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Note

Unusual and very long-chain fatty acids produced by Basidiomycetes

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The study of the metabolism of lower organisms has recently attracted considerable attention. Therefore, we decided to study a relatively less frequently investigated group of organisms, fungi of the family Basidiomycetes, the group of wood-decaying fungi in particular.

Fatty acids produced by fungi usually contain 14–20 carbon atoms. Saturated straight-chain fatty acids and branched and unsaturated acids with carbon chains longer than 20 are less frequent[1]. In *Saccharomyces cerevisiae*, in addition to common C_{10}–C_{18} acids, very long fatty acids representing up to 2% of all fatty acids were described[2,3].

Fatty acids, primarily acids with even carbon numbers and chain lengths of up to C_{26}, were detected on the surface of conidia and sporangiospores of various species of the genera Alternaria, Botrytis and Neurospora[4], but very long fatty acids of up to C_{32} were only detected in *Fomes igniarius*[5].

On the basis of our previous studies of fatty acids in green algae[6], fungi of the genus Claviceps[7], class Ascomycetes, we began to study other groups of lower organisms, *viz.*, fungi of the class Basidiomycetes. According to literature data[3–5] we assumed that these fungi should also contain very long fatty acids, and this assumption was fully confirmed.

EXPERIMENTAL

Mature fruit bodies of common wood-decaying fungi supplemented by growing fungal species were all collected in dry weather about 30 km south of Prague, within the period November 1985–October 1986. They were identified taxonomically; the fruit bodies are kept in our Department.

About 100 g of fungi were extracted according to Bligh and Dyer[8]. Total lipids (1–2%) were hydrolysed and the unsaponifiable fraction was extracted three times with diethyl ether, the pH of the aqueous solution was adjusted to 1 and fatty acids (1/2–1/3 of total lipids) were extracted again three times with diethyl ether. Fatty acids were esterified with a mixture of 14% boron trifluoride in methanol and subjected to thin-layer chromatography (TLC) on silica gel H (Merck, Darmstadt, F.R.G.) using hexane–diethyl ether (80:20).

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The positions of the double bonds in the methyl esters were determined on the basis of splitting of pyrrolidides (pyrrolidides were prepared by boiling the methyl esters in pyrrolidide and acetic acid) in the mass spectrum[9]. Methyl esters of fatty acids with more than 21 carbon atoms were separated by high-performance liquid chromatography (HPLC)[10]. Individual methyl esters of monoenoic fatty acids were oxidatively degraded[11] and the monocarboxylic and dicarboxylic acids obtained were converted into methyl esters by reaction with diazomethane and identified by gas chromatography–mass spectrometry (GC–MS)[11,12].

GC–MS of the methyl esters was performed on a HP 5995 instrument (Hewlett-Packard, Avondale, PA, U.S.A.) with an HP-1 fused-silica capillary column (25 m) as described previously[11]. Methyl esters of mono- and dicarboxylic acids were analysed according to Řezanka and co-workers[11,12]. Pyrrolidides were separated on an HP-1 fused-silica capillary column (25 m) according to the literature[13].

HPLC was carried out on an SP 8000 apparatus (Spectra-Physics, U.S.A.) on a 50 cm × 6 mm I.D. column with RP-1 as described previously[10].

RESULTS AND DISCUSSION

Fatty acids produced by fungi have previously been analysed on packed columns, where the identification of 10–15 acids has already been achieved successfuly[14] and usually even lower numbers of fatty acids have been detected.

In order to demonstrate very long-chain fatty acids it is necessary to analyse the sample for a sufficiently long time period and to perform chromatography on a capillary column. The use of such a column as described previously[6,7] made it pos-

