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Silver Ion-Complexation High-Speed Countercurrent Chromatography Coupled with Prep-HPLC for Separation of Sesquiterpenoids from Germacrene A Fermentation Broth

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Abstract: A silver ion high-speed counter-current chromatography ([Ag^+]-HSCCC) was developed to separate and purify five sesquiterpenoids from germacrene A fermentation broth. The solvent system was consisted of *n*-hexane-methanol-silver nitrate (3 mol/L) solution (10:9.5:0.5, *v*/*v*). By employing this chromatographic protocol, five sesquiterpenoids named *β*-elemene (**1**; 54.1 mg), germacrene A (**2**; 28.5 mg), *γ*-selinene (**3**; 4.6 mg), *β*-selinene (**4**; 3.4 mg), and *α*-selinene (**5**; 1.3 mg) were obtained successfully from 500 mg extracted crude sample with purities of 97.1%, 95.2%, 98.2%, 96.3% and 98.5%, respectively, combined with preparative HPLC. The results reveal that the addition of metal ion in biphasic solvent system significantly improved the HSCCC separation factor of sesquiterpenoids. Meanwhile, our study also provided an alternate approach to separate the compounds with less polarity, also geometrical isomers and various natural product classes.

Keywords: *β*-elemene; metal ion; high-speed counter-current chromatography; coordination complexation; DFT analysis; separation

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1. Introduction

β-Elemene (**1**; *β*-1-methyl-1-vinyl-2,4-diisopropenyl-cyclohexane), an active noncytotoxic class II antitumor drug derived from Chinese medicinal herb *Curcuma phaeocaulis* Val., has been reported to possess broad spectrum anti-cancer effects such as lung [1], breast [2], glioma [3], and gastric [4] etc. Pharmacological studies have proved that *β*-elemene (**1**) can play a role through a variety of pharmacological pathways including arresting the cell cycle [5], inhibiting cell proliferation [3], exerting antimetastasis and antiangiogenesis effects [4], and reversing multiple-drug resistance inducing cell apoptosis [6]. Because of the lipid solubility and small molecular weight, *β*-elemene (**1**) can break through the blood-brain barrier for the treatment of glioma [7]. Notably, 0*β*-elemene has been approved

by China's State Food and Drug Administration for the treatment of cancer as a second-class innovative drug [8] and applied for clinical studies in Food and Drug Administration department at USA. Besides the above anticancer effects, it also exhibited the properties like antioxidant [9], larvicidal [10], and for the treatment of various diseases including airway stenosis [11], neuroprotection [12], and rheumatoid arthritis [13]. Because of its broad-spectrum, safe, efficient, economical and other outstanding advantages in antitumor activities, this compound has numerous broad clinical applications.

The current production of β-elemene (**1**) is via extraction from plant sources. However, these plant sources are limited and the yield obtained is extremely low. In addition, biosynthesis is an important way to obtain β-elemene (**1**). Furthermore, the biosynthetic method has a short production cycle, is not limited by the time and supply of raw materials, and has relatively simple comparative products, which can be converted into become large-scale industrial production [14]. It has been used to biosynthesize the germacrene A (**2**) [15]. Germacrene A (**2**) was then further thermally converted to form β-elemene (**1**) [16]. However, there are usually non-quantitative concomitant impurities such as selinene analogues, which are generally difficult to separate by classic column chromatographic methods. Therefore, it is necessary to develop an efficient method for the preparative separation of β-elemene (**1**) from certain sources. Herein, germacrene fermentation broth was employed as the source for separation of β-elemene (**1**) which could provide more efficient way to obtain β-elemene for further drug development and quality control.

Techniques previously used for essential oils isolation include traditional silica gel column chromatography, silver ion coordination chromatography; macro porous resin chromatography, preparative-HPLC, GC-MS [17], GC-MS-FID [18], GC-FID [19] and supercritical fluid chromatography. High-speed counter-current chromatography (HSCCC), a unique liquid-liquid separation chromatography, is a popular separation method for the advantages such as higher sample recovery, less solvent consumption, and lower risk of sample denaturation. Previously HSCCC has been reported to separate alkaloids [20], flavonoids, acids, and furanocoumarins [21,22]. HSCCC has been used to separate the essential oils from plant material as well [23]. In consideration the similar chemical structures of the essential oils, it is hard to separate the compounds in the traditional HSCCC. Herein, a silver ion HSCCC ([Ag^+]-HSCCC) is developed. Recently silver ion HSCCC has been successfully applied for the separation of *cis*/*trans* asarone [24]. Thus by using [Ag^+]-HSCCC, the subtle structural differences between β-elemene and its analogues can be overcome by coordination forces with silver ions to enhance peak resolution. However, the separation of β-elemene and its analogues from Germacrene A fermentation broth by HSCCC has not been reported in the field of coordination chemistry. The separation parameters, HSCCC solvent system, ion concentrations and separation mechanism are discussed.

