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BIOSYNTHESIS OF ERGOT ALKALOIDS AND RELATED COMPOUNDS†

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INTRODUCTION

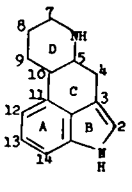
Ergot alkaloids, the metabolic products of various species of the parasitic fungus *Claviceps* (Clavicipitales), have many fascinating features which may account for the long-standing interest in this class of natural products. The toxic effects of these compounds have been the cause of mass poisonings in both animals and man throughout history. The animal poisonings usually resulted from feeding on grass infested with the ergot fungus, whereas the toxic effects in man stemmed from eating bread or other products made from rye flour which was contaminated with sclerotia of *Claviceps purpurea* (Fries) Tulasne, whose principal host is the rye plant. Ergot poisoning in man, known as "holy fire", "St Anthony's fire" or in more recent times as "ergotism", often took epidemic proportions in Central Europe during the Middle Ages, causing the death of thousands of people; yet it was not until the 17th Century that ergot was identified as the causative agent. In later years the useful pharmacological effects of ergot were recognized, making the ergot alkaloids a valuable pharmaceutical commodity. In addition to being useful medicinal agents themselves, ergot alkaloids have been a stimulus in the development of other drugs by providing the first structural prototype of a particular pharmacological activity. This is especially true for the pronounced psychotomimetic activity associated with the semisynthetic lysergic acid diethylamide (LSD), which led to the development of the broad field of psychopharmacology. Another interesting aspect of ergot alkaloids is their occurrence; from their structures they are clearly true alkaloids, yet their most prominent source is not a higher plant but a fungus. While this is of considerable advantage in terms of their production, both industrially and in biosynthetic studies, it does have the effect that ergot alkaloids are occasionally somewhat "disowned" by traditional workers in the alkaloid field. More recent views tend not to limit alkaloids to those compounds occurring in green plants, but to include microbial and animal constituents, and even the purist has to recognize the fact that ergot alkaloids do occur in higher plants. The chemistry of ergot alkaloids has presented many challenges and, even after the successful total synthesis of lysergic acid and of the intricate peptide portion, continues to pose fascinating problems and to provide unexpected reactions. Finally, as hopefully this review will show, the problem of their biological formation is most fascinating, unexpectedly complex and full of surprises.

It is the main purpose of this review to develop a picture of our current knowledge of the formation of ergoline derivatives in nature and to focus on unsolved questions which merit further investigation. To put the biosynthetic problem in perspective, the formation of some related groups of compounds will be discussed and some of the recent developments in the chemistry and pharmacology of ergolines will be outlined. The latter discussion of necessity will be far from complete. Since the chemistry and pharmacology of ergot alkaloids has been reviewed extensively in several books,[1-4] only some of the current trends and more significant developments of the last 10–12 years will be highlighted, essentially using Hofmann's book[3] as the baseline. Further information can be found in two general reviews by Gröger[5,6] and in several of the more specialized reviews quoted later on.

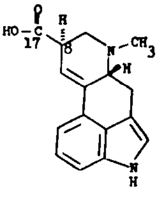
ERGOT ALKALOIDS AND RELATED COMPOUNDS

Naturally occurring ergolines and related structures

Lysergic acid derivatives. The structural characteristic of ergot alkaloids is the presence of the tetracyclic ergoline ring system (**1**) or, in a few cases, a slight modification of this ring system. Most of the naturally occurring alkaloids are derivatives of 6,8-dimethyl-$\Delta^{8,9}$- or $\Delta^{9,10}$-ergolene; they can be divided into two major classes. One of these consists of the derivatives of lysergic acid (**2**) and the other comprises the simpler, so-called "clavine" alkaloids. All the lysergic acid derivatives isolated to date are lysergic acid amides, in which the amide portion can be a small peptide or a simpler alkylamide. The "classical" peptide ergot alkaloids are characterized by a modified tripeptide containing proline and an α-hydroxy-α-amino acid which has undergone cyclol formation with the carboxyl carbon of proline. Structural variability



1: Ergoline



2: d-Lysergic acid

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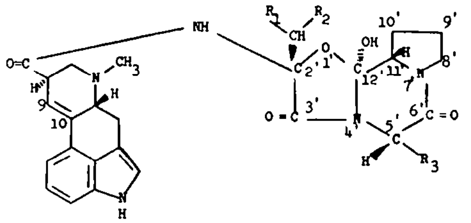
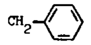
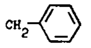
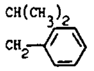
†Dedicated to Professor Matazo Abe, whose discovery of the clavines and pioneering fermentation work laid the foundation for biosynthetic studies on ergot alkaloids.

Table 1. Naturally occurring, cyclol-type peptide ergot alkaloids

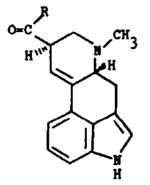


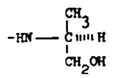
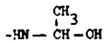
Name	R_1	R_2	R_3	Reference
Ergotamine	H	H		(7)
Ergosine	H	H	$CH_2CH(CH_3)_2$	(8)
9,10-Dihydroergosine[a]	H	H	$CH_2CH(CH_3)_2$	(9)
Ergocristine	CH_3	CH_3		(10)
α-Ergokryptine	CH_3	CH_3	$CH_2CH(CH_3)_2$	(11)
β-Ergokryptine	CH_3	CH_3	$CH(CH_3)CH_2CH_3$	(12)
Ergocornine	CH_3	CH_3	$CH(CH_3)_2$	(11)
Ergostine	H	CH_3		(13)

[a] $\Delta^{9,10}$-double bond in the lysergic acid moiety reduced.

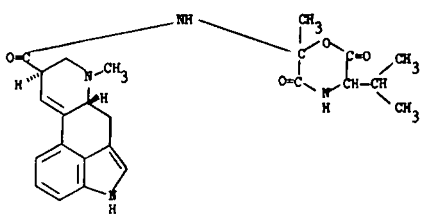
exists in the substituents at C-2 and C-5 of the cyclol peptide moiety, as shown in Table 1. In addition to the series of lysergic acid peptides shown in Table 1, which are typical constituents of *Claviceps purpurea*, one analogous derivative of dihydrolysergic acid, dihydroergosine, has been isolated from *Sphacelia sorghi*, a close relative of *Claviceps*. A few other amino acid derivatives of lysergic acid have been isolated from *Claviceps*. One compound, ergosecaline, isolated by Abe *et al.*[14] gave upon hydrolysis lysergic acid, pyruvic acid and valine and was, on that basis, tentatively assigned structure 3. Of particular biogenetic significance is the recent isolation, from an ergocristine-producing *Claviceps purpurea* strain, of a lysergyl peptide which was identified as N-[N-(d-lysergyl)-L-valyl]-L-phenylalanyl-D-proline lactam (4).[15] Two other compounds which had been isolated earlier, d-lysergyl-L-valine methyl ester[16] and d-lysergyl-L-valine amide[17] are now considered to be artefacts arising from the very readily occurring solvolytic cleavage of 4 during workup.[15] Table 2 lists some of the simpler, naturally occurring lysergic acid derivatives. Ergonovine was isolated simultaneously in four different laboratories (which accounts for the multitude of synonyms); it is the main constituent of the "water-soluble fraction" of the

Table 2. Naturally occurring lysergic acid derivatives other than peptides

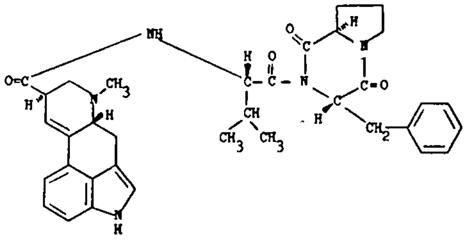


Name	R	Reference
Ergonovine (ergobasine, ergometrine)	 $-HN-C(CH_3)(H)(CH_2OH)$	(18-21)
Lysergic acid α-hydroxyethylamide	 $-HN-CH(CH_3)-OH$	(22)
Lysergic acid amide (ergine)	$-NH_2$	(22)
Lysergic acid	-OH	(23)
$\Delta^{8,9}$-Lysergic acid (paspalic acid)	-OH[a]	(24)

[a] $\Delta^{9,10}$ double bond shifted to $\Delta^{8,9}$ position.



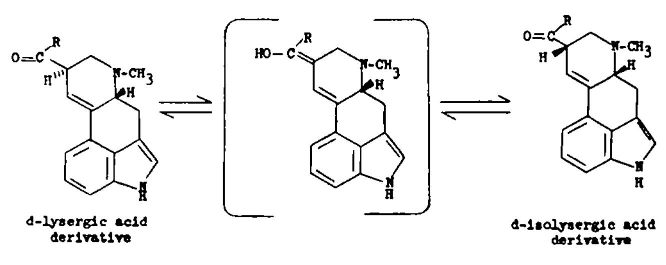
3· Ergosecaline



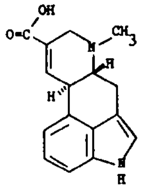
4: N-[N-(d-lysergyl)-L-valyl]-L-phenylalanyl-D-proline lactam

alkaloids of the ergot drug and is responsible for the pronounced oxytocic activity of this fraction. In addition to its common occurrence in *Claviceps purpurea*, the source of the ergot drug (Secale cornutum, the dried sclerotia of *C. purpurea*), ergonovine is also frequently found in *Claviceps paspali*. The other compounds listed in Table 2 are all primarily produced by Paspalum ergot. It is somewhat questionable whether lysergic acid amide is truely a natural alkaloid; it always co-occurs with the corresponding α-hydroxyethylamide which spontaneously decomposes into acetaldehyde and lysergic acid amide and may thus in a sense be an artefact. However, such a distinction is really only of some significance in biosynthetic considerations. Paspalic acid (**5**), which is the major alkaloid in Portuguese *C. paspalum* strains[24] but has also been observed in *C. purpurea*,[23a] occupies an intermediate position between the clavine alkaloids and the lysergic acid derivatives. It easily undergoes double bond isomerization at neutral or alkaline pH to give lysergic acid, which caused great problems in its isolation. Pure paspalic acid could only be obtained from the mixture by using the known photochemical hydration of the $\Delta^{9,10}$-lysergic acid into lumilysergic acid, which could then be readily separated from paspalic acid.[24] It is interesting to note that while paspalic acid occurs in abundance in certain *Claviceps* strains, free lysergic acid is never found in large quantities. This phenomenon may be of biogenetic significance and will be discussed later.

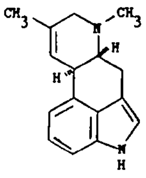
Finally, it should be mentioned that lysergic acid and its derivatives (but not dihydrolysergic acid) epimerize very readily at C-8 under a variety of conditions to give isolysergic acid and its derivatives:



Consequently, the d-lysergic acid derivatives listed usually co-occur with a matching partner in the d-isolysergic acid series. The latter are indicated by the ending "inine" instead of "ine"; thus, for example, ergotaminine is the C-8 epimer of ergotamine. The d-isolysergic acid derivatives are strongly dextrorotatory, whereas their d-lysergic acid counterparts are levorotatory or, occasionally, weakly dextrorotatory. Members of the two series can usually be separated easily by chromatography; in most systems the isolysergic acid derivatives have higher R_f values than the corresponding lysergic acid derivatives. Most importantly, while many of the compounds in the lysergic acid series exhibit pronounced pharmacological effects, the corresponding isolysergic acid derivatives are almost completely devoid of pharmacological activity.



5: Paspalic acid



6: Agroclavine

Simple ergolines and secoergolines. In 1951, Abe[26] isolated agroclavine (**6**) from ergot growing on *Agropyrum semicostatum* Nees and from saprophytic cultures of this *Claviceps* strain. This compound was the first member of a new class of ergot alkaloids, the so-called clavines. Sharing with the lysergic acid derivatives the characteristic tetracyclic ergoline ring system, the clavines carry C-17 in a lower oxidation state and the double bond in the D-ring may be in the 8,9- or 9,10-position or may be lacking altogether. Although originally isolated from *Claviceps* strains other than the typical producers of lysergic acid derivatives, clavines were soon found to invariably accompany lysergic acid derivatives in nature at least in traces (e.g., Ref. 27). The number of clavine alkaloids isolated has proliferated greatly over the years and new members continue to be added to the list. Table 3 presents the clavine alkaloids of known structure which have been isolated to-date. While most of the clavines contain only the carbon atoms of the 6,8-dimethylergoline system, a few exceptions can be seen in Table 3. An example is elymoclavine-O-β-D-fructoside, which has been isolated from saprophytic cultures of *Claviceps* strain SD 58, a producer of elymoclavine. The formation of this compound can be adequately explained by action of the enzyme invertase present in the organism, which transfers the fructose moiety from sucrose, an ingredient of the culture medium, to the hydroxyl group of water or alcohols, like elymoclavine.[30] Two other examples are fumigaclavine A, which is an acetate ester,[41] and the recently discovered paspaclavine,[40] which

Table 3. Naturally occurring clavines and secoergolines[a]

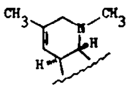
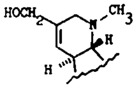
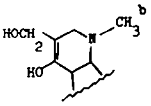
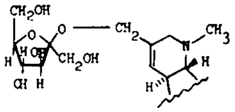
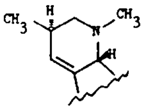
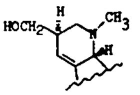
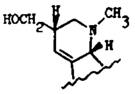
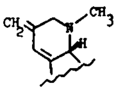
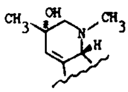
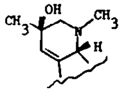
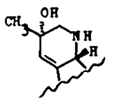
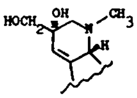
Name	Structure of ring D	Reference
$\Delta^{8,9}$-Ergolenes		
Agroclavine		(26)
Elymoclavine		(28)
Molliclavine		(29)
Elymoclavine-O-β-D-fructoside		(30)
$\Delta^{9,10}$-Ergolenes		
Lysergine		(31)
Lysergol		(31,32)
Isolysergol		(33)
Lysergene		(31)
Setoclavine		(34)
Isosetoclavine		(34)
Norsetoclavine		(35)
Penniclavine		(36)

Table 3 (*Contd*)

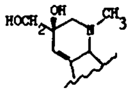
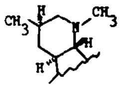
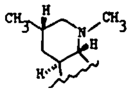
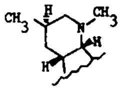
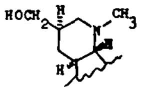
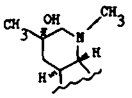
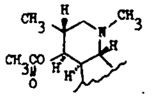
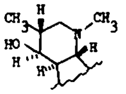
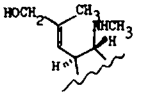
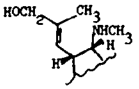
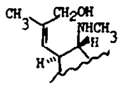
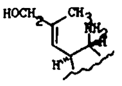
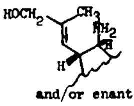
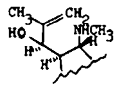
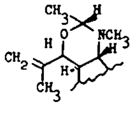
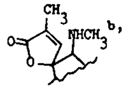
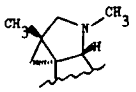
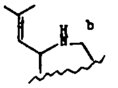
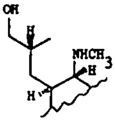
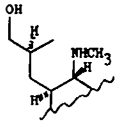
Name	Structure of ring D	Reference
Isopenniclavine		(34)
Ergolines		
Festuclavine		(37)
Pyroclavine		(38)
Costaclavine		(38)
Dihydrolysergol-I		(39)
Dihydrosetoclavine		(40)
Fumigaclavine A		(41)
Fumigaclavine B		(41)
6,7-Secoergolenes		
Chanoclavine-I		(34,38)
Chanoclavine-II	 + enantiomer	(42)
Isochanoclavine-I		(42)
Norchanoclavine-I		(43)
Norchanoclavine-II	 and/or enantiomer	(43,44)

Table 3 (*Contd*)

Name	Structure of ring D	Reference
Paliclavine		(40)
Paspaclavine		(40)
Rugulovasine A and B	 [b,c]	(45)
Other		
Cycloclavine		(46)
Clavicipitic acid	 [b]	(47,48)
dihydrochanoclavine-I		(249)
isodihydrochanoclavine-I		(249)

[a]Only ring D is shown; the remainder of the structure of all compounds is as in agroclavine (**6**).
[b]This structure should be considered tentative.
[c]The two compounds are considered to be diastereomers.

contains an extra acetaldehyde moiety. The stereochemistry of fumigaclavine A and B has recently been established[49,50] and the previous partial assignment[41] revised.

In addition to the tetracyclic ergolines and ergolenes, a number of compounds are found in nature in which the D-ring is not closed. The first compound in this class was chanoclavine-I, originally called chanoclavine,[34] which was later found to be one of a series stereoisomers.[42] The designation I or II after the name of an ergoline refers to compounds with *trans*- or *cis*-junction of ring C and D, respectively, or in the chanoclavine series to compounds with *trans* or *cis* arrangement, respectively, of the hydrogens at C-5 and C-10. The occurrence of both antipodes of chanoclavine-II, although not in equal quantity, is extremely interesting.[42] One of the enantiomers is the only example of a naturally occurring ergot alkaloid in which C-5 has *S* rather than *R* configuration. The levorotatory isomer predominates in nature, but since the absolute configuration of chanoclavine-II has not yet been determined it is unknown whether this enantiomer belongs to the 5*R* series. Several N-demethyl- or nor-analogs of normal clavines are found naturally in trace amounts and it appears likely that these are formed by demethylation of the parent compounds.[35,43,44]

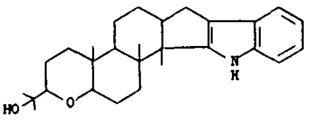
The structure of clavicipitic acid, a compound isolated from the amino acid fraction of ergot cultures[47,48] is still not entirely proven. In view of the possible biogenetic significance this compound will be discussed in more detail later.

Occurrence of ergolines in organisms other than the ergot fungus. Although most ergot alkaloids have been isolated from members of the genus *Claviceps*, some ergolines have also been found in other fungi. Spilsbury and Wilkinson[41] were the first to isolate three clavines, festuclavine and fumigaclavines A and B from *Aspergillus fumigatus*. The latter two have not yet been isolated from a *Claviceps* species. Yamano *et al.*[51] confirmed this observation and demonstrated in addition the presence of agroclavine, elymoclavine, chanoclavine and a new compound, fumigaclavine C, whose structure has not been determined. Agurell[52] isolated costaclavine from *Penicillium chermesinum* Biourge and Abe's group[53] has demonstrated the presence of various clavines in species of *Penicillium* and *Aspergillus* by paper chromatography. From *Penicillium concavo-rugulovasum* Abe *et al.*[54] isolated chanoclavine-I and two new interconvertible alkaloids, rugulovasines A and B, which are believed to be diastereoisomers.[45] Interestingly, no lysergic acid derivatives have so far been found in fungi outside the genus *Claviceps*.

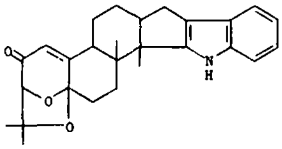
Even more exciting was the finding, in 1960, that ergoline derivatives occur in higher plants. Hofmann and Tscherter[55] made this discovery when they studied the active ingredients of "ololiuqui", a magic drug used by the Indians of Central America in their religious ceremonies, which consists of the seeds of two Convolvulaceae, *Ipomoea violaceae* L. and *Rivea corymbosa* (L.) Hall f. The first alkaloids isolated were lysergic acid amide, which is largely responsible for the hallucinogenic acitivity of ololiuqui, isolysergic acid amide and chanoclavine;[55] later Hofmann[32] also identified elymoclavine and lysergol. Since then, both the Swiss workers and numerous other groups have extended these investigations and have demonstrated the occurrence of many more ergoline alkaloids in *Ipomoea* and a few closely related genera. Gröger[56] found lysergic and isolysergic acid α-hydroxyethylamide in addition to the simple amides in several *Ipomoea* species, suggesting again that the lysergic acid amide may arise by breakdown of the α-hydroxyethylamide. Ergonovine has been isolated from *Ipomoea* species[56,57] and, interestingly, even peptide ergot alkaloids, ergosine and its epimer ergosinine, were found in *Ipomoea argyrophylla* Vatke.[58] Cycloclavine (cf. Table 3), representing a novel structural type and so far the only example of an ergoline not found in fungi, was isolated from *Ipomoea hildebrandtii* Vatke.[46] Following Hofmann's original discovery, many plants were screened for the presence of ergoline alkaloids, but their occurrence seems to be limited to some genera in the family Convolvulaceae. They are found in many, although not all, species of *Ipomoea* and *Argyreia* and in some species of *Rivea* and *Stictocardia* (cf. Refs 59, 60). An earlier report of the occurrence of agroclavine in *Cuscuta monogyna* Vahl[61] has more recently been refuted.[62] The surprising finding that ergoline alkaloids occur in the seeds of a higher plant initially raised the suspicion that the alkaloids might be produced by a contaminating fungus rather than by the plant tissue itself. However, a study by Taber and Heacock[63] showed that the alkaloids were located in the embryo, which was free of contaminating fungi, but not in the seed coat and membranes. Fungi were present in the seed coat only and they represented species of *Chaetomium* and *Fusarium* not known to produce ergot alkaloids. Additional evidence that the alkaloids are indeed formed by the plant tissue itself is the fact that they are also found in the vegetative tissue of the plant[56,64] and even in tissue cultures of *Ipomoea violacea* and *Rivea corymbosa.*[65]

Related compounds. Numerous indolic compounds have been isolated from natural sources and a survey of their structures is far beyond the scope of this review. However, a few compounds should be mentioned which have some special relationship to the ergot alkaloids. Two novel indole derivative, paspaline and paspalicine, have been isolated from mycelia of a strain of *Claviceps paspali* which produces paspalic acid.[66] Their structures were elucidated in Arigoni's laboratory. Paspaline was shown to have structure **7**[67,68] and paspalicine the related structure **8**.[69] In the course of the work on paspalicine, a third neutral indolic compound, paspalinine $C_{27}H_{31}NO_4$, was isolated from the same *Claviceps* strain.[69]

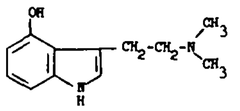
In view of the hallucinogenic activity of some lysergic acid derivatives, it was extremely interesting that the



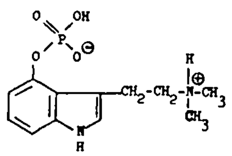
7: Paspaline



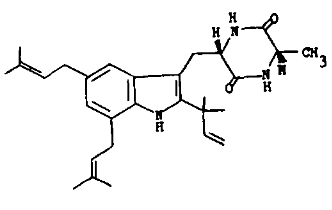
8: Paspalicine



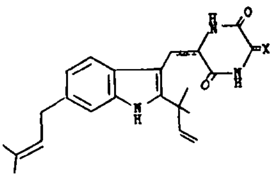
9: Psilocin



10: Psilocybin



11: Echinulin



X = O = **12a**: Neoechinuline
X = CH_2 = **12b**: Cryptoechinuline A

active ingredients responsible for the psychotropic activity of "teonanácatl", the sacred mushroom of the Mexican Indians, also turned out to be 4-substituted indoles. Teonanácatl comprises various species of fungi, largely belonging to the genus *Psilocybe*, and Hofmann *et al.*[70] identified psilocin (**9**) and psilocybin (**10**) as the hallucinogenic constituents. Recently, detailed crystal structures of these two compounds have been published.[71,72]

Isoprenylated indole derivatives are not too common in nature, but a number of examples besides the ergolines, are known, primarily as metabolic products of microorganisms. The oldest representative is echinuline (**11**), isolated in 1943 from *Aspergillus amstelodami*,[73] whose structure was elucidated by Quilico's group.[74] The absolute configuration of echinuline was a matter of debate at one time, but has been settled in terms of assigning both chiral centers *S* configuration.[75] Quite recently, two other isoprenylated indoles have been isolated from *A. amstelodami*, neoechinuline (**12a**)[76] and cryptoechinuline A (**12b**),[77] which, interestingly, carry an isoprenoid residue in the 6-position of the indole ring.† For a long time, echinuline seemed to be the only example of a diketopiperazine containing an isoprenylated tryptophan. However, the isolation of the brevianamides[78-80] (e.g. brevianamide E **13**) and the austamides[79] (e.g. austamide **14**), which are proline diketopiperazines, indicates that echinuline was only the first representative of a family of compounds. The occurrence of an "inverted" isoprene residue in the 2-position of the indole ring in these compounds is biogenetically intriguing. The structure of lanosulin (**15**), the major metabolite of *Penicillium lanosum* Westling[81] may provide a clue as to its formation.

