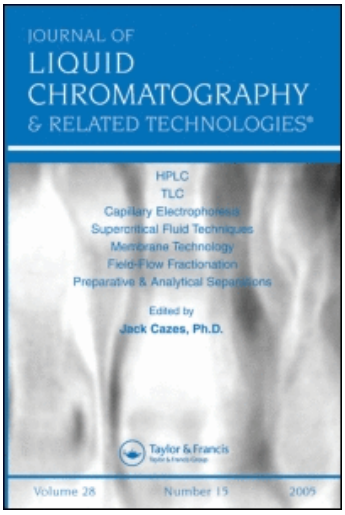

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Preparation of Triterpene Saponins from the Fruit of *Momordica Charantia L.* by High Speed Countercurrent Chromatography (HSCCC)

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Preparation of Triterpene Saponins from the Fruit of *Momordica Charantia L.* by High Speed Countercurrent Chromatography (HSCCC)

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Abstract: High speed countercurrent chromatography (HSCCC) was used for the separation of triterpene saponins of two components from silica gel column chromatography of bitter gourd crude extract (*Momordica charantia L*), with two biphasic solvent systems composed of methyl *tert*-butyl ether-n-butanol-methnaol-water in the proportions of 1 : 2 : 1 : 5 and 1 : 3 : 1 : 5 (v/v), respectively. Four compounds, goyaglycoside-e, momordicoside L, goyaglycoside-a, and momordicoside K were obtained and confirmed by means of ESI-MS, ^{1}H- and ^{13}C-NMR.

Keywords: *Momordica charantia*, Triterpene saponins, High speed countercurrent chromatography, Preparative isolation

INTRODUCTION

Bitter gourd is the fruit of *Momordica charantia L.*, which belongs to the cucurbiaceae family. It is cultivated in South China as a traditional vegetable. It is also commonly used as a traditional remedy for diabetes in China, India, Africa, and South America. The fruit, leaves, seeds, and roots of *M. charantia* have been used in the Indian system of medicine for a number of diseases, besides diabetes. The unripe fruits of this plant have been shown to produce a hypoglycemic effect in experimental models following oral administration.[1–6] Many clinical trials have also confirmed the hypoglycemic

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action.[7] Some investigators have attempted to purify the active fractions from fruits of *M. charantia* in order to establish the precise mechanism of its hypoglycemic effects.[8] Triterpene saponins, such as momordisides and goyaglycoside[9,10] are one kind of diagnostic components in the fruit of *M. charantia*, which are possibly bioactive components. In the present study, the preparative separation of triterpene saponins with high speed countercurrent chromatography (HSCCC)[11] is described. The structural identification of the obtained triterpene saponins are done by ESI-MS, ^{1}H- and ^{13}C-NMR experiments.

EXPERIMENTAL

Reagents

The organic solvents, n-butanol methyl tert-butyl ether and methanol used for HSCCC separation were of analytical grade. Water was nanopure quality.

Preparation of Crude Triterpene Saponins

Freeze-dried bitter gourd (500 g) was powdered and soaked two times with 90% methanol for 5 hours at 50°C. Each time 2.5 liters of 90% methanol was used. The methanolic extract solution was combined, evaporated, and freeze-dried to yield 63.5 g extract, which was subjected to silica gel column chromatography. After the extract was loaded to the column filled with 3 kg of silica gel (100 mesh), step-elution of chloroform-methanol-water (15 : 4 : 1, 15 : 6 : 1, 15 : 8 : 1) was performed to yield 1–10 fractions in which fraction 7 and fraction 8 contained triterpene saponins, demonstrated by TLC. Fraction 7 and fraction 8 were evaporated and freeze-dried to yield 170 mg of Fr. 7 and 90 mg of Fr. 8 for HSCCC separation.

Selection of Solvent System for HSCCC

TLC was used for checking the partition of the saponins of component 7 and component 9 in the two phases of a series of solvent systems composed of methyl tert-butyl ether, n-butanol, methanol, and water in different proportions. Finally, methyl tert-butyl ether-n-butanol-methanol-water (1 : 2 : 1 : 5, v/v) and methyl tert-butyl ether-n-butanol-methanol-water (1 : 3 : 1 : 5, v/v) were chosen as a solvent system for the separation of Fr. 7 and Fr. 8, respectively.

