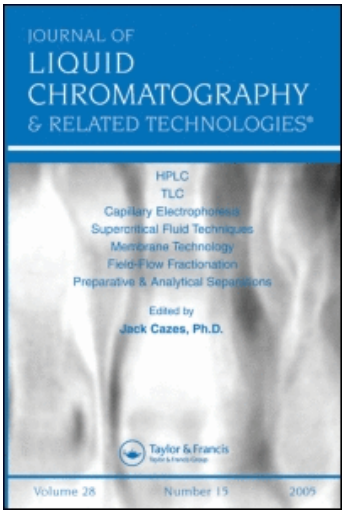

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

PREPARATIVE ISOLATION AND PURIFICATION OF PHENYLETHANOID GLYCOSIDES FROM THE EXTRACT OF FAECES OF BEAGLE DOGS BY HIGH-SPEED COUNTERCURRENT CHROMATOGRAPHY

Li Lei[a]; Fuquan Yang[b]; Tianyou Zhang[b]; Pengfei Tu[a]; Lijun Wu[c]; Fakui Chen[c]; Yoichiro Ito[d]
[a] Department of Natural Medicines, School of Pharmaceutical Sciences, Peiking University, Beijing, P.R. China [b] Beijing Institute of New Technology Application, Beijing, P.R. China [c] Shenyang Pharmaceutical University, Shenyang, P.R. China [d] Laboratory of Biophysical Chemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, U.S.A.

Online publication date: 31 August 2001

To cite this Article Lei, Li , Yang, Fuquan , Zhang, Tianyou , Tu, Pengfei , Wu, Lijun , Chen, Fakui and Ito, Yoichiro(2001) 'PREPARATIVE ISOLATION AND PURIFICATION OF PHENYLETHANOID GLYCOSIDES FROM THE EXTRACT OF FAECES OF BEAGLE DOGS BY HIGH-SPEED COUNTERCURRENT CHROMATOGRAPHY', Journal of Liquid Chromatography & Related Technologies, 24: 14, 2187 — 2195
To link to this Article: DOI: 10.1081/JLC-100104901
URL: http://dx.doi.org/10.1081/JLC-100104901

J. LIQ. CHROM. & REL. TECHNOL., 24(14), 2187–2195 (2001)

PREPARATIVE ISOLATION AND PURIFICATION OF PHENYLETHANOID GLYCOSIDES FROM THE EXTRACT OF FAECES OF BEAGLE DOGS BY HIGH-SPEED COUNTERCURRENT CHROMATOGRAPHY

Li Lei,[1,3] Fuquan Yang,[2,4] Tianyou Zhang,[2] Pengfei Tu,[1] Lijun Wu,[3] Fakui Chen,[3] and Yoichiro Ito[4,*]

[1] Department of Natural Medicines, School of Pharmaceutical Sciences, Peiking University, Beijing 100083, P.R. China
[2] Beijing Institute of New Technology Application, Beijing 100035, P.R. China
[3] Shenyang Pharmaceutical University, Shenyang 110015, P.R. China
[4] Laboratory of Biophysical Chemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA

ABSTRACT

High-speed countercurrent chromatography (HSCCC) was applied to the separation of acteoside and isoacteoside from a crude extract from canine faeces after the dogs were orally administered phenylethanoid glycosides (PhGs). Two-step separation

*Corresponding author. E-mail: itoy@gwgate.nhlbi.nih.gov

with a quaternary two-phase solvent system composed of ethyl acetate-n-butanol-ethanol-water (system 1, 35:6:6:50, v/v; system 2, 30:10:6:50, v/v) was used. HPLC analysis of final CCC fractions revealed that the two main PhGs showed purity of over 95%. Their chemical structures were identified by ^{1}HNMR, ^{13}CNMR, FAB-MS.

