CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200800292

Chemical Defence Strategies of Higher Fungi

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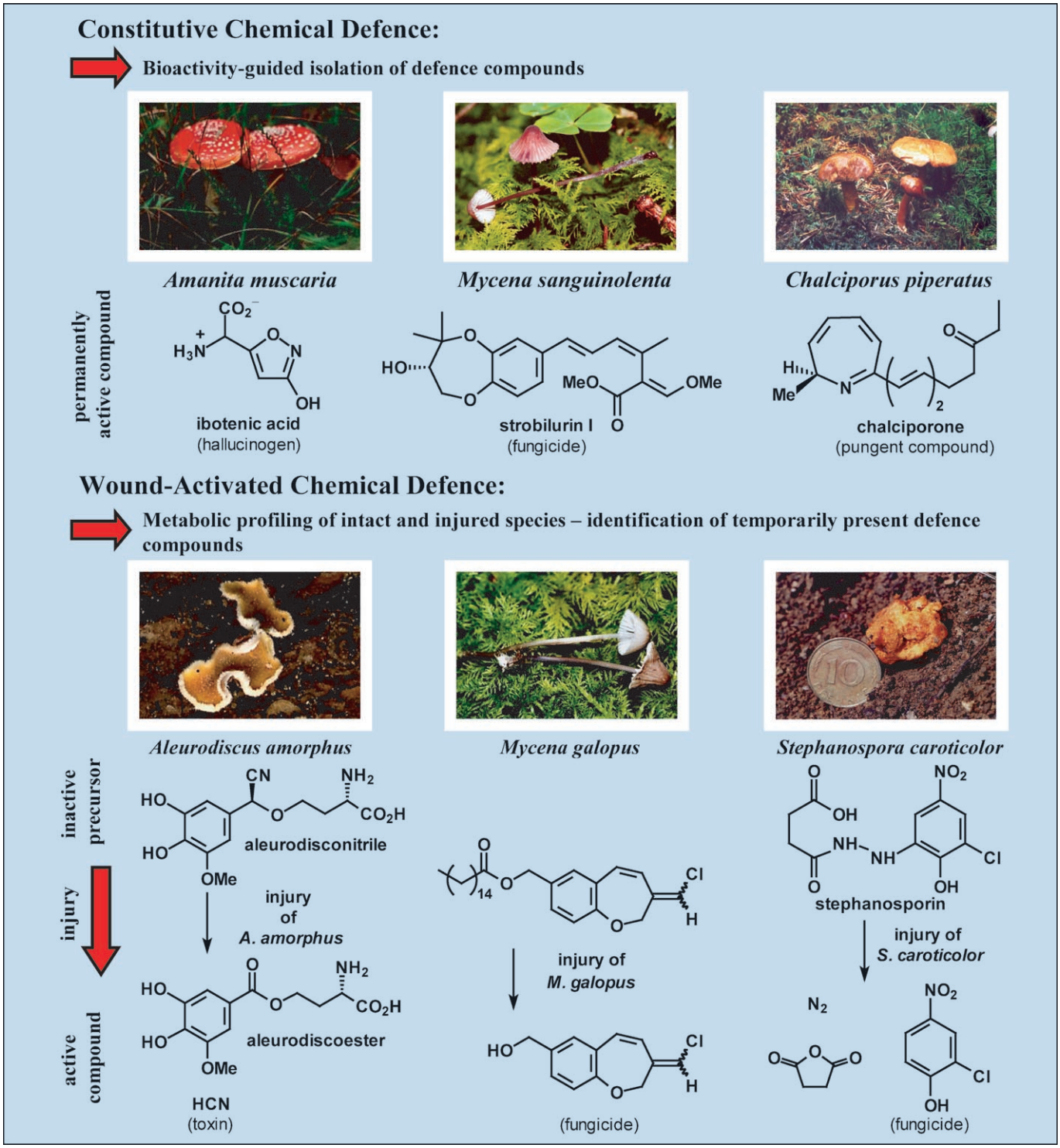
Dedicated to Professor Wolfgang Steglich on the occasion of his 75th birthday



Abstract: Like plants, fungi have evolved a variety of defence strategies in order to protect themselves against feeding mammals, insects and infection with parasitic fungi. In contrast to plants little is known on the chemical ecology of fruiting bodies of higher fungi, particularly those defence mechanisms which are induced upon wounding have only occasionally been recognised. Methods both for the detection of permanently present defence compounds and for the elucidation of wound-activated chemical defence mechanisms are discussed in this concept paper.

Keywords: bioorganic chemistry · chemical ecology · fungi · metabolism · natural products

Introduction

The inability of immobile organisms such as plants and higher fungi to escape from attack by herbivores or fungivores has lead to the evolution of a number of defence strategies to deter enemies. While the chemical ecology of a variety of plants including their chemical defence has already been studied in detail,[1] the chemical ecology of most higher fungi[2] including their fruiting bodies has not been investigated systematically so far. Rather, investigations on secondary metabolites from fungi have mostly been aiming to isolate bioactive compounds as potential lead structures for the development of new drugs or agents for crop protection.[3] During these investigations higher fungi have turned out to be a rich source of bioactive secondary metabolites.

Nowadays secondary metabolites—including those of higher fungi—are considered to be produced intentionally to provide the producer an advantage.[4] Both the mycelia and the fruiting bodies of higher fungi are exposed to various competitors and enemies. While the mycelia have to compete with other fungi and bacteria for nutrition and space,[5] the fruiting bodies are mainly endangered by mycoparasitic fungi and fungivores[6] ranging from insects to mammals including humans. For instance, fungal spores have been identified in the feces of game animals such as the roe deer, thus proving that these animals regularly consume mushrooms.[7] Although the fruiting bodies of higher fungi are usually short-lived entities, they are important for the reproduction since they produce spores.[8] Consequently, it can be expected that the reproduction of fungi is improved if the fruiting bodies are protected at least until the spores are fully developed.

Fruiting bodies of fungi often produce toxins and pungent or bitter compounds in order to deter fungivores. For mammals it has been shown that they learn to avoid these species even if intoxications occurred not immediately after ingestion.[9] In contrast, the mycelia of several saprophytic fungi contain fungicides to secure nutrition and space to themselves.[5]

Even if the ecological role of many fungal secondary metabolites is still obscure, the existing investigations clearly demonstrate that chemical defence strategies are not only used by plants but also by fungi. So far, at least three types of chemical defence mechanisms are known. Constitutive chemical defence mechanisms are based on secondary metabolites that are present permanently in their bioactive form. In contrast, wound-activated defence mechanisms rely on the enzymatic conversion of an inactive precursor to the active agent which occurs only transiently upon activation by injury.[10] Induced chemical defence mechanisms involve the de novo synthesis of bioactive compounds which is initiated on demand.[10]

Representative examples for the different types of chemical defence in fungi together with the underlying concept for their elucidation are presented in this concept article. The comparative metabolic screening of intact and wounded species has recently turned out to be a promising approach for the detection of wound-activated chemical defence mechanisms in this context.

Constitutive Chemical Defence

The elucidation of constitutive chemical defence mechanisms usually requires the observation of a response of an organism against its potential enemies. In general, bioactive compounds can be isolated by means of a bioassay-guided fractionation of extracts of the corresponding organism. After the structure elucidation of a bioactive compound, its potential ecological role may be evaluated by analysing its activity towards appropriate test organisms.

The means for the isolation, structure elucidation and the testing of compounds were mainly developed during the last hundred years. During the past century natural products research mainly concentrated on the isolation of bioactive constituents from plants,[11] while even the structures of important fungal toxins had not been elucidated by the middle of the last century.

Toxins as constitutive chemical defence: Even in ancient times some toxic, bitter or pungent properties directly linked to the chemical defence of certain mushrooms were known and exploited. For instance, the Roman emperor Claudius was probably poisoned with fruiting bodies of the death cap (*Amanita phalloides*) by his own wife, Agrippina,

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in the year 54.[12] The fruiting bodies of *A. phalloides*, the most poisonous toadstool in Europe, contain a variety of cyclic octapeptides, such as α-amanitin (**1**) which are responsible for its toxicity (Figure 1).[13] The toxicity of the amanitins is caused by an allosteric inhibition of the human RNA polymerase II.[14]

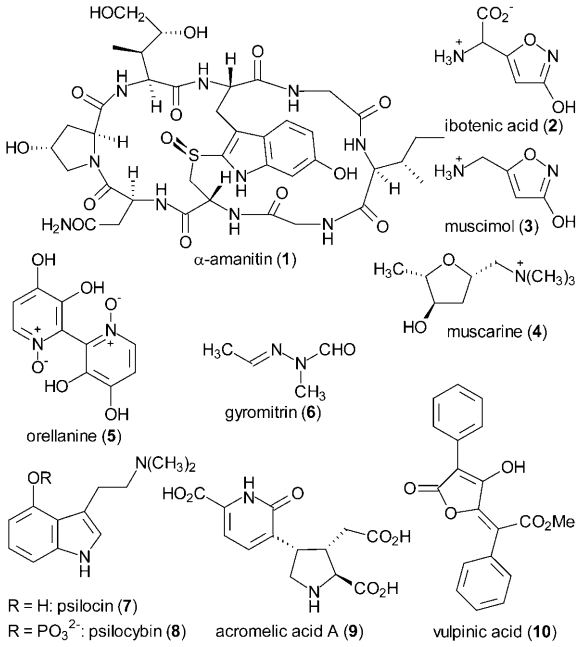


Figure 1. Toxins from fruiting bodies of macrofungi as constitutive defence compounds.

