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Note

Estimation of mimosine in ovine plasma

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The need for an accurate, sensitive and rapid method for the estimation of mimosine, β-[N-(3-hydroxy-4-oxopyridyl)]-α-aminopropionic acid, arose from studies in this laboratory[1] on the depilatory effect of mimosine on sheep.

This paper describes two procedures for measuring the concentration of mimosine in sheep plasma by ion-exchange chromatography, using a Technicon TSM amino acid analyser. The first, a four-buffer system, analyses all acidic and neutral amino acids, including mimosine. The second, faster procedure resolves only isoleucine, leucine, mimosine and tyrosine in plasma.

MATERIALS AND METHODS

Hydrazine sulphate was obtained from BDH (Poole, Great Britain). Other reagents were from Pierce (Rockford, Ill., U.S.A.). An analytically pure sample of L-mimosine[1] was provided by Mr. D. A. Tunks of this Division.

A TSM amino acid analyser with type C-3 (8% cross-linked) cation-exchange resin (Technicon, Tarrytown, N.Y., U.S.A.) was used in these studies. Details of the buffer compositions are shown in Table I.

Plasma samples were deproteinized with sulphosalicylic acid[2] and mimosine was added to provide 0.05, 0.075, 0.10 and 0.125 μmoles per 100 μl of plasma. Nor-

TABLE I

COMPOSITION AND pH OF THE LITHIUM CITRATE BUFFERS

Buffer	*Li conc. (N)*	*Citrate conc. (M)*	*Methyl cellosolve (%, v/v)*	*Thiodiglycol (%, v/v)*	*pH (± 0.01)*
System A					
1	0.30	0.50	4.0	0.1	2.75
2	0.29	0.48	4.5	0.1	2.60
3	0.30	0.50	0	0.1	3.35
4	0.29	0.48	0	0.1	4.30
System B					
1	0.30	0.50	1.0	0.1	3.25
2	0.28	0.47	1.5	0.1	3.95

leucine was used as an internal standard at a level of 0.05 μmoles per 100 μl of plasma.

In system A, a single column (41 × 0.5 cm I.D.) and four buffers were used with buffer changes after 34, 94, and 124 min. A column temperature of 42° and a flow-rate of 25.8 ml/h were maintained throughout the analysis.

System B involved only two buffers with a buffer change after 8 min. Other operating conditions were as in system A.

