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Chemical composition of fatty acids from some fungi

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SUMMARY

The chemical composition of eight higher fungal species from the class Basidiomycetes was studied using GC and GC/MS methods. 79 FAs including 43 saturated, 33 monoenoic (iso-, anteiso-, hydroxy and very long-chain acids) as well as 3 dienoic/trienoic acids were identified.

Introduction

Our previous GC and GC/MS examinations of the higher fungal FA composition [6, 7] indicated a broad diversity of FAs contained in the studied species. Similar indications are found in the works by other authors [1–4, 8–12, 20]. The bulk of hydroxy acids were identified from the acids having 16 to 25 carbons mainly within sphingolipids, ceramides and glycoglycerolipids [4, 10, 11, 17]. A set of unusual di- and tetrahydroxy acids was detected in various higher fungi [1, 9] and lichens [14]. The results obtained by GC/MS methods of FAs from previously unexamined fungal species are described in the present study and are supposed to provide an additional insight into the diversity of FAs produced by fungi.

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 [5, 6]. The lipid extracts were submitted to triple washing in cooled 0.9% KCl.

Methyl esters of corresponding FAs were prepared by alkaline hydrolysis and reaction of free acids with BF_3-methanol [13]. The oxazolines and /or their trimethylsilyl (TMS) ethers were prepared by modification of the reference methods [16, 21]: 5 mg dicyclohexylcarboiimide 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 silicagel. 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 × 0.32 mm ID/SPB-1; Supelco, USA), using splitless injection and helium as the carrier gas. Oven temperature was programmed from 100

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Table 1. The saturated fatty acids.

Class	Basidiomycetes							
Orders	Aphylo-phorales	Agaricales					Tremellales	Ustilaginineae
Families	Clavariaceae	Strophariceae		Tricholomataceae		Pleurotus	Tremellaceae	Thelephoraceae
Genera Species	Ramaria formosa	Stropharia aeruginosa	Pholiota lenta	Mycena polygramma	Clitocibe sp.	Pleurotus sp.	Exidia glandulosa	Thelephora sp.
iso-12:0	0.12	0.09	0.17	0.14	0.19	0.12	0.35	0.10
n-12:0	0.53	0.43	0.25	0.32	0.46	0.27	1.62	0.84
n-13:0	0.37	0.24	0.25	0.27	0.31	0.23	0.73	0.34
iso-12:0-OH	–	–	0.04	–	0.04	–	–	–
iso-14:0	0.57	0.57	0.34	0.59	0.34	0.50	1.32	0.79
n-14:0	1.15	1.18	1.22	1.55	1.19	1.31	9.27	1.23
anteiso-13:0-OH	0.08	–	–	0.05	0.08	0.04	–	0.05
iso-15:0	0.12	0.14	0.17	0.09	0.19	0.08	2.06	0.10
anteiso-15:0	0.29	0.28	0.34	0.32	0.23	0.27	3.31	0.25
n-15:0	0.78	0.76	0.88	0.68	0.65	0.58	1.59	0.88
iso-14:0-OH	0.08	0.05	–	0.09	–	0.08	–	–
n-14:0-OH	0.20	0.19	0.08	0.14	0.04	0.12	–	0.15
iso-16:0	1.39	1.00	1.55	1.45	1.11	1.24	7.26	3.19
n-16:0	19.80	21.67	17.65	21.07	17.42	17.91	58.42	16.26
anteiso-15:0-OH	0.12	0.05	0.13	0.09	–	0.12	–	0.10
n-15:0-OH	0.25	0.19	0.25	0.41	0.08	0.19	–	0.44
iso-17:0	0.25	0.47	0.25	0.23	0.42	0.58	1.71	0.25
anteiso-17:0	0.82	0.81	0.84	0.59	0.73	0.81	2.65	0.64
n-17:0	0.78	0.62	0.80	1.09	0.57	0.66	0.47	1.18
iso-16:0-OH	0.20	0.14	0.21	0.09	0.04	0.08	–	0.10
n-16:0-OH	0.45	0.47	0.46	0.55	0.23	0.35	–	0.59
n-18:0	7.98	11.43	8.18	5.77	5.54	7.30	1.07	6.24
anteiso-17:0-OH	0.04	0.14	0.29	0.14	0.08	0.27	–	0.10
anteiso-19:0	0.08	0.14	0.21	0.14	–	0.08	–	0.05
n-19:0	0.37	0.43	0.50	0.41	0.08	0.31	0.53	0.34
n-20:0	1.51	1.18	1.30	1.14	0.42	1.31	1.62	2.06
anteiso-19:0-OH	0.04	0.09	0.21	0.09	0.11	0.08	–	0.20
n-19:0-OH	0.37	0.52	0.71	0.50	0.61	0.12	–	0.39
n-21:0	0.25	0.24	0.38	0.23	0.34	0.12	–	0.39
iso-20:0-OH	0.70	1.00	0.59	0.95	0.54	0.50	–	0.29
n-20:0-OH	0.57	1.14	0.76	1.09	0.65	0.35	–	0.44
n-22:0	1.31	1.04	1.30	1.00	0.96	0.62	0.45	1,43
iso-21:0-OH	0.04	0.09	0.13	0.09	0.27	0.15	–	0.15
n-21:0-OH	0.20	0.33	0.21	0.32	0.31	0.35	–	0.29
n-23:0	0.65	0.90	0.59	0.86	0.50	0.66	–	0.88
iso-22:0-OH	0.08	0.19	0.38	0.18	0.04	0.23	–	0.15
n-22:0-OH	1.06	0.85	1.34	0.82	1.26	1.58	–	1.43
n-24:0	1.19	1.14	0.71	1.09	1.68	1.20	–	1.72
n-23:0-OH	0.25	0.38	0.21	0.36	0.08	0.35	–	0.20
n-25:0	0.25	0.33	0.21	0.32	0.15	0.35	–	0.39
n-24:0-OH	1.76	1.47	1.59	1.42	1.19	1.12	–	1.43
n-26:0	–	0.09	0.21	0.09	0.15	0.15	–	–
n-28:0	0.25	0.19	0.13	0.18	0.23	0.08	–	0.10
normal-acids	37.17	41.87	34.56	36.07	30.65	33.06	75.77	34.28
iso-acids	2.45	2.27	2.48	2.50	2.25	2.52	12.70	4.43
anteiso-acids	1.19	1.23	1.39	1.05	0.96	1.16	5.96	0.94
iso-acids-OH	1.10	1.47	1.35	1.40	0.93	1.04	–	0.69
anteiso-acids-OH	0.28	0.28	0.63	0.37	0.27	0.51	–	0.45
acids-OH	5.11	5.54	5.61	5.60	4.45	4.53	–	5.36
the sum HO-acids	6.49	7.29	7.59	7.37	5.65	6.08	–	6.50
Saturated	47.30	52.66	46.02	46.99	39.51	42.82	94.43	46.15