TLC Analysis

TLC of triterpene saponins was conducted on GF254 plates (Merck, Germany), which was developed with chloroform-methanol-water (15 : 4 : 1) and colorized with 5% H_2SO_4 in ethanol at 110°C.

HPLC-MS Analysis

A system composed of Waters 2690–996 Photodiode Array Detector (PAD)-Bruker Esquire ion trap multiple mass spectrometer (MS) was used for HPLC-electronic spray ionization (ESI)-MS analysis. HPLC separation was performed on a Waters Symmetry C18, 5 μ (3.9 × 150 mm) column at 30°C, eluted by a gradient solvent system composed of A (H_2O with 0.05% HCOOH (v/v)) and B (CH_3CN with 0.05% HCCOH (v/v)). Initial condition of the gradient was 95% A and 5% B, a linear gradient in 25 min to 0% A and 100% B, 100% B from 25 to 30 min, and back to initial condition from 30 to 35 min. All ESI-MS experiments were conducted in positive and negative ionization mode analyzing ions up to m/z 2200.

High Speed Countercurrent Chromatography (HSCCC)

The instrument was a Model GS10A high speed countercurrent chromatograph (Beijing Institute of New Technology Application, Beijing, China) equipped with a multilayer coil made of 130 m, 1.6 mm I.D. PTFE tubing. The standard HSCCC separation procedure was four steps. First, the solvent system was prepared thorough equilibration of methyl *tert*-butyl ether, n-butanol, acetonitrile, and water with the given proportion in a separatory funnel. The two resulting phases were separated shortly before use. Second, the multilayer coil column was entirely filled with the upper organic phase as the stationary phase, and then the apparatus was rotated at 800 rpm for injection of a sample. Third, the crude triterpene saponins dissolved in 10 mL of mobile phase was injected to the HSCCC system through a Teflon sample loop. Finally, the aqueous mobile phase was pumped at a flow rate of 1.5 mL/min by a Waters 510 pump (Waters, Milford, MA, USA). The effluent was collected with a fraction collector BS100 (Shanghai Puxi Instrument Factory, Shanghai, China). The composition in each fraction was analyzed with TLC analysis.

Nuclear Magnetic Resonance (NMR) Analysis

^{1}H- and ^{13}C-NMR experiments were recorded in pyride-d_5 on a Bruker Advance 400 MHz NMR spectrometer, respectively.

RESULTS AND DISCUSSION

HSCCC Separation

Figure 1 presents the TLC analysis of fractions from HSCCC separation of 170 mg Fr. 7 with the two-phase solvent system composed of methyl

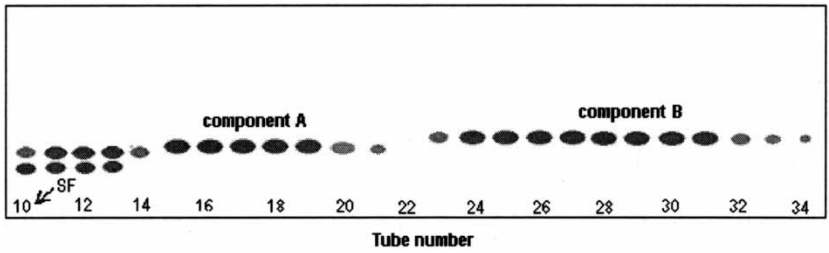

Figure 1. TLC analysis of fractions from HSCCC separation of 170 mg Fr. 7. SF: solvent front of mobile phase. TLC plate: GF254. Flow rate: 1.5 mL/min; Fraction volume: 10 mL.

tert-butyl ether-n-butanol-methanol-water (1 : 2 : 1 : 5, v/v). The HSCCC run yielded two components A and B with a single spot on the TLC plate (Figure 1). The combined fractions were evaporated under reduced pressure and subsequent lyophilization to yield 57 mg of component A (tubes 15–19) and 33 mg of component B (tubes 23–32). Figure 2 shows the TLC analysis of fractions from HSCCC separation of 90 mg Fr. 8 with the two-phase solvent system composed of methyl tert-butyl ether-n-butanol-methanol-water (1 : 3 : 1 : 5, v/v). The HSCCC separation yielded two components, A and B, with a single spot on the TLC plate (Figure 2). The combined fractions were evaporated under reduced pressure and subsequent lyophilization to yield 27 mg of component I (tubes 12–16) and 43 mg of component II (tubes 24–31). Components I, II, A, and B were very pure, demonstrated by analysis of HPLC-PDA-ESI-MS (Figure 3).

