Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 108 (2008) 402–406
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical Methods

Application of preparative high-speed counter-current chromatography for separation and purification of lignans from *Taraxacum mongolicum*

Shuyun Shi [a,*], Yuping Zhang [a], Kelong Huang [a], Suqin Liu [a], Yu Zhao [b]

[a] *School of Chemistry and Chemical Engineering, Central South University, Changsha 410083, People's Republic of China*
[b] *College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310031, People's Republic of China*

Received 13 September 2007; received in revised form 24 October 2007; accepted 25 October 2007

Abstract

Apart from being used as a pharmaceutical, the inflorescences, leaves and roots of *Taraxacum mongolicum* are processed into different food products. However, only few phytochemical investigations on this plant have been performed. In the present study, a preparative high-speed counter-current chromatography (HSCCC) for the separation and purification of bioactive compounds from *T. mongolicum* was developed. Two lignans, mongolicumin A and rufescidride, were obtained. The target compounds were finally isolated and purified with a solvent system composed of ethyl acetate–*n*-butanol–water (2:5:7, v/v/v). The lower phase was used as the mobile phase in the head to tail elution mode. By injecting 500 mg of the enriched extract of *T. mongolicum*, one-step HSCCC procedure yielded 36.7 mg of mongolicumin A and 43.9 mg of rufescidride with the purity of 98.7% and 98.5%, respectively, as determined by high-performance liquid chromatography (HPLC). The chemical structures of the two lignans were confirmed by UV, IR, HRESIMS, 1D and 2D NMR. Among them, mongolicumin A is a new compound, and rufescidride was obtained from genus *Taraxacum* for the first time. Furthermore, lignans were first isolated and identified from genus *Taraxacum*.

Keywords: High-speed counter-current chromatography; Preparative chromatography; *Taraxacum mongolicum*; Lignan; NMR

1. Introduction

The genus *Taraxacum* is a member of the family Asteraceae, and is widely distributed in the warmer temperature zones of the northern hemisphere, inhabiting fields, roadsides and ruderal sites. In China, the genus *Taraxacum* includes 70 species and one variety, while *Taraxacum mongolicum* Hand.-Mazz. is the famous traditional Chinese medicine (TCM), which was commonly used by Chinese local herbal physicians. The herb is frequently used to treat inflammatory disorders and viral infectious diseases in Pharmacopoeia Chinensis (Chinese National Pharmacopoeia Committee, 2000). Furthermore, fresh leaves of *T. mongolicum* have been used by local people as vegetable food in Northern China. Additionally, extracts are used as flavour components in various food products, including alcoholic beverages and soft drink, frozen dairy desserts, candy, baked goods, gelatins and puddings and cheese. Although *T. mongolicum* is a well-known traditional herbal remedy and food with a long history, until recently only limited scientific information is available to justify the reputed uses. In fact, medical plant therapy is mainly based on the empirical findings during hundreds and thousands of years (Gurib-Fakim, 2006). Moreover, only three references have reported on study of chemicals from the famous medical herb *T. mongolicum* (Ling et al., 1997; Ling et al., 1999; Yao, Lin, Zhou, & Zhao, 2007), whereas pharmacological properties of the compounds from *T. mongolicum* were still a neglected field until the last year. Therefore,

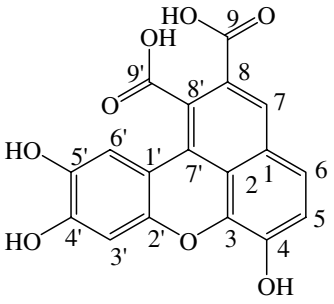
* Corresponding author. Tel./fax: +86 731 8879850.
E-mail address: shuyshi@gmail.com (S. Shi).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.10.069

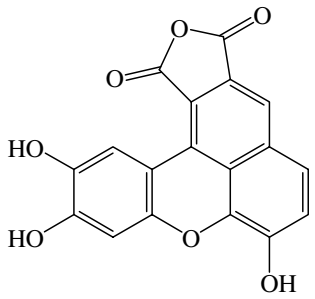
further chemical research from *T. mongolicum* is warranted for new TCM products and pharmacological test.