Table 2. The monoenes fatty acids.

Species	R. formosa	S. aeruginosa	P. lenta	M. polygramma	Cl. sp.	Pl. sp.	E. glandulosa	Th. sp.
12:1 (n–7)	0.12	0.14	0.21	0.05	0.23	0.04	–	0.10
anteiso-13:1 (n–8)	–	–	0.04	–	–	–	–	–
13:1 (n–8)	–	0.14	–	0.09	–	0.08	–	0.15
14:1 (n–9)	0.74	0.81	0.88	0.73	0.73	0.62	0.85	0.69
15:1 (n–8)	0.57	0.57	0.55	0.77	0.42	0.66	–	0.59
16:1 (n–9)	12.27	7.78	9.27	18.08	15.20	15.36	1.47	16.16
15:1-OH	0.16	0.14	0.17	0.23	0.11	0.08	–	0.25
iso-17:1 (n–8)	–	0.14	–	0.05	0.23	0.15	–	0.05
anteiso-17:1 (n–8)	0.12	0.28	0.13	0.18	0.31	0.42	–	0.20
17:1 (n–8)	0.20	0.24	0.21	0.18	0.46	0.35	–	0.20
17:1 (n–10)	0.45	0.38	0.46	0.55	1.19	0.97	–	0.59
iso-16:1 (n–9)-OH	0.04	–	0.04	–	–	–	–	–
16:1 (n–9)-OH	0.04	0.14	0.04	0.09	0.08	0.04	–	0.10
18:1 (n–9)	11.62	13.94	11.90	7.40	14.67	12.63	0.89	8.00
18:1 (n–11)	1.27	1.85	1.30	0.50	2.06	1.00	2.36	0.54
anteiso-17:1 (n–8)-OH	–	–	0.04	–	0.04	0.15	–	–
17:1 (n–8)-OH	–	0.05	0.13	0.05	0.08	0.08	–	0.05
19:1 (n–10)	0.29	0.28	0.38	0.27	–	0.19	–	0.10
18:1 (n–9)-OH	0.20	0.14	–	0.14	0.11	0.46	–	0.15
20:1 (n–9)	0.94	0.28	0.84	0.27	0.19	0.27	–	1.03
20:1 (n–11)	0.70	0.28	2.06	0.27	2.14	0.70	–	0.93
22:1 (n–13)	2.29	1.75	2.64	1.68	1.83	3.51	–	1.82
anteiso-21:1 (n–12)-OH	–	0.05	–	0.05	–	–	–	–
iso-22:1 (n–13)-OH	0.08	0.09	–	0.09	–	0.04	–	–
22:1 (n–13)-OH	0.20	0.57	0.55	0.55	–	0.54	–	–
24:1 (n–13)	0.78	1.28	0.50	1.23	0.42	0.39	–	1.87
24:1 (n–15)	0.74	1.61	1.72	1.55	1.19	1.08	–	1.28
iso-24:1 (n–15)-OH	0.08	–	–	–	0.08	0.15	–	–
24:1 (n–15)-OH	0.20	0.57	0.29	0.55	0.11	0.23	–	0.34
26:1 (n–17)	0.08	0.14	0.71	0.14	0.27	0.19	–	0.05
25:1 (n–16)-OH	0.04	–	–	–	–	–	–	–
26:1-OH	0.04	0.05	0.04	0.05	0.04	–	–	–
28:1-OH	0.04	–	–	–	–	–	–	–
iso-monoenes	–	0.14	–	0.05	0.23	0.23	–	0.05
anteiso-monoenes	0.12	0.28	0.17	0.18	0.31	0.42	–	0.20
iso-monoenes-OH	0.20	0.09	0.04	0.09	–	0.19	–	–
anteiso-monoenes-OH	–	0.05	0.04	0.05	0.04	0.15	–	–
monoenes-OH	0.92	1.66	1.22	1.66	0.53	1.43	–	0.89
the sum HO-acids	1.12	1.80	1.30	1.80	0.57	1.77	–	0.89
Monoenes	33.06	33.69	35.06	35.79	42.11	40.38	5.57	34.70

