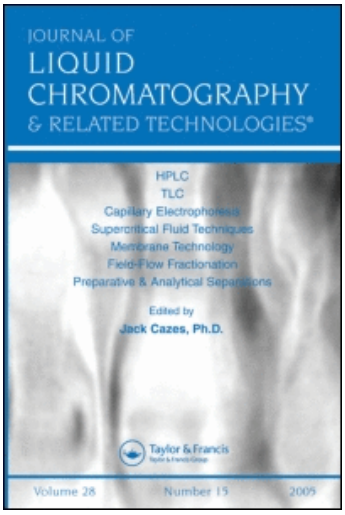

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Preparative Separation of a Minor Active Chromone from *Aloe vera* Leaves by CCC

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To cite this Article Cao, Xueli , Dong, Yinmao , Zhao, Hua , Pan, Xia and Ito, Yoichiro(2005) 'Preparative Separation of a Minor Active Chromone from *Aloe vera* Leaves by CCC', Journal of Liquid Chromatography & Related Technologies, 28: 12, 2005 — 2016

To link to this Article: DOI: 10.1081/JLC-200063655

URL: http://dx.doi.org/10.1081/JLC-200063655

Journal of Liquid Chromatography & Related Technologies®, 28: 2005–2016, 2005

ISSN 1082-6076 print/1520-572X online
DOI: 10.1081/JLC-200063655

Preparative Separation of a Minor Active Chromone from *Aloe vera* Leaves by CCC

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Abstract: HSCCC was applied successively in the preparative separation of a minor active chromone from an extract of *Aloe vera* leaves by combination with other traditional pretreatment, extraction, and partition methods. The chromone was finally identified as 8-C-β-D-(2′-O-(E)-cinnamoyl) glycopyranosyl-2-(2-hydroxy) propyl-7-methoxy-5-methyl-chromone by FAB-MS, ^{1}H NMR, and ^{13}C NMR. Both of the solvent systems composed of the normal and reversed phase systems, such as CH_2Cl_2-MeOH-H_2O (5 : 4 : 2, v/v/v) and hexane-ethyl acetate-methanol-water (1 : 5 : 1 : 5, v/v/v/v), can be used for the preparative separation of the target chromone compound. The later solvent system was superior regarding sample loading capacity, environmental concerns (no chlorinated solvent), and peak resolution.

Keywords: Active chromone, *Aloe vera*, CCC, Preparative separation

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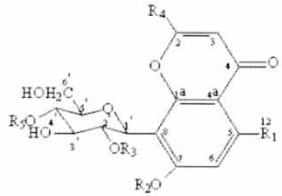
INTRODUCTION

In recent years, *Aloe vera* and related products have drawn a great deal of attention from cosmetic, food, and pharmaceutical industries. *Aloe vera* gel, which is generated in the mucilaginous cells of the inter central zone of the leaf, has been very well documented for its wound-healing, anti-inflammatory, immune-stimulatory, and hematopoietic activities[1–5] and protective activity on liver and kidney, from the damage caused by type-II diabetes.[6,7] However, the small phenolic compounds, which predominantly exist in the pericyclic cells underneath the leaf skin, have been ignored as impurities due to their color and gastrointestinal irritation.

One group of phenolic constituents contained in *Aloe* leaves is chromones. Scientific studies reveal that chromones have important biological activities such as anti-inflammation, anti-ulcer, tyrosinase inhibition, skin protection, laxative effect, and other antioxidant properties.[8–12] This has stimulated a great deal of research on identifying chromones from aloe plants. A number of aloe chromones with different structures have been identified,[13–18] which have been summarized in Figure 1.

Chromones are found in the aloe plant at a very low level. One chromone compound with a molecular weight of 540 Dalton (called "compound 540") was found in decolorized *Aloe vera* gel at a content of about 0.0001% by weight (1 ppm); in non-decolorized gel, the content was ten times higher or about 0.0011% by weight or 11 ppm; in decolorized whole leaf extract the content was about 20 ppm; in non-decolorized whole leaf extract, it was about 25 ppm.[16] The *in vivo* biological test data indicated that concentrations below about 15% by weight did not result in statistically significant anti-inflammatory activity.[16] Thus, it is required to obtain enriched or even pure "compound 540" to prepare formulations having the desired clinical properties, and to investigate further all biological activities. It is then necessary to develop an efficient extraction and separation method for "compound 540" and all other chromone compounds.

