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REVIEW PAPER

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Eight-carbon volatiles in mushrooms and fungi: properties, analysis, and biosynthesis

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Abstract Eight-carbon volatiles are ubiquitous among fungi and characteristic of the fungal aroma. They are the product of the oxidation and cleavage of the fatty acid linoleic acid and are classified as oxylipins, molecules taking part in a wide range of biological processes. Their involvement in the fungal aroma, interactions with pests and pathogens, and reproductive events are reviewed here, as well as the enzymic systems involved in their biosynthesis.

Key words Eight-carbon volatiles · Hydroperoxide · Linoleic acid · 1-Octen-3-ol · Oxidation

Eight-carbon volatiles and their properties

Eight-carbon volatile compounds are a key contributor to mushroom flavor and have been described by many in *Agaricus bisporus* and other fungi (Pyysalo and Suihko 1976, Pyysalo 1976; Grove 1981; Maga 1981; Macleod and Panchasara 1983; Mau et al. 1992b; Pfeil and Mumma 1992; Cruz et al. 1997; Mau and Hwang 1997; Venkateshwarlu et al. 1999). They account for 44.3%–97.6% of the total volatile fraction, depending on the extraction method used, and the eight-carbon volatile 1-octen-3-ol is the most abundant (Maga 1981; Tressl et al. 1982; Venkateshwarlu et al. 1999). The chemical structure of the main volatiles are illustrated in Fig. 1; their relative concentrations, threshold values, and aromas are listed in Table 1.

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Eight-carbon volatiles and fungal aroma

The aliphatic alcohol 1-octen-3-ol, discovered by Murahashi in *Tricholoma matsutake* and first called matsutake alcohol, is the principal compound contributing to the unique fungal aroma and flavor (Murahashi 1938). It has two optically active isomers, the naturally occurring (–) form having a stronger flavor than the (+) form (Fig. 2) (Dijkstra and Wiken 1976). The odor of the (–) isoform is said to be mushroom like, instead of the moldy grass note of the (+) form (Mosandl et al. 1986).

Each volatile compound has a particular odor or aroma, from mushroom like for 1-octen-3-ol to sweet and fruity for the ketone 3-octanone (see Table 1). Other volatile compounds have been identified, such as benzyl alcohol, which produces an almond-like aroma, and cyclo-octanol, which produces a leafy odor and could come from the cyclization of 1-octen-3-ol (Macleod and Panchasara 1983; Chen and Wu 1984; Wu and Chen 1986). Moreover, 1-octen-3-ol concentration can influence the resulting odor sensation (Cronin and Ward 1971; Dijkstra and Wiken 1976; Pyysalo 1976). Previous studies focused on volatile species emitted by fruit bodies or mycelium, mainly using methodologies such as solvent extraction, do not always reflect the true flavor profile of the organism studied because such techniques tend to concentrate the compounds and require destruction of the sample analyzed (Picardi and Issenberg 1973; Card and Avisse 1977; Chen and Wu 1984; Mau et al. 1992b). Using panel studies, Bernhard and Simone (1958) studied the locus of mushroom aroma and discovered that in *A. bisporus* this locus is mainly located in the central part of the cap and the stipe. However, according to enzymic studies, more 1-octen-3-ol is produced in the cap and gills than in the stipe, and that cap has a more desirable fresh and cooked aroma (Wurzenberger and Grosch 1983).

Table 1. Main eight-carbon volatile compounds present in mushrooms (after Manning 1985)

Compound	Relative concentration (%)	Threshold value (ppm)	Aroma
1-Octen-3-ol	33	0.010	Mushroom like, sweet
1-Octen-3-one	0.02	0.004	Boiled mushroom
3-Octanol	1	0.018	Cod liver oil, nutty, sweet
3-Octanone	4	0.050	Sweet, fruity, musty, lavender
Octanol	0.3	0.48	Detergent, soap, orange like

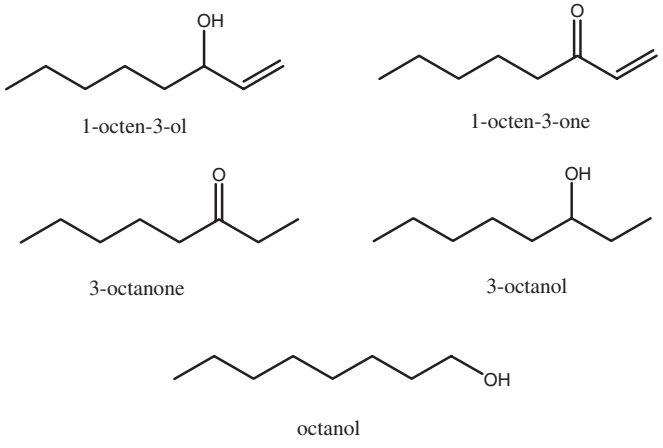

Fig. 1. Chemical structure of the main eight-carbon volatiles

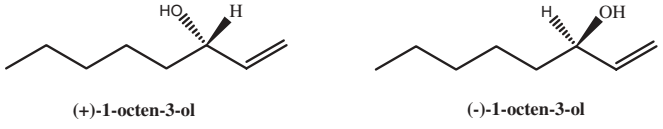

Fig. 2. Optical isomers of 1-octen-3-ol (after Mosandl et al. 1986)

Eight-carbon volatiles and fungal identification

With the development of electronic noses, which are volatile compound "mappers," eight-carbon volatiles, along with other fungal volatile metabolites, have been used as an indicator of food and feeds spoilage, and these were also used for the detection and classification of fungi at the species level in a chemosystematic study of *Penicillium* (Larsen and Frisvad 1995b; Keshri et al. 1998; Magan and Evans 2000; Schnürer et al. 2002; Canhoto et al. 2004). Moreover, by examining volatile components of several *Agaricus* species and comparing the ratios of benzoic compounds to eight-carbon volatile compounds, Callac and Guinberteau (2005) were able to identify a relationship between ratio and phylogenic closeness, indicating a potential importance of volatile compounds as a taxonomic marker.