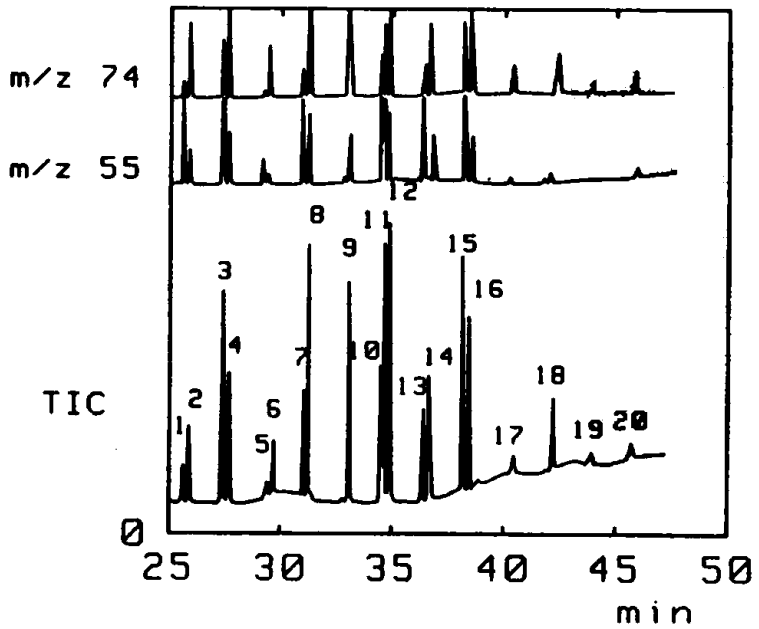

Fig. 1. GC–MS analysis of fatty acid methyl esters from *Trametes unicolor* (from 25 to 50 min; start after elution of dominant peaks, *e.g.*, C_{18} FAME). *m/z* 74: single ion monitoring (SIM) for *m/z* 74 = base peak for saturated fatty acid methyl esters. *m/z* 55: SIM for *m/z* 55 = base peak for monoenoic fatty acid methyl esters. TIC = total ion current. Peaks: 1 = 19:1; 2 = 19:0; 3 = 9–20:1; 4 = 20:0; 5 = 21:1; 6 = 21:0; 7 = 13–22:1; 8 = 22:0; 9 = 23:0; 10 = 13–24:1; 11 = 15–24:1; 12 = 24:0; 13 = 16–25:1; 14 = 25:0; 15 = 17–26:1; 16 = 26:0; 17 = 27:0; 18 = 28:0; 19 = 29:0; 20 = 30:0 FAME.

TABLE I

FATTY ACID COMPOSITION OF FUNGI (%)

i = isoacid; *ai* = anteisoacid; *br*, br_x = methyl groups on C_2 or C_x, respectively.

Fatty acid	*Armillaria mellea*	*Flammulina velutipes*	*Fomes fomentarius*	*Fomes igniarius*	*Fomitopsis pinicola*	*Ganoderma applanatum*	*Lactarius piperatus*	*Lactarius torminosus*	*Laetiporus sulphureus*	*Piptoporus betulinus*	*Pleurotus ostreatus*	*Russula xerampelina*	*Scleroderma citrinum*	*Stereum rugosum*	*Trametes unicolor*
i-12:0	1.30	0	0	0	0.94	0	0.07	0.41	0	0	0	0.47	0	0.56	0
12:1	0	0.24	0.11	0.11	0	0.37	0	0	0	0.34	0.28	0	0	0	0
12:0	1.36	0.24	0.11	0.11	0	2.82	0.09	6.33	1.62	0.34	0.28	0	0.58	1.04	0.61
ai-13:1	0	0.87	0	0.31	0	0	0	0	0	1.15	0	0	0	0	0.20
ai-13:0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13:0	0.27	0	0	0	0	0.14	0	0.74	0	0	0	0.29	0	0	0.04
br-14:0	0.31	0	0	0	0	0	0	0	0	0	0	0	0	0	0
i-14:0	3.25	0.51	0.20	0.11	0.46	0	0	0.32	0	0	0.20	0	0.27	0.75	0.04
9-14:1	2.06	0.16	0	0.42	0.08	0.18	0	0	0	0.40	0.13	0.85	0	0.41	0.04
14:0	2.07	1.86	1.31	1.62	1.16	3.92	0.72	1.42	0.30	1.28	0.83	0.60	0.78	1.27	0.52
i-15:0	1.83	0.10	0.41	1.07	0.51	0	0.06	0	0.37	0.55	0.31	0	0	0.48	0.14
ai-15:0	2.37	0.10	0.75	0.19	0.35	0.34	0.10	4.83	0.76	2.00	0.25	0.21	0.71	1.07	0.40
15:1	0	0.12	0	0	0	0.33	0	0.23	0	0	0	0.04	0	0	0
15:0	3.11	2.53	7.82	2.98	0.83	5.71	1.48	5.68	3.32	5.82	1.40	0.54	0.88	2.75	1.52
i-16:0	0.71	0.12	0	0	2.54	1.57	0	0.70	0	0.13	0.09	0	0	1.42	1.86
9-16:1	1.46	2.57	4.91	1.55	5.22*	2.56	0.44	1.17	2.41	9.18	1.64	1.04	8.50*	2.11	2.85
16:0	9.42	15.40	9.86	9.27	7.73	10.78	2.86	8.94	13.47	14.32	16.41	11.10	20.12	9.64	10.08
ai-17:1	0	0	0	0.31	0	0	0	0	0	0	0	0	0	0	0
i-17:0	0	0.18	0	0	0	0	0	0	0	0.15	0.09	0	0	0	0
ai-17:0	0.65	0.20	0	0	0	0	0	0.33	0	0.12	0.07	0	0	0	0
br_2-17:0	0	0	0	0	0	0	0	0	0	0	0.48	0	0	0	0
17:1	0.27	0.28	0.13	0.28	0	0	0.74	0	0	0	0	0	3.00	0	0
8-17:1	1.84	0	2.13	0.92	0.76	1.99	0	0.12	3.50	4.77	3.33	0.30	0.95	0.35	0.28
17:0	0.15	0.60	1.00	0.19	0	0.72	0.07	0.10	0.40	0.07	0	0	0	0.61	0.33
6,9,12-18:3	0	0.32	0	0	0	0	0	0	0	0.09	0	0	0	0	0
9,12,15-18:3	2.95	0.71	1.58	0.52	0.63	0.20	0.39	0.19	0.12	1.10	0.59	0.84	0.03	0.29	7.03
9,12-18:2	31.80	43.80	31.09	46.33	46.59	27.07	38.31	28.17	17.22	12.75	38.05	34.00	34.48	40.31	55.26
9-18:1	11.48	10.28	19.24	3.09	21.67*	9.82	26.34	8.68	34.85	7.97	15.86	38.46	21.47	24.18	9.27

