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Geosmin, a sesquiterpenoid compound responsible for the musty-earthy odor of *Cortinarius herculeus*, *Cystoderma amianthinum*, and *Cy. carcharias*

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Abstract: The fruiting bodies of 3 fresh wild mushrooms species were investigated for odorous volatile compounds by gas chromatography-mass spectrometry analysis using both headspace concentration and solvent extraction techniques. The mushrooms species studied, *Cortinarius herculeus, Cystoderma amianthinum* and *Cy. carcharias,* are well-known to possess musty-earthy odors. Geosmin, a sesquiterpenoid derivative, was identified as the key compound responsible for the musty-earthy aroma of these Basidiomycota.

Key Words: aroma, Basidiomycota

Many mushroom species (Basidiomycota) are well-known by mycologists to possess typical odors such as fungal, aniselike, fruity, or farinaceous. The fruit-body aromas may help species identification. On other hand, many authors have studied volatile molecules of fruit bodies without any correlation with their overall aromas. Basidiomycota produced oxygenated C_8-aliphatic, aromatic and heterocyclic compounds, i.e., sulfurous molecules, lactones and terpenic constituents (Breheret et al 1997a, Buchbauer et al 1993, Mau et al 1994, Rapior et al 1998). However only a few mushroom species have been investigated for odorous key components directly responsible for the corresponding fruit-body aroma (Breheret et al 1997b, Laatsch and Matthies 1992, Largent et al 1990, Rapior et al 1997, Watson et al 1986, Wood et al 1988, 1990, 1992, 1994).

The present work is the first study on the musty-earthy aroma of fresh mushroom species. According to the olfactory reference The Field of Odors (Jaubert et al 1995), and to the literature, three species were described as possessing an intensive, musty-earthy odor: *Cortinarius herculeus* Malençon (Courtecuisse and Duhem 1994, Trescol 1992, Moser 1978), *Cystoderma amianthinum* (Scop.) Fayod var. *Rugosoreticulatum* (Courtecuisse and Duhem 1994, Laskibar Urkiola and Palacios Quintano 1991, Lincoff 1988, Moser 1978), and *Cystoderma carcharias* (Pers.: Fr.) Fayod (Courtecuisse and Duhem 1994, Clémençon et al 1980, Moser 1978). These mushrooms were investigated for volatile constituents by dynamic headspace concentration and solvent extraction using gas chromatography, mass spectrometry, and sniffing evaluation to identify the key odorous compounds.

Materials and methods.—Fresh wild and odorous mushroom species were collected in the fall of 1995 in Languedoc-Roussillon (France). *Cortinarius herculeus, Cy. amianthinum, Cy. carcharias* were wrapped in waxed paper bags, after morphological identification by one of us (SR) according to the classification by Courtecuisse and Duhem (1994). The specimens were brushed clean of forest debris and treated immediately after collection using both techniques.

Aromas of the fresh fruit bodies were concentrated by the dynamic headspace method and solvent extraction, and analysed by gas chromatography and mass spectrometry (Breheret 1997). About 10 g of fresh fruiting bodies for each species were cubed and placed in a glass cell (0.25 L capacity) directly connected to a dynamic headspace injector. Volatiles were concentrated on TENAX trap with a stripping gas (Helium) flow rate of 30 mL/min for 20 min at room temperature. Solvent extraction was performed with 400, 40 and 19 g of fresh mushrooms cubed for *Co. herculeus, Cy. amianthinum* and *Cy. carcharias,* respectively. Volatile compounds were extracted with dichloromethane in a Soxhlet apparatus for 5 h. Organic extracts were gently concentrated under nitrogen stream and used directly for analysis.

