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快速制备液相色谱分离库拉索芦荟中 10-羟基芦荟大黄素苷

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摘　要:采用快速制备液相色谱从库拉索芦荟中高效分离制备 10-羟基芦荟大黄素苷 A 和 B。以库拉索芦荟药材粉甲醇提取物为原料,采用快速制备液相色谱,EYELA 柱(300 mm × 20 mm i. d.,20 ~ 45 μm),甲醇-水为流动相(35∶65,v/v)等度洗脱,流速 10 mL/min,检测波长 356 nm,对芦荟样品进行分离制备,得到 2 种化合物单体,经 UV、旋光度、HRMS 和 NMR 鉴定,HPLC 测定纯度,2 个化合物分别为 10-羟基芦荟大黄素苷 B(98.9%)和 10-羟基芦荟大黄素苷 A(98.2%)。该方法简便、快速,所得产物纯度较高,可用于对照品的制备和药理毒理活性研究。

关键词:库拉索芦荟;快速制备液相色谱;10-羟基芦荟大黄素苷 A;10-羟基芦荟大黄素苷 B

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Preparative Isolation of 10-hydroxyaloins from *Aloe vera* Using Reversed-phase Flash Chromatography

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Abstract: 10-hydroxyaloins A and B, two anthrones from *Aloe vera*, were successfully separated by reversed-phase flash chromatography on a C_{18} column (300 mm × 20 mm i. d., 20-45 μm) eluting with methanol-water (35∶65, v/v) at a flow rate of 10 mL/min. In a one-step operation, 6.5 mg of 10-hydroxyaloin B and 4.7 mg of 10-hydroxyaloin A were isolated from 200.0 mg of *A. vera* extract at purities of 98.9% and 98.2%, respectively. The structures of the two compounds were characterized on the basis of spectroscopic evidences (NMR, HRMS, UV and optical rotationdata) and literature data. The procedure offered a simple, fast and efficient method for the preparation of reference substances of 10-hydroxyaloins A and B which can be used for the further study on their analysis and biological properties.

Key words: *Aloe vera* L.; flash chromatography; 10-hydroxyaloin A; 10-hydroxyaloin B

Introduction

Aloe vera (*Aloe barbadensis* Mill) is a member of Asphodelaceae (Liliaceae) family which is widely distributed in Europe, Asia and southern parts of North America[1]. It has long been used as ingredients of food products, beverages, cosmetics and pharmaceuticals due to various biological properties and these biological activities lead to the studies of its composition[2]. Among its previously investigated chemical components, anthroneis the main secondary metabolites detected in *A. vera*[3]. Aloin, a common anthrone, is found in various aloe genuses and occurs as a mixture of the two diastereoisomeric aloin A and aloin B. As oxidation products of aloins, 10-hydroxyaloins were also reported as constituents of aloe species which presented in small a-

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mounts, naturally occurring as a pair of diastereoisomers as well as aloins A and B[4] (Fig. 1).

The popularity of *A. vera* owes to its multiple pharmacological properties including antiproliferative and antitumor activities, wound healing, anti-inflammatory effects, and purgative action. Aloin and other anthrones in *A. vera* have been shown to possess most of these properties[5]. To date, however, few research on the pharmacological activities of 10-hydroxyaloins A and Bwas reported. There is an urgent need to provide large quantities of pure 10-hydroxyaloins for in depth studies. The isolation of 10-hydroxyaloins was traditionally achieved by multiple column chromatographic procedures[6], which is time-consuming with low efficiency and yield. Therefore, it would be of great importance to develop an efficient method for the separation and purification of this diastereoisomers. Flash chromatography is basically an air pressure driven hybrid of medium pressure and short column chromatography which has been optimized for particularly rapid separation[7]. It can endure relatively high flow rate with low pressure, offering good separation in a short time at a proper chromatographic condition. Flash chromatography can be applied for normal phase separation and reversed phase separation[8].

In this paper, a method for preparative isolation of 10-hydroxyaloinsA and B using reversed-phase flash chromatography was developed. The method is simple, fast and efficient, and the compounds obtained are with high purity (over 98.0%), providing a basis for studies on the analysis and biological properties of 10-hydroxyaloins.

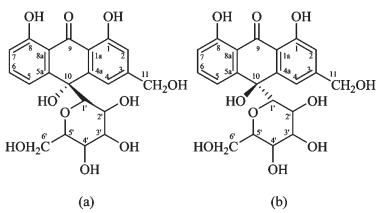

Fig. 1 Chemical structures of 10-hydroxyaloins A (a) and B (b)

Experimental

Apparatus

Preparative separation was carried outby an EYELA chromatography system (Kyoto, Japan), equipped with a low pressure gradientor, intellegent pumps, a UV-9000 UV-Vis detector, a PG-12 gastorr. HPLC analysis was carried out on a liquid chromatography system (Shimadzu, Kyoto, Japan) equipped with two LC-20AD pumps, a CTO-20A column oven and a SPD-M20A DAD detector. HRMS spectra were obtained with aLC-MS-IT-TOF mass spectrometer (Shimadzu, Kyoto, Japan), ^{1}H NMR and ^{13}C NMR spectra were acquired on a Bruker Avance III 400 Nuclear Magnetic Resonance Spectrometer and optical rotations were measured with a Perkin Eimer 341polarimeter. A SB25-12 DTD ultrasound machine (Scientz Biotechnical. Ltd, Ningbo, China), an electronic balance (KERN ABT 220-5DM, 0.1 mg, Germany) and RE-300 rotational vacuum concentrator (Shanghai YaRong biochemistry instrument factory, China) were utilized for sample preparation.

