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Review

Odd-numbered very-long-chain fatty acids from the microbial, animal and plant kingdoms

Tomáš Řezanka *, Karel Sigler

Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague, Czech Republic

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ABSTRACT

Very long chain fatty acids (FAs) are important components of different classes of lipids in all organisms from bacteria to man. They include also, usually as minor components, odd-numbered FAs. These have so far been given little attention because of technical difficulties inherent in their detection and identification. Current modern analytical methods such as GC–MS and/or LC–MS make this detection and identification possible, and should promote a study of their properties. This review brings, in a concise manner, most of the currently available information about these FAs, their occurrence in different organisms, their structure and other properties. It should provide an impetus for further research into these very interesting compounds whose chemical, biochemical and biological properties are poorly known.

Contents

Abbreviations: Ag^+-TLC, argentation TLC; FAs, fatty acids; GC–MS, gas chromatography–mass spectrometry; LC–MS/APCI, liquid chromatography–mass spectrometry with atmospheric pressure chemical ionization; MS, mass spectrometry; NMR, nuclear magnetic resonance; RP-HPLC, reversed phase high performance liquid chromatography; VLCFAs, very-long-chain fatty acids; VLCPUFAs, very-long-chain polyunsaturated fatty acids.

* Corresponding author. Tel.: +420 241 062 300; fax: +420 241 062 347.
E-mail address: rezanka@biomed.cas.cz (T. Řezanka).

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1. Introduction

Fatty acids (FAs) are an ever-present constituent of each genus and species of living matter. Usually, they are bound in lipids in cells of most organisms with the exception of oldest bacteria (*Archaebacteriae* spp.) that contain in cell walls isoprenoid chains bound to glycerol by the etheric bond.

The quantitative proportion and qualitative composition of fatty acids in various organisms are characteristic for every species and genus, and depend also on the environment. In living cells, fatty acids are mostly bound in lipids. Their qualitative composition is richer in eukaryotic than prokaryotic organisms and reflects the biological and biochemical roles they play in the cell. Fatty acids usually contain 12–22 carbon atoms (most often 16–20). In most organisms, the major acid is palmitic, followed by oleic and then by other acids typical of the species. Saturated and unsaturated fatty acids with carbon chains shorter than 12 and longer than 22 atoms are less common.

Many books and review articles deal with the fatty acids of the usual chain-lengths. In spite of this situation, only a few papers dealing with this topic refer to the composition of VLCFAs from different sources. The nearly 400 citations in this review concerned with one group of FAs represent a mere fragment of current knowledge, when compared with the number of papers dedicated to FAs and quoted by Chemical Abstracts (~84,000 papers from 1997 to the end of 2008). In this period alone, the mean yearly number of papers concerning FAs was about 7000. Therefore, only the most important papers concerning the VLCFAs in different organisms are included in this review and represent the main part of its contents.

Since FAs contain mostly from 12 to 22 carbon atoms, with the prevalence of the even-numbered of 16–20 [1–6], the majority of those papers are concerned with FAs with 12–20 or, at a maximum, 22 carbon atoms. By comparison, saturated or unsaturated VLCFAs with a chain longer than 20 carbon atoms are rare, though they have been reported in microbes [7], insects [8], plant waxes [9,10], plants and animals [11,12], their organs or products such as meibomian gland [13] and wool wax [14], or in humans suffering from pathobiochemical diseases (inability of α- and β-oxidation of FAs) [15].

Only three out of the many hundreds of reviews dealing with fatty acids concern directly odd-numbered FAs. They are either old [16], or the description of these compounds that they provide is somewhat limited [17,18].

Table 1
Examples of some of the more common VLCFAs.