A variety of methods are currently used for the isolation and purification of products from the aloe plant. Several commercial processes employ activated carbon to improve the color of slurries from aloe leaves. Treatment with activated carbon is commonly used in the so-called "whole-leaf processing"; the entire leaf is ground and processed. However, studies revealed that refinates obtained after treatment with activated carbon did not exhibit desired biological activity associated with chromone compounds. Extraction of decolorizing agent with organic solvent lead to the discovery that chromone compounds are absorbed by activated carbon with the colored material. The logical conclusion is that activated carbon can be utilized as the starting material for the extraction of chromone compounds. The further purification of aloe chromones has involved several methods such as Sephadex or neutral alumina column chromatography,[13,15] preparative



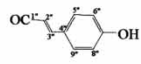
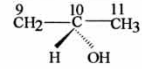
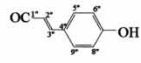
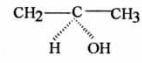
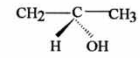
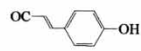
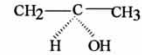
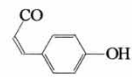
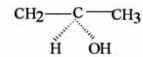
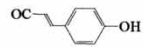
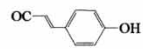
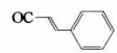
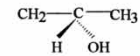
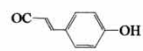
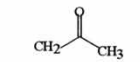
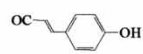
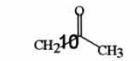
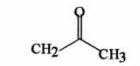
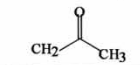
		R_1	R_2	R_3	R_4	R_5
1	aloeresin D	CH_3	CH_3			H
2	isoaloeresin D	CH_3	CH_3			H
3	aloeresin E	CH_3	H	H		H
4	8-C-glucosyl-7-O-methylaloediol	CH_3	CH_3	H	$CH(OH)CH(OH)CH_3$	H
5	8-C-glucosyl-noreugenin	OH	H	H	CH_3	H
6	4'-O-glucosyl-isoaloeresin DI	CH_3	CH_3			Glc
7	4'-O-glucosyl-isoaloeresin DII	CH_3	CH_3			Glc
8	8-C-glucosyl-(2'-O-cinnamoyl) -7-O-methyl-laloediol A	CH_3	CH_3		$CH(OH)CH(OH)CH_3$	H
9	8-C-glucosyl-(2'-O-cinnamoyl) -7-O-methyl-laloediol B	CH_3	CH_3		$CH(OH)CH(OH)CH_3$	H
10	8-C-(2'-O-cinnamoyl) glycopyranosyl-2-(2-hydroy) propyl-7-methoxy-5-methyl chromone	CH_3	CH_3			H
11	aloeresin A	CH_3	H			H
12	7-O-methylaloeresin A	CH_3	CH_3			H
13	7-O-methylaloesin	CH_3	CH_3	H		H
14	aloesin	CH_3	H	H		H

Figure 1. The structures of identified aloe chromones.

HPLC,[13,15] TLC,[14,17] column chromatography followed by countercurrent extraction,[19,20] and a combination of droplet countercurrent chromatography (DCCC), flash chromatography,[21] and preparative HPLC followed by flash chromatography.[22,23]

The present paper describes a simple method for preparative isolation and purification of a cinnamoyl-C-glycoside aloe chromone compound by high-speed countercurrent chromatography (HSCCC) combined with traditional pretreatment. The purified compound was analyzed and identified by HPLC, MS, and NMR.

EXPERIMENTAL

Reagents and Materials

Fresh *Aloe vera* leaves were provided by Tianjin Plantation, China. Activated carbon of analytical grade was brought from Tianjin Chemical Factory. All organic solvents used for crude extraction and HSCCC fractioning were of analytical grade and provided by Beijing Chemical Reagents Company. Methanol with HPLC grade was from Dikma Technologies Ltd., China.

Apparatus

All equipments used for the processing of aloe leaves were of pilot-scale and manufactured in China.