By far the most interesting compound in terms of its biogenetic relationship to the ergolines is cyclopiazonic acid (**16**), isolated by Holzapfel[82] as the major toxic metabolite from *Penicillium cyclopium* Westling. Cyclopiazonic acid essentially represents a different mode of cyclization of a 4-isoprenylated tryptophan than the ergot alkaloids. Two related compounds were subsequently isolated from the same organism, cyclopiazonic acid imine (**17**) and bissecodehydrocyclopiazonic acid (**18**).[83] All three compounds have also been isolated from *Aspergillus versicolor* (Vuill.) Tiraboschi.[84]

Finally it should be mentioned that 6-isoprenylindole {6-(3-methylbuta-1,3-dienyl)indole} has been isolated from the seeds of a higher plant, *Monodora tenuifolia* (Benth.).[85]

Some recent developments in the chemistry of ergot alkaloids

Following the structure elucidation of the major types of ergot alkaloids (cf. Refs. 3, 5, 86, 87), the total synthesis of lysergic acid by Kornfeld *et al.*[88] in 1954 and of ergotamine by Hofmann *et al.*[89] in 1961 marked the end of an era in research on the chemistry of ergot alkaloids. However, even after these landmark accomplishments, much work remains to be done on the chemistry of ergolines and interesting results continue to be published.

In the area of total synthesis, the general principle for elaborating the cyclol peptide moiety of the ergot alkaloids developed by the Sandoz group has been applied to the synthesis of numerous other naturally occurring ergot alkaloids and of unnatural analogs.[13,90-92] A second total synthesis of lysergic acid[93] and another synthesis of the dihydrolysergic acid system[94] have been reported. Julia's new lysergic acid synthesis takes a different approach from that of Kornfeld *et al.*, generating the

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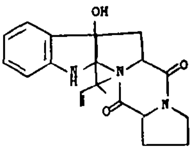
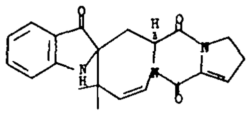
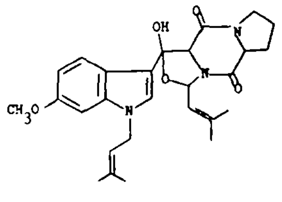
†Note added on proof: Three additional, related compounds have since been obtained from *A. amstelodami*: A. Dossena *et al.*, *Chem. Comm.* **1974**, 771; *Experientia* **31**, 1249 (1975).



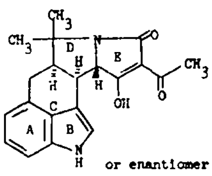
13: Brevianamide E



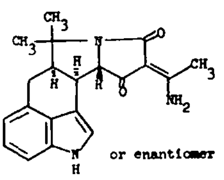
14: Austamide



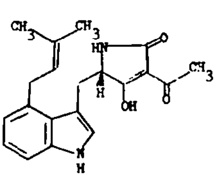
15: Lanosulin



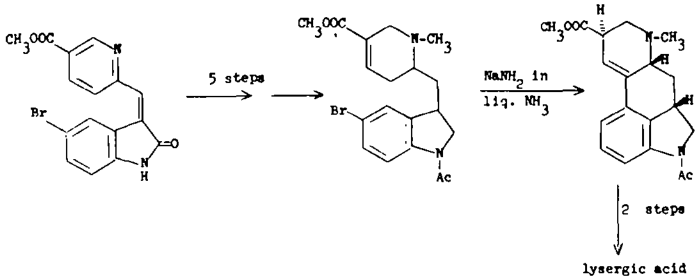
16: Cyclopiazonic acid



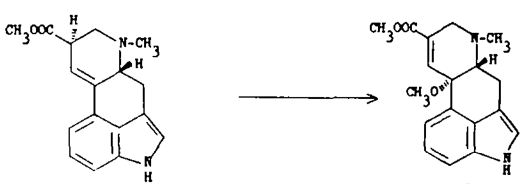
17: cyclopiazonic acid imine



18: Bissecodehydrocyclopiazonic acid tetracyclic ergolene system by an aryne coupling between C-5 of a tetrahydronicotinic acid and C-4 of a 2,3-dihydroindole portion:[93]

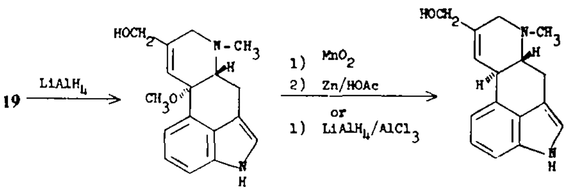


The synthesis of several clavines, isosetoclavine,[95,97] penniclavine[96,97] and elymoclavine,[96] from lysergic acid has been described. Since lysergic acid has been synthesized before and since elymoclavine has been converted into various other clavines, e.g. agroclavine, chanoclavine-I, and several others, this constitutes the total synthesis of a whole series of clavine alkaloids. A key intermediate in several of these syntheses was the 10-methoxy-$\Delta^{8,9}$-lysergic acid ester **19**, which is readily available by treatment of a methanol solution of methyl lysergate with mercuric acetate followed by alkaline sodium borohydride:[97]

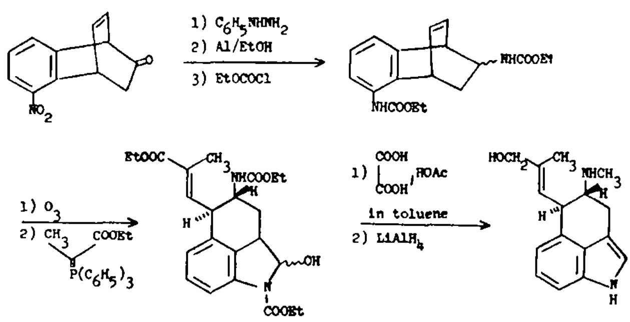


19

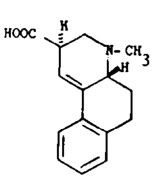
Since in derivatives of **19** the benzylic methoxy group can be removed reductively, this opens a route for the conversion of $\Delta^{9,10}$-ergolenes into $\Delta^{8,9}$-ergolenes:[96]



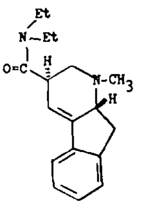
The reverse reaction, isomerization of the $\Delta^{8,9}$ to the $\Delta^{9,10}$-ergolene system, is readily achieved by base treatment.[24,98] An independent total synthesis of (±)-chanoclavine-I has just been accomplished by Plieninger's group:[99]



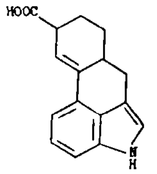
Various groups continue to focus on the synthesis of structural analogs of lysergic acid and its derivatives. Horii and his coworkers[100] have concentrated on analogs lacking the pyrrole ring (e.g. **20**), Craig *et al.*[101] have prepared the corresponding system in which the C ring has been contracted to a cyclopentane ring (e.g. **21**), and Rastogi *et al.*[102] have synthesized nor-6-deazalysergic acid (**22**). This subject has been reviewed by Campaigne and Knapp.[103]



20



21

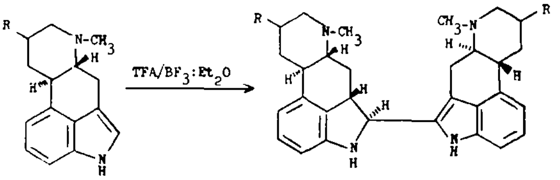


22

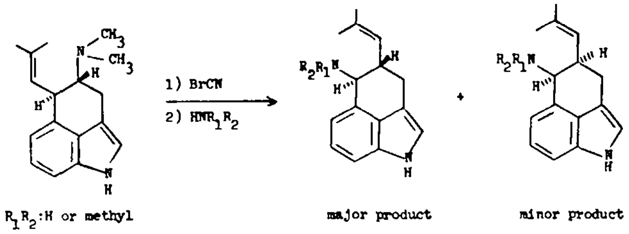
Structural modifications of the ergoline ring system continue to receive considerable attention, primarily for the purpose of establishing structure-activity relationships and obtaining new and superior medicinal agents. Among the transformations described during the last decade are the preparation of descarboxylysergic acid,[96,104] which, interestingly, shows the principal pharmacological properties of lysergic acid, although to a lesser extent,[104] the first synthesis of the $\Delta^{7,8}$-lysergic acid system,[105] the introduction of a methyl group at C-2 of lysergic acid[106] and the demethylation of the lysergic acid system.[107] 10-Methoxydihydrolysergic acid derivatives have been prepared and studied extensively,[108–110] leading to the discovery of nicergoline, a clinically useful antihypertensive agent. 6-Methyl-9-ergolene-8β-acetic acid and a number of its derivatives have been prepared in the Sandoz laboratories,[111] and Semonsky's group has prepared the corresponding 9,10-dihydro compound[112] and various of its derivatives. The latter work is part of an extensive program of Semonsky's laboratory on the synthesis of ergolines, which has resulted in a number of clinically interesting compounds.[113] The chemistry of

$\Delta^{8,9}$-lysergic acid was studied in some detail, leading to the preparation of various amides of this acid and its N-1 methyl derivative.[114] Metal hydride reduction of these amides is one route to 17-substituted $\Delta^{8,9}$-ergolenes;[114] an alternative, rather general route involves conversion of elymoclavine into its 17-chloride followed by reaction with suitable nucleophiles.[115]

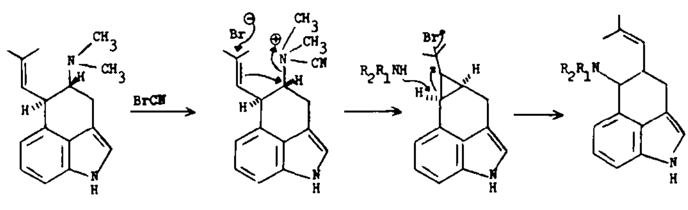
A number of transformations of ergot alkaloids have been studied. An acid-catalyzed isomerization of peptide ergot alkaloids discovered by Schlientz *et al.*[116] has been clarified. It was shown to involve a reversible epimerization at C-2 of the peptide moiety.[117,118] The epimeric *aci*-compounds are devoid of pharmacological activity. Bach and Kornfeld[119] noticed an interesting dimerization of various ergolenes and ergolines upon acid treatment, particularly on treatment with trifluoroacetic acid/BF$_3$ etherate:



An intriguing side-chain migration was observed by Fehr during attempts to carry out a von Braun demethylation on N-methyl-6,7-secoagroclavine:[120]



The mechanism of this reaction was rationalized in the following way:

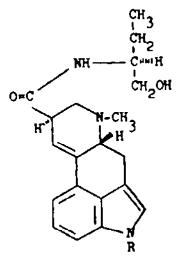


A number of spectroscopic and detailed structural studies on ergolines have been carried out in recent years. The mass spectral fragmentation patterns of ergot alkaloids have been investigated by Barber *et al.*[121] and these studies have recently been extended by Gröger's group[122] to include many additional compounds. The ^{13}C NMR spectra of representative ergot alkaloids have been assigned and the proton NMR spectra of some key clavines reinterpreted by Bach *et al.*[49] The conformation of lysergic acid diethylamide (LSD) in the solid state and in chloroform solution has been studied in detail by X-ray analysis[123] and proton NMR spectroscopy,[124] respectively, and compared to a theoretical prediction.[125]

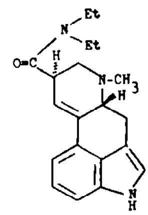
The analysis of ergot alkaloids is of considerable importance and numerous studies on their separation by various methods of chromatography have been carried out. A large number of publications deal with the paper and thin-layer chromatography of lysergic acid derivatives (cf. Ref. 126 and references therein) and clavines.[127-130] More recently, the techniques of gas–liquid chromatography (Ref. 131 and references therein) and high-speed liquid chromatography[132] have been used for the separation of ergot alkaloids.

Current interest in the pharmacology of ergolines

The problem with ergot alkaloids unlike that with many other compounds is not that they are devoid of pharmacological activity, but rather that they have too many pharmacological activities. The broad action spectrum (cf. Ref. 3) includes central, neurohumoral and peripheral effects. Fortunately, structural modification allows the manipulation of these activities to selectively enhance some and suppress others, leading to a number of clinically useful agents. To some extent this has already been accomplished in the naturally occurring ergot alkaloids. Ergotamine has perhaps the broadest spectrum of activity; although it has pronounced uterotonic effects, its use today, and that of its 9,10-dihydro derivative, is almost exclusively in the treatment of migraine. This application is presumably based on stimulation of vascular smooth muscle, leading to a constriction of the intracranial arteries. The uterocontractant activity is particularly prominent in ergonovine and its semisynthetic analog methylergonovine (lysergic acid butanolamide-(2)) (**23a**) and these two compounds are widely used in obstetrics for the treatment of postpartum hemorrhage. The serotonine-antagonistic activity of ergot alkaloids is selectively enhanced by methylation of the indole



23a: R = H, methylergonovine
23b: R = CH$_3$, methysergide



24: LSD

nitrogen; the 1-methyl derivative of methylergonovine (methysergide, **23b**) is one of the most potent compounds in this series and is used for the treatment of various conditions in which serotonine is believed to play a role. Another activity of ergot alkaloids, α-adrenergic blockade, is selectively enhanced in the 9,10-dihydro derivatives of some of the peptide alkaloids. Hydergine, the dihydro derivative of ergotoxine, a 1:1:1 mixture of ergocornine, ergocristine and ergokryptine, is used increasingly for the treatment of hypertension and to improve peripheral and cerebral circulation, particularly in older patients. An extremely potent hallucinogenic activity is exhibited by lysergic acid diethylamide (LSD, **24**) and some closely related compounds, and LSD has found limited application in experimental psychotherapy.

Despite the selectivity shown by some of the compounds currently in use, most of them still exhibit a broader spectrum of activity and resultant side effects than would be desirable. Particularly nausea and vomiting are frequent unpleasant side effects during therapy with ergot alkaloids. Thus there is a need for new agents in this series with further improved selectivity. This and the pursuit of new pharmacological activities is the main driving force behind continued and even expanding efforts in the area of preparation and pharmacological evaluation of ergolines. One current trend is towards more interest in the simpler ergoline derivatives as opposed to the amide derivatives of lysergic acid. It is becoming increasingly clear that most of the pharmacological activities of ergot alkaloids are not dependent on the peptide or even an amide moiety.

Present efforts in the development of new ergoline-based drugs seem to be aimed mainly in three directions:

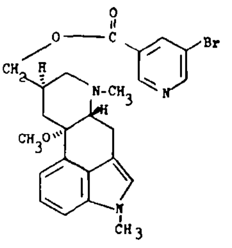
(1) Improved agents for the treatment of hypertension and of poor peripheral and cerebral blood circulation. One new compound, which was developed in recent years in the laboratories of Farmitalia in Milan, is nicergoline (**25**).[109,110] Under the tradename "Sermion" it has reached the market in Italy and in a number of other countries.

(2) More specific agents for the treatment of migraine. One problem in this area is the lack of good pharmacological test systems for the evaluation of antimigraine activity.

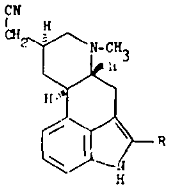
(3) Agents which affect the activity of the hypothalamic-pituitary system, particularly the release of prolactin from the pituitary gland. This is a new area of ergot pharmacology which currently commands considerable interest (cf. Ref. 133). Several agents in this series are currently undergoing clinical trial. These are 2-bromo-α-ergokryptine (CB 154),[134] developed at the Sandoz laboratories, VUFB 6605 (**26a**)[135] and VUFB 6683 (deprenone **27**)[136] which were synthesized by Semonsky's group in Prague, and lergotrile (**26b**),[137] a development of the Lilly laboratories. Their effectiveness in reducing prolactin levels in humans has been demonstrated and they show promise in the treatment of prolactin-dependent disorders, like galactorrhea, puerperal mastitis, prolactin-dependent mammary carcinoma, as well as other diseases, for example Parkinson's disease.

Industrial production of ergolines

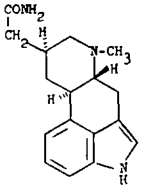
The current annual world production of ergot alkaloids is estimated at 4000 kg of peptide alkaloids and in excess of 12000 kg of lysergic acid. Lysergic acid presently sells at between $3000 and $4000 per kg.



25: Nicergoline



26a: R=H, VUFB 6605
26b: R=Cl, lergotrile



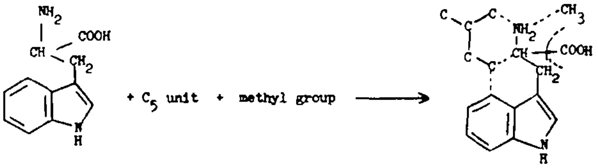
27: VUFB 6683
(deprenone)

The three principal ways to produce the commercially important ergot alkaloids are: (a) Isolation from field-cultivated ergot drug, (b) fermentation of the ergot fungus, and (c) chemical partial or total synthesis. Total synthesis is not used at all for commercial production because the chemical synthesis of lysergic acid is not economical. Partial synthesis from lysergic acid is the predominant route for the commercial production of all medicinally used ergolines with the exception of the peptide alkaloids. Most of the lysergic acid used for this purpose is produced by fermentation (cf. Ref. 138). Fermentation procedures were first established for some of the simple clavine alkaloids, e.g. agroclavine and elymoclavine (cf. Ref. 139), but these have so far not found industrial use. This could change with the increasing interest in simpler ergolines, since clavines might be alternative starting materials for their synthesis. The first practically useful fermentation procedure, the production of lysergic acid α-hydroxyethylamide by a strain of *Claviceps paspali*, was developed by Arcamone *et al.* in 1960.[140,22] The α-hydroxyethylamide can be readily hydrolyzed to give lysergic acid and this process is used by Farmitalia S.A. for the commercial production of lysergic acid. Yields of 2 g/l have been published and yields up to 5 g/l have been claimed for this fermentation. A second process for the production of lysergic acid was developed by Kobel *et al.* at the Sandoz laboratories in Switzerland and is being used at their fermentation plant in Kundl, Austria. It is based on production of $\Delta^{8,9}$-lysergic acid by another chemical race of *Claviceps paspali* and its isomerization to lysergic acid.[24] More recently, high-yielding fermentation procedures have also been developed for ergotamine[141] and other peptide alkaloids, using submerged cultures of various strains of *Claviceps purpurea*. However, while these may contribute increasingly to the commercial production of peptide alkaloids in the future, to the best knowledge of the author over 95% of the peptide alkaloids currently marketed are still obtained by extraction of field-cultivated ergot. Strain selection and mechanization of the infection process, largely due to the work of von Bekesy and of Stoll and Brack (cf. Ref. 142) have made field cultivation of ergot on rye an economical process which is widely used in several countries, particularly Switzerland and some East European countries. In terms of total quantity of all ergot alkaloids, the two main producers currently seem to be Farmitalia in Italy and Sandoz in Switzerland. Virtually no ergot alkaloids are produced in the United States.

BIOSYNTHETIC ORIGIN OF THE ERGOLINE RING SYSTEM

Early hypotheses

Following the structure elucidation of lysergic acid, thought was given to possible ways in which this structure could be formed in Nature. Some seven different hypotheses on the biogenetic origin of lysergic acid were published before the initiation of experiments to clarify this question. With the exception of one proposal by Plieninger,[143] who suggested that rings A and B might be formed by cyclization of prephenic acid, all the other hypotheses assumed that these two rings originate from the indole ring system of tryptophan. To account for the fact that indole does not readily undergo substitution in the 4-position, most authors invoked quinoid forms to account for increased reactivity at C-4. Van Tamelen[144] proposed that 5-hydroxytryptophan and dihydronicotinic acid are the building blocks of lysergic acid. Harley-Mason[145] also considered 5-hydroxytryptophan as one of the building blocks and proposed that the remainder of the molecule arises from acetonedicarboxylic acid and formaldehyde. The hypotheses of Wendler[146] and Feldstein[147] assumed that the tryptophan side chain provides ring C and that the D-ring arises from citric acid or from α-ketoglutaric acid and a C_1 unit, respectively. Robinson[148] postulated a Claisen condensation of tryptophan and succinic acid followed by reaction with formaldehyde. Finally, Mothes *et al.*[149] proposed the condensation of tryptophan with an isoprenoid C-5 unit as the origin of the ergoline ring system:



A similar hypothesis was advanced by Birch.[150] The subsequent experimental investigations have shown this last proposal to be correct.

