9. BIOTRANSFORMATIONS OF ERGOT ALKALOIDS

VLADIMÍR KŘEN

Laboratory of Biotransformation, Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, CZ-142 20 Prague 4, Czech Republic

9.1. INTRODUCTION

From all naturally occurring ergot alkaloids only two are used in therapy: ergotamine and ergometrine. The rest of the medicinally important ergot compounds underwent some chemical changes, e.g. halogenation, alkylation, 9, 10-double bond hydrogenation, etc.

Although total synthesis of various ergot alkaloids has been demonstrated, it is ruled out for economical reasons. On the other hand, chemical modifications of natural compounds possessing ergot skeleton are very important. Bioconversions can fulfil some of these tasks employing their advantages. Bioreactions often occur at chemically "nonactivated" positions. Biological systems exhibit regional selectivity upon polyfunctional molecules. This is of a great advantage over many chemical reagents that cannot distinguish among multiple similar functional groups. A high degree of stereoselectivity (both substrates and products) is a typical feature of bioconversions. This area is rapidly moving toward a more refined approach to choosing enzymes or organisms for their predictable type-reactions (Křen, 1991).

Much of the work on alkaloid transformations was motivated by the need to produce more effective drugs from naturally available compounds. Main effort has been devoted to specific oxidations of ergot alkaloids to produce desired amount of substrates for semisynthetic preparations. Many enzymatic reactions were employed for obtaining of new ergot derivatives, as e.g., their glycosides.

For biotransformation of ergot alkaloids various systems were used. Enzymes (Taylor and Shough, 1967; Shough and Taylor, 1969; Křen *et al.,* 1992), crude cell homogenates (Taylor *et al.,* 1966; Wilson *et al.,* 1971; Gröger, 1963), microbial prokaryotic (Béliveau and Ramstad, 1966; Yamatodani *et al.,* 1962; Davis, 1982) and eukaryotic cells (Béliveau and Ramstad, 1966; Brack *et at.,* 1962; Tyler *et al.,* 1965; Sieben *et al.,* 1984; Křen *et at.,* 1989) including the alkaloid producing strains of *Claviceps,* plant suspension cells (Ščigelová *et al.,* 1995; Křen *et al.,* 1996a; Křen *et al.,* 1996b) and also such unusual systems as plant seedlings (Taylor *et al.,* 1966). The biotransformation methods involved both free and immobilised cells (Křen *et al.,* 1989).

Although for many biotransformations the alkaloid producing *Claviceps* strains were used, some alkaloids fed to the organisms can be regarded as "xenobiotics". It is especially true in the case when added alkaloids are not naturally produced by the respective *Claviceps* strain used (e.g., feeding lysergols to *C. purpurea*). Similar situation occurs when the converted alkaloid is normally produced as a minor component and by its high concentration the alkaloid can enter alternative metabolic pathways affording thus new compounds (e.g., feeding high concentrations of chanoclavine into *C. fusiformis*). Part of the studies on ergot alkaloid bioconversions was stimulated also by problems of metabolism of ergot drugs in mammals.

Biotransformations of ergot alkaloids, as few other groups of natural products, offer rich variety of methodologies used and the results. Not only many important basic findings was obtained during the decades of this research but some of them can be exploited industrially.

9.2. BIOCONVERSIONS OF CLAVINE ALKALOIDS

Clavine alkaloids as the simplest ergot alkaloids are valuable starting material for many semisynthetic derivatives. The biotransformation studies in early sixties (Tyler *et al.,* 1965) were stimulated by not entirely known biogenesis of ergot skeleton. Later, mostly oxidative bioreactions were investigated aiming at the production of lysergol, elymoclavine and lysergic acid on an industrial scale.

9.2.1. Agroclavine

Agroclavine (**1**) can be produced in high yields by selected strains *C. fusiformis* and *C. purpurea*. However, till recently it was not considered to be a useful drug. Therefore, main effort in agroclavine bioconversions was targeted to its oxidations to elymoclavine (**6**) that is an important substrate for semisynthetic ergot alkaloid-based drugs. Chemical oxidation of **1** to elymoclavine is infeasible.

Enzyme systems from outside genus *Claviceps* transform agroclavine mostly to 8-hydroxyderivatives (Figure 1). These conversions are mediated mainly by peroxidases. The 8-oxidation of 8, 9-ergolenes is accompanied by the shift of double bond to 9, 10-position. Intermediates of this reaction are in some cases 10-hydroxy- or 8, 9-epoxy-derivatives.

More than 100 species of filamentous fungi and other microorganisms oxidise agroclavine to setoclavine (**2**) and isosetoclavine (**3**) (Béliveau and Ramstad, 1966, Yamatodani *et al.,* 1962) (Table 1). *Psilocybe semperviva* converts agroclavine with certain degree of stereoselectivity to setoclavine (Brack *et al.,* 1962). The same reaction is performed also by some prokaryotic microorganisms as, e.g., *Streptomycetes, Nocardias* (Béliveau and Ramstad, 1966; Yamatodani *et al.,* 1962) and *Pseudomonas aeruginosa* (Davis, 1982).

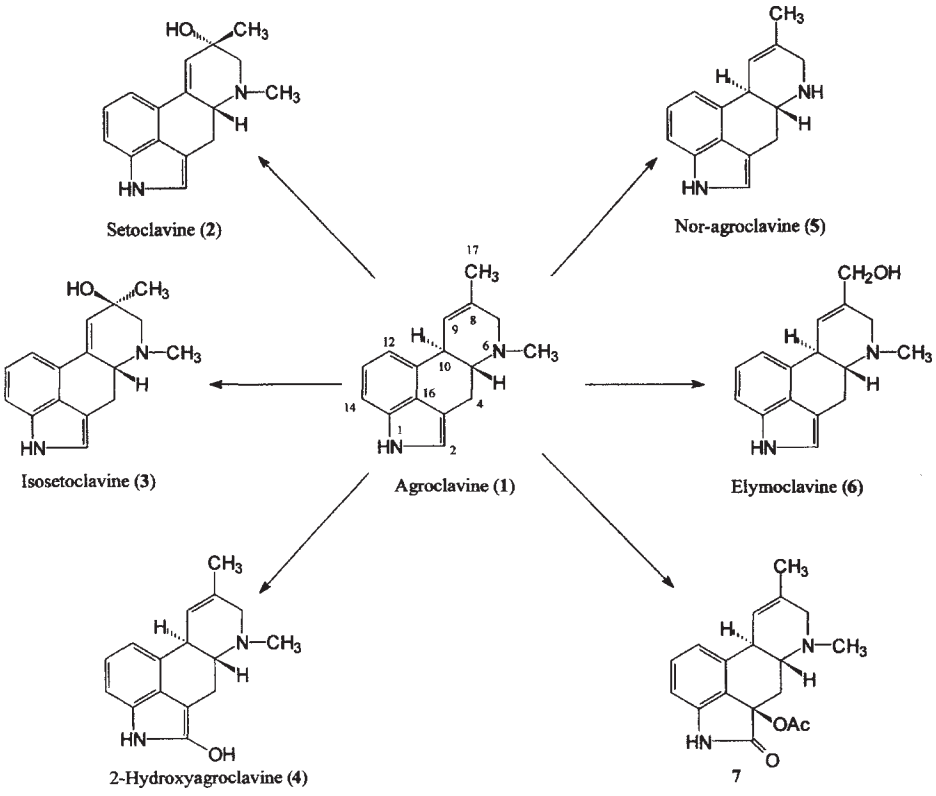


Figure 1 Typical bioconversions of agroclavine

Peroxidase rich homogenates from tomato fruits, potato sprouts, horse radish and morning glory *(Convolvulaceae)* seedlings catalyse the oxidation of agroclavine in présence of H_2O_2 to setoclavine and isosetoclavine (Taylor and Shough, 1967; Shough and Taylor, 1969; Taylor *et al.,* 1966). Shough and Taylor (1969) found that 10-hydroxyagroclavine and 10-hydroxy-8,9-epoxyagroclavine were intermediates of this reaction. The later compound was recently isolated as a product of agroclavine biotransformation (besides both setoclavines) by horse radish peroxidase. This biotransformation gave the same product under both aerobic and anaerobic conditions (Křen and Kawuloková, *unpublished results*).

Series of plant cell cultures exhibiting high peroxidase activity were tested for the agroclavine biotransformation (Ščigelová *et al.,* 1995). HPLC analyses revealed that some cultures gave rise to a mixture of setoclavine and isosetoclavine in nearly equimolar ratio *(Ajuga reptans, Atropa belladonna, Papaver somniferum),* whereas the other ones showed substantial degree of stereoselectivity *(Armoracia rusticana, Duboisia myoporoides, Euphorbia*

Table 1 Agroclavine bioconversions

Reaction	*Product*	*Conversion system (culture)*	*Reference*
8-Hydroxylation	Setoclavine	*Absidia spinosa* *ATCC-6648, *Aspergillus carbonarius* PCC-104, *Bispora effusa* CBS, *Cladosporium fulvus* ATCC-10391, *Fusarium solani* PCC-143, *Epicoccum* sp., *Giberella zeae* Ull, *Helminthosporium carbonum* Ull, *Mucor angulisporus* CBS, *Streptomyces annulatus* PCC-A-111, *S. griseus* PCC, *Nocardia rubra* PCC-252	Béliveau and Ramstad (1966)
		Psilocybe semperviva	Brack *et al.* (1962)
		Penicillium viridicatum	Tyler *et al.* (1965)
8-Hydroxylation	Setoclavine + Isosetoclavine	*Corticium sasakii*	Abe *et al.* (1963)
		Pseudomonas aeruginosa	Davis (1982)
		Horse radish peroxidase	Taylor and Shough (1967), Shough and Taylor (1969)
		Tomato homogenate, Potato sprouts homogenate, Morning glory seedlings homogenate	Taylor *et al.* (1966)
		C. purpurea (more strains)	Agurell and Ramstad (1962)
2-Hydroxylation	2-Hydroxyagroclavine	*Corticium sasakii*	Yamatodani *et al.* (1963)
2-Oxidation, 3-hydroxyacylation	2-Keto-3-*O*-acylagroclavines	Haloperoxidase from *Streptomyces aureofaciens*	Křen *et al.* (1997)
10-Hydroxylation	10-Hydroxyagroclavine	Horse radish peroxidase	Shough and Taylor (1969), Chan Lin *et al.* (1967b)

