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Volatile composition of *Clitocybe amoenolens*, *Tricholoma caligatum* and *Hebeloma radicosum*

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Abstract – The volatile extracts composition of fresh *Clitocybe amoenolens*, *Tricholoma caligatum* and *Hebeloma radicosum* were analysed by Gas Chromatography-Mass Spectrometry. Twenty-one, sixteen and twenty-three components were identified, respectively. Methyl-(*E*)-cinnamate was found in the three analysed mushrooms at various amounts. Methyl-(*E*)-cinnamate and methyl-benzoate as well as (*E*)-nerolidol and methyl-anthranilate were the key odorants of *C. amoenolens* floral odor. Combined methyl-(*E*)-cinnamate and indole derivatives should largely contribute to the complex floral odor of *T. caligatum* with a nauseous note when aged; the latter volatiles could be of chemotaxonomic interest for the genus *Tricholoma*. Various aromatic derivatives (benzaldehyde, 2-phenylethanal, 2-phenylethanol, phenylacetic acid) were responsible for the almond-like odor with a floral note of *Hebeloma radicosum*.

Basidiomycota / Benzaldehyde / Chemotaxonomy / Indole derivatives / Methyl benzoate / Methyl-(*E*)-cinnamate / (*E*)-nerolidol / Odor

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Résumé – La composition de l'extrait volatil de *Clitocybe amoenolens*, *Tricholoma caligatum* et *Hebeloma radicosum* a été analysée sur matériel frais par chromatographie en phase gazeuse couplée à la spectrométrie de masse. Vingt-et-un, seize et vingt-trois composés ont été identifiés respectivement pour les trois champignons. Le (*E*)-cinnamate de méthyle a été trouvé dans chacun d'eux en quantités variables. Le (*E*)-cinnamate de méthyle, le benzoate de méthyle, le (*E*)-nérolidol et l'anthranilate de méthyle sont les composés clés de l'odeur de *C. amoenolens*. L'association du (*E*)-cinnamate de méthyle et des dérivés indoliques semble contribuer largement à l'odeur florale complexe, devenant nauséeuse avec l'âge, de *T. caligatum*; les dérivés indoliques pourraient présenter un intérêt chimiotaxonomique pour le genre *Tricholoma*. Différents dérivés aromatiques (benzaldehyde, 2-phényléthanal, 2-phényléthanol, acide phenylacétique) sont responsables de l'odeur d'amande amère avec une note florale d'*Hebeloma radicosum*.

Basidiomycota / Benzaldéhyde / Chimiotaxonomie / Dérivés indoliques / Benzoate de méthyle / (*E*)-cinnamate de méthyle / (*E*)-nérolidol / Odeur

INTRODUCTION

Many mushrooms are well-known to exhale characteristic odors which highly contribute to their mycological identification, i.e., fruity flavor of *Cantharellus cibarius* (Fons *et al.*, 2003), anise-like smell of *Hydnellum suaveolens* (Wood *et al.*, 1988), of *Gyrophragmium dunalii* (Rapior *et al.*, 2000c), and of *Clitocybe odora* (Rapior *et al.*, 2002), sweet-odor of *Hebeloma sacchariolens* (Wood *et al.*, 1992), candy-like odor of *Nolanea fructufragrans* (Largent *et al.*, 1990), nutty and earthy flavor of *Tuber melanosporum* (Flament *et al.*, 1990), fenugreek odor of *Lactarius helvus* (Rapior *et al.*, 2000a), musky to disagreeable scent of *Laetiporus sulphureus* (Rapior *et al.*, 2000b) and even putrid odor of *Phallus impudicus* (Borg-Karlson *et al.*, 1994).

These pleasant or unpleasant aromas are generated by mixture of many volatile components which make them usually complex in their perception by various descriptors. Besides the aroma of a number of mushroom species can evolve or even turn when aged (Flament *et al.*, 1990; Chiron & Michelot, 2005). In this study we have been interested in the three species *Clitocybe amoenolens*, *Tricholoma caligatum* and *Hebeloma radicosum* for their major floral or fruity-like aroma.