Another prominent mushroom containing constitutive defence compounds is the fly agaric (*Amanita muscaria*) which has been used for centuries as hallucinogenic drug.[15] The name fly agaric (German: Fliegenpilz, French: amanite tuemouches) points to its insecticidal properties. Konrad von Megenberg reported as early as the year 1350 that a mixture of milk and pieces of the fly agaric can be used to kill flies.[16] These are stunned and drown subsequently in the milk. The toxic constituents of the mushroom were found to be ibotenic acid (**2**), muscimol (**3**) and muscarine (**4**).[15] While ibotenic acid interferes with the glutamic acid receptor, muscimol as structural analogue of γ-aminobutyric acid (GABA) binds to the GABA receptor.[15] Consequently, ibotenic acid and muscimol act as neurotoxins leading to confusion and dizziness.

In contrast to the well-known toxic properties of *A. phalloides* and *A. muscaria*, the deleterious effects of fruiting bodies of *Cortinarius orellanus* and *Cortinarius rubellus* were not recognised until the 1950s when a mass intoxication in Poland caused several casualties.[17] Probably, these toadstools had not been identified earlier as cause of the intoxication, since their ingestion leads to renal failure which only becomes evident after several days.[18] In rats, ingestion both of lethal and sublethal doses of orellanine led to symptoms of sickness already after several hours, while lethal doses caused death after three to five days.[19] The structure of the toxic principle, orellanine (**5**), was elucidated in 1979.[20] However, until recently, it had been overlooked that orellanine is present in form of its 4,4′-diglucoside in *C. rubellus*.[21] The glucoside is relatively unstable and it was evidently hydrolysed during the original work-up procedure.

Other known fungal toxins are gyromitrin (**6**)[22] occurring in *Gyromitra esculenta*, the hallucinogenic compounds psilocin (**7**) and psilocybin (**8**)[23] present in many *Psilocybe* species, acromelic acid A (**9**)[24] from *Clitocybe acromelalga* and vulpinic acid (**10**) from *Pulveroboletus ravenelii*.[25] Vulpinic acid, also occurs in the lichen *Letharia vulpina* which was used to kill foxes and wolves.[25]

Even today, mushroom species which were previously considered to be edible can turn out to be dangerous. For instance, *Tricholoma equestre* was considered to be a delicious species among edible mushrooms until 2001. However, in that year two casualties were reported from France which were linked to repeated ingestion of *T. equestre*.[18,26] So far, the toxic principle of this species remains unknown.

Although there have not been many investigations of the efficacy of toxins in protecting fruiting bodies of fungi from feeding animals, studies with the fungivorous opossum *Didelphis virginiana* provided evidence that this animal learns to avoid toxic fungi, such as the muscimol (**3**) containing *A. muscaria*.[27]

Bitter and pungent compounds as constitutive chemical defence: Aside from toxins, bitter or pungent tasting compounds are widespread in the fruiting bodies of many different genera of fungi (Figure 2) where they obviously serve fungi to deter predators. In general, not only humans but also many mammals consider the taste of those compounds to be unpleasant. For instance, the fungivorous opossum avoids most species which exhibit a bitter and pungent taste to humans.[27] The characteristic bitter taste of *Boletus calopus* and *Boletus radicans* is mainly caused by the sesquiterpenoid *O*-acetylcyclocalopin A (**11**).[28] The fungus *Sarcodon scabrosus* contains several bitter diterpenes, such as sarcodonin A (**12**).[29] Ganoderic acids A (**15**) and B are bitter triterpenes from the *Ganoderma lucidum*.[30] Bitter taste is not restricted to terpenes. For instance, the bitter taste of *Tricholoma lascivum* is attributed to the cyclohexenone derivative lascivol (**13**).[31] Bitterness is also characteristic of many alkaloids, for example, the indole alkaloid infractopicrin (**14**) is responsible for the highly bitter taste of *Cortinarius infractus*.[32]

The fruiting bodies of *Chalciporus piperatus* (German: Pfefferröhrling) exhibit a pungent, peppery taste which has been attributed to the azepine alkaloid chalciporone (**16**).[33] In particular, many *Lactarius* and *Russula* species are known to develop a pungent taste a few seconds after injury originating from sesquiterpenoid compounds. However, these pungent compounds are only produced upon injury and will therefore be discussed later.

Despite at least thirty years of research of flavour components from mushrooms, the bitter or pungent principles of

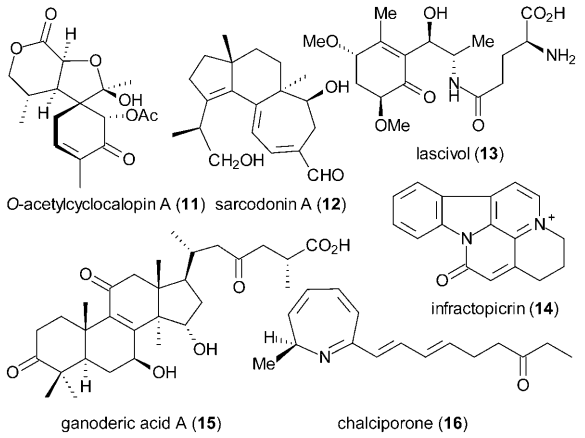


Figure 2. Selected bitter and pungent compounds from fruiting bodies of macrofungi.

some common species are still unknown. For instance, this is so for the compound responsible for the bitter taste of the inedible fruiting bodies of *Tylopilus felleus* which is often confused with the delicious mushroom *Boletus edulis*.

Pigments of fruiting bodies: Fruiting bodies of fungi are often very colourful, which attracted the interest of chemists early on.[34] The investigations on these pigments revealed that they represent a huge variety of different compounds[34] including alkaloids,[35] aromatics,[36] polyenes[37] and even metal complexes.[38] However, the ecological role of these compounds is probably linked rather to their bioactivity than to their colour, although the bright colours of some mushrooms might be interpreted as a warning signal. For instance, yellow and red anthraquinone pigments are known to exhibit laxative properties[39] thus repelling animals. Red pyrroloquinoline alkaloids occurring in several *Mycena* species possess an *ortho*-quinone moiety which is able to react with amine residues in proteins.[40] However, in general the ecological role of various pigments still remains obscure.

Fungicides: In the examples presented so far, isolation of secondary metabolites from fruiting bodies of mushrooms was based on the observation of certain properties (e.g. taste or toxicity) in their natural habitat. The detection of the antibiotic activity of the penicillins from the microfungus *Penicillium notatum* in the year 1928 had a great impact on natural product research in many respects.[41] Firstly, the experience obtained with the cultivation of bacteria[41] was also successfully applied to develop cultivation methods for plant cells[42] and the mycelia of macrofungi.[43] The cultivation of mycelial cultures of macrofungi made it possible to perform experiments throughout the whole year in contrast to investigations with fruiting bodies whose seasonally-dependent occurrence in nature is unreliable. Secondly, more and more microorganisms became available for bioactivity testing.[41] Hence, the bioactivity of fungal extracts and isolated new compounds could now be evaluated for a large panel of test organisms.[41] Typically, those tests were directed towards the isolation of compounds suitable as new lead structures for the development of drugs[41] and not towards the elucidation of the ecological role of secondary metabolites from higher fungi. Nevertheless, screening of natural products with respect to a diverse panel of test organisms is often also useful to decipher their original ecological roles.

Despite these advancements, a systematic cultivation and bioactivity-guided screening of macrofungi, such as basidiomycetes, was only begun thirty years ago, since their cultivation is often hampered due to their slow growth rate.[43]

On account of the differing functions of fruiting bodies and mycelia, mycelial cultures of a fungus often generate different natural products than the corresponding fruiting bodies.[6] Hence, the screening of mycelial cultures delivered a great variety of new bioactive natural products. One of the most prominent examples is the detection of the strobilurins, a class of antifungal compounds which was isolated first from mycelial cultures of the basidiomycete *Strobilurus tenacellus*.[44] The strobilurins, such as strobilurin A (**17**)[45] or strobilurin I (**18**),[46] contain a β-methoxy acrylate moiety as pharmacophore (Figure 3).

