Novel Sesquiterpene Ethers from Liquid Cultures of the Wood-Rotting Fungus *Lentinus lepideus*

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Dedicated to Professor Ewald Sprecher on the occasion of his 65th birthday

Lentinus lepideus, Lentideusether, Terrestrol, Fungal Sesquiterpenes, Fungal Fragrance Compounds

The brown-rot fungus *Lentinus lepideus* (Fr.: Fr.) Fr. FPRL 7B (Basidiomycotina) was cultivated on a defined synthetic liquid medium containing glucose (2%), isoleucine (0.15%), and mineral salts for 105 days. The steam distillate was separated by column chromatography. Three novel sesquiterpene ethers with muurolane skeleton (lentideusether, isolentideusether, and 10-hydroxylentideusether) were isolated and their structures elucidated by spectroscopic methods, in particular by ^{1}H NMR spectra and by two-dimensional $^{1}H/^{1}H$- and $^{13}C/^{1}H$-chemical shift correlation. These compounds are described for the first time as natural products. As a further metabolite, the acyclic sesquiterpene alcohol terrestrol was identified.

Introduction

Fruit-bodies of the wood-rotting basidiomycete *Lentinus lepideus* (Fr.: Fr.) Fr. produce a characteristic anise-like odour. In the past, a number of cinnamic acid derivatives have been isolated from natural material and from cultivated strains [1, 2]. Recently, several sesquiterpene hydrocarbons and alcohols have been identified in liquid cultures of *L. lepideus* FPRL 7B [3, 4]. Most of these constituents possess a 1,7-dimethyl-4-isopropyldecaline skeleton (cadinanes and related compounds). Sesquiterpene accumulation was stimulated in particular, when certain amino acids (isoleucine, phenylalanine, methionine) were offered as the sole nitrogen source in the culture medium [5]. From cultures of strain *L. lepideus* FPRL 7B grown on glucose-isoleucine-mineral salt medium, we have now isolated three novel muurolene ethers. As a minor compound, the acyclic sesquiterpene alcohol terrestrol (= 2,3-dihydro-6-[*E*]-farnesol) was identified.

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Material and Methods

Lentinus lepideus FPRL 7B (= ATCC 56985) was isolated in 1956 by the Forest Products Research Laboratory, Risborough (GB).

After mycelium inoculation, the basidiomycete was cultivated on a defined synthetic liquid culture medium containing glucose (2%), isoleucine (0.15%), and mineral salts [6]. Volatiles were determined after 15 weeks from 40 cultures (grown in 250 ml Erlenmeyer flasks containing 50 ml of culture broth) by circulation steam distillation [7]. The crude extract was further separated on a Si-60 column with a *n*-hexane/ethyl acetate gradient (changing from pure hexane to 19:1). If necessary, the collected fractions were further purified by preparative TLC using dichloromethane/acetone 19:1.

GLC analyses of the total distillate and of individual fractions 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, 200 °C; detector,

210 °C; carrier gas, N_2 at 1 ml/min; injection volume 1.0 µl. Sniffing GLC analysis of the total distillate was performed using a 60 m DB-1 silica capillary column, a linear temperature program (60–250 °C; 3 °C/min), and He (2 bar) as carrier gas.

MS analyses were carried out on a Hitachi-Perkin-Elmer RMU D 6 mass spectrometer (70 eV) coupled with a Perkin-Elmer F 21 fractometer using a glass capillary polypropylene glycol 5100 column (50 m; 0.25 mm i.d.) and a linear temperature program 60(80)–180 °C; 1.25 °C/min. High resolution mass spectrometry (7500) was performed on a Varian MAT MS 311 A instrument (70 eV) with PFK as a reference substance.