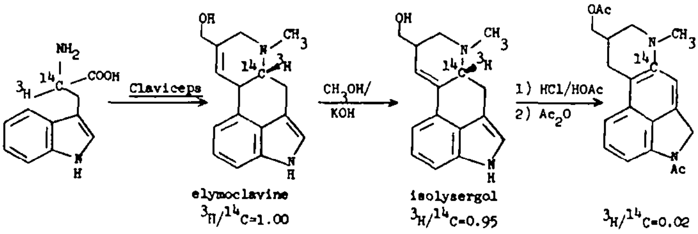
The biosynthesis of ergot alkaloids has been reviewed repeatedly;[151-159] the reader is referred to some of these reviews particularly for more detailed summaries of the earlier work.

Establishment of the biogenetic precursors by feeding experiments

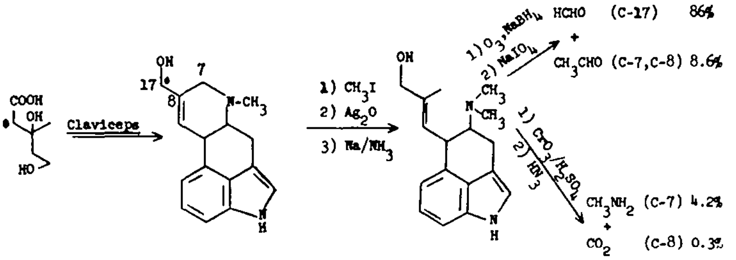
The first tracer experiments on the biosynthesis of ergot alkaloids were carried out with ergot growing parasitically on rye plants. Two groups injected tryptophan-^{14}C into the internodes of ergot-infected rye plants and analyzed the resulting alkaloids for radioactivity. Mothes *et al.*[149] found that tryptophan-β-^{14}C was incorporated into ergonovine and the peptide ergot alkaloids, labeling the lysergic acid moiety. Suhadolnik *et al.*[160] on the other hand, obtained extremely low incorporations of tryptophan-7a-^{14}C into ergonovine (0·003–0·014%) and, in contrast to Mothes *et al.*, concluded that tryptophan is not an ergoline precursor. The group of Mothes and Weygand repeated their experiments,[161] obtaining a 0·158% incorporation of tryptophan into ergokryptine under parasitic conditions. More importantly, they carried out the first radioactive feeding experiments with saprophytic cultures of *Claviceps*, obtaining incorporations of 10–39% of tryptophan-β-^{14}C into elymoclavine. The use of saprophytic cultures has since become the standard technique in biosynthetic studies on ergot alkaloids. Gröger *et al.*[161,162] also observed that tryptophan-(carboxyl-^{14}C) gave negligible incorporation, in accordance with their proposed hypothesis.[149] The positive incorporation results with tryptophan were soon confirmed by three other groups[163-165] and have been duplicated many times since, both for lysergic acid derivatives and for the simpler clavines. Only one degradation has been carried out to verify specific incorporation of tryptophan into the ergoline ring system; this involved elymoclavine biosynthesized from D,L-tryptophan-(alanine-2-^{14}C,2-^{3}H).[166]

Since it was independently shown that tryptophan-(alanine-3-^{14}C,3-^{3}H) is incorporated without change of the ^{3}H/^{14}C ratio,[165] the complete loss of tritium in the last step of the degradation indicated that at least 93% of the tritium must have been located at C-5 of elymoclavine. Further available evidence strongly indicates that the entire tryptophan molecule, with the exception of the carboxyl group and the hydrogen at C-4 is incorporated into the ergoline ring system. Plieninger's[167] experiments with tryptophan deuterated generally or specifically in the indole ring demonstrated incorporation of the indole moiety and, specifically, retention of the hydrogens at C-5 and C-6, but not that at C-4. Retention of the hydrogen at C-7 has been demonstrated more recently in Ramstad's laboratory[168] and incorporation of the amino nitrogen was shown by our work.[166]

Evidence for the involvement of the second postulated building block, an isoprenoid unit, came with the demonstration, almost simultaneously by three groups,[162,169,170] that mevalonic acid is efficiently incorporated into the ergoline ring system. These results were preceded[161] or accompanied[169] by demonstrations of the incorporation of acetate-^{14}C into ergot alkaloids. The efficiency of utilization of mevalonic acid for ergoline



biosynthesis (specific incorporation rates of 9–23% for D,L-mevalonate-2-^{14}C,2-^{3}H and 4-^{3}H were observed[162]) strongly suggested a direct precursor role. This work was the first demonstration that mevalonic acid is not only involved in the biosynthesis of typical isoprenoids, but also of alkaloids. Numerous other examples, of course, followed. Specificity of the incorporation was demonstrated by degradation of ergot alkaloids biosynthesized from mevalonate-2-^{14}C. Initial results from Birch's laboratory[169] seemed to indicate a different isotope distribution in agroclavine and elymoclavine, but later careful degradations showed that both compounds carried the majority of the label at C-17:[171]



The crucial step in this degradation, the cleavage of the 6,7-bond in the quaternized ergolene system with sodium in liquid ammonia, has been used repeatedly in subsequent biosynthetic studies. A second, different degradation carried out by Baxter's group,[172,173] involving the lysergic acid moiety of ergosine, confirmed that C-17 carries the majority of the label from C-2 of mevalonate. Both these degradations and numerous subsequent analyses in good agreement indicate that a small fraction of the label (5–10%) resides at C-7. Further evidence pointed to a mode of incorporation of mevalonate similar to that into terpenes, i.e. via isopentenylpyrophosphate and dimethylallylpyrophosphate. Baxter *et al.*[172] found that C-1 of mevalonate is not incorporated and that simultaneously fed isopentenyl- or γ,γ-dimethylallylpyrophosphate decreased the incorporation of mevalonate-2-^{14}C. Plieninger's group reported incorporation of deuterated isopentenylpyrophosphate[167] and ^{14}C-labeled dimethylallylpyrophosphate.[174] The latter findings are rather remarkable in view of the usual impermeability of cellular membranes for phosphate esters. We speculated that the pyrophosphates might be dephosphorylated to the alcohols before or during entry into the cell; however, γ,γ-dimethylallylalcohol-1-^{3}H was not incorporated.[175]

The origin of the N-methyl group was established by Baxter's group. Both formate-^{14}C and L-methionine-(methyl-^{14}C) were incorporated, the latter almost four times better than the former, and degradation of the resulting festuclavine showed that all the label (99–100%) was present in the N-methyl group. L-Methionine-(methyl-^{14}C,^{3}H) was incorporated into the clavine alkaloids without change in the ^{3}H/^{14}C ratio, suggesting that the methyl group is introduced as an intact unit.

Configuration of the precursors

The very high incorporation rates (up to 47·5%) obtained with D,L-tryptophan-β-^{14}C[162] and an experiment by Taber and Vining,[163] in which D- and L-tryptophan-β-^{14}C of different specific radioactivity were fed in the same experiment, suggested that not only L-tryptophan, but to some extent also the D-isomer is utilized for alkaloid synthesis. This was confirmed directly in a feeding experiment with generally tritiated D-tryptophan, which gave 28% incorporation. A more detailed analysis showed[166] that D-tryptophan is incorporated with complete loss (98%) of the α-hydrogen and predominant loss (90%) of the amino nitrogen of the side chain. D,L-Tryptophan was utilized with 43% and 44% retention of these labels, leading to the conclusion that L-tryptophan is incorporated with almost complete retention of the α-hydrogen and the amino nitrogen. This shows that L-tryptophan is the immediate alkaloid precursor and that D-tryptophan enters the pathway after conversion to the L-isomer, presumably via indolepyruvate. This result is interesting in view of the fact that the ergot alkaloids have *R*-configuration, corresponding to a D-amino acid, at C-5.[178,179] Consequently, the reaction sequence must, at some stage, involve inversion of configuration at the chiral center originating from C-2 of the tryptophan side chain and this step must occur with retention of the original hydrogen attached to this carbon atom.

The enantiomer of mevalonic acid active in ergoline biosynthesis is the 3*R*-isomer, the same which serves as precursor of squalene in rat liver. The *S*-isomer is biologically inactive in *Claviceps* as in other systems. This was demonstrated in two ways. One was to remove all the *R*-isomer from a sample of racemic mevalonate-2-^{14}C by reaction with the kinase from rat liver, which has absolute specificity for 3*R*-mevalonate, and to then feed the unreacted 3*S*-mevalonate-2-^{14}C to a *Claviceps* culture. In a parallel culture, *R*,*S*-mevalonate-2-^{14}C showed a 50 times higher incorporation rate than the 3*S*-mevalonate, indicating that 3*R*-mevalonate is an at least 100 times more efficient alkaloid precursor than the 3*S*-isomer.[180] The second proof involved the following steps; two samples of mevalonate, each labeled with a different isotope, were prepared, one as the racemate (isotope I) and the other as the 3*R*-isomer (isotope II). These two were mixed and fed to an ergot culture and both the precursor and the resulting alkaloids were analyzed for their isotope ratio. If both enantiomers of the precursor are utilized equally well, precursor and product will show the same isotope ratio. If only the 3*R*-isomer is utilized, the ratio of isotope II/I will double in going from precursor to product, because only half the isotope in the racemate will be in a biologically active form. In the actual experiments the racemate carried a ^{14}C label and the 3*R*-form carried tritium in the pro-5*S*-hydrogen. The results showed the expected doubling of the ^{3}H/^{14}C ratio.[181-183]

Figure 1 summarizes the biogenetic origin of the ergoline ring system.

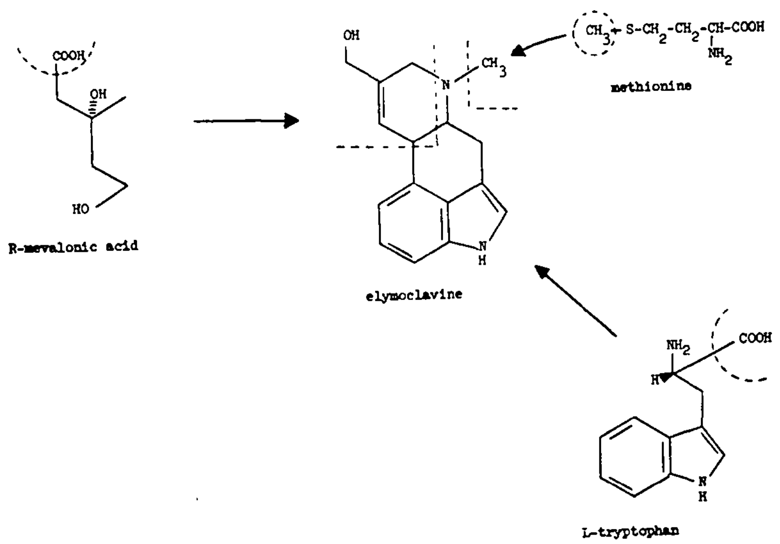


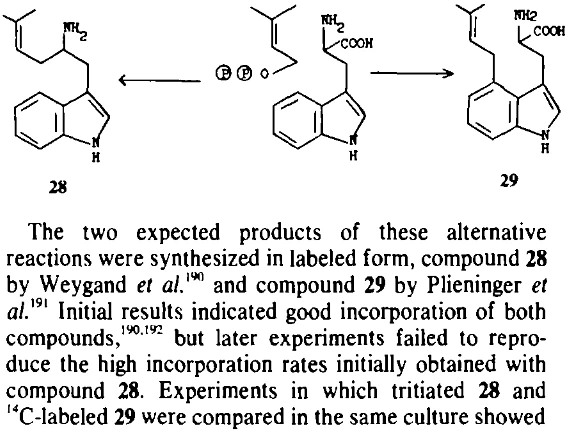
Fig. 1. Biosynthetic origin of the ergoline ring system.

PATHWAY AND MECHANISMS OF ERGOLINE RING FORMATION

Isoprenylation of tryptophan

Identification of the first pathway-specific reaction. From inspection of Fig. 1 it is obvious that the assembly of the ergoline ring system from its precursors requires decarboxylation, N-methylation and isoprenylation of the tryptophan moiety, and it was logical to ask which of these reactions occurs first. Tryptamine[176,184] and N$_\omega$-methyltryptamine[184] were not incorporated, excluding decarboxylation of tryptophan as the first reaction. N$_\alpha$-Methyltryptophan did give significant incorporation into elymoclavine (6·4% compared to 44% for tryptophan),[184] but closer examination by double labeling (^{14}C in the side chain, ^{3}H in the methyl group) showed that this incorporation was due to demethylation to tryptophan.[185] This left isoprenylation of tryptophan as the first pathway-specific step. Since the 4-position is not the most reactive position for electrophilic substitution of the indole ring, possible ways in which this position might be activated in nature were considered. Hydroxylation in the adjacent 5-position was excluded at an early stage both by Plieninger's experiments showing retention of H-5[167] and by the lack of incorporation of 5-hydroxytryptophan.[165] With the isolation of the 4-hydroxylated indoles psilocin and psilocybin[70] and the demonstration that these compounds are formed from tryptophan,[186] another proposal was made which envisioned a "tail-to-tail" condensation between dimethylallylpyrophosphate and a phosphorylated 4-hydroxy-indole derivative in analogy to the formation of squalene from farnesylpyrophosphate.[186] An obvious weakness of this proposal was that it equates an aromatic hydroxyl group to an allylic alcoholic hydroxyl in reactivity as a leaving group, and experimental evaluation by checking for incorporation of 4-hydroxytryptophan, despite an initial positive result,[187] ruled out this mechanism.[188,189]

These results left direct isoprenylation of tryptophan as the most likely possibility. Assuming that the initial reaction involves the allylic pyrophosphate group of the isoprenoid unit, an assumption which was supported by the structure of chanoclavine, this could be an alkylation at C-4 of the indole ring or at the α-carbon of the tryptophan side-chain. Because of the retention of the α-hydrogen the reaction in the latter case would have to occur with simultaneous decarboxylation:

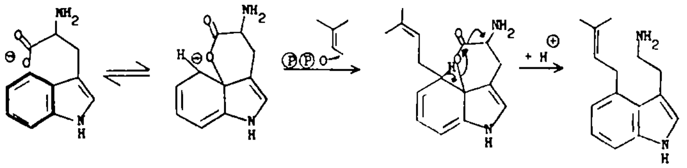


28 29

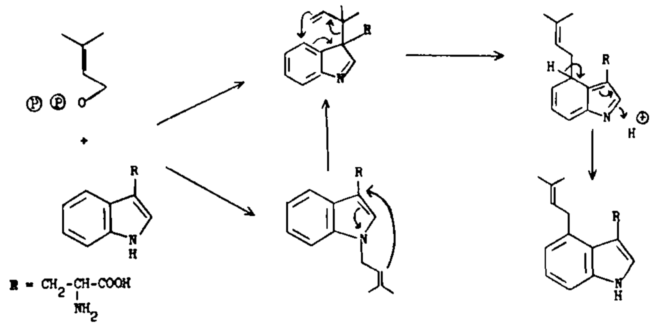
The two expected products of these alternative reactions were synthesized in labeled form, compound **28** by Weygand *et al.*[190] and compound **29** by Plieninger *et al.*[191] Initial results indicated good incorporation of both compounds,[190,192] but later experiments failed to reproduce the high incorporation rates initially obtained with compound **28**. Experiments in which tritiated **28** and ^{14}C-labeled **29** were compared in the same culture showed consistently much better incorporation of **29**.[193,194] Plieninger's group also showed[193] that both a label in the alanine side chain and in the isoprenoid moiety of γ,γ-dimethylallyltryptophan (**29**) is incorporated. Double-labeled **29**, carrying tritium at the α-carbon of the alanine side chain and ^{14}C at C-1 of the dimethylallyl group, gave elymoclavine without change in the ^{3}H/^{14}C ratio.[174] In most competition experiments **29** was utilized less efficiently for the biosynthesis of clavines than tryptophan itself,[193,194] but this may reflect differences in permeability and in a strain of *Claviceps paspali* the compound was found to be a decidedly better precursor of lysergic acid derivatives than tryptophan.[195] Later, dimethylallyltryptophan was isolated from ergot cultures in which alkaloid synthesis had been inhibited by exclusion of oxygen[196] or by addition of ethionine.[197] Finally, an enzyme catalyzing the formation of dimethylallyl-L-tryptophan from L-tryptophan and γ,γ-dimethylallylpyrophosphate has been isolated from *Claviceps*. All these results leave no doubt that the formation of **29** is the first pathway-specific reaction of ergoline biosynthesis.

Mechanism of the isoprenylation reaction. The mechanism of the isoprenylation at C-4 of the indole nucleus continued to puzzle the organic chemist. Plieninger[193] at one point speculated that the isoprenylation at C-4 may involve anchimeric assistance of the carboxylate group and lead, with loss of CO_2, directly to 4-(γ,γ-

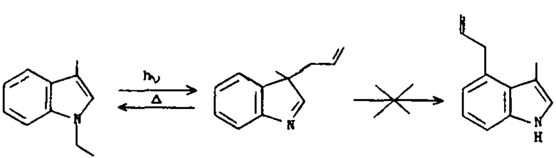
dimethylallyl)-tryptamine:



He later synthesized this amine in labeled form and found that it was incorporated into ergolines, although less efficiently than dimethylallyltryptophan.[174] The later work, however, particularly the enzymatic studies excluded this mechanism. After the exclusion of various modes of activation of the 4-position, two groups pursued the idea that the initial alkylation may occur at N-1 or C-3 followed by sigmatropic rearrangement of the side chain into the 4-position:[198]

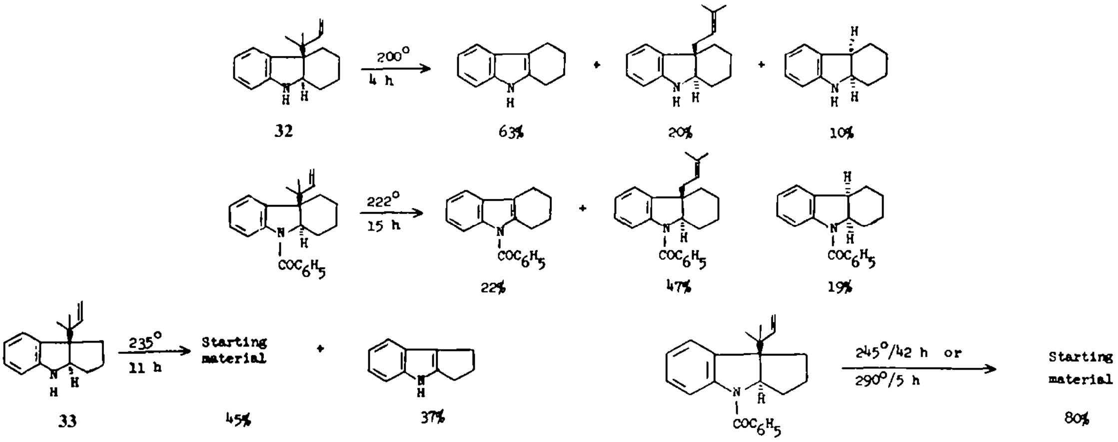


The rearrangement of an allyl residue from the N-1 to C-3 and particularly from C-3 to C-4 was studied in model systems by the laboratories of Arigoni and Wenkert. The Swiss workers[198] prepared N-allyl-3-methylindole (**30**) and 3-allyl-3-methylindolenine (**31**). Thermal treatment (3 h/250°) converted **31** into **30**, UV irradiation (254 nm) in cyclohexane effected the reverse reaction. Under no conditions did they observe any migration of the allyl group into the benzene ring:



30 **31**

Wenkert and Sliwa[199] synthesized the α,α-dimethylallyl systems **32** and **33** and carried out pyrolysis experiments on them with the following results:



Again, no formation of any benzene ring-alkylated material was observed. Of course, the negative outcome of these model experiments does not disprove the occurrence of such a rearrangement process in the enzymatic reaction, but it does at least render it less likely.

We have for some time held the view that a direct alkylation of the 4-position of the indole ring could occur readily, provided the enzyme in some way prevents attack at the more reactive positions. Our original idea was that the protein might fit tightly around the "bottom part" of the benzene ring and the pyrrole ring, leaving only the 4-position open for approach. With the isolation of the enzyme catalyzing this reaction[200] a preliminary test of this idea became possible.[201] If the "tight fit" idea is correct, it would be predicted that tryptophan analogs carrying methyl groups in the benzene ring should not bind to the enzyme if these substituents are located in "tight fit" areas of the molecule, i.e. in positions 5, 6 or 7. A series of experiments, in which tryptophan analogs were tested as alternate substrates of the enzymes (Table 4), showed, however, that 4-methyltryptophan, as expected, was a poor substrate, but that the substrate activity increased gradually as the methyl group was moved away from the site of reaction. This indicates that the "tight fit" idea cannot be correct, but suggests that the enzyme controls the reaction site specificity by binding the two substrates in such a geometrical arrangement relative to each other that reaction in the 4-position of the indole nucleus is optimized.