INTRODUCTION

Phenylethanoid glycosides (PhGs) are a group of water soluble natural products obtained from *Cistanche tubulosa* (Schenk) Wight, a parasitic plant native found in northwest China. As an important tonic in traditional Chinese medicine, the stem is used for kidney deficiency, female infertility, morbid leucorrhea, neurasthenin, senile constipation due to colonic ineria, etc.. It has been reported that the major active components are PhGs[1,2] which have activities of anti-fatigue, regulating immunity function, anti-aging, curing constipation, etc.

PhGs are characterized by cinnamic acid and hydroxyphenylethyl moieties attached to a ß-glucopyranose through ester and glycosidic linkages, respectively,[3] and their phenolic hydroxyl groups cause a tendency of irreversible adsorption onto the solid support during the separation using the conventional liquid-solid chromatography. So far, multiple-step chromatographic procedures are necessary for their purification using silica gel, polyamide, cellulose, Dianion HP-20, and Sephadex columns.

High-speed counter-current chromatography (HSCCC), being a support-free liquid-liquid partition chromatography, avoids the loss of material through irreversible adsorption onto the solid support,[4] and, therefore, it is an ideal alternative for separation of these compounds.

The present paper describes the HSCCC separation of acteoside and isoacteoside from the crude extract of canine faeces after the oral administration of PhGs obtained from *C. tubulosa* in the course of our studies on the metabolic regulation of PhGs in the gastrointestinal tract. The chemical structures of acteoside and isoacteoside are given in Fig. 1.

EXPERIMENTAL

The analytical HSCCC instrument employed in the present study is a Model GS 20 analytical high-speed countercurrent chromatography centrifuge which was designed and constructed at Beijing Institute of New Technology Application, Beijing, China. The apparatus holds a pair of column holders sym-

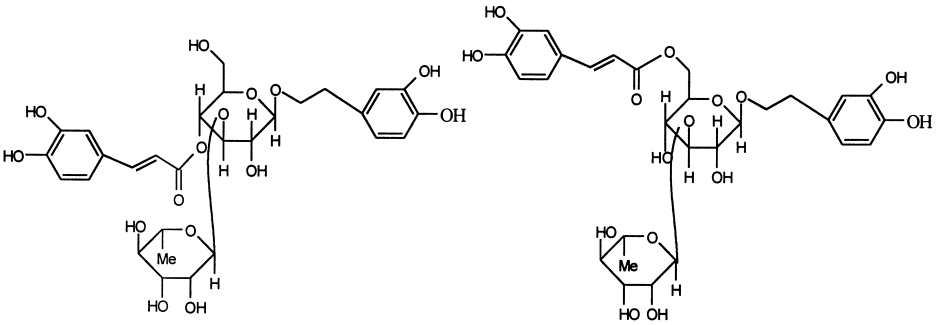

Figure 1. Chemical structures of acteoside and isoacteoside.

metrically on the rotary frame at a distance of 5 cm from the central axis of the centrifuge.

The multilayer coil separation column was prepared by winding a 50 m ? 0.85 mm I.D. PTFE (polytetrafluoroethylene) tube directly onto the holder hub, forming multiple coiled layers with a total capacity of 30 mL. The β value varied from 0.4 at the internal terminal to 0.7 at the external terminal (β=r/R where r is the distance from the coil to the holder shaft, and R, the revolution radius or the distance between the holder axis and central axis of the centrifuge).

The revolution speed of the apparatus could be regulated with a speed controller in the range between 0 to 2000 rpm, but an optimum speed of 1500 rpm was used in the present studies. Preparative HSCCC was performed using a Model GS10A2 multilayer coil planet centrifuge (Beijing Institute of New Technology Application, Beijing, China) equipped with a PTFE multilayer coil of 1.6 mm I.D. and 110 m in length with a total capacity of 230 mL. The β value of the preparative column ranges from 0.5 to 0.8. Revolution speed can be regulated between 0-1000 rpm, whereas the 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, Beijing, China). A Model 8823A-UV Monitor (Beijing Institute of New Technology Application, Beijing, China) was used to continuously monitor the effluent at 254 nm. A portable Yokogawa Model 3057 recorder (Sichuan Instrument Factory, Chongqin, China) was used to record the chromatogram.

