Journal of Chromatography, 558 (1991) 296–301
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 499

Short Communication

High-performance liquid chromatographic analysis of flavonol glycosides of *Solidago virgaurea*

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(First received February 22nd, 1991; revised manuscript received May 23rd, 1991)

ABSTRACT

Solidago virgaurea flavonols were separated by reversed-phase high-performance liquid chromatography (RPLC) on a C_8 column using 2-propanol–water (14:86) as eluent in the isocratic mode. The presence of rutin, kaempferol-3-O-rutinoside, kaempferol-3-O-robinobioside and isorhamnetin-3-O-rutinoside was confirmed. The identity of kaempferol-3-O-robinobioside was proved by acid hydrolysis followed by determination of kaempferol and rhamnose/galactose and mass spectrometry. A rapid isocratic separation of the flavonol aglycones obtained by acid hydrolysis of the *Solidago virgaurea* extract is also described.

INTRODUCTION

Solidago virgaurea herb is used in folk medicine for its diuretic [1] and hypotensive [2] properties. It has been reported that chlorogenic acid, caffeic acid, rutin (**I**), quercitrin (**II**), astragalin (**III**), kaempferol-3-rutinoside (**IV**), kaempferol-3-O-robinobioside (**V**) and isorhamnetin-3-O-rutinoside (**VI**) are the most representative phenolic compounds [3,4] (Fig. 1). Although thin-layer chromatographic methods have been described [5,6] for the identification of some of these substances, so far no high-performance liquid chromatographic (HPLC) method has been applied to *Solidago virgaurea*.

Owing to the great potential of reversed-phase HPLC in separating complex mixtures of phenolic compounds, we applied a recently reported technique [7] based on C_8 columns coupled with aqueous 2-propanol to evaluate *Solidago virgaurea* extracts. By this approach a valuable HPLC "fingerprint" of *Solidago virgaurea* has been obtained. In this paper we also describe an efficient HPLC procedure for the

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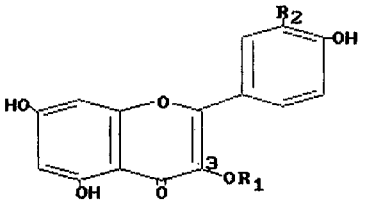

Rutin	(I)	R_1 = rutinose ; R_2 = OH
Quercitrin	(II)	R_1 = rhamnose ; R_2 = OH
Astragalin	(III)	R_1 = glucose ; R_2 = H
Kaempferol-3-O-rutinoside	(IV)	R_1 = rutinose ; R_2 = H
Kaempferol-3-O-robinobioside	(V)	R_1 = robinobiose ; R_2 = H
Isorhamnetin-3-O-rutinoside	(VI)	R_1 = rutinose ; R_2 = OCH$_3$

Fig. 1. Structures of *Solidago virgaurea* flavonol glycosides.

isolation of kaempferol-3-O-robinobioside (**V**), and its characterization by mass spectrometry and acid hydrolysis combined with chromatographic determination of the resulting aglycone and sugars.

EXPERIMENTAL

Materials

Solidago virgaurea herb was obtained from different commercial sources (Milanfarma, Milan, Italy; Birkenweg, Kleinostheim/Main, Germany; Galke, Gittelde/Harz, Germany). Quercetin-3-O-rhamnoglucoside (rutin, **I**), quercetin-3-O-rhamnoside (quercitrin, **II**), kaempferol-3-O-rhamnoglucoside (kaempferol-3-O-rutinoside, **IV**) and isorhamnetin-3-O-rhamnoglucoside (isorhamnetin-3-O-rutinoside, **VI**) were purchased from Extrasynthese (Genay, France). Kaempferol-3-O-rhamnogalactoside (kaempferol-3-O-robinobioside, **V**) was obtained in our laboratory from *Solidago virgaurea* by semi-preparative HPLC. 2-Propanol and water were of HPLC grade (Chromasolv; Riedel de Haën, Hannover, Germany).

Chromatographic conditions

Chromatographic analyses were performed on a system consisting of a Model 510 pump equipped with a Model U6K universal injector (Waters Assoc., Milford, MA, USA) and a Model HP 1040 A photodiode-array detector (DAD) (Hewlett-Packard, Waldbronn, Germany). Chromatographic runs were performed on a 7-μm spherical Aquapore C_8 column (250 × 4.6 mm I.D.) from Brownlee Labs. (Santa Clara, CA, USA). A semi-preparative 7-μm C_8 column (250 × 7.0 mm I.D.) was also supplied by Brownlee Labs. The eluent was 2-propanol–water (14:86) at a flow-rate of 1.8 ml/min. The eluent for the aglycones was 1-propanol–tetrahydrofuran–0.6% citric acid (12.5:7.5:80) at a flow-rate of 2 ml/min. The peaks were monitored at 254

nm and the UV spectra acquired for each peak were computer normalized and the plots superimposed.