Table 4. Tryptophan analogs as substrates for dimethylallyltryptophan synthetase

Substrate	Incorporation of dimethylallylpyrophosphate-1-^{14}C (nmoles/mg x min)	% of control
Tryptophan (control)	306	100
4-Methyltryptophan	2.6	0.8
5-Methyltryptophan	22.9	7.4
6-Methyltryptophan	29.8	9.8
7-Methyltryptophan	124	40.5
Bishomotryptophan	6.2	2.0
Thiotryptophan	0.7	0.2

Early hypotheses and experiments on the overall pathway

Interrelationships among clavines. Following the elucidation of the biosynthetic origin of the ergoline ring system, interest soon arose in the biogenetic relationships between the different ergot alkaloids, particularly among the various clavines and between clavines and lysergic acid derivatives. Various hypotheses had been put forward; the two extremes are perhaps represented by Rochelmeyer's[202] suggestion of a biogenetic sequence chanoclavine → agroclavine → elymoclavine → lysergic acid derivatives and Abe's[203] hypothesis that clavines and lysergic acid derivatives arise from a common precursor, lysergaldehyde:

$$\begin{tikzcd}
\text{elymoclavine} \arrow[d] & \text{lysergaldehyde} \arrow[l] \arrow[r] & \text{lysergic acid} \arrow[d] \\
\text{agroclavine} \arrow[d] & & \text{lysergic acid derivatives} \\
\text{chanoclavine} & &
\end{tikzcd}$$

The first and also most extensive experimental studies using labeled alkaloids were carried out by Agurell and Ramstad.[204,205] They initially established the irreversible oxidative conversion sequence agroclavine → elymoclavine → penniclavine.[204] Elymoclavine, in turn, was shown by Mothes *et al.*[206] to be a precursor of the lysergic acid derivatives. Further work by Agurell and Ramstad[205,154] and by Baxter's group[207] established the biogenetic relationships shown in Fig. 2. As can be seen, the main types of reactions are (a) reduction of the $\Delta^{8,9}$ double bond, (b) shift of the double bond from the 8,9 to the 9,10 position, and (c) hydroxylation at C-8 with shift of the double bond into the 9,10 position. The latter reaction is catalyzed by peroxidase; the C-8 epimers are formed independently and are not interconvertible. Abe and his coworkers[203] proposed a conversion cycle of agroclavine to elymoclavine and elymoclavine back to agroclavine and later presented experimental evidence for the occurrence of its individual steps in *Claviceps*:[208,209]

$$\begin{tikzcd}
\text{agroclavine} \arrow[r] & \text{elymoclavine} \arrow[d] & \text{unknown alkaloid PS} \arrow[l] \\
\text{lysergene} \arrow[u] & \text{lysergol} \arrow[l] \arrow[ur] &
\end{tikzcd}$$

This cycle can, however, not be universally operative in

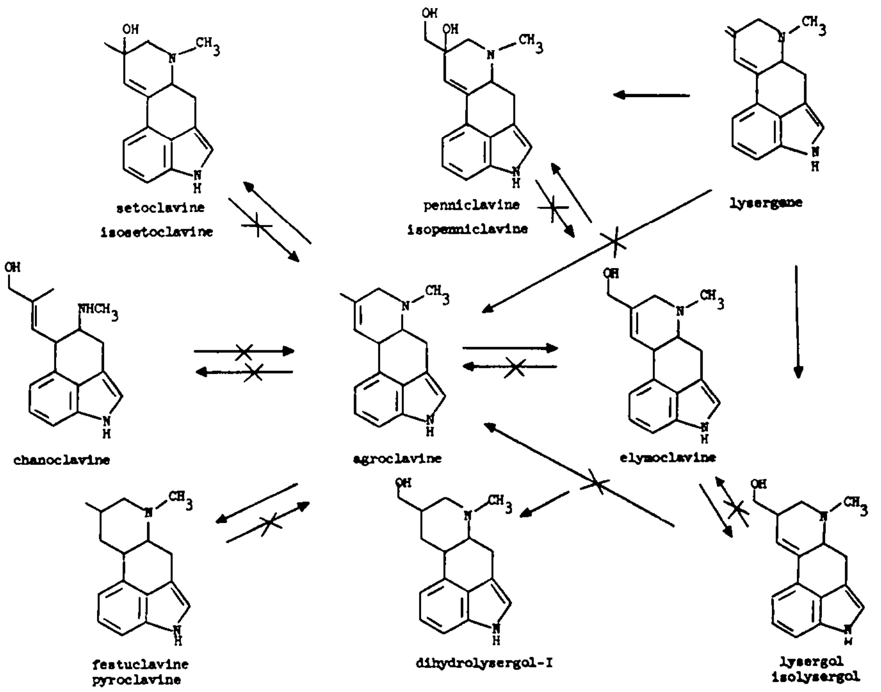


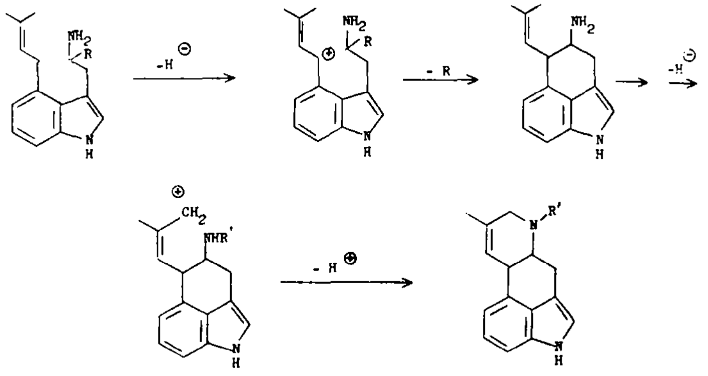
Fig. 2. Biogenetic relationships among clavines as established by Agurell and Ramstad,[205] Baxter *et al.*[207] and Agurell.[154]

Claviceps. Agurell and Ramstad[205] and Baxter *et al.*[207] failed to observe some of the conversions postulated and demonstrated by Abe, and furthermore, double-labeling experiments with mevalonate-2-^{14}C,2-^{3}H and -2-^{14}C,5-^{3}H[207,154] are incompatible with extensive operation of the cycle under the experimental conditions. The experiments with mevalonate-2-^{14},5-^{3}H showed that both agroclavine and elymoclavine contained one of the two hydrogens from C-5 of mevalonate. In the experiment with mevalonate-2-^{14}C,2-^{3}H agroclavine had essentially the same ^{3}H/^{14}C ratio as the precursor. This latter finding also rules out the common lysergaldehyde precursor postulated by Abe.[203]

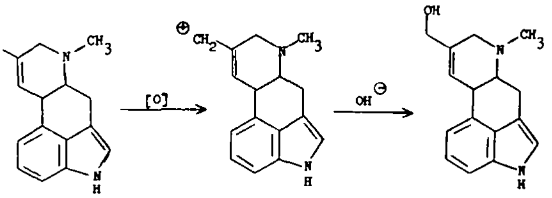
The most important outcome of these early studies was the establishment of the oxidative sequence agroclavine → elymoclavine → lysergic acid derivatives as the main biogenetic route; the other observed conversions can be considered secondary transformations. The methyl group hydroxylation of agroclavine is a rather specific reaction, which is largely, although not completely,[209] confined to *Claviceps* species, whereas, for example, the 8-hydroxylation of agroclavine and elymoclavine can be carried out by many fungi.[210] The role of chanoclavine in the biosynthesis remained largely obscure in these early studies.

Early mechanistic ideas. The experimental results establishing the biogenetic precursors of the ergoline ring system and some of the biogenetic interrelationships among ergot alkaloids placed further speculations on the mechanism of ergoline ring formation on a somewhat more rational basis. Trojanek[211] published a hypothesis, postulating the conversion of isopentenylpyrophosphate into 3-hydroxymethyl-2-butenylpyrophosphate which would react with tryptophan or a derivative thereof. The later identification of dimethylallylpyrophosphate as the enzyme substrate invalidated this proposal.

Following the initial isoprenylation of tryptophan, most authors invoked allylic oxidations[153,171,207] leading to allylic carbonium ions in the ring closure reactions, e.g.:[153]



The conversion of agroclavine to elymoclavine was also formulated as occurring through an allylic carbonium ion, as were some of the other oxidative clavine interconversions:[212]



While allylic oxidations may indeed be involved in the biosynthesis, the subsequent investigations have shown that these early ideas were rather naive and that the overall biosynthetic pathway is far more complex. The first clear indication of this came with the studies which led to a partial clarification of the role of the chanoclavines. The involvement of allylic carbonium ions in ergoline ring formation is now much more questionable; it can be clearly ruled out in the agroclavine → elymoclavine conversion because the oxygen atom in elymoclavine, as that in chanoclavine-I does not originate from water.[213]

The role of chanoclavines. As mentioned before, the role of chanoclavine in ergoline biosynthesis remained elusive in the early experiments. Both Agurell and Ramstad[205] and Baxter's group[207] concluded from their tracer experiments that chanoclavine was neither a precursor nor a metabolite of the tetracyclic ergolines. In the same year Mothes and Winkler[214] reported the conversion of radioactive elymoclavine into chanoclavine by sclerotia of *C. purpurea*, suggesting that chanoclavine arises by ring opening of clavines or lysergic acid derivatives. On the other hand, we noted[153] that in feeding experiments with labeled tryptophan the isolated chanoclavine had a higher specific radioactivity than the tetracyclic ergolines, indicating that most likely it had not arisen from the latter, and Voigt's group[215] found that addition of chanoclavine to ripening sclerotia of the ergot fungus significantly increased their ergotamine and ergonovine content. Clarification of this confusing situation was initiated by the isolation[42] of stereoisomers of chanoclavine, chanoclavine-I, (−)- and (±)-chanoclavine-II and isochanoclavine-I, from *Claviceps* (see Table 3). The assignments of structure and stereochemistry for these compounds[42] were confirmed by chemical correlation with elymoclavine.[216] The isolation of these compounds suggested that the conflicting results obtained so far might reflect the possibility that various investigators were dealing with different chanoclavine isomers in their experiments. In retrospect, for example, the failure of Agurell and Ramstad[205] to observe conversion of "chanoclavine" into tetracyclic ergolines can be accounted for by the fact that the major chanoclavine isomer in cultures of Pennisetum ergot, and the one they probably isolated, is chanoclavine-II.[217] Obviously, the role of chanoclavines had to be reinvestigated using pure isomers. It soon became clear that chanoclavine-I is a very efficient precursor of tetracyclic clavines and lysergic acid derivatives.[180,218–221] Incorporation rates into elymoclavine as high as 40%[218] and dilution factors as low as 1·23[221] have been observed. Significantly, chanoclavine-I was incorporated into agroclavine as well as elymoclavine and with higher dilution into the latter than into the former, in accordance with a pathway chanoclavine-I → agroclavine → elymoclavine;[218] no incorporation of agroclavine or elymoclavine into chanoclavine-I was observed. It was also found that neither chanoclavine-II, which has the "wrong" stereochemistry at C-5 or C-10, nor isochanoclavine-I, which from its stereochemistry would seem destined to be the precursor for the D-ring closure, are incorporated into agroclavine or elymoclavine.[219,220] The failure of chanoclavine-II and isochanoclavine-I to give rise to tetracyclic ergolines also implies that neither compound can be converted to chanoclavine-I. For isochanoclavine-I this has been confirmed by a direct feeding experiment.[180] Recent unpublished experiments by Acklin[43] demonstrate that isochanoclavine-I can arise from chanoclavine-I and unpublished experiments done several years ago in our laboratory are in accord with and support his conclusion.[222]

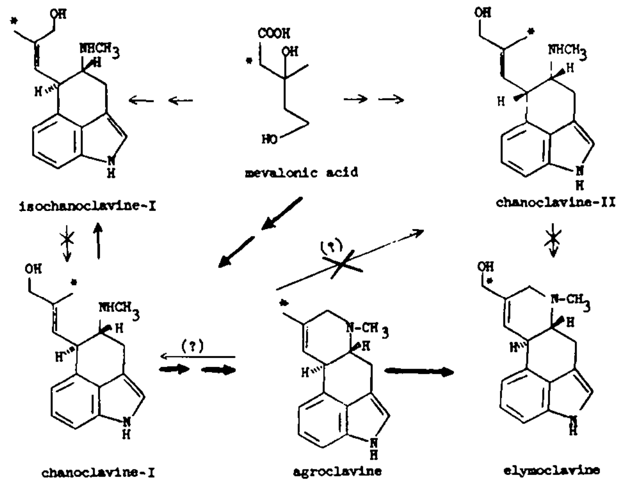
Finally, Abe[223] has presented additional evidence to support the notion that in certain *Claviceps* strains chanoclavine-I can arise by ring opening of agroclavine.

Evidence for a cis–trans *isomerization in the isoprenoid unit.* Following the isolation of the chanoclavine isomers, Arigoni's group determined the position of the label from mevalonic acid-2-^{14}C in these secoergolines[219] and found that in each case, irrespective of the geometry of the isoprenoid double bond, the C-methyl group carried the majority of the isotope. As in the tetracyclic ergolines there was also a slight scrambling of the label into the carbon originating predominantly from C-3′ of mevalonate, i.e. the hydroxymethyl group contained about 7% of the ^{14}C. The isotope distribution together with the finding that chanoclavine-I is converted to elymoclavine and agroclavine[218] suggested a *cis–trans* isomerization of the 8,9-double bond during the closure of ring D. Direct proof for this was obtained both in Zürich[219] and in our laboratory[220] by feeding chanoclavine-I-17-^{14}C or -7-^{14}C and degrading the resulting agroclavine and/or elymoclavine. In this way it was shown that the hydroxymethyl group of clanoclavine-I gives rise to C-7 and the C-methyl group to C-17 of agroclavine and elymoclavine. The established labeling pattern and biogenetic relationships can be summarized as below.

The conversions shown by heavy arrows are believed to represent the main pathway of ergoline biosynthesis.

Formation of ring C

Evidence for another cis–trans *isomerization.* The finding of Arigoni's group[219] that chanoclavine-I carried the label from C-2 of mevalonate predominantly in the C-methyl group, which occupies the *cis*-position relative to the carbon chain at the 8,9-double bond, raised another question. C-2 of mevalonate gives rise to C-4 of isopentenylpyrophosphate and the normal steric course of the isopentenylpyrophosphate isomerase reaction is known (cf. Ref. 224) to be such that the resulting dimethylallylpyrophosphate carries the label from C-2 of mevalonate in the *E*-methyl group. Since the same label appears in the *cis*-position in chanoclavine-I, the isopentenylpyrophosphate isomerase reaction in *Claviceps* must take an unusual steric course (Fig. 3, pathway a) or, alternatively, another *cis–trans* isomerization must take place between dimethylallylpyrophosphate and chanoclavine-I (Fig. 3, pathway b). Evidence supporting pathway b was obtained by tracing the fate of the two diastereotopic hydrogens at C-4 of mevalonate. The work of Cornforth and Popjak and others had shown (cf. Ref. 224) that both in the isopentenylpyrophosphate isomerase and in the prenyl transferase reaction the formation of a *trans* double bond correlates with loss of the *pro*-4*S* and retention of the *pro*-4*R* hydrogen from mevalonate, whereas the formation of a *cis* double bond, as in rubber or dolichol, involves loss of the *pro*-4*R* and retention of the *pro*-4*S* hydrogen of mevalonate. Consequently one would expect, assuming identical mechanism of the isomerase reaction, that pathway a should lead to the loss of the *pro*-4*R* and pathway b to loss of the *pro*-4*S* hydrogen from mevalonate. The experiment[180,225] showed 3% retention of the *pro*-4*S* and 70% retention of the *pro*-4*R* hydrogen in elymoclavine, indicating that pathway b is operative. The somewhat less than 100% retention of the *pro*-4*R* hydrogen has a bearing on the mechanism of the D-ring closure and will be discussed later. Unequivocal evidence proving the occurrence of another *cis–trans* isomerization between dimethylallylpyrophosphate and chanoclavine-I was recently obtained in Arigoni's laboratory.[226,226a] These authors synthesized 4-(γ,γ-dimethylallyltryptophan-(*Z*-^{14}CH$_3$) by the route shown in Fig. 4 and determined the labeling pattern in agroclavine and elymoclavine biosynthesized from this precursor. The results (Fig. 4) show that **29** is converted into agroclavine and elymoclavine with no, or an even



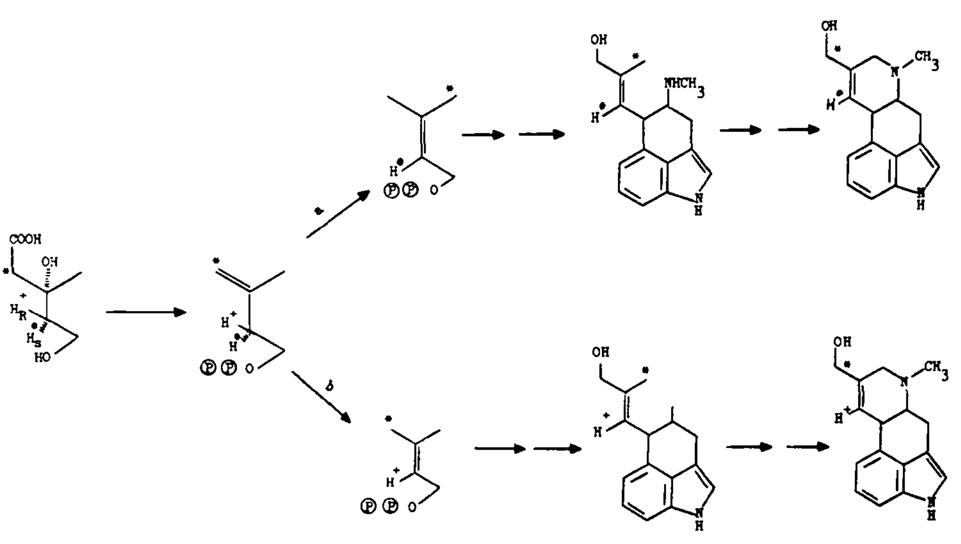


Fig. 3. Alternative stereochemical pathways of ergoline formation from mevalonate-2-^{14}C-4-^{3}H.

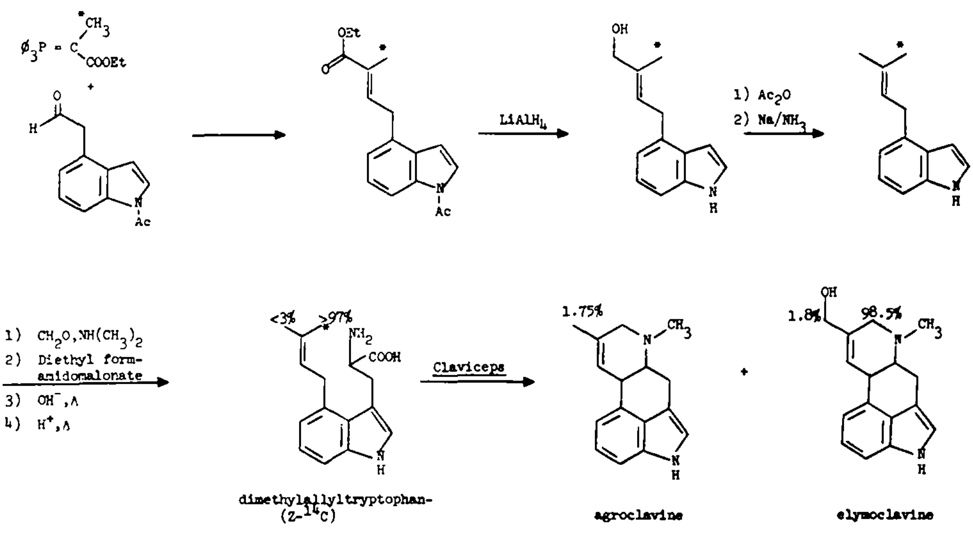


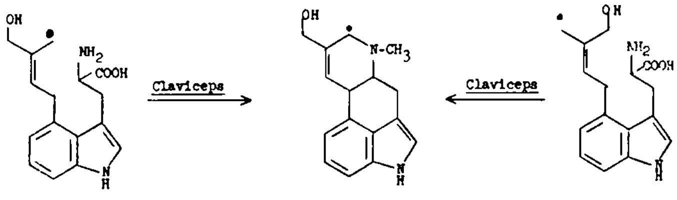
Fig. 4. Synthesis and biosynthetic utilization of dimethylallyltryptophan-(Z-^{14}C). The figures indicate the observed ^{14}C distribution.

number of, *cis–trans* isomerizations and, together with the already established isomerization occurring between chanoclavine-I and the tetracyclic ergoline, demonstrate the occurrence of two or 2n double bond isomerizations. They also show that the first of these isomerizations occurs between **29** and chanoclavine-I and not in the formation of **29**. Furthermore, the fact that the scrambling of the label between C-7 and C-17 observed in agroclavine and elymoclavine is within the limits of the stereochemical purity of the precursor shows that the approximately 7% scrambling of the label between these two atoms normally observed upon feeding mevalonate-2-^{14}C (see earlier discussion) or mevalonate-3′-D$_3$[227] must occur at a stage prior to **29**, possibly during the dimethylallyltryptophan synthetase reaction.

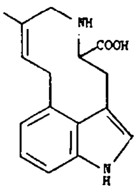
Hydroxylation of dimethylallyltryptophan. Chanoclavine-I carries a hydroxyl group at the carbon atom originating from C-3′ of mevalonate, indicating that this carbon, which was originally a methyl group, has undergone hydroxylation at some stage along the biosynthetic pathway. At an early stage in the investigations, Arigoni's group prepared labeled desoxychanoclavine-I, its N-nor-derivative and its N-methyl derivative and showed that neither was incorporated into agroclavine, elymoclavine or paspalic acid,[120,228] suggesting that the first methyl group hydroxylation takes place before the closure of ring C. The stereochemical results discussed in the previous section furthermore mandate that the methyl group undergoing hydroxylation must be the one occupying the *Z*-position in dimethylallyltryptophan. Although other reactions of **29** could conceivably precede the introduction of this oxygen function, the available information made 4-(4′-hydroxy-3′-methyl-2′-butenyl)-tryptophan (**34**) an extremely promising candidate for a biosynthetic intermediate and a desirable target for chemical synthesis. The first synthesis of **34**, labeled with ^{14}C in the hydroxymethyl group, was carried out by Plieninger's group.[229] The product was shown to have the *E*-configuration and was found to be incorporated into tetracyclic ergolines. The corresponding tryptamine derivative was also prepared and found to produce results similar to those obtained with dimethylallyltryptamine, i.e. the compound labeled the tetracyclic ergolines, but also inhibited alkaloid synthesis significantly. Recently, Anderson and Saini[230] isolated a compound from *Claviceps purpurea* cultures, which by mass spectral comparison with authentic material was identified as **34**. The double bond configuration of the isolated material was not established. While these various results might suggest a positive role of *E*-**34** in ergoline biosynthesis, closer examination changed the picture and, in fact, led to the perplexing conclusion that neither *E*- nor *Z*-**34** can be natural intermediates in the formation of ergot alkaloids.

Plieninger *et al.*[229,231] had observed that *E*-**34** was incorporated five times more efficiently into elymoclavine than into agroclavine and it was later even found that the thoroughly purified agroclavine was inactive.[232,233] Arigoni and coworkers degraded the elymoclavine obtained in Plieninger's experiment and found that the label resided at C-17 rather than at C-7, as would be expected.[233] This caused them to synthesize independently *E*-**34**-$^{14}\text{CH}_3$ and, by photoisomerization in the presence of acetophenone as sensibilizer, also *Z*-**34**-$^{14}\text{CH}_3$. Both compounds were fed to cultures of *Claviceps* and the resulting alkaloids were purified. In both cases agroclavine was essentially inactive whereas elymoclavine showed about 4% incorporation of radioactivity. Degradation established an essentially identical isotope distribution in the elymoclavine samples from the two precursors, C-7 carrying 97% and 98% of the radioactivity and C-17 0·9% and 1·0%:[226,226a] *E*-**34** thus appears to be an unnatural substrate analog, carrying an extra OH-group, which is treated like dimethylallyltryptophan, but in the step normally leading to agroclavine produces directly elymoclavine. The mode of incorporation of *Z*-**34** is particularly unexpected and even more difficult to rationalize. It would seem most plausible to assume that *Z*-**34** is converted into the *E*-isomer prior to its incorporation.

