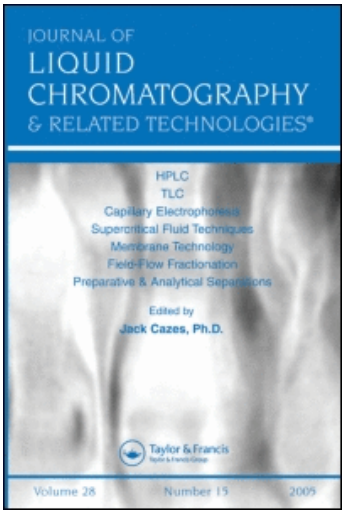

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SEMIPREPARATIVE SEPARATION OF ALKALOIDS FROM *CEPHALOTAXUS FORTUNEI HOOK. F.* BY HIGH-SPEED COUNTERCURRENT CHROMATOGRAPHY

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ABSTRACT

High-speed countercurrent chromatography was applied to the separation of harringtonine, isoharringtonine and homoharringtonine from Cephalotaxus fortunei Hook. f. The separation was performed with a two-phase solvent system composed of chloroform-0.07M sodium phosphate-0.04M citric acid buffer solution (pH 5.0). The fractionated components were identified with authentic pure compounds on TLC and PPC. The peak fraction of each component was analyzed with a mass spectrometer for structure identification. The results indicate that the method is suitable for semipreparative separations of these alkaloids.

INTRODUCTION

Being a liquid-liquid partition chromatographic system with advantages of eliminating the use of solid support matrix, countercurrent chromatography (CCC) is very suitable for separations of active principles from the traditional Chinese medicinal herbs and other natural products. In the past, we have successfully separated various components such as alkaloids (1,2), hydroxyanthraquinones (3), and flavonoids (4) by high-speed CCC.

Isoharringtonine (I), homoharringtonine (II) and harringtonine (III) isolated from Cephalotaxus possess the anticancer potency (5). Among those, (I) and (III) are the isomers, and (II) and (III) differ only by a $-CH_2$ group. They could hardly be separated by adsorption chromatography or other classic purification methods.

In order to produce (II) and (III) from C. fortunei Hook. f., the partition Si gel column chromatographic method had been used in China. But it needed several passages through the chromatographic column. Pewell et al. had reported that the mixture of (I), (II), and (III) were separated by countercurrent partition method (6). However, it also required a very long separation time and consumed a large volume of solvents.

In the present paper, we have isolated all three components with about 500ml of solvents with 3.5 hours by

high-speed CCC. The method yielded a high partition performance and rapid separation in semipreparation of these compounds.

EXPERIMENTAL

Apparatus: The multilayer coil planet centrifuge used in the present study was obtained from the Beijing Institute of New Technology Application, Beijing, China. The apparatus holds a pair of column holders symmetrically on the rotary fame at a distance of 8cm from the central axis of the centrifuge. A 130m-long, 1.5mm ID PTFE (polytetrafluoroethylene) tube was directly wound around the holder hub to form multiple coiled layers with a total capacity of 230ml. The revolution speed of the apparatus is adjustable with a speed controller in the range from 0 to 1000rpm; 700-800 rpm was used in the present separation.

The solvent was pumped into the column with a Milton Roy metering pump (Model 196-31, Milton Roy Company, U.S.A.). A uv detector (Model ZS2, Factory of the Academy of Military Medicinal Science, Beijing, china) and a strip-chart recorder (Model xwx 1042, Shanghai Dahua Instrument Factory, Shanghai, China) were used to monitor the effluent at 254nm.

Preparation of two-phase solvent system: Chloroform-0.07M sodium phosphate buffer solution (1:1, v/v) (pH

5.0) was selected. The solvent mixture was vigorously shaken in a separatory funnel and the two phases separated shortly before use.