Systematic name	Common name	Short notation
Tetracosanoic	Lignoceric	24:0
Hexacosanoic	Cerotic	26:0
Octacosanoic	Montanic	28:0
Triacontanoic	Melissic	30:0
Dotriacontanoic	Lacceroic	32:0
Tetratriacontanoic	Ghedoic	34:0
Pentatriacontanoic	Ceroplastic	35:0
15-Tetracosenoic	Nervonic	Δ^{15}-24:1
17-Hexacosenoic	Ximenic	Δ^{17}-26:1
21-Triacontenoic	Lumequeic	Δ^{21}-30:1

Biosynthesis of FAs, including VLCFAs and/or VLCPUFAs, has in the last years been studied by many authors and reviewed in many articles. Some of these deal with, e.g., chain elongation and desaturation of long-chain fatty acids in marine sponges [19], eukaryotic algae [20], biosynthesis of VLCFAs in plant cuticular wax [21] or in higher plants [22]. Other studies concern the biosynthesis of mycolic acids by mycobacteria [23] or yeast [24]. The general features of elongations of FAs are the subject of two reviews, one of which describes elongation in all organisms [25] while the other is focused on mammals [26]. A very important source of information about all areas of fatty acids, including nomenclature is to be found on the web page: http://www.lipidlibrary.co.uk/lipids.html.

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 is true for VLCFAs (Table 1). 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. According to this system, a fatty acid is designated by two numbers separated by a colon; the first number is the total number of carbon atoms in the FA and the second is the number of double bonds. Thus, 26:2 refers to a C26 acid with two double bonds, i.e. hexacosadienoic acid. The positions of unsaturation can be given by additional number(s). The position(s) of a double bond is counted from COOH (carboxyl group), and *cis* (*Z*) configuration is assumed in natural compounds. In a second system, the position(s) of a double bond (single methylene group between *cis* or *Z* double bonds) is counted from the end, i.e. from the CH_3 group (http://www.cyberlipid.org). Branched fatty acids are indicated by the abbreviation "br" and/or by the number of the carbon atom on which the chain is branched (e.g. br_2-26:0, 2-methyl-pentacosanoic acid). Special cases that occur quite frequently are penultimate (iso, i-) and antepenultimate (anteiso, ai-) isomers (the precursors for which are valine, leucine and isoleucine). The notation is always given for the methyl substitution of the chain. Cyclopropane rings can be best referred to as "c", e.g. c-11-19:0 (lactobacillic acid, 11,12-methylene octadecanoic acid). The position of the terminal double bond can be also denoted in the form (ω), where ω is the number of carbon atoms from the last double bond, assuming that all the other double bonds are methylene-interrupted. Thus 4,7,10,13,16,19-docosahexaenoic acid is 22:6ω3 acid.

2. Isolation and identification

In this chapter we made an effort to cover papers dealing with an enrichment of an original sample with VLCFAs; it quotes many papers in which a HPLC technique was in use. An extensive review on chromatography of very long-chain fatty acids from animal and plant kingdoms published 7 years ago still remains a valuable source of data about the chromatography of VLCFAs [27].

As already mentioned in the introductory part, the content of VLCFAs is rather variable and, therefore, it is suitable to employ the enriching methods in the first step [28]. However, this fact is very often neglected. Few papers deal with sample enrichment with VLCFAs in a complex way. In principle, two techniques of enrichment are used. The first one includes the separation of intact lipids containing VLCFAs and subsequent evaluation of the present

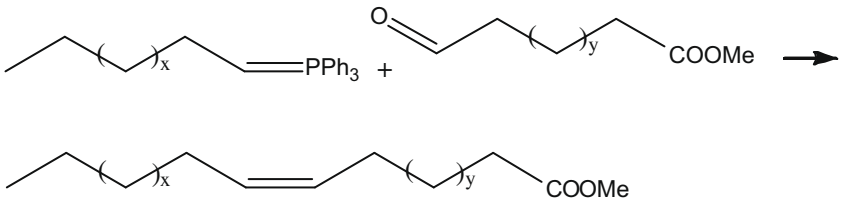
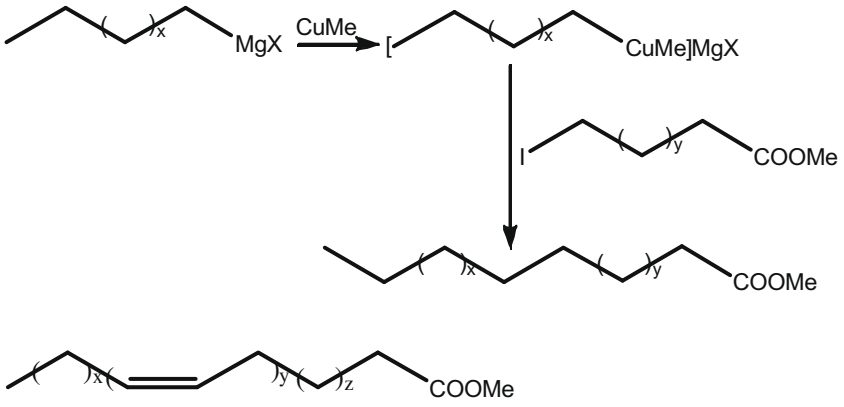


