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POLYUNSATURATED AND UNUSUAL FATTY ACIDS FROM SLIME MOULDS

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Key Word Index—Slime moulds; Myxomycetes; *Arcyria* sp.; *Fuligo* sp.; *Lycogala* sp.; *Physarium* sp.; *Trichia* sp.; polyunsaturated fatty acids; GC-mass spectrometry.

Abstract—Fatty acids of nine different slime moulds were analysed. In addition to the common fatty acids, polyunsaturated and methylene non-interrupted polyunsaturated fatty acids, for example, with 5, 9- and/or 5, 11-double bonds, were identified by GC-mass spectrometry of their corresponding oxazolines. The above-mentioned compounds have been identified in only one slime mould and, therefore, their presence offers a new concept of biosynthesis of these compounds and chemotaxonomy of the slime moulds.

INTRODUCTION

Slime moulds (Myxomycetes) are most interesting organisms having two distinct life forms, a mobile plasmodium and sporangiophores; they have long been classified either as protozoa (amoebae) or fungi.

From the slime mould fatty acid analyses reported in the literature, it would appear that practically all their lipids contain fatty acids with C_{14}–C_{20} chains. Fatty acids with non-methylene interrupted double bonds are predominant.

In our previous papers dealing with the analysis of fatty acids (FA) in scarce lower organisms [1–3], we focused our attention on the unusual. As lower fungi can also provide a source of essential polyunsaturated fatty acids (PUFA) [4], we decided to analyse Myxomycetes from this point of view. Non-methylene interrupted FAs and PUFA are known to occur in some marine invertebrates and gymnosperms [5]. Thus, Myxomycetes would be expected to contain a wide and varied spectrum of fatty acids.

As a result of the development of modern analytical methods like capillary gas chromatography-mass spectrometry (CGC-MS), it is possible to identify more fatty acids, especially minor ones such as branched and positional isomers, and/or those with unusual positions of double bonds. Also, by using suitable derivatives, especially those containing nitrogen, like picolinyl esters, pyrolidides and oxazolines, it is possible to identify the position of double bond(s), branched chain(s) and/or hydroxyls. Oxazolines give the best results as far as both their mass spectra [6] or chromatographic behaviour [7] are concerned. The present study is the first known report on fatty acids of more than one species of slime mould.

RESULTS AND DISCUSSION

The results of GC-MS analysis are summarized in Table 1. The individual Myxomycetes differ from one another by the presence of non-methylene interrupted polyunsaturated fatty acids. Such compounds have only been found so far in *Dictyostelium discoideum* [8] but not in *Physarum polycephalum* [9]. In contrast to the work of Weeks and Herring [9], we identified not only diunsaturated non-methylene interrupted FA but also tri- and tetraunsaturated ones, which added to our knowledge of the presence of these rare acids in nature. To date they have been described to occur commonly only in marine invertebrates and seeds of gymnosperms [5].

Similarly, Korn *et al.* [8] described two unidentified FA in slime moulds, characterizing them as saturated and, probably, branched. In our investigations, when a capillary column was used, we were able to identify such compounds, all being *iso* and/or *anteiso* FAs. Weeks and Herring [9] also mentioned the presence of two positional isomers of monounsaturated FAs. Up to five positional isomers were found amongst the C_{18}, C_{20} and C_{22} FA, which indicated a relatively complex biosynthesis. Thus, several different desaturases must be present, responsible for such a broad spectrum of monoenoic acids. The above-mentioned presence of non-methylene interrupted FAs and, even, the existence of PUFAs are in keeping with this fact. PUFAs are common components of lower fungi [4] and occur also in Myxomycetes. By using CGC-MS analysis, we were able to demonstrate the presence of a much higher level of PUFAs, compared to the results of other authors [8, 9].

Myxomycetes were also shown to synthesize ω-3 and ω-6 FAs, whose presence has not hitherto been reported,

Table 1. Fatty acid composition from slime moulds (Myxomycetes)