2. Materials and Methods

2.1. Reagents and Materials

All organic solvents used for HSCCC separation were of analytical grade (Nanjing Reagent Factory, Nanjing, China). Methanol and acetonitrile used for HPLC were of chromatographic grade (Merck, Whitehouse Station, NJ, USA). Water was produced by milli-Q system (18MΩ) (Millipore, Bedford, MA, USA).

The germacrene A fermentation broth used in present study was provided by the Institute of Chinese Medicine, Chinese Academy of Chinese Medical Sciences, and Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences. Synthetase (germacrene A synthase) and farnesyl pyrophosphate synthase fusion protein encoding nucleic acid was introduced into the host yeast (*Saccharomyces cerevisiae*) with the Chinese patent No.: CN201711064197.5, this recombinant strain can greatly improve the germacrene A production at the industrial application value.

2.2. Apparatus

TBE-300C HSCCC equipment was used with three multilayer column of a total 300 mL (diameter of the PTFE tube as 2.6 mm) and a 20 mL sample loop (Shanghai, Tauto Biotech, China). Four other instrument modules, including a 8823A-UV Monitor (Beijing Emilion Technology, Beijing, China), a 3057 portable recorder (Sichuan Instrument Factory, Sichuan, China), a Model DC-0506 low constant temperature bath and a TBP-5002 pump (Tauto Biotechnique, Shanghai, China). An Agilent 1120 HPLC system (Agilent Technologies, Santa Clara, CA, USA), equipped with an online vacuum degasser, a Binary-Pump, an automated injection valve, a thermostated column compartment, a DAD, and an Agilent ChemStation has been selected to analyze samples. The preparative HPLC system consisted of L-3245 quaternary pump, L-3500 UV-Vis detector (RIGOL Technologies, China), a 1 mL sample loop, a workstation (UltraChrom, Beijing, China), and an SilGreen reversed-phase C_{18} column (250 mm $\times$10 mm, 5 μm).

2.3. Preparation of the Crude Extract for HSCCC Isolation

The germacrene A fermentation broth (3.4 L) was added to a 5 L steam distillation bottle, steamed extraction was carried out for 15 h, and the condensate was collected. The upper oil phase of the condensate was transferred, while the lower aqueous phase was extracted with petroleum ether twice to extract the residual oil. The extract was combined, dried with anhydrous sodium sulfate, and evaporated under reduced pressure at 30 °C. The essential oil (33.37 g) was obtained with the main components of β-elemene (**1**) and germacrene A (**2**). The essential oil was placed in a round bottom flask, protected with nitrogen, heated to 150 °C, and kept at a reaction time for 1.0 h. Finally, a mixture of sesquiterpenoids (32.30 g) was obtained for further HSCCC separation.

2.4. Selection of Two-Phase Solvent System for HSCCC

Partition coefficient (K_D-values) of each target compound is one of the most important factors in a successful separation by HSCCC. In this study, *n*-hexane-ethyl acetate-methanol-water (HEMWat) with various solvent ratios was examined to optimize the K_D-values of the target compounds by HPLC analysis. Moreover, $AgNO_3$ was added to get a better separation. In brief, first suitable crude sample was weighed in a 10 mL centrifuge tube into which 4 mL of each phase of the pre-equilibrated two-phase solvent was added. The tube was capped and shaken vigorously for several minutes in order to thoroughly distribute the sample between the two phases. After two clear layers were formed, 0.5 mL of each phase was taken separately in 4 mL centrifuge tubes and diluted with 2 mL methanol each. The upper phase was analyzed by HPLC directly and the lower phase was added sodium chloride to remove the silver ion. The tube of the lower phase was shaken and then centrifuged at 5000 rpm for 10 min, the supernatant was analyzed by HPLC. The K_D-values of the target compounds were calculated according to the equation $K_D = A_L/A_U$, where A_U and A_L were the peak areas of target compound in the upper and lower phases, respectively.