Eight-carbon volatiles and biotic interactions

Eight-carbon volatiles emitted by mushroom sporophores play an important role in attracting flies and mosquitoes, which are likely to disseminate spores and therefore help in the reproduction process, outlining the importance of mushroom–insect mutualistic interactions (Chiron and Michelot 2005). 1-Octen-3-ol has been identified as an attractant to the tsetse flies *Glossina palidipes* and *G. moristans*, as well as an aggregation hormone for the beetles *Oryzaephilus surinamensis* and *O. mercator* (Hall et al. 1984; Pierce et al. 1989). Grove and Blight (1983) identified the volatiles 1-octen-3-ol, 3-octanone, and 1-octen-3-one as volatiles emitted from spawned compost, and suggested that 1-octen-3-ol is an attractant to the gravid female phorid fly *Megaselia halterata*, a pest of mushroom crops, because the larvae feed on the mycelia and the flies are potential carriers of the fungal pathogen *Verticillium fungicola*. However, Pfeil and Mumma (1993) showed that 1-octen-3-ol as a pure compound acts as a deterrent to the phorid flies, both males and females. Fäldt et al. (1999) also suggested that the eight-carbon volatiles could attract predator of fungal insect pests, as a defense mechanism. Moreover, eight-carbon volatiles are repulsive to predators such as the slug *Ariolimax columbianus* (Wood et al. 2001).

Eight-carbon volatiles and the reproductive function

In two bracket fungi, sporulation coincides with an augmentation in 1-octen-3-ol and 3-octanone levels, supposedly to recruit insects to disseminate the fungal spores (Fäldt et al. 1999). Such increases in eight-carbon volatiles have also been identified in the Ascomycetes *Penicillium* and *Aspergillus* (Borjesson et al. 1993).

As well as functioning as insect attractant, 1-octen-3-ol has been identified as a self-inhibitor in *Penicillium paneum* (Chitarra et al. 2004). The eight-carbon volatile blocks the germination process when spores are in high density, a condition known as the "crowding effect," a response characterized in Ascomycetes and Zygomycetes. Chitarra et al. (2005) studied the mode of action of 1-octen-3-ol and demonstrated that the volatile inhibits germ tube formation at low concentration in *P. paneum*, has a mild effect on membrane permeabilization, oxygen consumption, and intracellular pH in conidia, and affects the proteome by preventing the expression of certain proteins while promoting the expression of others. However, those proteins have not been identified yet. The authors concluded that 1-octen-3-ol acts as a fungal hormone, with transient effects.

The effects of 1-octen-3-ol and other eight-carbon volatiles on germination and sporophore initiation have not yet been well documented in Basidiomycetes; however, the oxo acid produced with 1-octen-3-ol was shown to have an

effect on *A. bisporus* growth (Mau et al. 1992a; Champavier et al. 2000). Moreover, eight-carbon volatiles were suggested to be involved with bacterial stimuli in *A. bisporus* sporophore formation (Hayes et al. 1969).

Analysis of eight-carbon volatile compounds

Analysis of flavor compounds requires sample preparation to concentrate the analyte of interest. The techniques described previously for sample concentration were purge and trap sampling, liquid–liquid extraction, conventional solid-phase extraction, and simultaneous distillation/extraction (Reineccius 1993). These methods each present some drawbacks, as they are time consuming, can involve organic solvents, and are not always representative of the headspace volatiles present at a given time. Moreover, a study by Jelen (2003) showed that the fungal volatile profile was highly dependent on the extraction method selected, and Larsen and Frisvad (1995a), when comparing methods for the collection of volatile chemical markers, noted that steam distillation extraction was producing volatile profiles dominated by the lipid degradation products 1-octen-3-ol and 3-octanone, probably not a true representation of the organism volatile profile.

Headspace analysis of volatiles emitted by a sample seems to be the method of choice to obtain an accurate and objective idea of the volatile species released at a given time without disrupting the sample (Zhang and Pawliszyn 1993). The term "headspace" refers to the volume left at the top of a jar or bottle before sealing, and by extension represents the volatile entities present in this volume. Direct sampling of the headspace with an airtight syringe is, however, not sensitive enough to detect most of the volatiles emitted in minute quantities. Analysis of fungal headspace volatiles have been conducted using Tenax traps or solid-phase microextraction (Jelen 2003; Da Costa and Eri 2006). Solid-phase microextraction (SPME), developed by Arthur and Pawliszyn (1990), is an adsorption/desorption technique that eliminates the drawbacks of sample preparation and concentrates volatile analytes in a solvent-free manner (Arthur et al. 1992; Zhang and Pawliszyn 1993; Yang and Peppard 1994; Zhang et al. 1994).

