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Identification of Volatile Compounds in Seven Edible Fresh Mushrooms

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About 50 volatile compounds were identified in each of the seven edible fresh mushrooms *Cantharellus, cibarius, Gyromitra esculenta, Boletus edulis, Lactarius trivialis, Lactarius torminosus, Lactarius rufus*, and *Agaricus bisporus*. The relative concentrations of the main aroma compounds were determined and their contribution to the mushroom aroma is discussed.

The studied mushrooms are rich in alcohols and carbonyl compounds containing eight carbon atoms. The main volatile compound in fresh wild mushrooms is 1-octen-3-ol and in cultivated *Agaricus bisporus*, benzyl alcohol. Among the volatiles of *Gyromitra esculenta*, three previously unknown toxic compounds were identified and their structures revealed.

The special aroma of mushrooms constitutes an essential part of their appeal, but mushrooms as foodstuff are difficult to handle, with the result that their aroma is easily changed during processing and storage.

Many mushroom species possess an odour that can be generally described as "mushroom-like". Other species have, in addition, distinct aromas with which they uniquely flavour foodstuffs.

Only a few studies on the volatile compounds in mushrooms have been made.

Tricholoma matsutake (S. Ito & Imai) Sing (called also *Armillaria matsutake*) contains 1-octen-3-ol,[1] the compound also responsible for the "mushroom-like" off flavour of some dairy products.[2]

The East Asian shiitake mushroom, *Lentinus edodes* (Berk.) Sing, contains the sulfur compound lenthionine, which helps to produce its specific flavour.[3]

About fifteen volatiles, including 1-octen-3-ol, 2-octen-1-ol, phenylacetaldehyde, and benzaldehyde, have been identified in cultivated English champignons, *Agaricus bisporus* (Lange) Sing.[4] Polish champignons have been reported to contain eight volatiles, including *cis*-2-octen-1-ol.[5]

Dried *Boletus edulis* Fr. is known to contain numerous lactones, pyrroles and pyrazines,[6] whereas six volatiles have been reported for the fresh mushroom.[7]

The sulfur compounds in *Phallus impudicus* Pers. give this nonedible mushroom its typical odour.[8]

Cantharelle, *Cantharellus cibarius* Fr., is enthusiastically collected in northern Europe because of its attractive aroma and taste and because it is seldom damaged by insects.

In spite of their toxicity when fresh, false morels, *Gyromitra esculenta* (Pers.) Fr., are likewise widely used. These mushrooms possess a delicious aroma distinctly different from that of cantharelle and other edible mushrooms. Since they contain the toxic compound acetaldehyde *N*-methyl-*N*-formyl hydrazone, known is gyromitrin, however, false morels must be cooked or dried before use.[9]

Boletus edulis is also a delicious wild mushroom and extensively collected.

Among wild edible mushrooms, many *Lactarius* species are abundant.

Lactarius trivialis Fr. is the wild mushroom most often collected in Finland.

Under favourable weather conditions high yields of *Lactarius rufus* Fr. are also obtained, and the mushroom has appeared in surprising amounts, for example, in connection with the drying of marshes.

Table 1. The volatile compounds identified in mushrooms.