HSCCC was performed with model GS10A3 multilayer coil planet centrifuge fabricated at Beijing Institute of New Technology Application, Beijing China. The column was prepared by winding about 110 m of 1.6 mm i.d. PTFE tubing in two coils of about 110 mL each. The average β ratio is 0.6 with a rotor radius of 8 cm. The GS10A3 machine volume is 220 mL. The revolution speed is adjustable between 0 and 1000 rpm.

The HPLC system used for analysis was an Agilent 1100 LC system containing a quaternary pump, an auto-sampler, a 25-cm ZORBAX SB-C_{18} (4.6 mm i.d.) column, and a diode-array detector (DAD).

Pretreatment of Aloe Leaves and Extraction

Whole fresh aloe leaves (35 kg) were washed, crushed, and ground to produce slurry, which was then treated with 2% activated carbon. After sufficient pummeling, stirring, and filtration, the spent decolorizing carbon was collected and dried at 60°C. This dried activated carbon (700 g) was extracted with 1.5 L methanol. The slurry was stirred under ultrasonication at room temperature for 2 h, and then was filtered through a 0.45 μm filter. The extraction was repeated twice. The two methanol extracts were combined and concentrated under vacuum. Then the residual syrup was partitioned between water and dichloromethane to remove a number of impurities,

particularly anthraquinones. Finally, the dichloromethane extract (1650 mg) was fractionated by HSCCC, searching for chromones.

HSCCC Separation

The solvent systems composed of chloroform–methanol–water, dichloromethane–methanol–water and hexane–ethyl acetate–methanol–water have been used for the separation of aloe chromones, either in the normal phase mode (aqueous polar stationary phase and organic apolar mobile phase) or in the reverse phase mode (organic apolar stationary phase).

The solvent system was thoroughly equilibrated in a separatory funnel and the two phases were separated shortly before use. In each separation, the CCC column was first entirely filled with the upper stationary phase, and then the lower mobile phase was pumped into the column at a flow-rate of 2 mL/min under 800 rpm. After the mobile phase front emerged and hydrodynamic equilibrium was established, the sample solution (sample dissolved in the mobile phase) was injected through the sample loop. Fractions were collected according to the recorded UV elution profile.

HPLC Analysis

Each extract and HSCCC fraction was analyzed by the Agilent HPLC system through a ZORBAX SB-C_{18} (250 × 4.6 mm i.d.) column. The mobile phase had a constant composition of methanol and water with 1% acetic acid (55 : 45, v/v). The flow rate was 0.7 mL/min. Detection: DAD, often the 284 nm line, was selected.

Structure Identification

The target compounds purified by HSCCC were identified by FAB-MS, ^{1}H NMR, and ^{13}C NMR. FAB-MS was performed on a Micromass ZabSpec mass spectrometer, and NMR on a Varian INOVA 600 MHz spectrometer (in CD_3OD), in National Center of Biomedical Analysis, Academy of Military Medical Sciences, Beijing.

RESULTS AND DISCUSSION

Investigation of Aloe Chromone Compounds in Crude Extract

There are two difficulties in the quest for chromone compounds in aloe leaf extract: one is their very low ppm level, and the second is a lack of

