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Journal of Chromatography A, 675 (1994) 240–243

JOURNAL OF CHROMATOGRAPHY A

Short Communication

High-performance liquid chromatographic determination of flavone C-glycosides in some species of the Cucurbitaceae family

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(First received December 27th, 1993; revised manuscript received February 22nd, 1994)

Abstract

Flavonoid complexes occurring in the medicinal plants *Bryonia alba*, *Bryonia dioica* and *Lagenaria siceraria* were found to be flavone C-glycosides. Flavonoids of these species were compared by HPLC and separation conditions were elaborated for C-glycosides using isocratic and gradient elution. The content of the major C-glycoside, saponarin, was determined. The highest saponarin level (2.481%) was found in *Bryonia dioica*.

1. Introduction

HPLC is one of the methods used for the separation and determination of naturally occurring mixtures of flavonoids (aglycones and glycosides) [1–5]. It permits rapid separations of the flavonoid complexes occurring in the plant material, a feature important in chemotaxonomic studies [6]. This study was carried out from the chemotaxonomic standpoint by utilizing the HPLC analysis of flavonoid C-glycoside complexes in some species of the Cucurbitaceae family.

So far, investigations of flavonoids in the Cucurbitaceae family have revealed C-glycoside compounds together with flavonoid O-glycosides [7–10]. The C-glycoside flavonoids saponarin and isovitexin, isolated and identified by us in three species of Cucurbitaceae [9,10], owing to their rare dissemination in the plant kingdom, can provide good markers for chemotaxonomic investigations of this family. Three species have now been studied, namely *Bryonia alba* L., *Bryonia dioica* Jacq. and *Lagenaria siceraria* L. As yet, flavonoids have not been surveyed in *Lagenaria siceraria* and *Bryonia alba*, whereas in the flavonoid complexes of *Bryonia dioica*, saponarin and vicenin-2 have been found [8]. The species are used in therapy as antirheumatics and in homeopathy [11,12].

The purpose of this study was to demonstrate, with the aid of HPLC, the chemical affinity of the three species and to determine the concentration of saponarin, the predominant C-glycoside in the flavonoid complexes.

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0021-9673/94/$07.00
SSDI 0021-9673(94)00269-F

2. Experimental

2.1. Equipment

An HPLC system from Knauer (Berlin, Germany) was used, consisting of two Model 64-00 pumps, a solvent dynamic mixing chamber and a Model 87-00 UV detector, equipped with a Model 7125 injection valve (Rheodyne, Cotati, CA, USA) with a 20-μl sample loop, under computer control (Knauer HPLC, version 211a). The flavonoids were separated on a LiChrospher RP-18 (5 μm) column (250 × 4 mm I.D.) (Merck, Darmstadt, Germany) connected to a guard column containing the same stationary phase (5 μm) (50 × 4 mm I.D.) (Merck).

2.2. Reagents

The organic solvents were of HPLC grade (acetonitrile and methanol; Merck) or analytical-reagent grade (acetic acid; POCH, Lublin, Poland). Redistilled water was used. After preparation of the mobile phase it was filtered through a 0.49-μm filter (J.T. Baker, Phillipsburg, NJ, USA).

2.3. Elution

The flavonoids were separated by isocratic elution using methanol–water–acetic acid (30:70:3) and by gradient elution using solvent A (acetonitrile) and solvent B [water–acetic acid (97:3)] with the following gradient programme: 0 to 20 min, from 12 to 15% A in (linear gradient), and from 20 to 45 min, 15% A (isocratic elution). A re-equilibration period of 10 min was used between individual runs. Elution was carried out at room temperature with a flow-rate of 1.3 ml/min and UV detection at 330 nm (sensitivity 0.008 AUFS).

2.4. Reference compounds

Flavone C-glycosides (Table 1) were isolated from plant material by preparative column and thin-layer chromatography. Their structures were established on the basis of classical (m.p., PC, TLC, hydrolysis) and spectroscopic methods (FD-MS, LSI-MS, EI-MS, UV, IR, ^{1}H NMR, ^{13}C NMR and 2D NMR) [9,10].

2.5. Calibration

A stock solution of saponarin was prepared by dissolving 4 mg of the flavonoid in 10 ml of water–methanol (7:3, v/v). The volumes injected (20 μl) corresponded to amounts of saponarin in the range 1–8 μg. A calibration graph was obtained by plotting peak area (y) against concentration of standard solutions (x) (regression equation: $y = 1.724x - 0.299$, correlation coefficient $r = 0.9998$).

Table 1
Flavone C-glycosides from *Bryonia alba*, *Bryonia dioica* and *Lagenaria siceraria*

Compound	Species		
	Bryonia alba	*Bryonia dioica*	*Lagenaria siceraria*
Vitexin	+	+	–
Isovitexin	+	+	+
Isoorientin	–	+	+
Saponarin	+	+	+
Lutonarin	+	+	–
Saponarin 4'-O-glucoside	–	–	+
Saponarin caffeic ester	–	+	–
Unidentified C-Glycoside flavonoid	–	+	–

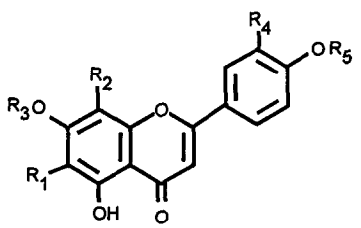
2.6. Sample preparation

The plant material was a flowering herb collected in the garden of Medicinal Plants, Medical Academy, Gdańsk, Poland. After drying, the material was powdered and purified by extraction with chloroform in a Soxhlet apparatus. The flavonoids were extracted from the material with methanol (Soxhlet apparatus, 3 h, 100 ml). For quantitative determinations, samples of either 0.5 g (*Bryonia dioica*) or 1 g (*Bryonia alba*, *Lagenaria siceraria*) were taken. The methanolic extracts were concentrated under reduced pressure and the dry residues were dissolved in water–methanol (7:3, v/v) (100 ml with *Bryonia* and 10 ml with *Lagenaria*). The solutions were then filtered through a 0.45-μm filter (J.T. Baker) and injected.