A manual sample injection valve with a 1.0 mL loop (for the analytical HSCCC) or a 20 mL loop (for the preparative HSCCC) (Tianjin High-New

Science & Technology Company, Tianjin, China) was used to introduce the sample into the column.

The high-performance liquid chromatography (HPLC) equipment used was a Shimadzu LC-10AVP system including two LC-10ATVP solvent delivery units, an SPD-M10AVP UV-VIS photodiode array detector, an SCL-10AVP system controller, a CTO-10ASVP column oven a DGU-12A degasser, a Model 7726 injection valve with a 20μL loop, and a Class-VP-LC workstation (Shimadzu Corporation, Kyoto, Japan).

Reagents

All organic solvents used for HSCCC were of analytical grade and were purchased from Beijing Chemical Factory, Beijing, China. Acetonitrile used for HPLC analysis was of chromatographic grade and was purchased from Tianjin Huaxi Special Reagent Factory, Tianjin, China.

Preparation of Two-Phase Solvent System

The two-phase solvent systems utilized in the present study were composed of ethyl acetate-n-butanol-ethanol-water at two different volume ratios (35:6:6:50 for system 1 and 30:10:6:50 for system 2). After thoroughly equilibrating the mixtures in a separatory funnel at room temperature, two phases were separated shortly before use; the organic phase was used as stationary phase and the aqueous phase as the mobile phase.

Preparation of Sample and Sample Solutions

Sample faeces were obtained from Beagle dogs at 24 hours after oral administration of PhGs (3g/kg) from the stems of *Cistanche tubulosa* (Schenk) Wight provided by the Department of Natural Products, School of Pharmaceutical Sciences, Peiking University, Beijing, China. The collected faeces were extracted three times with 200 mL of methanol for 30 min by sonication. The extracts were filtered, combined, and evaporated to dryness under reduced pressure. The residues were dissolved in methanol and partially purified by a Sephadex LH-20 column using methanol as a mobile phase. PhGs-containing fractions were combined, dried, and subjected to analytical and preparative HSCCC.

Separation Procedure

Analytical HSCCC was performed with a Model GS 20 analytical HSCCC instrument. The multilayer coiled column was first entirely filled with the upper stationary phase. Then, the lower mobile phase was pumped into the head end of the column at a flow-rate of 1.0 mL/min while the apparatus was rotated at 1500 rpm. After hydrodynamic equilibrium was reached, as indicated by a clear mobile phase eluting at the tail outlet, the sample solution (15 mg in 1 mL of lower aqueous phase) was injected through the injection valve. The effluent from the tail end of the column was continuously monitored with a UV detector at 254 nm. 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 after the separation was completed.

Preparative HSCCC was performed with a Model GS10A2 HSCCC instrument. The multilayer coiled column was first entirely filled with the upper stationary phase. Then, the lower mobile phase was pumped into the head end of the column at a flow-rate of 1.5 mL/min while the apparatus was rotated at 800 rpm. After hydrodynamic equilibrium was reached, the sample solution was injected through the injection valve.

The effluent from the tail end of the column was continuously monitored with a UV detector, at 254 nm, and peak fractions were collected according to the chromatogram. 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 after the separation was completed.

Because the sample was a very complex mixture, a two-step operation was employed in the present separation. In the first step, solvent system 1 was used. The fraction containing two main compounds was collected and concentrated, and then subjected to the second separation by HSCCC using solvent system 2.

HPLC Analyses and Identification of CCC Peak Fractions

The original PhGs given to Beagle dogs, the crude sample from their faeces after passing through a Sephedax LH-20 column, and each peak fraction from the second step of HSCCC separation were analyzed by HPLC. The HPLC analyses were performed with a Phenomenex PRODIGY-ODS column (250 ? 4.6 I.D. mm) at a column temperature at 40°C. The mobile phase was composed of MeCN-1% HAc(18:82, v/v) and was isocratically eluted at a flow-rate of 1.0 mL/min and the effluent monitored by a PAD detector.

