NPR

Cite this: *Nat. Prod. Rep.*, 2011, **28**, 496

www.rsc.org/npr

REVIEW

Ergot alkaloids: structure diversity, biosynthetic gene clusters and functional proof of biosynthetic genes

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Received 26th October 2010
DOI: 10.1039/c0np00060d

Covering: 2000 to 2010

Ergot alkaloids are toxins and important pharmaceuticals which are produced biotechnologically on an industrial scale. They have been identified in two orders of fungi and three families of higher plants. The most important producers are fungi of the genera *Claviceps*, *Penicillium* and *Aspergillus* (all belonging to the Ascomycota). Chemically, ergot alkaloids are characterised by the presence of a tetracyclic ergoline ring, and can be divided into three classes according to their structural features, *i.e.* amide- or peptide-like amide derivatives of D-lysergic acid and the clavine alkaloids. Significant progress has been achieved on the molecular biological and biochemical investigations of ergot alkaloid biosynthesis in the last decade. By gene cloning and genome mining, gene clusters for ergot alkaloid biosynthesis have been identified in at least 8 different ascomycete species. Functions of most structure genes have been assigned to reaction steps in the biosynthesis of ergot alkaloids by gene inactivation experiments or biochemical characterisation of the overproduced proteins.

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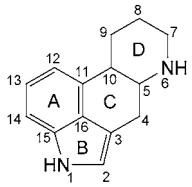
1 Introduction

Ergot alkaloids are a complex family of indole derivatives with diverse structures and biological activities.[1–4] They play important roles as pharmaceuticals, in the food industry and in ecological systems. The characteristic structural feature of all ergot alkaloids is the tetracyclic ergoline ring (**1**).



tetracyclic ergoline ring system (**1**)

Due to interactions with various receptors of the central nervous system, both natural and semi-synthetic ergot alkaloids are in widespread use in modern medicine, and exhibit a broad spectrum of pharmacological activities, including uterotonic activity, modulation of blood pressure, control of the secretion of pituitary hormones, migraine prevention, and dopaminergic and neuroleptic activities.[1,4,5] In contrast to their contribution to human health, ergot alkaloids are recognised as important natural toxins in the human history.[6,7] They are main toxins in ergots produced by members of the genus *Claviceps*, which parasitizes grass or grains. In Europe in the Middle Ages, ingestion of ergot-infected grass or grains by humans or animals caused severe epidemics, called St. Anthony's Fire. Two types can be recognised – ergotismus convulsivus, with paranoia and hallucinations, and ergotismus gangraenosus, with loss of infected tissues.[6] The first connection between ergotism and infected grain (especially rye) was discovered in 1853 by Tulasne.[4] Ergot-infected grasses continue to produce serious epidemics in livestock today.[4,8] On the other hand, infection by endophytic fungi confers several ecological benefits to infected plants, including resistance to invertebrate and vertebrate herbivory, as well as enhanced growth, mineral uptake, and resistance to drought.[9,10]

Ergot alkaloids are produced by fungi of the families Clavicipitaceae (*e.g. Claviceps* and *Neotyphodium*) and Trichocomaceae (including *Aspergillus* and *Penicillium*).[1,3] Ergot alkaloids have also been identified in plants of the families Convolvulaceae, Poaceae and Polygalaceae.[3] However, there is increased evidence that these compounds are produced by plant-associated fungi alone or together with the host plants.[11]

Due to their significant importance as toxins and drugs as well as their role in ecological systems, biosynthesis of ergot alkaloids has been an important research field in the secondary metabolism. A large number of feeding experiments, mainly carried out by the Gröger and Floss groups, were reported between the 1960s and 1990s.[1,3,12–14]

Identification of a biosynthetic gene cluster of ergot alkaloids in *Claviceps purpurea* in 1999[15] by genomic walking using the *dmaW* gene,[16] coding for the first pathway-specific enzyme dimethylallyltryptophan synthase (DMATS), provided the necessary background for molecular biological and biochemical investigations on structure genes of the biosynthetic enzymes.[1] Six years later, a gene cluster for the biosynthesis of fumigaclavine C was identified in the genome sequence of *Aspergillus fumigatus* Af293 by bioinformatic approaches.[17,18] The identification of this cluster for a clavine-type ergot alkaloid lacking the peptidyl moiety provided a convenient way to identify candidate genes, which are involved in the committed steps of the biosynthesis of ergot alkaloids by comparison of the two clusters in *C. purpurea* and *A. fumigatus*. Since then, especially in the last two years, significant progress has been achieved in the identification of structure genes involved in the reaction steps of ergot alkaloid biosynthesis. Therefore, the main focus of this review will be the results obtained in the last few years.

2 Diverse structures with broad biological and pharmacological activities

Ergot alkaloids are usually classified by their structures, *e.g.* clavine-type alkaloids, also called clavines, ergoamides or ergopeptines. Clavine-type alkaloids simply consist of the tetracyclic ergoline ring system (**1**) or the tricyclic precursors thereof. Ergoamides and ergopeptines are amides and peptides of D-lysergic acid (**2**), respectively. These compounds have the same ergoline ring system as the clavine-type alkaloids.[2]

Ergot alkaloids interact with different receptors of the central nervous system. Their effects are based on the similarity of the structures of ergot alkaloids with noradrenaline, dopamine and serotonine.[19] Some ergot alkaloids act as agonists whereas others act as antagonists.[20] The differences in the physiochemical, physiological and pharmacological properties of ergot alkaloids

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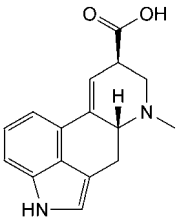
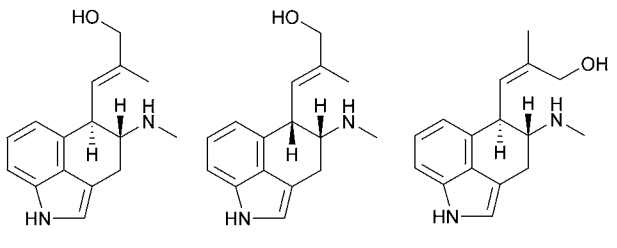
are caused by the various substituents attached to the carboxyl group of D-lysergic acid (**2**).[4]



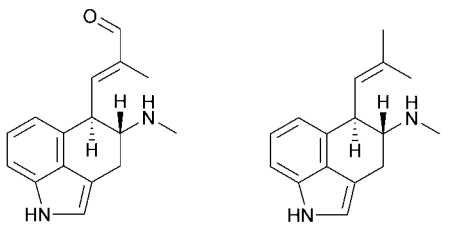
lysergic acid (**2**)

2.1 Tricyclic precursors of ergot alkaloids

Precursors of ergot alkaloids with a tricyclic structure carry a 6,7-seco-D-ring, and these compounds are therefore also called secoergolenes or tricyclic seco derivatives.[2] Important naturally occurring derivatives are chanoclavine-I (**3**), its two isomers chanoclavine-II (**4**) and isochanoclavine-I (**5**), as well as chanoclavine-I aldehyde (**6**) and 6,7-seco-agroclavine (**7**). So far, little is known about their biological and pharmacological activities.[21]



chanoclavine-I (**3**) chanoclavine-II (**4**) isochanoclavine-I (**5**)

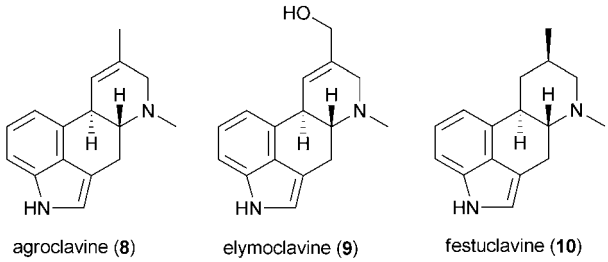


chanoclavine-I aldehyde (**6**) 6,7-seco-agroclavine (**7**)

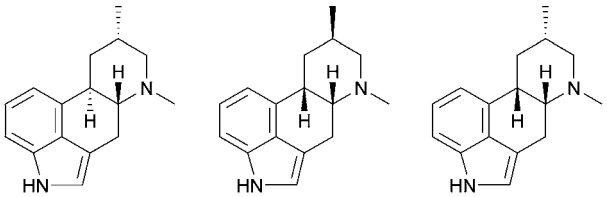
2.2 Clavine-type alkaloids

Clavine-type alkaloids, containing a tetracyclic ergoline ring, have been identified in various fungal strains, especially in the family Trichocomaceae. Agroclavine (**8**) and elymoclavine (**9**) contain a double bond between C8 and C9. Festuclavine (**10**) and pyroclavine (**11**) have a saturated ring D and differ from each other just in their stereochemistry at C8. Their stereoisomers costaclavine (**12**) and epicostaclavine (**13**) have also been identified in various fungi.[21] It has been shown that naturally occurring clavines, *e.g.* **8–11**, act as pure antagonists or partial agonists at 5-HT_{2A} receptors of rat tail artery, and as antagonists at α_1-adrenoceptors of rat aorta.[22]

In comparison to festuclavine or pyroclavine, fumigaclavines A (**14**), B (**15**) and C (**16**) carry additional substituents, *e.g.* OH or OAc group at C9 and a reverse prenyl moiety at C2. In the



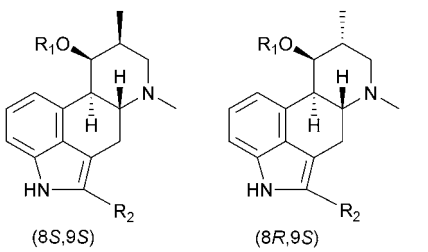
agroclavine (**8**) elymoclavine (**9**) festuclavine (**10**)



pyroclavine (**11**) costaclavine (**12**) epicostaclavine (**13**)

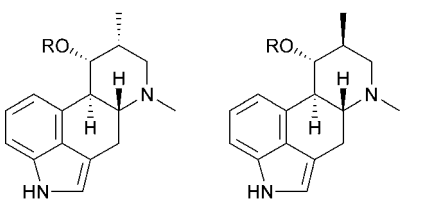
literature, the C8 stereochemistry of the fumigaclavines was somewhat confused. Structures with both (8*S*,9*S*) and (8*R*,9*S*) configurations (as clearly concluded from their NMR spectra[23,24]) were assigned to fumigaclavines A, B or C.[2,23,25–29] Furthermore, the (8*S*,9*R*) diastereomers of fumigaclavines A and B have been called isofumigaclavines A (**17**) and B (**18**)[30] or roquefortine A and B,[31,32] respectively. Isomers with a (8*R*,9*R*) configuration have not yet been reported. However, with the functional proof of biosynthetic enzymes, it should be possible to produce the corresponding derivatives. To better understand this type of compound and to avoid confusion in the future, we suggest that the names fumigaclavine A, B and C be applied to each group of four possible diastereomers, and that the stereochemistry at C8 and C9 be defined by prefixing them with (*R*) or (*S*) labels.

Biochemical investigations of the biosynthesis (see below) showed that fumigaclavine A (**14**) is formed from B (**15**) by acetylation and converted to C (**16**) by prenylation at C2. Interestingly, Ge *et al.*[23] reported recently the identification of



(8*S*,9*S*) (8*R*,9*S*)

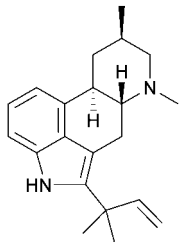
fumigaclavine A (**14**) $R_1 = CH_3CO$, $R_2 = H$
fumigaclavine B (**15**) $R_1 = H$, $R_2 = H$
fumigaclavine C (**16**) $R_1 = CH_3CO$, $R_2 =$



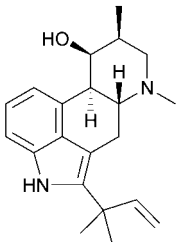
(8*R*,9*R*) (8*S*,9*R*)

isofumigaclavine A (**17**) R = CH_3CO
isofumigaclavine B (**18**) R = H

9-deacetoxyfumigaclavine C (**19**) and 9-deacetylfumigaclavine C (**20**) in *A. fumigatus*. Both substances showed selective and potent cytotoxicity against human leukemia cells.