SPME is sensitive, reproducible, and cost efficient, and becomes a powerful tool when combined with gas chromatography-mass spectrometry (GC-MS) analysis. It incorporates extraction, concentration, and sample introduction into a single step: the device consists of a short fiber of fused silica, coated with a stationary phase (often made of polydimethyl siloxane, divinylbenzene, Carboxen), in a syringe-like device (Supelco, Poole, UK); when volatiles have equilibrated in the headspace of the sample to analyze, the fiber is inserted in the chamber to allow volatile sampling. Analytes are subsequently partitioned between the stationary-phase coating and the gas phase and adsorb/absorb on the fiber. After a set time, the fiber is retracted and transferred to the gas chromatograph injection port, where the volatiles are thermally desorbed from the fiber and cold-trapped on the head of the capillary column (Zhang and Pawliszyn 1993; Yang and Peppard 1994).

Combination of SPME with separation by gas chromatography and detection by mass spectrometry makes the entire SPME-GS-MS procedure a very efficient approach to study the evolution of volatile compounds in biological system, and especially complex systems, without disrupting them (Diaz et al. 2003; Jelen 2003). It has been used successfully to identify the aroma components in truffles (Diaz et al. 2003; Piloni et al. 2005). However, when SPME was compared to dichloromethane solvent extraction, thermal desorption of volatile compounds entrained on Tenax traps, and solid-sample injection (SSI) for the identification of volatile organic compounds emitted by *Serpula lacrymans* and *Coniophora puteana* (two wood-rotting basidiomycete fungi), solid-sample injection and Tenax thermal desorption were found to be the most effective extraction methods, not SPME (Ewen et al. 2004).

Eight-carbon volatiles formation

Despite eight-carbon volatiles involvement in several key biological processes, from mushroom initiation to response to pathogens and biotic interactions, little is known about the biosynthetic pathway(s) leading to their formation in fungi (Fäldt et al. 1999; Okull et al. 2003; Chitarra et al. 2004, 2005; Chiron and Michelot 2005). The biochemistry of 1-octen-3-ol formation is better documented, and several hypotheses have been formulated without having being fully proven (Tressl et al. 1981, 1982; Wurzenberger and Grosch 1984b,c). Fungi, as do plants, utilize their fatty acids resources to produce volatile compounds; the fatty acid molecules are first oxidized and then cleaved to produce the short-chain volatiles.

Lipids and fatty acids in fungi

Fatty acids are precursors in the reaction of eight-carbon volatile synthesis and are key components in a variety of lipids. Lipids are present in mushrooms and fungi in small amounts, accounting only for 0.1%–0.3% of the fresh weight in mushrooms. The lipid content itself is highly variable, depending on the species and the age of the culture as well as the growing conditions. Neutral lipids tend to accumulate as the fungal culture ages whereas polar lipids vary with developmental stage and culture condition (Sumner 1973). Triacylglycerides are the main reserve lipids, used for energy supply and carbon skeletons during growth and development, whereas acylglycerides are the primary carbon substrate for spore germination in rusts, and sterols are major component of membranes, where they regulate permeability and may serve as precursors to steroid hormones involved in the sexual reproduction of some fungi (Weete 1980).

Two major studies concluded that the mycelium and the fruit body of *A. bisporus* contain free fatty acids, triglycer-

ides, phosphatidylcholine, and phosphatidylethanolamine (Holtz and Schisler 1971, 1972). Linoleic acid (18:2, $\Delta^{9,12}$) was detected in large amounts in both the mycelium and fruit body; however, free sterols were absent in the mycelium. Comparison of the composition of mycelia and fruit bodies concluded there are quantitative but no qualitative differences between the two (Byrne and Brennan 1975). The glycolipid fraction was composed of acylglucose, acylmannose, acyltrehalose, and glucosyloxy fatty acids whereas the phospholipid fraction was composed of phosphatidylethanolamine and phosphatidylserine; however, phosphatidylcholine found previously by Holtz and Schisler was absent (possibly the result of the occasional demethylation of phosphatidylcholine to phosphatidylethanolamine). More than 30% of the neutral lipid fatty acids have a chain length of 18 carbons at least (Byrne and Brennan 1975).

Linoleic acid represents 63%–74% of the fatty acids in mushrooms and is the most abundant, followed by palmitic and stearic acid (Griffin et al. 1971; Prostenik et al. 1978; Weete 1980). The totality of fatty acids (FA) detected in *A. bisporus* had a chain length between 12 and 18 carbons and were essentially unsaturated fatty acids (75.8%). Moreover, hydroperoxides of fatty acids (hydroperoxide) were present in low quantity (3.7% of the total fatty acids), had a carbon chain of variable length, from 15 to 24 carbons, and were mainly saturated (only 0.8% of hydroperoxide are unsaturated) (Prostenik et al. 1978).

Linoleic acid is the precursor to eight-carbon volatile formation in fungi, acting as substrate to a fatty acid oxygenase, thought to be a lipoxygenase (Tressl et al. 1982; Assaf et al. 1995; Husson et al. 2002). However, it is not known whether the linoleic acid is required to be present in the cell as a free fatty acid or if the oxidizing enzyme can target esterified fatty acids. If linoleic acid is required to be unesterified, phospholipases would play an important role in the bioavailability of the substrate of the reaction.

