Research Article

Separation of *cis*- and *trans*-Asarone from *Acorus tatarinowii* by Preparative Gas Chromatography

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A preparative gas chromatography (pGC) method was developed for the separation of isomers (*cis*- and *trans*-asarone) from essential oil of *Acorus tatarinowii*. The oil was primarily fractionated by silica gel chromatography using different ratios of petroleum ether and ethyl acetate as gradient elution solvents. And then the fraction that contains mixture of the isomers was further separated by pGC. The compounds were separated on a stainless steel column packed with 10% OV-101 (3 m × 6 mm, i.d.), and then the effluent was split into two gas flows. One percent of the effluent passed to the flame ionization detector (FID) for detection and the remaining 99% was directed to the fraction collector. Two isomers were collected after 90 single injections (5 μ L) with the yield of 178 mg and 82 mg, respectively. Furthermore, the structures of the obtained compounds were identified as *cis*- and *trans*-asarone by ^1H - and ^{13}C -NMR spectra, respectively.

1. Introduction

Isomers are quite common in the essential oils from natural plants. *Cis*-/*trans*- or β -/ α -asarones are the main active components in *Acorus tatarinowii* Schott, a traditional Chinese herbal medicine commonly used for improvement of learning and memory [1]. Asarones are known carcinogenic compounds [2, 3]. Actually, the neuroprotective effects of α -asarone [4, 5] and β -asarone [5–11] have been intensively studied, while the α -asarone was found to exhibit more potent effects than the β -asarone [5]. Furthermore, α -asarone was reported to reduce the incidence and duration of tonic seizures induced by maximal electroshock [12] and by pentylenetetrazole [13]. Further study indicated that activities of α -asarone in various animal seizure models [14] and against noise-stress effect [15] might be essentially accounted for by antioxidant properties. In addition, the hypolipidaemic [16–20] and radioprotective effects [21] of α -asarone were also been reported. Besides, β -asarone, which has toxic and sterilizing effects, shows potential for stored-product pest control [22].

On the other hand, the isomers usually have the similar mass spectra, which will make the accurate identification

of those compounds impossible only based on the mass data. For example, furanodienone could be completely transformed into curzerenone during GC-MS analysis of essential oils from *Curcuma* rhizome [23]. And the mass spectra of furanodienone and curzerenone were very similar, which induced the wrong identification of furanodienone as curzerenone without reference compound [24]. To date, HPLC [25–28] and GC-MS [29–32] are two most frequently used techniques for the analysis of asarone. Furthermore, asarone isomers also show similar mass spectra [33], which make the identification of those compounds difficult when the reference compounds were absence. Therefore, the separation and purification of chemical compounds for those isomers are important for the analytical purpose. However, conventional silica gel chromatography is often insufficient to resolve closely related substances and isomers [33]. Gas chromatography provides better separation for many organic compounds. Therefore, preparative gas chromatography (pGC) should be a promising alternative or supplement for fractionation of isomers from the essential oils. As the continuous study of our previous report [34], the pGC was applied for the isolation of *cis*- and *trans*-asarone from the essential oil of *Acorus tatarinowii*.

FIGURE 1: pGC-FID chromatogram for asarone isomers.

(a)

(b)

FIGURE 2: Capillary GC-FID chromatogram and MS spectra for the collected fractions (F1 and F2).

2. Materials and Methods

2.1. Materials. Essential oil of *Acorus tatarinowii* was purchased from Jiangxi ji'an FuDa Nature Medical Oil Factory (Jiangxi, China). Silica gel (100~200 mesh and 200~300 mesh) for column chromatography and silica gel (GF₂₅₄) for thin layer chromatography (TLC) were purchased from Branch of Qingdao Haiyang Chemical Plant (Branch of Qingdao Haiyang Chemical Co., Ltd. Shandong, China). Petroleum ether (PE) and Ethyl acetate (EA) were of analytical grade (Chuandong Chemical Co., Ltd. Chongqing,

China). The voucher specimen of *Acorus tatarinowii* oil was deposited at the Department of Pharmaceutics, College of Chemistry and Chemical Engineering, Chongqing University, Chongqing, China.

2.2. Sample Preparation-Silica Gel Chromatography. In brief, 53.6 g essential oil of *Acorus tatarinowii* was mixed with 100~200 mesh silica gel (the ratio was about 1 : 1.2), and then the mixed sample was subjected onto a column (60 cm × 6.0 cm, o.d.) packed with 800 g 200~300 mesh silica gel and washed by different ratios of PE and EA as gradient elution

FIGURE 3: TLC of collected fractions (F1 and F2). Developing solvent: PE : EA = 3 : 1. Colorizing agent: vanillin in concentrated sulfuric acid, 5% w/v.

FIGURE 4: Chemical structures for *cis*-asarone (a) and *trans*-asarone (b).

solvents. The higher polarity part of the fractions eluted by PE : EA = 20 : 1 was collected and we repeated the separation by silica gel chromatography until the pure mixture of the asarone isomers (a single claret-colored spot detected by TLC) was obtained. Then the target effluent was collected (about 2 g of the mixture of asarone isomers were obtained) and condensed before injected into pGC system.

2.3. pGC System. The pGC system was modified based on an SC-2000 GC instrument (Chuanyi Analyzer Co. Ltd. Chongqing, China) [34]. It is equipped with a stainless steel column packed with 10% OV-101 (3 m × 6 mm, i.d.), a flame ionization detector (FID), a special effluent splitter with minimum dead volume, and a home-made preparative fraction collector. The data was collected and analyzed on a HW-2000 Chromatographic Workstation (Nanjing Qianpu Software Co. Ltd., China).

