Phytochemistry, 1964, Vol. 3, pp. 23 to 26. Pergamon Press Ltd. Printed in England

N-CARBAMYLPUTRESCINE—AN INTERMEDIATE IN THE FORMATION OF PUTRESCINE BY BARLEY

T. A. SMITH and J. L. GARRAWAY*

Agricultural Research Council Unit of Plant Morphogenesis and Nutrition,
Wye College, near Ashford, Kent

(*Received* 24 *May*, 1963)

Abstract—N-Carbamylputrescine was detected in extracts of excised barley-seedling leaves which had been fed with agmatine. Synthetic N-carbamylputrescine fed similarly to leaves or incubated with leaf macerates resulted in the production of putrescine.

INTRODUCTION

IN MICRO-ORGANISMS, two routes are known by which putrescine may be formed from agmatine. The agmatine may be degraded directly to putrescine with the production of urea;[1] alternatively, it may first form an intermediate, N-carbamylputrescine, which is subsequently hydrolysed to putrescine[2,3] (Fig. 1). These pathways are analogous with the L-arginine ureohydrolase (arginase) and L-arginine iminohydrolase and L-citrulline amidohydrolase (arginine dihydrolase) systems respectively.[4]

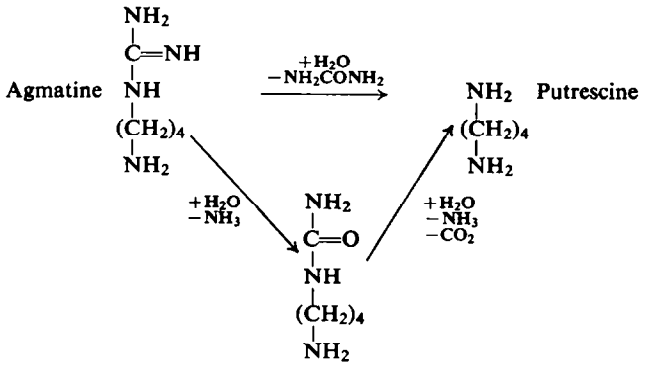

FIG. 1. PATHWAYS OF PUTRESCINE FORMATION FROM AGMATINE KNOWN TO OPERATE IN BACTERIA.

On being fed to excised leaves of barley seedlings (*Hordeum vulgare* L.) agmatine was converted to putrescine[5] and it was of interest to establish the route by which this degradation was effected.

* Department of Physical Sciences, Wye College, near Ashford, Kent.

[1] K. MIYAKI and H. MOMIYAMA, *Seikagaku*, **27**, 765 (1956).
[2] V. MØLLER, *Acta Pathol. Microbiol. Scand.* **36**, 158 (1955).
[3] F. LINNEWEH, *Hoppe-Seyl. Z.* **205**, 126 (1932).
[4] H. REINBOTHE and K. MOTHES, *Ann. Rev. Plant Physiol.* **13**, 129 (1962).
[5] T. A. SMITH and F. J. RICHARDS, *Biochem. J.* **84**, 292 (1962).

RESULTS

In extracts of the leaves of both Plumage Archer and Proctor barley seedlings which had been fed with agmatine by the method of Smith and Richards,[5] paper chromatography revealed, besides putrescine, a ninhydrin-positive substance which was not detected in the extracts of the control leaves. The behaviour of this substance was found to be indistinguishable from that of synthetic N-carbamylputrescine, both being ninhydrin-positive and giving a yellow colour with *p*-dimethylaminobenzaldehyde reagent,[6] and both having similar R_f values on paper chromatograms (Table 1).

Evidence that N-carbamylputrescine is a precursor of putrescine in barley was obtained by feeding leaves with the synthetic compound. These leaves were found to contain considerably more putrescine than leaves which were supplied with water alone, none being detected in the residual feeding solution. N,N'-Dicarbamylputrescine fed similarly did not appear to be metabolized to putrescine.

TABLE 1. R_f VALUES OF PUTRESCINE DERIVATIVES*

Solvent	N-Carbamyl-putrescine	N,N'-Dicarbamyl-putrescine
n-BuOH:CH_3COOH:H_2O (4:1:5 by vol.)	0·35	0·60
Phenol, sat. with H_2O	0·95	0·95
Butanol-ketone solvent[7]†	0·50	0·40

* On Whatman No. 1 paper.
† See Experimental; run ascending.

In vitro experiments were made by incubating extracts of the leaves of Proctor barley seedlings with N-carbamylputrescine. Chromatography in the butanol–ketone solvent of samples (50 μl) taken after 0, 24 and 66 hr showed a progressive increase in putrescine. No such increase occurred in similar samples which were boiled prior to the addition of the N-carbamylputrescine, nor in unboiled samples to which no N-carbamylputrescine had been added.

An attempt to detect naturally occurring N-carbamylputrescine in the leaves of Proctor barley seedlings was unsuccessful, the limit of the detection method being 1 μg/g fresh weight of leaf material. Although no urea was found in extracts of barley leaves which had been fed with agmatine it is not possible to exclude the direct degradation of agmatine with the production of urea as an alternative route for the formation of putrescine. Urea which may have been produced could have been decomposed by urease which was shown to be present in the tissue.