9-deacetoxyfumigaclavine C (**19**)

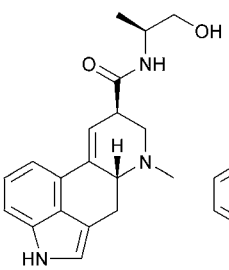


9-deacetylfumigaclavine C (**20**)

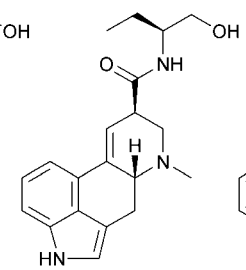
Other clavine-type alkaloids also show interesting pharmacological activities. For example, (8*R*,9*S*)-fumigaclavine C (**16**) causes relaxation of isolated rat aortic rings, and because of the vasorelaxant effect, it has potential capacity for vascular protection.[33] In addition, this compound has also been shown to have ameliorating effects on liver damage and colitis in animals.[29,34]

2.3 D-Lysergic acid amides: ergoamides

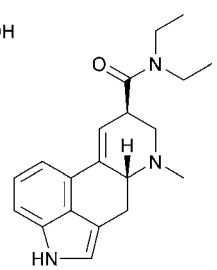
The important ergoamides are the naturally occurring ergometrine[35] (**21**) (synonymous with ergonovine and ergobasine) and its semi-synthetic derivative methylergometrine (**22**). In these structures, D-lysergic acid (**2**) is amidated with 2-aminopropanol or 2-aminobutanol, respectively. In comparison to the structures of agroclavine (**8**) and elymoclavine (**9**), the double bond in **2** is shifted to C9 and C10. Ergometrine (**21**) has a more pronounced uterotonic effect than ergotamine (**26**), and has been the most important drug for prevention and treatment of postpartum hemorrhage.[36] Methylergometrine (**22**) is still in clinical use for the same application.[37]



ergometrine (**21**)



methylergometrine (**22**)

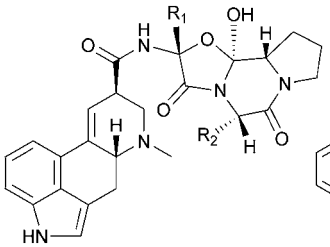


lysergic acid diethylamide (**23**)

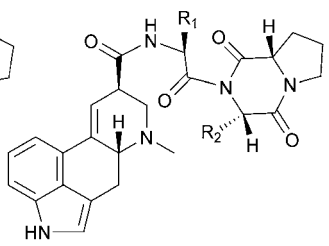
It should be mentioned that the semi-synthetic lysergic acid diethylamide (LSD, **23**) was initially developed for the treatment of various psychiatric disorders,[38] but due to its hallucinogenic effects, it remains an illegal drug.[39]

2.4 D-Lysergic acid peptides: ergopeptines

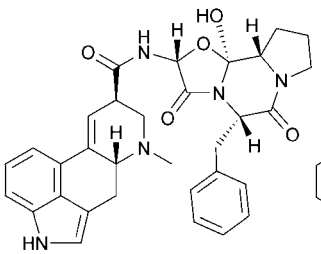
Ergopeptines (**24**) are lysergic acid derivatives with tripeptide moieties attached to its carboxy group *via* peptide-like amide bonds. The tripeptide residues of naturally occurring ergopeptines contain always proline and form a tricyclic ring system.[40] Ergopeptines (**24**) are derived from ergopeptams (**25**) of tripeptides with a bicyclic system, which have also been isolated as predominant alkaloids from sclerotia of *C. purpurea*.[40]



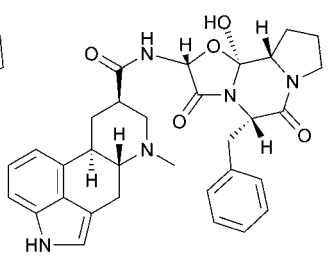
common structure of ergopeptines (**24**)



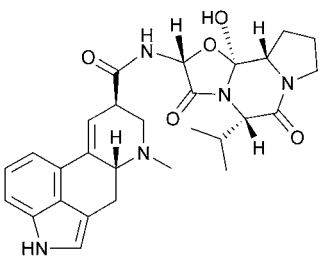
common structure of ergopeptams (**25**)



ergotamine (**26**)



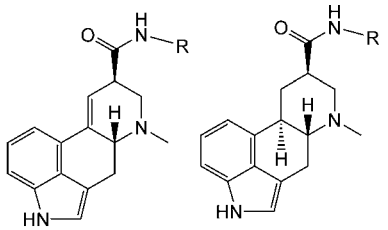
dihydroergotamine (**27**)



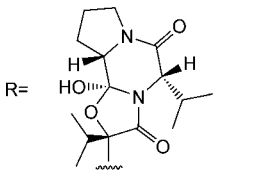
ergovaline (**28**)

Ergotamine (**26**) is undoubtedly the most important ergopeptine, and is produced by *C. purpurea* as the main ergot alkaloid.[7,41,42] Chemically, it is a peptide-like amide of D-lysergic acid (**2**) with a peptide derived from L-alanine, L-phenylalanine and L-proline. Ergotamine (**26**) and its semi-synthetic derivative dihydroergotamine (**27**) are clinically used for treatment of diverse diseases including acute migraine attacks and cluster headache.[43–46] In addition, dihydroergotamine is also for therapy of orthostatic hypotension.[47] Ergovaline (**28**), carrying a peptide moiety consisting of alanine, valine and proline, is implicated in livestock toxicoses caused by ingestion of endophyte-infected grasses.[9,48]

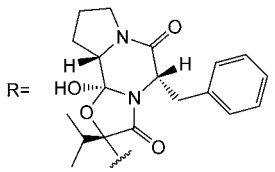
Ergotoxine is a mixture of ergopeptines containing L-valine (as the first amino acid) and L-proline (as the second amino acid) in their peptide moieties. The important components of ergotoxine are ergocornine (**29**), ergocristine (**30**), α-ergocryptine (**31**) and β-ergocryptine (**32**), which contain L-valine, L-phenylalanine, L-leucine and L-isoleucine as the third amino acid in their peptide moieties, respectively.[49] The naturally occurring ergotoxine is the raw material for the semi-synthesis of 9,10-dihydroergotoxine, consisting of dihydroergocornine (**33**), dihydroergocristine (**34**), α-dihydroergocryptine (**35**) and β-dihydroergocryptine (**36**). 9,10-Dihydroergotoxine, also named codergocrine or ergoloid, is used as its methanesulfonate (mesylate) in a ratio of 3 : 3 : 2 : 1 (w/w) to treat dementia and age-related cognitive impairment.[49,50]



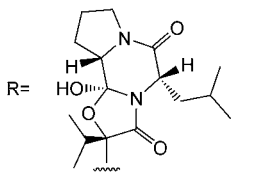
ergotoxine (**29-32**) dihydroergotoxine (**33-36**)



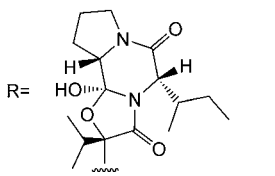
R= ergocornine (**29**) dihydroergocornine (**33**)



R= ergocristine (**30**) dihydroergocristine (**34**)



R= α-ergocryptine (**31**) α-dihydroergocryptine (**35**)



R= β-ergocryptine (**32**) β-dihydroergocryptine (**36**)

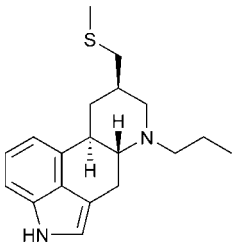
Pergolide (**37**) and cabergoline (**38**) are not only developed from ergopeptines (**24**) as leading structures, but can also be semi-synthesized from lysergic acid (**2**) and elymoclavine (**9**), which can be obtained from fermentation broths of *Claviceps*.[51]

α-Dihydroergocryptine (**35**) and bromocriptine (2-bromo-α-ergocryptine (**39**)), a semi-synthetic derivative of α-ergocryptine, as well as pergolide (**37**) and cabergoline (**38**), are used clinically for treatment of early-onset Parkinson's disease.[52,53,54]

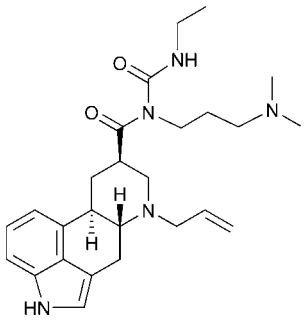
Furthermore, it has been shown that ergot alkaloids inhibit the release of the peptide hormone prolactin.[55] Therefore, bromocriptine and cabergoline are also used to treat prolactinemia.[56–58]

2.5 Other related structures

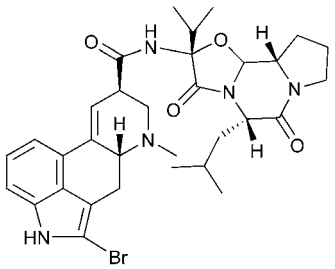
A number of indole alkaloids have been isolated from fungal strains, which are very likely derived from tryptophan (**49**) and dimethylallyl diphosphate, but carry a modified ergoline system or even clearly different structures.[2,59] Epoxyagroclavine (**40**) from *Penicillium kapuscinski*[2] and cycloclavine from *Aspergillus japonicus* (**41**)[60] can be considered as oxidation products of agroclavine (**8**). Rugulovasine A (**42**) and its stereoisomer rugulovasine B (**43**), as well as their 8-chlorinated derivatives (**44**) and (**45**), have been identified in various fungal strains, including *Penicillium* species,[2,61–63] while paspaclavine (**46**) was isolated



pergolide (**37**)

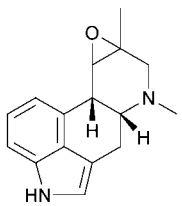


cabergoline (**38**)

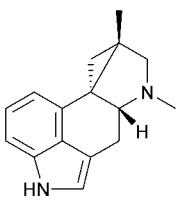


bromocriptine (**39**)

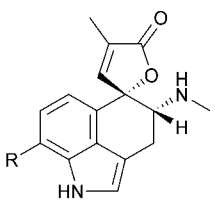
from *Claviceps paspali*.[64] Substances **42–46** have still the three-ring ABC system of ergolines (**1**), but differ from **1** in the fourth ring – a result of different cyclisation processes. Clavicipitic acid (**47**), from *Claviceps fusiformis*,[65,66] and its decarboxylated derivative aurantioclavine (**48**), from *Penicillium aurantiovirens*,[2,67] are likely formed by attack of the amino group of tryptophan (**49**) upon the C1 of the dimethylallyl diphosphate (DMAPP) moiety.



