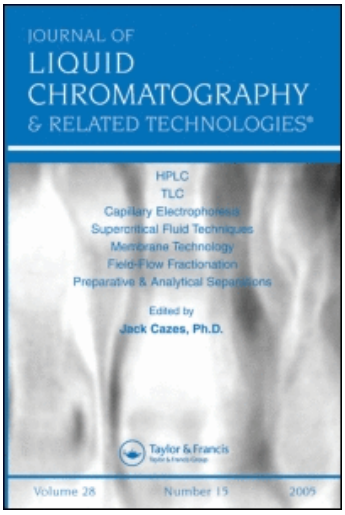

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Abstract: Aloin is the main anthraquinone in aloe leaf, which occurs naturally as a mixture of two diastereoisomers, aloin A and aloin B, and currently served as one of the important control constituents in most of the commercial aloe products. High speed countercurrent chromatography (HSCCC) has been employed for the preparative separation of individual isomers combined with preseparation on silica gel chromatography. Three solvent systems composed of chloroform-methanol-water (4:2:3), ethyl acetate-methanol-water (5:1:5), and butanol-ethyl acetate-water (1:3:4) have been used in HSCCC. 6 g of extract of dry aloe leaf exudates yielded 202 mg of aloin A (>98%) and 140 mg aloin B (>96%). Their structures have been confirmed by FAB-MS and ^{1}HNMR through gradient enhanced nuclear overhauser effect spectroscopy (Goesy).

Keywords: *Aloe vera*, Aloins A and B, High speed countercurrent chromatography

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INTRODUCTION

Aloe has been widely used for manufacturing food products and beverages, pharmaceuticals, and cosmetics during recent years, because of its aromatic properties, bitter taste, the cathartic activity of anthroquinones, and other pharmacological activities, such as anti-mutagenic, antiulcer, antidiabetic, anti-inflammatory, wound healing, and immunomodulatory activities.[1] The major active components of aloe leaf include the well documented polysaccharide contained in the gel and small molecular phenolic compounds mainly existing in the exudates of aloe leaf, of which aloin is the major component. Aloin, the C-glucoside of aloe emodin anthrone, has been reported to constitute up to 30% of the dried leaf exudates of aloe.[2] Main anthraquinones identified in aloe have been briefly listed in Table 1.

Aloin occurs naturally as a mixture of diastereoisomers, aloin A (configuration at C_{10}, C_1,: S, S) and aloin B (the C_{10}, C_1,: R, S). Studies of aloin's biosynthesis indicated that aloin B is preferentially formed. Non-enzymatic conversion to aloin A is thought to result in a mixture of aloins A and B, as observed for naturally derived aloin.[3,4]

Aloin has served as one of the restricted compounds in the quality control of aloe containing medicine, food, and retail products during recent years. Although aloin has been reported to be non-mutagenic, using in vitro assay, it has been shown that aloin can be metabolized to aloe emodin under pharmacological conditions.[5,6] In vitro studies have provided evidence of aloin emodin's toxicity, such as preferential toxicity to carcinoma cells, genotoxicity, and ability to promote malignant transformation of cells. In addition, it has been demonstrated that UV and visible light potentiate the toxicity of aloe emodin and structurally related anthraquinones.[7] There is a lack of standard aloin samples with high purity in China, and sometimes they are provided with a mixture of aloin A and aloin B.

The analytical methods for aloin A and aloin B have been well reported, including HPLC,[8,9] HPLC-MS,[10] micellar electrokinetic chromatography,[11] capillary zone electrophoresis,[12] and GC-MS.[13] However, the preparative separation of aloins is seldom reported. In this paper, the semi-preparative separation of aloin A and aloin B by high speed countercurrent chromatography (HSCCC) was conducted. HSCCC is a unique liquid-liquid partition chromatography using a liquid stationary phase without solid support, thus offering many advantages over the traditional chromatographic method, including no irreversible adsorption, low risk of sample denaturation, high recovery, large sample load capacity, and low cost.[14] In this paper, an amount of aloins A and B have been successively separated at high purity from the exudates of aloe leaf by HSCCC, combined with pre-separation by silica gel chromatography. HPLC and FAB-MS, ^{1}HNMR were employed for purity analysis and the elucidation of their structures.

