Journal of Chromatography, 477 (1989) 434–438
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 640

Note

Application of analytical high-speed counter-current chromatography to the isolation of bioactive natural products

Y. W. LEE* and C. E. COOK
Research Triangle Institute, P.O. Box 12194, Research Triangle Park, NC 27709 (U.S.A.)
Q. C. FANG
Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing (China)
and
Y. ITO
Laboratory of Technical Development, National Heart, Lung and Blood Institute, Bethesda, MD 20892 (U.S.A.)
(First received September 26th, 1988; revised manuscript received May 23rd, 1989)

Counter-current chromatography (CCC), based on the principle of liquid–liquid partition, has gained increasing popularity and a number of efficient systems have been developed for CCC performances[1,2]. Ito's planet centrifugal system represents one of the most convenient methods in fractionation of a variety of natural products, peptides and synthetic organic molecules[3–5]. The high resolving power of the system has been exemplified in the separation of antibiotics from actinomycete fermentation products[6,7]. Recently, further improvement of the system with a 0.85 mm I.D. multilayer coiled column and an operational speed of 2000 rpm has generated a highly efficient system. Its analytical capability has been demonstrated in the separation of plant alkaloids, plant indole hormones and herbicides[5,8,9]. In general, the observed resolution and speed are comparable to those of high-performance liquid chromatography (HPLC).

The study of bioactive principles from *Schisandra rubriflora* Rhed et Wils, a traditional Chinese herbal medicine for the treatment of chronic hepatitis, has led to the identification of nine lignans[10] (Fig. 1). Because of structural similarities among these bioactive lignans, the isolation of individual lignans for pharmacological evaluations has been a major challenge, particularly in the case of schisanhenol, which cannot be resolved from its acetate even with an analytical reversed-phase HPLC system (see Fig. 2).

In this paper, the analytical and semipreparative capabilities of the high-speed analytical CCC system are further demonstrated in the fractionation of bioactive schisanhenol and schisanhenol acetate. It is evident that analytical high-speed CCC provides a novel method complementary to HPLC for analytical applications.

EXPERIMENTAL

Apparatus

The apparatus employed is a newly developed analytical high-speed planet cen-

0021-9673/89/$03.50

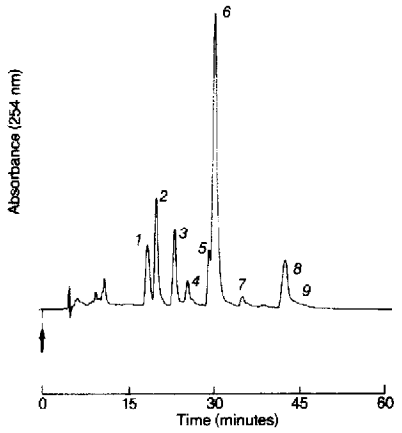

Fig. 1. HPLC trace of bioactive lignans from *Schisandra rubriflora* Rhed et Wils. Column: Zorbax-ODS, 250 × 4.6 mm I.D.; mobile phase: methanol–water (75:25); sample: ethanolic extract of *S. rubiflora* Rhed et Wils kernels; detection: UV absorbance at 254 nm. Peaks: 1 = wuweizisu C; 2 = (−)-rubschisandrin; 3 = rubschisantherin; 4 = deoxyschisandrin; 5 = schisanhenol acetate; 6 = schisanhenol; 7 = schisanhanol B; 8 = gomisin O; 9 = pregomisin.

