The Stage of *N*-Methylation in Ergot Alkaloid Biosynthesis

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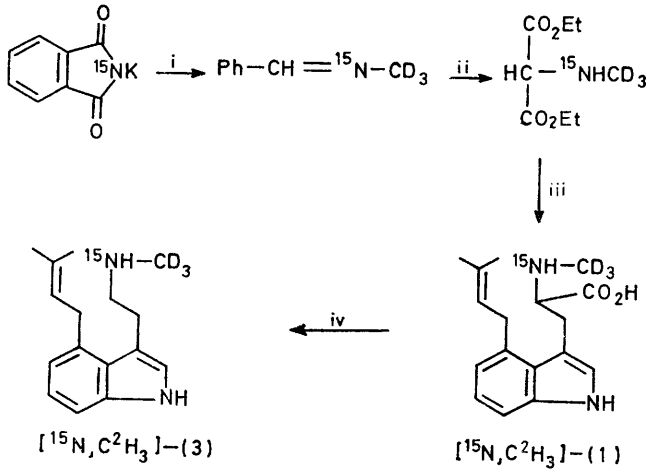
Summary Cultures of *Claviceps* species, strain SD 58, efficiently incorporate *N*-methyl-4-($\gamma\gamma$-dimethylallyl)-tryptophan (**1**) as an intact unit into elymoclavine (**2**), whereas the corresponding tryptamine (**3**) is not utilized, suggesting *N*-methylation as the second pathway-specific step in ergot alkaloid biosynthesis and rendering pyridoxal phosphate catalysis of C-ring closure unlikely.

ASSEMBLY of the tetracyclic ergoline ring system of the ergot alkaloids from the precursors L-tryptophan, dimethylallyl pyrophosphate, and a methyl group from methionine[1] involves a sequence of reactions leading from 4-($\gamma\gamma$-dimethylallyl)tryptophan (DMAT), the first intermediate in the pathway,[1] to chanoclavin-I, the first tricyclic compound, followed by closure of ring D. Methylation of the non-indolic nitrogen must occur prior to or during C-ring closure,[1] since we observed that *N*-demethylchanoclavine-I is not utilized in the biosynthesis.[2] Barrow and Quigley[3] reported the isolation of a new amphoteric indole derivative from oxygen-deprived cultures of *Claviceps fusiformis*,

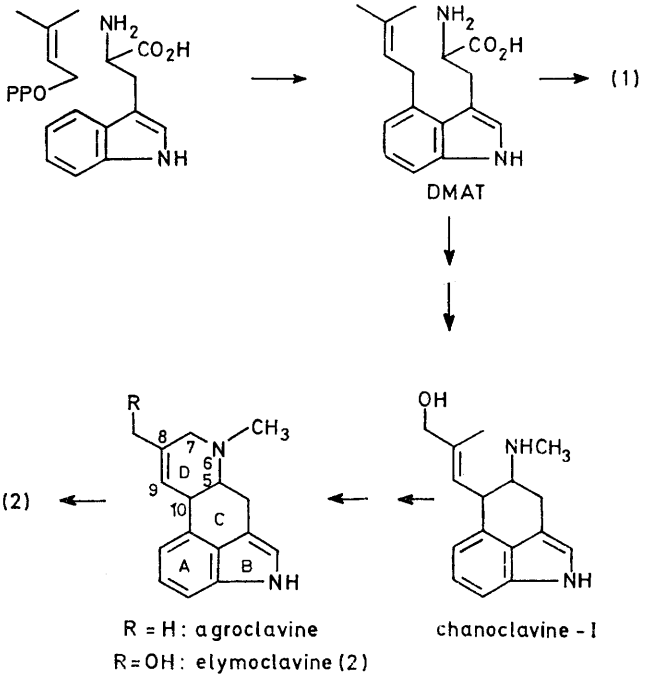
which they identified as N^{α}-methyl-DMAT (**1**). Compound (**1**), labelled with ^{14}C in the *N*-methyl group, gave labelled agroclavine with 1·4% incorporation when fed to normal cultures of *C. fusiformis*.[3] This rather low incorporation leaves serious doubts whether (**1**) is converted intact into ergot alkaloids. Significant incorporations (6·4%) were observed earlier for N^{α}-methyltryptophan[4] and were shown to be due to demethylation of the substrate to the true precursor, tryptophan.[5]



SCHEME 1. i, (a) CD_3Br, (b) H^+, (c) OH^-, (d) PhCHO; ii, (a) Et_2SiH_2, $(PPh_3)_3RhCl$, (b) $CHBr(CO_2Et)_2$, (c) H_2–Pd/C; iii, (a) HCO_2H–Ac_2O, (b) 4-(γγ-dimethylallyl)gramine, dimethyl acetylenedicarboxylate, (c) OH^-, (d) H^+, heat; iv, Ph_2O, heat.

