Production of Odd Chain Polyunsaturated Fatty Acids by *Mortierella* Fungi

Sakayu Shimizu*, Hiroshi Kawashima[1], Kengo Akimoto[2], Yoshifumi Shinmen[2] and Hideaki Yamada
Department of Agricultural Chemistry, Kyoto University, Sakyo-ku, Kyoto 606, Japan

A soil isolate, *Mortierella alpina* 1S-4, was found to show high production of odd chain polyunsaturated fatty acids (PUFAs) among various arachidonic acid-producing *Mortierella* strains tested. The fungus mainly accumulated 5,8,11,14-*cis*-nonadecatetraenoic acid. With 5% *n*-heptadecane and 1% yeast extract as growth substrates, the amount of $C_{19:4}$ acid accumulated reached 44.4 mg/g dry mycelia (0.68 mg/mL of culture broth). This value accounted for 11.2% of the total fatty acids in the extracted lipids from mycelia, and odd chain fatty acids comprised over 95% of the total mycelial fatty acids. The addition of sesamin, a specific inhibitor of Δ5 desaturation, caused an increase in $C_{19:3}$ acid and an accompanying decrease in $C_{19:4}$ acid. On the other hand, species of *Mortierella* that could not produce C-20 PUFAs accumulated C-17 acids, but no C-19 PUFAs, when grown with fatty substrates with an odd chain skeleton. The odd chain PUFAs were distributed in both neutral and polar lipids. The biosynthetic route to $C_{19:4}$ acid was presumed to mimic the n-6 route to arachidonic acid as follows: $C_{17:0} \rightarrow C_{17:1} \rightarrow C_{17:2} \rightarrow C_{17:3} \rightarrow C_{19:3} \rightarrow C_{19:4}$ acids.

KEY WORDS: Arachidonic acid, biosynthesis of polyunsaturated fatty acids, *Mortierella alpina*, nonadecatetraenoic acid, odd chain polyunsaturated fatty acids.

The natural occurrence of straight-chain, odd-numbered polyunsaturated fatty acids (PUFAs) has been reported in ruminant, fish and several other animal lipids. They can be considered as normal constituents of such fats. However, the odd chain PUFAs reported accounted for only a small percentage of the total cellular fatty acids. Only mullet oil so far has been reported to be a relatively rich source of these fatty acids, such as C-17 and C-19 PUFAs (1). The fatty acid methyl esters containing mainly 9,12-$C_{17:2}$ and 6,9,12-$C_{17:3}$ acids prepared from mullet oil, as well as linoleic acid methyl ester, were shown to cure the external symptoms of fat-deficient rats and to be converted to 5,8,11,14-$C_{19:4}$ acid in the rat liver (2,3). On the other hand, some mucoralean fungi (4) and *Candida* yeasts (5), grown with odd chain *n*-alkanes, have been shown to accumulate C-17 unsaturated fatty acids such as $C_{17:1}$, $C_{17:2}$ and $C_{17:3}$ acids. However, they do not produce C-19 PUFAs. In our recent studies (6–17), it was found that several fungal strains belonging to the genus *Mortierella* accumulate C-20 PUFAs, such as dihomo-γ-linolenic, arachidonic and eicosapentaenoic acids, in their mycelia. Here we report that these arachidonic acid producers accumulate specifically large amounts of C-17 and C-19 PUFAs in their mycelia when grown with odd chain *n*-alkanes. The identification of the odd chain PUFAs and their distribution in fractionated lipids are also described.

MATERIALS AND METHODS

Chemicals. *n*-Alkanes were purchased from Wako Pure Chemicals (Osaka, Japan). (+)Sesamin was prepared from unroasted sesame oil (S. Shimizu, K. Akimoto, Y. Shinmen, M. Sugano and H. Yamada, unpublished data). All other reagents used in this work were of analytical grade and commercially available.

Microorganisms. All fungal strains used were from our stock cultures (AKU Culture Collection, Faculty of Agriculture, Kyoto University). Each fungus was cultivated in a medium (10 mL, pH 6.0) containing glucose and/or odd chain fatty substrates, as the major carbon source, and 1% yeast extract in a 50-mL flask for 6–8 days at 28°C with reciprocal shaking (120 strokes/min).