Fatty acid	*A. c.**	*A. d.*	*A. n.*	*F. s.*	*L. e.*	*L. f.*	*P.* sp.	*T. f.*	*T. v.*
i–13:0	0.07	0.14	0.07	0.00	0.07	0.14	0.00	0.08	0.09
ai–13:0	0.00	0.00	0.00	0.15	0.00	0.00	0.18	0.00	0.00
13:0	0.22	0.14	0.21	0.20	0.29	0.22	0.09	0.17	0.28
i–14:0	0.07	0.14	0.14	0.00	0.14	0.08	0.00	0.17	0.09
14:0	0.51	0.35	0.42	0.39	0.29	0.37	0.41	0.17	0.36
i–15:0	0.15	0.14	0.21	0.00	0.14	0.08	0.00	0.17	0.09
ai–15:0	0.00	0.00	0.00	0.34	0.00	0.00	0.23	0.00	0.00
15:0	0.59	0.42	0.56	0.30	0.65	0.74	0.55	0.73	0.64
i–16:0	0.59	0.64	0.84	0.00	0.57	0.82	0.00	0.40	0.64
16:0	5.73	4.55	5.66	6.66	5.62	5.10	8.17	9.08	9.29
i–17:0	0.44	0.57	0.62	0.00	0.36	0.44	0.00	0.49	0.64
ai–17:0	0.00	0.00	0.07	0.44	0.00	0.00	0.41	0.00	0.00
17:0	0.51	0.57	0.49	0.59	0.29	0.44	0.59	0.40	0.55
i–18:0	0.22	0.14	0.21	0.00	0.22	0.22	0.00	0.17	0.28
18:0	1.32	1.42	1.46	2.17	1.37	1.63	2.53	2.51	2.67
i–19:0	0.07	0.07	0.14	0.00	0.14	0.14	0.00	0.17	0.28
ai–19:0	0.00	0.00	0.00	0.15	0.00	0.00	0.23	0.00	0.00
19:0	0.15	0.07	0.14	0.05	0.14	0.08	0.05	0.08	0.19
20:0	0.36	0.49	0.56	0.79	0.36	0.44	0.64	0.49	0.74
22:0	0.22	0.21	0.28	0.59	0.22	0.14	0.45	0.32	0.46
24:0	0.15	0.07	0.14	0.39	0.14	0.00	0.27	0.17	0.28
Σ saturated	11.37	10.13	12.22	13.21	11.01	11.08	14.08	15.77	17.57
14:1n–7	0.07	0.14	0.21	0.25	0.00	0.08	0.18	0.17	0.19
14:1n–5	0.15	0.21	0.21	0.30	0.14	0.22	0.14	0.24	0.36
16:1n–9	0.15	0.14	0.14	0.10	0.22	0.30	0.14	0.08	0.19
16:1n–7	1.61	2.34	1.88	1.72	1.58	1.78	1.85	1.46	1.48
16:1n–5	0.22	0.14	0.21	0.39	0.22	0.30	0.23	0.17	0.28
17:1n–8	0.22	0.35	0.28	0.44	0.22	0.14	0.36	0.32	0.36
18:1n–13	0.36	0.42	0.49	0.54	0.65	0.60	0.59	0.49	0.64
18:1n–11	0.22	0.14	0.35	0.44	0.51	0.52	0.23	0.40	0.55
18:1n–9	25.72	24.32	25.15	18.74	20.91	22.18	28.17	15.72	15.22
18:1n–7	0.59	0.49	0.42	0.25	0.43	0.37	0.23	0.57	0.74
18:1n–5	0.15	0.07	0.28	0.34	0.36	0.52	0.32	0.24	0.55
19:1n–10	0.00	0.00	0.00	0.05	0.00	0.08	0.09	0.00	0.00
19:1n–8	0.22	0.14	0.21	0.25	0.14	0.14	0.27	0.17	0.28
20:1n–15	0.07	0.00	0.00	0.05	0.00	0.00	0.05	0.00	0.00
20:1n–13	0.07	0.07	0.14	0.44	0.57	0.37	0.32	0.49	0.46
20:1n–11	0.15	0.21	0.14	0.34	0.29	0.14	0.23	0.32	0.46
20:1n–9	0.96	1.21	0.98	1.13	0.94	1.26	0.95	0.97	1.48
20:1n–7	0.36	0.28	0.42	0.25	0.22	0.22	0.14	0.24	0.19
21:1n–9	0.22	0.21	0.28	0.34	0.29	0.14	0.09	0.17	0.09
22:1n–15	0.00	0.00	0.00	0.05	0.00	0.00	0.05	0.00	0.00
22:1n–13	0.00	0.00	0.00	0.15	0.00	0.00	0.09	0.00	0.00
22:1n–11	0.15	0.07	0.14	0.30	0.14	0.08	0.23	0.17	0.09
22:1n–9	0.44	0.35	0.35	0.44	0.29	0.37	0.45	0.24	0.36
22:1n–7	0.15	0.14	0.07	0.20	0.00	0.08	0.23	0.00	0.09
23:1n–9	0.00	0.00	0.00	0.05	0.00	0.00	0.05	0.00	0.00
24:1n–9	0.00	0.07	0.07	0.20	0.00	0.08	0.09	0.00	0.00
Σ monoenic	32.25	31.51	32.42	27.75	28.12	29.97	35.77	22.63	24.06
5,9–16:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.15
16:2n–4	1.47	1.07	1.19	1.58	1.16	1.26	1.99	1.70	1.45
5,9–18:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.37	0.41
5,11–18:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.08
18:2n–9	1.99	2.49	2.17	2.17	1.30	1.70	2.71	2.03	1.34
18:2n–6	35.63	36.54	33.64	34.97	39.14	38.29	24.02	47.15	43.49
5,11–20:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.55
5,13–20:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.28
20:2n–9	0.59	0.64	0.49	0.59	0.65	0.82	0.95	0.24	0.74
20:2n–6	1.18	1.00	0.91	1.33	0.87	0.66	1.76	1.38	1.65
7,13–22:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.19

