Journal of Chromatography, 634 (1993) 135-137
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 822

Short Communication

High-performance liquid chromatography of thapsigargins

A.K. Jäger, L. Gudiksen, A. Adsersen and U.W. Smitt*

Department of Pharmacognosy, Royal Danish School of Pharmacy, Universitetsparken 2, DK-2100 Copenhagen Ø (Denmark)

(First received October 2nd, 1992; revised manuscript received December 21st, 1992)

ABSTRACT

A simple and rapid high-performance liquid chromatography method based on reversed-phase chromatography with isocratic elution [methanol-water (83:17)] and UV detection at 230 nm is described. The method permits the separation and determination of the main thapsigargins found in ***Thapsia garganica*** L. and ***Thapsia transtagana*** Brot.

INTRODUCTION

Thapsigargins are a group of highly oxygenated sesquiterpene lactones found in some species of the genus ***Thapsia,*** Apiaceae. They are tumour-promoting skin irritants with an unusual stereochemistry and a unique biological activity as specific inhibitors of Ca^{2+}-ATPases in animal cells [1,2]. No quantitative investigations of thapsigargins in plant material have previously been reported. However, the internationally increasing demand for thapsigargin as a pharmacological test compound has led to a requirement for a rapid, quantitative method to evaluate the plant material from which thapsigargin can be isolated. In this paper we describe a high-performance liquid chromatography (HPLC) method based on reversed-phase separation with isocratic elution, which permits the resolution and determination of the five main thapsigargins found in the two species ***Thapsia garganica*** and ***T. transtagana.***

EXPERIMENTAL

Reagents

All solvents were of analytical-reagent grade from Merck (Darmstadt, Germany); methanol for elution was of LiChrosolv grade and water was redistilled in glass.

The reference substances were all purified from plant material of ***T. garganica*** and ***T. transtagana*** in our laboratory [3,4].

Plant materials

Fruits of ***T. garganica*** L. were collected on Ibiza (Spain) in 1988 and fruits of ***T. transtagana*** Brot. in Portugal in 1988.

* Corresponding author.

0021-9673/93/$06.00

HPLC

All separations were carried out using Shimadzu (Kyoto, Japan) equipment: a C-R4A Chromatopac system with an SCL-6A system controller, LC-6A pumps, a CTO-6A column oven, an SPA-6A UV detector and a SIL-6A autoinjector.

The column was LiChrosorb RP-18, 5 μm (25 x 0.8 cm I.D.) (Merck), operated at 40°C. The mobile phase was methanol-water (83:17, v/v). A flow-rate of 4 ml/min and UV detection at 230 nm were applied. A volume of 10 μl was injected.

Standard solutions

Standard solutions of the thapsigargins 1-5 (Fig. 1) were prepared in the range 0.2-1.0 mg/ml, corresponding to the concentration range of the plant extracts.

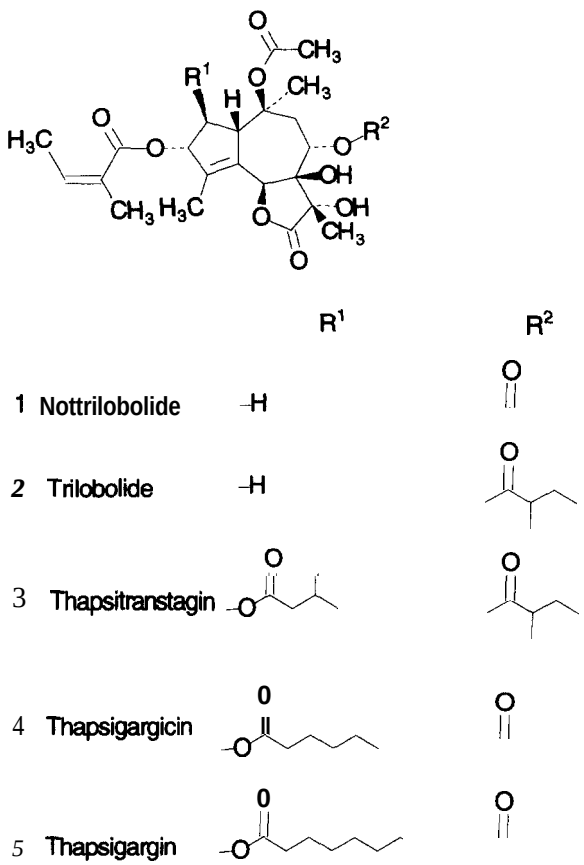

Fig. 1. Structures of the thapsigargins.

Calculation

Calibration graphs were generated by plotting the peak area versus the concentration of standard substances. In all instances the calibration graphs were linear in the concentration range 0.2-1.0 mg/ml with a correlation coefficient of 0.9999. Values of unknown sample concentrations were determined by comparison with the calibration graph.

