

18. PRODUCERS OF ERGOT ALKALOIDS OUT OF *CLAVICEPS* GENUS

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18.1. INTRODUCTION

It is known, that the ability to produce secondary metabolites is connected to the taxonomic position of the producers (Frisvad and Filtenborg, 1990). Traditional source of ergot alkaloids are fungi from the genus *Claviceps*. Up to now many organisms from filamentous fungi to higher plants were identified to be the producers of these biologically active compounds. It was established that strains belonging to fungi imperfecti, *Ascomycetes*, *Basidiomycetes* and *Phycomycetes* are able to synthesize the ergot alkaloids. In 1960, Hofmann and Tschertter described the occurrence of ergot alkaloids in higher plants. They succeeded in isolating of lysergic acid amide, isolysergic acid amide and chanoclavine from the seeds of *Ipomoea violacea* and *Rivea corymbosa* (Convolvulaceae).

Summary of all ergot alkaloids isolated from filamentous fungi and higher plants is given in [Table 1](#). For the structures of other fungal metabolites not given in this book see e.g., Turner and Aldridge (1983).

Most of ergot alkaloid producers outside of *Claviceps* genus produce clavine alkaloids. They can be classified in three groups:

- I. Producers "classical" type of clavines (elymoclavine, agroclavine, festuclavine etc.—for the structures see [Chapter 7](#)).
- II. Producers of the clavines having different configuration than those from *Claviceps* (Figure 1) (Kozlovsky *et al.*, 1983).

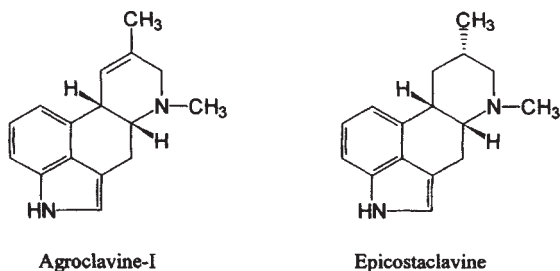


Figure 1

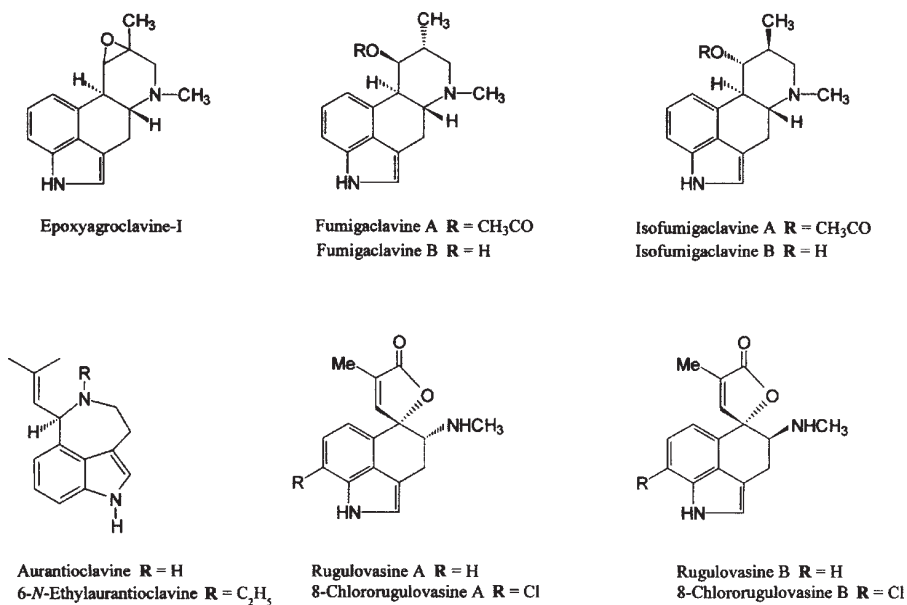


Figure 2

III. Producers of clavines which were never found in *Claviceps* (Figure 2) and clavine alkaloid dimers (Figure 3).

18.2. SCREENING OF THE ERGOT ALKALOID PRODUCERS AMONG THE FILAMENTOUS FUNGI

More than 1000 strains of fungi belonging to the *Ascomycetes*, *Phycomycetes*, *Basidiomycetes* and *Fungi imperfecti* were screened by Abe *et al.* (1967). Method of the screening included cultivation in submerged and surface culture, the use of Ehrlich reagent for detection of indolic compounds, and paper chromatography with the standard samples of ergot alkaloids. Several strains of fungi belonging to the genera *Penicillium* and *Aspergillus* were identified as producers of clavine alkaloids. The best alkaloid producers were strains of *Aspergillus fumigatus* synthesizing clavine alkaloids (Table 1).