Peak No.	Compound, proposed structure	%
	Cantharellus cibarius	
1	ethyl formate[a]	
3	ethyl acetate	
4	methylene chloride	
5	benzene	
6	2-pentanone	
7	chloroform	
8	3-methylbutanal[c]	
9	isobutanal	
10	2-hexanone	
11	butanol	0.1
12	2-methyl-2-penten-4-one	0.4
13	3-methylbutanol	0.1
14	pentanol	
16	3-octanone	0.2
20	1-octen-3-one	0.35
21	4-methylpentanol	
24	hexanol	1.0
28	1-octen-3-yl acetate	
29	3-octanol	0.4
31	*trans*-2-octenal	0.1
34	1-octen-3-ol	66
35	furfural[c]	
37	1-octen-3-yl propionate	
39	benzaldehyde[c]	0.4
42	octanol	0.9
47	*trans*-2-octen-1-ol	24
49	nonanol[c]	
55	2-decanone[c]	
58	1-phenylethanol	
60	benzyl alcohol	
62	2-phenylethanol	
64	β-ionone	0.1
66	benzothiazole	
67	epoxy-β-ionone[b]	
84	diethyl phthalate[b]	
86	dibutyl phthalate[b]	
	Gyromitra esculenta	
1	diethyl ether, pentane, and ethyl formate	
2	methylene chloride and ethyl acetate	
3	1-octen-3-yl formate	
4	3-methylbutanal	
5	pentanal[c]	
6	2-hexanone[c]	
7	hexanal	
8	isobutanol	
9	butanol	
10	3-methylbutanol	2.4
11	limonene	
12	3-octanone	0.4
13	*p*-cumene	
14	1-octen-3-one	0.4
16	2-methyl-3-octanone[c]	
19	hexanol	0.3
20	octanal[c]	
21	1-octen-3-yl acetate	
22	3-octanol	3.9
23	*trans*-2-octenal	0.7
24	1-octen-3-ol	72
25	heptanol[c]	
26	furfural[c]	
28	benzaldehyde	
29	isophorone	
30	acetaldehyde *N*-methyl-*N*-formyl hydrazone	0.9
31	octanol	0.3
36	*trans*-2-octen-1-ol	1.4
37	phenylacetaldehyde	3.0
38	nonanol	
39	lavandulol[c]	
40	benzyl acetate	
41	butyrophenone[c]	
43	3-methylbutanal *N*-methyl-*N*-formyl hydrazone	3.3
44	decanol[c]	
45	pentanal *N*-methyl-*N*-formyl hydrazone	0.1
48	benzyl alcohol	0.2
49	2,6-di-*tert*-butyl-4-methylphenol	
50	β-ionone	
51	hexanal *N*-methyl-*N*-formyl hydrazone	0.6
52	2-phenylethanol	0.6
57	benzothiazole	
58	4-hydroxynonanoic acid lactone[c]	
62	4-hydroxydecanoic acid lactone[c]	
65	diethyl phthalate[b]	
72	dibutyl phthalate[b]	
	Boletus edulis	
1	pentane and diethyl ether	
2	ethyl formate	
4	ethyl acetate	
5	methylene chloride	
10	3-methylbutanal	8
18	hexanal[c]	0.5
20	isobutanol	
22	butanol	
27	3-methylbutanol	17
28	pentanol	
29	3-octanone	0.8
35	1-octen-3-one	8
41	hexanol	
44	3-octanol	0.5
46	*trans*-2-octenal	1
47	1-octen-3-ol	49
51	1-octen-3-yl propionate	
52	benzaldehyde	0.5
53	linalool[c]	
54	octanol	
55	*trans*-2-octen-1-ol	11

Table 1. Continued.

56	phenylacetaldehyde	1
57	nonanol	
65	1-phenylethanol	
66	benzyl alcohol	
69	2,6-di-*tert*-butyl-4-methylphenol	1
71	2-phenylethanol	
72	2-phenyl-2-butenal	1
75	benzothiazole	
Lactarius trivialis		
1	pentane, diethyl ether, and 2-pentanone	
3	acetaldehyde[b]	
4	ethyl acetate	
5	methylene chloride	
6	1-octen-3-yl formate[b]	
7	2-hexanone	
10	hexanal	
13	isobutanol	
15	butanol	
16	2-heptanone[c]	
17	3-methylbutanol	
18	pentanol	
19	3-octanone	0.2
20	2-octanone[c]	
22	1-octen-3-one	0.2
26	4-methylcyclohexanone[c]	0.05
27	hexanol	0.2
28	1-octen-3-yl acetate	0.03
29	3-octanol	
30	*trans*-2-octenal	0.2
32	3,3,5-trimethylcyclohexanone	
34	1-octen-3-ol	70
36	furfural	
38	benzaldehyde	0.05
40	isophorone	
42	linalool	
43	linaloyl acetate	
44	octanol	0.4
46	*trans*-2-octen-1-ol	24
47	phenylacetaldehyde[c]	
53	2,4-nonadienal[b]	
55	naphthalene	
58	2,4-decadienal[b]	
59	citronellyl acetate[b]	
60	5-hydroxyheptanoic acid lactone[c]	
61	2-decanone[c]	
62	2,4-undecadienal	
63	*β*-ionone	
64	4-hydroxynonanoic acid lactone	
67	epoxy-*β*-ionone[c]	0.2
68	derivative of velleral[b]	0.1
Lactarius torminosus		
1	pentane	
2	diethyl ether	
3	ethyl formate	
4	ethyl acetate	
5	methylene chloride	
13	hexanal	
18	isobutanol[c]	
20	butanol	
25	3-methylbutanol[c]	1.5
26	pentanol	
28	3-octanone	0.5
34	1-octen-3-one	
38	3-octanol	0.5
39	*trans*-2-octenal	0.5
42	1-octen-3-ol	90
44	furfural[c]	
45	benzaldehyde[c]	
50	linalool	
51	linaloyl acetate	
52	octanol	
56	methyl decanoate	
58	*trans*-2-octen-1-ol[c]	
68	2,4-nonadienal[b]	
69	1-phenylethanol[c]	
74	2,4-decadienal[b]	
76	benzyl alcohol[c]	
84	4-hydroxynonanoic acid lactone	
93	epoxy-*β*-ionone[c]	
Agaricus bisporus		
1	pentane	
2	diethyl ether	
3	ethyl formate	
4	ethyl acetate	
5	methylene chloride	
7	1-octen-3-yl formate[b]	
8	3-methylbutanal	
12	hexanal	0.5
13	furfuryl acetone[c]	
14	isobutanol	
15	2-heptanone[c]	0.2
16	butanol	
19	3-methylbutanol	11
20	pentanol	
21	3-octanone	4
25	1-octen-3-one	
32	hexanol	
35	1-octen-3-yl acetate	
36	3-octanol	1
37	*trans*-2-octenal	
40	1-octen-3-ol	33
41	heptanol	
42	lavandulol deriv.[c]	
44	1-octen-3-yl propionate	
45	benzaldehyde	3
47	lavandulol deriv.[c]	
48	linalool[c]	
49	linaloyl acetate[c]	
50	octanol	
54	*trans*-2-octen-1-ol	6
56	nonanol	
57	lavandulol[c]	0.5
60	benzyl acetate	
65	1-phenylethanol	

Table 1. Continued.

