Volatile Fragrance Compounds from the Fungus *Gloeophyllum odoratum* (Basidiomycotina)

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The accumulation of volatile metabolites in liquid cultures of the basidiomycete *Gloeophyllum odoratum* CBS 444.61 was studied after cultivation on defined synthetic liquid culture medium containing glucose (2%), asparagine (0.15%), and mineral salts. The composition of the steam volatile compounds differed distinctly from that obtained with another, previously investigated strain of this species. Two major constituents, the bicyclic sesquiterpene alcohol drimenol and a linalool derivative, 3,7-dimethyl-3-hydroxy-6-octenic acid methyl ester, could be identified by mass spectral data and ^{1}H and ^{13}C NMR spectroscopy. Besides several monoterpene alcohols, other minor constituents were *trans*-linalool oxide and 1-octen-3-ol. 3,7-Dimethyl-3-hydroxy-6-octenic acid methyl ester is described for the first time as a natural product.

Introduction

The fruit-bodies of various basidiomycetes produce characteristic odours that are frequently mentioned in species descriptions and that are sometimes useful as taxonomic markers. *Gloeophyllum odoratum* (Wulfen ex Fr.) Imazeki [syn. *Osmoporus odoratus* (Wulfen ex Fr.) Singer] is a wood-inhabiting species causing brown-rot on conifers. Its fruit-bodies produce a pleasant scent resembling anise or fennel [1], other authors have described it as "strong, pleasant, like hay or anise" [2]. Malt agar and liquid cultures of *G. odoratum* emit also a fruity odour [3, 4]. During their studies on odoriferous substances from fungi, Halim and Collins identified several monoterpene alcohols (citronellol, nerol, and geraniol) and two constituents with an aromatic structure (methyl phenylacetate and methyl *p*-methoxyphenylacetate) from *G. odoratum* L 6330 [4]. These results were later confirmed identifying additionally anisaldehyde [5]. In the present communication, we describe the isolation and identification of volatile fragrance compounds from another strain, *G. odoratum* CBS 444.61, with a distinctly different spectrum of these metabolic products.

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Material and Methods

Gloeophyllum odoratum CBS 444.61 was obtained from Centraalbureau voor Schimmelcultures (CBS), Baarn (NL).

After mycelium inoculation, the basidiomycete was cultivated on a defined synthetic liquid culture medium containing glucose (2%), asparagine (0.15%), and mineral salts [6]. The volatile metabolites were determined after 19, 28, 42, 55 and 88 days. In each case, they were obtained from 10 cultures (grown in 250 ml Erlenmeyer flasks containing 50 ml of culture broth) by circulation steam distillation [7] in 2 ml pentane. For structure elucidation, 100 cultures (5 l) were grown under the same culture conditions and harvested after 42 days. Mycelia were dried at 80 °C to constant weight.

Steam distillates were further 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 program 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 on a Varian MAT 111 (GNOM) mass spectrometer (80 eV) using a 3 m packed Carbowax 20 M (3%) column and various isothermal conditions or a thermal program as described above. High resolution (7500) mass spectrometry was performed on a Varian MAT MS 311 A instrument (70 eV) with PFK as a reference substance.

For further structure elucidation of the major constituents, pentane was evaporated after drying with sodium sulfate, and the residue was separated with preparative TLC (Si-60, Merck). The mobile phase was n-hexane : ethylacetate 8:2.

NMR spectra were recorded on Bruker AM 300 in $CDCl_3$. Tetramethylsilane (TMS) was the internal standard for ^1H and the solvent signal ($\delta = 77.05$) for ^{13}C. IR spectra were recorded on a Perkin-Elmer PE 297 infrared spectrometer. Optical rotation was measured on a Perkin-Elmer PE 241 polarimeter.

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

The fruit-bodies of the brown-rot fungus *Gloeophyllum odoratum* (Wulfen ex Fr.) Imazeki (Aphyllophorales; Basidiomycotina) produce a pleasant anise- or fennel-like odour on natural substrates. Strain *G. odoratum* CBS 444.61 was cultivated on a defined synthetic glucose-asparagine-mineral salt liquid medium [6] for 88 days. Under these conditions, the fungus formed white mycelial mats becoming yellowish after 3 to 4 weeks of cultivation time. Mycelium weights and accumulation of volatile metabolites were determined five times (19, 28, 42, 55 and 88 days) during the culture period (Fig. 1). The maximum mycelium weight was obtained after 42 culture days, the maximum accumulation of volatiles after 55 days, however, there was a decrease in constituents with lower molecular weight after that time. For further structure elucidation, 6 weeks old cultures (5 l) producing a pleasant sweet odour with a mushroom-like note were analysed.

