CHEMISTRY OF THE AROMA BOUQUET OF THE EDIBLE MUSHROOM “LEBRE” (*Cortinarius lebre*, BASIDIOMYCOTA, AGARICALES) FROM CHILE

NORBERT ARNOLD¹*, GÖTZ PALFNER²*, JÜRGEN SCHMIDT¹,
CHRISTINE KUHN¹ & JOSÉ BECERRA²

¹ Leibniz Institute of Plant Biochemistry, Department of Bioorganic Chemistry, Weinberg 3, D-06120 Halle (Saale), Germany

² Universidad de Concepción, Facultad de Ciencias Naturales y Oceanográficas, Departamento de Botánica, Casilla 160-C, Concepción, VIII Región, Chile

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ABSTRACT

Cortinarius lebre Garrido, a common edible mushroom from central Chile, is distinct from other *Cortinarii* by its strong, naphthalene-like smell. The relevant volatile compounds were detected by gas chromatography – mass spectrometry and identified as indole together with 1-octen-3-ol, octane-3-one, and octan-3-ol.

Key words: *Cortinarius*, indole, 1-octen-3-ol, volatile compounds, scent.

INTRODUCTION

Cortinarius (Cortinariaceae, Agaricales, Basidiomycota) is the most diverse genus of ectomycorrhizal fungi in South American *Nothofagus* forests, with more than 250 species described to date (Moser & Horak 1975, Garrido 1988, Garnica et al. 2002). *Cortinarius lebre* Garrido is especially frequent in southern central Chile, where it is mainly associated to *N. obliqua*, but also grows beneath *N. antarctica*, *N. alessandrii*, *N. dombeyi* and *N. glauca* (Garrido 1988, pers. obs.). The epithet refers to the native Chilean name of this edible mushroom: “Lebre” can be encountered in large numbers in remnants of native forest along the coastal mountain range (“Cordillera de la Costa”), especially in the Biobío Region (36° 00’ – 38° 30’ s. l. approx.) during the rainy season which usually lasts from May to August. Major quantities are occasionally sold fresh on markets in regional urban centers like Angol, Nacimiento or Concepción. The most conspicuous property of *C. lebre*, apart from its tough consistence and violet - colored pileus, is its pungent and unpleasant smell of camphor and moth balls (naphthalene) which can often be perceived at several meters distance from the basidiomes and which has been described by other authors like gaseous fuel or *Tuber magnatum* (Garrido 1988), as well as reminding of “rabbit burrow” (Valenzuela & Moreno 1997). In an earlier description of *Rozites violacea* Horak (Moser & Horak 1975) from Argentina which is probably the same species, the authors described the smell as “strongly adstringent”. As to our knowledge, this very characteristic smell is not known for any other *Cortinarius* species, we analyzed its chemical composition using headspace gas chromatography-mass spectrometry (GC-MS) for the identification of the compounds, which are responsible for the strong aroma bouquet of *C. lebre*.

EXPERIMENTAL

Fungal Material

Cortinarius lebre Garrido (fig. 1) was collected in Chile, VIII Región del Bio Bio, Concepción, Universidad de Concepción, La Cantera y El Guindo, 36° 50’ 13’’/ 73° 01’ 28’’ s. l., 160 m.a.s.l., remnant of mixed native forest, dominated by *Nothofagus obliqua* (Mirb.) Oerst. and *Aextoxicon punctatum* Ruiz & Pav., on soil and litter beneath *N. obliqua*, 25-V-2011, CONC-F0781, leg. et det. N. Arnold, W. Helfer, G. Palfner. Voucher specimens are deposited in the Herbarium of Concepción University (CONC-F).

Extraction

Fresh collected fungal fruiting bodies (100 gr) were carefully removed from the substrate, crushed, and extracted with *n*-hexane (200 mL). After extraction, the light yellow coloured solution was evaporated at room temperature to 5 mL under slightly reduced pressure and exhibited further a strong gaseous fuel-like smell. This solution was immediately investigated by GC-MS. Commercially available indole (Acros) and 1-octen-3-ol (Fluka) were used as reference substances.

