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THE BIOSYNTHESIS OF COUMARYLAGMATINE IN BARLEY SEEDLINGS

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Key Word Index—*Hordeum vulgare*; Gramineae; barley seedlings; biosynthesis; coumarylagmatine; hordatines; coumaryl-coenzyme A.

Abstract—An enzyme present in extracts of the shoots of barley seedlings has been shown to synthesize coumarylagmatine from *p*-coumaryl-coenzyme A and [U-^{14}C]agmatine.

Coumarylagmatine and its antifungal dimers known as the hordatines are present in barley seedlings [1, 2] and the present communication describes the mode of biosynthesis of coumarylagmatine from coumaryl-CoA and agmatine. Although reaction of a phenylpropanoid-CoA conjugate with an amino group has been postulated in the biosynthesis of cinnamic acid/amine conjugates [3], this is the first report of an enzyme which catalyses this process.

Confirmation of the involvement of agmatine was obtained by feeding [U-^{14}C]agmatine monohydrochloride, prepared from [U-^{14}C]arginine by decarboxylation, to cut barley shoots harvested 3–6 days after sowing. Label was incorporated into coumarylagmatine, reaching a maximum after about 3 hr followed by a slow decline in the following 20 hr. Label was also incorporated into the hordatines, maximum activity being reached after about 6 hr with little change in the following 20 hr. This confirms the suggestion that agmatine is incorporated initially into coumarylagmatine which then dimerizes to the hordatines. A smaller incorporation of the label into *N*-carbamylputrescine and putrescine could also be detected.

Incubation of homogenized barley seedlings with K coumarate and [U-^{14}C]agmatine gave no detectable synthesis of coumarylagmatine. Neither did incorporation of coenzyme A (CoA), ATP and Mg^{2+} into incubation mixtures over a wide range of pH values give any formation of coumarylagmatine. However, buffered homogenates of 3-day-old barley seedlings incubated with coumaryl-CoA and [U-^{14}C]agmatine incorporated label into both coumarylagmatine and the hordatines. No label could be detected in coumarylagmatine or hordatines either with boiled barley extract or in the absence of barley extract. Enzyme activity was maximal when incubated at pH 8.5 in 0.1 M Tris, and activity was almost completely absent at pH 7. No activity was detectable in 0.1 M phosphate buffers in the range pH 5–7. Activity in 0.1 M Na_2CO_3/$NaHCO_3$ was less than in Tris and declined from pH 9 to pH 10.5. In optimum conditions over 90% of the label was incorporated into coumarylagmatine and hordatines. The decrease in label in free agmatine was almost complete after 30 min, when the label in the coumarylagmatine reached a peak, thereafter label in the coumarylagmatine declined and label in the hordatines increased. No label could be detected in putrescine or *N*-carbamylputrescine. After centrifugation of the crude barley extract at 10000 *g* for 20 min, activity was greater in the supernatant than in the resuspended precipitate. However, activity could still be detected in the precipitate even after repeated washing in the extraction medium.

These results suggest the formation of coumarylagmatine in barley seedlings by a *p*-coumaryl-CoA agmatine *N*-*p*-coumaryl transferase (EC 2.3.1.—) along the pathway shown in Scheme 1.

EXPERIMENTAL

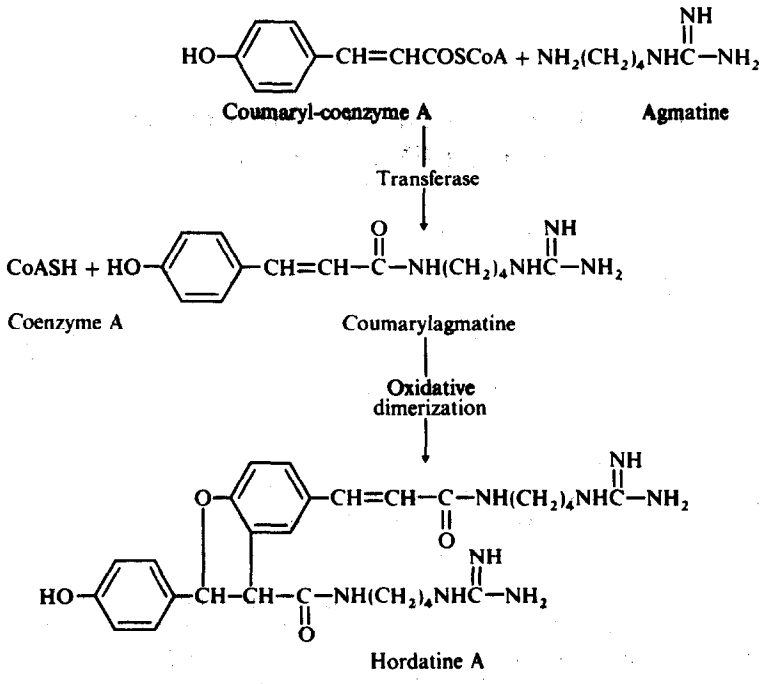
Barley (*Hordeum vulgare* L., cv Claret) was grown on muslin over a layer of polystyrene beads floating on deionized water in the dark at 22° [4].