8,9-Epoxidation	10-Hydroxy-8,9-epoxyagroclavine	Horse radish peroxidase	Shough and Taylor (1969), Chan Lin *et al.* (1967b)
17-Hydroxylation	Elymoclavine	*Penicillium roqueforti*	Abe *et al.* (1966)
		Rat liver homogenate	Wilson *et al.* (1971)
		Microsomal fraction from	Agurell and Ramstad (1962),
		C. purpurea PEPTY 695/S	Kim *et al.* (1981)
		C. purpurea	
		C. fusiformis	Sieben *et al.* (1984), Křen *et al.* (1989), Tyler *et al.* (1965)
		C. paspali	Tyler *et al.* (1965)
		Claviceps sp. strains KK-2, Se-134, 47A, SD-58	Sieben *et al.* (1984)
N-6 Demethylation	Noragroclavine	*Streptomyces roseochromogenes*, *S. punipalus*, *S. purpurascens*	Yamatodani *et al.* (1962)
		Rat liver homogenate	Wilson *et al.* (1971)
		Horse radish peroxidase ($+O_2$)	Chan Lin and Ramstad (1967)
N-6-Demethylation + 8-Hydroxylation	Norsetoagroclavine	Horse radish peroxidase	Shough and Taylor (1969)
8,9-Hydrogenation	Festuclavine + Pyroclavine	*C. purpurea*	Agurell and Ramstad (1962)

Table 1 (*Continued*)

Reaction	*Product*	*Conversion system* (*culture*)	*Reference*
17-Hydroxylation 1-Alkyl- 1-Benzyl- 1-Hydroxymethyl- 2-Bromo- 2,3-Dihydro- 6-Ethyl-6-nor-	Corresponding elymoclavines	*C. fusiformis* SD-58	Sieben *et al.* (1984)

* Abbreviations of culture collections:
ATCC=American Type Culture Collection, Washington, D.C., U.S.A.
CBS=Centralbureau voor Schimmelculturen, The Netherlands.
IMUR=Institute de Micologia Universitade Recife, Recife, Brazil.
LSHTM=London School of Tropical Medicine, Great Britain.
NRRL=Northern Regional Research Laboratory, Peoria, Illinois, U.S.A.
PCC=Purdue Culture Collection, Lafayette, Indiana, U.S.A.
U11=Collection of Dr. A.J.Ullstrup, Purdue University, Lafayette, Indiana, U.S.A.

Table 2 Plant cell cultures used in agroclavine biotransformation experiments. Yields of setoclavine and isosetoclavine were calculated using molar extinction coefficients (Ščigelová *et al.,* 1995)

Plant cell culture	*Setoclavine* (%)	*Isosetoclavine* (%)
Ajuga reptans	5	5
Armoracia rusticana	10	80
Atropa belladonna	10	12
Duboisia myoporoides	5	90
Euphorbia calyptrata	traces	85
Papaver somniferum	20	30
Solanum aviculare	20	70

calyptrata) producing isosetoclavine in high excess (Table 2). Previous experiments with plant homogenates did not reveal any stereospecifity of those systems (Taylor *et at.,* 1966).

Agroclavine can be also *N*-6 demethylated by peroxidase leading to the formation of nor-agroclavine (**5**) and formaldehyde (Chan Lin *et al.,* 1967b). Also mammalian tissue homogenates (rat liver, guinea pig adrenal) produce from agroclavine nor-agroclavine, elymoclavine (**6**) and small amount of both setoclavines (**2,3**) (Wilson *et al.,* 1991).

Abe (1967 and Abe *et al.,* 1963) found as a metabolite of agroclavine by *Corticium sasakii* besides setoclavines also 2-hydroxyagroclavine (**4**). The later compound must, however, exist in its keto-form.

Some peroxidase system are able to oxidise also the pyrrole part of ergoline skeleton while degrading it. So called "degradation products" of ergot alkaloids (**8, 9**) (Figures 2, 3) are obviously products of peroxidase attack to the previously produced agroclavine and elymoclavine (Flieger *et al.,* 1991). These compounds are products of enzymes not directly involved in the alkaloid biosynthesis. The same compound (**9**) was obtained as the oxidation product of agroclavine by halogenperoxidase from *Streptomyces aureofaciens* (Figure 3) (Křen *et al.,* 1997). The main product in this case was, however, 2, 3-dihydro-6, 8dimethyl-3*ß*-propionyloxy-8-ergolen-2-one (10), where the 3-propionyloxy group was introduced stereoselectively (3*ß*). When acetate instead of propionate buffer was used in this reaction, analogous product 2, 3-dihydro6, 8-dimethyl-3*ß*-acetoxy-8-ergolen-2-one (7) was produced without higher oxidation product as in the previous case (Křen *et al.,* 1997).

Some strains of *C. purpurea* are able, besides the main conversion product elymoclavine, to convert agroclavine to setoclavines and to festuclavine and pyroclavine (Agurell and Ramstad, 1962).

The most desired and the most economically important agroclavine conversion is its oxidation to elymoclavine or lysergol. Although minute amount of elymoclavine was found as agroclavine conversion product in rat liver homogenate system (Wilson *et al.,* 1971) and by *Penicillium roqueforti* (Abe,

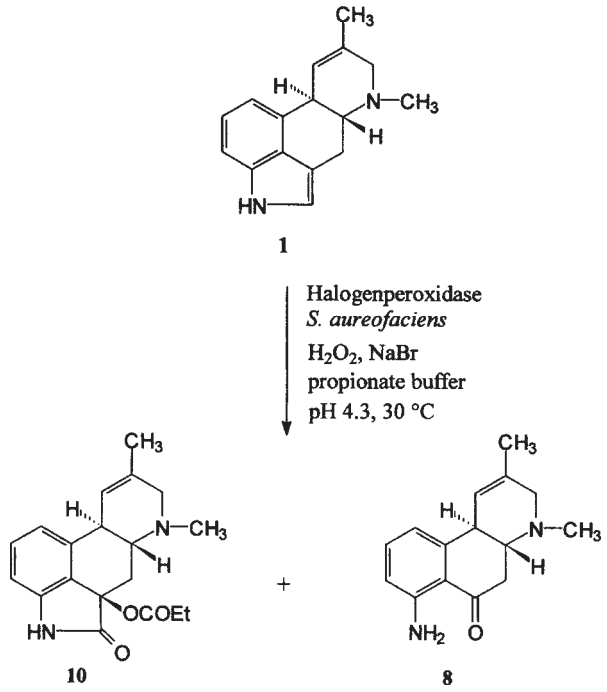


Figure 2 Biotransformation of agroclavine by halogenperoxidase from *Streptomyces aureofaciens*

1966), no organism, except for the *Claviceps* strains is able to perform this reaction at a reasonable rate.

Hsu and Anderson (1971) found agroclavine 17-hydroxylase activity in *C. purpurea* PRL 1980 cytosol, Kim *et al.* (1981) localised this activity to the microsomal fraction of the same strain. Sieben *et al.* (1984) conducted biotransformation of agroclavine derivatives with *C. fusiformis* SD-58 to obtain corresponding derivatives of elymoclavine. The strain was able to transform 1-alkyl-, 1-benzyl-, 1-hydroxymethyl-, 2-halo and 2, 3-dihydroagroclavine and 6-ethyl-6-noragroclavine to the corresponding elymoclavine derivatives. It was shown that the substrate specificity of the agroclavine 17-hydroxylase is high with respect to the 8,9-double bond and to the tertiary state of N-6, whereas the specificity is low for variations in the pyrrole partial structure (N-l, C-2, C-3). The N-1 alkylated agroclavines are hydroxylated faster probably due to better penetration of the substances into the cells because of more lipophilic attributes. Noragroclavine and lysergine were oxidised by the above system (Eich and Sieben, 1985) to the corresponding 8α-hydroxyderivatives, i.e. norsetoclavine and setoclavine. The system is highly stereospecific (to 8α-oxidation) in contrary to horse radish peroxidase that gives rise to a mixture of 8α and 8*ß* isomers.

For industrial bioconversion of agroclavine to elymoclavine, the high production strains *C. fusiformis* or selected strains *C. paspali* are the most suitable ones (Křen *et al.*, 1989). Use of immobilised and permeabilised *C. fusiformis* cells for this bioconversion was successfully tested (Křen *et al.*, 1989).

9.2.2. Elymoclavine

Most of the elymoclavine (6) bioconversions were focused to its oxidation to lysergic acid (14) or paspalic acid (15) (Figure 3). Similarly as in agroclavine biooxidations, the C-17 oxidation activity is confined to the *Claviceps* genus. Other biosystems as fungi (Béliveau and Ramstad, 1966; Abe, 1966; Abe *et al.*, 1963; Tyler *et al.*, 1965; Sebek, 1983), bacteria (Béliveau and Ramstad, 1966; Yamatodani *et al.*, 1963; Abe, 1966) and plant preparations (Taylor *et al.*, 1966; Chan Lin *et al.*, 1967a, b; Gröger, 1963) introduce hydroxy-group into position C-8 by peroxidase reaction—analogously to agroclavine—giving rise to penniclavine (11) and isopenniclavine (12). Production of complicated mixture

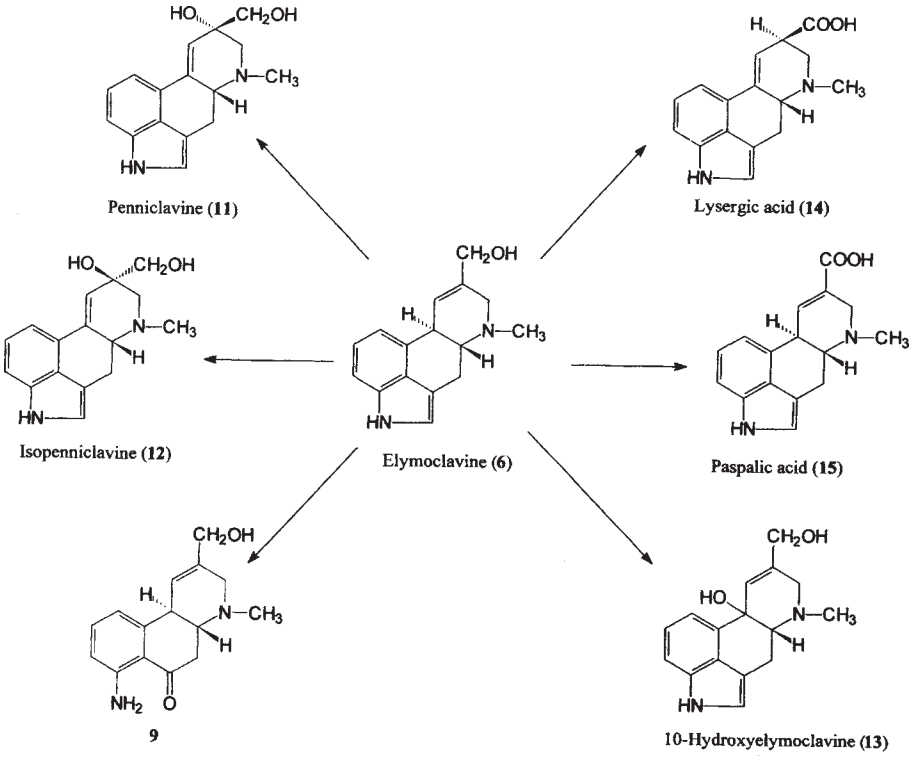


Figure 3 Typical bioconversions of elymoclavine

of penniclavine, isopenniclavine, lysergol (**20**) (Béliveau and Ramstad, 1966) and 10-hydroxyderivatives (Chan Lin *et al.,* 1967a, b) was frequently observed (Table 3).