(Table 1 *continued*)

Fatty acid	*A. c.**	*A. d.*	*A. n.*	*F. s.*	*L. e.*	*L. f.*	*P.* sp.	*T. f.*	*T. v.*
7,15–22:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.24	0.09
Σ dienoic	0.86	41.74	38.40	40.64	43.12	42.73	31.43	54.09	50.42
18:3n–6	2.50	2.56	2.03	5.00	5.83	6.81	4.43	0.52	0.31
18:3n–3	3.96	4.48	4.96	6.66	1.95	2.59	5.92	4.37	4.51
18:4n–3	0.36	0.49	0.42	0.59	0.36	0.52	0.64	0.49	0.64
20:3n–9	0.36	0.49	0.42	0.15	0.57	0.74	0.55	0.17	0.09
20:3†	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.57	0.46
20:3n–6	0.66	0.86	0.98	0.84	0.79	0.66	0.59	0.00	0.00
20:4n–6	4.77	5.15	5.36	2.11	4.18	0.34	4.09	0.40	0.28
20:3n–3	1.67	1.35	1.46	1.72	1.95	2.27	0.81	0.17	0.36
20:4‡	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.14	1.03
20:4n–3	0.36	0.49	0.56	0.59	1.01	1.11	0.36	0.24	0.19
22:3n–9	0.22	0.14	0.07	0.20	0.22	0.30	0.29	0.00	0.00
22:3n–6	0.15	0.14	0.14	0.25	0.29	0.37	0.09	0.00	0.00
22:4n–6	0.36	0.35	0.49	0.15	0.51	0.44	0.23	0.00	0.00
24:4n–6	0.15	0.02	0.07	0.14	0.09	0.07	0.00	0.15	0.08
Σ polyenoic	15.52	16.52	16.96	18.40	17.75	16.22	18.00	8.22	7.95

**A. c.*, *Arcyria cinerea*; *A. d.*, *A. denudata*; *A. n.*, *A. nutans*; *F. s.*, *Fuligo septica*; *L. e.*, *Lycogala epidendrum*; *L. f.*, *L. flavoscum*; *P.* sp., *Physarium* species; *T. f.*, *Trichia favoaiena*; *T. v.*, *T. varia*.

†5,11,14–20:3.

‡5,11,14,17–20:4.

as well as C_{22} PUFA and small amounts of 24:4n-6. PUFAs with more than four double bonds, however, were not detected, which suggested a high specificity of the desaturating enzymes. This finding is of great importance, especially if compared with the situation in lower fungi [4]. In conclusion our work on the FAs in Myxomycetes offers a first view of the complex FA content in such organisms and of its possible similarity to that in lower fungi. Such a comparison may have phylogenetic implications. The content of PUFA is also important from a practical point of view, since the cultivation time of Myxomycetes is very short (several hours), as compared to other moulds (several days).

EXPERIMENTAL

Isolation. Mature slime moulds (as sporangia) were collected in dry weather *ca* 30 km south of Prague, within the period November 1991–August 1992. They were identified taxonomically and samples are kept in our Department. About 1 g of the slime moulds was extracted according to ref. [10]. Total lipids were hydrolysed and the unsaponifiable fr. extracted × 3 with Et_2O, the pH of the aq. soln was adjusted to 1 and fatty acids ($\frac{1}{3}$–$\frac{1}{4}$ of total lipids) were extracted again × 3 with Et_2O.

Derivatization and GC-MS identification. Me esters of FAs were prepd by reaction with BF_3–MeOH [11]. Oxazolines and/or their TMSi ethers were prepared using a modification of published methods [12, 13]: 5 mg dicyclohexylcarbodiimide (Sigma) was added to a soln of 5 mg FA in 1 ml CH_2Cl_2. After stirring (10 min), 5 mg 2-amino-2-methylpropan-ol (Sigma) was added (20°, 4 hr). The evapd mixt. was dissolved in 1 ml Et_2O and treated with 0.5 ml $SOCl_2$ (20°, 1 hr), washed with ice-cold H_2O and dried (Na_2SO_4). The soln was evapd, dissolved in pyridine (0.5 ml) and heated with trimethylsilyl chloride (50°, 4 hr).