Structure and role of clavicipitic acid. In view of the results discussed earlier, the fact remains that the carbon atom originating from C-3′ must be oxygenated prior to the closure of ring C. However, this reaction may be preceded by other transformations of dimethylallyltryptophan. Clues as to their nature might be provided by the structure of clavicipitic acid, an amphoteric compound isolated from *Claviceps.*[47] This amino acid was originally detected in ethionine-inhibited ergot cultures, but was subsequently also found in normal cultures.[235] Largely on the basis of mass spectral and biosynthetic evidence, we proposed structure **35** for this compound.[47] One piece of evidence, obtained subsequently, which is not in accord with this structure, is the observation[236] that clavicipitic acid retains the *pro*-5*S* hydrogen of mevalonate essentially completely (95·5% retention), as do the other ergolines, but unlike the other ergolines also retains about half of the *pro*-5*R* hydrogen (52·5%). Two feeding experiments with (3*RS*, 5*RS*)-mevalonate-2-^{14}C-5-^{3}H both gave a tritium retention of 75%. Due to the insolubility of the compound, no useful NMR spectra could be obtained.



E-**34** elymoclavine *Z*-**34**



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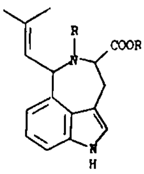
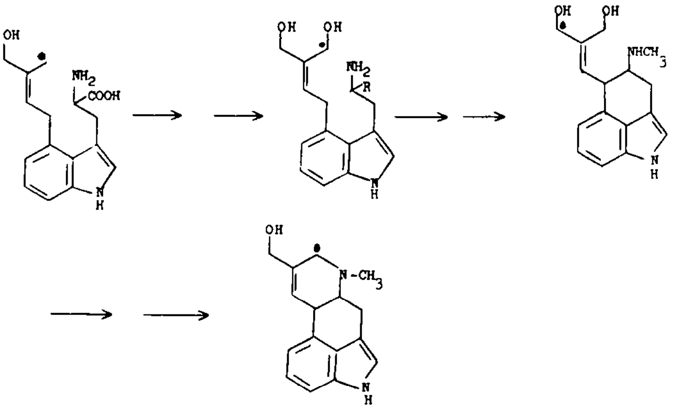


36: a. R = -COCH$_3$, R′ = CH$_3$
b: R = R′ = H

In view of the established intermediacy of chanoclavine-I and the fact that its methyl group gives rise to C-17 of agroclavine and elymoclavine, it is obvious that neither isomer of **34** can be an intermediate in the biosynthesis, since their methyl group gives rise to C-7 in elymoclavine and since they do not label agroclavine. For *E*-**34** this confirms the earlier observation, suggesting that the compound is treated by the organism as if the OH-group were not present. The following scheme or a variant of it can account for the formation of elymoclavine, but not agroclavine, and for the observed labeling pattern:[231,234]



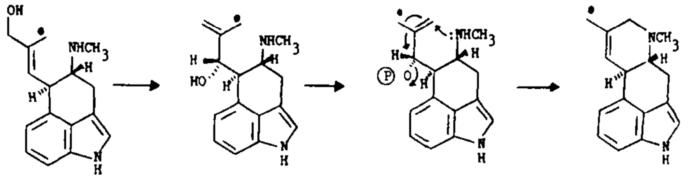
King *et al.*[235] succeeded in preparing a derivative, an N-acetyl methyl ester, which was soluble in chloroform, and which largely on the basis of its PMR spectrum was shown to have structure **36a**. Assuming that no rearrangement takes place during the derivatization, this would lead to structure **36b** for clavicipitic acid.[235] It will be noted that the above results with 5-tritiated mevalonic acid are not easily reconciled with this structure either. Taken at face value, they would suggest that clavicipitic acid must contain two isoprene residues, one of which retains both hydrogens from C-5 of mevalonate and the other only the *pro*-5*S* hydrogen. Conceivably, one of these might be lost during derivatization and during the recording of the electron-impact mass spectra, suggesting a structure such as **36b**. However, this possibility could be excluded, because chemical ionization and field desorption mass spectra[237] as well as determinations of the mass of the unit cell[238] clearly indicate a true molecular weight of 270. It is possible that the unusual results obtained with mevalonate-5-^{3}H are related to the observation of King *et al.*[235] that clavicipitic acid can be further resolved into two components which they presumed to be diastereomers. It is amusing to note that a 1 : 1 mixture of structures **35** and **36b** could produce all the data collected so far. In any event, the structure of this material obviously requires further examination.

In light of the above discussion it seems likely that clavicipitic acid or at least a component thereof is substituted at C-1 of the isoprenoid residue. Although clavicipitic acid is not converted into the tetracyclic ergolines,[239,240] it may arise from an early intermediate in ergoline biosynthesis, suggesting the possibility that the initial reaction in the biosynthetic sequence occurs at C-1 of the dimethylallyl residue of dimethylallyltryptophan. The following results from Anderson's group can be interpreted as supportive of this view. These workers recently observed conversion of dimethylallyltryptophan into clavicipitic acid by a microsomal fraction obtained from *Claviceps* mycelia.[240] The reaction is oxygen-dependent (although oxygen is most likely not incorporated into the product)[240] and results in the formation of hydrogen peroxide.[241] The same conversion, although in lower yield, was obtained non-enzymatically using a thioglycolate-Fe^{2+} system.[242] Whether these observations are related to the main biosynthetic pathway for the ergolines remains, of course, to be established.

Fate of the hydrogens from C-5 of mevalonate. Before the origin of isochanoclavine-I from chanoclavine-I was established, it seemed necessary to postulate a biogenetic scheme which would account for the independent formation of all the chanoclavine isomers, including the two enantiomers of chanoclavine-II. A mechanistically attractive scheme (Fig. 5), considered both in Arigoni's[198] and in our laboratory,[183] implies that a correlation exists between the stereochemistry of hydrogen abstraction from C-10 (ergoline numbering) and the stereochemistry of the chanoclavine resulting from the cyclization. As shown in Fig. 5, the scheme predicts that chanoclavine-I, and as a consequence possibly also agroclavine and elymoclavine should retain one of the diastereotopic hydrogens from C-5 of mevalonate and isochanoclavine-I the other. The scheme invokes an *sp*^{2} hybridization state for both C-10 and C-5, generated in the latter case from a Schiff base between pyridoxal phosphate and the amino group of the amino acid by decarboxylation or the amino group of the tryptamine analog by tautomerization. Circumstantial evidence for the involvement of pyridoxal phosphate in ergoline biosynthesis comes from early experiments showing that pyridoxal phosphate stimulates tryptophan incorporation into ergot alkaloids[161] and from Hsu and Anderson's finding[243] that isonicotinic acid hydrazide inhibits ergot alkaloid synthesis. Experimental evaluation of the scheme shown in Fig. 5 by feeding mevalonates tritiated stereospecifically at C-5, done both at the ETH[198] and in our laboratory,[183] showed clearly that all the alkaloids examined, agroclavine, elymoclavine, chanoclavine-I, isochanoclavine-I and (−)-chanoclavine-II, are formed with retention of the *pro*-5*S* and loss of the *pro*-5*R* hydrogen of mevalonate.[181-183] These results rule out the correlation between the stereochemistry of the clavines and the hydrogen removed during their formation which is predicted by the scheme shown in Fig. 5. They do not, however, disprove the principal mechanism of C-ring closure outlined in this scheme. In fact, the pathway labeled "a", modified to account for the fact that compound **34** is not a biosynthetic intermediate, might well be the obligatory route for the closure of ring C. If this is true, one would predict that (−)-chanoclavine-II has the absolute configuration 5*S*,10*R* and that (+)-chanoclavine-II, like isochanoclavine-I, might arise from chanoclavine-I.

Formation of ring D

Role of paliclavine. Several mechanisms have been considered in order to account for the closure of ring D and the *cis–trans* isomerization accompanying this process. The simplest possibility, formation of a carbonium ion at the hydroxymethyl carbon of chanoclavine-I[154,220] is ruled out by many of the results discussed below. A mechanistically more attractive alternative was considered by Arigoni's group.[120]



This proposal is also in conflict with some of the data obtained subsequently; however, it received strong support by the recent isolation, from *Claviceps* cultures, of paliclavine[40] (cf. Table 3), the C-9 epimer of the compound postulated above as a biosynthetic intermediate. This caused Acklin and coworkers[244] to examine the possible role of paliclavine in the formation of tetracyclic ergolines. He prepared chanoclavine-I-(N-^{14}CH$_3$) and paliclavine-(N-^{14}CH$_3$) and fed both precursors to cultures of *C. paspali* and a clavine-producing ergot strain. While chanoclavine-I was efficiently incorporated into the main alkaloids paspalic acid and agroclavine and elymoclavine, respectively, paliclavine was not utilized significantly. Examination of the minor alkaloids from these experiments further revealed that paliclavine does not arise from chanoclavine-I and is not converted into either chanoclavine-I or isochanoclavine-I. These data, in

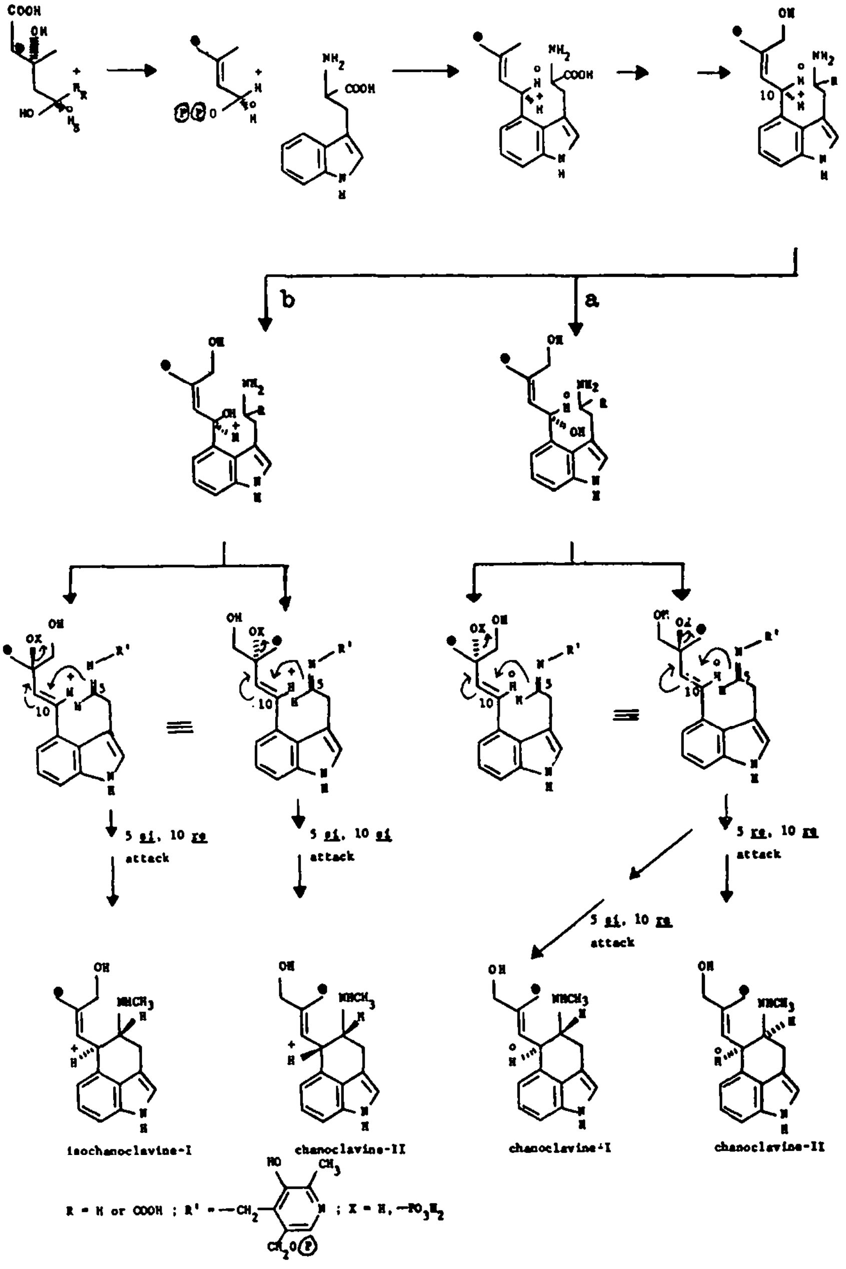


Fig. 5. Hypothetical scheme for C-ring formation during ergoline biosynthesis (from Ref. 183).

accord with the results discussed below, thus rule out paliclavine as an intermediate in the formation of ring D of the ergoline system. They furthermore necessitate a route for the formation of paliclavine independent of chanoclavine-I.

Chanoclavine-I-aldehyde. In the course of studies on the fate of the hydrogen at C-9 of chanoclavine-I during the conversion into tetracyclic ergolines we obtained data which suggested the possibility that one of the methylene hydrogens of the CH$_2$OH group is replaced by a different hydrogen in the process. This was verified by preparing chanoclavine-I-(17-^{3}H), mixing it with chanoclavine-I-4-^{14}C and feeding it to *Claviceps* strain SD 58. The elymoclavine isolated from this culture showed a tritium retention of 53%, confirming the loss of one of the labeled hydrogens from C-17 of chanoclavine-I.[245] This result suggested the intermediacy of a compound carrying only one hydrogen at C-17 and, as the most logical candidate, chanoclavine-I-aldehyde was prepared by MnO$_2$ oxidation of chanoclavine-I. Starting from chanoclavine-I-(17-^{3}H) and -(4-^{14}C-17-^{3}H) the data summarized in Fig. 6 were obtained.[245] They show that chanoclavine-I-aldehyde is incorporated into elymoclavine (a) specifically, all the tritium from the aldehyde group appearing at C-7, (b) efficiently, with an incorporation rate higher than that of chanoclavine-I, and (c) with essentially complete retention of the C-17 hydrogen. In a feeding experiment with mevalonate-3′-D$_3$ followed by mass spectral analysis of the resulting chanoclavine-I and elymoclavine it was confirmed that only one hydrogen at C-7 of elymoclavine, but both methylene hydrogens at C-17 of chanoclavine-I originate from C-3′ of mevalonate. NMR analysis of the

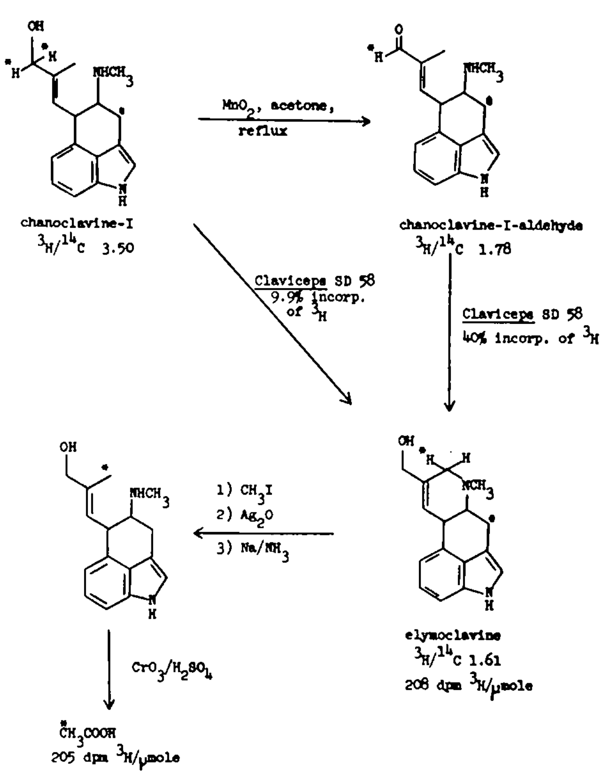


Fig. 6. Incorporation of chanoclavine-I-aldehyde into elymoclavine.

elymoclavine showed that the deuterium occupied the *pro*-7*S* position; thus the newly introduced hydrogen is the *pro*-7*R* hydrogen.[227] All these results are in accord with and support a mode of D-ring closure as outlined in Fig. 7, involving chanoclavine-I-aldehyde as an intermediate. Attempts to further evaluate this scheme by feeding the hypothetical intermediate isochanoclavine-I-aldehyde have been unsuccessful, because we have so far been unable to unequivocally prepare this compound.[246] Trapping experiments showed conversion of chanoclavine-I-aldehyde to chanoclavine-I, but failed to demonstrate the reverse reaction because we were unable to reisolate any aldehyde from the cultures.[227] Nevertheless, while various other explanations can be constructed which would account for the results, the most likely one seems to be that chanoclavine-I-aldehyde is an intermediate between chanoclavine-I and elymoclavine.

Intermolecular recycling of the C-9 hydrogen. Another very interesting aspect of the mechanism of ring D formation was uncovered when the fate of the hydrogen at C-9 of chanoclavine-I was traced. Our initial experiments on the cyclization of chanoclavine-I had shown[180] that the hydrogen at C-10 is completely retained in the process, but that chanoclavine-I-7-^{14}C-9-^{3}H is converted

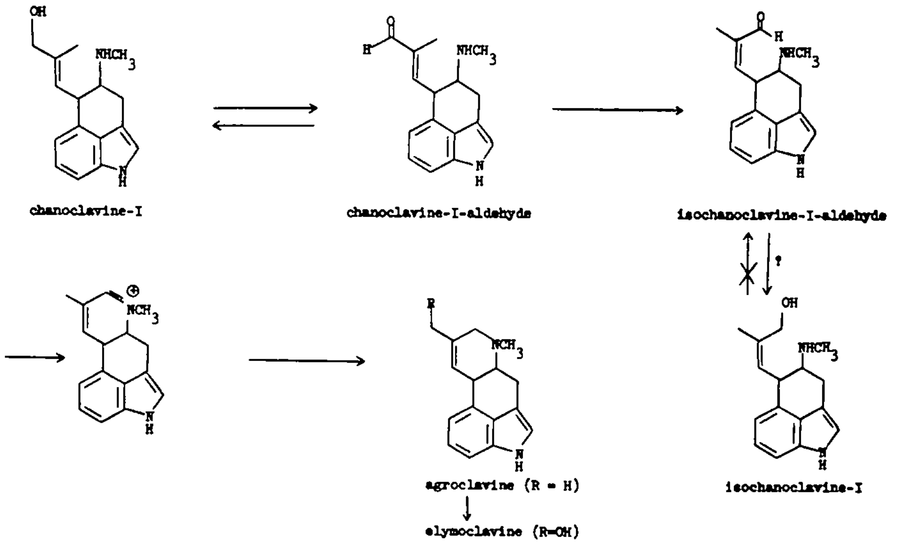


Fig. 7. Formation of tetracyclic ergolines via chanoclavine-I-aldehyde.

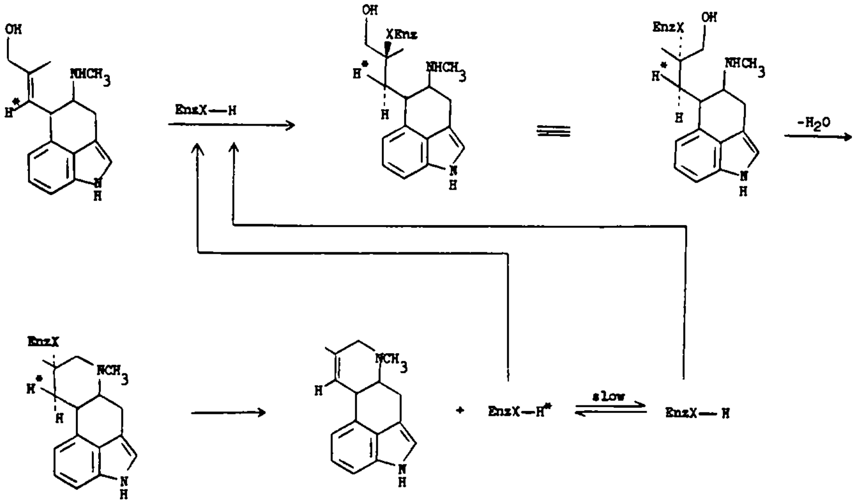
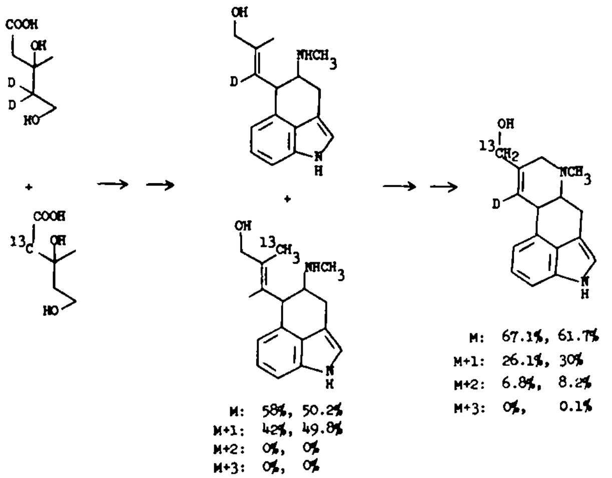


Fig. 8. Hypothetical mechanism for the formation of ring D of ergolines.

