Biosynthesis of 13-Hydroperoxylinoleate, 10-Oxo-8-decenoic Acid, and 1-Octen-3-ol from Linoleic Acid by a Mycelial-Pellet Homogenate of *Pleurotus pulmonarius*

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The compounds 13-hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acid (13-HPOD) and 10-oxo-*trans*-8-decenoic acid (10-oxo-acid) were quantified by HPLC and identified by FT-IR, GC, MS, and GC-MS as the major metabolites associated with the enzymatic cleavage of linoleic acid to 1-octen-3-ol by a homogenate of pellets of the mushroom *Pleurotus pulmonarius* grown in submerged culture. Whereas 13-HPOD was absent at linoleic acid concentrations below 1 mM, it was the major product of the enzymatic oxidation at linoleic acid concentrations above 1 mM. Nonetheless, 13-HPOD was found not to be the precursor of 1-octen-3-ol when supplied as the substrate instead of linoleic acid. Instead, 50% of it was reduced to 13-hydroxy-*cis*-9,*trans*-11-octadecadienoic acid. However, in the presence of linoleic acid, no hydroxy acid was detected, suggesting that linoleic acid itself inhibited 13-HPOD reduction. It appears, therefore, that 13-HPOD accumulation takes place in parallel with 1-octen-3-ol and 10-oxo-acid biosynthesis.

Keywords: *Edible mushroom mycelium; submerged culture; mushroom flavor; 1-octen-3-ol; linoleate hydroperoxide; linoleic acid oxidation; enzymatic oxidation*

INTRODUCTION

The fruiting bodies of edible mushrooms are known for their unique flavor and aromatic properties. The volatile fraction, and specifically a series of aliphatic compounds containing eight carbons, has been reported to be the major contributor to the characteristic mushroom flavor of each species (Hanssen and Klingenberg, 1983; Kinderlerer, 1989; Gross and Asther, 1989). Among the volatile compounds that constitute edible mushroom flavor, 1-octen-3-ol and its oxidation product, 1-octen-3-one, are considered to be the major contributors, and have been described as having a mushroom-like flavor (Maga, 1981; Fischer and Grosch, 1987). The biosynthetic pathway of 1-octen-3-ol in mushrooms is not known for certain. Moreover, no data have been reported on the intermediates of 1-octen-3-ol synthesis in fungal mycelium grown in liquid culture. Submerged culture of edible fungi for the production of fungal mycelium has been touted as a faster and more easily controllable method than the established growth of fruiting bodies by solid-state fermentation (Eyal, 1991). Many fungal strains adapt easily to different growth conditions and grow relatively rapidly in submerged culture (Jong and Birmingham, 1993). However, fungal mycelium produced by submerged fermentation has a very low level of flavor compounds and therefore this technique still requires significant improvement (Hadar and Dosoretz, 1991). Recently Belinky *et al.* (1994) have shown that the addition of soybean flour and soybean oil to the growth medium in submerged culture of *Pleurotus pulmonarius* enhances 1-octen-3-ol formation to a level similar to or even higher than that in fruiting bodies. This effect was attributed to enhanced linoleate lipoxygenase synthesis, due to the presence of fatty-acid precursors in the growth medium.

Lipoxygenase is thought to be a key enzyme in the biosynthesis of flavor compounds in both mushrooms and vegetables, through the formation of hydroperoxides (HPOD') of unsaturated fatty acids, mainly linoleic and linolenic acids, which in turn are cleaved to volatile aldehydes and alcohols (Zimmerman and Vick, 1970; Grossman and Zakut, 1978; O'Connor and O'Brien, 1991). Lipoxygenase activity, as well as the purification of lipoxygenase from fungi, as from vegetables, has been reported with the 9- and 13-HPOD isoforms only (Satoh *et al.*, 1976; de Lumen *et al.*, 1978). Tressl *et al.* (1982) proposed 13-HPOD to be the intermediate in the reaction that leads to 1-octen-3-ol in a homogenate of fruiting bodies of *Agaricus campestris*. Nonetheless, Wurzenberger and Grosch (1982, 1984a,b) reported that linoleic acid as well as 10-L-HPOD were cleaved by a protein fraction from fruiting bodies of *Agaricus bisporus* to 1-octen-3-ol and 10-oxo-*trans*-8-decenoic acid (10-oxo-acid). However, they did not detect 10-HPOD or 13-HPOD when radioactively labeled or cold linoleic acid was added. Moreover, no direct evidence has been reported as to the metabolites involved in 1-octen-3-ol formation in fungal mycelium grown in liquid culture.

In this work the major end products associated with the enzymatic cleavage of linoleic acid to 1-octen-3-ol by a homogenate of *P. pulmonarius* pellets grown in submerged culture were identified.

EXPERIMENTAL PROCEDURES

Organism. *P. pulmonarius* strain 3014 was obtained from Somycel (Langeais, France). The organism was maintained on 3% (w/v) malt extract agar at 28 °C.