Gas chromatography-mass spectrometry analyses were carried out using a gas chromatograph (5890-Hewlett-Packard) and a mass selective detector (5971-Hewlett-Packard) with a potential of 70 eV for ionization by electron impact. Headspace analyses were carried out by a headspace injector (CHISA device-SGE), directly connected to an analytical

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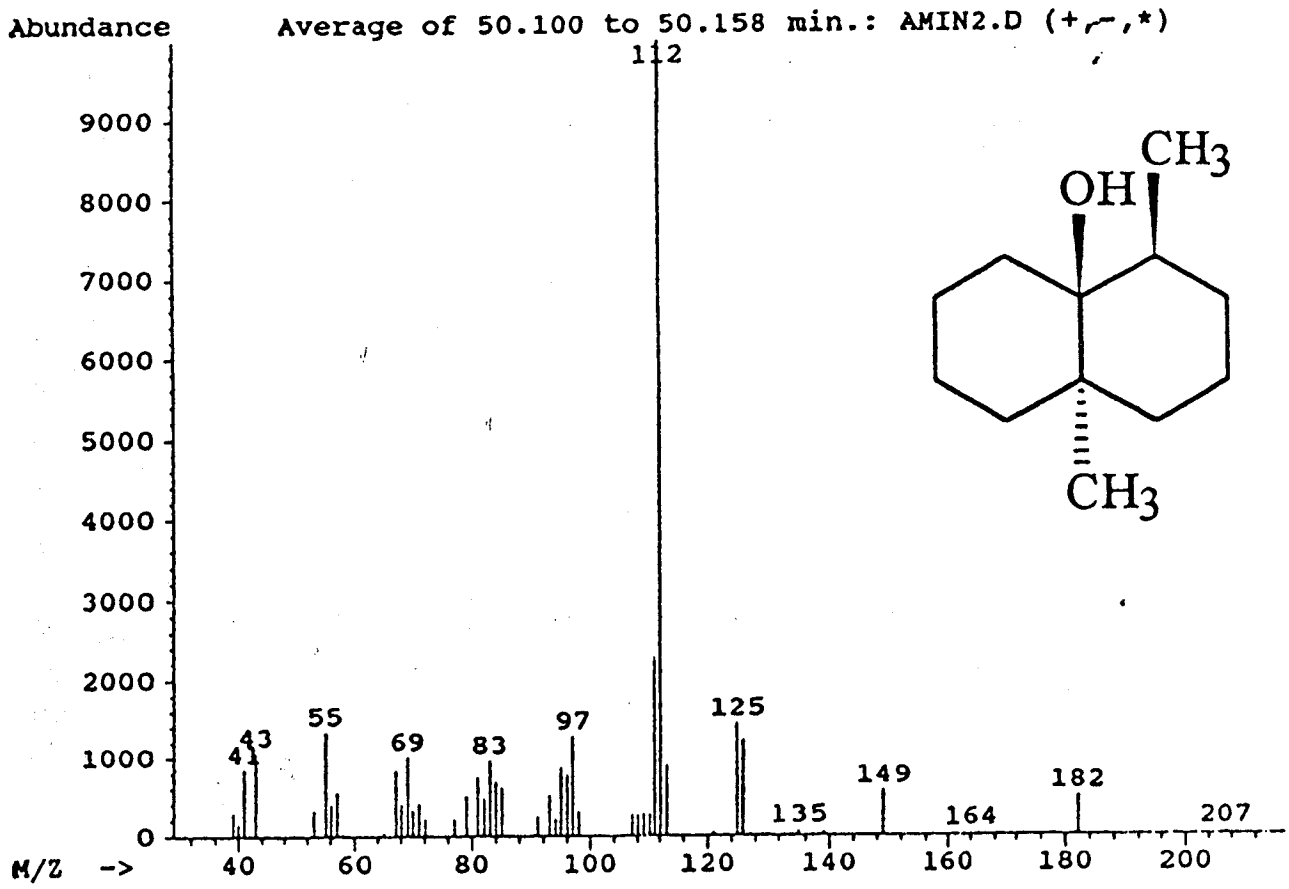

FIG. 1. Mass spectrum of geosmin obtained from *C. amianthinum* headspace extract.