Table 1. Main anthraquinones identified in aloe leaves.

Compounds	R_1	R_2	R_3	R_4	R_5	R_6	R_7
Aloe-emodin	H	H	C═O		H	H	H
Aloin A	H	H	C-glucopyranosyl	H	H	H	H
Aloin B	H	H	H	C-glucopyranosyl	H	H	H
8-O-methyl-7-hydroxyaloin A	CH_3	OH	C-glucopyranosyl	H	H	H	H
8-O-methyl-7-hydroxyaloin B	CH_3	OH	H	C-glucopyranosyl	H	H	H
10-hydroxyaloin A	H	H	C-glucopyranosyl	OH	H	H	H
4-hydroxyaloin	H	H	H	C-glucopyranosyl	OH	H	H
5-hydroxyaloin	H	H	H	C-glucopyranosyl	H	H	OH
Aloinoside A	H	H	C-glucopyranosyl	H	H	α-L-rhamnosyl	H
Aloinoside B	H	H	H	C-glucopyranosyl	H	α-L-rhamnosyl	H

EXPERIMENTAL

Apparatus

HSCCC was performed with a model GS10A3 multilayer coil planet centrifuge fabricated at the Beijing Institute of New Technology Application, Beijing China. The apparatus has a column holder and a counterweight symmetrically placed at a distance of 8 cm from the central axis of the centrifuge. The coil column was prepared with 1.6 mm ID PTFE (polytetrafluoroethylene) tubing with a total capacity of 220 mL. Beta values of the coiled column vary from 0.5 at the internal terminal to 0.75 at the outer terminal. The revolution speed is adjustable between 0–1,000 rpm with a speed controller.

The HPLC system used for analysis was an Agilent 1100 LC Series containing a quaternary pump, an auto-sampler, and a diode-array detector (DAD).

Reagents and Materials

A glass column (50 cm × 2.2 mm i.d.) was provided by Beijing Glass Apparatus, Beijing, China. Silica gel (200–300 mesh), analytical grade, was purchased from Qingdao Marine Chemical Factory, China.

All organic solvents used for crude extraction, column chromatography, and HSCCC fractionation were of analytical grade and were provided by Beijing Chemical Regents Company; HPLC grade methanol was from Dikma Technologies Ltd., USA.

Aloin A control sample was obtained from the National Institute for the Control of Pharmaceutical and Biological Products. *Aloe vera* exudate powder was purchased from a local drug store.

Extraction of Aloins

30 g dry powder was obtained by concentrating the yellow sap exuded from the aloe vera leaf and was used as the starting material. The crude powder was first distributed between a mixture of 500 mL of ethyl acetate and 500 mL of water under ultrasound treatment; the organic layer was collected, and the water later was repetitively extracted with ethyl acetate, 10 times. Finally, all organic extracts were combined, concentrated, and dried, yielding 11.5 g of crude extract.

Silica Gel Chromatography of Crude Extract

The glass column mentioned above was packed with a slurry of 95 g silica gel in methanol and, after cleaning with a column volume (Vc = 170 mL)

of methanol, the column was equilibrated with the starting mobile phase described below. Then, 3 g of the crude extract dissolved in a small volume of the same phase was delivered into the top of the column. The column was eluted successively with the lower phase of chloroform-methanol-water in three gradient volume ratios A(8.5:1.5:1)(2Vc), B(8:2:1)(1.5Vc), and C(7.5:2.5:1)(5Vc). At a flow rate of about 2.6 mL/min, the effluent was collected every 10 min. Aloin was mainly eluted by eluant C and was pooled into three fractions F_1, F_2, and F_3, according to the relative amount of aloins A and B, determined by HPLC analysis. Repeating this procedure, 6 g of the crude extract yielded F_1 (480 mg), F_2 (1557 mg), and F_3 (349 mg); these three fractions were subjected to HSCCC separation.

HSCCC Separation

Three solvent systems composed of chloroform-methanol-water, ethyl acetate- methanol-water, and butanol-ethyl acetate-water have been used for the separation and purification of aloins A and B.

The selected solvent system was thoroughly equilibrated in a separatory funnel and the two phases were separated shortly before use. In each separation, the coiled 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 rotation. 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. Peak fractions were collected according to the recorded UV elution profile.

