Volatiles from Liquid Cultures of *Lentinellus cochleatus* (Basidiomycotina)

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The basidiomycete *Lentinellus cochleatus* CBS 201.47 was cultivated on a defined synthetic liquid culture medium containing glucose (2%), asparagine (0.15%), malt extract (0.2%), and mineral salts. 12 weeks old cultures produced a "sweet" odour with an anise- or cinnamon-like note. 3 major volatiles could be identified from the steam distillates by their GC/MS- and ^{1}H NMR data. Predominant constituents were the acyclic sesquiterpene alcohols trans-nerolidol (41.4%) and fokienol (14.8%). 2,2-Dimethyl-6-formylchromene (6.1%) is described for the first time as a natural product. Besides these, 15 minor constituents could be identified or chemically characterized, respectively, the majority of them being sesquiterpene hydrocarbons and alcohols.

Introduction

Fruit-bodies of the wood-inhabiting basidiomycete *Lentinellus cochleatus* (Pers. ex Fr.) Karst. are mainly found on stumps and roots of various deciduous trees. Frequently, they produce a characteristic anise-like odour [1]. There is, however, a variety ("inodora") without such a distinct scent [2]. It has been reported that 7 weeks old malt-agar cultures produced an odour resembling "faintly of 5% anisaldehyde in proof spirit" [3]. Since no information was available on the chemistry of *L. cochleatus* volatiles, we decided to include this species in our screening programme on fragrance compounds from fungal liquid cultures. The isolation and identification of volatile metabolic products from strain *L. cochleatus* CBS 201.47 is subject of this communication.

Materials and Methods

Lentinellus cochleatus CBS 201.47 was obtained from Centraalbureau voor Schimmelcultures (CBS), Baarn (NL).

After mycelium inoculation, the basidiomycete was cultivated on a defined synthetic culture medium containing glucose (2%), asparagine (0.15%), malt extract (0.2%), and mineral salts [4].

Volatiles were determined after 12 weeks from 4 cultures (grown in 1 l Fernbach flasks containing 250 ml of culture broth) by circulation steam distillation [5] in 2 ml pentane. For structure elucidation, 12 Fernbach cultures (3 l) were grown under identical conditions and harvested likewise after 84 days. Mycelia were dried at 80 °C to constant weight. Determination of undepleted glucose and asparagine in the culture medium was performed by TLC [6].

Steam distillates were further separated into five fractions of different polarity by dry-CC [7] and analysed by GLC and GC/MS. GLC analyses were performed using a Perkin-Elmer F 22 gas chromatograph equipped with a glass capillary WG 11 column (22 m × 0.33 mm i.d.), a flame ionization detector (FID; range 1; attenuation 1:4; split 1:30), and a computing integrator (PE M-1). Operating conditions: linear temperature programme 80–200 °C, 2 °C/min; injector, 180 °C; detector, 180 °C; carrier gas, N_2 at 1 ml/min; injection volume: 1.0 µl.

MS analyses were carried out (a) on a Varian MAT 111 (GNOM) mass spectrometer (80 eV) using a 3 m packed Carbowax 20M (3%) column, and (b) on a Hewlett Packard HP 5985 Quadrupol instrument (70 eV) combined with a HP 5840A gas chromatograph using a silica capillary OV 1 column

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(25 m × 0.25 mm i.d.). He at 1 ml/min as carrier gas, and a thermal programme as described above.

For NMR analysis, the more polar fractions IV and V were combined, diethylether was evaporated after drying with sodium sulfate, and the residue was separated by preparative TLC (Si-60, Merck). The mobile phase was *n*-hexane–ethylacetate (8:2). NMR spectra were recorded on Bruker WM 400 in $CDCl_3$. Tetramethylsilane (TMS) was the internal standard for ^1H NMR.

Quantities of volatile constituents were calculated gaschromatographically via an internal standard (6-methyl-5-hepten-2-one) using FID-specific substance factors.

Results and Discussion

Liquid cultures of the basidiomycete *Lentinellus cochleatus* CBS 201.47 were cultivated on a defined glucose (2%)-asparagine (0.15%)-malt extract (0.2%)-mineral salt medium [4] for 12 weeks. Under these culture conditions, the fungus formed white to brownish mycelia becoming darker with increasing culture time. The slowly growing cultures produced a "sweet" odour with an anise- or cinnamon-like note. After 84 days, volatiles were obtained by circulation steam distillation [5] giving a slightly yellow distillate. At that time, C- and N-source (glucose and asparagine, respectively) were indepleted; the mycelium weight was 0.74 mg/l.

Fig. 1 shows the gas chromatogram of the total distillate revealing a composition of at least 140 components. Most of these are trace compounds with a quota of less than 0.3%. The percentage of the major constituents is given in Table I.

