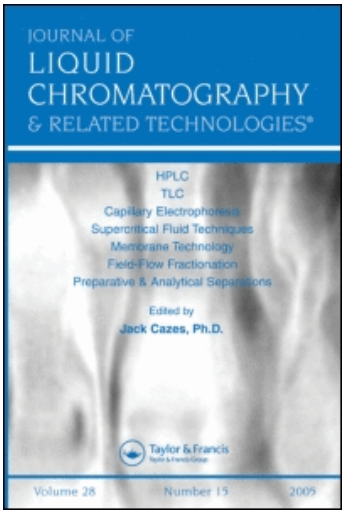


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Separation of Bioactive Quadri-Terpenic Acids from the Fruit of *Ligustrum Lucidum* Ait by High-Speed Countercurrent Chromatography

Qizhen Du[a]; Xinping Xiong[a]; Yolchiro Ito[b]
[a] Tea Research Institute Chinese Academy of Agricultural Sciences Hangzhou, Zhejiang, The People's Republic of China [b] Laboratory of Biophysical Chemistry National Heart, Lung, and Blood Institute National Institutes of Health Bethesda, Maryland

To cite this Article Du, Qizhen , Xiong, Xinping and Ito, Yolchiro(1995) 'Separation of Bioactive Quadri-Terpenic Acids from the Fruit of *Ligustrum Lucidum* Ait by High-Speed Countercurrent Chromatography', Journal of Liquid Chromatography & Related Technologies, 18: 10, 1997 — 2004
To link to this Article: DOI: 10.1080/10826079508013955
URL: http://dx.doi.org/10.1080/10826079508013955

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(10), 1997-2004 (1995)

SEPARATION OF BIOACTIVE QUADRI-TERPENIC ACIDS FROM THE FRUIT OF *LIGUSTRUM LUCIDUM AIT* BY HIGH-SPEED COUNTERCURRENT CHROMATOGRAPHY

QIZHEN DU[1], XINPING XIONG[1], AND YOICHIRO ITO[2]
[1]*Tea Research Institute*
Chinese Academy of Agricultural Sciences
Hangzhou, Zhejiang 310008, The People's Republic of China
[2]*Laboratory of Biophysical Chemistry*
National Heart, Lung, and Blood Institute
National Institutes of Health
Bethesda, Maryland 20892

ABSTRACT

High-speed countercurrent chromatography (HSCCC) was used for the separation of quadri-terpenic acids from the fruit of *Ligustrum lucidum Ait* using a two-phase solvent system composed of hexane/ethyl acetate/methanol/water (3:6:2:1, v/v). From 250mg of the crude extract, the method yielded 87 mg of oleanolic acid at 91.5% purity and 58 mg of ursolic acid at 93.2% purity in about 2.5 h.

INTRODUCTION

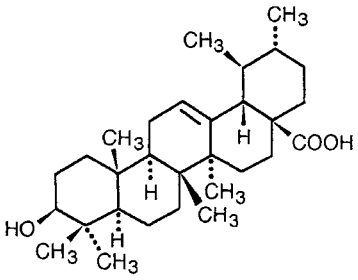
Oleanolic acid and ursolic acid are similar bioactive quadri-terpenic acids (Fig. 1), both being used

as anti-inflammatory and anti-tumor drugs (1). In the earlier studies (2-4), oleanolic acid was considered as a main bioactive component in *Ligustrum lucidum Ait.* Recently, HPLC analysis revealed that the plant extract also contained ursolic acid which often exceeds the amount of oleanolic acid (5). Wu et al. (6) also isolated ursolic acid from the fruit of *Ligustrum lucidum Ait* by column chromatography. In this paper, we conducted a separation of oleanolic acid and ursolic acid from the fruit of *Ligustrum lucidum Ait* by high-speed countercurrent chromatography (HSCCC) (7).