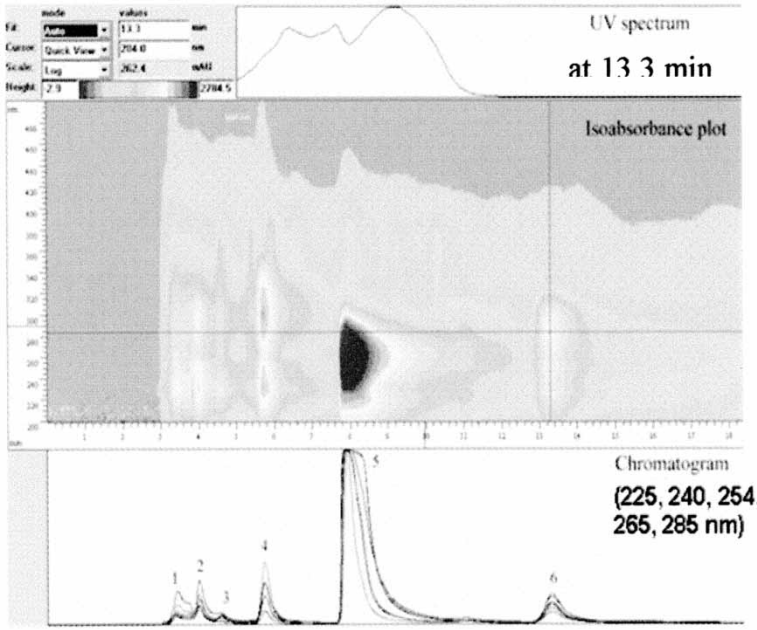


Figure 2. HPLC chromatogram of crude methanol extract from spent activated carbon. HPLC condition: Column: ZORBAX SB-C18 (250 × 4.6 mm I.D.); Mobile phase: methanol–water (55 : 45, v/v) with 1% acetic acid; flow rate: 0.7 mL/min; Detection: diode-array detector (DAD).

standard reference material. The only useful information is their UV absorbance spectrum with maxima at 228 nm,[24] 243–245 nm,[15,24] 252–255 nm,[15,24] 284 nm,[15] and 303 nm.[24]. So, the crude methanol extract was analyzed by HPLC and monitored with a DAD at different wavelengths to make sure all constituents in the extract can be detected. The HPLC column and mobile phase composition is similar to that in reference literature. Acetic acid (1%) was added to improve all peak profiles. Figure 2 gave the HPLC chromatogram of the crude methanol extract from spent activated carbon. Each peak in the chromatogram was examined for its UV spectrum, processing the data accumulated by the diode array (as illustrated in Figure 2), and compared with reported data. Preliminarily, two possible chromone peaks at 8.0 min (Peak 5) and 13.3 min (Peak 6) were locked as target peaks. But later identification revealed that Peak 5 was not a chromone. It was later identified as pentadienoic acid. Thus, only the second peak was purified as target chromone in the following procedure. Peak 6 corresponds to about 8% of the total HPLC peak area at 284 nm in methanol extract. This peak was selectively concentrated in organic phase after partition between dichloromethane and water (see Figure 3). It made

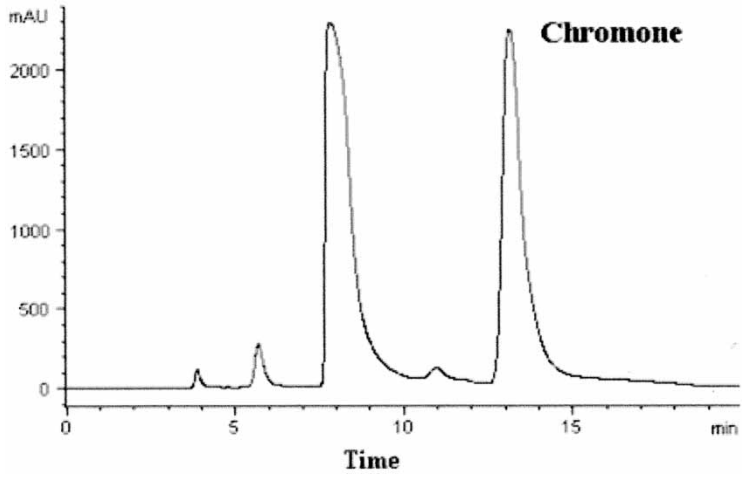


Figure 3. HPLC chromatogram of dichloromethane extract after the partition of methanol extract between dichloromethane and water. HPLC condition is the same as in Figure 2. Detection: 284 nm.

up approximately 44% of the dichloromethane extract. This improves the possibility of obtaining a significant amount of the chromone compound.