Bekmakhanova *et al.* (1975) screened potential alkaloid producers among 19 strains from the genus *Penicillium* using TLC. She found that strains *P. gorlenkoanum*, *P. sizovae*, *P. roqueforti*, *P. restrictum* and *P. paxilli* produced metabolites of the ergot alkaloid type. Later it was shown *P. gorlenkoanum*, *P. sizovae*, and *P. roqueforti* can synthesize ergot alkaloids (see Table 1) (Kozlovsky *et al.*, 1979; Kozlovsky *et al.*, 1981a; Kozlovsky *et al.*, 1986). Vining *et al.* (1982) examined several hundreds isolates of the fungi out of *Claviceps* genus for the

Table 1 Ergot alkaloid producers out of genus *Claviceps*

<i>Organism</i>	<i>Ergot alkaloid</i>	<i>Reference</i>
<i>Fungi imperfecti</i>		
<i>Aspergillus fumigatus</i>	festuclavine, fumigaclavine A, fumigaclavine B, elymoclavine, chano-clavine-I, ergotamine, ergocristine	Abe <i>et al.</i> (1967); Cole <i>et al.</i> (1977); Furuta <i>et al.</i> (1982); Narayan and Rao (1982); Spilsbury and Wilkinson (1961); Yamano <i>et al.</i> (1964)
<i>A. flavus</i>	elymoclavine, cyclopiazonic acid, ergokryptine	El-Refai <i>et al.</i> (1970); Hermansen <i>et al.</i> (1984); Naim (1980); Yokota <i>et al.</i> (1981)
<i>A. japonicus</i>	festuclavine, cycloclavine	Furuta <i>et al.</i> (1982); Stauffacher <i>et al.</i> (1969)
<i>A. nidulans</i>	clavines	Abe <i>et al.</i> (1967)
<i>A. oryzae</i>	cyclopiazonic acid	Hermansen <i>et al.</i> (1984); Yokota <i>et al.</i> (1981)
<i>A. tamarii</i>	fumigaclavine A, other unidentified clavine alkaloids	Janardhanan <i>et al.</i> (1984)
<i>A. versicolor</i>	cyclopiazonic acid, bis-secodehydrocyclopiazonic acid, imine cyclopiazonic acid	Ohmomo <i>et al.</i> (1973)
<i>Botrytis fabae</i>	elymoclavine, agroclavine, ergokryptine	Naim (1980)
<i>Curvularia lunata</i>	elymoclavine, agroclavine, ergokryptine	Naim (1980)
<i>Geotrichum candidum</i>	elymoclavine, agroclavine, ergosine, lysergic acid	El-Refai <i>et al.</i> (1970)
<i>Penicillium atramentosum</i>	rugulovasine A	Frisvad and Filtenborg (1989)
<i>P. aurantiovirens</i>	clavicipitic acid, chano-clavine-I, agroclavine, elymoclavine, penniclavine, isopenniclavine	Kozlovsky <i>et al.</i> (1981)
<i>P. biforme</i>	rugulovasine A, rugulovasine B, chlororugulovasine B	Dorner <i>et al.</i> (1980)
<i>P. camembertii</i>	cyclopiazonic acid	Hermansen <i>et al.</i> (1984); Le Bars (1979); Schoch <i>et al.</i> (1983)
<i>P. chermesinum</i>	costaclavine	Agurell (1964)
<i>P. chrysogenum</i>	rugulovasine A, rugulovasine B, fumigaclavine A, fumigaclavine B, pyroclavine	Kozlovsky <i>et al.</i> (1997); Reshetilova <i>et al.</i> (1995)

Table 1 (Continued)

<i>Organism</i>	<i>Ergot alkaloid</i>	<i>Reference</i>
<i>P. clavigerum</i>	isofumigaclavine A	Frisvad and Filtenborg (1989)
<i>P. commune</i> <i>chemotype-I</i>	rugulovasine A	Frisvad and Filtenborg (1989)
<i>P. commune</i> <i>chemotype-II</i>	cyclopiazonic acid, isofumigaclavine A	Frisvad and Filtenborg (1989); Hermansen <i>et al.</i> (1984)
<i>P. concavo-</i> <i>rugulosum</i>	chanoclavine-I, chanocla- vine-II, isochanoclavine-I, rugulovasine A, chlororugulovasine A, rugulovasine B, chloro- rugulovasine B, dihydro- rugulovasine A	Abe <i>et al.</i> (1969); Cole <i>et al.</i> (1976); Ohmomo <i>et al.</i> (1977)
<i>P. crustosum</i>	cyclopiazonic acid, fumi- gaclavine A, pyroclavine, festuclavine	Filtenborg <i>et al.</i> (1983); Hermansen <i>et al.</i> (1984); Kawai <i>et al.</i> (1992)
<i>P. citreo-viride</i>	cividiclavine, pyroclavine	Vining <i>et al.</i> (1982)
<i>P. cyclopium</i>	cyclopiazonic acid, bissecodehydrocyclo- piazonic acid, imine cyclopiazonic acid	Hermansen <i>et al.</i> (1984); Holzapfel (1968); Holzapfel (1970)
<i>P. expansum</i>	aurantioclavine	Kozlovsky (1990)
<i>P. gorlenkoanum</i>	rugulovasine A, rugulova- sine B, costaclavine, epicostaclavine, chano- clavine-I, isochanoclavine-I	Kozlovsky <i>et al.</i> (1981a); Reshetilova <i>et al.</i> (1995)
<i>P. griseofulvum</i>	cyclopiazonic acid, epicostaclavine	Hermansen <i>et al.</i> (1984); Kozlovsky (1990)
<i>P. implicatum</i>	epoxyagroclavine-I	Kozlovsky <i>et al.</i> (1997)
<i>P. islandicum</i>	rugulovasine A	Cole <i>et al.</i> (1976)
<i>P. kapuscinskii</i>	agroclavine-I, epoxy- agroclavine-I	Kozlovsky <i>et al.</i> (1982a)
<i>P. lanosoviride</i>	cyclopiazonic acid	Hermansen <i>et al.</i> (1984)
<i>P. mononematosum</i>	cyclopiazonic acid	Frisvad and Filtenborg (1989)
<i>P. oxalicum</i>	cyclopiazonic acid, fumiga- clavine A, fumigacla- vine B, pyroclavine, festuclavine, chanooclavine-I	Vinokurova <i>et al.</i> (1991)
<i>P. palitans</i>	cyclopiazonic acid, fumigaclavine A, fumiga- clavine B, pyroclavine, festuclavine, chanocla- vine-I, agroclavine	Reshetilova <i>et al.</i> (1995); Vinokurova <i>et al.</i> (1991)
<i>P. patulum</i>	cyclopiazonic acid	Filtenborg <i>et al.</i> (1983); Hermansen <i>et al.</i> (1984)

