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Note

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

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Leucaena leucocephala is a tropical legume that is used in many parts of the world for animal feeding. However, extensive use of leucaena is limited by the presence of the free amino acid mimosine {β-[N-(3-hydroxy-4-oxopyridyl)]-α-aminopropionic acid} and its metabolite 3-hydroxy-4(1*H*)-pyridone (DHP), with have been associated with the development of a number of abnormal growth and metabolic effects in ruminants[1,2]. Studies on the metabolism of mimosine have been hampered by the lack of a rapid method of analysis but recently the use of high-performance liquid chromatography (HPLC) has been shown to be a rapid and sensitive method for analysing both mimosine and DHP in leucaena leaf extracts and rumen urine[3,4]. Metabolic studies have also been carried out using plasma and this paper reports on the additional purification steps that need to be carried out to permit the analysis of mimosine and DHP in plasma by HPLC.

EXPERIMENTAL AND RESULTS

Blood was collected from the jugular vein of sheep that had been fed leucaena into a vacuum tube containing sodium heparin as an anticoagulant. The blood was centrifuged at 3000 *g* for 15 min and the plasma collected by decantation. It was necessary to deproteinize the plasma to prevent degradation of the HPLC column and this was carried out using 5-sulphosalicylic acid[5]. However, analysis of deproteinized plasma on a μBondapak C_{18} column using a solvent system of 0.2% (w/v) orthophosphoric acid and an absorbance detector (280 nm)[3] showed that while mimosine was eluted as a sharp peak, any DHP present was obscured by the large sulphosalicylic acid peak (Fig. 1).

A method for removing the sulphosalicylic acid was developed using small column chromatography. A cation-exchange resin (Dowex AG 50W-X4, 200–400 mesh, in the acid form) was packed into the plastic delivery tip of an Oxford Macro set dispenser pipette (Lancer Brunswick, St. Louis, MO, U.S.A.) to a height of 2.5 cm. Deproteinized plasma (1 ml) was placed on the column, which was washed with deionized water (8 ml) to elute the sulphosalicylic acid. The elution rate of the solvents was increased by applying air pressure to the column by pressing the pipette

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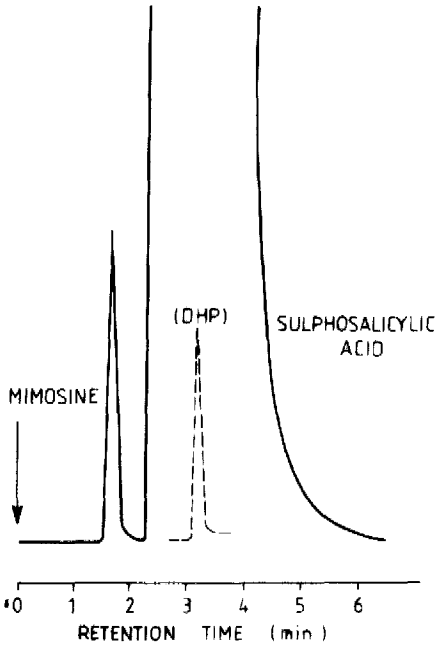

Fig. 1. Chromatogram of plasma from sheep that had been fed leucaena, after deproteinization with sulphosalicylic acid.

plunger. Mimosine and DHP were both eluted with 2 *N* sodium hydroxide solution (8 ml). The eluate was evaporated to dryness using a rotary evaporator, diluted with water (10 ml) and an aliquot (10 μl) injected on to the HPLC column for analysis. Complete and unhindered resolution of mimosine and DHP into sharp peaks was obtained, the level of detection being at the nanogram level. Recovery studies were conducted by adding mimosine and DHP at levels from 0 to 100 μg to deproteinized goat plasma (1 ml). Mimosine was recovered at about 95% and DHP at about 100% of that added; the concentration had no effect on the recovery.

Possible interferences in the analysis due to other free amino acids that absorb in the UV region were examined, as tyrosine and phenylalanine have been reported in the plasma of humans[6]. Tyrosine and tryptophan were eluted after mimosine and DHP and phenylalanine was not detected at 280 nm; hence they do not interfere.

The use of a small amount of cation-exchange resin was therefore found to be effective in purifying plasma of goat and sheep and the use of a pipette dispenser enabled the purification procedure to be rapid. The system would lend itself for preparative-scale purification by increasing the volume of resin.

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