HPLC Analysis

Each extract and HSCCC fraction was analyzed by the Agilent HPLC system with a Zorbax SB-C_{18} (250 × 4.6 mm ID) column. The mobile phase, composed of methanol and water (55:45, v/v) with 2% acetic acid, was isocratically eluted at a flow rate of 0.7 mL/min. Detection: DAD, 293 nm and 395 nm.

FAB-MS and ^{1}HNMR Identification

Aloins A and B, purified by HSCCC, were identified by FAB-MS and ^{1}HNMR. FAB-MS was performed with a Micromass ZabSpec mass spectrometer and NMR on a Varian INOVA 600 spectrometer (in CD_3OD), in

the National Center of Biomedical Analysis, Academy of Military Medical Sciences, Beijing.

RESULTS AND DISCUSSION

HPLC Analysis of Aloin Extract

HPLC analysis of both the crude extract and aloin A control sample indicated that the crude sample contained an abundant amount of aloin A (marked as A), and another compound which eluted at a retention volume close to that of component B (tentatively noted as aloin B) (see Fig. 1). In addition, there was a considerable amount of unknown polar impurities in the crude sample.

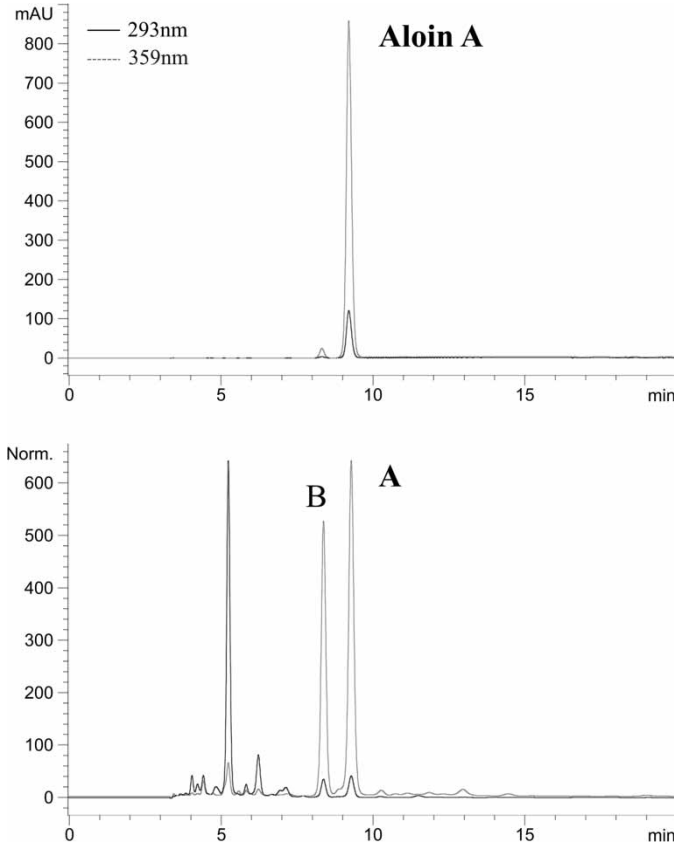

Figure 1. HPLC analysis of aloin A control sample (upper) and crude extract of aloe leaf exudates (lower). HPLC condition: column: ZORBAX SB-C_{18} (250 × 4.6 mm ID); Mobile phase:methanol and water (55:45, v/v) with 2% acetic acid; Flow rate: 0.7 mL/min; Detection: 293 nm and 395 nm.

HPLC Analysis of F_1, F_2, F_3 from Silica Gel Chromatography

The intention of silica gel chromatography is to achieve a certain degree of resolution between A and B, to facilitate their preparative separation by HSCCC, in addition to removing some of the polar impurities. The HPLC analysis of fractions F_1, F_2, and F_3, derived from silica gel chromatography, demonstrated that only a limited amounts of A and B can be resolved with this procedure, and most of them were coeluted. The chromatograms of F_1, F_2, and F_3 are shown in Fig. 2.