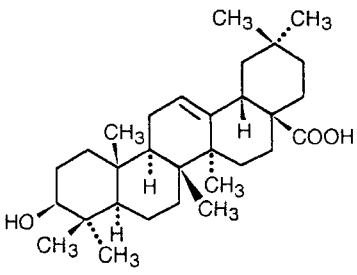
EXPERIMENTAL

Apparatus

HSCCC experiments were performed using a coil planet centrifuge equipped with a multilayer coil column that was designed and fabricated at the Beijing Institute of New Technology Application, Beijing, China. The multilayer coil was prepared by winding a 1.6mm ID PTFE (polytetrafluoroethylene) tube coaxially onto the column holder hub. The total capacity of the column measured 230 ml. The HSCCC centrifuge was equipped with an FMI pump (Zhejiang Instrument Factory, Hangzhou, China), an injection valve and a fraction collector.



Oleanolic acid



Ursolic acid

FIGURE 1. Chemical structures of oleanolic acid and ursolic acid.

Reagents

All organic solvents were of analytical grade and purchased from Shanghai Chemical Factory, Shanghai, China. Oleanolic and ursolic acids standards were purchased from Sigma Chemical Company, St. Louis, MO, U.S.A. Analytical grade phosphomolybdic acid was purchased from Beijing Chemical Factory, Beijing, China.

Extraction of Terpenic Acids

The extraction was initiated by degreasing 200g dried powder of *L. lucidum* fruit by petroleum ether followed by extracting twice each with 1000 ml of chloroform for 2 hours at 80°C. The chloroform extract was combined and evaporated to dryness. Then the residue

was dissolved in 50ml of ethanol and decolorized by filtering through active carbon. Ethanol was removed under reduced pressure at 60°C and the residue was in turn dried in vacuum. This procedure yielded 3.2g of light yellow powder which was subsequently subjected to HSCCC separation.

HSCCC Separation

HSCCC experiment was performed with a two-phase solvent system composed of hexane/ethyl acetate/ methanol/water (3:6:2:1, v/v). The solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and the two phases separated before use. In each separation, the multilayer coil was first entirely filled with the upper stationary phase. The lower mobile phase was pumped into the inlet of the column at a flow rate of 1.5 ml/min, while the apparatus was rotated at 800 rpm. After the mobile phase front emerged and the two phases had established hydrodynamic equilibrium, the sample solution (250 mg of the extract in 10ml of the mobile phase) was injected through the injection valve. The effluent from the outlet of the column was collected into test tubes with a fraction collector. Each fraction was subjected to color reaction analysis using phosphomolybdic acid (8) (Table 1).

TABLE 1
Color Reaction Analysis of HSCCC Fractions

Fraction No.[1]	Retention Time (min)	Color Reaction[2]	Component[3]
1	48	-	
3	54	-	
5	60	-	I
7	66	-	I
9	72	-	
11	78	-	
13	84	+	II
15	90	+	II
17	96	-	
19	102	++	III
21	108	+++	III
23	114	+++	III
25	120	++	III
27	126	-	
29	132	+	IV
31	138	++	IV
33	144	++	IV
35	150	++	IV
37	156	+	IV
39	162	-	

[1] Fraction volume: 4.5 ml/tube
[2] Reaction condition: Each fraction (5μl) on GF254 plate (Merck) was treated with 5% of phosphomolybdic acid in ethanol for 10 minutes at 110°C.
- negative; + light purple; ++ purple; +++ dark purple.
[3] Component I is itself yellow; Components II, III and IV are colorless.

HPLC Analysis

The crude extract and the HSCCC fractions were analyzed by HPLC using Shimadzu HPLC equipment (Shimadzu Corporation, Kyoto, Japan) consisting of an LC-10AD pump, an SPD-10A UV-VIS detector, a manual injector and a C-

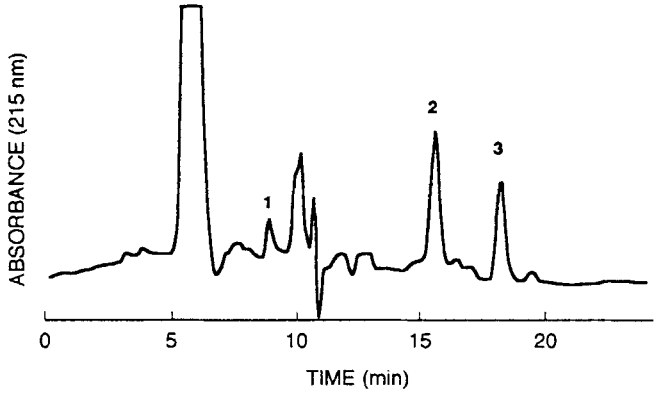


FIGURE 2. HPLC analysis of the crude extract of *L. lucidum* fruit. Mobile phase: methanol-water (9:1, v/v); flow-rate: 0.5 ml/min; detection 215 nm. Peak 1: unknown compound; peak 2: oleanolic acid; and peak 3: ursolic acid.

