

Chemical constituents of some higher fungi

I. Fatty acid and Phospholipid Compositions of Basidiomycetes

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SUMMARY

The 8 fungal species belonging to the class Basidiomycetes were examined for their FAs and PLs; 80 FAs were identified using capillary column and GC-MS. The identified FAs included 43 saturated (iso-, anteiso-, and hydroxyacids), 34 monoenoic (iso-, anteiso- and hydroxyacids); dienes and trienes were represented by 3 acids only. Hydroxyacids varied in the fungal species from 9.14 to 11.76%. PC and PE were identified as the major PL classes.

Introduction

The distinctive Kingdom of Fungi attracts the attention of chemists due to a great diversity of unusual FAs [1, 5, 20, 24] and lipids. The data accumulated in the studies on FAs from fungi and yeasts are reported in a number of reviews [3, 4, 11, 14, 23, 27, 29]. Diacylglycerotrimethylhomoserine was detected in some species [2, 25, 28, 29, 30]. Of interest are phytofluorols, novel lipids from *Phytophthora coctorum* [19], which are saturated and unsaturated alcohols having an odd number of carbons and 2 hydroxy groups forming 1,2-diols in each. Novel glycolipids were described from the 'unmoving' cells of rust fungi [13], which were 'cellobiose' lipids having 15,16-dihydroxy-, or 2', 15.16-trihydroxy decanoic acids linked via a glycoside bond with cellobiose units in positions 2' and 6. Recently, [21] have identified FAs from the Basidiomycetes with chains up to 30 carbons long. The fungal species *Enthomophthorales* and Basidiomycetes were found to possess polyunsaturated FAs C20, 16:3,

and 18:4 detected in Ascomycetes and Zycomycetes fungi [4, 12, 15]. Unusual 9,10-cis-epoxyocta-decanoic acid at 40% was found in *Cronartium immitis*, as well as in spores of rust fungi [4]. Previous data indicated a great diversity of lipids contained in fungi which may be used as sources of unusual lipid components.

Experiments

The fungal species to be studied were sampled in the nature reserve "Samarskaya Luka" near Togliatti in August 1990. Freshly collected species were thoroughly cleansed of extraneous matter and only clean fungal tissues were used for homogenization in a high-speed unit. Lipids were extracted in three stages and the extracts were combined as described previously [7, 10]. The lipid extracts were submitted to triple washing in cooled 0.9% KCl. Total lipids and PLs were determined as described elsewhere [8, 9].

Methyl esters of corresponding FAs were prepared by alkaline hydrolysis and reaction of free acids with BF_3 -methanol [22]. The oxazolines and/or their trimethylsilyl (TMS) ethers were prepared by modification of the reference methods [26, 32]: 5 mg dicyclohexylcarbodiimide was added to a solution of 5 mg FAs in 1 ml CH_2Cl_2 . After stirring (10 min) 5 mg 2-amino-2-methylpropanol was added (20 deg. C, 4 hr). The evaporated mixture was dissolved in 1 ml diethylether and treated with 0.5 ml SOCl_2 (20 deg. C, 1 hr), washed in ice cold water and eluted through a column with anhydrous sodium sulfate and silica gel. The eluate was evaporated, dissolved in pyridine, and heated with trimethylsilylchloride (50 deg. C, 4 hr). Methyl esters were identified in a Shimadzu QP-1000 apparatus with a fused silica capillary column (60 m $\times$ 0.32 mm ID/SPB-1; Supelco, USA), using splitless injection and helium as the carrier gas. Oven temperature was programmed from 100 to 320 deg. C, at the rate 4 deg. C/min. Ionization energy was 70 eV, and electron multiplier voltage was 2.5 kV. TMS-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 deg. C at the rate 5 deg. C/min.

Abbreviations

PL, phospholipid; FA, fatty acid; GC-MS, combined gas chromatographymass spectrometry; PC, phosphatidylcholine; PE, phosphatidylethanolamine; LPC, liso-PC; LPE, liso-PE; PI, phosphatidylinositol; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; TL, total lipids, mg/g dry wt.

