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Volatile constituents of wood-rotting basidiomycetes

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Abstract

Phytochemical investigation of the hydrodistillation products of the basidiomycetes *Fomitopsis pinicola*, *Piptoporus betulinus*, *Gloeophyllum odoratum* and *Trametes suaveolens* led to the identification of numerous mono- and sesquiterpenes as well as many aliphatic alcohols, aldehydes and ketones and some aromatic compounds. In addition, some diterpenes were identified as constituents of *Fomitopsis pinicola*. The absolute configuration of some terpenes was determined.

Keywords: *Piptoporus betulinus*; *Fomitopsis pinicola*; *Gloeophyllum odoratum*; *Trametes suaveolens*; Basidiomycete; Volatile metabolites; Sesquiterpene; Fungal terpenoids

1. Introduction

In the past little efforts have been made to investigate the volatile terpenoid constituents of naturally grown wood-rotting basidiomycetes. Linoleic acid derived short-chain alcohols like the "mushroom-alcohol" (*S*)-(+)-1-octene-3-ol, ketones like 2-nonanone and various fatty acids are well described in the literature (Turner and Aldridge, 1983; Tressl et al., 1982). If one looks at the volatile terpenoid constituents identified from fungi in the past, mainly abundant ones like drimenol from *Gloeophyllum odoratum* (Kahlos et al., 1994) or 6-protoilludene from the ascomycete *Ceratocystis piceae* were identified (Hanssen et al., 1986; Turner et al., 1983). It has to be noted that many of the latter compounds were isolated from liquid cultures and that their occurrence in nature was not confirmed. Only from a few species like *Lentinus lepideus*, a complete analysis of hydrodistillates of liquid cultures has been carried out (Hanssen, 1982, 1985). In the present paper the investigation of hydrodistillates of the species *Piptoporus betulinus* (Bull.: Fr.) P. Karst [syn. *Polyporus betulinus* (Bull.: Fr.) Fr.], *Fomitopsis pinicola* (Swartz ex Fr.) Karst (syn. *Polyporus pinicola* Fr., *Fomes pinicola*), *Gloeophyllum odoratum* (Wulf. ex Fr.) Imazeki [syn. *Osmoporus odoratus* (Wulf. ex Fr.) Singer, *Trametes odorata* (Wulf.) Fr.] and *Trametes suaveolens* (Fr.: Fr.) Fr. [syn. *Polyporus suaveolens* (Fr.) Fr.] is described.

2. Results and discussion

Fresh fruiting bodies of all species were cut in small pieces and submitted to circular hydrodistillation with *n*-hexane as collecting solvent. All compounds were identified by comparing their mass spectra and retention times with literature data (Joulain and König, 1998). The determination of absolute configuration of some compounds was carried out by enantioselective capillary gas chromatography (GC) by coinjection and comparison with previously isolated reference compounds using chiral stationary phases on the basis of cyclodextrin derivatives (König et al., 1999).