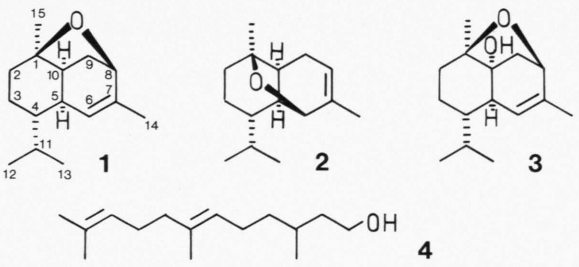
^{1}H NMR spectra were recorded at 400 MHz on a Bruker WM 400 spectrometer and the ^{13}C NMR spectra at 75.5 MHz on a Bruker AM 300 instrument. If not stated otherwise, $CDCl_3$ was used as solvent and TMS as an internal standard. Particulars of NOE and 2 D-^{1}H/^{1}H- and ^{13}C/^{1}H-chemical shift correlation experiments are described in [8]. Optical rotation was measured on a Perkin-Elmer PE 241 polarimeter.

Quantities of the newly identified metabolites were calculated gaschromatographically *via* internal standards (6-methyl-5-hepten-2-one, 1-octen-3-ol) using FID-specific substance factors.

Results and Discussion

The odour of cultures of *Lentinus lepideus* (Fr.: Fr.) Fr. has been described as "pleasant" or "like 10% anisaldehyde in proof spirit" [9]. Previously, we have shown that the production of volatiles by this fungus and the resulting flavour impression depend on the composition of the culture medium. Especially the nitrogen source influenced the formation of volatile metabolites distinctly [5].

Strain *L. lepideus* FPRL 7B was cultivated on a defined synthetic liquid glucose-isoleucine-mineral salt medium. 15-Weeks-old cultures produced a pleasant odour with a "sweet, woody note". The distillate of these cultures consisted almost exclusively of sesquiterpenes and of 2-methylbutan-1-ol [3, 4, 10]. After further separation of the total distillate by column chromatography, as major component a mixture of two sesquiterpene ethers was found which was very difficult to separate. Instead of separating the compounds in sufficient amount, we used a 3:1 mixture of **1** and **2** for two-dimensional ^{1}H/^{1}H- and



^{13}C/^{1}H-chemical shift correlation. This ratio between these two compounds proofed to be sufficient for the identification of the two sets of ^{13}C NMR data. The coupling between 5-H and 10-H is 5 Hz in **1** and smaller than 1 Hz in **2** requiring a *cis*-fused decaline. Irradiation at 5-H showed a NOE at 12-/13-H which is only possible in a *trans*-arrangement between 4-H and 5-H, thus the skeleton of both compounds is that of a muurolene. Compound **1** differs from compound **2** only in the positions of the double bond and the allylic ether bridge. The NMR data are listed in Tables I and II, the NOE experiments in Table III. We have named these new natural products lentideusether (**1**) ((1R*, 4S*, 5R*, 8R*, 10S*)-1,7-dimethyl-4-isopropyl-11-oxa-tricyclo-6.2.1.$0^{5,10}$-undecene-6 = 1,8-epoxymuurolene) and isolentideusether (**2**) (= 1,6-epoxymuurolene).

The mass spectrum of lentideusether (Fig. 1) shows a parent peak at *m/z* 220 (30%). High resolution mass spectra gave for M^+ 220.1843 (220.1827 calculated for $C_{15}H_{24}O$). The fragmentation pattern of **1** and **2** is very similar: GLC/MS (**1**): *m/z* (%) 220 (30), 205 ($M^+-CH_3\cdot$; 48), 202 (M^+-H_2O; 18), 177 ($M^+-C_3H_7\cdot$; 16), 159 ($M^+-C_3H_7\cdot-H_2O$; 100), 131 (38), 119 (28), 105 (40), 93 (63), 43 (60). GLC/MS (**2**): *m/z* (%) 220 (28), 205 (29), 202 (32), 177 (35), 159 (100), 131 (65), 105 (55), 93 (60), 91 (68), 43 (82). The quota of these compounds amounts to more than 50%, and 13- to 15-weeks-old cultures produce more than 100 mg/l culture medium of the mixture. The odour impression of these metabolites – determined by sniffing-capillary GLC – can be described as "warm, woody, reminding of cedar wood oil".

The mass spectrum of a third muurolene ether (**3**) with an additional hydroxy group (Fig. 2) shows a parent peak at *m/z* 236 (36%). Other prominent fragments are *m/z* 175 (40), 133 (17), 109 (100), 108 (74),

Table I. ^{13}C NMR data of lentideusether (**1**), isolentideusether (**2**), and terrestrol (**4**).