HSCCC Separation of Aloin from F_1, F_2, F_3

As aloins A and B are moderately polar compounds, their separation was initiated with a solvent system composed of chloroform-methanol-water (4:3:2, v/v/v), using the lower phase as a mobile phase. The separation profiles of F_1 and F_2 are presented in Fig. 3. HPLC analysis revealed that aloin A in F_1 was only partially purified through this solvent system. However, aloins A and B in F_2 can be almost completely separated from each other with a slightly longer separation time. A reversed phase solvent system, such as hexane-ethyl acetate-methanol-water (lower phase mobile), failed to resolve these two compounds. The HPLC chromatogram of sub-fractions I and II from F_2 are given in Fig. 3. As sub-fraction I from F_1 and F_2 have similar HPLC profiles, only one was given in Figure 3; they were combined as fraction I. For the same reason, sub-fraction II and F_3 were combined as fraction II. Both fractions I and II were subjected to further HSCCC purification.

Several solvent systems composed of hexane-ethyl acetate-methanol-water at various volume ratios were tested and, finally, the system composed of ethyl acetate-methanol-water (5:1:5, v/v/v) was found suitable to remove those polar impurities existing in fraction I, while another, more polar, system composed of butanol-ethyl acetate-water (1:3:4, v/v/v) was used to purify compound B in fraction II. The separation profiles of fractions I and II are given in Fig. 4. The target fractions A and B were collected, concentrated, and freeze dried. Finally, 202 mg of A and 140 mg of B were obtained, each at a high purity through the above separation procedure, among which aloin A is light yellow stellated small crystals and B is a yellow powder.

Purity Analysis and Structure Confirmation of Derived A and B

HPLC analysis demonstrated that compound A is of 98% purity at a wavelength of 293 nm and even higher at 359 nm, while B is about 96% pure at the two detection wavelengths, as shown in Fig. 5.

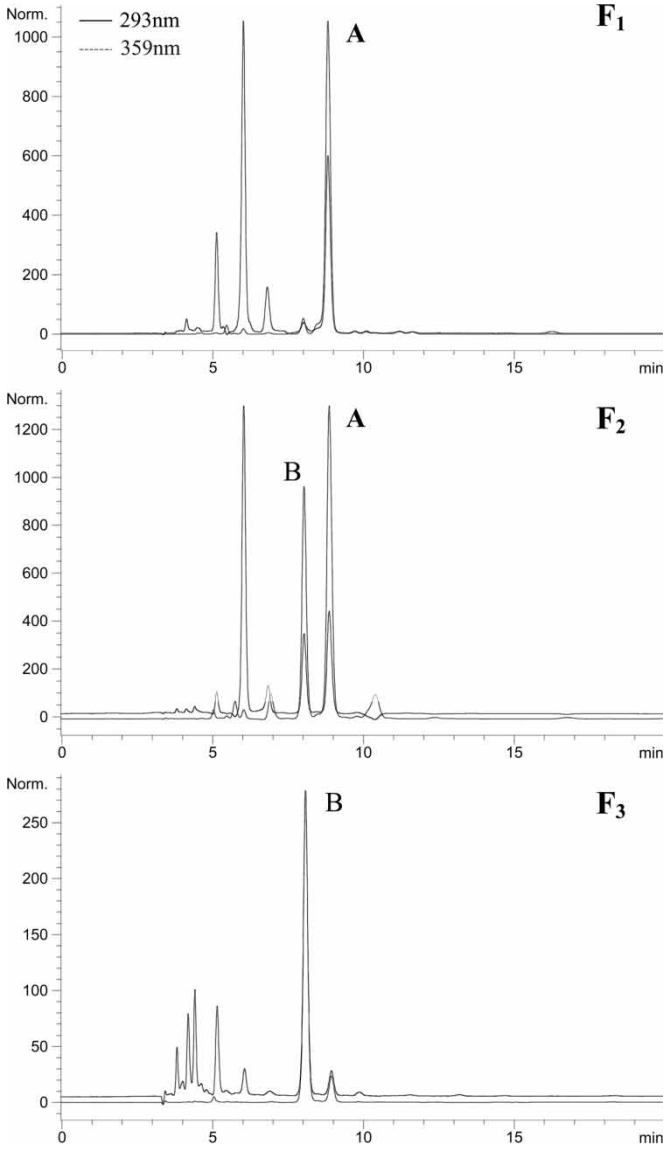

Figure 2. HPLC analysis of fraction F_1, F_2, and F_3 derived from silica gel chromatography. The condition is as Figure 1.

