REVIEW

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Very-Long-Chain Fatty Acids from Lower Organisms

T. Řezanka, J. Cudlín and M. Podojil

Department of Biogenesis of Natural Products, Institute of Microbiology, Czechoslovak Academy of Sciences, 142 20 Prague 4

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ABSTRACT. The qualitative occurrence and quantitative proportion of very-long-chain fatty acids (above C_{22}), mainly in lower organisms and briefly in higher plants and animals is described.

Contents

1 Introduction

The quantitative proportion and qualitative composition of fatty acids in various organisms is characteristic for every given species and genus and depends also on the environment. In microbial cells fatty acids are mostly bound in lipids. Their qualitative composition is richer than in higher plants or animals and reflects the biological and biochemical role they play in the cell. Microbial fatty acids usually contain 10—20 carbon atoms (most often 15—19) and can be divided into five types:

1. saturated with straight chain
2. unsaturated with straight chain (rarely found in bacteria, more abundant in photosynthesizing microorganisms)

3. branched
4. with cyclopropane ring
5. with oxygen in the molecule (mostly 2-hydroxy acids).

In most bacteria and other microorganisms the major acid is palmitic, followed by oleic and myristic acids, and then by other acids typical of the species. Saturated and unsaturated fatty acids with carbon chains shorter than ten and longer than twenty atoms are less common.

TABLE I. Nomenclature of the common very-long-chain fatty acids

Numerical symbol	Systematic name (acid)	Trivial name (acid)
24 : 0	tetracosanoic	lignoceric
15-24 : 1	15-tetracosenoic	nervonic
26 : 0	hexacosanoic	cerotic
28 : 0	octacosanoic	montanic
30 : 0	triacontanoic	melissic

2 Nomenclature

Because in the beginning of organic chemistry fatty acids were among the most intensively studied compounds they have been given, and still retain, trivial names: the same holds for very-long-chain fatty acids (Table I). Systematic names are formed by appending the suffix *-oic acid* to the stem of the name of the parent hydrocarbon; the carboxyl carbon is taken as number 1.

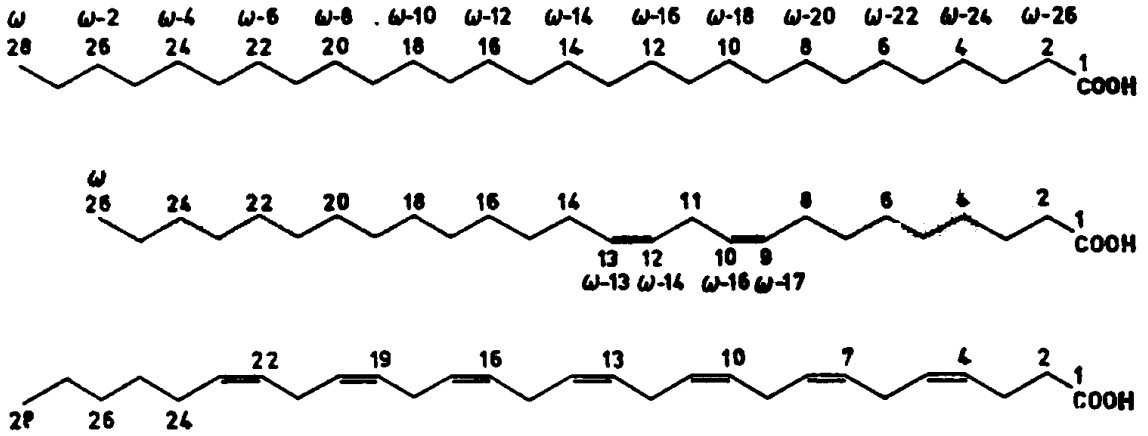


FIG. 1. Numbering and designation of fatty acids: *top*: octacosanoic acid (montanic acid; 28 : 0), *middle*: 9,12-hexacosadienoic acid (9,12-26:2), *bottom*: 4,7,10,13,16,19,22-octacosaheptaenoic acid (4,7,10,13,16,19,22-28:7).

According to the usual designation, 26:2 stands for an acid with 26 carbon atoms and with two double bonds (Fig. 1). Numerals preceding the hyphen give the position of the double bonds (*e.g.*, 5,9—26:2) beginning from the carboxyl. The double bonds are assumed to be in *cis*-configuration (*i.e.* *Z*-configuration; IUPAC 1970). The letters br denote branching (usually

with methyl group), c or cy a cyclopropane ring, prefix *i* (*iso*) or *ai* (*anteiso*) denote a methyl group substitution on the ω-1 or ω-2 end (precursors valine, leucine or isoleucine; *cf.* Table XI). The prefix ω denotes the position of the double bonds from the *methyl end*, the double bonds being assumed to be isolated ($CH_2-CH=CH-CH_2-CH=CH-CH_2$) (Thiele 1979).

This review deals particularly with fatty acids with 23 and more carbon atoms, usually called *very-long-chain fatty acids*.

3 Isolation, separation, identification

The conventional separation of very-long-chain fatty acids includes the hydrolysis of total lipids or their parts (*e.g.* phospholipids, glycolipids) and conversion to methyl esters (for GLC, GC—MS) or phenacyl esters (HPLC). This operation is sometimes preceded by a preliminary separation and accumulation of acids according to the number of double bonds (Hg adducts, impregnation of silica gel with $AgNO_3$) or according to the chain length (TLC, RP-TLC, RP-HPLC, CC on Sephadex LH-20). Separation of very-long-chain fatty acids is accomplished by HPLC or GLC on capillary or packed columns. Identification makes use of GLC (comparison of elution times with standards, use of Kovacz indexes) or mass spectrometry (GC—MS or a direct inlet into the mass spectrometer after isolation of individual components). The GC—MS method provides a much more complete information and makes a full identification of the acids possible. The position of double bonds is determined by their oxidative cleavage (with ozone or a permanganate—periodate mixture) or by means of conversion of the acids to pyrrolidides. Before the cleavage of double bonds individual compounds can be isolated by, *e.g.*, preparative GLC or by HPLC. In simpler mixtures the double bonds can be cleaved directly without previous isolation of individual compounds but the procedure yields a mixture of oxidative fragments.

In the separation of a complex of fatty acids with high molar mass the GLC can be used with methyl esters up to C_{40}. Fatty acids with $C_{30}-C_{56}$ and mycolic acids can be separated by RP-HPLC (Takayama *et al.* 1979; Qureshi *et al.* 1978). HPLC can be employed to separate acids having a difference of two CH_2 groups but the separation of the homologues is not always complete. Takayama *et al.* (1979) published a fundamental scheme of separation, isolation and identification of individual fatty acids of the non-mycolic type. Extraction of a mycelium with a chloroform—methanol mixture and a subsequent extraction of the evaporation residue with acetone transfers triacylglycerols into acetone and precipitates phospholipids (Walker *et al.* 1970). ^{14}C-Labelled fatty acids are added to facilitate detection and hydrolysis is performed to obtain free acids. The fractions of very-long-chain fatty acids are enriched on a silica gel column, the acids are separated according to the chain length on Sephadex LH-20 and derivatized to 4-bromophenacyl esters. Further separation is carried out by TLC with $AgNO_3$ impregnation, another TLC step, and RP-HPLC is finally used for separating the acids according to the chain length. Individual peaks are collected and very-long-chain fatty acids are identified by their mass spectra. The following types of acids were separated by this procedure (Takayama *et al.* 1979):

$CH_3(CH_2)_a-CH=CH-(CH_2)_b-CH=CH-COOH$ $\quad a+b=18, 20, 22$

$CH_3-(CH_2)_a-CH=CH-(CH_2)_b-COOH$ $\quad a+b=18-24, 26, 29, 35$

$$CH_3-(CH_2)_a-\left\{\begin{array}{l}-(CH\overset{CH_2}{\diagup\!\diagdown}CH)_x-\\ -(CH_2)_b-\\ -(CH=CH)_y-\end{array}\right\}-(CH_2)_c-COOH$$

$a+b+c=26-38$

$x, y = 1, 2$

The second of these three types included the longest monoenoic fatty acid identified so far, 39 : 1 (for $a + b = 35$).

The advances in GLC achieved at the end of the seventies and the beginning of the eighties marked the routine use of capillary columns with programmed temperature regime and a suitable stationary phase up to 350 °C and the appearance on the market of sophisticated and routinely applicable GC—MS instruments. The wide-spread use of HPLC also contributed to the separation and identification of very-long-chain fatty acids. For instance, Litchfield *et al.* (1976) were still using preparative GLC whereas Ayanoglu *et al.* (1983) already used HPLC for separation of individual acids.

