ELSEVIER

Journal of Chromatography A, 822 (1998) 316–320

JOURNAL OF CHROMATOGRAPHY A

Short communication

Preparative separation of alkaloids from the root of *Sophora flavescens* Ait by pH-zone-refining counter-current chromatography

Fuquan Yang[a], Jiang Quan[a], Tianyou Zhang[a], Yoichiro Ito[b,*]

[a]*Beijing Institute of New Technology Application, Beijing 100035, China*

[b]*Laboratory of Biophysical Chemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bldg. 10, Room 7N322, Bethesda, MD 20892, USA*

Received 28 April 1998; received in revised form 28 July 1998; accepted 29 July 1998

Abstract

pH-Zone-refining counter-current chromatography was applied to the separation of alkaloids from a crude extract of the root of *Sophora flavescens* Ait using a multilayer coil planet centrifuge. After methyl *tert*.-butyl ether and water were equilibrated, triethylamine (10 m*M*) was added to the organic phase as a retainer and hydrochloric acid (5–10 m*M*) to the aqueous phase as an eluter. The separation was performed by eluting the aqueous phase while the organic phase was used as the stationary phase. From 1.0 g of the crude extract, sophocarpine (170 mg) and matrine (600 mg) were separated within 4.5 h at high purity of over 98%. © 1998 Published by Elsevier Science B.V. All rights reserved.

Keywords: *Sophora flavescens*; Counter-current chromatography; Alkaloids; Matrine; Sophocarpine

1. Introduction

pH-Zone-refining counter-current chromatography (CCC) is a recently developed preparative method which provides many important advantages over the conventional CCC method including an over 10-fold increase in sample loading capacity, high concentration of fractions, concentration of minor impurities, etc. The method uses a retainer base (or acid) in the stationary phase to retain the analytes in the column and an eluter acid (or base) to elute the analytes according to their pK_a values and hydrophobicities [1–14]. It produces a succession of highly concentrated rectangular peaks with minimum overlap similar to those observed in displacement chromatography [15]. The method has been successfully applied to the separation of a variety of compounds including acidic [1,2,6,11–14] and basic [7] amino acid derivatives, many hydroxyxanthene dyes [1,3–5], alkaloids from natural sources [8], geometrical [9] and structural [10] isomers from synthetic products, chiral compounds, etc.

Sophora flavescens Ait, a typical traditional Chinese medicinal herb, is used for the treatment of various diseases in China as antifebrile, diuretic, anthelmintic and antidote [16]. The roots of *S. flavescens* Ait contain more than ten kinds of alkaloids, among which sophocarpine and matrine are the main effective constituents used for cancer treatment.

In this paper, pH-zone-refining CCC is applied to

*Corresponding author.

0021-9673/98/$ – see front matter © 1998 Published by Elsevier Science B.V. All rights reserved.
PII: S0021-9673(98)00624-4

the separation of these alkaloids from *S. flavescens* Ait.

2. Experimental

2.1. 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×1.6 mm I.D. PTFE tube directly onto a holder hub to form multiple coiled layers with a total capacity of 240 ml. Although the revolution speed of the apparatus could be regulated with a speed controller in the range between 0 and 1000 rpm, an optimum speed of 800 rpm was used in the present studies.

The solvent was pumped into the column with a Model NS-1007 constant-flow pump (Beijing Institute of New Technology Application). The continuous monitoring of the effluent was achieved with a Model 8823A-UV monitor (Beijing Institute of New Technology Application) operating at 254 nm and a Model 333 pH meter (ATI Orion Research, Boston, MA, USA). A manual sample injection valve with a 20- or 30-ml loop (Tianjin High-New Science and Technology, Tianjin, China) was used to introduce the sample into the column. A portable recorder (Yokogawa Model 3057, Sichuan Instrument Factory, Chongqin, China) was used to draw the chromatogram. A Model RE-90 rotary evaporator and a Model FC-95 auto fraction collector (both from Beijing Institute of New Technology Application) were also used.

2.2. Reagents

Triethylamine, hydrochloric acid and methyl *tert*.-butyl ether, chloroform, methanol (for recrystallization), bismuth subnitrate, potassium iodide and glacial acetic acid were of analytical grade and purchased from Beijing Chemical Factory (Beijing, China). Drangendorff reagent was prepared as follows: solution I: 0.85 g bismuth subnitrate was dissolved in 10 ml glacial acetic acid and 40 ml water. Solution II: 8 g potassium iodide was dissolved in 20 ml of water. Then, 1 ml solution I and 1 ml solution II were mixed with 4 ml of glacial acetic acid and 20 ml of water.

