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FORMATION OF β-ALANINE FROM SPERMINE AND SPERMIDINE IN MAIZE SHOOTS

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Key Word Index—*Zea mays*; Gramineae; maize; biosynthesis; β-alanine; 1,3-diaminopropane; spermidine; spermine.

Abstract—Spermine, spermidine or 1,3-diaminopropane fed to *Zea mays* shoots caused an accumulation of β-alanine. Furthermore, spermine 4 HCl [3-aminopropyl-3-^{3}H(C)] was metabolized in the shoots to β-alanine. These results are consistent with the formation of β-alanine from polyamines by oxidative degradation.

INTRODUCTION

Polyamine oxidase in the Gramineae produces 1,3-diaminopropane from polyamines such as spermine and spermidine [1–3]; however, the subsequent metabolism of 1,3-diaminopropane in higher plants is unknown. Razin *et al.* [4] reported that *Pseudomonas aeruginosa* accumulates β-alanine when it is incubated with spermine or spermidine. In this paper, we report the formation of β-alanine from spermine, spermidine, and 1,3-diaminopropane in maize seedlings.

RESULTS AND DISCUSSION

Time course for changes in the level of 1,3-diaminopropane and β-alanine

Excised shoots were allowed to absorb solutions of spermine, spermidine or 1,3-diaminopropane, and the change in the level of β-alanine and 1,3-diaminopropane was followed for 4-days (Table 1). In the controls, no detectable amounts of β-alanine and 1,3-diaminopropane were present. However, when spermine and spermidine were supplied to the shoots, a significant increase in the level of 1,3-diaminopropane and β-alanine occurred. This effect appeared clearly after about 3 days. A similar pattern for the increase in the level of 1,3-diaminopropane and β-alanine was found in etiolated shoots (Table 2). These results suggest that spermine, spermidine and 1,3-diaminopropane are precursors of β-alanine in maize shoots as well as in bacteria [4].

Table 1. Time course for changes in the level of 1,3-diaminopropane and β-alanine in excised shoots under light

Feeding substances	Substances formed	Feeding periods (days) 0	1	2	3	4
1,3-Diaminopropane	A	0	1.0	3.7	7.3	8.0
	B	0	0.3	0.8	1.4	1.5
Spermidine	A	0	0 3	1.0	6.0	3.5
	B	0	0.2	0.7	1.4	1.1
Spermine	A	0	0.7	2.7	7.7	6.4
	B	0	0.4	0.8	1.4	1.6

β-Alanine and 1,3-diaminopropane were estimated by GLC (see text), and contents were expressed with μmol/g fr. wt. A · 1,3-diaminopropane B. β-alanine.

Table 2. Time course for changes in the level of 1,3-diaminopropane and β-alanine in excised shoots in the dark

Feeding substances	Substances formed	Feeding periods (days) 0	1	2	3
1,3-Diaminopropane	A	0	1.5	4.5	3.0
	B	0.1	0.3	0.7	1.3
Spermidine	A	0	0.9	2.0	3.9
	B	0.1	0.4	0.7	1.1
Spermine	A	0	0.8	4.7	4.1
	B	0.1	0.3	0.8	1.2

Experimental conditions were similar to those of Table 1.

Metabolim of spermine-[^3H]

Excised shoots were allowed to absorb a solution of spermine-[^{3}H] and the distribution of radioactivity in the

Table 3. Distribution of radioactivity[^{3}H] in β-alanine and 1,3-diaminopropane plus 1-(3-aminopropyl)-pyrroline in excised maize shoots

Fraction	Days after feeding 1 dpm	(%)	2 dpm	(%)	3 dpm	(%)
Spermine	23 400	(52)	20 300	(41)	18 000	(36)
1,3-Diaminopropane plus 1-(3-aminopropyl)-pyrroline	11 000	(24)	15 400	(31)	19 200	(38)
β-Alanine	3100	(7)	3900	(8)	4100	(8)
Unknown substance	1700	(4)	1500	(3)	1200	(2)
Total activity	45 100	(100)	49 500	(100)	50 200	(100)

Extracts (20 mg fr. wt) were developed by PC.

