The Biogenesis of Ergot Alkaloids

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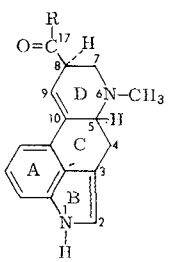
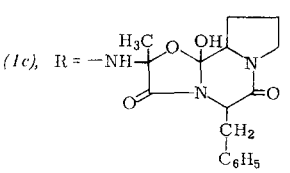
Dedicated to Professor Hans von Euler on the occasion of his 90th birthday

In this article the biogenesis of the ergoline ring in lysergic acid derivatives and the clavines is discussed. Tryptophan and mevalonic acid are the precursors. The N-methyl group is supplied by formate or methionine. Concepts and results dealing with the manner in which the compounds are formed are discussed. Finally, the known biogenetic relationships among the ergot alkaloids are discussed in connection with their biogenesis.

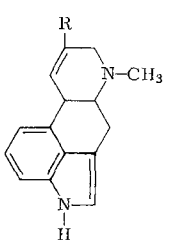
Taken in a wider sense, the ergot alkaloids include the amides of lysergic acid *(1a)* with their unusual cyclic peptides, *e.g.* ergotamine *(1c)*, on the one hand and on the other the clavines, of which agroclavine *(2a)*, elymoclavine *(2b)*, and chanoclavine *(3)* are the most important representatives. Knowledge of their structure is due to the work of *Barger*, *Jacobs*, and *Craig*, and, above



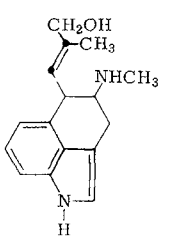
(1a), R = OH
(1b), R = NH_2



(1c), R = —NH—



(2a), R = CH_3
(2b), R = CH_2OH



(3)

all, to that of *Stoll* and *Hofmann* [1]. The clavines have been known only for the past 15 years and have been investigated principally by *Abe* and by *Stoll* and *Hofmann* [2]. Structurally, they are variants of 6,8-dimethylergoline without the peptide portion.

The peptide-containing ergot alkaloids of pharmaceutical interest are obtained even today from ergot cultivated on rye. Although the clavines are formed in fairly large quantities in saprophytic cultures, peptide derivatives are formed in only small quantities. Various attempts have been made to alter this situation by changing either the composition of the media or by other means but with little success so far. Very recently, a variety of *Claviceps paspali* was isolated which produces large quantities of lysergamide and the corresponding *N*-hydroxyethylamide [3].

Early Hypotheses Concerning the Biosynthesis of Ergot Alkaloids

All previous speculations concerning the biogenesis of ergot alkaloids assumed that tryptophan is the starting material, but otherwise they differed considerably. However, none has been proven to be correct.

In their considerations most authors took into account the fact that the 4-position of tryptophan undergoes electrophilic substitutions only with great difficulty. They therefore assumed that a quinoid system is first formed to which the missing carbons are linked. *Van Tamelen* [4] assumed further that an addition product is formed with a dihydronicotinic acid derivative or a similar compound, and that known biochemical reactions take place *via* this addition product.

Harley-Mason [5] also based his hypothesis on a quinoid compound, but then assumed that rings C and D were formed from acetonedicarboxylic acid and formaldehyde.

Plieninger [6] also suggested the addition of an active methylene group to a quinoid system, but gave no further details.

Wendler [7] and *Feldstein* [8], on the other hand, postulated that a ring was closed *via* the carboxyclic group of

[1] Reviews on the elucidation of structures: [a] *A. Stoll* in *L. Zechmeister:* Fortschritte der Chemie organischer Naturstoffe. Springer, Vienna 1952, Vol. IX, p. 114; [b] *A. L. Glenn*, Quart. Rev. (Chem. Soc., London) *8*, 192 (1954); Stereochemistry and absolute configuration; [c] *A. Stoll, Th. Petrzilka, J. Rutschmann, A. Hofmann*, and *H. H. Günthard*, Helv. chim. Acta *37*, 2039 (1954); [d] *J. B. Stenlake*, Chem. and Ind. *1953*, 1089; J. chem. Soc. (London) *1955*, 1626; [e] *H. G. Leemann* and *S. Fabbri*, Helv. chim. Acta *42*, 2696 (1959); Syntheses: [f] *E. C. Kornfeld, E. J. Fornefeld, G. B. Kline, M. J. Mann, D. E. Morrison, R. G. Jones*, and *R. B. Woodward*, J. Amer. chem. Soc. *78*, 3087 (1956); [g] *A. Hofmann, A. J. Frey*, and *H. Ott*, Experientia *17*, 206 (1961).