Fatty acids were analyzed as 3-methoxyphenacyl esters by HPLC (detection at 254 nm). Elution was performed with a water—acetonitrile gradient up to pure acetonitrile for 200 min; during this time acids of up to C_{32} were eluted from a column with phase μ-Bondapak C-18 (Busell *et al.* 1979). Aveldano *et al.* (1983) separated very-long-chain fatty acids either free or as methyl esters from phospholipids on phase RP-8, with elution using pure acetonitrile and detection at 192 nm. The method yielded both saturated (up to 28 : 0) and monoenoic (up to 32 : 1) acids.

The positions of double bonds were determined both by classical oxidative cleavage (Morales and Litchfield 1976) and by MS of pyrrolidides (Anderson 1978; Ayanoglu *et al.* 1982) and chemical ionization (Lankelma *et al.* 1983).

A novel approach to the determination of the double bond position made use of the MIKES/CID method (Cervilla and Puzo 1983) for identification of monoenoic acids from *Mycobacterium phlei*.

4 Occurrence

4.1 Autotrophic lower organisms

Among photosynthesizing microorganisms the largest variability in the chain length of fatty acids is displayed by photosynthesizing protozoa (Table II). The marine protozoan *Emiliana huxley* was found to contain two unusual acids, 36 : 2 and 36 : 3 (Volkman *et al.* 1981), the nearest lower homologues having 14 less carbon atoms. The marine diatom *Stauroneis amphioxys* (Gillan *et al.* 1981) contains acids of up to 30 : 0, the freshwater protozoan *Euglena gracilis* possesses fatty acids up to C_{28} (Rosenberg 1963) and the fresh-water green alga *Botryococcus braunii* has unsaturated acids 26 : 1, 28 : 2 (Douglas *et al.* 1969). Řezanka *et al.* (1983) described saturated fatty acids of up to C_{26} in the alga *Scenedesmus acuminatus*, acids of up to 28 : 0 in autotrophically cultivated alga *Chlorella kessleri* and 30 : 0 in the blue-green alga *Spirulina platensis*. Comparison of the occurrence of very long fatty acids in *C. kessleri* cultivated under autotrophic and heterotrophic conditions yielded interesting results (Řezanka and Podojil 1984) in that monoenoic acids up to 36 : 1 were found under the latter circumstances.

TABLE II. Content of very-long-chain acids in autotrophic microorganisms

Organism	Acid (%)	Reference
Botryococcus braunii[a]	24 : 0, 25 : 1, 28 : 1, 28 : 2, 23 : 0[b], 24 : 0[b], 26 : 0[b], 28 : 0[b], 30 : 0[b]	Douglas *et al.* 1969
Chlorella kesleri[a]	23 : 0 (0.11), 24 : 0 (0.49), 24 : 1 (0.16), 26 : 0 (0.53), 28 : 0 (0.23)	Řezanka *et al.* 1983
C. kesleri[f]	23 : 0 (0.29), 24 : 0 (2.38), 15-24 : 1 (0.66), 25 : 0 (0.28), br_2-25 : 0 (0.25), 26 : 0 (0.83), 8-26 : 1[c] (1.15), 27 : 0 (0.18), 28 : 0 (0.73), 10-28 : 1[c] (0.63),‖ 29 : 0 (0.10), br_2-29 : 0[d] (0.036), 11-29 : 1[c] (0.063), 30 : 0 (0.13), 12-30 : 1[c] (0.60), 13-31 : 1[c] (0.039), 14-32 : 1[c] (0.146), 15-33 : 1[c] (0.018), 16-34 : 1[c] (0.026), 17-35 : 1[c] (0.020), 18-36 : 1[c] (0.021)	
C. vulgaris	23 : 0 (0.36), 24 : 0 (1.76), 24 : 1 (0,07), 25 : 0 (0.96), br_2-25 : 0 (0.08), 26 : 0 (1.38), 27 : 0 (0.23), 28 : 0 (0.12)	
Euglena gracilis	23 : 1 + 22 : 2 (5.8), 24 : 1 (2.0), C_{25} (1.1), C_{26} (0.4)	Rosenberg 1963
Scenedesmus acuminatus	24 : 0 (2.77), 25 >0 (0.85), 26 : 0 (1.12)	Řezanka *et al.* 1983
Spirulina platensis	23 : 0 (0.13), 24 : 0 (0.46), 25 : 0 (0.12), 26 : 0 (0.22), 28 : 0 (0.17), 30 : 0 (0.11)	
Stauroneis amphioxys[e]	23 : 0 (0.13), 24 : 0 (0.08), 25 : 0 (0.07), 26 : 0 (tr), 27 : 0 (tr)	Gillan *et al.* 1981
S. amphioxys[h]	23 : 0 (0.10), 24 : 0 (0.09), 25 : 0 (0.11), 26 : 0 (0.14), 27 : 0 (0.12), 28 : 0 (0.12), 29 : 0 (0.09), 30 : 0 (0.10)	

[a] Per cent proportion not given.
[b] Found after hydrogenation.
[c] Unpublished data.
[d] Řezanka and Podojil (1984).
[e] Autotrophic cultivation.
[f] Heterotrophic cultivation.
[g] At 3 °C.
[h] At 20 °C.

TABLE III. Abundance of isomeric monoenoic very-long-chain fatty acids in the genus *Mycobacterium*[a] (100 = base peak)

Acid	Position of double bond																
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
22 : 1	9	100	6	—	—	—	—	—	—	12[b]	100[b] 100[c]	6[b] 45[c]	6[b] 27[c]	—	—	—	—
23 : 1	—	29	100	19	4	—	—	—	—	—	—	—	—	—	—	—	—
24 : 1	—	—	100	7	6	4	—	—	—	—	—	—	100[b] 100[c]	13[b] 12[c]	20[b] 6[c]	—	—
25 : 1	—	—	100	70	69	27	24	2	—	—	—	—	—	—	—	—	—
26 : 1	—	—	24	1	100	9	77	16	1	9	—	—	—	17[c]	100[c]	17[c]	34
27 : 1	—	—	4	4	32	55	100	39	9	3	—	—	—	—	—	—	—
28 : 1	—	—	—	—	14	2	100	7	15	11	—	—	—	—	—	—	—
29 : 1	—	—	—	—	—	4	70	100	69	65	10	25	—	—	—	—	—
30 : 1	—	—	—	—	—	—	7	tr	100	9	47	—	—	—	—	—	—
31 : 1	—	—	—	—	—	—	—	9	62	100	96	66	74	—	—	—	—
32 : 1	—	—	—	—	—	—	—	—	10	1	100	tr	61	—	—	—	—

[a] Unindexed data: *M. tuberculosis* (Takayama *et al.* 1978); Asselineau *et al.* (1970) found in *M. phlei:* 5-22 : 1, 5-24 : 1, br-27 : 1, 8-26 : 1 and 10-26 : 1 without giving the amounts.
[b] *M. bovis* (Hung and Walker 1970).
[c] *M. smegmatis* (Hung and Walker 1970).

4.2 Heterotrophic lower organisms

The genus *Mycobacterium* is characterized by a broad spectrum of non-mycolic acids C_{16}—C_{36} (Takayama *et al.* 1978) with a major proportion of palmitic acid. In mycobacteria, relatively short chain fatty acids (C_{14}—C_{24}) are an integral part of neutral lipids — triacylglycerols (Walker *et al.* 1970). As compared with other organisms, the phospholipids contain shorter-chain fatty acids than triacylglycerols do. The selectivity of position in the triacylglycerols is marked since in *Mycobacterium bovis* 76 % of acids with more than 20 carbon atoms are bound to carbon 3 of glycerol. The function of other fatty acids (above C_{26}) which were discovered in the last decade is as yet unknown and so is their association in the lipids. The monoenoic acids with the highest proportion are those with C_{24}—C_{32}.

The position of double bonds in monoenoic fatty acids of the genus *Mycobacterium* was studied by several authors with highly controversial results (Table III). Asselineau *et al.* (1970) found the double bonds in positions 5, ω-16 and ω-18, Takayama *et al.* (1978) in positions ω-18 and ω-19; the latter authors also reported on an enzyme that introduces a double bond into position 5 of a 24 : 0 acid, and an elongation of this acid by C_2-units. Hung and Walker (1970) found double bonds in positions 13, 15 and 17, i.e. in position ω-9 for 22 : 1, 24 : 1 and 26 : 1 acids. These results correspond to a simple elongation of palmitoleic acid (9-16:1) by C_2-units. These data stress the obviously incomplete knowledge of biosynthesis of monoenoic acids and also the probably large variability of enzyme systems in mycobacterial species.

M. phlei was found to contain unusual very-long-chain polyenoic fatty acids, *e.g.* 4,8,12,16,20-36:5 or 4,8,12,16,20,24-40:6 (Asselineau *et al.* 1969). In contrast to these polyenoic acids the presence of branched (methyl-substituted) acids is common. Asselineau *et al.* (1959) found 2,4,6,8-tetramethyloctacosanoic acid formed by a gradual elongation of eicosanoic acid by four propionate units (Ratledge 1976). Another type of branched fatty acids are homologues of tuberculostearic acid, *e.g.* 24-methylhexacosanoic and 26-methyltetracosanoic acids (Campbell and Naworal 1969).