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Media and Culture Conditions. Medium composition, inoculation procedure, and growth conditions were adapted from Belinky *et al.* (1994). The growth medium employed was number 6, based on soybean flour and soybean oil. It was autoclaved twice within 24 h to ensure a high level of sterility.

Fungal Biomass Harvesting. At the end of the growth period, the pellets were vacuum-filtered through a nylon net of 200-μm pore diameter, washed three times with double-distilled water (ddH_2O), and then used for the different experiments as indicated. In all experiments, only freshly harvested mycelium was used, because its ability to synthesize 1-octen-3-ol from linoleic acid decreases rapidly with storage, as was found in preliminary experiments.

Preparation and Determination 13-HPOD. 13-HPOD was prepared according to Vick (1991), using commercial soybean lipoxygenase (9,12-*cis*,*cis*-linoleate:oxygen oxidoreductase) type I-S, EC 1.13.11.12 (Sigma, St. Louis, MO). The reaction was run to completion, and the concentration of 13-HPOD was measured by both optical absorbance of the conjugated diene at 234 nm (ϵ = 25 000 mol^{-1} cm^{-1}) and by reaction with ferrous thiocyanate, followed by spectrophotometric determination of the complex formed, according to Sumner (1943). For incubation experiments, 13-HPOD was added directly from the aqueous solution. As a standard for high pressure liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC-MS), 13-HPOD was extracted as described below.

Characterization of Metabolites Produced by the Mycelium Homogenate Incubated with Linoleic Acid. The washed fungal pellets were resuspended in phosphate buffer (50 mM, pH 6.5) at a 1:6 (w/v) ratio and homogenized at room temperature for 2 min in a Polytron homogenizer (Kinematica, Germany), with or without linoleic acid, at the indicated concentrations. A stock solution of linoleic acid was prepared according to Grossman and Zakut (1978). The homogenate was extracted for GC and HPLC analyses, as described below. When indicated, homogenization with 13-HPOD instead of linoleic acid was performed under the above conditions.

Assay of 1-Octen-3-ol. 1-Octen-3-ol was extracted with cold *n*-pentane, using a modification of the method described by Wurzenberger and Grosch (1984a). To 4 mL of homogenate, 1-nonanol was added as an internal standard (25 μg/mL final concentration), and then 1 mL of *n*-pentane was added. The mixture was vortexed for 1 min and centrifuged for 6 min at 1900*g* in the cold, and the upper phase was removed. This step was repeated twice, and then 1 μL of the organic phase was analyzed by GC (Vista 6000, Varian, Palo Alto, CA) with a flame ionization detector, using a Stabilwax (Restek) column (30 m × 0.5 mm). Helium was used as the carrier gas, at a flow rate of 7 mL/min. A temperature program of 100–210 °C at 3 °C/min was applied. The injector and detector temperatures were 230 and 250 °C, respectively.

Assay of Linoleic Acid, 10-Oxo-acid, and HPOD. The method used was according to Tracey (1986). Diethyl ether (7 mL) containing butylated hydroxytoluene (BHT, 50 mg/L) and 100 μL of HCl (0.5 N) was added to 1 mL of homogenized mycelium and mixed well. The mixture was then centrifuged for 6 min at 1900*g*, and 5 mL of the upper phase was removed and evaporated to dryness with nitrogen gas. The residue was redissolved in 1 mL of acetonitrile, and the acetonitrile phase was analyzed by HPLC (Vista LC 54, Varian), using a Lichrospher 100 (Merck, Germany) RP-8 column (5 μm, 25 cm × 4 mm) at a flow rate of 1 mL/min. The solvent system consisted of acetonitrile:tetrahydrofuran:phosphoric acid (0.1%) at a ratio of 504:216:280. Monitoring was performed by UV detector, at 234 nm for the first 7 min and then at 210 nm. The concentrations of linoleic acid and HPOD were determined by the use of external standards (commercial linoleic acid from Sigma and synthesized 13-HPOD, purified as described above). The concentration of 10-oxo-acid was determined by an external standard method using a standard solution of the purified compound prepared for GC-MS. The concentration of the standard solution was determined by reaction with 2,4-dinitrophenylhydrazine [2.8% in sulfuric acid:ddH_2O:ethanol (15:20:70)] followed by spectrophotometric determination at 518 nm, according to Shriner *et al.* (1980), and using *n*-heptenal and *n*-octenal as reference compounds for the conjugated aldehyde.

Fractionation and Identification of 13-HPOD and 10-Oxo-acid Produced by the Mycelium Homogenate Incubated with Linoleic Acid. Washed fungal pellets (200 g) were mixed with phosphate buffer (50 mM, pH 6.5) at a ratio of 1:6 (w/v) and with linoleic acid solution (8 mM). The mixture was homogenized for 4 min at room temperature, and the pH was adjusted to 2 by adding HCl (0.5 N). Then, HPOD and 10-oxo-acid were extracted with diethyl ether containing BHT, the diethyl ether was further evaporated, and the residue was redissolved in hexane.