Table 3. The dienes and trienes fatty acids.

Species	R. formosa	S. aeruginosa	P. lenta	M. polygramma	Cl. sp.	Pl. sp.	E. glandulosa	Th. sp.
18:2 (n–9)	10.31	6.02	10.57	11.04	10.35	9.50	–	11.93
18:3 (n–6)	0.16	0.24	0.17	0.36	2.14	1.08	–	0.39
18:3 (n–9)	7.93	7.39	8.14	5.82	5.89	6.22	–	6.29
The sum of dienes and trienes	18.40	13.65	18.88	17.22	18.38	16.80	–	18.61

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

FA, fatty acid(s); GC, gas chromatography; GC/MS, combined gas chromatography-mass spectrometry. The fatty acids in the text and tables are designated by the number of carbon atoms and the number of double bonds respectively.

Results and discussion

Saturated FAs are the main constituents among the detected FAs, their content varies from 39.51 to 52.66% (Table 1). The only exception is Exidia glandulosa 94.43% saturated FAS with palmitic acid at 58.42%. Iso-acids are known to be invariably present in higher fungi. According to our results, they make up 2.27 to 4.43% in the majority of the studied species but 3 times as high in E. glandulosa. Hydroxy acids detected in 7 species are absent from E. glandulosa. All the detected hydroxy acids place the hydroxy group in position 2, including the present authors' studies [1, 4, 6, 7, 10]. However, certain fungal species possess hydroxy acids with hydroxy groups in different positions, as follows: 9, 10-di-hydroxy- [9] and 9, 10, 12, 13-tetrahydroxy stearic acid in lichen [14]. Very long-chain FAs C25–C28 have been identified in 7 species. Their amounts do not exceed 0.5%.

Second to saturated acids in quantity are 33 monoenoic FAs varying from 33.06 to 42.11% except E. glandulosa where monoenes make up 5.57%. Iso-, anteiso- and hydroxy acids are less numerous among monoenoic compounds than among saturated ones (Tables 2). Some authors report ten percent hydroxy acids [18–20] from sphingolipids and ceramides of higher fungi. Monoenoic compounds have also been found to contain very long-chain FAs C25–C28 having a hydroxy group. The unusual character of FA composition in Exidia glandulosa may be attributed to the wood substrate on which the species grow.

Polyenes are represented only by 3 acids: 18:2 (n–9), 18:3 (n–6) and 18:3 (n–9) (Table 3). The latter acids have not been detected in E. glandulosa. Solberg [15] and other workers [18, 19] report the presence of keto-FAs which have been discovered from hydrolisates of 2 fungal species – Lactarius rufus and L. chrysorrheus. In our study these acids have not been detected.

