Preparative Purification of Morroniside and Loganin from *Fructus corni* by Combination of Macroporous Absorption Resin and HSCCC

Liangpo Liu, Ailing Sun, Sujuan Wu, and **Renmin Liu***
College of Chemistry and Chemical Engineering, Liaocheng University, Liaocheng, 252059, PR China

Abstract

A method for preparative purification of loganin and morroniside from *Fructus corni* was established by combination of macroporous absorption resin column separation and high-speed counter-current chromatography (HSCCC). The crude extracts obtained from *Fructus corni* by ultrasonic extraction with 50% methanol were subjected to separation on a macroporous resin column and then eluted with 15% and 40% ethanol, respectively. A fraction of 40% ethanol was used as the sample for separation of morroniside and loganin by HSCCC. The two-phase solvent system used for HSCCC separation was *n*-butanol–methanol–1% acetic acid water (4:1:6, v/v). The upper phase was used as the stationary phase of HSCCC. Morroniside (28.7 mg) of 97.8% purity and loganin (11.5 mg) of 98.6% purity were obtained in a one-step HSCCC separation from 50 mg of sample. The structures of morroniside and loganin were identified by ^{1}H-NMR and ^{13}C-NMR.

Introduction

Fructus corni, the fruit of *Cornus officinalis* Sieb. et Zucc., is one of the more commonly used herbal medicines in China and officially listed in Chinese Pharmacopoeia (1). Pharmacological tests have revealed that *Fructus corni* can reduce blood sugar, improve immunity, and counteract inflammation and shock (2). Morroniside and loganin (Figure 1) are the main bioactive components of *Fructus corni*. Recent research has shown that loganin has the effects of immuno-regulation, anti-inflammatory, and anti-hemorrhagic shock, whereas morroniside has the effects of stomach strengthening and prevention of diabetic angiopathy (3–5). Thus, the separation and purification of morroniside and loganin is of great importance for pharmacological studies. A method for the preparation of loganin and morroniside from *Fructus corni* has been reported previously (6). Macroporous resin, silica gel column chromatography, and preparative high-performance liquid chromatography (HPLC) were employed, and many separation steps were needed in the method.

High-speed counter-current chromatography (HSCCC) is a kind of liquid–liquid partition chromatography which was first invented by Y. Ito (7). Being a support-free liquid–liquid partition chromatographic technique, HSCCC eliminates irreversible adsorption of the sample onto the solid support (8). Therefore, it is suitable for the preparative separation of natural products such as traditional Chinese medicinal herbs. Many successful applications of HSCCC have been reported for the separation of various components, such as alkaloids (6–12), flavonoids (13–17), coumarins (18–21), phenanthraquinones, and anthraquinones (22–27). But there is no report about the separation and purification of loganin and morroniside from *Fructus corni* by HSCCC.

In the present study, an HSCCC method for the preparative purification of morroniside and loganin from *Fructus corni* was established by using *n*-butanol–methanol–1% acetic acid water (4:1:6, v/v) as the two-phase solvent system. Macroporous absorption resin was used for the pretreatment of crude samples before the HSCCC separation. Morroniside and loganin were purified successfully in a one-step HSCCC separation. The present work established a successful HSCCC method for the separation and purification of morroniside and loganin from *Fructus corni* for the first time.

Experimental

Reagents and materials

All solvents used for preparation of crude samples and HSCCC separation were of analytical grade (Jinan Reagent Factory, Jinan, China). Methanol used for HPLC analysis was of chromatographic grade (Yucheng Chemical Factory, Yucheng, China), and water used was distilled water.

Fructus corni was purchased from a local drug store (Limin drugstore) and identified by Professor Yongqing Zhang (Shandong University of Traditional Chinese Medicine, Jinan, China).

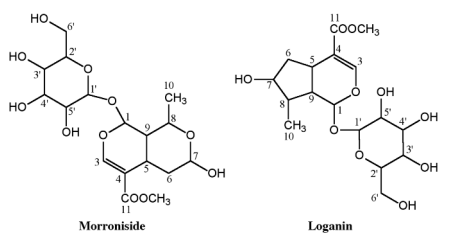

Figure 1. Chemical structures of morroniside and loganin.

* Author to whom correspondence should be addressed: email liurenmin@lcu.edu.cn.

