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Analysis of alkaloids in *Macleaya cordata* (Willd.) R. Br. using high-performance liquid chromatography with diode array detection and electrospray ionization mass spectrometry

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ABSTRACT

A method for the analysis of alkaloids in *Macleaya cordata* (Willd.) R. Br. using high-performance liquid chromatography with diode array detection and electrospray ionization mass spectrometry (HPLC–DAD–ESI/MS) was developed. Using protopine (PRO), allocryptopine (ALL), sanguinarine (SA), and chelerythrine (CHE) as the model components, different columns for the separation and different mobile phases for the signal intensities of alkaloids in ESI/MS were investigated, respectively. The results showed that good separation and high signal intensities can be obtained on a high carbon loading (17%) reversed-phase C_{18} column with 30 mM formic acid in mobile phase for the analysis of alkaloids. Under the optimal separation condition and UV detection (284 nm), linearity of the six alkaloids was obtained over concentration range from 0.05 to 100.00 μg/ml. The limit of detection (LOD) was 1.62, 1.87, 1.79, 1.76, 1.10, and 0.94 ng/ml for SA, CHE, PRO, ALL, dihydrosanguinarine (DHSA), and dihydrochelerythrine (DHCHE), respectively. The LODs with ESI/MS detection were lower three orders of magnitude than those obtained with UV detection. The proposed method could be used to control quality of the raw materials of the herb more comprehensively.

1. Introduction

Macleaya cordata (Willd.) R. Br. is a plant of Papaveraceae family and a traditional Chinese folk herbal medicine. Recently, it has been found that sanguinarine (SA) and chelerythrine (CHE) have antiseptic and antitumour activities [1–3]. The extract containing SA and CHE has been used as an antiplaque, gingivitis and antimicrobial agent. A number of pharmaceutical research results show that alkaloids in the herb are major bio-active components of the herb [4–7]. Except SA and CHE, many benzylisoquinoline alkaloids, such as protopine (PRO), allocryptopine (ALL), dihydrochelerythrine (DECHE) and dihydrosanguinarine (DHSA) were isolated from *M. cordata* (Willd.) R. Br. These alkaloids also have bio-activities. So, the quality of the herb should be evaluated based on the contents of several alkaloids. Therefore, the accurate determination of the alkaloids in the herb is very important for the more comprehensive quality control of the raw materials and the preparations of the herb.

A number of chromatographic methods for the determination of the alkaloids have been developed [8–10]. However, most of them have been focused solely on the determination of two alkaloids SA and CHE. In 2006, we proposed an HPLC–ESI/MS method for the analysis of the four alkaloids PRO, ALL, SA, and CHE in the fruits of the herb. Although the sensitivity of the method was high, the separation was unsatisfactory. Peak tailing of the alkaloids was obvious [9]. Suchomelova et al. developed a method for the simultaneous determination of seven quaternary benzo[*c*]phenanthridine alkaloids SA, CHE, chelirubine, chelilutine, sanguilutine, sanguirubine, and macarpine [8]. In their method, an RP C_{12} column and a mobile phase consisting of heptanesulfonic acid and triethanolamine were employed to separate the alkaloids. Under this condition, the separation and peak shape of the alkaloids were good. However, the mobile phase was unsuitable for the ESI/MS detection because there was non-volatile salt in it.

In this paper, a simple and accurate HPLC–DAD–ESI/MS method was proposed for the analysis of alkaloids in *M. cordata* (Willd.) R. Br. The analytical conditions were investigated in detail. The method is useful for the more comprehensive quality control of the raw materials of the herb and its preparations.

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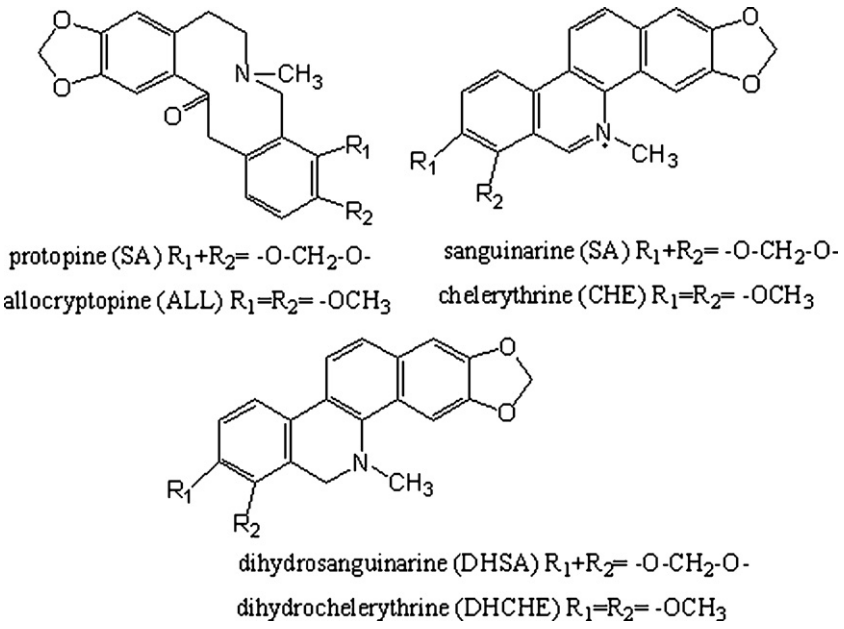

Fig. 1. Structures of six alkaloids.