Optimization of HSCCC Solvent System

Aloe chromones are hydrophobic compounds with a low polarity. The solvent system composed of $CHCl_3$–MeOH–H_2O (7 : 13 : 8 v/v/v) has been used for the separation of aloeresin D chromone by DCCC.[21] So, the above dichloromethane extract was subjected to HSCCC separation starting with a similar chlorinated system $CHCl_3$–MeOH–H_2O (4 : 3 : 2 v/v/v). The separation profile is shown in Figure 4A and three main peak fractions were collected. HPLC analysis indicated that the target compound was presented in Peak 2, but not resolved completely from other interfering compounds. In order to extend the retention of the target peak and improve its purity, several related solvent systems including $CHCl_3$–MeOH–H_2O (4 : 4 : 2 v/v/v), CH_2Cl_2–MeOH–H_2O (4 : 4 : 2 v/v/v), CH_2Cl_2–MeOH–H_2O (5 : 4 : 2 v/v/v) were tested, and finally, CH_2Cl_2–MeOH–H_2O (5 : 4 : 2 v/v/v) was found able to completely separate the target compound from all other compounds derived from the first HSCCC run (see Figure 4B). Further experiment showed that this solvent system can be used to produce a highly pure target chromone compound through a single-step separation, starting directly with the dichloromethane extract (see Figure 5). Using the later procedure, about 10 mg of almost 99% pure chromone could be obtained from 60 mg of crude extract.

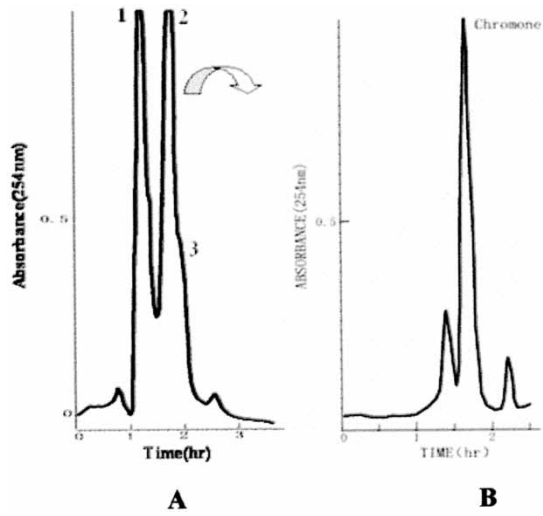


Figure 4. Optimization of HSCCC solvent systems. Experimental conditions: A. sample: dichloromethane extract 60 mg, solvent system: $CHCl_3$–MeOH–H_2O (4 : 3 : 2, v/v/v); B. sample: Peak 2 fraction from A, CH_2Cl_2–MeOH–H_2O (5 : 4 : 2, v/v/v); apparatus: model GS10A3 hydrodynamic CCC; column capacity: 220 mL; mobile phase: lower organic apolar phase; flow rate: 2 mL/min (H → T); revolution: 800 rpm; detection: 254 nm.

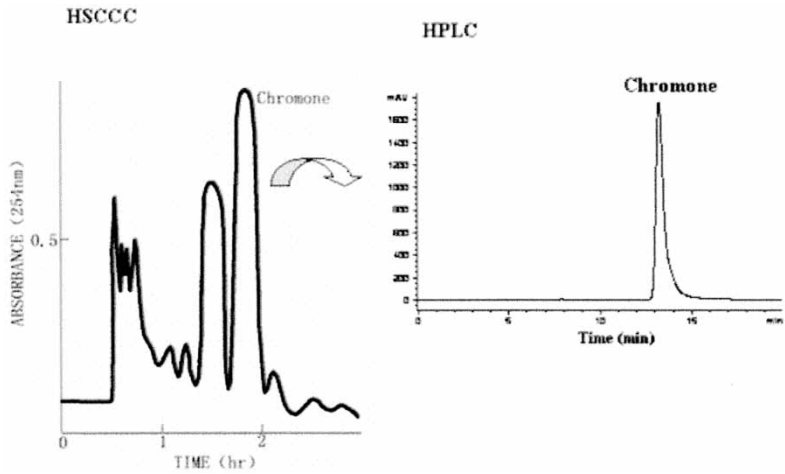


Figure 5. One-step HSCCC separation of target chromone compound by the apolar lower organic phase of the solvent system CH_2Cl_2–MeOH–H_2O (5 : 4 : 2, v/v/v) from dichloromethane extract 60 mg.