FAB-MS analysis indicated that both A and B are of similar molecular weight, i.e., 418, implying that they are two isomers, among which B is more like aloin B.

No significant difference was observed in the ^{1}HNMR spectra of A and B, and they all fit well with data reported in the literature as listed in Table 2. The

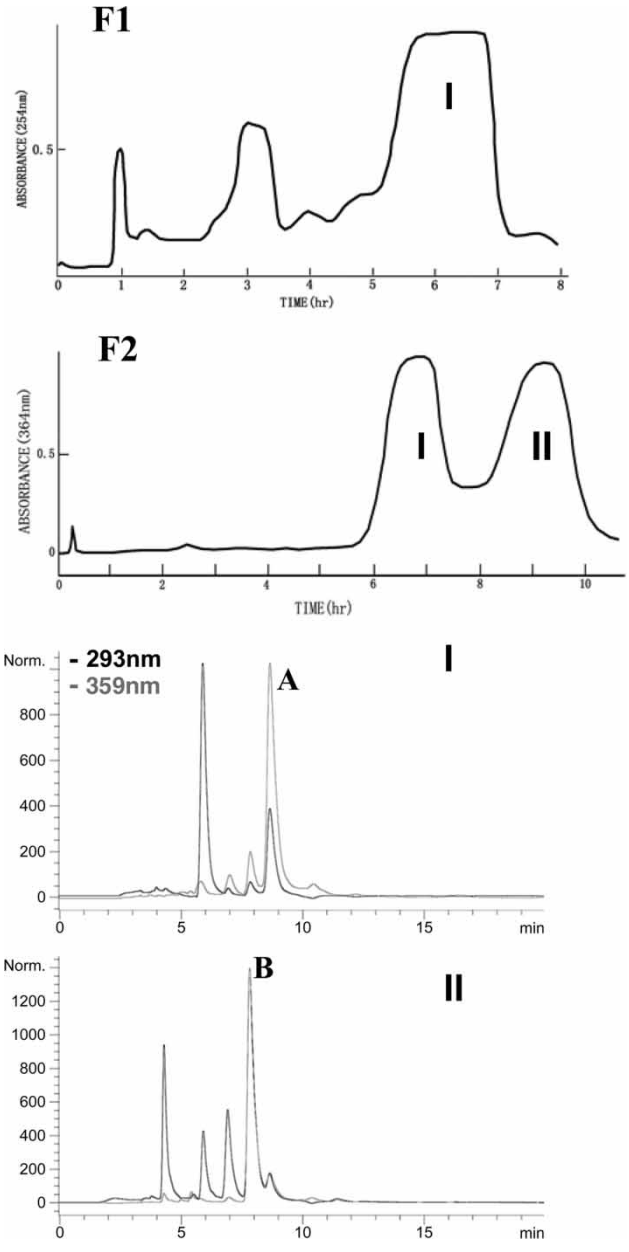

Figure 3. HSCCC separation of F_1 and F_2 and HPLC analysis of fraction I and II. Experimental conditions are as follows: apparatus: multilayer coil planet centrifuge at a 10 cm revolution radius; coiled separation column: 1.6 mm ID PTFE tubing, 220 mL capacity; solvent system: chloroform-methanol-water (4:3:2, v/v/v); mobile phase: lower organic phase; flow rate: 2 mL/min; revolution: 800 rpm; sample: F_1 210 mg; F_2 150 mg. HPLC condition is described in the Figure 1 caption.

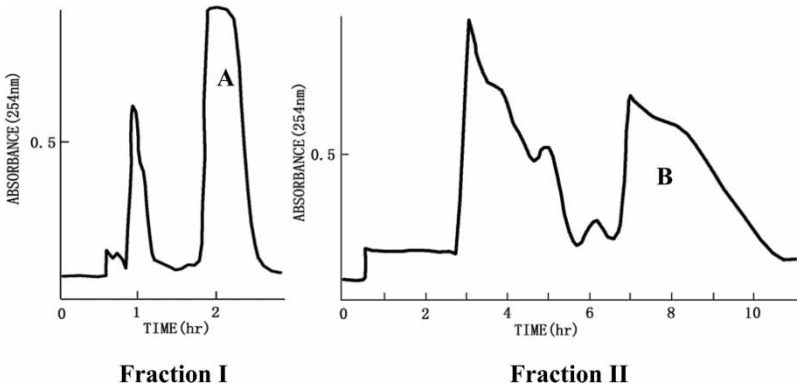

Figure 4. HSCCC separation of sub-fractions I and II. Fraction I, solvent system: ethyl acetate-methanol-water (5:1:5, v/v/v), lower phase mobile, sample: 120 mg; Fraction II, solvent system: butanol-ethyl acetate-water (1:3:4, v/v/v), lower phase mobile, sample: 150 mg. Other conditions are described in the Fig. 3 caption.

