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Preparative isolation and purification of four compounds from the Chinese medicinal herb *Rhizoma Anemarrhenae* by high-speed counter-current chromatography

Qinghua Sun, Ailing Sun, Renmin Liu *

College of Chemistry and Chemical Engineering, Liaocheng University, Liaocheng 252059, China

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Abstract

High-speed counter-current chromatography (HSCCC) was applied to the separation and purification of mangiferin, neomangiferin, *cis*-hinkiresinol and (-)-4′-*O*-methylnyasol from the Chinese medicinal herb *Rhizoma Anemarrhenae*. Five hundred milligrams of crude extracts were separated by using *n*-butanol–acetic acid (1%) (1:1, v/v) as the two-phase solvent system and yielded 35.3 mg of neomangiferin and 245.4 mg of mangiferin. During this separation, *cis*-hinkiresinol and (-)-4′-*O*-methylnyasol were still maintained in the stationary phase. The stationary phase was collected, evaporated to dryness and separated with light petroleum–ethyl acetate–methanol–water (1:1:1.2:0.8, v/v) and 1:1:1.4:0.6 (v/v) in gradient elution, which yielded 17.2 mg of *cis*-hinkiresinol and 12.4 mg of (-)-4′-*O*-methylnyasol. The purities of mangiferin, neomangiferin, *cis*-hinkiresinol and (-)-4′-*O*-methylnyasol were 96.3, 98.0, 97.3 and 98.2%, respectively, as determined by HPLC. The chemical structures of these components were identified by ^{1}H NMR and ^{13}C NMR.

Keywords: *Rhizoma Anemarrhenae*; HSCCC; Neomangiferin (7-*O*-β-D-glucopyranosyl-mangiferin); Mangiferin; *cis*-Hinkiresinol; (-)-4′-*O*-Methylnyasol

1. Introduction

Rhizoma Anemarrhenae, a well-known traditional Chinese medicinal herb and officially listed in the Chinese Pharmacopoeia [1], has the bioactive effects of anti-pathogenic microorganism, hypoglycemic effects, anti-inflammatory, antipyretic effects and anti-platelet aggregation [2]. Mangiferin neomangiferin, *cis*-hinkiresinol and (-)-4′-*O*-methylnyasol are the major bioactive constituents of *Rhizoma anemarrhenae*. The chemical structures of them were shown in Fig. 1.

The preparative separation and purification of neomangiferin and mangiferin from plant materials by conventional methods such as column chromatography has been reported previously [3]. But the method is tedious and requires multiple chromatography steps. Furthermore, the adsorbing effects on stationary phase material and artifact formation is serious. High-speed counter-current chromatography (HSCCC), invented by Ito [4], is a unique liquid–liquid partition chromatography method that uses no solid support matrix. It eliminates irreversible absorptive loss of samples onto the solid support matrix used in conventional chromatography. The technique allows complete recovery of the sample, and is suitable for separations in the gram range [5]. The present paper reported the successful preparative separation and purification of neomangiferin, mangiferin, *cis*-hinkiresinol and (-)-4′-*O*-methylnyasol from *Rhizoma Anemarrhenae* by HSCCC.

2. Experimental

2.1. Apparatus

The HSCCC instrument employed in the present study is TBE-300A high-speed counter-current chromatography (Shanghai Tauto Biotech Co., Ltd., Shanghai, China) with three multilayer coil separation column connected in series (I.D. of the tubing = 1.6 mm, total volume = 260 ml) and a 20 ml sample loop. The revolution radius or the distance between the holder axis and central axis of the centrifuge (*R*) was 5 cm,

* Corresponding author. Tel.: +86 6358230600
E-mail address: renminliu@lctu.edu.cn (R. Liu).