70	benzyl alcohol	37
71	benzyl butyl ether[c]	
73	2-phenylethanol	
74	benzothiazole	
75	benzyl octyl ether[c]	
76	ester of benzoic acid[c]	
77	dibutyl phthalate[c]	

Lactarius rufus

1	pentane and diethyl ether	
2	ethyl formate	
3	ethyl acetate	
4	methylene chloride	
11	hexanal	
16	isobutanol	
19	butanol	
22	3-methylbutanol	
27	heptanal[c]	
29	3-octanone	
37	1-octen-3-one	2
45	hexanol	8
52	3-octanol	1.5
53	*trans*-2-octenal[c]	3
57	1-octen-3-ol	72
58	furfural	
62	2-decanone[c]	
64	benzaldehyde	5
67	isophorone	
73	octanol	
80	*trans*-2-octen-1-ol[c]	
89	nonanol[c]	
90	ethyl benzoate[b]	1.5
92	2,4-nonadienal[b]	
99	naphthalene	
103	benzyl alcohol	
106	citronellyl acetate[b]	
114	4-hydroxyoctanoic acid lactone[c]	
120	2,6-di-*tert*-butyl-4-methylphenol[b]	
127	benzothiazole[b]	
132	epoxy-β-ionone[c]	
139	derivative of velleral[c]	

[a] Compounds without index: Identification based on the mass spectrum and comparison with reference compound in GLC. [b] Identification based on MS. [c] Tentative identification.

The attitude towards *Lactarius* species as edible mushrooms differs greatly from country to country. *Lactarius torminosus* (Fr.) Gray, for instance, is considered inedible and even poisonous in Germany. On the other hand, in eastern and northern European countries it is widely collected. Like most other *Lactarius* species, however, *Lactarius torminosus* is bitter tasting when fresh and must be boiled before being eaten.

Champignons, *Agaricus bisporus*, are cultivated on a large scale. Most of the commercial mushroom products are prepared from this mushroom. However, compared with the aroma of wild mushrooms, that of champignons can be considered mild and less attractive.

Wild, fresh or deep frozen *Cantharellus cibarius*, *Gyromitra esculenta*, *Boletus edulis*, *Lactarius trivialis*, *Lactarius rufus*, *Lactarius torminosus* and cultivated *Agaricus bisporus* were studied using a steam distillation, extraction and concentration procedure. The temperature was kept below +35 °C in order to obtain high yields of the volatile compounds.

RESULTS

The mushrooms studied contained, on average, 5 − 15 ppm volatiles, which amount does not include the acids. The percentages of the major components with respect to the total amount of volatiles are seen in Table 1. The areas (Fig. 1) of the minor peaks can be compared with the areas of the signals reported in Table 1. Table 2 presents the volatile acids and their relative concentrations in the mushroom samples.

The aroma concentrations also contained some unknown components for which proper mass spectra were recorded, but exact interpretation of the spectra failed.

Compound *29* in *Gyromitra esculenta*, MS: *m/e* 111(80), 91(15), 83(25), 70(60), 69(100), 55(85), and the compound *39*, MS: *m/e* 136(2), 124(7), 111(50), 93(14), 81(26), 69(100), 57(36), 55(54), 41(45) showed a similar type of mass spectra fragmentation. The spectrum of compound 39 is nearly identical to that of lavandulol.[10] The mass spectra of γ-isogeraniol and hydrated ionones also contain the fragments 111 and 69.

Based on the mass spectrum, compound *57* in *Agaricus bisporus* was tentatively identified as lavandulol,[10] and the compounds *42*, MS: *m/e* 111(83), 83(14), 81(13), 69(100), 57(38), 55(58), 41(46), and *47*, MS: *m/e* 151(10), 137(16), 111(95), 95(7), 81(12), 69(100), 55(45), 41(40) were tentatively identified as compounds whose structures are closely related to that of lavandulol.

Compound *68*, MS: *m/e* 232(75 %), 217(36), 202(25), 199(50), 189(53), 123(100), 105(21),

Table 2. The volatile acids and their relative concentrations (%) in steam distillates obtained from mushrooms.