Results and Discussion

A tentative description of fungal PLs and FAs is given in our previous work [9, 10]. The present work is a more detailed report of the fungal fatty acid analysis by GC-MS.

The above methods have allowed the identification of eighty FAs from the TL extracts of fungi. The distribution of saturated FAs has been examined in 8 fungal species (Table 1). The wide range of saturated FAs is very interesting. Normal FAs have been found to vary from 28.79 to 34.96%; iso-acids, from 4.93 to 6.85%; anteiso-acids have been detected in small amounts from 2.66 to 3.87%. Of interest are the presence and distribution of hydroxy acids, which are identified as iso-, anteiso-, and normal FAs with their totals among saturated acids varying from 5.71 to 7.65% (Table 1). Hydroxy acids often occur within the Kingdom of Fungi; they were identified in many species of sclerotial fungi [16–18] with the maximum amount observed in *Claviceps purpurea* (Rumania) at 35.5% [18]. For addition, 9,10-dihydroxyoctadecanoic acid was identified [17], this acid being detected in *Claviceps sulcata* at 63.6%.

The sphingolipids are found to contain straight chain hydroxyacids with C15–C25 [5, 20, 24]. Long-chain 2,3-di-OH FAs with C22–C26 have been found in ceramides of various fungi [1]. The variation in the number of free

hydroxyl groups in these compounds arises from the combination of normal 2-OH- and 2,3-di-OH-FAs with di-OH- and tri-OH long-chain bases [1]. Several other hydroxy FAs were found in fungi, for example, 17-HO-16:0, 17-HO-18:0 and 12-HO-18:1 [30]. The cited works report only hydroxy palmitic and stearic acids, while our own list of identified acids is extended with saturated acids 12:O-OH – 24:O-OH. Such a great diversity of hydroxy acids is rarely, if for the first time, reported; considering the presence of methyl groups in iso- and anteiso-positions of certain hydroxy acids, the examined fungal species can be suggested as sources of the unusual FAs indicated above.

Monoenoic fatty acids, similarly to saturated, have been found to contain iso-, anteiso-, and hydroxy acids (Table 2). The content of hydroxy acids in monoenoic compounds is lower than in saturated: their total amount varies from 3.5 to 4.11%. Monoenoic compounds included very long-chain FAs C24–C28. Acid 24:1 has been found to possess 4 isomers with a hydroxy group present in 2 isomers. Acid 26:1 is represented by 3 isomers, and, again, a hydroxy group is present in 2 isomers (Table 2). The longest acid, both among saturated and monoenoic compounds, is acid C28. This is true especially for the branched monoenoic HO-FA, e.g. iso-HO-9-16:1, anteiso-HO-17:1 etc.

The hydroxy group is in position 2 in all the acids. The chromatographic behaviour of these compounds is also very interesting; ECL of anteiso-HO-8-17:1 is greater than that of 17:0, whereas the behaviour of the couple anteiso-HO-12-21:1/22:0 is contrary to this.

Only 3 acids having more than 1 double bond have been found (Table 3). Most interesting is the change of the ratio of α -18:3 to γ -18:3 FAs and, also, the presence of hydroxyacids.

The PL composition from 8 fungal species is shown in Table 4. Main PL classes in all the examined species were PE and PC. PS was also detected at 4.8 to 21.4% in all the species. PI was present in 5 of the 8 species studied with the highest level in *C. comatus*. PG and DPG were found in 3 and 6 species, respectively. Liso-PE and liso-PC were detected in small amounts. In addition, the chromatograms showed the presence of unidentified lipids which did not contain phosphorus. These compounds are suggested to be diacyltrimethylhomoserines found in higher fungi [28], although the identification has not been attempted.

Thus, the analysis of the higher fungi has shown, that their FAs are dominated by saturated FAs with 2-hydroxy acids varying from 5.71 to 7.65%. The identified branched iso- and anteiso-acids containing a hydroxy group are present in small amounts. The sum of dienoic and trienoic acids varies from 11.34 to 15.09%. PLs are represented by 2 major classes, PE and PC, and several other PLs.

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Table 1. The saturated fatty acids.