Analysis of fatty acid composition and n-*pentadecane.* Extraction and determination of the mycelial fatty acids were described previously (11,12,14). *n*-Pentadecane was analyzed by gas liquid chromatography (GLC) in the same manner as for fatty acid methyl ester analysis except for the column temperature (140°C).

Lipid extraction and separation. Mycelia washed with water and *n*-hexane were treated twice with chloroform/methanol/water according to the procedure of Bligh and Dyer (18). The lipid extract was separated by thin-layer chromatography (TLC) on silica gel ($60F_{254}$, 200 × 200 × 0.25mm; E. Merck, Darmstadt, Germany). The spots were located under u.v. after spraying the plate with 0.2% dichlorofluorescein in ethanol. The solvent system used was petroleum ether/diethyl ether/acetic acid (82:18:1, v/v/v). The spots were directly scraped off for methanolysis.

Isolation of $C_{19:4}$ acid methyl ester from fungal mycelia and other methods. The procedures used for transmethylation and purification of $C_{19:4}$ acid were essentially the same as described previously (6). Measurements of mass and ^1H NMR (nuclear magnetic resonance) spectra, and other analyses were also carried out as described previously (6,11–14).

RESULTS

Formation of odd chain PUFAs from glucose. The mycelia of *Mortierella alpina* CBS 219.35 grown in a medium containing only glucose and yeast extract contained significant amounts of unusual fatty acids besides palmitic, stearic, oleic, linoleic, γ-linolenic, dihomo-γ-linolenic and arachidonic acids, which are commonly found in arachidonic acid-producing species of *Mortierella.* Two major components of these unusual fatty acids showed the same retention times as authentic $C_{15:0}$ and $C_{17:0}$ acid methyl esters, respectively, on both GLC and high performance liquid chromatography (HPLC). The others were also considered to be odd chain unsaturated fatty acids because of their retention times on GLC and HPLC, and the

*To whom correspondence should be addressed.
[1]On leave from Suntory Ltd.
[2]Present address: Laboratory of Microbial Science Institute for Fundamental Research, Suntory Ltd., Mishimagun, Osaka 618, Japan.

TABLE 1

Accumulation of Odd Chain PUFAs by Several Species of *Mortierella* in a Medium Containing Methyl Pentadecanoate or *n*-Pentadecane[a]

Strain	FAM[b] or NA[b] added (%)	Mycelial fatty acid composition[b] (%)												
		15:0	16:0	17:0	17:1	18:0	18:1	18:2	18:3	19:3	19:4	20:3	20:4	Others	Total odd chain FA
Subgenus *Mortierella*															
M. alpina 1S-4 AKU 3998	FAM 0.5	13.8	4.7	11.7	6.8	2.3	9.5	4.7	2.8	1.9	7.4	2.2	22.9	9.3	50.7
	NA 2.0	24.8	1.0	14.0	15.0	tr	5.0	3.1	2.8	1.8	11.6	tr	10.2	10.7	74.5
M. elongata 1S-5 AKU 3999	NA 2.0	47.4	1.7	9.5	12.3	tr	6.8	1.5	1.6	1.6	5.4	0.7	7.2	4.3	78.6
M. verticillata IFO 8575	NA 2.0	35.2	1.9	8.8	13.8	tr	7.2	5.3	2.3	1.0	3.7	tr	15.3	5.5	68.1
M. kuhlmanii CBS 157.71	NA 2.0	47.2	2.9	7.2	7.9	1.2	5.2	4.0	2.1	1.3	3.4	tr	8.2	9.4	75.6
M. alpina CBS 219.35	FAM 1.0	51.6	3.3	6.2	5.7	1.2	9.5	6.2	3.7	0.9	1.1	1.6	7.3	1.7	67.0
Subgenus *Micromucor*															
M. ramanniana	FAM 1.0	31.8	9.8	9.1	8.3	2.6	20.6	7.4	9.5	nd	nd	nd	nd	0.9	49.2
var. *angulispora* IFO 8187	NA 2.0	2.6	13.7	nd	nd	1.9	40.7	17.4	22.8	nd	nd	nd	nd	0.9	2.6
M. isabellina IFO 6739	FAM 1.0	28.1	10.1	2.5	2.7	1.8	29.1	13.7	11.7	nd	nd	nd	nd	0.3	33.3

[a]Each strain of *Mortierella* was grown in a medium containing 2% glucose, 1% yeast extract and methyl pentadecanoate or *n*-pentadecane, as indicated, pH 6.0, for 6-8 days at 28°C.

