed to be corydalmine. The fragmentation pathways proposed for the ions of compound **4** are shown in Fig. 5b.

The positive ion ESI mass spectrum of compound **5** gave the major ion at m/z 356. The major fragmentation product ions at m/z 192 and 206 are similar with the fragmentation product ions at m/z 178 and 192 generated from compound **4**. The difference between the major ions of compound **4** and compound **5** is 14, presuming that compound **5** has one more methylene group or one more methyl group. So compound **5** is presumed to be the quaternary ammonium salt of compound **4**. It is *N*-methylcorydalmine. The fragmentation pathways proposed for the ions of compound **5** are shown in Fig. 5c.

4. Conclusions

In conclusion, two alkaloids were isolated from the polar fraction of *S. yunnanensis* by CCC, and the compound stepharine was separated from the plant by CCC for the first time. The polar fraction of *S. yunnanensis* was analyzed by HPLC–MS^n. Three other alkaloid compounds were given promising structures through the analysis of the fragmentation. A method was established for the separation of the polar compounds from the crude sample by CCC coupled with the analysis of HPLC–MS^n.

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References

[1] D. Semwal, R. Badoni, R. Semwal, S. Kothiyal, G. Singh, U. Rawat, J. Ethnopharmacol. 132 (2010) 369.
[2] L. Zhang, G. Rao, Biochem. Syst. Ecol. 37 (2009) 622.
[3] I. Sutherland, D. Fisher, J. Chromatogr. A 1216 (2009) 740.
[4] Y. Chen, S. Fang, D. Liang, F. Jiang, Zhiwu Xuebao 31 (1989) 296.
[5] Y. Chen, Y. Chen, G. Song, H. Wang, Chin. Tradit. Herb Drugs 16 (1985) 1.
[6] R. Hu, X. Dai, Y. Lu, Y. Pan, J. Chromatogr. B 878 (2010) 1881.
[7] V. Berezhinskaya, E. Trutneva, Tr. Vses. Nauchno-Issled. Inst. Lek. Rast. 14 (1971) 66.
[8] K. Ingkaninan, P. Phengpa, S. Yuenyongsawad, N. Khorana, J. Pharm. Pharmacol. 58 (2006) 695.
[9] M. Camacho, G. Kirby, D. Warhurst, S. Croft, J. Phillipson, Planta Med. 66 (2000) 478.
[10] V. Bhat, B. Bhattacharya, N. De Souza, A. Dohadwalla, H. Kohl,;1; Ger. Offen. CODEN GWXXBX DE 2557282 19770707, 1977.
[11] Y. Ito, R. Bowman, Science 167 (1970) 281.
[12] A. Weisz, A.L. Scher, K. Shinomiya, H.M. Fales, Y. Ito, J. Am. Chem. Soc. 116 (1994) 704.
[13] Y. Ito, W.D. Conway, High-speed Counter-Current Chromatography, Wiley, New York, 1996.
[14] Y. Lu, W. Ma, R. Hu, A. Berthod, Y. Pan, J. Chromatogr. A 1216 (2009) 4140.
[15] Y. Lu, W. Ma, R. Hu, X. Dai, Y. Pan, J. Chromatogr. A 1208 (2008) 42.
[16] Y. Lu, R. Liu, A. Berthod, Y. Pan, J. Chromatogr. A 1181 (2008) 33.
[17] M. Gu, X. Wang, Z. Su, F. Ouyang, J. Chromatogr. A 1140 (2007) 107.
[18] G. Jerz, T. Skotzki, K. Fiege, P. Winterhalter, S. Wybraniec, J. Chromatogr. A 1190 (2008) 63.
[19] A. Weisz, E. Mazzola, Y. Ito, J. Chromatogr. A 1216 (2009) 4161.
[20] A. Yanagida, A. Shoji, Y. Shibusawa, H. Shindo, M. Tagashira, M. Ikeda, Y. Ito, J. Chromatogr. A 1112 (2006) 195.
[21] W. Liu, Z. Pi, X. Wang, F. Song, S. Liu, J. Sep. Sci. 33 (2010) 2898.
[22] Z. Spacil, L. Novakova, P. Solich, Food Chem. 123 (2010) 535.
[23] Y. Chen, G. Liu, Y. Shen, B. Chen, J. Zeng, J. Chromatogr. A 1216 (2009) 2104.
[24] Y. Ito, J. Chromatogr. 301 (1984) 387.
[25] M.J. Ruiz-Ángel, S. Carda-Broch, A. Levet, A. Berthodc, J. Chromatogr. A 1218 (2011) 6044.
[26] B. Charles, J. Bruneton, K. Pharadai, B. Tantisewie, H. Guinaudeau, M. Shamma, J. Nat. Prod. 50 (1987) 1113.
[27] W. Lin, H. Fu, J. Li, G. Cheng, R.A. Barnes, J. Chin. Pharm. Sci. 12 (2003) 117.
[28] W. Chuang, D. Young, L. Liu, S. Sheu, J. Chromatogr. A 755 (1996) 19.
[29] P. Deevanhxay, M. Suzuki, N. Maeshibu, H. Li, K. Tanaka, S. Hirose, J. Pharm. Biomed. Anal. 50 (2009) 413.