After separation by dry-CC [7] into five fractions of different polarity, several constituents were found in the most apolar fraction. Mass spectral analysis revealed most of them as sesquiterpene hydrocarbons. α-Copaene (**8**; 0.08 mg/l; 0.3%), δ-cadinene (**34**; 0.32 mg/l; 1.2%), and calacorene (**55**; 0.16 mg/l; 0.6%) were identified by comparison with literature spectra [8] and those obtained from authentic reference substances. Previously, these compounds have already been isolated from the brown-rot fungus *Lentinus lepideus* [9].

The majority of volatiles were found in the more polar fractions, especially in fraction IV. Non-ter-

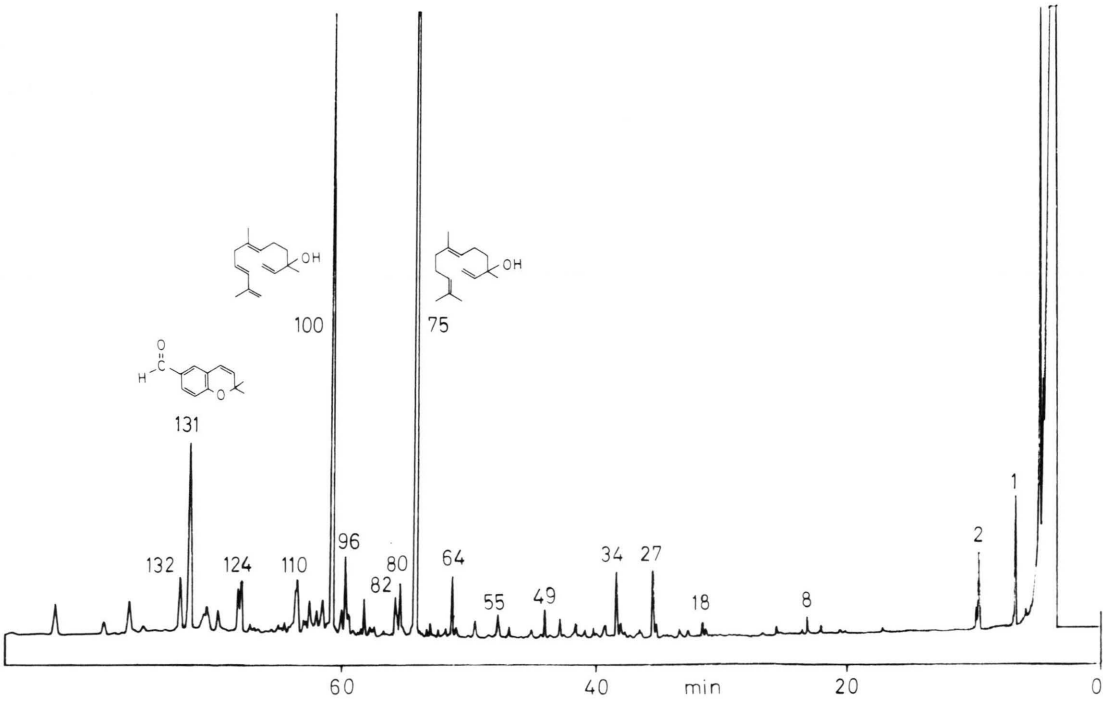

Fig. 1. Gas chromatogram of the volatiles in 12 weeks old cultures of *L. cochleatus* CBS 201.47. For culture and GLC conditions see under "Materials and Methods".

Table I. Composition of major volatile metabolites from 12 weeks old cultures of *Lentinellus cochleatus* CBS 201.47 grown on glucose-malt extract-asparagine-mineral salt medium.

Peak-No.	Compound	mg/l culture medium	% of GLC peak area
1	isobutyl alcohol	0.50	1.4
2	3-methyl-1-butanol	0.44	1.3
8	α-copaene	0.08	0.3
18	$C_{15}H_{22}$	0.06	0.2
27	$C_{15}H_{22}$	0.34	1.3
34	δ-cadinene	0.32	1.2
49	tetrahydro-nerolidol?	0.15	0.5
55	calacorene	0.16	0.6
64	$C_{15}H_{24}O$	0.33	1.2
75	*trans*-nerolidol	11.82	41.4
80	cubenol	0.34	1.2
82	epi-cubenol	0.30	1.0
96	$C_{15}H_{24}O$	0.46	1.6
100	fokienol	4.20	14.8
110	$C_{15}H_{24}O$	0.66	2.3
124	$C_{15}H_{24}O$	0.38	1.3
131	2,2-dimethyl-6-formylchromene	1.86	6.1
132	farnesol	0.49	1.7
	unidentified trace compounds		20.6

penoid substances are isobutyl alcohol (**1**; 0.5 mg/l; 1.4%) and 3-methyl-1-butanol (**2**; 0.44 mg/l; 1.3%), both being ubiquituous constituents in fungal liquid cultures and originating obviously from amino acid metabolism.