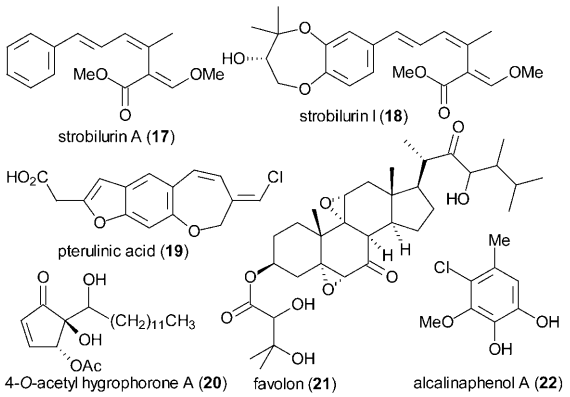


Figure 3. Selected fungicides from cultures and fruiting bodies of macrofungi.

The strobilurins are able to bind reversibly to the ubihydroquinone oxidation centre (Qp) of the cytochrome bc_1 complex of fungi, thus inhibiting the electron transfer in the fungal respiratory chain.[47] On account of their potent antifungal activity, the strobilurins served as lead structures for several commercially available products such as kresoxim-methyl and azoxystrobin which are currently broadly used as agricultural fungicides.[48]

In agreement with their bioactivity, investigations on the ecological role of the strobilurins demonstrated that *S. tenacellus* (German: Kiefernzapfenrübling), is able to prevent the growth of other fungi on the pine cone where the fungus grows.[5]

Apart from the strobilurins, a number of other fungicides, such as pterulinic acid (**19**),[49] 4-*O*-acetyl hygrophorone A (**20**),[50] favolon (**21**)[51] or alcalinaphenol A (**22**),[52] have been isolated from mycelial cultures or fruiting bodies of higher fungi (Figure 3). Obviously, fungicides are wide-

spread particularly in saprophytic fungi. The release of fungicides from their mycelia into their growth substrate means that they are able to prevent other species from growing in their surrounding, thus ensuring that other species do not compete with them for nutrients and space.

Both the detection of a new lead structure and the elucidation of a new chemical defence mechanism in higher fungi usually require a biological screening with appropriate test organisms. In contrast, the detection of bioactive compounds possessing a known structural element responsible for a certain bioactivity is sometimes also possible by a GC-MS or LC-MS screening. For instance, chlorinated phenols usually possess antifungal properties and exhibit characteristic EI mass spectra. Accordingly, a GC-MS screening of trimethylsilylylated methanolic extracts from different *Mycena* species led to the identification of new chlorinated phenols, such as alcalinaphenol A (**22**), from mycelial cultures of *Mycena alcalina*.[52]

Wound-Activated Chemical Defence

Since wound-activated chemical defence mechanisms are based on the activation of enzymes which convert an inactive precursor to the active agent, the elucidation of a wound-activated defence mechanism requires not only the elucidation of the chemical structure of the active compound but also of its inactive precursor, together with the conversion mechanism and the mode of action of the active agent towards potential parasites or fungivores.

History: It has been known for centuries that many mushrooms react with a change of their colour,[53] taste or odour, if they are mechanically injured. Especially colour changes, for instance, the blueing of certain boletes attracted the interest of chemists very early[53] and led to the elucidation of the structures of many mushroom pigments.[34] However, often the structure of the corresponding precursor remained unknown and a potential role of these compounds in the chemical defence was usually not recognised. Similarly, bioactivity-driven investigations on new compounds ended in many cases when the structure and the activity of the natural product had been elucidated. In contrast, the presence of the inactive precursor in the intact organism often remained unknown, mainly for two reasons. Firstly, a compound which does not show biological activity usually escapes detection, secondly it was often not recognised in the past that the enzymes which are responsible for the conversion of an inactive compound to an active one are activated instantly after injury during the work-up procedure. Therefore, even today only a limited number of examples are known of wound-activated defence mechanisms from higher fungi and many of these mechanisms were more or less detected by accident, for instance, when a known bioactive compound was missing in intact fungi. This is exemplified by the detection of a large number of sesquiterpene aldehydes and alcohols isolated from many *Lactarius* species in the 1970s (Scheme 1).[54] Reports in the 1980s, of qualitative and quantitative differences in the sesquiterpene content and on the occurrence of compounds which were considered to be extraction artefacts led to a reinvestigation of the sesquiterpenes of *Lactarius vellereus*.[55] An analysis of the extracts of intact and artificially wounded fruiting bodies by thin layer chromatography revealed significant differences in their metabolite profiles.[55] While the then unknown precursor stearoylvelutinal (**29**) (Scheme 1) turned out to be the predominant sesquiterpene present in intact fruiting bodies, the bioactive sesquiterpene aldehydes and alcohols only occurred in injured fruiting bodies.[55]

These observations had a considerable impact on the research of fungal natural products, since it demonstrated unambiguously that wound-activated chemical defence is not restricted to plants but also occurs in fungi. Consequently, many other *Lactarius* and *Russula* species were investigated for the presence of wound-activated chemical defence mechanisms.[56] However, systematic screening of large numbers of different fruiting bodies for wound-induced chemical defence mechanisms did not start until the 1990s.

Methods for the elucidation of wound-activated defence mechanisms: In general two methods are applicable both of which utilise the differences between intact and injured fruiting bodies.

The first method is based on comparison of the bioactivity of extracts from intact and injured fruiting bodies. Only species which show an increased bioactivity in the extracts of wounded fruiting bodies compared with those of intact ones are likely to contain compounds involved in a wound-activated chemical defence mechanism. So far, a large number of injured and intact fruiting bodies of fungi have been investigated for antifungal, antibiotic, nematicidal and insecticidal activity and several species have been identified which exhibit an increased bioactivity upon wounding.[6] However, this comparative biological screening only reveals the existence of wound-activated chemical defence mechanisms, but not the responsible compounds or the mechanism of the chemical defence. The bioactive agents can be identified by subsequent bioactivity-guided fractionation of the crude extract, but the inactive precursors still remain unknown.

In contrast to the bioactivity-guided comparative screening, the second method for the elucidation of wound-activated chemical defence mechanisms, namely comparative metabolic screening, is based on a comparison of the metabolite pattern of extracts from intact and injured fruiting bodies.[57] Secondary metabolites which are only present in significant amounts in injured fruiting bodies are potential candidates for defence compounds, while secondary metabolites which are only present in intact fruiting bodies might be their potential precursors. In contrast to the comparative biological screening of crude extracts, the comparative metabolic screening allows often recognition of the relationship between precursor and the corresponding defence compound, especially if the metabolic screening is performed by LC-UV, LC-ESIMS or GC-EIMS, since the precursor and the

corresponding active metabolite usually share structural elements which are reflected in similar UV spectra, MS/MS spectra or fragmentation patterns in the GC-EIMS. A recent example is the elucidation of the chemical defence mechanism of *Mycena galopus* (Figure 4).[57]

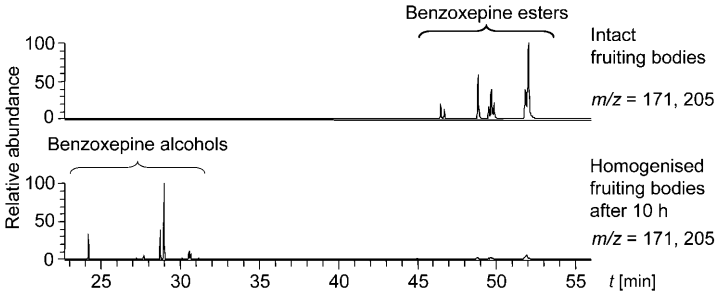


Figure 4. Comparative metabolic screening by GC-MS of trimethylsilylated methanolic extracts from intact and injured fruiting bodies of *Mycena galopus*.

The physiologically active benzoxepine alcohols such as **25** and (*E*/*Z*)-**26** were only present in injured fruiting bodies, while the corresponding inactive esters such as **23** and (*E*/*Z*)-**24** mainly occurred in intact fruiting bodies.[57] The close relationship of both compounds was easily recognisable since the EI spectra of the benzoxepine esters **23** and (*E*/*Z*)-**24** and of the trimethylsilyl derivatives **27** and (*E*/*Z*)-**28** of the benzoxepine alcohols exhibit the same key fragments (Figure 5).