[2] For bibliographies, see *e.g. M. Abe* in: 2. Arbeitstagung über Biochemie und Physiologie der Alkaloide, Halle (Saale) 1960. In the press.

[3] *F. Arcamone, E. B. Chain, A. Ferretti, A. Minghetti, P. Pennella, A. Tonolo*, and *L. Vero*, Proc. Roy. Soc. (London), Ser. B, *155*, 26 (1961).

[4] *E. E. van Tamelen*, Experientia *9*, 457 (1953).

[5] *J. Harley-Mason*, Chem. and Ind. *1954*, 251.

[6] *H. Plieninger*, Experientia *14*, 57 (1958).

[7] *N. L. Wendler*, Experientia *10*, 338 (1954).

[8] *A. Feldstein*, Experientia *12*, 475 (1956).

tryptophan. *Wendler* thought that the subsequent condensation involved citric acid, whereas *Feldstein* thought it was *via* α-ketoglutaric acid.

Finally, *Robinson* [9] suggested that tryptophan and succinic acid first undergo Claisen condensation, the missing carbon atom being supplied by formaldehyde.

Studies with Radioactive Tracers

Our starting point was the work by *Gröger* and *U. Mothes* [10] which showed that when tryptophan was injected into the phloem of rye plants, the yield of ergot alkaloids was greatly increased. In collaboration with *K. Mothes* and his group, we began our studies using compounds labelled with ^{14}C and ^{3}H.

Our first experiments consisted of injecting DL-[β-^{14}C]-tryptophan into the internodes of rye plants which had been raised in a greenhouse and which had been infected with *Claviceps purpurea*. We succeeded in isolating radioactive alkaloids and in hydrolysing them to radioactive lysergic and isolysergic acids [11].

At about the same time, *Suhadolnik* and coworkers [12] carried out similar experiments in the USA and concluded that tryptophan is not a precursor in the biosynthesis of ergot alkaloids. It has not been explained why they did not obtain positive results.

We then repeated our work with ergot grown on rye in the field infected with *C. purpurea*, strain XXVII, and obtained a rate of incorporation [*] of 0.16 % and a specific rate of incorporation [**] of 3.21 %. Undoubtedly these figures would have been higher if it had been possible to base them on the amount of tryptophan that had actually reached the sclera.

Using fungi cultures in the laboratory, we obtained the results shown in Table 1. The rate of incorporation of DL-[β-^{14}C]-tryptophan was raised to 47.5 % by the use of replacement cultures [***] [14].

These studies showed that the ergoline ring was made up not only from the L-form, but also, to some extent, from the D-form (racemase?) [15]. Apparently tryptophan undergoes decarboxylation in the course of the synthesis of the alkaloids, since tryptophan labelled with ^{14}C in the carboxyl group gave rise exclusively to non-radioactive alkaloids [13, 14]. Since then, other research teams have confirmed that tryptophan is the precursor of ergot alkaloids [16–20].

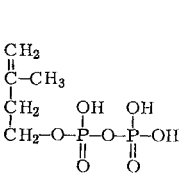
Table 1. Incorporation of radioactively labelled tryptophan and indole into ergot alkaloids by saprophytic cultures of *Claviceps purpurea*, strain SD 58. The strain preferentially forms elymoclavine in addition to agroclavine and penniclavine. The total alkaloids were isolated.

	Compound administered	Rate of incorporation
Surface cultures	DL-[β-^{14}C]-tryptophan	10.7-39.4 % on adding increasing quantities of pyridoxal phosphate [13]
	D-[Ar-^{3}H]-tryptophan	14 % [15]
	DL-[carboxyl-^{14}C]-tryptophan	no incorporation [13, 14]
Replacement cultures	DL-[β-^{14}C]-tryptophan	47.5 % [14]
	^{3}H-indole	5.5–24 % in elymoclavine [*] [15]

[*] Specific rate of incorporation.

