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Note

Separation of hydroxyanthraquinone derivatives extracted from rheum with analytical high-speed counter-current chromatography

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High-speed counter-current chromatography, which has been developed in recent years, is a high-efficiency speedy separation method[1,2]. The method provides excellent means for analytical-scale separations without complications arising from the use of solid support. In the analytical high-speed counter-current chromatographic system, application of a high revolutional speed up to 2000 rpm can promote counter-current flow of two solvent phases through a coiled tube of less than 1 mm I.D., resulting in highly efficient separations of microquantity of samples in a short period of time[3].

The present paper describes the separation of hydroxyanthraquinone derivatives extracted from rhizome of *Rheum palmatum* L. with an analytical high-speed counter-current chromatography instrument. In order to reduce the separation time of the compounds having a wide range of polarity, lower and upper phases of the selected solvent system were used as the mobile phase in succession. This method effected complete separation of five components present in 1 mg sample mixture in 70 min. The peak fraction of each compound was subjected to mass spectrometric analysis for structure identification.

EXPERIMENTAL

Apparatus

A Pharma-Tech (Baltimore, MD, U.S.A.) Model CCC-2000 analytical high-

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speed counter-current chromatograph was used in this experiment. It is a compact table top model of the synchronous flow-through coil planet centrifuge with a 2.5-in. revolutional radius. The rotary frame holds a multilayer coil which consists of about 70 m of 0.85 mm I.D. polytetrafluoroethylene (PTFE) tubing with a total capacity of about 40 ml. The ratio of the column radius to the revolutional radius (β value) ranges from 0.4 at the internal terminal to 0.75 at the external terminal of the multilayer coil. The maximum revolutional speed of this apparatus is 2000 rpm. The apparatus is equipped with an LDC/Milton Roy pump with a pressure gauge, a speed controller with digital rpm display, and a sample injection valve.

Preparation of solvent system and sample solution

In the present study, a two-phase solvent system composed of *n*-hexane–ethyl acetate–methanol–water at a 9:1:5:5 volume ratio was used. The solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and the two phases separated before use.

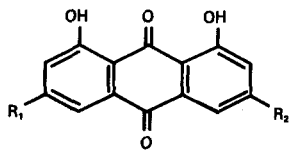
The mixture of hydroxyanthraquinone derivatives was extracted from the rhizome of *Rheum palmatum* L., a traditional Chinese medicinal herb. The extract contains free hydroxyanthraquinone at about 0.5%. Five major compounds are chrysophanol, emodin, rhein, physcion and aloe-emodin (Fig. 1). The use of conventional selective solvent extraction, column chromatography or thin-layer chromatography alone has failed to purify these compounds completely. In the present experiment, 1 mg of the crude powder was dissolved in 1 ml of the upper phase of the above two-phase solvent system and charged for each run.

Procedure

The coiled column was first entirely filled with the upper stationary phase followed by injection of 1 ml of the sample solution through the sample port. Then, the apparatus was rotated at the optimum revolutional speed of 1800 rpm while the lower mobile phase was pumped into the inlet of the column at a flow-rate of 60 ml/h. The maximum pressure at the outlet of the pump measured 160 p.s.i.

The UV absorbance of the effluent from the outlet of the column was continuously monitored with an LKB Uvicord S at 278 nm and the effluent was fractionated into test tubes (1 ml each tube) with an LKB fraction collector.

After 40 min of elution which produced three peaks on the chromatogram, the run was reversed without interrupting the centrifuge run by eluting with the upper phase through the external terminal of the multilayer coil at the same flow-rate of 60 ml/h. During this reversed run, a narrow capillary tube was applied at the outlet of the column to avoid surging of the effluent due to column pressure[1]. This created a



	R_1	R_2
chrysophanol	H	CH_3
emodin	OH	CH_3
rhein	H	COOH
physcion	OCH_3	CH_3
aloe-emodin	H	CH_2OH

Fig. 1. Structures of chrysophanol, emodin, rhein, physcion and aloe-emodin.

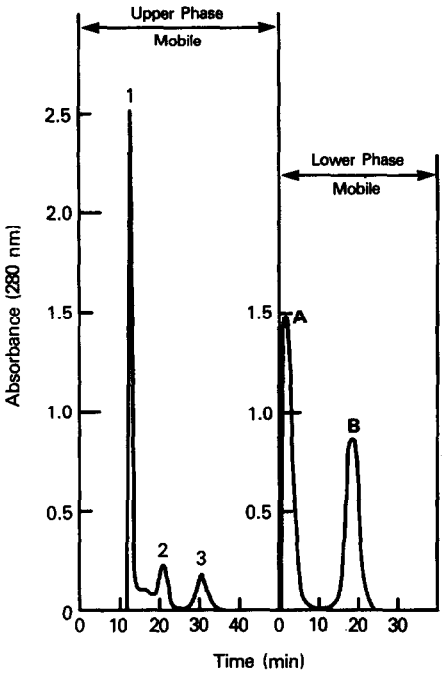


Fig. 2. Chromatogram of hydroxyanthraquinone derivatives from a crude extract of rhizome of *Rheum palmatum* L. Solvent system: *n*-hexane–ethyl acetate–methanol–water (9:1:5:5).

back pressure of about 200 p.s.i. at the outlet of the pump. The effluent of the reversed run was similarly monitored and fractionated as in the normal elution. This reversed mode of elution was continued until the retained two peaks were eluted, which took place in about 30 min. An aliquot of each fraction obtained from the normal and reversed runs was diluted with a known volume of methanol and the UV absorbance was determined at 280 nm with a Zeiss PM6 spectrophotometer.

RESULTS AND DISCUSSION

Fig. 2 shows the counter-current chromatogram of five major compounds in the crude extract. Three peaks (1, 2 and 3) were eluted with the lower phase in the normal elution mode in 40 min followed by two peaks (A and B) which were eluted with the upper phase in the reversed mode in additional 30 min. Fractions of all five peaks were analyzed with a Finnigan MAT mass spectrometer. The results indicated that peaks A, B, 1, 2 and 3 were corresponding to chrysophanol, emodin, physcion, aloe-emodin and rhein, respectively.

The above results clearly indicate superior performance of the analytical high-speed counter-current chromatography. A 1-mg amount of the complex mixture can be separated and purified in about 70 min. Solvent required for each phase was less than 100 ml and the experiment can be repeated several times a day.

One of the special advantages of counter-current chromatography is that either

phase of the two-phase solvent system can be used as the mobile phase. When the sample solution contains multiple components with a wide range of polarity, the separation usually requires a long elution time resulting in excessive dilution of later eluting peaks. In this situation, the combined use of normal and reversed elution modes will shorten the separation time and yield high solute concentration in fractions. In the present example, the lower aqueous phase was used to elute the polar compounds first, followed by the reversed elution with the upper non-aqueous phase to elute the nonpolar compounds retained in the column. In analytical high-speed counter-current chromatography, this advantage should be particularly emphasized, because the use of a narrow-bore coil under a high revolutional speed permits stable retention of the stationary phase in both normal and reversed elution modes.

The present results demonstrate the potential capability of analytical high-speed counter-current chromatography which will be useful for separation and purification of natural products. It enables rapid and efficient separations of microgram to milligram quantities of materials without adsorptive loss or deactivation caused by the solid supports.

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