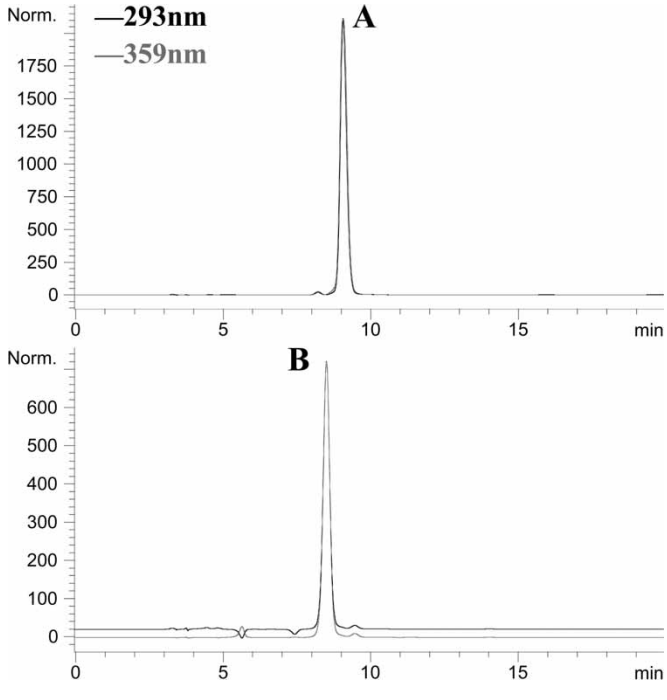

Figure 5. HPLC chromatograms of A and B obtained from separation. Experimental conditions are described in the Figure 1 caption.

Table 2. Comparison of ^{1}HNMR data (CD_3OD, 600 MHz) of A and B obtained from separation with that of aloins A and B from literature(DMSO, 300 MHz)[15]

Proton	A	Aloin A[16]	B	Aloin B[15]
2-H	6.87(s)	6.86(s)	6.85(s)	6.82(s)
4-H	7.04(s)	7.04(s)	7.05(s)	7.01(s)
5-H	7.05(d,7.2)	7.08(d,8.0)	7.07(d,7.8)	7.08(d,8.0)
6-H	7.48(dd,7.8)	7.57(dd,8.0)	7.46(dd,7.8)	7.54(dd,8.0)
7-H	6.85(d,7.8)	6.89(d,8.0)	6.84(d,8.4)	6.89(d,8.0)
10-H	4.59(d,2.0)	4.57(d,2.0)	4.59(d,2.0)	4.57(d,2.0)
11-H_2	4.64(d,7.2)	4.56(d,6.0)	4.63(d,4.2)	4.56(d,6.0)
1′-H	3.40(dd,9.6,2.0)	3.28(dd,9.5,2.0)	3.40(dd,9.6,2)	3.30(dd,9.5,2.0)
2′-H	3.00(dd,9.6)	2.79(dd,9.5)	3.01(dd,9.6)	2.77(dd,9.5)
3′-H	3.24(dd,8.4)	3.08(dd,9.5)	3.25(dd,8.4)	3.07(dd,9.5)
4′-H	2.87–2.94(m)	2.63–2.80(m)	2.87–2.90(m)	2.60–2.75(m)
5′-H	2.87–2.94(m)	2.63–2.80(m)	2.87–2.90(m)	2.60–2.75(m)
6′-H_a	3.37(dd,11.0,5.0)	3.16(dd,11.0,5.0)	3.37(dd,11,5)	3.08(dd,11.0,5.0)
6′-H_b	3.55(dd,11.0,2.0)	3.38(dd,11.0,1.8)	3.54(dd,12,2)	3.34(dd,11.0,1.8)

slight difference can be attributed to the difference of solvent used in ^{1}HNMR analysis. CD_3OD was used in our experiment, while DMSO and DMSO-$CDCl_3$ (1:3) were used in the literature.[15] To further confirm A and B from their conformation, GOESY (Gradient enhanced nuclear overhauser effect spectroscopy) ^{1}HNMR analysis was conducted.