R10A recording processor. The analyses were performed with Shim-pack CLC-ODS-C_{18} column, 15 X 0.60cm ID (Shimadzu). The mobile phase composed of methanol-water (9:1, v/v) was isocratically eluted at a flow-rate of 0.5 ml/min and the effluent was monitored at 215 nm.

RESULTS AND DISCUSSION

HPLC analysis (Fig. 2) of the crude extract from the fruit of *Ligustrum lucidum Ait* showed that it contained oleanolic acid (peak 2) and ursolic acid (peak 3) by comparison with authentic samples. A 250mg amount of the crude extract was separated by HSCCC. Tests with

phosphomolybdic acid of the CCC fractions revealed the colors shown in Table 1. Component I (which was itself yellow) failed to give a positive test while the other three components (themselves colorless) gave a purple color characteristic of quadri-terpenic acids (8). Component III (corresponding peak 2 in HPLC) and Component IV (corresponding to peak 3 in HPLC) were oleanolic acid (87 mg, 35% in weight, 91.5% pure by HPLC) and ursolic acid (58 mg, 23% in weight, 93.2% pure by HPLC), respectively. Recrystallization from methanol produced 41 mg of oleanolic acid that was 98.5% pure (HPLC) and 39 mg of ursolic acid, 97% pure (HPLC). Fractions containing component II (corresponding to peak 1 in HPLC) produced 7 mg of an unknown compound. On the basis of its short retention time in both HPLC and CCC together with a positive color reaction, this compound appears to be a polar derivative relating to the above products.

ACKNOWLEDGMENT

The authors wish to thank Dr. H.M. Fales for editing the manuscript with valuable suggestions.

REFERENCES

1. Monograph on Bioactive Compounds of Medicinal Plants, Information Center for Chinese Herbal Medicines, People's Health Publishing Agency, National Food and Drug Administration of China, p 786 and p 1101, 1986.

2. Ma, X.H., Zhao, Y.C., Yin, L. and Han, D.W., Studies on the effect of oleanolic acid on experimental liver injury, Chinese Journal of Pharmacology, 17, 917 (1982)

3. Da, Y., Hang, B.Q. and Mong, Q.Y., Inhibition of oleanolic acid on hypersensitivity reaction, Chinese Journal of Medicine, 9, 562 (1988).

4. Hang, B.Q., Dai, Y., Wu, G.Z., Dili, N., Zhao, L. and Tan, L.W., Protection of fructus ligustri lucidi and oleanolic acid on the chromosomal damage induced by cyclophosphamide and urethan, Journal of Chinese Pharmacological University, 18(3), 222 (1987).

5. Lu, X.H., Wang, Q., Xia, G.C. and Hei, W.L., HPLC analysis of oleanolic acid and ursolic acid in *Ligustrum lucidum Ait*, Chinese Journal of Pharmaceutical Analysis, 35, 291 (1993).

6. Wu, Q.J., Wang, D.X. and Sun, Y., Study on chemical components in the fruit of *Ligustrum lucidum Ait*, Chinese Traditional Herbal Drugs, 24(1), 4 (1993).

7. Ito, Y., High-Speed Countercurrent Chromatography, CRC Crit. Rev. Anal. Chem., 17, 65 (1986).

8. Zhang, G.Q., Liang, S.W., Du, T.S. and Zhang, T.W., Improved method for analysis of ursolic acid in luweidihuang pill. Chinese Journal of Pharmacology, 25, 283 (1990).

Received: January 10, 1995
Accepted: January 25, 1995