To probe for intact incorporation of this compound we synthesized intramolecularly labelled [α-^{15}N,N^{α}-*Me*-$^2H_3$]-(**1**) from potassium [^{15}N]phthalimide (99% ^{15}N, Stohler Isotope Chemicals Co.) and [$^2H_3$]methyl bromide (99·5% ^2H, Stohler) (Scheme 1). Mass spectral analysis (chemical ionization, isobutane, Dupont 492 BR instrument) of the product indicated the presence of at least 89% of molecules containing both ^{15}N and three atoms of deuterium. Since no unlabelled reference sample was available for mass spectral comparison, this represents a minimum value; the actual percentage of ^{15}N,$^2H_3$-labelled molecules most likely is higher. A sample (52 mg) of this material was fed to a 25 ml shake culture of *Claviceps* spec., strain SD 58 in medium NL 406 on day 6. Five days later the culture was harvested, and elymoclavine (**2**) was isolated[4] and analysed by chemical ionization mass spectrometry. The results (Table, expt. 1) indicated the presence of only two species: unlabelled molecules from endogenously synthesized precursor, and species which have arisen from intact conversion of the added precursor. Hence, (**1**) is efficiently converted into (**2**) and in the process the N–CH_3 bond is not cleaved.

To probe further if decarboxylation is the next step in the biosynthetic sequence, we converted a sample of [^{15}N,C^2H_3]-(**1**) into the corresponding amine, [^{15}N,C^2H_3]-(**3**), by heating in diphenylether.[6] Feeding of both [^{15}N,C^2H_3]-(**1**) and [^{15}N,C^2H_3]-(**3**) to replacement cultures in m/15 phosphate buffer, pH 7·3, under identical conditions again indicated efficient and intact incorporation of [^{15}N,C^2H_3]-(**1**), but no significant conversion of [^{15}N,-C^2H_3]-(**3**), into (**2**) (Table, expts. 2 and 3). Thus, loss of the carboxy-group of *N*-methyl-DMAT, which must occur prior to or concerted with the closure of ring c,[7] is apparently preceded by modification of the isoprenoid side chain. The efficiency and specificity of the conversion of (**1**) into (**2**) makes it likely that (**1**) is a normal biosynthetic intermediate. It is therefore suggested that methylation of the amino group of DMAT is the second pathway-specific step in ergoline biosynthesis (Scheme 2).



SCHEME 2

The finding that methylation of the amino nitrogen precedes c-ring closure has important mechanistic implications. The latter process and the decarboxylation step have generally been thought to involve pyridoxal phosphate as the mechanistically most plausible cofactor.[1] Formation of a pyridoxal phosphate–Schiff's base with the amino group would allow generation of a carbanion at C-5, either

TABLE. Mass spectral analysis of precursors and products of feeding experiments with *Claviceps sp.*, strain SD 58.

Expt.	Compound analysed	MH^+	$(MH+1)^+$	$(MH+2)^+$	$(MH+3)^+$	$(MH+4)^+$
	[^{15}N,C^2H_3]-(**1**)	0	0	<3%	<8%	>89%
	[^{15}N,C^2H_3]-(**3**)	0	0	<6%	<12%	>82%
1	(**2**) from [^{15}N,C^2H_3]-(**1**) in normal submerged culture	69%	1%	0	1%	29%
2	(**2**) from [^{15}N,C^2H_3]-(**1**) in replacement culture	<44%	<1%	<2%	<8%	>45%
3	(**2**) from [^{15}N,C^2H_3]-(**3**) in replacement culture	>98%	0	0	0	<2%

† Ergoline numbering.

by decarboxylation or loss of a proton, which could react with a potential carbonium ion site at C-10. However, such a classical pyridoxal phosphate catalysis requires coplanarity of the imine double bond and the pyridine ring,[8] which is not possible in an *N*-methylated amino acid or amine. While the involvement of a simple Schiff's base is still possible, and hence a general process as described is still feasible, one should now also consider a mechanism which would be initiated by an oxidative decarboxylation of the amino acid to an imine as in other alkaloid biosyntheses.[9]

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