The conventional methods of preparative separating and purifying products were to utilize repeated chromatographic steps on silica gel, polyamide and Sephadex LH-20 column, which is easy to enlarge the scale of separation and separate different compounds entirely. However, it is tedious and time consuming, and the overall yield of these methods is usually poor because the target compounds tend to be irreversibly absorbed onto the solid support during separation. Then, for setting up an easy system capable of separating the bioactive compounds is necessary. HSCCC is a support-free liquid–liquid chromatographic technique that was first invented by Ito (Ito, 1981), and has been successfully applied to separation and isolation of various natural products (Cao, Huang, Dong, Zhao, & Ito, 2007; Frighetto, Welendorf, Nigro, Frighetto, & Siani, 2007; Lu, Liu, Sun, & Pan, 2007; Salas et al., 2005; Shi, Jiang, Zhao, & Tu, 2007).

The aim of the present paper, therefore, describes a very convenient and successful method to prepare two lignans: mongolicumin A and rufescidride with high purity from *T. mongolicum* (Fig. 1). The best isolation conditions were optimized after investigating the effects of two-phase solvent system. The chemical structures of the two target compounds were elucidated by UV, IR, HRESIMS, 1D and 2D NMR. To our best knowledge, this is the first report of lignans presented in *Taraxacum* species and mongolicumin A was a novel lignan.



mongolicumin A



rufescidride

Fig. 1. The chemical structures of mongolicumin A and rufescidride from *T. mongolicum*.

2. Experimental

2.1. Apparatus

HSCCC was performed with a seal-free high-speed counter-current chromatography (Designed and manufactured by Prof. Qizhen Du in his lab, Institute of Food and Biological Engineering, Zhejiang Gongshang University, Hangzhou, China). The apparatus was equipped with one layer coil column by winding 2.6 mm i.d. polytetrafluoroethylene (PTFE) tubing coaxially onto the column holder with a total volume of 420 ml and a 20 ml sample loop. The HSCCC system used in the present study was equipped with a FMI pump (Zhejiang Instrument Factory, Hangzhou, China), a variable wavelength PC300 detector operating at 254 nm and a model SCJS-3000 workstation (Tianjin Scientific Instrument Ltd., Tianjin, China).

HPLC equipment was a Waters Alliance 2695 system including a quaternary pump with a degasser, a column temperature control module, an automatic sampler, a 2699 photodiode array detector and *Empower pro* data handling system (Waters Corporation, Milford, MA01757, USA).

2.2. Chemicals and reagents

All organic solvents for preparation of enriched extract and HSCCC separation were of analytical grade (The Second Institute of Oceanography, Hangzhou, China). Methanol used for HPLC was of chromatographic grade (Merck, Darmstadt, Germany). All aqueous solutions were prepared with pure water produced by Milli-Q water (18.2 MΩ) system (Millipore, Bedford, MA, USA). The reversed-phase C_{18} resin was purchased from Merck (Darmstadt, Germany).

The aerial part of *T. mongolicum* Hand.-Mazz. (including leaves and inflorescences) was purchased from Bozhou, Anhui province in January, 2004, and identified by Prof. Liurong Chen. A voucher specimen (TM200401-02) was deposited in Department of Traditional Chinese Medicine and Natural Drug Research, College of Pharmaceutical Sciences, Zhejiang University.

2.3. Preparation and pre-purification of the crude extract

The pulverized material of *T. mongolicum* (2.0 kg) was extracted with 95% ethanol under reflux for 2 h and concentrated under reduced pressure to give brown syrup (253 g). A portion of this syrup (200 g) was then suspended in 5 l water by sonication and extracted three times with ethyl acetate. The ethyl acetate extract (18 g) was then subjected to a reversed-phase C_{18} open column chromatography (40 cm × 5 cm, i.d., contained 250 ml C_{18} resin) and eluted with 50% aqueous ethanol and 70% aqueous ethanol, respectively. The eluant of 70% aqueous ethanol (1.2 g) was concentrated to dryness by rotatory evaporator, which was then stored in a refrigerator (4 °C) for the further isolation by HSCCC.

The sample solution was prepared by dissolving 500 mg of the enriched extract in 20 ml of the lower phase of the solvent system.