Sample Fractionation. The hexane solution containing HPOD and 10-oxo-acid was fractionated by HPLC, using a C-18 Hypersyl 5ODS (Hplc Technologies Ltd., U.K.) semi-preparative column (25 cm × 8 mm) at a flow rate of 4 mL/min. The solvent system consisted of: (a) acetonitrile; (b) phosphoric acid (0.1%); (c) tetrahydrofuran. The following gradient was applied:

Time (min)	0-3	8	10	12	15-18
a (%)	40	42	42	47	60
b (%)	45	43	40	35	20
c (%)	15	15	18	18	20

HPOD and 10-oxo-acid were monitored by UV detector at 234 nm and/or 210 nm, respectively. The peaks were collected into test tubes containing BHT. The organic phase was then evaporated in a rotary evaporator, and the remaining water phase was further extracted with dichloromethane.

Identification by Thin-Layer Chromatography (TLC) and Infrared (IR) Spectroscopy. The dichloromethane solutions containing HPOD and 10-oxo-acid were further subjected to TLC on silica gel 60 F_{254} analytical plates. The solvent system consisted of isooctane:ethyl ether:acetic acid (50:50:1). The spots were identified in three ways: (i) UV lamp at 254 nm; (ii) spray with phosphomolybdic acid; (iii) spray with potassium iodine–starch for HPOD or with 2,4-dinitrophenylhydrazine (0.4% in 2 N HCl) for 10-oxo-acid (Joseph and Murrel, 1983). In parallel, the HPLC peak in BHT containing 10-oxo-acid was redissolved in hexane and scanned by IR spectroscopy (Nicolet 60-5XB).

Identification by MS and GC-MS. The HPOD samples in dichloromethane were reevaporated in a rotary evaporator, redissolved in ethanol, then reduced with $NaBH_4$, according to Zimmerman and Vick (1970), and silylated with bis-(trimethylsilyl)trifluoroacetic acid (BSTFA), according to Smith *et al.* (1976). The 10-oxo-acid samples in dichloromethane were reevaporated in a rotary evaporator, redissolved in pyridine, and a methoxime derivative with CH_3ONH_2·HCl was first prepared, according to Thenot and Horning (1972). Thereafter, the samples were also silylated with BSTFA as described above.

The samples were analyzed by GC-MS (Finnigan 4020 LR, Quadrupool Instrument) equipped with a silicone phase SE-30 capillary column (30 m × 0.25 mm). The column temperature was programmed to stay 1 min at 180 °C and then to increase up to 250 °C at a rate of 4 °C/min. The samples were analyzed by electron ionization (EI, ionization voltage 70 eV) and chemical ionization (CI) with CH_4 as a reagent gas. The 10-oxo-acid was also identified by direct inlet, chemical ionization mass spectrometry (MS-CI-NH_3).

RESULTS

Identification of 13-HPOD Formed during Enzymatic Oxidation of Linoleic Acid by *P. pulmonarius*. The HPOD which accumulated during homogenization of mushroom pellets with linoleic acid (8 mM) was fractionated by semipreparative HPLC, analyzed

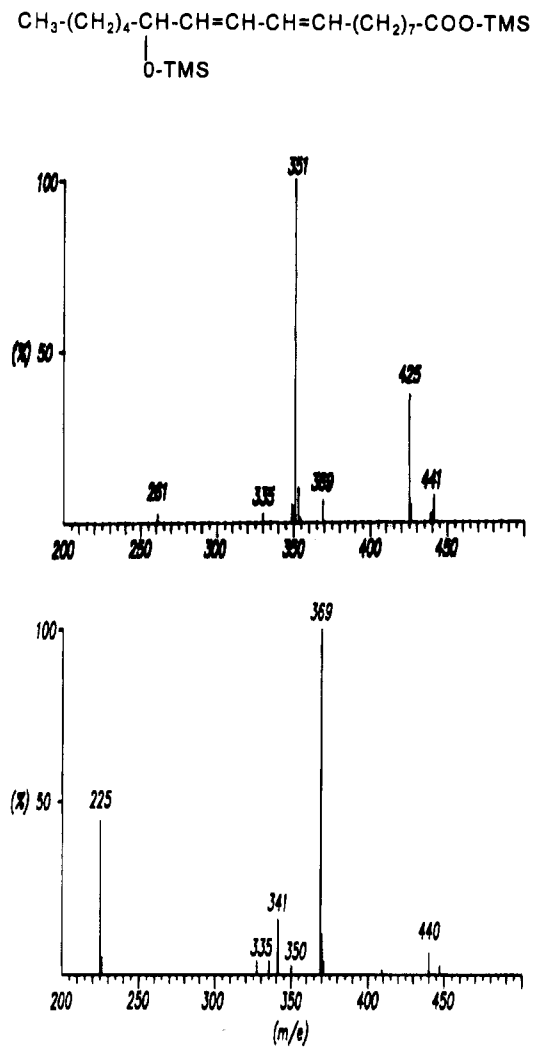


Figure 1. GC-MS of trimethylsilyl 13-*O*-(trimethylsilyl)-*cis*-9,*trans*-11-octadecadienoate (13-HPOD) formed by incubating a mycelial pellet homogenate of *P. pulmonarius* with linoleic acid: (top) CI spectrum; (bottom) EI spectrum.

for its chemical composition, and compared with the HPOD obtained by incubating pure soybean lipoxygenase, type I-S, with linoleic acid (standard 13-HPOD). The isolated HPOD, analyzed by TLC displayed a single band ($R_f = 0.46$) when visualized with phosphomolybdic acid, a band with UV absorbance (254 nm), and a blue-violet band, specific to the peroxide group, when sprayed with a potassium iodine–starch solution.