EXPERIMENTAL

Preparation of N-Carbamylputrescine Hydrochloride and N,N'-Dicarbamylputrescine

Putrescine dihydrochloride (3·2 g) was dissolved in water (5·0 ml) and half neutralized with 5 N potassium hydroxide (4·0 ml). Potassium cyanate (2·0 g, excess) in water (5·0 ml)

[6] I. SMITH, *Nature* **171**, 43 (1953).
[7] M. WOLFE, *Biochim. biophys. Acta* **23**, 186 (1957).

was added and the solution heated in a small conical flask on a boiling-water bath. After ½ hr a small quantity of decolourizing charcoal was added and the heating continued for a further ¼ hr. The solution was filtered and allowed to stand overnight. The crystals (A) which had formed were filtered off, washed with water and dried. The filtrate was evaporated to dryness and the residue (B) left for 18 hr over calcium chloride in a vacuum desiccator.

The product (A) (0·7 g) was recrystallized from water to yield colourless plates (0·6 g), decomposing 225–226° (Linneweh[3] gives m.p. 225° for N,N'-dicarbamylputrescine). (Found: N, 32·40. Calc. for $C_6H_{14}O_2N_4$: N, 32·18%.) The product was ninhydrin-negative and gave a yellow colour with the *p*-dimethylaminobenzaldehyde reagent.

The residue (B) was extracted with absolute alcohol (50 ml), the solution filtered and evaporated to dryness. The residue was redissolved in absolute alcohol (25 ml), filtered, and dry hydrogen chloride passed slowly into the solution with cooling. The white precipitate produced was filtered off, dried, and the product (0·8 g) recrystallized from rectified spirit to yield colourless needles (0·5 g), m.p. 187–188° (Linneweh[3] gives m.p. 185° for N-carbamylputrescine hydrochloride). The product on chromatography was found to be contaminated with a small quantity of N,N'-dicarbamylputrescine. As recrystallization failed to remove the contaminant, a chromatographic technique was employed for further purification. A portion of the product (200 mg) was dissolved in water (2 ml) and the solution was applied in a horizontal band near the base of five 20 × 20 cm squares of Whatman No. 3 chromatography paper. The chromatograms were developed by the method of Datta, Dent and Harris[8] with butan-1-ol:acetic acid:water (4:1:5 by vol., upper phase) as ascending solvent. After about 4 hr, when the solvent front had reached the top of the paper, the chromatograms were removed and dried. The N-carbamylputrescine was located with the *p*-dimethylamino benzaldehyde reagent on a vertical strip cut from the side of one of the sheets. The regions of the chromatograms containing the N-carbamylputrescine were cut out and eluted with water, using three portions of 50 ml each. The solutions were combined and the water removed using a rotary film evaporator. The residue was placed over calcium chloride in a vacuum desiccator for 18 hr and recrystallized from rectified spirit to which conc. HCl (1 drop) had been added. Poorly-defined crystals (120 mg) were obtained which, after leaving for 18 hr over calcium chloride in a desiccator and recrystallizing from rectified spirit, yielded colourless needles (50 mg), m.p. 185–186°. (Found: N, 25·40. Calc. for $C_5H_{13}O_1N_3$,HCl: N, 25·05%.) The product was ninhydrin-positive and gave a yellow colour with the *p*-dimethylaminobenzaldehyde reagent.

R_f values obtained on paper chromatography are given in Table 1.

Investigation of the Metabolism of the Putrescine Derivatives by Barley

Proctor barley seedlings were grown for 12 days in a glasshouse, being then about 6 cm high. Leaves (2 g samples) were excised, and the cut ends were placed in three phials containing 5 ml of 25 mM N-carbamylputrescine hydrochloride, 5 ml of approx. 20 mM (saturated) N,N'-dicarbamylputrescine and 5 ml of water respectively, the depth of liquid in each phial being about 1 cm. After 24 hr in continuous light, the samples were washed with water and extracted separately in ethanol (10 ml). Each extract was centrifuged and the supernatant concentrated to 2 ml in a stream of warm air. Samples (100 μl) of these solutions were chromatographed on Whatman No. 1 paper in a solvent composed of

[8] S. P. DATTA, C. E. DENT and H. HARRIS, *Biochem. J.* **46**, xlii (1950).

butan-1-ol:ethyl methyl ketone:aq. NH_3 (sp. gr. 0·88):water (5:3:1:1 by vol., the butanol–ketone solvent).

Extracts of 2-week-old pre-frozen Proctor barley leaves were used for the *in vitro* experiments. These leaves were macerated with two vol. (w/v) of 0·1 M di-sodium hydrogen phosphate. The extract, pH 7·0, was filtered through nylon, centrifuged at $2000 \times g$ for 5 min, and samples (1 ml) incubated with N-carbamylputrescine hydrochloride (2 mg) at $20 \pm 2°$. Toluene (1 ml) was added as an antiseptic.