Fig. 1. Synthesis of VLCFAs by Wittig reaction.



x	y	z
16	1	7
7	1	14
7	1	10
17	0	10
7	1	22
5	1	24
4	2	22
4	2	14
1	3	22

Fig. 2. Synthesis of VLCFAs by Grignard reagents.

FAs. The second approach derives benefit from a selective isolation of VLCFAs from the total FAs fraction. Unfortunately, neither method can enrich the fraction of odd carbon chain FAs at the expense of even-numbered ones. In further chapters, this problem is therefore addressed separately in each concrete case.

The current progress in analytical methods and the current trends in the prices of special instrumentation have spurred a wide use of techniques such as LC–MS/APCI [29], which were available to few laboratories only a decade ago. These techniques can be used to identify without any problems odd-numbered VLCFAs the content of which is one to two orders of magnitude lower than that of the even-numbered VLCFAs.

3. Standards

In general, all the saturated straight-chain fatty acids up to 31:0 are commercially available; their price increases with the chain length and, also, the odd-number fatty acids are a bit more expensive. Only some unsaturated FAs can be bought from specialized companies (to be found on the Internet). A problem exists with the acids higher than C_{31}, whose standards are not available on the market. Two approaches can be used to overcome this situation. Firstly, a natural mixture possibly enriched with VLCFAs can be used. Secondly, one has to synthesize those compounds in the laboratory. The same holds, to a still higher degree, for polyunsaturated VLCFAs.

Rare and unusual fatty acids were predominantly synthesized [30] via boranes. Convenient and simple syntheses of long straight chain carboxylic acids were performed with the help of organoborane chemistry. One method entails the thermal isomerization of long-chain alkyldicyclohexylboranes, followed by oxidation. An alternative procedure involves the isomerization of internal alkynes to the terminal derivative, which can be oxidized directly to the carboxylic acid [31,32], i.e. alkyltriphenylphosphonium bromides (Wittig reaction) with lithium hexamethyldisilazide, reacted with ω-oxo esters to give the corresponding methyl *Z*-FAs (Fig. 1). Still another method, treatment of ω-iodo esters with the complexes formed in reactions of alkylcopper(I) and Grignard reagents (Fig. 2), yields saturated methyl esters, *Z*-monoenoic esters, and methylene-interrupted *Z,Z*-esters and *Z,Z,Z*-esters of FAs [32]. Yet another approach [33] involves stereoselective synthesis of unsaturated very long chain fatty acid methyl esters based on the new transition metal-catalyzed coupling reaction between an organomagnesium reagent and a functionalized vinyl bromide.

An interesting piece of information found in Chemical Abstracts concerns the successful synthesis of an even-numbered FA with extremely long chain, viz. 192:0. This longest known fatty acid with 192 carbon atoms was synthesized as early as 1996 [34] by a series of very elegant reactions starting with commercially available 12-chlorododecanol whose elongation by Wittig type reactions was used to synthesize it.

Although the syntheses described in the literature [35] are predominantly focused on even-numbered FAs, they can be fairly simply modified by using a suitable starting unit, or elongating the starting even-numbered alkyl-derivative by an odd number of carbon atoms. This approach is illustrated below. When trying

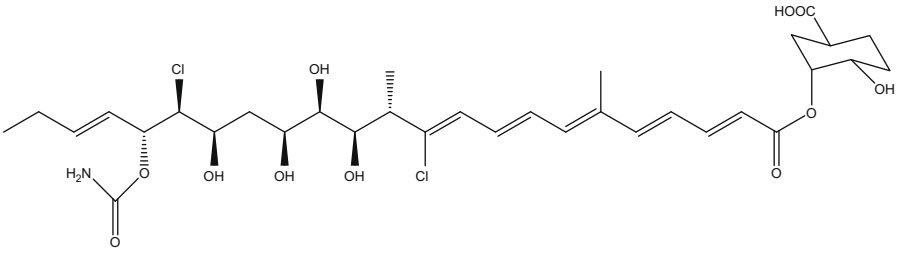


Fig. 3. Structure of enacyloxin IV.