The isolated HPOD was further derivatized and identified by GC-MS, CI and EI mode (Figure 1). In the mass spectrum of chemical ionization (Figure 1, top), the ion peak at m/e 441 $(M + 1)^+$ confirmed the molecular weight of the HPOD derivative after reduction and silylation to be 440. The ion peak at m/e 369 came from the loss of C_5H_{11}, suggesting that the position of the OOH group in the fatty acid chain was carbon 13. The ion peak at m/e 425 came from the loss of the CH_3 fragment and the ion peak at m/e 335 came from the elimination of both CH_3 and trimethylsilyloxy (OTMS) fragments. The ion peaks at m/e 351 and 261 came from the elimination of one and two OTMS fragments, respectively, from the molecular ion.

In the mass spectrum of electron ionization (Figure 1, bottom), the ion peak at m/e 440 confirmed the molecular weight of the derivative to be 440. The ion peak at m/e 369 [molecular mass minus $CH_3(CH_2)_4$] indicated that the position of the OTMS group in the fatty-acid chain (corresponding to the OOH) was carbon 13. The ion peak at m/e 225 was obtained as a result of the loss of $(CH_2)_7COOTMS$; this fragment is α to the double bond and is typical of a breakdown in EI. The ion peaks at m/e 350 and 335 came from the loss of OTMS and both CH_3 and OTMS fragments, respectively. Both CI and EI mass spectra of the supposed 13-HPOD were compared to the corresponding mass spectra of the authentic sample of 13-HPOD, and the spectra were found to be identical, confirming once again that the identified compound was in fact trimethylsilyl 13-*O*-(trimethylsilyl)-*cis*-9,*trans*-11-octadecadienoate. Moreover, the same retention times (5.5 min) and λ_{max} (234 nm) were found for both standard and isolated 13-HPOD by HPLC analysis of the nonderivatized samples, as well as by GC analysis of the derivatized ones.

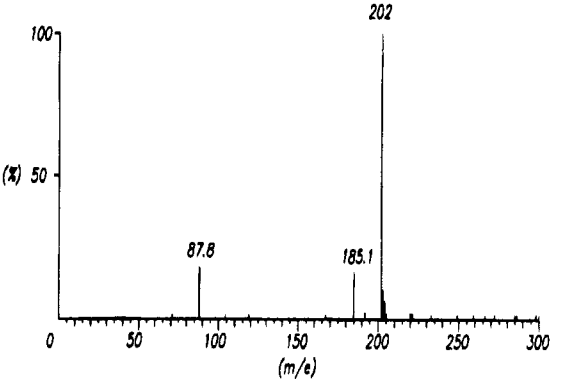


Figure 2. CI-NH_3-MS of 10-oxo-*trans*-8-decenoic acid formed by incubating a mycelial pellet homogenate of *P. pulmonarius* with linoleic acid.

Identification of 10-Oxo-acid Formed during Enzymatic Oxidation of Linoleic Acid by *P. pulmonarius*. As a result of the homogenization of mushroom pellets with linoleic acid (8 mM), a peak with retention time of 2.75 min was obtained by HPLC analysis with a slightly higher specific absorbance at 210 than at 234 nm with λ_{max} at 218 (in hexane), typical of the oxo-ene structure, reported by Wurzenberger and Grosch (1982). This compound was formed in parallel with 1-octen-3-ol and it was assumed to be 10-oxo-acid. A sample containing the supposed 10-oxo-acid was fractionated by semipreparative HPLC and first analyzed by TLC: a single band ($R_f = 0.15$) was displayed when visualized with phosphomolybdic acid, a band with UV absorbance (254 nm), and a yellow-brown band, specific to the carbonyl group, when sprayed with 2,4-dinitrophenylhydrazine.

Scanned by FT-IR, the isolated compound showed the characteristic band of conjugated aldehyde (1692 cm^{-1}) and carboxylic carbonyl (1705 cm^{-1}). A band at 1633 cm^{-1} indicated a double bond, and a band at 970 cm^{-1} indicated a *trans*-double bond, in agreement with the FT-IR spectrum reported by Wurzenberger and Grosch (1982) and Tressl *et al.* (1982). The isolated peak containing the 10-oxo-acid was further identified by MS-CI-NH_3 (Figure 2). The ion peak at m/e 185 $(M + 1)^+$ confirmed the molecular weight of the 10-oxo-acid to be 184. The base peak at m/e 202 resulted from the addition of NH_4 to the molecular ion. These results are in agreement with the MS spectrum of methyl 10-oxo-