	1	Correlated with	**2**	Correlated with	**4**
C-1	76.4 0[a]	–	84.0 0	–	26.9 –
C-2	30.9 –	1.67 and 1.56	37.0 –	1.49 and 1.45	40.1 –
C-3	19.7 –	1.61 and 1.40	22.0 –	1.81 and 1.45	135.0 0
C-4	45.4 +	1.11	44.5 +	1.37	124.7 +[b]
C-5	39.3 +	2.60	46.3 +	2.27	25.5 –
C-6	127.6 +	4.96	81.9 +	3.74	37.3 –
C-7	140.4 0	–	142.7 0	–	29.4 +
C-8	76.4 +	4.04	119.9 +	5.11	39.8 –
C-9	35.7 –	1.71 and 2.33	29.4 –	2.24 and 2.42	61.3 –
C-10	38.9 +	2.00	40.8 +	1.94	124.5 +[b]
C-11	25.9 +	1.72	29.5 +	1.65	131.2 0
C-12	21.1 +	0.94	21.9 +	0.95	25.7 +
C-13	21.1 +	0.93	20.7 +	0.93	17.7 +
C-14	21.0 +	1.72	21.4 +	1.74	16.0 +
C-15	29.7 +	1.18	22.9 +	1.27	19.7 +

[a] Amplitude of signals in DEPT-135 spectrum (CH_3 or CH = +; CH_2 = –; quat. C = 0.
[b] Assignments may be interchanged (assigned by comparison with ^{13}C NMR data of citronellol [11] and *trans*-nerolidol [12, 13]).

Table II: ^1H NMR chemical shifts and coupling constants of lentideusether (**1**), isolentideusether (**2**), and 10-hydroxy-lentideusether (**3**); in $CDCl_3$.

	1	**2**	**3**
2-H	m 1.67	m 1.49	m 1.7
2′-H	m 1.56	m 1.45	to
3-H	m 1.61	m 1.81	m 1.4
3′-H	m 1.40	m 1.45	
4-H	m 1.11	m 1.37	m 1.2
5-H	m 2.60	d(br) 2.27	dqdd 2.82
6-H	ddq 4.96	d 3.74	dqd 5.13
8-H	dd 4.04	m 5.11	dd 3.98
9α-H	d 1.71	dddq 2.24	d 1.84
9β-H	ddd 2.33	ddq 2.42	dd 2.32
10-H	dd 2.00	dd 1.94	–
11-H	m 1.72	mm 1.65	dqq 1.95
12-H	d 0.94	d 0.95	d 1.00
13-H	d 0.93	d 0.93	d 0.91
14-H	dd 1.72	m 1.74	dd 1.75
15-H	s 1.18	s 1.27	s 1.19

J [Hz]: **1**: 5, 6 = 1.3; 5, 10 = 5.0; 5, 14 = 2.3; 6, 8α = 1.5; 6, 14 = 1.5; 8α, 9β = 5.5; 9α, 9β = 10.8; 9β, 10 = 5.0; 11, 12 = 6.5; 11, 13 = 6.5.
2: 4, 5 = 3; 5, 6 > 0 < 1; 5, 15 > 0; 6, 8 > 0; 6, 10 = 1.5; 8, 9α = 3.2; 8, 9β = 3.5; 8, 10 > 0; 9α, 9β = 18.5; 9α, 10 = 5; 9α, 14 = 2.7; 9β, 14 = 2; 11, 12 = 6.5; 11, 13 = 6.5.
3: 3α, 5 = 1.5; 4, 5 = 1.5; 4, 11 = 10.5; 5, 6 = 3; 5, 14 = 2; 6, 8 = 1.8; 6, 14 = 2; 8, 9β = 5.7; 9α, 9β = 10; 11, 12 = 6.4; 11, 13 = 6.4.

Table III. Results of NOE experiments on lentideusether (**1**) and isolentideusether (**2**).

Compound	Resonance irradiated	Resonances enhanced
1	5-H	10-H, 6-H, 12- or 13-H
	12- and 13-H	5-H
	15-H	9β-H, 10-H, 2-H
2	6-H	5-H, 4-H
	12- and 13-H	5-H
	15-H	9β-H, 10-H

105 (18), 58 (19), 43 (83). HR-MS spectra gave for M^+ 236.1743 (236.1776 calculated for $C_{15}H_{24}O_2$).

