

Characterization of the Hydroxy Fatty Acid Content of *Basidiomycotina*

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ABSTRACT. Fatty acids (FA) of nine fungal species belonging to the subphylum *Basidiomycotina* were identified by using capillary GC–MS, MS and HPLC. The identified fatty acids included 45 saturated (iso-, anteiso-, and 19 hydroxy acids) and 42 monoenoic acids (including 14 hydroxy acids); dienes and polyenes were represented by 13 fatty acids. The proportion of hydroxy acids in the total fatty acids in the fungal species ranged from 4.3 to 10.2 %. Very long-chain fatty acids (C₂₄–C₃₀) were also determined. Four fatty acids 16:0 (8.8–14.3 %), 18:1(11) (3.9–14.9 %), 18:1(9) (7.7–19.0 %) and 18:2(6) (7.6–19.4 %), were found as major acids. Of the identified acids, 17 were detected in *Basidiomycotina* for the first time.

Studies of lipids and fatty acids (FA) of fungi have recently attracted considerable attention (Kock and Botha 1998; Lösel 1988). Therefore, we decided to study a relatively less frequently investigated group of organisms, viz. fungi of the *Basidiomycetes* class.

Fatty acids produced by fungi usually contain 14–20 carbon atoms (Cosovic and Prostenik 1981; Dembitsky and Pechenkina 1991; Dembitsky *et al.* 1992, 1993a–d; Řezanka *et al.* 1987b, 1990). Saturated straight-chain fatty acids and branched and unsaturated acids with carbon chains longer than 20 are less frequent (Muller *et al.* 1994a,b). There have been numerous studies of the fatty acid composition of fungi and many included the use of fatty acids in taxonomic and phylogenetic investigations (Tyrrel 1971; Jabaji-Hare 1988; Kerwin *et al.* 1995; Martinez *et al.* 1991; Řezanka and Dembitsky 1999).

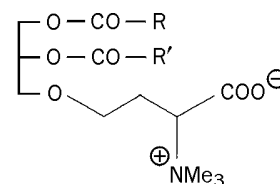
Hydroxy fatty acids make up an interesting group of natural compounds widespread among fungi. They were identified in many species of fungi (Mantle *et al.* 1969; Morris 1966, 1967) with the maximum amount of 35.5 % total acids observed in *Claviceps purpurea* (Morris 1966).

Dembitsky *et al.* (1993) had studied nine fungal species belonging to the *Ascomycetes* and *Basidiomycetes* for their fatty acid composition, and some novel unusual hydroxy fatty acids were identified, including four homologues, three minor and one major acid, from C₁₈ to C₂₄. As a rule, the major hydroxy acids are 2-hydroxy acids and, in particular, 2-hydroxystearic acid which constitutes over 30 % of total fatty acids in a number of fungal species. Dihydroxy acids also occur in ceramides and sphingolipids of various fungal species (Kock and Botha 1998).

The sphingolipids are found to contain straight-chain hydroxy acids with C₁₅–C₂₅ (Cosovic and Prostenik 1981; Prostenik *et al.* 1978; Shibata *et al.* 1964). Long-chain 2,3-dihydroxy fatty acids C₂₂–C₂₆ have been found in ceramides of various fungi (Ahlquist and Pascher 1984). The variation in the number of free hydroxyl groups in these compounds arises from the combination of normal 2-hydroxy and 2,3-dihydroxy fatty acids with dihydroxy and trihydroxy long-chain bases (Ahlquist and Pascher 1984).

Previous data indicated a great diversity of fatty acids contained in fungi, which may be used as a source of unusual lipid components and for chemotaxonomical purposes (Dembitsky *et al.* 1993a; Vaskovsky *et al.* 1991; Yamada and Nozawa 1979), including betaine ether-linked glycerolipid diacylglycerol-4'-O-(N,N,N-trimethyl)homoserine (DGTS) (Dembitsky 1996; Vaskovsky *et al.* 1991; Yamada and Nozawa 1979).

On the basis of our previous studies of fatty acids in fungi of the genus *Claviceps*, subphylum *Ascomycotina* (Dembitsky *et al.* 1993b; Křen *et al.* 1985), we began to study other groups of organisms, viz. fungi of the subphylum *Basidiomycotina*. The present work is a more detailed report on the fatty acid content of nine different *Basidiomycotina*.