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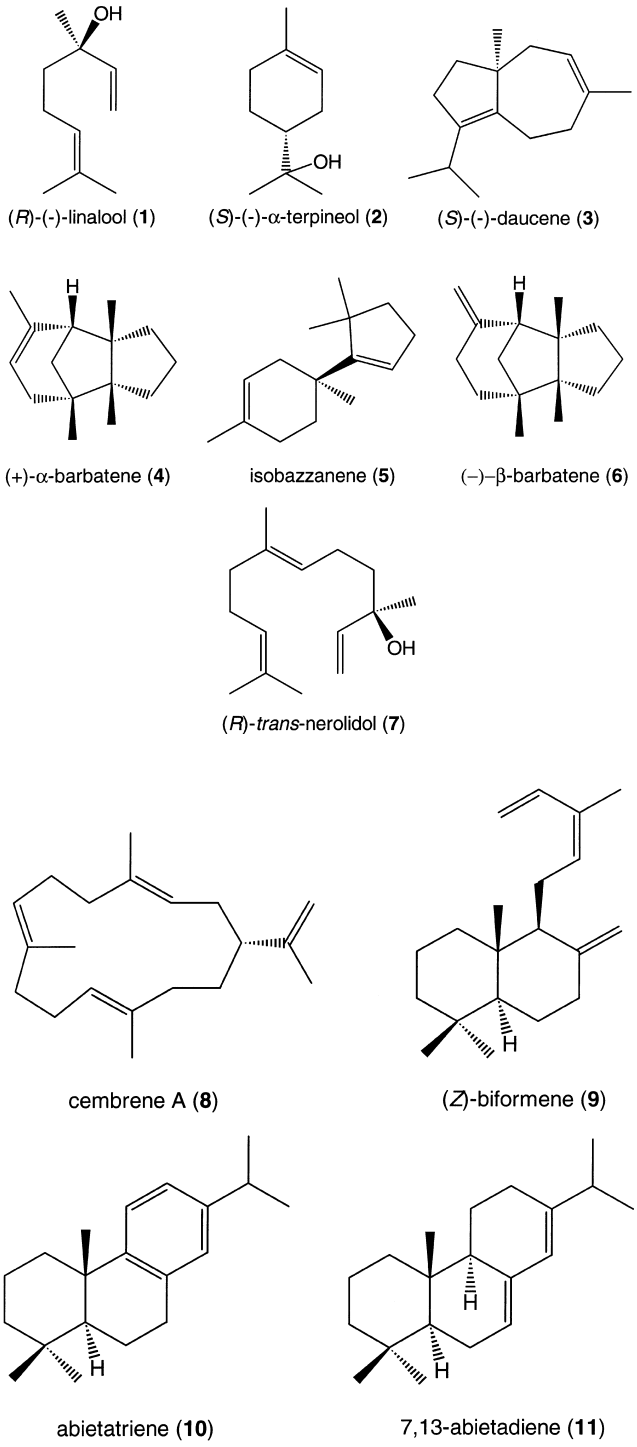
2.1. Piptoporus betulinus

The hydrocarbons from the crude hydrodistillate were separated from oxygenated compounds by dry column chromatography on silica gel with *n*-hexane as eluent (three fractions). The oxygenated compounds were then eluted with ethyl acetate. In addition, fraction 1 was purified by preparative GC. The identified compounds and their quantities are listed in Table 1. The hydrocarbon fractions were both separated by preparative GC. This yielded pure α-barbatene (**4**) and isobazzanene (**5**). Coinjection of all fractions and the isolated compounds with authentic samples on chiral stationary phases led to the determination of the absolute configurations of (+)-α-barbatene (**4**), (*S*)-(−)-daucene (**3**) and (*R*)-*trans*-nerolidol (**7**). **3**, **4**, **5** and (−)-β-barbatene (**6**) are reported here for the first time as fungal constituents. As the barbatenes, daucene and *trans*-nerolidol have been found in some of the other investigated species, they appear to be important constituents of fungi.

Table 1
Constituents from *P. betulinus*

Compound	Means of identification	% in the fractions
Aliphatic alcohols, ketones and aldehydes		
1-Octen-3-ol	MS	45.0
3-Octanol	MS	27.0
(*Z*)-2-octen-1-ol	MS	0.8
1-Octanol	MS	4.7
(*Z*)-1,5-octadien-3-ol	MS	3.2
3-Octanone	MS	6.1
Terpenes		
Linalool (**1**)	MS, R_i	0.9
α-Terpineol (**2**)	MS, R_i	tr.
α-Pinene[a]	MS, R_i	tr.
Δ-3-Carene[a]	MS, R_i	tr.
Pentalenene	MS, R_i	tr.
α-Cubebene[b]	MS, R_i	tr.
(S)-(−)-Daucene (**3**)	MS, R_i	tr., 1.2
β-Cubebene[b]	MS, R_i	0.8
β-Elemene[c]	MS, R_i	0.6
Thujopsene	MS, R_i	1.6, 2.9
(+)-α-Barbatene (**4**)	MS, R_i	6.4, 1.8
Isobazzanene (**5**)	MS, R_i	8.2, 15.7
(−)-β-Barbatene (**6**)	MS, R_i	0.2, 0.3
Cadina-1(6),4-diene	MS, R_i	tr., 0.3
β-Chamigrene	MS, R_i	1.2, 0.9
Selina-4,11-diene	MS, R_i	0.3
α-Cuprenene	MS, R_i	0.4, 8.7
α-Chamigrene	MS, R_i	1.5, 7.3
δ-Cadinene	MS, R_i	tr., 3.4
β-Bazzanene	MS, R_i	0.5, 4.3
Cyclobazzanene	MS, R_i	0.4, 4.2
(*R*)-*trans*-nerolidol (**7**)	MS, R_i	0.8
T-cadinol	MS, R_i	tr.
1-*epi*-cubenol	MS, R_i	tr.
Aromatic compounds		
Benzaldehyde	MS	0.1
Methyl anisate	MS	tr.