2.3. Extraction of crude alkaloids

Raw roots of *S. flavescens* Ait (ca. 1.5 kg) were extracted three times each with 2.1 l of 95% ethanol. Then, the extracts were combined and evaporated to dryness under reduced pressure. The residue obtained from the combined extracts was dissolved with 500 ml of 2% HCl. After filtration, the acidic aqueous solution was extracted three times each with 500 ml of chloroform. The pH of the aqueous solution was adjusted to 9.6 with NaOH solution and the total alkaloids were extracted three times again each with 500 ml of chloroform. The chloroform extracts were combined and evaporated to dryness. Portions of this crude alkaloid extract of *S. flavescens* Ait were subjected to pH-zone-refining CCC.

2.4. Preparation of two-phase solvent system and sample solutions

The solvent pairs used for CCC separations were prepared as follows: about equal volumes of methyl *tert*.-butyl ether and distilled water were thoroughly equilibrated in a separatory funnel at room temperature and the two phases were separated. Then, triethylamine (retainer) was added to the upper organic phase at a concentration of 10 m*M*, while the lower aqueous phase was acidified with hydrochloric acid (eluter) at 5 or 10 m*M*.

The sample solutions were prepared by dissolving the crude alkaloid extract of *S. flavescens* Ait in 20~30 ml of a phase mixture consisting of equal volumes of each phase. The sample solutions were sonicated for several minutes before injecting into the column.

2.5. Separation procedure

For each separation, the column was first entirely filled with the organic stationary phase. Then the sample solution was injected through the sample port

and the aqueous mobile phase was pumped through the column at a flow-rate of 1.5 ml/min while the column was rotated at 800 rpm. The effluent from the outlet of the column was continuously monitored with a UV detector at 254 nm and a pH monitor, and then collected into test tubes at 2 min intervals (3.0 ml/tube) with a fraction collector. After the desired peaks were eluted, the rotation and elution were stopped and the column contents were collected into a graduated cylinder by connecting the inlet of the column to a nitrogen line pressurized at approximately 80 p.s.i. (1 p.s.i.=6894.76 Pa). The retention of the stationary phase relative to the total column capacity was computed from the volume of the stationary phase collected from the column.

2.6. Analysis of CCC fractions

All collected fractions were analyzed by using silica gel G thin-layer chromatography (TLC) plates (100×50 cm, 0.20–0.25 mm thick) (Qingdao Haiyang Chemical Factory, Qingdao, China) by developing with a solvent mixture composed of C_6H_6–EtOAc–CH_3COCH_3–25% aqueous NH_3 (2:3:4:0.2, v/v/v/v) and staining with a Dragendorff reagent to detect the alkaloids.

3. Results and discussion

Fig. 1A shows a typical pH-zone-refining counter-current chromatogram obtained for the separation of 1.0 g of crude alkaloid extract of *S. flavescens* Ait. Two pure alkaloids were obtained in this separation: matrine (I in Fig. 2) from peak I and sophocarpine (II in Fig. 2) from peak II. Peak I showed a normal Gaussian shape without affecting pH of the mobile phase while peak II was eluted in a rectangular shape that coincided with a sharp drop in pH followed by a flat pH zone. As shown in the chromatogram peak II is associated with a sharp impurity peak (1~2 ml) at its rear boundary where pH abruptly drops from 8.3 to 2.2.

Fig. 1B shows a similar chromatogram obtained from the separation of 2.0 g sample of the same alkaloid extract of *S. flavescens* Ait under otherwise identical experimental conditions. Increasing the sample size resulted in broadening of both peaks without loss of peak resolution.

Fig. 1C shows a chromatogram obtained for the separation of 1.0 g of the same extract of *S. flavescens* Ait by pH-zone-refining CCC using a mobile phase containing a lower concentration of HCl (5 m*M*). The reduction of the eluter acid concentration in the mobile phase produced a remarkable change in the elution profile of peak II which became broader, loosing its rectangular shape and being totally separated from the sharp impurity peak. However, pure alkaloid were still obtained from each of these two peaks: matrine (600 mg) and sophocarpine (170 mg) both over 98% pure.