Table 1 (Continued)

<i>Organism</i>	<i>Ergot alkaloid</i>	<i>Reference</i>
<i>P. puberulum</i>	cyclopiazonic acid	Filtborg <i>et al.</i> (1983); Hermansen <i>et al.</i> (1984)
<i>P. roqueforti</i>	festuclavine, isofumigaclavine A, isofumigaclavine B	Frisvad and Filtborg (1989); Kozlovsky <i>et al.</i> (1979); Ohmomo <i>et al.</i> (1975); Ohmomo <i>et al.</i> (1977); Polonsky <i>et al.</i> (1977); Scott <i>et al.</i> (1976); Scott <i>et al.</i> (1977)
<i>P. rubrum</i>	rugulovasine A, chlororugulovasine, chlororugulovasine B	Dorner <i>et al.</i> (1980)
<i>P. rugulosum</i>	rugulovasine A, rugulovasine B	Abe <i>et al.</i> (1969)
<i>P. sizovae</i>	agroclavine-I, epoxyagroclavine-I, agroclavine, chanoclavine-III, chanoclavine-I, 6,7-secoagroclavine, elymoclavine, dimer of epoxyagroclavine-I, dimer of agroclavine-I, mixed dimer of agroclavine-I and epoxyagroclavine-I, dimer of chanoclavine	Kozlovsky (1990); Kozlovsky and Reshetilova (1984); Kozlovsky <i>et al.</i> (1995a); Zelenkova <i>et al.</i> (1992); Kozlovsky <i>et al.</i> (1996)
<i>P. solitum</i>	rugulovasine A, rugulovasine B, chlororugulovasine A	Reshetilova <i>et al.</i> (1995)
<i>P. viridicatum</i>	cyclopiazonic acid, rugulovasine A, rugulovasine B	Filtborg <i>et al.</i> (1983); Hermansen <i>et al.</i> (1984); Reshetilova <i>et al.</i> (1995)
<i>P. vulpinum</i>	cyclopiazonic acid	Kozlovsky <i>et al.</i> (1997)
<i>Ascomycetes</i>		
<i>Balansia cyperi</i>	ergobalansine, ergobalansinine	Powell <i>et al.</i> (1990)
<i>B. claviceps</i>	chanoclavine-I, ergonovine, ergonovinine	Bacon <i>et al.</i> (1979); Porter <i>et al.</i> (1979)
<i>B. epichloe</i>	chanoclavine-I, isochanoclavine-I, agroclavine, elymoclavine, penniclavine, ergonovine, ergonovinine, 6,7-secoagroclavine	Bacon <i>et al.</i> (1979); Porter <i>et al.</i> (1979); Porter <i>et al.</i> (1981)
<i>B. henningsiana</i>	chanoclavine-I, dihydro-elymoclavine	Bacon <i>et al.</i> (1979); Porter <i>et al.</i> (1981)
<i>B. obtecta</i>	ergobalansine, ergobalansinine	Powell <i>et al.</i> (1990)
<i>B. strangulans</i>	6,7-secoagroclavine, chanoclavine-I	Bacon <i>et al.</i> (1979); Porter <i>et al.</i> (1981)

Table 1 (Continued)

<i>Organism</i>	<i>Ergot alkaloid</i>	<i>Reference</i>
<i>Epichloe typhina</i>	6,7-secoagroclavine, agroclavine, elymoclavine, penniclavine, festuclavine, ergovaline, ergovalinine, ergotamine, ergosine, β -ergosine, ergostine, ergoptine, β -ergoptine, ergonine, ergocristine, α -ergokryptine, β -ergokryptine, ergocornine	Porter <i>et al.</i> (1979a); Porter <i>et al.</i> (1981); Yates <i>et al.</i> (1985)
<i>Hypomyces aurantius</i>	ergokryptine, ergokryptinine, agroclavine, elymo-clavine, chanoclavine	Yamatodani and Yamamoto (1983)
<i>Sepedonium</i> sp.	elymoclavine, agroclavine, ergocornine	Naim <i>et al.</i> (1980)
Basidiomycetes		
<i>Corticium caeruleum</i>	clavines	Abe <i>et al.</i> (1967)
<i>Lenzites trabea</i>	clavines	Abe <i>et al.</i> (1967)
<i>Pellicularia filamentosa</i>	clavines	Abe <i>et al.</i> (1967)
Phycomycetes		
<i>Cunninghamella blakesleana</i>	elymoclavine, agroclavine, ergosine, lysergic acid	Naim (1980)
<i>Mucor hiemalis</i>	ergosine	El-Refai <i>et al.</i> (1970)
<i>Rhizopus arrhizus</i>	agroclavine	Sallam <i>et al.</i> (1969)
<i>Rh. nigricans</i>	agroclavine, ergosine, ergosinine	El-Refai <i>et al.</i> (1970); Sallam <i>et al.</i> (1969)
Higher plants		
Convolvulaceae		
<i>Argyreia</i> (20 species)	agroclavine, chanoclavine-I, chanoclavine-II, rac.chanoclavine-II, elymo-clavine, festuclavine, lysergine, lysergine, lysergol, isolysergol molliclavine, penniclavine, setoclavine, isosetoclavine, ergine, isoergine, ergometrine, ergometrinine, lysergic acid 2-hydroxyethylamide, iso-lysergic acid 2-hydroxy-ethylamide, ergosine, ergosinine, cycloclavine	Chao and DerMarderosian (1973)