[b]Abbreviations used: FAM, methyl pentadecanoate; NA, *n*-pentadecane; 15:0, pentadecanoic acid; 16:0, palmitic acid; 17:0, heptadecanoic acid; 17:1, heptadecenoic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, γ-linolenic acid; 19:3, nonadecatrienoic acid; 19:4, nonadecatetraenoic acid; 20:3, dihomo-γ-linolenic acid; 20:4, arachidonic acid; FA, fatty acids; tr, trace; nd, not detectable.

TABLE 2

Comparison of Mycelial Fatty Acid Composition of *M. alpina* 1S-4 Grown With Odd Chain Fatty Compounds[a]

FAM or NA added[b]	Mycelial fatty acid composition[b] (%)												
	15:0	17:0	17:1	17:2	17:3 19:0	19:1	19:3	19:4	16:0	18:1	20:4	Others	Total odd chain FA
C15 FAM	13.8	11.7	6.8	tr	1.3	6.2	1.9	7.4	4.7	9.5	22.9	13.8	50.7
C19 FAM	tr	tr	tr	nd	20.9	nd	nd	nd	13.2	8.6	42.2	15.1	20.9
C11 NA	0.5	0.7	0.2	nd	0.3	nd	nd	nd	17.5	25.0	28.3	27.5	1.7
C13 NA	1.1	1.2	0.3	nd	0.2	nd	nd	nd	16.7	16.7	33.9	29.9	2.8
C15 NA	16.8	8.4	5.6	tr	0.8	tr	1.2	3.3	8.2	11.4	25.2	19.1	36.6
C17 NA	2.0	9.4	4.6	1.2	1.0	tr	0.7	0.8	16.6	23.6	18.5	21.6	19.7
C19 NA	0.8	2.8	1.2	tr	2.4	tr	0.3	0.4	16.0	26.4	20.1	29.6	9.6

[a]*M. alpina* 1S-4 was grown in a medium containing 2% glucose, 1% yeast extract and each of the indicated fatty acid methyl esters (0.5%) or *n*-alkanes (1.0%), pH 6.0, for 6-8 days at 28°C.

[b]Abbreviations used: C15 FAM, methyl pentadecanoate; C19 FAM, methyl nonadecanoate; C11 NA, *n*-undecane; C13 NA, *n*-tridecane; C15 NA, *n*-pentadecane; C17 NA, *n*-heptadecane; C19 NA, *n*-nonadecane; 17:2, heptadecadienoic acid; 17:3, heptadecatrienoic acid; 19:0, nonadecanoic acid; 19:1, nonadecenoic acid. For other abbreviations, see Table 1.

results described later regarding their GLC-mass and ^{1}H NMR spectra. These odd chain fatty acids accounted for 17.7% of the total mycelial fatty acids, and comprised $C_{15:0}$ (4.0%, by weight), $C_{17:0}$ (7.2), $C_{17:1}$ (5.2), $C_{19:3}$ (0.8) and $C_{19:4}$ (0.5) acids, whereas the odd chain fatty acids in mullet oil have been reported to account for about 25% of the total fatty acids, and to be comprised of $C_{15:0}$ (11.2%), $C_{17:0}$ (4.6), $C_{17:2}$ (2.5), $C_{19:4}$ (1.7) acids, plus others (1). Similarly, *M. alpina* var. *renispora* CBS 210.32, *M. hyalina* NRRL 6427 and *M. minutissima* IFO 8573 accumulated detectable amounts of C-17 and C-19 PUFAs.