2.4. Selection and preparation of two-phase solvent system for HSCCC

The selection of the two-phase solvent system for isolation and purification of the target compounds is the most important step in HSCCC, which may account for 90% of the entire work in HSCCC (Ito, 2005). The solvent system for HSCCC separation was selected according to the difference of partition coefficients (K) of each target compound between the two-phase systems. Successfully separation requires a suitable choice of the two-phase solvent system, which provides an ideal partition coefficient. A series of experiments were performed to determine the partition coefficient in different solvent systems. The values of partition coefficient were calculated by HPLC peak area of them in the upper phase and lower phase when the enriched extract was separated in a two-phase solvent system.

Ethyl acetate–*n*-butanol–water (2:5:7, v/v/v) was finally used as the two-phase solvent system for the HSCCC separation. The solvent mixture (2 l) was thoroughly equilibrated in a separation funnel at room temperature. The two phases were separated and let to reach the equilibrium (5–10 min) just before being used in the HSCCC experiment.

2.5. HSCCC separation procedure

In each separation, the multiplayer coil column was first entirely filled with the upper stationary phase. Then the lower mobile phase was pumped into the inlet of the column at the flow rate of 1.5 ml/min, while the column was rotated at 800 rpm. After the mobile phase was eluted from the tail outlet and the two phases had established the hydrodynamic equilibrium throughout the column, a sample (500 mg) dissolved in 20 ml of the lower phase was loaded into the injection valve. The effluent from the outlet of the column was continuously monitored with a UV detector at 276 nm and the peak fractions were collected manually according to the chromatographic profile. After total elution of the compounds, the column was stopped and its content was exhausted by continuously washing with the mobile phase.

2.6. HPLC analysis and identification of the fractions

The enriched extract and each fraction peaked from HSCCC were analyzed by analytical HPLC, which was performed with a Symmetry® C_{18} column (150 mm × 3.9 mm i.d., 5 μm). The mobile phase was 0.1% acetic acid-methanol (50:50, v/v). Before delivering in to the system it was filtered through 0.45 μm PTEE filter. The analysis was carried out using a flow rate of 0.8 ml/min at 30 °C. Chromatograms were recorded at 276 nm.

IR spectra were recorded as KBr disks on a Bruker Vector-22 spectrometer. ESIMS data were measured on a Bruker Esquire 3000+ instrument. HRESIMS were recorded on a Bruker Bio Apex 70 eV FT-ICR (Bruker Daldonis, USA). The ^{1}H NMR, ^{13}C NMR and 2D NMR experiments were performed on a VARIAN INOVA-400 (Varian Corporation, USA) NMR spectrometer using DMSO-d_6 as solvent.

3. Results and discussion

3.1. Selection of solvent system

The HPLC chromatogram of enriched extract from *T. mongolicum* is given in Fig. 2. Two major peaks were detected and separated.

In the experiment, the solvent systems based on ethyl acetate–*n*-butanol–water at different volume ratios were tested (Table 1). Among the solvent systems, ethyl acetate–*n*-butanol–water (2:5:7, v/v/v) was found to be satisfactory for the separation of mongolicumin A and rufescidride from the enriched extract within a short retention time.

Other factors such as the revolution speed of the separation column and the flow rate of the mobile phase were also investigated. The result showed that when the flow rate was 1.5 ml/min, revolution speed was 800 rpm, retention percentage of the stationary phase could reach 48.6% and good separation results could be achieved by using a 420 ml capacity column.

Under the optimum conditions, two fractions (I–II) were obtained in one-step elution and less then 6.5 h, which is 36.7 mg of fraction I (collected between 150 and 200 min)

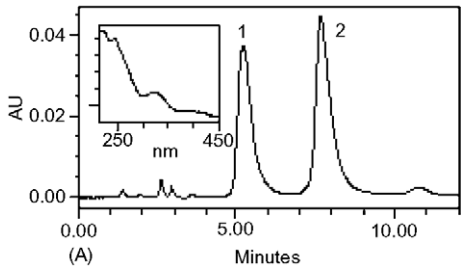


Fig. 2. HPLC chromatogram of the enriched extract from *T. mongolicum* and the UV absorption spectra of the lignans. Peaks 1 and 2 correspond to mongolicumin A and rufescidride.