The ^1H NMR spectrum of **3** is very similar to that one of **1**. Instead of the threefold doublet of 9β-H of **1**, the 9β-H of **3** displayed only a double doublet. This observation together with the deshielding of 5-H, 6-H and 12-H points to an α-oriented 10-hydroxy group, thus this compound was identified as 10-hydroxy-lentideusether (= 10-hydroxy-1,8-epoxy-muurolene). Determination of the optical rotation of the colourless crystals (m.p. 115 °C) gave [α(c = 0.3, $CHCl_3$) = +25.3 °C (589 nm), +27.3 °C (578 nm), +30.7 °C (546 nm), +49.7 °C (436 nm), +70.7 °C (365 nm)].

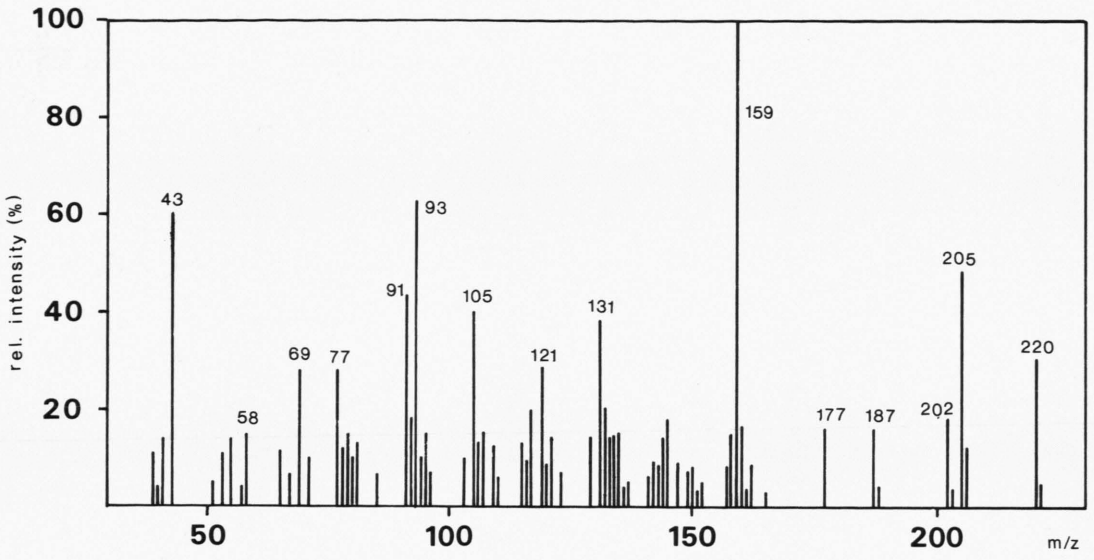


Fig. 1. Mass spectrum of lentideusether (= 1,8-epoxymuurolene).

