Expulsion of water and electron pair migrations as depicted would then give rise to the alkaloid *1*.

Synthetic work is under progress.

References and Notes

(1) Khuong-Huu, F., A. Chiaroni, and C. Riche: Tetrahedron Lett. *22*, 733 (1981). Liu, C. T., Q. W. Wang, and C. H. Wang, *J. Am. Chem. Soc. 103*, 4634 (1981).
(2) The alkaloid was first isolated from the Chinese drug Kou Wen, *Gelsemium elegans* Benth. by Chou T. Q. Chinese J. Physiol. *5*, 345 (1931), ref. Chem. Abstr. *20*, 806 (1932).
(3) Chi, Y. F., Y.-S. Kao and Y.-T. Huang: J. Am. Chem. Soc. *60*, 1723 (1938).
(4) Janot, M.-M., R. Goutarel and M. C. Perezamador Y Barron: Ann. Pharm. Fr. *11*, 602 (1953).
(5) Sakai, S., A. Kubo and J. Haginiwa: Tetrahedron Lett. 1485 (1969).

Address: Prof. Mauri Lounasmaa, Technical University of Helsinki, Department of Chemistry, SF-02150 Espoo 15, Finland

Qualitative Changes in Peptide Alkaloid Biosynthesis of Claviceps purpurea by Environmental Conditions

K. Breuel*, K. Braun*, Ch. Dauth* and D. Gröger**

* VEB Pharmazeutisches Kombinat GERMED Dresden,
** Institute of Plant Biochemistry, Academy of Sciences of the GDR, Halle (Saale), German Democratic Republic

Key Word Index:

Claviceps purpurea; Alkaloid Pattern; Saprophytic Culture; Parasitic Cultivation.

The spectra of saprophytically synthesized ergot alkaloids may be changed either by mutation [1, 2] or by externally applied substrates. Addition of appropriate amino acids or their analogues to nutrient media may alter to a varying degree the composition of the alkaloid mixture [3–5]. Mycelial dry weight and total alkaloid yield are practically not influenced. Obviously the biosynthesis of the cyclol part of ergopeptines is here partially controlled by the relative concentrations of amino acids in the internal pool. The competition seems to be restricted to structurally related amino acids e.g. branched chain amino acids or aromatic amino acids and their analogues but not inter alia. Exogenously administered amino acids also strongly influenced the alkaloid spectrum in the maturing sclerotia under parasitic conditions [3].

We observed an internally controlled reversible change in the alkaloid spectrum under different environmental conditions with a particular *Claviceps purpurea* (Fr.) Tul. strain, designated DM 595. Strain DM 595 produces saprophytically a mixture of ergotoxines (ergocornine: ergokryptine, ratio 2:1) besides clavines and simple lysergic acid derivatives. Culture conditions using a sucrose/ammoncitrate fermentation medium according to [6] were adopted. Spores of this strain were used to inoculate ears of *Secale cereale*. The infected ears were isolated by paper bags. A careful investigation of about 50 mature single sclerotia revealed the absence of ergocornine and ergokryptine.

Instead of these ergotoxines two other peptide-type alkaloids were found as main ergolines. The mixture was composed of ergotamine and ergocristine. The ratio of these alkaloids to each other varied considerably from sclerotium to sclerotium, but the percentage of ergopeptines in the total alkaloid content was nearly constant. Saprophytic cultures derived from these particular sclerotia again produced ergocornine and ergokryptine (Table I). These experiments were performed throughout two consecutive years, with essentially the same results.

Table I

Alkaloid formation in *C. purpurea*, strain DM 595, under various environmental conditions

	Saprophytic cultivation →	Parasitic cultivation →	Saprophytic cultivation
Total alkaloid yield (mg/g dry mycelium)	25	5	25
Ergopeptines:			
– Alkaloid spectrum	ergocornine ergokryptine	ergotamine ergocristine	ergocornine ergokryptine
– Total alkaloid content %	50	75	50
– Alkaloid ratio to each other	constant	variable	constant

Alkaloids were identified by UV, IR, MS, co-TLC and amino acid analysis of the peptide moiety. Quantitation of ergopeptines was performed by fluorodensitometry after TLC on formamide impregnated cellulose plates. Solvent systems: ethylacetate-n-heptane-diethyl-amine (5:6:0.02) and benzene with 1 % ethanol.

Consequently the genotype of this strain possesses the capability to synthesize four different peptide alkaloids. It depends largely on the actual environmental complex which alkaloids may be formed. A strict pair formation regarding the alkaloid quality was observed. It is still an open question whether the internal concentration only of a particular amino acid (e.g. phenylalanine in the case of parasitic cultivation) or if other factors also control the alkaloid spectrum.

References

(1) Kobel, H. and J. J. Sanglier, in Vaněk, Z., Z. Hŏsfalek and I. Cudlin (eds.): "Genetics of Industrial Microorganisms", Vol. II, p. 421, Amsterdam, London, New York, 1973, Elsevier Publishing Comp.
(2) Marnati, M. P., A. Minghetti and C. Spalla, in: "Abstracts, Conference on Medicinal Plants with International Participation" p. 68, Marianske Lazne, 1975.
(3) Kobel, H. and J. J. Sanglier, in Hütter, R., T. Leisinger, J. Nüesch and W. Wehrli (eds.): "Antibiotics and other Secondary Metabolites", p. 233, London, New York, San Francisco, 1978, Academic Press.
(4) Beacco, E., M. L. Bianchi, A. Minghetti and C. Spalla: Experientia *34*, 1291 (1978).
(5) Spalla, C., in Phillipson, I. D. and M. H. Zenk (eds.): "Indole and Biogenetically Related Alkaloids", p. 271, London, New York, Toronto, Sydney, San Francisco, 1980, Academic Press.
(6) Borowski et al.: Process for the fermentative production of ergoline derivatives, US-Patent 4, 086, 141.

