Chemistry of the earthy odour of basidiomata of *Cortinarius hinnuleus* (*Basidiomycota, Agaricales*)

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Accepted 13. January 2016

Key words: *Cortinarius hinnuleus*. – Geosmin, ß-caryophyllene, ß-barbatene, 1-octen-3-ol, volatile compounds.

Abstract: *Cortinarius hinnuleus* (Earthy Webcap), a common mycorrhizal mushroom in Central Europe, is characterized by a mouldy earthy odour. The relevant volatile compounds were detected by gas chromatography-mass spectrometry using headspace-solid phase microextraction technology and identified as geosmin, ß-caryophyllene and ß-barbatene together with the C_8-volatiles 1-octen-3-ol, 1-octen-3-one, octan-3-ol, octan-3-one, and 2-octen-1-ol.

Zusammenfassung: *Cortinarius hinnuleus* (Erdigriechender Gürtelfuß), ein in Mitteleuropa häufiger Mykorrhiza-Pilz, zeichnet sich durch schimmelig-erdigen Geruch aus. Die relevanten flüchtigen Verbindungen wurden durch Gaschromatographie-Massenspektrometrie (GC-MS) unter Verwendung der „headspace-solid phase microextraction" (HS-SPME)-Technologie ermittelt und als Geosmin, ß-Caryophyllen und ß-Barbaten zusammen mit den flüchtigen C_8-Verbindungen 1-Octen-3-ol, 1-Octen-3-on, Octan-3-ol, Octan-3-on und 2-Octen-1-ol identifiziert.

Cortinarius (PERS.) GRAY (*Cortinariaceae, Agaricales, Basidiomycota*) is the most diverse genus of ectomycorrhizal fungi with about 5000 epithets listed in the database www.indexfungorum.org. *Cortinarius hinnuleus* FR. (Earthy Webcap) is especially frequent in Central Europe, where it occurs in mixed and deciduous forests and parks on rich, acidic to calcareous soil, mainly associated with *Quercus, Fagus*, *Tilia*, *Carpinus*, but also near *Betula*, *Populus* and *Corylus* (GMINDER 2010, NISKANEN & KYTÖVUORI 2012). However, *C. hinnuleus* is not cultivable on artificial media so far. The basidiomata appear between late summer and late autumn and the species is common in nemoral to hemiboreal zones in Europe, therefore less common in northern Europe (BRANDRUD & al. 1990, GMINDER 2010, NISKANEN & KYTÖVUORI 2012). This is one of the classical European *Cortinarii* developing reddish brown to ochraceous brown carpophores, defined by a universal veil forming a white ring and incomplete girdles on the stem, distant pale yellow to red brown gills and, most conspicuously, by a pungent, unpleasant earthy odour. We analysed its chemical composition from collected basidiomata using headspace-solid phase microextraction gas chromatography-mass spectrometry (HS-SPME/GC-MS) for the identification of the compounds responsible for the strong earthy aroma of *C. hinnuleus*.

Materials and methods

Experimental: The 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* 40–500; source temperature 200 °C; column: ZB-5MS (Phenomenex, 30 m x 0.25 mm, 0.25 µm film thickness), interface temperature 270 °C, carrier gas Helium, column flow 1.02 ml/min, constant flow mode, column temperature program: 40 °C for 2 min, then raised to 260 °C at a rate of 10 °C min^{-1} and then held on 260 °C for 5 min.

The following operating conditions for the HS-SPME (headspace-solid phase microextraction) were used: SPME fibre: 50/30 µm divinylbenzene(DVB)/carboxen/polydimethylsiloxane(PDMS), adsorption temperature: 65 °C, adsorption time: 5 min; split injection (split ratio 1:5), injector port temperature: 250 °C, desorption time: 2 min. The volatiles were identified by comparing their EI mass spectra with the databases NIST 11 (National Institute of Standard and Technology), FFNSC 2 (Shimadzu) and MassFinder 4.0 (Dr. Hochmuth Scientific Consulting, Hamburg, Germany). The relative abundances of components **1-10** were calculated from the peak areas of the total ion chromatogram (TIC).

Fungal material: *Cortinarius hinnuleus:* Germany, Saxony-Anhalt, near Freyburg (Unstrut), 18. October 2012, leg. & det. N. Arnold & G. Palfner, near *Quercus robur*, voucher deposited at the Leibniz Institute of Plant Biochemistry (Halle/Saale).