column 50 m × 0.22 mm × 1 μm dimethylpolysiloxane BP1 fused silica capillary column (SGE). The carrier gas (Helium) was fixed at 22 psi. Volatile compounds were cryofocused at −20 C in the column's head before to be injected in the analytical column with a temperature desorption of 210 C. The detector temperature was 250 C. The column was temperature programmed as follows: 50 C to 220 C at 3 C/min. Solvent extraction analyses were performed by a 25 m × 0.25 mm × 0.13 μm dimethylpolysiloxane DB1, fused silica capillary column (J & W). The carrier gas was Helium with a flow rate close to 0.9 mL/min. The injector and detector temperatures were 200 C and 220 C, respectively. The column was temperature programmed as follows: 60 C (2 min) to 200 C at 4 C/min.

Gas chromatography-sniffing evaluation (with headspace concentration) analyses were carried out by a purge and trap injector (DCI device-PERICHROM), connected to a gas chromatography (DELSI Instruments 30), and performed with a 50 m × 0.32 mm × 1 μm dimethylpolysiloxane SPB1 fused silica capillary column (SUPELCO). Pressure of carrier gas (Helium) was fixed at 14 psi. The column was temperature programmed as follows: 50 C to 220 C at 4 C/min. Temperatures of the trap system for concentration and desorption were respectively −20 C and 250 C. The detector temperature was 230 C. Odor-profile description was obtained using a sniffing-port (Olfactory Detector-SGE) with a ratio FID 30%/Sniffing 70%, and performed by olfactory test (Jaubert et al 1995).

Results.—The headspace extracts were investigated with a sniffing evaluation which was carried out on both *Cystoderma* species. Gas chromatograms and sniffing evaluation profiles of *Cy. amianthinum* and *Cy. carcharias* show the same zone (retention time around 50 min) that does square with an unique molecule, associated with an intensive, musty-earthy odor.

The odorous volatile constituent was identified as geosmin (1,10-*trans*-dimethyl-*trans*-9-decalol), a sesquiterpenoïd derivative molecule with a molecular mass of 182 ($C_{12}H_{22}O$). Geosmin was determined by its retention indice, calculated using n-alcanes (C_{17}-C_{18}), and the experimental mass spectrum (FIG. 1). Both data were compared to those available from the literature. The mass spectrum of geosmin is characterized by the intense base peak at m/z = 112 (Kikuchi et al 1981, 1984, Matthies and Roberts 1992). The calculated retention indice of geosmin is 1421, which is very close to the data (1423) reported by Kondjoyan and Berdagué (1996).

Amounts of geosmin were calculated by a semi-quantitative evaluation based on gas chromatography areas (TABLE I). Aromas of *Cy. amianthinum* and *Cy. carcharias* contained 2.5% of geosmin in the headspace extracts. The solvent extracts of the three odor-

TABLE I. Relative percentage[a] of geosmin in mushroom extracts

	Headspace concentration	Solvent extraction
C. herculeus	—[b]	17.7%
C. amianthinum	2.5%	10.2%
C. carcharias	2.5%	4.0%

[a] Based on GC/MS chromatographic area and refers to the total fraction eluted in GC.

[b] Not evaluated.

ous mushroom species, *Co. herculeus, Cy. amianthinum* and *Cy. carcharias* contained 17.7, 10.2 and 4.0% of geosmin, respectively (TABLE I).

Discussion.—Geosmin is the key characteristic compound responsible for the musty-earthy odor of the mushroom species, *Co. herculeus, Cy. amianthinum* and *Cy. carcharias*. Indeed, this volatile compound is well known for possessing a characteristic musty-earthy odor (Jaubert et al 1995, Maga 1987), and its sensory treshold was evaluated to 0.1 ppb (Börjesson et al 1993), which is unusually low. That confers on geosmin a high odorous potential. Geosmin is often considered as an off-flavor to some food, potable water and aquaculture (Dionigi et al 1992, Gerber 1983, Maga 1987, Persson 1980). However this odorous molecule could be used as an aroma component (Maga 1987) and has generated much interest in the flavor industry.