Clitocybe amoenolens Malençon is a toxic species responsible for the acromelalgic syndrome; several intoxications occurred recently in France (Maurienne valley) (Saviuc *et al.*, 2001, 2003; Moreau *et al.*, 2001; Bessard *et al.*, 2004) and in Italy (Abruzzo) (Leonardi *et al.*, 2002) after confusion with *Lepista inversa*. The odor of *C. amoenolens* has been described as a strong reminiscent smell with sweet floral, fruity note (pear) related to that of *Inocybe corydalina*, *I. piriodora* and *I. bongardii* (Malençon & Bertault, 1975; Bon, 1983, 1997; Charignon & Garcin, 1998), of *Hebeloma sacchariolens* (Bon, 1987; Contu *et al.*, 1999), of *Tricholoma caligatum* (Charignon & Garcin, 1998; Moreau, 2002). The *C. amoenolens* smell was also described as closed to that of benzoin (balsamic note), jasmine, orange blossom, iris and seringa or fruit drop odors sickly towards the end (Poumarat & Neville, 1993; Bon, 1997; Contu *et al.*, 1999; Moreau *et al.*, 2001). *C. amoenolens* taste has been described as sweet to slightly earthy or floury (Malençon & Bertault, 1975; Contu *et al.*, 1999). No volatile constituents of *C. amoenolens* have been previously identified.

Tricholoma caligatum (Viviani) Ricken (Fragrant *Tricholoma*) is better known, more widely found than *C. amoenolens* and consequently more described. The former has a dual odor reported as a pleasant spicy (cinnamon), balsamic (Peru or Tolu balsam), floral (narcissus, jasmine, pear blossom, whitethorn blossom) or fruity flavor (bergamot orange, orange, pear) followed by a nauseous note (Moser, 1983; Marchand, 1986; Riva, 1997). Its jasmine and pear to overripe pear scent is comparable to that of *I. bongardii*, *I. corydalina* and *I. piriodora* (Claus, 1978, Schmitt 1978; Mazza, 1998). According to Bessette *et al.* (1997), *T. caligatum* is a complex of several forms with a range of odor and taste. Lincoff (1998) states that varieties ground under oak in North America have pungent odor and disagreeable taste or slight odor and bitter taste whereas varieties found under conifers have fragrant cinnamon-like odor. Even, if it tastes the way it smells when fresh, *T. caligatum* is considered an edible species; the unpleasant-smelling varieties of the Fragrant *Tricholoma* are enjoyable once cooked (Fischer & Bessette, 1992). Other mycologists describe its taste as nutty, slightly or slowly bitter or not distinctive (Bessette *et al.*, 1997; Courtecuisse, 1999).

Hebeloma radicosum (Bulliard:Fries) Ricken (Rooting Fairy Cake) is a common inedible mushroom. Its odor is less complex and so better determined than that of the two previously reported mushrooms. *H. radicosum* exhales a strong pleasant odor of marzipan, cherry laurel or bitter almonds and has a bitter taste (Claus, 1978; Læssøe *et al.*, 1996; Bessette *et al.*, 1997; Courtecuisse, 1999). Despite their wide occurrence, *T. caligatum* and *H. radicosum* have been poorly investigated for secondary volatile metabolites.

In the present work, wild and fresh basidiocarps of *Clitocybe amoenolens*, *Tricholoma caligatum* and *Hebeloma radicosum* were investigated for volatile compounds by solvent extraction using Gas Chromatography-Mass Spectrometry (GC-MS) to identify the compounds responsible for their typical odors. The volatile compositions were compared and the chemotaxonomic value of some constituents was discussed therein.

MATERIALS AND METHODS

Materials and isolation of volatile extracts

Fresh and wild mushroom materials were collected in the fall of 2004 in Savoie (France) for *Clitocybe amoenolens*, and in the fall of 2003 in Languedoc Roussillon (France) for *Tricholoma caligatum* and *Hebeloma radicosum*. The fresh sporophores were brushed clean, crushed to fine particles (average particle size was 0.5 cm) and then immediately covered with diethyl ether (w/4v) to stop enzymatic activity and so maintain the freshness of the fungal specimens at the time of collection in the field. Solvent extraction was performed at room temperature in the darkness. The sample extracts were gently concentrated to a small volume (0.5 mL) under nitrogen stream.