Apparatus

The HSCCC instrument used in the present study was a TBE-300A high speed counter-current chromatography (Tauto biotechnique Company, Shanghai, China) with three multiplayer coil separation columns connected in series (i.d. of the tubing = 1.6 mm, total volume = 260 mL) and a 20-mL sample loop. The revolution radius was 5 cm, and the β values of the multilayer coil varied from 0.5 at the internal terminal to 0.8 at the external terminal. 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 Co., Ltd., Beijing, China) was used to control the separation temperature. An ÄKTA prime (Amersham Pharmacia Biotechnique Group, Uppsala, Sweden) was used to pump the two-phase solvent system and perform the UV absorbance measurement. It contained a switch valve and a mixer, which were used for gradient formation. The data were collected with a Sepu 3000 chromatography workstation (Hangzhou Puhui Science Apparatus Co., Ltd., Hangzhou, China).

A FZ102 plant disintegrator (Taisite Instrument Company, Tianjin, China) was used for disintegration of the sample. The HPLC equipment used was Agilent 1100 HPLC system including a 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, Waldbronn, Germany). The nuclear magnetic resonance (NMR) spectrometer used here was a Mercury Plus 400 NMR (Varian, Palo Alto, CA).

Preparation of sample

Fructus corni was dried constant at 60°C and pulverized to ~ 30-mesh with a disintegrator. The power (500 g) was extracted with 3000 mL methanol (50%) by ultrasonic for 15 min. The extraction procedure was then repeated twice (2000 mL each time), and the extracts were combined together. After filtration with a ceramic filter, the filtrate was concentrated to remove methanol in vacuum to give water fluid. The water fluid was then subjected to separation with a glass column (5 × 80 cm) packed with macroporous resin (D-101, 400 g) and eluted with 5000 mL of 15% methanol and 5000 mL of 40% methanol, respectively. The eluent of 40% methanol was concentrated in vacuum using a rotary evaporator to give dried powder (11 g). The dried powder was stored in a refrigerator (–4°C) for subsequent HSCCC separation.

Selection of the two-phase solvent system

The composition of the two-phase solvent system was selected according to the partition coefficient (K) of the target compounds of sample extracted from the *Fructus corni*. The K values were determined by HPLC as follows: a suitable amount of crude sample 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, an 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: $K = (A_1 - A_2)/A_2$.

Preparation of two-phase solvent system and sample solution

The two-phase solvent system selected for the separation was *n*-butanol–methanol–1% acetic acid water (4:1:6, v/v). It was prepared by adding the solvents to a separation funnel according to the volume ratios and thoroughly equilibrated by shaking repeatedly. Then, the upper phase and the lower phase were separated and degassed by sonication for 30 min prior to use.

The sample solution for HSCCC separation was prepared by dissolving 50 mg of sample powder in the mixture of 2 mL of the upper phase and 2 mL of the lower phase of *n*-butanol–methanol–1% acetic acid water (4:1:6, v/v).

HSCCC separation procedure

The upper phase (stationary phase) and the lower phase (mobile phase) of *n*-butanol–methanol–1% acetic acid water (4:1:6, v/v) were pumped into the multiplayer-coiled separation columns of HSCCC instrument simultaneously by using the ÄKTA prime system, according to the volume ratio of 50:50. After the columns were entirely filled with the two phases, only the lower phase was pumped at the flow rate of 2 mL/min, and at the same time, the HSCCC apparatus was run at the revolution speed of 800 rmp. After hydrodynamic equilibration was reached (~ 30 min), the sample solution was injected into the separation column. The separation temperature was controlled at 25°C. The chromatogram was recorded 50 min after sample injection. Different fractions were collected according to the obtained chromatogram and evaporated to dryness. The residuals were dissolved in methanol for subsequent HPLC analysis.