Table 1 (Continued)

<i>Organism</i>	<i>Ergot alkaloid</i>	<i>Reference</i>
<i>Cuscuta monogyna</i>	agroclavine	Chao and DerMarderosian (1973)
<i>Ipomoea</i> (11 species)	agroclavine, chanoclavine-I, chanoclavine-II, rac.chanoclavine-II, elymoclavine, festuclavine, lysergol, isolysergol, penniclavine, ergine, isoergine, ergometrine, ergometrinine, lysergic acid 2-hydroxyethylamide, isolysergic acid 2-hydroxyethylamide, ergosine, ergosinine, cycloclavine	Chao and DerMarderosian (1973)
<i>I. rubro-caerulea</i>	chanoclavine-I, lysergic acid amide, lysergic acid 2-hydroxyethylamide, isolysergic acid 2-hydroxyethylamide	Gröger <i>et al.</i> (1963)
<i>I. piurensis</i>	chanoclavine-I, ergine, lysergic acid 2-hydroxyethylamide, ergobalasinine, ergobalansinine	Jenettisiems <i>et al.</i> (1994)
<i>Rivea corymbosa</i>	agroclavine, chanoclavine-I, chanoclavine-II, elymoclavine, lysergine, lysergol, molliclavine, setoclavine, isosetoclavine, ergine, isoergine, ergometrine, ergometrinine, lysergic acid 2-hydroxyethylamide, isolysergic acid 2-hydroxyethylamide, ergosine	Chao and DerMarderosian (1973)
<i>Stictocardia tiliifolia</i>	chanoclavine-I, chanoclavine-II, festuclavine, lysergol, ergine, ergometrine, ergometrinine, lysergic acid 2-hydroxyethylamide	Hofmann (1961)

production of ergot alkaloids. Only one, *Penicillium citreoviride* gave positive reaction for indolic compounds. The main component named as cividiclavine was isolated and its structure was elucidated as a new type of clavine alkaloid dimer. It contains a pyroclavine moiety linked through its indolic nitrogen to a hydroxypyroclavine. The linkage in the latter is tentatively placed at C-13' and

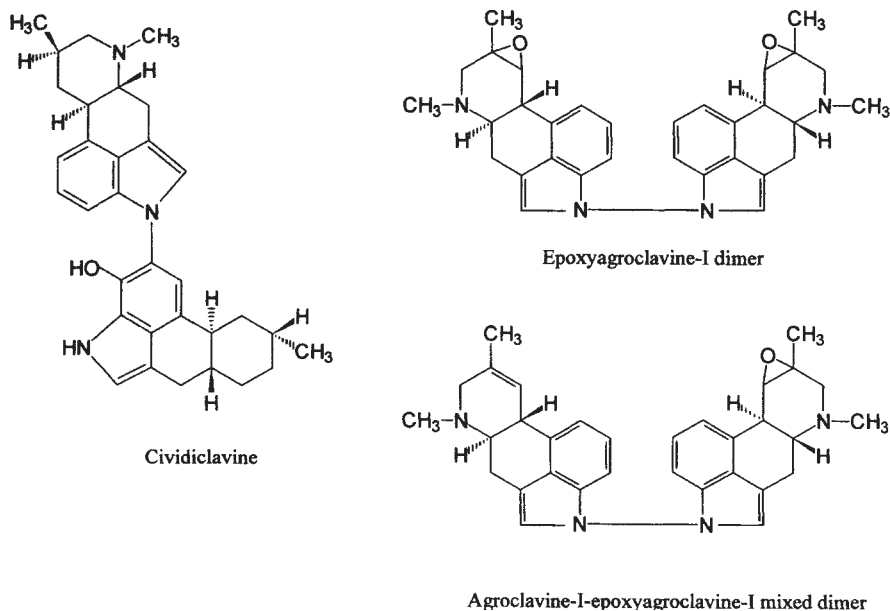


Figure 3

the hydroxy group is assigned to C-14'. Free pyroclavine was also isolated from the culture broth (Figure 3). A screening method for alkaloid producing fungi was developed (Kozlovsky and Solov'eva, 1985) including cultivation under the optimal conditions for the secondary metabolite production, sampling during growth, analysis of the culture liquid and mycelium for alkaloids, isolation of metabolites of the alkaloid origin (alkaline, neutral and acid), and TLC of extracts with standard samples. Seven strains of *Aspergillus*, 3 belonging to *Chaetomium*, 10 of *Fusarium*, 6 of *Helminthosporium*, 2 of *Rhizopus* and 36 of *Penicillium* genera were examined. *P. aurantiovirens*, *P. kapuscinskii*, and *P. palitans* were identified as ergot alkaloid producers (see Table 1) and their structures were elucidated (Kozlovsky *et al.*, 1981; Kozlovsky *et al.*, 1982b; Kozlovsky *et al.*, 1990; Vinokurova *et al.*, 1991).