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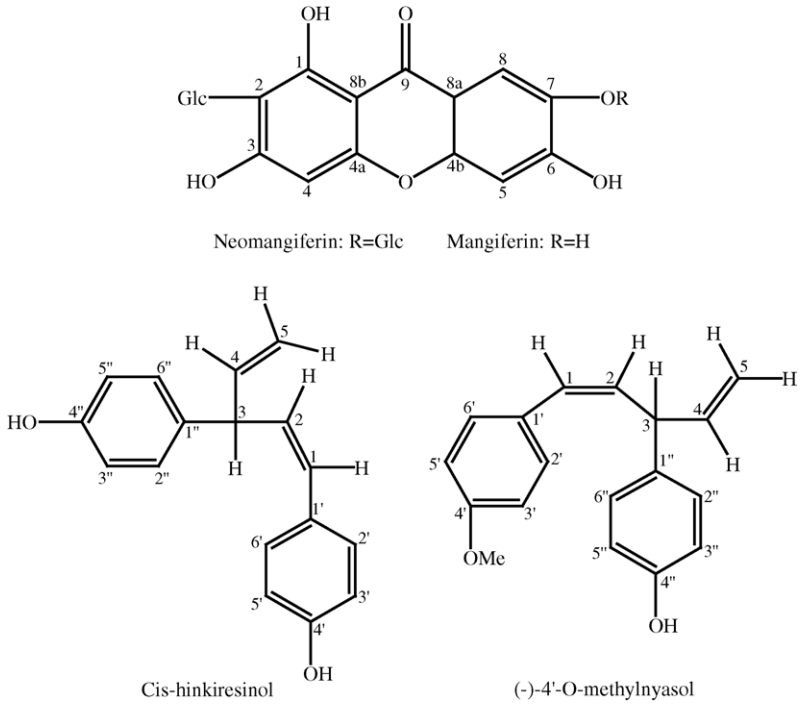

Fig. 1. Chemical structures of target compounds from *Rhizoma Anemarrhenae*.

and the β-values of the multilayer coil varied from 0.5 at internal terminal to 0.8 at the external terminal ($\beta = r/R$, where r is the distance from the coil to the holder shaft). The revolution speed of the apparatus can be regulated with a speed controller in the range between 0 and 1000 rpm. An HX 1050 constant-temperature circulating implement (Beijing Boyikang Lab Instrument Company, Beijing, China) was used to control the separation temperature. A ÄKTA prime system (Amersham Pharmacia Biotechnique Group, Sweden) was used to pump the two-phase solvent system and perform the UV absorbance measurement. It contains a switch valve and a mixer, which were used for gradient formation. The data were collected with Sepu 3000 chromatography workstation (Hangzhou Puhui Science Apparatus Company, Hangzhou, China).

The HPLC equipment used was Agilent 1100 HPLC system including G1311A QuatPump, G1315B UV–vis photodiode array detector, Rheodyne 7725i injection valve with a 20 μl loop, G1332 degasser and Agilent HPLC workstation (Agilent Technologies, Germany).

The nuclear magnetic resonance (NMR) spectrometer used here was a Mercury Plus 400 NMR system (Varian, USA).

A FZ102 plant disintegrator (Taisite Instrument Company, Tianjin, China) was used for disintegration of the sample.

2.2. Reagents and materials

All solvents used for preparation of crude extracts and HSCCC separation were of analytical grade (Jinan Reagent Factory, Jinan, China). The boiling point range of the light petroleum used for all experiments was 60–90 °C). Methanol used for HPLC was of chromatographic grade (Yucheng Chemical Factory, Yucheng, China), and water used was distilled water.

Rhizoma Anemarrhenae was purchased from a local drug store and was identified as the dried roots of *Anemarrhena asphodeloides Bge.* by Professor Yongqing Zhang (Shandong University of Traditonal Chinese Medicine, Jinan, China).

2.3. Preparation of crude sample

The dried *Rhizoma Anemarrhenae* was ground to powder (about 40 mesh) by using FZ102 plant disintegrator. The powder (100 g) was extracted with 500 ml of methanol under sonication for 30 min. The extraction procedure was repeated three times. The extracts were combined and evaporated under reduced pressure by rotary vaporization at 45 °C, which yielded 13.6 g of crude extracts. It was stored in a refrigerator for the subsequent HSCCC separation.

2.4. Selection of the two-phase solvent systems

The composition of the two-phase solvent system was selected according to the partition coefficient (K) of the target compounds of crude example. The partition coefficients were determined by HPLC as follows: suitable amount of crude example was dissolved in 2 ml of aqueous phase of the pre-equilibrated two-phase solvent system. The solution was determined by HPLC and the peak area was recorded as A_1. Then equal volume of the organic phase was added to the solution and mixed thoroughly. After the equilibration was established, the aqueous phase was determined by HPLC again and the peak area was recorded as A_2. The partition coefficient (K) was obtained by the following equation: $K = (A_1 - A_2)/A_2$.

