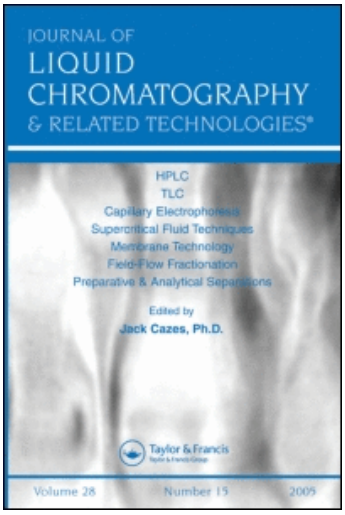

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SEPARATION OF ALKALOIDS FROM DATURA METE L. AND SOPHORA FLAVESCENS AIT BY HIGH-SPEED COUNTERCURRENT CHROMATOGRAPHY

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ABSTRACT

High-speed countercurrent chromatography was successfully applied to the separation of alkaloids from two medicinal herbs. Matrine and oxymatrine were isolated from the root of Sophora flavescens Ait with a two-phase solvent system composed of chloroform-0.07 M sodium phosphate (pH 6.4), while atropine and scopolamine were purified from the flowers of Datura mete L. with a similar solvent system by modifying the phosphate buffer pH at 6.5. Identification of each alkaloid was made by either TLC or paper partition chromatography using authentic pure compounds.

INTRODUCTION

High-speed countercurrent chromatography (HSCCC) is a newly developed technology particularly suitable for separation of

chemical components from medicinal herbs and other natural products. The capability of the method has been demonstrated in our laboratory in separations of hydroxyanthraquinones (1, 2), alkaloids (3), etc. This paper describes the separation of alkaloids from the root of Sophora flavescens Ait and flowers of Datura mete L. with HSCCC. Datura mete L. is said to be effective for relieving asthma, a cold cure and alleviating pain while Sophora flavescens Ait is used for the treatment of various diseases in China as antifebrile, diuretic, anthelmintic, and antidote.

EXPERIMENTAL

Apparatus

The present studies were performed with a multilayer coil planet centrifuge constructed at the Beijing Institute of New Technology Application, Beijing, China. The apparatus holds a pair of column holders symmetrically on the rotary frame at a distance of 8 cm from the central axis of the centrifuge. The multilayer coil separation column was prepared by winding a 110 m long, 1.5 mm I.D. PTFE (polytetrafluoroethylene) tube directly onto the holder hub making multiple coiled layers between a pair of flanges spaced 7.4 cm apart. The total capacity measured 230 ml. One column was used for separation while a counterweight was mounted on the other holder to balance the centrifuge system. The revolutional speed of the apparatus was regulated with a speed controller in a range between 0 and 1000 rpm while 700-800 rpm was applied in the present experiments.

The solvent was pumped with a Milton Roy metering pump (Model 196-31) (Milton Roy Co., USA), and the effluent was monitored at 254 nm with a uv detector (Model ZS2, Factory of the Academy of Military Medical Science, Beijing, China) with a strip-chart recorder (Model XWX-1042, Shanghai Dahua Instrument Factory, Shanghai, China). Combination of these instruments provided an efficient elution system for performing HSCCC.

Extraction of Crude Alkaloids

Raw roots of Sophora flavescens Ait (ca 250 g) were extracted with methanol three times, then the methanol extracts were combined and acidified to pH 3-4 with 6 M hydrochloric acid. The acidic liquid was diluted to about 500 ml with distilled water and then concentrated to 50 ml by evaporation under a reduced pressure. The pH of the extract was adjusted to 13 with NaOH so that the alkaloids could be extracted with dichloromethane, using a separatory funnel, from the aqueous extract. After evaporation of the dichloromethane, the crude alkaloids were then redissolved in about 30 ml of chloroform.

The flowers of Datura mete L. (ca 250 g) were first baked in an oven at 80°C for two hours and then ground into a fine powder with a mortar. A moderate amount of ethanol was added to extract the alkaloids using a water bath heated to 70°C. This extraction was repeated three times and the ethanol was evaporated at the same temperature. The extracts were then acidified with dilute hydrochloric acid to pH 3-4 and filtered to remove the insoluble

material. Sodium hydroxide was added to the acidic extract to raise the pH to 9.0 followed by extraction of the alkaloids with chloroform as described above.

Identification of Alkaloids

Identification of alkaloids in both crude extracts and HSCCC fractions was performed by either TLC and/or paper partition chromatography (PPC) analyses. Three different solvent systems were used for the TLC analyses: chloroform-methanol-7% ammonium hydroxide (5:0.4:0.6, v/v/v) for the alkaloids extracted from S. flavenscens Ait, and ethyl acetate-methanol-7% ammonium hydroxide (17:2:1) or chloroform-methanol-7% ammonium hydroxide (8:2:0.25, v/v/v) for the alkaloids from D. mete L. After TLC development, the alkaloids on the TLC plate were identified with the Dragendorf reagent.

