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Short communication

Preparative separation of flavonoid glycosides in leaves extract of *Ampelopsis grossedentata* using high-speed counter-current chromatography

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Abstract

Preparative separation of flavonoid glycosides in leaves extract of *Ampelopsis grossedentata* was conducted using high-speed counter-current chromatograph (HSCCC) with a solvent system composed of *n*-hexane–ethyl acetate–methanol–water (1:6:1.5:7.5, v/v). In a single operation, 28 mg of 5,7-dihydroxy-3′,4′-trihydroxyflavone-3-*O*-6″-rhamnose and 18 mg of 5,7-dihydroxy-3′,4′-dihydroxyflavone-3-*O*-6″-rhamnose was obtained from 150 mg of the extract. The chemical structure of the two compounds was elucidated by electrospray ionization (EIS) MS and NMR.

Keywords: Preparative chromatography; Counter-current chromatography; *Ampelopsis grossedentata*; Plant materials; Flavonoids; Glycosides

1. Introduction

The leaves of *Ampelopsis grossedentata* are used for beverages in southern China, which can inhibit the contraction of rabbit aortic strips evoked by noradrenaline and the ethanol-induced muscle relaxation in rats [1,2]. In our previous report, the purification of (+)-dihydromyricetin, the main component in the leaves, was successfully performed using a high-speed counter-current chromatograph (HSCCC) equipped with three scale-up columns [3]. Several papers reported that flavonoid glycosides also occur in the plant [4–6]. In this paper we describe the preparative separation of two flavonoid glycosides of *A. grossedentata* using HSCCC and elucidation of their chemical structure with electrospray ionization (EIS) MS and NMR.

2. Experimental

2.1. Apparatus

A HSCCC instrument, Model GS-10A was manufactured by Beijing Institute of New Technology Application, Beijing, China. It was equipped with a 280 ml multilayer coil column made of a 1.6 mm i.d. PTFE (polytetrafluoroethylene) tubing. The separation was run at a revolution speed of 750 rpm. The mobile phase was delivered with a Waters 510 HPLC pump (Waters, Milford, MA, USA). An injection loop was used for injection of samples. The effluent was monitored at 254 nm by a 8823A-UV detector (Beijing Institute of New Technology Application) and collected with a BS-100 fraction collector (Shanghai Puxi Instrument Factory, Shanghai, China).

2.2. Reagents

n-Hexane, methanol and ethyl acetate were of an analytical grade and were purchased from Shanghai Chemical Co., Shanghai, China.

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2.3. Preparation of two-phase solvent system

The HSCCC experiments were performed with a two-phase solvent system composed of *n*-hexane–ethyl acetate–methanol–water (1:6:1.5:7.5, v/v). After thoroughly equilibrating the mixtures in a separtory funnel at room temperature, two phases were separated shortly before use. The upper organic phase was used as stationary phase, and the lower aqueous phase as mobile phase.

2.4. Preparation of sample

A 200 g amount of leaves of *A. grossedentata* was extracted with 2 × 1.5 l 60 °C water. The extracting solution was evaporated in reduced pressure to 500 ml. The concentrated solution was extracted with 500 ml chloroform to remove the pigments, and lyophilized to yield 53 g of extract product. The 53 g of extract product was dissolved in 250 ml of 60 °C hot water, then kept at 4 °C overnight. The supernatant solution was obtained by filtrating with 40 μm size filtration film under decreased pressure (0.08 MPa) and extracted with equivoluminal *n*-butanol. The extracting solution was evaporated in vacuum to remove *n*-butanol, then the residue was lyophilized to yield 1.3 g crude sample for separation of flavonoid glycosides.

2.5. HSCCC procedure

The multiplayer coiled column was first entirely filled with the upper phase organic phase as the stationary phase. Then the apparatus was started. The sample solution was injected through the injection loop before the mobile phase was pumped into the column at a flow rate of 1.5 ml/min after run at 750 rpm. The effluent was collected with a fraction collector in order to analyze and collect needed fractions.

2.6. HPLC analysis

An Agilent 1100 HPLC system composed of a quaternary pump with a degasser, a thermostatted column compartment, a variable wavelength detector, a manual injector, and 1100 ChemStation software. The HPLC separation was performed on a Zorbax-ODS column (5 μm, 25 cm × 4.6 mm i.d.) with a gradient elution. The gradient elution initiated with 35% methanol, then linearly increased to 80% methanol in 30 min at a flow-rate of 1.0 ml/min by monitoring at 254 nm.