The present work provides the first example of geosmin production from Basidiomycota metabolites. Nevertheless, this compound was previously detected as a volatile constituent of *Streptomyces* species (Bacteria) (Pollak and Berger 1996, Dionigi et al 1992, Bentley and Meganathan 1982), Cyanobacteria (Izaguirre et al 1982, Naes et al 1989), Myxomycota (Kikuchi et al 1984), Actinomycetes (Aoyama 1990, Gerber 1983) and Ascomycota species, i.e., *Penicillium expansum* (Matthies and Roberts 1992) and *Chaetomium globosum* (Kikuchi et al 1981), according to the fungi classification of Hawksworth et al (1995).

Regarding future prospects on the optimization of culture conditions, *Co. herculeus, Cy. amianthinum* and *Cy. carcharias* are potentially good sources for the bioproduction of natural geosmin. Indeed, the aroma and perfume industries have a great demand for such odorous and natural molecules.

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The fenugreek odor of *Lactarius helvus*

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Abstract: *Lactarius helvus* was investigated for volatile compounds by GC/MS. The volatile components proportion corresponds to 0.04% of dry weight. Thirty-eight components were identified. The major constituents were capric acid (25.6%), 3-amino-4,5-dimethyl-2(5H)-furanone (15.8%, [1]) and 2-methylbutyric acid (12.9%). 3-Hydroxy-4,5-dimethyl-2(5H)-furanone (= sotolon, [2], 1.4%), the hydroxy furanone derivative molecule with the characteristic aroma of *Trigonella foenum graecum* seeds, is the key compound responsible for the fenugreek odor of *L. helvus*. Sotolon is widely used as an aroma component in food and tobacco industries due to its strong reminiscent odor.

Key Words: Basidiomycota, furanone, Russulaceae, sotolon, *Trigonella foenum graecum*, volatile

Many mushroom species are well known by mycologists and chemists to possess typical odors such as fungal (Maga 1981, Rapior et al 1997b, Talou et al 1995, Tressl et al 1982), aniselike (Wood et al 1988, 1990), cucumber (Wood et al 1994), garlic (Rapior et al 1997a) or coal tar (Rapior et al 1998, Watson et al 1986).

The odor of *Lactarius helvus* (Fr.: Fr.) Fr. (Russulales, Russulaceae) has been described as a strong spicy smell reminiscent of chicory and fenugreek while drying (Benjamin 1995, Bresinsky and Besl 1990, Claus 1978, Læssøe et al 1996, Mazza 1998). The tawny *Lactarius* is a European species (Bresinsky and Besl 1990, Courtecuisse and Duhem 1994, Moser 1978, Sălăgeanu and Sălăgeanu 1985) which does not occur in North America (Ammirati et al 1985, Bessette et al 1997).

The food industry makes use of fenugreek odor due to sotolon in tobacco flavorings (Matsukura et al 1985), artificial maple syrup, and curry (Girardon et al 1985). Sotolon is one of the most important high-value flavor chemicals. Sotolon is a key flavor compound in the French flor-sherry wine (Dubois et al 1976, Guichard et al 1993), and gives the burnt flavoring to old sake (Takahashi et al 1976). This molecule was also found in soy sauce (Nunomora et al 1976), sugar molasses (Kobayashi 1989, Okada et al 1983), and barley malt used in the manufacturing of beer (Fickert and Schieberle 1998).

The present work is the first chemical study on the fenugreek odor of dried *L. helvus*. The mushroom was investigated for volatile components by solvent extraction using gas chromatography/mass spectrometry (CG/MS). Specimens of *L. helvus*, representing a combination of young and old basidiocarps (24 g), were collected in the field in summer 1998. Dehydratation of mushroom occurs within 2 h after harvest by air-drying at room atmosphere (30–32 C, 2 h). Dried mushroom was ground, then hydrated (100 mL) and immediately treated with diethyl ether (120 mL) to stop enzymatic activity. Organic extract was concentrated under nitrogen stream and used directly for GC/MS analysis.