Fig. 2 shows the gas chromatogram of the steam distillate of 6 weeks old cultures consisting of about 50 components. Most of these are trace compounds

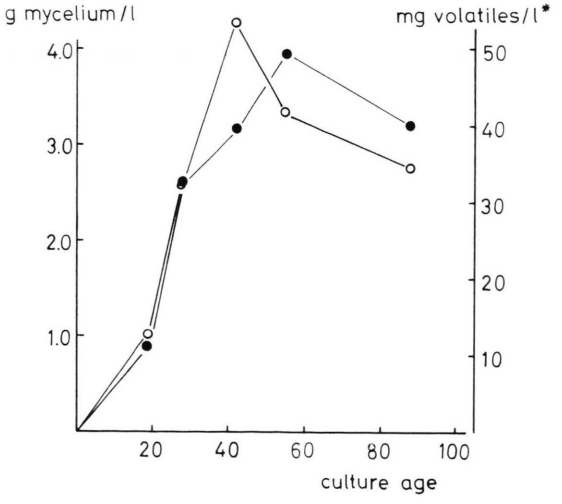

Fig. 1. Mycelium dry weights and amounts of volatiles in liquid cultures of *G. odoratum* CBS 444.61 during a culture period of 88 days grown on a glucose-asparagine-mineral salt medium. ○——○ Mycelium weights; ●——● volatiles.

* Total yields of volatiles are estimated on the basis of the major constituents (approx. 90%).

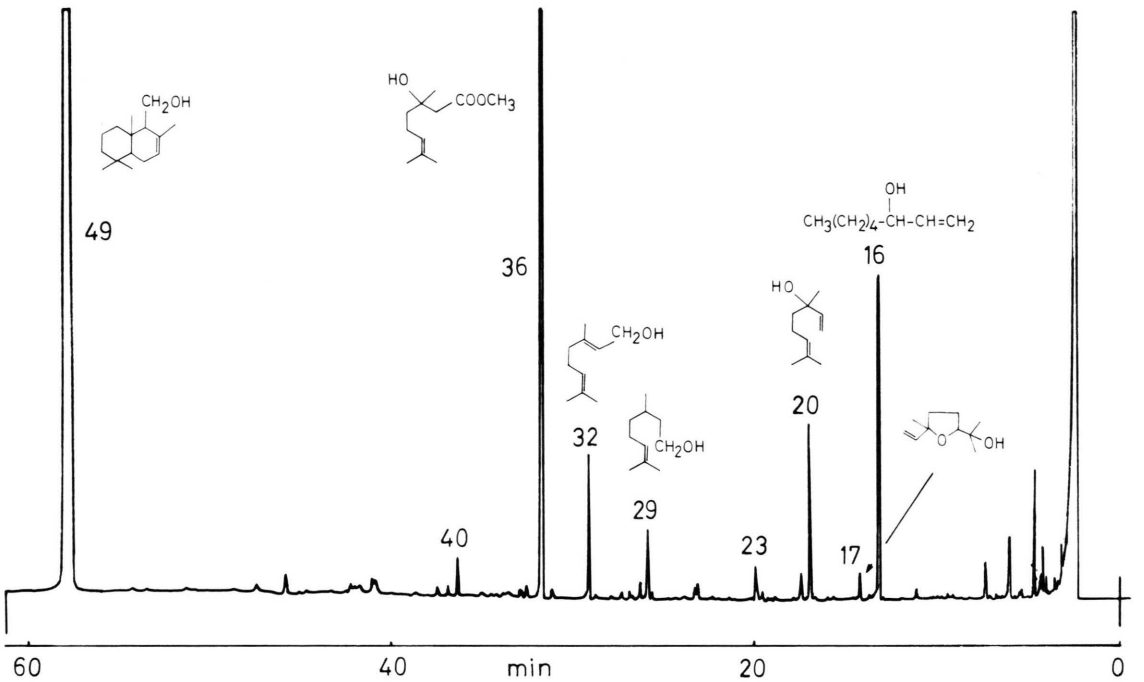

Fig. 2. Gas chromatogram of the volatiles in 6 weeks old cultures of *G. odoratum* CBS 444.61. For gaschromatographic conditions see under Material and Methods.

with a quota of less than 0.5%. The percentage of the major constituents is given in Table I. Predominant compound is the bicyclic sesquiterpene alcohol drimenol (**49**; 20.42 mg/l; 55.8%). Mass spectra of this constituent were in good agreement with the literature spectrum [8], ^{1}H and ^{13}C NMR spectra agreed well with data published by Aasen *et al.* [9] for drimenol. Comparison with authentic material confirmed our results. Recently, drimenol has also been isolated from other basidiomycetes [10, 11].

A second major component, compound **36** (10.19 mg/l; 22.5%), was separated by preparative TLC. The ^{1}H NMR spectrum showed an AB system of two protons with a coupling constant of 16 Hz which is characteristic of a geminal coupling near a carbonyl function. The IR spectrum (in $CHCl_3$) revealed an absorption at 1740 cm^{-1}, so the carbonyl function was identified as an ester. ^{1}H and ^{13}C NMR spectra (Table II) were only in agreement with structure **36** which was supported by comparison with

Table I. Composition of major volatile metabolites from 6 weeks old cultures of *G. odoratum* CBS 444.61 grown on a glucose-asparagine-mineral salt medium.