It was found that the signal of 4-H was enhanced by irradiation of 1′-H for compound B, while the signal of 5-H was enhanced by the same irradiation of 1′-H for compound A. According to the literature,[15] compounds A and B were identified as aloin A and aloin B, respectively. Their structures are presented in Fig. 6.

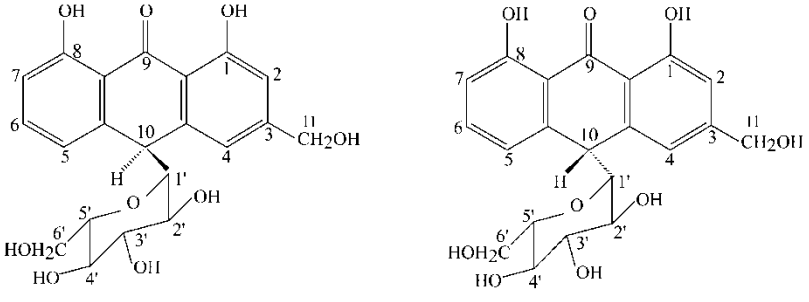

Figure 6. The conformation structures of aloin A and aloin B.

These assignments are consistent with their chromatographic behavior in HPLC and HSCCC. As aloin A is less polar than aloin B, it is eluted out after aloin B in reverse HPLC, as shown in the above figures. In normal phase HSCCC separation with the chloroform-methanol-water solvent system, the fraction I, rich in aloin A, is eluted before aloin B. This analysis further confirmed the above conformational assignment.

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REFERENCES

1. Saleem, R.; Faizi, S.; Siddiqui, B.S.; Ahmed, M.; Hussain, S.A.; Qazi, A.; Dar, A.; Ahamed, S.I.; Qazi, M.H.; Akhtar, S.; Hasnain, S.N. Planta Med. **2001**, *67*, 757.
2. Groom, Q.J.; Reynolds, T. Planta Med. **1986**, *52*, 354.
3. Grun, M.; Franz, G. Mill. Arch. Pharm. **1982**, *315*, 231.
4. Zonta, F.; Bogoni, P.; Masotti, P.; Micali, G. J. Chromatogr. A **1995**, *718*, 99–106.
5. Brown, J.P.; Dietrich, P.S. Mutat. Res. **1979**, *66*, 3.
6. Ishii, Y.; Tanizawa, H.; Takino, Y. Bio. Pharm. Bull. **1974**, *17*, 651.
7. Wamer, W.G.; Vath, P.; Falvey, D.E. Free Rad. Biol. Med. **2003**, *34*, 233.
8. Yamamoto, M.; Ishikawa, M.; Masui, T.; Nakazawa, H.; Kabasawa, Y. JAOAC **1985**, *68*, 493.
9. Park, M.K.; Park, J.H.; Kim, N.Y.; Shin, Y.G.; Choi, Y.S.; Lee, J.G.; Kim, K.H.; Lee, S.K. Phytochem. Anal. **1998**, *9*, 186.
10. Rebecca, W.; Kayser, O.; Hagels, H.; Zessin, K.H.; Madundo, M.; Gamba, N. Phytochem. Anal. **2003**, *14*, 83.
11. Kuzuya, H.; Tamai, I.; Beppu, H.; Shimpo, K.; Chihara, T. J. Chromatogr. B **2001**, *752*, 91.
12. Wang, D.X.; Yang, G.L.; Wang, L.Y.; Song, X.R. Chin. J. Chromatogr. **2000**, *18*, 423.
13. Elsohly, M.A.; Gul, W.; Murphy, T.P. Intl. Immunopharmacol. **2004**, *4*, 1739.
14. Ito, Y. CRC Crit. Rev. Anal. Chem. **1986**, *17*, 65.
15. Manitto, P.; Monti, D.; Speranza, G. J. Chem. Soc. Perkin. Trans. **1990**, *1*, 1297.

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