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Note

Gas–liquid chromatographic determination of the iridoid content in *Harpagophytum procumbens* D.C.

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Several methods for the determination of the iridoid content (harpagoside, harpagide, procumbide) in *Harpagophytum procumbens* D.C. (*Pedaliaceae*) have been described. Among these, high-performance liquid chromatography (HPLC) with UV detection[1–4] appears to be very useful for the rapid and accurate determination of harpagoside in crude extracts of the drug. However, it does not allow the determination of harpagide which could be, together with harpagoside, implicated in the biological activity of the plant; moreover, the presence of great amounts of harpagide in the drug or in the extracts could indicate that an enzymatic or a chemical degradation has occurred during sample collection, extract preparation or storage.

A colorimetric method[5] can also be applied to the quantitative analysis of the crude extracts. The final results of this procedure, which concern the total iridoid content (principally, harpagoside and harpagide), are expressed as harpagoside but the linearity of the colorimetric response of harpagide with the vanillin–sulphuric reagent was not investigated.

On the other hand, gas–liquid chromatographic (GLC) analysis of a great number of iridoids (except harpagoside and harpagide) as silyl derivatives has been performed by Inouye *et al.*[6].

This paper describes a rapid GLC method for the determination of harpagide, and consequently of harpagoside, in crude extracts of *H. procumbens*.

EXPERIMENTAL

High-performance liquid chromatography

The liquid chromatograph supplied by Waters Assoc. (Milford, MA, U.S.A.) was equipped with a pump (Model 6000A), a sample loop (Model U6K), a UV detector (Model 440) operating at 254 or 280 nm and a 300 × 3.9 mm I.D. stainless-steel column pre-packed with μBondapack C_{18} (mean particle size 10 μm). The mobile phase was ethanol–water (4:6) at a flow-rate of 1 ml/min (retention time of harpagoside, 7 min).

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Gas–liquid chromatography

The Packard-Becker (Delft, The Netherlands) Model 421 gas chromatograph was equipped with a packed column (1 m × 0.125 in. I.D.) of 3% SE-30 on Chromosorb W HP (100–120 mesh).

The internal standard solution was prepared by dissolving 50 mg of cholesterol in 25 ml of anhydrous pyridine.

The reference solutions were prepared by dissolving 4, 5 and 6 mg of harpagide in 5 ml of the internal standard solution; 0.5 ml of N-trimethylsilylimidazole (TSIM, Macherey, Nagel & Co., Düren, G.F.R.) was added to 0.5 ml of each of the three reference solutions. Before injection, the mixtures were heated for 30 min in a sealed vial equipped with a Mininert® valve (Pierce, Rockford, IL, U.S.A.).

To prepare sample solutions, 20 mg of the crude dried extract (aqueous or methanolic) of the drug (corresponding to *ca.* 0.5 mg of harpagide) were dispersed in a mixture of 0.5 ml of the internal standard solution and of 0.5 ml of TSIM, then treated as described for the reference solutions.

Another amount (20 mg) of the crude extract was treated by 1 ml of an ammonia solution at 25% ($d = 0.91$) and allowed to stand at room temperature for 4 h; the solution was then evaporated at 45°C *in vacuo* and the residue silylated as described for the non-hydrolysed extract.

RESULTS AND DISCUSSION

Even as its silyl derivative, harpagoside is extensively decomposed during GLC analysis, probably because of the presence of the ester bond between cinnamic acid and harpagide. This problem can be resolved as follows. Harpagide is determined in the crude extract using cholesterol as internal standard; harpagide yields a single peak under these silylation and chromatographic conditions. The crude extract is hydrolysed under alkaline conditions in order to transform harpagoside quantitatively into harpagide. The total harpagide content is finally determined by the proposed GLC procedure (Table I, Fig. 1). In this way, the content of harpagoside and harpagide can be calculated separately without interferences: the crude extract (before or after alkaline hydrolysis) gives no peak before silylation at the retention time of the silyl

TABLE I

THE PERCENTAGES OF HARPAGOSIDE AND HARPAGIDE IN A COMMERCIALLY AVAILABLE AQUEOUS SPRAY-DRIED EXTRACT OF *H. PROCUMBENS*: A COMPARISON BETWEEN GLC, HPLC AND COLORIMETRIC METHODS

	Crude extract without treatment	*Crude extract after alkaline hydrolysis*
% total iridoids (harpagoside + harpagide) by colorimetry	2.70 ± 0.10	Irreproducible results
% harpagoside by HPLC	2.45 ± 0.03	<0.03
% harpagide by GLC	0.50 ± 0.02	2.30 ± 0.08
Calculated % harpagoside following GLC procedure	$\frac{(2.30 - 0.50) \text{ mol. wt. harpagoside}}{\text{mol. wt. harpagide}} = 2.44$	

derivative of harpagide. The comparison between HPLC and GLC methods does not show any significant difference with respect to the content in harpagoside (Table I). The results obtained with the colorimetric method could not be interpreted as absolute values. The reaction of harpagide with the vanillin–sulphuric reagent, in contrast to that of harpagoside under the same experimental conditions, gives a poor consistency; so, in comparison with the chromatographic methods, the results are systematically too low (Table I).

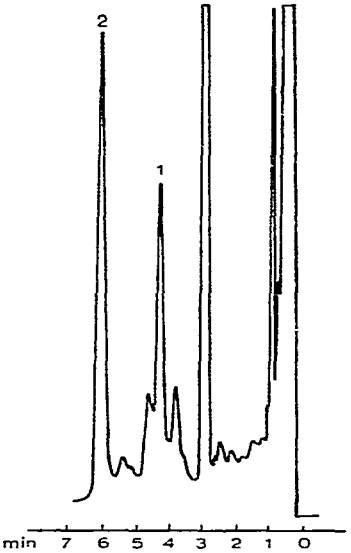

Fig. 1. Gas–liquid chromatogram of a silylated aqueous spray-dried extract of *H. procumbens* after alkaline hydrolysis. Conditions: detector and injector temperature, 280°C; oven temperature (isothermal), 250°C; nitrogen flow-rate, 30 ml/min. Peaks: 1 = harpagide (silyl derivative); 2 = cholesterol (silyl derivative) as internal standard.

For these reasons, GLC seems to be the only reliable method for the separate determination of harpagide and harpagoside in *H. procumbens* and in other drugs which contain the same iridoids.

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