GC-MS analysis

The organic extracts were analysed (1.0 µL) in duplicate by Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS analyses were carried out using a gas chromatograph Hewlett-Packard (5890) and a mass selective detector

Hewlett-Packard (5971) with a potential of 70 eV for ionization by electron impact. Solvent extract analyses were performed using a 25 m × 0.20 µm × 0.13 µm polydimethylsiloxane BPX5 (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 230°C (3 C/min). The carrier gas was helium with a constant flow rate set close to 0.9 mL/min. All volatile components were identified by comparison with mass spectral library NBS (MacLafferty & Stauffer, 1989), literature spectra (Shibamoto, 1980; The Mass Spectrometry Data Centre, 1986; Jennings & National Institute of Standard and Technology, 1994; Adams, 1995) and our own data bank.

RESULTS AND DISCUSSION

The percentages of the volatile constituents and the retention indices are listed in Table 1. Chromatographic profiles of the organic extracts revealed, 21, 16 and 23 volatile components, which represented, 95.9, 98.2 and 94.5% of the volatile fraction for *Clitocybe amoenolens*, *Tricholoma caligatum* and *Hebeloma radicosum*, respectively (Table 1).

The GC-MS analysis revealed that *C. amoenolens* contained odorous aromatic derivatives, i.e., methyl-(*E*)-cinnamate (42.6%) and methyl benzoate (9.5%), and sesquiterpenes, i.e., β-barbatene (18.3%) and (*E*)-nerolidol (3.8%). For *T. caligatum*, we observed nine indole derivatives (84.4%), i.e., 2,4-dimethyl-1*H*-indole (29.4%) and 5-methoxy-2,4-dimethyl-1*H*-indole (29.5%) as well as methyl-(*E*)-cinnamate (10.5%). The *H. radicosum* extract was characterized by high amount of aromatic derivatives (52.6%), i.e., benzaldehyde (29.6%), 2-phenylethanal (9.0%), 2-phenylethanol (8.4%), β-barbatene (10.7%) and methyl-(*E*)-cinnamate (2.0%).

Methyl-(*E*)-cinnamate was found in the three analysed mushrooms at various levels; it seemed to be the key compound responsible for the pleasant aroma of *C. amoenolens* and *T. caligatum*. This cinnamic derivative is well-known in perfume and flavor industries for its fruity-balsamic scent (Arctander, 2003). It is responsible in perfume for "oriental" or sweet notes in high dilutions and strawberry/cherry-like fruity notes in lower dilutions. Arctander (2003) notices its frequent use in flavor for grape, cherry and strawberry notes. This ester was really found in strawberries (Ducruet *et al.*, 2001) and plums, blended with benzaldehyde (Ismail *et al.*, 1981); consequently, presence of methyl-(*E*)-cinnamate explains the fruity first note of *C. amoenolens* and *T. caligatum* usually compared to the note of several species of *Inocybe*. Moreover, the odorous substance of *I. corydalina* and *I. pyriodora* was also identified as methyl-(*E*)-cinnamate (Schmitt, 1978). These *Inocybe* species as well as *C. amoenolens* and *T. caligatum* exhale also a floral note (the second note of their scent) reported as corydale- and jasmine flowers-like. Methyl-(*E*)-cinnamate and methyl benzoate identified in *C. amoenolens* were commonly found in scent profile of *Corydalis cava* (Olesen & Knudsen, 1994) and jasmine (Christensen *et al.*, 1997); furthermore, it should be noted that methyl anthranilate detected in *C. amoenolens* was determined as one of the key odorants of the jasmine tea (Ito *et al.*, 2002). In addition, according to the flavoring properties published by Arctander (2003), methyl-(*E*)-cinnamate could also be responsible for the third balsamic note of *C. amoenolens* and *T. caligatum*

Table 1. Volatile composition (percentage) of fresh *Clitocybe amoenolens, Tricholoma caligatum* and *Hebeloma radicosum.*

