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Note

Analysis of mimosine and 3-hydroxy-4(1H)-pyridone by high-performance liquid chromatography

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Mimosine, β-[N-(3-hydroxy-4-oxopyridyl)]-α-aminopropionic acid, is a non-protein amino acid that occurs in the tropical plants, *Mimosa pudica* and more importantly, in *Leucaena leucocephala*. Its presence in *Leucaena* has prevented the widespread use of this legume for intensive animal feeding as mimosine induces the depilatory and other toxic effects in ruminants and monogastric animals[1,2]. 3-Hydroxy-4(1H)-pyridone (DHP), a metabolite of mimosine in both plants[3] and animals[4], has also been associated with the development of various abnormal growth or metabolic effects in ruminants[5,6].

A range of methods has been developed for the analysis of mimosine and/or DHP utilising ion-exchange and paper chromatography[7], gas chromatography[8], an amino acid analyser[9], and colorimetry with an auto-analyser[10]. All these methods are unsatisfactory for use as a standard routine method as they are either specific for only mimosine or DHP, are not suitable for analysis of both plant and animal extracts, are tedious and time-consuming, or are subject to variable losses during analysis. This paper describes a sensitive and simple method for the simultaneous analysis of mimosine and DHP in plant material and urine by high-performance liquid chromatography (HPLC).

METHODS AND RESULTS

HPLC analyses were performed on a μBondapak C_{18} column in a Waters liquid chromatograph (Model No. ALC/GPC 244) using a single wavelength UV (280 nm) absorbance detector. Rapid elution and good separation of mimosine and DHP in standard solutions was obtained using a solvent system of 0.2% (w/v) orthophosphoric acid in double distilled water at a flow-rate of 1 ml/min (Fig. 1a). There was a linear response of both peak height and peak area to concentration of mimosine and DHP with the limits of detection being 1 ng mimosine and 2 ng DHP.

Leaf samples of *Leucaena* were prepared for analysis by initially holding the leaf at 20°C for 24 h, to ensure the production of some DHP[3], and then freeze dried. Dried leaf (25 mg) was ground in a mortar with 0.1 *N* hydrochloric acid (10 ml) to extract mimosine and DHP[7], the mixture was then centrifuged for 10 min at 7500 *g* and the supernatant was filtered under nitrogen (60 p.s.i.) through a membrane ultrafilter. Analysis of the leaf extract (10-μl aliquot) showed that sharp resolution of mimosine and DHP was retained and there were no major components in the extract that interfered with the analysis (Fig. 1b).

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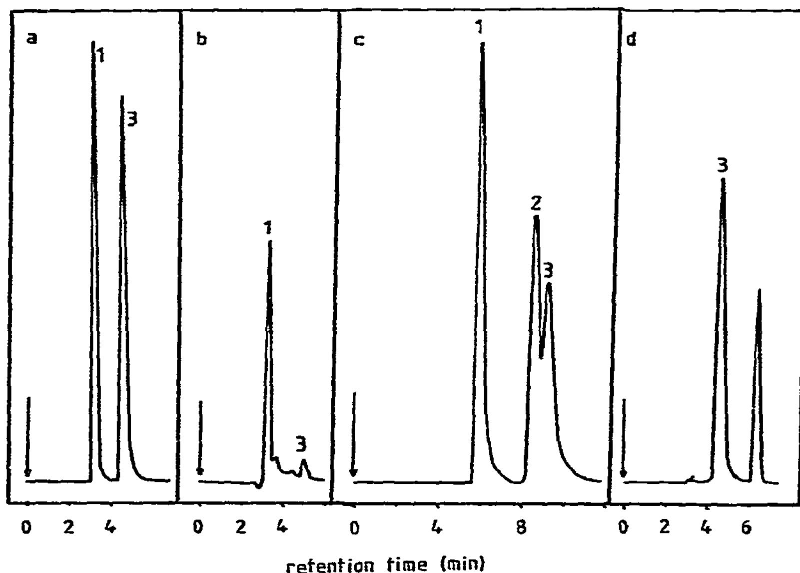

Fig. 1. Chromatograms of the separation of mimosine and DHP. (a): Standards; (b): extract from *Leucaena* leaf; (c): standards at reduced flow-rate (0.5 ml/min); (d): hydrolysed urine. Sample composition: 1 = mimosine; 2 = DHP-glucoside; 3 = DHP.

Urine obtained from ruminants that have been fed *Leucaena* can contain mimosine, DHP and DHP-glucoside[4]. DHP and DHP-glucoside in a standard solution were partially resolved when the solvent flow was reduced to 0.5 ml/min (Fig. 1c). However for most studies only a total estimate of DHP is required and a quantitative conversion of glucoside to DHP can be achieved by acid hydrolysis[7]. Fresh urine was mixed with an equal volume of 10 *N* hydrochloric acid and heated at 110°C for 4 h, the pH was adjusted to pH 3 with sodium hydroxide, the solution filtered and made up to volume. Analysis of the urine extract (10-μl aliquot) showed that DHP gave a sharp peak with only a small amount of mimosine present and no DHP-glucoside (Fig. 1d). There were no major interfering compounds in the extract.

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