2.5. HSCCC separation

In each separation process, the upper phase (stationary phase) and the lower phase (mobile phase) were pumped into the multiplayer-coiled column simultaneously by using ÄKTA prime system, according to a suitable volume ratio. When the column was totally filled with the two phases, only the lower phase was pumped at a flow rate of 2.0 ml min^{-1}, and at the same time, the HSCCC apparatus was run at a suitable revolution speed. After hydrodynamic equilibrium was reached (about half an hour), the sample solution was injected into the separation column. The separation temperature was controlled at 25 °C. The effluent from the outlet of the column was continuously monitored at 254 nm. Each peak fraction was manually collected according to the chromatogram and evaporated under reduced pressure. The residuals were dissolved in methanol for subsequent HPLC analysis.

2.5.1. Separation of neomangiferin and mangiferin from crude extracts

A solvent system consisting of *n*-butanol–acetic acid (1%) (1:1, v/v) was used as the two-phase solvent system of HSCCC for separation of neomangiferin (I) and mangiferin (II). In this separation process, the upper phase (stationary phase) and the lower phase (mobile phase) were pumped into the multiplayer-coiled column simultaneously by using ÄKTA prime system, according to the volume ratio of 40:60. When the column was totally filled with the two phases, only the lower phase was pumped at a flow rate of 2.0 ml min^{-1}, and at the same time, the HSCCC apparatus was run at a revolution speed of 700 rpm. After hydrodynamic equilibrium was reached (about half an hour), the sample solution (500 mg of crude extracts dissolved in 5 ml of the lower phase of the two-phase solvent system) was injected into the separation column. The separation temperature was controlled at 25 °C. The effluent from the outlet of the column was continuously monitored at 254 nm. The chromatogram was recorded 80 min after sample injection. Each peak fraction was manually collected according to the chromatogram and evaporated under reduced pressure. The residuals were dissolved in methanol for subsequent HPLC analysis.

After the separation procedure finished, the two-phase solvent in the separation column of HSCCC was blown out. The upper phase was separated and evaporated to dryness by rotary vaporization. The residual (refined sample) was used for HSCCC separation of *cis*-hinkiresinol and (-)-4′-*O*-methylnyasol.

2.5.2. Separation of cis-hinkiresinol and (-)-4′-O-methylnyasol

The gradient elution with the two-phase solvent system of light petroleum–ethyl acetate–methanol–water at volume ratios of 1:1:1.2:0.8 and 1:1:1.4:0.6 was employed for the separation of *cis*-hinkiresinol and (-)-4′-*O*-methylnyasol from the refined sample. In this separation process, the upper phase (stationary phase) and the lower phase (mobile phase) of light petroleum–ethyl acetate–methanol–water (1:1:1.2:0.8, v/v) were pumped into the multiplayer-coiled column simultaneously by using ÄKTA prime system, according to the volume ratio of 50:50. When the column was totally filled with the two phases, only the lower phase was pumped at a flow rate of 2.0 ml min^{-1}, and at the same time, the HSCCC apparatus was run at a revolution speed of 850 rpm. After hydrodynamic equilibrium was reached (about half an hour), the sample solution (80 mg of refined sample dissolved in 3 ml of the upper phase of the two-phase solvent system) was injected into the separation column. The gradient elution was as follows: in the first 100 min, only the lower phase of light petroleum–ethyl acetate–methanol–water (1:1:1.2:0.8, v/v) was pumped into the column. Then the volume ratio of the lower phase of light petroleum–ethyl acetate–methanol–water (1:1:1.2:0.8, v/v) and (1:1:1.4:0.6, v/v) was continuously changed from 100:0 to 0:100 in 30 min. The separation temperature was controlled at 25 °C. The effluent from the outlet of the column was continuously monitored at 254 nm. The chromatogram was recorded 60 min after the sample injection. Each peak fraction was manually collected according to the chromatogram and evaporated under reduced pressure. The residuals were dissolved in methanol for subsequent HPLC analysis.