HPLC analysis and identification of HSCCC peak fractions

The crude extract of *Fructus corni*, the sample powder obtained after macroporous absorption resin pretreatment of crude extract, and each HSCCC peak fraction were analyzed by HPLC. Analysis was accomplished with YWG C_{18} column (200 ×

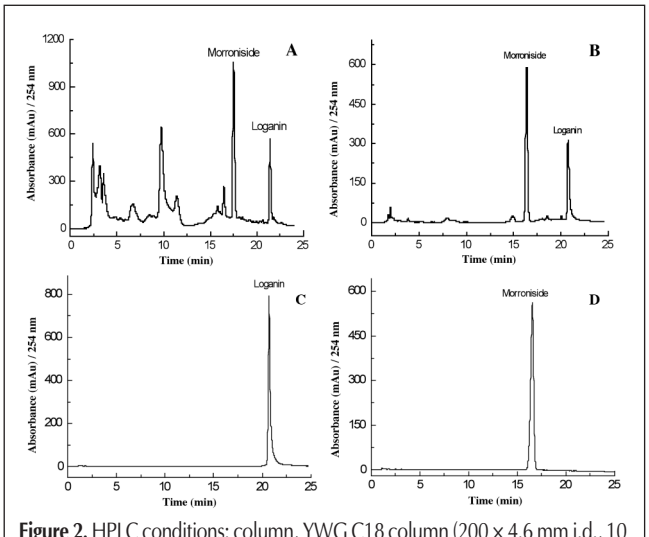

Figure 2. HPLC conditions: column, YWG C18 column (200 × 4.6 mm i.d., 10 µm); mobile phase, methanol–water in gradient as follows: 0–10 min, 20% methanol; 10–20 min, 20% methanol to 60% methanol; after 20 min, 60% methanol; flow rate, 1.0 mL/min; detection wavelength, 254 nm. Crude extract from Fructus corni (A); sample obtained from the eluent of 40% methanol of crude sample on D-101 macroporous resin column (B); HSCCC peak fraction (peak II, loganin) (C); HSCCC peak fraction (peak I, morroniside) (D).

4.6 mm i.d, 10 µm) at room temperature. Methanol–water was used as the mobile phase in gradient mode as follows: 0–10 min, 20% methanol; 10–20 min, 20% methanol to 60% methanol; after 20 min, 60% methanol. The flow-rate was 1.0 mL/min. The effluents were monitored at 254 nm.

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

Results and Discussion

Optimization of HPLC method

In present work, an HPLC method for analysis of crude sample was established. In order to select an appropriate elution system for the HPLC separation of morroniside and loganin, different kinds of solvents were employed. When methanol–water was used as the mobile phase in gradient mode as follows: 0–10 min, 20% methanol; 10–20 min, 20% methanol to 60% methanol; after 20 min, 60% methanol; morroniside and loganin could be separated well with other components. Chromatograms are shown in Figure 2.

Optimization of the separation conditions of macroporous absorption resin column

The crude sample extracted directly from *Fructus corni* contains many types of compounds. This can be seen from the HPLC chromatogram shown in Figure 2A. The contents of morroniside and loganin are very low, and the impurities present in the crude sample also interfere in the HSCCC purification of morroniside and loganin. Thus, the crude sample was pretreated with macroporous absorption resin before HSCCC separation. The crude sample was subjected to a D-101 macroporous resin column and washed with different concentrations of methanol. When the sample was eluted with 15% methanol first and then with 40% methanol, most impurities present in the crude sample could be removed, and morroniside and loganin were mainly present in the eluent of 40% methanol. The HPLC chromatogram of the eluent of 40% methanol is shown in Figure 2B.

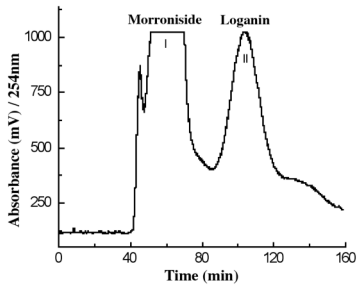

Figure 3. HSCCC chromatograms of sample from *Fructus corni*. Two-phase solvent system: *n*-butanol–methanol–1% acetic acid water (4:1:6, v/v); mobile phase: the lower phase; flow rate: 2.0 mL/min; revolution speed: 800 rpm; detection wavelength: 254 nm; sample size: 50 mg of crude sample dissolved in the mixture of 2 mL of the upper phase and 2 mL of the lower phase of the two-phase solvent system; separation temperature: 25°C; retention of the stationary phase: 50%. Morroniside (collected during 50–72 min); loganin (collected during 92–115 min).