Extraction and fractionation of plant material

A 100-mg amount of dried, pulverized plant material was extracted twice with 3.0 ml of ethylacetate (EtOAc) in a sonication bath for 30 min. The extract was centrifuged, the supernatant was evaporated to dryness and the residue was dissolved in 1 ml of dichloromethane (CH_2Cl_2) and this was loaded on a Bond Elut 1

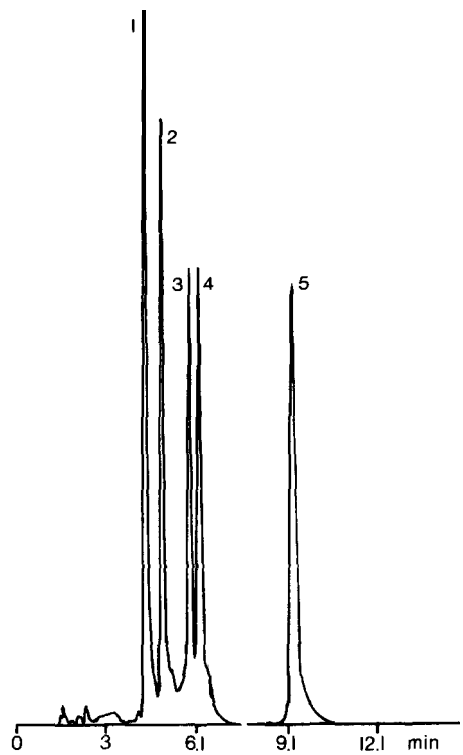

Fig. 2. High-performance liquid chromatogram of a mixture of thapsigargin standards. LiChrosorb RP-18, 5 μm, column (25 x 0.8 cm I.D.); eluent, methanol-water (83:17); flow-rate, 4 ml/min; UV detection at 230 nm. Peaks: 1 = nortrilobolide; 2 = trilobolide; 3 = thapsitranstagin; 4 = thapsigargicin; 5 = thapsigargin.

CC, SI 1210-2010 column (Varian, Harbor City, CA, USA). The column was eluted with 1 ml of CH_2Cl_2–EtOAc (19:1) (fraction 1) and then with 2 ml of CH_2Cl_2–EtOAc (1:1) (fraction 2). The thapsigargins were eluted completely in fraction 2. This fraction was evaporated to dryness and the residue was dissolved in 1 ml of methanol and used for injection into the HPLC system.

RESULTS AND DISCUSSION

A reversed-phase system allowed the best separation of the thapsigargins. The thapsigargins (Fig. 1) have UV maxima between 215 and 220 nm. Owing to the variable quality of methanol, which causes fluctuations in the interference of the mobile phase, it proved in practice to be satisfactory to run the HPLC analysis at 230 nm. As illustrated in Fig. 2, separation of the thapsigargins can be achieved by the use of an isocratic solvent system consisting of methanol–water (83:17, v/v), giving retention times less than 10 min. It was not necessary to obtain a better resolution of peaks 3 and 4, as the two compounds occur in different species [5]. To avoid changes in the retention times, it was essential to stabilize the column temperature. The relative retention times of the thapsigargins were nortrilobolid 0.42, trilobolid 0.47, thapsitranstagin 0.56, thapsigargicin 0.59 and thapsigargin 1.00.

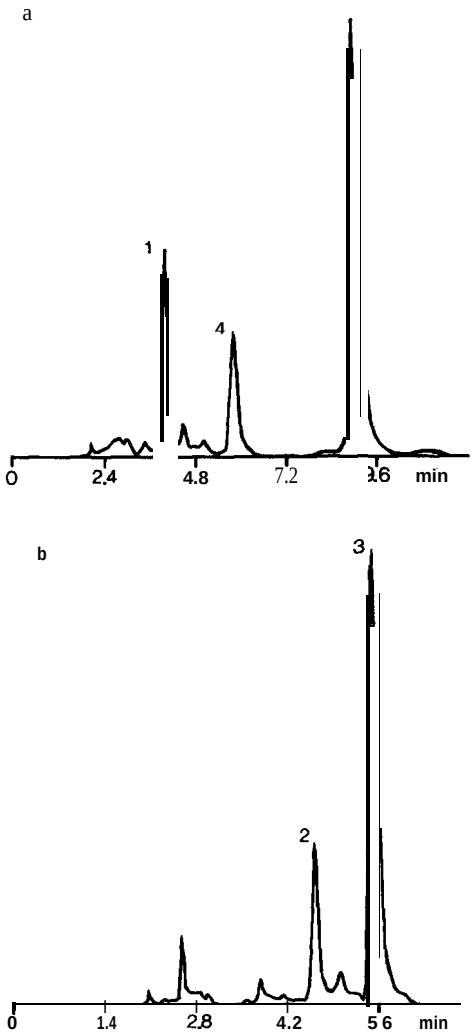

Fig. 3. Chromatograms of fractionated fruit extracts of (a) *Thapsia garganica* L. and (b) *Thapsia transtagana* Brot. Conditions and peak numbers as in Fig. 2.

Fractionation of the plant extract prior to the HPLC analysis results in the separation of the thapsigargins from almost all other compounds in the extract. Fig. 3 shows the chromatograms of fraction 2 from fruit extracts of *T. garganica* and *T. transtagana.*

It can be concluded that the described procedure of extraction, fractionation and HPLC analysis is applicable to the determination of the five main thapsigargins in plant material of *T. garganica* and *T. transtagana,* and could be the basis for the development of a procedure for the determination of other thapsigargins.

REFERENCES

1 0. Thastrup, A.P. Dawson, 0. Scharff, B. Foder, P.J. Cullen, B.K. Drøbak, P.J. Bjerrum, S.B. Christensen and M.R. Hanley, *Agents Actions, 27 (1989) 17.*

2 S.B. Christensen, A. Andersen, A. Lauridsen, P. Moldt, U.W. Smitt and 0. Thastrup, in P. Krogsgaard-Larsen, S.B. Christensen and H. Kofod (Editors), *Alfred Benzon Symposium 33,* Munksgaard, Copenhagen, 1992, p. 243.

3 S.B. Christensen, E. Norup, U. Rasmussen and J.O. Madsen, *Phytochemistry, 23 (1984) 1659.*

4 U.W. Smitt and S.B. Christensen, *Planta* Med., 57 (1991) 196.

5 U.W. Smitt, L. Gudiksen, A. Adsersen and A.K. Jäger, in preparation.