2.6. HPLC analysis and identification of HSCCC peak fractions

The crude extracts and each HSCCC peak fraction were analyzed by HPLC. The analysis was accomplished with a YWG ODS C_{18} column (200 × 4.6 mm I.D., 10 μm) at room temperature. Methanol-phosphoric acid (0.1%) was used as the mobile phase in gradient elution mode as follows: methanol: 0–5 min, 10%; 5–18 min, 10–50%; 18–28 min, 50–100%. The flow-rate of the mobile phase was 1.0 ml min^{-1}. The effluents were monitored at 254 nm by a photodiode array detector.

Identification of the HSCCC peak fractions was performed by ^{1}H NMR and ^{13}C NMR. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Mercury Plus 400 NMR.

3. Results and discussion

3.1. Optimization of HPLC conditions

Several elution systems, such as methanol–water, acetonitrile–water, methanol–phosphoric acid, were tested for HPLC analysis separation of crude extracts. When methanol–phosphoric acid (0.1%) was used as the mobile phase in gradient elution mode (methanol: 0–5 min, 10%; 5–18 min, 10–50%; 18–28 min, 50–100%), good results could be obtained. The crude extracts and peak fractions separated by HSCCC were analyzed by HPLC under the optimum analytical conditions. The chromatograms were shown in Fig. 2.

3.2. Selection of two-phase solvent system and other conditions of HSCCC

Successful separation by HSCCC depends upon the selection of a suitable two-phase solvent system, which provides an ideal range of the partition coefficient (*K*) for the targeted compounds.

