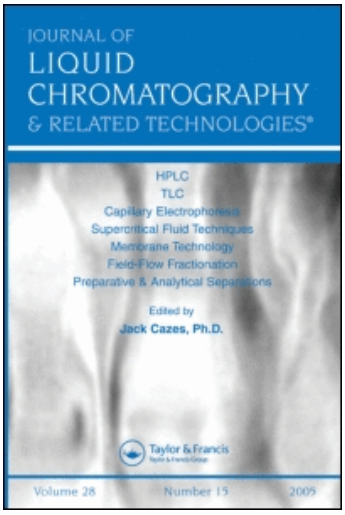

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

SEPARATION AND ISOLATION OF TERPENE LACTONES FROM *GINKGO BILOBA* L. BY DIRECT HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Tang Yuping[a]; Lou Fengchang[a]
[a] China Pharmaceutical University, Nanjing, P. R. China

Online publication date: 11 July 2000

To cite this Article Yuping, Tang and Fengchang, Lou(2000) 'SEPARATION AND ISOLATION OF TERPENE LACTONES FROM *GINKGO BILOBA* L. BY DIRECT HIGH PERFORMANCE LIQUID CHROMATOGRAPHY', Journal of Liquid Chromatography & Related Technologies, 23: 18, 2897 — 2900
To link to this Article: DOI: 10.1081/JLC-100101241
URL: http://dx.doi.org/10.1081/JLC-100101241

J. LIQ. CHROM. & REL. TECHNOL., 23(18), 2897–2900 (2000)

SEPARATION AND ISOLATION OF TERPENE LACTONES FROM *GINKGO BILOBA* L. BY DIRECT HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Tang Yuping,* Lou Fengchang

China Pharmaceutical University
No. 1 Shennong Road
Box G-05
Nanjing 210038, P. R. China

ABSTRACT

Isolation of terpene lactones, i.e., Bilobalide, Ginkgolides A, B, C and J in pure form from *Ginkgo biloba* leaves by a preparative high performance liquid chromatography procedure is described.

INTRODUCTION

In recent years, *Ginkgo biloba* L. (*Ginkgoaceae*) has become one of the most popular medicinal plants, and phytopharmaceuticals containing *G. biloba* leaf extracts belonging to the best selling drugs in several countries, in particular Germany and France.[1] They are widely used in the treatment of cerebrovascular and peripheral circulatory disorders of the elderly, and to cure asthma.[2]

The most important constituents from a medicinal point of view are the ginkgolides. They are very potent platelet activating factor (PAF-acether) antagonists and unique to *G. biloba*. The most active compound is ginkgolide B, and this co-occurs with ginkgolides A, C and J.[3] A closely related sesquiterpene bilobalide lacks anti-PAF activity but has a neuroprotective effect.[4]

Because ginkgolides A, B, C, J, and Bilobalide have closely similar structures, common column chromatography of the extracts from *G. biloba* is very difficult. In some publications[5-7] HPLC analysis of terpene lactones using reverse phase column has been carried out, but the compounds were not isolated.

Herein, we report the actual isolation of various individual terpene lactones present in *G. biloba* leaves by preparative HPLC procedures.

EXPERIMENTAL

Preparative High Performance Liquid Chromatography was carried out using a Shimadzu LC 8A HPLC system linked to CR 4A data processor and the peaks were detected at 220 nm. Shimpack reverse phase (C_{18}) preparative column (25 cm × 20 mm i.d.) was used for preparative runs and Phenomenex reverse phase column (C_{18}) (25 cm × 4.6 mm) was used for analysis.

Freshly powdered leaves (1.0 kg) of *G. biloba* were extracted three times with cyclohexane at room temperature and the defatted leaf powder was extracted with ethanol. The ethanol extract, after removal of solvent (85.3 gm), was suspended in water and extracted with ethyl acetate. The residue (40.5 gm) was subjected to silica gel column chromatography[8] using cyclohexane and increasing quantities of ethyl acetate (10-50%) and then, finally, with ethyl acetate.

The 40-50% ethyl acetate fraction was concentrated to a small volume; when crystals appeared they were filtered. They were subjected to preparative HPLC for the isolation of terpene lactones.

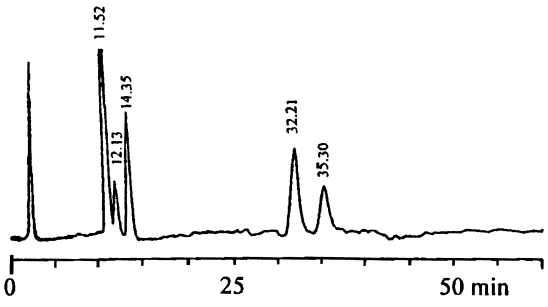

Figure 1. Preparative high performance liquid chromatogram of the terpene lactones from the leaves of *Ginkgo biloba*.

Table 1

Isolation of Terpene Lactones from the Leaves of *G. Biloba*

Peak	RT (Min)	Compound	Amount Obtained (mg)
1	11.52	Bilobalide	230
2	12.13	Ginkgolide J	40
3	14.35	Ginkgolide C	200
4	32.21	Ginkgolide A	210
5	35.30	Ginkgolide B	130

RESULTS AND DISCUSSION

For each preparative run, 1500 mg of the crystals from 40-50% ethyl acetate fraction was dissolved in 2mL of methanol, filtered through a Millipore filter (0.25μm), and then injected into the preparative column (25 cm × 20 mm i.d.). The eluent system was MeOH:*i*-PrOH:H_2O 15:10:75, and the eluent flow rate was 20 mL/min. throughout the run.

The individual peaks (Figure 1) were collected and evaporated. The purity of the compounds recovered from the peaks was checked by analytical HPLC. Identification of the compounds was established by spectral methods, especially ^{1}H-NMR and ^{13}C-NMR spectrum, and comparison with literature data.[3,9]

The peaks with retention times 11.52 min.(230 mg), 12.13 min.(40 mg), 14.35 min.(200 mg), 32.21 min.(210 mg), and 35.30 min.(130 mg) were identified to be Bilobalide, Ginkgolide J, Ginkgolide C, Ginkgolide A and Ginkgolide B, respectively, (Table 1).

REFERENCES

1. O. Sticher, Planta Med.,**59(1)**, 2-11 (1993).

2. H. Oberpichler-Schwenk, J. Krieglstein, Pharm. Unserer Zeit, **21(5)**, 224-235 (1992).

3. P. Braquet, Drugs Future, **12(7)**, 643-699 (1987).

4. C. Bruno, R. Cuppini, S. Sartini, T. Cecchini, P. Ambrogini, E. Bombardelli, Planta Med., **59(4)**, 302-307 (1993).

5. A. Lobstein-Guth, F. Briancon-Scheid, R. Anton, J. Chromatogr., **267(2)**, 431-438 (1983).

6. P. G. Pietta, P. L. Mauri, A. Rava, Chromatographia, **29(5-6)**, 251-253 (1990).

7. T. A. Van Beek, H. A. Scheeren, T. Rantio, W. Ch. Melger, G. P. Lelyveld, J. Chromatogr.. **543(2)**, 375-387 (1991).

8. Y. Song, Y. Xinsheng, C. Chengbin, Y. Tezuka, T. Kikuchi, Zhongguo Yaowu Huaxue Zazhi, **5(4)**, 258-265 (1995).

9. K. Weinges, M. Hepp, H. Jaggy, Liebigs Ann. Chem., **6**, 521-526 (1987).

Received May 31, 1999
Accepted August 16, 1999

Author's Revisions June 22, 2000
Manuscript 5087