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Separation and purification of two new and two known alkaloids from leaves of *Nitraria sibirica* by pH-zone-refining counter-current chromatography

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ABSTRACT

The total alkaloids from *Nitraria sibirica* leaves have been confirmed to exhibit significant protective effects against inflammatory renal injury, hypertension and albuminuria in angiotensin II-salt hypertension. In the present study, a separation method of pH-zone-refining counter-current chromatography was established for separation of the alkaloids from *N. sibirica*. The separation was performed with a solvent system of MtBE-*n*-BuOH-H_2O (2:2:5, *v*/*v*) at a flow rate of 2.0 mL/min. And 15 mM triethylamine (TEA) was added to the upper organic phase, while 10 mM hydrochloric acid was added to the lower aqueous phase. As a result, a new alkaloid, schobemine (5.6 mg), and a known alkaloid, nitraramine (5.0 mg), together with fractions A and B were obtained from the total alkaloids of *N. sibirica*. The fractions A and B were further purified by means of pH-zone-refining counter-current chromatography with solvent systems of *n*-hexane-*n*-BuOH-H_2O (1.5:3.5:5, *v*/*v*) and (2:3:5, *v*/*v*), respectively. TEA (10 mM) was added to the upper phase, and 10 mM of HCl was added to the lower phase in above two solvent systems, respectively. As a result, a known alkaloid, schoberidine (5.0 mg), and a new alkaloid, schoberimine (3.0 mg) were obtained from fractions A and B, respectively. The purities of the compounds were measured by HPLC–ELSD, and their structures were identified by ESI–MS, 1D and 2D NMR.

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1. Introduction

Alkaloids have been extensively investigated for decades due to their wide range of bioactivities and structural diversity [1,2]. Separation of alkaloids from medicinal materials by conventional column chromatographic techniques usually encounters a series of practical problems, such as the limited amount of stationary phase and the irreversible adsorption of the sample by solid support [3,4]. To cope with these problems, pH-zone-refining counter-current chromatography (pH-zone-refining CCC) was developed and employed as a large-scale preparative technique for separating ionizable analytes based on the all-liquid chromatographic technique [5–10], which provided great advantages over the conventional column chromatography by eliminating the use of solid support. This method presents its unique advantages on preparation of particular basic or acidic compounds, such as highly concentrated rectangular peaks of targets fused together with minimum overlapping impurities [7,11], and had been widely used for preparative separation of basic or acidic compounds with potent bioactivity from medicinal materials [12–17].

Nitraria sibirica Pall. (NSP), belonging to the Zygophyllacea family, is one of the dominant species in Xinjiang, China, which plays a key role of improving the ecological environment due to its superior tolerance to severe drought and high salinity. As a traditional Uyghur herb, the fruits and leaves of NSP were used to treat hypertension, menstrual disorders and gestroenterities [18,19]. Up to date, only a few phytochemical investigations of NSP have been reported [20–23], although alkaloids and flavonoids were found from the other species of *Nitraria* genus in the past several years [24–27]. The alkaloids from the genus of *Nitraria*

Abbreviations: pH-zone-refining CCC, pH-zone-refining counter-current chromatography; NSP, *Nitraria sibirica* Pall; NSPL, *Nitraria sibirica* Pall. leaves; HSCCC, high-speed counter-current chromatography.

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have been reported to possess hypotensive activity [28], and the total alkaloids from *N. sibirica* Pall. leaves (NSPL) have also been confirmed to exhibit significant protective effects against inflammatory renal injury, hypertension and albuminuria in angiotensin II-salt hypertension in our previous studies [29]. In the present study, a pH-zone-refining CCC method was established for separation and purification of two new alkaloids, schobemine and schoberimine, together with two known alkaloids, nitraramine and schoberidine (Fig. 1) from NSPL for the first time. Which imply that the pH-zone-refining CCC can be applied in the separation of trace amounts of new bioactive alkaloids.

2. Experimental

2.1. Apparatus

The pH-zone-refining CCC was carried out using a model of TBE-300A high-speed countercurrent chromatography (HSCCC) instrument (Shanghai, Tauto Biotech, China) with a total volume of 290 mL three polytetrafluoroethylene multilayer coil (tubing ID: 2.6 mm). The β-value ($\beta = r/R$, where r was the rotation radius or the distance from the coil to the holder shaft, and R was the revolution radius or the distance between the holder axis and central axis of the centrifuge) varied from 0.5 (internal) to 0.8 (external).

The solvent was pumped into the column with a model P230 constant flow pump (Dalian Elite Analytical Instruments Co., Ltd., China). The effluent was continuously monitored by a 8823A-UV detector at 254 nm (Beijing Binta Instrument Technology Co., Ltd., China), and the results were recorded by N2000 workstation (Zhejiang University, Hangzhou, China). The sample was injected into the column manually with a 20 mL injection loop. The pH of each collected fraction was measured manually by a PSH-3C pH meter (Shanghai Hongyi Instrumentation Co., Ltd.)