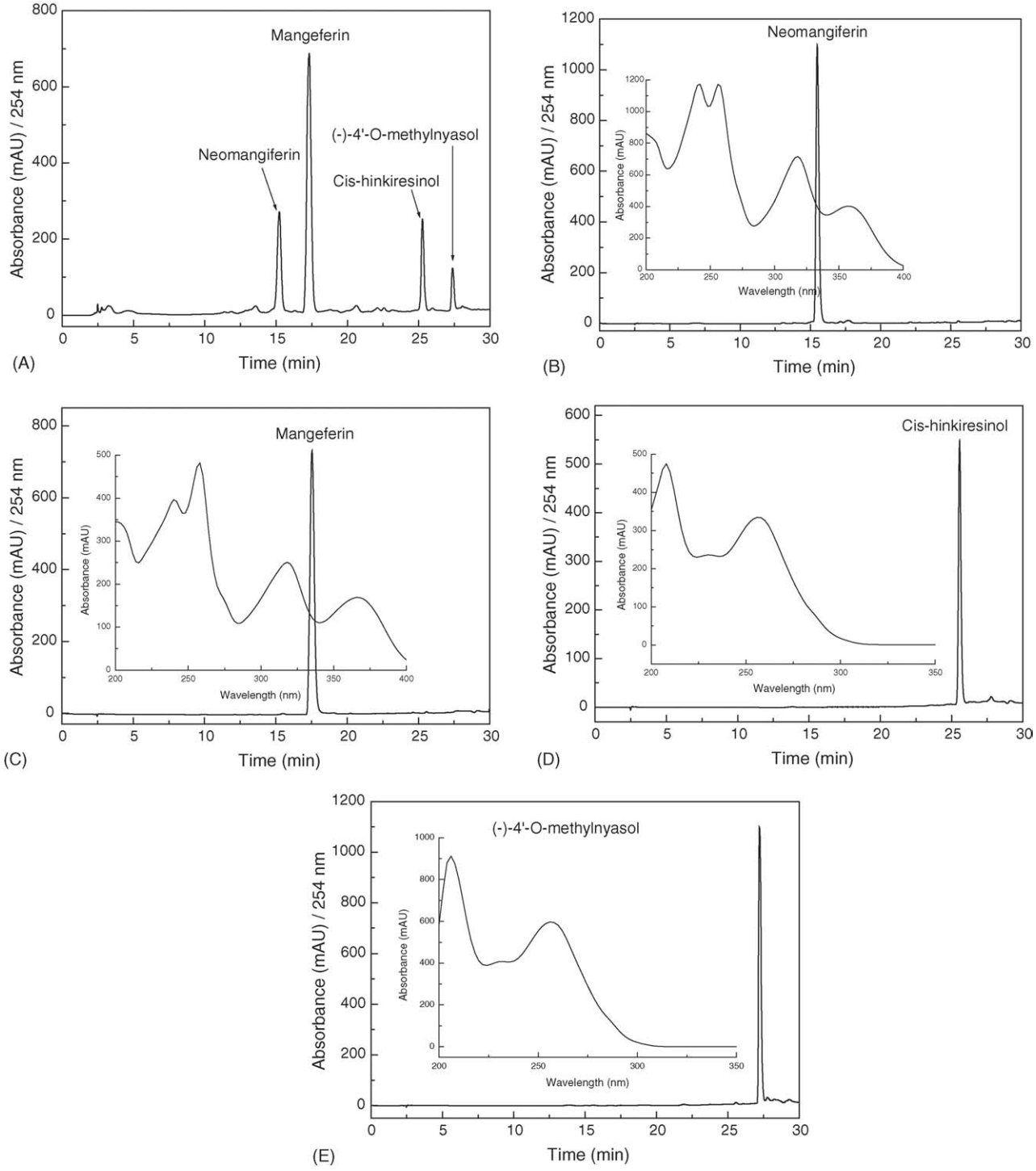

Fig. 2. HPLC chromatograms of crude extracts from *Rhizoma Anemarrhenae* (A) and HSCCC peak fractions (B–E). Column: YWG ODS C_{18} column (200 × 4.6 mm I.D., 10 μm); mobile phase: methanol-phosphoric acid (0.1%) (Methanol: 0–5 min, 10%; 5–18 min, 10–50%; 18–28 min, 50–100%); flow rate: 1.0 ml min^{-1}; detection wavelength: 254 nm.

Several two-phase solvent systems were tested and the *K*-values were measured and summarized in Table 1.

The ideal *K*-value of the compound separated by HSCCC is between 0.5 and 5.0. According to the *K*-values shown in Table 1, it can be seen that no two-phase solvent system is suitable for separation of the target compounds by a one step HSCCC separation. The *K*-values of neomangiferin and mangeferin were very small and that of *cis*-hinkiresinol and (-)-4′-*O*-methylnyasol were very big when ethyl acetate-methanol-water systems in different volume ratios were used as the two-phase solvent systems. When *n*-butanol–methanol–acetic acid systems in different volume ratios were used as the two-phase solvent systems, the *K*-values of neomangiferin and mangeferin were suitable but that of *cis*-hinkiresinol and (-)-4′-*O*-methylnyasol

Table 1
The *K*-values of target components measured in several solvent systems

Solvent system (v/v)	*K*			
	Neomangiferin	Mangiferin	*cis*-Hinkiresinol	(-)-4′-*O*-Methylnyasol
Ethyl acetate–water (1:1)	0.02	0.05	∞	∞
Ethyl acetate–methanol–water (1:0.1:1)	0.06	0.09	15.71	29.0
Ethyl acetate–methanol–water (1:0.3:1)	0.09	0.29	10.39	13.20
n-Butanol–acetic acid (1%) (1:1)	0.97	3.89	∞	∞
n-Butanol–methanol–acetic acid (1%)(1:0.2:1)	0.74	2.38	35.65	∞
Light petroleum–ethyl acetate–methanol–water (1:1:0.8:1.2)	<0.01	<0.01	8.84	∞
Light petroleum–ethyl acetate–methanol–water (1:1:1:1)	<0.01	<0.01	2.50	14.07
Light petroleum–ethyl acetate–methanol–water (1:1:1.2:0.8)	<0.01	<0.01	0.81	4.78
Light petroleum–ethyl acetate–methanol–water (1:1:1.4:0.6)	<0.01	0.02	0.22	1.56

The symbol '∞' means the partition coefficient is too big that cannot be evaluated.

very big. When light petroleum–ethyl acetate–methanol–water systems in different volume ratios were used, the *K*-values of *cis*-hinkiresinol and (-)-4′-*O*-methylnyasol were suitable but that of neomangiferin and mangeferin very small. So a two-step separation procedure was used for separation of the four target compounds. A two-phase solvent system, *n*-butanol–acetic acid (1%), was chosen to separate neomangiferin and mangeferin, and another two-phase solvent system, light petroleum–ethyl acetate–methanol–water, was chosen to separate *cis*-hinkiresinol and (-)-4′-*O*-methylnyasol.