Acid	*Cantharellus cibarius*	*Gyromitra esculenta*	*Boletus edulis*	*Lactarius trivialis*	*Lactarius torminosus*	*Lactarius rufus*	*Agaricus bisporus*
Acetic	26	9	4	8	8	35	76
Propionic	5	+	1	0.5	+	3.5	+
Isobutyric	+	6	+	0.5	3	+	+
Butyric	8	+	1	1	+	4.5	+
Isovaleric	+	31	+	1	+	3	+
Valeric	5	+	3	0.5	+	2.5	+
Isocaproic	+	2	+	0.5	–	5.0	+
Caproic	32	23	53	8	2	17	7
Heptanoic	+	7	2	1	1	3.5	+
Octanoic	8	11	2	4	6	8	7
Nonanoic	2	+	2	10	1	2.5	+
Decanoic	1	+	+	57	72	8	4
trans-2-Octenoic	7	7	9	1	+	+	+

+ Small amounts tentatively identified.

95(37), 91(29), 81(51), 43(55) in *Lactarius trivialis*, and compound *139*, MS: m/e 232(70 %), 217(35), 199(45), 189(50), 123(100), 95(59), 81(50), 57(60), 43(90), in *Lactarius rufus* were tentatively identified as compounds with structures closely related to those of velleral and isovelleral.[11] These bitter tasting compounds were originally identified from *Lactarius vellereus* (Fr.) Fr.[11,12]

Three new toxic compounds were found among the volatiles of *Gyromitra esculenta.* Structurally (Fig. 2) these compounds proved to be higher homologues of acetaldehyde *N*-methyl-*N*-formyl hydrazone (gyromitrin). In preliminary tests, rabbits fed with 350 mg of pentanal, 3-methylbutanal, or hexanal *N*-methyl-*N*-formyl hydrazone per 1 kg body weight died within 24 h.

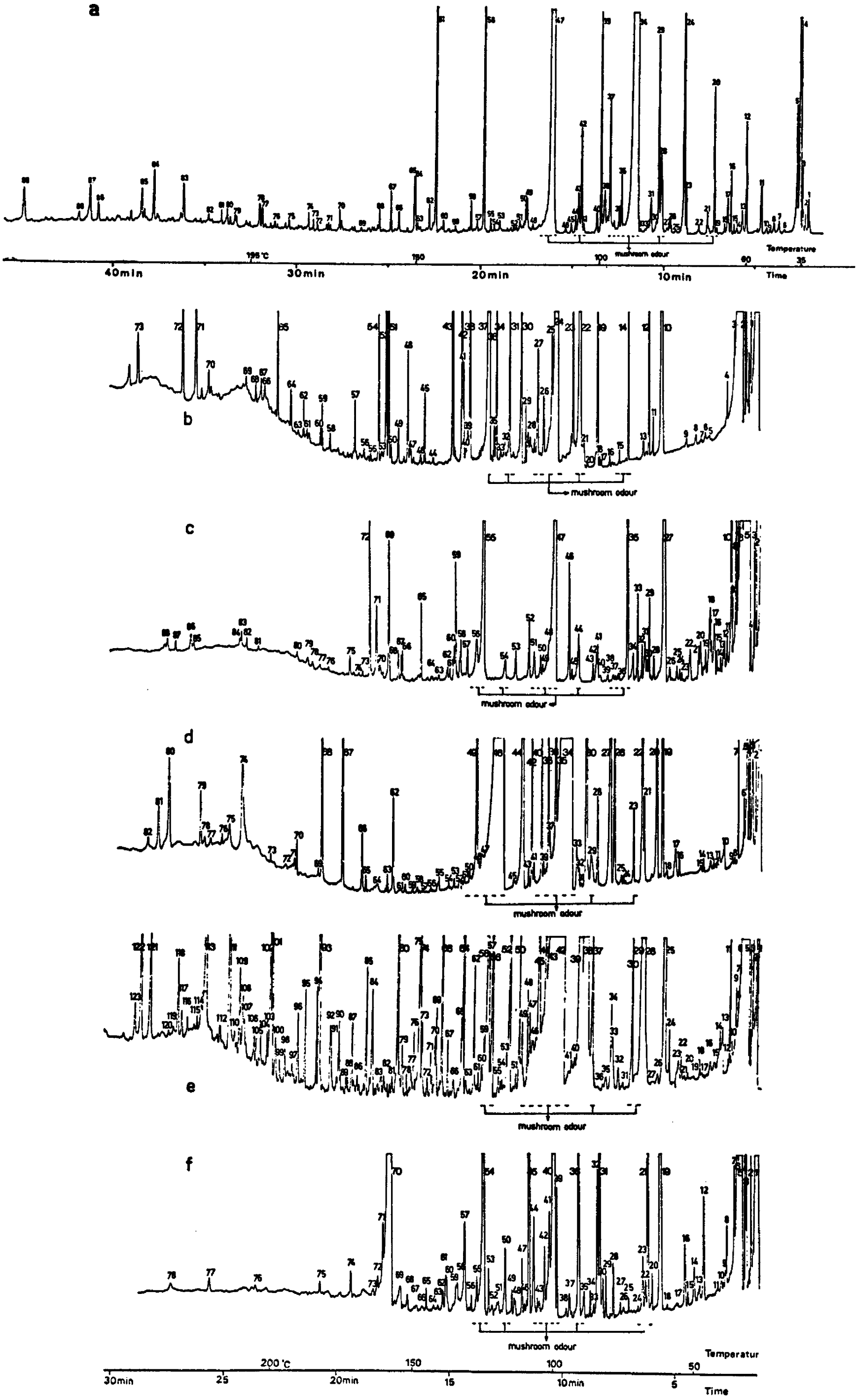
Pentane, diethyl ether and methylene chloride were used as solvents. The phthalates and 2,6-di-*tert*-butyl-4-methylphenol may have originated from the packing materials. Naphthalene, chloroform and benzene probably did not originate from mushrooms.