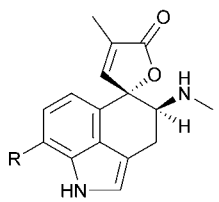
epoxyagroclavine-I (**40**)



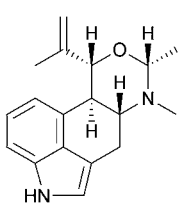
cycloclavine (**41**)



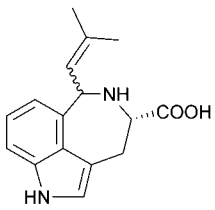
rugulovasine A (**42**) R = H
8-chlororugulovasine A (**44**) R = Cl



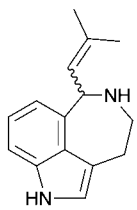
rugulovasine B (**43**) R = H
8-chlororugulovasine B (**45**) R = Cl



paspaclavine (**46**)



clavicipitic acid (**47**)



aurantioclavine (**48**)

3 Producers of ergot alkaloids

3.1 Ascomycota as producers of ergot alkaloids

3.1.1 Producers of clavines. Producers with clavines as the main products are *A. fumigatus*,[68] *Penicillium* strains including *P. roquefortii*,[31] *P. verrucosum*[69] and *P. commune*,[70] as well as *Claviceps* species including *C. fusiformis*, *C. paspali* and *C. hirtella*.[2] In *Aspergillus fumigatus*, it seems that ergot alkaloid production is associated with conidiation, as it was shown that a conidiation-deficient mutant produced no ergot alkaloids.[18]

Fumigaclavines A (**14**) and B (**15**) have been identified in both *Aspergillus* and *Penicillium* strains.[26,70–72] In contrast, fumigaclavine C (**16**) was only found in *Aspergillus*, but not in *Penicillium* species.[68,73] Festuclavine (**10**) has so far only been identified in *Aspergillus fumigatus*,[73,74] while *Penicillium* strains produced both festuclavine (**10**) and its stereoisomer pyroclavine (**11**).[27,32,70,75,76] Agroclavine is mainly produced by *Claviceps* strains,[77–81] but also by *Penicillium commune*.[70] *Claviceps hirtella* mainly produced clavines, together with ergometrine as a minor (3–8%) component of the total ergot alkaloids.[82] *C. fusiformis* is the only known clavicipitaceaous fungus that produces clavines but no D-lysergic acid derivatives.[78]

3.1.2 Producers of ergoamides and ergopeptines. Producers of ergoamides and ergopeptines are fungi of the family Clavicipitaceae. *Claviceps purpurea* is undoubtedly the most important of all the ergot alkaloid producers. In *C. purpurea*, ergot alkaloids are found in the sclerotia,[82] and the spectrum of ergot alkaloids varies strongly between different *C. purpurea* strains.[82] For example, the strain P1 produces elymoclavine (**9**), ergotamine (**26**), ergocryptine (**31** + **32**) and ergosine, with **26** as the major product.[41] The strain ECC93, in contrast, produces ergocristine (**30**) and ergotamine (**26**) as the major components, and ergometrine (**21**) as a minor component.[42,83]

In addition to fungi of the genus *Claviceps*, endophytic fungi of the *Epichloë* species and their asexual counterpart *Neotyphodium* as well as the genus *Balansia* produce ergot alkaloids when they are associated with their host plants. For example, ergovaline (**28**) and simple lysergic acid amide (synonymous with ergine) have been identified in *Epichloë festucae*.[84] These fungi are recognized as symbionts of many grasses of the subfamily Pooideae of the family Poaceae (see below).

3.2 Plants as producers of ergot alkaloids

For a long time, it was believed that not only fungi, but also plants of the families Convolvulaceae, Poaceae and Polygalaceae produced ergot alkaloids and that horizontal gene transfer from fungi to higher plants had taken place during the evolutionary process.[3,11] Recent investigations on the molecular level have revealed that, at least in Poaceae and Convolvulaceae, the plant-associated fungi are likely responsible for ergot alkaloid production.[11,85] Treatment of *Ipomoea asarifolia* (Convolvulaceae) with two fungicides inhibited the growth of the fungi and resulted in complete abolishment of ergot alkaloid production.[85] Production of volatile oil in the treated plants was in contrast not significantly changed. Therefore, it seems that the accumulation of ergot alkaloids in plants depended on the presence of plant-associated fungi, which are seed-transmitted.[86] Ahimsa-Müller *et al.*[87] showed that four different plants of the family Convolvulaceae are infected by closely related fungi of the family Clavicipitaceae, and that all fungi contain genes for ergot alkaloid biosynthesis.

3.3 Symbiotic communities

Grasses of the family Poaceae and fungi of the family Clavicipitaceae have a long history of symbiosis. Fungi gain shelter, nutrition, and dissemination *via* host propagules, and can contribute an array of host fitness enhancements, including protection against insect and vertebrate herbivores and root nematodes, enhancements of drought tolerance and nutrient status, and improved growth, particularly of the root.[88] Tests of several ergot alkaloids revealed that ergovaline (**28**) and α-ergocryptine (**31**) were nematicidal at 5 and 50 μg ml^{-1}, respectively. Ergocornine (**29**) and ergometrine (**21**) showed also nematistatic activity.[89]

Fleetwood *et al.*[48] identified in 2007 a gene cluster for ergovaline (**28**) biosynthesis from the endophyte *Epichloë festucae*. They reported that all of the genes from the ergovaline (**28**) cluster were only highly expressed during biotrophic growth in association with the plant. In mycelia from axenic culture, the expression was very low or even undetectable. The authors concluded therefore that special signals or components of the plant were necessary for the induction of the gene expression.[48]

Faeth *et al.*[90] showed that in native grass populations, ergot alkaloid production by an endophytic infection has no advantages for the growth or seed production of the plant, and that ergot alkaloids have a negative effect. However, it seems that ergot alkaloids protect the plant against below-ground herbivory and harsh winter conditions.

In the case of plant-associated fungi in Convolvulaceae, it has been shown[86] that the genes for ergot alkaloid production are only found in the fungi, but not in the plants. However, ergot alkaloids were not detectable in the fungal mycelium, but in the plant leaves. The authors speculated therefore that a system must exist for transport of the produced alkaloids from the fungus into the plants.[86] More interestingly, until now it was not possible to cultivate the *Ipomoea asarifolia*-associated fungus *in vitro*. It seems that the host plant is also important for the fungus, and may provide components for fungal growth, as in the case of grasses for *Neotyphodium lolii*.[11,48]

4 Investigations into the biosynthesis of ergot alkaloids by feeding experiments and crude enzyme extracts

Feeding experiments with isotopically labelled precursors and by enzyme extracts from the producers[1,3,12,14] have clearly revealed that the ergoline moiety is derived from tryptophan (**49**) and DMAPP.[12,14] It has also been demonstrated that chanoclavine-I aldehyde (**6**) must be an intermediate in the conversion of chanoclavine-I (**3**) to elymoclavine (**9**) and that isomerisation of the double bond between C8 and C9 in **6** must take place before cyclisation.[91] Several excellent reviews have summarized the results of feeding experiments on ergot alkaloids and enzymatic characterisation of certain reactions.[1,3,12,14,92] This area of study will therefore not be discussed in this review.

5 Identification of biosynthetic gene clusters and comparison of genetic information

5.1 Cloning and identification of biosynthetic gene clusters

In fungi and bacteria the genes for the biosynthesis of secondary metabolites are usually clustered as a continuous segment on the chromosome.[93–95] This feature provides a convenient way to identify genes that are involved in the biosynthesis of a certain compound or compound group.

The first biosynthetic gene cluster of ergot alkaloids was identified in *Claviceps purpurea* strain P1 by genomic walking using the *dmaW* gene.[15] This cluster was extended later to a region covering over 68.5 kb and containing 14 genes that are co-ordinately induced under ergot alkaloid production conditions (Fig. 1).[41,96] It is unclear why the sequences of two genes, *i.e. easH1* and *easH2*, are not available in public databases.

To discover the reason for accumulation of different ergot alkaloids in *Claviceps purpurea* and *C. fusiformis*, a cosmid library from *C. fusiformis* SD58 was constructed and screened with *dmaW* cDNA as a probe. Sequencing of a positive clone provided a contig of a 35.4 kb segment. A 19.6 kb region of this contig contains nine homologues of the *C. purpurea* gene cluster (Tables 1 and 2). The identified cluster contains no homologous of *lpsA1*, *lpsA2* and *lpsC*, coding for three non-ribosomal peptide synthetases (NRPS) in *C. purpurea*.[97] NRPSs are large multifunctional enzymes, which are often encoded by genes within biosynthetic gene clusters of secondary metabolites in bacteria and fungi.[95,98] They are responsible for the biosynthesis of peptidyl moieties in these compounds. These enzymes comprise one or more modules, which are usually responsible for activation of one (amino) acid each, its modification and connection with other reaction partners. Each module consists of several domains, namely adenylation (A), thiolation (T), condensation (C) and epimerization (E) domains, which catalyse different reactions. A thioesterase (TE) domain at the C-terminus of most NRPSs releases the newly synthesized peptide from the enzyme.[99–101]

Five homologous genes of the *C. purpurea* cluster were identified in a segment from a small-insert genomic library of the ryegrass pathogen *Neotyphodium lolii* Lp19. These genes belong to a putative cluster of the ergopeptine alkaloid ergovaline (**28**).[48] Together with the genes obtained from its sexual counterpart *Epichloë festucae* and from a previous study, 12 homologous genes to those from the gene cluster of *C. purpurea* have been identified (Tables 1 and 2).[10]

Penicillium commune produces the clavine-type alkaloid fumigaclavine A (**14**),[24,70] lacking a prenyl moiety at position C2 of fumigaclavine C (**16**), found in *A. fumigatus*.[73] To identify candidate genes for the biosynthesis of fumigaclavines, we constructed a cosmid library with genomic DNA from *P. commune* NRRL2033. 3000 cosmid clones were screened by PCR amplification of *fgaPT*, a homologous gene of *dmaW*. The sequence of *fgaPT* from *P. commune* NRRL2033 was obtained by PCR amplification of genomic DNA using degenerate primers and subsequent sequencing of the cloned plasmid. Sequencing and analysis of three positive cosmids revealed a putative cluster containing homologous genes to the cluster from *A. fumigatus* (see below and Tables 1 and 2).[102]

5.2 Identification of gene clusters from genome sequences by genome mining

The availability of genome sequences of bacteria and fungi, which has been increased tremendously in recent years (www.genomesonline.org),[103] accelerates the identification of genes involved in the biosynthesis of secondary metabolites.[104,105] The genome of *A. fumigatus* strain Af293 was the first to be sequenced of the *Aspergillus* species,[106] and its sequence was released to the public in 2003. By blasting the genome sequence with *dmaW* from *C. purpurea*, a putative gene cluster covering a DNA range of 27 kb and comprising 11 genes was identified on chromosome 2 (Fig. 1).[17] Three years later, a very similar cluster was identified in the genome sequence of another *A. fumigatus* strain A1163.[107,108] The putative genes of this cluster show high homology to those of strain Af293. The genes are located in the same order to each other and with the same orientation as in the case of Af293. Blasting the genome sequences of fungi from the family Arthrodermataceae, *e.g. Microsporum canis*, *Anthroderma benhamiae* and *Trichophyton verrucosum*, in GenBank revealed the presence of five putative genes, which share significant homology to those involved in the biosynthesis of ergot alkaloids in *A. fumigatus* and *P. commune*, as well as in clavicipitaceous fungi (Table 1).

