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Mimoside: A Glucosidic Metabolite of Mimosine in *Mimosa pudica* and *Leucaena leucocephala*

Mimosine is found in large quantities in the seeds and shoots of the legume species *Mimosa pudica*[1] and *Leucaena leucocephala*[2]; it shows various physiological activities, *i.e.* growth retardation, alopecia, cataract and infertility, in experimental animal,[3–10] and also inhibits the growth of *Escherichia coli*[11] and mung bean seedlings(*Phaseolus aureus*).[12]

Hylin[13] and Notation, Tiwari, Penrose and Spenser[14] studies the biosynthesis of mimosine in *Mimosa pudica* and *Leucaena leucocephala* by ^{14}C-labelled precursor techniques: the pyridone ring and the side chain of mimosine could originate from lysine and serine, respectively.

The enzymic synthesis of mimosine from 3-hydroxy-4-pyridone (3,4-dihydroxypyridine) and O-acetylserine using an extract of *Leucaena* seedlings was reported by Murakoshi, Kuramoto, Haginiwa and Fowden[15]; neither serine nor O-phosphoserine could serve as a direct substrate in the enzymic reaction.

In continuing our study of mimosine metabolism in plants, we now report that the amino acid is rapidly converted into mimosine-O-glucoside(I), which we have named mimoside.

When *Mimosa pudica* plants were supplied ^{14}C-mimosine (labelled either universally or specifically in the alanyl side chain) *via* a cotton wick inserted through the stem, mimoside (I) represented the major metabolic product. I is normally a minor component of *Mimosa pudica* plants in the period before flowering.

Fig. 1 shows the distribution of ^{14}C in the amino acid fraction of the upper part (stem and attached leaves) of two-month-old *Mimosa pudica* at different times after feeding label-

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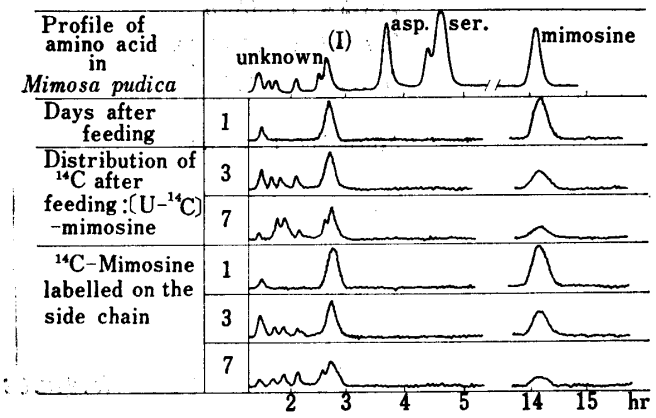


Fig. 1. Flow Diagram to Illustrate the Distribution of ^{14}C in the Amion Acid Fraction of *Mimosa pudica*[a] at Various Times after Feeding [U-^{14}C]-mimosine and ^{14}C-Mimosine labelled only on the Side Chain

a) standard operating condition: 150 cm column, 50°, 0.2N sodium citrate buffer, pH 3.25 followed by 4.25, flow rate 0.5 ml/min. Mimoside (I) was eluted in the area between S-methylcysteine-sulfoxide and methioninesulfoxide under this condition.

led mimosine: the illustration shows the profile of amino acids eluted from an automatic analyzer (Shibata model AA-500, Tokyo) coupled to a Packard flow monitor system (model 3002) and ratemeter (model 282 A).

Three days after feeding U-^{14}C-mimosine, 85% of radioactivity still present in the plant (*i.e.* 59% of that administered) was in the amino acid fraction of the upper stem and leaves: at this time, radioactivity associated with organic acids (oxalic, citric, malic, succinic and glutaric acid), sugars (glucose, fructose and two unknowns), and the insoluble residue fraction were 1.1, 5.9, and 6.2%, respectively. The roots contained only 1.6% of the total activity.

