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THE UTILIZATION OF TYROSINE FOR MESCALINE AND PROTEIN BIOSYNTHESIS IN *LOPHOPHORA WILLIAMSII*

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Key Word Index—*Lophophora williamsii*; Cactaceae; tyrosine utilization; protein formation; mescaline; peyote alkaloids; metabolic compartmentation; biochemical channeling.

Abstract—Studies on the comparative utilization of tyrosine for protein and alkaloid biosynthesis indicate that this amino acid is incorporated into peyote alkaloids at three times the rate at which it is incorporated into protein. In addition, the biosynthetic pathway for tyrosine formation appears to be compartmented into two channels; one supplying the needs for alkaloid biosynthesis and the other providing tyrosine for protein biosynthesis. The latter compartment is possibly under a negative feedback control mechanism.

INTRODUCTION

THE BIOSYNTHESIS of alkaloids is closely related to the metabolism of amino acids[1] and in plants elaborating alkaloids, the demand for a particular amino acid must be shared by both protein and alkaloid synthesis. Yet, to our knowledge, no data has been published that related to this utilization of an amino acid for both alkaloid and protein biosynthesis.

Lophophora williamsii (Lem.) Coult., commonly known as peyote, is a small, fleshy, spineless cactus that grows wild on the Mexican plateau and in the arid regions of Southwestern United States. This plant yields many alkaloids that are either of the β-phenethylamine or tetrahydroisoquinoline structure and are derived from the amino acid tyrosine.[2,3] In the present study we have investigated the comparative incorporation of uniformly labeled L-tyrosine into alkaloids and soluble protein in peyote.

In another aspect of the study, we wished to determine whether the tyrosine biosynthetic pathway is compartmented in the sense that one channel serves the needs of alkaloid biosynthesis, and another supplies tyrosine for protein synthesis. To do this, the cactus plants were provided with uniformly labeled D-fructose. Thus, all carbon containing compounds including tyrosine and its immediate precursor, *p*-hydroxyphenylpyruvic acid are labeled. In the absence of competition from an unlabeled exogenous pool, the radioactive *p*-hydroxyphenylpyruvic acid formed from D-fructose is transaminated to tyrosine which is in turn incorporated into alkaloids and protein. On the other hand, when unlabeled *p*-hydroxyphenylpyruvic acid is administered to the cactus, the activity incorporated into alkaloids and protein is a measure of the competition between the endogenously formed and externally added intermediate. If the effect of this competition on alkaloid and protein formation is different, then the possibility of compartmentation does exist. Determination of

[1] PELLETIER, S. W. (1970) *Chemistry of the Alkaloids*, pp. 670, Van Nostrand, New York.
[2] KAPADIA, G. J. and FAYEZ, M. B. E. (1970) *J. Pharmac. Sc.* **59**, 1699.
[3] LUNDSTROM, J. (1971) *Acta Pharm. Suecica* **8**, 275.

radioactivity in protein and alkaloid fractions within the same plant was carried out by blending the cactus plants in chilled acetone, thus fractioning the plants into an acetone powder containing the protein and the acetone filtrate containing the alkaloids.

TABLE 1. UTILIZATION OF TYROSINE IN ALKALOID AND PROTEIN BIOSYNTHESIS

Fraction	Radioactivity (μC)	% Radioactivity recovered
Total alkaloids	1·999	5
Mescaline	0·238	0·59
Soluble protein	0·685	1·71
Tyrosine (protein)	0·606	1·51

The fractions listed in the table represent the distribution of activity following the injection of 40 μCi (483 mCi/mM) of L-tyrosine-UL-^{14}C into 2 cactus plants which were subsequently harvested 4 weeks after the injection.

RESULTS AND DISCUSSION

The data obtained from the *in vivo* study on the incorporation of labeled tyrosine into protein and alkaloids are summarized in Table 1. From the total of 40 μCi administered to two plants, 5% of the radioactivity was recovered in the total alkaloid fraction, 0·59% in mescaline, 1·71% in soluble protein and 1·51% in protein tyrosine. These results indicate that tyrosine was incorporated into alkaloids at approximately 3 times the rate of its incorporation into protein. The total activity present in protein tyrosine is slightly less than that present in the soluble protein. This suggests that some of the injected tyrosine was metabolized and its catabolic products recycled to form amino acids which were then incorporated into protein.