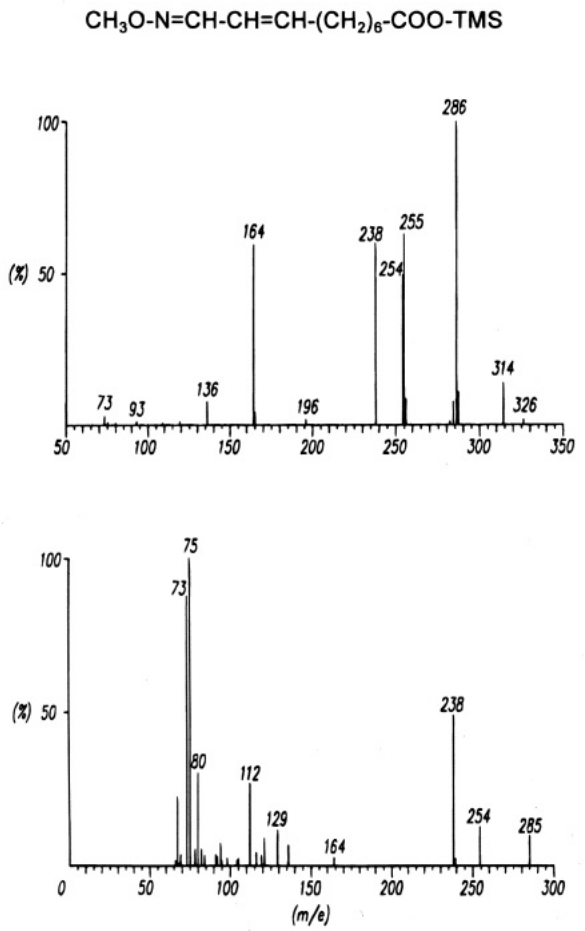


Figure 3. GC-MS of the methoxime-trimethylsilyl derivative of 10-oxo-acid formed by incubating a mycelial pellet homogenate of *P. pulmonarius* with linoleic acid: (top) CI spectrum; (bottom) EI spectrum.

trans-8-decenoate reported by Wurzenberger and Grosch (1984c) and Tressl *et al.* (1982).

The methoxime-trimethylsilyl derivative of the 10-oxo acid sample was injected for CI-CH_4 and EI GC-MS analysis (Figure 3). In the CI spectrum (Figure 3, top), the ion peak at m/e 286 $(M + 1)^+$ confirmed the molecular weight of the 10-oxo-acid derivative to be 285. The ion peaks at m/e 255 and 254 came from a loss of CH_3O and CH_3OH fragments, respectively, the ion peak at m/e 238 came from the loss of CH_3OH and CH_3, and the ion peak at m/e 164 came from the loss of OTMS and CH_3OH from the molecular ion. In the EI spectrum (Figure 3, bottom), the molecular ion peak at m/e 285 confirmed the molecular weight of the 10-oxo-acid derivative. The ion peak at m/e 238 came from the loss of CH_3 and CH_3OH, the ion peak at m/e 254 came from the loss of CH_3O, from the molecular ion, indicating that it was in fact a 10-oxo-acid molecule, and the ion peaks at m/e 73 and 75 corresponded to OTMS and $(CH_3)_2$-SiOH fragments, respectively. The results of the GC-MS spectra agree with those of the methyl 10-hydroxy-8-decenoate trimethylsilyl ester reported by Tressl *et al.* (1982).

Effect of Initial Linoleic Acid Concentration on the Enzymatic Oxidation. The formation of 1-octen-3-ol, 10-oxo-acid, and 13-HPOD by mycelial homogenate of *P. pulmonarius* at three different initial concentrations of linoleic acid was studied (Figure 4). The 1-octen-3-ol and 10-oxo-acid increased with an increase in initial linoleic acid concentration. At a 1 mM concentration of linoleic acid, both compounds were

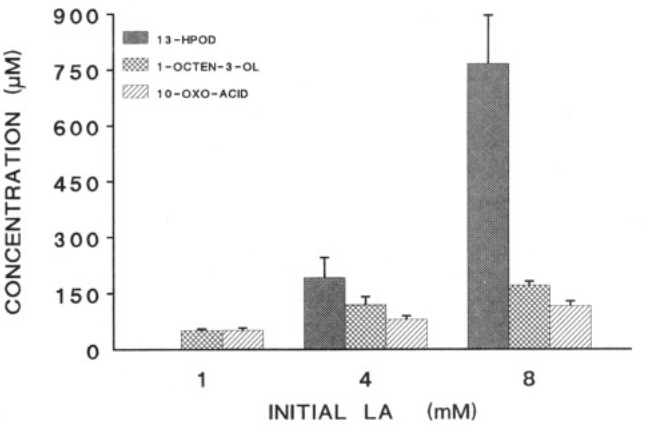


Figure 4. Formation of 1-octen-3-ol, 10-oxo-acid, and 13-HPOD at three different concentrations of initial linoleic acid (LA) by a mycelial pellet homogenate of *P. pulmonarius*. Values indicate mean ± standard deviation.