The oxidation step

1-Octen-3-ol can be formed via autoxidation of polyunsaturated fatty acids and/or via enzyme-catalysed oxidation and cleavage of polyunsaturated fatty acids (Badings 1970). Enzyme-catalyzed oxidation of linoleic acid, the most likely route leading to the formation of eight-carbon volatiles, relies on the oxidation of the polyunsaturated fatty acid linoleic acid by a lipoxygenase (a non heme, iron-containing dioxygenase), and the further cleavage of the intermediate fatty acid hydroperoxide by a hydroperoxide lyase, as described in two different pathways, according to Tressl et al. (1982) (Fig. 3), and Wurzenberger and Grosch (1984c) (Fig. 4). Both lipoxygenase and hydroperoxide lyase are enzymes present in plants and involved in short-chain volatile synthesis.

Although both pathways agree on the precursor compound to the reaction, linoleic acid, and the end product, 1-octen-3-ol, they differ on which intermediate compound is the product of the oxidation step: Tressl et al. (1982) proposed a 13-hydroperoxide as intermediate compound (see Fig. 3), whereas Wurzenberger and Grosch (1984a,c) established that 1-octen-3-ol is derived from a 10-hydroperoxide [(8*E*,12*Z*)-10-hydroperoxyoctadeca-8,12-dienoic acid] intermediate and not a 13-hydroperoxide (see Fig. 4).

By measuring the different rates of 1-octen-3-ol and 13-hydroperoxide formation according to change in the substrate (linoleic acid) availability, Assaf et al. (1995, 1997) demonstrated that 13-hydroperoxide and 1-octen-3-ol come from two distinct pathways, and suggesting the presence of two different lipoxygenases in the mushroom *Pleurotus pulmonarius*, one responsible for 13-hydroperoxide production and the other directed toward the production of 1-octen-3-ol. These results reinforced the hypothesis proposed by Grosch and Wurzenberger (1984a,c), dismissing 13-hydroperoxide as an intermediate. The study concluded that the 1-octen-3-ol precursor is likely to be 10-hydroperoxide, for the main pathway, with the possibility of 1-octen-3-ol being produced via other secondary ways such as 1-octen-3-one reduction, or autoxidation (Assaf et al. 1995, 1997).

A study of the enzymatic reduction of 1-octen-3-one in the Shiitake mushroom *Lentinus edodes* and in *A. bisporus* demonstrated that 1-octen-3-one was effectively reduced to 1-octen-3-ol, as well as 3-octanone (Chen and Wu 1984; Wu and Chen 1986). 1-Octen-3-one had been proposed to be a precursor to 1-octen-3-ol through reduction an alcohol dehydrogenase (Tressl et al. 1982). However, the enzyme(s) involved in the reaction reducing 1-octen-3-one could in fact also break down the double bond in 1-octen-3-ol to form 3-octanol. This contradiction in the pathway leading to 1-octen-3-ol formation from 1-octen-3-one could be explained by the presence of two enzymes, one reducing the ketone to 1-octen-3-ol and the other one producing the 3-octanol; however, it was concluded that 1-octen-3-ol is likely to originate from another pathway than the one described by Tressl et al. (1982).

The intermediate compound identified by Wurzenberger and Grosch (1984a,c), a 10-hydroperoxide, is an unlikely product of lipoxygenase-catalyzed oxidation of linoleic acid, mainly because of its nonconjugated double-bond system: lipoxygenases are nonheme dioxygenases that recognize a *cis*, *cis*-1,4-pentadiene motif in their fatty acid substrate, and carry out an oxidative attack on the only saturated carbon of the structure, carbon-11, before provoking a radical movement (+2/–2) and double-bond rearrangement leading to the formation of either 9- or 13-hydroperoxide, but not a 10-hydroperoxide. The enzymic reaction leading to the formation of a 10-hydroperoxide from linoleic acid in fungi has not been characterized.

Cleavage of the hydroperoxide compound(s)

The fate of the hydroperoxide formed, such as the 10-hydroperoxide, depends on the enzyme present alongside the lipoxygenase: hydroperoxide compounds have a short half-life and act as substrate to a range of cytochrome

