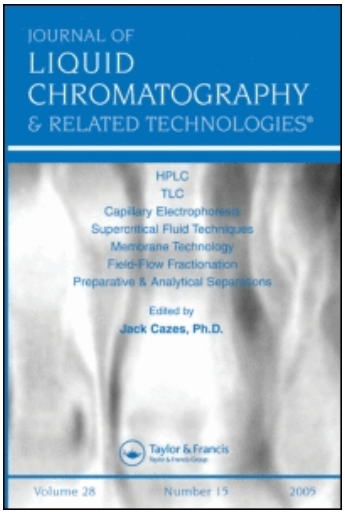


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# Large Scale Purification of Apocarotenoids from Cochlospermum Tinctorium by Countercurrent Chromatography

B. Diallo[a]; M. Vanhaelen[a]
[a] Laboratoire de Pharmacognosie, Institut de Pharmacie, Bruxelles, Belgium

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# LARGE SCALE PURIFICATION OF APOCAROTENOIDS FROM COCHLOSPERMUM TINCTORIUM BY COUNTER-CURRENT CHROMATOGRAPHY

B. Diallo and M. Vanhaelen
*Laboratoire de Pharmacognosie*
*Institut de Pharmacie, U. L. B. B205-4*
*Bd Triomphe, 1050 Bruxelles, Belgium*



## ABSTRACT

Carotenoids are well-known for their instability so as usual analytical methods such as HPLC or preparative TLC applied to the separation of the constituents of Cochlospermum tinctorium failed to the isolation of pure pigments for further chemical characterization. Thus high-speed CCC using a horizontal flow-through coil planet centrifuge was used to conveniently and efficiently achieve this separation for quantities up to 500mg of the crude plant extract.

## INTRODUCTION

In a search for the biologically active constituents from Cochlospermum tinctorium A. Rich (Cochlospermaceae), large amounts of carotenoids were detected in the rhizome. The presence of these pigments has been previously mentioned (1) but probably because of purification difficulties, they were not characterized. Separation

of the pigments was not efficient by usual chromatographic methods as HPLC and preparative TLC. To our knowledge, counter-current chromatography has not been applied until now for the separation of carotenoids. Therefore, attempts to use this method were carried out and led to pure compounds which were further characterized as cochloxanthin and dihydrocochloxanthin by spectroscopic methods (2).

## MATERIALS AND METHODS

Apparatus. Counter-current chromatography was performed using a horizontal flow-through planet centrifuge (3,4) with an Ito Multi-layer coil (5) (P.C. Inc., Potomac, MD, U.S.A.) equipped with a 2.6mm I.D. column. A LDC Milton Roy Mini pump (Riviera Beach, FL, U.S.A.) was used to pump the solvents through the column. The rotational speed was 800 rpm. A manual sample injection valve (Lobar columns accessories, Merck) equipped with a 10ml loop was used to introduce the samples into the column. The continuous monitoring of the eluent was achieved with a Shimadzu spectrophotometer UV 120-02 operating at 440nm in a 1mm through flow cell and the fractions (20ml) were collected with a LKB Ultrorac 7000 fraction collector.

Reagents. All chemicals were analytical grade.

Preparation of samples. A methanol extract of *Cochlospermum tinctorium* was absorbed on cellulose and extracted by percolation with petroleum ether then with dried peroxide-free diethyl ether. The dried diethyl ether extract was evaporated under reduced pressure and directly used for the CCC separation. The extract (500mg) was dissolved in 11ml of a 1:1 mixture of each phase, filtered and 10ml of this solution was loaded on the column.

Separation procedure. The two phase solvent system was prepared by equilibrating equal volumes of carbon tetrachloride and a methanol-water 4:1 mixture in a separatory funnel. After phase separation, they were degassed in an ultrasonic bath. The lower phase used as stationary phase was pumped into the column at

6ml/min . The sample solution was introduced through the injection port. As rotation began, the upper phase used as mobile phase was pumped into the column at 4ml/min. The separations were performed at room temperature.

Fractionation monitoring. The purity of the fractions was checked by TLC either on silica gel using toluene-chloroform-methanol 5:4:1 as mobile phase or on RP-18 silica gel using methanol-water 9:1 as mobile phase.