Conversion of fatty substrates with an odd chain skeleton to odd chain PUFAs. To obtain greater amounts of odd chain PUFAs, several species of *Mortierella* were grown with *n*-pentadecane or methyl pentadecanoate, as shown in Table 1. All the arachidonic acid-producing species of *Mortierella,* which belong to the subgenus *Mortierella,* accmulated $C_{19:4}$ and $C_{19:3}$ acids together with C-15 and C-17 fatty acids, such as $C_{15:0}$, $C_{17:0}$ and $C_{17:1}$ acids. C-15 unsaturated fatty acids were not detected. On the other hand, species of *Mortierella* belonging to the subgenus *Micromucor,* which cannot produce C-20 PUFAs (10), accumulated C-17 PUFAs but not C-19 PUFAs. The total accumulation of odd chain fatty acids in arachidonic acid-producing species of *Mortierella* accounted for more than half of the total mycelial fatty acids. Both *n*-pentadecane and methyl pentadecanoate were efficiently converted to odd chain fatty acids, and exhibited essentially the same mycelial fatty acid profile. We selected *M. alpina* 1S-4 for the following experiments because of its high mycelial accumulation of $C_{19:4}$ acid.

M. alpina 1S-4 was grown with glucose and odd chain fatty acid methyl esters or odd chain *n*-alkanes, as shown in Table 2. C-15 and C-17 alkanes were efficiently converted to C-19 PUFAs. But fatty substrates with shorter and longer carbon chains (i.e., C-11, C-13 and C-19) were

TABLE 3

Production of Nonadecatetraenoic and Arachidonic Acids by *M. alpina* 1S-4 Grown with *n*-Pentadecane or *n*-Heptadecane[a]

	n-Pentadecane					*n*-Heptadecane				
	Mycelial mass	Mycelial PUFA content (mg/g dry mycelia)		PUFA yield (mg/mL)		Mycelial mass	Mycelial PUFA content (mg/g dry mycelia)		PUFA yield (mg/mL)	
(%)	(mg/mL)	19:4[b]	Ara[c]	19:4	Ara	(mg/mL)	19:4	Ara	19:4	Ara
3	14.8	31.5	8.7	0.47	0.13	14.3	27.4	5.9	0.39	0.08
4	13.0	24.1	5.3	0.31	0.07	14.7	37.8	5.0	0.55	0.07
5	13.0	19.8	4.4	0.26	0.06	15.4	44.4	5.9	0.68	0.09

[a] *M. alpina* 1S-4 was grown in a medium containing 3–5% *n*-pentadecane or *n*-heptadecane, as indicated, and 1% yeast extract, pH 6.0, for 7 days at 28°C.

[b] 19:4, 5,8,11,14-*cis*-Nonadecatetraenoic acid.

[c] Ara, arachidonic acid.

TABLE 4

Comparison of Mycelial Fatty Acid Composition of *M. alpina* 1S-4 Grown in the Presence or Absence of Sesamin[a]

Addition of sesamin	Mycelial fatty acid composition[b] (%)													
	15:0	16:0	17:0	17:1	18:0	17:2	18:1	18:2	18:3	19:3	19:4	20:3	20:4	Others
No	15.1	9.1	8.9	5.1	5.3	1.6	9.6	4.5	3.3	1.0	3.4	3.8	26.3	3.0
Yes	20.0	9.8	7.2	3.1	2.8	1.1	6.7	1.7	4.4	3.8	1.7	14.1	19.6	4.0

Addition of sesamin	19:3/19:4	20:3/20:4	Odd chain fatty acids (%)	Even chain fatty acids (%)	S+M[b] (%)	P[b] (%)
No	0.29	0.14	36.0	61.9	53.1	43.9
Yes	2.24	0.72	37.3	59.1	49.6	46.4

[a] *M. alpina* 1S-4 was grown in a medium containing 2% glucose, 2% *n*-pentadecane and 1% yeast extract supplemented with 0.01% of sesamin or unsupplemented, pH 6.0, for 6 days at 28°C.

[b] Abbreviations used: S, saturated fatty acids; M, monounsaturated fatty acids; P, fatty acids having more than two double bonds. For other abbreviations, see Tables 1 and 2.

not efficiently converted. When it was grown with C-15 or C-17 fatty substrates, the predominant odd chain fatty acids were $C_{15:0}$, $C_{17:0}$, $C_{17:1}$, $C_{17:2}$, $C_{17:3}$, $C_{19:3}$ and $C_{19:4}$ acids. C-15 unsaturated fatty acids and odd chain fatty acids of more than 21 carbons were not detected.