Me esters were identified and quantified (by total ion current) in GC-MS apparatus equipped with a fused-silica capillary column (60 mm × 0.32 mm i.d. × 0.25 μm SPB-1, Supelco) using splitless injection with He carrier gas. The oven temp. was prog. from 100 to 320° at 4° min^{-1}. The ionization energy was 70 eV with an electron-multiplier voltage of 2.5 kV.

Oxazolines were identified under similar conditions as Me esters, only at a different column temp., which was programmed from 150 to 330° at 5° min^{-1}.

MS (*m/z*, rel. int.) of non-methylene interrupted fatty acids (as oxazoline derivatives). 5,9–16:2. 305 ($[M]^+$ 4.1), 290 (3.6), 276 (2.1), 262 (1.9), 248 (2.1), 234 (6.2), 220 (18.3), 206 (0.5), 194 (3.2), 180 (27.1), 166 (20.2), 153 (1.4), 152 (1.3), 140 (35.8), 126 (84.3), 113 (100), 98 (67.3), 5, 9–18:2. 333 ($[M]^+$ 3.9), 318 (2.8), 304 (2.2), 290 (2.6), 276 (1.8), 262 (1.3), 248 (2.5), 234 (5.3), 220 (11.9), 206 (0.2), 194 (3.9), 180 (35.6), 166 (42.8), 153 (1.0), 152 (0.8), 140 (63.2), 126 (88.1), 113 (100), 98 (75.2). 5,11–18:2. 333 ($[M]^+$ 4.0), 318 (1.7), 304 (1.2), 290 (0.9), 276 (1.2), 262 (1.3), 248 (8.9), 234 (0.3), 222 (6.7), 208 (4.2), 194 (5.1), 180 (24.2), 166 (37.4), 153 (0.8), 152 (0.7), 140 (52.7), 126 (75.9), 113 (100), 98 (49.3). 5,11–20:2. 361 ($[M]^+$ 3.4), 346 (1.8), 332 (1.8), 318 (1.2), 304 (1.0), 290 (1.0), 276 (0.9), 262 (1.4), 248 (5.7), 234 (0.2), 222 (5.9), 208 (3.5), 194 (6.3), 180 (18.1), 166 (25.7), 153 (0.6), 152 (0.7), 140 (48.5), 126 (63.1), 113 (100), 98 (52.8). 5,13–20:2. 361 ($[M]^+$ 3.2), 346 (1.9), 332 (1.3), 318 (0.7), 304 (0.8), 290 (1.3), 276 (1.8), 262 (0.3), 250 (4.8), 236 (3.3), 222 (4.1), 208 (3.7), 194 (8.3), 180 (23.2), 166 (32.5), 153 (0.5),

152 (0.6), 140 (49.2), 126 (87.6), 113 (100), 98 (43.7). 7,13–22:2. 389 ($[M]^+$ 2.8), 374 (1.4), 360 (0.8), 346 (0.5), 332 (0.4), 318 (0.5), 304 (0.4), 290 (1.2), 276 (2.7), 262 (0.2), 250 (4.6), 236 (3.1), 222 (4.5), 208 (3.6), 194 (4.9), 180 (0.7), 168 (18.3), 154 (29.3), 140 (45.8), 126 (83.2), 113 (100), 98 (39.4). 7,15–22:2. 389 ($[M]^+$ 2.3), 374 (1.2), 360 (0.5), 346 (0.3), 332 (0.9), 318 (1.2), 304 (2.8), 290 (0.2), 278 (2.3), 264 (3.5), 250 (5.1), 236 (4.6), 222 (4.7), 208 (5.1), 194 (4.0), 180 (0.8), 168 (12.7), 154 (22.6), 140 (37.4), 126 (63.7), 113 (100), 98 (42.6). 5,11,14–20:3. 359 ($[M]^+$ 6.9), 344 (1.8), 330 (0.9), 316 (1.3), 302 (1.8), 288 (4.2), 274 (0.3), 262 (4.0), 248 (5.3), 234 (0.6), 222 (6.7), 208 (7.5), 194 (7.2), 180 (8.3), 166 (14.7), 153 (1.2), 152 (1.3), 140 (24.7), 126 (65.1), 113 (100), 98 (69.0). 5,11,14,17–20:3. 357 ($[M]^+$ 12.6), 342 (6.5), 328 (4.3), 314 (0.3), 302 (1.7), 288 (3.9), 274 (0.5), 262 (4.5), 248 (5.7), 234 (0.8), 222 (6.2), 208 (6.4), 194 (7.0), 180 (8.5), 166 (12.5), 153 (1.7), 152 (0.9), 140 (35.2), 126 (60.3), 113 (100), 98 (72.8).

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