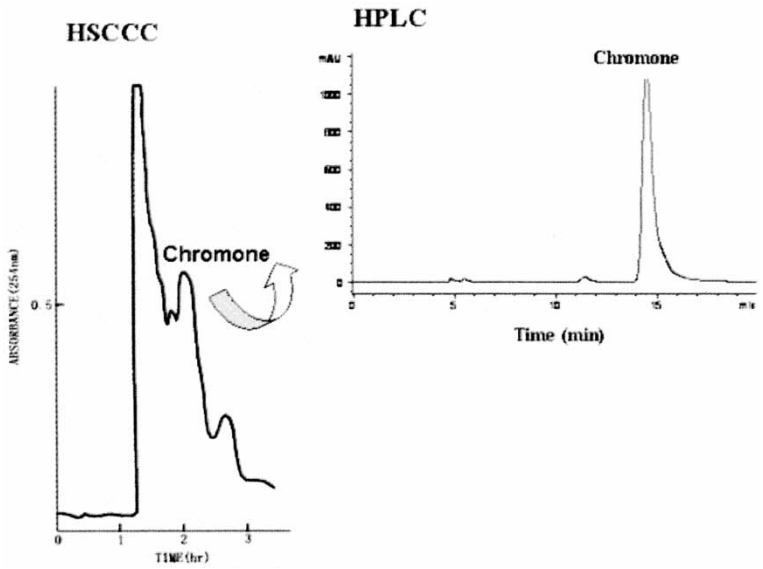


Figure 6. Preparative separation of target chromone by the apolar lower organic phase of the solvent system: CH_2Cl_2–MeOH–H_2O (5 : 4 : 2,v/v/v). Injected sample weight: 400 mg.

Preparative Separation of Individual Chromones

The solvent system CH_2Cl_2-MeOH–H_2O (5 : 4 : 2) was, therefore, employed for preparative separation of target chromone (see Figure 6). As the sample loading is increased, the peak resolution decreases rapidly. However, highly

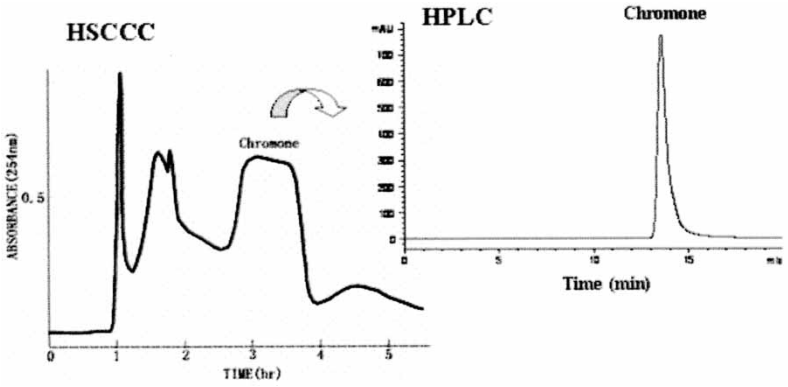


Figure 7. Preparative separation of target chromone by the polar lower aqueous phase of the solvent system (reverse-phase mode) composed of hexane–ethyl acetate–methanol–water (1 : 5 : 1 : 5 v/v/v/v). Injected sample weight: 700 mg.

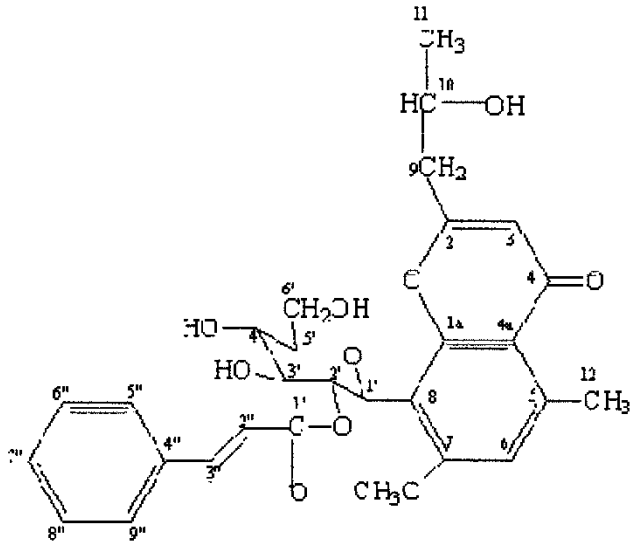


Figure 8. Identified chemical structure of the target chromone compound purified by HSCCC.

pure target compounds (about 95% purity) can still be obtained. Thus, a non-chlorinated solvent system composed of hexane–ethyl acetate–methanol–water (1 : 5 : 1 : 5 v/v/v/v) was tested in the reversed phase mode (polar aqueous lower mobile phase pushed in the head to tail direction and apolar organic upper stationary phase). The results obtained with this environmental friendly liquid system indicated that a higher resolution could be achieved with almost twice the loading amount (Figure 7), compared to the results obtained with the chlorinated solvent systems. The purity of the target compound was excellent, reaching almost 99% (by area in HPLC).