Elymoclavine transformation was tested also with plant cell cultures as in the case of agroclavine (Table 2) (Ščigelová *et al.,* 1995). Mixture of penniclavine (**11**) and isopenniclavine (**12**) was obtained with *Atropa belladonna* and *Papaver somniferum* cultures. *Euphorbia calyptrata* culture revealed a strict stereoselectivity, yielding almost exclusively **11**. Penniclavine and isopenniclavine mixture produced by *A. belladonna* was accompanied with a polar substance not detected in other systems. The compound was isolated and identified as 10-hydroxyelymoclavine (**13**). This compound was suggested previously to be a tentative intermediate of peroxidase conversion of elymoclavine to penniclavine and isopenniclavine by plant homogenates (Chan Lin *et al.,* 1967a).

Biological reduction of elymoclavine leading to agroclavine was accomplished by several fungi (Yamatodani *et al.,* 1963; Abe, 1966; Abe *et al.,* 1963; Sebek, 1983). Oxidation of elymoclavine to lysergic acid and to its derivatives (**18, 19**) is practicable only by selected strains *C. purpurea* and *C. paspali* (Sebek, 1983; Maier *et al.,* 1988; Kim *et al.,* 1983; Mothes *et al.,* 1962; Philippi and Eich, 1984). Enzyme system responsible for this reaction is localised in microsomal fraction (Maier *et al.,* 1988; Kim *et al.,* 1983) and belongs obviously to the cytochrome P-450 family. Derivatives of elymoclavine are analogously converted by *C. paspali* to respective lysergic acid derivatives (Philippi and Eich, 1984). Biotransformations of elymoclavine leading to its glycosides are described separately.

9.2.3. Lysergol, Lysergene, and Lysergine

Lysergol (**21**), lysergine (**24**), and their isomers occur as minor alkaloids in the *Claviceps* cultures. Their biogenetic relations to other alkaloids remain still unclear. Most of lysergolns bioconversio were aimed at discovering their biogenetic relations to other alkaloids.

Lysergol and lysergine are oxidised by *C. fusiformis* by similar mechanism as elymoclavine to penniclavine and setoclavine, respectively (Eich and Sieben, 1985). However, lysergol is not converted by elymoclavine 17-oxygenase from *C. purpurea* to paspalic acid (Maier *et al.,* 1988). Lysergols were also enzymatically glycosylated—*vide infra*.

Lysergene (**23**) is the only ergot alkaloid containing an exo-methylene group. Owing to its conjugation with the $\Delta^{9,10}$-double bond, this molecule is rather susceptible to oxidation or addition. The compound can be prepared easily and in good yield from elymoclavine (Křen *et al.,* 1996b) that makes **23** a favourite starting material for various semisynthetic ergot alkaloids. Its biotransformation using plant cell culture of *Euphorbia calyptrata* gave a unique spirooxadimer that can be formally derived from a putative intermediate, 8, 17-epoxylysergene (**26**) produced probably by action of peroxidase.

Table 3 Elymoclavine bioconversions

Reaction	*Product*	*Conversion system (culture)*	*Reference*
8-Hydroxylation	Penniclavine	*Aspergillus fumigatus*, *Mucor corticolus* *CBS, *Rhizopus arrhizus* CBS, *Streptomyces rimosus* NRLL-2234, *S. scabies* ATCC-3352, *Streptomyces* sp. PCC	Béliveau and Ramstad (1966)
		Penicillium viridicatum	Tyler *et al.* (1965)
		Euphorbia calyptrata plant cell culture	Ščigelová *et al.* (1995)
		Ipomoea (leaves)	Gröger (1963)
8-Hydroxylation	Isopenniclavine	*Absidia spinosa* ATCC-6648, *Fusarium graminearum* PCC-140	Béliveau and Ramstad (1966)
8-Hydroxylation	Penniclavine + Isopenniclavine	*Colletatrislium graminicolum*, *Cunninghamela echinulata* CBS, *Giberela zeae* U11, *Helmithosporium victorie*, *Rhizopus circinans* CBS, *R. nigricans* PCC-199	Béliveau and Ramstad (1966)
		Streptomyces lipanii, *Fusarium niveum*, *Corticium sasakii*	Yamatodani *et al.* (1963), Abe (1966)
		Atropa belladonna, *Papaver somniferum* plant cell cultures	Ščigelová *et al.* (1995)
		Tomato homogenate, Potato sprouts homogenate, Morning glory seedlings homogenate	Taylor *et al.* (1966)
		Horse radish peroxidase	Chan Lin *et al.*, 1967a, b
10-Hydroxylation	10-Hydroxy-elymoclavine	Horse radish peroxidase	Chan Lin *et al.*, 1967a, b
		Atropa belladonna plant cell culture	Ščigelová *et al.* (1995)
8,9-Epoxidation	10-Hydroxy-8,9-epoxyelymoclavine	Horse radish peroxidase	Chan Lin *et al.* 1967a, b

Table 3 (*Continued*)

Reaction	*Product*	*Conversion system (culture)*	*Reference*
17-Oxidation	*Paspalic acid*	*Claviceps* spp.	Sebek (1983)
		Claviceps sp. PCCE1 (*purpurea?*) mycelial fraction	Kim *et al.* (1983)
17-Oxidation	Lysergic acid	*C. purpurea* PEPTY 695/S microsomal fraction	Maier *et al.* (1988)
		C. paspali SO 70/5/2	Philippi and Eich (1984)
17-OH-Reduction	Agroclavine	*Aspergillus fumigatus*, *Corticium sasakii*	Abe (1966), Abe *et al.* (1963), Sebek (1983)
8,9-Double bond isomerisation	Lysergol	*Beauveria bassiana* PCC-122, *Fusarium lini* ATCC-9593, *F. lycopersici* PCC-141, *F. roseum* PCC-142, *Mucor adventitius* CBS, *Monascus pitosus* IMUR-165, *Rhizopus chinensis* CBS, *Streptomyces parvus* NRRL-B-1456, *S. griseus* (3 strains)	Béliveau and Ramstad (1966)
Isomerisation	Lysergol + isolysergol	*Claviceps* spp.	Agurell and Ramstad (1962)
Isomerisation + hydroxylation	Lysergol + penniclavine + isopenniclavine	*Aspergillus chevalieri* NRRL-78, *A. fumigatus* (3 strains), *A. niger* ATCC-6277, *A. quadrilineatus* PCC-115, *Botryosporium* sp. PCC-284, *Ceratocystis ulmi*, *Fusarium graminearum* PCC-144, *Mucor corticolus* CBS, *M. flavus* PCC-256, *M. globosus* CBS, *Melanospora destruens* LSHTM	Béliveau and Ramstad (1966)

		BB-168, *Sclerotinia sclerotiorum*, *Nocardia convoluta* PCC-109, *Streptomyces rimosus* NRRL-2234, *S. rubrireticuli* NRRL-B-1484, *S. scabies* ATCC-3352	
17-Oxidation	Ergine	*C. paspali* LI 189+	Mothes *et al.* (1962)
17-Oxidation + derivatization	Ergotamine	*C. purpurea* (sclerotia)	Mothes *et al.* (1962), Winkler and Mothes (1962)
17-Oxidation Elymoclavine derivatives 1-Alkyl- 1-Benzyl- 1-Hydroxymethyl- 2-Bromo- 2,3-Dihydro- 6-Ethyl-6-nor-	Corresponding lysergic acid α-hydroxyethyl-amides	*C. paspali*	Philippi and Eich (1984)

*For the abbreviations of culture collections—see footnote of Table 1.

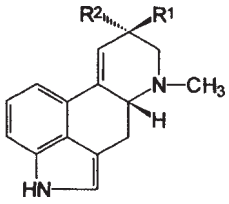


Figure 4 9, 10-Ergolenes, lysergic acid derivatives
(**14**) Lysergic acid R^1=COOH, R^2=H
(**16**) *iso*-Lysergic acid R^1=H, R^2=H
(**17**) Ergine R^1=$CONH_2$, R^2=H
(**18**) Erginine R^1=H, R^2=$CONH_2$
(**19**) Ergometrine R^1=$CONHCH(CH_3)CH_2OH$, R^2=H
(**20**) Lysergic acid α-hydroxyethylamide R^1=$CONHCH(CH_3)OH$, R^2=H
(**21**) Lysergol R^1=CH_2OH, R^2=H
(**22**) *iso*-Lysergol R^1=H, R^2=CH_2OH
(**23**) Lysergene $R^{1,2}$= =CH_2
(**24**) Lysergine R^1=CH_3, R^2=H
(**25**) 8-Hydroxyergine R^1=$CONH_2$, R^2=OH

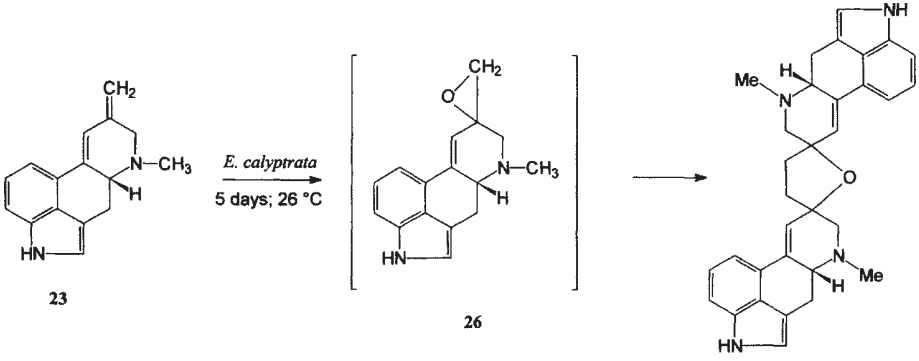


Figure 5 Dimerisation of lysergene by plant cell culture of *Euphorbia calyptrata*

Addition of this compound to **23** (presumably by radical mechanism) could then lead to the dimer **27** (Křen *et al.,* 1996b).