Class	Basidiomycetes							
Order	Agaricales							
Family	Agaricaceae		Tricholomataceae			Coprinaceae		Russulaceae
Genera	Agaricus		Clitocybe	Laccaria	Marasmius	Coprinus		Lactarius
Species	arvensis	silvaticus	inversa	amethystina	oreades	atramentarius	comatus	flexuosus
n-12:0	1.41	1.58	2.05	1.60	2.19	1.35	1.25	1.75
iso-12:0	0.25	0.34	0.55	0.66	0.82	0.45	0.70	0.80
iso-12:0-OH	0.25	0.19	0.11	0.39	0.05	0.05	0.15	0.15
n-13:0	0.76	0.91	0.72	0.88	0.66	0.95	0.50	0.85
anteiso-13:0-OH	—	0.05	—	0.17	—	0.10	0.05	0.10
n-14:0	3.78	3.83	4.71	4.37	3.83	4.75	4.00	3.26
iso-14:0	0.96	0.86	1.39	1.05	0.93	1.15	0.85	1.20
iso-14:0-OH	0.15	0.05	0.06	0.11	0.11	0.15	0.10	0.15
n-14:0-OH	0.15	0.24	—	0.06	0.16	0.20	0.05	0.10
n-15:0	2.22	2.25	2.16	2.82	2.30	2.35	2.23	2.26
iso-15:0	1.11	1.39	1.39	1.55	0.93	1.30	1.00	1.55
anteiso-15:0	1.71	1.72	1.99	1.88	1.26	1.90	1.35	1.90
anteiso-15:0-OH	0.15	0.10	0.11	—	0.05	0.05	0.10	0.05
n-15:0-OH	0.35	0.14	0.17	0.28	0.22	0.10	0.15	0.45
n-16:0	9.82	9.63	11.74	10.47	10.67	12.26	11.72	9.85
iso-16:0	2.01	1.87	2.66	2.76	2.40	2.35	2.70	2.41
iso-16:0-OH	0.10	0.14	0.22	0.17	0.16	0.15	0.10	0.25
n-16:0-OH	0.65	0.77	0.99	0.77	0.38	0.50	0.60	0.60
n-17:0	1.81	1.82	2.22	2.32	1.91	2.05	2.10	1.70
iso-17:0	0.60	0.53	0.72	0.83	0.93	0.95	0.50	0.70
anteiso-17:0	0.70	0.81	1.22	1.27	1.26	1.00	1.05	1.25
anteiso-17:0-OH	0.10	0.05	0.11	0.17	—	0.05	0.10	0.15
n-18:0	1.96	1.96	1.94	2.32	2.62	2.25	2.70	2.36
n-19:0	0.30	0.38	0.50	0.28	0.55	0.40	0.80	0.15
anteiso-19:0	0.25	0.19	0.66	0.06	0.66	0.35	0.65	0.10
anteiso-19:0-OH	0.10	0.05	0.11	0.17	0.05	0.05	0.10	—
n-19:0-OH	0.10	0.24	0.28	0.17	0.11	0.10	0.15	0.20
n-20:0	0.86	1.20	1.33	0.94	1.31	0.50	1.20	0.95
iso-20:0-OH	0.10	0.05	0.17	0.22	0.05	0.10	—	0.10
n-20:0-OH	0.70	0.57	1.00	0.88	0.66	0.95	0.65	0.70
n-21:0	0.50	0.29	0.39	0.28	0.71	0.45	0.75	0.60
iso-21:0-OH	0.30	0.19	0.28	0.33	0.27	0.35	0.35	0.25
n-21:0-OH	0.30	0.34	0.33	0.44	0.38	0.55	0.25	0.25
n-22:0	2.52	1.39	2.16	1.93	2.95	3.15	3.05	2.01
iso-22:0-OH	0.25	0.14	0.33	0.22	0.22	0.30	0.20	0.10
n-22:0-OH	0.76	0.72	0.95	0.72	0.98	0.60	0.60	0.70
n-23:0	0.35	0.62	0.55	0.66	0.66	0.60	0.50	0.35
n-23:0-OH	0.40	0.34	0.44	0.28	0.38	0.35	0.25	0.30
n-24:0	1.86	2.11	2.27	2.76	2.51	2.35	3.20	2.66
n-24:0-OH	1.46	1.96	1.99	1.66	1.48	1.70	1.85	1.65
n-25:0	0.25	0.14	0.66	0.55	0.44	0.85	—	0.45
n-26:0	0.30	0.34	0.55	0.50	0.60	0.35	0.45	0.60
n-28:0	0.30	0.34	0.55	0.50	0.38	0.35	0.45	0.60
normal-acids	29.00	28.79	34.50	33.18	34.29	34.96	34.90	34.40
iso-acids	4.93	4.99	6.71	6.85	6.01	6.20	5.75	6.66
anteiso-acids	2.66	2.72	3.87	3.21	3.18	3.25	3.05	3.25
iso-acids-OH	1.15	0.76	1.17	1.44	0.86	1.10	0.90	1.00
anteiso-acids-OH	0.35	0.25	0.33	0.51	0.10	0.25	0.35	0.30
acids-OH	4.87	5.32	6.15	5.26	4.75	5.05	4.55	4.95
the sum HO-acids	6.37	6.33	7.65	7.21	5.71	6.40	5.80	6.25
Saturated	42.96	42.83	52.73	50.45	49.19	50.81	49.50	46.56