The data in Table 3 show that *M. alpina* 1S-4 could grow in a medium containing an odd chain *n*-alkane as a major carbon source and that the $C_{19:4}$ acid content reached 44.4 mg/g dry mycelia (0.68 mg/mL of culture broth) when it was grown in a medium containing 5% *n*-heptadecane and 1% yeast extract. The total odd chain fatty acids accumulated reached over 95% of the mycelial fatty acids. The $C_{19:4}$ acid accounted for 11.2%, whereas arachidonic acid accounted for only 1.5%.

Inhibitory effect of sesamin on desaturation of $C_{19:3}$ acid to $C_{19:4}$ acid. *M. alpina* 1S-4 was grown with *n*-pentadecane supplemented with 0.01% of (+)sesamin or unsupplemented, as shown in Table 4. (+)Sesamin and related lignan compounds are specific inhibitors of Δ5 desaturation (S. Shimizu *et al.*, unpublished data). Table 4 shows that the mycelial "19:3/19:4" ratio increased from 0.29 to 2.24 with the supplementation of sesamin. The mycelial "20:3/20:4" ratio increased from 0.14 to 0.72, without a change in the percentage of odd chain fatty acids (36% with no supplementation and 37% with supplementation) or that of PUFAs (44% with no supplementation and 46% with supplementation).

Distribution of odd chain PUFAs in extracted lipids. The lipids extracted from mycelia of *M. alpina* 1S-4 grown with 3% *n*-pentadecane (NA medium) or 2% glucose (G medium) were separated into the triacylglyceride (TG)-fraction (Rf=0.6), the polar lipid (PL)-fraction (Rf=0.02) and the *n*-alkane (NA)-fraction (Rf=0.85) (Table 5). The *n*-pentadecane remaining in the NA medium amounted to 6.1% of the supplemented *n*-pentadecane and mycelial *n*-pentadecane represented by the NA-fraction to 12.6%, so about 80% of the supplemented *n*-pentadecane was consumed during the cultivation and 12% of the consumed *n*-pentadecane was converted to mycelial fatty acids.

In the case of the NA medium, every odd chain fatty acid was found in both the TG- and PL-fractions, but the fatty acid compositions of these two fractions were different. Table 5 shows that the percentage of saturated

TABLE 5

Comparison of Mycelial Fatty Acid Composition of the TG[a]-Fraction and PL[a]-Fraction of *M. alpina* 1S-4 Grown in the Presence or Absence of *n*-Pentadecane[b]

Medium[c]	Fra[d]	FA (mg/g dry mycelia)	Fatty acid composition[d] (%)											
			15:0	16:0	17:0	17:1	18:1	17:3	18:3	19:3	19:4	20:3	20:4	Others
NA	TG	218.7	56.8	1.2	21.0	7.7	1.9	2.3	0.4	1.0	1.7	tr	0.4	5.6
NA	PL	28.9	15.4	2.4	7.5	19.7	12.5	3.4	8.7	2.1	14.0	0.5	3.9	9.9
G	TG	173.6	nd	18.2	nd	nd	17.5	nd	5.5	nd	nd	2.3	38.7	17.8
G	PL	27.5	nd	16.0	nd	nd	15.8	nd	5.7	nd	nd	2.5	42.4	17.6

[a]Abbreviations used: TG, triacylglyceride; PL, polar lipid.

[b]*M. alpina* 1S-4 was grown in the NA or G medium for 6 days at 28°C.

[c]The NA medium contained 3% *n*-pentadecane and 1% yeast extract, pH 6.0. The G medium contained 2% glucose and 1% yeast extract, pH 6.0.

[d]Fra, fraction. For other abbreviations used, see Tables 1 and 2.

odd chain fatty acids in the TG-fraction was high ($C_{15:0}$, 56.8%; and $C_{17:0}$, 21.0%) and the percentage of PUFAs was low ($C_{19:4}$, 1.7%; and $C_{20:4}$, 0.4%). On the other hand, the percentage of saturated odd chain fatty acids in the PL-fraction was relatively lower ($C_{15:0}$, 15.4%; and $C_{17:0}$, 7.5%) and that of PUFAs was higher ($C_{19:4}$, 14.0%; and $C_{20:4}$, 3.9%). In the case of the G medium, the fatty acid compositions of both fractions were almost the same, the $C_{20:4}$ acid content reaching about 40%. The ratio of the TG- and PL-fractions of the lipids from mycelia grown in the NA medium was 88:12, while that of the lipids from mycelia grown in the G medium was 86:14.