Confirmation of Chemical Structures

Components I, II, A, and B were analyzed by ESI-MS and ^{1}H-NMR and ^{13}C-NMR experiments. The data demonstrated the four components were

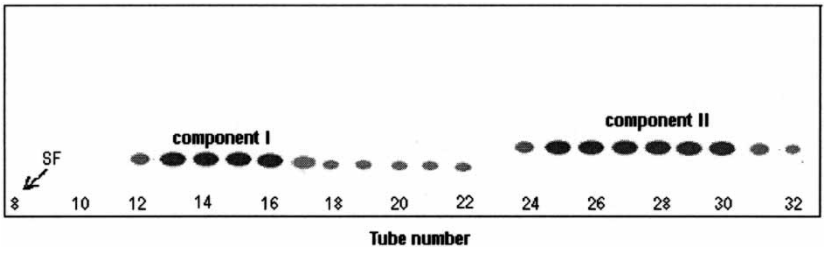

Figure 2. TLC analysis of fractions from HSCCC separation of 90 mg Fr. 8. SF: solvent front of mobile phase; TLC plate: GF254. Flow rate: 1.5 mL/min; Fraction volume: 10 mL.

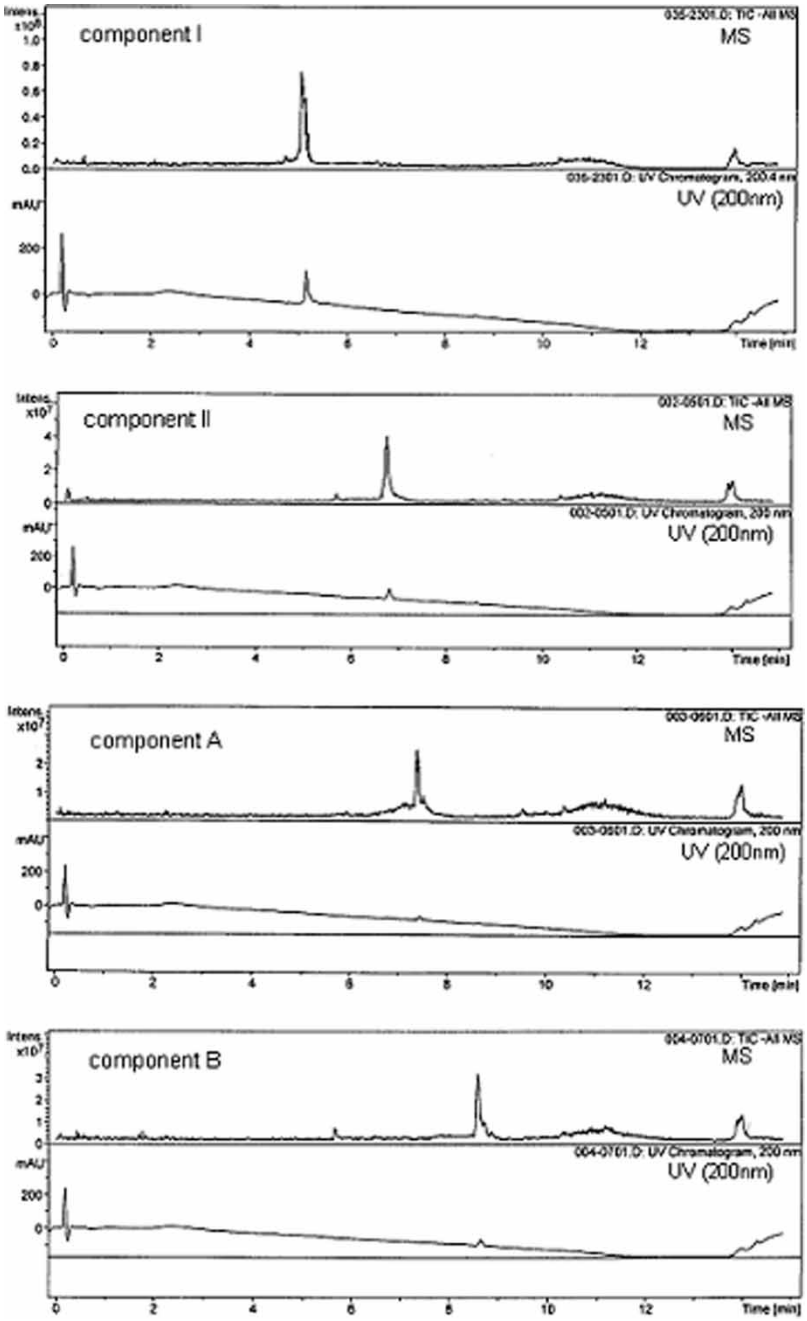

Figure 3. HPLC-PAD-MS analysis of the four components from HSCCC separations.