to obtain a required odd-numbered FA one should start by consulting Chemical Abstracts or Beilstein, to learn if this FA has not been synthesized. If it was, then one should try to repeat the synthesis. From our own experience we should like to caution: the procedure may not be as simple as described in the relevant paper. If the synthesis has not been described one should get a suitable analog one carbon atom shorter or longer. Since saturated odd-numbered VLCFAs are commercially available (see above) we give only examples of the preparation of unsaturated FAs. Some examples of the synthesis of saturated as well as monoenoic and polyunsaturated VLCFAs are illustrated in our three papers [36–38].

4. Bacteria

Bacteria are the largest and most widespread group of organisms. The total number of species can only be suspected and the estimates are between 10^7 and 10^9 species. Bacteria can be found in soil, water, air, and as symbionts inside and on the surface of multicellular organisms. One gram soil contains some 40 millions of bacteria, one milliliter of fresh water about a million. Some species, on the other hand, are specialized to environments in which other organisms should hardly survive (boiling water in volcanic lakes, highest layers of atmosphere, and others). One of the most frequent taxonomical classifications is based on Gram stain – in principle the staining of cells based on the structure of the cell wall composed among other constituents also of lipids. The number of species so characterized is negligible in view of the overall number of species mentioned above. In general, bacteria can be stated to contain odd-numbered FAs, as a rule long-chain ones, most often C15:0 and C17:0 (i-, ai- and n-) or C19, but not VLCFAs. Despite this, however, VLCFAs have been identified in several species.

Careful analyses have revealed traces or a maximum of tenths of percent of VLCFAs *e.g.* in *Streptomyces cinnamonensis* [39], in which the content of 23:0 was around 0.1% total FAs.

Nichols et al. [40] found that *Franchisella tularensis*, an important pathogenic bacterium, contains monoenoic FAs up to C26 with traces of 23:0 and 25:0.

VLCFAs, unfortunately only even-numbered ones, have been identified in *Lactobacillus heterohiochi* 35 years ago. They included 24:0 (4.9), 17-24:1 (4.0), 26:0 (1.1), 19-26:1 (0.3), 28:0 (0.3), 28:0 (0.3), 21-28:1 (tr); percent fraction of total FAs is in brackets [41].

Sulfate reducing bacteria are an interesting group of microorganisms capable of dissimilatory sulfate respiration, a process whereby inorganic sulfur compounds are utilized as terminal acceptors of respiration. As a result of this electron transport, sulfides are the normal end products. Fatty acids of two strains of the sulfate-reducing, spore forming bacterium *Desulfotomaculum ruminis* were found to contain, apart from the common fatty acids, VLCFAs up to C40, including odd-numbered saturated acids with straight chains up to ai- and n-25:0 and monoenoic up to 25:1. None of these compounds have so far been described in sulfate-reducing bacteria and, therefore, their presence offers a new concept of biosynthesis of these compounds and chemotaxonomy of the sulfate-reducing bacteria [42,43].

Some bacteria were found to contain compounds having a long aliphatic chain and a carboxyl group, though these cannot be simply classed as FAs. Apart from the major even-numbered dicarboxylic acids, *Thermoanaerobacter ethanolicus* was reported to contain also odd-numbered dicarboxylic acids (C29 and C31) [44]. Enacyloxins, unique, yellow-colored polyhydroxylated polyenes having as the main structural unit a richly substituted C25 acid (see the formula of enacyloxin IV in Fig. 3) with antibiotic properties, are produced by *Frateuria* sp. W-315 in Czapek-Dox medium spent by *Neurospora crassa*. Enacyloxin IVa and its extracellular enzymatic oxidation product, enacyloxin IIa, are the main products showing antibiotic activity against Gram-positive and -negative bacteria, but are inactive against yeasts and fungi. Their mode of action was considered to be inhibition of peptide biosynthesis by hindering the release of the elongation factor Tu (EF-Tu)·GDP from ribosomes. Enacyloxins have also attracted considerable attention because of their inhibitory activity toward organelle protein synthesis in *Plasmodium falciparum* [45].