Optimization of two-phase solvent system of HSCCC

Several two-phase solvent systems, such as ethyl acetate–water, ethyl acetate–methanol–water, *n*-butanol–methanol–water, *n*-butanol–acetic acid water, and *n*-butanol–methanol–acetic acid water were employed to optimize the HSCCC separation condition. When ethyl acetate–water and ethyl acetate–methanol–water were used as the two-phase solvent systems, the target compounds mainly partitioned in the aqueous phase. So these systems were unsuitable for the HSCCC separation. When *n*-butanol–methanol–water was used as the solvent system, the stationary phase loss was serious. But the maintenance of the stationary phase could be improved greatly by using acetic acid water solution instead of water. The partition coefficients of the target compounds in *n*-butanol–1% acetic acid water and *n*-butanol–methanol–1% acetic acid water were listed in Table I. According to the partition coefficients listed in Table I, *n*-butanol–methanol–1% acetic acid water (4:1:6, v/v) was used as the two-phase solvent system.

The sample from *Fructus corni* was separated and purified under the optimum HSCCC conditions. A typical HSCCC chromatogram is shown in Figure 3. Morroniside (28.7 mg) and loganin (11.5 mg) were obtained from 50 mg of sample in a one-step HSCCC separation. The purity of morroniside and loganin was 97.8% and 98.6%, respectively, as determined by HPLC. The HPLC chromatograms of these compounds are shown in Figure 2.

The structural identification

The chemical structures of the peak fractions of HSCCC were identified according to its ^{1}H-NMR and ^{13}C-NMR data.

Peak I of Figure 3: ^{1}H-NMR (DMSO-d_6) δ ppm: 5.32 (^{1}H, d, C_1-H), 7.40 (^{1}H, s, C_3-H), 1.85 (^{1}H, m, C_5-H), 1.85, 1.61 (each ^{1}H, m, C_6-H), 5.32 (^{1}H, m, C_7-H), 3.74 (^{1}H, m, C_8-H), 2.50 (^{1}H, m, C_9-H), 1.20 (^{3}H, d, C_{10}-H), 3.74 (^{3}H, s, –OCH_3), 4.93 (^{1}H, d, C_1'-H). ^{13}C-NMR (DMSO-d_6) δ ppm: 94.6 (C_1), 152.3 (C_3), 109.2 (C_4), 27.4 (C_5), 34.7 (C_6), 94.6 (C_7), 63.3 (C_8), 39.5 (C_9), 20.4 (C_{10}), 166.2 (C_{11}), 52.0 (–OCH_3), 98.1 (C_1'), 77.1 (C_2'), 70.9 (C_3'), 72.6 (C_4'), 73.8 (C_5'), 61.7 (C_6'). Compared with the data given in the literature (31), the compound obtained from peak I was identified as morroniside.

Peak II of Figure 3: ^{1}H-NMR (C_5D_5N) δ ppm: 5.64 (1H, d, C_1-H), 7.64 (^{1}H, s, C_3-H), 3.2 (^{1}H, q, C_5-H), 1.70 (^{1}H, m, C_6-H), 4.23 (^{1}H, m, C_7-H), 2.01 (^{1}H, m, C_8-H), 2.33 (^{1}H, m, C_9-H), 11.11 (^{3}H, d, C_{10}-H), 2.54 (^{1}H, m, C_6'-H), 3.50 (3H, s, OCH_3), 5.32 (1H, d, C_1'-H). ^{13}C-NMR δ ppm: 96.6 (C_1), 150.3 (C_3), 112.3 (C_4), 31.2 (C_5), 42.9 (C_6), 73.9 (C_7), 41.0 (C_8), 45.3 (C_9), 13.1 (C_{10}), 166.7 (C_{11}), 50.2 (–OCH_3), 100.1 (C_1'), 72.6 (C_2'), 77.8 (C_3'), 71.4 (C_4'), 78.2 (C_5'), 62.1 (C_6'). Compared with the data given in literature (32), the compound obtained from peak II was identified as loganin.