2.5. Preparation of Solvent System and Sample Solutions

In HSCCC separation, two-phase solvent system consisted of *n*-hexane-methanol-silver nitrate solution (3 mol/L) (10:9.5:0.5, *v*/*v*) were placed into a separating funnel. After being shaken vigorously, the solution was kept still for several minutes. Then, it was separated into two phases for the experiment. The upper and lower phases were degassed by ultrasound before use. The sample solutions were prepared by dissolving 500 mg of the essential oils in 10 mL isometric upper and lower phases.

2.6. HSCCC Separation

In each separation, the coil column was first filled with the lower phase (stationary phase) at a flow rate of 30 mL/min, and then the apparatus was rotated at 800 rpm, while the upper phase (mobile phase) was pumped into the column at a flow rate of 5.0 mL/min

with tail-head mode. After the mobile phase front emerged and hydrodynamic equilibrium was established in the column, the sample solution was injected into the HSCCC system through the 20 mL sample loop. The effluent of the column was continuously monitored with a UV-Vis detector at 210 nm. The fractions were collected into 12 mL tubes every 2 min with an automatic sampler. After separation, the solvent in the CCC column was blown-out with nitrogen. The blowout solvent was concentrated under vacuum at 40 °C, and the residue was extracted with equal volume of *n*-hexane three times. The upper layer was discarded, and the lower aqueous phase was concentrated under vacuum at 40 °C to dryness to recover silver nitrate.

2.7. Sample Analysis and Structural Identification

HPLC was used to analyze the crude sample and separated compounds peaks. The mobile phase was methanol and water (95:5, *v*/*v*) with a flow rate of 1.0 mL/min, a wavelength of 210 nm, and injection volume of 5.0 µL. Thin layer chromatography was developed to detect the samples with *n*-hexane as developing solvent and iodine vapor as color reagent. The identification of HSCCC peak fractions were performed by HPLC, electron impact mass spectrometry (EI/MS) (Agilent Technologies, Santa Clara, CA, USA) and NMR spectra with a Bruker AV-400 spectrometer (Bruker BioSpin, Rheinstetten, Germany) with $CDCl_3$ as solvent.

3. Results

3.1. Two-Phase Solvent System in HSCCC

One of the most important steps in performing an HSCCC separation is the selection of an appropriate solvent system, which is not an exception in complexation HSCCC. According to the golden rules proposed by Ito partition coefficient of the target compounds between two phases should be in the range of 0.2–2.0 and α values between two compounds should be higher than 1.5 [25]. A lower K_D value elutes with lower resolution and a larger K_D results in broader peaks because of the prolonged elution time. The choice of a suitable biphasic system is guided by the chemical nature of target compound such as the sample polarity, solubility, ionic form and ability to form complexes. Due to the small polarity of the target compounds, *n*-hexane-ethyl acetate-methanol-water was found as the ideal solvent system, which could cover a wide range of polarity by modifying the volume ratio of the four solvents. Different volume ratios of this system were tested and the K_D values of target compounds were determined by HPLC. However, the K_D values are too small no matter what the volume ratio is and they cannot be separated at all by conventional HSCCC.

According to the Hund's rule, the silver ion (Ag^+) in $AgNO_3$ is d^{10} type ([Kr] $4d^{10}$), occupying the empty *s* and *p* orbitals (5*s*, 5*p*x, 5*p*y and 5*p*z), and can react with bond compounds to form the different stability coordination complex [26]. Based on this phenomenon, silver ion chromatography was developed and applied in thin-layer chromatography, high-performance liquid and supercritical fluid chromatography [27]. So silver ion HSCCC was used in this experiment.

Different concentrations of silver nitrate, including 0–3 mol/L were added into the water which was composed of *n*-hexane-ethyl acetate-methanol-water (9:1:9:1, *v*/*v*) and (10:0:9.5:0.5, *v*/*v*). The K_D values of target compounds (Figure 1) were investigated under the same conditions and summarized in Table 1. For determination of K_D values, each phase (upper and lower) of the selected solvent system was taken in a tube in equal volumes. Approximately 2 mg of the crude sample was added, and dissolved very well. After the phases had fully separated, 1 mL of each layer was taken and dried with nitrogen, separately. Both the samples were dissolved in 1 mL of methanol each, in different tubes, and analyzed by analytical HPLC. The partition coefficients (K_D-values) of the compounds were calculated according to the formula $K_D = A_S/A_M$, where A_S and A_M were the peak areas of target compound in upper and lower phases, respectively

