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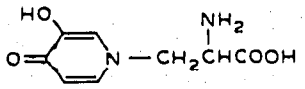
Gas–liquid chromatography of mimosine*

Mimosine (I), also known as leucenol, is a naturally occurring free amino acid found in tropical leguminous plants, *e.g. Leucaena leucocephala* (Lam.) de Wit,



(I)

L. glauca Benth., and *Mimosa pudica* L. The structural formula is 3-hydroxy-4-oxo-1(4H)-pyridinealanine. As a tyrosine analogue, this amino acid is of interest because of its toxic effect on non-ruminants[1,2]. DEWREEDE AND WAYMAN[1] were also concerned about the use of seeds and succulent tips of these plants for human food in Southeast Asia.

In the course of studies on the availability of protein amino acids by gas–liquid chromatography (GLC), it was found that mimosine appears at the shortest retention time which is distinct from commonly known amino acids when assayed as their N-trifluoracetyl (N-TFA) *n*-butyl ester by GLC on an EGA column. The purpose of this report is to show that mimosine can be analyzed by GLC as a non-protein amino acid in a unique separation of the same column along with the protein amino acid.

Experimental

Mimosine was extracted from finely ground dry *L. glauca* with cold 0.1 *N* HCl. After filtration, an aliquot of 0.1 ml (equivalent to 5 mg dry material) was introduced directly into a cone-shaped micro-vial (0.5 ml volume) without clean-up. The derivatization procedure was (1) direct esterification and (2) acylation according to ROACH AND GEHRKE[3]. An MT-220, four-column oven, equipped with Coulson electrolytic conductivity detector, was used in this study. A 6 in. × 1/4 in. I.D. U-glass column packed with 0.325 w/w% EGA on 80–100 mesh a.w. HT Chromosorb G (preheated

TABLE I

GLC (N DETECTION) CONDITIONS FOR MIMOSINE AND AMINO ACIDS SEPARATION

Column liquid phase	0.325 w/w% EGA
Column solid support	80/100 mesh a.w. HT Chromosorb G (preheated at 140° for 12 h)
Helium flow, ml/min	
Carrier	60
Pyrolyzer	10
Hydrogen flow, ml/min	
Pyrolyzer	50
Pyrolyzer temp., °C	820
Initial temp., °C	80
Programmed temp., °C/min	5
Final temp., °C	230
Inlet temp., °C	220
Chart speed, in./min	0.5

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at 140° for 12 h) was used for the separation of amino acids. DL-α-Aminocaprylic acid was used as internal standard.

Various parameters used in GLC operation in this study are outlined in Table I.

Results and discussion

The elution times and temperatures for the various amino acids studied on the EGA column are presented in Fig. 1. Among the protein amino acids (Fig. 1A) alanine always elutes as the first peak at 127° after 9 min. Tryptophan usually shows 30 min later as the last peak at 225°. In acid hydrolysate (6 *N* HCl), however, tryptophan is destroyed and lysine becomes the final peak at 220°. CASAGRANDE[4] recently reported that a complex mixture of 35 amino acids including some novel bacterial non-protein amino acids can be analyzed by GLC on a similar EGA column. These acids were all chromatographed after alanine.

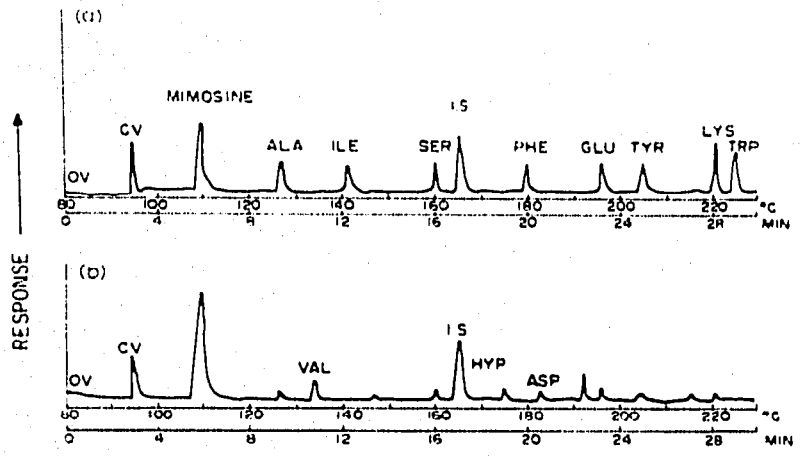

Fig. 1. Separation of mimosine and amino acid N-TFA *n*-butyl esters. Column: 0.325 w/w %, EGA on 80/100 mesh a.w. HT Chromosorb G, 6 in. × $\frac{1}{8}$ in. I. D. U-glass; initial temp., 80°, program, 5°/min. (a) Mimosine and amino acid mixture; the injected mixture contained *ca.* 0.2–0.4 μg of each acid. (b) *L. glauca* extract; the injected mixture contained 25 μg of sample. I.S. was α-aminocaprylic acid. OV = open vent, CV = close vent.

Mimosine so far is the first non-protein amino acid N-trifluoroacetyl *n*-butyl ester found with shorter retention than that of alanine on EGA. It elutes at 110° for 6 min (or 90° at 0.5 min isothermally) indicating presumably a di-acyl derivative with markedly increased volatility. If excess mimosine (more than 100 g) was present in the reaction micro-vial, a monoacyl product was formed also giving a relatively smaller peak at 176° after 19 min. Under these conditions, it would superimpose on the proline peak (Fig. 1).

The diacyl derivative is of interest because the 3-hydroxy group on pyridone-4 ring is acetylated in addition to trifluoroacetylated amino, imino, hydroxy, phenolic, sulfhydryl, guanido and imidazole groups reported. The diacyl tyrosine ester (TFA on amino and phenolic groups), however, elutes at 205°/25 min under the same conditions.

The stability of mimosine to hot, concentrated acid is also of interest to this study because mimosine is subjected to heat in the course of initial drying of aliquot extract in 0.1 *N* HCl medium. The ready hydrolysis of mimosine to dihydroxypyridine and alanine by boiling 0.1 *N* HCl reported by HEGARTY[4], however, has not been ob-

served in any of our studies. This is due to the fact that no alanine residue was indicated by nitrogen detector (Fig. 1A).

A quantitative GLC analysis of mimosine can be readily achieved by directly adding a known amount of an internal standard (I.S.) to the extract prior to derivatization. This, in turn, can be calibrated against a mixture of mimosine standard and internal standard run according to GEHRKE *et al.*[6]. An internal standard permits correction for volatization of solvent, dilution of final derivative mixture, and losses during sample manipulation.

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Department of Animal Sciences, University of Hawaii, Honolulu, Hawaii 96822 (U.S.A.)

JOHN M. L. MEE
COY C. BROOKS

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