To establish which compounds contribute to the characteristic odours of the mushrooms, the concentrates were analyzed by preparative GLC with packed FFAP column and four persons familiar with mushrooms were asked to describe the odours eluting from the column. The retention times at which the panelists considered the odours to be "mushroom-like" are seen in Fig. 1.

DISCUSSION

In the mushrooms studied here the main volatiles all have eight carbon atoms. From the descriptions of the odours (Fig. 1) and the tests with synthetic reference compounds,[13] the fresh mushroom-like aroma of the studied mushroom species can be attributed mainly to 1-octen-3-ol, 1-octen-3-one, *trans*-2-octen-1-ol, octanol, nonanol, esters of 1-octen-3-ol, *trans*-2-octenal, and 3-octanol. Many others of the identified compounds, *e.g.*, benzaldehyde, 2,4-dienals, hexanal, and dihydroisophorone, possess strong odours, too. 1-Octen-3-ol gives the general mushroom-like aroma to all mushrooms, while 1-octen-3-one seems to be important in producing the more specific aroma of wild mushrooms.

Dried and cooked *Boletus edulis* contains a great number of pyrazines, pyrroles and lactones,[6] none of which was found in the fresh mushroom. The main volatile in fresh *Boletus edulis*, 1-octen-3-ol, was present in the dried product only in low concentration, while 1-octen-3-one and *trans*-2-octen-1-ol were not found at all in dried *Boletus edulis.*[6] 2-Phenyl-2-butenal was not found in any of the mushrooms studied here except *Boletus edulis*. It has also been isolated from *Phallus impudicus*,[8] a mushroom with an odd odour. 2-Phenyl-2-butenal was synthesized and found to possess


a
mushroom odour
Temperature
195 °C
150
100
60
35
40min
30min
20min
10min
Time
b
mushroom odour
c
mushroom odour
d
mushroom odour
e
mushroom odour
f
mushroom odour
Temperatur
200 °C
150
100
50
30min
25
20min
15
10min
5
Time

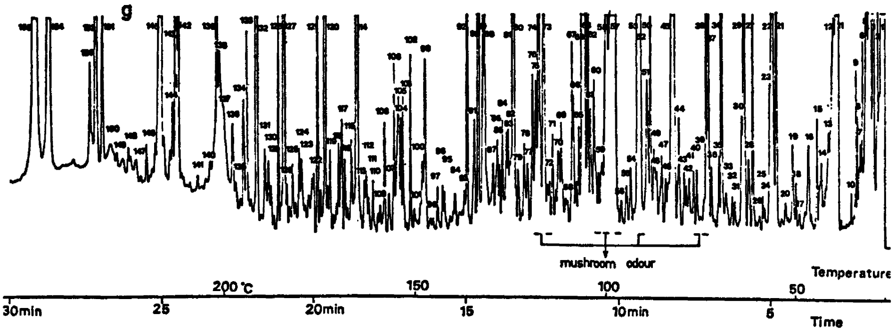


Fig. 1. Gas chromatogram of the volatile extract obtained from (a) fresh *Cantharellus cibarius*, (b) deep frozen, fresh *Gyromitra esculenta*, (c) deep frozen, fresh *Boletus edulis*, (d) deep frozen, fresh *Lactarius trivialis*, (e) fresh *Lactarius torminosus*, (f) fresh *Agaricus bisporus*, and (g) deep frozen, fresh *Lactarius rufus*. Liquid phase FFAP, 40 m × 0.3 mm i.d. glass capillary column, constructed by A. Hesso, University of Helsinki.