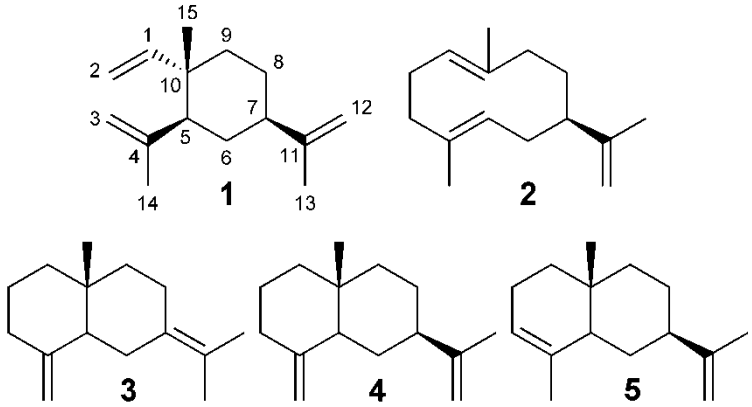

Figure 1. The chemical structures of compounds **1**–**5**.

Table 1. The partition coefficient (K_D values) of target compounds in different solvent systems.

n-Hexane:Ethyl Acetate:Methanol:Water	K_D Value						
	1	**2**	**3**	**4**	**5**	α_{12}	α_{23}
5:5:5:5	<0.01	<0.01	<0.01	<0.01	<0.01	1.00	1.00
8:2:8:2	0.02	0.02	0.02	0.02	0.02	1.00	1.00
5:5:8:2	0.07	0.07	0.04	0.06	0.05	1.00	1.00
9:1:9:1	0.05	0.05	0.05	0.06	0.05	1.03	0.99
10:0:9.5:0.5	0.11	0.11	0.08	0.09	0.09	1.00	1.00
9:1:9:1 1 mol/L [Ag^+]	0.68	0.87	0.06	0.13	0.05	1.51	1.27
9:1:9:1 2 mol/L [Ag^+]	0.77	1.07	0.05	0.10	0.05	1.44	1.39
9:1:9:1 3 mol/L [Ag^+]	1.82	2.86	0.11	0.27	0.14	1.55	1.57
10:0:9.5:0.5 1 mol/L [Ag^+]	0.42	0.45	0.14	0.22	0.16	1.47	1.06
10:0:9.5:0.5 2 mol/L [Ag^+]	0.77	0.91	0.14	0.26	0.15	1.51	1.19
10:0:9.5:0.5 3 mol/L [Ag^+]	1.33	1.74	0.16	0.38	0.18	1.50	1.30

Without [Ag^+] in the system, all compounds would be eluted very quickly and could not be separated at all. As the [Ag^+] concentration increased, all peaks eluted later because the partition coefficients increased. The separation factors between the peaks were also improved except for the compound **3** and **5** (α_{35}). As we can see in Table 1, for *n*-hexane-ethyl acetate-methanol-water (9:1:9:1, *v*/*v*/*v*/*v*), when the silver ion concentration in water was 2 mol/L, the K_D values of compounds **3**, **4** and **5** are too small, when the silver ion concentration in water was increased to 3 mol/L, the K_D value of compound **2** was too high, caused it difficult to be eluted. For *n*-hexane-ethyl acetate-methanol-water (10:0:9.5:0.5, *v*/*v*), when the silver ion concentration in water was kept 3 mol/L, the K_D values of five compounds were almost in line with the golden rule. Therefore, *n*-hexane-ethyl acetate-methanol-silver nitrate solution (3 mol/L) (10:0:9.5:0.5, *v*/*v*) was selected finally for HSCCC experiment.

3.2. Scheme of Complexation via Computational Study

To complete an applauding complexation scheme, simulations using the density functional theory (DFT) approach were accomplished. For this end, geometrical optimization computations particularly with no imaginary frequencies were obtained for all possible coordinated complexes based on the active sites (double bonds) in five sesquiterpenoids with Ag metal ion. All calculations were performed in Gaussian 16 tool using the CAM-B3LYP/6-311++G (2d,p) level of theory including the universal solvation model SMD [28] for water solvent with a dielectric constant of ε = 78.36 [29]. Additionally, the relativistic effective core potentials (LanL2DZ) basis set was used to get the optimization of Ag metal [30].