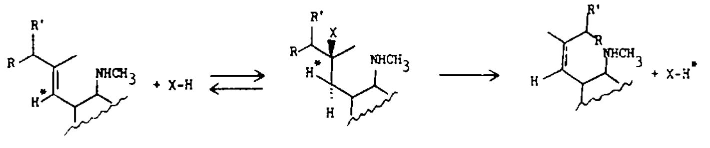
into elymoclavine and lysergic acid α-hydroxyethylamide with only about 70% tritium retention. Likewise, mevalonate-(2-^{14}C-4R,4-^{3}H) gave elymoclavine with about 70% tritium retention. Subsequent experiments[227] indicated that the degree of retention of this hydrogen can vary within the range of 40–80% depending on the rate of alkaloid synthesis and that a low rate of alkaloid formation correlates with low retention of the C-9 hydrogen. In order to rationalize these unusual data, we originally considered as a working hypothesis the mechanism shown in Fig. 8. The[105] crucial element of this mechanism is the addition of a second hydrogen at C-9, removal of the original hydrogen from this position and its transfer to the same carbon atom of another substrate molecule. Initial experiments with mevalonate-3′,4-$^{2}H_5$ tentatively supported such a mechanism, with the modification of invoking an aldehyde intermediate. Unequivocal proof for an intermolecular transfer of the C-9 hydrogen was obtained by feeding a 1:1 mixture of mevalonate-(2-^{13}C) (86% ^{13}C) and mevalonate-(4-$^{2}H_2$) (100% ^{2}H) to *Claviceps* strain SD 58 followed by mass spectral analysis of the resulting chanoclavine-I and elymoclavine for the percentage excess of M + 1 and M + 2 species:

The results show that chanoclavine-I only contains single labeled (^{2}H or ^{13}C) species, whereas elymoclavine shows an appreciable percentage of double labeled molecules, which, as shown by high-resolution peak-matching, contained both ^{13}C *and* deuterium in the same molecule. High-resolution NMR analysis of a sample of elymoclavine biosynthesized from mevalonate-(4-$^{2}H_2$) showed that all the deuterium was located at C-9, establishing that the hydrogen is transferred back into the 9-position.

A second, independent line of evidence also provides proof for the addition of a second hydrogen at C-9 and transfer of the original hydrogen. In feeding experiments with mevalonate-(2-^{14}C-4R,4-^{3}H) both Arigoni's laboratory[233] and we[227] observed that of the various alkaloids isolated, isochanoclavine-I and (−)-chanoclavine-II had about the same ^{3}H/^{14}C ratios as the precursor (in a recent careful experiment, however, Acklin[43] observed an 86·7% tritium retention for isochanoclavine-I), agroclavine and elymoclavine showed the usual decrease in the ^{3}H/^{14}C ratio to 63% and 52%, respectively, but chanoclavine-I was actually enriched in tritium (146% tritium retention). This finding indicated the operation of an isotope effect in the further metabolism of chanoclavine-I leading to



enrichment of tritium at C-9 in the unreacted material. This requires the addition of a second hydrogen at C-9, followed by competition between the two hydrogens in a subsequent elimination step and complete reversibility of the entire reaction sequence from chanoclavine-I to this elimination step. The following system or a modification thereof would satisfy these requirements without having to invoke non-stereospecific reactions:



To verify this explanation, mevalonate-2-^{14}C-4R,4-^{3}H was fed to cultures in the presence of a large excess of mevalonate-4-$^{2}H_2$ or a mixture of mevalonate-4-$^{2}H_2$ and unlabeled mevalonate. If the postulated intermolecular transfer of the C-9 hydrogen does indeed occur, the competition between the two hydrogens in the elimination step should now be largely between tritium and deuterium rather than between tritium and normal hydrogen, leading to a lower tritium enrichment in the unreacted chanoclavine-I. In accord with this prediction, the tritium retention in clanoclavine-I was reduced to 114–117% in the presence of mevalonate-4-$^{2}H_2$ and to 125–130% in the presence of the mixture of deuterated and unlabeled mevalonate.[227]

All these experiments leave no doubt that the hydrogen at C-9 of chanoclavine-I is recycled during the process of D-ring formation in a manner similar to that outlined in our original working hypothesis (Fig. 8). Whether this intermolecular transfer occurs as a proton or as a hydride cannot be decided at the present time. In the first case, a mechanism as outlined in Fig. 9 can be envisioned, leading either to isochanoclavine-I-aldehyde (path b) or to the corresponding carbinolamine (path a).[227] An interesting variation of this scheme, which is considered by Acklin,[43] involves thiamine pyrophosphate as a cofactor in this reaction, which is used to "mask" the carbonyl group of chanoclavine-I-aldehyde. This would allow an actual "repolarization" of the carbonyl group rather than a mere masking. The second option could, for example, involve a reversible 1,4-reduction of the α,β-unsaturated carbonyl system (Fig. 10). In possible support of a redox mechanism, we recently found that our cell-free system for the conversion of chanoclavine-I to agroclavine and elymoclavine is flavine-dependent.[247]

Cyclization of dihydrochanoclavines. An altogether different line of thought regarding the cyclization of chanoclavine-I to tetracyclic ergolenes has been pursued in Voigt's laboratory in Berlin. In chemical model studies this group found that various oxidative reactions with chanoclavine-I did not lead to identifiable tetracyclic

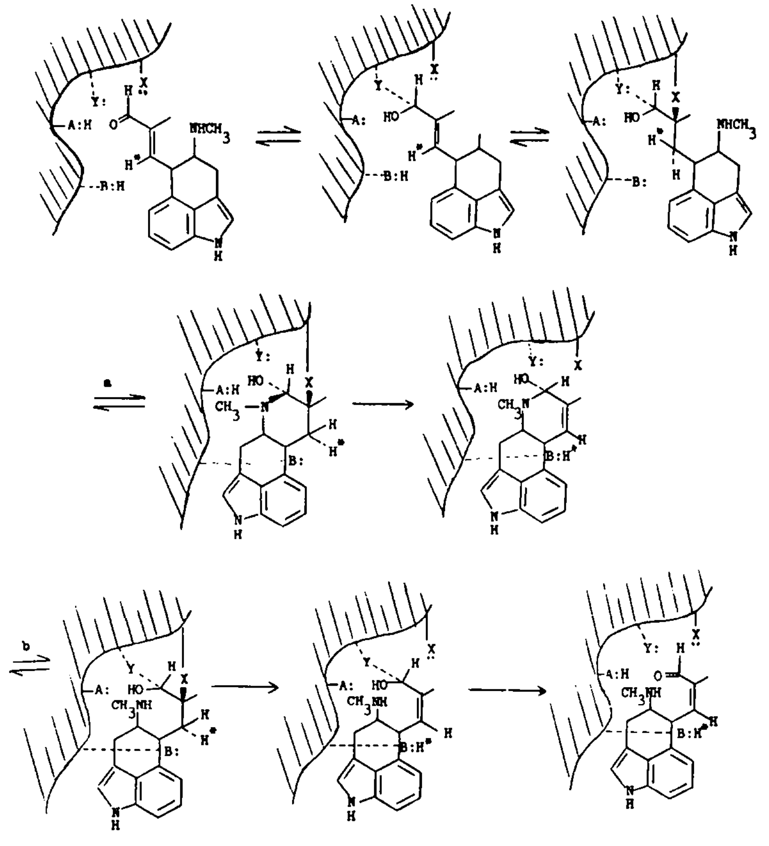


Fig. 9. Isomerization mechanism involving transfer of the C-9 hydrogen of chanoclavine-I-aldehyde as a proton.

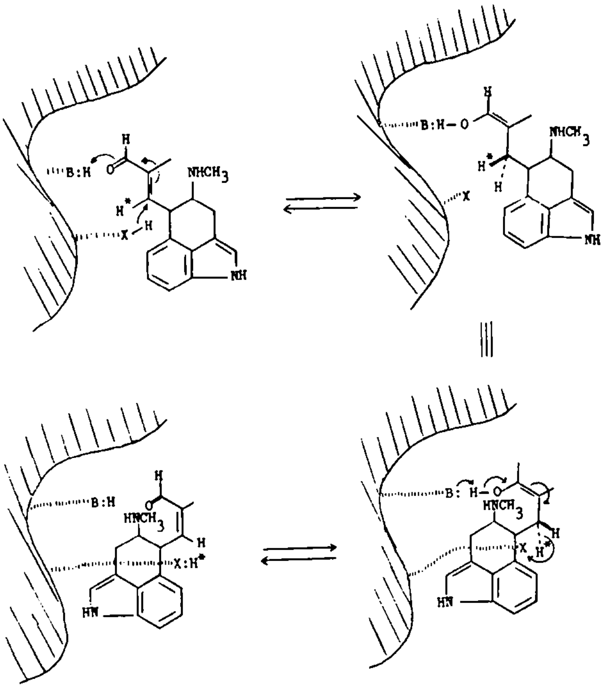
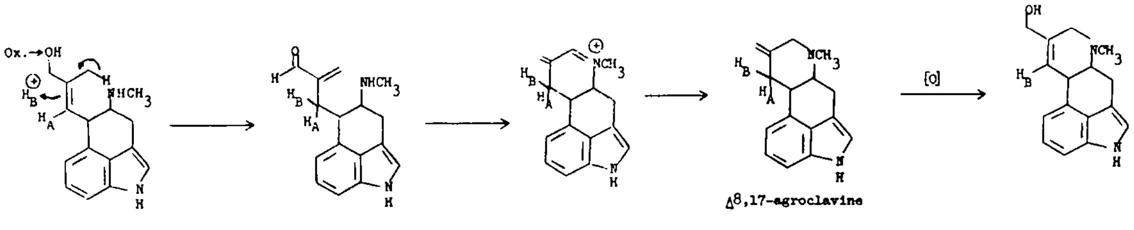


Fig. 10. Isomerization mechanism involving transfer of the C-9 hydrogen of chanoclavine-I-aldehyde as a hydride.

ergolines,[248] but that catalytic hydrogenation under various conditions produced consistently significant amounts of the two C-8 epimeric dihydroagroclavines, festuclavine and pyroclavine (see Table 3), in addition to the two C-8 epimers dihydrochanoclavine-I and isodihydrochanoclavine-I.[249,250] The same products were obtained upon hydrogenation of isochanoclavine-I.[251] Treatment of the dihydrochanoclavines with zinc chloride produced the corresponding dihydroagroclavines.[249,250] Both dihydrochanoclavine-I and isodihydrochanovlavine-I were detected as naturally occurring alkaloids in a *Claviceps paspalum* strain and in rye ergot.[249] These findings led Voigt and coworkers to suggest that the biosynthesis of tetracyclic ergot alkaloids can occur by a reductive pathway,[250] for which they considered the sequence dihydrochanoclavine → dihydroagroclavine → agroclavine → elymoclavine.[251] Initial non-isotopic feeding experiments with the dihydrochanoclavine mixture using ripening sclerotia of rye ergot, indicating about 10% conversion into dihydroagroclavines and an approximately 25% increase in the amount of peptide alkaloids,[250] were soon repeated with Wilzbach-tritiated dihydrochanoclavines.[252] These experiments showed 7·7% conversion into dihydroagroclavines, but no labeling of the peptide alkaloids and little or no conversion into agroclavine. The latter result is in accord with earlier findings by Agurell and Ramstad[205] and Baxter *et al.*[207] who had shown that the conversion of agroclavine to festuclavine and pyroclavine is irreversible. Evaluation of the utilization of the dihydrochanoclavines-(G-^{3}H) in saprophytic cultures of *Claviceps* strain SD 58 gave a somewhat different picture.[253] In this case, no incorporation into dihydroagroclavines was observed, but chanoclavine-I, agroclavine and elymoclavine were labeled, although the specific incorporation rates were very low (0·005–0·01%). Chanoclavine-I had a higher specific radioactivity than the two tetracyclic ergolenes, suggesting that the dihydrochanoclavines entered the pathway by dehydrogenation to chanoclavine-I. In summary, these results do not support a reductive pathway via dihydrochanoclavines as the major or even a significant route to the tetracyclic $\Delta^{8,9}$-ergolenes.

Other observations. In 1970, Ramstad *et al.*[254] reported the preparation of a cell-free system from *Claviceps* mycelia capable of converting chanoclavine-I into elymoclavine in the presence of ATP, Mg^{2+}, NADPH and oxygen. No detectable quantity of agroclavine was formed, although a small amount of material of similar chromatographic behaviour was observed, and added agroclavine was poorly converted to elymoclavine. This led the authors to postulate a pathway from chanoclavine-I to elymoclavine not involving agroclavine as an intermediate. This pathway would be a second one, in addition to the well-established route chanoclavine-I → agroclavine → elymoclavine. A reasonable route, modified from Ramstad's original proposal[254] to accommodate some of the more recent data, could involve the $\Delta^{8,17}$-isomer of agroclavine as an intermediate, e.g.:[226]

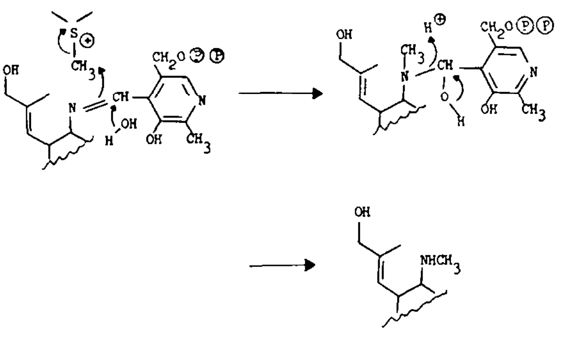


To test this mechansim, Arigoni *et al.*[226] prepared $\Delta^{8,17}$-agroclavine by dehydrobromination of 17-bromofestuclavine, which had been obtained from O-tosyl-dihydrolysergol-I with LiBr in acetone. The 17-tritiated compound was prepared analogously and fed to cultures of *Claviceps* alone and in competition with agroclavine-(17-^{14}C). No significant incorporation of tritium into elymoclavine was observed, ruling out a pathway via $\Delta^{8,17}$-agroclavine.

This finding does, of course, not disprove the operation of a second pathway to elymoclavine not proceeding through agroclavine. There is other circumstantial evidence which can be interpreted to support two pathways. In feeding experiments with mevalonate-(2-^{14}C-4*R*,4-^{3}H), agroclavine and elymoclavine usually show somewhat different tritium retentions. Likewise, we found[255] that after feeding mevalonate-(2-^{14}C-2-^{3}H) chanoclavine-I and agroclavine had quite different tritium retentions (92% vs 69%) whereas elymoclavine was relatively close to agroclavine (62% tritium retention), and Agurell[256] has made similar observations. This could indicate that the methyl group of chanoclavine-I must be functionalized, losing one hydrogen, during the conversion to agroclavine. None of this evidence, however, is compelling, because both the hydrogens at C-2 and the *pro*-4*R* hydrogen of mevalonate are subject to partial exchange at some stage along the pathway, the latter during D-ring formation and the former in the isopentenylpyrophosphate isomerase reaction. The rate of this exchange, relative to the rate of alkaloid synthesis, may change during the culture period and the various alkaloids have quite different turnover rates. It is therefore not unlikely that the alkaloids isolated at the end of the experiment will have suffered tritium loss by exchange to different extents. The enzymatic experiments of Ramstad's group[254] are not compelling evidence for two pathways to elymoclavine either. Gröger *et al.*[257,258] have obtained an enzyme system which is not dependent on oxygen and produces only agroclavine but not elymoclavine from chanoclavine-I. In our laboratory, we[247] have obtained a cell-free system from *Claviceps* which converts chanoclavine-I into agroclavine and elymoclavine. In the opinion of this author, the observed differences may well reflect differences in the amount of agroclavine hydroxylase[259,260] present in these preparations and different degrees of structural association of the hydroxylase with the chanoclavine-I-cyclase. The ATP-dependence observed with Ramstad's and with Gröger's enzyme system, but not with ours, is somewhat more puzzling. However, it has not been shown that the reaction proceeds with stochiometric consumption of ATP, and nucleotides like ATP are known to occasionally stabilize enzymes. Thus, the ATP may serve to protect the enzyme from inactivation rather than as a reaction partner.

The N-methylation step

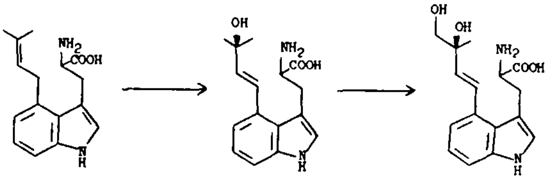
At some stage along the pathway the amino nitrogen of the ergolene precursor must be methylated by transfer of a methyl group from S-adenosylmethionine. As mentioned earlier this reaction must occur after the initial isoprenylation of tryptophan, but its precise location in the sequence has so far not been determined. Mechanistic considerations suggested that methylation might take place at the N-demethylchanoclavine-I stage, because this would allow for the ready involvement of a pyridoxalphosphate-N-6 Schiff base in the closure of ring C. Both N-demethylchanoclavine-I and -II were detected as natural alkaloids in *Claviceps*[43,44] but feeding experiments with either compound in tritiated form showed no incorporation of label into tetracyclic ergolenes.[44] In accordance with this result, Acklin[43] found that N-norchanoclavine-I arises from chanoclavine-I. The ability of *Claviceps* to non-specifically demethylate various N-methylated bases in the tryptophan and clavine series is well documented.[35,185,261] In contrast, the introduction of the N-methyl group during ergolene biosynthesis seems to be a reaction of high substrate specificity. Fehr[120] had found earlier that N-noragroclavine is not methylated by the fungus. The fact that N-norchanoclavine-I is not an intermediate in the biosynthesis suggests that the N-methylation must take place either prior to or during the formation of ring C or at the stage of an as yet unknown (and unsuspected) product of C-ring formation. Acklin[43] considers the possibility that methylation takes place as part of the reaction leading to closure of ring C. Assuming that C-5 of the precursor is activated by Schiff base formation with pyridoxal phosphate, the reaction of necessity leads to an enzyme-bound pyridoxal phosphate imine, the decomposition of which could be initiated by addition of $CH_3^{\oplus}$ instead of $H^{\oplus}$:



A possible drawback of this mechanistically attractive proposal is that it implies a very complicated enzyme active site.

Current working hypotheses

It is not possible at this time to write a complete pathway and detailed mechanisms of ergolene biosynthesis with any degree of confidence. While some intermediates and reaction mechanisms are well secured, others are still very hypothetical. Figure 11 is an attempt to draw up a complete pathway which represents the minimum number of steps required to accommodate most or all of the presently available data. Many variations of this scheme are, of course, possible. The initial hydroxylation of dimethylallyltryptophan, for example, could lead to compound **38** rather than **37** or **37** could rearrange to **38**, and the subsequent second hydroxylation would produce directly **39**:



38 **39**

Another interesting variation, discussed by Pachlatko[226] involves C-ring closure through an epoxide, postulated to

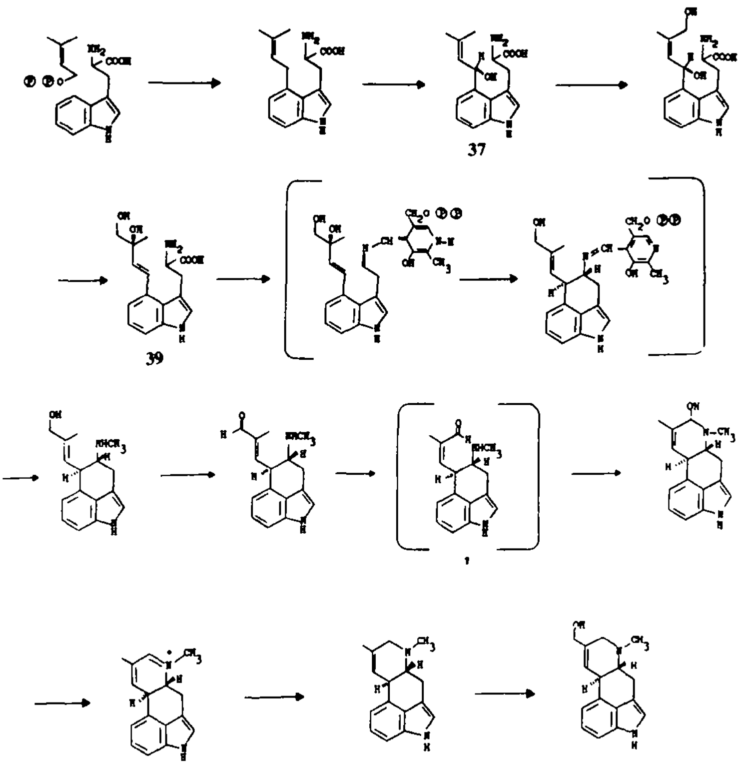
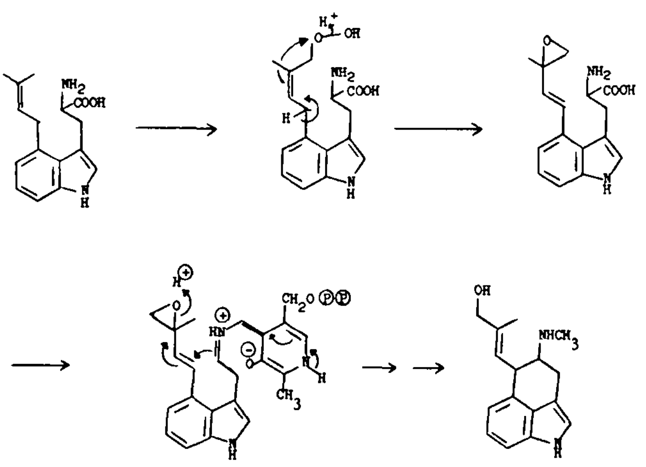


Fig. 11. Hypothetical pathway for the formation of tetracyclic ergolenes from tryptophan and dimethylallyl-pyrophosphate.

be formed via a hydroperoxide:

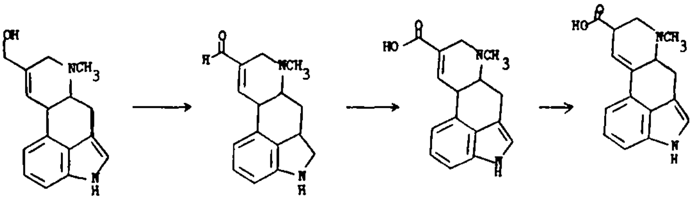


An analogous cyclization through an epoxide formed at C-9/C-10 (ergoline numbering) could readily explain the independent formation of paliclavine. In the scheme shown in Fig. 11 it is not clear whether isochanoclavine-I-aldehyde is an intermediate or whether the cyclization leads directly to the carbinolamine (see Fig. 9). However, it seems likely now that isochanoclavine-I is formed via this aldehyde. As discussed above, the evidence for more than one pathway to elymoclavine is by no means compelling, and while this possibility has to be kept in mind, it is quite feasible to account for all presently available data on the basis of one single biogenetic pathway.