DGTS

MATERIAL AND METHODS

Sampling and lipid analyses. The fungal species to be studied were sampled in the nature reserve near Lake Baikal (Bolshye Koty, Western Siberia, Russia) and Kuzovatovo (Ulyanovsk region, Volga river basin, Russia) in August–September 1992. Freshly collected species were thoroughly cleansed of extraneous matter and only clean fungal tissues were used for homogenization in a high-speed unit. The fungi were extracted within 4 h after harvest. Lipids were extracted in a three-stage process and the extracts were combined as described previously (Dembitsky *et al.* 1993a,b). The lipid extracts were submitted to triple washing in cooled 0.9 % KCl.

Preparation of fatty acids. Methyl esters of the corresponding fatty acids were prepared by alkaline hydrolysis and reaction of free acids with BF_3 –methanol (Řezanka *et al.* 1990). The oxazolines and/or their trimethylsilyl (TMS) ethers were prepared by a modification of the method reported by Tulloch (1985) and Yu *et al.* (1988): 5 mg dicyclohexylcarbodiimide was added to a solution of 5 mg of fatty acids in 1 mL CHCl_3 . After stirring (10 min), 5 mg 2-amino-2-methylpropanol was added (20 °C, 4 h). The evaporated mixture was dissolved in 1 mL diethyl ether and treated with 0.5 mL SOCl_2 (20 °C, 1 h), washed in ice-cold water and eluted through a column with anhydrous sodium sulfate and silica gel (1:1 V/V). The eluate was evaporated, dissolved in pyridine, and heated with trimethylchlorosilane (50 °C, 4 h). Total hydroxy acid methyl esters were separated on a semipreparative column (RP-C18, 250 × 10 mm, 5 µm particle size) by elution with a gradient of MeOH–tetrahydrofuran from 3:2 to 2:3 over 25 min (flow 0.9 mL/min). Methyl esters of the corresponding non-hydroxy fatty acids were separated by HPLC on a silica gel column (250 × 10 mm, 5 µm particle size) with hexane–tetrahydrofuran (4:1, V/V). After elution of non-hydroxy-methyl esters, the hydroxymethyl esters were collected and analyzed by GC–MS as the corresponding picolinyl–TMS esters (Dembitsky *et al.* 1993a–d).

Gas chromatographic–mass spectrometric analyses of fatty acids. GC–MS of the corresponding methyl ester mixture was done using a Finnigan 1020 B single-state quadrupole GC–MS instrument in the electron ionization mode. The temperature program was as follows: 100 °C for 1 min with a subsequent increase to 185 °C at a gradient of 20 K/min and to 330 °C at 2 K/min, the last temperature being maintained for 1 min. A fused silica capillary column (60 m × 0.32 mm, ID/SPB-1 Supelco), using splitless injection and hydrogen as the carrier gas (a linear velocity of 600 mm/s) was used. The temperature program was 100–320 °C, at a gradient of 4 K/min, ionization energy was 11 eV (70 eV), and electron multiplier voltage was 2.5 kV. All spectra were scanned within the range of m/z 50–600.

Trimethylsilyl ethers of oxazolines were identified under conditions similar to those of the methyl ester identification, the only difference being the column temperature programmed from 150 to 300 °C at a gradient of 5 K/min (Dembitsky *et al.* 1993c,d).

Picolinyl esters–TMS ethers of hydroxy acids were separated on a fused silica capillary column (60 m × 0.32 mm i.d.) coated with a 0.25 mm layer of SPB-1 (Supelco, Bellefonte, USA) using splitless injection and He as carrier gas.

Identification of mass spectra was done according to Řezanka *et al.* (1990) and confirmed with the help of a library (Wiley Library, 6th ed.). Methyl esters were identified and quantified (by total ion current). The presence of the hydroxy group in position 2 in all the hydroxy fatty acids was established by means of MS fragmentation (the ion $M^+ - 59$, i.e. $M^+ - \text{MeOCO}$ is base peak) and using the library.

RESULTS AND DISCUSSION

A total of 100 fatty acids were identified in the total lipid extracts of nine *Basidiomycetes*. The distribution of hydroxy acids was determined in nine fungal species (Tables I and II). Saturated hydroxy acids were found to range from 2.4 to 5.8 % of total hydroxy fatty acids, including iso- and anteiso-acids (Tables I and V).