5.3 The structural similarities and differences of ergot alkaloids are perfectly reflected by genetic organisation

As mentioned previously, the most ergot alkaloids share the tetracyclic ergoline system (**1**) derived from tryptophan (**49**) and dimethylallyl diphosphate. Differing from ergopeptines (**24**) produced by the *Claviceps* genera, fumigaclavines from *Aspergillus* and *Penicillium* strains bear no peptide moiety in their structures. Instead, they carry a hydroxyl or an acetoxy group at position C9 of the ergoline ring. Furthermore, fumigaclavine C (**16**) carries an additional reverse prenyl moiety at position C2 of the indole ring. These similarities and differences are perfectly reflected by the genetic organisation in their gene clusters. Comparison of the gene clusters from *C. purpurea*, *A. fumigatus* and *P. commune* revealed that seven orthologous/homologous genes are found in all the clusters, which could be speculated to be responsible for the formation of the ergoline scaffold.[17] The NRPS genes from the cluster of *Claviceps* would catalyse the synthesis and attachment of the peptidyl moiety to the ergoline system. The presence of the putative acetyltransferase genes in the fumigaclavine clusters in *A. fumigatus* and *P. commune* would be used for the transfer of an acetyl group from acetyl CoA to a hydroxyl group at C9. The second prenyltransferase gene *fgaPT1* in the cluster of *A. fumigatus* could be assigned to the conversion of fumigaclavine A (**14**) to C (**16**).[24] This gene is absent in the cluster of *P. commune*, indicating that fumigaclavine A (**14**) instead of C (**16**) is the end product of the gene cluster. This was proven by isolation and identification of fumigaclavine A (**14**) as the main ergot alkaloid in *P. commune* NRRL 2033.[24,102]

Whereas *C. purpurea* proceeds *via* clavine intermediates to lysergic acid (**2**) and the complex ergopeptines (**24**), *C. fusiformis* produces only agroclavine (**8**) and elymoclavine (**9**). Analysis of the ergot alkaloid biosynthetic gene cluster in *C. fusiformis*

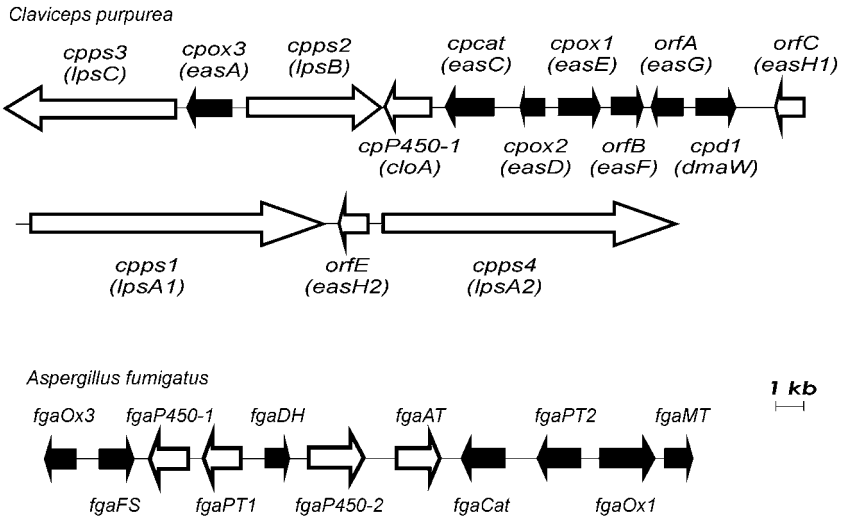


Fig. 1 Ergot alkaloid clusters of *Claviceps purpurea* and *Aspergillus fumigatus*. The black arrows indicate the homologous genes of both clusters (modified after Li & Unsöld[143] and Lorenz *et al.*[82]).

revealed the absence of orthologous of *lpsC*, *lpsA1* and *lpsA2*, but the presence of homologous of other *C. purpurea* genes, including those of *lpsB* and *cloA*. LpsB and CloA from *C. purpurea* are involved in the steps after clavine biosynthesis.[97] Cross-complementation analyses demonstrated that *cloA* and *lpsB* from *C. fusiformis* were expressed in the fungus, but did not encode functional enzymes. The authors proposed that the *C. fusiformis* ergot alkaloid biosynthetic cluster evolved from a more complete cluster by loss of some *lps* genes and by rearrangements and mutations resulting in inactivated *lpsB* and *cloA*.

Table 1 Homologous genes in the gene clusters of different fungi involved in the early, common steps of the ergot alkaloid biosynthesis. In addition to the accession number, the length of the proteins in amino acids (aa) and the sequence identity with *Claviceps pupurea* (*C.p.*) or *Aspergillus fumigatus* (*A.f.*) in percent are given.

	Protein name						
For *Claviceps purpurea*: For *Aspergillus fumigatus*:	DMATS FgaPT2	EasF/CpOrfB FgaMT	EasE/CpOx1/CcsA FgaOx1	EasD/CpOx2 FgaDH	EasA/CpOx3 FgaOx3	EasG/CpOrfA FgaFS	EasC/CpCat FgaCat
Claviceps purpurea	CAB39314[a] 448 aa 55% with *A.f.*	AAW57090[b] 344 aa 53% with *A.f.*	CAB39328[a] 483 aa 47% with *A.f.*	CAB39316[a] 261 aa 66% with *A.f.*	CAG28312[a] 369 aa 57% with *A.f.*	AAW57089[b] 290 aa 46% with *A.f.*	CAG28311[a] 473 aa 56% with *A.f.*
Claviceps fusiformis SD58[97]	ABV57826 455 aa 68% with *C.p.*	ABV57824 355 aa 73% with *C.p.*	ABV57823 581 aa 66% with *C.p.*	ABV57822 261 aa 79% with *C.p.*	ABV57819 382 aa 79% with *C.p.*	ABV57825 289 aa 60% with *C.p.*	ABV57821 479 aa 76% with *C.p.*
Neotyphodium loliil Epichloë festucae	AAP81206[c] 450 aa 65% with *C.p.*	ABM91451[d] 344 aa 61% with *C.p.*	ABM91450[d] 605 aa 54% with *C.p.*	ACM47225[e] 262 aa 71% with *C.p.*	ABM91449[d] 380 aa 75% with *C.p.*	ABM91452[d] 309 aa 52% with *C.p.*	ACM47224[e] 476 aa 69% with *C.p.*
Penicillium commune NRRL 2033[102]	480 aa 56% with *A.f.*	339 aa 60% with *A.f.* 54% with *C.p.*	588 aa 42% with *A.f.* 39% with *C.p.*	261 aa 78% with *A.f.* 68% with *C.p.*	379 aa 64% with *A.f.* 51% with *C.p.*	286 aa 62% with *A.f.* 42% with *C.p.*	466 aa 66% with *A.f.* 57% with *C.p.*
Aspergillus fumigatus Af293[106]	EAL94103 451 aa 55% with *C.p.*	EAL94105 339 aa 53% with *C.p.*	EAL94104 628 aa 47% with *C.p.*	EAL94099 261 aa 66% with *C.p.*	EAL94095 376 aa 57% with *C.p.*	EAL94096 290 aa 46% with *C.p.*	EAL94102 520 aa 56% with *C.p.*
Microsporum canis CBS 113480 (GenBank)	EEQ33236 446 aa 65% with *A.f.* 58% with *C.p.*	EEQ33234 340 aa 65% with *A.f.* 60% with *C.p.*	EEQ33235 612 aa 51% with *A.f.* 50% with *C.p.*	EEQ33233 264 aa 69% with *A.f.* 63% with *C.p.*	—	—	EEQ33232 482 aa 63% with *A.f.* 59% with *C.p.*
Arthroderma benhamiae CBS 112371 (GenBank)	EFE37121 385 aa 62% with *A.f.* 57% with *C.p.*	EFE37119 340 aa 65% with *A.f.* 59% with *C.p.*	EFE37120 500 aa 49% with *A.f.* 49% with *C.p.*	EFE37118 246 aa 65% with *A.f.* 59% with *C.p.*	—	—	EFE37117 478 aa 63% with *A.f.* 57% with *C.p.*
Trichophyton verrucosum HKI 0517 (GenBank)	EFE43383 375 aa 63% with *A.f.* 57% with *C.p.*	EFE43381 382 aa 64% with *A.f.* 58% with *C.p.*	EFE43382 604 aa 49% with *A.f.* 49% with *C.p.*	EFE43380 265 aa 65% with *A.f.* 60% with *C.p.*	—	—	EFE43379 478 aa 63% with *A.f.* 57% with *C.p.*

Sequences were obtained from: [a] *C.p.* strain P1 (ref. 15); [b] *C.p.* isolate ATCC20102; [c] *Epichloe typhina* × *Neotyphodium lolii* isolate Lp1 (ref. 112); [d] *Neotyphodium lolii* strain Lp19 (ref. 48); [e] *Epichloe festucae* strain E2368.

Table 2 Homologous genes in the gene clusters of different fungi involved in the later steps of the ergot alkaloid biosynthesis, which differ in the families Clavicipitaceae and Trichocomaceae. In addition to the accession number, the length of the proteins in amino acids (aa) and the sequence identity with *Claviceps pupurea* (*C.p.*) or *Aspergillus fumigatus* (*A.f.*) in percent are given.

	Protein name							
For *Claviceps purpurea*: For *Aspergillus fumigatus*:	FgaP450-1	FgaP450-2	FgaAT	FgaPT1	CloA/CpP450-1	LpsA1/Cpps1	LpsA2/Cpps4	LpsB/Cpps2	LpsC/Cpps3
Claviceps purpurea P1[15]	—	—	—	—	CAI59266 507 aa	CAB39315 3232 aa	CAI59268 3524 aa	CAD28788 1308 aa	CAI59267 1633 aa
Claviceps fusiformis SD58[97]	—	—	—	—	ABV57820 511 aa	—	—	ABV57818 644 aa (truncated)	—
Neotyphodium lolii/*Epichloe festucae*	—	—	—	—	68% with *C.p.* ACM47223[e] 506 aa 65% with *C.p.*	AAL26315[f] 3589 aa 53% with *C.p.*	AAL26315[f] 3589 aa 50% with *C.p.*	ABM91454[d] 1351 aa 49% with *C.p.*	AAL26315[f] 3589 aa 46% with *C.p.*
Penicillium commune NRRL 2033[102]	—	520 aa 63% with *A.f.*	491 aa 67% with *A.f.*	—	—	—	—	—	—
Aspergillus fumigatus Af293[106]	EAL940097 338 aa	EAL94100 519 aa	EAL94101 494 aa	EAL94098 436 aa	—	—	—	—	—

Sequences were obtained from: [d] *Neotyphodium lolii* strain Lp19 (ref. 48); [e] *Epichloe festucae* strain E2368; [f] *Neotyphodium lolii* (ref. 10).