Table 1
The partition coefficients (K) of the target components in different ratio of volume in ethyl acetate–*n*-butanol–water solvent system

Solvent system		Mongolicumin A	Rufescidride
Ethyl acetate–*n*-butanol–water	2:7:9	2.15	3.48
Ethyl acetate–*n*-butanol–water	2:6:8	1.43	2.31
Ethyl acetate–*n*-butanol–water	2:5:7	0.69	1.23
Ethyl acetate–*n*-butanol–water	2:3:5	0.28	0.47
Ethyl acetate–*n*-butanol–water	2:2:4	0.13	0.24

Table 2
NMR data of mongolicumin A in DMSO-d_6 (TMS, δ ppm; ^{13}C NMR[a]: 100 MHz; ^{1}H NMR: 400 MHz)

Position	δ_C	δ_H	HMBC (H → C)	Position	δ_C	δ_H	HMBC
1	125.8			1′	109.7		
2	123.3			2′	146.4		
3	136.7			3′	103.9	6.62 (1H, s)	C-1′, C-5′
4	141.8			4′	148.6		
5	120.0	7.25 (1H, d, $J = 8.8$ Hz)	C-1, C-3	5′	142.1		
6	121.4	7.46 (1H, d, $J = 8.8$ Hz)	C-2, C-4, C-7	6′	112.5	7.40 (1H, s)	C-2′, C-4′, H-7′
7	128.4	8.05 (1H, s)	C-2, C-6, C-8′, C-9	7′	123.0		
8	126.2			8′	122.3		
9	167.8			9′	171.6		

[a] Assignments confirmed by decoupling, 1H-1H COSY, NOESY, HMQC and HMBC.

and 43.9 mg of fraction II (collected between 235 and 300 min). Each HSCCC fraction revealed that two pure lignans could be obtained from the enriched extract. The purity of two compounds was 98.7% and 98.5%, respectively, as indicated by HPLC analysis.

3.2. Identification of the isolated compounds

The structural identification of compound was all performed with UV, IR, HRESIMS, 1D and 2D NMR spectra.

Fraction I was isolated as red amorphous powder and exhibited a molecular ion $[M + H]^+$ peak at m/z 355.0441 (calcd for $[C_{18}H_{10}O_8 + H]^+$, 355.0454) in the HRESIMS, corresponding to the molecular formula $C_{18}H_{10}O_8$. UV λ_{max} (MeOH) nm: 196, 226, 276, 317 and 373. The IR spectrum displayed absorption bands for hydroxyl (3400 cm^{-1}) and two carboxyl functions (1796, 1721 cm^{-1}). The ^{1}H NMR spectrum (Table 2) showed the presence of three singlet signals δ_H 8.05 (1H, s, H-7), 7.40 (1H, s, H-6') and 6.62 (1H, s, H-3') and two doublets at δ_H 7.46 (1H, d, $J = 8.8$ Hz, H-6) and 7.25 (1H, d, $J = 8.8$ Hz, H-5). The ^{13}C NMR and DEPT spectra showed 18 carbon resonances including five aromatic methines and thirteen quaternary carbons (including five oxygenated aromatic carbons and two carboxyl groups). In the HMBC experiment, long-rang correlations (H → C) of the two doublets (δ 7.46, 7.25) and one singlet (δ 8.05) proton signals suggested the presence of a dicarboxyl naphthalene structure. The remaining two singlet signals (δ 7.40, 6.62) were attributable to aromatic protons of a tri-oxygenated benzene ring attached to the naphthalene nucleus. These indicated the presence of an arylnaphthalene type lignan skeleton that was agreement with the lignan part of a triterpene–lignan ester isolated from *Rhoiptelea chiliantha* (Jiang, Tanaka, & Kouno, 1996). Therefore, mongolicumin A was concluded to be a novel compound, as shown in Fig. 1.

Fraction II was a known compound, which had the identical UV, IR, ESI-MS, ^{1}H NMR and ^{13}C NMR data to those of rufescidride previously isolated from *Cordia rufescenes* (da Silva et al., 2004), and was isolated from *Taraxacum* species for the first time.