The PPC analyses were performed with a solvent system composed of chloroform saturated with sodium phosphate buffer (pH 6.5). Before development, the filter paper was thoroughly saturated with the stationary phase, i.e., the phosphate buffer solution mentioned above. Then the chromatograms were developed with the chloroform-rich mobile phase at room temperature.

Preparation and Evaluation of Two-Phase Solvent Systems

For HSCCC separations of alkaloids, we have selected two-phase solvent systems composed of chloroform-0.07 M sodium phosphate buffer solution (1:1, V/V) in which pH of the buffer solution was

adjusted at 6.4 for S. flavescens Ait extract and at 6.5 for D. mete L. extract. Each solvent mixture was thoroughly equilibrated in a separatory funnel and the two phases separated shortly before use.

In order to ensure the satisfactory retention of the stationary phase in the multilayer coil, preliminary experiment was performed in the following way without loading the sample solution in the column: The entire multilayer coil and the flow tubes were filled with the stationary upper aqueous phase, and the lower nonaqueous phase was eluted through the column in the head-to-tail elution mode while the apparatus was rotated at a selected revolution speed. After the solvent front emerged from the column, the centrifuge run was stopped and, by connecting the inlet of the column to a pressured N_2 line, the column contents were collected into a graduated cylinder to measure the volume of the stationary phase retained in the column. The results showed that a high retention level of 80% was obtained at the revolution speed of 800 rpm.

HSCCC Procedure

In each separation, the column was entirely filled with the stationary aqueous phase. This was followed by injection of chloroform containing 10 mg of the crude sample. Then the apparatus was rotated at 800 rpm while the nonaqueous mobile phase was pumped into the head of the column at a flow rate of 2 ml/min. The effluent from the outlet of the column was continuously

monitored with a uv monitor at 254 nm and then fractionated with a fraction collector at 2 min intervals or 4 ml per tube. When a group of nonpolar components was eluted, the centrifuge run was stopped while pumping was continued to fractionate polar components retained in the column. Finally, the coiled column was cleaned by elution of distilled water and then methanol. The column was finally dried by passing N_2 through it for several min.

RESULTS AND DISCUSSION

Fig. 1A shows a chromatogram of crude alkaloids extracted from S. flavescens Ait obtained by HSCCC. Separation was performed with a two-phase solvent system composed of chloroform-0.07 M sodium phosphate buffer solution (pH 6.4) (1:1, v/v) using the lower nonaqueous phase as the mobile phase. As indicated in the diagram, peaks 1, 2, and 3 were eluted during the centrifuge run and peaks 4 and 5 from the column contents after stopping centrifugation.

By means of the TLC and PPC analyses, fractions corresponding to peaks 1 and 5 were identified as matrine and oxymatrine, respectively (Fig. 1B). Fractions corresponding to peaks 2 and 3 each gave a single monochromatic spot on the TLC and PPC plates with the Dragendorf reagent, while the nature of these compounds remains to be identified in the future. Fractions from peak 4 contained a mixture of oxymatrine and one minor impurity of unknown nature as illustrated in Fig. 1B.

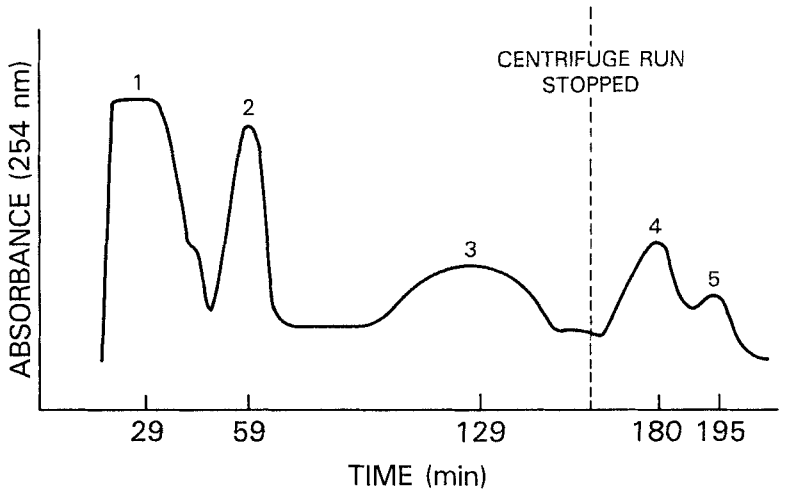

FIGURE 1A. HSCCC separation of crude alkaloids from Sophora flavescens Ait.