goyaglycoside-e, momordicoside L, goyaglycoside-a, and momordicoside K (Figure 4), compared to those data in ref. 9 and 10, respectively. Our data were listed as follows:

Momordicoside K (**A**): ESI-MS: positive, m/z 687 $[M + K]^+$; negative, m/z 693 $[M + HCOO]^-$. ^{1}H-NMR(pyridine-d_5): 0.77, 0.88, 1.15, 1.45, 1.53, (all s, 29, 30, 18, 21, 28, CH_3), 3.81 (brs, C3-H), 5.92 (brs, C23-H, C24-H), 10.42 (s, CHO). ^{13}C-NMR (pyridine-d_5): 22.8 (C-1), 30.3 (C-2), 78.6 (C-3), 42.9 (C-4), 148.4 (C-5), 123.9 (C-6), 73.9 (C-7), 51.8 (C-8), 51.54 (C-9), 36.07 (C-10), 23.7 (C-11), 30.5 (C-12), 47.0 (C-13), 47.4 (C-14), 37.9 (C-15), 28.9 (C-16), 47.1 (C-17), 15.9 (C-18), 210.7 (C-19), 36.3 (C-20), 19.7 (C-21), 40.7 (C-22), 126.3 (C-23), 141.4 (C-24), 71.47 (C-25), 30.6 (C-26), 30.6 (C-27), 26.5 (C-28), 28.3 (C-29), 19.4 (C-30), 38.1 (19-OMe).

Goyaglycoside-a (**B**): ESI-MS: positive, m/z 687 $[M + K]^+$; negative, m/z 693 $[M + HCOO]^-$. ^{1}H-NMR (pyridine-d_5): 0.86, 0.89, 0.90, 0.97, 1.47 (all s, 29, 30, 18, 21, 28, H_3), 1.54 (s, 26, 27-H_3), 3.12 (dd, 8-H), 3.44 (s, 19-OMe), 3.73 (brs, 3-H), 4.84 (s, 119-H), 5.62 (dd, J = 3.6, 9.6 Hz, 7-H), 5.92 (m, 23, 24-H), 6.16 (dd, J = 2.0, 9.6 Hz, 6-H). ^{13}C-NMR (pyridine-d_5): 18.7 (C-1), 27.4 (C-2), 83.8 (C-3), 39.4 (C-4), 85.5 (C-5), 133.2 (C-6), 131.6 (C-7), 42.3 (C-8), 48.2 (C-9), 41.7 (C-10), 23.4 (C-11), 30.8 (C-12), 45.3 (C-13), 48.3 (C-14), 33.9 (C-15), 28.2 (C-16), 50.4 (C-17), 14.9 (C-18), 112.4 (C-19), 36.6 (C-20), 18.9 (C-21), 39.6 (C-22), 124.4 (C-23), 141.7 (C-24), 69.7 (C-25), 30.9 (C-26), 30.8 (C-27), 21.2 (C-28), 24.9 (C-29), 20.0 (C-30), 57.6 (19-OMe), 105.4 (C′-1), 76.2 (C′-2), 78.3 (C′-3), 72.1 (C′-4), 78.0 (C′-5), 63.1 (C′-6).

Goyaglycoside-e (**I**): ESI-MS: positive, m/z 819$[M + K]^+$; negative, m/z 827 $[M + HCOO]^-$. ^{1}H-NMR (pyridine-d_5): 0.87, 0.90, 0.92,0.98, 1.49