Ohmomo *et al.* (1989) have screened for indole alkaloid producing fungi and he isolated some new producers from the genus *Aspergillus*. One of them, a thermophilic strain No. 2-18, was identified as *Aspergillus fumigatus* producing mainly fumigaclavine B (Figure 2).

From the culture liquid of *P. sizovae*, known as a producer of agroclavine-I (Figure 1) and epoxyagroclavine-I, a group of new dimeric ergot alkaloids was recently isolated (Zelenkova *et al.*, 1992; Kozlovsky *et al.*, 1995a). Specific feature of these dimers is a linkage between indolic nitrogens of the ergoline moieties. Several derivatives of these dimer were obtained by opening of the oxiran ring of epoxyagroclavine-I (Zelenkova *et al.*, 1992). Later, Kozlovsky *et al.* (1995a) isolated dimer of agroclavine-I and a mixed dimer of agroclavine-I

and epoxyagroclavine-I (Figure 3). Based on the preliminary data (retention time in HPLC in comparison with the same for other dimers) they supposed also that chanoclavine dimer can be present in the culture broth.

Clavicipitic acid and its plausible decarboxylation product aurantioclavine were detected as metabolites *P. aurantiovirens* (Kozlovsky *et al.*, 1981). Later, *N*-6-ethylaurantioclavine has been isolated and its structure was elucidated (Kozlovsky *et al.*, 1997a). It is the first case of natural ergot alkaloid containing *N*-6-ethyl group (Figure 2).

Rugulovasine A and B (Figure 2) originally found by Abe *et al.* (1967) in *P. concavorugulosum* differs from clavine alkaloid by irregular cyclisation of D ring. Later, also chlororugulovasine A and B were found that are probably single natural ergot alkaloids containing halogen atom (see Table 1).

18.3. PHYSIOLOGY OF THE PRODUCERS AND SOME ASPECTS OF THE REGULATION OF ERGOT ALKALOID BIOSYNTHESIS

The data on the physiology of ergot alkaloid biosynthesis by the fungi, besides *Claviceps* strains are quite scarce. Extensive studies have been done with the strains of *P. sizovae*, *P. gorlenkoanum*, *P. kapuscinskii* and *P. aurantiovirens*.

Production kinetics of agroclavine-I and epoxyagroclavine-I, the main components of alkaloid mixture of *P. sizovae*, was studied by Kozlovsky *et al.* (1986). Accumulation and degradation of alkaloids took place in two stages which coincide with two growth phases. During growth in a medium containing succinic acid and mannitol sequential substrate utilization by the culture and biphasic growth was observed. For the alkaloid biosynthesis with *P. sizovae* high residual phosphate concentrations was necessary, compared to *Claviceps* strains, where high phosphate concentrations inhibited the alkaloid production.

An influence of carbon sources on the growth of *P. sizovae* and biosynthesis of agroclavine-I and epoxyagroclavine-I, as well as the activity of key enzymes of the Krebs cycle, the pentose phosphate pathway and glyoxalate cycle were studied (Kozlovsky and Vepritskaya, 1987). The best alkaloid productivity was observed with mannitol and fumaric acid as the carbon sources. A combination of sorbitol with fumaric acid stimulated epoxyagroclavine-I synthesis. A high alkaloid production was accompanied by high activity of the pentose phosphate cycle and low activity of the Krebs cycle.

Tryptophan is a precursor of ergot alkaloids in *Claviceps* and in some cases plays also the role of an inducer and derepressor (Bu'Lock and Barr, 1968; Vining, 1970; Robbers *et al.*, 1972). Isotopically labelled tryptophan shown a high level of incorporation into ergot alkaloids (41%) in the strain of *P. sizovae* indicating that it is also here a direct precursor Kozlovsky *et al.* (1985). In *Claviceps* not only L- but also D-tryptophan are utilised for the ergot alkaloid biosynthesis (Robbers *et al.*, 1972; Floss, 1976). The effect of both L- and D-tryptophan and also of their analogue, D,L-6-methyltryptophan on the alkaloid

production kinetics in *P. sizovae* was studied (Kozlovsky *et al.*, 1985a). In most of our experiments L- and D-tryptophan were added at concentrations of 0.1, 0.25, 0.4 and 2 mM together with the inoculum because their induction effect in *Claviceps* occurs only when fed during the first 24 hours of production cultivation (Bu'Lock and Barr, 1968; Robbers *et al.*, 1978). Feeding of both D- and L-tryptophan to the *P. sizovae* culture at the beginning of the production cultivation did not increase the alkaloid production. The production was even lowered to one half of the control. However, feeding both L- and D-tryptophan on the 6th day of the production stage increased the alkaloid yield 2.4 times with D-tryptophan, and 1.9 times with L-tryptophan. Additions of 6-methyltryptophan did not exert any stimulating effect on the production of ergot alkaloids in *P. sizovae*, moreover, an inhibition of their biosynthesis was observed. Thus it can be concluded, that the induction of ergot alkaloid biosynthesis by tryptophan is absent in *P. sizovae*. The absence of the induction effect in some strains of the *Claviceps* has also been established earlier by some authors (Gröger and Tyler, 1963; Řičicová *et al.*, 1982).

