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1,3-DIAMINOPROPANE AND SPERMIDINE IN *CUCUMIS SATIVUS* (CUCUMBER)

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Key Word Index—*Cucumis sativus*, Cucurbitaceae; cucumber; 1,3-diaminopropane; spermidine; amines.

Abstract—1,3-Diaminopropane has been detected, together with spermidine, in cucumber seeds. Quantitative estimation of the two metabolites in cucumber seeds and germinating seedlings (up to 21 days old) showed that the concentrations of both metabolites increased, then decreased with age. A metabolic sequence linking spermidine, 1,3-diaminopropane and the non-protein amino acid β-pyrazol-1-yl alanine was suggested.

INTRODUCTION

1,3-Diaminopropane has been detected in leaf extracts of wheat, barley [1–3], pea seeds [4], bovine brain [5] and, on oxidation of polyamines by *Mycobacterium smegmatis* and *Serratia marcescens* [6, 7] and by polyomine oxidase found in many Gramineae [8]. Spermidine is well established as a constituent of microorganisms and animals [9, 10]. It has also been detected in wheat embryos [11, 12], pea roots, marrow leaves [1], barley seedlings [3] and seeds of *Cucumis sativus* [13, 14]. 1,3-Diaminopropane and spermidine, like some other di- and polyamines, have recently attracted interest due to their ability of interact with nucleic acids and their growth-promoting effects.

It has recently been shown that 1,3-diaminopropane is a precursor of the pyrazole moiety of the non-protein amino acid β-pyrazol-1-yl-alanine in *Cucumis sativus* [15]. Preliminary studies suggest that spermidine is a precursor of 1,3-diaminopropane in *C. sativus* [15]. The aim of the present work was to establish the presence of 1,3-diaminopropane and spermidine in *C. sativus* (cucumber) and to examine the production of these two metabolities during the growth of these plants.

RESULTS AND DISCUSSION

Spermidine and 1,3-diaminopropane were detected on paper chromatograms of the amine fraction of cucumber seeds, seedlings (up to 21 days old) and fruits (fr. wts 19 g, 25 g and 29 g). Two spots corresponded exactly to standard spermidine and 1,3-diaminopropane both with respect to R_f and ninhydrin colour. The R_f values of these two metabolites in the above solvent systems were (i) 0.11 and 0.20, (ii) 0.12 and 0.18, and (iii) 0.04 and 0.01 respectively. Paper chromatograms run in solvent (i) for 48 hr separated spermidine and 1,3-diaminopropane from other ninhydrin positive components present in the amine fraction of cucumber. Three other major unidentified spots were detected on this chromatogram. For further confirmation, the purified spermidine and 1,3-diaminopropane from several paper chromatographic separations were eluted and pooled, then subjected to thin layer electrophoresis at pH 3.4 (see Experimental). The metabolites migrated with authentic samples of spermidine or 1,3-diaminopropane at 16.9 and 19.9 cm per hr respectively, towards the cathode at 30 V/cm.

Gas chromatography of the isolated 1,3-diaminopropane and spermidine (see Experimental) showed peaks corresponding to those of standards 1,3-diaminopropane and spermidine (retention times 4.2 and 13 min respectively).

Investigation of the amine fraction of cucumber extract by paper chromatography of the basic fraction in solvent (i) showed a spot corresponding to that of authentic putrescine (R_f 0.13). Bands corresponding to authentic 1,3-diaminopropane, putresine and spermidine were eluted from the chromatogram of the basic fraction of cucumber extract run in solvent (i) for 48 hr. When the concentrated eluates were rechromatographed in a mixture of phenol and KCl–HCl, as described in ref. [16], spots corresponding to those of standard 1,3-diaminopropane, putrescine and spermidine were detected (R_f 0.16, 0.29 and 0.2 respectively). Gas chromatography of the amine fraction of cucumber extracts, under the conditions described in the Experimental, showed a peak similar to that shown by authentic putrescine (retention time 5 min).

The detection of 1,3-diaminopropane in cucumber supports the studies on the biosynthetic origin of the pyrazole ring of the non-protein amino acid β-pyrazol-1-yl-alanine in the same plant, which implicated 1,3-diaminopropane as a precursor [15].