Identification of HSCCC peak fractions was carried out by FAB-MS, UV, ^{1}H-NMR, and ^{13}C-NMR spectra.

RESULTS AND DISCUSSION

In order to select a suitable two-phase solvent system, the analytical HSCCC was first used in the separation with a two-phase system composed of EtOAc-n-BuOH-EtOH-H_2O (35:6:6:50, v/v, system 1). The results indicated that acteoside and isoacteoside were well resolved (Fig. 2). Fig. 3A shows the result obtained from 214mg of the crude sample by preparative HSCCC with solvent system 1. Baseline separation for acteoside and isoacteoside was not obtained. Reseparation of the shaded fraction in Fig. 3A using solvent system 2 (ethyl acetate-n-butanol-ethanol-water, 30:10:6:50, v/v), gave a good separation as shown in Fig. 3B.

HPLC analyses indicated that the purity of two target compounds was over 95% (Fig.2 C and D). The structural identification of HSCCC peak fractions was carried out by FAB-MS, ^{1}H-NMR, and ^{13}C-NMR spectra.

The results of our studies clearly demonstrate that HSCCC may be successfully used in the purification of acteoside and isoacteoside from a crude extract

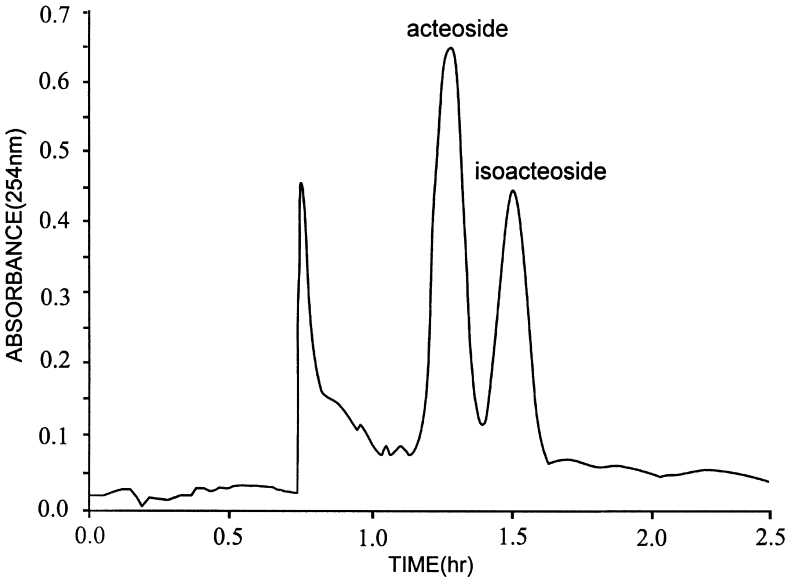

Figure 2. Chromatogram of the crude sample from canine faeces by analytical HSCCC. Solvent systems: ethyl acetate-n-butanol-ethanol-water (35:6:6:50, v/v); stationary phase: upper organic phase; mobile phase lower aqueous phase; flow rate: 1.0 mL/min; revolution speed: 1500 rpm; sample size: 10 mg dissolved in 1 mL lower aqueous phase; retention of the stationary phase: about 30%.