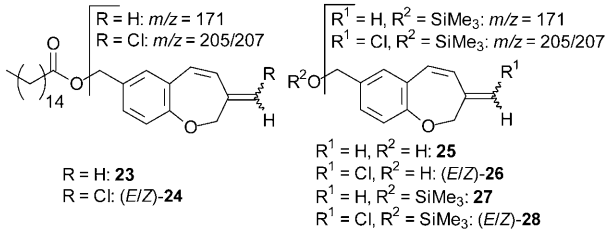


Figure 5. Key ions in the EI-MS of benzoxepines from *Mycena galopus*.

The comparative metabolic screening had already been used in the 1970s and 1980s, for instance, to elucidate the chemical defence mechanism of *L. vellereus* by comparison of thin layer chromatograms. However, the comparative metabolic screening became only recently a very sensitive and powerful tool for the elucidation of wound-activated chemical defence mechanisms as a result of the development of new analytical methods, such as sensitive LC-ESI mass spectrometry. Hence, now only small fragments of a fruiting body are required to detect differences in the metabolite pattern of intact and injured species. Moreover, sensitive LC-HR-ESIMS instruments now permit the direct determination of the molecular composition of whole molecules and of key fragments, thus confirming the relationship of a potential precursor and the corresponding bioactive defence compound. LC-NMR might also be applied in the future, but so far the method is expensive and not very efficient due to the relatively low sensitivity of NMR instruments compared to that of mass spectrometers.

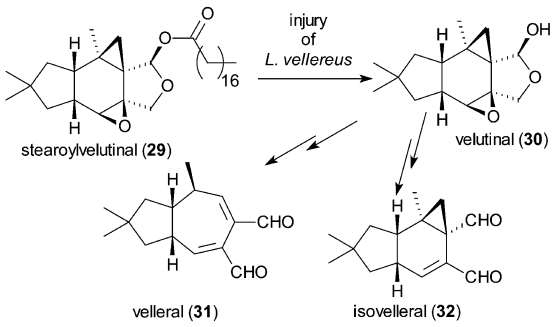
Although comparative metabolic screening allows the detection of the conversion of a compound to another one upon wounding additional bioactivity tests are required to confirm that an inactive precursor is transformed to a compound which exhibits activity against potential parasites or predators.

Classification of wound-activated defence mechanisms: In general, wounding of any organism leads to activation of a number of different types of enzymes. This is also the case with fruiting bodies of mushrooms. Three classes of enzymes in particular play an important role: Hydrolytic enzymes (esterases, proteases or glucosidases), phenol oxidases and lipoxygenases. As a consequence, there are three different main types of wound-activated chemical defence strategies known for fungi:

- hydrolysis of esters,
- oxidation of phenols and
- lipid peroxidation

Hydrolysis of esters: Wound-activated defence mechanisms based on the hydrolysis of esters are widespread among many fungi.

As already mentioned many *Lactarius* and *Russula* species contain stearoylvelutinal (**29**). Upon injury, **29** is saponified and the instable free velutinal (**30**) is transformed to pungent tasting aldehydes such as velleral (**31**), isovelleral (**32**) or the corresponding alcohols vellerol and isovellerol (Scheme 1).[55]



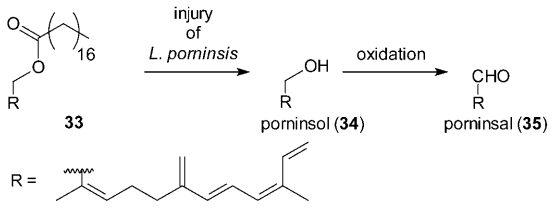
Scheme 1. Wound-activated chemical defence mechanism of *Lactarius vellereus*.

The efficacy of this defence mechanism was demonstrated in a study with the opossum *Didelphis virginiana* which avoids isovelleral-generating mushrooms.[27] In addition, the dialdehydes possess potent antibiotic and cytotoxic properties.[58] Moreover, isovelleral (**32**) is a potent selective insecticide against *Tribolium confusum*.[59] These effects are apparently based on the general ability of α,β-unsaturated alde-

hydes, such as velleral (**31**) and isovelleral (**32**), to react with nucleophiles, for instance with the basic ε-amino group of lysine residues in proteins, thus inactivating enzymes.[60]

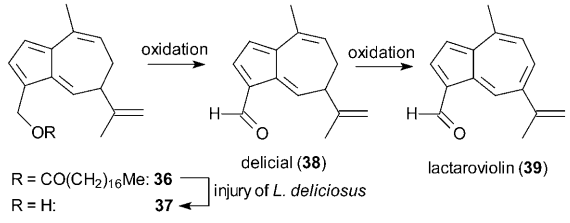
The ability of 1,4-dialdehydes to covalently bind primary amine residues in proteins has also been linked to their hot taste.[61] Moreover, the chemical defence by α,β-unsaturated dicarbonyl compounds is relatively widespread in nature. These compounds are not only produced upon injury of mushrooms (Scheme 1) but also upon wounding of terrestrial plants,[62] algae[63] and even of animals.[64] Thus, upon injury the algae *Caulerpa taxifolia* transforms the inactive acetate caulerpenin to the reactive aldehyde oxytoxin 2,[63] while the mollusc *Dendrodoris limbata* converts olepupane to the hot tasting polygodial.[64]

In general, terpene esters are widespread in fruiting bodies of *Russulaceae*.[65] For instance, the fruiting bodies of *Lactarius porninsis* contain mild flavoured farnesane esters such as **33**.[66] Upon injury, the fruiting bodies develop a bitter taste and the farnesane esters are hydrolysed to porninsol (**34**) which is subsequently oxidised to the reactive α,β-unsaturated aldehyde porninsal (**35**) (Scheme 2).



Scheme 2. Wound-activated hydrolysis of stearoylporninsol in *Lactarius por*ninsis.

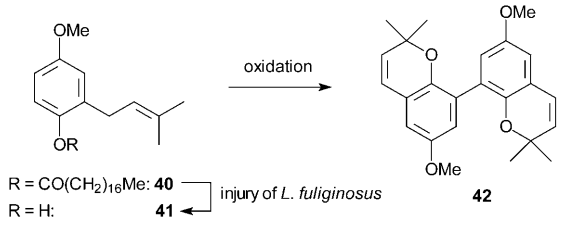
Wounding the fruiting bodies of *Lactarius deliciosus* and *Lactarius deterrimus* leads to the hydrolysis of the guaiane ester **36** and subsequent conversion of the free alcohol **37** to the aldehydes delicial (**38**) and lactaroviolin (**39**) (Scheme 3).[67]



Scheme 3. Wound-activated hydrolysis of a dihydroazulene ester in *Lactarius deliciosus.*

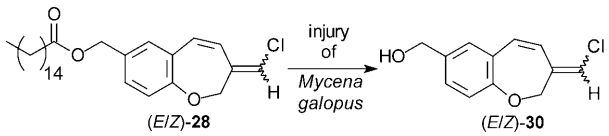
In contrast to the bioactive dialdehydes of *L. vellereus*, the bioactivity of porninsal (**35**), delicial (**38**) and lactaroviolin (**39**) is less well investigated. Moreover, *L. deliciosus* and *L. deterrimus* are edible mild flavoured mushrooms which are consumed in large quantities by humans.[67] Therefore, further investigations are required to confirm the presence of a wound-activated defence mechanism in these mushrooms.

Unlike many *Lactarius* species which contain terpene esters, in intact fruiting bodies of *Lactarius fuliginosus* and *Lactarius picinus* a tasteless phenol ester **40** turned out to be the precursor of the acrid tasting antifungal-active free phenol **41** which is gradually oxidised to a mixture of benzofuran and red chromene pigments such as **42** (Scheme 4).[68]



Scheme 4. Wound-activated hydrolysis of a phenol ester in *Lactarius* fuligino*sus.*

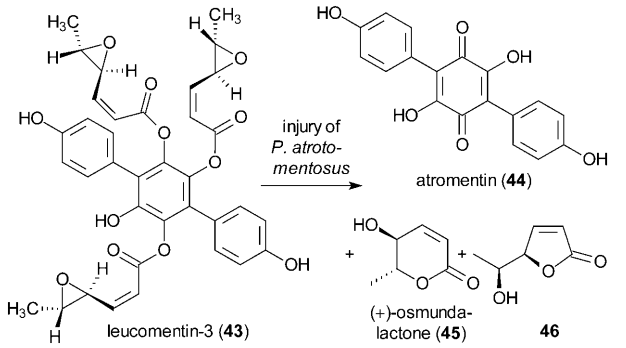
Wound-activated defence mechanisms based on the hydrolysis of esters are not restricted to *Lactarius* and *Russula* species but also occur in *Mycena galopus* (German: Weißmilchender Helmling) which releases a white latex if the fruiting bodies are bruised.[57] As already mentioned, a GC-MS screening of the ethyl acetate extracts of intact and injured fruiting bodies recently revealed that the latex of intact fruiting bodies contains inactive benzoxepine esters such as (*E*/*Z*)-**28** which are hydrolysed to the corresponding antifungally active free alcohols such as (*E*/*Z*)-**30**, thus protecting the tiny fruiting bodies from yeasts and fungi (Figures 4 and 5 and Scheme 5).