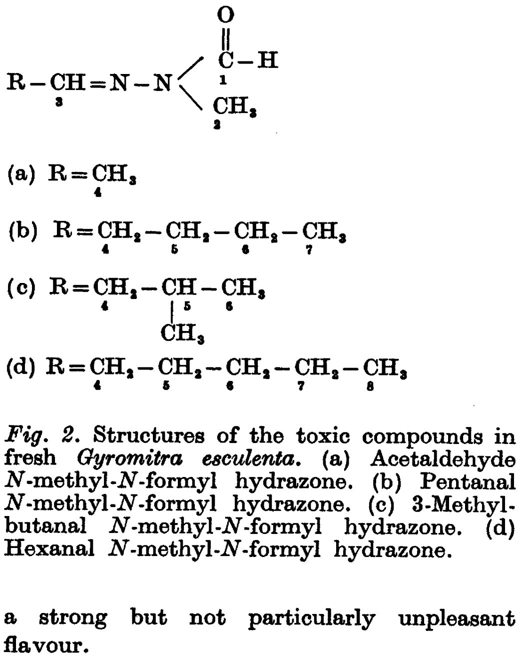


Fig. 2. Structures of the toxic compounds in fresh *Gyromitra esculenta*. (a) Acetaldehyde *N*-methyl-*N*-formyl hydrazone. (b) Pentanal *N*-methyl-*N*-formyl hydrazone. (c) 3-Methylbutanal *N*-methyl-*N*-formyl hydrazone. (d) Hexanal *N*-methyl-*N*-formyl hydrazone.

a strong but not particularly unpleasant flavour.

1-Octen-3-ol is the main volatile component in all three *Lactarius* mushrooms. *trans*-2-Octen-1-ol is present in *Lactarius rufus* and *Lactarius torminosus* in very low concentration, if at all, although it is among the main volatiles in *Lactarius trivialis* and in all the other mushrooms studied. The aroma of *Lactarius rufus* is considered to be milder than that of *Lactarius trivialis* and *Lactarius torminosus*. Hence, the great number of signals in Fig. 1 g does not reflect the higher total concentration of volatiles in *Lactarius rufus* but greater sensitivity of the gas chromatograph used in recording the chromatogram of this mushroom.

The composition of aroma compounds in *Agaricus bisporus* differs from that in wild mushrooms mostly with respect to the high concentration of benzyl alcohol and benzaldehyde. These compounds are the main volatiles only in champignons. The total amount of volatiles in wild mushrooms and in *Agaricus bisporus* was found to be of the same level, 5 – 15 ppm and the mild mushroom-like aroma of champignons can thus be attributed to low concentrations of 1-octen-3-ol, 1-octen-3-one, and *trans*-2-octen-1-ol.

It is evident that processing quickly changes the aroma composition of mushrooms. The steam distillate of English champignons obtained at atmospheric pressure was reported to contain a high concentration of phenylacetaldehyde,[4] which compound was not found in the Finnish champignons. Polish champignons have been reported to contain *cis*-2-octen-1-ol as one of the main volatile components.[5] According to this work, 2-octen-1-ol is in *trans*-form in *Agaricus bisporus* and all the other mushrooms studied. It is possible, however, that the mushrooms also contain low concentrations of *cis*-2-octen-1-ol.

It should be noted that the concentration of 3-methylbutanal *N*-methyl-*N*-formyl hydrazone in *Gyromitra esculenta* is high, even higher than the concentration of acetaldehyde *N*-methyl-*N*-formyl hydrazone. Since hydrazones easily hydrolyse, the concentrations in the sample made from the steam distillate do not correspond exactly to the concentrations of hydrazones in fresh false morels.

The quantitative determinations of the volatiles (Table 1) are based on peak areas in GLC. Although the GLC showed satisfactory separation power, peak areas determined by integration do not correspond exactly to original concentrations. The volatiles are lost to some extent during preparation of the concentrates and consequently the ratio of the concentrations of the components is changed. Moreover, the splitter in GLC does not split the compounds in exactly the original ratio.

The results of analyses on acids do not exclude the presence of formic acid. Formic acid gives no signal in FID though there is no reason to believe it absent, especially since esters of formic acid were found.

Acids containing an even number of carbon atoms are present in considerably higher concentration than those with odd numbers of carbon atoms. However, isovaleric acid is one of the main acidic components in *Gyromitra esculenta*.

EXPERIMENTAL

The volatile concentrate of *Cantharellus cibarius* was prepared from fresh mushrooms collected in early September in eastern Finland. *Gyromitra esculenta* was collected in May and deep frozen and stored a few months at − 30 °C. *Agaricus bisporus* was purchased from a local cultivator. The aroma concentrate was made from fresh champignons, diameter of the pileus less than 3 cm. *Boletus edulis* was collected in August in northern Finland and stored a few days at − 30 °C. The aroma concentrate of *Lactarius torminosus* was prepared from fresh mushrooms, collected in August in eastern Finland. *Lactarius trivialis* and *Lactarius rufus* were collected in southern Finland in August and stored a few weeks at − 30 °C. Only mushrooms with a diameter of the pileus less than 8 cm were used.

General methods and instruments. Mushrooms (5 kg) were pressed into juice, which was steam distilled in vacuum, not exceeding a temperature of 35 °C. The pH of the distillate was adjusted with sodium bicarbonate to 8 and the volatiles were extracted during 24 h into an ether − pentane mixture 1:1. The extract was concentrated in vacuum to 50 μl. Instruments described by Honkanen *et al.* and Suomalainen *et al.* were used.[14,15] The solvents were commercial chemicals of *pro analysi* quality and were freshly distilled. Cool traps in dry ice or in liquid nitrogen were used to avoid the loss of light volatiles.