Saturated non-hydroxy fatty acids were present in all examined species as $\text{C}_{12:0}$ – $\text{C}_{28:0}$, in good agreement with our earlier results (Dembitsky *et al.* 1992, 1993a–d), where 16:0, 18:0, and 20:0 are almost always reported as major FA (Table III).

Monoenoic, similarly to the saturated ones, were found to include iso-, anteiso-, and hydroxy acids (Tables II and IV). The content of hydroxy acids in monoenoic compounds was lower than in saturated ones: their total amount ranged from 1.4 to 4.4 % of total hydroxy acids (Table V). Monoenoic compounds included very long-chain fatty acids C_{24} – C_{30} . The 24:1 acid was present as four isomers, with a hydroxy group present in two isomers. Acid 26:1 was represented by 3 isomers, and a hydroxy group was present in one of them. The longest acid, among both saturated and monoenoic compounds, was the 30:1 acid. The hydroxy

group was established to be located in position 2 in all the hydroxy acids. The structure of nine main hydroxy fatty acids is shown in Fig. 1.

Table I. Saturated hydroxy fatty acids of nine *Basidiomycotina*^a (% of total hydroxy acids)

Fatty acid	<i>S.h.</i>	<i>C.v.</i>	<i>L.s.</i>	<i>M.g.</i>	<i>G.l.</i>	<i>P.b.</i>	<i>L.p.</i>	<i>M.e.</i>	<i>S.l.</i>
OH-14:0	6.57	16.0	3.43	8.91	12.1	4.69	9.72	5.02	10.8
OH-15:0	7.42	1.41	0.81	0.29	1.99	0.67	0.47	1.52	2.61
OH-16:0	24.8	35.7	26.9	23.3	31.5	37.7	29.0	24.9	26.3
OH-19:0	0.85	0.89	3.43	3.62	0.18	2.69	2.51	0.91	1.74
OH-20:0	1.48	0	0	0	1.00	0	0	0.61	0
OH-21:0 ^b	0.64	0	0	0.29	1.63	0	0.47	0.15	0
OH-22:0 ^b	1.27	0	0	0.39	3.44	0	0.63	0.15	0.70
OH-23:0 ^b	2.12	0	0.00	0	1.81	0	0	1.37	0
OH-24:0 ^b	1.91	1.17	2.63	1.18	1.91	2.02	2.04	0	0
i-OH-12:0	0	1.88	0.20	3.33	1.27	0.17	2.41	5.78	2.78
i-OH-14:0	0	0.94	0.81	0.59	0.72	0.17	0.63	8.81	2.96
i-OH-16:0	8.90	0	0	0	0.18	0	0	0.15	0
i-OH-20:0	1.91	1.88	4.46	3.92	1.63	3.70	3.13	2.13	1.22
i-OH-21:0 ^b	1.91	0	0	0	1.09	0	0	0.61	0
i-OH-22:0 ^b	0	0	0	0.10	2.54	0	0.16	0.46	0
ai-OH-13:0 ^b	0	0	0	0.69	0	0	0.94	1.82	0.70
ai-OH-15:0	0.42	0.94	0	0.88	0.72	0	1.10	3.95	5.22
ai-OH-17:0	3.00	5.40	7.27	7.34	4.17	5.56	5.64	1.98	4.09
ai-OH-19:0 ^b	0	0	1.41	1.86	0	2.01	1.41	0.15	2.61
Saturated	63.2	66.2	51.3	56.7	67.9	59.4	60.3	60.5	61.7
<i>n</i> -OH	47.1	55.1	37.2	38.0	55.6	47.8	44.8	34.6	42.1
<i>iso</i> -OH	12.72	4.70	5.47	7.94	7.43	4.04	6.33	17.94	6.96
<i>anteiso</i> -OH	3.42	6.34	8.68	10.8	4.89	7.57	9.09	7.90	12.6

^a*S.h.* *Stereum hirsutum* (Polyporaceae)

C.v. *Coriolus versicolor* (Polyporaceae)

L.s. *Lachnea scutellata* (Pezizaceae)

M.g. *Mycena galericulata* (Tricholomataceae)

G.l. *Ganoderma lueidum* (Ganodermataceae)

P.b. *Piptoporus betulinus* (Polyporaceae)

L.p. *Lycoperdon perlatum* (Lycoperdaceae)

M.e. *Morchella esculenta* (Morchellaceae)

S.l. *Suillus luteus* (Boletaceae)

^bFatty acids detected in *Basidiomycotina* for the first time.