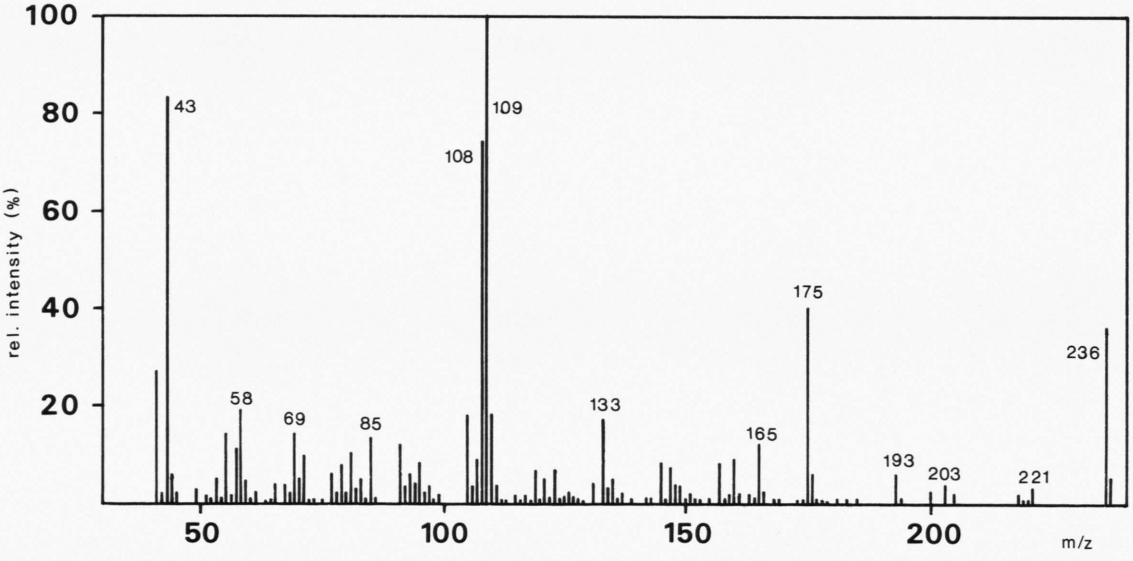


Fig. 2. Mass spectrum of 10-hydroxy-lentideusether (= 10-hydroxy-1,8-epoxymuurolene).

So far, ethers with a muurolane skeleton have been found in brown algae [14] and tentatively in cultures of the basidiomycete *Poria xantha* [15]. The isolated compound, however, is described as a 1,7-epoxymuurolane differing from the lentideusethers also in the lack of the double bond.

Screening the individual fractions from column chromatography, we could also isolate a further sesquiterpene alcohol which could be identified especially by its ^{13}C NMR (Table II) and its MS data as terrestrol (**4**) (= 2,3-dihydro-6[*E*]-farnesol). This compound – isolated for the first time as a marking

substance of male bumble bees [16] – has already been found in liquid cultures of yeasts [17], in *Ceratocystis coerulescens* (Ascomycotina) [18], and cultures of the basidiomycete *Poria xantha* [15].

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[1] W. B. Turner, Fungal Metabolites, Academic Press, London 1971.
[2] W. B. Turner and D. C. Aldridge, Fungal Metabolites II, Academic Press, London 1983.
[3] H.-P. Hanssen, Phytochemistry **21,** 1159 (1981).
[4] H.-P. Hanssen, Phytochemistry **24,** 1293 (1985).
[5] H.-P. Hanssen and E. Sprecher, in: Flavour '81 (P. Schreier, ed.), p. 547, Walter de Gruyter, Berlin, New York 1981.
[6] E. Sprecher, Planta **53,** 565 (1959).
[7] E. Sprecher, Dtsch. Apoth. Ztg. **103,** 213 (1963).
[8] W.-R. Abraham, L. Ernst, L. Witte, H.-P. Hanssen, and E. Sprecher, Tetrahedron **42,** 4475 (1986).
[9] E. C. Badcock, Trans. Br. Mycol. Soc. **23,** 188 (1939).
[10] H.-P. Hanssen and W.-R. Abraham, in: Proc. 4th European Congress on Biotechnology (O. M. Neijssel, R. R. van der Meer, and K. Ch. A. M. Luyben, eds.), p. 291, Elsevier, Amsterdam 1987.
[11] F. Bohlmann, R. Zeisberg, and E. Klein, Org. Magn. Resonance **7,** 426 (1975).
[12] H.-P. Hanssen and W.-R. Abraham, Z. Naturforsch. **41c,** 959 (1986).
[13] W.-R. Abraham, unpublished data.
[14] V. Amico, G. Oriente, M. Piattelli, C. Tringali, E. Fattorusso, S. Magno, and L. Mayol, Experientia **35,** 450 (1979).
[15] U. Krahn, Ph.D. thesis, Technical University of Berlin (1986).
[16] S. Ställberg-Stenhagen, Acta Chem. Scand. **24,** 358 (1970).
[17] H.-P. Hanssen, E. Sprecher, and A. Klingenberg, in: Progress in Essential Oil Research (H. Brunke, ed.), p. 395, Walter de Gruyter, Berlin, New York 1986.
[18] E. Sprecher, K.-H. Kubeczka, and M. Ratschko, Arch. Pharm. **308,** 843 (1975).