In 1951 Bergmann and Swift (1951) first described the presence of acids with high molar mass in two marine sponges, *Spheciospongia vesparia* and *Suberites compacta*. Fraction distillation was used to isolate 17,20-26:2 and 9-26:1 acids from the former and 28:3 and 28:1 acids from the latter. A logical extension of this work was the examination of other genera of marine sponges from the class *Demospongiae*.

From the sponge *Microciona prolifera* (Jeffers *et al.* 1974; Morales and Litchfield 1976) tens of fatty acids have been successfully isolated; they were called demospongic fatty acids by Litchfield (Table IV). They are highly unsaturated very-long-chain fatty acids with typical positions of double bonds (mostly isolated) 5,9-26:2 and 27:2,9,19-26:3 and 5,9,21-28:3 acids with possible chain branching (Tables IV and V).

A simple screening was used to determine the proportion of very-long-chain fatty acids in subclasses of the class *Demospongiae* (Litchfield *et al.* 1976). Extraction of total lipids, isolation of acids and GLC after preceding hydrogenation was performed in 17 fungal species. Fatty acids with C_{22}—C_{30} made up 34—79 % of all fatty acids (Table V) with major proportion of a C_{26}-acid. C_{28}-acids (8—17 %) were present in four genera, C_{30}-acids have a relative proportion of up to 38 % in the genus *Chondrilla* and 11 % in

TABLE IV. The content of very-long-chain fatty acids in marine sponges from the Atlantic

Organism	Acid (%)	Reference
Aplysina fistularis		Walkup *et al.* 1981
PL	br_{26}-5,9-26 : 2 (0.5), 5,9,21-28 : 3 (2.1), 5,9,23--28 : 3 (0.4), br_{28}-5,9-28 : 2 (6.1), 5,9,23-29 : 3 (0.6), 5,9,23-30 : 3 (5,6), 5,9-28 : 2 (tr), 5,9-31 : 2 (tr)	
PI	5,9,21-28 : 3 (1.0), br_{28}-5,9-28 : 2 (1.6), 5,9,23-30 : 3 (1.5)	
PS	br_{26}-5,9-26 : 2 (2.2), 5,9,21-28 : 3 (8.3), 5,9,23-28 : 3 (3.1), br_{28}-5,9-28 : 2 (20.0), 5,9,23-29 : 3 (1.4), 5,9,23-30 : 3 (24.5)	
PC	br_{28}-5,9-28 : 2 (0.6), 5,9,23-30 : 3 (0.6)	
PG	br_{28}-5,9-28 : 2 (1.1), 5,9,23-30 : 3 (1.0)	
PE + DPG	br_{26}-5,9-26 : 2 (2.4), 5,9,21-28 : 3 (3.6), 5,9,23-28 : 3 (3.0), br_{28}-5,9-28 : 2 (17.8), 5,9,23-29 : 3 (1.2), 5,9,23-30 : 3 (18.4)	
Calyx nicaeensis	5,9-26 : 2(6.1), *i*-5,9-27 : 2 (9.9), *ai*-5,9-27 : 2 (20.1), c-19-27 : 0 (0.6), 4-23 : 1 (tr)	Lankelma *et al.* 1983

Cliona celata	15, 18, 21, 24-30 : 4 (3.4), 15, 18, 21, 24, 27-30 : 5 (7.0)	Lichtfield *et al.* 1979
Higginsia tethyoides	2-MeO-23 : 0 (0.6), 2-MeO-17-24 : 1 (1.9), 2-MeO-24 : 0 (3.2), 2-MeO-18-25 : 1 (0.5), 2-MeO-5, 19-26 : 2 (2.7), 2-MeO-19--26 : 1 (1.3), 2-MeO-7, 21-28 : 2 (2.7), 2-MeO-21-28 : 1 (13.7), 5, 9, 23-30 : 3 (34.2), 2-MeO-16-23 : 1 (tr), 2-MeO-26 : 3 (tr), 2-MeO-20-25 : 1 (tr), 2-MeO-28 : 3 (tr), 30 : 2 (tr)	Ayanoglu *et al.* 1983
Microciona prolifera	5, 9-26 : 2 (14), 5, 9, 19-26 : 3 (31)	Jeffers *et al.* 1974
Microciona prolifera	*i*-23 : 0 (0.2), 23 : 0 (0.2), 23 : 1 (0.1), 23 : 1 (0.1), 24 : 0 (1.9), 7-24 : 1 (tr), 9-24 : 1 (0.1), 15-24 : 1 (1.6), 17--24 : 1 (2.2), 5, 9-24 : 2 (0.4), 15, 18-24 : 2 (0.3), 17, 20-24 : 2 (tr), 24 : 3 (0.1), 15, 18, 21-24 : 3 (0.2), 25 : 0 (0.1), 25 : 1 (0.1), 25 : 1 (0.1), 5, 9-25 : 2 (1.3), 25 : 2 (0.1), 25 : 3 (0.3), 26 : 0 (0.1), 9-26 : 1 (0.6), 15-26 : 1 (0.1), 17-26 : 1 (0.4), 19-25 : 1 (4.0), 5, 9-26 : 2 (18.1), 17, 20-26 : 2 (1.6), 19, 22-26 : 2 (tr), 5, 9, 17-26 : 3 (0.7), 5, 9, 19-26 : 3 (12.2), 17, 20, 23-26 : 3 (0.4), 26 : ? (0.1), 27 : 1 (0.1), 27 : 2 (0.1), 27 : 3 (0.1), 5, 9, 19-27 : 3 (0.1), 5, 9, 20-27 : 3 (0.2), 28 : 1 (0.1), 28 : 2 (0.1), 28 : 3 (0.1)	Morales and Litchfield 1976
Petrosia ficiformis[a]		Ayanoglu *et al.* 1982
TL	*i*-5, 9-26 : 2 (3.8), 5, 9-26 : 2 (1.4), *i*-5, 9-27 : 2 (8.4), *ai*-5, 9-27 : 2 (18.4), br_{15}-23 : 0 (tr), br_{15}-24 : 0 (tr), 17-24 : 1 (tr)	
PI + PS	*i*-5, 9-26 : 2 (4.8), 5, 9-26 : 2 (2.1), *i*-5, 9-27 : 2 (11.8), *ai*-5, 9-27 : 2 (26.2)	
PC	*i*-5, 9-26 : 2 (1.4), *i*-5, 9-27 : 2 (6.6), *ai*-5, 9-27 : 2 (14.8)	
PG	*i*-5, 9-26 : 2 (1.9), 5, 9-26 : 2 (1.6), *i*-5, 9-27 : 2 (6.2), *ai*-5, 9-27 : 2 (14.2)	
PE	*i*- 5, 9-26 : 2 (4.4), 5, 9-26 : 2 (2.9), *i*-5, 9-27 : 2 (7.9), *ai*-5, 9-27 : 2 (20.8)	
Xestospongia halichondroides	17-26 : 1 (21), 5, 9-26 : 2 (27), 5, 9, 19-26 : 3 (13)	Litchfield and Marcantonio 1978

[a] PL phospholipids, PI phosphatidylinositol, PS phosphatidylserine, PC phosphatidylcholine, PG phosphatidylglycerol, PE phosphatidylethanolamine, DPG diphosphatidylglycerol, TL total lipids.

TABLE V. Distribution of very-long-chain fatty acids according to chain length (%) in sponges of the class *Demospongiae*

Fungus	Number of carbon atoms in chain									Reference
	23	24	25	26	27	28	29	30	Total	
Anthosigmella varians	tr	5	1	28	—	—	—	—	34	Litchfield *et al.* 1976
Axinella polycapella	—	7	2	50	15	1	—	—	75	
Cliona celata	tr	8	4	54	1	1	—	11	70	
Chondrilla nucula	—	1	tr	2	—	1	—	38	42	
Dysidea camera	tr	8	11	20	tr	—	—	—	39	
Halichondria panicea	tr	5	2	27	tr	1	—	—	35	
Haliclona oculata	—	11	tr	43	3	9	—	—	66	
Ictrochota birotulata	—	3	5	55	1	—	—	—	64	
Isodyctya deichmannae	—	1	2	55	—	3	—	—	61	
Lissodendoryx sp.	tr	16	3	41	tr	1	—	—	61	
Microciona prolifera	1	7	2	38	tr	tr	—	—	48	Morales and Litchfield 1976
Mycale fibrexilis	tr	8	1	52	—	—	—	—	61	Litchfield *et al.* 1976
Spheciospongia vesparia	—	4	—	39	—	15	—	—	58	Bergmann and Swift 1951
Spongia sp.	1	12	25	1	—	—	—	—	39	Litchfield *et al.* 1976
Spongilla lacustric	tr	18	6	30	tr	1	—	—	55	
Stelletta grubii	—	3	2	34	5	1	—	—	45	
Suberites compacta	—	12	—	22	—	17	—	—	51	Bergmann and Swift 1951
Tedania ignis	tr	11	4	46	1	—	—	—	62	Litchfield *et al.* 1976
Xestospongia halichondroides	—	2	1	39	6	15	—	—	64	
Xytopsene sigmatum	—	4	1	42	tr	—	—	—	47	