Table II. Monoenoic hydroxy fatty acids of nine *Basidiomycotina*^a (% of total hydroxy acids)

Fatty acid	<i>S.h.</i>	<i>C.v.</i>	<i>L.s.</i>	<i>M.g.</i>	<i>G.l.</i>	<i>P.b.</i>	<i>L.p.</i>	<i>M.e.</i>	<i>S.l.</i>
OH-15:1	2.91	6.57	6.67	9.01	5.07	0.05	7.21	1.82	4.54
OH-16:1(9)	24.79	18.84	29.70	13.32	13.41	23.23	17.71	15.50	13.30
OH-17:1(8)	1.06	1.41	2.63	4.90	1.99	2.02	2.88	3.80	6.09
OH-18:1(9)	0.85	0	0	0.20	3.62	0	0.16	0.46	0.17
OH-22:1(13) ^b	0	0	0	0.49	0	0	0.63	0	0.17
OH-24:1(15) ^b	0.21	0	0	0	2.17	0	0	1.52	1.22
OH-25:1(16) ^b	0	0	0	0.98	0	0	1.41	0.46	0.17
OH-26:1 ^b	0.42	0.23	0	0.20	0.54	1.35	0.63	0.15	0
OH-28:1 ^b	0.39	0	0	0.09	0.18	0.51	0.31	0	0
i-OH-16:1(9)	5.30	5.16	7.47	6.27	3.99	6.89	6.43	13.22	6.78
i-OH-22:1(13) ^b	0	0	1.01	2.74	0	0.67	0.80	1.06	2.09
i-OH-24:1(15) ^b	0	0	0.60	1.96	0	0.17	0.47	0	0
ai-OH-17:1(8) ^b	0.85	1.64	0	2.45	1.09	0	0.63	1.37	2.78
ai-OH-21:1(12) ^b	0	0	0.61	0.69	0	0.72	0.47	0.15	1.04
Monoenoic	36.78	33.85	48.69	43.30	32.06	35.61	39.74	39.51	38.35
<i>n</i> -OH	30.63	27.05	39.00	29.19	26.98	27.16	30.94	23.71	25.66
<i>iso</i> -OH	5.30	5.16	9.08	10.97	3.99	7.73	7.70	14.28	8.87
<i>anteiso</i> -OH	0.85	1.64	0.61	3.14	1.09	0.72	1.10	1.52	3.82

^{a,b}See footnote to Table I.

Table III. Saturated fatty acids of nine *Basidiomycotina*^a (% of total fatty acids)

Fatty acid	<i>S.h.</i>	<i>C.v.</i>	<i>L.s.</i>	<i>M.g.</i>	<i>G.l.</i>	<i>P.b.</i>	<i>L.p.</i>	<i>M.e.</i>	<i>S.l.</i>
12:0	0.29	0.83	0.41	0.31	1.06	1.00	0.55	0.48	0.31
13:0	0.03	0	0.03	0.07	0.10	0.03	0	0.11	0
14:0	1.34	1.68	1.49	1.34	1.62	1.77	1.72	1.97	2.30
15:0	0.22	0.10	0.17	0.27	0.20	0.16	0.22	0.25	0.31
16:0	12.35	11.34	14.29	8.79	9.54	11.17	10.59	10.99	13.36
17:0	1.00	0.47	0.68	0.55	1.27	0.59	0.64	0.87	1.09
18:0	2.97	3.56	1.19	2.70	1.99	2.65	1.44	3.22	2.20
19:0	0.51	0.55	0.85	0.65	0.69	0.80	0.46	1.13	0.92
20:0	1.49	1.25	1.84	1.40	1.16	2.90	1.81	2.08	2.68
21:0	0.55	1.24	2.19	1.77	1.41	1.29	1.66	2.43	1.77
22:0	0.67	0.91	0.30	0.55	0.57	0.80	0.24	0.77	0.48
23:0	0.07	0.22	0.10	0.07	0.10	0.13	0.03	0.04	0.00
24:0	0.51	0.33	0.44	0.45	0.51	0.45	0.61	0.49	0.61
25:0	0.11	0.07	0.10	0.07	0.10	0.06	0.09	0.07	0.03
26:0	0.11	0.14	0	0.07	0.10	0.03	0.02	0.07	0.02
28:0	0.07	0.11	0	0	0.03	0.03	0.06	0.07	0.04
i-12:0	0.41	0.33	0.13	0.21	0.21	0.31	0.34	0.21	0.14
i-13:0	0.15	0.10	0.10	0.17	0.10	0.14	0.06	0.11	0.06
i-14:0	0.67	0.68	0.71	0.31	0.61	0.50	0.53	0.84	0.41
i-15:0	0.03	0	0.17	0.07	0.03	0.13	0.09	0.11	0.27
i-16:0	0.45	0.61	0.65	0.62	0.51	0.59	0.65	0.88	0.99
i-17:0	0.26	0.14	0.30	0.27	0.44	0.50	0.21	0.33	0.55
ai-13:0	0.26	0.22	0.28	0.17	0.24	0.20	0.24	0.22	0.27
ai-15:0	0.85	0.61	0.65	0.55	0.48	0.41	0.46	0.51	0.58
ai-17:0	0.63	0.44	0.58	0.44	0.51	0.59	0.58	0.92	0.96
ai-19:0	0.07	0.04	0.10	0.07	0.10	0.14	0.06	0.07	0.10
Saturated	26.07	25.97	27.75	21.94	23.68	27.37	23.36	29.24	30.45