Lysergene was also converted by various strains *C. purpurea* to lysergol, isolysergol, penniclavine and isopenniclavine (Chan Lin *et al.,* 1967a; Agurell and Ramstad, 1962),

9.2.4. Chanoclavine

Chanoclavine-I (**28**) is a common precursor of most ergot alkaloids in the *Claviceps* genus and its conversion to agroclavine and elymoclavine by enzymatic

system of various *Claviceps* strains has been clearly proved (Ogunlana *et al.*, 1970; Erge *et al.*, 1973; Sajdl and Řeháček, 1975). Ogunlana *et al.* (1969) reported cyclisation of chanoclavine-I to elymoclavine as a sole product by pigeon-liver acetone-powder (+ATP+Mg^{2+}). This is probably the only report referring to this reaction in a system of *non-Claviceps* origin.

The conversion of exogenous chanoclavine by intact mycelia of *C. fusiformis* strain W1 gave chanoclavine-I aldehyde, elymoclavine and agroclavine. However, comparing with analogous agroclavine biotransformation in the same system, the conversion proceeded slowly due to, presumably low, transport rate of chanoclavine into cells. More polar alkaloids like chanoclavine and elymoclavine enter cells in a lower rate than less polar agroclavine. Beside these products also mono- and difructoside of chanoclavine were identified (Flieger *et al.*, 1989). These glycosides are formed by the action of *C. fusiformis* ß-fructofuranosidase using as a ß-fructofuranosyl donor sucrose (Figure 8).

Suspension plant cell cultures of *Euphorbia calyptrata, Atropa belladona, Armoracia rusticana,* and *Solanum aviculare* were tested for biotransformation of chanoclavine (**28**). All tested cultures produced similar spectrum of biotransformation products from **28** (Figure 6). *Euphorbia calyptrata* gave the highest yields and, therefore, it was chosen for the preparatory purposes (Křen *et al.*, 1996a). Observed oxidative biotransformation of chanoclavine mimics the synthetic process in the reversed order. It also probably demonstrates typical biooxidative pattern of secoclavines by peroxidases.

8,9-Dihydrochanoclavine and isochanoclavine are converted by *Claviceps* sp. SD-58 to festuclavine and pyroclavine, respectively (Johne *et al.*, 1972).

9.2.5. Semisynthetic Clavine Alkaloids

Recent interest in lergotrile (**33**) (Figure 7) stems from its putative dopaminergic activity and inhibition of prolactin secretion. Toxicity in clinical trials prompted the exploration of microbial transformation for producing less toxic derivatives.

Davis *et al.* (1979) screened nearly 40 organisms for their ability to produce metabolites of lergotrile. Five microorganisms (*Cunninghamela echinulata* UI 3655, *Streptomyces rimosus* ATCC 23955, *S. platensis* NRRL 2364, *S. spectabilis* UI-C632, *S. flocculus* ATCC 25435) biotransformed lergotrile (**33**) to nor-lergotril (**34**) by *N*-demethylation. *S. platensis* exhibited complete conversion, and preparative-scale incubation was accomplished with an isolated yield of 50%. Additional organisms have been screened for their ability to produce hydroxylated metabolites of lergotrile (**35, 36**) that has been found in humans and in mammals but these efforts have been unsuccessful (Smith and Rosazza, 1982). Microbial *N*-demethylation is important because chemical demethylation of the compounds like lergotrile is rather difficult (Smith and Rosazza, 1982). In guinea pig liver, lergotrile is demethylated, and hydroxylated on C-13 and on nitrile group (Parli and Smith, 1975).

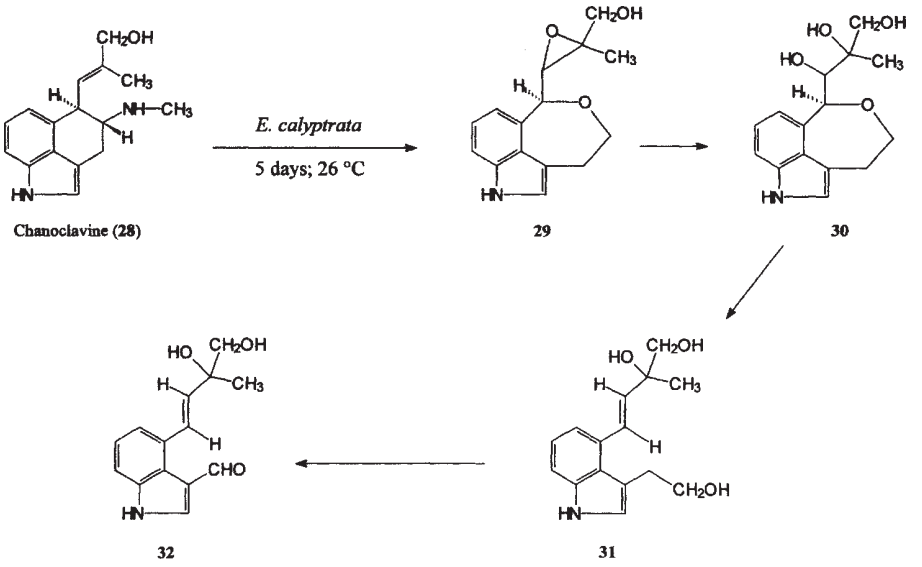


Figure 6 Oxidative sequential biotransformation of chanoclavine by plant cell culture of *Euphorbia calyptrata*

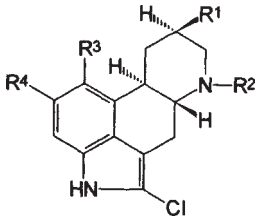


Figure 7 Semisynthetic ergot drugs and their metabolites
(**33**) Lergotrile R^1=CH_2CN, R^2=CH_3, R^3=R^4=H
(**34**) Nor-lergotrile R^1=CH_2CN, R^2=R^3=R^4=H
(**35**) 12-Hydroxylergotrile R^1=CH_2CN, R^2=CH_3, R^3=OH, R^4=H
(**36**) 13-Hydroxylergotrile R^1=CH_2CN, R^2=CH_3, R^3=H, R^4=OH

9.2.6. Ergot Alkaloid Glycosylations

Ergot alkaloid fructosylation

Glycosides of ergot alkaloids were isolated as naturally occurring products (Floss *et al.*, 1967) and recently large series of them was prepared by chemical and enzymatic methods (Křen, 1997). Their promising physiological effects stimulate future research in this field. Most of the work with the enzymatic ergot alkaloid glycosylations has been performed with clavine alkaloids.

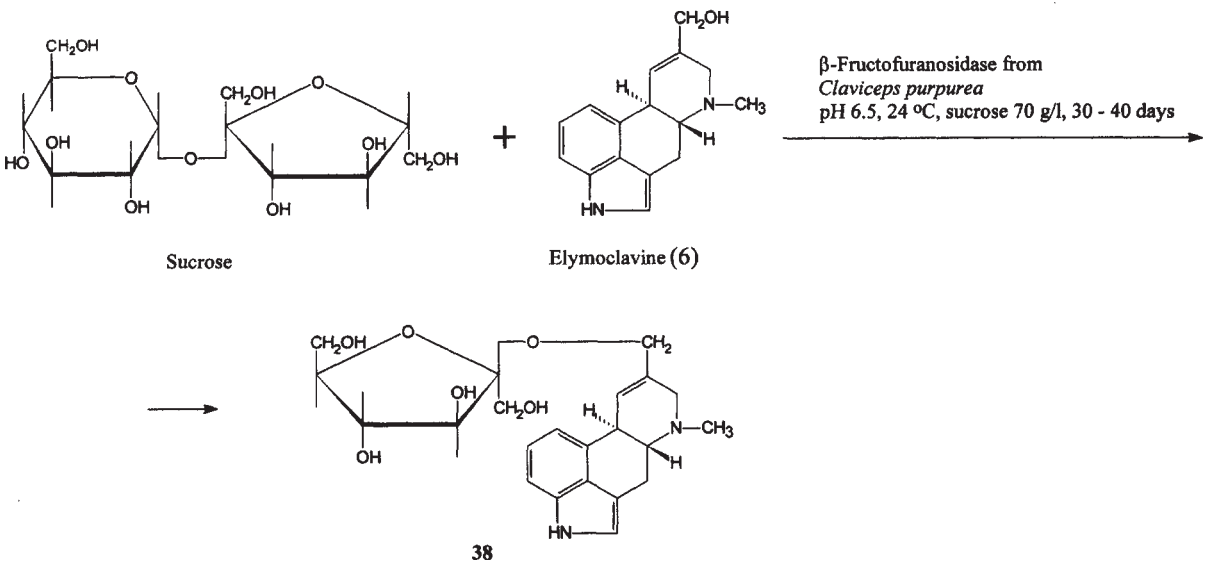


Figure 8 *ß*-Fructosylation of elymoclavine by *Claviceps fusiformis*

Fructosylation of elymoclavine by *C. purpurea* was described (Křen *et al.*, 1990). This reaction is mediated by transfructosylating activity of *ß*-fructofuranosidase in sucrose containing media. Beside mono- (38) and difructoside (Figure 8), probably higher fructosides (tri- and tetra-) (39) are formed (Figure 9) (Havlíček *et al.*, 1994).

The reaction is strongly dependent on pH (optimum 6.5) and substrate concentration (optimum 75 g sucrose/1). Similar reaction occurs with chanoclavine (Flieger *et al.*, 1989) and also with other clavines bearing primary OH group (lysergols). This bioconversion can be effectively performed in a culture of *C. purpurea* with alkaloid production selectively blocked by 5-fluorotryptophan (Křen *et al.*, 1993). The bioconversions of ergot alkaloids by *Claviceps* strains are often complicated by production of alkaloids *de novo* that might compete with added "xeno" alkaloids and make the mixture after conversion rather complex. Strains *C. purpurea* with glycosylation activity produce normally high amount of elymoclavine that is glycosylated at a high rate and thus competes with added lysergoles. 5-Fluorotryptophane blocks the alkaloid production at the first reaction—dimethylallyltryptophan synthase and consequent steps remain active (Pazoutová *et al.*, 1990). The growth and differentiation of the culture was not impaired, so the bioconversion proceeds in "physiologically" normal culture and without any endogenous alkaloid production.