5. Mycobacteria

Mycolic acids, present in mycobacteria and related bacteria, are major and specific components of their cell envelope and essential for mycobacterial survival [23,46]. They play a critical role in the resistance of these organisms to both antibiotics and host cell defense mechanisms. The review of Minnikin et al. [47] details the history, structure, and genetic aspects of the biosynthesis of these methyl-branched components, good examples of which are the phthiocerols and the mycocerosic and mycolipenic acids.

Mycobacteria also contain structurally related long chain compounds but the metabolic relationships between these various classes of lipids remain obscure. A series of C35–C54 non-hydroxylated fatty acids (mycobacteric acids), ketones and alcohols structurally related to the C70–80 dicyclopropanated or diethylenic mycolic acids have been characterized. The relationships between long chain acids and mycolic acids were established by following the *in vivo* traffic of ^{14}C labelled α-mycolic acids purified from the same mycobacterial species. The labeling was found exclusively in mycobacteric acids. The mechanism of this degradation was established by incorporation of $^{18}O_2$ into long chain lipids and shown to consist of the rupture of mycolic acids between carbon 3 and 4 by a Baeyer–Villiger-like reaction. It was also demonstrated that mycobacteric acids occur exclusively in the triacylglycerol fraction where one molecule of these acids esterifies one of the three hydroxyl groups of glycerol [48]. The selectivity of position in the triacylglycerols is considerable since, in *Mycobacterium bovis,* 76% of acids with more than 20 carbon atoms are bound to carbon 3 of glycerol. The function of FAs above C26 and their association in the lipids are not yet known. The monoenoic acids in the highest proportion are C24–C32 [48].

A crude phenolic glycolipid extract from *M. bovis* was fractionated by HPLC and glycolipids containing mycocerosic acids (branched C26, C27 and C29) were identified by desorption chemical

ionization-mass spectrometry [49]. The enzyme short chain mycocerosic acid synthase in *M. bovis*, which catalyzes the synthesis of such acids, was purified and the native enzyme was shown to be a 537-kDa protein. This enzyme is composed of three subunits or two 280-kDa monomers with an unusual susceptibility to proteolysis. It utilizes methylmalonyl-CoA with C12 to C20 acyl-CoA as primers and with either NADH or NADPH as the reductant to synthesize the short mycocerosic acids [50].

The structure of a novel antigenic glycolipid that distinguishes the opportunistic pathogen *Mycobacterium haemophilum* from all other mycobacteria was established as a complex mixture of multi-methyl branched mycocerosic acids, C27 (2.4% of total FAs), C30, C32, C34 and C37 (8.7% of total FAs). The methyl branches of the mycocerosates have the *R* absolute configuration. The glycolipid is highly antigenic and appears to be specific for *M. haemophilum* [51].

M. tuberculosis H37Ra was incubated with [$^{2-14}$C] malonate to produce labeled long-chain fatty acids which were derivatized to the *p*-bromophenacyl esters and separated into the nonmycolic saturated, monounsaturated, and polyunsaturated esters by Ag^+-TLC and further by RP-HPLC. The results showed that the cell-free system is able to synthesize both saturated and monounsaturated fatty acids of the sizes C30–C40 and C48–C56. This latter series was strikingly similar to meromycolic acid, a putative precursor of mycolic acid. When acetate or oleate was used as the labeled substrate, the major products were not longer than C32. When palmitate was used as the labeled substrate, the saturated acids ranged in size from C18 to C32, whereas the monounsaturated products contained C18, C26–C30, and C32–C40 FAs. Fatty acids > C40 were also detected. When methyl-labeled *S*-adenosyl-*L*-methionine was used as the substrate, the methyl group was incorporated into short-chain and C48–C56 fatty acids [52].

A mixture of VLCFAs (C30–C56) from *M. tuberculosis* was separated into their individual components by means of Sephadex LH-20. VLCFAs were obtained and derivatized as *p*-bromophenacyl esters, which were separated by Ag^+-TLC and further separation into individual components was achieved by RP-HPLC. NMR, MS and chemical analyses were utilized to detect a series to C40 FAs containing two *cis*-cyclopropane rings, a series of even-numbered FAs with two cyclopropane rings but of different structure, a further series with two cyclopropane rings but with chain-length C43–C56, and a series of FAs with methoxy- and cyclopropane-groups and double bond. These FAs appear to be structurally related to the mycolic acids [53].