Table 2. The monoenes fatty acids.

Species	<i>A. arvensis</i>	<i>A. silvaticus</i>	<i>C. inversa</i>	<i>L. amethystina</i>	<i>M. oreades</i>	<i>C. atramentarius</i>	<i>C. comatus</i>	<i>L. flexuosus</i>
12:1 (n-7)	0.50	0.24	0.28	0.33	0.55	0.35	0.40	0.35
13:1 (n-8)	0.10	0.05	0.11	0.17	0.27	0.05	0.10	0.25
anteiso-13:1 (n-8)	0.20	0.24	0.28	0.17	0.16	0.10	0.15	0.10
14:1 (n-9)	1.21	1.25	1.22	1.27	1.15	1.25	1.35	1.25
15:1 (n-8)	1.51	1.96	2.33	2.05	1.69	2.05	1.75	2.11
15:1-OH	0.10	0.10	0.22	0.06	0.11	0.05	0.10	0.10
16:1 (n-9)	10.05	9.91	5.43	6.30	5.30	5.09	5.24	5.26
iso-16:1 (n-9)-OH	0.30	0.38	0.11	0.17	0.11	0.25	0.10	0.25
16:1 (n-9)-OH	—	0.19	0.06	0.11	0.05	0.05	0.05	0.15
17:1 (n-8)	0.96	1.63	0.94	1.87	1.40	1.20	1.40	1.41
iso-17:1 (n-8)	0.35	0.48	0.50	0.55	0.55	0.35	0.45	0.60
anteiso-17:1 (n-8)	0.45	0.43	0.61	0.77	0.38	0.40	0.30	0.35
anteiso-17:1 (n-8)-OH	0.10	—	0.17	0.06	0.05	0.10	0.10	0.05
17:1 (n-8)-OH	0.10	0.05	0.06	0.06	0.05	0.10	0.05	0.10
17:1 (n-10)	1.21	1.34	1.77	1.33	0.98	1.45	1.00	1.25
18:1 (n-9)	15.24	15.02	9.91	11.03	11.12	15.36	15.02	13.77
18:1 (n-9)-OH	0.45	0.38	0.22	0.33	0.22	0.35	0.30	0.35
18:1 (n-11)	0.81	1.10	0.28	1.11	1.80	0.70	0.70	0.95
19:1 (n-10)	0.35	0.24	—	0.06	0.44	0.95	0.60	0.20
20:1 (n-9)	0.86	0.85	0.66	2.27	1.15	0.80	1.60	1.30
20:1 (n-11)	0.25	0.35	0.39	—	0.44	0.60	—	0.30
anteiso-21:1 (n-12)-OH ^a	0.45	0.38	0.28	0.39	0.22	0.40	0.30	0.30
22:1 (n-13)	0.91	0.48	1.61	1.44	1.64	0.35	1.75	1.85
iso-22:1 (n-13)-OH ^b	0.45	0.29	0.66	0.22	0.38	0.30	0.45	0.40
22:1 (n-13)-OH	0.86	0.72	0.83	0.94	0.66	0.65	0.95	0.80
24:1 (n-13)	1.21	1.96	3.49	1.77	2.02	1.90	2.10	1.80
24:1 (n-15)	0.60	0.72	0.05	0.50	1.04	0.35	0.90	0.90
iso-24:1 (n-15)-OH ^b	0.35	0.19	0.33	0.22	0.27	0.25	0.40	0.25
24:1 (n-15)-OH	0.30	0.43	0.55	0.44	0.49	0.35	0.30	0.35
25:1 (n-16)-OH	0.25	0.10	0.22	0.28	0.27	0.15	0.20	0.30
26:1 (n-17)	0.60	0.86	0.94	0.61	0.77	0.75	0.85	0.70
iso-26:1 (n-17)-OH ^b	0.05	0.10	0.17	0.11	0.11	0.05	0.10	—
26:1-OH	0.20	0.24	0.17	0.11	0.33	0.20	0.05	0.20
28:1-OH ^c	—	0.10	0.06	0.11	0.11	0.10	0.05	0.05
iso-monoenes	0.35	0.48	0.50	0.55	0.55	0.35	0.45	0.60
anteiso-monoenes	0.65	0.67	0.89	0.94	0.54	0.50	0.45	0.45
iso-monoenes-OH	1.15	0.96	1.27	0.72	0.87	0.85	1.05	0.90
anteiso-monoenes-OH	0.55	0.38	0.45	0.45	0.27	0.50	0.40	0.35
monoenes-OH	2.26	2.31	2.39	2.44	2.29	2.00	2.05	2.40
the sum HO-acids	3.96	3.65	4.11	3.61	3.43	3.35	3.50	3.65
Monoenes	41.33	42.76	34.91	37.21	36.28	37.40	39.16	38.35