In our experiments with saprophytic cultures, we also found that [1-^{14}C]-acetate is utilized in the biosynthesis of the alkaloids with rates of incorporation of up to 0.3 % [13]. This result was in accordance with our hypothesis [11] that the five missing carbon atoms are derived from an isoprenoid compound. This hypothesis received further support from the elucidation of the structure of chanoclavine *(3)*, accomplished a short time earlier [21]. Its open D-ring structure is in good agreement with our assumption.

Isopentenyl pyrophosphate *(4)* and its isomer dimethylallyl pyrophosphate *(5)* have been recognized by *Bloch* and by *Lynen* as "active isoprene"; these originate from mevalonic acid *(6)*, the acetate replacing factor discovered by *Folkers* [22]. We were able to show that [2-^{14}C]-mevalonic acid is utilized for alkaloid synthesis with a specific rate of incorporation of 16 % [23]. This was the first demonstration that mevalonic acid is used



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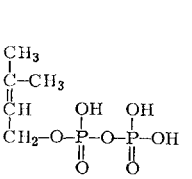

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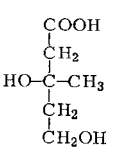

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[9] *R. Robinson*, The Structural Relations of Natural Products. The Clarendon Press, Oxford 1955, p. 7.

[10] *D. Gröger* and *U. Mothes*, Pharmazie *11*, 323 (1956).

[11] *K. Mothes, F. Weygand, D. Gröger*, and *H. Grisebach*, Z. Naturforsch. *13b*, 41 (1958).

[12] *R. J. Suhadolnik, L. M. Henderson, J. B. Hanson*, and *Y. H. Loo*, J. Amer. chem. Soc. *80*, 3153 (1958).

[*] The rate of incorporation is defined as $\frac{\text{total activity of product} \times 100}{\text{total activity of the precursor}}$ [%]

[**] The specific rate of incorporation is defined as $\frac{\text{specific molar activity of product} \times 100}{\text{specific molar activity of the precursor}}$ [%]

[***] A replacement culture is washed mature mycelium suspended in a medium which suppresses further substantial vegetative growth.

[13] *D. Gröger, H. J. Wendt, K. Mothes*, and *F. Weygand*, Z. Naturforsch. *14b*, 355 (1959).

[14] *D. Gröger, K. Mothes, H. Simon, H.-G. Floß*, and *F. Weygand*, Z. Naturforsch. *15b*, 141 (1960).

[15] *D. Gröger, K. Mothes, H. Simon, H.-G. Floß*, and *F. Weygand*, Z. Naturforsch. *16b*, 432 (1961).

[16] *W. A. Taber* and *L. C. Vining*, Chem. and Ind. *1959*, 1218.

[17] *R. M. Baxter, S. I. Kandel*, and *A. Okany*, Chem. and Ind. *1960*, 266.

[18] [a] *H. Plieninger, R. Fischer, W. Lwowski, A. Brack, H. Kobel*, and *A. Hofmann*, Angew. Chem. *71*, 383 (1959).
[b] *H. Plieninger, R. Fischer, G. Keilich*, and *H. D. Orth*, Liebigs Ann. Chem. *642*, 214 (1961).

[19] *L. R. Brady* and *V. E. Tyler jr.*, Planta med. 7, 225 (1959).

[20] *F. Arcamone, E. B. Chain, A. Ferretti, A. Minghetti, P. Pennella*, and *A. Tonolo*, Biochim. biophysica Acta *57*, 174 (1962).

[21] *A. Hofmann, R. Brunner, H. Kobel*, and *A. Brack*, Helv. chim. Acta *40*, 1358 (1957).

[22] Review: *A. F. Wagner* and *K. Folkers*, Endeavour *20*, 177 (1961).

[23] *F. Weygand*, Angew. Chem. *71*, 383 (1959).

not only for the synthesis of typical isoprenoid compounds, but also for the biosynthesis of alkaloids.

Further experiments have shown that both [2-^{3}H]-mevalonic acid and [4-^{3}H]-mevalonic acid are incorporated into clavines [14]. It therefore seems probable that mevalonic acid is utilized without prior decomposition into smaller compounds.