The composition of cellular FAs of *M. paratuberculosis* of bovine, ovine and caprine origin served for chemotaxonomic purposes by distinguishing the three strains. The VLCFAs found in the mycobacteria (24:0, 25:0, 26:0 and 28:0) were identified by using capillary GC–MS. From a comparison with another 19 species of the *Mycobacterium* genus, these four VLCFAs were present in more than a half of the species tested [54].

Analysis of cellular fatty acids in 165 isolates of mycobacteria revealed significant differences between individual groups of bacteria. The isolated acids included odd chain length FAs such as 2-Me-20:0 or 2,4,6-triMe-24:0, *e.g.* in *M. malmoense* [55].

The position of double bonds in monoenoic FAs of the genus *Mycobacterium* was studied by several authors with variable results; see also Table 2 below. Asselineau et al. [56] found double bonds in positions Δ^5, ω16 and ω18, Takayama et al. [57] in positions ω18 and ω19. The latter authors also described an enzyme that introduces a double bond into position 5 of a 24:0 acid, and an elongation of this acid by C2-units. Other authors [58] found double bonds in positions 13, 15 and 17, i.e. in position ω9 for 22:1 and 26:1 acids. These results correspond to a simple elongation of oleic acid by C2-units. These data stress an obviously incomplete knowledge of the biosynthesis of monoenoic acids and also the probable large variability of enzyme systems in mycobacterial species.

Table 2
Relative abundances of isomeric monoenoic VLCFAs in *M. tuberculosis* [59] (100 = base peak).

Acid	Position of double bond											
	4	5	6	7	8	9	10	11	12	13	14	15
23:1	29	100	19	4	–	–	–	–	–	–	–	–
25:1	–	100	70	69	27	24	2	–	–	–	–	–
27:1	–	–	4	32	55	100	39	9	3	–	–	–
29:1	–	–	–	–	4	70	100	9	47	–	–	–
31:1	–	–	–	–	–	–	9	62	100	96	66	74

Isoniazide, a well-known drug for the treatment and prevention of tuberculosis, was found to inhibit the synthesis of VLCFAs, both saturated (above 26:0) and unsaturated (above 24:1). Since these acids probably serve as precursors of mycolic acids, a hypothesis was formulated about the effect of isoniazide during the tuberculosis treatment. The researchers made use of a current procedure for the separation and partial enrichment of VLCFAs employing Sephadex LH-20 chromatography. The concentrate was further separated by silver nitrate column chromatography. Of total FAs, 0.1% had the chain-length of C27–40 and 0.03% had the chain-length of C39–56. The acids having more than 30 carbon atoms contained either double bonds or 1–3 cyclopropane rings. The compounds were identified by using preparative GC and mass spectrometry [60].

Sathyamoorthy et al. [61] described the isolation and identification of nonmycolic FAs (up to C55). The VLCFAs fraction was enriched by using Sephadex LH-20 as usual, the saturated and unsaturated *p*-bromophenacyl esters were separated by the use of Ag^+-TLC and subsequently identified by RP-HPLC. Each peak from the RP-HPLC was collected and identified by using MS. No cyclopropane ring was detected in the FA by NMR but double bonds were present, as was confirmed by Ag^+-TLC. The longest branched FA so far discovered, namely 49:1 and 55:2, were identified using modern analytical techniques. Unfortunately, neither the type of the alkane chain nor the position(s) of the double bonds have been identified [61].

Several new FAs have been identified in three genera of *Nocardia*. The authors have described a series of C30–50 ω9 polyenes with both odd and even chain-lengths [62].

6. Fungi

Fungi *sensu stricto* are mycelial organisms of different shapes and sizes, without assimilation dyes (i.e. without plastids), with heterotrophic nutrition and chitin-containing cell wall. Reserve substances are oils and glycogen. Fungi reproduce either vegetatively through disintegration of the mycelial filaments, or by sexual or asexual spores. Their representatives are spread all over the Earth and include important degraders, parasites or species used in food and other industries. Many species are mutualists that live in symbiosis with vascular plants or algae (lichens – see the following section).