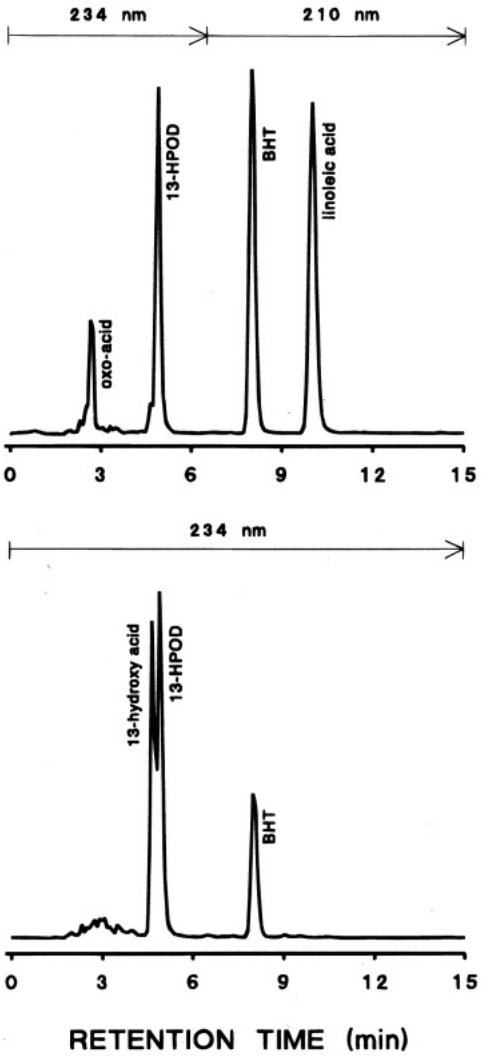
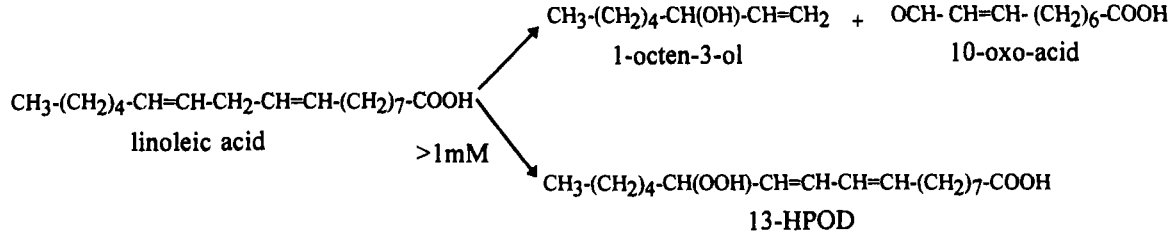


Figure 5. Typical reversed phase HPLC chromatograms of the ethyl ether extraction of a mycelial pellet homogenate of *P. pulmonarius* after incubation with linoleic acid (top) and 13-HPOD (bottom). Arrows indicate monitoring wavelength.

formed to an almost identical concentration (around 51 μM each), whereas at 4 and 8 mM linoleic acid, 1-octen-3-ol concentration became approximately 1.5 times higher than that of 10-oxo-acid, concomitant with the accumulation of 13-HPOD. 13-HPOD, which was not detectable at 1 mM linoleic acid, accumulated to almost 200 μM at 4 mM initial linoleic acid and reached 766

Scheme 1



μM at 8 mM linoleic acid, thereby becoming the major end product of the reaction.

Incubation of Mycelial Pellet Homogenate of *P. pulmonarius* with 13-HPOD. To further clarify whether 13-HPOD is an intermediate in the formation of 1-octen-3-ol from linoleic acid or whether it accumulates in a parallel reaction at high substrate concentration, 13-HPOD synthesized with soybean lipoxygenase type 1 was added as the substrate (1 mM) during homogenization, instead of linoleic acid (Figure 5). As a control, homogenization in the presence of linoleic acid was performed (Figure 5, top). The results showed no production of 1-octen-3-ol or 10-oxo-acid when 13-HPOD was added (unlike the control). However about 50% of the initial 13-HPOD was converted to another compound, having a conjugated diene (λ_{max} = 234 nm), which was detected by HPLC analysis (Figure 5, bottom). This compound was assumed to be 13-hydroxy-*cis*-9,*trans*-11-octadecadienoic acid, as determined by cochromatography against an authentic standard in HPLC (t_R = 4.6 min). These results agree with those reported by Wurzenberger and Grosch (1982) for *A. bisporus* and strongly suggest that 13-HPOD is not an intermediate in the formation of 1-octen-3-ol.

DISCUSSION

In the present work, a mycelial pellet homogenate of *P. pulmonarius* grown in submerged culture converted linoleic acid into 13-HPOD, 1-octen-3-ol, and 10-oxo-acid. The 13-HPOD was analyzed by HPLC, TLC, and GC-MS and determined to be identical to 13-hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acid synthesized from linoleic acid and pure soybean lipoxygenase, type I-S. HPLC, UV, FT-IR, MS, and GC-MS data pertaining to 10-oxo-acid were in agreement with those reported for the 10-oxo-*trans*-8-decenoic acid identified upon incubation of a homogenate of fruiting bodies from *A. bisporus* and *A. campestris* with linoleic acid (Wurzenberger and Grosch, 1982, 1984c; Tressl *et al.*, 1982).