Table I. Partition Coefficients of Morroniside and Loganin

n-Butanol–methanol–1% acetic acid water (v/v/v)	*K* (morroniside)	*K* (loganin)
5:0:5	0.46	5.9
5:1:5	0.73	3.3
4:1:6	0.74	1.3

Conclusion

In present work, preparative purification of loganin and morroniside from *Fructus corni* by HSCCC was studied. The results of our study clearly demonstrated that HSCCC could provide highly efficient preparative separation of loganin and morroniside from Fructus corni. In combination with pre-separation of macroporous absorption resin column prior to HSCCC separation, loganin and morroniside with high purity were obtained.

Acknowledgments

This work was supported by The Project of Taishan Scholarship of Shandong Province.

References

1. China Pharmacopoeia Committee, Pharmacopoeia of People's Republic of China, the first division of 2005 edition, China Chemical Industry Press, Beijing, p. 20.
2. Y. Zhang and Y. Gao. Recent development of chemical component and pharmacological study of *Cornus officinalis*. *Chin. J. Info. TCM* **9(10):** 81–82 (2002).
3. T.S. Wang, Y. Pan, and F. Yin. Effect of loganin and synephrineon rabbit model with severe hemorrhagic shock. *J. Nanjing Univ. Trad. Chin. Med.* **15(6):** 345–46 (1999).
4. L.L. Guo, Y. Zhou, and X.D. Wang. Effect of the extract of the fruit of Cornus of ficinalis on rat splenic lymphocytic proliferation. *J. Beijing Univ. Trad. Chin. Med.* **24(3):** 38–40 (2001).
5. H.Q. Xu, H.P. Hao, X. Zhang, and Y. Pan. Morroniside protects cultured human umbilical vein endothelial cells from damage by high ambient glucose. *Acta Pharmacol. Sin.* **25(4):** 412–15 (2004).
6. X.N. Li, Ch. H. Huo, W. Yin, and L.T. Zhang. Preparation of loganin and morroniside reference substances from Fructus Corni. *Chin Trad Herbal Drugs.* **37(8):** 1168–70 (2006).
7. Y. Ito. Efficient preparative counter-current chromatography with a coil planet centrifuge. *J. Chromatogr.* **214:** 122–25 (1981).
8. Y. Ito. High-speed countercurrent chromatography. *CRC Crit. Rev. Anal. Chem.* **17:** 65–143 (1986).
9. T.Y. Zhang, L.K. Pannell, D.G. Cai, and Y. Ito. Separation of alkaloids extracted from *Stephania tetrandra* S. Moore by analytical high-speed counter-current chromatography. *J. Liq. Chromatogr. Relat. Technol.* **11:** 1661–71 (1988).
10. T.Y. Zhang, D.G. Cai, and Y. Ito. Separations of flavonoids and alkaloids in medicinal herbs by high-speed counter-current chromatography. *J. Chromatogr.* **435:** 159–66 (1988).
11. J.Y. Zhou and Q.C. Fang. The application of high-speed counter-current chromatography to the semipreparative separation of vincamine and vincine. *Phytochem. Analysis* **11:** 74–76 (1990).
12. R.M. Liu, X. Chu, A.L. Sun, and L.Y. Kong. Preparative isolation and purification of alkaloids from the Chinese medicinal herb *Evodia rutaecarpa* (Juss.) Benth by high-speed counter-current chromatography. *J. Chromatogr. A* **1074:** 139–44 (2005).
13. F. Yang, J. Quan, T. Zhang, and Y. Ito. Preparative separation of alkaloids from the root of *Sophora flavescens* Ait by pH-zone-refining counter-current chromatography. *J. Chromatogr. A* **822:** 316–20 (1998).
14 Y. Ma, Y. Ito, E. Sokolosky, F. Edward, and M. Henry. Separation of alkaloids by pH-zone-refining counter-current chromatography. *J. Chromatogr. A* **685:** 259–62 (1994).
15. D.G. Cai, M.J. Gu, J.D. Zhang, G.P. Zhu, T.Y. Zhang, N. Li, and Y. Ito. Separation of alkaloids from *Daturamete* L. and *Sophora flavesens* ait by high-speed counter-current chromatography. *J. Liq. Chromatogr. Relat. Technol.* **13:** 2399–2408 (1990).