[a] Most likely rearrangement products of α-terpineol.
[b] Most likely rearrangement product of 1-*epi*-cubenol.
[c] Possibly COPE-rearrangement product of germacrene A.



2.2. Fomitopsis pinicola

Again, the crude hydrodistillate was filtered through dry silica gel with *n*-hexane as eluent (2 fractions). The polar constituents were then eluted with ethyl acetate.

All identified compounds and their quantities are listed in Table 2. For the first time the diterpene hydrocarbons cembrene A (**8**), (*Z*)-biformene (**9**), abietatriene (**10**) and 7,13-abietadiene (**11**) are reported to be constituents of fungi.

2.3. Gloeophyllum odoratum and Trametes suaveolens

The hydrodistillates were not further separated. The identified compounds and their quantities are listed in Table 3 (*G. odoratum*) and Table 4 (*T. suaveolens*). In the case of *G. odoratum* the enantiomeric excesses of linalool (**1**) and α-terpineol (**2**) were determined by coinjection of the crude hydrodistillate with racemic standard solutions using enantioselective capillary GC.

Table 2
Constituents from *F. pinicola*

Compound	Means of identification	% in the fractions
Aliphatic alcohols, ketones and aldehydes		
3-Decanone	MS	0.2
3-Undecanone	MS	0.4
3-Dodecanone	MS	1.1
n-Heptanal	MS	1.5
1-Octen-3-ol	MS	49.6
3-Octanol	MS	1.5
(*Z*)-2-octen-1-ol	MS	2.9
1-Octanol	MS	3.2
(*E,E*)-2,4-decadienal	MS	0.5
Terpenes		
α-Pinene	MS, R_i	11.5
Camphene	MS, R_i	5.5
Limonene	MS, R_i	0.8
Camphor	MS, R_i	0.2
α-Cubebene	MS, R_i	0.5
α-Longipinene	MS, R_i	0.9
α-Ylangene	MS, R_i	0.5
α-Copaene	MS, R_i	1.9
6-Protoilludene	MS, R_i	1.6
Sativene	MS, R_i	0.6
Longifolene	MS, R_i	6.3
α-Barbatene **(4)**	MS, R_i	6.2
β-Caryophyllene	MS, R_i	0.9, 0.7
β-Copaene	MS, R_i	1.1
β-Barbatene **(6)**	MS, R_i	6.0, 0.4
(*E*)-β-farnesene	MS, R_i	14.7
γ-Muurolene	MS, R_i	2.0
γ-Amorphene	MS, R_i	0.6
α-Muurolene	MS, R_i	2.0
γ-Cadinene	MS, R_i	1.0
δ-Cadinene	MS, R_i	6.0, 2.1
Trans-calamenene	MS, R_i	4.0
β-Calacorene	MS, R_i	1.6
Trans-nerolidol **(7)**	MS, R_i	1.7
1-*epi*-cubenol	MS, R_i	0.5
a-Cadinol	MS, R_i	0.2
Cembrene A **(8)**	MS, R_i	0.4
(*Z*)-Biformene **(9)**	MS, R_i	1.0
Abietatriene **(10)**	MS, R_i	0.4
7,13-Abietadiene **(11)**	MS, R_i	0.1
Aromatic compounds		
2-Pentylfurane	MS	3.4
Furfural	MS	1.3
Benzaldehyde	MS	4.0
Phenylacetaldehyde	MS	3.3
Methyl 4-methoxyphenylacetate	MS	0.1