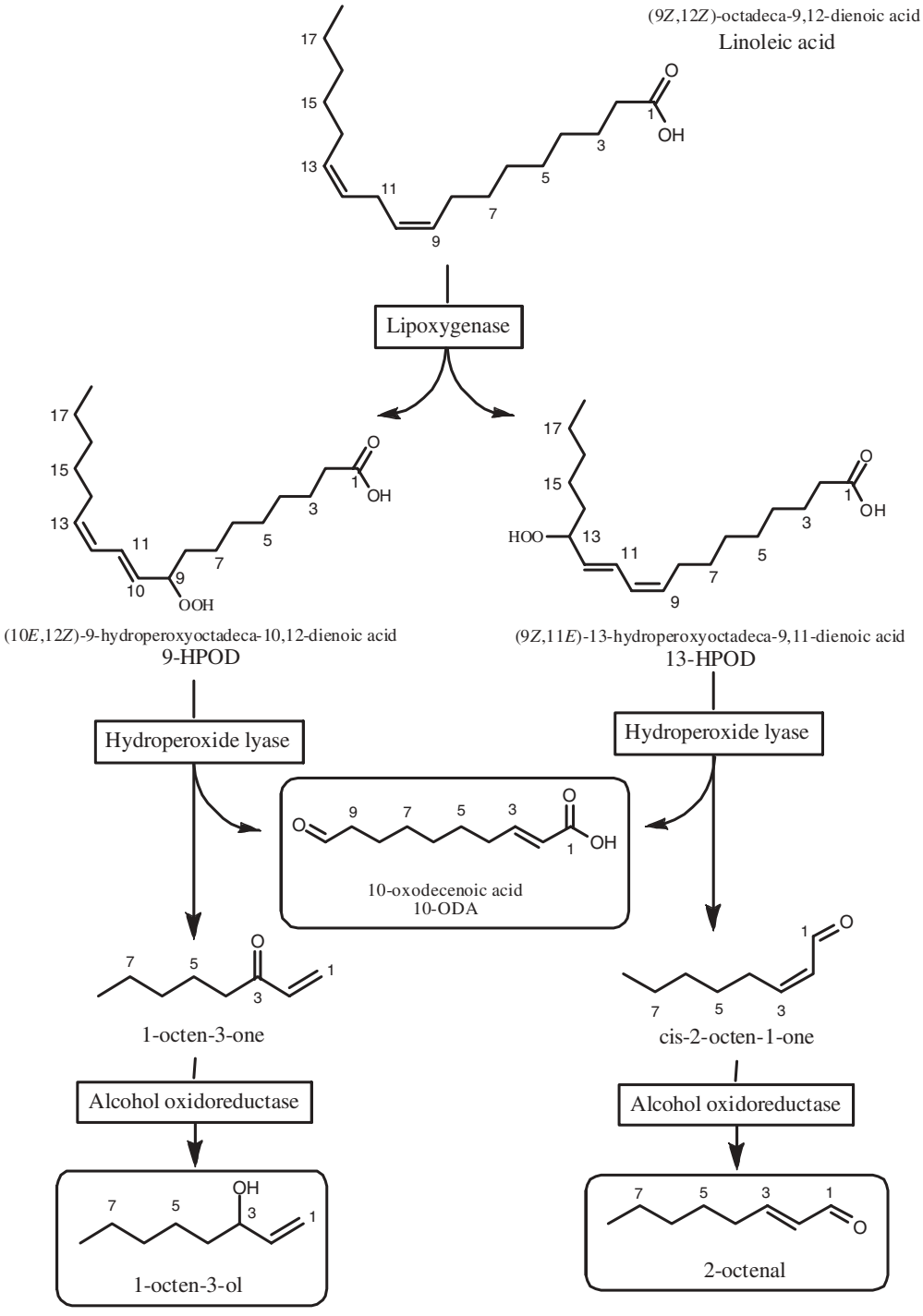

Fig. 3. Formation of 1-octen-3-ol as proposed by Tressl et al. (1981)

P450 enzymes, including the enzyme hydroperoxide lyase (HPOL), which is responsible for the cleavage of hydroperoxide molecules into short volatile compounds. Hydroperoxide lyases have been recently reclassified as hydroperoxide isomerases by Grechkin and Hamberg (2004), who demonstrated that the end product of the reaction was in fact an hemiacetal, once thought to be an intermediate compound, having a very short half-life of 20s at 0°C; spontaneous decomposition of the hemiacetal yields short-chain volatiles that were previously thought to be the end products of the reaction (Grechkin and Hamberg 2004). Hydroperoxide lyases are said to perform either a homolytic or heterolytic cleavage of hydroperoxides, yielding different short-chain volatiles, depending on their cleavage mode.

The first cleavage mechanism, heterolytic, is found in most plants: the enzyme cleaves the hydroperoxide between the carbon bearing the hydroperoxide group and the unsaturated carbon. If the substrate is a 9-hydroperoxide, a 9-carbon volatile and a 9-carbon oxoacid will be formed; if it is a 13-hydroperoxide, a 6-carbon volatile and a 12-carbon oxoacid will be formed. The second mechanism is called