Fresh aloe leaves (35 kg) were submitted to the whole process: extraction, partition and subsequent HSCCC separation. About 288 mg of ~98% pure chromone was obtained in white powder. The extracted chromone was subjected to structure identification. Assuming that 100% of the chromone was extracted, it can be calculated that the isolated chromone compound was at the level of 8 ppm (0.0008% w/w) in the whole fresh aloe leaves used. This content is lower than what was reported in literature (25 ppm or 0.0025% w/w).[16] Of course, it is likely that our method did not produce a 100% recovery in each step of the separation.

Structure Elucidation

The compound obtained showed UV absorption maximum at: 225 nm, 246 nm, 254 nm, 284 nm.

Table 1. ^{13}CNMR and ^{1}HNMR data of target chromone compound in CD_3OD

Atoms	δ_C	δ_H	Atoms	δ_C	δ_H
2	167.4	—	**1′**	72.0	5.20 *d* (10.2)
3	112.1	6.13 *s*	**2′**	74.2	5.73 *dd* (10.2)
4	182.2	—	**3′**	77.7	3.70–3.80 *m*
4a	117.2	—	**4′**	72.6	3.61 *dd* (10)
5	144.6	—	**5′**	82.8	3.40–3.50 *m*
6	112.6	6.80 *s*	**6′a**	63.0	3.70–3.80 *m*
7	161.9	—	**6′b**		4.00 *m*
8	111.8	—	**1″**	167.6	—
1a	159.5	—	**2″**	118.3	6.26 *d* (16)
9	44.5	2.84 *dd* (14,7.2)	**3″**	146.4	7.46 *d* (16)
10	66.7	4.38 *m*	**4″**	135.5	—
11	23.6	1.33 *d* (6)	**5″,9**	129.9	7.37–7.50 *m*
12	23.5	2.75 *s*	**6″,8″**	129.1	7.37–7.50 *m*
7-OMe	57.0	3.89 *s*	**7″**	131.5	7.37–7.50 *m*

The positive FAB-MS showed two main peak fragments at m/z 541 and 131, which correspond to $[M+H]^+$ and $[cinnamoyl]^+$, respectively, and several other minor peak fragments at m/z 522 $[M\text{-}H_2O]^+$ ($[M\text{-}18]^+$), 496 $[M\text{-}CH_3CHO]^+$ ($[M\text{-}44]^+$), 392 $[M\text{-cinnamoyl-}H_2O]^+$ ($[M\text{-}130\text{-}18]^+$), 277 $[M\text{-}130\text{-}133]^+$, 259 $[M\text{-}130\text{-}133\text{-}18]^+$, 233 $[M\text{-}130\text{-}133\text{-}44]^+$, and 103 $[C_6H_5CHCH]^+$.

The NMR data (CD_3OD) was listed in Table 1.

All the above spectrum data were quite close to those of 8-C-β-D-(2′-O-(E)-cinnamoyl)glycopyranosyl-2-(2-hydroxy)propyl-7-methoxy-5-methylchromone (Compound 10 in Figure 1).[13–16] It is called the 540 cinnamoyl-C-glycoside chromone in abbreviation, as its molecular weight is 540 Dalton ($C_{29}H_{32}O_{10}$). This "compound 540" chromone has been reported to exhibit potent anti-inflammatory activity, as measured by *in vivo* assays. This compound has also been shown to inhibit EGF-induced DNA synthesis *in vitro* testing in epithelial cell lines.[15,16] The development of this CCC efficient preparative isolation method may promote further exploration of its biological activity.

CONCLUSION

HSCCC has been used successfully in the preparative separation of an active cinnamoyl–C-glycoside chromone compound from an extract of *Aloe vera* leaves by combination with other traditional pretreatment, extraction, and partition methods. The CCC method proved one more time that it can produce difficult-to-extract compounds with an interesting purity and using a minimum amount of solvent. The availability of this particular chromone may trigger further bioactive investigation.

