# Biosynthesis of Ergot Alkaloids. Lysergylalanine as Precursor of Amide-type Alkaloids

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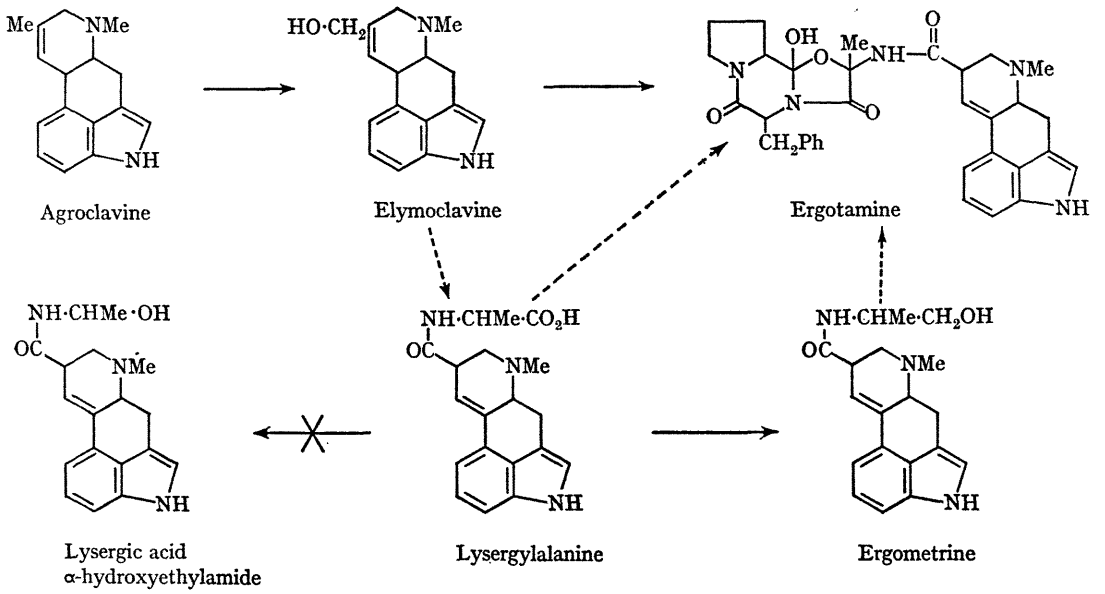
THE lysergic acid moiety of the amide-type ergot alkaloids arises from the structurally simpler clavines, *i.e.* from agroclavine *via* elymoclavine, although the detailed sequence of reaction steps leading from the latter to lysergic acid amide derivatives is not clear.[1] Based on findings that lysergic acid amide was not converted into the corresponding α-hydroxyethylamide by *Claviceps paspali*, and that L-alanine (but not ethylamine) was incorporated into the latter, Agurell[1a] has suggested that lysergylalanine might be an intermediate in the formation of lysergic acid α-hydroxyethylamide and, because of the structural analogy, also of ergometrine and of the ergotamine-type peptide alkaloids. Further work by several groups is compatible with this suggestion.[2-4]

In the present study, D-lysergyl-L-alanine labelled with $^{14}C$ at the 2-position of alanine was prepared by reaction of D-lysergic acid chloride hydrochloride with [2-$^{14}C$]-DL-alanine benzyl ester, t.l.c. resolution of the ether-soluble fraction of the reaction mixture [silica gel G. (1) chloroform–ethanol, 9 : 1; (2) benzene–ethyl acetate–methanol, 7 : 2 : 1 :], and hydrolysis of the D-lysergyl-L-alanine benzyl ester. The labelled material ($3{\cdot}90 \times 10^6$ d.p.m., 1·2 μc/μmole) was added to two 100 ml. cultures of *Claviceps paspali*.[3] After 4 days of fermentation, the two radioactive cultures and a nonradioactive parallel culture were combined, and the alkaloids (201 mg. total) were isolated by extraction and crystallization as described earlier.[3] The resulting crude crystalline alkaloid mixture had a specific radioactivity of 651 d.p.m./mg. and, according to t.l.c., contained an unusually high percentage (50—60%) of ergometrine in addition to the lysergic acid α-hydroxyethylamide. This agreed with the result of an enzymic determination[5] of the amount of acetaldehyde released on treatment with buffer,[3] which indicated the presence of 43—44% lysergic acid α-hydroxyethylamide. The acetaldehyde had only very little radioactivity (Table). A

*Incorporation of D-lysergyl-L-alanine labelled with $^{14}C$ at the alanine 2-position into ergometrine and lysergic acid α-hydroxyethylamide by* Claviceps paspali.

| | D.p.m. total | Spec. radioactivity | % Incorpn. |
|---|---|---|---|
| Ergometrine (113 mg.) .. .. .. | $6{\cdot}92 \times 10^4$ | 199 d.p.m./μmole | 1·77 |
| C-2 + C-3 of alaninol .. .. .. | $6{\cdot}75 \times 10^4$ | 194 d.p.m./μmole | 1·72 |
| Lysergic acid + C-1 of alaninol .. .. | $0{\cdot}17 \times 10^4$ | 5 d.p.m./μmole | max. 0·05 |
| Lysergic acid α-hydroxyethylamide (88 mg.) | n.d.[a] | n.d. | n.d. |
| Acetaldehyde portion .. .. .. | $0{\cdot}34 \times 10^4$ | 11·9 d.p.m./μmole | max. 0·09 |

[a] n.d. = not determined.




*Biogenetic relationships among ergot alkaloids (full arrows: demonstrated; broken arrows: postulated).*

portion of the crude alkaloid was crystallized to constant specific radioactivity with carrier ergometrine and degraded by Kuhn–Roth oxidation to give acetic acid from C-2 and C-3 of the alaninol moiety.

The data (Table) indicate that lysergylalanine is incorporated into ergometrine but not into lysergic acid α-hydroxyethylamide. This incorporation is very specific in that extremely little radioactivity is scrambled into the ergoline portion but almost all of it is present in the expected position in the side-chain. This contrasts with the utilization of alanine in *Claviceps paspali*, which gives rise to substantial labelling of the ring system,[2,3] and suggests that lysergylalanine is incorporated into ergometrine as a unit. This result favours one part of Agurell's suggestion, [1a] that lysergylalanine is an intermediate in the formation of ergometrine. The role of lysergylalanine in ergotamine formation has yet to be assessed. Its involvement in the formation of lysergic acid α-hydroxyethylamide, however, has become doubtful as a result of this experiment as well as of unpublished experiments by Agurell, who was also unable to demonstrate conversion of lysergylalanine (or its methyl ester) into the α-hydroxyethylamide.[6]

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[1] For recent review *cf.*: (a) S. Agurell, *Acta Pharm. Suecica*, 1966, **3**, 71; (b) R. Voigt, *Pharmazie* (*Berlin*), 1968, **23**, 285; 335; 419.
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[5] H. U. Bergmeyer, "Methods of Enzymatic Analysis," Academic Press, New York and London, 1963, p. 290.
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