TLC was on cellulose CC41 using solvents: A, *n*-BuOH–MeCOEt–18M NH_4OH–H_2O (5:3:1:1) [5] (arginine, agmatine and putrescine had R_f values 0.10, 0.30 and 0.46 respectively); B, *n*-BuOH–HOAc–H_2O (4:1:5 upper) [2] (coumarylagmatine, hordatines A/B and hordatines M had R_f values of 0.77, 0.59 and 0.47 respectively); C, *n*-BuOH–EtOH–H_2O (4:1:2) (coumarylagmatine, hordatines A/B, hordatines M and agmatine had R_f values of 0.63, 0.49, 0.40 and 0.06 respectively). Radioactive spots were visualized using a Panax Betagraph and quantified by removing the cellulose, eluting with 1 M HCl, evaporating and taking up in 5 ml EtOH for scintillation counting.

[U-^{14}C]*Agmatine* was prepared from [U^{14}C]-arginine monohydrochloride by incubating with arginine decarboxylase (arginine carboxy-lyase, EC 4.1.1.19)–Sigma (0.2 mg/ml) in 0.1 M Na citrate, pH 5.2, at 38° for 1 hr. The product was identified by chromatography in solvent 1, as a single spot corresponding to agmatine.

p-*Coumaryl-coenzyme A* was prepared by trans-esterification of coumaryl-*N*-hydroxysuccinimide with coenzyme A by the method of ref. [6]. The product gave a single fluorescent spot on TLC in solvent 1, *n*-BuOH–HOAc–H_2O (5:2:3); 2, *iso*-butyric acid–18 M NH_4OH–H_2O (66:1:33); 3, EtOH–0.1 N Na acetate, pH 4.5 (1:1). It also showed the characteristic UV spectrum in 0.1 M Pi buffer, pH 7.0, with λ_{max} 262 and 333 nm.

In vivo *feeding of agmatine*. Seventy complete shoots were cut from barley seedlings 3, 4, 5 and 6 days after sowing. [U-^{14}C]Agmatine (20 μCi) in a 2 μl drop was applied to each cut



Scheme 1. Pathway of coumarylagmatine biosynthesis in barley.

end and the shoots were placed in a draught until all of the soln had been absorbed, when they were transferred to damp filter paper in a Petri dish and kept at 22° in the dark. Ten seedlings were taken at regular intervals and immersed in 5 ml 17 M HOAc for 24 hr, then washed in a further 5 ml HOAc. The combined HOAc was evaporated, the residue taken up in 1 ml 50% HOAc and 20 μl samples chromatographed separately in solvents A and B. The agmatine (solvent A), coumarylagmatine and hordatines (solvent B) were quantified as described in *TLC*.

In vitro *enzyme activity*. Three-day-old barley seedlings were homogenized with a pestle and mortar with sand in 4 vol. of extraction medium containing 0.1 M Tris, pH 8.5, 2 mM EDTA 10 mM mercaptoethanol (ME), 1% BSA and 1% Polyclar AT. The extract was centrifuged at 10000 *g* for 20 min and the supernatant dialysed against 0.1 M Tris, pH 8.5, 10 mM ME. In the investigation of the pH optimum, dialysis was against 0.1 M Pi, pH 5.0–7.0; 0.1 M Tris, pH 7.0–9.0; 0.1 M Na_2CO_3–$NaHCO_3$, pH 9.0–10.5 — all with 10 mM ME. The assay mixture containing *p*-coumaryl-CoA (3.28 nmol), [U-^{14}C]agmatine (50 nCi) and enzyme in a final vol. of 0.2 ml was incubated at 30° for 30 min. The reaction was stopped by the addition of 0.1 ml 17 M HOAc and 20 μl samples were separated in solvent C. The products were quantified as described in *TLC*. The products were identified by co-TLC with known samples of agmatine, coumarylagmatine and hordatines A/B in solvents B and C and also by electrophoresis in pyridine–HOAc–H_2O (4:5:200), pH 4.6 on 3 MM paper (80 V/cm) for 30 min.

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