Tetrahedron Letters No. 15, pp. 501-505, 1961. Pergamon Press Ltd. Printed in Great Britain.

BIOGENETIC INTERRELATIONSHIPS OF ERGOT ALKALOIDS

Stig Agurell and Egil Ramstad

Department of Pharmacognosy, School of Pharmacy,

Purdue University, West Lafayette, Indiana

(Received 24 July 1961)

THERE is now good evidence for the fact that tryptophan[1,2] and tryptophan precursors[3] as well as mevalonic acid[4,5,6] and isopentenyl pyrophosphate[3] serve as precursors of the ergoline portion of the ergot alkaloids. As the next step in the elucidation of the biogenesis of these alkaloids, it appeared logical to establish their biogenetic kinship, suggestions for which have been put forth.[7,8] Allowing the fungus to act on C-14-labeled clavine alkaloids, we have now found living ergot to bring about the following conversion:

agroclavine ⟶ elymoclavine ⟶ penniclavine

---

[1] D. Gröger, H. J. Wendt, K. Mothes, F. Weygand, Z. Naturf. 14b, 355 (1959)

[2] W. A. Taber and L. C. Vining, Chem. and Ind. 1218 (1959); and others

[3] H. Plieninger, R. Fisher, et al., Liebigs Ann. Chem. 642, 214 (1961)

[4] A. J. Birch, B. J. McLoughlin, H. Smith, Tetrahed. Letters No. 7, 1 (1960)

[5] D. Gröger, K. Mothes, H. Simon, H.-G. Floss and F. Weygand, Z. Naturf. 15b, 141 (1960)

[6] E. H. Taylor and E. Ramstad, Nature 188, 494 (1960)

[7] H. Rochelmeyer, Pharm. Ztg. 103, 1269 (1958)

[8] M. Abe, A Consideration Concerning the Biosynthesis of Ergot Alkaloids, 1960, Osaka, Japan

In the present investigation ergot strain 47 A, originally isolated from Pennisetum typhoideum, was used. It produces clavine-type alkaloids and small amounts of lysergic acid. It was grown in darkness on a sterilized medium consisting of 1% yeast extract and 1% glucose in tap water. On this medium, 47 A rapidly synthesizes alkaloids that are largely of the oxidized type. Labeled clavine alkaloids were produced in flasks containing 40 ml of medium by addition of 20 µC of mevalonic acid-2-C-14 ten days after inoculation with the organism. Two weeks later the cultures were harvested and the extracted alkaloids were chromatographed on formamide-impregnated Whatman-3-MM paper with benzene-pyridine (6:1) as the mobile phase.[9] The alkaloids produced by strain 47 A were separated by this system in the following sequence: secaclavine (chanoclavine), unknown-1, unknown-2, penniclavine, elymoclavine, lysergol, isopenniclavine, unknown-3, setoclavine, agroclavine and isosetoclavine. The major alkaloids, viz. elymoclavine, penniclavine, agroclavine, and isopenniclavine, were eluted separately and purified, and each alkaloid (spec. activities 0.51 - 1.47 x 10^6 d.p.m./mg) was dissolved in 5.00 ml of 0.25% aqueous succinic acid. Aliquots of each radioactive solution were introduced by means of a Millipore Hypodermic Adapter equipped with sterile filter into flasks containing 15 ml of culture medium that had been inoculated 9-12 days earlier with strain 47 A. For each alkaloid an aliquot of the solution was also introduced into a flask containing sterile medium alone (=blank) to serve as a check on the sterility of the introduced solution and on any possible conversion of the introduced alkaloid by the medium itself. Ten to twenty days after the introduction of the single labeled alkaloid, the resulting alkaloid mixture (250 - 500 γ / culture