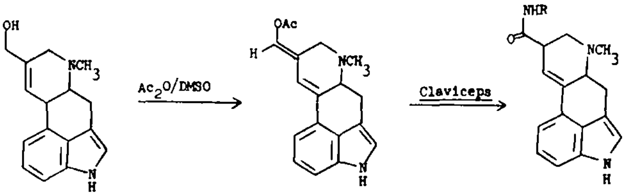
FORMATION OF LYSERGIC ACID DERIVATIVES

Conversion of clavines into $\Delta^{8,9}$- and $\Delta^{9,10}$-lysergic acid

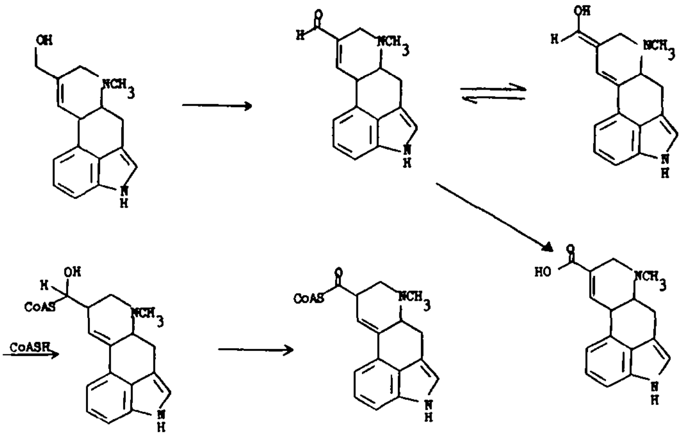
The second part of the problem of ergot alkaloid biosynthesis is that of the formation of lysergic acid and its derivatives. As mentioned earlier elymoclavine has been shown[206] to be the precursor of lysergic acid derivatives both in sclerotia and in cultures of the ergot fungus. This finding has since been confirmed repeatedly.[209,262-267] Gröger *et al.*[268] demonstrated by chemical degradation that elymoclavine is incorporated specifically into lysergic acid amides and Eich[269] recently showed that addition of elymoclavine to cultures of *Claviceps paspali* substantially increases the yield of lysergic acid amides. The detailed sequence of steps involved in this conversion has not yet been fully elucidated, but it has been shown[262,264] that the reaction does not proceed through penniclavine or lysergol. The latter excludes shift of the double bond prior to oxidation of the hydroxymethyl group. Labeled $\Delta^{8,9}$-lysergic acid, the naturally occurring paspalic acid, was converted into lysergic acid amides with an efficiency approaching that of elymoclavine.[266,270] This is widely regarded as proof that the double bond isomerization takes place at the oxidation stage of the acid:



However, this conclusion is not inescapable, because $\Delta^{8,9}$-lysergic acid is known to isomerize slowly spontaneously to the $\Delta^{9,10}$-acid and the positive outcome of the incorporation experiment could be due to slow spontaneous conversion of the added labeled $\Delta^{8,9}$-lysergic acid to the $\Delta^{9,10}$-isomer, a known precursor, rather than to an enzymatic process. The alternative possibility that the double bond is shifted at the aldehyde stage is therefore still a viable option, particularly since the lysergaldehydes are likely to exist almost entirely in the enol form. Many attempts in our laboratory to synthesize $\Delta^{8,9}$- or $\Delta^{9,10}$-lysergaldehyde have so far been unsuccessful. We did succeed in oxidizing elymoclavine with acetic anhydride/DMSO, but obtained as product the enol acetate **40** rather than the desired aldehyde:[267]



Attempts to generate the aldehyde from this derivative were again totally unsuccessful, but upon feeding a labeled sample to *Claviceps purpurea* incorporation into lysergic acid derivatives was observed[267] with an efficiency comparable with that of elymoclavine-O-acetate (the organism is known to have esterase activity[263]). While this does not prove the intermediacy of $\Delta^{9,10}$-lysergaldehyde, it does indicate that the question of the mechanism of conversion of elymoclavine to lysergic acid derivatives cannot yet be considered completely settled. Another unresolved question relates to the further derivatization of lysergic acid. Essentially all natural derivatives of lysergic acid are amides and their formation requires an activation of the carboxyl group, most likely in the form of a coenzyme A thiolester. Free lysergic acid is converted to lysergic acid amides[263] and the fact that lysergic acid-(8-^{3}H) gives rise to lysergic acid amides without loss of tritium indicates that this conversion does not proceed through the $\Delta^{8,9}$-isomer.[270] Gröger *et al.*[271] demonstrated that cell-free extracts of *Claviceps* can catalyze the formation of lysergyl-coenzyme A. This evidence can be taken to indicate that activation takes place at the lysergic acid stage, but again, the evidence is not compelling. Gröger's work showed[271] little correlation between the ability to activate lysergic acid and alkaloid production and, in fact, some strains which do not produce lysergic acid derivatives still gave cell-free extracts able to activate lysergic acid. This enzyme system thus may not be specific for lysergic acid. For this reason one should still keep in mind the possibility that the oxidation of elymoclavine leads directly to an activated form of lysergic acid, e.g. the coenzyme A ester, and that the free acid is not an intermediate in the formation of lysergic acid derivatives. This could involve a pathway similar to one we have proposed some time ago:[264]



$\Delta^{8,9}$-Lysergic acid would in this scheme occupy the position of an alternate endproduct formed in strains which are unable to carry out the "energy-conserving" dehydrogenation of the lysergaldehyde-CoA hemiacetal. This proposal would explain the facts that free lysergic acid is not accumulated by ergot strains to any significant extent, and that no amide derivatives of $\Delta^{8,9}$-lysergic acid are found. Obviously, however, this pathway is at the moment entirely speculative.

A slightly different route has been found to operate in the formation of the dihydrolysergic acid peptide alkaloid dihydroergosine (see Table 1) in *Sphacelia sorghi*.[272] A feeding experiment with mevalonate-(2-^{14}C-5-^{3}H) showed that one hydrogen from C-5 of mevalonate was retained in this alkaloid, excluding the intermediacy of $\Delta^{9,10}$-lysergic acid derivatives. Further experiments demonstrated good incorporation of festuclavine, dihydroelymoclavine and dihydrolysergic acid, but only poor incorporation of agroclavine. This indicates that in this case the entire sequence from the clavines to the peptide alkaloid takes place in the absence of a double bond in ring D and that the reduction of the allylic double bond must occur early in the biosynthetic sequence, possibly, at the agroclavine stage or even earlier, e.g. at the chanoclavine-I stage. Dihydrochanoclavines could not be detected in these cultures, but were not evaluated as precursors.[272]

Origin of the non-ergoline moiety of simple lysergic acid amides

The two simple lysergic acid amides which have been studied extensively are lysergic acid α-hydroxyethylamide and ergonovine (see Table 2), and in both cases the mode of formation of the side chain is not entirely clarified. Agurell[273] showed that tritiated lysergic acid amide and isolysergic acid amide were not incorporated into the α-hydroxyethylamide. Complementing this finding, Kleinerova and Kybal[274] recently presented evidence suggesting that lysergic acid α-hydroxyethylamide is the only native amide produced and that the co-occurring lysergic acid amide and the two corresponding isolysergic acid derivatives are formed secondarily. Agurell also reported non-incorporation of ethylamine-(1-^{14}C)[273] and acetamide-(1-^{14}C)[154] into the α-hydroxyethylamide, but found that L-alanine-(U-^{14}C) was incorporated.[154] The incorporation of alanine has been confirmed repeatedly[275-277] and the majority of the label from alanine-(2-^{14}C) has been shown to reside in the carbinolamide moiety.[275,277] Alanine-(1-^{14}C) was not incorporated and pyruvate-(2-^{14}C) and -(1-^{14}C) gave results very similar to those obtained with the corresponding alanines.[277] Alanine-^{15}N labeled predominantly the amide nitrogen.[275,277] Interestingly, L-alanine-(U-^{14}C-^{15}N) was incorporated into the carbinolamide moiety consistently with an increase in the ^{15}N/^{14}C ratio considerably above that predicted for loss of one carbon.[277] This could mean that alanine is not the direct precursor, but might on the other hand simply reflect the metabolic activity of this amino acid.

The origin of the alaninol side chain of ergonovine has also been traced to L-alanine,[276,278] whereas α-methylserine was ruled out as a precursor.[278] The role of L-alaninol itself as a precursor of the ergonovine side chain is still somewhat unclear. Two groups reported no incorporation of L-alaninol-(U-^{14}C) or -^{3}H into ergonovine,[278,279] but a third group found much higher incorporation of L-alaninol than L-alanine into the ergonovine side chain.[276]

Based on his early results and on structural comparisons, Agurell[154] proposed an attractive general hypothesis for the formation of lysergic acid amide derivatives involving lysergylalanine as an intermediate (Fig. 12). Experimental evaluation of this scheme by feeding of d-lysergyl-L-alanine-(2-^{14}C) to *Claviceps paspali* showed no significant incorporation of this compound into the

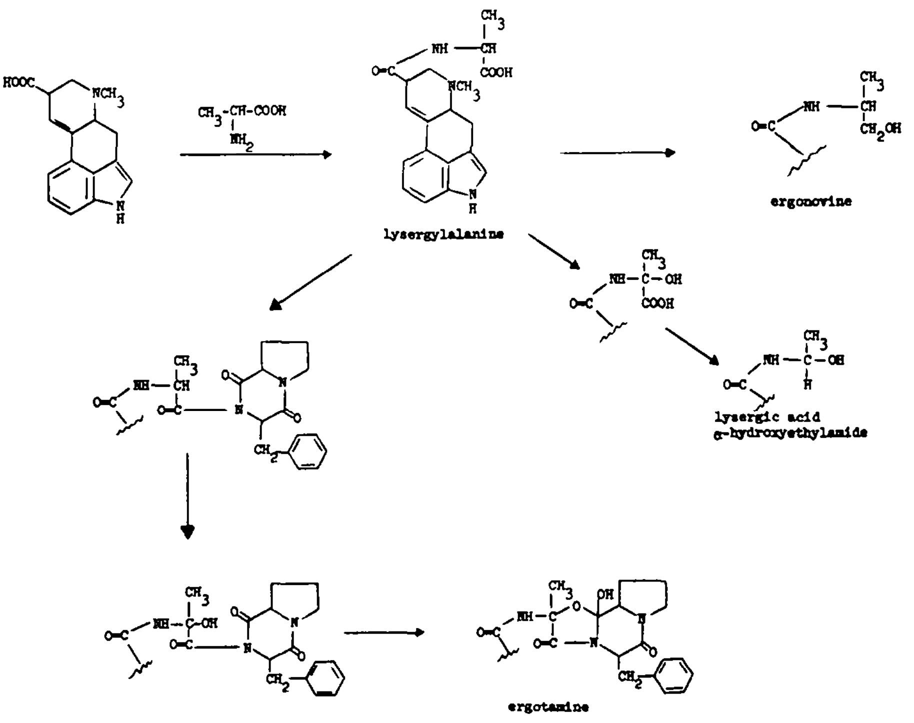


Fig. 12. Hypothetic scheme for formation of lysergic acid amide derivatives according to Agurell.[154]

carbinolamine side chain, but gave a small (1·77%) and specific (97·4% of radioactivity at C-2 + C-3 of alaninol side chain) conversion into ergonovine.[280] However, the low incorporation rates and failure to demonstrate the formation of lysergylalanine in this organism by trapping experiments led us to question that this compound is a normal intermediate in the biosynthesis of ergonovine.[281]

Origin and formation of the peptide moiety

The formation of the peptide moiety of peptide ergot alkaloids has received increasing attention in recent years. As would be expected, lysergic acid[276,284] and the amino acids phenylalanine,[282,286] proline,[283,286] valine,[284,285] and leucine[284] are specifically incorporated into the appropriate constituents of the respective peptide alkaloids. The available evidence also suggests strongly that the α-hydroxyamino acid moieties of ergotamine, ergocornine and ergokryptine originate from the corresponding amino acids. Degradation[287] of ergotoxine obtained from a feeding experiment with D,L-valine-(1-^{14}C) showed that the α-hydroxyvaline moiety of the alkaloid mixture had 59% of the specific radioactivity of the parent alkaloid (another 37% were accounted for by the valine moiety of ergocornine) and that 92% of this label resided at C-1 of the α-hydroxyvaline.[285] Similar results were obtained in numerous experiments.[284,285] Valine-^{15}N gave significant labeling of the lysergamide nitrogen, but the experiments were not entirely conclusive because of the presence of substantial amino acid transaminase activity.[284] Nevertheless, together with the failure of lysergic acid amide-^{15}NH$_2$ to label the alkaloid they do suggest that the nitrogen of the α-hydroxyamino acid moiety originates from valine. Studies on ergotamine formation using L-alanine-(U-^{14}C) showed incorporation of label into the α-hydroxyalanine portion, but a large percentage of the radioactivity was scrambled over the rest of the molecule.[276,279,286,288] Feeding L-alanine-(U-^{14}C) at high concentrations improved the specificity of incorporation; up to 74% of the label were recovered in the α-hydroxyalanine portion under these conditions.[288] Even better results were obtained with alanine-(1-^{14}C), which gave ergotamine carrying about 90% of the label in the α-hydroxyalanine moiety.[286] This leaves little doubt of the direct origin of this portion of the molecule from alanine.

Considerable efforts have been made to elucidate the mode of assembly of the peptide alkaloids. Voigt and Bornschein[289] concluded from feeding experiments with non-labeled ergonovine, which gave substantial increases in the levels of peptide alkaloids, that ergonovine is an intermediate in the formation of the peptides. However, experiments with ergonovine labeled in the lysergic acid[290] or alaninol moiety[288] showed little or no incorporation of radioactivity into ergotamine. According to Agurell's hypothesis (Fig. 12) d-lysergyl-L-alanine should be a precursor of ergotamine and, in an extension of it, d-lysergyl-L-valine should be the precursor of ergocornine and ergokryptine. The latter was postulated by Abe[223] and seemed to be supported by the isolation of lysergylvaline methyl ester from ergot.[16] Experimental evaluation of this question in ergotamine biosynthesis during d-lysergyl-L-alanine-(alanine-U-^{14}C) gave incorporations not exceeding those obtained with alanine-U-^{14}C and labeling patterns similar to those produced by alanine-U-^{14}C. Both d-lysergyl- and d-isolysergyl-L-alanine were incorporated to about the same extent (0·26–0·52%), whereas d-lysergyl-D-alanine was utilized much more poorly (0·03%). All the data are compatible with incorporation of the label only after hydrolysis of the precursor to alanine; they do not support the lysergylamino acid hypothesis.[288] More conclusive evidence was obtained in studies on ergocornine and ergokryptine biosynthesis using lysergylvaline-(1-^{14}C).[285] Again, both d-lysergyl- and d-isolysergyl-L-valine were about equally effective, but their incorporation rates (ca. 5%) did not exceed those of valine itself, and their D-valine analogs were about an order of magnitude less efficiently utilized. Most importantly, the labeling pattern of the alkaloids obtained from lysergylvaline matched closely that produced by valine itself. In particular, both the hydroxyvaline and the valine moiety of ergocornine were labeled in about the same ratio as in the valine experiment (59%/37% vs 51%/33%). This showed clearly that lysergylvaline was not incorporated as an intact unit but only after breakdown to valine. Positive results with d-lysergyl-L-valine-methyl ester-(G-^{3}H) in a cell-free system prepared from an ergokryptine-producing strain were obtained by Abe's group.[291] The ester gave about 1·2% incorporation into ergokryptinine/inine and, in a competition experiment, reduced the incorporation of elymoclavine-^{14}C by about a factor of three. However, no degradation of the labeled peptide alkaloid was carried out and the results therefore do not prove intact incorporation of lysergylvaline. The notion that the latter is only incorporated following degradation to its components is supported by recent work from Gröger's laboratory.[292] The authors showed that both intact cells and cell-free extracts of *Claviceps purpurea* readily degraded lysergyl-L-alanine methyl ester and lysergyl-L-valine methyl ester to the corresponding lysergylamino acids and to lysergic acid and alanine or valine, respectively. Trapping experiments in peptide alkaloid-producing strains failed to detect any formation of lysergylalanine or lysergylvaline. The evidence thus suggests strongly that lysergyl amino acids are not free intermediates in the formation of peptide ergot alkaloids.

All the degradations of ergocornine obtained from labeled valine or valine precursors had always consistently shown a higher specific radioactivity of the hydroxyvaline compared to the valine moiety.[284,285] This suggested that the peptides are built up starting from the proline end.[285] At the same time Abe[223,293] reported the isolation of prolyldiketopiperazines corresponding to the peptide portions of peptide alkaloids from ergot cultures, in particular L-leucyl-D-proline-lactam and L-phenylalanyl-D- and L-proline-lactam. The time-course of their appearance suggested a connection with alkaloid formation.[293] Using cell-free extracts and intact cells of *Claviceps purpurea* they[223,294] then showed incorporation of tritiated L-leucyl-D- and L-proline-lactam into ergokryptine/inine (0·06–1·45%) and L-phenylalanyl-D- and L-proline-lactam into ergotamine/inine (0·04–0·3%). No degradations were carried out and the almost equal incorporations of the D- and L-proline lactams into the alkaloids in which the proline has L-configuration suggests that the precursors may have been hydrolyzed prior to incorporation of the label. This is the conclusion reached in extensive feeding experiments with various open-chain and cyclic dipeptides and tripeptides followed by degradation of the alkaloids. The labeling pattern of ergocornine from valine-(1-^{14}C) was not changed by feeding the amino acid together with a large excess of unlabeled L-valyl-L-proline.[285] Labeled L-valyl-L-leucine, L-leucyl-L-proline-lactam and L-valyl-L-proline-lactam were incorporated not only into the alkaloid for which they should be precursors, but into both ergocornine and ergokryptine and the isotope distributions in the products were indicative of cleavage of the precursors prior to incorporation.[295] A similar result was obtained with the tripeptide L-valyl-(1-^{14}C)-L-valyl-L-proline.[296] Further experiments of Gröger *et al.*[297] showed that in addition to the three dipeptides studied earlier[295] the dipeptides L-prolyl-U-^{14}C-L-alanine and L-prolyl-U-^{14}C-glycine, which do not correspond to any sequence in the peptide ergot alkaloids, were incorporated, as was the tripeptide L-valyl-L-leucyl-L-proline-U-^{14}C. The latter labeled both ergokryptine, for which it could be a precursor, and ergocornine, for which it cannot be a direct precursor. This work also showed that each of the precursors labeled the mycelial protein and the appropriate amino acid in the soluble amino acid pool, and demonstrated that washed mycelia of *Claviceps purpurea* cleaved the added peptides, e.g. leucyl-proline-lactam.[297]

The available evidence thus excludes virtually all the plausible free intermediates in the formation of the peptide chain of these alkaloids. This led to the suggestion that the assembly of the peptide chain takes place in a concerted fashion on the surface of a multienzyme complex.[296] A way in which this could occur is outlined in Fig. 13. The chain could grow starting from the lysergic acid end, analogous to the direction of chain growth in gramicidin S and tyrocidin biosynthesis,[298] or from the proline end, as suggested by the higher specific radioactivity of the α-hydroxyvaline compared to the valine moiety of ergocornine. The reaction sequence would produce a lysergyltripeptide covalently linked to a group on the enzyme, e.g. an SH-group, through the carboxyl group of proline. Release from the enzyme by internal displacement of the sulfur would lead simultaneously to formation of the lactam ring. A principally similar mode of lactam ring formation has been proposed earlier by Ramstad.[156] α-Hydroxylation either directly or by a dehydrogenation/hydration sequence via the 2,3-dehydroamino acid or the imino acid[299] followed by enzymatic or spontaneous cyclol formation would complete the reaction sequence. The proposal is supported by the isolation from ergot of the D-proline isomer of a compound of the type resulting from the postulated release from the multienzyme (**40**).[15] The isolated compound **4** is thought to arise by the readily occurring epimerization of L-proline lactams from the corresponding L-proline isomer, which would be a logical intermediate in the biosynthesis of ergocristine.[15] **4** on treatment with methanol readily produces lysergylvaline methyl ester and L-phenylalanyl-D-proline, explaining the isolation of compounds of this type from *Claviceps*. The validity of this proposed route will have to be checked by further experimentation.

