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BIOSYNTHESIS OF MIMOSINE*

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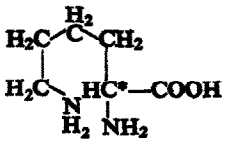
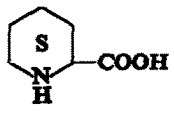
Abstract—Administration of [2-^{14}C]-DL-lysine to *Leucaena glauca* results in incorporation of radioactivity into mimosine, pipecolic acid and 5-hydroxypipecolic acid. Degradation of the mimosine reveals that 95% of the radioactivity is present in the pyridone ring.

INTRODUCTION

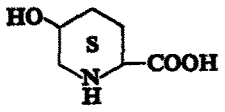
THE TROPICAL legume *Leucaena glauca* Benth. has been found to contain relatively large amounts of three piperidine compounds namely pipecolic acid (II), 5-hydroxypipecolic acid (III),[1] and mimosine (IV).[2] These compounds occur free and may be readily extracted from the leaves with alcohol, water, dilute acid or alkali. As part of a study on the biochemistry of mimosine, its biosynthesis in *L. glauca* was investigated.



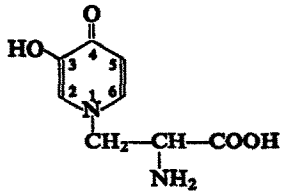
(I)
Lysine



(II)
Pipecolic Acid



(III)
5-Hydroxypipecolic Acid



(IV)
Mimosine

In view of the simultaneous occurrence of three similar compounds and the known biosynthetic pathway leading to pipecolic acid[3] it was felt that ^{14}C lysine would be the most profitable compound to use in feeding experiments.

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[1] M. P. HEGARTY, *Australian J. Chem.* **10**, 484 (1957).
[2] M. MASCRE, *Compt. rend.* **204**, 890 (1937).
[3] P. H. LOWY, *Arch. Biochem. Biophys.* **47**, 228 (1952).

RESULTS AND DISCUSSION

Radioactive mimosine, pipecolic acid, and 5-hydroxypipecolic acid have been isolated from *Leucaena glauca* following administration of [2-^{14}C]-lysine monohydrochloride. The distribution of radioactivity in these compounds isolated at various times after administration of isotope is shown in Table 1. The high specific activity of pipecolic acid confirms the results

TABLE 1. DISTRIBUTION OF RADIOACTIVITY AT VARIOUS TIMES AFTER FEEDING [2-^{14}C]-DL-LYSINE TO *Leucaena glauca*

Compound	Radioactivity found (dpm/μM)		
Days after feeding	1	3	7
Lysine	783	344	75
Pipecolic acid	680	515	255
5-OH pipecolic acid	131	296	335
Mimosine	188	315	283

already cited.[3] The rapid formation of radioactive mimosine indicates that lysine is an important precursor of this compound. The relatively high radiochemical yield (*ca.* 3·6%) shown in Table 2 supports this conclusion. The incorporation of radioactivity into 5-hydroxypipecolic acid would indicate that lysine is a significant precursor of this compound also. Lindstedt and Lindstedt[4] have demonstrated the formation of 5-hydroxypipecolic acid from

TABLE 2. INCORPORATION OF RADIOACTIVITY INTO MIMOSINE SEVEN DAYS AFTER FEEDING [2-^{14}C]-DL-LYSINE TO TWO *Leucaena glauca* PLANTS

	Radioactivity (dpm × 10^{-4})	
[2-^{14}C]-Lysine administered	240	240
0·1 N HCl leaf extract	120	90
C^{14} in mimosine*	9·1	8·5
% Incorporation	3·8	3·5

* Calculated from specific activity of the isolated mimosine and the quantity found present in the original plant extract.

5-hydroxylysine in a mammalian system and whether a similar system is present in plants is not known. To date, 5-hydroxylysine has not been observed in *L. glauca*.

The radioactivity present in mimosine is almost exclusively localized in the pyridone ring. This was shown by pyrolysis of a radioactive sample using the method of Adams *et al.*[5] The isolated 3,4-dihydroxypyridine contained 95% of the activity present in the mimosine.