Other major components of these fractions could be characterized almost exclusively as oxygenated sesquiterpenes. After separation and purification by preparative TLC using *n*-hexane–ethylacetate (8:2) as mobile phase, the predominant constituents were identified as acyclic sesquiterpene alcohols by GC/MS and ^{1}H NMR data: trans-nerolidol (**75**; 11.82 mg/l; 41.4%) which has already been shown to be present in cultures of *Ceratocystis coerulescens* [10], *Penicillium decumbens* [11], and in various yeast strains [12]. Mass spectra for component **100** (4.2 mg/l; 14.8%) gave an M^+ signal at m/z = 220 (2%). Base peak was m/z = 93. The fragmentation pattern was in good agreement with literature data for fokienol [13], a fourfold unsaturated sesquiterpene alcohol previously isolated from the wood oil of *Fokienia hodginsii* [14]. NMR spectroscopy confirmed our results. Hitherto, fokienol has not been described as a fungal metabolite.

Another acyclic alcohol, farnesol (**132**; 0.49 mg/l; 1.7%), has already been isolated from various other fungal sources [10, 12, 15], whereas the isomeric bicyclic alcohols cubenol (**80**; 0.34 mg/l; 1.2%) and epi-cubenol (**82**; 0.3 mg/l; 1.0%) have been identified only in liquid cultures of *Lentinus lepideus* [15].

Mass spectra of component **131** (1.86 mg/l; 6.1%) showed an M^+ peak at m/z = 188 (18%) and a base peak at m/z = 173 $[M\text{-}Me]^+$ (Fig. 2). Other more prominent peaks, m/z = 159 (4%) $[M\text{-}COH]^+$ and m/z = 144 (11%) $[M\text{-}44]^+$ are typical for aldehydes. ^{1}NMR data (Table II) are only in agreement with

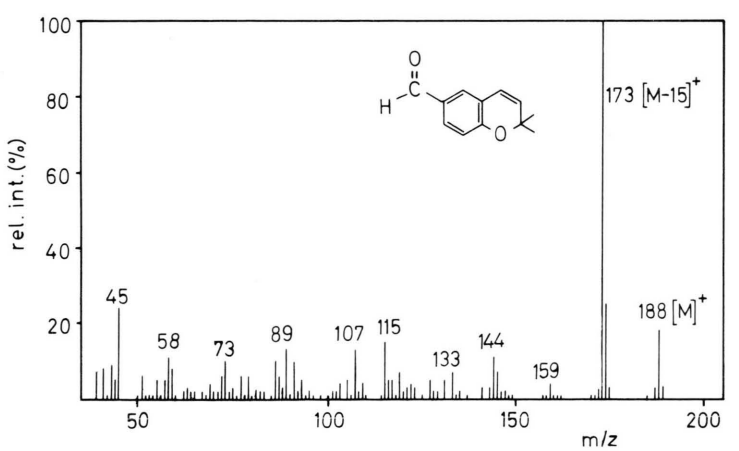

Fig. 2. Mass spectrum of 2,2-dimethyl-6-formylchromene (**131**).

Table II. ^{1}H NMR data of 2,2-dimethyl-6-formylchromene (**131**; $CDCl_3$, 400 MHz).

H	δ, multiplicity	*J* [Hz]
3	5.69 d	10
4	6.38 d	10
5	7.54 d	2
7	7.64 dd	8.2
8	6.87 d	8
CH_3	1.48 (6H)	
CHO	9.76 s	

2,2-dimethyl-6-formylchromene. This compound is described for the first time as a natural product. So far, other fungal chromenes have only been reported from *Lactarius* species [16].

The fruit-bodies of many basidiomycetes emit anise-like odours. Our results show that the chemical composition of volatiles obtained from liquid cultures of such species may reveal distinct differences: While *Lentinus lepideus* FPRL 7B produces mainly cinnamic acid derivatives and – under certain culture conditions – considerable amounts of cadinane-type sesquiterpenes [9, 15], we found monoterpenoids and the bicyclic sesquiterpene alcohol drimenol to be the major constituents of *Gloeophyllum odoratum* CBS 444.61 [17]. The spectrum of volatiles from wood-inhabiting fungi including the newly investigated strain, *Lentinellus cochleatus* CBS 201.47, show often surprisingly much likeness with certain wood oils (*e.g.* [14]). This circumstance implies the question on coevolutionary tendencies between host and wood-inhabiting fungus in the formation of enzymatic reactions leading to these secondary metabolites.

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