Claviceps hirtella produced mainly clavine alkaloids, and ergometrine (**21**) as a minor component (3–8% of total ergot alkaloids). Its gene cluster contains no homologous of *lpsA1* and *lpsA2*, which are responsible for the biosynthesis of ergopeptines (**24**) in *C. purpurea*.[83] In contrast to that of *C. fusiformis*, the gene cluster of *C. hirtella* contains functional LpsB and LpsC, which are responsible for the production of ergometrine (**21**) (see below).[82]

6 Investigations into the biosynthesis of ergot alkaloids by gene inactivation experiments and biochemical approaches with recombinant enzymes

Based on the information obtained from gene clusters mentioned above,[15,17,109] significant progress has been achieved in the identification of the structural genes in the biosynthesis of ergot alkaloids. The function of most genes has been proven by gene deletion experiments or heterologous expression and biochemical characterisation.[4,110]

6.1 Formation of the ergoline scaffold

Functions of six of the seven genes found in all of the known ergot alkaloid clusters have been proven experimentally. Their roles in the biosynthesis were assigned to reaction steps from L-tryptophan (**49**) to agroclavine (**8**) or festuclavine (**10**).[111]

The biosynthesis of ergot alkaloids begins with the prenylation of L-tryptophan (**49**) at position C4 of the indole ring in the presence of DMAPP (Scheme 1). This reaction is catalysed by a prenyltransferase, *i.e.* 4-dimethylallyltryptophan synthase (4-DMATS), encoded by the gene *dmaW*.[1,17] A number of experiments were carried out with this first pathway-specific enzyme from different sources. Knockout of *dmaW* in the fumigaclavine C (**16**) producer *A. fumigatus* abolished the ergot alkaloid accumulation. Complementation of the mutation restored the ergot alkaloid production, proving its essential role in the biosynthesis.[109] Similar experiments were also carried out in *Neotyphodium* sp. and *C. fusiformis*.[112]

Cloning of the orthologous gene *fgaPT2* from *A. fumigatus* and expression in *E. coli* resulted in the formation of substantial amount of soluble recombinant protein, which was studied biochemically[17,113,114] and structurally.[115] Isolation and structure elucidation of the enzyme product from the incubation mixture of FgaPT2 with L-tryptophan (**49**) and DMAPP revealed clearly the C4-prenylation of **49**.[17] Several other 4-dimethylallyltryptophan synthases were also cloned, overexpressed and characterised biochemically.[86,116]

4-DMATSs were the first characterised members of the prenyltransferase class, which catalyse the transfer reactions of a prenyl moiety, usually from dimethylallyl diphosphate, onto different positions of indole ring of diverse structures or the hydroxyl group of L-tyrosine.[108,117] These enzymes are soluble proteins, and are usually involved in the biosynthesis of fungal secondary metabolites.[110] In contrast to other prenyltransferases,[118,119] these enzymes contain no (N/D)DXXD motifs in their sequences, and their reactions are independent of metal ions such as Mg^{2+} or Mn^{2+}.[107,108] In recent years, 16 new members of this so-called DMATS superfamily have been cloned, expressed and characterised biochemically.[110,120] These enzymes

accept almost only DMAPP as a prenyl donor, but show significant flexibility towards their aromatic substrates. In general, the prenylation reactions are regiospecific and stereospecific. For example, FgaPT2 accepted not only simple indole derivatives, but also tryptophan-containing cyclic dipeptides as prenylation substrates, which was also observed with another 4-DMATS from *Malbranchea aurantiana*.[116] The prenylation pattern and position were for all of the compounds identical, *i.e.* regular C4-prenylation.[113,114] These features were successfully used for production of prenylated derivatives.[121–124]

Prenyltransferases of the DMATS superfamily show no sequence similarity to another group of aromatic prenyltransferases from bacteria, which catalyse the prenylation of diverse aromatic substrates such as hydroxynaphthalenes,[125,126] 4-hydroxyphenylpyruvate[127] or phenazines.[128] Therefore, it was interesting to obtain the crystal structure of a member of the DMATS superfamily and to compare it with that of the prenyltransferases from bacteria.[125] X-ray structural analysis of the 4-DMATS FgaPT2 from *A. fumigatus* revealed that FgaPT2 exhibits a PT fold (β/α barrel fold), which was only found in the group of bacterial prenyltransferases.[115,125,129] This somewhat surprising result could be explained by the common evolution of the prenyltransferases of the both groups.

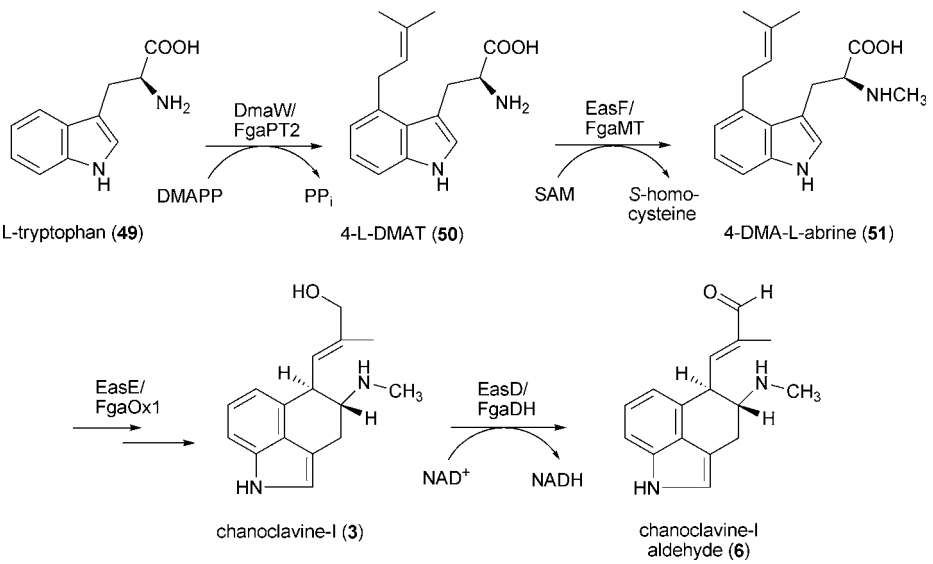
The availability of the FgaPT2 structure, especially of the soaking experiments with its substrate L-tryptophan (**49**) and DMAPP analogue, provided a convenient way to understand the reaction mechanism of C-prenylation.[115] Based on the crystal structure, a three-step reaction mechanism was proposed that involves the formation of a dimethylallylic cation by interaction of basic amino acids such as lysine with phosphate residues of DMAPP, subsequent nucleophilic attack of this cation by the indole nucleus to form the σ-complex, and deprotonation to form the final product (Scheme 2).[115] The importance of basic amino acids for the enzyme activities of prenyltransferases of the DMATS superfamily including FgaPT2 has been proven by site-directed mutagenesis.[115,130] Evidence for the presence of a dimethylallyl cation in the reaction mixture of FgaPT2 was provided by incubation of L-tryptophan (**49**) and [1-^{18}O]-DMAPP with FgaPT2 and analysis of the ^{31}P NMR spectrum of DMAPP in the reaction mixture.[131]

From results of the phylogenetic analysis, Liu *et al.*[132] suggested that *fgaPT2* from *A. fumigatus* has the same origin as the corresponding genes from clavicipitaceous fungi.

In the biosynthesis of ergot alkaloids, the product of 4-DMATS is methylated at the amino group. Using purified recombinant protein, our group has demonstrated that a methyltransferase FgaMT from *A. fumigatus* was responsible for the transfer of a methyl group from *S*-adenosyl methionine to 4-DMAT (**50**), resulting in the formation of *N*-methyl-dimethylallyl tryptophan (**51**) (4-dimethylallyl-L-abrine).[133] FgaMT also showed broad substrate specificity towards its aromatic substrates, and accepted a number of C4-prenylated tryptophan derivatives, even 4-methyltryptophan, as substrates.[133] FgaMT belongs to a new enzyme group of SAM-dependent methyltransferases. With the exception of its orthologues in other ergot alkaloid clusters, FgaMT shows no significant sequence similarity to known entries in the database. No conserved motifs for methyltransferases[134,135] could be found in the sequence of FgaMT.

The next detected intermediate in the biosynthesis of ergot alkaloids is chanoclavine-I (**3**).[91,136] Conversion of 4-dimethylallyl-L-abrine (**51**) to chanoclavine-I (**3**) would include at least three reactions, *i.e.* decarboxylation, cyclisation and hydroxylation. Mutants of *C. purpurea* strain P1 deficient in *ccsA* (also termed *easE*) accumulated *N*-methyl-4-dimethylallyltryptophan (**51**) and traces of 4-DMAT (**50**).[137] Complementation of the deletion mutants with a gene construct that expresses a CcsA:GFP fusion protein restored the ergot alkaloid production. The authors proposed that CcsA is responsible for or at least involved in the formation of chanoclavine-I (**3**). Further biochemical studies with recombinant protein would provide direct evidence of the function of this gene.

Feeding experiments in cultures of *Claviceps* sp. with isotopically labelled precursors in the 1970s and 1980s indicated that chanoclavine-I aldehyde (**6**) is also an intermediate in the



Scheme 1 Common steps of ergot alkaloid biosynthesis in *Aspergillus fumigatus* and *Claviceps purpurea* (modified after Wallwey *et al.*[111]).

conversion of 4-DMA-L-abrine (**51**) to agroclavine (**8**).[13,91] Using purified recombinant protein after expression in *E. coli*, we have shown that FgaDH from *A. fumigatus* catalyses the conversion of chanoclavine-I (**3**) to its aldehyde (**6**) in the presence of NAD^+ and functions as a short-chain dehydrogenase/reductase (SDR).[138] However, FgaDH shows sequence similarity neither to known SDRs, nor to other known proteins in the database. Therefore, FgaDH represents a new group of SDRs.

Chanoclavine-I aldehyde (**6**) is the branch point for the biosynthetic pathways of ergot alkaloids in *Aspergillus* and *Claviceps*. It seems that an old yellow enzyme EasA (also termed FgaOx3) controls this branch point, at least for pathways in *A. fumigatus* and *C. purpurea*.[139,140] Disruption of *easA* in *A. fumigatus* resulted in the accumulation of chanoclavine-I (**3**) and chanoclavine-I aldehyde (**6**).[139] Augmentation of the *A. fumigatus* mutant with a homologue of *easA* from *C. purpurea* leads to production of ergot alkaloids typical for clavicipitaceous fungi, like agroclavine (**8**), setoclavine and isosetoclavine.[139]

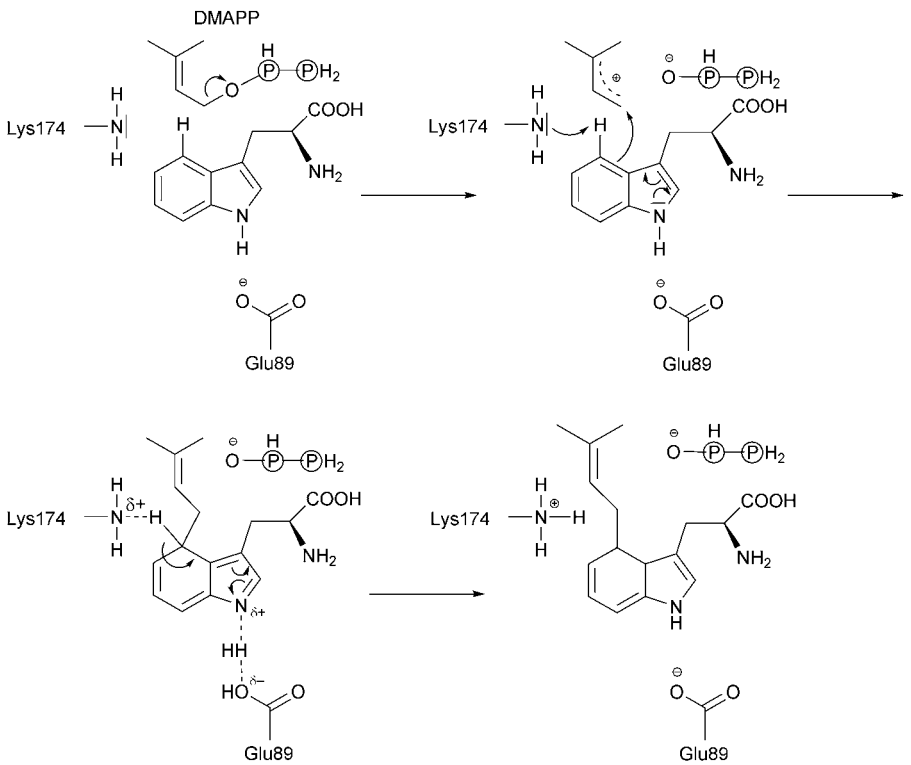
Cheng *et al.*[141] reported that EasA from *A. fumigatus* was involved in the formation of festuclavine (**10**), which was demonstrated by incubation of chanoclavine-I aldehyde (**6**) with EasA and subsequent reduction of the reaction mixture with $NaCNBH_3$. Using purified recombinant proteins, we have demonstrated that two enzymes, FgaOx3 and FgaFS, were necessary for the conversion of chanoclavine-I aldehyde (**6**) to festuclavine (**10**) in *A. fumigatus*.[111] The structure of festuclavine (**10**) including the stereochemistry was unequivocally elucidated by NMR and MS analyses. Festuclavine (**10**) formation was only observed when chanoclavine-I aldehyde (**6**) was incubated with FgaOx3 and FgaFS simultaneously, or as a tandem-reaction with a sequence of FgaOx3 before FgaFS.