Sample preparation

Solidago virgaurea powdered samples (1 g) were treated with ethanol–water (50:50) (50 ml) at 70°C for 60 min. After filtration, the solution was concentrated, then extracted with ethyl acetate (3 × 20 ml). The combined extracts were dried over sodium sulphate, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in methanol (3 ml).

Acid hydrolysis

A 0.5-ml volume of the sample solution was refluxed with 5 ml of methanol and 10 ml of 2 *M* hydrochloric acid for 30 min. The reaction mixture was then diluted to 25 ml with methanol. A 10-μl aliquot of the filtered solution (Spartan 13 filters, 0.45 μm; Schleicher and Schüll, Dassel, Germany) was injected into the HPLC system.

*Isolation of kaempferol-3-O-rhamnogalactoside (**V**)*

Aliquots of 50 μl of the sample solution were chromatographated on the semi-preparative column using 2-propanol–water (14:86) at a flow-rate of 4 ml/min. Peak V was collected by means of a Gilson Model 201 fraction (Biolabo Instruments, Milan, Italy) and the combined fractions were freeze-dried. This compound was hydrolysed with 2 *M* hydrochloric acid–methanol, (1:1) at 100°C for 10 min. Galactose and rhamnose were detected by gas chromatography as acetyl derivatives [8]. Kaempferol was detected by HPLC as described under *Chromatographic conditions* for aglycones.

Mass spectrometry

Fast atom bombardament (FAB) mass spectra were obtained on a VG Analytical Model 70-70 EQ instrument, employing argon atoms with kinetic energy 7 keV. Recordings in the negative-ion mode were taken at a resolution of 3000, with a speed of 20 s per decade. Data were processed with a Digital PDP 8/A computer system.

RESULTS AND DISCUSSION

Preliminary experiments using C_{18} columns showed that a methanol–water–acetic acid mobile phase in the isocratic mode would not provide the optimum peak shape and separation of *Solidago virgaurea* flavonols. On the other hand, a short analysis time (14 min) with good peak resolution was obtained by isocratic elution on a C_8 column using 2-propanol–water (14:86) as the mobile phase (Fig. 2). Four major flavonoids were present (peaks I, IV, VI and V), while the previously reported [2] quercitrin (**II**) was present only in a trace amount and astragalin (retention time *ca.* 25 min) was absent. Peaks I, II, IV and VI were identified by co-chromatography with standards. In addition, the identity of peaks I, IV and VI was confirmed by comparing their UV spectra with those of corresponding standards. Peak II was small, and its spectrum was not significant.

The spectrum of peak V with diode-array detection presented a slope of the maximum in the short-wavelength region typical of kaempferol derivatives (Fig. 3).