2.7. Spectroscopic analysis

^{1}H and ^{13}C NMR spectra were recorded in [$^{2}H_5$]pyridine on a Bruker AMX 300 spectrometer (Karlsruhe, Germany) with 300 MHz for ^{1}H and 75.5 MHz for ^{13}C measurements, respectively. Electrospray ionization ion trap multiple mass spectrometry (ESI-MS) experiments were performed on a Bruker Esquire LC–MS system in positive and negative mode using a syringe pump. Drying gas was nitrogen with a gas flow of 7 l/min (330 °C), nebulizer pressure was set to 30 psi (1 psi = 6894.76 Pa), parameters for negative ESI mode were as follows: capillary, −3500 V; end plate, −3000 V; capillary exit, 90 V; skim 1, 30 V; skim 2, 10 V; MS–MS fragmentations were done with different fragmentation amplitudes; for positive ESI mode: capillary, 3500 V; end plate, 3000 V; capillary exit, −90 V; skim 1, −30 V; skim 2, −10 V.

3. Results and discussion

3.1. HSCCC separation

Fig. 1 shows the HSCCC separation result of 150 mg crude sample, which gave five peaks I–V corresponding to peaks 1–5 in HPLC analysis of the crude sample, respectively (Fig. 2). The five sections of the fractions corresponding peaks I–V were combined and freeze-dried to yield 5 mg component I, 28 mg component II, 36 mg component III, 18 mg of component IV and 9 mg of component V. Component III was (+)-dihydromyricetin, the same compound obtained in our previous separation [1]. Components I–V were used for ESI-MS and NMR analysis.

3.2. Elucidation of chemical structure

It is difficult to elucidate the chemical structure of components I and V since their NMR gave inconsistent results with ESI-MS. Component II and IV showed obvious characters of flavonoid glycosides as below.

3.2.1. Component II

Positive ESI-MS, *m/z* 487 (*M* + Na); negative ESI-MS, *m/z* 463 (*M* − H). ^{13}C NMR: 134.2 (C-2), 119.6 (C-3), 177.7 (C-4), 161.2 (C-5), 93.4 (C-6), 164.1 (C-7), 98.6 (C-8), 157.4 (C-9), 101.1 (C-10), 129.4 (C-1′), 107.9 (C-2′), 146.3 (C-3′), 136.4 (C-4′) 104.0 (C-1″), 70.4 (C-2″), 70.3 (C-3″), 71.2 (C-4″), 69.9 (C-5″) and 17.4 (C-6″). ^{1}H NMR: 6.35, 1H (C-6); 6.18, 1H (C-8), 6.91, 2H (C-2′, C-6′); 12.63, 1H (C-5, –OH); 4.01, 1H (C-1″) and 0.85, 3H (C-6″). The

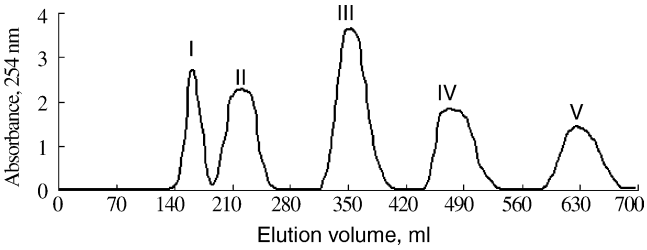

Fig. 1. HSCCC separation chromatogram of 150 mg crude sample. Solvent system: *n*-hexane–ethyl acetate–methanol–water (1:6:1.5:7.5, v/v); stationary phase: upper phase; flow-rate of the mobile phase: 1.5 ml/min; revolution: 750 rpm; retention of stationary phase: 45%.