The most stable coordinated complexed was carefully chosen based on their energies and bond lengths. For instance β-elemene, there were three possible exposed double

bonds to make a complex. However, the given isomer in Figure 2 showed the most appropriate one. Similarly, for germacrene total two isomers were examined on account of the unsaturated bonds in the ring together participated to build chelation, however, the exo-double exhibited stronger bond relatively owing to the active donation of the π-electrons for the ultimate complexation as shown in Figure 2(2). For other three sesquiterpenoids such as γ-selinene, β-selinene and α-selinene there were two possible double bonds for metal-π bond interactions and the most stable complexes are shown in Figure 2(3,4,5) respectively.

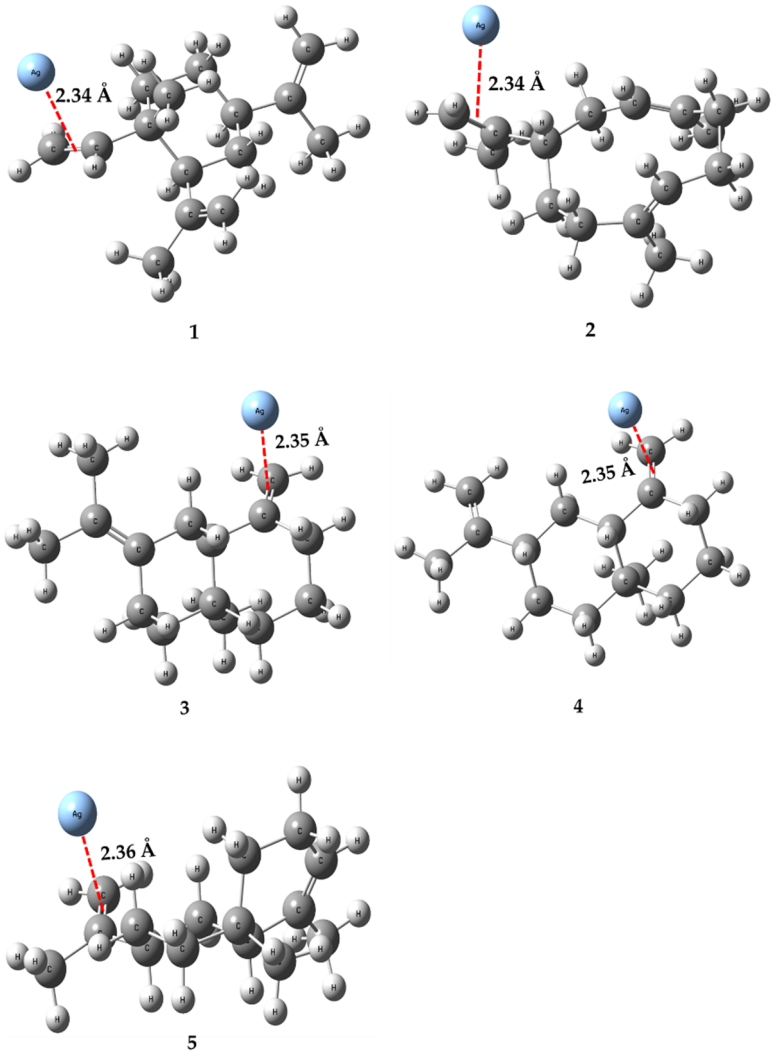

Figure 2. Complexation scheme for compounds **1**–**5**.

From the results it was concluded that all the compounds have shown the potential affinity towards the Ag ion which lead to separate them effectively.

3.3. HSCCC Procedure

Under optimized conditions, the crude sample (500 mg) was completely dissolved in the selected two-phase solvent system (20 mL) for the separation by HSCCC with the following parameters: rotation speed, 800 rpm; two-phase solvent system, *n*-hexane-methanol-silver nitrate solution (3 mol/L) (10:9.5:0.5, *v*/*v*); flow rate, 2.0 mL/min; column temperature, 25 °C. The effluent was collected by an automatic sampler. Each fraction obtained by HSCCC was analyzed by HPLC. Under the optimum conditions, compound **1** (54.1 mg), compound **2** (28.5 mg), mixture of compound **3** and **5** (11.9 mg), compound **4** (3.4 mg), were obtained from 500 mg crude extract (Figure 3). The mixture of compounds **3**

and **5** was further separated using prep-HPLC (Figure 4). Finally, 4.6 mg of compound **3** and 1.3 mg of compound **5** were obtained. As shown in Figure 5, the purities of compounds **1**–**5** were 97.1%, 95.2%, 98.2%, 96.3% and 98.5%, respectively.

