Lepistirones, Major Volatile Metabolites from Liquid Cultures of *Lepista irina* (Basidiomycotina)

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The basidiomycete *Lepista irina* (Fr.) Bigelow (strains CBS 366.47 and CBS 458.79) was cultivated on a biomalt (2%)-liquid medium for 28 days. The steam distillate was analyzed by GLC and GLC-MS. The structure elucidation of the predominant constituent resulted from spectroscopic methods, particularly from its ^{13}C NMR spectrum, nuclear Overhauser enhancement ^{1}H NMR data, and from 2d ^{1}H homonuclear shift correlation. The bisabolane derivative was named lepistirone and is described for the first time as a natural product. Two minor related compounds could be characterized by their MS data.

Introduction

Lepista irina (Fr.) Bigelow (Basidiomycotina, Agaricales) is an edible fungal species which can be recognized by its typical odour reminding of "iris oil" or "orange blossoms" [1, 2]. Recently, several terpenoid constituents including α- and β-bisabolene have been identified in fruit-bodies of a related species, *L. nuda* (Bull.: Fr.) Cooke [3]. Therefore, we decided to include *Lepista* strains into our screening programme for micro-organisms producing novel flavour or fragrance chemicals. We report here on the isolation and identification of new bisabolane derivatives from liquid cultures of *L. irina* CBS 366.47 and CBS 458.79.

Material and Methods

Lepista irina CBS 366.47 and CBS 458.79 were obtained from Centraalbureau voor Schimmelcultures (CBS), Baarn (NL).

After mycelium inoculation, the basidiomycete was cultivated on a liquid medium containing 2% malt extract for 28 days. The volatile metabolites were obtained from 10 cultures grown in 250 ml Erlenmeyer flasks with 50 ml of culture broth by circulation steam distillation in 2 ml pentane. For structure elucidation, 20 Fernbach cultures (4 l) were grown under identical conditions and harvested likewise after 4 weeks. Mycelia were dried at 80 °C to constant weight.

Steam distillates were further analyzed by GLC and GLC-MS. GLC analyses were performed using a Siemens L 402 gas chromatograph equipped with a glass capillary SE 54 column (25 m × 0.32 m i.d.), a flame ionization detector (FID; split 1:50), and a computing integrator (PE M 3). Operating conditions: linear temperature programme 80–220 °C, 2 °C/min; injector, 275 °C; detector, 275 °C; carrier gas, He (0.7 bar); injection volume: 1.0 μl.

MS analyses were carried out on a Varian MAT 112 S mass spectrometer (80 eV) combined with a Perkin-Elmer F 22 gas chromatograph using a SE 54 glass capillary column under the conditions as described above. High resolution mass spectrometry (HR-MS; 7500) was performed on a Varian MAT MS 311 A instrument (70 eV) with PFK as a reference substance.

^{1}H NMR spectra were recorded at 400 MHz on a Bruker WM 400 spectrometer and the ^{13}C NMR spectra at 75.5 MHz on a Bruker AM 300 instrument. $CDCl_3$ was used as solvent and TMS as an internal standard. Particulars of NOE and chemical shift correlation experiments are described in [4]. Optical rotation data were obtained on a Perkin-Elmer PE 241 polarimeter in methanol. Infrared spectra were measured in chloroform on a Perkin-Elmer PE 297 instrument.

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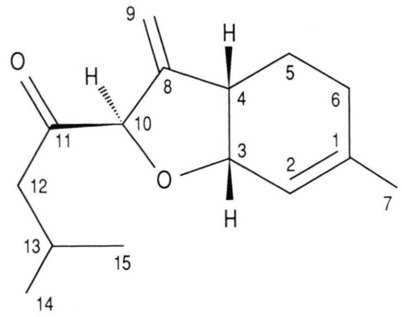
Quantities of the newly identified metabolites were calculated gas chromatographically *via* an internal standard (tridecane) using FID-specific substance factors.

Results and Discussion

Strains of *Lepista irina* CBS 366.47 and CBS 458.79 were cultivated on a liquid malt extract medium for 4 weeks. By that time, the cultures had formed white surface mycelia (0.87 g/l) producing a pleasant odour with a "mushroom-like, nutty" note, whereas younger cultures exhibited a more "yeast-like" flavour impression. The distillates consisted of more than 100 constituents, most of them being trace components, however. The mass spectrum (EI; Fig. 1) of the predominant compound **I** (3.81 mg/l culture broth) showed a molecular peak at *m/z* 234 giving first hints of an oxygenated sesquiterpene with the composition $C_{15}H_{22}O_2$. This assumption was confirmed by its HR-MS spectra (M^+ 234.1617; 234.1614 calculated for $C_{15}H_{22}O_2$). The IR spectra revealed a strong absorption of a carbonyl group (1715 cm^{-1}). The NMR data of compound **I** are summarized in Table I.