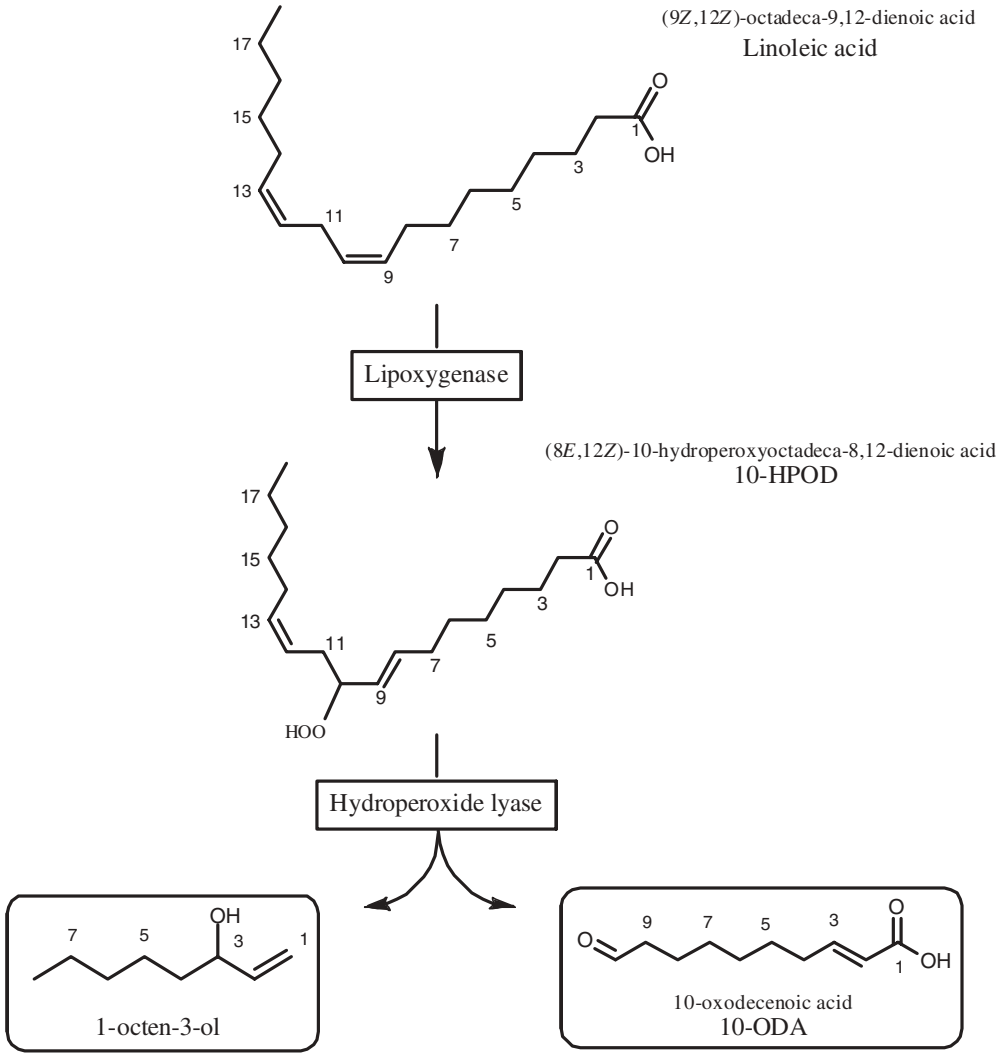

Fig. 4. Formation of 1-octen-3-ol as proposed by Wurzenberger and Grosch (1984a–c)

homolytic and involves the cleavage of the hydroperoxide between the carbon bearing the hydroperoxide group and the saturated carbon. This mechanism has been observed in algae, grass, and mushrooms (Wurzenberger and Grosch 1984a; Berger et al. 1986; Vick and Zimmerman 1989). Hydroperoxide lyases present in grass or algae perform the homolytic cleavage on products of the lipoxygenase pathway, 13-hydroperoxide molecules, resulting in the generation of 5-carbon compounds, whereas in mushrooms, Grosch and Wurzenberger showed that the reaction has for substrate a 10-hydroperoxide [(8E,12Z)-10-hydroperoxyoctadeca-8,12-dienoic acid], an unlikely product of any lipoxygenase pathway. Both cleavage mechanisms are described in Fig. 5.

To this date, no 10-hydroperoxide-specific hydroperoxide lyase has been identified in fungi. Over recent years, researchers have been reporting lipoxygenase activity in several fungi, including *Penicillium* sp., *Morchella esculenta*, and *Fusarium proliferatum* (Bisakowski et al. 1995, 1998, 2000; Perraud and Kermasha 2000; Husson et al. 2002). Moreover, a novel lipoxygenase, a manganese lipoxygenase, has been identified in the fungus *Gaeumannomyces graminis*, and other lipoxygenase-like genes have been identified in *Neurospora crassa* (Hornsten et al. 1999). However, none of those studies could establish a strong link between a lipoxygenase encoding gene and eight-carbon volatile synthesis.

A novel fungal oxidation model

It appears more and more that the fungal eight-carbon volatile synthesis cannot be modeled on the plant system, despite obvious similarities (Fig. 6). The oxidation step occurring in fungi is the principal difference, yielding an unusual nonconjugated diene (10-hydroperoxide), an unsuitable product of lipoxygenase action. Eight-carbon volatile formation is unique to fungi and is likely to involve a fungal-specific pathway. It is evident that lipid and fatty acid metabolism have been underinvestigated in the fungal kingdom, with very few genes and enzymes yet identified. This lack adds to the difficulty in understanding unique systems such as eight-carbon compound production, having to rely