Very recently, Cheng *et al.*[140] reported the conversion of chanoclavine-I aldehyde (**6**) to agroclavine (**8**) by EasA from *Neotyphodium lolii* and FgaFS, an orthologue of EasG, from *A. fumigatus.* The enzyme product was identified as agroclavine (**8**) by comparison of its behaviour in LC–MS with that of a standard. Results from feeding experiments with isotopically labelled precursors showed that an isomerisation of chanoclavine-I aldehyde (**6**) must take place before the formation of the D-ring.[142] Cheng *et al.* proposed that reversible reduction of the double bond would be involved in the EasA reaction.[140]

So far, only one gene from the gene clusters, *easC* or *fgaCat*, has not been assigned to a reaction step in the biosynthesis of ergot alkaloids. EasC/FgaCat shows significant homology to catalases. It has been also shown that EasC has catalase activity, and an *easC* deletion mutant produced no detectable ergot alkaloids.[4]

6.2 Biosynthesis of fumigaclavines

In comparison to festuclavine (**10**), the structure of fumigaclavine C bears an acetoxy group at position C9 and a reverse prenyl moiety at position C2. These substituents are also absent in the structures of ergoamides or ergopeptines (**24**). Therefore, comparison of the gene clusters from *C. purpurea* and *A. fumigatus* would help to find candidate genes involved in the conversion of festuclavine (**10**) to fumigaclavine C (**16**), because the responsible genes should only exist in the gene cluster of fumigaclavine C (**16**) in *A. fumigatus*. Considering the chemical reactions, three steps are necessary for this conversion, *i.e.* hydroxylation, acetylation and prenylation. Three genes in the cluster from *A. fumigatus*, *fgaP450-2*, *fgaAT* and *fgaPT1* (which



Scheme 2 Hypothetical reaction mechanism of FgaPT2 (modified after Metzger *et al.*[115]).

are absent in the cluster from *C. purpurea*, and encode a putative hydroxylase, a putative acetyltransferase and a putative prenyltransferase, respectively) are proposed to be responsible for these reactions.[143]

Gene cloning, expression and biochemical investigations demonstrated that FgaAT catalyses the acetylation of fumigaclavine B in the presence of acetyl-CoA, resulting in the formation of fumigaclavine A, which is then converted to fumigaclavine C by FgaPT1 in the presence of DMAPP (Scheme 3).[24,144]

We speculate that the cyctochrome P450 enzyme FgaP450-2 from *A. fumigatus* and its orthologue from *P. commune* would catalyse the conversion of festuclavine to fumigaclavine B. However, no experimental data is yet available for these enzymes.

6.3 Formation of D-lysergic acid

Agroclavine (**8**) is expected to be converted in *Claviceps* to lysergic acid, which serves as the acyl component of ergoamides and ergopeptines (**24**). It was proposed that agroclavine (**8**) would be converted *via* elymoclavine (**9**) to paspalic acid by two oxidation steps. The latter would be isomerized to lysergic acid (**2**) (Scheme 4).[145] Disruption of *cloA*, encoding a cytochrome P450 monooxygenase, abolished the ergopeptine production in the resulting deficient mutant. Agroclavine (**8**), elymoclavine (**9**) and chanoclavine (**3**) were instead detected by thin-layer chromatography. Feeding the mutant with D-lysergic acid (**2**) restored ergopeptine synthesis. Therefore, the authors concluded that CloA is involved in the conversion of elymoclavine (**9**) to paspalic acid (Scheme 4).[145] Expression of *cloA* from *Claviceps purpurea* in *C. fusiformis*, which accumulates only agroclavine (**8**) and elymoclavine (**9**) due to a non functional CloA protein, resulted in the formation of lysergic acid.[97] This experiment confirmed the function of CloA as elymoclavine oxidase. No information on the structural genes is available, which are responsible for the conversion of agroclavine (**8**) to elymoclavine (**9**) as well as that for paspalic acid to lysergic acid (**2**) (Scheme 4).

6.4 Biosynthesis of ergoamides and ergopeptines

C. purpurea produces both ergopeptines (**24**) and simple D-lysergic acid alkylamides (ergoamides). In the ergopeptines (**24**), D-lysergic acid (**2**) is linked to a tricyclic tripeptide in an amide-like fashion, whereas in the D-lysergylalkanolamides it is linked to an amino alcohol derived from alanine.

The biosynthetic gene cluster of ergot alkaloids in *C. purpurea* strain P1 contains four NRPS genes – *cpps1* (also called *lpsA1*), *cpps2* (*lpsB*), *cpps3* (*lpsC*) and *cpps4* (*lpsA2*).[96] *cpps1* and *cpps4* encode two trimodular lysergylpeptidyl synthetases – LPS1 (LpsA1) ($A_1TC_1A_2TC_2A_3TC_3$) and LPS4 (LpsA2) ($A_1TC_1A_2TC_2A_3TC_3$), while products of *cpps2* and *cpps3* are two monomodular NRPSs LPS2 (ATC) and LPS3 (ATCR, R: reduction),† respectively.[41,83]

To prove the function of *cpps1* (termed *lpsA1* in that study), gene inactivation experiments were carried out in *C. purpurea* strain P1.[42] Mutants lacking the *cpps1* gene did not accumulate ergotamine (**26**) carrying a tripeptide consisting of phenylalanine, alanine and proline, but still produce ergocryptine (**31** + **32**) with a tripeptidyl moiety of valine, proline and leucine or isoleucine, demonstrating that LPS1 (LpsA1), with a molecular weight of 370 kDa, is involved in the biosynthesis of ergotamine (**26**), but not ergocryptine (**31** + **32**). The authors concluded that the type of the ergopeptines (**24**) produced by a *Claviceps* strain is mainly determined by the specificity of the NRPSs, not by the composition of the amino acid pool.

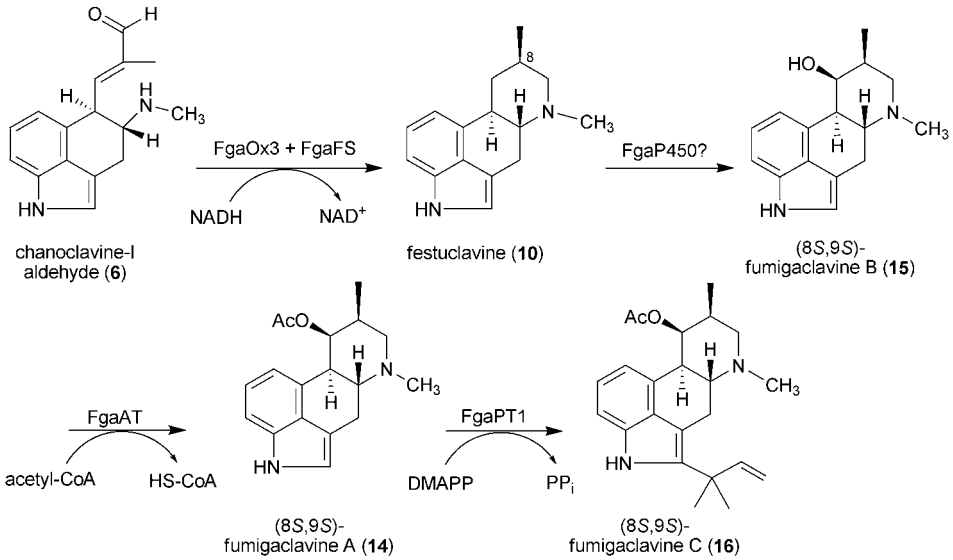
Transcriptional analysis, targeted inactivation, analysis of disruption mutants and heterologous expression revealed that LPS2 with a molecular weight of 140 kDa is responsible for D-lysergic acid (**2**) activation and incorporation into the ergopeptine (**24**) backbone.[41] A deletion mutant of *cpps2* of the producing strain *C. purpurea* P1 did not produce any ergopeptine (**24**) on solid or in liquid medium, but accumulated D-lysergic acid (**2**), which is absent in the extract of strain P1.[41] Using partially purified protein fraction after expression in *E. coli*, Correia *et al.*[41] reported that LPS2 activated only D-lysergic acid (**2**), but not tryptophan (**49**) or any of the amino acids present in the peptide moiety of ergopeptines (**24**).

It was proposed that D-lysergic acid (**2**) is activated by LPS2 and bound to the T-domain of LPS2. LPS1 (LpsA1) activates alanine, phenylalanine and proline by its A_1, A_2 and A_3-domains, respectively, and is transferred to the 4′-phosphopantetheinyl residues of the three T-domains.[41,83] D-Lysergic acid (**2**) is then progressively elongated to the D-lysergyl mono-, di-, and tripeptides by the trimodular LPS1. Enzyme-bound D-lysergyl tripeptide is finally released as lysergyl peptide lactam (L-ergopeptam (**25**)) (Scheme 5).[146] One further heterocyclisation step results in the formation of the final ergopeptine (**24**) product.[147] It was speculated that a cyctochrome P450 enzyme catalyses this reaction.[96,97]

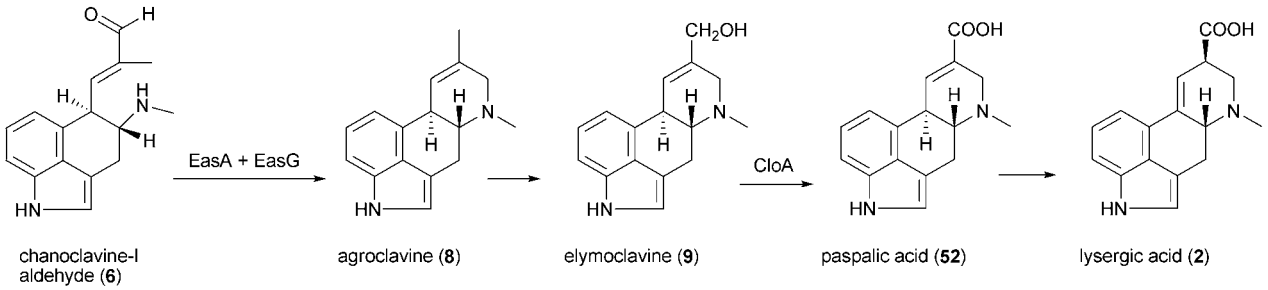
By monitoring the thioester formation with ^{14}C-labelled phenylalanine, alanine and dihydrolysergic acid, Ortel and Keller[83] identified three distinct activity peaks after gel filtration of a partially purified protein extract from the producer *C. purpurea* strain ECC93, which produces ergopeptines, *e.g.* **30** and **26**, as main components and ergometrine (**21**) as minor components of the alkaloid mixture. Protein peaks at molecular weights of about 400 kDa, 200 kDa and 150 kDa showed thiolation activity, activating phenylalanine, alanine and D-lysergic acid, respectively. These proteins corresponded well to the molecular masses of LPS1, LPS3 and LPS2, respectively. Ergopeptam (**25**) formation was found by combination of the fractions at 400 kDa and 150 kDa, while ergometrine (**21**) was synthesized by mixing of the fractions at 200 kDa and 150 kDa. The authors concluded that LPS2 activated D-lysergic acid not only for ergopeptines (**24**), but also for ergometrine (**21**). Furthermore, they proposed that the fraction at 200 kDa contained LPS3 with a molecular weight of 179 kDa, and that the reductase domain at the C-terminus of LPS3 is responsible for the formation of the amine component of ergometrine (**21**), *i.e.* 2-aminopropanol, from alanine. This conversion would take place in the presence of NADPH during the release of the D-lysergylalaninyl residue from LPS3.