ACKNOWLEDGMENTS

The authors thank National Natural Science Foundation of China and Beijing Municipal Education Committee for financial support. We also thank the National Center of Biomedical Analysis, Academy of Military Medical Sciences (Beijing) for their FAB-MS and NMR analysis.

REFERENCES

1. Grindlay, D.; Reynolds, T. Ethnopharmacology **1986**, *16*, 117.
2. Davis, R.H.; Leitner, M.G.; Russo, J.M.; Byrne, M.E. J. Amer. Podiatric Med. Assoc. **1989**, *79*, 263.
3. Udupa, S.L.; Udupa, A.L.; Kulkarni, D.R. Fitoterapia LXV **1994**, 141.
4. Woomble, D.; Helderman, J.H. Int. J. Immunopharmac. **1988**, *10*, 967.
5. Talmadge, J.; Chavez, J.; Jacobs, L.; Munger, C.; Chinnah, T.; Chow, J.T.; Williamson, D.; Yates, K. Int. Immunopharmacol. **2004**, *4* (14), 1757.
6. Can, A.; Akev, N.; Ozsoy, N.; Bolkent, S.; Arda, B.P.; Yanardag, R.; Okyar, A. Biol. Pharm. Bull. **2004**, *27* (5), 694.
7. Bolkent, S.; Akev, N.; Ozsoy, N.; Sengezer-Inceli, M.; Can, A.; Alper, O.; Yanardag, R. Indian J. Exp. Biol. **2004**, *42* (1), 48.
8. Hutter, J.A.; Salman, M.; Stavinoha, W.B.; Satangi, N.; Williams, R.F.; Streeper, R.T.; Weintraub, S.T. J. Nat. Prod. **1996**, *59*, 541.
9. Hirata, T.; Suga, T. Bull. Chem. Soc. Jap. **1978**, *51*, 842.
10. Okamura, N.; Hine, N.; Harada, S.; Fujiokam, T.; Mihashi, K.; Yagi, A. Phytochem. **1996**, *43*, 495.
11. Grollier, J.F.; Lang, G.; Gratien, S.; Forestler, S.; Rosenbaum, G US Patent 4,656,029, 1987.
12. Hottori, M.; Akao, T.; Kobashi, K.; Namba, T. Phamacology **1993**, *47*, S125.
13. Okamura, N.; Hine, N.; Tateyama, Y.; Nakazawa, M.; Fujioka, T.; Mihashi, K.; Yagi, A. Phytochemistry **1998**, *49*, 219.
14. Bisrat, D.; Dagne, E.; Wyk, B-E.; Viljoen, A. Phytochemistry **2000**, *55*, 949.
15. Waller, T.A.; Jia, Q.; Padmapriya, A. US patent 5,675,000, 1997.
16. Waller, T.A. US patent 5,965,540, 1999.
17. Lee, K.Y.; Weintraub, S.T.; Yu, B.P. Free Radical Biol. Med. **2000**, *28*, 261.
18. Park, M.K.; Park, J.H.; Kim, N.Y.; Shin, Y.G.; Choi, Y.S.; Lee, Y.G.; Kim, K.h.; Lee, S.K. Phytochem. Anal. **1998**, *9*, 186.
19. Holdsworth, H. Chromones in aloe species, Part I-Aloesin. P.M. **1972**, *19*, 322.
20. Holdsworth, H. Chromones in aloe species, Part II-Aloesone. P.M. **1972**, *22*, 54.
21. Speranza, G.; Dada, G.; Lunazz, L.; Gramatic, P.; Manitto, P. Phytochemistry **1986**, *25*, 2219.
22. Speranza, G.; Gramatic, P.; Dada, G.; Manitto, P. Phytochemistry **1985**, *24*, 1571.
23. Gramatic, P.; Monti, D.; Speranza, G.; Manitto, P. Tetrahedron lett. **1982**, *23*, 2423.
24. Zonta, F.; Bogoni, P.; Masotti, P.; Micali, G. J. Chromatogr. A. **1995**, *718*, 99.

Received September 19, 2004
Accepted December 21, 2004
Manuscript 6591C