18:0	1.23	5.63	0.16	0.30	0.42	0.25	22.29	15.64	0.09	0.18	7.37	2.41	0.95	2.62	0.75
i-19:0	0	0.24	0	2.19	0	0	0	0	0	0	0.09	0	0	0	0
ai-19:0	0	0.22	0	0.20	0	0	0	0	0	0	0.13	0	0	0	0
19:1	1.98	0	0.42	0.16	0.48	0.31	0.13	0.08	4.20	1.69	0	0.25	0.08	0.31	0.12
19:0	1.42	0.26	0.11	0.89	0.44	0.74	0.14	0.06	0.16	0.09	0.15	0.37	0.06	0.12	0.27
20:2	0	0.18	0	0	0	0	0	0	0	0.24	0	0	0	0	0
20:1	0	0.16	0.34	0	0	0	0	0	0	0	0.07	0	0	0	0
11-20:1	1.11	0.79	0	0.83	0.27	0.96	0.71	1.71	0	0	0.61	0.36	0	0	0
9-20:1	0.83	0	4.52	0	0.94	1.80	0	0	2.65	6.04	0.09	0.20	0.88	0.56	0.73
20:0	1.10	0.63	0.39	0.89	0.47	1.73	2.02	2.56	0.49	0.99	0.52	0.63	0.51	0.47	0.45
21:1	0.43	0.10	0	0	0	0.18	0	0	0	0.57	0	0	0	0	0
21:1	0.18	0.08	0.22	0	0	0.29	0	0	0	0	0.09	0	0	0.13	0.05
21:0	0.47	0.12	0.30	1.95	0.11	1.76	0.07	0.30	0.12	0.74	0.15	0.08	0.10	0.27	0.19
br$_x$-22:0	0	0	0	2.71	0	0	0	0	0	0	0	0.06	0	0	0
22:1	0.18	0.08	0	0	0	0	0	0	0	0	0.09	0.16	0	0	0
13-22:1	0.50	0.30	1.15	0.27	0.12	1.27	0	0.99	0.25	2.46	0.09	0.88	1.33	1.47	0.38
22:0	2.10	1.13	1.18	4.76	0.68	3.45	1.05	2.27	0.83	2.28	1.18	2.02	0.98	1.13	0.89
14-23:1	0.68	0.75	1.06	0.72	0.07	3.28	0	0.16	0.13	2.22	0.63	0.19	0	0.29	0
23:0	1.02	0.51	0.86	4.46	0.32	3.04	0.11	0.89	0.64	1.80	0.44	0.08	0.06	0.56	0.76
13-24:1	0.15	0.89	0	0	0.06	0	0	0	0	0	0	0.10	0	0.21	0.47
15-24:1	1.75	1.72	1.56	1.97	0.44	2.79	0.05	0.63	0.70	3.06	1.82	0.84	1.46	1.37	0.90
24:0	2.90	2.94	2.17	4.45	0.92	3.19	1.34	2.57	5.08	3.18	3.41	1.56	0.65	0.95	0.98
16-25:1	0.43	0.30	2.62	0.80	0.11	1.84	0	0	0.22	4.15	0.33	0	0	0	0.32
25:0	0.89	0.34	0.81	0.99	0.42	1.47	0.13	1.18	2.41	1.94	0.48	0.29	0.03	0.51	0.43
17-26:1	1.17*	0.47	1.34	0.94	0.11	2.08	0.04	0	1.33	3.61	0.96	0.31	0.37	0.48	0.81
26:0	0.54	0.22	0.25	1.03	0.18	0.61	0.11	0.42	1.55	0.72	0.88	0.17	0.75	0.92	0.62
18-27:1	0	0.28	0	0	0	0.25	0	0	0	0.75	0.09	0	0	0.06	0
27:0	0	0.20	0	0	0	0	0	0	0.15	0.87	0	0.03	0	0.07	0.06
19-28:1	0.14	0	0	0	0	0	0	0	0.66	0	0	0	0	0	0
28:0	0.14	0.51	0	0.22	0	0.19	0	0.29	0	0.27	0.28	0.13	0	0.14	0.24
29:0	0	0	0	0	0	0	0	0	0	0	0	0.04	0	0.03	0.04
30:0	0	0	0	0	0	0	0	0	0	0	0	0.08	0	0.09	0.07

