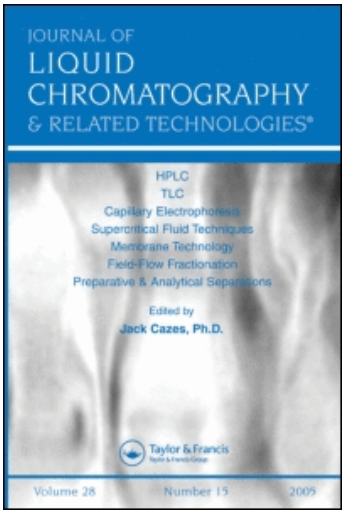

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Some New Developments in Centrifugal Partiton Chromatography and Applications in the Separation of Natural Products

Andrew Marston[a]; Ivan Slacanin[a]; Kurt Hostettmann[a]
[a] Institute of Pharmacognosy and Phytochemistry School of Pharmacy University of Lausanne Rue Vuillermet 2, Lausanne, Switzerland

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SOME NEW DEVELOPMENTS IN CENTRIFUGAL PARTITON CHROMATOGRAPHY AND APPLICATIONS IN THE SEPARATION OF NATURAL PRODUCTS

ANDREW MARSTON, IVAN SLACANIN, AND KURT HOSTETTMANN*
Institute of Pharmacognosy and Phytochemistry
School of Pharmacy
University of Lausanne
Rue Vuillermet 2
CH-1005 Lausanne, Switzerland

ABSTRACT

A two-pump method for the control of stationary to mobile phase ratios in a cartridge centrifugal partition chromatography system is described. This cartridge CPC set-up and a rotating coil instrument are used in the separation of natural products from complex mixtures contained in crude plant extracts.

INTRODUCTION

Centrifugal partition chromatography (CPC), for diverse reasons, is attracting a great deal of interest as a mild and efficient separation method [1]. The technique is capable of operation with both aqueous and non-aqueous solvent systems and with samples of a wide range of polarities. This versatility has led to an ever-increasing number of applications in the field of natural products [2].

The present paper reports an extension to the operation capacity of a cartridge CPC instrument and certain separations of crude plant mixtures on a preparative scale.

EXPERIMENTAL

Separations were performed at ca. 20°C with either a cartridge or a rotating coil CPC instrument. The cartridge instrument was a Sanki CPC model LLN (Sanki Engineering, Kyoto, Japan), containing 12 partition cartridges (type 250W; total volume 256 ml) and fitted with a 3 ml sample loop. For control of phase composition in the cartridges, two Waters Assoc. 6000A pumps were connected, one for dosing mobile phase and one for stationary phase. Solvent and sample introductions were carried out as previously reported [3]. For coil separations, an Ito multilayer coil separator-extractor (P.C. Inc., MD, U.S.A.) equipped with a 2.6 mm I.D. coil (volume 360 ml) was used. Included were also a 10 ml sample loop (with six-way valve) and a valve to permit rapid switching of the solvent between "head" or "tail" ends of the coil. Two Waters Assoc. 6000A pumps were attached for delivery of each phase of a biphasic solvent system, as already described [4].

Both instruments were connected to a Büchi 683 detector (254 nm), chart recorder (W + W Scientific, Basle, Switzerland, model 600) and Pharmacia Ultrorac II fraction collector.

RESULTS AND DISCUSSION

Changing solvent phase ratios in cartridges of a Sanki instrument

There are certain ways of changing the elution times and sequences in centrifugal partition chromatography. One possibility is to alter the solvent system and another way is to change the proportions of the lower and upper phases of a biphasic solvent system in the instrument. For the rotating coil system, a procedure for achieving the latter, by use of two pumps, has already been described [4]. The control of phase ratios in the cartridge instrument has now also proved possible with a two-pump arrangement, using a similar filling arrangement to that employed in the rotating coil. The lower phase pump and the upper phase pump are allowed to run simultaneously at flow rates which give the