A specific relationship between exogenous tryptophan and alkaloid level was found in *P. roqueforti* (Kozlovsky *et al.*, 1982; Reshetilova and Kozlovsky, 1985). This strain produces two types of alkaloids, clavines (festuclavine, isofumigaclavine A and isofumigaclavine B) (Figure 2) and diketopiperazines (roquefortine and 3,12-dihydrodroquefortine) (Figure 4) (Kozlovsky *et al.*, 1979) that have both a common precursors—tryptophan and mevalonic acid. Exogenous tryptophan has different effect on these alkaloid types. Biosynthesis of diketopiperazines was enhanced by the precursor addition, whereas the production of clavines did not depend on the precursor and sometimes it was even inhibited by its addition (Reshetilova and Kozlovsky, 1985).

Effect of various concentrations of inorganic phosphate, microelements, temperature, pH, and sources of carbon, nitrogen on the yield of epoxyagroclavine-I dimer was studied in *P. sizovae* (Kozlovsky *et al.*, 1995). Active biosynthesis of the dimer occurred upon the cultivation on the media containing mannitol, α -ketoglutarate, ammonium, KH_2PO_4 in concentration of 0.1 g L^{-1} , and microelements (Fe^{2+} , Mn^{2+}) at 28°C and initial pH 7.0.

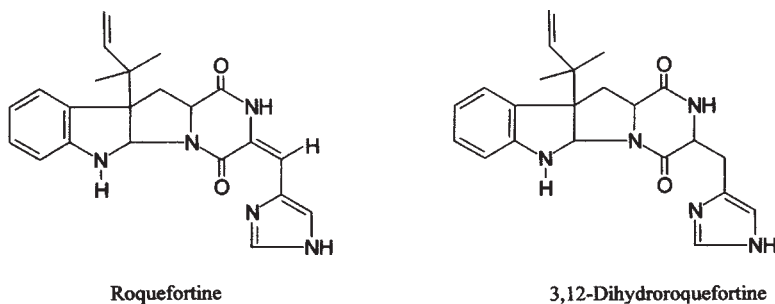


Figure 4

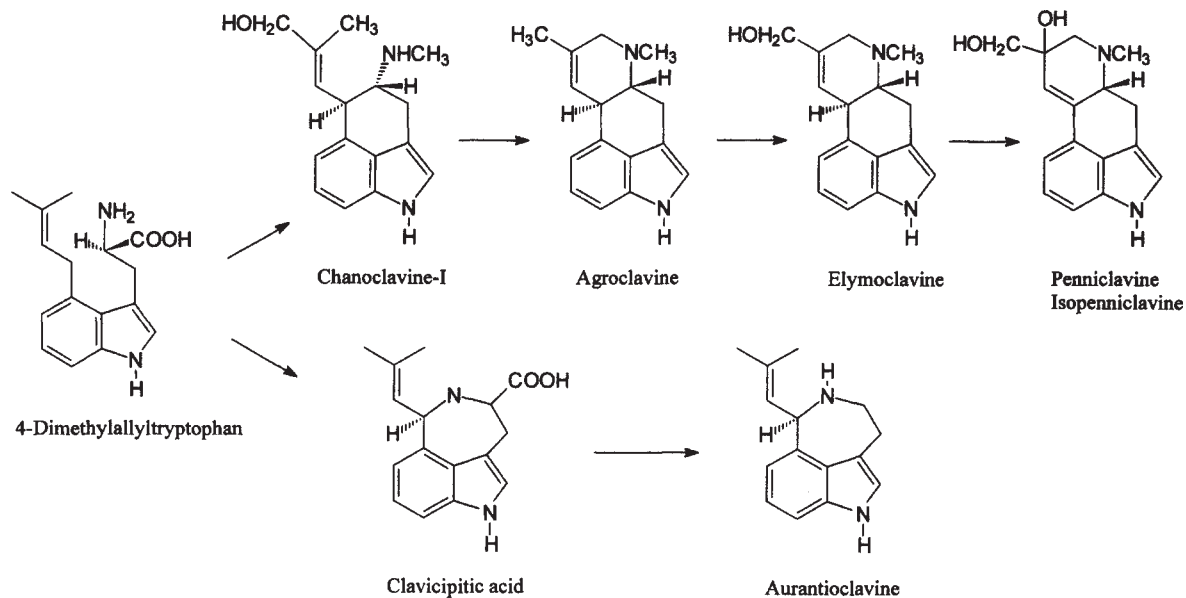
Active alkaloid production (agroclavine-I and epoxyagroclavine-I) by *P. kapuscinskii* was observed on the media containing mannitol and succinic or malic acids as carbon sources, and ammonium sulfate, asparagine, and tryptophan as nitrogen sources (Kozlovsky and Solov'eva, 1986a). The optimum of the phosphate concentration for alkaloid biosynthesis was $1 \text{ g L}^{-1} \text{ KH}_2\text{PO}_4$. Ascorbic acid (1 M) stimulated the yield of epoxyagroclavine-I and agroclavine-I.

The optimum medium for epicostaclavine (Figure 1) synthesis by *P. gorlenkoanum* contained mannitol, succinic acid and 1% KH_2PO_4 . Change in the carbohydrate or organic acid concentration, or variation in the phosphate concentration, altered the costaclavine and epicostaclavine ratio (Kozlovsky *et al.*, 1981b). Glucose together with fructose as carbon source inhibited alkaloid synthesis, addition of microelements as well as lowered aeration stimulated the alkaloid biosynthesis (Stefanova-Avramova and Kozlovsky, 1984).