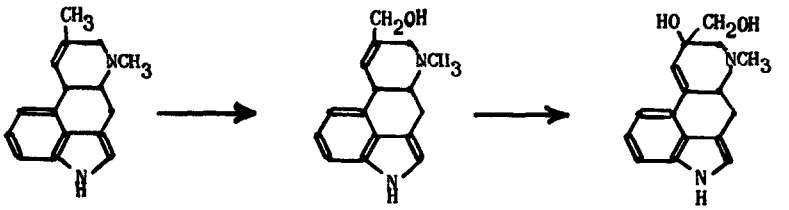
---

[9] M. Pöhm, Arch. Pharm. 291, 468 (1958)

flask) was extracted and, after chromatographic separation of the mixture, the total radioactivity of each individual alkaloid was determined with a Forro Radiochromograph. Benzoic acid-C-14 was used as a standard both for the calibration of the chromatogram scanner and for the counting with a Tri-Carb Liquid Scintillation Spectrometer. Quantitative estimations of the individual alkaloids were carried out according to the method of Pöhm and Fuchs,[10] the absorbance being measured at 550 mμ in the case of agroclavine and elymoclavine, and at 400 mμ in the case of penniclavine and isopenniclavine.

The table illustrates the distribution of radioactivity (per 0.5 ml of introduced alkaloid solution) among the alkaloids recovered from blank and from each 47 A culture.

Exposure of labeled agroclavine in the liquid medium to the alkaloid-producing ergot strain gave rise to labeled elymoclavine, labeled penniclavine and labeled isopenniclavine, whereas exposure of radioactive elymoclavine to the same treatment caused formation of radioactive penniclavine and isopenniclavine but not of radioactive agroclavine. Labeled penniclavine and isopenniclavine did not give rise to radioactive elymoclavine nor to radioactive agroclavine. The following biogenetic scheme is thus well supported by our experimental findings:



Agroclavine Elymoclavine Penniclavine
Iso-penniclavine

[10] M. Pöhm and L. Fuchs, Naturwiss. 40, 244, (1953)

DISTRIBUTION OF RADIOACTIVITY FROM 0.50 ml INTRODUCED ALKALOID SOLUTION AMONG RECOVERED ALKALOIDS

Introduced Labeled Alkaloid	Exposed to	Recovery of Radio-activity in Percent of Blank	Radioactivity of Isolated Alkaloids d.p.m.			
			Agro-clavine	Elymo-clavine	Penni-clavine	Isopenni-clavine
Agroclavine	Blank	100.0	12,650	<200	<200	<200
	Medium+47 A	51.2	1,230	2,350	1,900	680
Agroclavine	Blank*	100.0	18,200	<200	<200	<400
	Medium+47 A*	58.0	400	7,140	1,230	730
	Medium+47 A*	57.7	300	8,540	1,230	530
Elymoclavine	Blank	100.0	<200	14,550	<400	<300
	Medium+47 A	41.2	<200	2,500	2,790	740
	Medium+47 A	42.0	<200	2,360	3,010	560
Elymoclavine	Blank*	100.0	<400	21,950	<400	<300
	Medium+47 A*	59.6	<200	10,300	1,820	850
Penni- and Isopenni-clavine	Blank	100.0	<200	<300	6,620	2,000
	Medium+47 A	59.5	<200	<200	2,950	1,380
	Medium+47 A	51.8	<200	<200	2,800	1,320

*An elymoclavine-accumulating medium consisting of 1% yeast extract and 2% glucose in tap water.

The total recovery of alkaloidal radioactivity from the blanks amounted to 88.8 - 96.4% of the introduced activity.

We found no reversibility of these reactions under our experimental conditions.

A considerable net loss in radioactivity occurred in the experiments. However, the possibility of a breakdown of the radioactive alkaloids followed by a re-incorporation of radiolabel from the breakdown products into the new alkaloids is very unlikely since, in the experiments with labeled elymoclavine, no incorporation into agroclavine had occurred, and with penniclavine, no incorporation into agroclavine or elymoclavine.

Preliminary conversion experiments with labeled elymoclavine indicate that some of the deficit radioactivity is present in lysergic acid, which was not extracted by the above method.

Acknowledgements: This investigation was supported by a grant from The National Institutes of Health, Contract No. RG-6606. We wish to express our appreciation to Dr. V. E. Tyler Jr., College of Pharmacy, University of Washington, for the culture 47 A and to Dr. A. Hofmann, Sandoz, Ltd., Basel, and Dr. M. Abe, Takeda Pharmaceutical Industries, Ltd., Osaka, for samples of pure ergot alkaloids, and to Mr. Adjit Shirodkar for technical assistance.