GC-MS Analysis

The static headspace GC-EIMS measurements were performed on a QP-2010 Ultra (Shimadzu Corporation, Kyoto, Japan) by using the following

conditions: electron energy 70 eV, detected mass range *m/z* 50-500; source temperature 200°C; column: ZB-5MS (Phenomenex, 30 m x 0.25 mm, 0.25 µm film thickness), injector temperature 220 °C, interface temperature 300 °C, carrier gas Helium, column flow 1.02 ml/min, constant flow mode, column temperature program: 40 °C for 1 min, then raised to 300 °C at a rate of 10 °C min⁻¹ and then held on 300 °C for 5 min. Static headspace conditions: split injection (split ratio 1:5), incubation temperature 40 °C; incubation time 30 min; syringe temperature 45 °C; injection volume 500 µL. The relative composition of the main components **1-4** were calculated from the peak areas of the total ion chromatogram (TIC).

Figure 1: *Cortinarius lebre* in situ (CONC-F0781).

RESULTS AND DISCUSSION

A GC-EIMS analysis of the crude *n*-hexane extract from *C. lebre* demonstrates, that a mixture of indole (**1**, 29.3% of identified compounds, RT 29.3 min), 1-octen-3-ol (**2**, 43.2% of identified compounds, RT 7.23 min), together with octane-3-one (**3**, 18.7% of identified compounds, RT 18.7 min) and octan-3-ol (**4**, 8.7% of identified compounds, RT 8.7 min) are responsible for the gaseous fuel-like or camphor and moth balls-like odour of *C. lebre*. Compounds **1-4** were identified by comparison of the obtained EI mass spectra with those in the NIST 08 database.

Indole (**1**) and indole derivatives are widespread-occurring natural products. Indole (**1**) can be produced by bacteria as a degradation product of the amino acid tryptophan. It also occurs naturally in human feces and has an intense fecal odour. However, it has a flowery smell at very low concentrations

and is a constituent of many flower scents. It was isolated from etheric oils of various plants like the jasmines and orange blossoms. Hesse (1904) isolated 2.5 % from jasmine, while 0.1 % indole (**1**) was obtained from orange-blossom (Hesse & Zeitschel, 1902). This ambiguous behaviour, with a large whiff of indole (**1**) smelling putrid, whereas a trace of the same chemical smells flowery, may be explained by the fact that large amounts of the chemical bind to a wider variety of receptors than do small amounts of the same chemical (Leffingwell, 1999). This olfactory ambiguity may explain why the odor of *Rozites purpurea*, probably conspecific with *C. lebre*, was described as flowery, reminding of hyacinths by the author (Moser & Horak 1975) and is also mentioned in literature for toadstools of *Tricholoma lascivum*, *T. inamoenum*, and *T. caligatum* (Fons et al., 2006). The complex odor is described with a fruity sweet note (jasmine, seringa), followed by a nauseous insecticidal or sulphurated note.

Although rare in Cortinariaceae, indole (**1**) and its derivatives are common components of fungal scents. In some mushrooms, indole (**1**) is present in a mixture along with other constituents, especially 1-octen-3-ol (**2**, also known as mushroom alcohol), the main compound being responsible for their unpleasant odour (Table 1).

In the volatile fraction of *T. caligatum*, high levels of indole derivatives together with methyl-(E)-cinnamate, benzaldehyde and (E) nerolidol were found and are responsible for the second nauseous note of this species (Fons et al., 2006). Additionally, in some other species skatole (**5**) and 3-chloroindole (**7**) smelling fecal-like (Wood et al. 2003), respectively like coal-tar or gas-like (**5**, **6**, **7**) (Hilber 1968; Rapior et al., 1998), can be obtained.