^aSee footnote to Table I.

Oligoenoic fatty acids were found in all the nine studied species. Their proportion in total acids ranged from 13.4 % in *Lycoperdon perlatum* to 32.4 % in *Coriolus versicolor* (Table V). Several other hydroxy acids were found in fungi, *e.g.*, 17-OH-16:0, 17-OH-18:0 and 12-OH-18:1 (Shibata *et al.* 1964). The cited papers report only hydroxypalmitic and hydroxystearic acids, while our own list of identified acids contain also saturated acids OH-12:0–OH-24:0. Such a great diversity of hydroxy acids has never been reported before.

Previously, we have found very long-chain fatty acids (C₂₄–C₃₀) in 15 fungal species belonging to *Basidiomycetes* (Řezanka and Mareš 1987; Řezanka *et al.* 1987a). The fatty acids indicated above were found as minor components, except for the 24:0 acid, ranging from 0.6 to 5.1 % and the 17–26:1 acid (0–3.6 %).

According to Lösel (1988), *Coriolus versicolor* contains one major fatty acid, 18:2, which corresponds very well with our results (see Table V); 21.9 % of oleic acid was found in *Piptoporus betulinus*, this value again corresponding well with our data (17.1 %; Table IV). Also the genus *Suillus* was found to contain 12.0 % of major fatty acid (palmitic), which is in keeping with our data (Table III). *Mycena polygramma* was found to contain similar hydroxy acids, *e.g.*, 2-OH-ai-19:0, 2-OH-i-20:0, but also unsaturated 2-OH-25:1(16), 2-OH-i-24:1(15) a 2-OH-ai-21:1(12) (Lösel 1988).

All the above listed fatty acids have already been found in living organisms but 17 hydroxy fatty acids were identified for the first time in *Basidiomycotina*.

Thus, this analysis of some higher fungi showed that their fatty acids are dominated by saturated fatty acids with 2-hydroxy groups. Branched iso- and anteiso-hydroxy acids are present in small amounts.

In general, the fatty acids of the lipids of the *Basidiomycotina* are not good taxonomic markers because of the great similarity in fatty acid composition of members of the various taxa. Some species, however, are characterized by some very specific acids, as documented in studies describing the composition of hydroxy fatty acids from different species of *Basidiomycotina* (Kock and Botha 1998; Lösel 1988).

Table IV. Monoenoic fatty acids of nine *Basidiomycotina*^a (% of total fatty acids)