Analyses of volatile constituents from *L. helvus* were carried out in triplicate using a gas chromatograph (5890-Hewlett-Packard) and a mass selective detector (5971-Hewlett-Packard) with a potential of 70 eV for ionization by electron impact. Solvent extract analyses were performed by a 25 m × 0.20 μm × 0.13 μm dimethylpolysiloxane Optima 5 (Macherey-Nagel), fused silica capillary column. The injector and detector temperatures were 200 C and 270 C, respectively. The column was temperature programmed as follows: 50 C (2 min) to 200 C (3 C/min). The carrier gas was helium with a constant flow rate set close to 0.6 mL/min (Breheret et al 1999, Rapior et al 1997a).

The volatile compound proportion corresponds to 425 $\mu g.g^{-1}$ of dry weight. Thirty-eight volatile components were identified by GC/MS (TABLE I). All compounds were identified by comparison with mass spectral library NBS (MacLafferty and Stauffer 1989), literature spectra (Adams 1989, Jennings and Shibamoto 1980, Stenhagen et al 1976) and our own data bank. The major compounds were quantitatively capric acid (25.6%), 3-amino-4,5-dimethyl-2(5H)-furanone (15.8%, [1], FIG. 1) and 2-methylbutyric acid (12.9%).

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TABLE I. Volatile composition of *Lactarius helvus*

Volatile compounds	RI[a]	Percentage[b]
3-Methylbutanol	716	1.4
2-Methylbutanol	720	3.0
Isobutyric acid	769	2.7
o-Xylene	861	1.1
3-Methylbutyric acid	876	4.6
2-Methylbutyric acid	891	12.9
Tiglic acid	906	0.1
Angelic acid	921	0.1
Benzaldehyde	953	0.3
3-Methylthiopropanol	977	0.3
2-Pentylfurane	997	0.4
Decane	1000	0.1
Hexanoic acid	1003	1.6
2-Phenylethanal	1033	0.1
n-Octanol	1051	0.5
2-Nonanone	1090	0.1
Dihydromaltol	1094	0.3
Undecane	1100	0.1
3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone [2][c]	1105	1.4
2-Phenylethanol	1106	1.4
Methyl 3-methylthiopropanoate	1110	0.1
Compound A (isomer of [2])	1158	1.5
Naphtalene	1170	0.9
Benzoic acid	1182	1.1
3-Amino-4,5-dimethyl-2(5*H*)-furanone [1][c]	1223	15.8
5-Methylcytosine	1237	0.8
3-Methylcinnamaldehyde	1249	0.3
Phenylacetic acid	1257	4.1
Unidentified	1260	0.4
Nonanoic acid	1269	2.4
Compound B (isomer of [1])	1277	2.3
Dodecanal	1283	0.3
2-Methyldodecane	1298	0.1
Tridecane	1300	0.7
Capric acid	1366	25.6
Unidentified	1368	0.5
2,2'-Bithiophene	1400	0.4
Compound C	1419	0.1
Unidentified	1460	0.3
Pentadecane	1500	0.8
Lauric acid	1565	0.3
Myristic acid	1764	1.7
Pentadecanoic acid	1867	6.6
Nonadecane	1900	0.1

[a] Retention indices due to the GC column used.
[b] Relative percentage of the identified volatile component based on the GC/MS chromatographic area.
[c] Unidentified enantiomer.

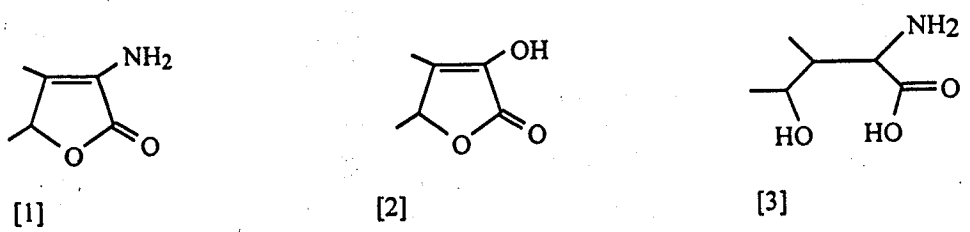
Among the flavor volatile components, C4-aliphatic alcohols (3-methylbutanol, 2-methylbutanol) and short-chain acids (isobutyric acid, 3-methylbutyric acid, 2-methylbutyric acid, tiglic acid, angelic acid) were also identified from *L. helvus* as well as several sulfur compounds (3-methylthiopropanol, methyl 3-methylthiopropanoate, 2,2'-bithiophene). Large amount of palmitic acid was found in *L. helvus* (166 $\mu g.g^{-1}$).