Preparation of sample solution: A crude methanol extract from the leaves and branches of C. fortunei Hook. f. was acidified with dilute hydrochloric acid. After removing the precipitated impurities by filtration, the extract was treated with a base to extract the alkaloids with chloroform. The alkaloids in the chloroform were extracted by dilute hydrochloric acid. Then, by adding a base to the extract to raise the pH to 6.7, the alkaloids were again extracted with chloroform. The chloroform solution was evaporated to obtain a crude alkaloid powder, 10 to 20mg of which were dissolved in 1 ml of the solvent mixture and used as the sample solution for each experiment.

Separation procedure: The column was first entirely filled with the stationary aqueous phase followed by injection of the sample solution containing 10-20mg of the crude extract in the lower chloroform phase. The apparatus was rotated at 800rpm while the nonaqueous mobile phase was pumped into the column at a flow rate of 2ml/min. The effluent from the outlet of the column was continuously monitored with a uv monitor at 254nm and collected with a fraction collector at 2min intervals per tube. After a group of nonpolar components was eluted,

the centrifuge was stopped while pumping was resumed to collect a polar component still retained in column.

Identification of alkaloids: Both crude sample solution and high-speed CCC fractions were subjected to TLC and PPC (paper partition chromatography) for tentative identification of the alkaloids. The TLC plate was prepared with SiO_2-Gel and 2% NaOH and activated at 105°C for one hour. TLC was developed with ethyl acetate-acetone (6:2.5) and stained with a Dragendorff reagent to detect the alkaloids.

For PPC, a chromatographic filter paper was dipped into 0.07M Na_2HPO_4-0.04M citric acid buffer solution saturated with n-butanol before chromatography, and dried at room temperature. n-Butanol was chosen to develop the plate and a modified Dragendorff reagent was used to detect the compounds. As shown in Fig. 1, harringtonine, isoharringtonine, and homoharringtonine are all cephalotaxin-derived alkaloids.

RESULTS AND DISCUSSION

Fig. 2 shows a chromatogram obtained from the crude extract of Cephalotaxus fortunei Hook. f. The separation was performed with a two-phase solvent system composed of chloroform/0.07M sodium phosphate-0.04M citric acid buffer (pH 5.0) (1:1, v/v) by eluting with the upper aqueous phase at 2ml/min. After peaks 1 - 5 were eluted,

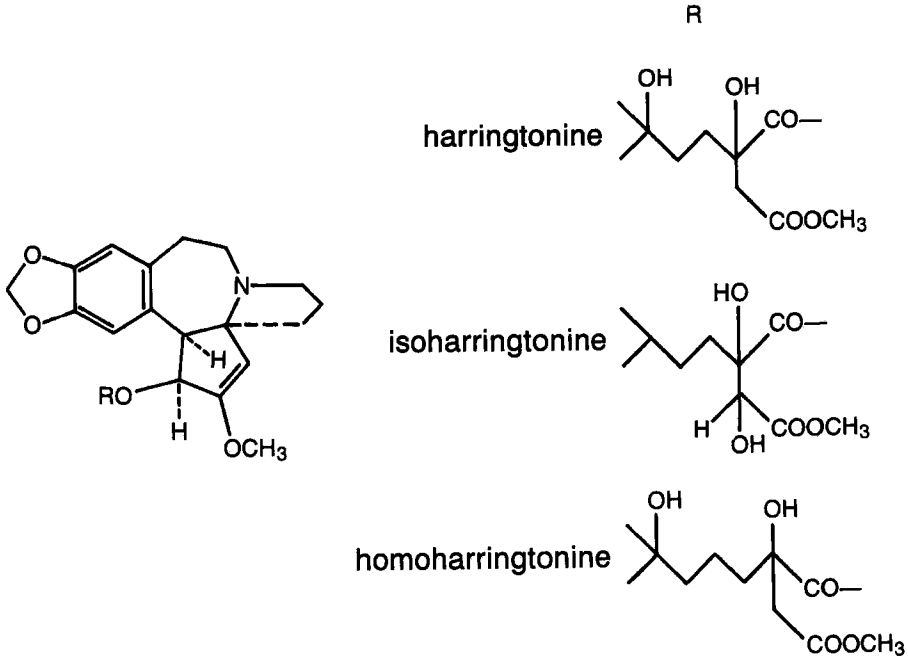

FIGURE 1. Chemical structures of harringtonine, isoharringtonine, and homoharringtonine.

the centrifuge run was terminated to elute peak 6 from the column.