Other workers [24–27] have confirmed our results with mevalonic acid. *Birch* also found that [2-^{14}C]-mevalonic acid is incorporated into agroclavine and elymoclavine. By Kuhn-Roth oxidation and degradation of the resulting acetic acid the activities of the CH_3 and the $-CH_2OH$ groups were determined; the latter was first reduced to a methyl group. *Birch* obtained the surprising result that C-17 of elymoclavine contained 90% of the total radioactivity, whereas C-17 of agroclavine contained only 33%. In a later paper [25], however, he showed that in both compounds C-17 contained the bulk of the activity. *Baxter* and coworkers [27] obtained similar results by degradative studies on the lysergic acid derivative ergosine, obtained biosynthetically from [2-^{14}C]-mevalonic acid.

The experiments of *Baxter et al.* [27a] and of *Plieninger* [18b] furthermore lead to the conclusion that mevalonic acid is incorporated into ergot alkaloids *via* isopentenyl or dimethylallyl pyrophosphate, as is the case with terpenes. In *Baxter*'s experiments, the presence of non-radioactive isopentenyl and dimethylallyl pyrophosphate lowered the rate of incorporation of [2-^{14}C]-mevalonic acid. In addition, experiments with [1-^{14}C]-mevalonic acid demonstrated the non-incorporation of the carboxyl group. *Plieninger* found that deuterated isopentenyl pyrophosphate is incorporated into clavines.

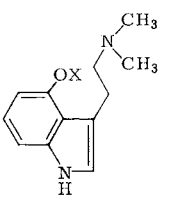
The origin of the carbon atom at the *N*-methyl group could be shown with a great degree of certainty by *Baxter* [28]. The methyl group of methionine labelled with ^{14}C as well as [^{14}C]-formate were incorporated. Degradation of festuclavine obtained from these labelled precursors showed that the *N*-methyl group contained all of the radioactivity [29]. The higher rate of incorporation of [Me-^{14}C]-methionine and the incorporation of [Me-^{14}C,^{3}H]-methionine without alteration of the ratio ^{14}C:^{3}H indicated that the *N*-methyl group can result from methionine by transmethylation. Probably, the biosynthesis also takes this course.

The experiments described so far have elucidated the origin of the entire carbon skeleton of the ergot alkaloids. It was now of interest to determine the manner in which the known precursors are joined together and also to determine the biogenetic interrelationships of the various ergot alkaloids, particularly between the clavines and the lysergic acid derivatives.

Formation of Rings C and D

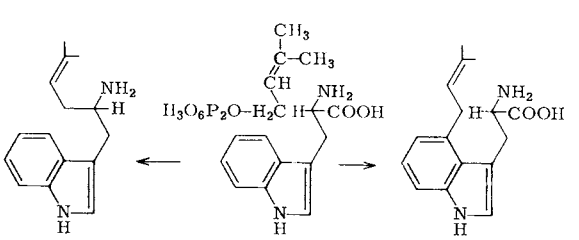
The formation of rings C and D poses a question that is particularly difficult to answer, as it seemed reasonable to believe that electrophilic substitution of the indole ring in position 4 is difficult. Whereas many 5-substituted indoles do occur in nature the number of natural 4-substituted derivatives is extremely small. Both *Plieninger* [18b] and *Baxter* [17] were able to disprove the earlier supposition that the 4-position is activated by a hydroxyl group in position 5.

The discovery of the fungal metabolites psilocine *(7)* and psilocybine *(8)* [30] was of particular interest in this connection, as it was shown shortly afterwards that these indole derivatives, which are hydroxylated in the 4-position, are derived from tryptophan [31].



(7), X = H
(8), X = PO_3H_2

These findings therefore demonstrated that tryptophan can be hydroxylated in the 4-position in nature, and gave rise to the supposition that such a reaction may also play a part in the biosynthesis of the ergot alkaloids [31]. Another possibility is the occurence of a direct condensation of tryptophan with "active isoprene", presumably dimethylallyl pyrophosphate. Such a reaction may occur by one of two ways, either by attack at the 4-position of the indole ring system, leading to the formation of 4-dimethylallyltryptophan *(10)* [25] or by attack at the α-carbon atom of the tryptophan side-chain with simultaneous decarboxylation giving compound *(9)*. In the first case, the condensing enzyme must in some way cause the reaction to occur at position 4 instead of at the electronically favored positions 5 or 7. In the second, preference for position 4 results from arrangement of the carbon atoms in *(9)*, since for stereochemical reasons, position 5 cannot be attacked during cyclization.



(9) *(10)*

[24] *A. J. Birch, B. J. McLoughlin*, and *H. Smith*, Tetrahedron Letters *1960*, No. 7, p. 1.