Address: Dr. K. Breuel, VEB Arzneimittelwerk Dresden, Wilhelm-Pieck-Str. 35, DDR-8122 Radebeul

Incorporation of Thiazolidine-4-Carboxylic Acid Into Ergosine by Claviceps purpurea

A. Baumert, D. Erge and D. Gröger

Institute of Plant Biochemistry, Academy of Sciences of the GDR, DDR-4010 Halle (Saale), German Democratic Republic

Key Word Index:

Thiazolidine-4-carboxylic acid; Ergosine Analogue, Claviceps purpurea.

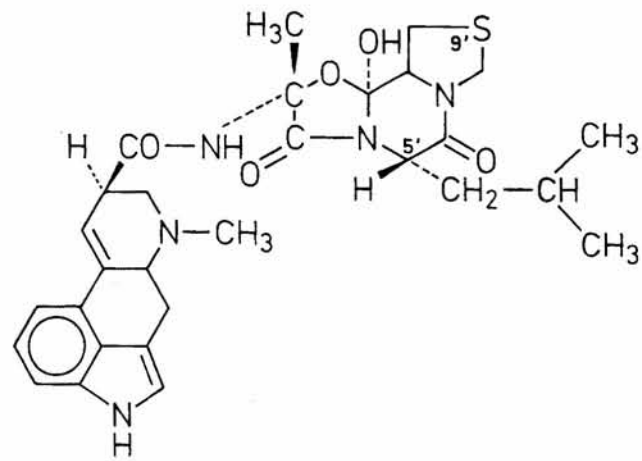
An ergosine analogue with a modified proline moiety can be obtained by feeding an appropriate Claviceps strain with L-thiazolidine-4-carboxylic acid.

The peptide ergot alkaloids = ergopeptines [1] are composed of lysergic acid and the cyclol group containing peptide moiety. The cyclol part of the molecule is biosynthetically derived from three amino acids. The single ergopeptines differ in the composition of the L-amino acids derived side chain. L-Proline seems to play a key role and is found in all peptide type ergot alkaloids.

The biosynthesis of the peptide moiety is at least partially controlled by the relative amino acid concentration in the internal pool of the cells. This has been demonstrated by several groups [2–5]. The addition of appropriate amino acids or their analogues to the nutrient medium determines significantly the proportions of the alkaloid mixture in a given strain. Pronounced effects were observed by using amino acid auxotrophic mutants [3]. Also under parasitic conditions the alkaloid spectrum in a mature sclerotium may be altered by a given amino acid.

Interesting to note that in submerged culture the competition between various amino acids is restricted to structurally related compounds, e. g. phenylalanine can not be replaced by branched-chain amino acids in an ergocristine producing strain. Recently [4] the amino acid adjacent to lysergic acid in ergotamine could be replaced by α-aminobutyric acid giving the alkaloid ergostine. We wish to report the first directed biosynthesis of an ergot alkaloid with an altered proline moiety.

For these studies the C. purpurea strain MUT 168/2 was used, which produces ergosine in submerged culture. The culture technique of this strain was described previously [6, 7]. Alkaloid production of strain 168/2 was achieved in an ammoncitrate/saccharose medium NL 833. After the first day of incubation ten flasks each containing 100 ml NL 833 were supplemented with 5 g/l of L-thiazolidine-4-carboxylic acid [8, 9]. After further ten days' incubation the cultures were harvested, the mycelium filtered off and the filtrate extracted. The filtrate contained 290 µg/ml ergosine/ergosinine and 60 µg/ml of an unknown alkaloid X and its apparent isomer X_I. X and X_I were interconvertible.



2'β–Methyl–5'α–isobutyl–9'–thiaergopeptine

Both compounds exhibit a bright fluorescence under UV-light and give a blue color reaction with van Urk's reagent. 30 mg of compound X as amorphous substance was obtained after succesive TLC-separation (silica gel PF_{254} Merck). The following solvent systems were used; I. Chloroform : methanol (9:1); II. chloroform: methanol : acetic acid (80:15:5); III. ethylacetate : methanol (95:5), R_F-values in system I, II, III respectively: ergosine 0.45; 0.52, 0.22; X 0.51, 0.54, 0.44: ergosinine 0.76, 0.66, 0.59. X_I 0.85, 0.77, 0.69.

Alkaloid X was identified as 2'β-Methyl-5'α-isobutyl-9'-thiaergopeptine = "Thiaergosine". It showed UV absorption characteristic of lysergic acid derivatives, UV_{max}^{EtOH} 240, 312 nm and an minimum at 270 nm. IR ν̃ OH, NH 3470, 3150–3300, ν̃ CO 1725, 1660, 1645, 1545 cm^{-1}, identical between 3500–750 cm^{-1} with the IR spectrum of ergosine. The mass spectrum gave the following peaks: m/e no M^+ peak, 337, 298