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SHORT COMMUNICATION

BIOSYNTHESIS OF MIMOSINE: INCORPORATION OF SERINE AND OF α-AMINOADIPIC ACID

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Abstract—Serine serves as a precursor of the alanyl side-chain of mimosine. Activity from α-aminoadipic acid is incorporated into the γ-pyridone nucleus. Pipecolic acid and 5-hydroxypipecolic acid occur in *Mimosa pudica*.

THE γ-pyridone nucleus of mimosine (I) is derived from lysine.[1,2] The origin of the alanyl side-chain of (I) has not been established. We now present evidence which indicates that this side-chain is derived from serine.

Labelled mimosine, isolated[1] from the sap of *Mimosa pudica* L. plants to which DL-[1-^{14}C]-serine had been administered (Table 1) by infusion through a cotton wick,[3] was diluted with

TABLE 1. ^{14}C-LABELLED COMPOUNDS ADMINISTERED TO *Mimosa pudica* L.

Precursor	Weight (mg)	Specific activity (counts min^{-1} mmole^{-1} × 10^{-9})	Mimosine: Yield (mg)	Mimosine: Specific activity (counts min^{-1} mmole^{-1} × 10^{-5})
DL-[1-^{14}C]-Serine[a]	2·1	2·70 ± 0·05	15·0	0·89 ± 0·05
DL-[2-^{14}C]-Aspartic acid[b]	7·8	0·94 ± 0·02	11·5	0·93 ± 0·03
DL-[4-^{14}C]-Aspartic acid[a]	3·9	1·96 ± 0·04	14·0	0·49 ± 0·02
DL-[6-^{14}C]-α-Aminoadipic acid[c]	3·7	1·94 ± 0·03	10·5	0·46 ± 0·01
DL-[6-^{14}C]-δ-Hydroxylysine[a]	4·7	3·34 ± 0·05	12·5	inactive

[a] New England Nuclear Corp.
[b] Daiichi Pure Chemicals, Tokyo.
[c] Calbiochem.

carrier and degraded, by the reaction sequences shown in Fig. 1, to establish the distribution of activity (Table 2). The pyridone nucleus, isolated as 3-hydroxy-4-pyridone (IV),[3] contained 29 per cent of the activity of the intact mimosine. Since the nucleus has been shown to be derived from lysine,[1,2] the presence of label, derived from [1-^{14}C]-serine, in the pyridone ring serves as an index of the random scatter of activity over individual carbon atoms of the molecule (~6 per cent/carbon atom) due to *de novo* incorporation after metabolic breakdown of

[1] H. P. TIWARI and I. D. SPENSER, *Can. J. Biochem.* **43**, 1687 (1965).
[2] J. W. HYLIN, *Phytochem.* **3**, 161 (1964).
[3] A. D. NOTATION and I. D. SPENSER, *Can. J. Biochem.* **42**, 1803 (1964).

serine (presumably by conversion to pyruvate, followed by decarboxylation). The major part of the label was recovered in the mimosine side-chain, which was isolated as α,β-diaminopropionic acid (II).[4] The carboxyl group of this degradation product contained 80 per cent of its total activity (II minus III) (Table 2). This non-random incorporation of label from the carboxyl group of serine into the carboxyl group of mimosine indicates that the alanyl side-chain of mimosine is derived from serine and suggests that mimosine is a further example of a plant amino acid which, like β-cyanoalanine[5] and orcylalanine,[6] arises by nucleophilic substitution at the β-carbon of serine or, more probably, of phosphoserine.

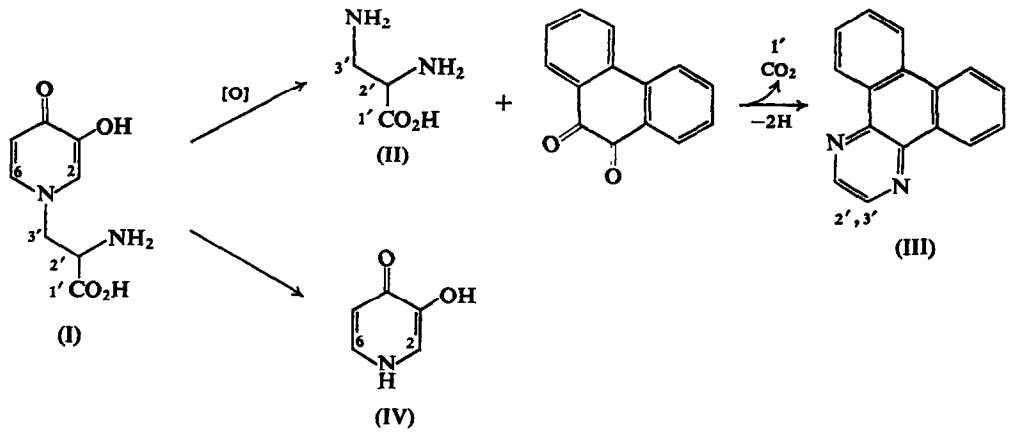

FIG. 1. DEGRADATION OF MIMOSINE.