The HPLC (Shimadzu, Kyoto, Japan) equipment used was a system including an LC-20AT pump, an SIL-20A automated sample injector, a CTO-20AC Thermostatted column compartment, a CBM-20A communications Bus Module and an ELSD-LTII low temperature-evaporative light scattering detector. The analysis was carried out on an InertSustainR C_{18} column (5 μm, 4.6 × 150 mm i.d.) from Shimadzu, Japan.

2.2. Reagents

Methyl tert-butyl ether (MtBE) and acetonitrile (CH_3CN) were HPLC-grade (Dikma Technologies Inc., USA). Methonal (MeOH), *n*-butanol (*n*-BuOH), *n*-hexane, hydrochloric acid (HCl), formic acid and triethylamine (TEA) were analytical grade and purchased from Baishi Chemical Co., Ltd (Tianjin, China). Methanol and acetonitrile used for HPLC were HPLC-grade and purchased from Fisher Scientific company (Fair Lawn, NJ, USA).

The NSPL were collected from Hetian city of Xinjiang, China, and authenticated by Prof. Nurbay Abdusalih (Key Laboratory of Oasis Ecology, College of Resources & Environment Science, Xinjiang University, China).

2.3. Preparation of the crude sample

The NSPL (2 kg) were refluxed with 70% EtOH (10 L × 3, each time for 1 h) at 80 °C. The pooled extract (400 g) were dissolved in 2% HCl and then filtered. The filtrate was extracted with $CHCl_3$ to remove non-alkaloid impurities. Then, the obtained acid solution was basified with saturated Na_2CO_3 solution to pH 12, and extracted with $CHCl_3$ to give crude alkaloids extract (1.0 g). The crude alkaloids extract (1.0 g) was dissolved in 2% HCl (50 mL) again, and further repurified with EtOAc (50 mL × 4). The acid solution was basified with NH_4OH to pH 10, and extracted with $CHCl_3$ to give the total alkaloids (650 mg).

2.4. Selection and preparation of two-phase solvent systems

In the pH-zone-refining CCC separation, the two-phase solvent systems of MtBE-CH_3CN-H_2O (2:2:3, *v*/*v*), MtBE-*n*-BuOH-CH_3CN-H_2O (6:4:5:5, *v*/*v*), MtBE-*n*-BuOH-CH_3CN-H_2O (2:2:1:5, *v*/*v*) and MtBE-*n*-BuOH-H_2O (2:2:5, *v*/*v*) were tested to select the optimal solvent composition [7,11]. The values of K_{acid} and K_{base} of these two-phase solvent systems need to meet $K_{acid} \ll 1$ and $K_{base} \gg 1$, which was observed from the TLC plates. The solvent system of MtBE-*n*-BuOH-H_2O (2:2:5, *v*/*v*) was finally selected for the pH-zone-refining CCC experiments of the total alkaloids. The solvent system of MtBE-*n*-BuOH-H_2O (2:2:5, *v*/*v*) was totally equilibrated in a separating funnel and separated into two phases. The upper phase (organic phase) was used as stationary phase after added 15 mM of triethylamine, and the lower phase (aqueous phase) was used as mobile phase after added 10 mM of HCl. The total alkaloids (500 mg) were dissolved in 5 mL of upper phase with 15 mM of TEA, and 5 mL of lower phase without HCl was added into the sample solution, then totally equilibrated.

In addition, the two-phase solvent systems, *n*-hexane-*n*-BuOH-H_2O (1.5:3.5:5, *v*/*v*) and *n*-hexane-*n*-BuOH-H_2O (2:3:5, *v*/*v*), were selected for further purification of fractions A and B, respectively, according to the results of the pH-zone-refining CCC separation of the total alkaloids. In these two solvent systems 10 mM of TEA was added to the upper organic phase and 10 mM of HCl was added to the lower aqueous phase.

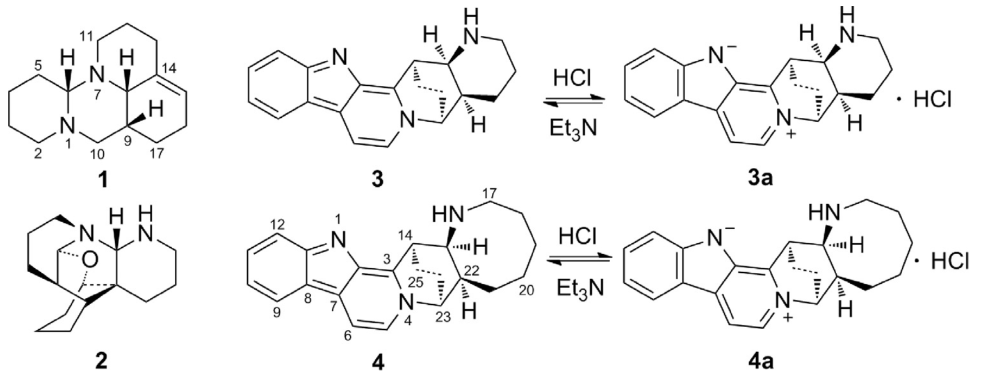

Fig. 1. Structures of the alkaloids from the leaves of *Nitraria sibirica* Pall.

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