TABLE VII. Content of branched fatty acids (%) in phospholipids of the marine sponge *Stongylophora durissima*[a]

Acid	Total PL	PI + PS	PC	PG	PE
br_{19}-25 : 0	0.5	0.5	—	0.7	0.9
br_{19}-5-26 : 1	5.0	5.2	10.1	2.4	2.7
br_{19}-26 : 0	4.7	4.5	9.6	2.2	2.5
br_{19}-5-27 : 1	19.3	24.7	30.6	9.9	11.3
br_{19}-27 : 0	7.5	5.3	4.1	7.7	13.0
br_{19}-5-28 : 1	7.8	5.5	8.2	7.1	12.1
br_{19}-28 : 0	12.3	10.4	10.8	11.8	12.9
$br_{19,21}$-29 : 0	2.9	3.9	—	3.0	4.5
br_{20}-29 : 0	1.9	2.5	—	2.2	2.7
$br_{20,22}$-30 : 0	1.9	1.8	3.1	0.9	2.0

[a] For abbreviations see Table IV, footnote *a*.

the genus *Cliona*. The major part of the 3—8 % branched fatty acids with equivalent chain length (ECL) 23,41—23,47 is due to *i*-24:0 acid which is present in five genera.

Dembitsky (1981*a*,*b*), Dembitsky and Nebylitsyn (1980) and Dembitsky *et al.* (1977) found in marine sponges from the Asiatic coast of the Pacific ocean similar proportions of fatty acids as reported by other authors from the Atlantic (Tables IV and VI) with major amounts of 5,9,19-26:3, 5,9-26:2 and 5,9-28:2 acids. Interesting results were obtained with sponges from the Baikal lake; these sponges contain more odd-number fatty acids 23:0 and

TABLE VIII. Content of very-long-chain fatty acids (%[a]) in *S. cerevisiae* in different growth phases

Acid	Growth phase			
	mid log	late log	early stationary	late stationary
23 : 0	—	—	—	—
23 : 1	3	2	1	0.5
24 : 0	5	4	2	2
24 : 1	1	1	1	tr
25 : 0	4	1	4	4
25 : 1	2	1	1	tr
26 : 0	38	64	49	58
26 : 1	3	tr	4	tr
27 : 0	6	4	5	6
27 : 1	2	1	0.5	tr
28 : 0	4	5	12.5	12
28 : 1	3	1	tr	tr
29 : 0	1	1	1	1
30 : 0	1	0.5	0.5	2
C_{31}	1	0.5	0.5	tr
C_{32}	1	0.5	0.5	tr
C_{33}	tr	tr	tr	tr
C_{34}	tr	tr	tr	tr

[a] 100 % = C_{19}—C_{34} (Welch and Burlingame 1973).

TABLE IX. Content of very-long-chain fatty acids in yeast fractions (%)

Acid	Cells[a]	Sphingolipids[a]	Neutral lipids[a]	Neutral glycolipids[a]	Glycerophosphatides[a]	Acid glycolipids[a]	Waxes[b,c]		Sterol esters[b,c]		Glycolipids[b,d]
							CC	PM	CC	PM	
23 : 0	0.2	—	3.8	—	4.3	2.4	8.8	3.8	3.2	0.4	1.3
24 : 0	0.8	0.9	2.2	2.0	25.9	2.0	4.8	2.9	2.9	0.3	7.5
2-OH-24 : 0	2.4	4.0	—	2.0	—	0.9	—	—	—	—	—
25 : 0	2.0	0.6	4.0	2.0	1.3	2.0	2.8	tr	1.9	0.3	tr
26 : 0	12.9	21.1	16.0	6.0	4.9	7.5	—	—	1.1	—	—
2-OH-26 : 0	42.6	68.3	—	7.0	—	31.5	—	—	—	—	—

[a] Nurminen and Suomalainen (1971).
[b] Baraud *et al.* (1970).
[c] CC cell content, PM protoplast membrane.
[d] Protoplast membrane.

TABLE VI. Very-long-chain fatty acids (%) found in Pacific coast and Baikal lake sponges

Acid	*Adocia cinerea*[a]	*Baicalospongia bacillifera*[d]	*B. intermedia*[d]	*Cornacuspongia* gen. sp.[a]	*Halichondria panicea*[a]	*H. panicea*[b]	*Homaxinella rubiloa*[a]	*Lubomirskia abietina*[a]	*L. baicalensis*[a]	*L. fursifera*[a]	*Myxilla incrustans*[a]	*Ophlitaspongia pennata*[a]	*Phakellia ribosa*[a]	*Suastochemokia papyracea*[d]	*Suberites domuncula*[a]	*Tylodesma* aff. *rosea*[a]
23 : 0	—	0.4	0.4	—	—	—	—	0.3	0.3	0.2	—	—	—	0.4	—	—
24 : 0	1.2	0.8	0.7	5.0	2.6	0.8	0.9	0.8	1.1	0.9	1.8	1.1	1.6	0.9	4.0	1.2
24 : 1	0.8	—	—	1.1	2.0	1.0	10.3	—	—	—	2.8	4.4	0.8	—	5.4	0.5
25 : 0	0.7	0.3	0.6	1.8	0.9	—	0.5	0.3	0.4	0.4	0.4	—	4.6	0.4	—	5.6
25 : 1	0.3	—	—	—	0.8	0.1	—	—	—	1.2	—	1.1	—	—	—	0.9
25 : 2	—	1.1	0.6	—	—	0.3	—	0.9	1.0	1.4	—	—	—	4.9	—	—
26 : 0	—	0.5	0.4	—	—	—	—	0.3	0.6	0.3	—	—	—	0.5	—	—
26 : 1	1.1	1.1	0.8	—	—	—	1.4	0.9	0.5	1.2	—	—	—	1.3	0.7	—
26 : 2[e]	5.6	1.9	6.1	16.3	31.4	4.9	17.8	3.0	2.0	3.7	5.3	1.8	21.1	2.4	24.9	19.4
26 : 3[e]	—	21.7	20.3	8.1	17.4	26.7	2.4	18.1	16.7	15.9	7.7	20.1	6.3	19.1	—	3.7
27 : 0	—	—	0.3	—	0.8	—	—	0.1	0.2	0.1	—	—	—	0.2	—	—
28 : 0	1.3	0.5	0.4	—	2.2	—	—	0.6	0.7	0.5	0.8	—	0.3	0.8	2.2	—
28 : 1	18.2	0.9	0.5	—	0.1	—	1.1	0.4	0.6	0.8	4.5	—	5.7	1.1	3.1	0.8
28 : 2[e]	4.2	5.8	0.3	—	0.9	—	1.2	0.4	0.5	0.2	1.7	—	0.5	1.1	0.9	5.9
28 : 3	—	—	—	—	—	—	12.3	—	—	—	—	—	—	—	17.8	—

[a] Dembitsky and Nebylitsyn (1990).
[b] Dembitsky *et al.* (1977).
[c] Dembitsky (1981*a*).
[d] Dembitsky (1981*b*).
[e] Major: 5, 9-26 : 2; 5,9,19-26:3; 5,9-28:2; minor: 17,20-26:2; 19,22-26:2; 5,9,23-26:3; 17,20,23-26:3; 5,9,17-26:3.