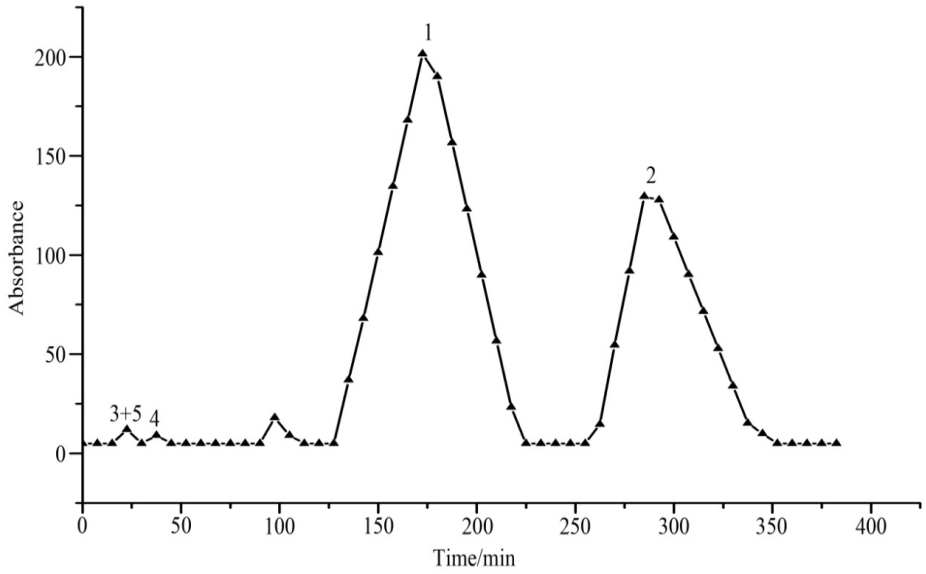

Figure 3. Simulated HSCCC separation chromatogram of Germacrene A fermentation broth by adding [Ag^+]. Two-phase solvent system: *n*-hexane-methanol-silver nitrate solution (3 mol/L) (10:9.5:0.5, *v*/*v*); stationary phase: The lower phase; mobile phase: the upper phase; elution mode: tail to head; flow rate: 5.0 mL/min; revolution speed: 800 rpm; sample size: 500 mg of crude extract dissolved in10 mL isometric upper and lower phases; retention percentage of the stationary phase: 73.3%.

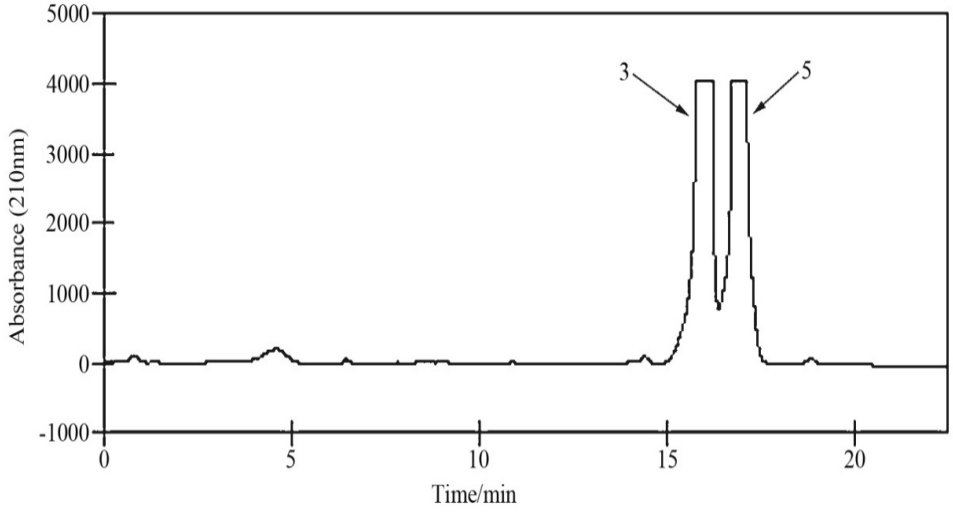

Figure 4. Prep-HPLC chromatogram of compounds **3** and **5**. Mobile phase: methanol-water (98:2, *v*/*v*); wavelength: 210 nm; flow rate: 3 mL/min.