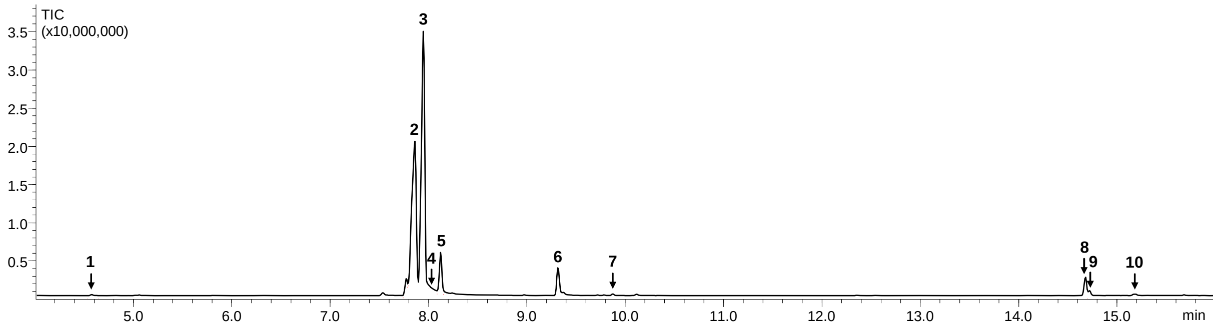
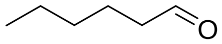
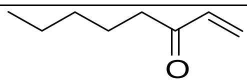
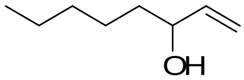
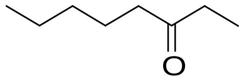
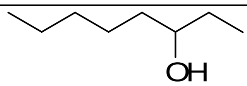
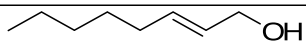
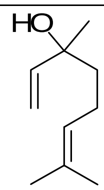
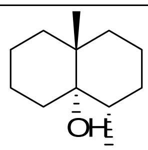
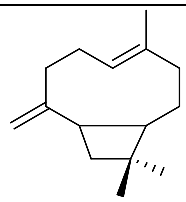
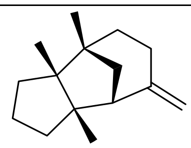


Fig. 1. Gas chromatogram of main volatile compounds of *Cortinarius hinnuleus.*

Results and discussion

Headspace-solid phase microextraction (HS-SPME) analysis of volatiles emitted by the cap of *C. hinnuleus* revealed several aliphatic alcohols and ketones, viz.: hexanal (**1**), a mixture of the C_8-volatiles 1-octen-3-one (**2**), 1-octen-3-ol (**3**), octan-3-one (**4**), octan-3-ol (**5**), 2-octen-1-ol (**6**) and the monoterpene linalool (**7**) (Tab. 1, Fig. 1). Besides these compounds, geosmin (**8,** 3% of identified compounds, RT 14.67 min) as well as the sesquiterpenes β-caryophyllene (**9**, 0.6 % of identified compounds, RT 14.69 min) and ß-barbatene (**10**, 0.3% of identified compounds, RT 15.17 min) could be identified.

Tab. 1. GC data of main volatile compounds of *Cortinarius hinnuleus*, obtained by GC-MS measurements.

No.	RT (min)	Structure	Compound	Relative Composition (%)
1	4.55		Hexanal	0.2
2	7.77		1-Octen-3-one	1.2
3	7.80		1-Octen-3-ol	35.7
4	7.91		Octan-3-one	49.4
5	8.10		Octan-3-ol	5.4
6	9.28		2-Octen-1-ol	3.9
7	9.81		Linalool	0.2
8	14.67		Geosmin	3.0
9	14.69		β-Caryophyllene	0.6
10	15.17		β-Barbatene	0.3
			Others (not identified)	0.1

Compounds **1**–**8** represent 96 % of total volatiles in *C. hinnuleus*. In particular, the volatiles **2**–**6** are responsible for the typical mushroom smell of fungi and have been known for some time (e.g. BELTRAN-GARCÍA & al. 1997, CHO & al. 2008, COMB & al. 2006, MALHEIRO & al. 2013). The occurrence of the alkyl aldehyde hexanal (**1,** 0.2 % of identified compounds, RT 4.55 min) could be interpreted as a degradation product by oxidation of fatty acids or a biosynthetic side product during formation of 1-octen-3-ol (**3**). Hexanal (**1**) has been shown to inhibit spore germination of different fungi and to act against microorganisms (SPITELLER 2002). The eight-carbon volatiles **2-6** seem to possess two biological functions. On one side, they play an important role in attracting mycophilous diptera, which are likely to disseminate spores (CHIRON & MICHELOT 2005). On the other side, it has been demonstrated that 1-octen-3-ol (**3**) inhibits germ tube formation in spores of parasitic fungi like *Penicillium paneum* and exhibits bactericidal effects at the same time (COMB & al. 2006), protecting the fungal basidioma against ubiquitous moulds and bacteria in its natural habitat.