Reagents

A. vera powder (Batch No:20110601) was purchased from Yunnan Yuanjiang Evergreen Biological Co., Ltd. (Yuxi, China) and authenticated by Associate Professor Jinzhi Wan (School of Pharmaceutical Sciences, SunYat-Sen University). Preparative C_{18} reversed phase silica gel was purchased from TianjinBonna-Agela Technologies Co., Ltd (Lot:BL0001L2201, size:20-45 μm, Qty: 100 g, Tianjin, China). Methanol used for HPLC was of HPLC grade manufactured by SK Chemicals (Korea). Ultrapure water obtained from Milli-QRG purification unit (Millipore, Bedford, MA, USA) was used for all solutions, dilutions and HPLC analysis. Other chemicals were of analytical grade and purchased from Tianjin Damao Chemical Reagent Factory (Tianjin, China).

Sample preparation

200.0 mg of dried *A. vera* powder was extracted under sonication with 500 mL of methanol at room temperature for 30 min. The extraction procedure was repeated for five times. The extracts were combined and evaporated to dry by rotary evaporator at 40 ℃ under reduced pressure. The extract was yielded after rotary evaporator and dissolved in 50% methanol, affording a sample solution.

Analytical HPLC method

An Agilent TC-C_{18} column (250 mm × 4.6 mm, 5 μm) preceded by a Phenomenex C_{18} guard column was used; The binary mobile phase was composed of ultrapure water (A) and methanol (B). The elution was run with a gradient program at 1.0 mL/min: 0-25 min, 45% B→50% B. Detection wavelength was set at 356 nm. The sample injection volume was 10 μL. The column temperature was ther mostated at 30 ℃.

Preparative flash chromatography method

A reversed-phase C_{18} column (manually packed, 300 mm × 20 mm, particle size: 20-45 μm). The mobile phase was composed of water (A) and methanol (B) = 65∶35. The flow rate was 10 mL/min and the effluent was monitored at 356 nm. 1.0 mL of the sample solution was injected into the reversed-phase C_{18} column and the effluent from the column was collected into test tubes with a fraction collector set at 5 mL for each tube. Fractions within the same peak were combined, concentrated and dried under reduced pressure. Peak 1 and peak 2 were collected separately (Fig. 2).

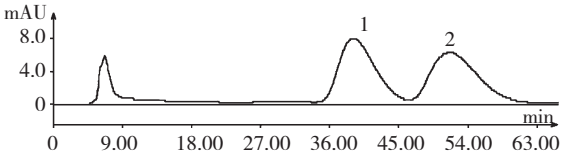

Fig. 2 Chromatogram of the sample solution eluted with flash chromatography

Results and Discussion

Selection of the extraction solvent

In this study, 10-hydroxyaloins in *A. vera* powder were extracted by ultrasonic extraction method. According to the solubility of the two targeted compounds, three solvents including methanol, ethyl acetate and acetone were selected to extract 10-hydroxyaloins. After HPLC analysis, both methanol and acetone were found to give higher extraction efficiencies. Due to the lower cost of methanol, it was selected as the extraction solvent (Fig. 3).

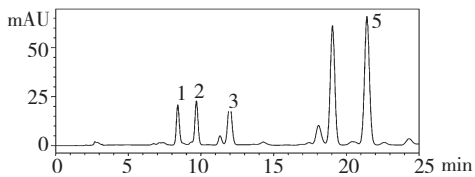

Fig. 3 HPLC chromatogram of the methanol extract of *A. vera* powder

Optimization of flash chromatography conditions

The effects of particle size and injection volume on chromatographic separation were investigated in this study. As the polarity of 10-hydroxyaloinsA and B were similar, C_{18} reversed-phase silica gel with 20-45 μm of the particle size was selected, and the result gave a satisfactory resolution. Regarding the sample injection volume, it gave rise to low yield and purity when loading 0.5 mL of the sample solutions onto the manually packed column, while bad resolution was found when enlarging the injection volume more than 1.0 mL. Finally the sample injection volume was set at 1.0 mL. The gradient of the mobile phase was optimized as well, and isocratic elution with methanol-water (35∶65, v/v) as the mobile phase gave an ideal resolution and a relatively short analysis time.