5: R¹ = CH₃

6: R¹ = CHO

7

8: R¹ = CH₃; R² = H

9: R¹ = CH₂OH; R² = H

10: R¹ = CH₃; R² = OCH₃

11: R¹ = CH₂OCH₃; R² =

H

12: R¹ = CH₂OCH₃; R² = OCH₃

An overview of the so-far known occurrence of indole (**1**) and indole derivatives from volatile fraction of mushrooms is given in Table 1.

Table 1: Occurrence of indole derivatives in the volatile fraction of mushrooms constituting the unpleasant scent of certain mushrooms: 1*H*-indole (**1**), 3-methyl-1*H*-indole (skatole) (**5**), 3-formyl-1*H*-indole (**6**), 3-chloro-1*H*-indole (**7**), 2,4-dimethyl-1*H*-indole (**8**), 4-hydroxymethyl-2-methyl-1*H*-indole (**9**), 5-methoxy-2,4-dimethyl-1*H*-indole (**10**), 4-methoxymethyl-2-methyl-1*H*-indole (**11**), 4-methoxymethyl-5-methoxy-1*H*-indole (**12**).

Species	1	5	6	7	8	9	10	11	12	References
<i>Boletus calopus</i>	+	-	-	-	-	-	-	-	-	Rapior et al. (1997)
<i>Gyrophragmium dunalii</i>	+	-	-	-	-	-	-	-	-	Rapior et al. (2000)
<i>Morchella conica</i>	+	-	-	-	-	-	-	-	-	Audouin et al. (1989)
<i>Lepiota bucknallii</i>	+	-	-	-	-	-	-	-	-	Hilber (1968)
<i>Hebeloma radicosum</i>	+	-	-	-	-	-	-	-	-	Fons et al. (2006)
<i>Tricholoma bufonium</i>	+	-	-	-	-	-	-	-	-	Hilber (1968)
<i>Tricholoma inamoenum</i>	+	+	-	-	-	-	-	-	-	Hilber (1968), Watson et al. (1986)
<i>T. lascivum</i>	+	+	-	-	-	-	-	-	-	Hilber (1968) Eizenhöfer et al. (1990)
<i>T. sulphureum</i>	+	+	+	-	-	-	-	-	-	Hilber (1968) Rapior et al. (1998) Rapior et al. (2000)
<i>T. sciodes</i>	-	-	-	-	+	+	+	+	-	Garlaschelli et al. (1994) Pang & Sterner (1996)
<i>T. virgatum</i>	-	-	-	-	+	+	-	+	-	Garlaschelli et al. (1994)
<i>T. caligatum</i>	-	-	-	-	+	-	+	+	+	Fons et al. (2006)
<i>Coprinus picaceus</i>	+	+	-	-	-	-	-	-	-	Laatsch & Matthies (1992)
<i>Hygrophorus paupertinus</i>	+	-	-	-	-	-	-	-	-	Wood et al. (2003)
<i>Scytinostroma portentosum</i>	+	-	-	+	-	-	-	-	-	Arnold et al. (2007)

Besides their function as odorous compounds, monomeric indole derivatives as well as unstable pungent and non-pungent bisindolic derivatives are contributing to the taste of some mushrooms, especially species in *Tricholoma* and *Collybia* (Sterner, 1994; Pang & Sterner, 1994; Pang & Sterner, 1996; Fons et al., 2006).

Lascivol, the bitter principle of *Tricholoma lascivum*, degrades to dimethyl L-glutamate and 5-methoxy-2,4-dimethylindole (**9**) by acid methanolysis (Eizenhöfer et al., 1990). The latter compound was also isolated from *T.*

sciodes and *T. virgatum*, two species which are characterized by their bitter and acrid taste (Garlaschelli et al., 1994), and from *T. caligatum* (Fons et al., 2006).

In pure state, indole (**1**) is a colourless crystalline solid, which melts at 52 °C. It is volatile with steam and soluble in hot water. This may be the reason that *C. lebre* is favored as an excellent edible mushroom after cooking in water and discarding the broth, which reduces the content of the unpleasant indole (**1**).

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