Scheme 5. Chemical defence by hydrolysis of esters in *Mycena galopus.*

Interestingly, the benzoxepine esters and the esterase responsible for their saponification are evidently stored in separate compartments in the intact fruiting bodies, since the benzoxepine esters in the latex were only hydrolysed if the latex was mixed with homogenised fungal tissue.[57]

In contrast to the wound-activated defence mechanisms discussed so far, the bioactive compound in *Paxillus atrotomentosus* is derived from the acid moiety.[69] Upon injury of the fruiting-bodies the inactive leucomentins such as leucomentin-3 (**43**) are hydrolysed and subsequently converted to atromentin (**44**), (+)-osmundalactone (**45**) and butenolide **46** (Scheme 6).[69] Butenolide **46** and (−)-osmundalactone are known as feeding deterrents from leaves of the fern *Osmunda japonic*a.[70] Although atromentin (**44**) had already



Scheme 6. Wound-activated hydrolysis of leucomentin esters in *Paxillus atrotomentosus*.

been identified in 1928 as the brown constituent of the mushroom's surface,[71] it was not recognised until 1989 that the flesh of intact fruiting bodies contains precursors of atromentin as decisive constituents of a wound-activated chemical defence mechanism.[69]

Oxidation of phenols: Fungi have developed various, sometimes even unique wound-activated chemical defence reactions which involve the oxidation of phenols.

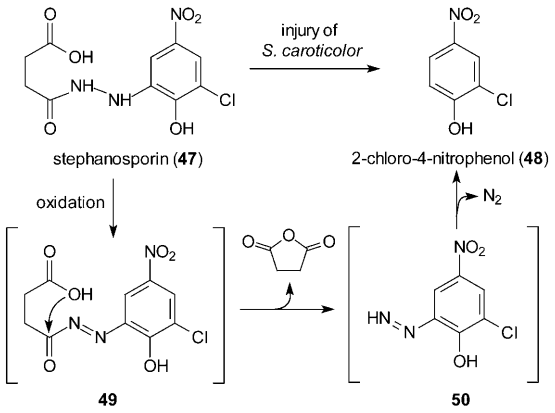
While hydrolysis but not the oxidation of the above mentioned flavomentins to atromentin is the initial step in the wound-activated chemical defence of *P. atrotomentosus*,[69] in some other fungi an oxidation reaction is the key step to activate their chemical defence.

For instance, this applies to the defence of the orange fruiting bodies of the carrot truffle (*Stephanospora caroticolor*) which is a rare gasteromycete.[72] The antifungal properties of the fungus were attributed in 1989 to the presence of 2-chloro-4-nitrophenol (**48**) in injured fruiting bodies. Some years later, a comparative HPLC-UV screening of intact and injured fruiting bodies revealed that intact fruiting bodies exclusively contain the inactive precursor stephanosporin (**47**) which is structurally related to 2-chloro-4-nitrophenol (**48**).[72] In the year 2001 the mechanism of the wound-activated chemical defence reaction was elucidated by oxidation of synthetic stephanosporin (**47**) under different conditions and by analysis of the products generated. According to these investigations stephanosporin is first oxidised to the diazene **49**. Subsequently, this activated acid derivative looses succinic acid anhydride. The 2-chloro-6-diazenyl-4-nitrophenol (**50**) generated is decomposed to nitrogen and 2-chloro-4-nitrophenol (**48**) (Scheme 7).[72]

The "traceless" loss of the side chain in stephanosporin is a principle which is also exploited in the form of hydrazide anchors in solid phase synthesis.[73]

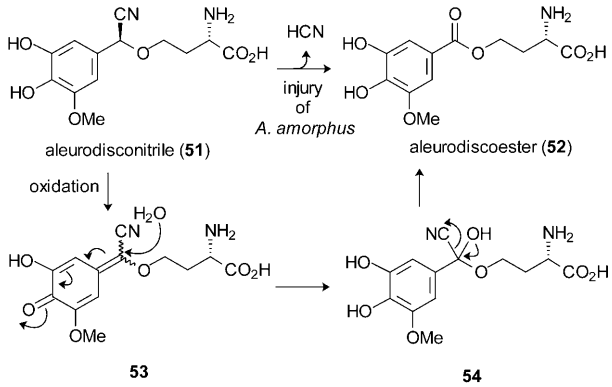
A similar oxidative degradation mechanism yields the bioactive 4-diazo-2,5-cyclohexadien-1-one from the 4-hydroxyphenylhydrazone leucoagaricone.[74]

Similarly, the chemical defence of the crust fungus *Aleurodiscus amorphus* is initiated by enzymatic oxidation of the tailor-made cyanohydrin ether aleurodisconitrile (**51**).[75] Upon injury hydrocyanic acid and aleurodiscoester (**52**) are



Scheme 7. Oxidative chemical defence mechanism in *Stephanospora caroticolor*.

generated from **51** via an oxidative mechanism so far unknown in nature. Artificial oxidation of model compounds with MnO_2 revealed the mechanism of the wound-activated defence reaction. In the initial step aleurodisconitrile (**51**) is oxidised to the corresponding *ortho*-quinone which subsequently rearranges to quinonemethide **53**. Formal addition of water leads to the intermediate **54**. By elimination of hydrocyanic acid aleurodiscoester (**52**) then is generated (Scheme 8).[75]



Scheme 8. Oxidative chemical defence mechanism in *Aleurodiscus amorphus*.

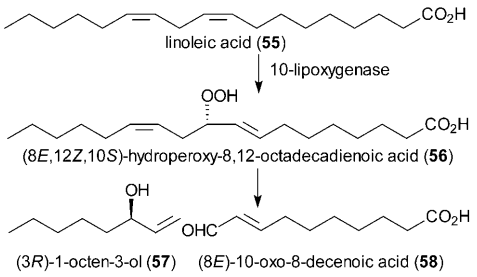
Oxidation reactions in mushrooms are often initiated by rather unspecific tyrosinases or other enzymes with a similar function which are widespread in fungi and which only become active upon injury.[76] The oxidative degradation mechanism of aleurodisconitrile (**51**) to aleurodiscoester (**52**) and HCN differs fundamentally from that via oxidation of glycine in bacteria,[77] but also from the hydrolytic degradation of cyanogenic glycosides in plants.[78] While the ease of hydrolysis of the glycosidic bond by glycosidases is essential in plants, the high oxidisability of the aromatic residue is decisive in *A. amorphus*.[75]

Oxidases are also responsible for many colour reactions which occur upon injury of mushrooms.[34] For instance, the

blueing of many boletes upon bruising of the fruiting bodies has been attributed to the oxidation of variegatic acid,[79] xerocomic acid[80] or of gyrocyanin[81] to hydroxyquinonemethide anions. However, it is not yet known in most cases whether these reactions also play a role in the wound-activated chemical defence.

Lipid peroxidation: In general, eukaryotic organisms respond upon wounding not only by activation of hydrolysing enzymes but also by activation of lipoxygenases.[82] These enzymes usually oxidise unsaturated fatty acids to hydroperoxy fatty acids which are then converted to a variety of degradation products.[82]

Fungi possess a typical mushroom lipoxygenase which oxidises linoleic acid (**55**) upon injury to (8*E*,12*Z*,10*S*)-10-hydroperoxy-8,12-octadecadienoic acid (**56**).[83] The latter then suffers enzymatic cleavage to (3*R*)-1-octen-3-ol (**57**) and (8*E*)-10-oxo-8-decenoic acid (**58**) (Scheme 9).[83] The substrate for the lipoxygenase, linoleic acid (**55**), is generated from lipids upon wounding by the activation of hydrolases.



Scheme 9. Generation of (3*R*)-1-octen-3-ol by lipid peroxidation in mushrooms.

(3*R*)-1-Octen-3-ol (**57**) has been found in many mushrooms[84] and is responsible for the typical odour of fresh fruiting bodies.[85] Upon injury of fruiting bodies the odour intensifies, since the production of **57** increases dramatically.[86]

(8*E*)-10-Oxo-8-decenoic acid (**58**), possessing an α,β-unsaturated aldehyde moiety, was also detected in increased amounts after the wounding of mushrooms, such as *Marasmius oreades*, *Cantharellus tubaeformis* or *Lepista nebularis*.[87] (8*E*)-10-Oxo-8-decenoic (**58**) seems to stimulate the stipe elongation and the mycelial growth of *Agaricus bisporus*,[88] while both (3*R*)-1-octen-3-ol and (8*E*)-10-oxo-8-decenoic acid also exhibit antifungal activities for instance against mycelia of *Penicillium expansum*[89] indicating that these compounds might play a role in the wound-activated chemical defence of mushrooms.