## RESULTS AND DISCUSSION

Figure 1 shows the separation of the two major carotenoids (cochloxanthin and dihydrocochloxanthin) from a crude diethyl ether extract of Cochlospermum tinctorium rhizomes. The solvent system was chosen on the basis of the partition coefficient of the pigments in the liquid phases. Partition coefficients were determined using the measurement of the absorbance at 440nm and TLC on silica gel or RP-18 silica gel. Chloroform-methanol-water mixtures were first tested but unsuccessfully ; carbon tetrachloride was used instead of chloroform to obtain an acceptable partition coefficient value (1.1) and a short settling time of the solvent pair. It was observed that the use of a restriction connected at the outlet of the column was essential to avoid bubbling in the cell of the UV detector, an excessive elution of the stationary phase and the consecutive rebalancing of the system during the run (large density difference between the solvent pair). Unlike usual chromatographic methods as TLC or HPLC, high-speed CCC was found very convenient for the efficient separation of the pigments. The structure determination by spectroscopic measurements of cochloxanthin and dihydrocochloxanthin was possible without any further purification procedure.Evident advantages of high-speed CCC for the isolation on a preparative scale of carotenoids are the high resolution within a short time (120min) in the dark and the suppression of irreversible binding on adsorbents and of degradation on solid supports.

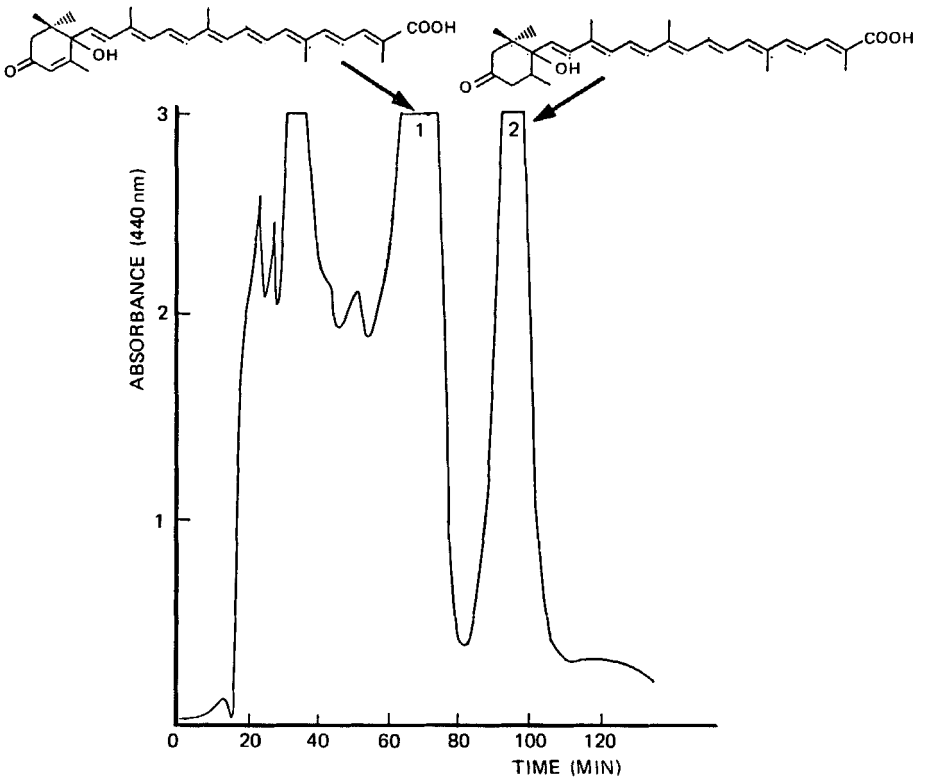


Figure 1. Preparative CCC of the diethyl ether extract from Cochlospermum tinctorium rhizomes. Sample : 500mg of the extract dissolved in 10ml (5ml of each phase) ; solvent system : $CCl_4$-$CH_3OH$-$H_2O$ (5:4:1) ; stationary phase : $CCl_4$ (lower phase) ; flow-rate : 4ml/min ; column : 2.6mm I.D., 335ml capacity ; detection at 440nm.

1 . Cochloxanthin ; 2 . Dihydrocochloxanthin

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