The flavours were retained during preparation of the samples, and the final concentrates, as detected by several persons familiar with mushrooms, had the characteristic aromas of the original mushrooms. After extraction of the neutral volatiles, the steam distillate was made acidic (pH = 3) with hydrochloric acid and the acids were extracted into ether − pentane as above. The aroma concentrates were studied with a Perkin-Elmer 270B GLC-MS system equipped with an 8 m stainless steel packed FFAP column and with a Jeol JMS-D100 GLC-MS system equipped with a 90 m glass capillary FFAP column. The gas chromatographs were programmed from 60 to 200 °C, 2 °C per min. The gas chromatograms (Fig. 1) were recorded with a Carlo-Erba Mod. GI-instrument, carrier gas and flow 4 ml H_2 per min, splitting ratio 1:30.

The quantitative estimates of the total amounts of the volatiles were made so that known amounts of the 5 − 10 largest components were added to the original juices and the peak areas in the GLC made from these samples were compared with those obtained from the samples prepared in a similar way but without added components.

^1H NMR spectra were recorded at 60 or 100 MHz in $CDCl_3$ and the scales are in ppm downfield from internal TMS.

Proton noise decoupled ^{13}C NMR spectra were recorded at 25 MHz with a Jeol JNM PFT-100 spectrometer and the chemical shifts from internal TMS in $CDCl_3$ were determined with a 20k EC-20 data system. The assignments in the proton noise decoupled ^{13}C NMR spectra were confirmed through the undecoupled ^{13}C NMR spectra.

The mass spectra (70 eV) were compared with those in standard collections of mass spectra and the retention times in a 90 m FFAP glass capillary column were compared with those of commercially available or synthetic compounds.

The acidic fractions were studied by GLC with a 50 m FFAP glass capillary column. The retention times of the original acids and in some cases also their methyl esters were compared with reference compounds. The mass spectra of the main acidic components were measured as well. The quantitative estimations of the acids (Table 2) are based on their peak areas in GLC.

Preparation of the reference compounds. 1-Octen-3-one was obtained by a potassium dichromate oxidation from commercial 1-octen-3-ol.[16] The mass spectrum of synthetic 1-octen-3-one was identical to that reported by Stark *et al.*[17] *trans*-2-Octenoic acid, b.p. 140 − 143 °C/15 mmHg, was prepared by Knoevenagel reaction from hexanal and malonic acid.[18]

From the splitting due to the coupling $J_{H_2-H_3}$ = 16Hz in the ^{1}H NMR spectrum, the compound was identified as the *trans*-isomer.

trans-2-Octen-1-ol, b.p. 95 °C/13 mmHg, was prepared from *trans*-2-octenoic acid by reduction with lithium aluminium hydride in ether. ^{1}H NMR spectrum: δ 5.35 (m, 2 H, C2-H, C3-H), 3.8 (m, 2 H, C1-H), 2.6 (s, 1 H, OH), 1.8 (m, 2 H, C4-H), 1.3 (m. 6 H, C5-H, C6-H, C7-H), 0.9 (t, 3 H, C8-H). With the aid of tris(dipivalomethanato)-europium shift reagent, the coupling $J_{H_2-H_3}$ was revealed to be 16 Hz, which indicates the compound to be the *trans*-isomer. The IR spectrum was identical to that reported for *trans*-2-octen-1-ol.[5]

trans-2-Octenal, b.p. 90–95 °C/15 mmHg, was prepared from the corresponding alcohol by oxidation with fresh manganese dioxide,[18] and from the coupling $J_{H_2-H_3}$ = 16 Hz in ^{1}H NMR spectrum identified as the *trans*-isomer.

Acetaldehyde *N*-methyl-*N*-formyl hydrazone was synthesized according to the method described by List.[9] The structure of this compound was confirmed by MS, IR, ^{1}H NMR and ^{13}C NMR experiments.[19]

2,4-Undecadienal was obtained from heptanal and malonic acid, from which the compounds 2-nonenic acid, 2-nonen-1-ol, and 2-nonenal were obtained analogously to the method described by Nobuhara *et al.*[18] 2-Nonenal and malonic acid in dry pyridine, piperidine as catalyst, gave 2,4-undecadienoic acid, b.p. 170–175 °C/mmHg.

2,4-Undecadienoic acid was reduced with lithium aluminium hydride in dry ether to 2,4-undecadienol, b.p. 130–135 °C/9 mmHg. 2,4-Undecadienol was oxidized with fresh manganese dioxide in dry ether to 2,4-undecadienal, b.p. 130–135 °C/11 mmHg. ^{1}H NMR δ 0.95 (t, 3 H, CH_3), 1.35 (m, 8 H, C7-H, C8-H, C9-H, C10-H), 1.35 (m, 2 H, C6-H), 5.5–7.0 (m, 4 H, C2-H, C3-H, C4-H, C5-H) 9.3 (d, 1 H, CHO).