* Two isomers, mainly Δ^9.

sible to increase significantly the number of peaks identified, as is also demonstrated here. For instance, Takagi *et al.*[15] found a 24:6 *n*-3 acid using only a capillary column in an analysis that proceeded for a further 75 min after elution of the last known acid, *i.e.*, 24:1 *n*-9.

The pyrrolidide method for the determination of double bond positions is limited primarily by the chromatographic behaviour of pyrrolidides. For the elution of these derivatives a temperature up to 50°C higher than that for the elution of methyl esters must be used. In addition, pyrrolidides of fatty acids with more than 24 carbon atoms can only be chromatographed by means of on-column injection, which is not suitable when using GC–MS.

On the basis of the above findings and useful experience[11] in the determination of double bonds after oxidation as mono- and dicarboxylic acids, we decided to use this method in studies of fatty acids with more than 21 carbon atoms.

Linoleic acid was detected as a major acid in all fifteen species studied (see Table I). Its content did not decrease below 10% and sometimes it exceeded more than 50% of the total fatty acids present. The occurrence of other fatty acids, in addition to linoleic acid, *i.e.*, palmitic acid and oleic acid, is in agreement with literature data[16,17]. The most significant inter-genic and inter-species differences were detected with stearic acid, where they varied over a range of up to two orders of magnitude, which is again in agreement with literature data[17,18]. In contrast to published data, we were able to detect a wide range of fatty acids differing primarily in chain length, from C_{12} to C_{30}. Only Epstein *et al.*[5] identified acids of up to C_{28} (traces of C_{30} and C_{32}).

In contrast to all other published data, we detected acids of up to C_{30} as common lipid components; their concentrations were of the order of 1% and 0.1% for C_{24} and C_{26} acids, respectively (Fig. 1).

We also identified branched-chain fatty acids(iso- and anteiso-C_{12}–C_{17} acids) and unsaturated acids, in which three and two positional isomers were detected in C_{20} and C_{24}, respectively.

On the basis of literature data[5], our earlier results[7] and those obtained here, it can be concluded that very long-chain fatty acids are common in fungi, where they play a biochemical role that has not yet been clarified.

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