The effect of the composition of the culture medium on the biosynthetic spectrum of ergot alkaloids in *P. aurantiovirens* was studied by Solov'eva *et al.* (1995). Addition of methionine and replacement succinic acid in Abe's medium by asparagic acid caused that besides aurantioclavine also chanoclavine-I was produced. When glucose was used as a sole source of carbon and energy only chanoclavine-I was produced, indicating stimulation of N-6-methyl transferase. Decrease of the dissolved oxygen from 75% to 2% at the end of the exponential phase, aurantioclavine and intermediates of the ordinary pathway of the ergot alkaloid biosynthesis, e.g., chanoclavine-I, agroclavine, elymoclavine, penniclavine and isopenniclavine were produced. Thus, *P. aurantiovirens* is able to produce clavine alkaloids by two pathways—through clavicipitic acid to aurantioclavine and from chanoclavine-I through agroclavine, elymoclavine to penniclavine and isopenniclavine (Figure 5).

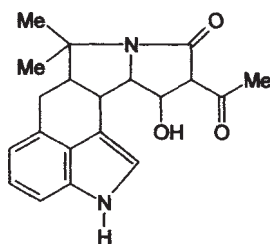
Effect of the culture age, the medium composition, various concentrations of phosphate and possible precursors, tryptophan, mevalonic acid and methionine, on the clavine alkaloids production by *A. fumigatus* was examined more authors (Rao and Patel, 1974; Rao *et al.*, 1977; Narayan and Rao, 1982). Tryptophan, mevalonic acid and methionine stimulated the clavine alkaloid production. Correlation between the alkaloid production and the culture growth was not established. Quality of the results obtained by these authors (Rao and Patel, 1974; Narayan and Rao, 1982; Ohmomo *et al.*, 1989) can be, however, hampered by questionable methodology.

Ohmomo *et al.* (1989) investigated the effect of carbon and nitrogen sources on the alkaloid synthesis with thermophilic strain *A. fumigatus* producing fumigaclavine B. Optimum medium was the combination of mannitol (5%), glucose (5%) and ammonium succinate (2%). Alkaloids were produced in good yields at 37°C, while the highest growth rate was attained at 41°C. The maximum alkaloid yield—20 mg/L was reached. After 10th day of cultivation, alkaloid degradation started.



Ergot alkaloid biosynthesis by *Penicillium aurantiovirens*

Figure 5



Cyclopiazonic acid

Figure 6

18.4. CYCLOPIAZONIC ACID BIOSYNTHESIS

Cyclopiazonic acid, a toxic metabolite of *P. cyclospium*, which can belong in some extent to ergot alkaloids, was isolated and identified by Holzapfel (1968) (Figure 6). Stereochemical aspects of D-ring formation of cyclopiazonic acid were investigated by Chalmers *et al.* (1982).

Sixty two isolates of *Penicillium* and *Aspergillus* were screened for cyclopiazonic acid production in surface and submerged culture on different media (Hermansen *et al.*, 1984). The production of this mycotoxin is restricted to *P. camembertii*, *P. griseofulvum* and *A. flavus* (and its domesticated form *A. oryzae*).

Best yield of cyclopiazonic acid was obtained with *P. griseofulvum* but several strains of *P. camembertii* were also found to be good producers. Submerged cultures gave best yields of cyclopiazonic acid, but in some cases the production occurred only in a surface culture. Hermansen *et al.* (1984) described also a simplified procedure for isolation of cyclopiazonic acid.

Effect of carbon and nitrogen sources on the production of cyclopiazonic acid by *P. griseofulvum* was studied by Reddy and Reddy (1988). Glycerol supported cyclopiazonic acid production, while citric acid and lactose were poor substrates. L-Asparagine, potassium nitrate and D,L-alanine supported good production of cyclopiazonic acid, while L-histidine did not.

18.5. HIGHER PLANTS AS THE PRODUCERS OF THE ERGOT ALKALOIDS

As mentioned above, for the first time ergot alkaloids have been found, in the higher plants by Hofmann and Tschertter (1960). They established that Mexican crude drug "Ololiuqui" consists of the seeds of *Rivea corymbosa* and *Ipomoea violacea* belonging to the family Convolvulaceae. They identified three main alkaloid components of the drug to be lysergic acid amide, isolysergic acid amide and chanoclavine-I. Later, Hofmann (1961) found elymoclavine in it. Stauffacher *et al.* (1969) isolated festuclavine and cycloclavine from *Ipomoea hildebrandtii* (Figure 7). First ergot alkaloids of the peptide type have been found

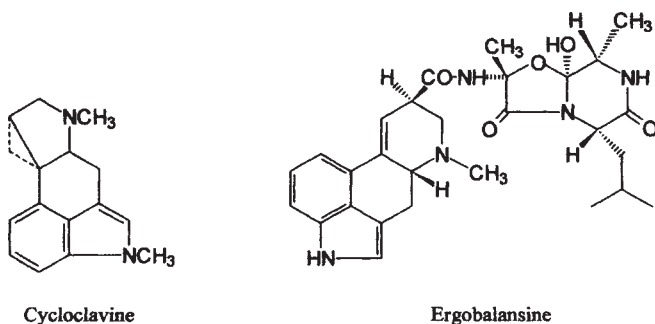


Figure 7

in higher plants by Stauffacher *et al.* (1965). They have found in the seeds of *Ipomoea argyrophylla* ergosine, ergosinine and also agroclavine (for the structures see Chapter 7). Jenettsiems *et al.* (1994) isolated from *Ipomoea piurensis* and elucidated the structures proline-free peptide ergot alkaloids, ergobalansine and ergobalansinine, and three simple ergoline alkaloids, chanoclavine-I, ergine and lysergic acid 2-hydroxyethylamide.

