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Short communication

Preparative isolation and purification of germacrone and curdione from the essential oil of the rhizomes of *Curcuma wenyujin* by high-speed counter-current chromatography

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Abstract

High-speed counter-current chromatography (HSCCC) was applied to isolate and purify bioactive compounds in the essential oil of the rhizomes of *Curcuma wenyujin*. Two sesquiterpenes germacrone and curdione were successfully isolated and purified using two-phase solvent system composed of petroleum ether–ethanol–diethyl ether–water (5/4/0.5/1, v/v) in tail to head elution mode. 62 mg of germacrone and 93 mg of curdione were obtained from 658 mg of the essential oil each at over 95% purity. Their structures were identified by ^{1}H NMR and EI MS.

Keywords: Counter-current chromatography; Purification; *Curcuma wenyujin*; Germacrone; Curdione

1. Introduction

Curcuma wenyujin is a traditional medicinal plant in China, and its rhizomes and tubers specified as two herbal medicines in Chinese Pharmacopoeia, namely Rhizoma Curcumae and Radix Curcumae, are of wide medicinal value [1]. The essential oil is considered as an effective part of Rhizoma Curcumae, and it is suggested that germacrone is selected as one of major bioactive constituents for control of quality of Rhizoma Curcumae [2]. Reports show that germacrone and curdione are two important bioactive compounds, having many pharmaceutical actions such as anticancer activity, antimicrobial activity, activity on treatment for endotoxin-caused hepatitis and so on [2–4].

High-speed counter-current chromatography (HSCCC) is an all-liquid chromatographic technique based on partitioning of compounds between two immiscible liquid phases. HSCCC does not employ a solid phase support and thus there is no irreversible adsorption associated with the solid supports. In addition, it operates under very gentle conditions and allows non-destructive separation of compounds. As a result, this technology has recently gained increasingly importance in the separation of naturally occurring products, and has been successfully applied to the separation of a number of natural products [5–10]. No report has been seen on the use of HSCCC for the isolation and purification of sesquiterpenes from essential oil. In this paper, we introduce an efficient method for preparative isolation and purification of two sesquiterpenes germacrone and curdione (Fig. 1) from the essential oil of the rhizomes of *C. wenyujin* using HSCCC.

2. Experimental

2.1. Apparatus

A Model TBE-300A high-speed counter-current chromatography (Shanghai Tauto Biotech, Shanghai, China)

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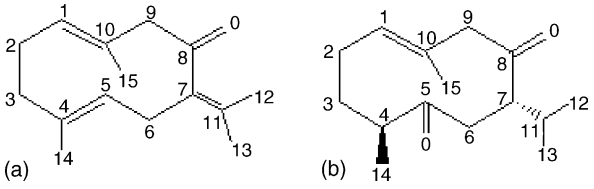

Fig. 1. Chemical structures of germacrone (a) and curdione (b).

equipped with three preparative multilayer coils (290 ml, wound with 2.0 mm i.d. PTFE tubing) was employed in the present research. The β values of this preparative column range from 0.46 to 0.73 ($\beta = r/R$, $R = 9.5$ cm, where r is the distance from the coil to the holder shaft, and R, the revolution radius or the distance between the holder axis and central axis of the centrifuge). The columns of HSCCC were installed in a vessel which was retained at 25 °C by a Model HX-1050 constant-temperature controller (Beijing Detianyou Technology, Beijing, China). The two-phase solvent was pumped into the column with a Model NS-1007 constant-flow pump (Beijing Institute of New Technology Application, Beijing, China). Continuous monitoring of the effluent was achieved with a Model UV-II detector (Shanghai Institute of Biochemistry, Academy of Science, Shanghai, China) set at 254 nm. A manual sample injection valve with a 20 ml loop (Shanghai Tauto Biotech, Shanghai, China) was used to introduce the sample into the column. A Sepu3000 workstation (Hangzhou PuHui Technology, Hangzhou, China) was employed to record the chromatogram. Eluate was collected with a Model BSZ-100 fraction collector (Shanghai Huxi Tech, Shanghai, China).

The high-performance liquid chromatography (HPLC) used was a CLASS-VP Ver.6.1 system (Shimadzu, Japan) comprised of a Shimadzu SPD10Avp UV detector, a Shimadzu LC-10ATvp Multisolvent Delivery System, a Shimadzu SCL-10Avp controller, a Shimadzu LC pump, and a Shimadzu CLASS-VP Ver.6.1 workstation.