16. H.T. Lu, Y. Jiang, and F. Chen. Application of high-speed counter-current chromatography to the preparative separation and purification of baicalin from the Chinese medicinal plant *Scutellaria baicalensis*. *J. Chromatogr. A* **1017:** 117–23 (2003).
17. S.J. Wu, A.L. Sun, and R.M. Liu. Separation and purification of baicalin and wogonoside from the Chinese medicinal plant *Scutellaria baicalensis* Georgi by high-speed counter-current chromatography. *J. Chromatogr. A* **1066:** 243–47 (2005).
18. Q. Du, M. Xia, and Y. Ito. Purification of icariin from the extract of *Epimedium segittatum* using high-speed counter-current chromatography. *J. Chromatogr. A* **962:** 239–41 (2002).
19. F. Yang, Y. Ma, and Y. Ito. Separation and purification of isoflavones from a crude soybean extract by high-speed counter-current chromatography. *J. Chromatogr. A* **928:** 163–70 (2001).
20. X. Cao, Y. Tian, T. Zhang, X. Li, and Y. Ito. Separation and purification of isoflavones from *Pueraria lobata* by high-speed counter-current chromatography. *J. Chromatogr. A* **855:** 709–13 (1999).
21. R. M. Liu, Q. H. Sun, A. L. Sun, and J. C. Cui. Isolation and purification of coumarin compounds from *Cortex fraxinus* by high-speed counter-current chromatography. *J. Chromatogr. A* **1072:** 195–99 (2005).
22. R.M. Liu, A.F. Li, and A.L. Sun. Preparative isolation and purification of coumarins from *Angelica dahurica* (Fisch. ex Hoffm) Benth, et Hook. f (Chinese traditional medicinal herb) by high-speed counter-current chromatography. *J. Chromatogr. A* **1052:** 223–27 (2004).
23. R.M. Liu, L. Feng, A.L. Sun, and L.Y. Kong. Preparative isolation and purification of coumarins from *Cnidium monnieri* (L.) Cusson by high-speed counter-current chromatography. *J. Chromatogr. A* **1055:** 71–76 (2004).
24. R.M. Liu, A.F. Li, A.L. Sun, and L.Y. Kong. Preparative isolation and purification of psoralen and isopsoralen from *Psoralea corylifolia* by high-speed counter-current chromatography. *J. Chromatogr. A* **1057:** 225–28 (2004).
25. H.B. Li and F. Chen. Preparative isolation and purification of six diterpenoids from the Chinese medicinal plant *Salvia miltiorrhiza* by high-speed counter-current chromatography. *J. Chromatogr. A* **925:** 109–14 (2001).
26. G. Tian, T. Zhang, Y. Zhang, and Y. Ito. Separation of tanshinones from *Salvia miltiorrhiza* Bunge by multidimensional counter-current chromatography. *J. Chromatogr. A* **945:** 281–85 (2002).
27. G. Tian, Y. Zhang, T. Zhang, F. Yang, and Y. Ito. Separation of tanshinones from *Salvia miltiorrhiza* Bunge by high-speed counter-current chromatography using stepwise elution. *J. Chromatogr. A* **904:** 107–11(2000).
28. F. Yang, T. Zhang, G. Tian, H. Cao, Q. Liu, and Y. Ito. Preparative isolation and purification of hydroxyanthraquinones from *Rheum officinale* Baill by high-speed counter-current chromatography using pH-modulated stepwise elution. *J. Chromatogr. A* **858:** 103–107 (1999).
29. T.Y. Zhang, L.K. Pannell, Q.L. Pu, D.G. Cai, and Y. Ito. Separation of hydroxyanthraquinone derivatives extracted from rheum with analytical high-speed counter-current chromatography. *J. Chromatogr.* **442:** 455–58 (1988).
30. R.M. Liu, A.F. Li, and A.L. Sun. Preparative isolation and purification of hydroxyanthraquinones and cinnamic acid from the Chinese medicinal herb *Rheum officinale* Baill. by high-speed counter-current chromatography. *J. Chromatogr. A* **1052:** 217–21 (2004).
31. L.J. Naggar and J.L. Beal. Iridoids. A review. *J. Nat. Prod.* **43:** 649–707 (1980).
32. L. Xu, H. Li, L. Tian, and K. Li. Studies on the chemical constituents of common macrocarpium (*Cornus officinalis*). *Chin. Trad. Herbal Drugs.* **26(2):** 62–65 (1995).

Manuscript received May 9, 2008;
revision received September 26, 2008.