TABLE 2. DEGRADATION OF MIMOSINE DERIVED FROM [1-^{14}C]-SERINE

Compound	Carbon atoms of mimosine	Specific activity (counts min^{-1} mmole^{-1} × 10^{-3})	Relative specific activity (%) (Mimosine = 100)	Relative specific activity (%) (Side-chain = 100)
Mimosine (I) (diluted)	All	6·53 ± 0·35	100 ± 6	—
3-Hydroxy-4-pyridone (IV)	2–6	1·91 ± 0·06	29 ± 2	—
α,β-Diaminopropionic acid (II)	1′–3′	4·53 ± 0·19	69 ± 5	100 ± 4
Dibenzoquinoxaline (III)	2′, 3′	0·90 ± 0·05	14 ± 1	20 ± 1
By difference	1′		55 ± 5	80 ± 4

In an earlier study[3] we reported that activity from [3-^{14}C]-aspartic acid was recovered almost entirely from the nucleus of mimosine (90 ± 2, 87 ± 3 per cent), whereas the incorporation of label from [1-^{14}C]- and from [4-^{14}C]-aspartic acid into the pyridone nucleus (66 ± 2 and 57 ± 3 per cent, respectively) closely corresponded to that calculated for random distribution (62·5 per cent). Re-examination of the incorporation of [4-^{14}C]-aspartic acid into the nucleus of mimosine (61 ± 3 per cent) (Table 3) confirms the earlier finding. As expected, incorporation of activity from [2-^{14}C]-aspartic acid into the pyridone nucleus is almost quantitative

[4] H. C. BEYERMAN, L. MAAT and M. P. HEGARTY, *Rec. Trav. Chim.* **83**, 1078 (1964).
[5] S. N. NIGAM and C. RESSLER, *Biochim. Biophys. Acta* **93**, 339 (1964).
[6] L. A. HADWIGER, H. G. FLOSS, J. R. STOKER and E. E. CONN, *Phytochem.* **4**, 825 (1965).

(96 ± 4 per cent) (Table 3). Since it has been shown that lysine serves as a precursor of the pyridone nucleus of mimosine, these findings suggest that in *M. pudica* lysine is generated by the α-aminoadipic pathway (cf. Ref. 3), an inference which is strengthened by the observation that activity from [6-^{14}C]-α-aminoadipic acid is localized in the pyridone ring of mimosine (89 ± 4 per cent) (Table 3).

TABLE 3. INCORPORATION OF RADIOACTIVITY INTO THE PYRIDONE NUCLEUS

Precursor	Specific activity (counts min^{-1} mmole^{-1} × 10^{-3})		Relative specific activity of the pyridone nucleus (mimosine = 100)
	Diluted mimosine	3-hydroxy-4-pyridone	
DL-[2-^{14}C]-Aspartic acid	11·88 ± 0·38	11·46 ± 0·32	96 ± 4
DL-[4-^{14}C]-Aspartic acid	5·43 ± 0·22	3·33 ± 0·09	61 ± 3
DL-[6-^{14}C]-α-Aminoadipic acid	7·01 ± 0·22	6·21 ± 0·17	89 ± 4
DL-[1-^{14}C]-Serine	6·53 ± 0·35	1·91 ± 0·06	29 ± 2
Uniform labelling	8	5	62·5

Activity from δ-hydroxylysine is not incorporated into mimosine (Table 1). This finding indicates that hydroxylation occurs at a late stage in the biosynthesis. Radiochromatography of the amino acid fraction from the sap of *Mimosa* plants to which [6-^{14}C]-δ-hydroxylysine had been administered revealed the presence of a labelled component. The R_f value of this radioactive material, whose concentration was too low for detection by ninhydrin, matched that of 5-hydroxypipecolic acid in three solvent systems.

The same compound, together with pipecolic acid, was detected in the methanol extract of *Mimosa* plants to which [6-^{14}C]-lysine had been administered. Pipecolic acid and 5-hydroxypipecolic accompany mimosine in *Leucaena glauca* Benth.[2] They had not hitherto been shown to occur in *M. pudica* L.

EXPERIMENTAL

Administration of Labelled Precursors to Mimosa pudica *and Isolation of Labelled Mimosine*

The labelled compounds which were administered to 7-week-old *Mimosa* plants by the wick method[3] are listed in Table 1. The plants were allowed to remain in contact with the tracer for 48 hr, and mimosine was then isolated from the sap of the plants, in the manner described previously.[1] The radioactive mimosine from each experiment was mixed with carrier, crystallized to constant activity, and degraded to 3-hydroxy-4-pyridone (IV).[3] The mimosine from the experiment with [1-^{14}C]-serine was further degraded as described below.