TABLE X. Content of very-long-chain fatty acids in heterotrophic microorganisms

Organism	Acid (%)	Reference
Alternaria tenuis	22 : 0 (1.8), 24 : 0 (3.0), 25 : 0 (1.2), 26 : 1 (tr), 26 : 0 (tr), 28 : 1 (0.8)	Fisher *et al.* 1972
Botrytis fabae[a]	22 : 0 (16.3), 23 : 1 (8.6), 24 : 0 (3.5)	
B. fabae[b]	22 : 0 (2.1), 24 : 0 (1.4), 25 : 0 (3.4), 25 : 1 (1.2), 26 : 0 (2.1), 26 : 1 (1.6)	
Cochliobolus miyabeanus	24 : 0 (4.9), 26 : 0 (2.1)	Wassef 1977
Cryptococcus albidus	22 : 0 (3.3), 24 : 0 (6.9)	Zviagintseva *et al.* 1975
Curvularia sp.	24 : 0 (2.4, 24 : 1 (9.0)	Wassef 1977
Erysiphe graminis	24 : 0 (7.6), 24 : 1 (6.5)	
Fomes igniarius	22 : 0 (1), 24 : 0 (26), 25 : 0 (4.4), 26 : 0 (15.4), 27 : 0 (1.9), 28 : 0 (23.9), 30 : 0 (tr), 32 : 0 (tr)	Wassef 1977
Lactobacillus heterohiochi H1	24 : 0 (1.9), 17-24 : 1 (3.6), 26 : 0 (0.2), 19-26 : 1 (tr)	Uchida 1974
L. heterohiochi 814	24 : 0 (4.2), 17-24 : 1 (4.9), 26 : 0 (1.4), 19-26 : 1 (0.7), 28 : 0 (0.8), 21-28 : 1 (0.3), 30 : 0 (0.2), 23-30 : 1 (0.1)	
L. heterohiochi 846	24 : 0 (4.9), 17-24 : 1 (4.0), 26 : 0 (1.1), 19-26 : 1 (0.3), 28 : 0 (0.3), 21-28 : 1 (tr)	
Lipomyces anomalus L-386	22 : 0 (3.3), 24 : 0 (6.9)	Zviagintseva *et al.* 1975
Neurospora crassa[b]	22 : 0 (1.6), 24 : 0 (1.2), 24 : 1 (1.6)	Fisher *et al.* 1972
N. crassa[a]	22 : 0 (1.8)	
Penicillium pulvillorum	22 : 0 (1), 24 : 0 (4.9), 26 : 0 (1.1)	Wassef 1977
Phycomyces blakesleanus	24 : 1 (2.9), 26 : 0 (1.3), 26 : 1 (4.9)	Shaw 1966
Rhodotorula glutinis A-328	22 : 0 (2.7), 24 : 0 (6.3)	Zviagintseva *et al.* 1975
R. rubra 1527	24 : 0 (0.4)	

R. rubra 1649	24 : 0 (2.2), 26 : 0 (1.9)	
R. rubra 1836	24 : 0 (2.5), 26 : 0 (2.4)	
Streptomyces cinnamonensis	23 : 0 (0.1), 24 : 0 (0.1)	Řezanka *et al.* 1984

[a] Cell wall.
[b] Surface.

27:0, otherwise the relative proportions of fatty acids are the same as in samples from other locations. This indicates that there may be no difference between marine and fresh-water sponges. The group of Djerassi (Walkup *et al.* 1981; Ayanoglu *et al.* 1982) separated individual types of phospholipids and determined the content of acids in each type, in spongal genera *Aplysia* and *Petrosia*. No marked changes were observed in *Petrosia* whilst *Aplysia* exhibits an uncommonly low content of the fatty acids under study in phosphatidylcholines and phosphatidylglycerols. Table IV points out the main types of desmospongic acids. In genus *Aplysia* they include $C_{(20)}$- and $C_{(22)}$-methyl substituted dienoic acids C_{26} and C_{28} or the typical 5,9,21-28:3 and 5,9,23-28:3 acids. The sponge *Higginsia tethyoides* was found to contain 2-methoxy acids in addition to common demospongic acids (Ayanoglu *et al.* 1983) whereas the sponge *Calyx niceaensis* contains a cyclopropanoic acid c-19-27:0 (Lankelma *et al.* 1983). Litchfield and co-workers (1979) found in the sponge *Cliona celata* tetra- and pentaenoic C_{30}-acids double bonds in positions atypical (*i.e.* isolated) for sponges but otherwise commonly found, *i.e.* ω-6 30:4 and ω-3 30:5.

The data obtained by Dasgupta *et al.* (1984) in their study of very-long-chain fatty acids, mostly branched, from the marine sponge *Strongylophora durissima* are summarized in Table VII.

The content of fatty acids in yeasts and yeast-like organisms was investigated in the genera *Lipomyces*, *Saccharomyces* and *Rhodotorula*. *S. cerevisiae* was reported to contain, apart from the common fatty acids C_{10}—C_{18}, also very-long-chain fatty acids (Table VIII) which make up about 1—2 % of all fatty acids. Up to 64 % of fatty acids with chain length above C_{18} was found to be represented by a 26 : 0 acid. Baraud *et al.* (1970) and Nurminen and Suomalainen (1971) determined the proportion of very-long-chain fatty acids and 2-hydroxy acids up to C_{26} in different parts of the cell and in different lipid fractions (Table IX). The major acid is again 26:0 even though, *e.g.* in sphingolipids, the 2-OH-26:0 acid makes up 68.3 % of all fatty acids longer than C_{18}. The highest amount of very-long-chain fatty acids is obviously found in phospholipids or glycolipids. Of interest is the occurrence of very-long-chain fatty acids in the form of esters with sterols (Baraud *et al.* 1970) which are known to form an important part of the yeast cell wall.

The presence of other hydroxy acids (*e.g. erythro*-2,3-dihydroxyhexacosanoic acid) was reported by Prostenik *et al.* (1973). The genus *Rhodotorula* was found to contain frequently acids above C_{22} (Table X). All studied species of genera *Rhodotorula*, *Lipomyces* and *Cryptococcus* contain very-long-chain fatty acids C_{22}—C_{26}. Acids with more than 22 carbon atoms are most often found in *Saccharomyces*, to be followed by *Rhodotorula*, *Lipomyces* and *Cryptococcus*.

Fatty acids were detected on the surface of conidia and sporangiospores of various fungi from genera *Alternaria*, *Botrytis* and *Neurospora*. They include usually saturated even-number acids up to C_{26}. Very-long-chain fatty acids up to C_{32} were found in *Fomes igniarius* (Epstein *et al.* 1966).

Both saturated and monoenoic fatty acids up to C_{30} are found also in *Lactobacillus heterohiochi* (Table X) (Uchida 1974) whereas the related species *L. homohiochi* does not contain acids above C_{20}.

Nichols *et al.* (1985), found that *Franchisella tularensis* an important pathogenic bacterium contains monoenoic fatty acids up to C_{26}. The position of the double bond was determined elegantly by means of cis- and *trans* dihydroxylation and also a Diels-Alder addition to the double bond. The resulting derivatives were analyzed by GC—MS. The double bonds were shown to be in position *cis*-9. The proportion of the corresponding acids (in per cent) was: 23:0 (tr), 9-24:1 (9.8), *trans*-15-24:1 (tr), 25:0 (tr), 9-26:1 (0.3), 26:0 (0.5).

The studies of Dormaar (1982) and Ketola *et al.* (1981) document the fact that little attention has been paid to the occurrence of very-long-chain fatty acids in soil microorganisms. Soil extracts contain acids up to C_{30}, peat was found to contain acids C_{14}—C_{30} (Ketola *et al.* 1981).

4.3 Higher plants

Waxes from higher plants contain very-long-chain fatty acids mostly saturated either conjugated as esters with long-chain aliphatic alcohols (up to C_{32} in the wax from *Cecropia adenopus;* Neidlein and Koch 1980) or as esters of sterols *e.g.* in lemon fruit which was used to isolate 24 : 0, 24 : 1, *i*-25 : 0, 25 : 0 *i*-26 : 0, 26 : 0, 26 : 1, *i*-27 : 0, *ai*-27 :.0, 27 : 0, *i*-28 : 0, 28 : 1, *ai*-29 : 0 and 29 : 0 acids (Nordby and Nagy 1974). The wax of sunflower seed coats yielded acids in the range of 24 : 0 to 30 : 0 (Popov and Stefanov 1968).

Cutin from spinach membranes (Holloway 1974) was found to contain even-number saturated acids up to C_{32}.

Plant-seed oils were found to contain fatty acids to a maximum of C_{22} (erucic acid) (Miller *et al.* 1965). Exception is represented by, *e.g.*, genera *Tropaeolum* (Litchfield 1970) and *Ximenia* (Ligthelm 1954) which were found to contain monoenoic acids ω-9 C_{24-30}.

Schmidt *et al.* (1984) found in the bark of *Schefflera octophylla* esters of 3α-hydroxylup-20(29)-ene-23,28-dioic acid with very-long-chain fatty acids, mostly branched in position 2, with the major proportion (in per cent) of 24 : 0 (50.6), br_2-25 : 0 (29.1), 20 : 0 (4.8), br_2-27 : 0 (5.2), br_2-29 : 0 (1.3). In their study of tissues of 18 kinds of higher plants (potato, oat, wheat, maize, cucumber) Murata *et al.* (1984) found higher fatty acids (saturated up to C_{26} and monoenoic up to C_{24}) in trace amounts bound in phosphatidylserines. In a condensate of marijuana smoke Maskarinec *et al.* (1976) identified 23 : 0, 24 : 0, 26 : 0, 26 : 2, and 28 : 0 acids.

Study of very-long-chain fatty acids in garlic (Agrawal *et al.* 1984) revealed that the elongation system is localized in microsomes and only saturated acids can be utilized as acceptors of malonyl-CoA as C_2-donor in the presence of this compound.