A similar conversion of mimosine into the ralated glucoside(I) was demonstrated in *Leucaena leucocephala* plants.

The concentration of I in dry *Leucaena* seed is very low, but its concentration increased rapidly during the first 3—5 days growth of seedlings at 30° in the dark. In the later stages of the plant's growth, the concentration of I diminished and it again became a minor component. Therefore I was isolated from 4 or 5-day-old seedlings of *Leucaena leucocephala* by a Dowex 50W (×4) resin column using 0.2M ammonium formate buffer, pH 2.4, followed on paper, using butan-1-ol-acetic acid-water (3,1,1, by vol.), in 0.013% yield. The isolated compound was identified as a glucoside of mimosine from the profile on an automatic amino acid analyzer.

I shows an absorption peak at 277 nm (ε 11400) in water; mimosine absorbed maximally at 284 nm (ε 15000) in water. I has mp 178—179°, $[\alpha]_D^{25}$ −60.9 (c=0.46, water); it is very soluble in water, gives a fluorescent spot with the aniline-phthalate reagent for sugars (a general characteristic of glucosides), and a violet spot with ninhydrin on paper. I did not give an immediate colour with 0.1% ferric chloride in 0.1N hydrochloric acid, but a violet colour gradually developed during 25—30 hr at room temperature. This behaviour suggested that slow hydrolysis occurred in the presence of dil. hydrochloric acid to yield mimosine as described below.

I was hydrolyzed by 5% (w/v) hydrochloric acid (95—100°, 1 hr) to give mimosine and glucose in quantitative yield. Mimosine was detected by paper chromatographic procedures, using ninhydrin or ferric chloride as chromogenic reagents, as described in previous report.[15]

Further confirmation of the identity of the aglycone as mimosine was obtained using an automatic amino acid analyzer: mimosine eluted from the column at a position close to an isoleucine reference peak under standard operating conditions (Fig. 1).

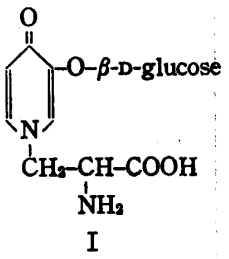
Glucose present in the hydrolysate was similarly identified by co-chromatography with authentic material; it was detected as a brown spot after spraying with aniline-phthalate and ammoniacal silver nitrate reagents. The following solvent systems were employed: 1, butan-1-ol-acetic acid-water (90,10,29, by vol.); 2, ethyl acetate-water-pyridine (4,4,2, by vol., upper phase); 3, ethyl acetate-water-pyridine (10,3,4, by vol., upper phase); 4, benzene-butan-1-ol-pyridine-water (1,5,3,3, by vol., upper phase). *Rf* values determined for glucose in these solvents were 0.13, 0.25, 0.18, and 0.17, respectively.

For further confirmation of the glycone moiety as glucose, a specific β-D-glucose oxidase (Worthington) was employed. When the enzymic system was coupled to O-dianisidine as a chromogenic reagent (colour maximum at 530 nm[16]), colour production was theoretical for glucose. Quantitative analysis showed that glucose was conjugated with mimosine in equimolar amounts.

Conjugation was assumed to involve the C-1 of glucose, because I (mimoside) lacked reducing properties, and the C-3 hydroxy group of mimosine, since mimoside (I) gave no immediate colour with 0.1% ferric chloride.

The glucoside (I) also could be degraded to mimosine and glucose (30% yield in 1 hr at 30°) by the β-D-glucosidase of sweet almonds (Miles).

Therefore the structure of mimoside (I) was considered to be 2-N-(3-β-D-glucosyloxy-4-pyridone)-1-aminopropionic acid (formula I).



I

Mimoside (I) was synthesized by an extract of *Leucaena* seedlings from mimosine and uridine diphosphate-glucose, and also conversely was degraded into mimosine and D-glucose by the hydrolytic enzyme in *Leucaena* seedlings. Those results will be reported in the following paper.

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