Agroclavine Hydroxylase of *Claviceps purpurea*

By JOAN CHEN HSU and JOHN A. ANDERSON*
(*Chemistry Department, Texas Tech University, Lubbock, Texas* 79409)

Summary The NADPH-dependent hydroxylation of agroclavine to elymoclavine takes place in a cell-free system from *Claviceps purpurea* PRL 1980.

OGUNLANA *et al.*[1] recently reported the conversion of chanoclavine I into elymoclavine in a crude supernatant fraction from *Claviceps*, strain 231. No labelled agroclavine was found after incubation, and the extract was inactive for the conversion of agroclavine into elymoclavine. The authors concluded that, contrary to the generally accepted pathway, agroclavine is not an intermediate between chanoclavine I and elymoclavine in the pathway of clavine alkaloid biosynthesis.

The 60—80% ammonium sulphate fraction from *Claviceps purpurea* PRL 1980 catalyses the conversion of tryptophan into chanoclavines I and II, agroclavine, and elymoclavine.[2] Aerobic incubation of this enzyme system, [^{14}C]- or [^{3}H-] agroclavine, and liver concentrate or an NADPH-generating system (NADP, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase), was carried out for 18 h at 25°. After extraction, t.l.c., and radioautography, elymoclavine was identified as the major product. Small amounts of setoclavine and a Van Urk's-negative yellow fluorescent compound were also formed. The conversion of agroclavine into elymoclavine, determined by liquid scintillation counting, was 15%. The crude supernatant fraction, prior to ammonium sulphate fractionation, had no measurable activity.

The agroclavine-dependent oxidation of NADPH was measured spectrophotometrically. The rate of NADPH oxidation in the absence of added agroclavine was subtracted from the rate for the complete system. The rate was proportional to the enzyme concentration. With 1·8 μmoles agroclavine, 0·70 μmole NADPH, and 0·51 mg protein, in 3·0 ml of 0·020 M-potassium phosphate buffer pH 7·0, the specific activity was 2·3 μmoles NADPH/h/mg at 25°. The amount of activity was 8·1 μmoles/h/l of original fungus culture. The maximum rate of synthesis of elymoclavine in shake cultures was 10 μmoles/h/l. The amount of agroclavine hydroxylase activity is therefore similar to the rate of elymoclavine synthesis *in vivo*. The results indicate that agroclavine is a direct precursor of elymoclavine *in vitro* and *in vivo*.

The results of Ogunlana *et al.*[1] indicate that there is a pathway from chanoclavine I to elymoclavine which does not involve agroclavine. It may be significant that their system does not catalyse the synthesis of agroclavine from chanoclavine I, since the amount of agroclavine is about equal to the amount of elymoclavine produced by *Claviceps* strain 231 *in vivo*.[3] It is evident that further experiments with cell-free preparations from various *Claviceps* strains are needed to clarify the relationships between chanoclavine I, agroclavine, and elymoclavine, as well as other reactions in the pathway of clavine alkaloid biosynthesis.

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