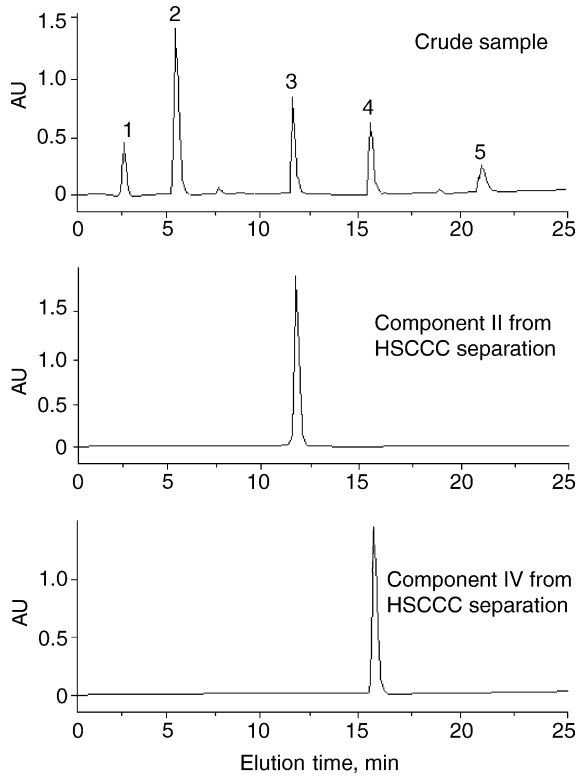

Fig. 2. HPLC analysis of crude sample and the components obtained from HSCCC separation. Experimental conditions: HPLC column: Zorbax-ODS column, 5 μm, 25 cm × 4.6 mm i.d.; gradient elution: 35–80% methanol in water from 0 to 30 min; flow-rate: 1.0 ml/min; detection wavelength: 254 nm.

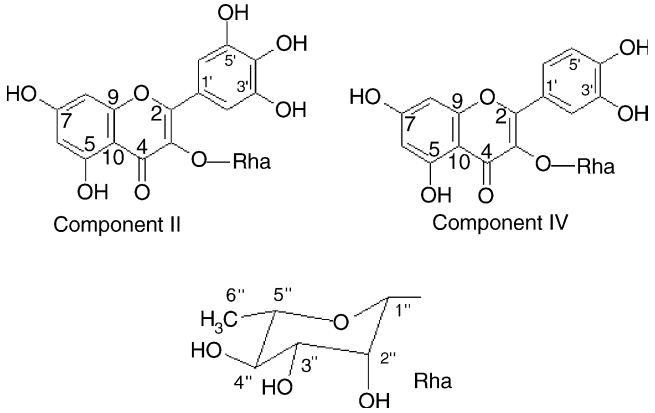

Fig. 3. Chemical structures of components II and IV. Component II: 5,7-dihydroxy-3′,4′-trihydroxyflavone-3-*O*-6″-rhamnose; component VI: 5,7-dihydroxy-3′,4′-dihydroxyflavone-3-*O*-6″-rhamnose.

ESI-MS and NMR data indicate compound II is a typical flavonoid glycoside with three hydroxyl groups in C-cycle, which is 5,7-dihydroxy-3′,4′,5′- trihydroxyflavone-3-*O*-6″-rhamnose (myricitrin) (Fig. 3).

3.2.2. Component IV

Positive ESI-MS, *m*/*z* 461 (*M* + Na); negative ESI-MS, *m*/*z* 447 (*M* − H). ^{13}C NMR: 134.1 (C-2), 115.4 (C-3), 177.6 (C-4), 161.2 (C-5), 93.5 (C-6), 164.1 (C-7), 98.6 (C-8), 157.2 (C-9), 101.8 (C-10), 129.0 (C-1′), 114.2 (C-2′), 145.1 (C-3′) and 145.6 (C-4′) 104.0 (C-1″), 70.4 (C-2″), 70.3 (C-3″), 71.1 (C-4″), 70.0 (C-5″), 17.4 (C-6″). ^{1}H NMR: 6.38, 1H (C-6); 6.19, 1H (C-8); 12.68, 1H (–OH, C-5); 6.92, 1H (C-5′); 7.27, 1H (C-2′); 7.23, 1H (C-6′); 3.98, 1H (C-1″); 1.08, 3H (C-6″). The ESI-MS and NMR data indicate compound IV also is a typical flavonoid glycoside with two hydroxyl groups in C-cycle, which is 5,7-dihydroxy-3′,4′-dihydroxyflavone-3-*O*-6″-rhamnose (Fig. 3), a un-reported compound in the chemical studies of *A. grossedentata*.

The above results demonstrate that HSCCC is an excellent technology to separate flavonoid glycosides in *A. grossedentata*.

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