3. Results and discussion

The conditions for the HPLC analysis of the complexes of flavone C-glycosides occurring in some species of the Cucurbitaceae family were established. The structures of the C-glycosides isolated from the plant material are shown in Fig. 1.

The procedures leading to the separation of the complexes of flavonoid C-glycosides are based on solvents typical for the RP-HPLC of flavonoids [13,14]. The best results were obtained with methanol–water–acetic acid (30:70:3, v/v/v). Under these conditions, the complex of flavone C-glycosides of *Lagenaria siceraria* was separated (Fig. 2). For the remaining species, a good separation of lutonarin, saponarin and isoorientin was obtained, whereas vitexin and isovitexin gave poorly resolved



compound	R_1	R_2	R_3	R_4	R_5
vitexin	H	glucose	H	H	H
isovitexin	glucose	H	H	H	H
saponarin	glucose	H	glucose	H	H
saponarin 4'-O-glucoside	glucose	H	glucose	H	glucose
saponarin caffeic ester	glucose	H	caffeoylglucose	H	H
isoorientin	glucose	H	H	OH	H
lutonarin	glucose	H	glucose	OH	H

Fig. 1. Structure of flavone C-glycosides from *Bryonia alba*, *Bryonia dioica* and *Lagenaria siceraria*.

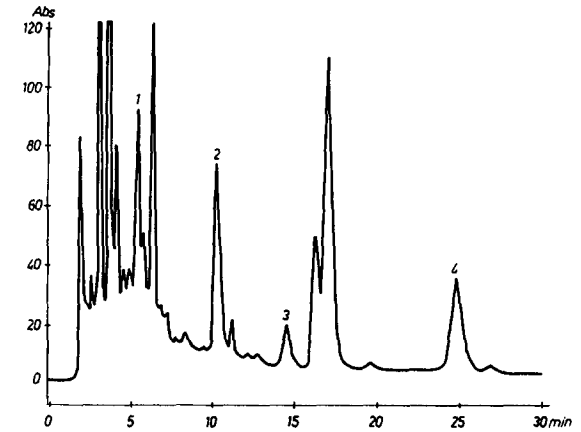


Fig. 2. HPLC of flavone C-glycosides from *Lagenaria siceraria* (isocratic elution). Peaks: 1 = 7,4'-O-diglucosyl-6-C-glucoside of apigenin; 2 = saponarin; 3 = isoorientin; 4 = isovitexin.

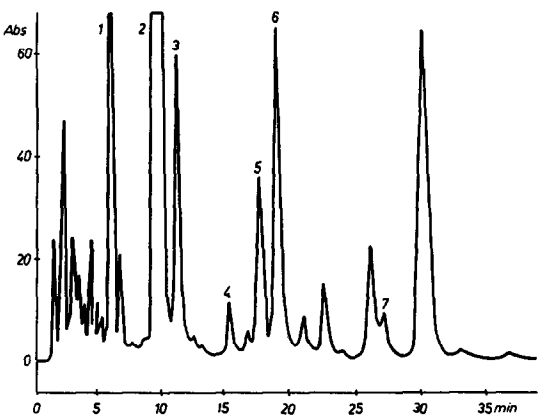


Fig. 3. HPLC of flavone C-glycosides from *Bryonia dioica* (gradient elution). Peaks: 1 = lutonarin; 2 = saponarin; 3 = isovitexin; 4 = vitexin; 5 = isovitexin; 6 = caffeic ester of saponarin; 7 = unidentified.

Table 2
Determination of saponarin in plant material

Species	Amount of saponarin (% dry material) ($n = 5$)	Standard deviation (%)
Bryonia alba	1.142	0.0495
Bryonia dioica	2.481	0.0280
Lagenaria siceraria	0.064	0.0037

peaks. To separate flavone C-glycosides from *Bryonia dioica* and *Bryonia alba*, programme for gradient elution were developed with the acetonitrile–3% acetic acid system. The best separations of the C-glycosides from *Bryonia dioica* (Fig. 3) and *Bryonia alba* were achieved using a linear gradient of acetonitrile in 3% acetic acid followed by isocratic elution. The determination of saponarin in the aforementioned species was carried out by isocratic elution using methanol–water–acetic acid (30:70:3). The results of quantitative analysis are given in Table 2. The high saponarin content in *Bryonia* allows it to be classified among those rich in flavonoids.

The flavonoid complexes occurring in the plants of the Cucurbitaceae family are mixtures that are difficult to separate and identify. The species investigated here, although fairly common, have been poorly surveyed for their flavonoid dyes. The present HPLC method permits good separations and identification of the flavonoids in these plants.

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