Despite the fact that numerous volatiles derived from plants—which are often either lipid peroxidation products or terpenoid compounds—serve defence purposes,[90] there have only been a few investigations on the ecological role of volatiles detected in mushrooms. For instance, (3*R*)-1-octen-3-ol seems to attract certain wood-living beetles.[91]

Induced Chemical Defence

Induced chemical defence is usually associated either with the de novo synthesis or a drastic increase in the production of constitutive defence compounds. In contrast to a wound-activated chemical defence which instantly activates enzymes in order to convert a precursor to the corresponding defence compound, in an induced defence mechanism the response often takes several hours, since it involves signal transduction leading to the activation of genes and an induction of the *de novo* biosynthesis of a defence compound. An induced chemical defence mechanism has the advantage that the energy-consuming biosynthesis of defence compounds is only initiated in response to an external danger thus saving valuable resources which can be used for an increased growth rate.

In general, induced chemical defence mechanisms can be detected by a comparative metabolite profiling as used for the investigation of wound-activated chemical defence strategies. In contrast to a wound-activated chemical defence mechanism, in an induced chemical defence mechanism no precursor can be detected in an unaffected organism, but monitoring of the metabolite pattern of the stressed organism after different periods of time shows an increase of defence compounds.

Induced chemical defence is widespread in plants. Upon wounding hormones, such as jasmonic acid,[92] ethylene[93] and salicylic acid[92] are generated and trigger induced chemical defence of plants[94] by activation of genes leading to the induction of the *de novo* biosynthesis of phytoalexins[95] for their chemical defence and of volatiles such as terpenes for their communication.[94] Moreover, predators of herbivores can be attracted by volatiles emitted from injured plants, thus serving as a kind of bodyguard.[94]

So far, little is known about induced chemical defence in fungi and it has not been reported whether fungal volatiles such as **57**, which are produced upon injury, may be able to stimulate induced chemical defence reactions. However, **57** induces defence responses in plants.[96] Even the upregulation of the production of constitutive defence compounds in fungi in dependence on external stress has usually not been investigated, thus requiring future research. However, in the case of the strobilurins, it has been shown, that the production of strobilurins increases significantly, if competing fungi are present in the mycelial cultures suggesting the presence of an induced chemical defence in the mycelium of *Strobilurus tenacellus*.[97]

Conclusion

Like plants, higher fungi have evolved a huge variety of responses to injury. Besides potent toxins as constitutive defence compounds wound-activated defence mechanisms are common in fruiting bodies of higher fungi. These are based on the enzymatic transformation of an inactive precursor molecule to a biological active compound. However, in the

past wound-activated defence mechanisms have often been overlooked, as a result of inappropriate work-up procedures and a strong bias towards bioactive compounds, but not on their generation or their ecological role. Hence, some more known bioactive natural products now regarded as constitutive defence compounds might turn out to be generated only upon wounding. A consequent comparative metabolic screening of intact and injured fruiting bodies is therefore expected to reveal more wound-activated chemical defence mechanisms in the future. Moreover, comparative metabolic profiling will also contribute to the identification of new natural products.

Acknowledgements

I am very grateful to my co-workers Dr. Silke Peters, Dr. Bernhard Kindler and Robert Jaeger for their enthusiastic work in the laboratory and in the field, to Dr. Dieter Spiteller (Max-Planck-Institut für chemische Ökologie, Jena, Germany) for helpful discussions and to Dr. Birgit Schulze (Botanisches Institut, Basel, Switzerland) and Dr. Norbert Arnold (Institut für Pflanzenbiochemie, Halle, Germany) for photos of some mushrooms. Our work has been generously supported by an Emmy Noether Fellowship for young investigators of the Deutsche Forschungsgemeinschaft (SP 718/1-2), by the Fonds der Chemischen Industrie and by the Otto-Röhm-Gedächtnisstiftung.