4.4 Animals

Kishimoto and Radin (1963) described unsaturated fatty acids in bovine

brain sphingolipids. They succeeded in identifying 24 monoenoic and dienoic acids up to C_{26} and determining their precursors (C_{16-18} acids).

Cerebrosides and sulfatides isolated from bovine brain were hydrolyzed to obtain fatty acids and 2-hydroxy acids in the elution sequence (capillary column with OV-101 phase): 23 : 0, OH-22 : 0, 24 : 1, 24 : 0, OH-23 : 0, 25 : 0, OH-24 : 1, OH-24 : 0, 26 : 1, 26 : 0, OH-25 : 1, OH-25 : 0, OH-26 : 1, OH-26 : 0 (Abe and Tamai 1982).

Ox and human eye glands were found to contain fatty acids up to C_{38} (Nicolaides *et al.* 1984) including *n*-, *iso*-, *anteiso*-acids, both saturated and monoenoic.

In the blood of patients suffering from adrenoleucodystrophy, HPLC was used to determine very-long-chain fatty acids 24 : 0—26 : 0, this assay was methodologically applied to the diagnosis of this disease (Kobayashi *et al.* 1983; Antoku *et al.* 1984). Very-long-chain fatty acids were also found in humans with other congenital metabolic disorders, *e.g.* the Refsum disease (Poulos and Sharp 1984) or the Zellweger syndrome (Bakkeren *et al.* 1984; Govaerts *et al.* 1985; Poulos *et al.* 1986).

GC—MS was employed to identify fatty acids, saturated, with both straight and branched chain (*iso*-, *anteiso*-) up to C_{29}, and monoenoic acids (15-24 : 1, 17-24 : 1) in the meibomian gland (Harvey and Tiffany 1984). Even-number monoenoic acids up to C_{34} were found on the surface of mouse skin (Wilkinson 1970).

Autotrophic algae serving as nutrition for higher animals can be traced as a direct source of very-long-chain fatty acids in the fat of sea fishes. Ferguson (1976) documented the direct dependence of the presence of fatty acids in the fat on the nutrition of the fish *Echinaster* sp. (algae to fish, %: *i*-23:0 0.4/1.6; 4,7,10,13,16,19-24:6 1.1/0.5; 16-25:1 0.9/0.1; 16-26:1 8.5/16.6). The herring (*Clupea harengus*) was found to contain very-long-chain polyenoic fatty acids (12,15,18,21-24:4 1.3 %; 9,12,15,18,21-24:5 1.4 %; 6,9,12,15,18,21-24:6 0.9 %; 11,14,17,20,23-26:5 0.5 %, 8,11,14,17,20,23-26:6 0.7 %; 4,7,10,13,16,19,22-28:7 0.4 %; the formula of the last acid is given in Fig. 1) (Linko and Karinkanta 1970).

The insect species studies so far are very few; in contrast to plants very-long-chain fatty acids are found predominantly as a component of waxes, *e.g.* in beeswax. Beeswax was found to contain esterified acids C_{24-34} (Downing *et al.* 1961; Stránský *et al.* 1972).

5 Biosynthesis

Biosynthesis of very-long-chain fatty acids proceeds in two stages:

1. synthesis of the "basic" fatty acid (usually C_{16}—C_{18});
2. elongation of this fatty acid by a gradual extension of the chain with C_2-units.

5.1 Biosynthesis of C_{16}—C_{18}-fatty acids

This stage includes the biosynthesis of common fatty acids on a multienzyme complex (fatty acid synthase; Vagelos 1973*a*). The basic building unit — in unbranched even-number acids the only one — is acetylcoenzyme A which is incorporated into the acid chain in the form of malonyl-[acyl-carrier protein]. The first step of synthesis was originally thought to be the

simple carboxylation of acetyl-CoA to malonyl-CoA, catalyzed by acetyl-CoA carboxylase (EC 6.4.1.2) (Utter 1961):

$$AcSCoA + CO_2 \xrightarrow{2.3.1.39} MaSCoA$$

Actually, the reaction is far more complex and involves, apart from biotin, at least three other enzymes (Dimroth *et al.* 1970; Guchhait *et al.* 1971)

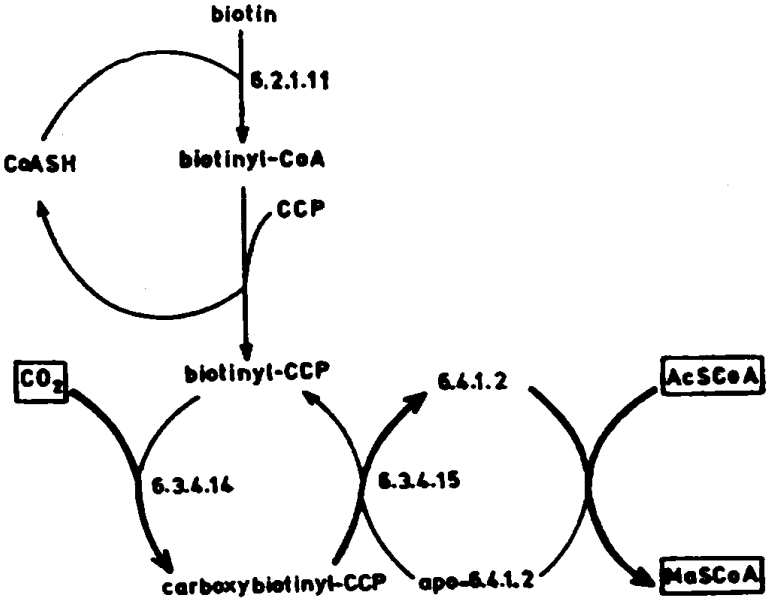


FIG. 2. Carboxylation of acetyl-CoA to malonyl-CoA; *thick arrows* denote the actual resulting reaction; CCP — carboxyl-carrier protein;
6.2.1.11 — biotin-CoA ligase,
6.3.4.14 — biotin carboxylase,
6.3.4.15 — biotin-[acetyl-CoA-carboxylase] ligase,
6.4.1.2 — acetyl-CoA carboxylase.

(Fig. 2). The next step includes the transfer of malonyl from acetyl-CoA to acyl-carrier protein (ACP) catalyzed by acyl-carrier-protein malonyl transferase (EC 2.3.1.39):

$$MaSCoA + ACP \xrightarrow{6.4.1.2} MaACP + CoASH$$

This acyl-carrier protein is the site of a repeating "merry-go-round" of four reactions and represents the actual "body" of fatty acid synthase (Fig. 3). The four reactions include:

1. condensation of MaACP (under its concomitant decarboxylation) with a "starter" unit; in the case of the first round and if the ensuing acid is an unbranched even-number acid, the starter unit is AcACP;
2. reduction of a keto group to hydroxy group;
3. dehydration (removal of hydroxy group) giving rise to a double bond;
4. reduction of the double bond.

During the synthesis of palmitic acid the cycle is repeated seven times, in stearic acid eight times.

Whereas the elongation unit during the building of C_{16}—C_{18}-acids is

TABLE XI. Five types of fatty acids synthesized after a 7-fold repetition of the fatty acid synthase reaction