The spermidine and 1,3-diaminopropane contents of seeds and young seedlings of cucumber were determined at various stages of germination and growth. Spermidine and 1,3-diaminopropane were detected in cucumber fruits; however their concentrations were not determined, due to difficulty of obtaining the fruits. It was found that the content of spermidine was greater than that of 1,3-diaminopropane in cucumber seeds and seedlings up to 21 days old. It was also found that the contents of these two metabolites increased rapidly during the first 9 days and decreased sharply during the following 2 days. However after that, and, up to 21 days, the decrease in the contents of these metabolites were very gradual. The rise in the contents of 1,3-diaminopropane here (Table 1) could be attributable to catabolism of spermidine. However, the

Table 1. Content of 1,3-diaminopropane and spermidine in seeds and growing seedlings of cucumber

Age of seedlings (days)	Fresh wt of 40 seedlings (g)	1,3-Diaminopropane (μmol/ 40 seedlings)	1,3-Diaminopropane (μmol/g)	Spermidine (μmol/ 40 seedlings	Spermidine (μmol/g)
0	1.4*	0.5	0.36	1.8	1.3
4	9.0	11.5	1.27	23.2	2.6
7	24.1	32	1.33	91.0	3.7
9	25.5	38	1.5	102	4.0
11	26.8	15	0.56	43	1.6
15	33.5	14	0.42	41	1.2
18	36.5	12.5	0.34	40	1.1
21	43.7	11	0.25	31	0.7

*Dry wt of seeds.

later decrease in the contents of the two metabolites may be attributed to their involvement in the formation of pyrazole and β-pyrazol-1-yl-alanine [15]. Thus, the three metabolites (spermidine, 1,3-diaminopropane and pyrazole) may be linked in a metabolic sequence. Finally the presence of spermidine and 1,3-diaminopropane in *Cucumis sativus* may provide a clue to a possible role (see for example refs. [17, 18]) of the non protein amino acid β-pyrazol-1-yl-alanine which is synthesized in the same plant.

EXPERIMENTAL

Materials. Seeds of *Cucumis sativus* (cucumber) var. asgrow vigorpak obtained from Asgrow seed company, Kalamazoo, U.S.A., were germinated in moist vermiculite and the seedlings grown at 25° with 16 hr light (6 kl ×) and 8 hr dark. Spermidine trihydrochloride, 1,3-diaminopropane dihydrochloride and putrescine dihydrochloride, were obtained from Aldrich.

Extraction and purification of 1,3-diaminopropane and spermidine. The method used was that described in ref. [1] with minor modifications. Cucumber plants (10 g) samples were homogenized in 5% trichloroacetic acid (80 ml). The extract was left for 18 hr at 2° and then filtered. After measuring the vol., the filtrate was added to 1 g of Dowex 50 W-X8 (20–50 mesh) ion exchange resin (H^+ form) and was shaken for 1 hr. The soln was then decanted. After washing the resin with H_2O (10 ml), the H_2O was decanted and discarded. To the resin, 36% HCl (10 ml) was added and shaken for 2 hr. The eluate was removed and the resin washed with H_2O (3 ml), the washings being combined with the acid eluate. The soln (amine fraction) was concd to dryness *in vacuo* at 0°. The dried extract was dissolved in H_2O (1 ml). A sample of this soln (500 μl) and standards (60 μmol each) were chromatographed on Whatman No. 1 paper, in solvent (i) Me cellosolve–propionic acid–H_2O (14:3:3) satd with NaCl, for 48 hr. The bands corresponding to standard 1,3-diaminopropane and spermidine were eluted and rechromatographed in (ii) *n*-BuOH–HOAc–H_2O (4:1:5) subsequently the isolated 1,3-diaminopropane and spermidine were eluted and rechromatographed in solvent (iii) *n*-BuOH–MeEtCO–NH_4OH–H_2O (5:3:1:1). The purified 1,3-diaminopropane and spermidine from several separations were pooled and subjected to thin-layer electrophoresis, at pH 3.4 (0.1 M citrate buffer 30 V/cm) for 22 min on microcrystalline cellulose plates. The locating agent normally used was ninhydrin (1% ninhydrin and 1% collidine in acetone).

Gas chromatography. The column (1.5 m long × 5 mm i.d.) contained carbowax 20 M-2% KOH on chromosorb W (100–120 mesh) prepared as described in ref. [9]. The detector oven was heated to 200°. The N_2 carrier gas flow rate was 40 ml/min. Samples (20 μl) of MeOH–KOH amine fraction were injected for analysis.

Concns of spermidine and 1,3-diaminopropane were measured by the ninhydrin procedure [20]. Standard curves were constructed for both authentic spermidine and 1,3-diaminopropane.

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