Inconsistent with this hypothesis is that the *cpps3* gene of the ergometrine (**21**) producer *C. purpurea* strain ECC93 shows

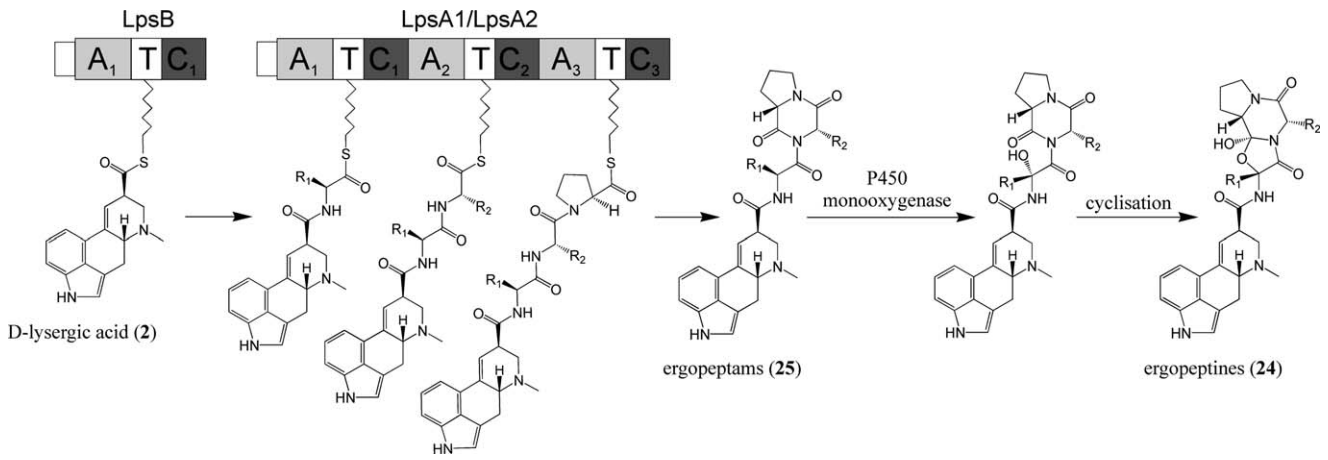
† A: adenylation, T: thiolation, C: condensation, R: reduction.



Scheme 3 Late steps of the biosynthesis of fumigaclavines in *Aspergillus fumigatus* (modified after Wallwey *et al.*[111]).



Scheme 4 Late steps of the biosynthesis of lysergic acid in *Claviceps purpurea* (modified after Haarmann *et al.*[145]).



Scheme 5 Formation of ergopeptines by LpsB and LpsA1 and LpsA2, respectively (modified after Haarmann *et al.*[4]).

a very high sequence identity (99%) to its orthologue in strain P1, which produces practically no ergometrine (**21**). The authors reported that *cpps3* transcript was detected by reverse PCR in the strain P1. In contrast, no hybridised signal was obtained from protein extract of strain P1 by using antibodies obtained with LPS3 from strain ECC93 in an immunoblot analysis.[83] The authors speculated that this would be caused by long-term selection for high production for ergopeptines (**24**). The authors suggested the disruption of both *lpsA1* (*cpps1*) and *lpsA2* (*cpps4*) genes in strain P1 and investigation of the ergometrine (**21**) synthesis in the absence of interfering LpsA-type enzymes.[83]

Homologous genes of *cpps1* and *cpps2* from *C. purpurea* strain P1, *lpsA* and *lpsB*, were also identified in the endophyte *Neotyphodium lolii* on Poaceae. The infected grasses accumulated an

ergopeptine, ergovaline (**28**), and an ergoamide lysergyl alanine.[48,148] To prove gene function, *lpsA* in *Neotyphodium lolii* and *lpsB* in its sexual progenitor *Epichloë festucae* were inactivated. The resulting mutants were used for subsequent infection of ryegrass plants. LC–MS analysis demonstrated that the *lpsA*- and *lpsB*-deficient mutants produced neither ergovaline (**28**) nor lysergyl alanine, but instead lysergic acid (**2**) and its stereoisomer, proving their roles in the biosynthesis of ergoamides and ergopeptines.[48,148]

7 Conclusion and outlook

Significant progress in the biosynthesis of ergot alkaloids has been achieved after identification of the biosynthetic gene clusters from different fungal strains. Almost all of the early steps in the biosynthesis have been clarified by molecular biological and biochemical investigations in the last five years.

Using the obtained genetic information, it should be possible to obtain ergot alkaloids with new structural features or to improve the yield of a desired compound by directed biosynthesis, *e.g.* by combinatorial biosynthesis or by feeding of synthetic precursors in blocked mutants (mutagenesis).

8 Acknowledgements

Work in the author's laboratory was supported by a grant from the Deutsche Forschungsgemeinschaft (Li844/3-1).

9 References

1 C. L. Schardl, D. G. Panaccione and P. Tudzynski, *Alkaloids: Chem. Biol.*, 2006, **63**, 45–86.
2 M. Flieger, M. Wurst and R. Shelby, *Folia Microbiol.*, 1997, **42**, 3–30.
3 D. Gröger and H. G. Floss, *Alkaloids: Chem. Biol.*, 1998, **50**, 171–218.
4 T. Haarmann, *et al.*, *Mol. Plant Pathol.*, 2009, **10**, 563–577.
5 A. N. de Groot, *et al.*, *Drugs*, 1998, **56**, 523–535.
6 M. R. Lee, *J. R. Coll. Physicians Edinbugh*, 2009, **39**, 179–184.
7 M. R. Lee, *J. R. Coll. Physicians Edinburgh*, 2009, **39**, 365–369.
8 K. Pedrosa and K. Grießler, Feed Intern., September–October 2010, pp. 28–30.
9 C. L. Schardl, A. Leuchtmann and M. J. Spiering, *Annu. Rev. Plant Biol.*, 2004, **55**, 315–340.
10 D. G. Panaccione, *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 12820–12825.
11 E. Leistner and U. Steiner, 'Fungal Origin of Ergoline Alkaloids Present in Dicotyledonous Plants (Convolvulaceae)', in *The Mycota XV: Physiology and Genetics*, Springer-Verlag, Berlin/Heidelberg, 2009, pp. 197–208.
12 H. G. Floss, *Tetrahedron*, 1976, **32**, 873–912.
13 H. G. Floss, *et al.*, *J. Am. Chem. Soc.*, 1974, **96**, 1898–1909.
14 R. M. Williams, E. M. Stocking and J. F. Sanz-Cervera, *Top. Curr. Chem.*, 2000, **209**, 97–173.
15 P. Tudzynski, *et al.*, *Mol. Gen. Genet.*, 1999, **261**, 133–141.
16 H. F. Tsai, *et al.*, *Biochem. Biophys. Res. Commun.*, 1995, **216**, 119–125.
17 I. A. Unsöld and S.-M. Li, *Microbiology*, 2005, **151**, 1499–1505.
18 C. M. Coyle, *et al.*, *Mycologia*, 2007, **99**, 804–811.
19 B. G. Katzug, 'Histamine, Serotonin and the Ergot Alkaloids', in *Basic and Clinical Pharmacology*, ed. B. G. Katzung, S. B. Masters, and A. J. Trevor, McGraw-Hill Medical, 2009, pp. 271–292.
20 A. Sinz, *Pharm. Unserer Zeit*, 2008, **37**, 306–309.
21 R. J. Cole and M. A. Schweikert, 'Indole Alkaloids', in *Handbook of secondary fungal metabolites*, Elsevier Science, 2003, pp. 1–143.
22 H. Pertz, *Planta Med.*, 1996, **62**, 387–392.
23 H. M. Ge, *et al.*, *J. Nat. Prod.*, 2009, **72**, 753–755.
24 I. A. Unsöld and S.-M. Li, *ChemBioChem*, 2006, **7**, 158–164.
25 J. C. Frisvad, *et al.*, *Med. Mycol.*, 2008, 1–19.
26 S. Ohmomo, M. Kaneko and P. Atthasampunna, *MIRCEN J. Appl. Microbiol. Biotechnol.*, 1989, **5**, 5–13.
27 K. Kawai, *et al.*, *Mycotoxins*, 1992, **36**, 19–24.
28 R. J. Cole, *et al.*, *Ann. Nutr. Aliment.*, 1977, **31**, 685–691.
29 Y. Zhao, *et al.*, *J. Pharm. Pharmacol.*, 2004, **56**, 775–782.
30 T. Möller, K. Åkerstrand and T. Massoud, *Nat. Toxins.*, 1997, **5**, 86–89.
31 M. O'Brien, *et al.*, *J. Agric. Food Chem.*, 2006, **54**, 9268–9276.
32 R. J. Cole, *et al.*, *J. Agric. Food Chem.*, 1983, **31**, 655–657.
33 H. Y. Ma, *et al.*, *Planta Med.*, 2006, **72**, 387–392.
34 X.-F. Wu, *et al.*, *Int. Immunopharmacol.*, 2005, **5**, 1543–1553.
35 P. W. van Dongen and A. N. de Groot, *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 1995, **60**, 109–116.
36 C. De Costa, *Lancet*, 2002, **359**, 1768–1770.
37 P. V. Rajan and D. A. Wing, *Clin. Obst. Gyn.*, 2010, **53**, 165–181.
38 M. R. Lee, *J. R. Coll. Physicians Edinburgh*, 2010, **40**, 77–80.
39 A. Hofmann, *LSD – My Problem Child*, McGraw-Hill, New York, 1980.
40 S. Uhlig and D. Petersen, *Toxicon*, 2008, **52**, 175–185.
41 T. Correia, *et al.*, *Chem. Biol.*, 2003, **10**, 1281–1292.
42 T. Haarmann, N. Lorenz and P. Tudzynski, *Fungal. Genet. Biol*, 2008, **45**, 35–44.
43 C. E. Robertson, D. F. Black and J. W. Swanson, *Semin. Neurol.*, 2010, **30**, 201–211.
44 R. Halker, B. Vargas and D. W. Dodick, *Semin. Neurol.*, 2010, **30**, 175–185.
45 J. R. Saper and S. Silberstein, *Headache*, 2006, **46**(Suppl. 4), S171–S181.
46 B. V. Lovell and M. J. Marmura, *Curr. Opin. Neurol.*, 2010, **23**, 254–258.
47 G. Jourdan, *et al.*, *Fundam. Clin. Pharmacol.*, 2007, **21**, 45–53.
48 D. J. Fleetwood, *et al.*, *Appl. Environ. Microbiol.*, 2007, **73**, 2571–2579.
49 P. L. Schiff, *Am. J. Pharm. Educ.*, 2006, **70**, 1–10.
50 I. Setnikar, *et al.*, *Arzneimittelforschung.*, 2001, **51**, 2–6.
51 W. Cabri, *et al.*, *Org. Process Res. Dev.*, 2006, **10**, 198–202.
52 U. Bonuccelli, P. Del Dotto and O. Rascol, *Parkinsonism Relat. Disord.*, 2009, **15S**, S44–S53.
53 P. Martinez-Martin and M. M. Kurtis, *Parkinsonism Relat. Disord.*, 2009, **15**(Suppl. 4), S58–S64.
54 M. P. Curran and C. M. Perry, *Drugs*, 2004, **64**, 2125–2141.
55 J. M. Cassady, *et al.*, *J. Med. Chem.*, 1974, **17**, 300–307.
56 P. Chanson, *et al.*, *Ann. Endocrinol. (Paris)*, 2007, **68**, 113–117.
57 S. Motazedian, *et al.*, *Indian J Med. Res.*, 2010, **131**, 670–674.
58 A. Colao, *et al.*, *Nat. Clin. Pract. Endocrinol. Metab.*, 2006, **2**, 200–210.
59 J. C. Frisvad, *et al.*, *Stud. Mycol.*, 2004, **49**, 201–241.
60 T. Furuta, M. Koike and M. Abe, *Agric. Biol. Chem.*, 1982, **46**, 1921–1922.
61 J. W. Dorner, *et al.*, *Appl. Environ. Microbiol.*, 1980, **40**, 685–687.
62 R. J. Cole, *et al.*, *Tetrahedron Lett.*, 1976, **17**, 3849–3852.
63 S. Yamatodani, *et al.*, *Agric. Biol. Chem.*, 1970, **34**, 485–487.
64 H. Tscherter and H. Hauth, *Helv. Chim. Acta*, 1974, **57**, 113–121.
65 G. S. King, *et al.*, *J. Chem. Soc., Perkin Trans. 1*, 1977, 2099–2103.
66 J. E. Robbers and H. G. Floss, *Tetrahedron Lett.*, 1969, **10**, 1857–1858.
67 A. G. Kozlovskii, *et al.*, *Dokl. Akad. Nauk SSSR*, 1981, **260**, 230–233.
68 J. C. Frisvad, *et al.*, *Med. Mycol.*, 2009, **47**, S53–S71.
69 V. P. Zhelifonova, *et al.*, *Microbiology (Moscow)*, 2009, **78**, 350–354.
70 N. G. Vinokurova, *et al.*, *Microbiology (Moscow)*, 2003, **72**, 149–151.
71 K. K. Janardhanan, A. Sattar and A. Husain, *Can. J. Microbiol.*, 1984, **30**, 247–250.
72 R. J. Cole, *et al.*, *J. Agric. Food Chem.*, 1977, **25**, 826–830.
73 D. G. Panaccione and C. M. Coyle, *Appl. Environ. Microbiol.*, 2005, **71**, 3106–3111.
74 J. F. Spilsbury and S. Wilkinson, *J. Chem. Soc.*, 1961, 2085–2091.
75 N. G. Vinokurova, *et al.*, *Appl. Biochem. Microbiol.*, 1991, **27**, 644–648.
76 N. G. Vinokurova, L. V. Boichenko and M. U. Arinbasarov, *Appl. Biochem. Microbiol.*, 2003, **39**, 403–406.