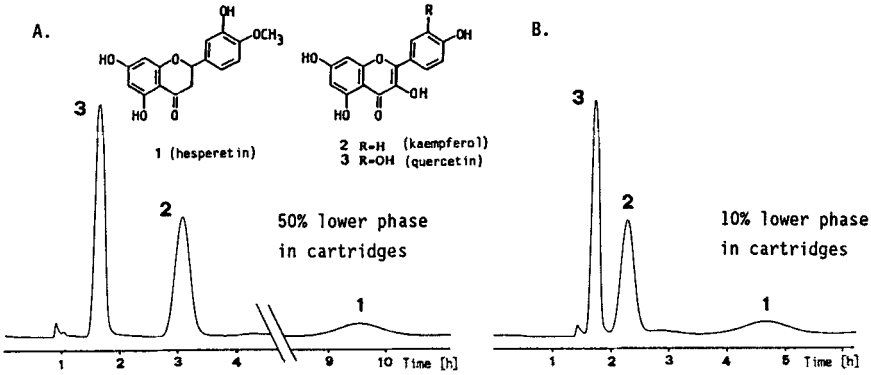


FIGURE 1. Separation of flavonoids on a Sanki LLN CPC instrument with different ratios of phases in the cartridges. Solvent $CHCl_3$-MeOH-H_2O 5:6:4 (mobile phase:upper phase); flow-rate 2.5 ml/min; 400 rpm; sample 15 mg.

proportion of phases desired in the cartridges e.g. running both pumps at 5 ml/min gives 50% of each phase in the instrument.

A demonstration of the effectiveness of varying solvent phase ratios is given in Figure 1. When the cartridges contain 50% lower (stationary) phase, the three flavonoids (1-3) require over 10 h for complete separation with the solvent system shown. However, diminishing the proportion of lower phase to 10% accelerates the separation time to just over 5 h.

Fractionation of crude plant extracts

One of the major advantages of CPC is the ability to cope with crude plant extracts without any of the major problems associated with irreversible adsorption, common to chromatography on solid supports. Until the present time, however, relatively modest sample loads have been introduced into the apparatus. Provided a suitable solvent system is chosen and the extract is not too complex, the capacity of the CPC instruments can be considerably extended.

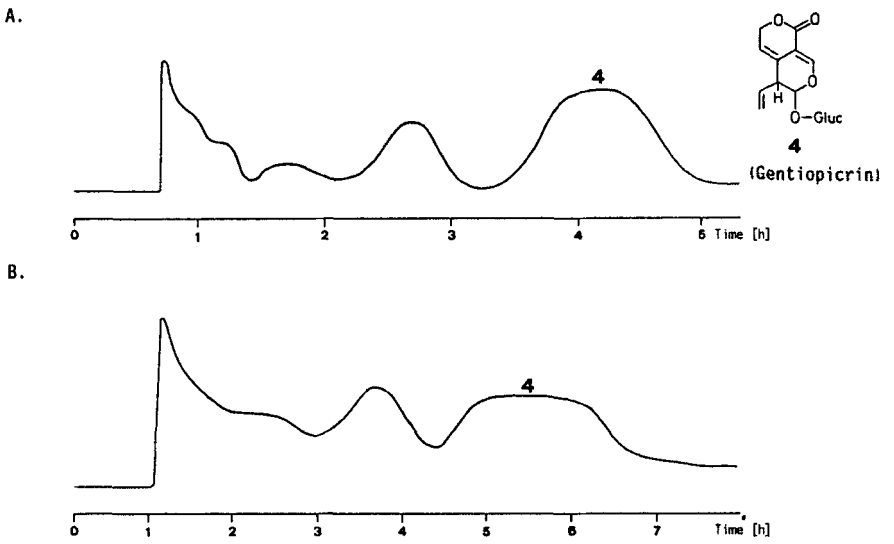
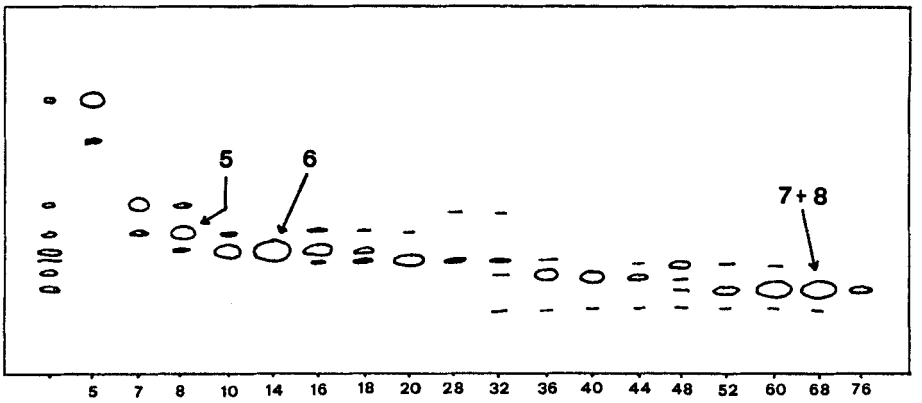


