be quantitatively converted into XII, contrary to the results of the pyrolysis of *p*-methoxyphenylazide. The reason why electrolyses of III, IV, and VIII do not give the corresponding phenazines is not clear at present. Detailed studies are now in progress.

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The Enzymatic Conversion of (−)-Lupinine to (−)-(*trans*-4′-Hydroxycinnamoyl) lupinine by Extracts of *Lupinus* Seedlings

(−)-(*trans*-4′-Hydroxycinnamoyl) lupinine (III) was found to be synthesized from (−)-lupinine (I) and *trans*-4-hydroxycinnamic acid (II) by enzymes in *Lupinus* seedlings in the presence of adenosine triphosphate and co-enzyme A as cofactors. The conversion of *trans*-cinnamic acid (IV) to (−)-(*trans*-cinnamoyl) lupinine (V) by the enzyme systems was negligible although the cinnamoyl-CoA ligase activity was clearly observed.

Keywords——enzymatic synthesis; *Lupinus luteus* seedlings; leguminosae; lupin alkaloid; lupinine; cinnamic acid derivatives; cinnamoyl lupinine derivatives; CoA; ATP

In the course of our investigations on the lupin alkaloids in the family leguminosae, we have established the presence of a new alkaloid, (−)-(*trans*-4′-hydroxycinnamoyl) lupinine (III), in the extracts of young seedlings of *Lupinus luteus*.[1] No detectable amount of III was found in the mature and immature seeds, and in the later stages of the plant growth.

However, its concentration increased rapidly during the first 4—8 days growth of *Lupinus* seedlings grown in both the dark and the daylight at 25—30°; during further growth of the plant the concentration of III fell gradually to a very low level.

Cinnamic acid and a variety of closely related hydroxycinnamic acids have been postulated for a long time to be involved in a great variety of important metabolic pathway in higher plants, including the formation of flavonoids, lignin, chlorogenic acid and many other plant products.

This communication reports new enzymatic systems in *Lupinus* seedlings capable of forming III from (−)-lupinine (I) and *trans*-4-hydroxycinnamic acid(II) in the presence of ATP and CoA as cofactors, as shown in Fig. 1.

Enzyme preparations were obtained from the hypocotyls of seedlings of *Lupinus luteus* grown in the dark for 5—6 days at 30° essentially as described in previous papers[2—4]: the hypocotyls were homogenized in a mortar with Si sand and 0.1 M K-phosphate buffer, pH 7.5,

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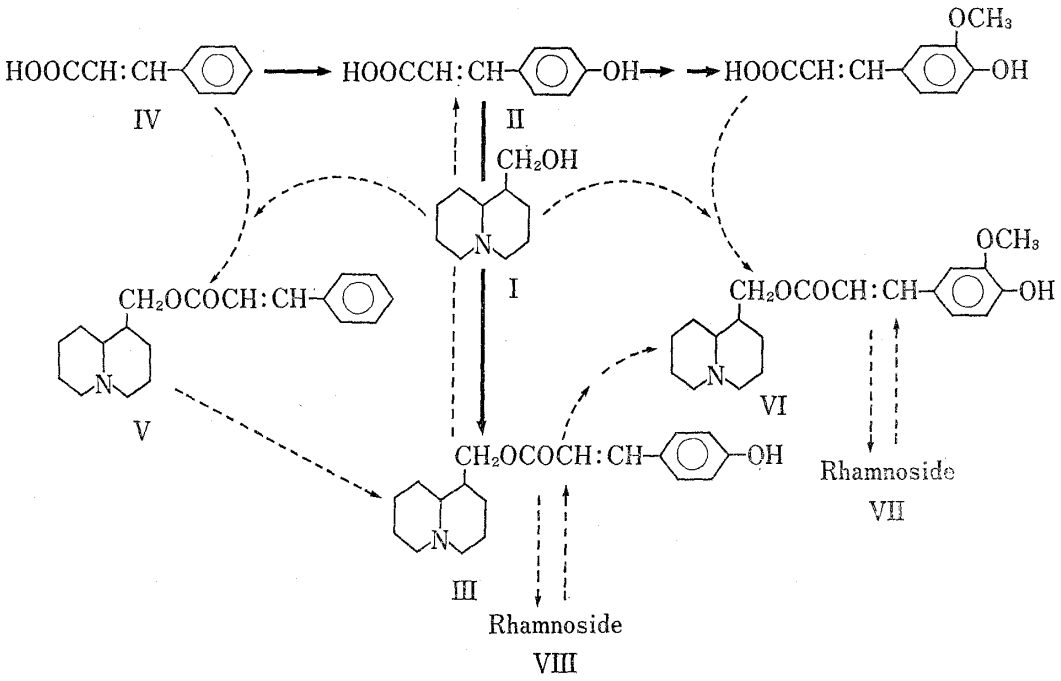

Fig. 1. Scheme for the Biosynthesis of (−)-(*trans*-4′-Hydroxycinnamoyl)-lupinine (III) by Enzymes in *Lupinus* Seedlings

------: possible biosynthetic pathways

containing 0.5% (v/v) 2-mercaptoethanol, 1.0 mM EDTA and 0.25 M sucrose using 0.5 ml buffer for each gram of the seedlings. The clear supernatant solutions, recovered by centrifugation at 25000 ***g*** for 30 min, partially purified by ammonium sulfate precipitation, Dowex 1-treatment and desalting on a Sephadex G-25 (fine) column, were used as the source of enzyme preparation.

Reaction mixtures used to demonstrate the formation of III contained (−)-lupinine (12.5 μ moles), *trans*-4-hydroxycinnamic acid (2.5 μ moles), co-enzyme A (CoA) (2.5 μ moles), adenosine triphosphate (ATP) (12.5 μ moles), $MgSO_4$ (12.5 μ moles), KF (48 μ moles) and 0.5 ml of enzyme preparation in a final volume of 1.2 ml. The pH of incubation mixture was normally maintained at pH 7.5 by 0.1 M K-phosphate buffer. The incubation was usually carried out at 30° for 120 min and terminated by the addition of 0.5 ml of 20% K-carbonate.

The identity of the reaction product as III was confirmed by chromatographic comparison with natural material: the reaction product, extracted from the supernatant of terminated reaction mixtures with CH_2Cl_2, was applied to a high-speed liquid chromatography employing a monitoring flow system (310 nm) coupled to recorder, using Lichrosorb SI 100 (Merck, particle size 10 μ m) and 3% MeOH–CH_2Cl_2: 28% NH_4OH (500: 1, v/v), and also to Si gel TLC as described in our previous paper.[1] These methods indicated clearly the presence of a product, reacting positively with Dragendorff's reagent, that was inseparable from added natural compound (III).

The reaction product (III) was not formed in reaction mixture lacking ATP or CoA, nor was the product formed when the enzyme preparation was preheated at 100° for 15 min. It can be seen that the enzymatic potential for the conversion of *trans*-4-hydroxycinnamic acid (II) to (−)-(*trans*-4′-hydroxycinnamoyl) lupinine (III) requires a heat-labile factor which has an absolute requirement for ATP and CoA, and a partial requirement for Mg ion.

The present results, together with the formations of chlorogenic acid in cell suspension cultures of *Nicotiana alata* by Stockigt and Zenk[5] and of *p*-coumarylquinic acid and chlorogenic acid in tomatoes by Rhodes and Wooltorton,[6] strongly suggest the CoA thioester also has a

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