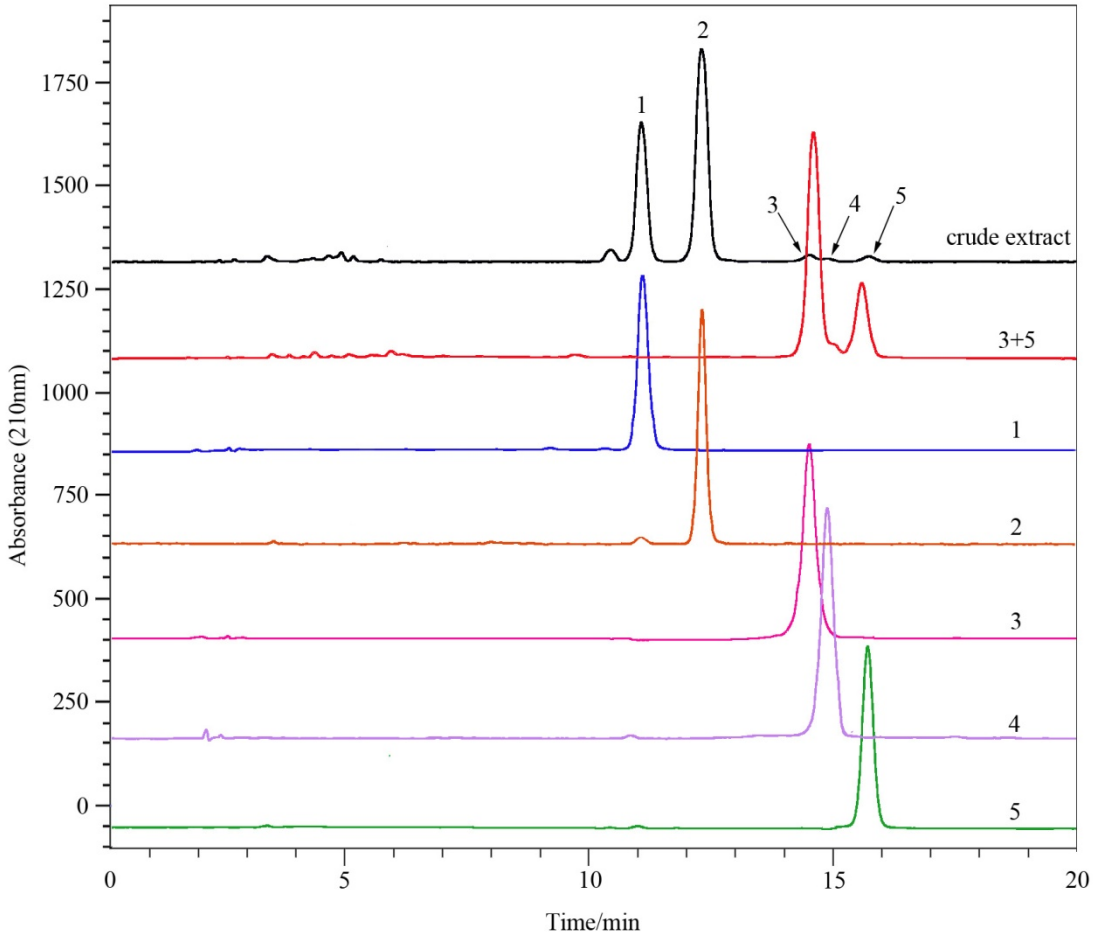

Figure 5. HPLC chromatograms of crude extract and HSCCC fraction of compounds **1**–**5**. Column: waters C_{18} column (4.6 × 250 mm, 5 μm); mobile phase: methanol-water (95:5, *v*/*v*); wavelength: 210 nm; flow rate: 1 mL/min.

3.4. Identification of Compounds

Compound **1** (Peak 1 in Figure 4): colorless oil, EI-MS *m*/*z* 204 $[M]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.93 (3H, s, H-15), 1.64 (3H, s, H-14), 1.68 (3H, s, H-13), 4.52 (1H, s, H-2a), 4.63 (1H, s, H-12a), 4.65 (1H, s, H-2b), 4.75 (1H, s, H-12b), 4.82 (1H, d, *J* = 11.0 Hz, H-3a), 4.83 (1H, d, *J* = 17.2 Hz, H-3b), 5.75 (1H, dd, *J* = 17.2, 11.0 Hz, H-1). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.6 (C-15), 20.0 (C-13), 23.7 (C-14), 25.8 (C-8), 31.9 (C-6), 38.8 (C-10), 38.9 (C-9), 44.7 (C-7), 51.7 (C-5), 107.2 (C-12), 108.8 (C-2), 111.1 (C-3), 146.7 (C-4), 149.3 (C-1), 149.4 (C-11). The data was found in comparison with the literature [31], and the compounds was identified as *β*-elemene.