77 L. V. Boichenko, *et al.*, *Appl. Biochem. Microbiol.*, 2003, **39**, 294–299.
78 G. T. Banks, P. G. Mantle and C. A. Szczyrbak, *J. Gen. Microbiol.*, 1974, **82**(Pt. 2), 345–361.
79 S. Pažoutová, *et al.*, *J. Nat. Prod.*, 1981, **44**, 225–235.
80 B. Schumann, *et al.*, *Planta Med.*, 1982, **45**, 11–14.
81 V. Kren, *et al.*, *FEMS Microbiol. Lett.*, 1986, **37**, 31–34.
82 N. Lorenz, *et al.*, *Phytochemistry*, 2009, **70**, 1822–1932.
83 I. Ortel and U. Keller, *J. Biol. Chem.*, 2009, **284**, 6650–6660.
84 C. L. Schardl, B. Scott, S. Florea and D. Zhang, '*Epichloë* Endophytes: Clavicipitaceous Symbionts of Grasses', in *The Mycota V: Plant Relationships*, Springer-Verlag, Berlin/Heidelberg, 2009, pp. 275–306.
85 S. Kucht, *et al.*, *Planta*, 2004, **219**, 619–625.
86 A. Markert, *et al.*, *Plant Physiol.*, 2008, **147**, 296–305.
87 M. A. Ahimsa-Müller, *et al.*, *J. Nat. Prod.*, 2007, **70**, 1955–1960.
88 C. L. Schardl, *Fungal. Genet. Biol.*, 2001, **33**, 69–82.
89 A. A. Bacetty, *et al.*, *Phytopathology*, 2009, **99**, 1336–1345.
90 S. H. Faeth, C. J. Hayes and D. R. Gardner, *Plant–Microbe Interact.*, 2010, DOI: 10.1007/s00248-010-9643-4.
91 S. B. Hassam and H. G. Floss, *J. Nat. Prod.*, 1981, **44**, 756–758.
92 H. G. Floss and J. A. Anderson, 'Biosynthesis of Ergot Toxins', in *The biosynthesis of mycotoxins. A study in secondary metabolism*, ed. S. S. Pieter, 1980, pp. 17–67.
93 D. Hoffmeister and N. P. Keller, *Nat. Prod. Rep.*, 2007, **24**, 393–416.
94 M. Luzhetska, J. Härle and A. Bechthold, *Fortschr. Chem Org. Naturst.*, 2010, **93**, 211–237.
95 A. Osbourn, *Trends Genet.*, 2010, **26**, 449–457.
96 T. Haarmann, *et al.*, *Phytochemistry*, 2005, **66**, 1312–1320.
97 N. Lorenz, *et al.*, *Appl. Environ. Microbiol.*, 2007, **73**, 7185–7191.
98 C. T. Walsh and M. A. Fischbach, *J. Am. Chem. Soc.*, 2010, **132**, 2469–2493.
99 D. Stack, C. Neville and S. Doyle, *Microbiology*, 2007, **153**, 1297–1306.
100 H. von Döhren, *Adv. Biochem. Eng. Biotechnol.*, 2004, **88**, 217–264.
101 L. Du and L. Lou, *Nat. Prod. Rep.*, 2010, **27**, 255–278.
102 I. A. Unsöld, *Dissertation Universität Tübingen*, 2006.
103 M. G. Jones, *Microbiology*, 2007, **153**, 1–6.
104 N. P. Keller, G. Turner and J. W. Bennett, *Nat. Rev. Microbiol.*, 2005, **3**, 937–947.
105 C. Corre and G. L. Challis, *Nat. Prod. Rep.*, 2009, **26**, 977–986.
106 W. C. Nierman, *et al.*, *Nature*, 2005, **438**, 1151–1156.
107 S.-M. Li, *Phytochemistry*, 2009, **70**, 1746–1757.
108 N. Steffan, *et al.*, *Curr. Med. Chem.*, 2009, **16**, 218–231.
109 C. M. Coyle and D. G. Panaccione, *Appl. Environ. Microbiol.*, 2005, **71**, 3112–3118.
110 S.-M. Li, *Nat. Prod. Rep.*, 2010, **27**, 57–78.
111 C. Wallwey, *et al.*, *Org. Biomol. Chem.*, 2010, **8**, 3500–3508.
112 J. Wang, *et al.*, *Fungal Genet. Biol.*, 2004, **41**, 189–198.
113 N. Steffan, I. A. Unsöld and S.-M. Li, *ChemBioChem*, 2007, **8**, 1298–1307.
114 N. Steffan and S.-M. Li, *Arch. Microbiol.*, 2009, **191**, 461–466.
115 U. Metzger, *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 14309–14314.
116 Y. Ding, R. M. Williams and D. H. Sherman, *J. Biol. Chem.*, 2008, **283**, 16068–16076.
117 A. Kremer and S.-M. Li, *Microbiology*, 2010, **156**, 278–286.
118 P. H. Liang, T. P. Ko and A. H. Wang, *Eur. J. Biochem.*, 2002, **269**, 3339–3354.
119 K. Yazaki, K. Sasaki and Y. Tsurumaru, *Phytochemistry*, 2009, **70**, 1739–1745.
120 Y. Ding, *et al.*, *J. Am. Chem. Soc.*, 2010, **132**, 12733–12740.
121 W.-B. Yin, J. Cheng and S.-M. Li, *Org. Biomol. Chem.*, 2009, **7**, 2202–2207.
122 S.-M. Li, *Appl. Microbiol. Biotechnol.*, 2009, **84**, 631–639.
123 W.-B. Yin, *et al.*, *Org. Biomol. Chem.*, 2010, **8**, 1133–1141.
124 W.-B. Yin, *et al.*, *Org. Biomol. Chem.*, 2010, **8**, 2430–2438.
125 T. Kuzuyama, J. P. Noel and S. B. Richard, *Nature*, 2005, **435**, 983–987.
126 Y. Haagen, *et al.*, *FEBS Lett.*, 2007, **581**, 2889–2893.
127 F. Pojer, *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 2316–2321.
128 O. Saleh, *et al.*, *J. Biol. Chem.*, 2009, **284**, 14439–14447.
129 S. Keller, *et al.*, *Acta Crystallogr., Sect. F: Struct. Biol. Cryst. Commun.*, 2006, **62**, 1153–1155.
130 E. Stec, *et al.*, *ChemBioChem*, 2008, **9**, 2055–2058.
131 L. Y. Luk and M. E. Tanner, *J. Am. Chem. Soc.*, 2009, **131**, 13932–13933.
132 M. Liu, D. G. Panaccione and C. L. Schardl, *Evol. Bioinform.*, 2009, **5**, 15–30.
133 O. Rigbers and S.-M. Li, *J. Biol. Chem.*, 2008, **283**, 26859–26868.
134 R. M. Kagan and S. Clarke, *Arch. Biochem. Biophys.*, 1994, **310**, 417–427.
135 P. Z. Kozbial and A. R. Mushegian, *BMC Struct. Biol.*, 2005, **5**, 19–44.
136 M. Abe, *et al.*, *Agric. Biol. Chem.*, 1969, **33**, 469–471.
137 N. Lorenz, *et al.*, *Appl. Environ. Microbiol.*, 2010, **76**, 1822–1830.
138 C. Wallwey, M. Matuschek and S.-M. Li, *Arch. Microbiol.*, 2010, **192**, 127–134.
139 C. M. Coyle, *et al.*, *Appl. Environ. Microbiol.*, 2010, **76**, 3898–3903.
140 J. Z. Cheng, *et al.*, *J. Am. Chem. Soc.*, 2010, **132**, 12835–12837.
141 J. Z. Cheng, *et al.*, *J. Am. Chem. Soc.*, 2010, **132**, 1776–1777.
142 H. Plieninger, *et al.*, *Justus Liebigs Ann. Chem.*, 1978, **1978**, 813–817.
143 S.-M. Li and I. A. Unsöld, *Planta Med.*, 2006, **72**, 1117–1120.
144 X. Liu, *et al.*, *ChemBioChem*, 2009, **10**, 2325–2328.
145 T. Haarmann, *et al.*, *ChemBioChem*, 2006, **7**, 645–652.
146 B. Walzel, B. Riederer and U. Keller, *Chem. Biol.*, 1997, **4**, 223–230.
147 F. R. Quigley and H. G. Floss, *J. Org. Chem.*, 1981, **46**, 464–466.
148 D. G. Panaccione, *et al.*, *J. Agric. Food Chem.*, 2003, **51**, 6429–6437.