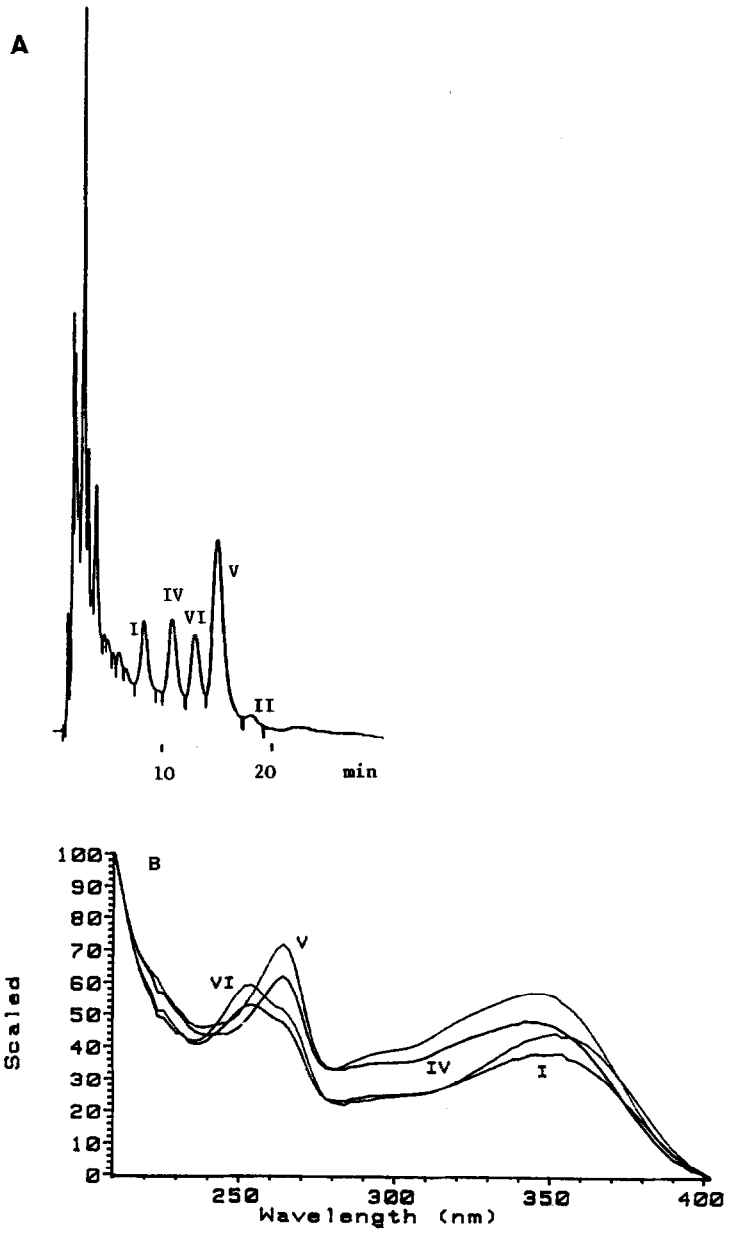

Fig. 2. (A) Typical chromatogram of a *Solidago virgaurea* extract. Peak numbers correspond to compound numbers in Fig. 1. Column, C_8 Aquapore RP 300; eluent, 2-propanol–water (14:86); flow-rate, 1.8 ml/min; UV detection at 254 nm. (B) Spectra obtained with diode-array detection. Numbers on lines correspond to compound numbers in Fig. 1.

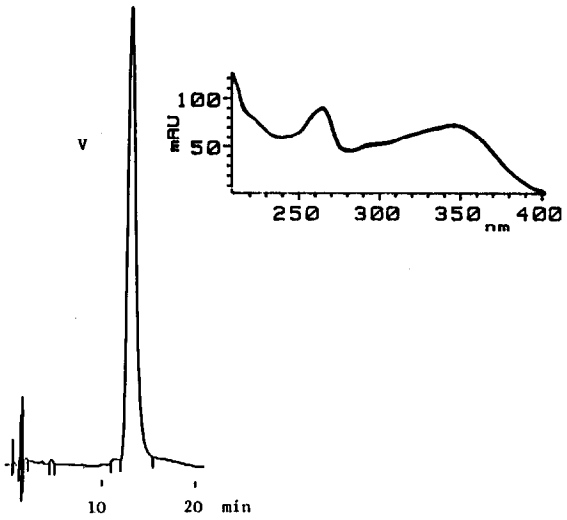

Fig. 3. HPLC and spectrum obtained with diode-array detection of compound V purified from peak V.

On acid hydrolysis of the isolated compound, kaempferol was obtained from **V**; in addition, galactose and rhamnose in the ratio 1:1 were detected. The negative-ion mass spectrum (Fig. 4) of **V** showed a deprotonated molecular ion at *m/z* 593 and an abundant fragment ion at *m/z* 285. The latter corresponds to the aglycone kaempferol originating from the molecular ion by loss of a rhamnosylgalactose residue. These data represent further positive evidence that peak V was the previously reported [4] kaempferol-3-O-rhamnogalactoside (**V**).

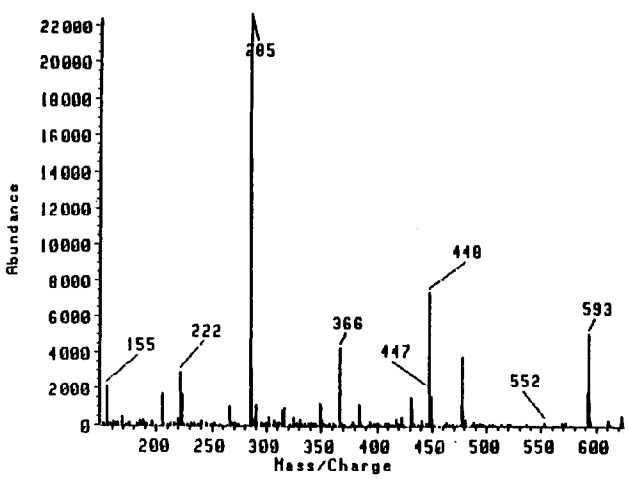

Fig. 4. Mass spectrum of V.

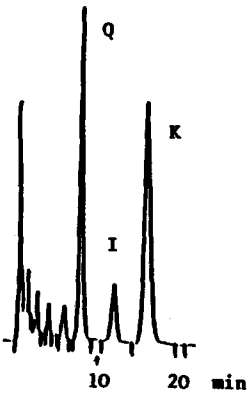

Fig. 5. Typical chromatogram of acid-hydrolysed *Solidago virgaurea* extract. Q = Quercetin; I = isorhamnetin; K = kaempferol.

During this study a rapid procedure for the determination of quercetin, isorhamnetin and kaempferol was also elaborated. *Solidago virgaurea* extract was hydrolysed under acid conditions, and the resulting aglycones were directly separated in the isocratic mode (Fig. 5). This procedure represents an improvement over the gradient approach [9], and it may be usefully applied to the determination of these widespread flavonol aglycones.

ACKNOWLEDGEMENTS

This work was financially supported by the P.S. "Innovazione Produttiva nella P&MI", CNR. The technical assistance of Annamaria Pietta is gratefully acknowledged.

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