These chromatograms demonstrate the characteristic feature of pH-zone-refining CCC. As described elsewhere [2,13], the method requires some critical relationship between the respective partition coefficients (K) of the analyte and the retainer. In brief, the K value of the retainer should fall within a range of K values of the analyte that are altered by the pH of the solvent. If this requirement is met, the analyte forms a particular elution curve that is characteristic of pH-zone-refining CCC: the main components elute in a train of highly concentrated rectangular peaks while minor components are concentrated at the boundaries of the main peaks. In Fig. 1A, matrine in peak I has K values always smaller than that of the retainer base and therefore it elutes earlier than the retainer border forming a normal Gaussian shape. In contrast, sophocarpine in peak II satisfies the above requirement so that it formes a characteristic rectangular peak associated with its own specific pH. Once this condition is established, an increase of sample size results in a proportional increase of the peak width without affecting the peak resolution as shown in Fig. 1B. In Fig. 1C decreased HCl concentration in the mobile phase substantially increased the K value of the retainer resulting in disruption of the proper relationship between the K values of the retainer and that of the second peak. Consequently, peak II was eluted earlier than the retainer border losing its rectangular shape and completely separated from the succeeding impurity peak which still maintained the favorable relationship with the K value of the retainer thus preserving its sharp profile [2,13].

Fig. 2 shows the result of the TLC analysis of various samples including the crude alkaloid extract

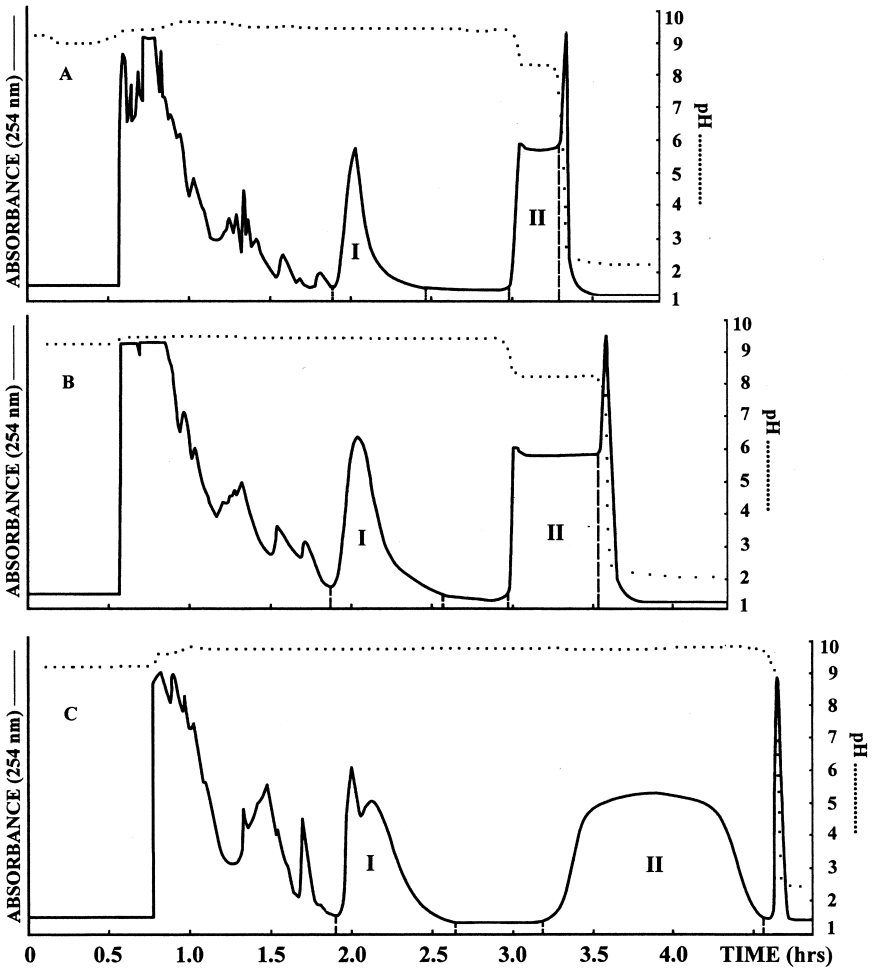

Fig. 1. Chromatograms of the crude alkaloid extract from the root of *Sophora flavescens* Ait obtained by pH-zone-refining CCC. Experimental conditions were as follows: solvent system: methyl *tert*.-butyl ether–water (1:1); stationary phase: upper phase (10 m*M* triethylamine); mobile phase: lower phase (10 m*M* HCl for A and B, 5 m*M* for C); flow-rate: 1.5 ml/min; sample size: 1.0 g (A), 2.0 g (B) and 1.0 g (C) dissolved in 20 ml (A), 30 ml (B) and 20 ml (C) of each phase, respectively; revolution speed: 800 rpm; retention of stationary phase: 66%. I (matrine), II (sophocarpine).

of *S. flavescens* Ait, combined fractions of peak I (I), matrine standard (S-1), combined fractions of peak II (II) and sophocarpine standard (S-2) using silica gel G TLC developed with a solvent mixture composed of C_6H_6–EtOAc–CH_3COCH_3–25% aqueous NH_3 (2:3:4:0.2, v/v/v/v) and stained with a Dragendorff reagent to detect the alkaloids. The result indicates that peaks I and II each contained one alkaloid compound (R_F values: 0.63 for sophocarpine and 0.55 for matrine). Although peak I contained minor brown impurities (as it was only partially resolved from the main peak in Fig. 1C), they were easily removed by recrystallization using a mixture of chloroform–methanol (10:1, v/v).