Each peak fraction was analyzed by TLC and PPC. As shown in Fig. 3, the fraction corresponding to Peaks 2, 4, and 6 were identical with isoharringtonine, homoharringtonine, and harringtonine, respectively. A JMS-D 300 type mass spectrometer was used to analyze the peak fractions. The mass spectrum obtained from the fraction of peak 2 showed important fragments at m/z 531 ($M^{+\cdot}$), 315, 314, 298 (B^{+}), 284, 282, 266, 150, 137, 133,

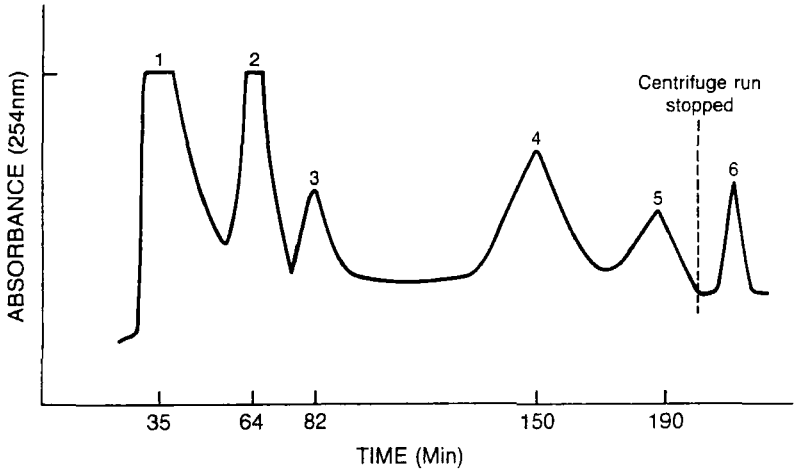

FIGURE 2. High-seed CCC separation of harringtonine, isoharringtonine and homoharringtonine from a crude extract of Cephalotaxus fortunei Hook. f.

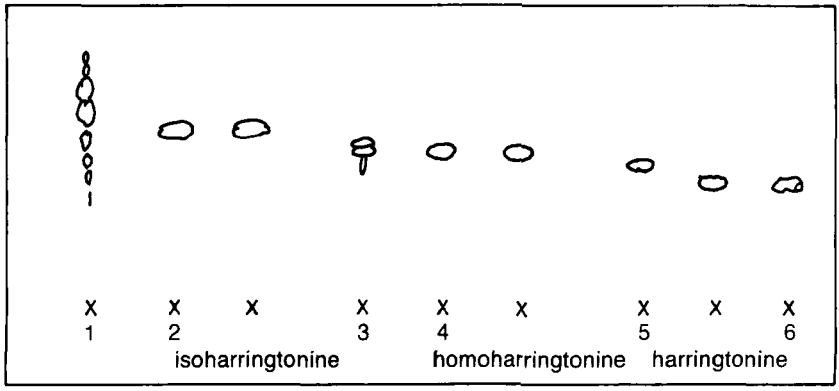

FIGURE 3. TLC analysis of high-speed CCC fractions obtained from a crude extract of Cephalotaxus fortunei Hook. f.

indicating that isoharringtonine is the major component in peak 2. The mass spectrum from the fraction of peak 4 shows important fragments at m/z 545 ($M^{+\cdot}$), 512, 315, 314, 298 (B^{+}), 284, 282, 265, 150, 137, 133, 59, indicating that homoharringtonine is the major constituents in peak 4. The important fragments of the compound from the fraction of peak 6 were similarly determined at m/z 531 ($M^{+\cdot}$), 315, 314, 298 (B^{+}), 284, 282, 266, 150, 137, 133, 59, according to the mass spectrum which indicates that harringtonine is the major compound in peak 6.

In order to increase the yield of homoharringtonine, we applied 300mg of the crude extract which produced 70mg of the pure product. The present method will be useful for semipreparative separation of alkaloids and other natural products with similar polarity.

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