Volatile compounds	*RI*[a]	*Clitocybe amoenolens*	*Tricholoma caligatum*	*Hebeloma radicosum*
		(%)[b]	*(%)*	*(%)*
Hexanal	790	0.8	–	1.8
Ethylbenzene	861	–	0.2	–
Styrene	887	–	0.1	–
Butyric acid	930	–	–	1.5
Benzaldehyde	949	0.1	1.0	29.6
2,3-Octadione	968	0.3	–	5.6
1-Octen-3-ol	974	–	–	2.6
3-Octanol	986	–	–	0.5
Limonene	1 020	0.3	–	–
2-Phenylethanal	1 032	1.0	–	9.0
Acetophenone	1 060	0.2	–	–
Linalool	1 093	0.3	–	–
Undecane	1 100	1.3	–	0.2
2-Phenylethanol	1 102	–	–	8.4
Methyl benzoate	1 130	9.5	–	–
Octanoic acid	1 160	–	–	1.6
Nonanoic acid	1 260	–	–	0.8
Benzoic acid	1 270	–	–	2.6
(*Z*,*E*)-2,4-Decadienal	1 279	–	–	0.3
Methyl-(*Z*)-cinnamate	1 292	0.1	0.9	–
Tridecane	1 300	0.8	–	1.1
(*E*,*E*)-2,4-Decadienal	1 309	–	–	3.9
1*H*-Indole	1 320	–	–	1.9
Methyl anthranilate	1 331	0.2	–	–
Phenylacetic acid	1 445	0.8	–	3.0
Methyl-(*E*)-cinnamate	1 365	42.6	10.5	2.0
α-Barbatene	1 409	0.6	–	0.2
(*E*)-Cinnamic acid	1 430	2.5	–	–
β-Barbatene	1 440	18.3	–	10.7
2,4-Dimethyl-1*H*-indole [7]	1 485	–	29.6	–
Pentadecane	1 500	1.8	0.2	0.5
NI[c]	1 537	–	–	3.5
(*E*)-Nerolidol	1 556	3.8	0.9	–
Myristic acid	1 578	8.1	-	4.8
NI (indole derivative)	1 639	–	0.9	–
NI (indole derivative)	1 660	–	3.0	–
4-Methoxymethyl-2-methyl-1*H*-indole [10]	1 695	–	11.6	–
5-Methoxy-2,4-dimethyl-1*H*-indole [8]	1 719	–	29.5	–
NI (indole derivative)	1 732	–	2.8	–
4-Hydroxymethyl-2-methyl-1*H*-indole [9]	1 750	–	3.9	–
NI (indole derivative)	1 778	–	0.8	–
NI (M = 272)	1 798	2.5	-	–
5-Methoxy-4-methoxymethyl-2-methyl-1*H*-indole [11]	1 800	–	2.3	–

[a] Retention indices on polydimethylsiloxane BPX5 column.
[b] Percentage of total ion current (TIC).
[c] Not identified.

smell reported by a few descriptors. Lastly, it should be taken into account that this cinnamate derivative was previously recorded as responsible for the flavor of *Tricholoma matsutake* (Yajima *et al.*, 1981; Ahn & Lee, 1986; Wood & Fesler, 1986), a related Asian species of *T. caligatum*.

In *C. amoenolens*, methyl benzoate, the compound isolated from benzoin (Fernandez *et al.*, 2003) with heavy floral or fruity tones (Arctander, 2003) strengthened the three notes (floral, fruity and balsamic) already noticed for methyl-(*E*)-cinnamate. The floral and fruity notes of *C. amoenolens* were also enriched with (*E*)-nerolidol (apple, berry, lily, rose flavors), methyl anthranilate (grape, jasmine, orange blossom odors), linalool (floral, lemon notes) and limonene (fresh sweet citrusy smell) (Breheret *et al.*, 1997; Ito *et al.*, 2002; Jirovetz *et al.*, 2002; Arctander, 2003).