4. Conclusion

The results obtained in the present study demonstrate that HSCCC is a powerful technique for isolation of compounds from plant materials with a two-phase solvent system. The compounds obtained may be used as reference substances for chromatographic purposes without additional clean-up. Furthermore, with the method described here, the two lignans can be isolated on a large scale with high purities which may then be used for bioactivity studies. Mongolicumin A is a new compound and rufescidride is isolated from genus *Taraxacum* for the first time.

Acknowledgement

The authors wish to acknowledge the assistance of Prof. Qizhen Du for his excellent technical support.

References

Cao, X. L., Huang, D. F., Dong, Y. M., Zhao, H., & Ito, Y. (2007). Separation of aloins A and B from Aloe vera exudates by high speed countercurrent chromatography. *Journal of Liquid Chromatography & Related Technology, 30*(11), 1657–1668.

Chinese National Pharmacopoeia Committee. (2000). Pharmacopoeia Chinensis (Zhong Hua Ren Min Gong He Guo Yao Dian). Beijing: Chemistry Engineering Publishers, p. 289.

Da Silva, S. A. S., Souto, A. L., Agra, M. F., da-Cunha, E. V. L., Barbosa-Filho, J. M., Da Silva, M. S., et al. (2004). A new arylnaphthalene type lignan from Cordia rufescens A DC Boraginaceae. *ARKIVOC*, 54–58.

Frighetto, R. T. S., Welendorf, R. M., Nigro, E. N., Frighetto, N., & Siani, A. C. (2007). Isolation of ursolic acid from apple peels by high speed counter-current chromatography. *Food Chemistry, 106*(2), 767–771.

Gurib-Fakim, A. (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine, 27*(1), 1–93.

Ito, Y. (1981). Efficient preparative counter-current chromatography with a coil planet centrifuge. *Journal of Chromatography, 214*(1), 122–125.

Ito, Y. (2005). Golden rules and pitfalls in selecting optimum conditions for high-speed counter-current chromatography. *Journal of Chromatogray A, 1065*(2), 145–168.

Jiang, Z. H., Tanaka, T., & Kouno, I. (1996). Chilianthins A-F, six triterpene esters having dimeric structures from *Rhoiptelea chiliantha* Diels et Hand.-Mazz. *Chemical and Pharmaceutical Bulletin, 44*(9), 1669–1675.

Ling, Y., Bao, Y. Y., Zhu, L. L., Zheng, J. H., Cai, S. Q., & Xiao, Y. (1997). Chemical constituents of *Taraxacum mongolicum*. *Zhongguo Yaoxue Zazhi, 32*(10), 584–586.

Ling, Y., Bao, Y. Y., Guo, X. F., Xu, Y., Cai, S. Q., & Zheng, J. H. (1999). Isolation and identification of two flavonoids from *Taraxacum mongolicum*. *Zhongguo Zhongyao Zazhi, 24*(4), 225–226.

Lu, Y. B., Liu, R., Sun, C. R., & Pan, Y. J. (2007). An effective high-speed countercurrent chromatographic method for preparative isolation and purification of mollugin directly from the ethanol extract of the Chinese medicinal plant *Rubia cordifolia*. *Journal of Separation Science, 30*(9), 1313–1317.

Salas, E., Dueñas, M., Schwarz, M., Winterhalter, P., Cheynier, V., & Fulcrand, H. (2005). Characterization of pigments from different high speed countercurrent chromatography wine fractions. *Journal of Agricultural and Food Chemistry, 53*(11), 4536–4546.

Shi, S. P., Jiang, D., Zhao, M. B., & Tu, P. F. (2007). Preparative isolation and purification of triterpene saponins from *Clematis mandshurica* by high-speed counter-current chromatography coupled with evaporative light scattering detection. *Journal of Chromatography B, 852*(1–2), 679–683.

Yao, W., Lin, W. Y., Zhou, C. X., & Zhao, Y. (2007). Studies on constituents from *Taraxacum mongolicum*. *Zhongguo Zhongyao Zazhi, 32*(10), 926–928.