Glycosylating *Claviceps* strains (mostly *C. fusiformis*) produce most of elymoclavine in the form of *ß*-fructofuranosides that complicates isolation of elymoclavine. Hydrolysis of fructosides by HCl is not suitable for a large scale process due to aggression of the acid solution and losses of elymoclavine. A more elegant method is a bioconversion employing high invertase activity of *Saccharomyces cerevisiae*. At the end of the production cultivation, a suspension of baker's yeast is added to the fermentation broth (without the product isolation). The hydrolysis is completed within 1 hour (37°C) (Křen *et al.*, 1990).

For high submerged production of elymoclavine it is advantageous to screen for the strains having high transfructosylation activity. Most of the elymoclavine produced is fructosylated (extracellulary) and due to higher polarity it does not re-enter the cell avoiding thus the feedback inhibition of agroclavine oxygenase (cytochrome P-450). Fructosylated elymoclavine can be eventually hydrolysed to its aglycone by the above method.

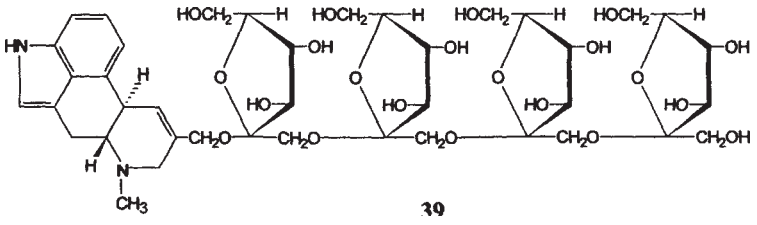


Figure 9 Elymoclavine tetrafructoside

Ergot alkaloid glycosylation using glycosidases

Interesting physiological effects of alkaloid fructosides (Křen, 1997) stimulated preparation of other glycosides, as e.g., galactosides, glucosides, *N*-glucosaminides and complex alkaloid glycosides.

ß-Galactosylation of elymoclavine (**6**), chanoclavine (**28**), lysergol (**21**), 9, 10dihydrolysergol, and ergometrine (**19**) was accomplished by *ß*-galactosidase from *Aspergillus oryzae* using *p*-nitrophenyl-*ß*-D-galactopyranoside or lactose as a glycosyl donors. Transglycosylation yields ranged from 13 to 40% (Křen *et al.,* 1992). This enzymatic method enabled for the first time to glycosylate ergometrine bearing in the molecule amidic bond.

Aminosugar-bearing alkaloids had been expected to have immunomodulatory activities and also this glycosylation would create basis for further extension of carbohydrate chain (introduction of LacNAc or sialyl residue—*vide infra*).

This task was accomplished by tranglycosylation using *ß*-hexosaminidase from *A. oryzae*. Representatives of each class of ergot alkaloids, e.g. clavines—elymoclavine (**6**), secoclavines—chanoclavine (**28**) and lysergic acid derivatives—ergometrine (**19**) were chosen to demonstrate the wide applicability of this method. As a donor *p*-nitrophenyl-*ß*-*N*-acetylglucosaminide or -galactosaminide were used, the yields ranged from 5 to 15% (Křen *et al.,* 1994a).

Enzymatic mannosylation of the alkaloids by α-mannosidase from *Canavalia ensiformis* (Jack beans) was accomplished by two different strategies—transglycosylation using *p*-nitrophenyl-α-D-mannopyranoside or reversed glycosylation using high concentration of mannose (Ščigelová *et al.,* 1994). In the case of chanoclavine (**28**) higher yield of respective α-mannoside (**30**) was obtained in shorter time by using of transglycosylation concept (Figure 10). Lower yields in reversed glycosylation are, however, compensated by considerably cheaper mannosyl donor (mannose). Unreacted aglycone can be nearly quantitatively recuperated.

Series of other glycosides of ergot alkaloids was prepared by enzymatic transglycosylations using activated *p*-nitrophenylglycosides as donors. *ß*-Glucosides of, e.g., elymoclavine and dihydrolysergol, were prepared by *ß*-glucosidase from *Aspergillus oryzae* (Kčen *et al.,* 1996c), α-glucosides of e.g., elymoclavine and chanoclavine were prepared by α-glucosidase from *Bacillus stearothermophilus* or by α-glucosidase from rice, α-galactosides of the same alkaloids were prepared by α-galactosidase from *Coffea arabica* (green coffee beans) or that one from *A. niger*.

Ergot alkaloid glycosylations by glycosyltransferases—complex glycosides

Complex alkaloid glycosides bearing e.g., lactosyl (Lac), lactosaminidyl (LacNAc) or sialyl (Neu5Ac) moieties were required for immunomodulation tests *(vide infra)*. Because of paucity of starting material (alkaloid monoglycosides) and because of the need of regioselective glycosylation only enzymatic reactions were practicable.

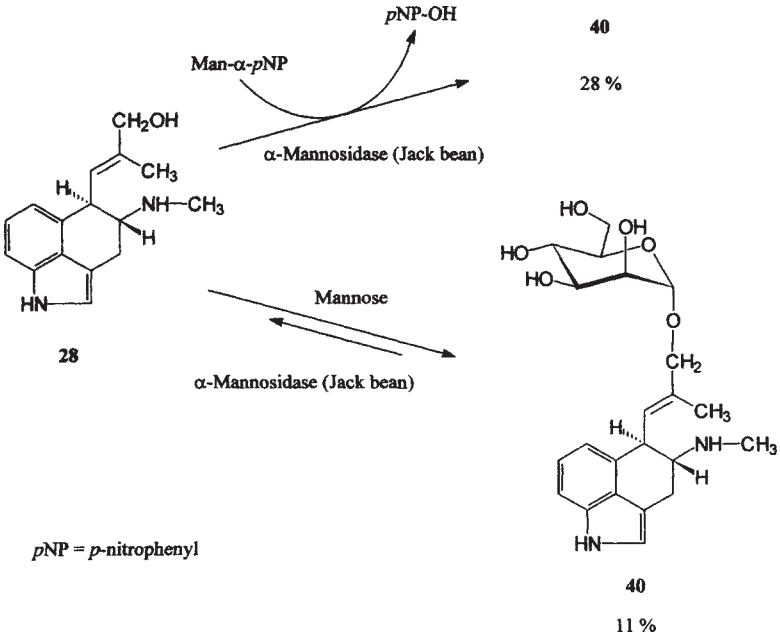


Figure 10 Mannosylation of chanoclavine with α-mannosidase from jack bean: transglycosylation using *p*-nitrophenyl-α-mannoside and reversed mannosylation using high concentration of free mannose.

For preparation of D-galactopyranosyl (1→4)-2-acetamido-2-deoxy-ß-Dglucopyranosyl-(l→O)-elymoclavine (**32**) the extension of previously prepared 2-acetamido-2-deoxy-ß-D-glucopyranosyl-(1→O)-elymoclavine (**31**) by the use of bovine ß-1, 4-galactosyltransferase was chosen. Uridine 5´-diphosphogalactose (UDP-Gal) served as a substrate (Křen *et at.*, 1994b).

ß-Lactosyl elymoclavine (**32**) was prepared from respective ß-glucoside by the use of bovine ß-1, 4-galactosyltranferase in présence of α-lactalbumine. Analogously, ß-Lac and ß-LacNAc derivatives of other ergot alkaloids, e.g., 9, 10-dihydrolysergol were prepared (Křen *et al.*, 1994b).

Attachment of 5-*N*-acetylneuraminic acid to ß-LacNAc-elymoclavine (**32**) yielding 33 was accomplished by the use of α-2, 6 sialyltransferase from rat liver (Figure 11) (Křen *et al.*, 1994b).

9.3. BIOCONVERSIONS OF LYSERGIC ACID DERIVATIVES

9.3.1. Hydrolysis of Lysergic Acid Amides

This group includes simple lysergic acid amides and peptide ergot alkaloids. Most of these substances are directly used in pharmaceutical preparations. However, reports referring to their biotransformation are scarce.

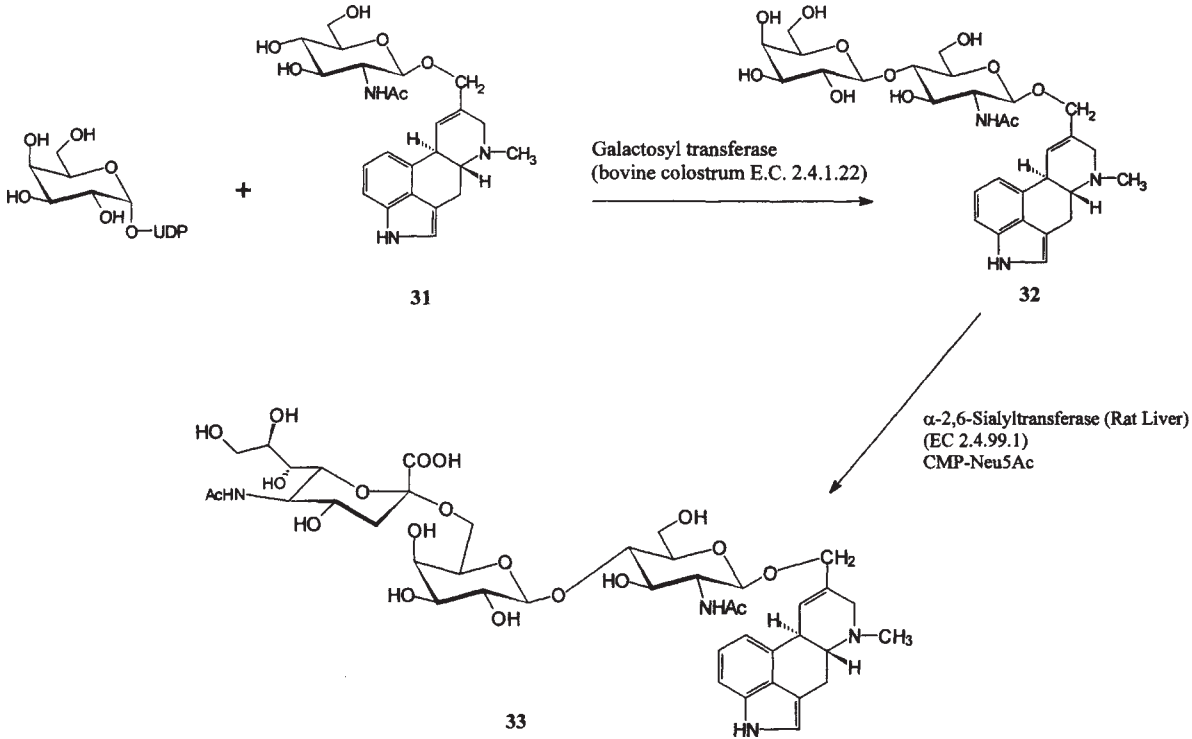


Figure 11 Preparation of complex ergot alkaloid glycosides using glycosyltransferases

One of the most desired transformations is a hydrolysis of above substances resulting in free lysergic acid, the substrate for many semisynthetic preparations. Chemical hydrolysis of its derivatives gives low yields (50–65%). Fermentative production of lysergic acid is somehow complicated. Enzymatic hydrolysis of peptide alkaloids is still impracticable. Common proteolytic enzymes (papain, subtilisin, chymotrypsin, termolysin) do not attack peptidic bond in ergokryptine (**34**) probably due to the steric reasons (Křen—unpublished results).