Compound **1** and compound **2** were isolated by the optimized reversed-phase flash chromatography conditions. After dried under reduced pressure, 6.5 mg of 10-hydroxyaloin B and 4.7 mg of 10-hydroxyaloin A were yielded, with their chromatographic purities of 98.9% and 98.2% (Fig. 4), respectively.

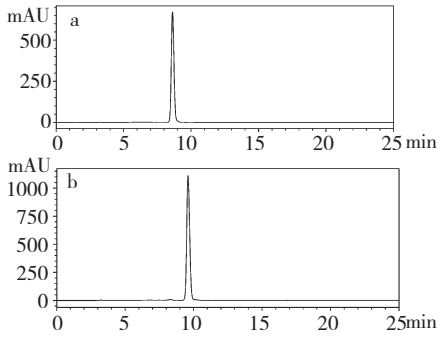

Fig. 4 HPLC chromatogramof isolated 10-hydroxyaloin B (a) and 10-hydroxyaloin A (b)

Confirmation of the two compounds

The structural data of compound **1** were listed as follows, which were the same as 10-hydroxyaloin B[4,6,9]. Yellow amorphous powder; UV (MeOH) λ_{max} nm: 269, 301, 364; HRMS (ESI) calcd. for $C_{21}H_{22}O_{10}$ [M + Na]$^+$ 457.0439, found 457.0448; $[\alpha]_D^{20}$ -49.7° (*c*

0.95, MeOH); ^{1}H NMR and ^{13}C NMR spectral data were summarized in Table 1.

The structural data of compound 2 were listed as follows, which matched the data of 10-hydroxyaloin A[4,6,10]. Yellow amorphous powder; UV (MeOH) λ_{max} nm: 269, 301, 364; HRMS (ESI) calcd. for $C_{21}H_{22}O_{10}$ $[M+H]^+$ 435.1015, found 435.1012; $[\alpha]_D^{20}$ + 102.8° (*c* 1.07, MeOH); ^{1}H NMR and ^{13}C NMR spectral data were summarized in Table 1.

Table 1 ^{13}C and ^{1}H NMR data of 10-hydroxyaloin Band 10-hydroxyaloin A (CD_3OD, *J* in Hz, δ in ppm)

No.	10-hydroxyaloin B		10-hydroxyaloin A	
	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR
1	163.17		163.36	
1a	115.96		116.61	
2	115.46	6.91 (1H, br, d, *J* = 1.5 Hz)	115.54	6.98 (1H, br, d, *J* = 1.5 Hz)
3-α	152.45	4.68 (1H, d, *J* = 14.5 Hz)	151.73	4.75 (1H, d, *J* = 14.8 Hz)
3-β		4.63 (1H, d, *J* = 14.5 Hz)		4.70 (1H, d, *J* = 14.5 Hz)
4	116.41	7.47 (1H, d, *J* = 1.1 Hz)	116.99	7.41 (1H, s)
4a	146.48		146.89	
5	118.95	7.36 (1H, m)	118.13	7.50 (1H, dd, *J* = 7.7, 0.9Hz)
5a	149.19		148.87	
6	136.48	7.55 (1H, t, *J* = 8.0 Hz)	137.24	7.60 (1H, t, *J* = 8.0 Hz)
7	118.37	6.92 (1H, dd, *J* = 6.8, 1.3 Hz)	118.08	6.95 (1H, dd, *J* = 8.3, 0.9 Hz)
8	163.12		162.67	
8a	117.87		117.37	
9	194.50		194.53	
10	76.97		76.83	
11	64.83		64.75	
1′	85.25	3.26 (1H, d, *J* = 9.2 Hz)	85.33	3.30 (1H, d, *J* = 9.8 Hz)
2′	73.06	2.99 (1H, t, *J* = 9.1 Hz)	73.03	3.00 (1H, m)
3′	79.65	3.24 (1H, d, *J* = 8.9 Hz)	79.63	3.28 (1H, d, *J* = 8.9 Hz)
4′	71.73	2.83 (1H, t, *J* = 10.0 Hz)	71.76	2.85 (1H, t, *J* = 9.3 Hz)
5′	81.65	2.92 (1H, m)	81.73	2.96 (1H, m)
6′-α	63.36	3.55 (1H, dd, *J* = 11.7, 2.5 Hz)	63.68	3.61 (1H, dd, *J* = 11.8, 2.5 Hz)
6′-β		3.37 (1H, t, *J* = 6.0 Hz)		3.41 (1H, t, *J* = 5.9 Hz)

Conclusion

10-hydroxyaloin B and 10-hydroxyaloin A were successfully separated by reversed-phase flash chromatography on a manually-packed C_{18} column, with purities of 98.9% and 98.2%, respectively. The established method was proved to be simple, fast and reproductive. It can be applied to the preparation of reference substances of 10-hydroxyaloins, providing a basis for studies on the analysis and biological properties.

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