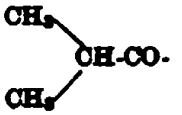
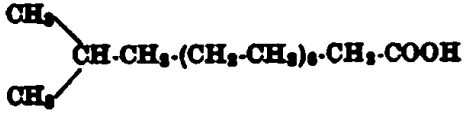
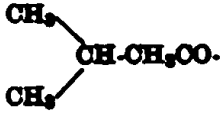
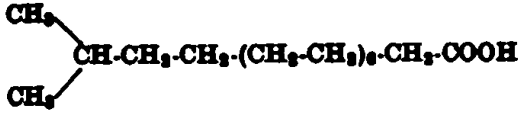
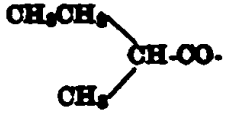
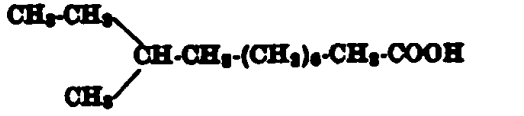
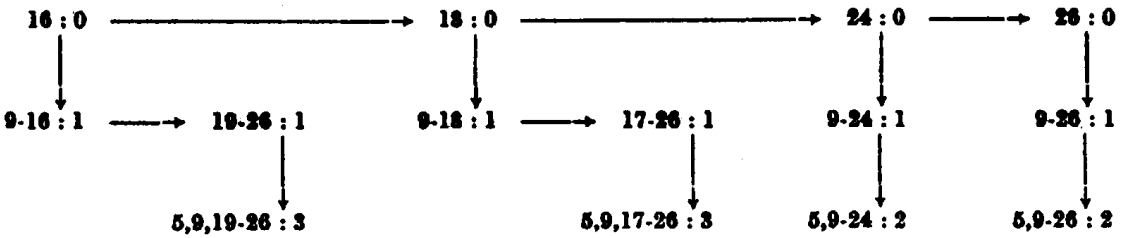
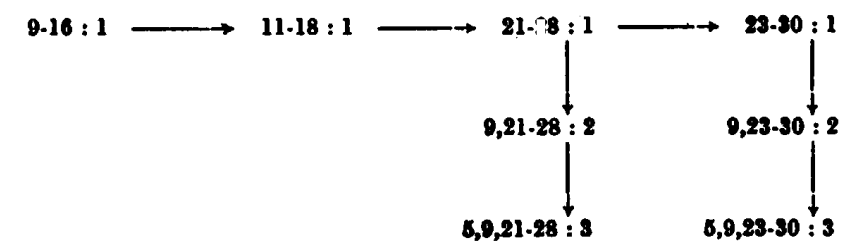
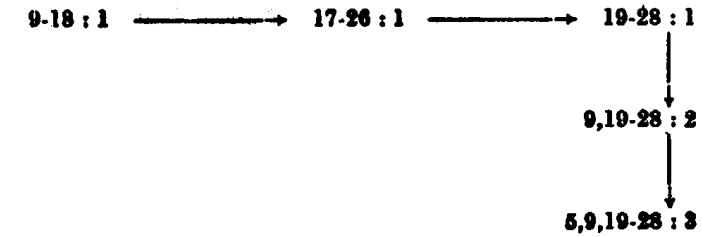
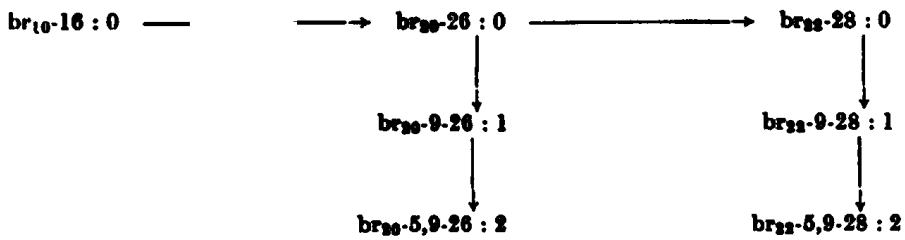
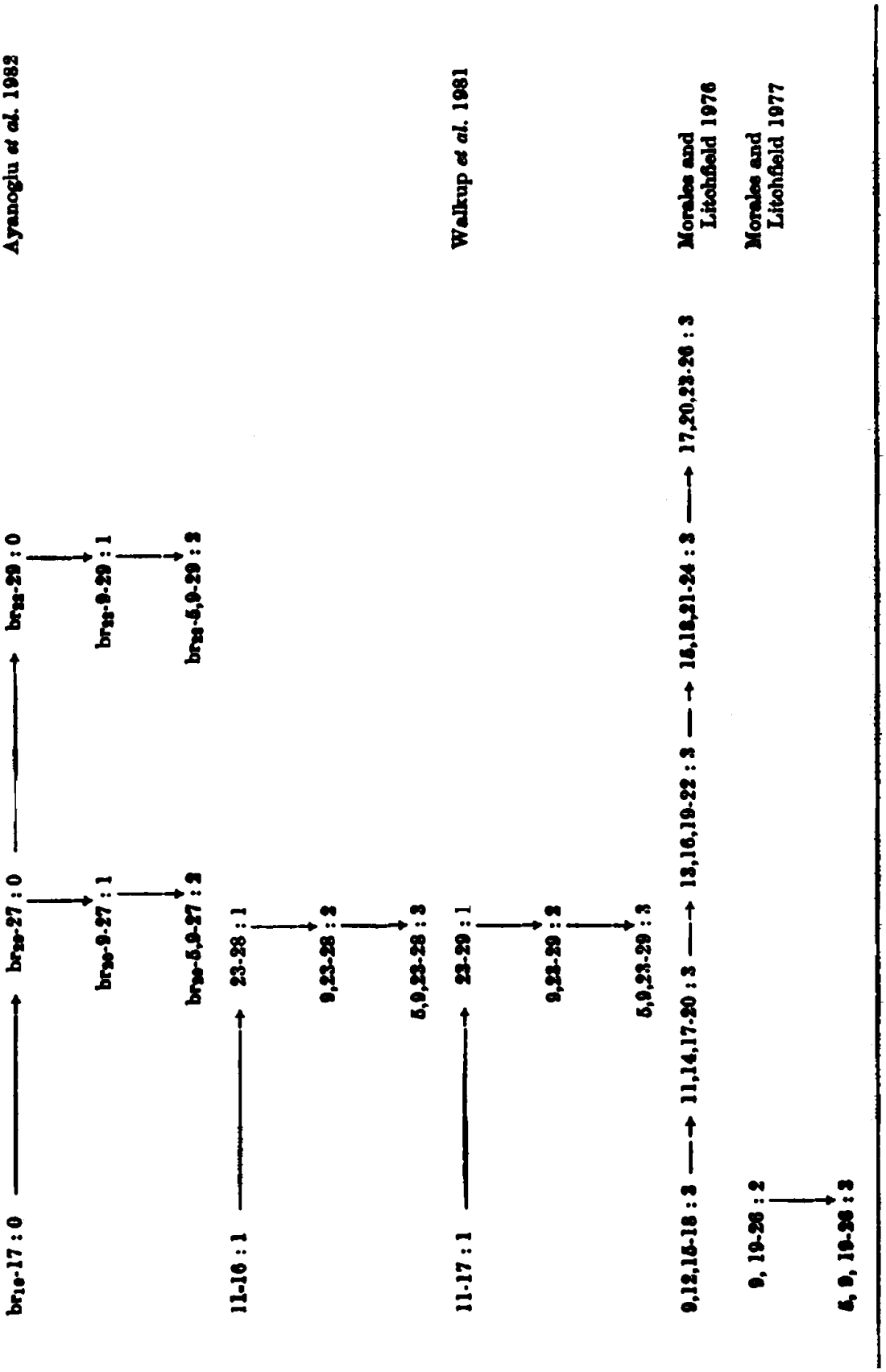
Starter		Resulting acid	Type	Trivial name
Acetyl	CH_3CO-	CH_3-CH_2-$(CH_2$-$CH_2)_6$-CH_2-COOH	even (C_{16})	palmitic acid
Propionyl	CH_3CH_2CO-	CH_3-CH_2-CH_2-$(CH_2$-$CH_2)_6$-CH_2-COOH	odd (C_{17})	margaric acid
Isobutyryl (valine)			even, *iso*- (C_{18})	—
Isovaleryl (leucine)			odd, *iso*- (C_{19})	—
2-Methylbutyryl (isoleucine)			odd, *anteiso*- (C_{19})	—

TABLE XII. Biosynthesis of very-long-chain fatty acids of marine sponges

Type of elongation (*horizontal arrows*) and desaturation (*vertical arrows*)	Reference
	Morales and Litchfield 1976
	Ayanoglu *et al.* 1982
	Litchfield and Marcantonio 1978
	Walkup *et al.* 1981


Ayanoglu *et al.* 1982
Walkup *et al.* 1981
Morales and Litchfield 1976
Morales and Litchfield 1977
br_{16}-17 : 0
brs-27 : 0
brs-29 : 0
brs-9-29 : 1
brs-6,9-29 : 3
brs-9-27 : 1
brs-6,9-27 : 3
11-16 : 1
23-28 : 1
9,23-28 : 3
5,9,23-28 : 3
11-17 : 1
23-29 : 1
9,23-29 : 3
5,9,23-29 : 3
9,12,15-18 : 3
11,14,17-20 : 3
13,16,19-22 : 3
15,18,21-24 : 3
17,20,23-26 : 3
9, 19-26 : 2
6, 9, 19-26 : 3

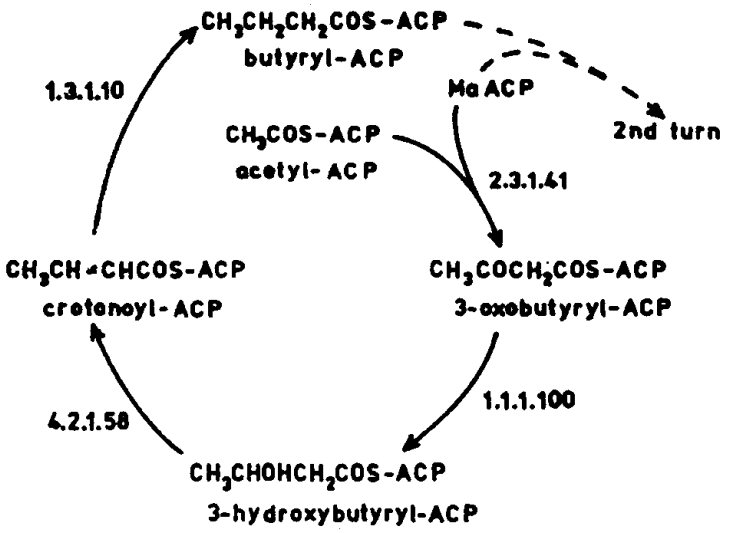


FIG. 3. The first round of synthesis of unbranched even number fatty acids;
2.3.1.41 — 3-oxoacyl-[acyl-carrier-protein] synthase,
1.1.1.100 — 3-oxoacyl-[acyl-carrier-protein] reductase,
4.2.1.58 — crotonoyl-[acyl-carrier-protein] hydratase,
1.3.1.10 — enoyl-[acyl-carrier-protein] reductase (NADPH).

always malonate (incorporated as a C_2-unit), the starter unit may be *acetyl*, *propionyl*, *isobutyryl*, (arriving from valine degradation), *isooaleryl* (from leucine) or *2-methylbutyryl* (from isoleucine). The type of the starter unit determines the type of the ultimate C_{16}—C_{18}-fatty acid (Table XI).