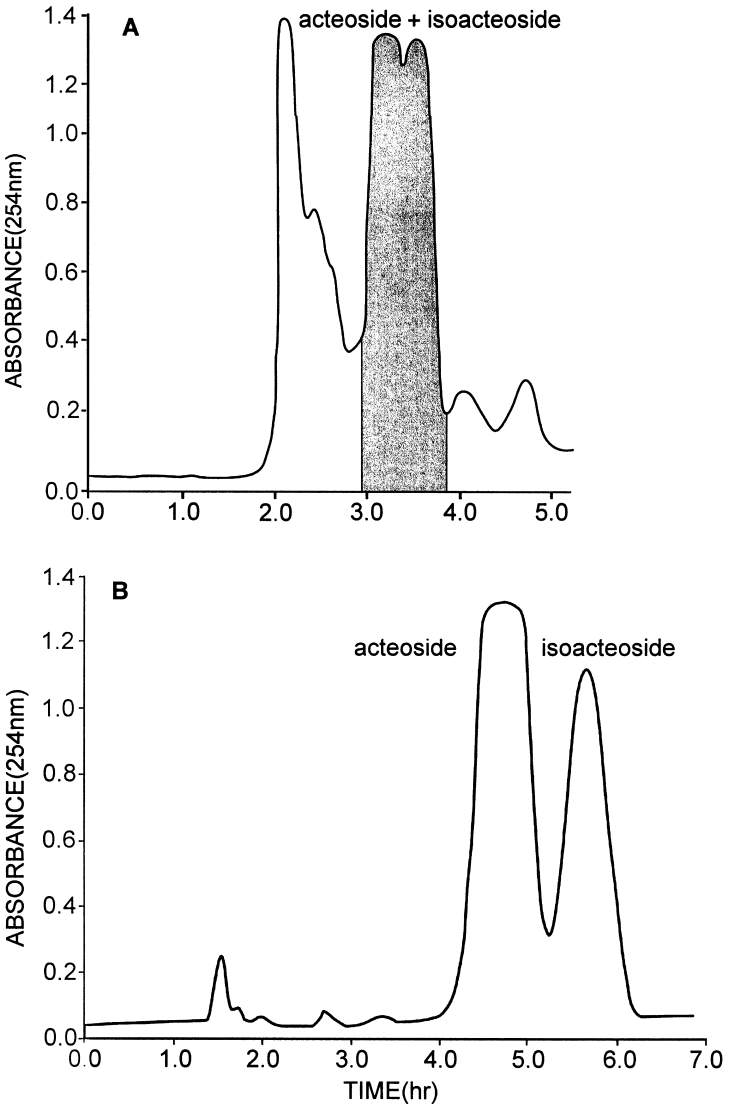

Figure 3. Chromatogram of 214 mg of the crude sample from the dog's faeces by preparative HSCCC with solvent system 1 (ethyl acetate-n-butanol-ethanol-water at 35:6:6:50, v/v) (A) and chromatogram of shaded peak fraction in Fig. 3A by preparative HSCCC with solvent system 2 (ethyl acetate-n-butanol-ethanol-water at 30:10:6:50, v/v); stationary phase: upper organic phase; mobile phase: lower aqueous phase; flow rate: 1.5 mL/min; revolution speed: 800 rpm; retention of the stationary phase: 16% (A) & 20% (B).