[25] *S. Bhattacharji, A. J. Birch, A. Brack, A. Hofmann, H. Kobel, D. C. C. Smith, H. Smith*, and *J. Winter*, J. chem. Soc. (London) *1962*, 421.

[26] [a] *E. H. Taylor* and *E. Ramstad*, Nature (London) *188*, 494 (1960).
[b] *E. H. Taylor* and *E. Ramstad*, J. pharmac. Sci. *50*, 681 (1961).

[27] [a] *R. M. Baxter, S. I. Kandel*, and *A. Okany*, Tetrahedron Letters *1961*, 596.
[b] *R. M. Baxter, S. I. Kandel*, and *A. Okany*, J. Amer. chem. Soc. *84*, 2997 (1962).

[28] *R. M. Baxter, S. I. Kandel*, and *A. Okany*, Chem. and Ind. *1961*, 1453.

[29] *S. I. Kandel, R. M. Baxter, A. Okany*, and *K. L. Tam*, Lecture delivered at the International Symposium on Organic Chemistry of Natural Products, Brussels 1962.

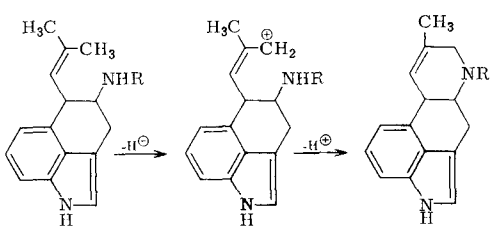
[30] *A. Hofmann, R. Heim, A. Brack, H. Kobel, A. Frey, H. Ott, Th. Petrzilka*, and *F. Troxler*, Helv. chim. Acta *42*, 1557 (1959).

[31] *A. Brack, A. Hofmann, F. Kalberer, H. Kobel*, and *J. Rutschmann*, Arch. Pharmaz. Ber. dtsch. pharmaz. Ges. *294*, 230 (1961).

Although some experimental studies have been carried out, the question of the formation of the ergoline ring system is still largely unexplored. Experiments by *Baxter* [28,29] as well as ours [32] have shown that probably neither tryptamine nor *N(α)*-methyltryptophan nor *N(ω)*-methyltryptamine take part in the biosynthesis of the alkaloids. Hence, the condensation of tryptophan with an active isoprene is preceded by neither decarboxylation nor by methylation. Experiments using 4-hydroxytryptophan have, as yet, not been published. Recently, *Plieninger* [33] synthesized 4-dimethylallyl-[^{14}C]-tryptophan *(10)* and observed that this compound is utilized in the formation of elymoclavine with a specific rate of incorporation of 4.2 %. We in turn synthesized compound *(9)* with a tritium label in the indole nucleus. This compound, too, led to labelled elymoclavine with a rate of incorporation of 20–33 % [34]. Because the results with these two precursors are not comparable, ^{14}C-labelled compound *(10)* and ^{3}H-labelled compound *(9)* were added together to cultures of ergot fungus. The experiments were carried out under different experimental conditions in Heidelberg, Munich, and, by *D. Gröger*, in Halle (Germany). All of them showed that *(10)* is always utilized better than *(9)*. The ratio at which these two precursors were utilized varied between 1.6:1 and 87:1 [expressed in terms of *(10)*:*(9)*]. The rates of incorporation for *(10)* varied between 3.5 and 20 %, and those for *(9)* between 0.04 and 5 %. So far we have been unable to reproduce the high rates of incorporation previously found for compound *(9)*. The reasons for this are still unknown [*]. On the other hand, the rates of incorporation of dimethylallyltryptophan into alkaloids are not higher than those commonly encountered under comparable conditions with tryptophan. If compound *(10)* is synthesized subsequent to tryptophan in the biosynthetic pathway, one would expect it to be utilized to a greater extent than the latter. Problems of permeability and the like may of course play a role here.

One may imagine that rings C and D are formed from a condensation of tryptophan and active isoprene, *i.e.* compound *(9)* oder *(10)* or a similar compound, and that this condensation product undergoes dehydrogenation at the methylene group in the allyl position, and that the allyl cation adds on electrophilically in position 4 or in the side chain to give compound *(11)*. Another allyl cation can be formed at the *cis*-methyl group *(12)* and ring closure with the amino group would then lead to ring D.