FIGURE 2. Isolation of gentiopicrin (4) from *Gentiana lutea* (Gentianaceae) roots by CPC.
Solvent $CHCl_3$-MeOH-H_2O 9:12:8 (mobile phase:lower phase); sample 500 mg.
A. Sanki LLN instrument: 300 rpm; flow-rate 2.4 ml/min.
B. Rotating coil instrument: 700 rpm; flow-rate 3 ml/min; 20% lower phase in coil.

An example of a useful one-step separation of a natural product from a crude plant extract is shown in Figure 2. Gentian roots (*Gentiana lutea*, Gentianaceae) are very bitter and find extensive popular use for their tonic and gastric stimulant properties. Details of preparations of this plant drug are to be found in many European pharmacopoeia. The roots were extracted with methanol and after evaporation of solvent, the residue was taken up in water and partitioned with petroleum ether, ethyl acetate and butanol (in that order). Centrifugal partition chromatography of the butanol extract (500 mg) with a chloroform-methanol-water system by both the cartridge and rotating coil instruments gave similar elution curves, with the multilayer coil separator-extractor requiring a slightly longer separation time. Pure gentiopicrin (4), which

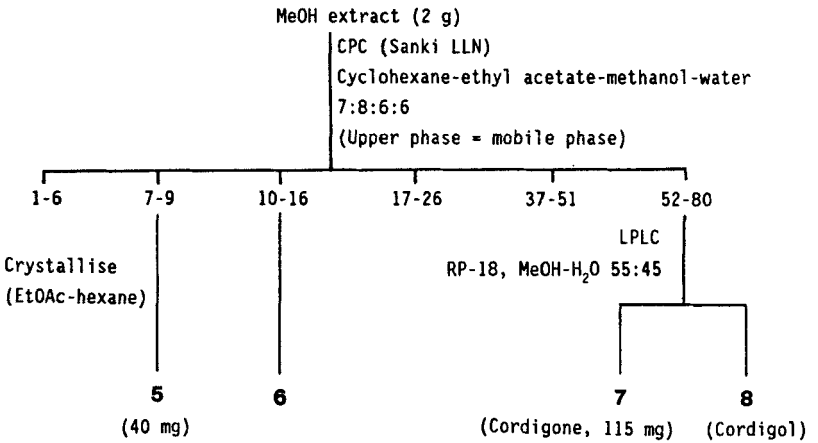


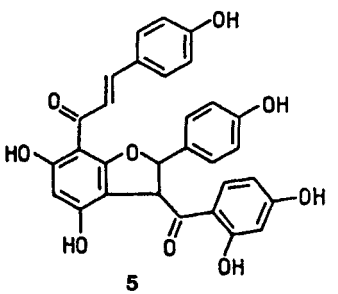
Solvent: $CHCl_3$-MeOH-H_2O 13:7:4 (lower phase)

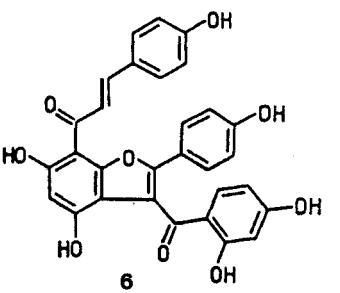
FIGURE 3. TLC monitoring of the CPC separation of *Cordia goetzei* (Boraginaceae) stem bark constituents.