The presence of 13-HPOD depended on the initial linoleic acid concentration, as summarized in the chemical reactions presented in Scheme 1.

Both 1-octen-3-ol and 10-oxo-acid were formed to similar molar ratios regardless of the linoleic acid concentration studied. In contrast, 13-HPOD, which was absent at linoleic acid concentrations below 1 mM, accumulated and became the major nonvolatile product of the enzymatic oxidation above 1 mM linoleic acid. 1-Octen-3-ol and 10-oxo-*trans*-8-decenoic acid were found at an equal molar ratio upon incubation of a homogenate of *A. bisporus* fruiting bodies with 1 mM linoleic acid (Wurzenberger and Grosch, 1982, 1984a,b). Furthermore, these authors proposed 10-HPOD to be the intermediate in the cleavage of linoleic acid to 1-octen-3-ol because tests of a series of four hydroperoxides of linoleic acid showed that only incubation with 10-HPOD led to the formation of 1-octen-3-ol and 10-oxo-acid. However, they did not report any data on HPOD formation during the oxidation of linoleic acid by mushroom fruiting bodies. In contrast, Tressl *et al.* (1982) found 13-HPOD during the cleavage of linoleic acid to 1-octen-3-ol in a homogenate of fruiting bodies of the mushroom *A. campestris* incubated with almost 2 mM linoleic acid. The basis for these differences may be explained by the fact that linoleic acid cleavage by mushroom, as found in our work, depends on its initial concentration, and thus, at a low fatty-acid concentration (*i.e.* at physiological levels) no HPOD accumulates, whereas at intermediate and high concentrations it does.

Despite its accumulation, 13-HPOD was found not to be the precursor of 1-octen-3-ol when supplied as substrate instead of linoleic acid, as it was in plants and vegetables (Vick and Zimmerman, 1989; Gardner *et al.*, 1991; O'Connor and O'Brien, 1991). Furthermore, 50% of the 13-HPOD was reduced to the corresponding hydroxy fatty-acid. Interestingly, 13-hydroxy-*cis*-9,*trans*-11-octadecadienoic acid was not detected when linoleic acid was added as substrate at any concentration studied, suggesting that linoleic acid itself inhibits the reduction of 13-HPOD. Wurzenberger and Grosch (1982, 1984a,b) also found no 1-octen-3-ol formation but a 20% conversion to the corresponding 13-hydroxy fatty acid, when a homogenate of *A. bisporus* fruiting bodies was incubated with 13-HPOD.

The results of the present work further indicate that 13-HPOD accumulation takes place in parallel with 1-octen-3-ol and 10-oxo-acid biosynthesis, suggesting that these are two distinct biosynthetic pathways. Two parallel pathways for the cleavage of linoleic acid in green beans have been proposed by de Lumen *et al.* (1978): one for 1-hexanol, involving 13-HPOD as the precursor, and a second for 1-octen-3-ol, that does not involving 13-HPOD. In addition, the fact that 13-HPOD accumulated at high linoleic acid concentrations whereas it was undetectable at low concentrations of the latter, suggests that two parallel pathways are present, one catalyzing 13-HPOD with low substrate affinity, and a second catalyzing the formation of the 1-octen-3-ol/10-oxo-acid precursor, with high substrate affinity. Further work should be done to clarify these issues.

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LITERATURE CITED

Belinky, P. A.; Masaphy, S.; Levanon, D.; Hadar, Y.; Dosoretz, C. G. Effect of medium composition on 1-octen-3-ol formation in submerged cultures of *Pleurotus pulmonarius*. *Appl. Microbiol. Biotechnol.* **1994**, *40*, 629–633.

de Lumen, B. O.; Stone, E. J.; Kazeniac, S. J.; Forsythe, R. H. Formation of volatile flavor compounds in green beans from linoleic and linolenic acids. *J. Food Sci.* **1978**, *43*, 698-702.

Eyal, J. Mushroom mycelium grown in submerged culture: potential food applications. In *Biotechnology and Food Ingredients*; Goldberg, I., Williams, R., Eds.; Van Nostrand Reinhold: New York, 1991; pp 31–64.

Fischer, K. H.; Grosch, W. Volatile compounds of importance in the aroma of mushrooms (*Psalliota bispora*). *Lebensm. Wiss. Technol.* **1987**, *20*, 233–236.

Gardner, H. W.; Weisleder, D.; Plattner, R. D. Hydroperoxide lyase and other hydroperoxide-metabolizing activity in tissue of soybean, *Glycine max*. *Plant Physiol.* **1991**, *97*, 1059–1072.

Gross, B.; Asther, M. Aromas from basidiomycetes: characteristics, analyses and productions. *Sci. Aliment.* **1989**, *9*, 427–454.