According to the *K*-values shown in Table 1, *n*-butanol–acetic acid (1%) (1:1, v/v) and *n*-butanol–methanol–acetic acid (1%) (1:0.2:1, v/v) were suitable for the separation of neomangiferin and mangeferin. So these two solvent systems were tested for HSCCC separation of neomangiferin and mangeferin. When *n*-butanol–methanol–acetic acid (1%) (1:0.2:1, v/v) was used as the two-phase solvent system, the stationary phase of HSCCC lost seriously and the purity of mangiferin was only 85.6%. When *n*-butanol–acetic acid (1%) (1:1,v/v) was used as the two-phase solvent system, good separation results could be obtained and the purity of neomangiferin and mangeferin was 96.3 and 98.0%, respectively. So *n*-butanol–acetic acid (1%) (1:1,v/v) was used as the two-phase solvent system for the separation and purification of neomangiferin and mangeferin. Under this condition, *cis*-hinkiresinol and (-)-4′-*O*-methylnyasol were still maintained in the stationary phase.

Light petroleum–ethyl acetate–methanol–water in different volume ratios was chosen as the two-phase solvent systems to separate *cis*-hinkiresinol and (-)-4′-*O*-methylnyasol. The *K*-value of *cis*-hinkiresinol and (-)-4′-*O*-methylnyasol in light petroleum–ethyl acetate–methanol–water (1:1:1.2:0.8, v/v) was 0.65 and 3.98, and 0.22 and 1.56 in light petroleum–ethyl acetate–methanol–water (1:1:1.4:0.6, v/v). When light petroleum–ethyl acetate–methanol–water (1:1:1.2:0.8, v/v) was used as the two-phase solvent system, the separation time of (-)-4′-*O*-methylnyasol was too long. But when light petroleum–ethyl acetate–methanol–water (1:1:1.4:0.6, v/v) was used as the two-phase solvent system, the *K*-value of *cis*-hinkiresinol was too small and the purity of *cis*-hinkiresinol is lower than 90%. So the gradient elution mode was tested. The upper phase of light petroleum–ethyl acetate–methanol–water (1:1:1.2:0.8, v/v) was used as the stationary phase. The lower phase of light petroleum–ethyl acetate–methanol–water (1:1:1.2:0.8, v/v) and light ppetroleum–ethyl acetate–methanol–water (1:1:1.4:0.6, v/v) was used as the mobile phase in gradient elution mode as follows: 0–100 min, only the lower phase of 1:1:1.2:0.8 system; 100–130 min, the volume ratio of 1:1:1.2:0.8 and 1:1:1.4:0.6 system was continuously changed from 100:0 to 0:100. Under this separation condition good separation results could be obtained.

Other conditions such as the revolution speed of the separation column, the flow rate of the mobile phase and the separation temperature, were also investigated. For separation of neomangiferin and mangeferin from crude extracts, the stationary phase lost seriously when the revolution speed was high. Reducing the revolution speed of the separation column could improve the maintenance of the stationary phase in this experiment. Ultimately, a flow rate of 2.0 ml min^{-1}, revolution speed of 700 rpm and separation temperature of 25 °C was employed for separation of neomangiferin and mangeferin. For separation of *cis*-hinkiresinol and (-)-4′-*O*-methylnyasol, a flow rate of 2.0 ml min^{-1}, revolution speed of 850 rpm and separation temperature of 25 °C was employed.

The crude extracts was separated and purified under the optimum HSCCC conditions. The typical HSCCC chromatogram was shown in Fig. 3A. 35.3 mg of neomangiferin (I) and 245.4 mg of mangeferin (II) were obtained from 500 mg of crude extracts in a one-step separation. After the separation of mangeferin (II) was finished, the stationary phase was blown out and evaporated to dryness. 80 mg of refined sample was obtained. The refined sample was then separated in the gradient HSCCC separation. The HSCCC chromatogram was shown in Fig. 3B. 17.2 mg of *cis*-hinkiresinol (III), and 12.4 mg of (-)-4′-*O*-methylnyasol (IV) were obtained from the refined sample. The purity of neomangiferin, mangeferin, *cis*-hinkiresinol and (-)-4′-*O*-methylnyasol was 96.3, 98.0, 97.3 and 98.2%, respectively, as determined by HPLC. The chromatograms of HPLC and UV spectra of these components were shown in Fig. 2.