^a The FA have equal ECL, and they can be determined after HPLC separation FA-OH from n-FA.^b FA with ECL lower than in the saturated straight chain FAs.^c The GC-MS method does not allow the determination of the position of C = C.

Table 3. The dienes and trienes fatty acids of fungi.

Species	<i>A. arvensis</i>	<i>A. silvaticus</i>	<i>C. inversa</i>	<i>L. amethystina</i>	<i>M. oreades</i>	<i>C. atramentarius</i>	<i>C. comatus</i>	<i>L. flexuosus</i>
18:2 (n-9)	5.29	4.98	5.82	7.08	6.72	7.69	7.39	6.82
18:3 (n-6)	0.55	0.57	0.28	2.27	2.62	2.90	2.70	3.36
18:3 (n-9)	9.87	8.86	6.26	2.99	5.19	1.20	1.25	4.91
The sum of dienes and trienes	15.71	14.41	12.36	12.34	14.53	11.79	11.34	15.09

Table 4. Phospholipid composition of some fungi.

Species	PE	LPE	PS	PC	LPC	PI	DPG	PG	TL	PI
<i>Agaricus arvensis</i>	39.0	1.7	16.0	35.6	—	7.0	0.3	0.4	17.0	22.1
<i>A. silvaticus</i>	20.7	—	17.7	28.0	1.3	16.0	7.0	9.3	14.0	19.2
<i>Clitocybe inversa</i>	32.2	—	11.7	43.7	1.0	7.5	—	—	40.0	34.6
<i>Coprinus atramentarius</i>	34.1	1.6	9.4	50.5	3.1	1.3	—	—	13.0	41.1
<i>C. comatus</i>	27.1	1.4	4.8	38.3	2.3	23.7	2.4	—	30.4	37.5
<i>Laccaria amethystina</i>	25.8	2.6	8.6	39.5	2.1	—	12.2	9.2	35.0	29.8
<i>Lactarius flexuosus</i>	22.7	—	21.4	49.5	4.8	—	1.6	—	40.0	19.4
<i>Marasmius oreades</i>	19.7	—	21.0	34.7	2.0	—	10.0	—	21.0	47.2

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