[4] S. LINDSTEDT and G. LINDSTEDT, *Arch. Biochem. Biophys.* **85**, 565 (1959).
[5] R. ADAMS, S. J. CRISTOL, A. A. ANDERSON and A. A. ALBERT, *J. Am. Chem. Soc.* **67**, 89 (1945).

EXPERIMENTAL

Materials

DL-Lysine and DL-pipecolic acid were commercial products. Mimosine was isolated from *Leucaena glauca* seed.[6] DL-5-Hydroxypipecolic acid was prepared as described by Hegarty.[1] [2-^{14}C]-DL-Lysine monohydrochloride was a commercial product.

Assays

Mimosine was determined by the method of Matsumoto and Sherman.[7] Lysine, pipecolic acid and 5-hydroxypipecolic acid were assayed by the modified ninhydrin method of Moore and Stein.[8] Radioactivity measurements were made with a gas flow geiger counter with a micro-mil window and having an efficiency of 27%.

Feeding Experiments with Radioactivity Lysine

Leucaena glauca Benth. was grown from seed in pots containing soil and the plants were 24 weeks old when used. [2-^{14}C]-Lysine monohydrochloride (0·3 ml, 465 μg, $2{\cdot}4 \times 10^6$ dpm) was administered to each of four plants in the following manner. The leaflets and petiolules of a newly matured leaf were removed, the petiolule tip cut and immediately inserted into a glass tube (0·3 mm × 30 mm) containing the solution to be fed. The glass tube was taped to the end of a glass rod inserted into the potting medium. This permitted adjustment of the height of the tube to minimize strain on the bent petiolule. The plant was illuminated by one 40-W and one 32-W circline fluorescent lamps closely encircling the leaves. The radioactive solution was absorbed in ½ to 1½ hr and was followed by the administration of three 0·3-ml portions of distilled water to wash in residual radioactive lysine. When the water had been absorbed, the plant was returned to the greenhouse.

Isolation and Purification of Compounds

One plant was studied after 24 hr, another after three days and the remaining two plants were examined seven days after administration of the radioactive solution. In each case the leaves were removed, dried, ground, weighed and extracted.[7] An aliquot of the extract was assayed for mimosine content and the remainder was concentrated to dryness. The residue was suspended in a small volume of 80% ethanol–1% conc. HCl. Mimosine, lysine, pipecolic acid and 5-hydroxypipecolic acid were separated and purified by paper chromatography. The ethanolic solution was applied as a streak to sheets of Whatman 3 mm paper and subjected to descending chromatography using *n*-butanol, *n*-propanol, water, diethylamine (10:10:5:2) as the developing solvent. The bands containing the compounds of interest were detected with the aid of guide strips containing the pure compounds. Mimosine was located by spraying the paper with 0·5% $FeCl_3$ in 0·1 N HCl and the amino acids were detected with ninhydrin. The bands thus located were cut out and eluted with 80% ethanol–1% HCl. The process of chromatographic separation and elution was repeated three more times using the following sequence of solvents: *n*-butanol, glacial acetic acid, water (4:1:1); *n*-butanol, pyridine, water (6:4:3); *n*-butanol, *n*-propanol, water, diethylamine. The eluates from this fourth separation were made to volume and assayed for their respective components and radioactivity. The specific activities of the compounds were the same after the third and fourth chromatographic separations.

[6] D. Kostermans, *Rec. trav. chim.* **66**, 93 (1947).
[7] H. Matsumoto and G. D. Sherman, *Arch. Biochem. Biophys.* **33**, 195 (1951).
[8] S. Moore and W. H. Stein, *J. Biol. Chem.* **211**, 907 (1954).

Pyrolysis of Mimosine

The residual mimosine eluates (*ca.* 10 mg mimosine) from the seven-day experiments were combined and a solution containing 90 mg mimosine was added. The mixture was concentrated until crystals appeared. After standing overnight at 4° the separated mimosine was isolated. The compound was recrystallized to a constant specific activity of 283 dpm/μmole, after correction for carrier dilution. Fifty mg of the mimosine were pyrolyzed[5] to 3,4-dihydroxypyridine. The purified product melted at 239–240° (undepressed when mixed with known 3,4-dihydroxypyridine) and had a specific activity of 270 dpm/μmole after correction for carrier dilution.

Note added in press: It has come to the author's notice that *L. glauca* Benth. is now *L. leucocephala* (Lam.) de Wit.