High-purity nitrogen (N₂) was used as carrier gas at a flow rate of 25 mL/min. The inlet and FID temperature were 230°C and 250°C, respectively. The column temperature was isocratic at 220°C. The effluent was splitted into two

flows, one (1%) towards the FID and the other (99%) to the fraction collector using a special gas effluent splitter. Two restrictor valves were used to control the split flow. In order to supply sufficient gas flow for the FID detection, a supplementary gas (N₂, 10 mL/min) was added before arrived at the detector. Volumes of 5 μL asarone isomers mixture were injected. After being separated by the column, the fractions were collected in 2 mL traps filled with ethyl acetate. The trapping time and peak retention time were synchro. The isolated fractions were analyzed by capillary GC and GC-MS.

2.4. GC-FID and GC-MS Analysis. The purity identification was performed on an SC-6000 GC instrument (Chuanyi Analyzer Co. Ltd. Chongqing, China). Compounds were separated on an AC-5 fused silica capillary column (30 m × 0.32 mm, 0.25 μm, SGE). The inlet and FID temperature were 250°C and 270°C, respectively. Injection volume was 0.1 μL. Carrier gas was N₂. The column temperature was maintained at 105°C for 10 min, and ramped at 10°C min⁻¹ to 150°C, kept at 150°C for 1 min, then ramped at 5°C min⁻¹

to 180°C, eventually to 230°C with the speed of 10°C min⁻¹, and kept at 230°C for 2 min.

GC-MS was performed on a Trace GC Ultra gas chromatography instrument coupled to a DSQ II mass spectrometer and an Xcalibur Version 2.0.7 software (Thermo Fisher Scientific, Boston, MA, USA). Compounds were separated on an HP-5MS (30 m × 0.25 mm, i.d.) capillary column coated with 0.25 µm film 5% phenyl methyl siloxane. The temperature of the column was maintained at 80°C for 2 min and then ramped at 8°C min⁻¹ to 220°C. Split injection with a split ratio of 1:19 and high-purity helium was used as carrier gas with the flow rate of 0.9 mL/min. The spectrometer was operated in electron-impact (EI) mode, the scan range was 10–900 amu, the ionization energy was 70 eV, and the scan rate was 0.34 s/scan. The injection temperature and ionization source temperature were 300 and 230°C, respectively.

3. Results and Discussion

3.1. Isolation of Asarone Isomers by pGC. The GC chromatogram of asarone isomers mixture recorded by pGC with FID detection is given in Figure 1. It was used as a basis for the collection of two fractions (F1 and F2) that were analyzed by the capillary GC and GC-MS system for an evaluation of resolution and yields of the pGC. After 90 injections, it was resulting in amounts of 178 mg (F1) and 82 mg (F2) for the compounds in the respective traps, respectively.

3.2. Identification and Yield of Collected Fractions. Capillary GC chromatograms as well as mass spectra of peaks of every collected fraction are given in Figure 2. It is indicated that the two isomers can be well separated by pGC and result in high purity products (Figure 2). Actually, the isomers are difficult to be separated by conventional method such as silica gel chromatography. As shown in Figure 3, the two separated isomers (F1 and F2) have the same retention factor (R_f) values and similar color (claret-colored). Therefore, pGC shows advantages over the conventional silica gel chromatography in the separation and preparation of volatile isomers.

Furthermore, the obtained fractions were identified by MS (Figure 2), ¹H and ¹³C NMR spectra (shown in the appendix), and fractions 1 and 2 were identified as *cis*- and *trans*-asarone, respectively (Figure 4).

4. Conclusions

Preparative GC on a 3 m × 6 mm peaked column using a FID, an effluent splitter, and a fraction collector was shown with an appropriate resolution (resolution factor (R_s) = 1.49) and yield to obtain pure volatile isomers at milligram level.

Appendix

NMR Data of *cis*- and *trans*-Asarone: Analyzed by AV500 NMR (Bruker, Switzerland), Solvent: CDCl₃, Internal Standard: TMS

cis-Asarone (F1) [35–37]. ¹H-NMR (CDCl₃) δ: 6.85 (1 H, s, H-6), 6.54 (1 H, s, H-3), 6.49 (1 H, dd, J = 1.5, 11.5 Hz, H-7),

5.77 (1 H, dq, J = 7.0, 11.5 Hz, H-8), 1.84 (3 H, dd, J = 2.0, 7.0 Hz, H-9), 3.90 (3 H, s, 2-OCH₃), 3.84 (3 H, s, 5-OCH₃), 3.81 (3 H, s, 4-OCH₃).

¹³C-NMR (CDCl₃) δ: 151.6 (C-4), 148.6 (C-2), 142.5 (C-1), 125.9 (C-7), 124.9 (C-8), 118.1 (C-5), 114.2 (C-6), 97.6 (C-3), 56.7 (5-OCH₃), 56.5 (2-OCH₃), 56.2 (4-OCH₃), 14.8 (C-9).

trans-Asarone (F2) [35, 37]. ¹H-NMR (CDCl₃) δ: 6.95 (1 H, s, H-6), 6.48 (1 H, s, H-3), 6.65 (1 H, dd, J = 1.6, 16.0 Hz, H-7), 6.09 (1 H, dq, J = 7.2, 16.0 Hz, H-8), 1.89 (3 H, dd, J = 1.6, 7.2 Hz, H-9), 3.90 (3 H, s, 2-OCH₃), 3.88 (3 H, s, 5-OCH₃), 3.84 (3 H, s, 4-OCH₃).

¹³C-NMR (CDCl₃) δ: 150.8 (C-4), 148.9 (C-2), 143.5 (C-1), 125.2 (C-7), 124.5 (C-8), 119.2 (C-5), 98.1 (C-6), 56.8 (5-OCH₃), 56.6 (2-OCH₃), 56.2 (4-OCH₃), 18.9 (C-9).

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