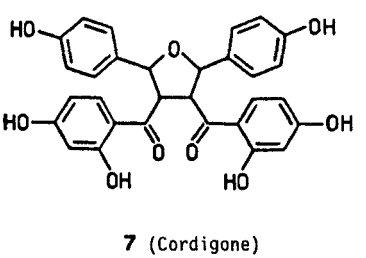
is both bitter and a gastric stimulant, was obtained in both cases (113 mg from the Sanki instrument) after this single chromatographic step.

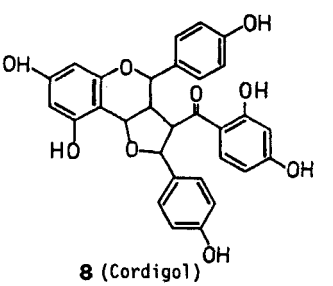
Antifungal polyphenolic compounds (**5-8**) have previously been isolated and characterised from the stem bark of the African medicinal plant *Cordia goetzei* (Boraginaceae) [5]. CPC has now proved to be a fast and efficient method of obtaining these compounds in large quantities, as shown in Scheme I. Methanol extract (2g; dissolved in 10 ml of a mixture of upper and lower phases of the biphasic solvent and introduced via the mobile phase solvent inlet) was chromatographed into six fractions over 8 h on a cartridge instrument. TLC monitoring of the progress of the separation is shown in Figure 3. Orange pigment **5** was obtained in pure form by direct crystallisation of the second fraction, from test tubes 7-9. The third fraction (test tubes 10-16) contained the second pigment (**6**). Pure cordigone (**7**), the most interesting compound from the point of view of bioactivity, was separated from cordigol (**8**) by low-pressure liquid chromatography on a Lobar B column.



