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On the Biosynthesis of Peptide Ergot Alkaloids

Recent investigations into the biosynthesis of the peptide ergot alkaloids have clarified the origin of the various components. Lysergic acid, phenylalanine, proline, valine and leucine are incorporated into the appropriate constituents of the peptides[1–5] and the α-hydroxyamino acid portions of ergotamine, ergocornine and ergokryptine are derived from the corresponding amino acids[3–7]. The nitrogen in the amide linkage between lysergic acid and the α-hydroxyamino acid does not originate from lysergic acid amide but rather, most likely, from the precursor amino acid, i.e., alanine or valine, respectively[4,5]. Other than that, however, there is little positive information on the pathway and mechanisms involved in this biosynthesis. Neither D-lysergyl-L-valine nor D-lysergyl-L-alanine were incorporated as intact units into the appropriate peptide alkaloids[3,7] and likewise, L-valyl-L-leucine, L-leucyl-L-proline lactam and L-valyl-L-proline lactam can be excluded as free intermediates in the biosynthesis of ergokryptine and ergocornine, respectively.

In order to test for the possible involvement of free tripeptide intermediates, we synthesized L-valyl-(1-^{14}C)-L-valyl-L-proline. L-Valyl-L-proline (Miles Ltd.) was converted into the methyl ester with dimethoxypropane/HCl and condensed with N-carbobenzoxy-L-valine-1-^{14}C by the anhydride method as described by TILAK[8]. Removal of the protecting groups by mild alkaline hydrolysis and hydrogenolysis gave the radioactive tripeptide (spec. act. 23 μCi/μmole), which was fed to cultures of the ergocornine/ergokryptine-producing *Claviceps purpurea* strain, Fb 299. Two 50 ml cultures received 3.55×10^6 dpm precursor on day 4 and were harvested 10 days later. The alkaloid was extracted from the mycelium, ergocornine and ergokryptine were purified by chromatography and crystallization with carrier material[4] and were degraded as described earlier[9]. The results (Tables I and II) clearly show that radioactivity from L-valyl-(1-^{14}C)-L-valyl-L-proline is incorporated only after breakdown of the precursor into its component amino acids because (a) not only ergocornine (as expected) but also ergokryptine is labeled and (b) ergocornine is labeled not only in the α-hydroxyvaline moiety (as expected) but also in the valine moiety[10]. This suggests that L-valyl-L-valyl-L-proline is not a free intermediate in ergocornine biosynthesis. Analogous results have been obtained in experiments with L-valyl-L-leucyl-L-proline[11].

Table I. Incorporation of L-valyl-(1-^{14}C)-L-valyl-L-proline into ergot alkaloids of the ergotoxine group by cultures of *Claviceps purpurea*

Amount of precursor fed	0.07 μmoles
Specific radioactivity of precursor	5.06×10^7 dpm/μmole
Amount of alkaloid formed	17.6 μmoles
Specific radioactivity of ergocornine	1.05×10^4 dpm/μmole
Specific radioactivity of ergokryptine	7.5×10^3 dpm/μmole

Table II. Degradation of ergocornine and ergokryptine from experiments with L-valyl-(1-^{14}C)-L-valyl-L-proline

	Specific radioactivity in dpm/μmole (% of total)	
	Ergocornine	Ergokryptine
Starting alkaloid	264 (100) [304 (100)][a]	515 (100)
α-Hydroxyvaline	122 (46) [128 (42)][a]	500 (97)
Valine	100 (38) [110 (36)][a]	—
Leucine	—	n.d.

n.d., not determined. [a] For comparison, given in square brackets are the figures obtained in a feeding experiment with D, L-valine-(1-^{14}C) from ref. [3].