Amici *et al.* (1964) reports the hydrolysis of ergine (**17**) and erginine (**18**) by a strain *C. purpurea* yielding 80–90% lysergic acid. The bioreaction is aerobic, cultivation medium was supplemented by ergine dissolved in organic solvent up to final cone. 1000 mg/1.

Ergotoxine producing strain *C. purpurea* is able to split lysergyl-amino acidmethyl esters into corresponding lysergyl-amino acids as well as into lysergic acid and alanine and valine (Maier *et al.*, 1974). This reaction proceeds inside the cells, so the transport is involved. Ergotoxine producing strains are more active than the nonproducing strains. Also lysergyl-oligopeptides (*d*-lysergyl-L-valyl-L-leucine-OMe, *d*-lysergyl-L-valyl-L-valine-OMe and *d*-lysergyl-L-valyl-L-proline-OMe) are split into their components after feeding to the mycelium of *C. purpurea* Pepty 695 (ergotoxine producing) (Maier *et al.*, 1978).

9.3.2. Oxidation of Lysergic Acid Derivatives

Ergotamine (**36**) and ergometrine (**19**) are partially isomerised on C-8 (but not hydroxylated) to ergotaminine and ergometrinine by *Psilocybe semperviva* and partially decomposed (Brack *et al.*, 1962).

Lysergic acid derivatives can be oxidised, analogously to most clavine alkaloids, on C-8 due to action of peroxidases. 8α-Hydroxy-α-ergokryptine (**35**) has been found in α-ergokryptine (**34**) producing *C. purpurea* strain (Cvak *et al.*, 1997). Oxidation of exogenous ergine (**17**) to 8-hydroxyergine (**25**) by *C. paspali* MG-6 was accomplished by Bumbová-Linhartová *et al.* (1991).

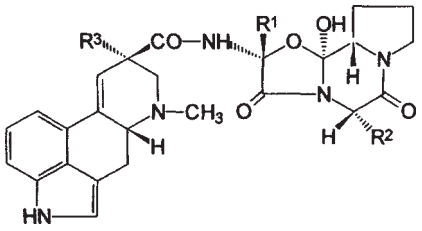


Figure 12 Peptide ergot alkaloids and their biotransformation products
(**34**) α-Ergokryptine R^1=$CH(CH_3)_2$, R^2=$CH_2CH(CH_3)_2$, R^3=H
(**35**) 8α-Hydroxy-α-ergokryptine R^1=$CH(CH_3)_2$, R2=$CH_2CH(CH_3)_2$, R^3=O
(**36**) Ergotamine R^1=CH_3, R^2=$CH_2C_6H_5$, R^3=H
(**36a**) 8α-Hydroxyergotamine R^1=CH_3, R^2=$CH_2C_6H_5$, R^3=OH

9.3.3. Biotransformations of LSD and its Homologues

Lysergic acid amides, most notably lysergic acid diethylamide (LSD) (**37**), are of interest because of their hallucinogenic activity. Microbial metabolites of LSD might serve as more active medical preparations. Another biotransformation products correspond to LSD metabolites found in humans. Both these facts led Ishii *et al.* (1979a,b; 1980) to an extensive study of microbial bioconversions of the LSD and its derivatives.

Initial studies with LSD showed that many cultures were capable of attacking the *N*-6 and amide *N*-alkyl substituents (Ishii *et al.*, 1979a,b; 1980). *Streptomyces lavendulae* IFM 1031 demethylated only the *N*-6 position yielding nor-LSD. Conversely, *Streptomyces roseochromogenes* IFM 1081 attacked only the *N*-amide alkyl group to yield lysergic acid ethylamide (**38**), lysergic acid ethylvinylamide (**39**), and lysergic acid ethyl 2-hydroxyethylamide (**40**) (Figure 13). Other *Streptomycetes* and *Cunninghamella* strains produced all four metabolites. The high degree of substrate stereoselectivity in *Streptomyces roseochromogenes* was proved by the fact that this organism could not metabolise *iso*-LSD while, in contrary, *S. lavendulae* yielded *iso*-nor-LSD.

C-17-Amide dealkylations were examined in greater detail using a series of lower and higher alkyl homologues of LSD (Figure 14). The following results were observed: Lysergic acid dimethylamide (**41**) was only dealkylated to the monomethylamide (**42**), lysergic acid diethylamide (**37**) was also dealkylated to yield monoethyiamide (**38**), and other metabolites like ethylvinylamide (**39**) and ethylhydroxyethylamide (**40**). Neither lysergic acid di-*n*-propylamide (**43**) nor lysergic acid di-*n*-butylamide (**47**) were dealkylated, but rather yielded two epimeric alcohols resulting from ω-1 hydroxylation, i.e. (**44**) and (**46**) from (**43**), and (**48**) and (**50**) from (**47**), as well as the further oxidation products, ketones (**45**) and (**49**), respectively. Based on these results, the authors proposed that the chain length regulates the site of oxygenation, with ω-1 hydroxylation occurring, if possible. The dimethyl-derivative (**41**) has no ω-1 position, thus C-α-(methyl)hydroxylation yields the carbinolamide with resulting *N*-demethylation.

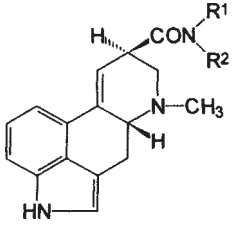


Figure 13 LSD and its metabolites
(**37**) Lysergic acid diethylamide (LSD) $R^1=R^2=CH_2CH_3$
(**38**) Lysergic acid ethylamide R1=H, $R^2=CH_2CH_3$
(**39**) Lysergic acid ethylvinylamide $R^1=CH_2CH_3$, $R^2=CH=CH_2$
(**40**) Lysergic acid ethyl 2-hydroxyethylamide $R^1=CH_2CH_3$, $R^2=CH_2CH_2OH$

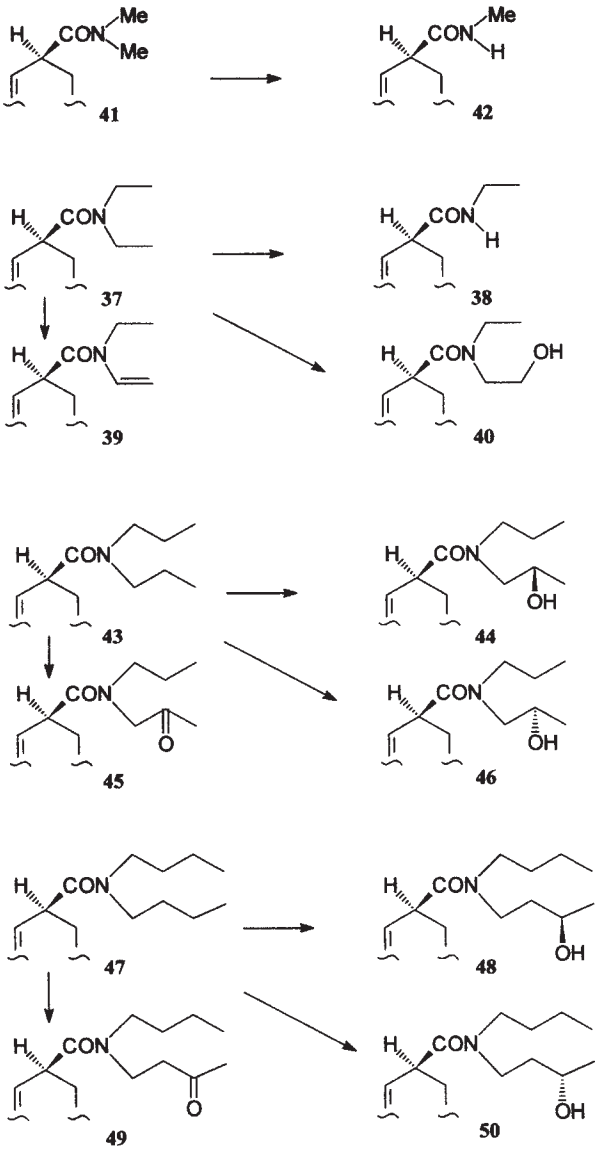


Figure 14 Metabolism of lysergic acid dialkylamide homologues by *Streptomyces roseochromogenes*

These studies resulted in a proposed active site for the hydroxylase (*N*-dealkylase) of *S. roseocbromogenes* that accounts for the general mode of metabolism of the homologues. Authors also use the diagram to explain the stereochemical control of the system, based on the observation that one epimeric

alcohol predominates in the hydroxylation of (**43**) or (**47**). This argument is based on a favoured binding of one alkyl group over the other, implying non-equivalence of two alkyl groups (Ishii *et al.,* 1979b; Davis, 1984). Microsomes from mammalian liver (+NADPH+O_2) oxidise LSD to 2-hydroxyLSD (Axelrod *et al.,* 1956).