Compound **2** (Peak 2 in Figure 4): colorless oil, EI-MS *m*/*z* 204 $[M]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.31 (3H, s, H-14), 1.40 (3H, br s, H-15), 1.49–1.56 (1H, m, H-8a), 1.61 (3H, s, H-12), 1.67–1.72 (1H, m, H-8b), 1.75–2.37 (9H, m, H-2ab, 3ab, 6ab, 7, 9ab), 4.52 (1H, d, *J* = 9.98 Hz, H-5), 4.67 (1H, m, H-13a), 4.72–4.77 (1H, m, H-1), 4.79 (1H, s, H-13b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 16.8 (C-14), 16.9 (C-15), 20.4 (C-12), 26.8 (C-2), 33.8 (C-8), 35.0 (C-6), 39.8 (C-3), 42.4 (C-9), 51.5 (C-7), 108.2 (C-13), 126.5 (C-1), 129.1 (C-4), 131.8 (C-5), 138.3 (C-10), 153.90 (C-11). The spectral information was found in accordance with the reported values [32], and the compound was identified as germacrene A.

Compound **3** (Peak 3 in Figure 4): colorless oil, EI-MS *m*/*z* 204 $[M]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.04 (3H, s, H-15), 1.61 (3H, s, H-12), 1.75 (3H, s, H-13), 4.70 (1H, s, H-14a), 4.72 (1H, s, H-14b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.1 (C-2), 19.3 (C-12), 20.9 (C-13), 24.7 (C-15), 27.7 (C-6), 30.8 (C-8), 33.2 (C-3), 34.5 (C-10), 40.3 (C-1), 42.3 (C-9), 46.8 (C-5),

108.1 (C-14), 124.5 (C-11), 134.9 (C-7), 150.8 (C-4). The spectral data were in accordance with the previous report [33], and the structure was identified as γ-selinene.

Compound **4** (Peak 4 in Figure 4): light yellow oil, EI-MS m/z 204 $[M]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.73 (3H, s, H-15), 1.31 (3H, m, H-1a, H-6a, H-9a), 1.46 (2H, m, H-1b, H-9b), 1.57 (5H, m, H-2, H-6b, H-8), 1.75 (3H, s, H-13), 1.82 (1H, d, J = 12.08 Hz, H-5), 1.99 (3H, m, H-3a, H-7), 2.31 (1H, d, J = 13.12 Hz, H-3b), 4.44 (1H, s, H-14a), 4.70 (2H, brs, H-12a, H-14b), 4.72 (1H, s, H-12b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 16.4 (C-15), 21.0 (C-13), 23.5 (C-2), 26.8 (C-8), 29.7 (C-6), 36.0 (C-10), 36.9 (C-3), 41.2 (C-9), 42.0 (C-1), 45.9 (C-7), 49.9 (C-5), 105.4 (C-14),108.1 (C-12), 150.8 (C-4), 151.0 (C-11). The structure of the compound was identified as β-selinene, and the data was compared with the literature [34].

Compound **5** (Peak 5 in Figure 4): colorless oil, EI-MS m/z 204 $[M]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.80 (3H, s, H-15), 1.19 (2H, m, H-6a, H-9a), 1.35 (2H, m, H-1), 1.46 (1H, m, H-9b), 1.56 (2H, m, H-8), 1.61 (3H, s, H-14), 1.75 (3H, s, H-13), 1.78 (1H, m, H-6b), 1.95 (3H, m, H-2a, H-5, H-7), 2.09 (1H, m, H-2b), 4.71 (2H, d, J = 8.0 Hz, H-12), 5.32 (1H, 3, H-3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.6 (C-15), 20.9 (C-13), 21.2 (C-14), 23.0 (C-2), 36.9 (C-8), 28.9 (C-6), 32.3 (C-10), 38.0 (C-1), 40.3 (C-9), 46.7 (C-7), 46.8 (C-5), 108.3 (C-12), 120.9 (C-3), 135.2 (C-4), 151.0 (C-11). The spectral data was found in accordance with the previous report [34], and the compound was identified as α-selinene.

4. Conclusions

In this study, β-elemene, germacrene A and three selinene analogues were obtained in high purity from crude extract of fermentation broth by silver ion high-speed counter-current chromatography combined with preparative high-performance liquid chromatography. The purities were all over 94%. A two-phase solvent system, *n*-hexane-methanol-silver nitrate solution (3 mol/L) (10:9.5:0.5, v/v) was applied. This silver ion HSCCC technique may offer an alternative method for other separation of isomers with unsaturated bonds.

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