3.3. The structural identification

The chemical structure of each peak fraction of HSCCC was identified according to its ^{1}H NMR and ^{13}C NMR data.

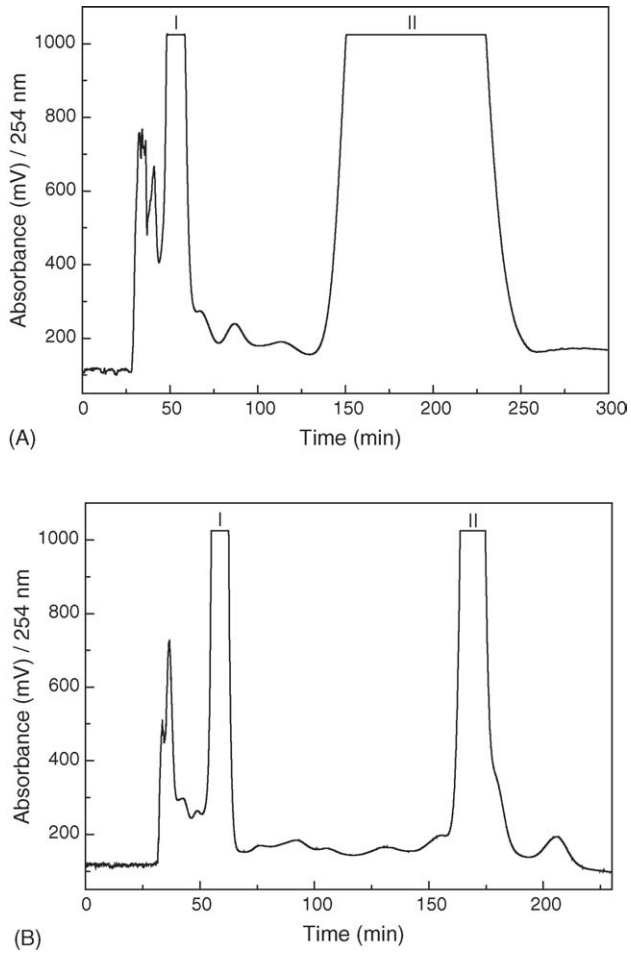

Fig. 3. HSCCC chromatograms of crude extracts (A) and refined sample (B) from *Rhizoma Anemarrhenae*. Conditions of: (A) Two-phase solvent system: *n*-butanol–acetic acid (1%) (1:1, v/v); mobile phase: the lower phase; flow rate: 2.0 ml min^{-1}; revolution speed: 700 rpm; detection wavelength: 254 nm; sample size: 500 mg of crude extracts dissolved in 5 ml of the lower phase; separation temperature: 25 °C. (I) neomangiferin (collected during 47–61 min); (II) mangiferin (collected during 144–238 min). Conditions of: (B) Two-phase solvent system: light petroleum–ethyl acetate–methanol–water (1:1:1.2:0.8, 1:1:1.4:0.6, v/v) in gradient elution; Stationary phase: upper organic phase of 1:1:1.2:0.8; mobile phase: lower phase of 1:1:1.2:0.8 and 1:1:1.4:0.6 used in gradient elution mode as follows: 0–100 min, only the lower phase of 1:1:1.2:0.8 system; 100–130 min, the volume ratio of 1:1:1.2:0.8 and 1:1:1.4:0.6 system was continuously changed from 100:0 to 0:100; flow rate: 2.0 ml min^{-1}; revolution speed: 850 rpm; detection wavelength: 254 nm; sample size: 80 mg of refined sample dissolved in 3 ml of the upper phase of light petroleum–ethyl acetate–methanol–water (1:1:1.2:0.8, v/v); separation temperature: 25 °C. (I) *cis*-hinkiresinol (collected during 54–63.5 min); (II) (-)-4′-*O*-methylnyasol (collected during 162–176 min).