Peak-No.	Compound	% of GLC peak area	mg/l culture medium
16	1-octen-3-ol	4.3	1.71
17	*trans*-linalool oxide	0.4	0.15
20	linalool	2.2	0.84
23	not identified	0.7	
29	citronellol	1.2	0.47
32	geraniol	2.2	0.84
36	3,7-dimethyl-3-hydroxy-6-octenic acid methyl ester	22.5	10.19
40	$C_{15}H_{24}O$	0.6	0.20
49	drimenol	55.8	20.42

Table II. ^{1}H NMR and ^{13}C NMR data of 3,7-dimethyl-3-hydroxy-6-octenic acid methyl ester (**36**; 300 MHz resp. 75.5 MHz, $CDCl_3$, TMS internal standard).

H	δ, multiplicity	*J* [Hz]	C	δ, multiplicity
2	2.55 d	2.2′ = 16	1	173.4 s
2′	2.46 d	5,6 = 7	2	44.8 t
4	1.54 m	5′,6 = 7	3	71.0 s
4′	1.59 m		4	41.9 t
5	2.06 m		5	22.7 t
5′			6	124.1 d
6	5.11 t		7	131.8 s
8	1.68 s (br)		8	25.7 q
9	1.62 s (br)		9	17.6 q
10	1.26 s		10	26.7 q
OCH_3	3.73 s		OCH_3	51.7 q

data of related compounds. The mass spectrum (Fig. 3) shows no parent peak due to loss of H_2O, but *m/z* 182 $[M-H_2O]^+$ (34%); base peak is *m/z* 109 $[M-H_2O-CH_2-COOCH_3]^+$. High resolution mass spectrometry gave for $[M-H_2O]^+$ 182.13086 (calc. 182.13067). The sign of the optical rotation [α(c = 0.5, $CHCl_3$) = +3.3° (589 nm); +3.6° (578 nm); +3.7° (546 nm); +5.4° (436 nm); +8.8° (365 nm)] agreed with derivatives of mevalonic acid and points to a 3*R*-configuration [12]. To our knowledge, this is the first description of 3,7-dimethyl-3-hydroxy-6-octenic acid methyl ester, a linalool derivative, as a natural product.

As minor metabolites, several monoterpenes could be identified by comparison of their MS spectra with literature data [13, 14] or authentic reference substances: trans-linalool oxide (**17**; 0.15 mg/l; 0.4%), linalool (**20**; 0.84 mg/l; 2.2%), citronellol (**29**; 0.47 mg/l; 1.2%), and geraniol (**32**; 0.84 mg/l; 2.2%). Citronellol and geraniol have already been isolated from *G. odoratum* L 6330 [4, 5]. Another related monoterpene alcohol, nerol, could not be detected in the distillates of our strain. A further metabolite, 1-octen-3-ol (**16**; 1.71 mg/l; 4.3%) has a mushroom-like flavour and has been shown to be an ubiquitous fungal constituent [15].

Using culture media with altered composition (glucose replaced by fructose; asparagine replaced by leucine or isoleucine) the spectrum of volatiles was similar to the above. However, when asparagine was replaced by 0.15% phenylalanine, we found additionally several compounds with an aromatic structure (2-phenylethanol, 3-phenylpropanol, methyl 2-phenylacetate). Our results confirm that even closely related fungal strains are not only able to produce volatiles in sometimes distinctly different yields, but that, moreover, they may also differ in regard to a strain-dependent production of individual components [16].

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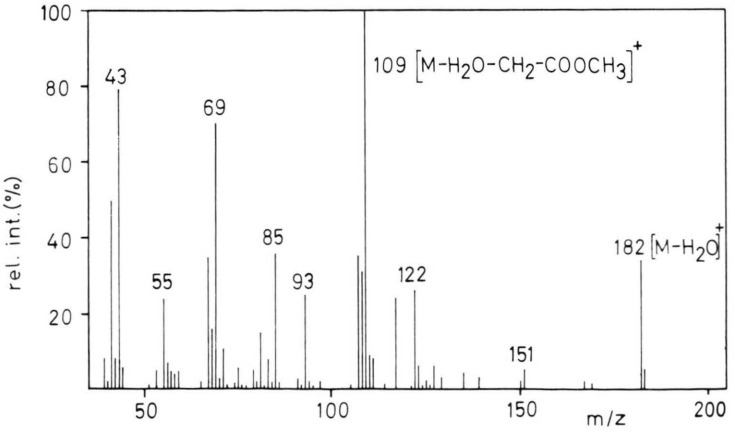

Fig. 3. Mass spectrum of 3,7-dimethyl-3-hydroxy-6-octenic acid methyl ester (**36**).

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