

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, Besette 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 (GC/MS). Specimens of *L. helvus*, representing a combination of young and old basidiocarps (24 g), were collected in the field in summer 1998. Dehydration 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 μg·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%).

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
<i>o</i> -Xylene	861	1.1
3-Methylbutyric acid	878	4.8
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-Pentylfuran	997	0.4
Decane	1000	0.1
Hexanoic acid	1003	1.6
2-Phenylethanol	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(5H)-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(5H)-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-methylthiopropoate, 2,2'-bithiophene). Large amount of palmitic acid was found in *L. helvus* (166 $\mu\text{g}\cdot\text{g}^{-1}$).

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, Bessièrè 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, Haefelè 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 biosynthesized 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

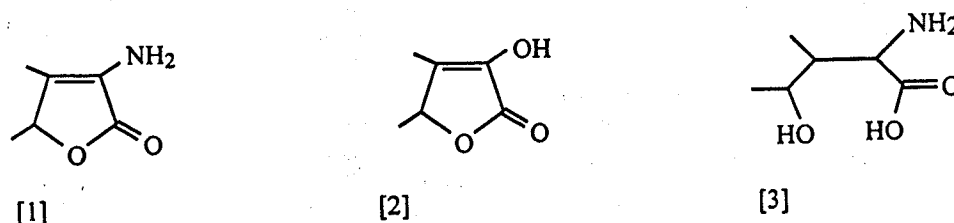


FIG. 1. Structures of 3-amino-4,5-dimethyl-2(5H)-furanone [1], sotolon, [2], and 4-hydroxyisoleucine [3].

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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