TABLE 2. EFFECT OF *P*-HYDROXYPHENYLPYRUVIC ACID AND TYROSINE ON THE INCORPORATION OF ^{14}C-FRUCTOSE INTO PROTEIN AND ALKALOIDS

	Total alkaloids		Mescaline		Tyrosine (protein)	
Experiment	Radioactivity recovered per gram protein (%)	Competition (%)	Radioactivity recovered per gram protein (%)	Competition (%)	Radioactivity recovered per gram protein (%)	Competition (%)
Fructose-U-^{14}C	0·614		0·068		0·397	
Fructose-U-^{14}C + *p*-Hydroxyphenylpyruvic acid	0·546	11·07	0·057	16·17	0·207	47·85
Fructose-U-^{14}C + L-Tyrosine	0·596	2·93	0·061	10·29	0·239	39·80

Approximately 100 μCi (275 mCi/mM) of D-fructose-U-^{14}C were injected into each of 3 pairs of cactus plants. After 1 week, one of the pairs of fructose-injected plants received 20 mg of *p*-hydroxyphenylpyruvic acid, while another pair received a like amount of L-tyrosine. The plants were harvested 4 weeks after the injection of the labeled fructose.

Previous studies on the biosynthetic pathway leading to mescaline using DL-tyrosine as precursor reported incorporations of approximately one-half that obtained in our investigation with labeled L-tyrosine.[4] Since only L-isomers of amino acids are usually biochemically active, it would appear that peyote does not possess a tyrosine racemase or a non-specific decarboxylase.

Table 2 presents the results of the competition experiments. These results show that both the exogenously added *p*-hydroxyphenylpyruvic acid and tyrosine caused a greater dilution of the activity channeled for protein synthesis than for alkaloid synthesis. This is suggestive of a two compartment system for tyrosine biosynthesis and metabolism in peyote. The evidence for this metabolic compartmentation is based upon the fact that endogenously generated *p*-hydroxyphenylpyruvic acid competes successfully with its exogenously added counterpart in the formation of alkaloids, but not in the formation of protein. Perhaps, only the protein compartment is under a negative control mechanism and when tyrosine accumulates it either feedback inhibits an early enzymic step in the tyrosine pathway and/or it represses the enzymes in the entire sequence. A lack of such control in the alkaloid compartment may possibly explain why alkaloids accumulate in the cactus as well as other plants.

EXPERIMENTAL

Administration of test compounds. Living plants of *L. williamsii* were maintained in a greenhouse. The procedure employed for the introduction of test compounds into peyote tissue was previously described.[4,5] In the initial study, 40 μCi (483 mCi/mM) of uniformly labeled L-tyrosine were aseptically injected into 2 locations, namely the root and the stem, of each of 2 plants. In the subsequent investigation, approximately 100 μCi (275 mCi/mM) of uniformly labeled D-fructose was similarly injected into each of 3 pairs of cactus plants. After 1 week, one of the pairs of fructose-injected plants received 20 mg of *p*-hydroxyphenylpyruvic acid, while another pair received a similar quantity of L-tyrosine. After each injection the plants were repotted, watered and returned to the greenhouse.

Isolation procedure. Four weeks after the injection of the labeled test compounds, the plants were removed from the greenhouse in pairs and sliced and blended with 5 vol. of chilled acetone ($-15°$), at high speed, for 1 min in a Waring blender. The resulting slurry was filtered, washed with excess chilled acetone, and the residue air dried. The acetone powder was then extracted with 10 vol. of 0·1 M phosphate buffer (pH 7·5) with mechanical shaking for 4 hr. The resulting slurry was filtered and part of the protein extract (30 ml) was hydrolyzed by heating in a sealed tube with concn HCl for 24 hr at 110°. Fifty mg of L-tyrosine was added as carrier to the hydrolysate which was then evaporated with the aid of stream of N_2 to incipient crystallization of tyrosine. The isolated tyrosine was recrystallized to constant sp. act. The remaining protein extract that was not hydrolyzed was employed in the determination of total protein by the method of Lowry *et al.*[6] Per cent incorporations of radioactivity in protein and protein tyrosine were determined. The acetone filtrate was evaporated to dryness and 100 ml of 1N HCl added. After filtration, the acid extract was extracted with $CHCl_3$ until the organic layer was colorless. The aq. soln was then made basic to a pH 9·5 (NH_4OH) and extracted several times with equal portions of $CHCl_3$. The combined $CHCl_3$ extract (total alkaloid fraction) was divided into two equal volumes, to one of which 100 mg of mescaline HCl was added as carrier. Both parts were then evaporated to dryness and passed through anion exchange column to separate non-phenolic from phenolic fractions.[7] The addition of methanolic HCl to the condensed non-phenolic extract followed by ether caused the crystallization of mescaline HCl which was also recrystallized to constant specific activity. The quantity of mescaline present in the plant was calculated by means of the isotope dilution technique and per cent incorporations were determined.

Radioactive assay. The method employed for the preparation of samples for scintillation counting was described previously.[4] All samples were counted in a Beckman LS-230 liquid scintillation system at a preset 2σ statistical counting error of 1%. The external standard ratio method was used to determine losses due to quenching. All counts were corrected for counter efficiency.

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[5] Rosenberg, H., McLaughlin, J. L. and Paul, A. G. (1967) *Lloydia* **30**, 100.
[6] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biological Chemistry* 265.
[7] McLaughlin, J. L. and Paul, A. G. (1966) *Lloydia* **29**, 315.