Identification of $C_{19:4}$ acid and the other odd chain PUFAs. $C_{19:4}$ acid methyl ester (13 mg) was isolated from the lipids extracted from 40 g of wet mycelia of *M. alpina* 1S-4 grown in a medium containing 4% *n*-pentadecane and 1% yeast extract, pH 6.0, for 8 days at 28°C as described previously (6). The mass spectrum of the isolated methyl ester showed peaks at *m/z* 304 (M^+; relative intensity, 2%), 250, 220, 180, 163, 150, 136, 119, 106, 93, 79 (base peak), 67, 55 and 41. The ^1H NMR spectrum in $CDCl_3$, with tetramethylsilane as an internal standard, showed signals at 0.90 (*t*, 3H, CH_3), 1.31 (*m*, 4H, CH_2), 1.68 (*m*, 2H, CH_2), 2.09 (*m*, 4H, CH_2), 2.30 (*t*, 2H, CH_2), 2.83 (*m*, 6H, CH_2), 3.63 (*s*, 3H, CH_3) and 5.38 ppm (*m*, 8H, C=C), which are the same as those in the case of authentic methyl arachidonate except that the peak at 1.31 ppm of methyl arachidonate, corresponding to methylene protons at the 17, 18 and 19 carbon atoms, indicates 6H. These data suggest that the structure of the isolated methyl ester was 5,8,11,14-*cis*-nonadecatetraenoic acid methyl ester. The other odd chain PUFA methyl esters were partly purified and determined by GLC-mass spectra to be as follows: nonadecatrienoic acid methyl ester, *m/z* 306 (M^+; relative intensity, 17%), 275, 222, 177, 163, 149, 136, 121, 107, 93, 79 (base peak), 67, 55 and 41; heptadecatrienoic acid methyl ester, *m/z* 278 (M^+, 13%), 194, 163, 149, 136, 120, 107, 93, 79, 67 (base peak), 55 and 41; heptadecadienoic acid methyl ester, *m/z* 280 (M^+, 22%), 249, 206, 164, 150, 136, 123, 109, 95, 81, 67 (base peak), 55 and 41.

DISCUSSION

Hoffmann and Rehm reported that three fungi belonging to the Mucorales, *Absidia spinosa, Cunninghamella echinulata* and *Mortierella isabellina*, which could not produce C-20 PUFAs, accumulated C-17 PUFAs but no C-19 PUFAs when grown with odd chain *n*-alkanes (4). Here we also show that *M. isabellina* and *M. ramanniana* var. *angulispora*, belonging to the subgenus *Micromucor*, which could not produce C-20 PUFAs, could accumulate some C-17 acids but no C-19 PUFAs. Some *Candida* yeasts, which are unable to form C-20 PUFAs, were also reported to produce C-17 acids but no C-19 PUFAs when grown on odd chain *n*-alkanes (5). These data indicate that only microorganisms that can produce C-20 PUFAs can produce C-19 PUFAs, and that microorganisms that are unable to produce C-20 PUFAs cannot produce C-19 PUFAs.

One of the predominant C-19 PUFAs produced by arachidonic acid-producing species of *Mortierella* was identified as 5,8,11,14-*cis*-nonadecatetraenoic acid, corresponding to arachidonic acid minus one carbon unit at the ω-terminal. The other odd chain PUFAs formed by these fungi were considered to be $C_{19:3}$, $C_{17:3}$ and $C_{17:2}$ acids, according to the mass spectra of their methyl esters. C-21 PUFAs have not been detected. These data suggest that the odd chain PUFAs were biosynthesized through the proposed pathway in Figure 1, which mimics the pathway for the biosynthesis of even chain PUFAs, the following successive reactions being involved: Oxidation of *n*-pentadecane to $C_{15:0}$ acid, elongation to $C_{17:0}$ acid, desaturation to 6,9,12-*cis*-$C_{17:3}$ acid *via* $C_{17:1}$ and $C_{17:2}$ acids as successive intermediates, elongation of $C_{17:3}$ acid to $C_{19:3}$ acid, and further desaturation of $C_{19:3}$ acid to $C_{19:4}$ acid. These odd chain PUFAs correspond to the equivalent even chain PUFAs minus one carbon unit at the ω-terminal. This is supported by the fact that only microorganisms that can produce C-20 PUFAs can produce C-19 PUFAs. The reports (2,3) that 9,12-$C_{17:2}$ and 6,9,12-$C_{17:3}$ acids were probably converted to 5,8,11,14-$C_{19:4}$ acid in the rat liver, and the results in Table 3 that show a significant decrease in the accumulation of arachidonic acid in *M. alpina* 1S-4 when a large amount of $C_{19:4}$ acid is produced also support this assumption.