9.4. BIOCONVERSION OF ERGOT ALKALOIDS AS A TOOL FOR STUDY OF THEIR METABOLISM IN MAMMALS

Elucidation of mammalian metabolic pathways is important in attempting to rationalise detoxification, and to evaluate potentially active metabolites. In case of some ergot alkaloids with strong hallucinogenic activity (e.g. LSD) it is desirable to detect its metabolites for use in anti-doping screening and forensic chemistry. However, such studies have been hampered by a lack of minor metabolite availability. For this reason, Ishii *et al.* (1979a, b, 1980) examined a series of microorganisms and animals for parallel routes of metabolism of LSD and of the related compounds. They assumed that the metabolism of these xenobiotics would proceed in a similar manner in both mammals and microorganisms (Ishii *et al.,* 1980). Both nor-LSD and lysergic acid ethylamide produced by the microbial conversion of LSD are known metabolites of LSD in mammals, and the authors were able to use the lysergic acid ethylvinyl amide generated in the microbial studies to determine its présence in mammals.

This work has allowed a high degree of predictability regarding the *N*-(amide) -alkyl oxidation of lysergic acid amide derivatives using *Streptomyces roseochromogenes* (see above). Analysis of a series of homologues from lysergic acid dimethylamide through lysergic acid dibutylamide resulted in a proposal for the enzyme active site, as well as rationalisation of the resulting metabolites. This study also enabled preparation of the minor metabolites to be made for further investigation.

Lergotrile (**33**) (see above) was hydroxylated at various positions in mammal systems (C-13, C-12, hydrolysis of nitrile group) and demethylated at *N*-6 (Smith and Rozassa, 1982; Axelrod *et al.,* 1956). To find a parallel for this reaction and to prepare some standards of the metabolites more than 30 microorganisms have been screened for their hydroxylation ability (Davis *et al.,* 1979). Only *N*-6 demethylation activity has been found in *Streptomyces platensis.*

Microbial systems parallel mammalian metabolism with an other dopaminergic ergoline, e.g., pergolide (**53a**) (Figure 16). Metabolism in mammals centres on the methyl sulfide moiety, which is sequentially oxidised to the sulfoxide (**53b**) and the sulfone (**53c**)—*vide infra*. Similarly, *Aspergillus alliaceus* UI-315 catalysed the same sequential oxidative transformation. In contrast, *Helminthosporium* sp. NRRL 4761 stops at the sulfoxide stage and also catalyses reduction of the sulfoxide back to pergolide (Davis, 1984). No stereoselectivity in sulfoxide formation was observed, in contrast to the high degree of product

stereoselectivity often observed in this microbial-type reactions (Davis, 1984; Auret *et al.,* 1981).

9.4.1. Metabolism of the Therapeutically Used Ergot Alkaloids in Human Organism

Ergot alkaloids undergo many metabolic changes in human organism. The study of their metabolites is important both from pharmacodynamic and pharmacokinetic views. Liver is probably the most active organ in ergot alkaloid detoxification. Oxidative and conjugation systems in liver catalyse many reaction analogous to those discussed in previous parts (e.g., cytochrome P-450, oxygenases, peroxidases, glucuronyltransferases, etc.). Typical degradative reactions are *N*-demethylation, alkylamide dealkylations, hydroxylations (oxidative), epimerization at C-8, oxidation at C-2 followed by oxidative ring B opening and *N*-6 oxidation. The complex knowledge of the "weak" positions in alkaloid structures enables predicting of possible metabolic transformations of respective ergot alkaloid derivative.

Nicergoline

Nicergoline (**50**) used as cerebrovascular dilatans with strong α-adrenolytic activity (see the chapter 14 in this book) is metabolised in human organism into two products (Arcamone *et al.,* 1971; Arcamone *et al.,* 1972; Gabor *et al.,* 1995). The hydrolysis yields 5-bromonicotinic acid and 1-methyl-10α-methoxydihydrolysergol (**51**) which is further demethylated via 1-hydrohymethylderivative **51a** into 10α-methoxydihydrolysergol (**52**) (Banno *et al.,* 1991) (Figure 15). The metabolite **51** can be, due to its free hydroxyl group, a subject of further *ß*-glucuronidation.

Pergolide

Pergolide (**53a**) is one of the strongest dopamine agonists and prolactin inhibitors. It is metabolised into four products resulting from oxidation and *N*-6 dealkylation (Clemens *et al.,* 1993; Kerr *et al.,* 1981). Pergolide sulfoxide (**53b**)

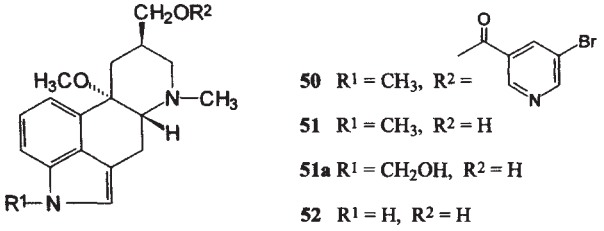
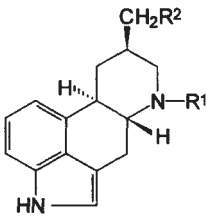


Figure 15 Nicergoline (**50**) and its metabolites



	R1	R2
53a	C_3H_7	SCH_3
53b	C_3H_7	$SOCH_3$
53c	C_3H_7	SO_2CH_3
53d	H	SCH_3
53e	H	$SOCH_3$

Figure 16 Pergolide (53a) and its metabolites

and pergolide sulfone (**53c**) are formed by the oxidation of **53a.** Dealkylation of **53a** yields in despropylpergolide (**53d**) and analogously metabolite **53b** is dealkylated into despropylpergolidesulfoxid (**53e**) (Figure 16).

2-Bromo-ß-ergokryptine

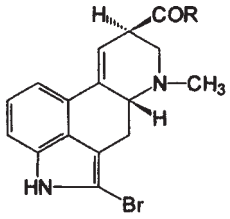
2-Bromo-*ß*-ergokryptine (**54a**) is very strong dopaminergic agonist and inhibits secretion of pituitary hormones, e.g., prolactine, somatotropic hormone (growth hormone) and ACTH (adrenocorticotropic hormone). It is, therefore, used for the treatment of disorders associated with the pathological overproduction of these hormones, as, e.g., hyperprolactinemia, acromegaly and Parkinson's disease. Metabolism of **54a** was studied in rats, and from their bile 18 metabolites were isolated (Maurer *et al.,* 1982) (Figure 17). From their structure following metabolic changes can be expected:

— epimerisation at C-8 of lysergyl moiety is quite common in its derivatives and can be also spontaneous. Therefore, occurrence of metabolites **54b** (bromokryptinine), 2-bromoisolysergic acid (**54g**) and its amide (**54c**) need not to be a result of the enzymatic processes.
— oxidation in the position 8′ of proline fragment of the peptide part is non-stereoselective and yields a mixture of four isomers at C-8 and C-8′ (**54j-m**).
— further oxidation of 8′-hydroxylated isomers leads either to introduction of second OH-group at the position 9′ and followed by their glucuronidation (**54q-s**). Non-conjugated 8′, 9′ dihydroxyderivative can undergo rearrangement leading to opening of proline ring (**54e**).

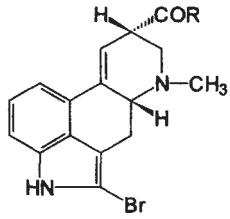
Occurrence of the above metabolites was also monitored in human urine. As the main metabolites 2-bromolysergic acid and its 8-epimer and the respective amides were found (**54c, d, g, h**) (Maurer *et al.,* 1983). It is interesting that oxidative attack did not start at ergoline ring, especially in pos. 2 or 3. Bromination probably improves the drug stability.

Terguride and lisuride

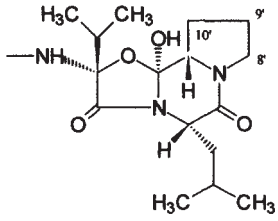
Terguride (**55**) and lisuride (**56**) are structurally very similar ergoline derivatives with urea side chain (Figure 18). Lisuride is selective D_2-agonist and together

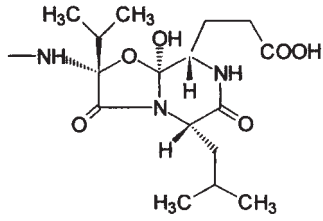


Type A (8β COR)



Type B (8α COR)

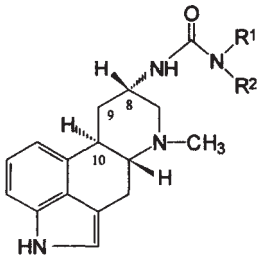




R: X Y

Metabolite	Type	R	Other substituents
54a	A	X	
54b	B	X	
54c	B	NH_2	
54d	A	NH_2	
54e	B	Y	
54f	A	Y	
54g	B	OH	
54h	A	OH	
54i	A	X	8′-oxo, 10′-hydroxy
54j	A	X	8′α-OH
54k	A	X	8′β-OH
54l	B	X	8′α-OH
54m	B	X	8′β-OH
54n	B	X	8′,9′-didehydro
54o	A	X	8′β-*O*-glucuronide
54p	B	X	8′β-*O*-glucuronide
54q	A	X	8′α-OH, 9′β-*O*-glucuronide
54r	B	X	8′α-OH, 9′β-*O*-glucuronide
54s	B	X	8′β-*O*-glucuronide, 9′β-OH

Figure 17 Bromokryptine (**54a**) and its metabolites



55a R^1 = Et, R^2 = Et
55b R^1 = H, R^2 = Et
55c R^1 = H, R^2 = H
56a 8,9-didehydro, R^1 = Et, R^2 = Et
56b 8,9-didehydro, R^1 = H, R^2 = Et
56c 8,9-didehydro, R^1 = H, R^2 = H

Figure 18 Terguride (**55a**) and lisuride (**56a**) metabolites

with its transdihydroderivative terguride, having partial agonistic activity towards CNS-dopamine receptor, they are both used as prolactin inhibiting drugs. They are extensively metabolised in mammal organisms. The studies of their biotransformation both *in vivo* and *in vitro* (Gieschen *et al.*, 1994, Hümpel *et al.*, 1989) revealed that the main metabolic changes are *N*-dealkylations at urea moiety leading to, e.g., deethylterguride (**55b**) and deethyllysuride (**56b**). Monodealkylation is catalysed by human cyt P450 2D6 (Rauschenbach *et al.*, 1997). Further dealkylation yielded dideethylderivatives (**55c, 56c**) (Hümpel *et al.*, 1989). These reactions resemble to those of LSD metabolism—*vide supra*.