RESULTS AND DISCUSSION

Mimosine was eluted immediately before tyrosine, with a norleucine equiva-

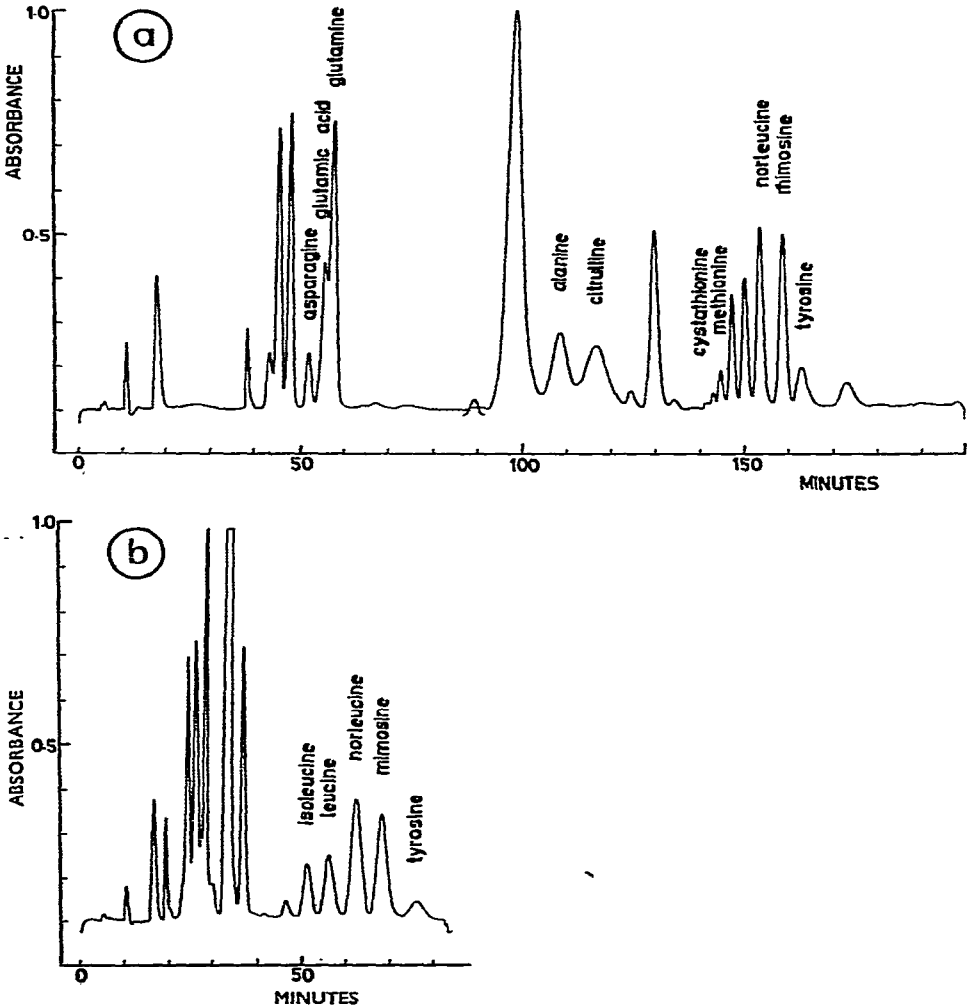

Fig. 1. Chromatograms of 0.25 ml deproteinized sheep blood plasma, containing 0.05 μmoles of mimosine. (a) determination of mimosine and all acidic and neutral amino acids, using buffer system A; (b) determination of mimosine, isoleucine, leucine, norleucine and tyrosine only, using buffer system B.

lent* of 1.06 (standard error of the mean from 12 samples, ±0.02). Recovery of mimosine added to sheep plasma (triplicate samples in the range of 0.05 to 0.125 μmoles) with both systems was 100.3 (±1.6%), using the peak height method[3,4] for quantitation. With either system mimosine concentrations in the range of 0.005–0.15 μmoles per 100 μl can be analysed.

The absorbance properties of mimosine enabled it to be distinguished easily from tyrosine and norleucine. The ratio of absorbance at 570 nm to that at 440 nm was 4.65 ± 0.25 for mimosine whereas the ratios for norleucine and tyrosine were 7.00 and 6.25, respectively.

Elution of mimosine is very sensitive to both pH and methyl cellosolve concentration and these factors were used to alter the elution time. The rate of elution was accelerated with increased pH and ionic strength but retarded by high concentrations of methyl cellosolve.

System A allowed a complete analysis of acidic and neutral plasma amino acids to be performed without affecting resolution in the areas asparagine–glutamic acid–glutamine, alanine–citrulline and cystathionine–methionine (Fig. 1a). Initial attempts to separate mimosine from tyrosine indicated an optimal pH for the fourth buffer of 4.30 but this disturbed the separation of other amino acids. The resolution of mimosine and the cystathionine–methionine separation were improved by decreasing the ionic strength, the flow-rate and the column temperature. The relatively low temperature (42°) adversely affected the resolution of glutamic acid and glutamine, but this was rectified by increasing the pH of the first buffer from 2.7 to 2.75. In this system all compounds were analysed within 203 min, and mimosine was eluted after 158 min.

System B, designed for the rapid determination of mimosine (68 min), gave complete resolution of isoleucine, leucine, norleucine, mimosine and tyrosine (Fig. 1b). In this system the volume of equilibrating buffer was decreased by one third to shorten the elution times for the first amino acids. This was preferable to increasing the pH of the equilibrating buffer which would affect mimosine separation.

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* Norleucine equivalent is defined as the ratio of norleucine peak area to mimosine peak area with isomolar quantities of both amino acids.