amino acids and amines was examined. Table 3 shows the estimated incorporations of ^3H from spermine into 1,3-diaminopropane and β-alanine. Considerable radioactivity was incorporated into the fraction of 1,3-diaminopropane plus 1-(3-aminopropyl)-pyrroline and β-alanine. The sp. act. of β-alanine found after 24 hr incubation from 0.5 μCi/μmol spermine was 0.18 μCi/μmol (Table 3). Theoretically this value should be 0.18 μCi/μmol. However, 2–4% of the radioactivity derived from spermine-[^3H] was recovered in an unidentified compound (Table 3). The biosynthetic route of β-alanine in higher plants as suggested here seems attractive, since spermine and spermidine are widespread in various Gramineae [5]. The formation of 1,3-diaminopropane from both polyamines has been established convincingly at the enzyme level [1]. Our results leave open the question of the mechanism by which the 1,3-diaminopropane is oxidised.

EXPERIMENTAL

Plant material. Seeds of maize (*Zea mays.* L. cv Goldencross Bantam T 51) were germinated in moist vermiculite at 25° for 3 days in the dark. The seedlings were then transferred to plastic trays containing Hoagland's soln. They were grown hydroponically under continuous light (*ca* 2 klx at plant level) at 25° for 11 days. Etiolated seedlings were raised in moist vermiculite at 25° for 7 days in the dark.

Feeding experiment. 4 to 5 shoot tips, comprising the 3 developed leaves, were used. Cut ends (2 cm above the seed) of excised shoot tips were placed in a flask containing 30 ml of 1/2 strength Hoagland's soln with or without 35 mM spermine, spermidine or 1,3-diaminopropane and were allowed to absorb the soln at 25° in the dark or under light (*ca* 2 klx). The solns in the flasks were renewed every day.

Extraction and fractionation. The amino acids and amines from green shoots were determined in the EtOH-soluble fraction obtained by extracting with 99% EtOH (8 ml per g fr. wt). After centrifugation (10 min at 1000 *g*), the chlorophyll and other pigments were removed from the supernatant by extraction with toluene [6]. The amino acids and amines from the etiolated shoots were obtained according to the method ref. [7]. The extracts obtained were treated with Amberite IR-120 (H^+) [7]. Partially purified fractions of amino acids and amines were trimethylsilyated (TMSi) [8].

GLC (FID) was carried out with 3 mm id × 2 m glass columns packed with Chromosorb W coated with 10% OV-17 or SE-30.

Tracer experiments. 11-days-old shoots absorbed 35 mM radioactive spermine [3-aminopropyl-3-[^3H(C)] : sp. act. 0.5 μCi/μmol] and 1/2 strength Hoagland's soln under continuous light (as above). After 24 hr, they were transferred to another flask containing 35 mM spermine and 1/2 strength Hoagland's soln. At the end of the experimental periods, they were separated by descending PC, with *n*-BuOH–HOAc–H_2O (4:1:2) (solvent 1); and methyl cellosolve–HOPr–H_2O (14:3:3) satd with NaCl (solvent 2) [9]. After spraying with ninhydrin, spermine, 1,3-diaminopropane, 1-(3-aminopropyl)-pyrroline, and β-alanine were determined. R_fs in solvent 1 were spermine, 0.05; 1,3-diaminopropane, 0.13; β-alanine, 0.40; and 1-(3-aminopropyl)pyrroline, 0.11: R_fs in solvent 2 were 0.09, 0.27, 0.62 and 0.45 resp. 1-(3-Aminopropyl)pyrroline was obtained in the polyamine oxidase reaction. The radioactivity of the amino acids and amines was measured directly from the papers by liquid scintillation counter at 48% efficiency, with a PPO-dimethyl-POPOP-toluene scintillator.

Polyamine oxidase. Partially purified polyamine oxidase from maize shoots was prepared using the method of ref. [10].

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A SECOISOLARICIRESINOL BRANCHED FATTY DIESTER FROM *SALVIA PLEBEIA* SEED

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Key Word Index—*Salvia plebeia*; Labiatae; lignan; diester; *anteiso* fatty acid; 12-methyltetradecanoic acid; secoisolariciresinol.

Abstract—The structure of a second new lignan diester from *Salvia plebeia* seed has been determined. Hydrolysis of this diester yields two compounds, 12-methyltetradecanoic acid and secoisolariciresinol.

INTRODUCTION

We recently characterized a unique lignan diester (1) which was isolated by crystallization from the hexane extract of *Salvia plebeia* seeds [1]. Further examination of the hexane extract of this seed revealed the presence of a