Grossman, S.; Zakut, R. Determination of the activity of lipoxygenase (lipoxidase). In *Methods of Biochemical Analysis*; Glick, D., Ed.; Wiley-Interscience: New York, 1978; Vol. 25, pp 308–322.

Hadar, Y.; Dosoretz, C. G. Mushroom mycelium as a potential source of food flavour. *Trends Food Sci. Technol.* **1991**, *2*, 214–218.

Hanssen, H. P.; Klingenberg, A. Determination of some important flavour compounds in commercial mushroom concentrates. *Z. Lebensm. Unters. Forsch.* **1983**, *177*, 333–335.

Jong, S. C.; Birmingham, J. M. Mushrooms as a source of natural flavor and aroma compounds. In *Mushroom Biology and Mushroom Products*; Chang, S., Buswell, J. A., Chin, S., Eds.; Chinese University Press: Hong Kong, 1993; pp 345–366.

Joseph, C. T.; Murrell, F. D. Visualization procedures. In *Practice of Thin Layer Chromatography*; Joseph, C. T., Murrell, F. D., Eds.; Wiley-Interscience: New York, 1983; pp 163–224.

Kinderlerer, J. L. Volatile metabolites of filamentous fungi and their role in food flavour. *J. Appl. Bacteriol. Symp. Suppl.* **1989**, *18*, 133S–144S.

Maga, J. A. Mushroom flavor. *J. Agric. Food Chem.* **1981**, *29*, 1–4.

O'Connor, T. P.; O'Brien, N. M. Significance of lipoxygenase in fruits and vegetables. In *Food Enzymology*; Fox, P. F., Ed.; Elsevier Applied Science: London, 1991; Vol. 1, pp 337–397.

Satoh, T.; Matsuda, Y.; Takashio, M.; Satoh, K.; Beppu, T.; Arima, K. Isolation of lipoxygenase-like enzyme from *Fusarium oxysporum*. *Agric. Biol. Chem.* **1976**, *40*, 953–961.

Shriner, L. R.; Fuson, C. R.; Curtin, Y. D.; Morrill, C. T. The detection and confirmation of functional groups: complete structure determination. In *The Systematic Identification of Organic Compounds*; Shriner, L. R., Fuson, C. R., Curtin, Y. D., Morrill, C. T., Eds.; Wiley: New York, 1980; pp 145–355.

Smith, A. G.; Gaskell, S. J.; Brooks, C. J. W. Trimethylsilyl group migration during electron impact and chemical ionization mass spectrometry of the trimethylsilyl ethers of 20-hydroxy-5α-pregnan-3-ones and 20-hydroxy-4- pregnen-3-ones. *Biomed. Mass Spectrom.* **1976**, *3*, 161–165.

Sumner, R. J. Lipoid oxidase studies, a method for the determination of lipoxygenase activity. *Ind. Eng. Chem. Anal. Ed.* **1943**, *15*, 14–15.

Thenot, J. P.; Horning, E. C. MO-TMS derivatives of human urinary steroids for GC and GC-MS studies. *Anal. Lett.* **1972**, *5*, 21–33.

Tracey, B. Lipids. In *HPLC of Small Molecules: a Practical Approach*; Lim, C. K., Ed.; IRL Press: Oxford, U.K., 1986; pp 69–102.

Tressl, R.; Bahri, D.; Engel, K. H. Formation of eight-carbon and ten-carbon components in mushrooms (*Agaricus campestris*). *J. Agric. Food Chem.* **1982**, *30*, 89–93.

Vick, B. A. A spectrophotometric assay for hydroperoxide lyase. *Lipids* **1991**, *26*, 315–320.

Vick, B. A.; Zimmerman, D. C. Metabolism of fatty acid hydroperoxide by *Chlorella pyrenoidosa*. *Plant Physiol.* **1989**, *90*, 125–132.

Wurzenberger, M.; Grosch, W. The enzymic oxidative breakdown of linolenic acid in mushrooms (*Psalliota bispora*). *Z. Lebensm. Unters. Forsch.* **1982**, *175*, 186–190.

Wurzenberger, M.; Grosch, W. Stereochemistry of the cleavage of the 10-hydroperoxide isomer of linoleic acid to 1-octen-3-ol by a hydroperoxide lyase from mushrooms (*Psalliota bispora*). *Biochim. Biophys. Acta* **1984a**, *795*, 163–165.

Wurzenberger, M.; Grosch, W. The formation of 1-octen-3-ol from 10-hydroperoxide isomer of linoleic acid by a hydroperoxide lyase in mushrooms (*Psalliota bispora*). *Biochim. Biophys. Acta* **1984b**, *794*, 25–30.

Wurzenberger, M.; Grosch, W. Origin of the oxygen in the products of the enzymatic cleavage reaction of linoleic acid to 1-octen-3-ol and 10-oxo-*trans*-8-decenoic acids in mushrooms (*Psalliota bispora*). *Biochim. Biophys. Acta* **1984c**, *794*, 18–24.

Zimmerman, D. C.; Vick, B. A. Specificity of flaxseed lipoxidase. *Lipids* **1970**, *5*, 392–397.

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