Peak I in Fig. 3A: ^{1}H NMR (400 MHz, DMSO): 6.37 (1H, s, H-4), 6.93 (1H, s, H-5), 7.69 (1H, s, H-8), 4.57 (1H, d, 2-Glu, H-1′, J = 9.2 Hz), 4.87 (1H, d, 7-Glu, H-1′, J = 7.6 Hz); ^{13}C NMR (400 MHz, DMSO): 162.5 (C-1), 108.3 (C-2), 164.5 (C-3), 94.0 (C-4), 103.3 (C-5), 156.9 (C-6), 144.4 (C-7), 112.4 (C-8), 179.8 (C-9), 154.7 (C-4a), 151.5 (C-4b), 108.8 (C-8a), 102.0 (C-8b); 2-Glu: 73.8 (C-1′), 71.3 (C-2′), 79.7 (C-3′), 71.0 (C-4′), 82.2 (C-5′), 61.4 (C-6′); 7-Glu: 103.4 (C-1′), 73.5 (C-2′), 76.1 (C-3′), 69.6 (C-4′), 77.3 (C-5′), 60.7 (C-6′). Compared with the data given in reference [6], it was identified as neomangiferin.

Peak II in Fig. 3A: ^{1}H NMR (400 MHz, DMSO): 6.34 (1H, s, H-4), 6.84 (1H, s, H-5), 7.35 (1H, s, H-8), 4.56 (1H, d, H-1′, J = 9.6 Hz); ^{13}C NMR (400 MHz, DMSO): 162.5 (C-1), 108.3 (C-2), 164.5 (C-3), 93.8 (C-4), 103.3 (C-5), 154.7 (C-6), 144.4 (C-7), 108.8 (C-8), 179.8 (C-9), 156.9 (C-4a), 151.5 (C-4b), 112.4 (C-8a), 102.0 (C-8b); 2-Glu: 82.3 (C-1′), 73.8 (C-2′), 71.3 (C-3′), 70.9 (C-4′), 79.7 (C-5′), 62.2 (C-6′). Compared with the data given in reference [3], it was identified as mangiferin.

Peak I in Fig. 3B: ^{1}H NMR (400 MHz, $CDCl_3$): 6.51 (1H, d, H-1, J = 11.6 Hz), 5.67 (1H, t, H-2, J = 10.6 Hz), 4.48 (1H, t, H-3, J = 15.6 Hz), 6.00 (1H, m, H-4), 5.17 (2H, t, H-5, J = 8.4 Hz), 7.17 (2H, d, H-2′, H-6′, J = 8.0 Hz), 6.78 (2H, s, H-3′, H-5′), 7.10 (2H, d, H-2″, H-6″, J = 8.0 Hz), 6.78 (2H, s, H-3″, H-5″); ^{13}C NMR (400 MHz, $CDCl_3$): 128.6 (C-1), 131.7 (C-2), 46.8 (C-3), 140.7 (C-4), 115.0 (C-5), 129.8 (C-1′), 130.0 (C-2′, C6′), 115.1 (C-3′, C-5′), 154.6 (C-4′), 135.5 (C-1″), 128.9 (C-2″, C-6″), 115.4 (C-3″, C-5″), 154.1 (C-4″). Compared with the data given in reference [7], it was identified as *cis*-hinkiresinol.

Peak II in Fig. 3B: ^{1}H NMR (400 MHz, $CDCl_3$): 6.53 (1H, d, H-1, J = 11.2 Hz), 5.68 (1H, t, H-2, J = 10.6 Hz), 4.50 (1H, t, H-3, J = 7.8 Hz), 6.00 (1H, m, H-4), 5.14–5.19 (2H, m, H-5), 7.22 (2H, d, H-2′, H-6′, J = 8.0 Hz), 6.86 (2H, d, H-3′, H-5′, J = 7.6 Hz), 7.10 (2H, d, H-2″, H-6″, J = 7.6 Hz), 6.78 (2H, d, H-3″, H-5″, J = 8.0 Hz), 3.81 (3H, s, H-OCH_3); ^{13}C NMR (400 MHz, $CDCl_3$): 128.6 (C-1), 131.7 (C-2), 46.8 (C-3), 140.7 (C-4), 115.0 (C-5), 129.8 (C-1′), 129.7 (C-2′, C-6′), 113.6 (C-3′, C-5′), 158.5 (C-4′), 135.6 (C-1″), 128.9 (C-2″, C-6″), 115.4 (C-3″, C-5″), 154.1 (C-4″), 55.2 (C-OCH_3). Compared with the data given in reference [8], it was identified as (-)-4′-*O*-methylnyasol.

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