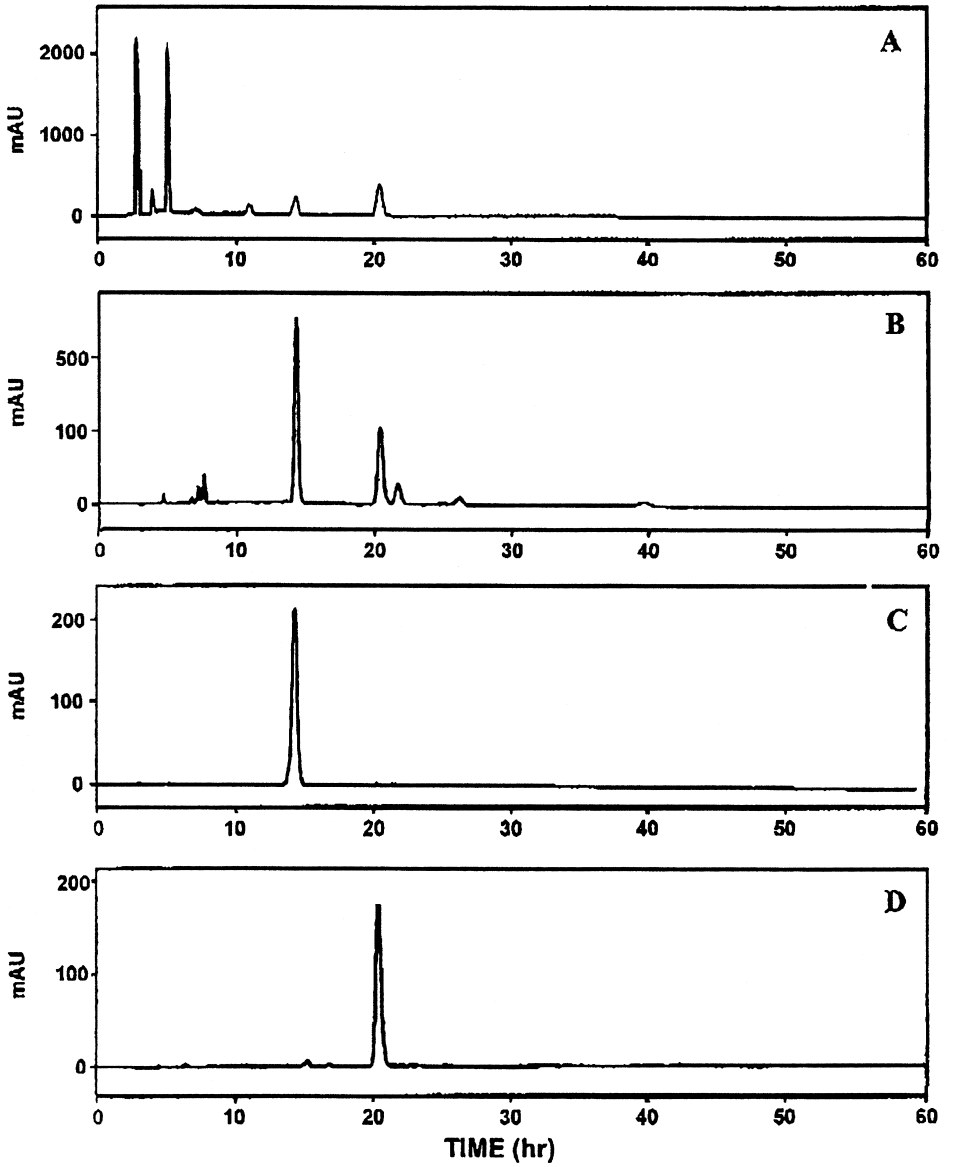

Figure 4. The results of HPLC analyses of the crude PhGs sample from *C. tubulosa,* the crude extract of canine faeces from each of the purified CCC peak fractions in Fig. 3B. Column: Phenomenex ODS column (250 x 4.6 mm I.D.); column temperature: 40 degrees Centigrade; mobile phase: acetonitrile-1% acetic acid (18:82, v/v); flow-rate: 1.0 mL/min; (A) the crude PhGs sample from *C. tubulosa*; (B) the crude extract of canine faeces; (C): peak I in Fig. 3B; (D): peak II in Fig. 3B.

from canine faeces during the course of our research on the metabolic regulation of PhGs in the gastrointestinal tract.

ACKNOWLEDGMENTS

Financial support from Beijing Academy of Science & Technology and Beijing Commission of Science & Technology is gratefully acknowledged. We also thank senior engineer Xining Li for his excellent technical assistance.

REFERENCES

1. Sato, T.; Kozima, S.; Kobayashi, K.; Kobayashi, H. Studies on the Pharmacology of Cistanchis Herba. Yakugaku Zasshi **1985**, *105* (12), 1131.
2. Cometa, S.; Tomassini, I.; Nicoletti, M. Phenylethanoid Glycosides: Distribution and Pharmacological Activity. Fitoterapia **1993**, *64* (3), 195.
3. Jimenez, C.; Riguera, C. Phenylethanoid Glycosides in Plants: Structure and biological Activity. Nat. Prod. Rep. **1994**, *11* (6), 591.
4. Ito, Y. High-Speed Countercurrent Chromatography. CRC Crit. Rev. Anal. Chem. **1986**, *17*, 65.

Received January 15, 2001 Manuscript 5503
Accepted February 10, 2001