[1] a) D. Spiteller, "Chemical Defense Strategies of Plants" in *Encyclopedia of Ecology* (Eds.: S. E. Jorgensen, B. Fath), Elsevier, **2008**; b) J. B. Harborne, "Plant Chemical Ecology" in *Comprehensive Natural Products Chemistry*, *Vol. 8* (Eds.: D. Barton, K. Nakanishi, O. Meth-Cohn; Vol. Ed.: K. Mori), Elsevier, Amsterdam, **1999**, pp. 137–196.
[2] W. Steglich, "Some Chemical Phenomena of Mushrooms and Toadstools" in *Biologically Active Molecules* (Ed.: U. P. Schlunegger), Springer, Berlin, **1989**, pp. 1–8.
[3] T. Anke, E. Thines, *Br. Mycol. Soc. Symp. Ser.* **2007**, *26*, 45–58.
[4] T. Hartmann, *Proc. Natl. Acad. Sci. USA*, **2008**, *105*, 4541–4546.
[5] T. Anke, *Can. J. Bot.* **1995**, *73 (Suppl. 1)* S940-S945.
[6] M. Stadler, O. Sterner, *Phytochemistry* **1998**, *49*, 1013–1019.
[7] B. Pokorny, S. Al Sayegh-Petkovšek, C. Ribarič-Lasnik, J. Vrtačnik, D. Z. Doganoc, M. Adamič, *Sci. Total Environ.* **2004**, *324*, 223–234.
[8] a) T. Kamada, *BioEssays*, **2002**, *24*, 449–459; b) A. J. Brown, L. A. Casselton, *Trends Genet.* **2001**, *17*, 393–400.
[9] C. C. Palmerino, K. W. Rusiniak, J. Garcia, *Science* **1980**, *208*, 753–755.
[10] a) D. Spiteller, P. Spiteller, "Defense Strategies of Fungi" in *Encyclopedia of Ecology* (Eds.: S. E. Jorgensen, B. Fath), Elsevier, Amsterdam, **2008**; b) T. Wiesemeier, M. Hay, G. Pohnert, *Aquat. Sci.* **2007**, 403–412.
[11] M. H. Zenk, M. Juenger, *Phytochemistry* **2007**, *68*, 2757–2772.
[12] C. Plinius Sec. , L. von Jan, K. F. T. Mayhoff, *C. Plini Secundi Naturalis historiae libri XXII, 92*, Teubner, Leipzig, **1892**, *Vol. 3*, p. 468.
[13] a) H. Faulstich, T. Wieland, *Adv. Exp. Med. Biol.* **1996**, *391*, 309–314; b) T. Wieland, H. Faulstich, *Crit. Rev. Biochem.* **1978**, *5*, 185–260.
[14] X. Q. Gong, Y. A. Nedialkov, Z. F. Burton, *J. Biol. Chem.* **2004**, *279*, 27422–27427.
[15] a) D. Michelot, L. M. Melendez-Howell, *Mycol. Res.* **2003**, *107*, 131–146; b) W. Z. Antkowiak, "The Chemistry and Toxicology of Mushroom Alkaloids" in *Chemistry and Toxicology of Diverse Classes of Alkaloids* (Ed.: M. S. Blum), Alaken, Ft. Collins, **1996**, pp. 185–300.
[16] K. von Megenberg in *Buch der Natur—Die erste Naturgeschichte in Deutscher Sprache* (Ed.: F. Pfeiffer), Karl Aue Verlag, Stuttgart, **1861**, p. 402.
[17] S. Grzymala, *Zeitschr. Pilzk.* **1957**, *23*, 139–142.
[18] C. Karlson-Stiber, H. Persson, *Toxicon* **2003**, *42*, 339–349.
[19] H. Prast, W. Pfaller, *Arch. Toxicol.* **1988**, *62*, 89–96.
[20] W. Z. Antkowiak, W. P. Gessner, *Tetrahedron Lett.* **1979**, *20*, 1931–1934.
[21] P. Spiteller, M. Spiteller, W. Steglich, *Angew. Chem.* **2003**, *115*, 2971–2974; *Angew. Chem. Int. Ed.* **2003**, *42*, 2864–2867.
[22] S. Franke, U. Freimuth, P. H. List, *Arch. Toxikol.* **1967**, *22*, 293–332.
[23] A. Hofmann, R. Heim, A. Brack, H. Kobel, A. Frey, H. Ott, T. Petrzilka, F. Troxler, *Helv. Chim. Acta* **1959**, *42*, 1557–1572.
[24] a) K. Konno, K. Hashimoto, Y. Ohfune, T. Matsumoto, *J. Am. Chem. Soc.* **1988**, *110*, 4807–4815; b) K. Konno, H. Shirahama, T. Matsumoto, *Tetrahedron Lett.* **1983**, *24*, 939–942.
[25] R. Marumoto, C. Kilpert, W. Steglich, *Z. Naturforsch. C* **1986**, *41*, 363–365.
[26] R. Bedry, I. Baudrimont, G. Deffieux, E. E. Creppy, J. P. Pomies, J. M. Ragnaud, M. Dupon, D. Neau, C. Gabinsky, S. De Witte, J. C. Chapalain, J. Beylot, P. Godeau, *New Engl. J. Med.* **2001**, *345*, 798–802.
[27] a) S. M. Camazine, J. F. Resch, T. Eisner, J. Meinwald, *J. Chem. Ecol.* **1983**, *9*, 1439–1447; b) S. Camazime, *J. Chem. Ecol.* **1983**, *9*, 1473–1481; c) S. Camazime, *J. Chem. Ecol.* **1985**, *11*, 1289–1295.
[28] V. Hellwig, J. Dasenbrock, C. Gräf, L. Kahner, S. Schumann, W. Steglich, *Eur. J. Org. Chem.* **2002**, 2895–2904.
[29] H. Shibata, T. Tokunaga, D. Karasawa, A. Hirota, M. Nakayama, H. Nozaki, T. Tada, *Agric. Biol. Chem.* **1989**, *53*, 3373–3375.
[30] T. Kubota, Y. Asaka, I. Miura, H. Mori, *Helv. Chim. Acta* **1982**, *65*, 611–619.
[31] T. Eizenhöfer, B. Fugmann, W. S. Sheldrick, B. Steffan, W. Steglich, *Liebigs Ann. Chem.* **1990**, 1115–1118.
[32] W. Steglich, L. Kopanski, M. Wolf, M. Moser, G. Tegtmeyer, *Tetrahedron Lett.* **1984**, *25*, 2341–2344.
[33] a) O. Sterner, B. Steffan, W. Steglich, *Tetrahedron* **1987**, *43*, 1075–1082; b) P. Spiteller, D. Hamprecht, W. Steglich, *J. Am. Chem. Soc.* **2001**, *123*, 4837–4838.
[34] a) M. Gill, *Nat. Prod. Rep.* **2003**, *20*, 615–639; b) M. Gill, W. Steglich, *Progr. Chem. Org. Nat. Prod.* **1987**, *51*, 1–317.
[35] a) A. Bartsch, M. Bross, P. Spiteller, M. Spiteller, W. Steglich, *Angew. Chem.* **2005**, *117*, 3016–3019; *Angew. Chem. Int. Ed.* **2005**, *44*, 2957–2959; b) B. Fugmann, B. Steffan, W. Steglich, *Tetrahedron Lett.* **1984**, *25*, 3575–3578.
[36] a) P. Spiteller, W. Steglich, *J. Nat. Prod.* **2002**, *65*, 725–727; b) P. Spiteller, N. Arnold, M. Spiteller, W. Steglich, *J. Nat. Prod.* **2003**, *86*, 1402–1403.
[37] a) L. Kahner, J. Dasenbrock, P. Spiteller, W. Steglich, R. Marumoto, M. Spiteller, *Phytochemistry* **1998**, *49*, 1693–1697; b) K. Aulinger, H. Besl, P. Spiteller, M. Spiteller, W. Steglich, *Z. Naturforsch. C* **2001**, *56*, 495–498.
[38] a) F. von Nussbaum, P. Spiteller, M. Rüth, W. Steglich, G. Wanner, B. Gamblin, L. Stievano, F. E. Wagner, *Angew. Chem.* **1998**, *110*, 3483–3485; *Angew. Chem. Int. Ed.* **1998**, *37*, 3292–3295; b) P. Spiteller, M. Rüth, F. von Nussbaum, W. Steglich, *Angew. Chem.* **2000**, *112*, 2864–2866; *Angew. Chem. Int. Ed.* **2000**, *39*, 2754–2756.
[39] S. O. Mueller, M. Schmitt, W. Dekant, H. Stopper, J. Schlatter, P. Schreier, W. K. Lutz, *Food Chem. Toxicol.* **1999**, *37*, 481–491.
[40] a) S. Peters, P. Spiteller, *Eur. J. Org. Chem.* **2007**, 1571–1576; b) S. Peters, P. Spiteller, *J. Nat. Prod.* **2007**, *70*, 1274–1277; c) S. Peters, R. J. R. Jaeger, P. Spiteller, *Eur. J. Org. Chem.* **2008**, 319–323; d) C. Baumann, M. Bröckelmann, B. Fugmann, B. Steffan, W. Steglich, W. S. Sheldrick, *Angew. Chem.* **1993**, *105*, 1120–1121; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1087–1089.
[41] a) M. S. Butler, *J. Nat. Prod.* **2004**, *67*, 2141–2153; b) D. J. Newman, G. M. Cragg, K. M. Snader, *Nat. Prod. Rep.* **2000**, *17*, 215–234.
[42] a) M. H. Zenk, *Phytochemistry*, **1991**, *30*, 3861–3863; b) V. Bisaria, A. Panda, *Curr. Opin. Biotechnol.* **1991**, *2*, 370–374.
[43] K. Lorenzen, T. Anke, *Curr. Org. Chem.* **1998**, *2*, 329–364.
[44] T. Anke, F. Oberwinkler, W. Steglich, G. Schramm, *J. Antibiot.* **1977**, *30*, 806–810.