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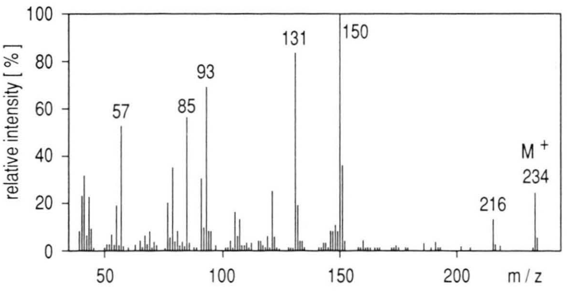

Fig. 1. Mass spectrum of lepistirone.

Table I. NMR data of Lepistirone from *Lepista irina* CBS 458.79 (TMS internal standard, $CDCl_3$).

C-1	140.3	0[a]		
C-2	120.2	+	2-H	dq 5.48
C-3	76.6	+	3-H	ddq 4.64
C-4	40.8	+	4-H	m 2.74
C-5	24.0	−	5-H	m 1.78
			5′-H	
C-6	26.8	−	6-H	m 2.00
			6′-H	m 1.81
C-7	23.5[b]	+	7-H	t 1.70
C-8	148.7	0		
C-9	107.0	−	9-H	t 5.13
			9′-H	dd 5.06
C-10	84.6	+	10-H	ddd 4.68
C-11	209.6	0		
C-12	46.7	−	12-H	dd 2.53
			12′-H	dd 2.34
C-13	23.7[b]	+	13-H	ddqq 2.14
C-14	22.6	+	14-H	d 0.91
C-15	22.5	+	15-H	d 0.87

J(Hz): 2, 3 = 3.3; 2, 7 = 1.6; 3, 4 = 3.3; 3, 7 = 1.6; 4, 9 = 2.7; 4, 9′ = 4, 10 = 2.3; 9, 10 = 9′, 10 = 2.3; 12, 12′ = 17.3; 12, 13 = 6,5; 12′, 13 = 7.1; 12, 15 > 0; 12′, 14 > 0; 13, 14 = 6.7; 13, 15 = 6.6.

[a] Amplitude of signals in DEPT-135 spectrum (CH_3 or CH = +; CH_2 = −; quat. C = 0).

[b] Assignments may be interchanged.

The ^{13}C NMR spectrum showed four resonances between $\delta_C = 148.7$ and 107.0 belonging to carbons of two double bonds and a singlet at $\delta_C = 209.6$ pointing to a ketone. These fragments account to three of the required five double bond equivalents, therefore the molecule has to be bicyclic. One of the rings must be a cyclic ether because of resonances at $\delta_H = 4.64$ and 4.68 and $\delta_C = 84.6$ and 76.6 and the absence of a hydroxyl absorption band in the infra-red. 2D ^1H homonuclear shift correlation (COSY) led to a sesquiterpene with constitution **I** with the bisabolane skeleton. The small coupling constant of $J_{HH} = 3.3$ Hz between 3-H and 3-H needs a *cis*-fusion of the rings. The configuration of **I** was determined by DNOE: Irradiation at the resonance at $\delta_H = 2.74$ (4-H) led to an enhancement of the signals at $\delta_H = 5.06$ (9′-H) and 2.34 (12′-H) requiring an *anti*-configuration of 3-H and 10-H. The observed positive Cotton effect and the application of the octant rule pointed to the absolute configuration of (3*S*,4*S*,10*R*)-3,10-epoxy-11-oxo-bisabola-1,8-diene for **I** which we named lepistirone.

Determination of the optical rotation gave $[\alpha](c = 1.0, CH_3OH) = +31.7°$ (600 nm), +50.3° (500 nm), +101,6° (400 nm), +129,5° (380 nm), +101,7° (365 nm).

Two minor compounds, **II** (1.95 mg/l culture broth) and **III** (0.18 mg/l culture broth) appear to be related to lepistirone. The mass spectra of **II** showed a molecular peak at m/z 236, those of **III** at m/z 232. From their fragmentation patterns, we suppose that these lipistirones differ in the degree of saturation of the cyclohexane ring in their molecules. Further studies are under way to confirm this assumption.

So far, sesquiterpenes with a bisabolane skeleton have been reported only rarely from fungi or fungal cultures. Sydowic acid, sydonic acid, and hydroxysydonic acid have been identified in *Aspergillus sydowii* (Bain & Sartory) Thom & Church [5, 6]. From another, but not truly identified *Aspergillus* strain, Nukina *et al.* [7] described the isolation of sydonol. Recently, α-bisabolol was found in *Phlebia radiata* Fr.: Fr. [8] and in liquid cultures of *Dichomitus squalens* (Karsten) Reid [9]. Sanson *et al.* [10] have isolated 4,5,10,11-tetrahydroxybisaboline from *Fusarium sambunicum* Fuckel, and α- and β-bisabolene have been described – as mentioned above – as constituents of *L. nuda* [3], and only β-bisabolene from liquid cultures of *Pleurotus ostreatus* (Jacq.: Fr.) Kummer [9]. The additional finding of a bisabolene derivative with a benzofuran nucleus in *Cystostereum murraii* (Berk. & Curtis) Pouzar [11] leads us to the assumption that these sesquiterpenes are more widespread in fungi. It should be further mentioned that Errington *et al.* have recently isolated a sesquiterpene with a nordrimane skeleton in cultures of *L. glaucocana* (Bres.) Singer [12].

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