Fatty acid	<i>S.h.</i>	<i>C.v.</i>	<i>L.s.</i>	<i>M.g.</i>	<i>G.l.</i>	<i>P.b.</i>	<i>L.p.</i>	<i>M.e.</i>	<i>S.l.</i>
12:1(5)	0.11	0.14	0.07	0.09	0.17	0.25	0.09	0.18	0.21
13:1(5)	0.18	0.22	0.10	0.27	0.34	0.10	0.15	0.33	0.10
14:1(5)	0.33	0.18	0.16	0.24	0.14	0.20	0.22	0.07	0.25
15:1(9)	0.19	0.08	0.13	0.10	0.18	0.26	0.26	0.19	0.21
15:1(7)	1.25	1.49	1.53	1.67	1.81	1.97	1.41	1.75	1.95
16:1(9)	2.52	3.27	3.36	2.68	2.95	2.37	2.73	2.45	2.68
16:1(7)	1.67	0.91	1.84	1.14	2.04	1.63	1.13	1.43	1.09
17:1(9)	0.45	1.16	0.78	0.85	0.79	1.76	0.95	1.28	1.05
17:1(7)	0.26	0.14	0.14	0	0.07	0.14	0.09	0.21	0.06
i-17:1(9)	0.37	0.61	0.66	0.62	0.55	0.52	0.52	0.73	0.72
ai-17:1(9)	0.33	0.10	0.17	0.04	0	0.66	0.36	0.14	0.11
18:1(11)	4.83	7.27	8.35	13.30	11.02	9.87	9.21	14.92	10.91
18:1(9)	15.52	16.39	18.54	18.98	15.65	17.06	15.35	7.71	16.41
18:1(7)	1.15	0.77	1.08	0.89	1.41	1.32	1.13	0.55	1.47
19:1(8)	0.22	0	0.24	0.13	0.20	0.28	0.27	0.25	0.31
20:1(11)	2.16	2.57	3.02	2.67	4.06	0.87	0.80	1.76	1.99
20:1(9)	1.08	1.49	0.81	0.72	0.93	1.16	1.39	1.77	0.92
21:1(8)	0	0	0	0	0	0	0	0	0
22:1(11)	0.74	0.91	0.47	0.58	0.65	0.41	0.61	0.95	0.99
22:1(9)	1.52	1.58	0.91	1.38	1.06	1.00	1.32	1.28	1.65
24:1(11)	0.45	0.29	0.51	0.50	0.61	0.69	0.43	0.70	0.72
24:1(9)	0.71	0.65	0.71	0.79	0.86	0.66	0.53	0.49	0.49
25:1(9)	0	0	0.06	0.03	0.06	0	0.02	0.03	0.06
26:1(11)	0.03	0.04	0.10	0.07	0.10	0	0.03	0.11	0.07
26:1(9)	0.55	0.73	0.41	0.79	0.61	0.42	0.27	0.29	0.33
27:1	0.04	0.07	0.04	0.04	0	0.00	0.06	0.07	0
28:1	0.06	0.26	0	0.06	0.13	0.16	0.01	0.04	0.08
30:1	0.11	0.04	0	0.03	0	0.06	0.09	0.07	0.03
Monoenoic	36.83	41.36	44.19	48.66	46.39	43.82	39.43	39.75	44.86

^aSee footnote to Table I.**Table V.** Di-, tri-, and tetraenoic fatty acids and total non-hydroxy and hydroxy fatty acids of nine *Basidiomycotina*^a (% of total fatty acids)

Fatty acid	<i>S.h.</i>	<i>C.v.</i>	<i>L.s.</i>	<i>M.g.</i>	<i>G.l.</i>	<i>P.b.</i>	<i>L.p.</i>	<i>M.e.</i>	<i>S.l.</i>
14:2(5)	0.15	0.26	1.22	0.21	0.10	0.16	0.02	0.15	0.04
16:2(4)	0.67	2.21	8.05	0.92	0.72	1.05	1.11	1.24	0.65
18:2(5)	18.96	17.63	0.06	11.03	10.30	9.36	1.94	12.61	7.60
20:2(6)	0.41	0.33	0	0.07	0.27	0.17	0.27	0.18	0.14
22:2(6)	0.03	0.09	0	0	0.05	0.02	0.03	0.04	0
16:3(6)	0	0.04	3.47	0	0.03	0.07	0.02	0.03	0.06
18:3(6)	2.49	2.84	8.79	1.68	2.51	4.42	2.02	5.82	3.44
18:3(3)	8.68	4.03	0	3.42	9.16	8.15	6.93	3.62	6.21
20:3(9)	0.22	0.04	0	0.17	0.10	0	0.24	0.11	0.22
20:3(6)	0.11	0.10	0.24	0	0.16	0.10	0.04	0.06	0.05
20:3(3)	0.18	0.08	1.26	0.07	0.12	0.04	0.06	0	0.06
18:4(3)	0.45	0.76	0.01	0.62	0.86	0.32	0.66	0.53	0.40
20:4(6)	0.03	0	23.13	0	0.04	0.01	0.01	0.04	0.07
Di- and oligoenoic	32.38	28.41	46.23	18.19	24.42	23.87	13.35	24.43	18.94
Non OH-FA	95.28	95.74	95.05	89.79	94.78	94.06	93.62	93.42	94.25
OH-FA	4.72	4.26	4.95	10.21	5.52	5.94	6.38	6.58	5.75
Saturated	2.98	2.82	2.54	5.79	3.75	3.53	3.84	3.98	3.55
Monoenoic	1.74	1.44	2.41	4.42	1.77	2.41	2.54	2.60	2.30

^aSee footnote to Table I.