2.2. Reagents and materials

All organic solvents used for HSCCC were of analytical grade and purchased from Hangzhou HuiPu Chemical Factory (Hangzhou, China). Petroleum ether used for HSCCC was alkane mixture with boiling point from 60 to 90 °C. Methanol used for HPLC analysis was of chromatographic grade.

The essential oil obtained by steam distillation from the rhizomes of *C. wenyujin* was purchased from Shuinan Yaoyong Baicaoyou Factory (Jishui, Jiangxi, China).

2.3. Preparation of two-phase solvent system and sample solutions

For HSCCC, we selected a two-phase solvent system composed of petroleum ether–ethanol–diethyl ether–water (5/4/0.5/1, v/v), which was selected by a partition experiment of the essential oil in a series of solvent systems composed of petroleum ether–ethanol–diethyl ether–water at different volume ratios using TLC analysis. The solvent mixture was thoroughly equilibrated in a separation funnel at the same temperature as in the vessel of HSCCC and the two phases were separated shortly before use.

The sample solutions were prepared by diluting essential oil in the mixture solution of lower phase and upper phase (1:1, v/v) of the solvent system used for HSCCC separation.

2.4. Separation procedure

HSCCC was performed as follows: the multilayer coiled column was first entirely filled with the lower phase as a stationary phase. The upper mobile phase was then pumped into the tail end of the column inlet at a flow-rate of 1.0 ml/min, while the apparatus was run at a counter clockwise revolution speed of 670 rpm. After hydrodynamic equilibrium was reached, as indicated by a clear mobile phase eluting at the head outlet, the sample solution (658 mg of the essential oil diluted in 18 ml mixture solution of lower phase and upper phase (1:1, v/v) of the solvent system) was injected through the sample port. The eluent from the head end of the column was continuously monitored with the UV detector at 254 nm. Each peak fraction was manually collected according to the elution profile and determined by HPLC. In order to save solvents and time, the compounds eluting after curdione were removed by forcing out the stationary phase with pressurized air instead of eluting them with the mobile phase, because the stationary phase was used only once. The retention of the stationary phase relative to the total column capacity was computed from the volume of the stationary phase collected from the column after the separation was completed.

2.5. HPLC analysis and identification of HSCCC peak fractions

The essential oil of the rhizomes of *C. wenyujin* and each peak fraction from HSCCC were analyzed by the HPLC. The analyses were performed with a Shim-Pack CLC-ODS C_{18} column (250 mm × 4.6 mm i.d.). The mobile phase, a methanol-water mixture (85:15, v/v), was eluted at a flow-rate of 0.8 ml/min, and the eluent monitored by a Shimadzu SPD10Avp UV detector at 254 nm.

Identification of the HSCCC peak fractions was conducted by EI MS and ^{1}H NMR spectra. The NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer with TMS (tetramethylsilane) as internal standard.

3. Results and discussion

In HSCCC, there are two different elution modes: head to tail elution mode and tail to head elution mode. The former is often selected because its flow rate can be kept more stably than that in the latter. In this work, when

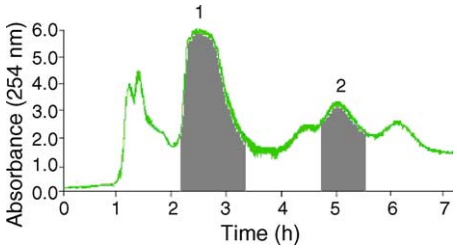

Fig. 2. Chromatogram of the essential oil of the rhizomes of *Curcuma wenyujin* by preparative HSCCC. Peak 1: germacrone; Peak 2: curdione. Solvent system: petroleum ether–ethanol–diethyl ether–water (5/4/0.5/1, v/v); stationary phase: lower aqueous phase; mobile phase: upper organic phase; flow-rate: 1.0 ml/min; revolution speed: 670 rpm; sample: 658 mg diluted in 18 ml mixture solution of lower phase and upper phase (1:1, v/v); retention of the stationary phase: 85%.

the lower phase was used as mobile phase in head to tail elution mode, excessively broad peak containing germacrone was eluted with a relatively long elution time, and the low recovery of target compounds was yielded because they were easy taken away by steam in subsequent process. When the tail to head mode was applied, the above issue was overcome. Therefore, the tail to head mode was employed in subsequent experiments. In order to obtain good resolution of target compounds, four solvent systems composed of petroleum ether–ethanol–diethyl ether–water (6:4:0:1, 5:4:0:1, 5:4:0.75:1, 5:4:0.5:1, v/v) were selected by the partition experiment of the essential oil in a series of different solvent systems using TLC analysis, the four solvent systems were further tested by HSCCC. Among those the solvent system composed of petroleum ether–ethanol–diethyl ether–water at volume ratio of 5:4:0.5:1 gave the best separation of target compounds with good resolution.