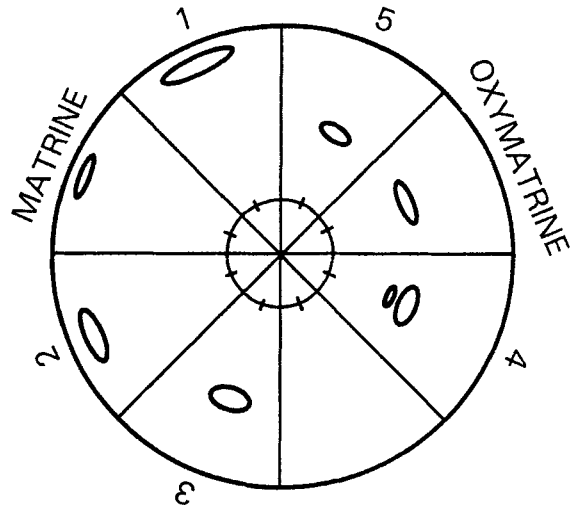

FIGURE 1B. PPC analysis of HSCCC fractions from Sophora flavescens Ait.

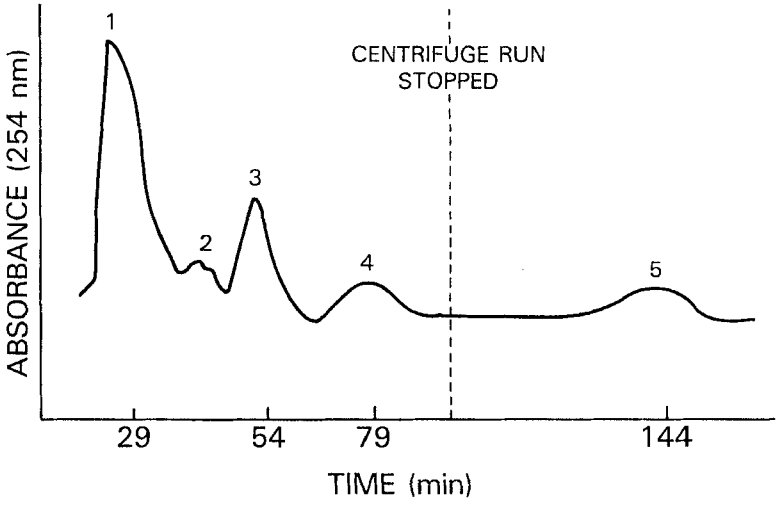

FIGURE 2A. HSCCC separation of crude alkaloids from Datura mete L.

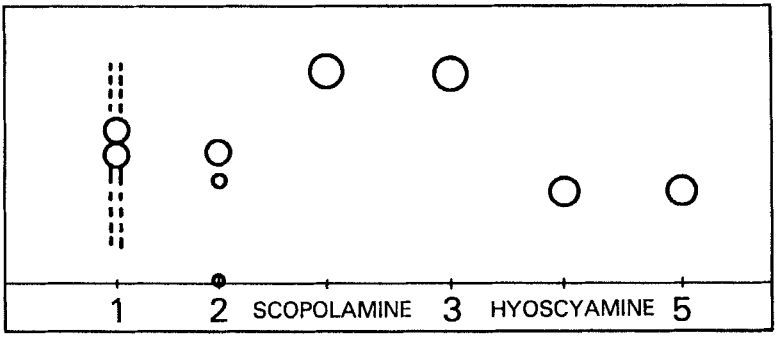

FIGURE 2B. TLC analysis of HSCCC fractions from Datura mete L.

Fig. 2A shows a similar chromatogram of crude alkaloids extracted from D. mete L. obtained by HSCCC. The separation was performed with a two-phase solvent system composed of chloroform-0.07 M sodium phosphate buffer solution (pH 6.5) (1:1, v/v) by eluting the lower nonaqueous phase as the mobile phase. Peaks 1 to 4 were eluted during the centrifuge run and peak 5 from the column contents. By means of TLC analysis (Fig. 2B), fractions corresponding to peaks 3 and 5 were identified as scopolamine and hyoscyamine, respectively. Fractions corresponding to peak 2 gave a monochromatic color reaction with the Dragendorf reagent. Fractions of peak 4 also produced a single spot on the TLC plate while the negative color reaction to the Drangendorf reagent suggested a pure non-alkaloid compound of unknown nature. Fractions corresponding to peak 1, which was eluted near the solvent front, contained a mixture of alkaloids as shown on the TLC plate (Fig. 2B).

The overall results of the present studies demonstrated that various alkaloids in the plant extracts can be efficiently separated by HSCCC using a two-phase solvent system composed of chloroform-sodium phosphate buffer solution simply by adjusting the pH of the buffer solution. The present method may also be useful for separations of various other alkaloids and similar compounds from the natural products.

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