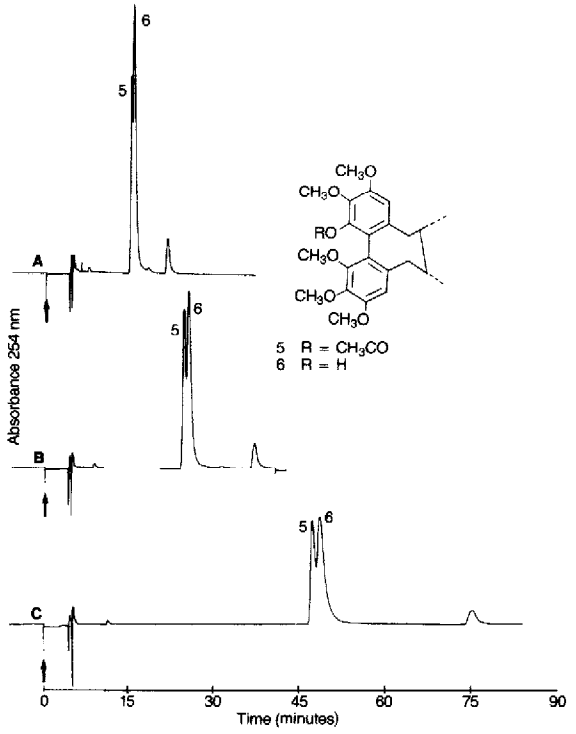

Fig. 2. HPLC traces of schisanhenol (6) and schisanhenol acetate (5). Column and detection as in Fig. 1; mobile phases: methanol–water (80:20) (A), (75:25) (B) and (70:30) (C).

trifuge with a 5-cm revolutional radius and a 0.85-mm-diameter multilayer coiled column. The details of this apparatus have been described in ref. 9. The holder shaft is equipped with a plastic planetary gear which is coupled to an identical stationary sun gear rigidly mounted on the central axis of the centrifuge. This gear coupling produces a desired synchronous planetary motion of the column holder. The holder revolves around the central axis of the centrifuge and simultaneously rotates about its own axis at the same angular velocity. As described elsewhere[4], this particular type of planetary motion permits the flow tubes to rotate around the central axis of the centrifuge without twisting, thus facilitating continuous elution of the mobile phase through the rotating column. The revolutional speed of the centrifuge is continuously adjustable up to 2000 rpm with a speed control unit.

The multilayer coiled column was prepared by winding a long piece of PTFE tubing (0.85 mm I.D.) onto the holder with a 5-cm hub diameter, making multiple coiled layers. The β value (the ratio between the helical radius, r, and the revolutional radius, R:$\beta = r/R$) varied from 0.5 at the internal terminal to 0.8 at the external terminal. The total column capacity of the multilayer coil measured approximately 39 ml.

The HPLC system consisted of a Model 6000A pump, a Model 46K injector and a Model 440 UV detector (all from Waters Assoc.). HPLC separations were performed on a 25 cm × 4.6 mm Zorbax-ODS column (DuPont).

Reagents and materials

Organic solvents used for preparation of the two-phase solvent systems, including *n*-hexane and ethanol, are glass-distilled chromatographic grade purchased from Burdick & Jackson Labs., Muskegon, MI, U.S.A. Experiments were performed with the two-phase system *n*-hexane–ethanol–water (6:5:5 v/v/v), which gives better peak resolution than the 6:5:1 solvent system. The two-phase solvent system was prepared by thoroughly equilibrating the solvent mixture in a separatory funnel at room temperature followed by filtration through a 5-μm filter and degassing. The samples of *Schisandra rubriflora* Rhed et Wils were kindly provided by Professor Y. Y. Chen (Institute of Materia Medica, Chinese Academy of Medical Sciences). The ethanolic extract of the kernels of *S. rubriflora* was filtered and concentrated to provide the crude sample for HPLC analysis. The semipurified sample for CCC analysis containing mainly schisanhanol acetate and schisanhanol was obtained from preparative silica gel column chromatography.

Methods

The analytical CCC was performed with an analytical high-speed planet centrifuge system equipped with a multilayer coil column of 0.85 mm I.D. and at a revolutional speed of 1500 rpm.

In each separation the multilayer coiled column was first filled with the upper phase solvent as the stationary phase. The mobile phase solvent (lower phase) was then pumped into the column head inlet while the apparatus was run at a revolutional speed of 1500 rpm. After equilibrium was reached, as indicated by a clear mobile phase eluting at the tail outlet, the sample solution (0.15 mg in 0.2 ml of lower mobile phase) was injected through the head inlet. The effluent from the tail outlet of the column was continuously monitored with a UV detector at 254 nm and fractionated into test tubes with an LKB fraction collector.