2. Materials and methods

2.1. Chemicals and plant materials

Standards of PRO, ALL, SA, CHE, DHCHE and DHSA were prepared in the laboratory by authors. Structures of the alkaloids are given in Fig. 1. The identity was verified by ESI/MS, ^{1}H NMR and ^{13}C NMR [7,11,12]. The purity was not less than 96.5% (HPLC and ^{1}H NMR) [13]. Methanol was HPLC grade. Water was purified with a Milli-Q system (Millipore, Bedford, MA, USA). Sodium borohydride ($NaBH_4$) was from Sigma. Other chemicals were analytical grade.

Samples of fresh *M. cordata* (Willd.) R. Br. were collected at one site: 20 km south from Changsha (28°12′N, 112°55′E) (located in the northeast part of Hunan Province, China), during June–August 2007. The plant materials were identified as *M. cordata* (Willd.) R. Br. by Prof. Jian-Zhong Li, Department of Botany, Hunan Normal University, China. The roots, leaves, flowers and fruits of the herb were separated, washed and sucked dry with absorbent papers. Then, they were dried in the shade. Voucher specimens are deposited in the Key Laboratory of Chemical Biology & Traditional Chinese Medicine Research, Ministry of Education, Hunan Normal University. Five samples were collected during this study.

Twelve samples of *M. cordata* (Willd.) R. Br. preparation including five killing oncomelania preparations and seven anti-fungal infection preparations were kindly presented by Prof. Qiong-Yao Huang (College of Medicine, Hunan Normal University, China).

2.2. Preparation of standard solutions

Stock solutions of PRO, ALL, SA, CHE, DHCHE and DHSA were prepared in methanol. The concentration of all solutions was 1.00 mg/ml. 2.5 ml aliquots of each stock solution were transferred into a 25-ml volumetric flask, mixed and diluted to volume to yield a mixed standard solution. Then, the mixed standard solutions with different volume were transferred to six 10-ml volumetric flasks, and diluted to volume with methanol to yield a series of working solutions. The concentration was 0.05, 0.10, 5.00, 10.00, 20.00, 50.00, and 100.00 μg/ml.

2.3. Pre-treatment of samples

2.3.1. Preparation of sample solution

The dried roots, leaves, stems, flowers and fruits were pulverized in a mill (20 mesh, particle size <830 μm). 1.0 g of the powder of the herb or the solid preparation was extracted with 8 ml of methanol in an ultrasonic bath for 15 min. The extraction was filtrated. The residue was extracted again according to same conditions. The filtrates were combined and made up to 20 ml with the methanol. The solution was filtered through 0.45 μm membrane. Then a 20 μl aliquot of the solution was injected into the LC–DAD–ESI/MS system. For the accurate analysis of high content alkaloids, the sample solutions must be diluted to avoid a concentration that lies outside of the linear range.

2.3.2. Reduction of quaternary alkaloids in root of M. cordata (Willd.) R. Br.

100 mg of solid $NaBH_4$ was added in 10 ml of methanol extraction solution, the solution was refluxed for 30 min in a water-bath. After cooling, the solution was evaporated to dry under a nitrogen stream. The residue was extracted by chloroform (5 ml 3×) in an acoustic wave bath. Combining the chloroform extractions, the extraction was evaporated to dry under a nitrogen stream. Then, the residue was dissolved in 10 ml of methanol. The solution was filtered through 0.45 μm membrane. 20 μl of the solution was injected into the LC–DAD–ESI/MS system.

2.4. HPLC–DAD–ESI/MS analysis

Analysis were carried out using a Waters alliance 2695 liquid chromatographic system (Milford, MA, USA), interfaced to a 996 DAD system and a Micromass ZQ2000 electrospray mass spectrometer (Manchester, UK). LC separations were accomplished on a 5 μm, 250 mm × 4.6 mm Ultimate XB C_{18} column (Welch Materials, Ellicott City, USA) at 25 °C. Mobile phases consisted of (A) water containing 30 mM formic acid and (B) acetonitrile. Gradient elution program was as follows: 0–12 min 20% B, 12–22 min 20–30% B, 22–32 min 30–45% B, 32–40 min 45–75% B, 40–45 min 75–27% B,