The results of our studies demonstrated that pH-zone-refining CCC produced efficient separations of two main alkaloids from gram quantities of crude alkaloid extracts from *S. flavescens* Ait. The present method may be applied to purification of various other alkaloids from natural products.

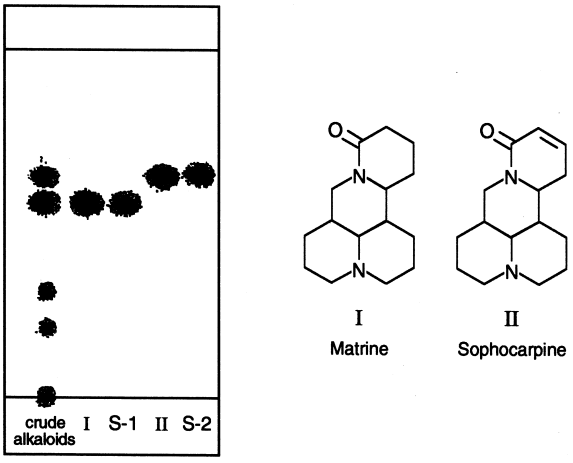

Fig. 2. The results of TLC analyses. Left: the crude alkaloid extract of *S. flavescens* Ait; I: peak I; S-1: matrine standard; II: peak II; S-2: sophocarpine standard. The analyses were made by a silica gel G TLC plate developed with a solvent system composed of C_6H_6–EtOAc–CH_3COCH_3–25% aqueous NH_3 (2:3:4:0.2, v/v/v/v) and stained with a Dragendorff reagent to detect the alkaloids. Chemical structures of matrine (I) and sophocarpine (II) are illustrated on the right.

Acknowledgements

Financial support from Beijing Academy of Science and Technology and Beijing Commission of Science and Technology is gratefully acknowledged. We also thank senior engineer Xining Li for his excellent technical assistance, and Mr. Xu Lang and Ms. Ren Zhang for their help during the experiment.

References

[1] A. Weisz, A.L. Scher, K. Shinomiya, H.M. Fales, Y. Ito, J. Am. Chem. Soc. 116 (1994) 704.
[2] Y. Ito, in: Y. Ito, W.D. Conway (Eds.), High-Speed Countercurrent Chromatography (Chemical Analysis, Vol. 132), Wiley Interscience, New York, 1996, Ch. 6, pp. 121–178.
[3] A. Weisz, in: Y. Ito, W.D. Conway (Eds.), High-Speed Countercurrent Chromatography (Chemical Analysis, Vol. 132), Wiley Interscience, New York, 1996, Ch. 12, pp. 337–384.
[4] A. Weisz, D. Andrzejewski, Y. Ito, J. Chromatogr. A 678 (1994) 77.
[5] A. Weisz, D. Andrzejewski, R.J. Highet, Y. Ito, J. Chromatogr. A 658 (1994) 505.
[6] Y. Ito, Y. Ma, J. Chromatogr. A 672 (1994) 101.
[7] Y. Ma, Y. Ito, J. Chromatogr. A 678 (1994) 233.
[8] Y. Ma, Y. Ito, E. Sokoloski, H.M. Fales, J. Chromatogr. A 685 (1994) 259.
[9] C. Denekamp, A. Mandelbaum, A. Weisz, Y. Ito, J. Chromatogr. A 685 (1994) 253.
[10] Y. Ma, Y. Ito, D.S. Torok, H. Ziffer, J. Liq. Chromatogr. 17 (1994) 3507.
[11] Y. Ma, Y. Ito, J. Chromatogr. A 702 (1995) 197.
[12] A. Weisz, A.L. Scher, Y. Ito, J. Chromatogr. A 732 (1996) 283.
[13] Y. Ito, Y. Ma, J. Chromatogr. A 753 (1996) 1.
[14] Y. Shibusawa, Y. Hagiwara, Z. Chao, Y. Ma, Y. Ito, J. Chromatogr. A 759 (1997) 47.
[15] Cs. Horváth, A. Nahum, J.H. Frenz, J. Chromatogr. 218 (1981) 365.
[16] D.-C. Cai, M.-J. Gu, J.-D. Zhang, G.-P. Zhu, T.-Y. Zhang, N. Li, Y. Ito, J. Liq. Chromatogr. 13 (1990) 2399.