Thus, our analysis of FAs using TMS-oxasolines of FAs and GC/MS methods has shown that higher fungi contain a broad diversity of iso-, anteiso-, hydroxy and very long-chain FAs some of which are new species.

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References

1 Ahlquist, L. and Pascher, I. (1984): On the stereochemistry of 2,3-dihydroxy fatty acids of fungi sphingolipids. Resolution and configuration analysis erythro-2,3-dihydroxyoctadecanoic acid. Chem. Phys. Lipids *35*, 209–215.

2 Chopra, A. and Khuller, G. K. (1983): Lipid of patogenic fungi. Progress in Lipid Res. *22*, 161–184.

3 Chopra, A. and Khuller, G. K. (1985): Lipid metabolism in fungi. CRC, Crit. Rev. Microbiol. *11*, 209–271.

4 Cosovic, C. and Prostenik, M. (1981): Lipids of higher fungi. VI. Long-chain erythro-2,3-dihydroxy fatty acids as structural moieties of ceramides from Lactarius vellereus. Periodicum biologorum *83*, 239–244.

5 Dembitsky, V. M., Rozentsvet, O. A. and Pechenkina, E. E. (1990): Glycolipids, phospholipids and fatty acids of some brown algae species from the Black sea. Phytochemistry *29*, 3417–3421.

6 Dembitsky, V. M., Shubina, E. E. and Kashin, A. G. (1992): Phospholipid and fatty acid composition of some fungi (Class Basidiomycetes). Phytochemistry *31*, in press.

7 Dembitsky, V. M., Rezanka, T. and Shubina, E. E. (1992): Chemical constituents of some fungi. J. Hattori Bot. Lab., in press.

8 Mantle, P. G., Morris, L. J. and Hall, S. W. (1969): Fatty acid composition of sphacelial and sclerotial growth forms of Claviceps purpurea in relation to production of ergoline alkaloids in culture. Trans Br. Mycol. Soc. *53*, 441–450.

9 Morris, L. J. (1967): Fatty acid composition of Claviceps species occurrence of (+)-threo-9,10-dihydroxystearic acid. Lipids *3*, 260–263.

10 Prostenik, M., Burcar, I., Castek, A., Cosovic, C., Golem, J., Jandric, Z., Kljaic, K. and Ondrusek, V. (1978): Lipids of higher fungi. III. The fatty acids and 2-hydroxy fatty acids in some species of Basidiomycetes. Chem. Phys. Lipids *22*, 97–103.

11 Prostenik, M. and Cosovic, C. (1981): Long-chain erythro-2,3-dihydroxy fatty acids as structural moieties of ceramides from Lactarius vellereus. Period. Biol. *83*, 239–244.

12 Rezanka, T. and Mures, P. (1987): Unusual and very long-chain fatty acids produced by Basidiomycetes. J. Chromatography *409*, 390–395.

13 Rezanka, T., Sokolov, M. Y. and Viden, I. (1990): Unusual and very long fatty acids in Desulfotomaculum, a sulfate reducing bacterium. FENS Microbiol. Ecol. *73*, 231–238.

14 Solberg, Y. (1960): Studies on the Chemistry of Lichens. 3. Long-chain tetrahydroxy fatty acids from some Norwegian lichens. Acta Chem. Scand. *14*, 2152–2160.

15 Solberg, Y. (1989): A literature review of the lipid constituents of higher fungi new investigations of Agaricales species. Int. J. Mycol. Lichenol. *4*, 137–154.

16 Tulloch, A. P. (1985): Mass spectra of pyrrolidides of oxo, hydroxy and trimethylsilyloxy ocyadecanoic acids. Lipids *20*, 652–663.

17 Tulloch, A. P. (1990): Glycosides of hydroxy fatty acids. In: Glycolipid, phosphoglycolipids, and sulfoglycolipids (M. Kates, Ed.) Plenum Publ. Co. pp. 463–487.

18 Turner, W. B. (1971): Fungal metabolites, pp. 254. Academic Press, London.

19 Turner, W. B. and Aldridge, D. C. (1983): Fungal metabolites II, pp. 306. Academic Press, London.

20 Wassef, M. K. (1977): Fungal lipids. Adv. Lipid Res. *15*, 159–232.

21 Yu, Q. T., Liu, B. N., Zhang, J. Y. and Hung, Z. H. (1988): Location of methyl branchings in fatty acids: Fatty acids in uropygial secretion of shanghai duck by GC–MS of 4,4-dimethyloxazoline serivatives. Lipids *23*, 804–810.

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