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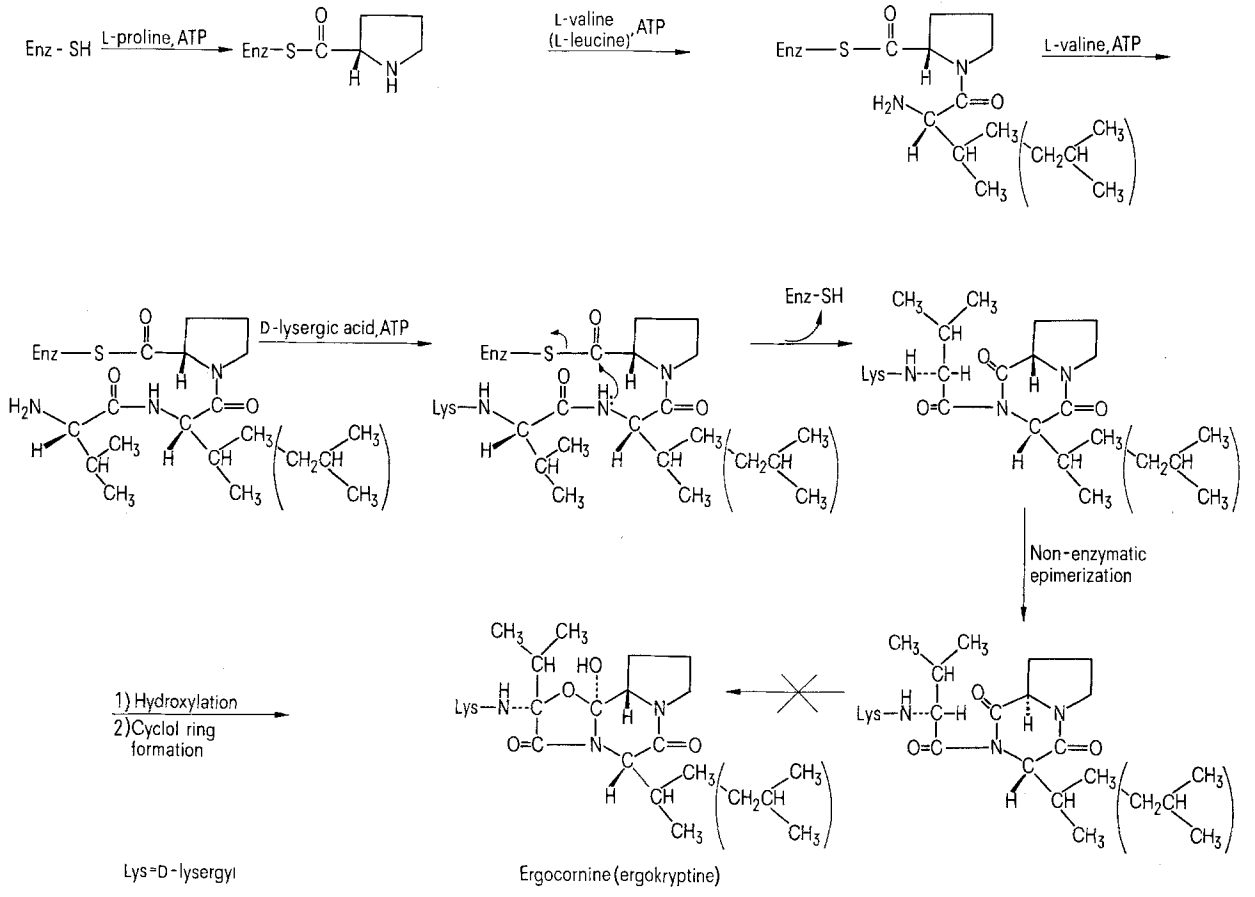

Based on the sum of the negative evidence, which seems to exclude virtually all the plausible free intermediates, it is more than likely that the peptide chain formation takes place in a concerted fashion on a multienzyme complex. The chain could grow starting from the lysergic acid end, analogous to the direction of chain growth in gramicidin S biosynthesis, or starting from the proline end, as is suggested by the finding that in ergocornine derived from valine-^{14}C the α-hydroxyvaline moiety has a higher specific radioactivity than the valine moiety[3] (cf. Table II). In any event, the reaction sequence would produce a lysergyltripeptide which is covalently linked to a group on the enzyme, possibly an SH-group, through the carboxyl group of proline. Release from the enzyme could plausibly occur by intramolecular attack of the nitrogen of the amino acid adjacent to the proline, leading directly to formation of the lactam ring. A principally similar mode of lactam ring formation has been proposed some time ago by RAMSTAD[12]. The D-proline analog of a compound of the type resulting from this cleavage, which would be the first free intermediate in the biosynthesis, has recently been isolated from ergot[13]. α-Hydroxylation of the amino acid adjacent to the lysergic acid, possibly via a 2,3-dehydro intermediate, followed by cyclol formation would complete the reaction sequence. The Scheme outlines the proposed biosynthetic sequence, which provides the basis for further experimental investigations[14].

Zusammenfassung. Fütterung von L-Valyl-(1-^{14}C)-L-valyl-L-prolin an *Claviceps purpurea* und Abbau des erhaltenen Ergocornins und Ergokryptins zeigt, dass dieses Tripeptid, ebenso wie andere früher untersuchte Peptide, sehr wahrscheinlich kein freies Zwischenprodukt in der Biogenese der Mutterkornalkaloide vom Peptidtyp ist. Es wird vorgeschlagen, dass die Biosynthese dieser Peptidalkaloide bis zur Stufe eines (D-Lysergyl-L-valyl)-L-valyl(oder leucyl)-L-prolin-lactams an einem Multienzym-Komplex ohne freie Zwischenprodukte abläuft.

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