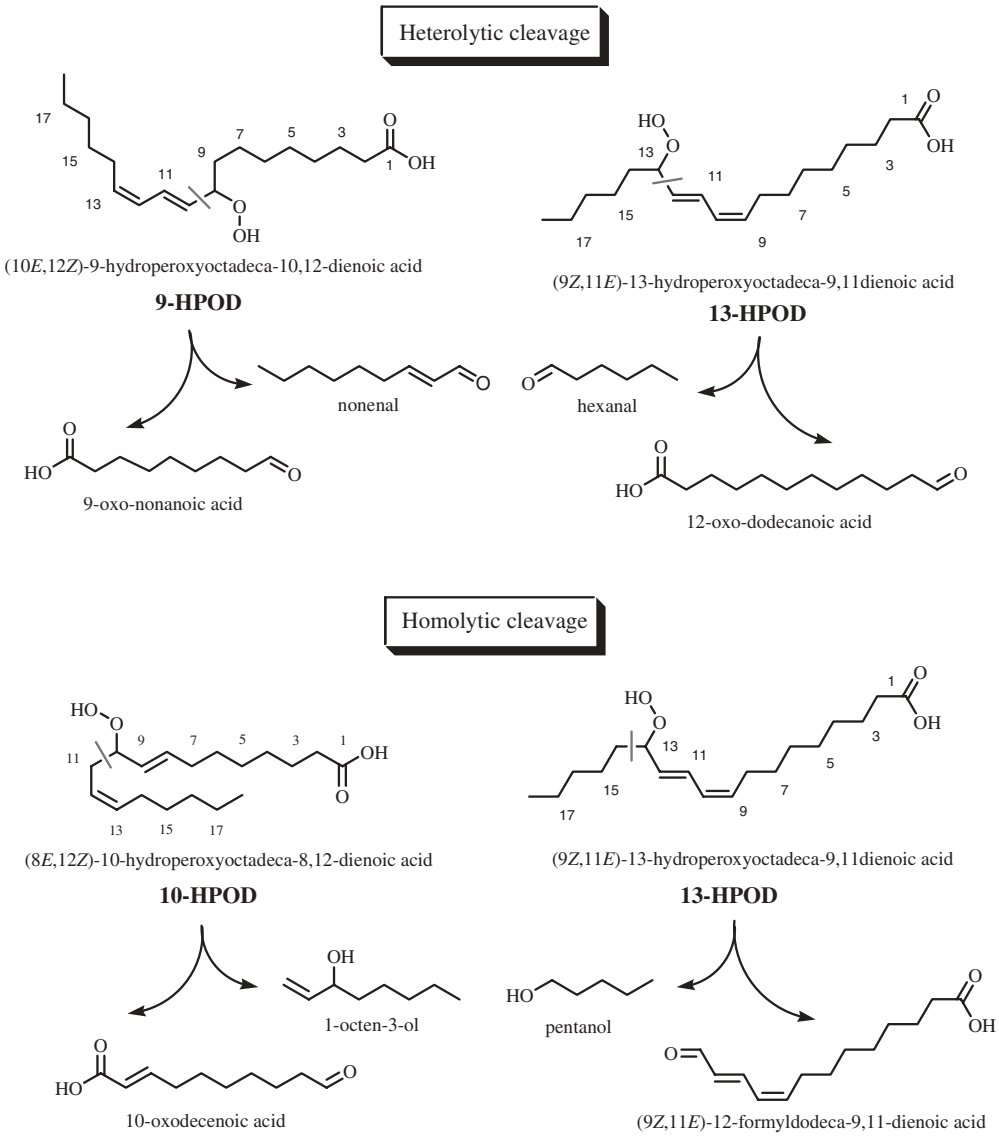

Fig. 5. Homolytic and heterolytic cleavage of hydroperoxides by the enzyme hydroperoxide lyase (based on Wurzenberger and Grosch 1984a; Matsui 1998; Noordermeer et al. 2001)

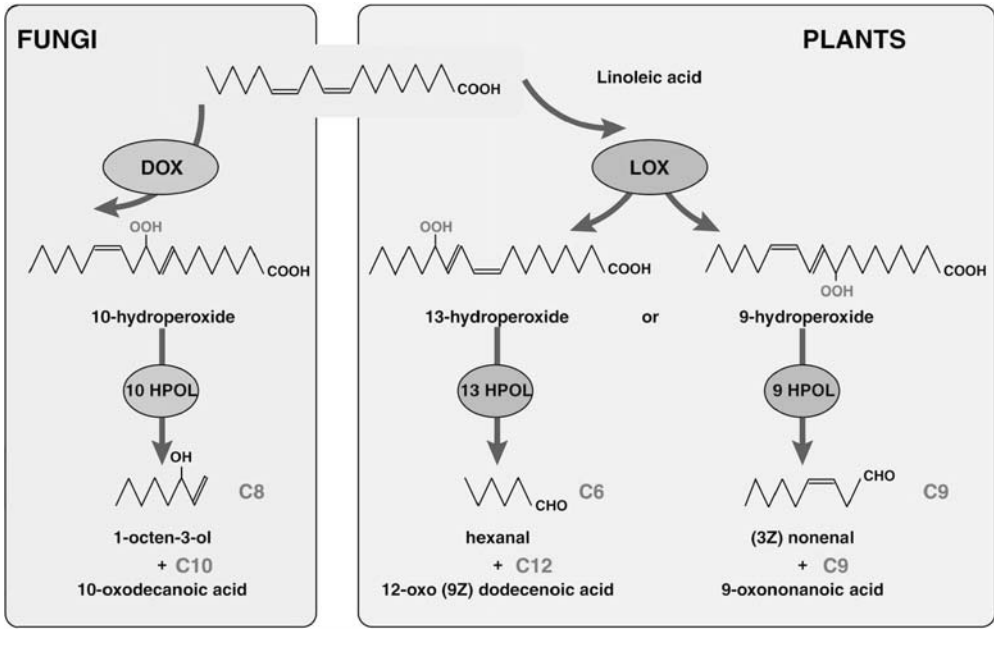

Fig. 6. Differences in fungal and plant volatiles synthesis pathways (based on Wurzenberger and Grosch 1984c; Gardner 1995; Feussner and Wasternack 1998; Grechkin 1998; Feussner and Wasternack 2002). *DOX*, haem dioxygenase; *LOX*, lipoxygenase; *HPOL*, hydroperoxide lyase

heavily on animal and plant resources, from sequence information to model biochemical pathways, complicating the characterization of such unique systems.