While methyl-(*E*)-cinnamate, benzaldehyde and (*E*)-nerolidol also justified the fragrant odor of *T. caligatum*, high levels of nine indole derivatives (84.4% entirely) were found in its volatile fraction. The latter compounds well-known for various disagreeable odors (Hilber, 1968; Rapior *et al.*, 1998; Arctander, 2003) could explain the second nauseous note of *T. caligatum*. Indole derivatives, i.e., indole, 2,4-dimethyl-1*H*-indole and 5-methoxy 2,4-dimethyl-1*H*-indole were previously reported from several *Tricholoma* species as *T. lascivum*, *T. inamoenum*, *T. sulfureum*, *T. sciodes* and *T. virgatum* (Hilber, 1968; Eizenhöfer *et al.*, 1990; Garlaschelli *et al.*, 1994; Pang & Sterner, 1996). Unlike Watson *et al.* (1986), Hilber (1968) and Rapior *et al.* (1998) stated that indole derivatives (indole, skatole, formyl indole) were responsible for the coal-tar or gas-like odor of the three first *Tricholoma* species reported above. It is worth to notice the complex odor of *T. lascivum* and *T. inamoenum* (as that of *T. caligatum*) with a fruity sweety note (jasmine, seringa) followed by a nauseous insecticidal or sulphurated note (Bon, 1988; Courtecuisse & Duhem, 1994; Gerhardt, 1999). Karagül-Yüceer *et al.* (2003) considered that indole and 3-methyl-1*H*-indole (skatole), described as mothball odor were key compounds for the unpleasant smell of rennet caseine.

In addition to odor, monomeric indole derivatives as bisindolic compounds also contribute to the taste of *Tricholoma* and *Collybia* species. Bisindolic derivatives are unstable pungent and non pungent compounds isolated from *T. scalpturatum*, *T. sciodes*, *T. virgatum* and *Collybia peronata* (Pang & Sterner, 1994; Sterner, 1994). Besides, in *T. lascivum*, the bitter component named lascivol release indolic derivatives after methanolysis according to Eizenhöfer *et al.* (1990) and Garlaschelli *et al.* (1994). As tentatively reported in Fig. 1, the volatile indole metabolites [7-11] listed in Table 1 for *T. caligatum* as well as lascivol could derive i) from the non-volatile intermediate components [1, 2] and then ii) from one of the intermediate components [3-6], biosynthesized in the polycetidic pathway. Hence, these unusual indole derivatives in the Higher Fungi Kingdom but reported for several *Tricholoma* species could be of chemotaxonomic interest for this genus. Aromatic derivatives, i.e., ethyl benzene and styrene were also detected from the *T. caligatum* extract by GC-MS as previously reported from headspace extract by GC-Olfactometry evaluation (Talou *et al.*, 2000); these very light volatile components should contribute to the mushroom aroma.

Concerning the organic extract of fresh *H. radicosum*, GC-MS analyses revealed minor amount of methyl-(*E*)-cinnamate and high level of odorous aromatic compounds derived from the shikimic pathway at various oxidized stages (benzaldehyde: almond odor; 2-phenylethanal: reminiscent odor of lilac and hyacinth; 2-phenylethanol: rose odor; phenyl acetic acid: honey-like note predominant at low concentration; Arctander, 2003). These results are slightly different from those reported by Rapior *et al.* (1996) from frozen material; thus, it is well