[45] a) T. Anke, G. Schramm, B. Schwalge, B. Steffan, W. Steglich, *Liebigs Ann. Chem.* **1984**, 1616–1625; b) G. Schramm, W. Steglich, T. Anke, F. Oberwinkler, *Chem. Ber.* **1978**, *111*, 2779–2784.
[46] V. Hellwig, J. Dasenbrock, D. Klostermeyer, S. Kroiß, T. Sindlinger, P. Spiteller, B. Steffan, W. Steglich, M. Engler-Lohr, S. Semar, T. Anke, *Tetrahedron*, **1999**, *55*, 10101–10118.
[47] P. Kraiczy, U. Haase, S. Gencic, S. Flindt, T. Anke, U. Brandt, G. von Jagow, *Eur. J. Biochem.* **1996**, *235*, 54–63.
[48] H. Sauter, W. Steglich, T. Anke, *Angew. Chem.* **1999**, *111*, 1416–1438; *Angew. Chem. Int. Ed.* **1999**, *38*, 1328–1349.
[49] a) M. Engler, T. Anke, O. Sterner, *J. Antibiot.* **1997**, *50*, 330–333; b) M. Engler, T. Anke, O. Sterner, U. Brandt, *J. Antibiot.* **1997**, *50*, 325–329.
[50] T. Lübken, J. Schmidt, A. Porzel, N. Arnold, L. Wessjohann, *Phytochemistry*, **2004**, *65*, 1061–1071.
[51] a) T. Anke, A. Werle, S. Zapf, *J. Antibiot.* **1995**, *48*, 725–726; b) P. Aqueveque, T. Anke, H. Anke, O. Sterner, J. Becerra, M. Silva, *J. Antibiot.* **2005**, *58*, 61–64.
[52] S. Peters, P. Spiteller, *J. Nat. Prod.* **2006**, *69*, 1809–1812.
[53] G. Bertrand, *Compt. Rend.* **1901**, *133*, 1233–1236.
[54] a) G. Magnusson, S. Thorén, *Acta Chem. Scand.* **1973**, *27*, 2396–2398; b) G. Magnusson, S. Thorén, T. Drakenberg, *Tetrahedron* **1973**, *29*, 1621–1624.
[55] O. Sterner, R. Bergman, J. Kihlberg, B. Wickberg, *J. Nat. Prod.* **1985**, *48*, 279–288.
[56] G. Vidari, P. Vita-Finzi, "Sesquiterpenes and Other Secondary Metabolites of Genus *Lactarius* (*Basidiomycetes*): Chemistry and Biological Activity in Studies" in *Natural Products Chemistry*, *Vol. 17* (Ed.: Atta-ur-Rahman), Elsevier, **1995**, pp. 153–206.
[57] S. Peters, R. J. R. Jaeger, P. Spiteller, *Eur. J. Org. Chem.* **2008**, 1187–1194.
[58] H. Anke, O. Sterner, W. Steglich, *J. Antibiot.* **1989**, *42*, 738–744.
[59] W. M. Daniewski, M. Gumułka, D. K. Ptaszyńska, E. Błoszyk, B. Drożdż, *Phytochemistry* **1995**, *38*, 1161–1168.
[60] a) M. Jonassohn, O. Sterner, *Trends Org. Chem.* **1997**, *6*, 23–43; b) I. Aujard, D. Röme, E. Arzel, M. Johansson, D. de Vos, O. Sterner, *Bioorg. Med. Chem.* **2005**, *13*, 6145–6150; c) M. Andersson, F. Bocchio, O. Sterner, A. Forsby, L. Lewan, *Toxicol. in Vitro* **1993**, *7*, 1–6.
[61] G. Cimino, G. Sodano, A. Spinella, Tetrahedron, 1987, 5401–5410.
[62] a) K. Konno, C. Hirayama, H. Yasui, M. Nakamura, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 9159–9164; b) H. Inouye, S. Saito, H. Taguchi, T. Endo, *Tetrahedron Lett.* **1969**, *10*, 2347–2350.
[63] S. Adolph, V. Jung, J. Rattke, G. Pohnert, *Angew. Chem.* **2005**, *117*, 2866–2869; *Angew. Chem. Int. Ed.* **2005**, *44*, 2806–2808.
[64] a) M. Gavagnin, E. Mollo, G. Calado, S. Fahey, M. Ghiselin, J. Ortea, G. Cimino, *Chemoecology* **2001**, *11*, 131–136; b) M. Gavagnin, E. Mollo, F. Castelluccio, M. T. Ghiselin, G. Calado, G. Cimino, *Tetrahedron* **2001**, *57*, 8913–8916; c) G. Cimino, G. Sodano, A. Spinella, *J. Nat. Prod.* **1988**, *51*, 1010–1011; d) G. Cimino, S. De Rosa, S. De Stefano, R. Morrone, G. Sodano, *Tetrahedron* **1985**, *41*, 1093–1100.
[65] W. M. Daniewski, G. Vidari, *Progr. Chem. Org. Nat. Prod.* **1999**, *77*, 69–171.
[66] A. Gamba-Invernizzi, L. Garlaschelli, A. Rossi, G. Vidari, P. Vita-Finzi, *J. Nat. Prod.* **1993**, *56*, 1948–1953.
[67] O. Bergendorff, O. Sterner, *Phytochemistry* **1988**, *27*, 97–100.
[68] M. De Bernardi, G. Vidari, P. Vita Finzi, G. Fronza, *Tetrahedron* **1992**, *48*, 7331–7344.
[69] a) M. Holzapfel, C. Kilpert, W. Steglich, *Liebigs Ann. Chem.* **1989**, 797–801; b) H. Besl, A. Bresinsky, G. Geigenmüller, R. Herrmann, C. Kilpert, W. Steglich, *Liebigs Ann. Chem.* **1989**, 803–810; c) M. S. Buchanan, T. Hashimoto, S. Takaoka, Y. Asakawa, *Phytochemistry* **1995**, *40*, 1251–1257.
[70] A. Numata, K. Hokimoto, T. Takemura, T. Katsuno, K. Yamamoto, *Chem. Pharm. Bull.* **1984**, *32*, 2815–2820.
[71] F. Kögl, Fritz, H. Becker, A. Detzel, G. de Voss, *Liebigs Ann. Chem.* **1928**, *465* 211–242.
[72] M. Lang, P. Spiteller, V. Hellwig, W. Steglich, *Angew. Chem.* **2001**, *113*, 1749–1751; *Angew. Chem. Int. Ed.* **2001**, *40*, 1704–1705.
[73] F. Stieber, U. Grether, H, Waldmann, *Angew. Chem.* **1999**, *111*, 1142–1145; *Angew. Chem. Int. Ed.* **1999**, *38*, 1073–1077.
[74] S. Hilbig, T. Andries, W. Steglich, T. Anke, *Angew. Chem.* **1985**, *97*, 1063–1064; *Angew. Chem. Int. Ed. Engl.* **1985**, *24*, 1063–1065.
[75] B. L. J. Kindler, P. Spiteller, *Angew. Chem.* **2007**, *119*, 8222–8224; *Angew. Chem. Int. Ed.* **2007**, *46*, 8076–8078.
[76] a) H. Decker, T. Schweikardt, F. Tuczek, *Angew. Chem.* **2006**, *118*, 4658–4663; *Angew. Chem. Int. Ed.* **2006**, *45*, 4546–4550; b) S.-Y. Seo, V. K. Sharma, N. Sharma, *J. Agric. Food Chem.* **2003**, *51*, 2837–2853; c) A. M. Mayer, *Phytochemistry* **2006**, *67*, 2318–2331; d) S. Halaouli, M. Asther, J.-C. Sigoillot, M. Hamdi, A. Lomascolo, *J. Appl. Microbiol.* **2006**, *100*, 219–232.
[77] C. Blumer, D. Haas, *Arch. Microbiol.* **2000**, *173*, 170–177.
[78] M. A. Hughes, "Biosynthesis and Degradation of Cyanogenic Glycosides" in *Comprehensive Natural Products Chemistry*, *Vol. 1* (Ed.: U. Sankawa), Elsevier, Amsterdam, **1999**, pp. 881–895.
[79] P. C. Beaumont, R. L. Edwards, G. C. Elsworthy, *J. Chem. Soc. C* **1968**, 2968–2974.
[80] W. Steglich, W. Furtner, A. Prox, *Z. Naturforsch. B* **1968**, *23*, 1044–1050.
[81] a) H. Besl, A. Bresinsky, W. Steglich, K. Zipfel, *Chem. Ber.* **1973**, *106*, 3223–3229; b) W. Steglich, A. Thilmann, H. Besl, A. Bresinsky, *Z. Naturforsch. C* **1977**, *32*, 46–48.
[82] A. Grechkin, *Progr. Lipid Res.* **1998**, *37*, 317–352.
[83] a) M. Wurzenberger, W. Grosch, *Biochim. Biophys. Acta* **1984**, *795*, 163–165; b) M. Wurzenberger, W. Grosch, *Z. Lebensm.-Unters. Forsch.* **1982**, *175*, 186–190.
[84] a) S. Rapior, S. Cavalié, P. Croze, C. Andary, Y. Pélissier, J.-M. Bessière, *J. Essent. Oil Res.* **1996**, *8*, 63–66; b) P. Audouin, J. P. Vidal, H. Richard, *Sci. Aliments* **1989**, *9*, 185–193; c) G. Venkateshwarlu, M. V. Chandravadana, R. P. Tewari, *Flav. Fragr. J.* **1999**, *14*, 191–194; d) R. Zawirska-Wojtasiak, *Food Chem.* **2004**, *86*, 113–118.
[85] A. Mosandl, G. Heusinger, M. Gessner, *J. Agric. Food Chem.* **1986**, *34*, 119–122.
[86] C.-M. Wu, W. Ziyang, *Food Sci. Technol. Res.* **2000**, *6*, 166–170.
[87] a) Z. Pang, O. Sterner, *Nat. Prod. Lett.* **1993**, *3*, 193–196; b) Z. Pang, O. Sterner, H. Anke, *Acta Chem. Scand.* **1992**, *46*, 301–303; c) Z. Pang, H. Anke, O. Sterner, *Acta Chem. Scand.* **1994**, *48*, 408–410.
[88] J.-L. Mau, R. B. Beelman, G. R. Ziegler, *Phytochemistry* **1992**, *31*, 4059–4064.
[89] D. O. Okull, R. B. Beelman, H. Gourama, *J. Food Prot.* **2003**, *66*, 1503–1505.
[90] a) Z.-G. Yan, C.-Z. Wang, *Phytochemistry* **2006**, *67*, 34–42; b) J. A. Gatehouse, *New Phytol.* **2002**, *156*, 145–169.
[91] J. Fäldt, M. Jonsell, G. Nordlander, A.-K. Borg-Karlson, *J. Chem. Ecol.* **1999**, *25*, 567–590.
[92] V. A. Halim, A. Vess, D. Scheel, S. Rosahl, *Plant. Biol.* **2006**, *8*, 307–313.
[93] a) K. L.-C. Wang, H. Li, J. R. Ecker, *Plant Cell*, **2002** *(Suppl.)*, S131–S151.
[94] a) G.-i. Arimura, C. Kost, W. Boland, *Biochim. Biophys. Acta* **2005**, *1734*, 91–111; b) B. Schulze, C. Kost, G.-i. Arimura, W. Boland, *Chem. Unserer Zeit*, **2006**, *40*, 366–377.
[95] H. Grisebach, J. Ebel, *Angew. Chem.* **1978**, *90*, 668–681; *Angew. Chem. Int. Ed. Engl.* **1978**, *17*, 635–647.
[96] K. Kishimoto, K. Matsui, R. Ozawa, J. Takabayashi, *J. Gen. Plant Pathol.* **2007**, *73*, 35–37.
[97] M. Kettering, O. Sterner, T. Anke, *Z. Naturforsch.* **2004**, *59C*, 816–823.

Published online: July 7, 2008