Table 3
Constituents from *G. odoratum*

Compound	Means of identification	% hydrodistillate
Aliphatic alcohols, ketones and aldehydes		
1-Octen-3-ol	MS	24.7
(*E,E*)-2,4-decadienal	MS	tr.
Terpenes		
(*R*)-(−)-linalool **(1)**	MS, R_i	13.1
(*S*)-(+)-linalool **(1)**	MS, R_i	1.6
(*R*)-(+)-α-terpineol **(2)**	MS, R_i	0.6
(*S*)-(−)-α-terpineol **(2)**	MS, R_i	0.3
Geraniol	MS, R_i	0.1
Geranial	MS, R_i	0.1
Daucene	MS, R_i	0.3
γ-Calacorene	MS, R_i	0.4
Trans-nerolidol **(7)**	MS, R_i	0.4
Drimenol	MS, R_i	2.8
Aromatic compounds		
Methyl phenylacetate	MS	0.1
Methyl 4-methoxyphenylacetate	MS	46.4

3. Experimental

3.1. Plant material

Fruiting bodies were collected in the Sachsenwald near Hamburg (*Piptoporus betulinus*, October 1997;

Table 4
Constituents from *T. suaveolens*

Compound	Means of identification	% hydrodistillate
Aliphatic alcohols, ketones and aldehydes		
1-Octen-3-ol	MS	6.0
(*Z*)-1,5-octadien-3-ol	MS	0.2
3-Octanone	MS	0.7
n-Heptanal	MS	0.8
(*E,E*)-2,4-nonadienal	MS	0.1
(*E,E*)-2,4-decadienal	MS	0.6
Terpenes		
β-Barbatene **(6)**	MS, Ri	1.1
Trans-nerolidol **(7)**	MS, Ri	0.6
Aromatic compounds		
Benzaldehyde	MS	0.6
Methyl anisate	MS	77.9
Ethyl anisate	MS	2.0
2-Pentylfurane	MS	3.1
Phenylacetaldehyde	MS	0.1
Acetophenone	MS	0.1

Gloeophyllum odoratum, September 1997), near Zachersmühle, Göppingen, southern Germany (*Trametes suaveolens*; November 1997) and *Fomitopsis pinicola* on several locations in Germany (Gerhardt, 1996).

3.2. Hydrodistillation and pre-separation

Fresh fruiting bodies were cut in small pieces and submitted to circular hydrodistillation. The steam was condensed in a special apparatus and led through a layer of 1 ml of *n*-hexane as collection solvent and back into the flask with the plant material. The hydrocarbons from *P. betulinus* and *F. pinicola* were separated from the more polar components by silica dry column chromatography with *n*-hexane as eluent.

3.3. Capillary GC

Orion Micromat 412 instrument equipped with two fused silica capillaries (25 m) coated with CPSil-5CB and CPSil-19CB, respectively, split injection and flame ionization detection.

3.4. Enantioselective capillary GC

Carlo Erba Fractovap 2150, 4160 equipped with fused silica capillaries (25 m) coated with 50% heptakis(2,6-dimethyl-3-pentyl)-β-cyclodextrin and heptakis(6-*tert*-butyldimethylsilyl-2,3-methyl)-β-cyclodextrin, respectively, 50% in polysiloxane OV 1701, w/w, split injection and flame ionization detection.

3.5. Preparative GC

Modified Varian 1400 equipped with a stainless steel column (Amchro Silcosteel) packed with Chromosorb W-HP (Merck) and coated with 10% polydimethylsiloxane (SE30).

3.6. GC–MS

Hewlett-Packard HP 5890 gas chromatograph coupled to a VG Analytical VG 70-250S mass spectrometer [electron impact (70 eV)].

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