From dry *L. helvus* described with a fenugreek smell, we identified 3-hydroxy-4,5-dimethyl-2(5H)-furanone (= sotolon, 1.4 %, [2], FIG. 1), the key odorous compound of *Trigonella foenum graecum* (*Fabaceae*); sotolon is responsible for the characteristic odor of *L. helvus*. Sotolon is directly related to 3-amino-4,5-dimethyl-2(5H)-furanone (= quabalactone III, [1]) because structure similarity of both volatile components and detection in high amounts in *L. helvus* (TABLE I) as well as in the seeds of *T. foenum graecum* (Rijkens and Boelens 1975, Bessière et al unpubl) and the fragrant flowers of *Quararibea funebris* (Raffauf et al 1984, Zennie and Cassady 1990).

Several biosynthetic pathways were reported for sotolon formation (Blank et al 1995, Cheetham 1997, Fronza et al 1992, Kobayashi 1989, Lerch and Ambühl 1995). Within the hypotheses, it was suggested that this volatile component might originate from 4-hydroxyisoleucine [3, FIG. 1], a hydroxylated insulin-stimulating amino acid identified in both *T. foenum graecum* (Fowden et al 1973, Girardon et al 1986, Haefelé et al 1997, Sauvaire et al 1998) and *Q. funebris* (Raffauf et al 1984); on the other hand, Takahashi et al (1976) and Kobayashi (1989) reported that sotolon formation is due to a condensation of osidic molecules in cane sugar and aged sake productions, respectively. Now, these precursors have never been described in *L. helvus*. So, the key compound 4-hydroxyisoleucine could be biosynthetized in mushroom from 3-methylidene-2-aminovaleric acid, an unsaturated amino acid identified from *L. helvus* (Levenberg 1968), through an allylic hydroxylation.

Unidentified compounds A and B with molecular weight and mass spectrum close to those of sotolon [2] and 3-amino-4,5-dimethyl-2(5H)-furanone [1] were also reported in *L. helvus*. The difference between the retention indices of compounds A and B is the same as that of [2] and [1] (TABLE I). So, we stated that compounds A and B are analogs of [2] and [1], respectively. Another unidentified amino furanone (compound C) with a higher molecular weight and the same spectral characteristics as both precursors, i.e., compound B and 3-amino-4,5-dimethyl-2(5H)-furanone [1], was also reported in TABLE I.

Sotolon has been identified as the key characteristic compound responsible for the fenugreek odor of dried *L. helvus*. According to Bresinski and Besl (1990), Claus (1978), and Mazza (1998), the strong



[1] [2] [3]

FIG. 1. Structures of 3-amino-4,5-dimethyl-2(5H)-furanone [1], sotolon, [2], and 4-hydroxyisoleucine [3].

reminiscent fenugreek odor of *L. helvus* developed on drying. On the other hand, sotolon was previously reported as the dominant odorous constituent responsible for the typical flavor of *T. foenum graecum* seeds (Girardon et al 1986). The authors mentioned that the characteristic smell of fenugreek occurred during the dehydration process of seeds. Thus, the drying process produced the fenugreek odor in both *L. helvus* (Basidiomycota) and *T. foenum graecum* (Fabaceae).

This study highlighted for the first time the fenugreek odor of *L. helvus* due to sotolon, the volatile compound responsible for the odor of *T. foenum graecum*. The present work also provided the first example of sotolon production from Basidiomycota higher fungi. That could be of wider biotechnological significance for the production of "natural" flavor compounds.

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