In evolutionarily lower fungi, polyenoic fatty acids are the major ones (Lösel 1988), while the acids detected in higher fungi were found to include saturated, and also branched and hydroxy fatty acids. Higher fungi were also found to completely lack, or to contain a maximum of 0.1 %, C₂₀ oligoenoic fatty acids, while the content of the same fatty acids in lower fungi is several tenths of a percent. The higher fungi were

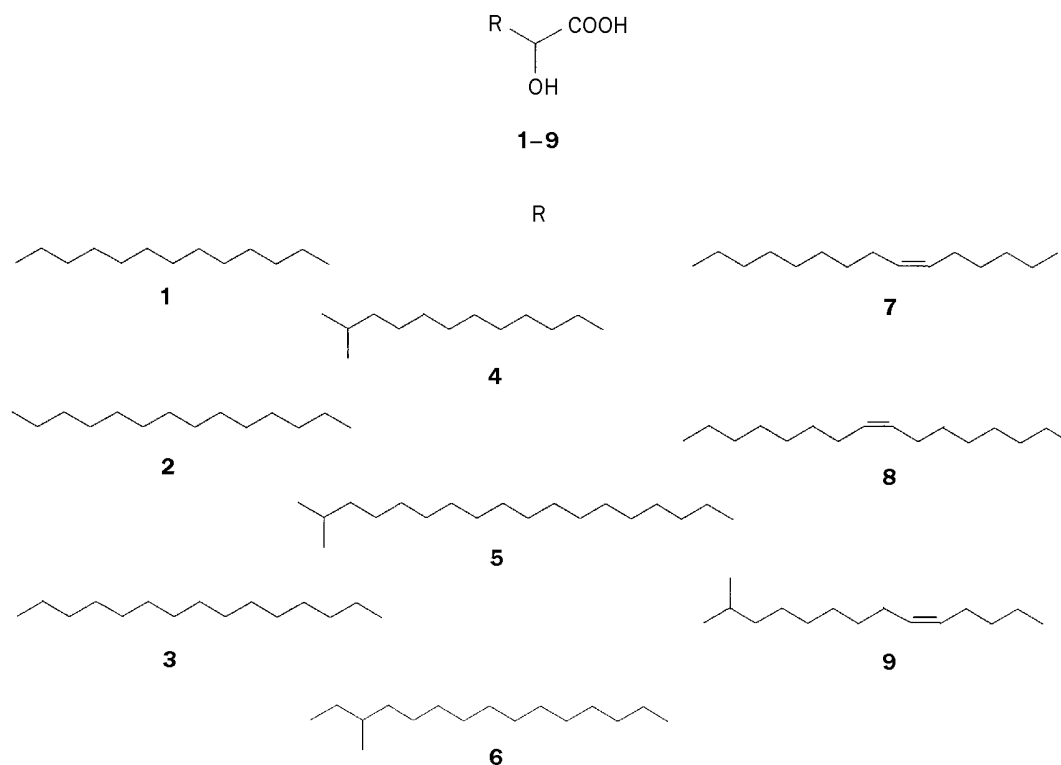


Fig. 1. Nine main hydroxy fatty acids found in high fungal species; saturated: **1** – OH-14:0; **2** – OH-15:0; **3** – OH-16:0; branched: **4** – iso-OH-14:0; **5** – iso-OH-20:0; **6** – anteiso-OH-17:0; monoenic: **7** – OH-16:1(9), **8** – OH-17:1(8) and **9** – iso-OH-17:1 (9).

found to contain very long-chain fatty acids (*i.e.* fatty acids with more than 24 carbon atoms) and also branched fatty acids. Another group of fatty acids comprises hydroxy fatty acids, which were never found in lower fungi with the exception of yeast. Their presence in higher fungi was not documented, probably owing to the difficult process of detection, not to their absence in higher fungi.

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