RESULTS AND DISCUSSION

The capabilities of high-speed analytical CCC have been successfully demonstrated in the separation of plant alkaloids, plant indole hormones and herbicides[9]. A recent study of bioactive lignans from *S. rubriflora* Rhed et Wils has identified nine lignans[10] (Fig. 1). These bioactive lignans are similar in structure, thus it is difficult to obtain individual lignans in pure form for pharmacological evaluations. As shown in Fig. 2, the schisanhenol and its acetate could not be totally resolved even with a reversed-phase analytical HPLC system (12 000 plates) using several solvent systems; *i.e.*, methanol–water (80:20), (75:25) or (70:30). It is postulated that the hydroxyl group in schisanhenol is ideally situated for intramolecular hydrogen bonding with a neighboring methoxy group and that therefore the resulting hydrogen-bonding complex behaves similarly to its acetate in terms of adsorption and desorption process while passing through the reversed-phase HPLC column. This is also supported by the observed reverse sequence of elution in which the acetate eluted first followed by schisanhenol under reversed-phase HPLC conditions.

On the other hand, the application of analytical CCC which eliminates the complications arising from solid supports and based on the partition coefficients of the two molecules has resulted in a baseline separation of schisanhenol and its acetate (Fig. 3). The semipreparative capability of the analytical CCC system is also evidenced in the sample size which is 25 times higher than that generally used in HPLC.

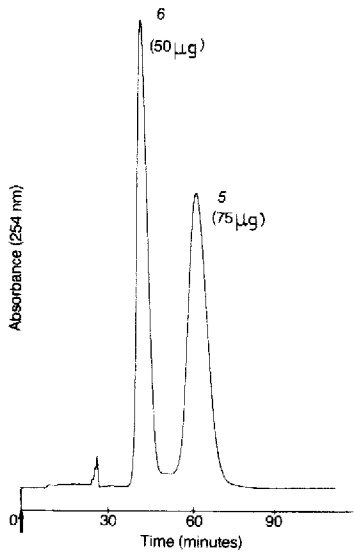

Fig. 3. High-speed analytical counter-current chromatographic separation of schisanhenol (6) and schisanhenol acetate (5). Solvent system: *n*-hexane–ethanol–water (6:5:5), mobile phase: lower phase, volume retention ratio: upper phase:lower phase = 19 ml:20 ml, flow-rate: 0.8 ml/min; column pressure: 165 p.s.i.; detection: UV absorbance at 254 nm.

CONCLUSIONS

The complete resolution of the bioactive lignans schisanhenol and schisanhenol acetate demonstrates that high-speed analytical CCC can be a complementary method to HPLC for analytical and semipreparative applications. The major advantages are (1) high resolution and speed, (2) avoidance of complications arising from solid adsorbance, (3) total recovery of bioactivity and sample, (4) inexpensive operation, and (5) analytical and semipreparative applications.

ACKNOWLEDGEMENT

We express our appreciation to Dr. Robert Bowman of National Heart, Lung and Blood Institute for the loan of some equipment.

REFERENCES

1 Y. Ito and W. D. Conway, *Anal. Chem.*, 56 (1984) 534A.
2 Y. Ito, *CRC Crit. Rev. Anal. Chem.*, 17 (1986) 65.
3 M. Knight, *J. Liq. Chromatogr.*, 8 (1985) 2281.
4 N. B. Mardava, Y. Ito and J. M. Ruth, *J. Liq. Chromatogr.*, 8 (1985) 2221.
5 Y. Ito and Y. W. Lee, *J. Chromatogr.*, 391 (1987) 290.
6 G. M. Brill, J. B. McAlpine and J. E. Hochlowski, *J. Liq. Chromatogr.*, 8 (1985) 2259.
7 D. G. Martin, R. E. Peltonen and J. W. Nielsen, *J. Antibiot.*, 39 (1986) 721.
8 Y. Ito, J. Sandlin and W. G. Bowers, *J. Chromatogr.*, 244 (1982) 247.
9 Y. W. Lee, Y. Ito, Q. C. Fang and C. E. Cook, *J. Liq. Chromatogr.*, 11 (1988) 75.
10 H. J. Wang and Y. Y. Chen, *Acta Pharm. Sin.*, 20 (1985) 832.