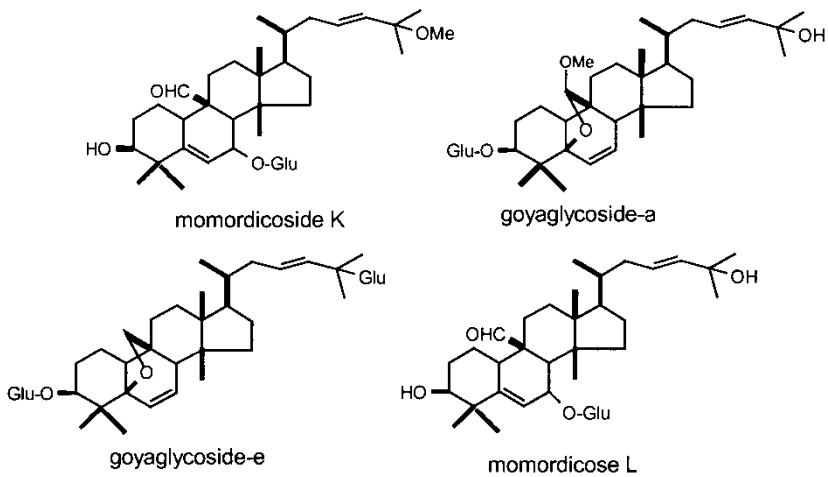

Figure 4. Chemical structures of components. Glu: glucoside; momordicose K(**A**), goyaglycoside-a (**B**), goyaglycoside-e (**I**) and momordicose L (**II**).

(all s, 29, 30, 18, 21, 28, H_3), 2.31 (brs, 8-H), 3.64(brs, 3-H), 3.65, 3.71(ABq, J = 7.9 Hz, 19-H2), 5.56 (dd, J = 3.3, 9.9 Hz, 7-H), 5.79 (23-H), 6.01(d, J = 15.8 Hz, 24-H), 6.18 (d, J = 9.9 Hz, 6-H). ^{13}C-NMR (pyridine-d_5): 18.9 (C-1), 27.6 (C-2), 85.1 (C-3), 39.0 (C-4), 86.0 (C-5), 134.2 (C-6), 130.0 (C-7), 52.3 (C-8), 45.2 (C-9), 40.2 (C-10), 23.8 (C-11), 31.2 (C-12), 45.5 (C-13), 48.8 (C-14), 33.5 (C-15), 28.2 (C-16), 50.5 (C-17), 15.1 (C-18), 80.2 (C-19), 36.6 (C-20), 19.1 (C-21), 39.8 (C-22), 128.4 (C-23), 138.7 (C-24), 77.7 (C-25), 28.7 (C-26), 27.8 (C-27), 21.1 (C-28), 25.6 (C-29), 20.2 (C-30), 103.8 (C′-1), 73.1 (C′-2), 72.3 (C′-3), 69.3 (C′-4), 76.0 (C′-5), 63.4 (C′-6), 99.8 (C″-1), 75.3 (C″-2), 78.7 (C″-3), 71.9 (C″-4), 78.0 (C′-5), 63.2 (C′-6).

Momordicoside L (**II**): EIS-MS: positive, m/z 673 [$M + K^+$]; negative, m/z 679 [$M + HCOO^-$]. ^{1}H-NMR (pyridine-d_5): 0.78, 0.87, 0.95, 1.12, 1.31, (all s, 29, 30, 18, 21, 28, CH_3), 3.21 (3H, 3, -OCH_3), 3.79 (H, brs, C3-H), 5.57 (2H, brs, C23-H), 6.17 (H, brd, J = 6 Hz, C6-H), 10.43 (s, -CHO). ^{13}C-NMR(pyridine-d_5): 22.6 (C-1), 30.1 (C-2), 77.4 (C-3), 42.7 (C-4), 148.2 (C-5), 123.7 (C-6), 73.8 (C-7), 51.6 (C-8), 51.5 (C-9), 36.1(C-10), 23.5(C-11), 30.3(C-12), 46.9(C-13), 49.1(C-14), 37.7(C-15), 28.7 (C-16), 47.3 (C-17), 15.7 (C-18), 210.6 (C-19), 36.1 (C-20), 19.5 (C-21), 40.5 (C-22), 126.1 (C-23), 141.2 (C-24), 71.5 (C-25), 30.4 (C-26), 30.4 (C-27), 26.3 (C-28), 28.1 (C-29), 19.16 (C-30), 102.5 (C′-1), 75.3 (C′-2), 78.3 (C′-3), 72.0 (C′-4), 77.3 (C′-5), 63.1 (C′-6) 102.6 (C′-1), 75.5 (C′-2), 78.5 (C′-3), 71.7 (C′-4), 77.5 (C′-5), 63.3 (C′-6).

CONCLUSION

HSCCC is an effective methodology for isolation of the triterpene glycosides on a laboratory scale, which can be used for preparation of pure triterpene glycosides in *Momordica charantia*, available for further biological studies.

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