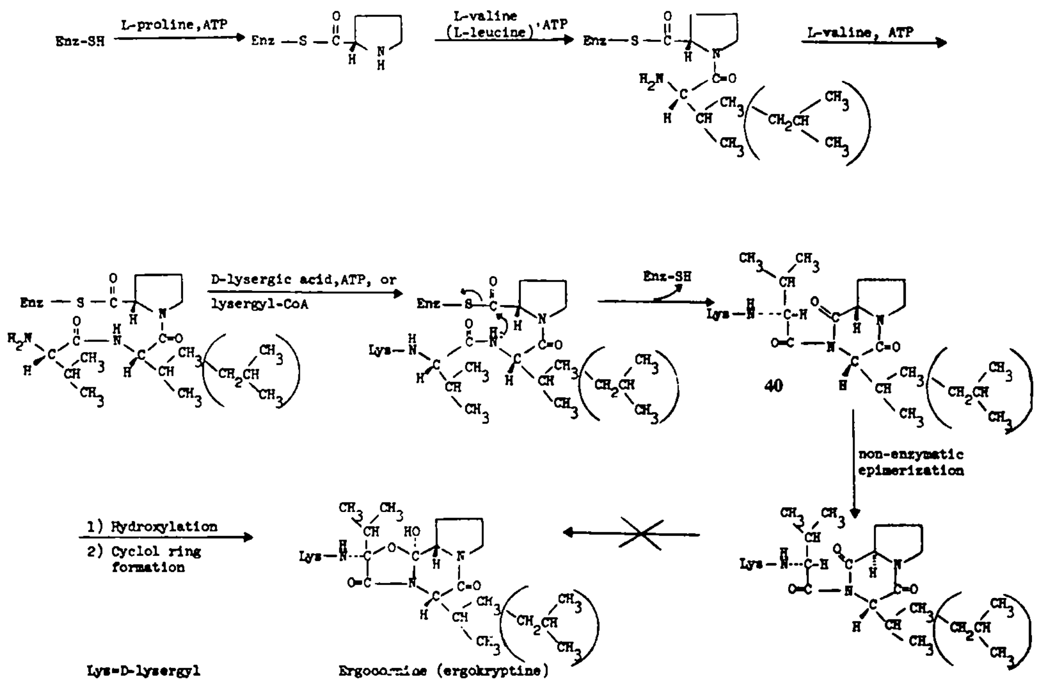
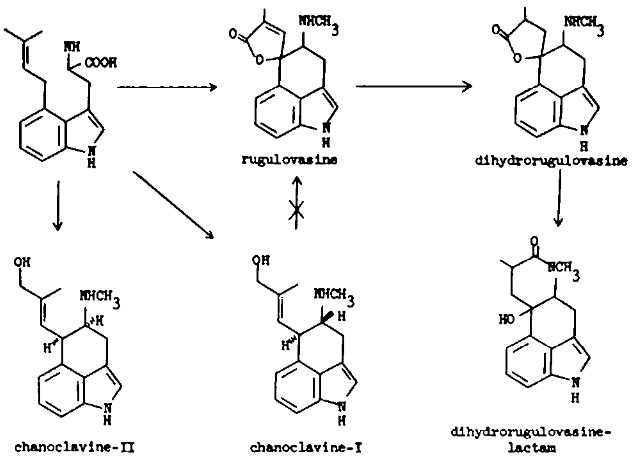


Fig. 13. Hypothetical formation of peptide ergot alkaloids on a multienzyme complex according to Floss *et al.*[296]

FORMATION OF ERGOLINES AND SECOERGOLINES IN ORGANISMS OTHER THAN *CLAVICEPS*

Rugulovasine biosynthesis in Penicillium concavo-rugulovasum

The structure of the rugulovasines[45] suggested a close biogenetic relationship to the ergolines, particularly the chanoclavines. Feeding experiments with cultures of *Penicillium concavo-rugulosum* indicated efficient incorporation of tryptophan-(3-^{14}C) (30–41·8%) and mevalonic acid-(2-^{14}C) (10·9–14·2%) into rugulovasine, as well as into chanoclavines-I and -II.[300] Likewise, tritiated dimethylallyltryptophan was incorporated into all three compounds; however, chanoclavine-I was not converted into either rugulovasine or chanoclavine-II. Rugulovasine was converted into dihydrorugulovasine and dihydrorugulovasinelactam and dihydrorugulovasine into the lactam. These experiments suggest the following sequence of conversions:



Ergoline formation in higher plants

The striking discovery of Hofmann and Tscherter[55] that higher plants in the family *Convolvulaceae* contain ergoline alkaloids raised the question whether the alkaloids in the plant were biosynthesized in the same way as in the ergot fungus. This question was studied in feeding experiments with L-tryptophan-(indole-^{3}H) and D,L-mevalonic acid-(2-^{3}H) using *Ipomoea rubro-caerulea* Hook.[301] The precursors were fed through the cut stems to four-month-old plants and eight days later the plants were dried and the alkaloids were isolated separately from stems, leaves, seed capsules and seeds. Leaves and capsules were virtually free of alkaloid. The stem alkaloids were significantly labeled; dilution factors were 50 and 7·7 for tryptophan and mevalonate, respectively. Interestingly the seeds, where most of the alkaloids are found, showed less labeling; the corresponding dilution factors here were 769 and 4760. This suggested that the alkaloids are synthesized in the stems and then transported to the seeds, an interpretation which is supported by the failure to obtain incorporation of tryptophan into the alkaloid when the precursor was introduced directly into the seed capsules or the seeds.[301] An explanation for the low alkaloid content of the leaves is provided by the observation that the leaves were able to convert added elymoclavine into penniclavine and predominantly into non-indolic constituents.[302]

These results indicate that the ergolines in the fungus and in the higher plant are formed from the same precursors. This raises the fascinating question whether this specialized biogenetic pathway has evolved independently twice in nature, or whether it has evolved once and the genetic information has been transferred from the fungus to the plant or vice versa. A third possibility, that this genetic information is essentially universal but usually not expressed, seems considerably less likely. A very detailed comparison of the biosynthetic pathway in the plant and the fungus seems necessary to obtain information on this question.

ENZYMOLOGY AND PHYSIOLOGY OF ERGOT ALKALOID FORMATION

Ergoline biosynthesis in cell-free extracts

The enzymology of ergot alkaloid biosynthesis, as the enzymology of alkaloid biosynthesis in general, has been slow in coming, but progress is finally being made in this area. Two groups have obtained cell-free systems from *Claviceps* which are capable of synthesizing ergoline derivative *de novo* from simple precursors. Cavender and Anderson[303] found that the 60–80% ammonium sulfate precipitate from the 105,000 × *g* supernatant of a homogenate of *Claviceps purpurea* strain PRL 1980 cells catalyzes the formation of chanoclavine-I and -II and, to a lesser extent, of agroclavine and elymoclavine from tryptophan-^{14}C and isopentenylpyrophosphate. The efficiency of conversion was low (0·15%) and the reaction required a long incubation time (12 h). Identification of the radioactive products was done by autoradiography; dependence of product formation on isopentenylpyrophosphate, methionine, ATP and enzyme was demonstrated. The reaction required addition of a liver concentrate to the incubation mixture, presumably to provide additional cofactors. Abe's group[223,304] obtained cell-free systems from two *Claviceps* strains which catalyzed the formation of setoclavine from tryptophan-^{14}C, mevalonate and methionine. Their system was considerably more efficient (4·4% conversion) and required shorter incubation times (3 h) for optimal synthesis. The radioactive product was identified by chromatography and by co-crystallization with authentic carrier material.[304] Abe's system also converted tritiated dimethylallyltryptophan, but not compound **28**, into setoclavine.[223,304] This system and cell-free systems from other ergot strains were used by Abe's group to study various ergot alkaloid interconversions and the formation of peptide ergot alkaloids.[223,265,266,291,305] The work of other groups was increasingly focussed on the isolation and study of single enzymes or enzyme systems catalyzing individual reactions in the pathway. Several of these have been obtained to-date.

Dimethylallyltryptophan synthetase

This enzyme was isolated in our laboratory[200] from mycelia of *Claviceps* strain SD 58 and has been purified 63 fold to apparent homogeneity as judged by polyacrylamide disc gel electrophoresis.[201] It catalyzes the first pathway-specific reaction of ergoline biosynthesis, the conversion of L-tryptophan and dimethylallylpyrophosphate to L-dimethylallyltryptophan and pyrophosphate. The enzyme (systematic name dimethylallylpyrophosphate: L-tryptophan dimethylallyl transferase) has a molecular weight of 73000, a broad pH optimum between 7 and 8 and an isoelectric point at pH 5·8, seems to contain only two half-cystine residues, and consists of only one subunit. It is activated by Fe^{2+}, Mg^{2+} and particularly Ca^{2+}; K_m values for L-tryptophan and dimethylallylpyrophosphate were determined to be 0·067 and 0·2 mM, respectively. Kinetic analysis indicated that the dimethylallyltryptophan synthetase reaction proceeds by a random or ordered sequential rather than a ping-pong mechanism. The substrate specificity of the enzyme has already been discussed earlier. Of particular interest was the finding that the enzyme is inhibited by the endproducts agroclavine and elymoclavine.[200] The inhibition is uncompetitive with either substrate and occurs at alkaloid concentrations which are reached in a typical fermentation.[306] This inhibition may thus be of physiological significance in terminating alkaloid synthesis. The enzyme is absent in growing cells and starts to appear at the end of the growth phase just preceding the appearance of alkaloids. Interestingly, in old mycelia which no longer produce alkaloid the enzyme level decreases, raising the possibility that repression by the endproduct may be a factor controlling the level of this enzyme.

Chanoclavine cyclase

Three groups have obtained enzyme systems from *Claviceps* which catalyze the cyclization of chanoclavine-I to tetracyclic ergolines. Ramstad's group first reported[254] conversion of chanoclavine-I into elymoclavine, but not agroclavine, by the 12,000 × *g* supernatant of an extract from *Claviceps* strain 231 prepared in the presence of 0·25 M sucrose. The reaction required ATP, Mg^{2+}, NADPH and oxygen. Conversions in excess of 20% were obtained in 9 h incubations. Agroclavine was poorly converted into elymoclavine. Gröger's laboratory[257] obtained a cell-free system from two alkaloid-producing strains, but not from one which produced no alkaloids in culture, which converted chanoclavine-I into agroclavine, but not elymoclavine, using ATP, Mg^{2+} and NADPH as cofactors. Conversion rates were in the order of 10–30%. Unpublished work in our laboratory[247] resulted in the isolation of a cell-free preparation from *Claviceps* strain SD 58 which converted chanoclavine-I into a mixture of agroclavine and elymoclavine. Further studies by Gröger's group showed that the reaction catalyzed by their enzyme, which they termed chanoclavine-I-cyclase, was dependent on ATP and NAD^+ or $NADP^+$, but not oxygen and added FAD.[258] Our enzyme preparation, on the other hand, exhibited no strict dependence on ATP.[247] Chanoclavine-I-cyclase also converts chanoclavine-I-aldehyde, but not isochanoclavine-I or dihydrochanoclavine, into agroclavine.[258] The time-course of the appearance and decline of this enzyme in the cultures closely resembles that determined for dimethylallyltryptophan synthetase,[247,258] and as the latter, the cyclase is inhibited by elymoclavine, as well as by lysergic acid, although perhaps somewhat less strongly.[258] Attempts to purify the enzyme or enzyme system have so far only met with limited success. It was found that the enzyme is stabilized by glycerol,[247,258] but that most of the activity is lost during column chromatography on Sephadex[247,258] or DEAE cellulose.[247] Addition of FAD to appropriate fractions from the column, however, restored activity.[247] This and the observation of a typical flavine difference spectrum upon reduction of the extract tentatively suggests the possibility that a flavine coenzyme may be involved in the reaction.

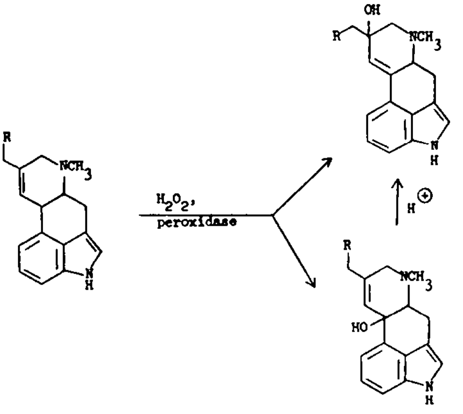
Agroclavine hydroxylase

The conversion of agroclavine to elymoclavine, which occurs readily in intact *Claviceps* mycelia, has long been searched for in cell-free extracts of the fungus.[154,307,308] Hsu and Anderson finally succeeded in demonstrating this conversion in the 60–80% ammonium sulfate fraction of a *Claviceps* cell-free extract.[259] The reaction seems to be catalyzed by a mixed-function oxygenase; it required NADPH or an NADPH-generating system and was inhibited by EDTA or cyanide. It could be followed spectrophotometrically by following the disappearance of NADPH at 340 nm. The enzyme had a pH optimum for reaction at pH 7; it resided entirely in the 100,000 × *g* supernatant of the cell-free extract whereas the pellet was devoid of activity. However, the significance of the latter observation is unclear, because the uncentrifuged crude extract also showed no activity.[260] It is interesting to note that although agroclavine hydroxylase activity seems to be largely restricted to ergoline-producing organisms,[210] a mammalian microsomal enzyme system has been found to convert agroclavine to noragroclavine and elymoclavine.[309] The reaction is dependent upon cytochrome P-450.

Other enzymes possibly involved in alkaloid formation

Anderson *et al.*[240] have observed the conversion of dimethylallyltryptophan into clavicipitic acid by the microsomal fraction of a *Claviceps* cell-free extract. The reaction was oxygen-dependent, but not dependent on NADPH, and was not inhibited by CO, suggesting that cytochrome P-450 is not involved.[240] Further studies[241] showed that hydrogen peroxide was the second product of the reaction. The enzyme could be solubilized by treatment with 1% Triton X-100.[241] Saini and Anderson[310] recently found a small (0·1–0·3%) conversion of dimethylallyltryptophan to 4-(4′-hydroxy-3′-methyl-2′-butenyl)- tryptophan (**34**) using the 60–80% ammonium sulfate fraction of the *Claviceps* PRL 1980 supernatant.

Extensive studies have been done by Ramstad and Taylor and their coworkers on the enzymology of the conversion of agroclavine and elymoclavine into their 8-hydroxy-$\Delta^{9,10}$-analogs. They demonstrated that peroxidase will catalyze the conversion of agroclavine into setoclavine and isosetoclavine and of elymoclavine into penniclavine and isopenniclavine.[156,311-313] The reaction involves initial abstraction of a hydrogen from C-10 and produces both 8-hydroxy-$\Delta^{9,10}$- and 10-hydroxy-$\Delta^{8,9}$-clavines. The latter, e.g. 10-hydroxyelymoclavine, rearrange readily to the 8-hydroxy-$\Delta^{9,10}$ compounds[314] and must be considered facultative intermediates in the formation of 8-hydroxyclavines:



Conversion of agroclavine to setoclavine and isosetoclavine was also obtained in a thioglycolate-Fe^{2+} model system.[242]

Among other enzymatic studies, the activation of lysergic acid by *Claviceps* extracts[271] has already been discussed earlier. An enzyme preparation which hydrolyzes lysergic acid amide to the free acid has been obtained from *Claviceps purpurea*.[315] Finally it should be mentioned that extensive studies have been carried out on enzymes involved in the biosynthesis of ergot alkaloid precursors (e.g. Refs. 316–318 and references therein), mainly in order to investigate their regulatory properties.

Physiology and regulation of ergot alkaloid biosynthesis

This topic has commanded great interest for years and continues to be one of considerable fundamental as well as practical importance. An understanding of the factors governing alkaloid synthesis will hopefully help us define the role of these compounds in the life of the producing organism, i.e. will help answer the question why these compounds are formed. In addition such information may eventually lead to more rational approaches to the development of industrial fermentation procedures. With the increasing success in the isolation of enzymes from *Claviceps*, emphasis in this field has shifted increasingly from physiological studies in whole cells to the investigation of regulatory mechanisms at the enzymatic level. Attention in recent years has focussed on such topics as the genetic requirements of alkaloid synthesis, the regulation of the formation of the alkaloid precursors, the induction of alkaloid synthesis by one of the precursors, tryptophan, and the inhibition of alkaloid formation by the endproducts of the pathway. The interested reader is referred to two recent reviews[318,319] for an overview of this field and for references.

BIOSYNTHESIS OF OTHER ISOPRENYLATED INDOLES

Echinulin

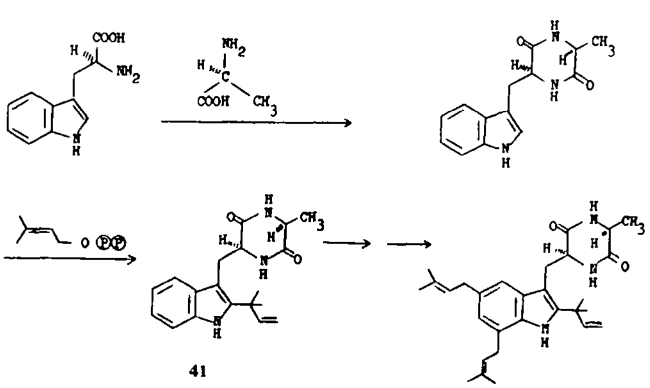
Biosynthetic theory predicts that echinulin (**11**) should be formed from tryptophan, alanine and mevalonic acid, the latter providing the three isoprene units. This was indeed found to be the case. Birch *et al.*[320] fed alanine-1-^{14}C to *Aspergillus amstelodami* and showed by degradation that the radioactive **11** produced (0·12% incorporation) contained the majority of its label in the alanine moiety. Mevalonate-(2-^{14}C) was also incorporated efficiently (4·25%) and degradation gave radioactive acetone (from the γ,γ-dimethylallyl moieties) and α,α-dimethylvaleric acid (from the α,α-dimethylallyl portion). D,L-Tryptophan-(3-^{14}C) gave a 1·36% incorporation and degradation results were consistent with the expected labeling of echinulin.[321] The incorporation of tryptophan into **11** was further studied by MacDonald and Slater,[322] who showed that tryptophan samples labeled specifically in various positions were all incorporated into **11**, that the incorporation of D,L-tryptophan-(1-^{14}C) proceeded without randomization of label, and that L-tryptophan-3-^{14}C was incorporated more efficiently than the D-isomer. The latter finding identified L-tryptophan as the immediate precursor, in agreement with the absolute configuration established for **11**.

Subsequent experiments[323] showed that L-alanyl-L-tryptophanyl-^{14}C-lactam was efficiently (9·9–16·2%) and specifically incorporated into **11** and competition experiments with tryptophan clearly indicated that this incorporation was not preceded by cleavage of the precursor. This indicated that the formation of the lactam ring occurs prior to the introduction of the isoprene residues, a conclusion which was born out by enzymatic experiments. Allen[324] found that crude ammonium sulfate fractions of a cell-free extract from *A. amstelodami* catalyzed the transfer of the 3,3-dimethylallyl group from dimethylallylpyrophosphate to L-alanyl-L-tryptophanyl-lactam. Tryptophan, alanyltryptophan and tryptophanylalanine were not substrates. The reaction product contained only one isoprene residue per molecule of cyclic dipeptide and was tentatively identified as L-alanyl-L-2-(α,α-dimethylallyl-tryptophanyl-lactam (**41**).[324] Feeding experiments showed[325] that the product of this enzymatic reaction was incorporated into echinulin and in double labeling experiments (^{3}H in isoprene residue, ^{14}C in tryptophan moiety), no change in the ^{3}H/^{14}C was seen during incorporation. The sum of the data thus supports a pathway of echinulin biosynthesis as below.

The "inverted" isoprenoid substituent at C-2 is thought to arise by initial isoprenylation at N-1, followed by allylic rearrangement into the 2-position. This assumption is supported by model studies,[326] but no other evidence to prove or disprove it is currently available. The formation of neochinuline (**12a**) must involve a dehydrogenation between C-2 and C-3 of the tryptophan moiety at some stage; half the tritium from C-3 of tryptophan is lost in the process.[77]†

Cyclopiazonic acid

The structure of cyclopiazonic acid (**16**) suggested a biosynthetic origin very similar to that of ergot alkaloids. Its formation could either involve tryptophan, one isoprenoid unit from mevalonate and two acetate units or tryptophan, losing its carboxyl group, and two isoprenoid C$_5$ units.[327] Careful feeding experiments and chemical



41 **11**

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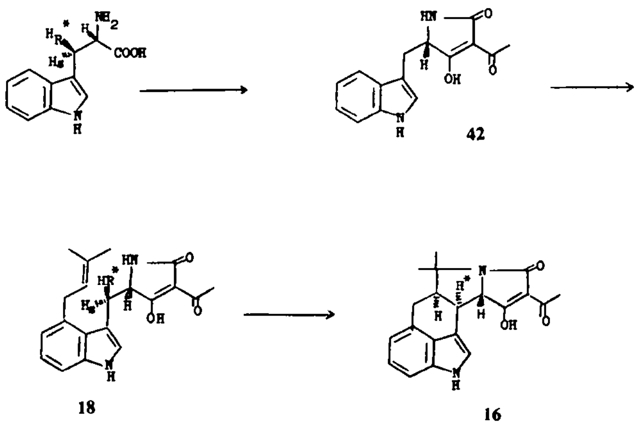
† Note added on proof: The stereochemistry of this dehydrogenation has recently been shown to be *cis* (R. Cardillo *et al.*, Chem. Comm. **1975**, 778) and doubly labeled L-alanyl-L-tryptophanyl lactam was incorporated intact into various echinulins (R. Marchelli, *et al.*, Chem. Comm. **1975**, 779).

degradations showed that **16** is formed in *Penicillium cyclopium* from tryptophan, acetate and mevalonate in accordance with the first option. Incorporation rates for these three precursors were 24·7%, 3·5% and 7%, respectively. Mevalonate-(2-^{14}C) specifically labeled one or both of the C-methyl groups attached to ring D and acetate-(1-^{14}C) labeled the two carbonyl carbon atoms of the acetyltetramic acid unit in addition to two carbon atoms in the isoprenoid moiety.[327] Holzapfel and Wilkins[327] also found that bis-secodehydrocyclopiazonic acid (β-cyclopiazonic acid) (**18**), another constituent of *P. cyclopium*, is a precursor of cyclopiazonic acid. The enzymatic formation of this compound in a cell-free extract of *P. cyclopium* has been demonstrated.[328] The substrates for its formation are dimethylallylpyrophosphate and α-acetyl-γ-(β-indolyl)methyltetramic acid (**42**), a new compound isolated from this fungus. Tryptophan itself did not act as acceptor of the dimethylallyl unit. The enzyme catalyzing the cyclization of β-cyclopiazonic acid (**18**) to cyclopiazonic acid (**16**), β-cyclopiazonate oxidocyclase, has been isolated from *P. cyclopium*.[329] Purification showed that it consists of five isoenzymes, all of which were obtained in pure and homogeneous form. Detailed studies on the composition of the enzyme(s) and the mechanism of the reaction have been done.[330–332] The enzyme is a flavoprotein and the kinetics of the reaction indicate a Ping-Pong-Bi-Bi mechanism. Recently, the stereochemistry of the proton removal from the original C-3 of the tryptophan side chain during this reaction has been studied. The *pro-S* hydrogen from this position is removed in the process.[333] It was also found that tritium at C-2 of tryptophan is retained throughout the biosynthesis,[334] which is consistent with cyclization via a 1,4-, but not a 4,5-didehydro derivative of **18**. The stereochemical results indicate that in the β-cyclopiazonate oxidocyclase reaction formation of the new C–C bond occurs from the opposite side of the molecule to proton removal.[333] This conclusion seems to be dependent on the, undoubtedly justified, assumption that **16** and **18** have the same absolute configuration at C-5. The pathway of cyclopiazonic acid formation can be summarized as follows:



CONCLUDING REMARKS

The foregoing review has hopefully shown that the biosynthesis of the ergoline ring system, which at one time looked like a simple sequence of mostly non-specific reactions, involves a substantial number of very specific and rather complicated transformations. It has also pointed out that the ability to generate the isoprenylated indole system is not restricted to the ergot fungus but that both the mode of isoprenylation and the fate of the product differ in different organisms. One of the great debates in the field of alkaloids and secondary metabolites in general regards the question whether, to name the extremes, these compounds are luxuries which the producing organism can afford but which really serve no critical purpose, whether they are waste products, or whether every one of them represents an evolutionary advantage to the producer, or they would not be formed, and thus must have some role in the life of the producing organism. If we consider ergot alkaloid biosynthesis in the light of this question it becomes apparent that some of the compounds found must have such a role, but not necessarily all of them. We can clearly discern a series of ergot alkaloids which lie on the main pathway leading to the lysergic acid derivatives. The specificity of the reactions involved in this pathway leaves little doubt in the mind of this author that it must have evolved by a selection process and that these compounds must thus have some role in the normal life of the fungus or in its interaction with its environment. However, many other ergot alkaloids arise by secondary transformations of these compounds by enzymes which are present in the organism without being related to ergoline biosynthesis, but which may have other important functions. The formation of the latter, secondary alkaloids may not necessarily reflect an evolutionary advantage, but may well be a minor luxury the organism can afford.

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