Gröger *et al.* (1963) have shown that in *Ipomoea rubro-caerulea* producing ergot alkaloids chanoclavine-I, lysergic acid amide, 2-hydroxylysergic acid amide, and 2-hydroxyisolysergic acid amide, tryptophan and mevalonic acid were their precursors analogously as in *Claviceps*.

Topics of the evolutionary relationship of ergoline biosynthesis in fungi and in higher plants has been discussed by Boyes-Korkis and Floss (1992). They raised the questions whether the genetic information coding for ergoline biosynthesis developed two times independently in nature, or it evolved only once and then was passed from the fungus to the plant or *vice versa*. If the latter is the case, are the pathway genes in the plant and/or fungus clustered or scattered throughout the genom? Is the genetic information coding for the ergoline pathway perhaps ubiquitous, but genetically silent in some other organisms? These questions, in their opinion, not yet answered, will be possibly solved in the future by the help of molecular biology.

18.6. ENVIRONMENTAL AND HAZARD PROBLEMS WITH UNTRADITIONAL PRODUCERS OF ERGOT ALKALOIDS

Safety methods and understanding of the danger from traditional source of ergot alkaloids, fungi of the genus *Claviceps* were developed very well. But now new challenge connected with a change of the relationship between bacterial and fungal microflora in environmental. In many cases it can be explained by a pollution of an antropogenic nature such, as wide using of herbicides, pesticides, another xenobiotics, heavy metals, etc. As a rule, fungi are more resistant than bacteria against the influence of these factors. Fungi of the genera *Penicillium* and *Aspergillus* are widely distributed in our environment. They occur in a soil,

on the food and feed, on the plants etc. As a result of these processes an rearrangement and narrowing of the diversity of the fungal community take place. New species of fungi became dominant strains. It is important to know the toxigenic potential of these strains, especially dominant, in the respect to their ability to produce such toxic secondary metabolites as ergot alkaloids, to evaluate the scale of the danger.

Nineteen strains of the twelve species of the genus *Penicillium*, isolated from the polluted soils and extreme places of habitation were examined for their ability to produce mycotoxins, included ergot alkaloids (Kozlovsky *et al.*, 1997). It was established that strains *P. chrysogenum* isolated from the city soil, can produce fumigaclavine A, fumigaclavine B and pyroclavine. *P. implicatum* isolated from Turkmenistan soil can synthesize epoxyagroclavine-I. One of the dominant strains, *P. vulpinum*, common in the city soil, can produce in considerable quantities cyclopiazonic acid and its imine. Cyclopiazonic acid belong to mycotoxins that should be strictly controled.

Ability to synthesize ergot alkaloids was tested in 31 fungal strains belonging to the genera *Aspergillus* and *Penicillium* that were isolated from Uzbekistan soils treated with pesticides for a long time (Kozlovsky *et al.*, 1990). It was shown, that one of the examined strains—strain *P. verrucosum* var. *cyclopium* can produce cyclopiazonic acid.

About 15 years ago a limited case of feed contamination by ergot alkaloids in Czechoslovakia was identified (V.Křen—personal communication). Clavine alkaloids (agroclavine, elymoclavine) were found in eggs and hen's meat and later it was found that this was caused by the grain, contaminated by some *Aspergilli* during the ship transport from South America (hot/humid conditions). Hens also suffered from ovaria degradation. They, in the first days of toxications, laid 2–3 eggs per day, however, later within ca 14 days stopped egg production and their ovaria were found severely damaged.

18.7. OUTLOOK ON USING OF ERGOT ALKALOID PRODUCERS OUT OF THE GENUS *CLAVICEPS* IN PRACTICE

Filamentous fungi, especially those belonging to genus *Penicillium* can be a source of the new ergot alkaloids with “unusual” structures (Skryabin and Kozlovsky, 1984). It is known that variations in the substitution and configuration of the ergoiline moiety may result into the considerable changes in the biological activity (Fluckiger, 1980). From this point of view epoxyagroclavine-I, N, N'-dimer epoxyagroclavine-I, mixed dimer epoxyagroclavine-I and agroclavine-I, metabolites of *P. sizovae* and *P. kapuscinskii*, are the most perspective as the base for the obtaining of the new biologically active compounds. Due to the high reactivity of the epoxy-group, these compounds are readily converted into some new ergoline derivatives under mild conditions using classical epoxide chemistry (Kozlovsky *et al.*, 1982a; Kozlovsky *et al.*, 1983; Zelenkova *et al.*, 1992; Kozlovsky *et al.*, 1995a).

Analogous reactions were performed with *N, N'*-dimer of epoxyagroclavine-I (Zelenkova *et al.*, 1992) and new derivatives were obtained.

18.8. CONCLUSIONS

Producers of ergot alkaloids are widely distributed among the various genera belong to various taxons. These organisms, especially filamentous fungi, can be dangerous for people and animals and their level must be controled. They can produce ergot alkaloids with a great variety of structural types including those which have not been found in *Claviceps*. Fungi of genus *Penicillium* are perspective as a source of the new ergot alkaloids and their derivatives, for production of the new biologically active compounds.

In these organisms, precursors of the ergot alkaloids tryptophan and mevalonic acid are the same as in *Claviceps*. Composition of the media and the cultivation conditions are rather specific for the production of the alkaloids by these strains.

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