The terpene linalool (**7**) together with other constituents could be partially related to the characteristic odours of some fungi (BREHER & al. 1997, MALHEIRO & al. 2013). Linalool (**7**) shows several biological activities, among them also bactericidal and fungicidal effects (PATTNAIK & al. 1997).

The natural bicyclic sesquiterpene ß-caryophyllene (**9**), occurs in more than 50 % of all plant families, representing a very common constituent of essential oils of various aromatic plants such as *Syzygium aromaticum*, *Cannabis sativa* and *Rosmarinus officinalis* (KNUDSEN & al. 2006). Recently, ß-caryophyllene could also be identified as VOC (Volatile Organic Compounds) from a fungal strain of *Talaromyces wortmannii* (YAMAGIWA & al. 2011) and *Cladosporium cladosporioides* (DIBY & PARK 2013). We can therefore conclude that ß-caryophyllene (**9**) could also be a naturally occurring constituent of the basidioma of *C. hinnuleus*. Derivatives of ß-caryophyllene (**9**) have so far been reported from the culture broth of the basidiomycetes *Hebeloma longicaudum* (WICHLACZ & al. 1999) and *Hypholoma fasciculare* (SHIONO & al. 2004).

The sesquiterpene ß-barbatene (**10**) was so far only known as VOC of *Fomitopsis pinicola* (FÄLDT & al. 1999) and could be also detected after inoculation experiments of *Trametes versicolor* on *Fagus sylvatica* (THAKEOW & al. 2006). The authors suggested that two beetle species might use this compound to discriminate the host odours of chopped *F. pinicola* and *Fomes fomentarius* (FÄLDT & al. 1999).

The unpleasant, musty, earthy odour of *C. hinnuleus* is caused by geosmin (**8**) and was previously reported as the key compound also responsible for the musty-earthy aroma of *Cortinarius herculeus*, *Cystoderma amianthinum* and *Cy. carcharias* (BREHER & al. 1999). Geosmin (**8**), normally accompanied by 2-methylisoborneol, is common in nature, being produced by blue-green algae (Cyanobacteria), some vascular plants, mosses and protozoan symbionts (WATSON 2003), but is mainly synthesized by terrestrial actinomycetes (genus *Streptomyces)* and some filamentous fungi such as *Penicillium* or *Aspergillus* spp., giving soil its characteristic smell (BUTTERY & GARIBALDI 1976). The geosmin (**8**) biosynthesis investigated by feeding experiments to *Streptomyces* and the liverwort *Fossombronia pusilla* demonstrates that it is synthesized by cyclization of farnesyl diphosphate (SPITELLER & al. 2012). The odour threshold concentrations (OTCs; minimum detectable concentrations) is about 10 ng l^{-1} or less (WATSON & al. 2000) and its presence in drinking water creates consumer complaints. Objectionable soily taste and odour in drinking water mainly caused by geosmin (**8**) and 2-methylisoborneol is a common problem for water suppliers using surface water sources (BAO & al. 1997). Furthermore, geosmin (**8**) is also resistant to conventional water treatment, and its bioaccumulation in fish and shellfish causes off-flavours in farmed and wild stocks (WATSON 2003). The occurrence of geosmin (**8**) also indicates mould-related spoilage (SCHNÜRER & al. 1999).

It is not surprising that in a large genus like *Cortinarius*, several species are known to produce conspicuous and diagnostic odours, although only very few of them have been chemically analysed, such as the anise-like odour of *C. odorifer* which was very recently identified as methyl p-anisate (KLEOFAS & al. 2015) or the naphthalene-like smell of *C. lebre* from Chile which is caused by indol (ARNOLD & al. 2012). Other scents like that of fruits in *C. traganus*, or of *Pelargonium* leaves in *C. flexipes,* among others are used as characters in species discrimination, but remain to be chemically identified.

In general, VOCs play an important role for fungi in their natural environments. They act as signals between fungi and plants, arthropods, bacteria, and other fungi (BENNETT & al. 2012). Such fungal VOCs can further developed for use in biotechnological applications (MORATH & al. 2012). But it has to be taken in account, that for this purposes the fungal organism must be cultivable on artificial media, which is not the case in highly specialized ectomycorrhizal species such as *C. hinnuleus*.

G. PALFNER and N. ARNOLD are grateful for support by CONICYT-BMBF [bilateral cooperation project CONICYT (no. PCCI 2011-609) – German Ministry of Education and Research and (no. 01DN12107)].

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