We reported that (+)sesamin and some related lignans are specific inhibitors of the Δ5 desaturation reaction in *M. alpina* 1S-4 and rat liver (S. Shimizu, *et al.*, unpublished data). Mycelial dihomo-γ-linolenic acid, which is a substrate for Δ5 desaturation, increases with an accompanying decrease in mycelial arachidonic acid, a product

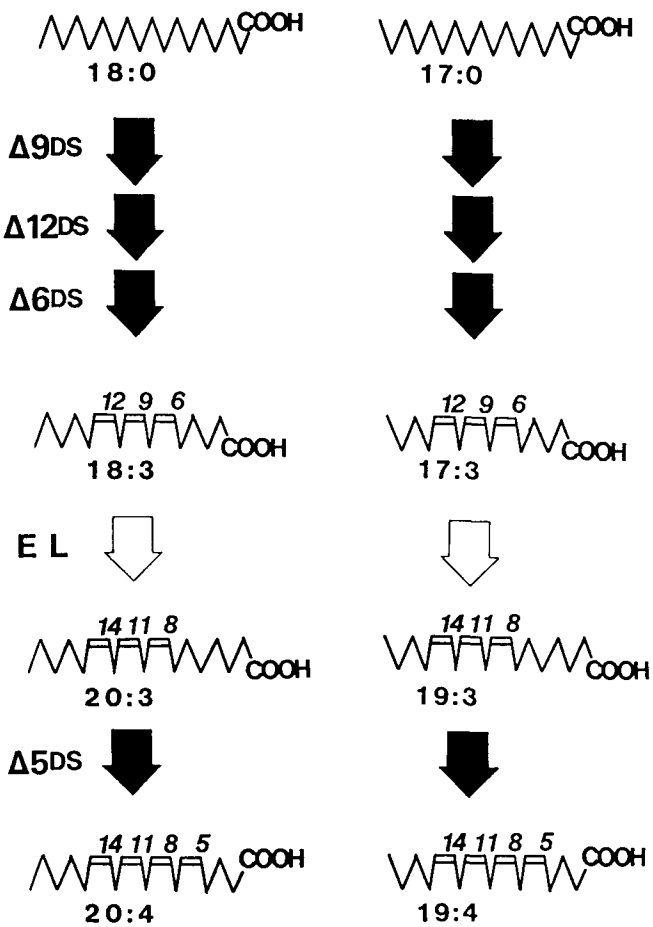

FIG. 1. Proposed pathway for the biosynthesis of odd chain PUFAs in *Mortierella*. Abbreviations used: DS, desaturation; EL, elongation. For other abbreviations, see Tables 1 and 2.

of Δ5 desaturation. The composition of the other mycelial fatty acids does not change when the fungus is grown with (+)sesamin. Here we show that mycelial $C_{19:3}$ acid also increased with an accompanying decrease in $C_{19:4}$ acid when *M. alpina* 1S-4 was grown with *n*-pentadecane in the presence of (+)sesamin. This phenomenon suggests that both the desaturation of $C_{19:3}$ acid to $C_{19:4}$ acid and that of dihomo-γ-linolenic acid to arachidonic acid are catalyzed by the same enzyme. This also supports the above assumption that the biosynthesis of odd chain PUFAs mimics that of even chain PUFAs.