Fig. 2 shows the result obtained from 658 mg the essential oil of the rhizomes of *C. wenyujin* by preparative HSCCC. After this separation, the fractions containing germacrone and curdione were collected, respectively. The analysis of these fractions indicated that the Peak 1 fraction contained germacrone which weighed 62 mg, at over 97% purity, and the Peak 2 fraction contained curdione which weighed 93 mg, at over 95% purity, as assayed by HPLC (Fig. 3B and C).

The structural elucidation of the first component germacrone was carried out by ^{1}H NMR and EI MS spectra as follows: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.44 (3H, s, H-14), 1.63 (3H, s, H-15), 1.73 (3H, s, H-13), 1.78 (3H, s, H-12), 2.06–2.40 (4H, m, H-2α, 2β, 3α, 3β), 2.86 (1H, br d, J = 11.2 Hz, H-6β), 2.94 (2H, m, H-6α, 9α), 3.41 (1H, d, J = 10.5 Hz, H-9β), 4.71 (1H, br d, J = 8.6 Hz, H-5), 4.99 (1H, br d, J = 11.7 Hz, H-1). EI MS *m/z* (rel. int.): 218 (5), 176 (9), 175 (22), 136 (64), 135 (75), 107 (100). Its ^{1}H NMR and EI MS signals are in agreement with the data of germacrone [11–12].

The structural elucidation of the second component curdione was carried out by ^{1}H NMR and EI MS spectra as follows: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 0.89, 0.95 (3H × 2, each d, J = 6.6 Hz, H-12, 13), 0.98 (3H, d, J = 6.9 Hz, H-14),

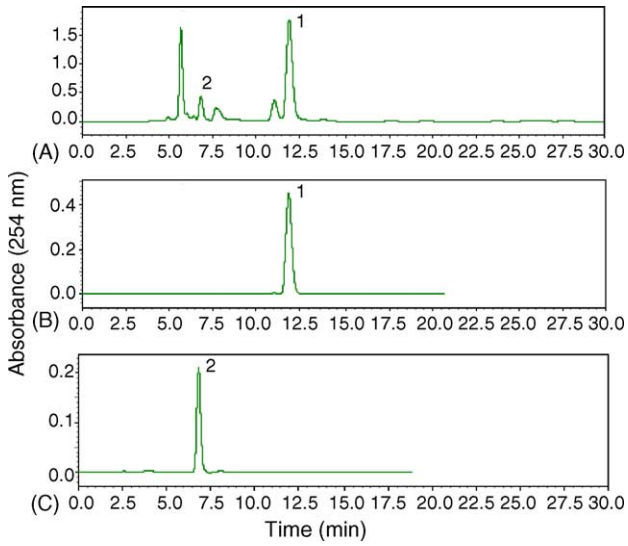

Fig. 3. The result of HPLC analyses of the essential oil and the partially purified sample from the essential oil. Column: Shim-Pack CLC-ODS C_{18} column (250 mm × 4.6 mm i.d.); mobile phase: methanol–water (85:15, v/v); flow-rate: 0.8 ml/min; (A) The essential oil; (B) HSCCC fraction from peak 1 (Fig. 2); (C) HSCCC fraction from peak 2 (Fig. 2).

1.58 (1H, m, H-3α), 1.66 (3H, s, H-15), 1.87 (1H, m, H-11), 2.09–2.14 (3H, m, H-3β, 2α, 2β), 2.34 (1H, m, H-4α), 2.40 (1H, dd, J = 16.6 Hz, J = 2.0 Hz, H-6β), 2.69 (1H, m, H-6α), 2.84 (1H, m, H-7β), 2.94 (1H, d, J = 10.9 Hz, H-9α), 3.07 (1H, d, J = 10.9 Hz, H-9β), 5.17(1H, br s, H-1). EI MS *m/z* (rel. int.): 236 (2), 208 (1), 180 (33), 167 (28), 109 (52), 95 (23), 83 (13), 69 (100), 55 (76). Comparing with the reported data, the ^{1}H NMR and EI MS data are in agreement with curdione [13–14].

After only one-step operation by HSCCC, 62 mg of germacrone and 93 mg of curdione with high purity were obtained from 658 mg of the essential oil of the rhizomes of *C. wenyujin*. The result clearly demonstrated that HSCCC is a fast and effective technique for the preparative separation of germacrone and curdione from the essential oil.

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