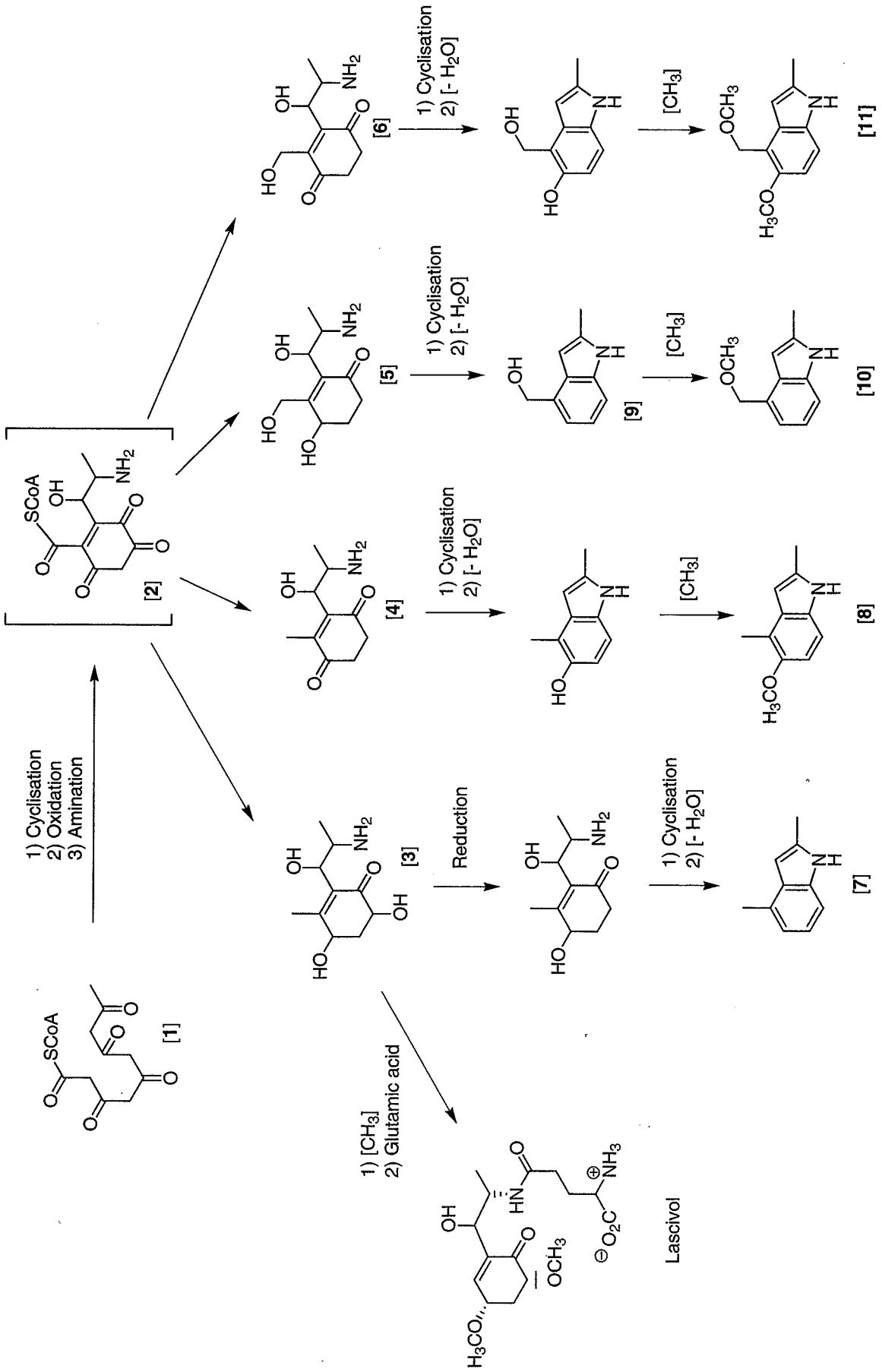

Fig. 1. Tentatively indole derivative biosynthesis from *Tricholoma* species.

known that the freezing changes considerably the volatile composition of fungal specimens as reported for *Hydnellum suaveolens* (Wood *et al.*, 1988). The fact that the chemical constituents responsible for the fungus odor change after freezing as well as during maturation (Flament *et al.*, 1990; Wu *et al.*, 2005) emphasizes the importance of recording the volatile extract at the time of collection in the field as it was carried out here for *C. amoenolens*, *T. caligatum* and *H. radicosum*.

This study highlighted for the first time the reminiscent floral odor of *Clitocybe amoenolens* due to methyl-(*E*)-cinnamate and methyl benzoate. Methyl-(*E*)-cinnamate as well as indole derivatives were reported as the dominant odorous constituents responsible for the complex odor of *Tricholoma caligatum*. The strong almond-like odor of *Hebeloma radicosum* was due to the major benzaldehyde but various aromatic derivatives with floral note (methyl-(*E*)-cinnamate, 2-phenylethanal, phenylacetic acid) enriched the chemical profile of this mushroom. Increasing requirements of the food and cosmetic industries for natural flavor and odor materials, respectively, higher fungi resources are of interest to naturally obtained aroma ingredients and in biotechnology to achieve nature-identical volatile components.

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