Other transformations of the clavine alkaloids can also be explained by such allyl dehydrogenations and subsequent steps, *e.g.* allyl rearrangement and hydroxylation.



(11) *(12)* *(2c)*

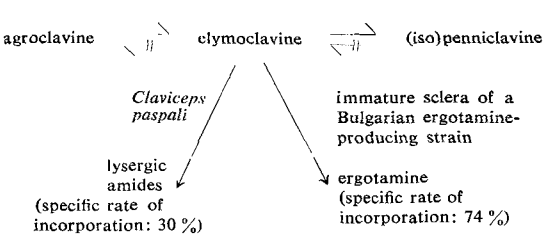
Biogenetic Interrelationships

It is much simpler to explain biogenetic interrelationships between ergot alkaloids by means of isotope studies. There are originally two mainpoints of view: According to *Abe* [2], all ergot alkaloids are supposed to originate from a common precursor, the C-17 aldehyde, which is oxidized to lysergic acid on the one hand and which undergoes reduction to elymoclavine, agroclavine, and chanoclavine on the other. The other opinion, formulated for example by *Rochelmeyer* [35], considers the clavines to be precursors of lysergic acid. *Abe*'s opinion became untenable when it was found that [2-^{3}H]-mevalonic acid is incorporated into the clavines nearly as readily as [2-^{14}C]-mevalonic acid [14]. Since carbon 2 of mevalonic acid becomes C-17 of the clavines, then if *Abe*'s hypothesis were correct, the loss of tritium should have been substantial. That this is not the case was recently confirmed by *Baxter* [29]. Some clarification then came from work by *Agurell* and *Ramstad* [36], who were able to demonstrate the following sequence of reactions by means of studies with a strain of *Claviceps*:

agroclavine → elymoclavine → (iso)penniclavine.

Each of these steps proved irreversible. This eliminated the possibility of clavine-formation by way of reduction.

Recently, it has been demonstrated [37] that transformation of elymoclavine into lysergic acid derivatives can be accomplished biologically. This transformation was demonstrated by application of [^{3}H]-elymoclavine to the sclera of a strain of rye ergot, using the method of *Winkler* and *Mothes* [38], which gave rates of incorporation of up to 74 %. It was also achieved using submerged cultures of a strain of *Claviceps paspali*, which forms simple lysergic amides. The specific rates of incorporation of elymoclavine were up to 30 %. These experiments make it probable that lysergic acid derivatives can be formed oxidatively from clavines (Scheme 1).



Scheme 1. Formation of lysergic acid derivatives from clavines

[32] *D. Gröger, K. Mothes, H.-G. Floß*, and *F. Weygand*, unpublished data.

[33] *H. Plieninger, R. Fischer*, and *V. Liede*, Angew. Chem. internat. Edit. *1*, 399 (1962).

[34] *F. Weygand, H.-G. Floß*, and *U. Mothes*, Tetrahedron Letters *1962*, 873.

[*] It should be mentioned in this connection that the fungus strain we used practically stopped producing alkaloids soon after our first experiments with compound *(9)*. Because of this we are unable to reproduce accurately the conditions of our earlier experiments.

[35] *H. Rochelmeyer*, Pharmaz. Ztg. *103*, 1269 (1958).