Radioactivity was assayed on samples of finite thickness, using a low-background gas flow system (Nuclear Chicago Corp.). The usual corrections for background and self-absorption were applied. Confidence limits shown in the results are standard deviation of the mean.

Degradation of Mimosine

α,β-Diaminopropionic acid (II) from mimosine (I).[4] Bromine (0·4 g) in water (2 ml) was added dropwise to a stirred, ice-cold suspension of mimosine (120 mg) in water (5 ml). Stirring was continued for 1 hr. The mixture was concentrated *in vacuo* to a small volume, ethanol added, and the mixture kept at 0° overnight. The crystalline product was filtered, washed (ice-cold ethanol), redissolved in water (2 ml), and clarified with charcoal. On addition of ethanol α,β-diaminopropionic acid hydrobromide, m.p. 239°, crystallized (reported m.p. 240°).[4]

Dibenzo(f,h)quinoxaline (III) from α,β-diaminopropionic acid (II). Phenanthraquinone (40 mg) in ethanol (2 ml) was added to a boiling solution of α,β-diaminopropionic acid hydrobromide (35 mg) in water (5 ml), and refluxed for 4 hr, water being added, if necessary, to keep the solution homogeneous. The ethanol was distilled off, the solution cooled, and the product which precipitated applied to an alumina column and eluted with

[7] M. P. HEGARTY, *Australian J. Chem.* 10, 484 (1957).

benzene. Solvent was evaporated, the residue sublimed at 120° and 3×10^{-3} mm, and the product crystallized from ethanol to yield colourless needles (20 mg), melting at 180°, which showed NMR, i.r. and mass spectra, identical with those of authentic dibenzoquinoxaline (*vide infra*).

Dibenzo(f,h)quinoxaline (III) from ethylenediamine.[8] A solution of ethylenediamine (120 mg) and phenanthraquinone (416 mg) in ethanol was refluxed for 4 hr. Addition of water caused precipitation of product, which was purified by elution with benzene from an alumina column, followed by high vacuum sublimation and recrystallization from ethanol. Dibenzoquinoxaline (310 mg) which was obtained melted at 183° (reported melting point 180°).[8] The mass spectrum showed a molecular ion at 230 mass units. The NMR spectrum showed only aromatic protons.

Detection of pipecolic and 5-hydroxypipecolic acid in M. pudica. The dried leaves (8 g) from twenty 7-week-old *Mimosa* plants, to which DL-[6-^{14}C]-lysine had been administered and from whose sap labelled mimosine had been isolated,[1] were ground in a mortar with two portions of 50 per cent aqueous methanol (70 ml and 30 ml). The combined extracts were clarified by centrifugation, the methanol was evaporated, Dowex 2-X4 (20–50 mesh, OH^- form, 15 ml) was added and the mixture stirred for 10 min. The resin was filtered, washed (water) and the amino acids were eluted with 0·5 N HCl. The eluate was neutralized with resin in the HCO_3^- form and filtered. The filtrate was evaporated and the residue dissolved in a little water, and chromatographed on Whatman No. 1 paper in three solvent systems. Four radioactive zones were detected by radioscanning (Radiochromatogram scanner, Packard Instrument Co., Model No. 7200) of the chromatograms (Table 4). The R_f values of these matched lysine, 5-hydroxypipecolic acid, pipecolic acid and 3-hydroxy-4-pyridone, respectively, on co-chromatography with authentic samples. The identity of pipecolic acid was confirmed by elution of the radioactive zone, R_f 0·56 (butanol/acetic acid/water), addition of carrier DL-pipecolic acid and crystallization to constant activity.

TABLE 4. R_f VALUES

	Butanol/acetic acid/water 4:1:1·8	Phenol/ethanol/ water 3:1:1	Methanol/0·880 ammonia 99:1
Lysine	0·15	0·37	0·29
5-Hydroxypipecolic acid	0·36	0·69	0·53
Pipecolic acid	0·56	0·90	0·65
3-Hydroxy-4-pyridone	0·70	0·95	

The amino acid fraction from the sap of the plants to which [6-^{14}C]-δ-hydroxylysine had been administered was separated, in the usual manner,[1] by adsorption onto Dowex 50W-X4 (H^+ form), and elution with 0·2 N ammonia. The mimosine (12·5 mg), obtained from the eluate, was not radioactive (Table 1). Radiochromatography of the eluate indicated the presence of a radioactive zone whose R_f value corresponded to that of authentic 5-hydroxypipecolic acid, on co-chromatography in the three solvent systems.

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[8] A. T. MASON, *Chem. Ber.* **19**, 112 (1886); *J. Chem. Soc.* **55**, 97 (1889).