About 90% of the supplemented *n*-pentadecane was incorporated into the mycelia of *M. alpina* 1S-4, and about 80% of that was consumed by the fungus. Twelve percent of the consumed *n*-pentadecane was converted and accumulated as mycelial fatty acids, but the rest of the consumed *n*-pentadecane is considered to have also been converted to pentadecanoic acid, which is then metabolized to various cellular components. This seems to be reflected by the fact that the percentage of $C_{15:0}$ acid in the TG-fraction derived from the mycelia grown in the NA medium was very high (56.8%) (Table 5). The PUFA contents then were low ($C_{19:4}$, 1.7%; and $C_{20:4}$, 0.4%). On the other hand, the PL-fraction of the same mycelia contained relatively lower percentages of $C_{15:0}$ (15.4%) and $C_{17:0}$ (7.5%) acids and higher percentage of $C_{19:4}$ (14.0%) and $C_{20:4}$ (3.9%). Since all odd chain fatty acids were found not only in the TG-fraction but also in the PL-fraction, they are considered to occur and function as constituents in biomembranes. The higher percentages of PUFAs such as $C_{19:4}$ and $C_{20:4}$ acids in the PL-fraction show that these PUFAs are necessary for the functions of a biomembrane and are probably selectively incorporated into the biomembrane.

ACKNOWLEDGMENT

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. Sen, N., and H. Schlenk, *J. Am. Oil Chem. Soc. 41*:241 (1964).
2. Schlenk, H., D.M. Sand and N. Sen, *Biochim. Biophys. Acta 84*:361 (1964).
3. Schlenk, H., and D.M. Sand, *Ibid. 144*:305 (1967).
4. Hoffmann, B., and H.J. Rehm, *Eur. J. Appl. Microbiol. 5*:189 (1978).
5. Mishina, M., S. Yanagawa, A. Tanaka and S. Fukui, *Agric. Biol. Chem. 37*:863 (1973).
6. Yamada, H., S. Shimizu and Y. Shinmen, *Ibid. 51*:785 (1987).
7. Yamada, H., S. Shimizu, Y. Shinmen, H. Kawashima and K. Akimoto, *J. Am. Oil Chem. Soc. 64*:1254 (1987).
8. Yamada, H., S. Shimizu, Y. Shinmen, H. Kawashima and K. Akimoto, in *World Conference on Biotechnology for the Fats and Oils Industry*, edited by T. Applewhite, American Oil Chemists' Society, Champaign, IL, 1988, pp. 173-177.
9. Shimizu, S., K. Akimoto, H. Kawashima, Y. Shinmen and H. Yamada, *J. Am. Oil Chem. Soc. 66*:237 (1989).
10. Shinmen, Y., S. Shimizu, K. Akimoto, H. Kawashima and H. Yamada, *Appl. Microbiol. Biotechnol. 31*:11 (1989).
11. Shimizu, S., H. Kawashima, Y. Shinmen, K. Akimoto and H. Yamada, *J. Am. Oil Chem. Soc. 65*:1455 (1988).
12. Shimizu, S., H. Kawashima, Y. Shinmen, K. Akimoto and H. Yamada, *Ibid. 66*:342 (1989).
13. Shimizu, S., H. Kawashima, K. Akimoto, Y. Shinmen and H. Yamada, *Appl. Microbiol. Biotechnol. 32*:1 (1989).
14. Shimizu, S., Y. Shinmen, H. Kawashima, K. Akimoto and H. Yamada, *Biochem. Biophys. Res. Commun. 150*:335 (1988).
15. Yamada, H., S. Shimizu, Y. Shinmen, H. Kawashima and K. Akimoto, *J. Dispersion Sci. Technol. 10*:561 (1989).
16. Shimizu, S., and H. Yamada, in *Biotechnology of Vitamin, Growth Factor and Pigment Production*, edited by E. Vandamme, Elsevier Science Publishers, London and New York, 1989, pp. 105-121.
17. Shimizu, S., K. Akimoto, H. Kawashima, Y. Shinmen, S. Jareonkitmongkol and H. Yamada, *Agric. Biol. Chem. 53*:1437 (1989).
18. Bligh, E.G., and W.J. Dyer, *Can. J. Biochem. Physiol. 37*:911 (1959).

[Received October 18, 1990; accepted December 6, 1990]