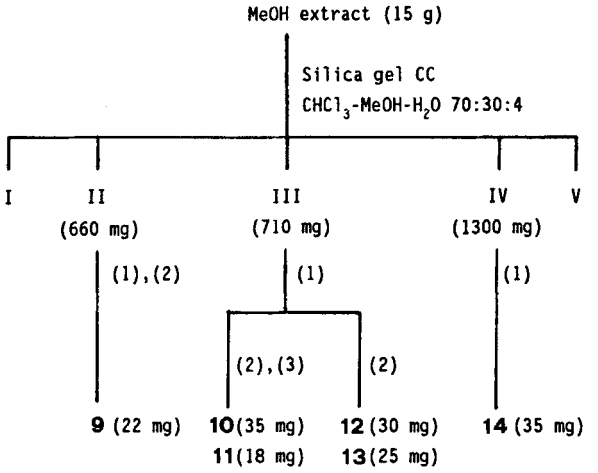




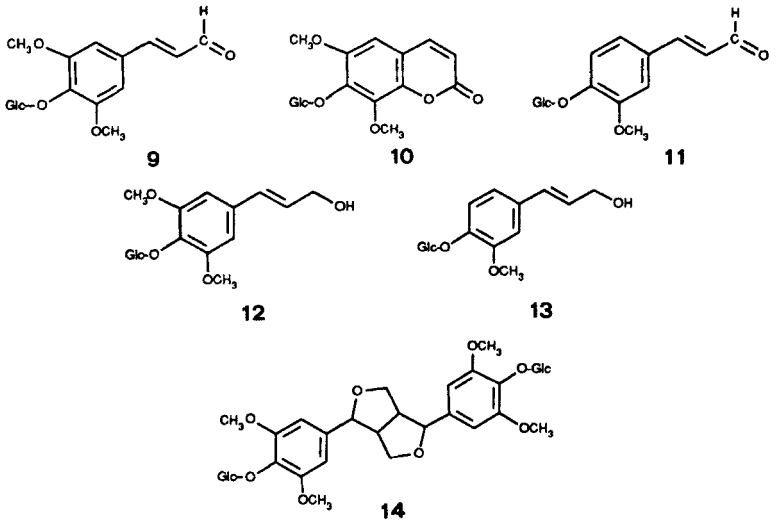




SCHEME I
Isolation of polyphenolic compounds from *Cordia goetzei* stem bark



(1) CPC, $CHCl_3$-MeOH-H_2O 7:13:8 (mobile phase=lower phase)
(2) LPLC, RP-18, MeOH-H_2O
(3) HPLC semi-prep, RP-18, MeOH-H_2O 15:85



SCHEME II
Isolation of constituents of *Eleutherococcus senticosus* roots

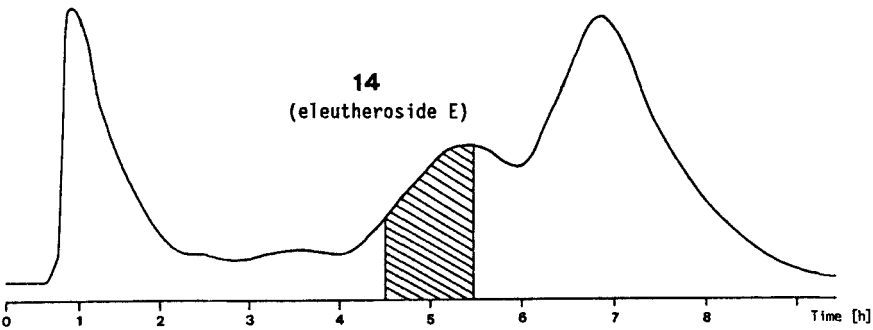


FIGURE 4. CPC separation of eleutheroside E (14) from *Eleutherococcus senticosus* (Araliaceae) roots (multilayer coil separator-extractor). Solvent $CHCl_3$-MeOH-H_2O 7:13:8 (mobile phase:lower phase); 50% lower phase in coil; flow-rate 3.5 ml/min; 700 rpm; sample 730 mg of fraction IV from silica gel CC.

Centrifugal partition chromatography has proved of value in the isolation of constituents from the roots of *Eleutherococcus senticosus* (Araliaceae). The characterisation of phenolics found in preparations of this plant, important for its stimulant and adaptogenic properties [6], is necessary for standardisation purposes. A total of 6 components (9-14) of the methanol extract of the roots were isolated by a combination of open-column chromatography, CPC (with the multilayer coil separator-extractor), low-pressure and high-pressure liquid chromatography (Scheme II). Eleutherosides B_1 (10), B (12) and E (14) are known constituents of *E. senticosus* roots, while sinapaldehyde glucoside (9), coniferaldehyde glucoside (11) and coniferin (13) have not previously been described from the plant. In one instance, CPC of a fraction from silica gel column chromatography led directly to pure eleutheroside E (14). The elution profile of fraction IV is shown in Figure 4. A total of 730 mg of this fraction was loaded onto the coil, filled with 50% of each phase of the chloroform-methanol-water solvent system by the two-pump method [4].

CONCLUSION

The possibilities open to centrifugal partition chromatographic methods have been further extended by the introduction of certain innovations: the ability to reverse phases and elution modes [4], the use of mobile phase gradients in both the rotating coil [7] and cartridge [8] systems, and the regulation of mobile phase : stationary phase ratios in the rotating coil apparatus [4]. By operating with two pumps, this latter option is now possible with the cartridge CPC instrument. Therefore, if elution of a sample is too slow, a change in the phase ratio can be introduced in order to speed up separation times.

Another advantage of this modern liquid-liquid chromatography technique is the ability to separate complex mixtures without material losses; the effectiveness of the utilisation of CPC for the efficient separation of crude plant extracts is also demonstrated. Loads of over 2 g can be handled without problem.

ACKNOWLEDGEMENTS

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