5.2 Chain elongation

The chain of palmitoyl-ACP formed after the seven-fold repetition of the fatty acid synthase cycle cannot be further elongated by this enzyme system (Vagelos 1973*b*); it is apparently converted to palmitoyl-CoA which is then elongated sequentially by C_2-units till the final length of the very-long-chain fatty acid. Examples in Table XII point to the possible formation of a wide spectrum of very-long-chain fatty acids found in nature.

All these examples are based merely on a generally valid hypothesis (Thiele 1979) supported by analytical data about the presence of homologues (Table XII). Only very few studies, however, have been devoted to the investigation of biosynthesis of very-long-chain fatty acids by incorporation of radioactive precursors. Thus Morales and Litchfield (1977) derived the course of biosynthesis of unsaturated C_{26} fatty acids from the incorporation of 1-^{14}C-acetate into the marine sponge *Microciona prolifera*. The presumed precursors of 5,9-26 : 2 and 5,9,19-26 : 3 acids exhibited a high radioactivity, indicating the biosynthetic pathways of formation of C_{26} acids summarized in Table XII.

Degradation of these acids at the site of the double bonds showed that the radioactive parts of the chain were localized near the carboxyl end. This confirms the hypothetical picture of the biosynthesis of very-long-chain fatty acids by elongation of existing C_{16-18} chains.

Incorporation of radioactive precursors was further used to study the biosynthesis of mycolic acids (Qureshi *et al.* 1978) which form the main component of the cell wall of mycobacteria.

From the incorporation of different ^{14}C-precursors (acetate, malonate, palmitate, S-adenosylmethionine) into very-long-chain fatty acids (C_{26}—C_{30}, C_{30}—C_{40}, C_{48}—C_{56}) from the homogenate of *Mycobacterium tuberculosis* cells, Qureshi *et al.* (1984) inferred that the resulting meromycolic acids

C_{48}—C_{56} are direct precursors of mycolic acids formed by their alkylation in position 2. From the analogy of the presence of the cyclopropane ring in position ω-19 of mycolic acids and some very-long-chain fatty acids (*e.g. cis*-3,4-*cis*-15,16-dimethylenetetratriacontanoic acid) Qureshi *et al.* (1980) assumed that very-long-chain fatty acids of this type are precursors of mycolic acids. Similar considerations form the basis of the proposed scheme of biosynthesis of very-long-chain fatty acids in *Lactobacillus heterohiochii* with double bond in position ω-7 from *cis*-vaccenic acid (*cis*-11-18 : 1) (Uchida 1974).

As shown by Rosenthal and Hill (1984), in vascular endothelial cells from human umbilical vein incorporation of ^{14}C-labelled fatty acid, *i.e.* administration of ω-6 20 : 3 acid, leads to elongation as well as desaturation which yields oligoenoic acids (24 : 4, 24 : 5, 26 : 4 and 26 : 5).

^{14}C-Arachidonic acid incorporated into rat testicles serves as a precursor for the synthesis of fatty acids with double bonds in position ω-6 (24 : 4, 24 : 5, 26 : 4, 26 : 5, 28 : 5 and 30 : 5) (Grogan 1984).

Rainwater and Kolattukudy (1985) described the purification and characterization of an enzyme responsible for fatty acid elongation (or hexanoyl-CoA to eicosanoyl-CoA). The enzyme has two subunits, each with molar mass of 238 kg/mol, and a total molar mass of 490 kg/mol. The enzyme is specific for methylmalonyl-CoA (or $^{2}H_{3}$-methylmalonyl-CoA); malonyl-CoA is not a substrate and the resulting acid is thus only the 2,4,6,8-tetramethyl-octacosanoic acid.

After centrifugation at 105 000 *g* the supernatant from *Mycobacterium smegmatis* desaturates lignoceroyl-CoA to the corresponding 15-monoenoic derivative. Separation on DEAE cellulose, salting-out, gel filtration and affinity chromatography yields three components: NADPH-oxidase, ferredoxin fraction and desaturase (Kikuchi and Kusaka 1986).

6 Significance in organism

The quantitative occurrence of very-long-fatty acids cannot be taken as a species- or genus-specific feature. Their percent proportion in the sum of fatty acids ranges from tenths of percent to tens of percent, the latter contents being similar to those of common fatty acids with 16 and 18 carbon atoms.

The maximum qualitative frequency of very-long-chain fatty acids in the whole plant and animal realm is displayed by bacteria. Welch and Burlingame (1973) published the dependence of changes in the fatty acid content on the ageing of the culture. The absolute amount increases with age whereas distribution according to the chain length decreases. The authors explain the presence of these acids by their participation in membrane functions or in hormone and regulatory activities. The content and type of fatty acids is also regulated by temperature. Thus in the marine diatom *Stauroneis amphioxys* (Table II) — in keeping with the general knowledge of the membrane structure and the types of acids found in phospholipids — the chain length decreases and unsaturation increases at 3 °C while at 20 °C the situation is opposite (Gillian *et al.* 1981). Hence the very-long-chain fatty acids bound in phospholipids can affect membrane permeability by their chain length and unsaturation.

The significance of very-long-chain fatty acids in lower organisms has so

far received only marginal attention and the pertinent considerations are based on analogies and hypotheses. It is not yet clear why the acids are synthesized. In marine sponges and probably also in fresh-water sponges their synthesis appears to be strictly genus-specific. In mycobacteria, very-long-chain fatty acids appear almost certainly to be precursors of mycolic acids. In higher plants they are a component of waxes and increase thereby the hydrophobicity of the plant body surface.

According to Murata *et al.* (1984) the localization and biological role of very-long-chain fatty acids in plants is not yet clear. On the basis of analyses it is assumed that phosphatidylserines containing these acids could serve as precursors or a transport form of epidermal lipids (waxes).

As seen from the above survey, fairly many data exist on the occurrence of very-long-chain fatty acids, less being known about their biosynthesis and still less about their functions in the organism.

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Note added in proof

The phospholipids of the sponge *Polymastia gleneni* contain C_{22}—C_{26} 2-acetoxy fatty acids (Ayanoglu *et al.* 1985), and from the sponge *Halichondria panicea* (*Z*)-17-tetracosenal (Carballeira 1986) was isolated.

Branched fatty acids *i*-5,9-27 : 2, *ai*-5,9-27 : 2 and 5,9-26 : 2 in the marine sponge *Jaspis stellifera* (Carballeira *et al.* 1986) were shown by means of ^{14}C incorporation experiments to originate from the short-chain precursors *i*-15 : 0, *ai*-15 : 0 and 16 : 0, respectively. Examination of fractionated sponge tissue shows that very long chain fatty acids occur in high proportions in cell membranes. This conclusion refutes a recent suggestion that sponge membranes would contain convential fatty acids similar to those found in membranes from other organisms (Lawson *et al.* 1986).

In antartic diatoms (plankton) saturated and monoenoic (24 : 0, 26 : 0, 13-24 : 1, 15-26 : 1 and 17-26 : 1; Nichols *et al.* 1986) fatty acids are reported.

Very long chain fatty acids (saturated and monounsatured up to C_{30}) were described in wax from cells of green freshwater alga *Chlorella kessleri* (Řezanka and Podojil 1986) and they were also separated by means HPLC on a phase RP-1 (Řezanka and Podojil 1985). Long chain saturated and monoenoic (ω-5) hydroxy fatty acids have been found in seeds *Grevillea decora* (Kleiman *et al.* 1985).

Oligoenoic fatty acids (*e.g.* 28 : 6ω-3, 32 : 7ω-3, 29 : 5ω-6, 34 : 5ω-6) were detected in mammalian spermatozoa (Poulos *et al.* 1986).

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