3,3,5-Trimethylcyclohexanone (dihydroisophorone), b.p. 189–189.5 °C, was synthesized from isophorone *via* catalytic reduction with Raney nickel in 1:1 tetrahydrofuran–ethanol mixture. ^{1}H NMR spectrum: δ 0.9 (d, 3 H, C5-CH_3). 1.05 (s, 6 H, C3-CH_3), 1.1–1.6 (m, 1 H, C3-H), 1.7–2.4 (m, 4 H, C2-H, C4-H). MS: *m/e* 149(9 %), 125(10), 97(4), 83(100), 69(57), 56(31), 55(35), 41(35).

The aliphatic chain γ-lactones were obtained from the corresponding 2-alkenoic acids in 70 % sulfuric acid,[20] and the aliphatic chain δ-lactones from their corresponding δ-hydroxyacids.[20]

Following List,[8] 2-phenyl-2-butenal, b.p. 120 °C/14 mmHg, was prepared in water–ethanol mixture (1:1) by condensing 24 g phenylacetaldehyde with 8.8 g acetaldehyde in the presence of 10 g sodium acetate during 24 h in an autoclave at 120 °C. The ^{1}H NMR spectrum was identical to that reported in the literature.[8] Mass spectrum: *m/e* 146(100 %), 118(18), 117(90), 115(64), 91(30), 78(13), 77(8), 65(10), 51(13).

Pentanal, 3-methylbutanal, and hexanal *N*-methyl-*N*-formyl hydrazones were prepared analogously to acetaldehyde *N*-methyl-*N*-formyl hydrazone.[9] Methylhydrazine (0.3 mol) was treated at –20 °C with ethyl formate (0.3 mol) to give 19 g (85 % of theory) *N*-methyl-*N*-formyl hydrazine, which upon addition of the corresponding aldehyde (0.1 mol:0.1 mol) at –20 °C gave *N*-methyl-*N*-formyl hydrazones of pentanal, 3-methylbutanal, and hexanal in 50 % yield, respectively. Pentanal *N*-methyl-*N*-formyl hydrazone: Mass spectrum: *m/e* 142(16), 113(8), 100(48), 85(100), 84(39), 71(25), 69(21), 67(8), 60(19), 59(73), 57(25), 43(50), 41(34). ^{1}H NMR spectrum: δ 8.5 (s, 1 H, C1-H), 7.1 (t, 1 H, C3-H), 3.1 (s, 3 H, C2-H), 2.25 (m, 2 H, C4-H), 1.45 (m, 4 H, C5-H, C6-H), 0.95 (t, 3 H, C7-H). Proton noise decoupled ^{13}C NMR spectrum. The scales are in ppm downfield from internal TMS in $CDCl_3$ (δ). C1: 164.7, C2: 26.5, C3: 144.8, C4, C5 and C6: 32.4, 28.9 and 22.3, C7: 13.8. IR spectrum: 2970, 1660–1700, 1625, 1450, 1400, 1370, 1325, 1100, and 1030 cm^{-1}.

3-Methylbutanal *N*-methyl-*N*-formyl hydrazone: Mass spectrum: *m/e* 142(13 %), 127(9), 100(10), 85(89), 84(52), 71(31), 60(19), 59(100), 57(28), 43(80), 41(56). ^{1}H NMR spectrum: δ 8.45 (s, 1 H, C1-H), 7.15 (t, 1 H, C3-H), 3.1 (s, 3 H, C2-H), 2.2 (m, 2 H, C4-H), 1.7–2.3 (m, 1 H, C5-H), 1.0 (d, 6 H, C6-H). ^{13}C NMR spectrum: C1: 164.7, C2: 26.5, C3: 144.0, C4: 41.4, C5: 26.9, C6: 22.4. IR spectrum: 2950, 1660–1700, 1615, 1450, 1395, 1360, 1315, 1205, 1195, 1020, 910, 830, 695 cm^{-1}.

Hexanal *N*-methyl-*N*-formyl hydrazone: Mass spectrum *m/e* 156(12 %), 139(4), 127(3), 113(10), 100(57), 98(54), 85(100), 71(20), 60(41), 59(78), 55(25), 43(68). ^{1}H NMR spectrum: δ 8.45 (s, 1 H, C1-H), 7.1 (t, 1 H, C3-H), 3.1 (s, 3 H, C2-H), 2.25 (m, 2 H, C4-H), 1.45 (m, 6 H, C5-H, C6-H, C7-H), 0.95 (t, 3 H, C8-H). ^{13}C NMR spectrum: C1: 164.7, C2: 26.5, C3: 144.8, C4, C5, C6 and C7: 32.6, 31.4, 26.5 and 22.4, C8: 13.9. IR spectrum: 2840–2950, 1660–1700, 1615, 1445, 1390, 1355, 1315, 1205, 1135, 1020, 890, 690 cm^{-1}.

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