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Structural analyses of protoberberine alkaloids in medicine herbs by using ESI–FT-ICR-MS and HPLC–ESI–MS^n

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Abstract

The Electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI–FT-ICR-MS) using sustained off-resonance irradiation (SORI)/collision-induced dissociation (CID) method at high mass resolution has been first applied to investigate the characteristic fragment ions of four protoberberine alkaloids in medicine herbs. The ESI–FT–ICR SORI-CID experiment results demonstrate that the unambiguous elemental composition of fragment ions can be obtained at high mass resolution, then the logical fragmentation pathways of the protoberberine alkaloids has been proposed. The characteristic fragment ions of CID and MS^n of protoberberine alkaloids have been discussed, which are specific and useful for the identification of some protoberberine alkaloid compounds. Then, the extracts of four kinds of medicine herbs have been analyzed by HPLC–ESI–MS^n. According to these characteristic fragmentation pathways, the retention time (t_R) of HPLC and mass spectra of product ion, the structures of six kinds of protoberberine alkaloids have been identified. And, in the present paper, the selected ion monitoring (SIM) method has been used to separate and identify the alkaloid isomers.

Keywords: Structural analyses; Protoberberine alkaloids; ESI–FT-ICR-MS; HPLC–ESI–MS^n

1. Introduction

Protoberberine alkaloids belong to benzyltetrahydroisoquinolines alkaloids, which widely distribute in the plant kingdom, e.g. in Ranunculaceae plant *Coptis chinensis* Franch., Rutaceae plant *Phellodendron amurense* Rupr., Berberidaceae plant *Berberis poiretii* Schneid and B. *amurensis* Rupr. These four herbs are effectively used in Traditional Chinese Medicine (TCM) for heat clearing, damp drying, and so on [1]. Recently, the most of research results demonstrated that protoberberine alkaloids have wide biological activities [2–5]. In addition, it is very significant to modify the natural alkaloid's structure [1], for example, the natural coralyne has the activity of lowering blood pressure while its derivation compounds can be used for antileukaemia. Thus, it appeared important for us to investigate the constituents of herbs in order to search for new bioactive compounds. Up to now, the identification and investigation of protoberberine alkaloids have been reported by using high-performance liquid chromatography (HPLC) [6,7], high-speed counter current chromatography (HSCCC) [8], and capillary electrophoresis-mass spectrometry (CE–MS) [9,10]. With the development of "soft" ionization technique, electrospray ionization tandem mass spectrometry (ESI–MS^n) has been widely employed to characterize alkaloids owing to its high sensitivity, rapid analysis time and low levels of sample consumption, and meanwhile, the useful structure information for the identification of compounds can be obtained by HPLC–ESI–MS^n [11], which is a powerful analysis tool in the field of phytochemistry [12–14].

In the present paper, the alkaloid standards have been firstly determined by ESI–FT-ICR-MS^n and ESI–MS^n in the positive ion mode, the ions of molecular species M^+ and their MS^n spectra data have been obtained. As a result, the useful

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and characteristic fragment ions have been found. The identification and elucidation of the structure of the protoberberine alkaloids in the four medicine herbs have been done by ESI–MS^n and HPLC–ESI–MS/MS. By comparing the retention time (t_R) and mass spectra of product ions of the alkaloids with authentic standards or literature data, the structures of six kinds of protoberberine alkaloids have been identified. Finally, the quantitation analyses of berberine and palmatine have been done by UV technique. The HPLC–UV–ESI–MS^n method for the identification and quantitative analyses of protoberberine alkaloids in TCM is established, which is important and useful for controlling medicine herbs quality.

2. Experimental

2.1. Apparatus

HPLC system is consisted of a Waters (Milford, MA, USA) 2690 HPLC with a photodiode-array detector set at 277 nm. The chromatographic conditions are as follows: column, Dikma Diamonsil C18, 250 mm × 4.6 mm, 5 μm; eluent, (A) water with 0.0034 mol/l ammonium acetate and 0.2% acetic acid (v/v) and (B) acetonitrile. The linear gradient is 0–60 min 30–88% B; the flow-rate is 0.5ml/min; and the temperature is 23 °C.

The mass spectrometry determination was performed on an LCQ ion trap instrument (Finnigan MAT, San Jose, CA, USA) with an electrospray source in the positive ion mode. The electrospray voltage is set to 5.0 kV. The capillary temperature is 260 °C. HPLC is connected to the mass spectrometer via the UV cell outlet. Selected ion monitoring (SIM) of *m*/*z* 336 and *m*/*z* 338 was employed for further confirmation of alkaloid isomers, respectively.

The high-resolution mass spectrometry was performed using IonSpec Ultima 7.0 T FTICR–MS (IonSpec, USA) with an electrospray source in the positive ion mode. Probe heater is 120 °C. Source heater is 80 °C. Probe HV is set to 3.4 kV. Sample cone voltage is set to 30 V. Extractor cone is set to 5.0 V. Desolvent gas is set to 3.0 V. Cone gas is set to 0.5 V. SORI R_f is set to 2.6 V, and collision gas is N_2 with 100 ms pulse (40 Torr).

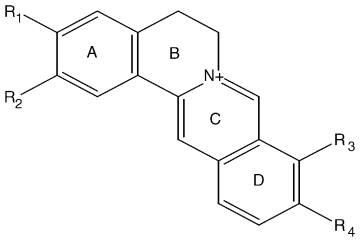
2.2. Standards of alkaloids

Standards of berberine (1), palmatine (2), and jatrorrhizine (3) were purchased from the Chinese Authenticating Institute of Material Medical and Biological Products (Peking, China) and coptisine (4) was kindly provided by Pharmaceutical College of Jilin University. The structures of alkaloids in this study were shown in Fig. 1.

2.3. Sample preparation

2.3.1. Quantitation

The external standard method of calibration was used to the quantitative analyses of the protoberberine alkaloids. Standard solution of berberine was prepared in methanol at the concentrations of 8, 18, 14, 30, and 36 μg/ml, the pamaltine standard solution was prepared in methanol at the concentrations of 5, 10, 15, 20, and 25 μg/ml. The calibra-



	Name	M.W.	R_1	R_2	R_3	R_4
1	berberine	336	-OCH_2O-		OCH_3	OCH_3
2	palmatine	352	OCH_3	OCH_3	OCH_3	OCH_3
3	jatrorrhizine	338	OCH_3	OH	OCH_3	OCH_3
4	coptisine	320	-OCH_2O-		-OCH_2O-	
5	columbamine	338	OH	OCH_3	OCH_3	OCH_3
6	epiberberine	336	OCH_3	OCH_3	-OCH_2O-	

Fig. 1. Structures of alkaloids (1–6) studied.