Analogous metabolic changes, e.g., side chain dealkylation undergoes semisynthetic alkaloid CQA 206–291 (*N,N*-diethyl-*N′*-(1-ethyl-6-methylergoline-8α-*yl*)-sulfamide that is effective against Parkinson's disease. Human liver microsomes rich in cytochrome P-450 deethylated this compound exclusively to *N*-monodeethylated metabolite (Ball *et al.*, 1992).

Proterguride

Proterguride (**57a**) is a new ergoline derivative with a strong agonistic activity towards dopamine receptors reducing thus prolactine levels. *In vitro* studies revealed similar metabolic changes analogous to terguride, e.g., *N*-dealkylation yielding **57b** (Figure 19). Further oxidative changes lead to *N*-6 oxide (**57c**) and oxidative degradation of indole ring starting with 2-oxoderivative (**57d**) *via* 3-hydroxy-2-oxoderivative (**57e**). **57e** is further oxidised to 2-oxoderivative which is unique by the full aromatisation of C-ring. Another oxidative degradation of **57e** yields despyrrole derivative (**57f**) (Krause *et al.*, 1993). This pathway, reminding kynurenine pathway of tryptophan degradation, has its parallel in the oxo-derivatives of agroclavine and elymoclavine (**8, 9**) (Figures 2, 3) obtained mostly by peroxidase action—*vide supra*.

Cabergoline

Cabergoline (**58a**) is another modern prolactine inhibitor with prolonged effect (Figure 20). This drug is metabolised in human organism similarly as the above

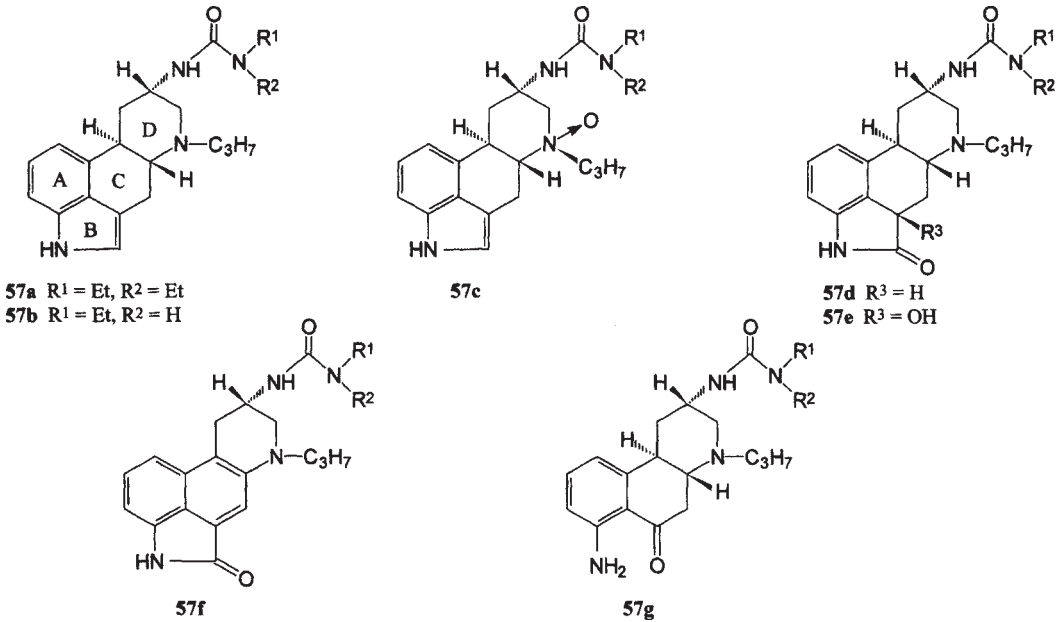


Figure 19 Proterguride (57a) and its metabolites

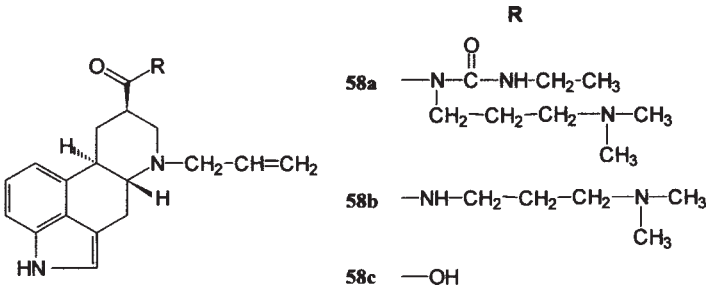


Figure 20 Cabergoline (**58a**) and its metabolites

compounds undergoing, primarily dealkylation (**58b**) (minor metabolite). Dealkylated metabolites are finally hydrolysed giving dihydrolysergic acid derivative (**58c**) (major metabolite) found in urine (Cocchiara and Benedetti, 1992).

Dihydroergotamine

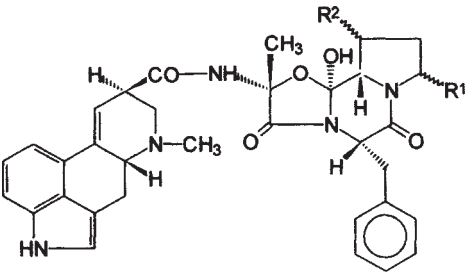
Dihydroergotamine (**59a**) has been used for a long time for a treatment of migraine and orthostatic hypotension. This compound is oxidised in human organism analogously as bromokryptine at 8´-position of the proline part of cyclopeptide moiety to the mixture of hydroxyderivatives (**59b**). Also dihydrolysergic acid and its amide (dihydroergine) are produced in large quantities (Maurer and Frick, 1984) (Figure 21).

In vitro transformations produced further oxidative products, e.g., dihydroxyderivative **59c** and derivative **59d** arisen by oxidative opening of the proline ring. Glucuronidation of dihydroxyderivatives could be also expected as in the case of bromokryptine. Oxidative attack to the pyrrole moiety results in its opening. Metabolites **59e, f** are obvious intermediates of the next oxidative product found, e.g., in the case of proterguride metabolism (**57g**).

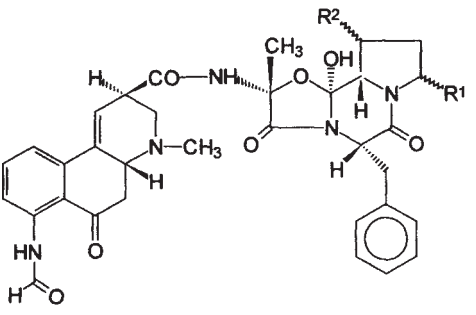
Other peptide ergot alkaloids and their dihydroderivatives are metabolised analogously as bromokryptine or dihydroergotamine. Human cytochrome *P*450s 3A exhibits high affinity to the cyclopeptide moiety of ergopeptines catalysing its hydroxylation (Peyronneau *et al.,* 1994). Metabolism of ergopeptines is inhibited by erythromycin and oleandromycin and stimulated by co-administration of dexamethasone that is *P*450S 3A inducer (Peyronneau *et al.,* 1994).

6-Methyl-8ß-(2, 4-dioxo-1-imidazolidinylmethyl) ergoline

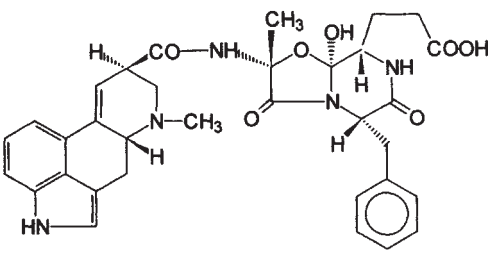
This compound belongs to the intensively studied α_1-adrenoreceptors and S_2-receptors blockers. It is probably first ergoline derivative influencing the



59a R^1= H, R^2 = H
59b R^1 = OH, R^2 = H
59c R^1 = OH, R^2 = OH



59e R^1 = OH, R^2 = H
59f R^1 = OH, R^2 = OH



59d

Figure 21 Dihydroergotamine (**59a**) and its metabolites

cardiovascular system without substantial side effects (Salvati *et al.*, 1989) observed in its analogues. Metabolic studies with it demonstrated that this compound is excreted almost unchanged. Its *N*-6 oxide is probably reduced in the organism back to the original drug. Small quantities of *N*-6 demethyl derivative are formed as well.

9.5. PROSPECTS TO THE FUTURE

Production of most desired ergot substances, e.g. elymoclavine, paspalic acid and lysergol, used mainly for semisynthetic ergot preparatives, is rather complicated. Bioconversion of their precursors may improve the effectivity of their preparation.

The most important ergot alkaloid bioconversion from industrial point of view is ergoline oxidation at C-17. The precursors in the lower oxidation degree, i.e., agroclavine and chanoclavine, can be produced in considerably high amounts. Elymoclavine acts as a feedback inhibitor of its production in *Claviceps,* so its yield in the batch fermentation is limited. Another highly desired reaction is the bioconversion of clavine alkaloids to paspalic acid. Both these biooxidations seem to be performed only by organisms within *Claviceps* genus. Although some work on these problems has already been done, both processes should be amended and adopted for large scale application.

In some cases, lysergic acid is produced by alkaline hydrolysis of peptide ergot alkaloids or lysergic acid derivatives. Harsh conditions of this chemical reaction cause a drop in the yields due to decomposition of ergoline skeleton. This could be another challenge for a further search for bioconversion methods.

Immobilised cells are applicable for most bioconversions. Mixed cultures, either free or immobilised, could simplify performance of these bioreactions. However, meeting different nutritional requirements of mixed cultures is a problem that must be overcome.

Ergot alkaloids may be also industrial pollutant. Their biodegradation has been, therefore, addressed as well. Various yeasts were tested for reduction of ergot alkaloid in industrial wastes and some of them *(Hansenula anomala, Pichia kluyveri, Candida utilis)* degraded up to 70–80% of the alkaloid content. The best degraders were isolated directly from the wastes containing ergot alkaloids (Recek *et al.,* 1984).

Finally, thorough and detailed study of various products of alkaloid biotransfromations can help to predict their most plausible bioconversion in animal organisms. From the large set of data on various ergot alkaloid biotransformations it can be concluded that the most sensitive parts of ergoline skeleton are positions 1, 2 and 3 (analogy to kynurenine degradation), *N*-6 (dealkylation, oxidation) and 8, 9, and 10—depending on the double bond position. Contrary to that, position C-4 is extremely resistant to any substitution (till now no chemical or biological modification of this position has been reported), and also position 7 is very stable. The above general conclusions can be very helpful for estimation of chemical and/or metabolic reactivity of various ergot alkaloids.

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