The recent discovery of novel oxidizing enzymes in fungi, such as linoleate diol synthase (LDS) from the wheat pathogen *Gaeumannomyces graminis* or *Ppo A* from the model ascomycete *Aspergillus nidulans,* cast a new light on fungal fatty acid oxidation (Hornsten et al. 1999; Tsitsigiannis et al. 2005). Linoleate diol synthase (LDS) combines a dioxygenase and an isomerase activity and uses linoleic acid as a substrate to produce a 8-hydroperoxide intermediate, which is then converted to a diol (Brodowski et al. 1992; Su et al. 1998). The gene coding for this enzyme shows high similarity to the prostaglandin endoperoxide synthase H (PGHs) family, present in mammals, and opens new perspectives for fungal fatty acid metabolism (Hornsten et al. 1999). Other enzymes belonging to a heme-oxygenase family could be performing a wide range of oxidation reactions in fungi. However, the main difficulty toward the identification of this novel gene family resides in the lack of genetic and genomic information for fungi.

Fatty acid oxidation and oxylipin production

Fatty acid oxidation provides plants, mammals, and fungi with a pool of biologically active compounds named oxylipins (Hamberg and Gardner 1992; Hamberg 1993; Blee 2002). Oxylipins in fungi include fatty acid hydroperoxides as well as their breakdown products, eight-carbon volatile compounds. In plants, oxylipins act as signal molecules and protective compounds against bacteria and fungi, as well as wound-healing compounds, whereas in mammals, the arachidonic acid cascade provides most of the oxylipins (the eicosanoids, 20-carbon fatty acids including prostaglandins, thromboxanes, and leukotrienes), which play a major role in the inflammatory process, stress response to infections, and allergies (Zimmerman and Coudron 1979; Turini et al. 1990; Hamberg 1993; Smith and Dewitt 1996; Blee 2002). Prostaglandins and thromboxanes are derived from a common pathway, involving the enzyme PGHS (prostaglandin endoperoxide synthase H), whereas leukotrienes are derived from arachidonic acid via a different pathway involving a lipoxygenase (Smith and Dewitt 1996; Feussner and Wasternack 1998; Brash 1999). They have a major role in the immune response of higher organisms: prostaglandins are involved in inflammation, and thromboxanes induce platelet aggregation at the onset of blood clot formation (Brash 1999; Smith et al. 2000; Simmons et al. 2004).

In fungi, there is an emerging role for 18:2 polyunsaturated fatty acids and their oxidation products in fungal development, reproduction, and biology: from the presence and function of *psi* factors (precocious sexual inducers, a mixture of three hydroxylated linoleic molecules that have an impact on spore formation) in *Aspergillus nidulans* to the antifungal action of compounds such as (8*R*)-hydroxylinoleate in *Laetisaria arvalis*, and 10-oxodecanoic acid (10-ODA) and 1-octen-3-ol on *Penicillium* mycelium in potato dextrose agar (Bowers et al. 1986; Calvo 2001; Okull et al. 2003; Tsitsigiannis et al. 2004).

In most fungi, it is understood that linoleic acid is oxidized to form a 10-hydroperoxide intermediate, which is then cleaved to form a eight-carbon volatile (1-octen-3-ol) and a ten-carbon oxoacid (10-ODA) (Wurzenberger and Grosch 1984c). 10-Oxodecanoic acid (10-ODA) possesses hormone-like properties toward growth of the mushroom stipe and development of fungal structures, and it is suggested that both 1-octen-3-ol and 10-ODA could work together to regulate the transition between vegetative and reproductive growth (Mau et al. 1992a; Champavier et al. 2000). Fungal oxylipins are at the crossroads of several biologically significant domains, such as reproduction and growth, pathogen interactions, taxonomy, and crop quality, justifying the need to investigate further their biosynthesis.

For a better understanding of eight-carbon volatiles biosynthesis and regulation, it is essential to identify and characterize the enzymes involved in their biosynthesis, as well as the origin and availability of the reaction substrate, linoleic acid, and intermediate compounds, the 10-hydroperoxy fatty acid. Lipid peroxidation and fungal volatile synthesis is more than a mushroom crop quality issue. The pathway is at the heart of fungal biology, and acts at several levels, showing how the fungal organism interacts with its surroundings, through emission of volatiles to defend itself against pest and pathogens, or by regulating its reproduction, through inhibition of sporophore formation and control of sporulation. The total elucidation of the processes involved in this pathway, from substrate synthesis and mobilization, to oxidation, cleavage, and distribution/release of the reaction products, should provide exciting research opportunities, leading to the understanding of the origin, role, and function of fungal oxylipins as a family of biologically active compounds.

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