Biosynthesis of Ergot Alkaloids. Enzymatic Closure of Ring D of the Ergolene Nucleus

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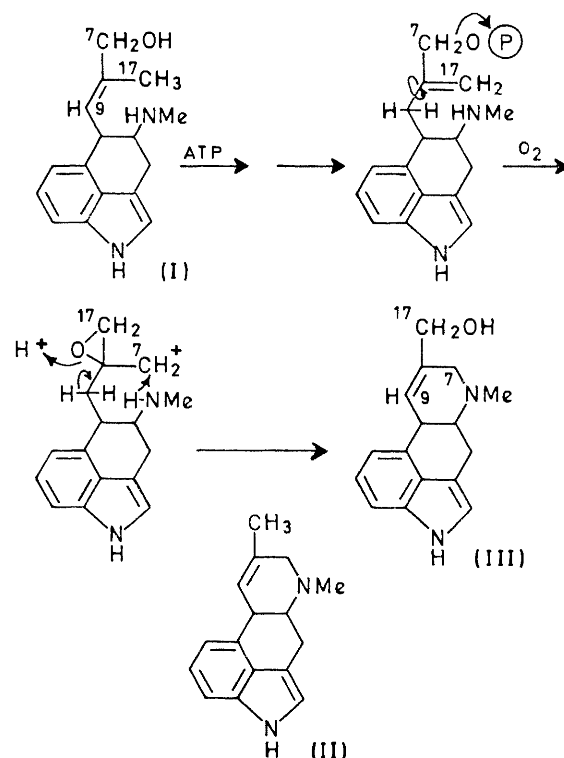
Summary Oxidative closure of ring D of the ergolene nucleus has been accomplished by *in vitro* conversion of chanoclavine-I into elymoclavine.

CHANOCLAVINE-I (I) is one of the three isomers of chanoclavine isolated from *Claviceps*.[1] Except for the chanoclavines, all known ergot alkaloids possess a closed D ring. The biosynthetic sequence: agroclavine (II) → elymoclavine (III) → lysergic acid has been generally accepted,[2] but, although *in vivo* conversion of (I) into (III) has been established repeatedly,[3,4] the precise position of (I) in the biosynthetic scheme of ergot alkaloids has remained obscure. The assumption has invariably been that this conversion occurs by way of (II).

In order to clarify the place of (I) in the biosynthetic pathway of the ergolenes, we have undertaken experiments to develop a cell-free system capable of closing ring D enzymatically. For this we have used mycelium from saprophytically grown *Claviceps*, strain 231,[5] carefully washed, frozen, then ground in a mortar and suspended in 0·25M-sucrose and dialysed. The supernatant from 12,100 × g centrifugation was used as crude-enzyme fraction. Other strains, *e.g.* SD/58, have also been used.

The enzyme fraction operating in a 0·05M-phosphate buffer at pH 7·4, requires, besides substrate (I), adenosine triphosphate, Mg^{2+}, reduced nicotinamide adenine dinucleotide phosphate, and free oxygen as co-factors. The system was maintained on a metabolic shaker at 26—28° for 9 h. The isolated alkaloids were separated and identified by t.l.c.† Co-crystallization of the product formed from [^{14}C]chanoclavine with elymoclavine to constant activity confirmed the identification.

In some preparations, incorporation of (I) into (III) exceeded 20%. No detectable quantity of (II) was obtained. However, a small amount (*ca.* 0·5%) of a compound 'x' formed which exhibited similar chromatographic behaviour to that of (II) but was eventually separated from co-spotted (II) in two-dimensional t.l.c. by use of suitable solvent systems.



Since it is conceivable that (II) might be an intermediate in the conversion and might metabolize too rapidly for accumulation to detectable levels in the reaction mixture, (II) was tested as substrate. It was converted poorly into

† Silica Gel G (Merck); solvent systems: chloroform–ethanol (5 + 1), chloroform–methanol– conc. ammonium hydroxide (80 + 20 + 0·2), chloroform–diethylamine (9 + 1).

(III), indicating that (II) could not function as a rapidly metabolizing intermediate.

The need for free oxygen and for NADPH in the conversion *in vitro* indicate that a mono-oxygenase is implicated in the process. Oxygenase has previously been indicated to participate in the ergot-alkaloid biosynthesis.[6] Since a *cis–trans* isomerization about the allylic double bond occurs during conversion of (I) into (III) *in vivo*[3] and a loss of ^{3}H from position C-9 of labelled (I) has been observed,[7] one can envisage that the transformation includes a $\Delta^{8(9)} \rightarrow \Delta^{8(17)}$‡ isomerization followed by 8,17-epoxidation and final opening of the epoxide ring through the loss of a proton at C-9 (or at 7 with subsequent shift of a double bond). The need for ATP in the reaction calls attention to a ring-closure mechanism involving a carbonium ion generated by loss of phosphate ion from (I) phosphate.

In some cases, biosynthetically prepared [5-^{14}C]-(I) was used and the conversion product then identified by radiographic coincidence of radioactivity and reference (III). Scintillation counting was used to assess percentage of conversion. Blanks without substrate (I) were always run concomitantly.

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‡ Numbering analogous to that of ergolene.

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