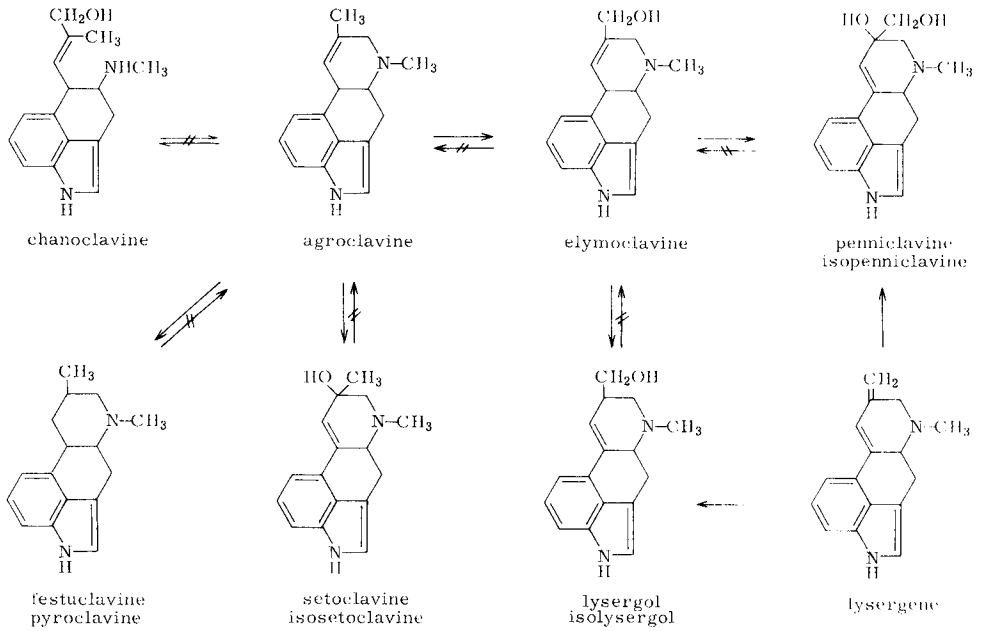
[36] *S. Agurell* and *E. Ramstad*, Tetrahedron Letters *1961*, 501.

[37] *K. Mothes, K. Winkler, D. Gröger, H.-G. Floß, U. Mothes*, and *F. Weygand*, Tetrahedron Letters *1962*, 933.

[38] *K. Winkler* and *K. Mothes*, Planta med. *10*, 208 (1962).

In the meantime, *Agurell* and *Ramstad* [39] have succeeded in elucidating the biogenetic interrelationships between numerous clavine alkaloids; *Baxter et al.* [29] have come to similar conclusions (Scheme 2).

Accordingly, most clavines are derived from agroclavine and elymoclavine. The epimers at C-8, which are formed



Scheme 2. Biogenetic interrelationships between some clavine alkaloids

in the course of these transformations, *e.g.* penniclavine and isopenniclavine, cannot be interconverted. They therefore appear to originate independently. It is also interesting to note that the transformation of agroclavine into setoclavine, and of elymoclavine into penniclavine and isopenniclavine can be accomplished not only with the aid of various strains of *Claviceps*, but also with the Mexican fungus *Psilocybe semperviva* [40].

Finally, some remarks should be made concerning the peculiar position of chanoclavine among the ergot alkaloids. It has been considered both as a precursor and as a by-product of the alkaloids of the clavine type, but may also represent the product resulting from the opening of the D-ring. *Baxter* [29] has reported that chanoclavine did not arise from agroclavine in the course of his experiments, nor did it give rise to the latter. *Agurell* and *Ramstad* [39] arrived at similar conclusions. Consequently, in the cases studied, chanoclavine does not appear to be a precursor in the biosynthesis of clavines which possess a closed D-ring.

Mothes and *Winkler* [41] recently obtained quite remarkable results. They found that after application of [^{3}H]- and [^{14}C]-elymoclavine to ripening sclera not only was the ergotamine labelled, but also the chanoclavine, which had a specific rate of incorporation of 40 %. This demonstrates that chanoclavine can arise by ring opening.

Attention is called to the fact that, on the basis of our work, this pathway is probably not the only one leading to chanoclavine. We found that after addition of tritium-labelled tryptophan to a surface culture of *Claviceps* and isolation of the resulting alkaloids, *viz.* chanoclavine, agroclavine, elymoclavine, and penniclavine, the chanoclavine had a much higher specific activity than any of the other three alkaloids [42]. Hence chanoclavine cannot possibly have originated from the other three compounds. It is noteworthy in this connection that, according to *Arigoni* [43], the hydroxymethyl group of chanoclavine is *trans* with respect to ring C. We therefore assume that chanoclavine can also be formed by a side reaction, *i.e.* that oxidation which normally serves to convert agroclavine into elymoclavine occurs before ring D is closed.

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[39] *S. Agurell* and *E. Ramstad*, Arch. Biochem. Biophysics *98*, 457 (1962).

[40] *A. Brack*, *R. Brunner*, and *H. Kobel*, Helv. chim. Acta *45*, 276 (1962).

[41] *K. Mothes* and *K. Winkler*, Tetrahedron Letters *1962*, 1207.

[42] *F. Weygand*, *H.-G. Floß*, and *U. Mothes*, unpublished results.

[43] *D. Arigoni*, personal communication.