Both VLCFAs and 2-hydroxy-VLCFAs were detected in mushrooms, the proportion of the hydroxy VLCFAs being much higher. 2-Hydroxy VLCFAs were identified in a number of species and their amount reached as much as 66% of the total hydroxy acids; these acids are bound mostly in sphingolipids [63].

Fatty acids were detected on the surface of conidia and sporangiospores of various fungi from genera *Alternaria*, *Botrytis* and *Neurospora*. They usually include saturated even-numbered acids up to C26. VLCFAs up to C32 were found in *Fomes igniarius* [64].

FAs isolated from basidiomycetes in the form of FAMEs were detected by using GC–MS [65]. The double bond positions were

Table 3
Content of VLCFAs in fungi.

Organism	Acid (%)	References
Alternaria tenuis	23:0 (1.8), 25:0 (1.3), 25:1 (tr),	[68]
Botrytis fabae[a]	23:0 (16.3), 23:1 (8.6)	[68]
B. fabae	23:0 (2.1), 25:0 (3.4), 25:1 (1.2)	[68]
Cryptococcus albidus	23:0 (3.3)	[69]
Fomes igniarius	23:0 (1), 25:0 (4.4), 27:0 (1.9)	[69]
Lipomyces anomalus	23:0 (3.3)	[70]
Neurospora crassa	23:0 (1.6)	[68]
Penicillium pulvillorum	23:0 (1)	[69]
Rhodotorula glutinis	23:0 (2.7)	[70]

[a] Cell wall.

Table 5
Compositions of VLCFAs (%)[a] in *S. cerevisiae* in different growth phases.

Acid	Growth phases			
	Mid-log	Late log	Early stationary	Late stationary
23:1	3	2	1	0.5
25:0	4	1	4	4
25:1	2	l	1	tr
27:0	6	4	5	6
27:1	2	1	0.5	tr
29:0	1	l	1	1
C31	1	0.5	0.5	tr
C33	tr	tr	tr	tr

[a] 100% = C19–C34.

determined by oxidative degradation after monoenoic FAMEs had been separated by using RP-HPLC. This method was used especially for FAs with more than 21 carbon atoms. FAs up to 30:0 were often detected; the data shown in Tables 3 and 4 suggest that VLCFAs represent a common component of fungal lipids even though their biochemical role is unclear.

Similarly, VLCFAs were detected in the mycelium of *Claviceps*, a producer of ergot alkaloids [66]. The largest amount of VLCFAs was observed in *Claviceps purpurea* grown in a liquid culture containing sucrose as the carbon source, where C23–C26 components were identified (23:0, 0.07%; 25:0, 0.03%).

Cerebrosides, including compounds that contain in their molecule odd-chain-length 2-OH-VLCFAs (e.g. 2-OH-23:0) were isolated from the fruiting bodies of the basidiomycete *Cortinarius tenuipes* [67].

The iso-butyl ester of the unusual fatty acid xanthomonadin I is produced by the walnut pathogen *Xanthomonas juglandis* [71] (Fig. 4).

7. Yeasts

Yeasts are unicellular fungal microorganisms, with about 1500 species currently described. Most yeasts belong to ascomycetes, some belong to basidiomycetes. For this reason the yeasts do for form a single taxonomical group. They do not form fruiting bodies and reproduce mostly asexually, predominantly by budding. Most yeasts reproduce also sexually and form spores. Yeast cells capable of sexual reproduction sporulate under conditions of nutritional deficiency. Yeasts are intensively used in the food and biotechnological industries.

One of the first and most comprehensive reviews about yeast lipids, including also odd-numbered FAs, was published as early as 1975. To our knowledge, this review is unfortunately also the last to cover in a complex way the problem of lipids in yeast [72]. A review of sphingolipids in bacteria and fungi published in 2001 describes their content, including VLCFAs, in tens of microbial genera and species [73]. Another review of functions and metabolism of sphingolipids in *Saccharomyces cerevisiae* summarizes recent advances in understanding sphingolipid functions and metabolism in baker's yeast [24,74].

The FA content in yeasts and yeast-like organisms was investigated in many genera of yeasts. *S. cerevisiae* was reported to contain, apart from the common C10-C18 FAs, also VLCFAs [75] (Table 5), which make up about 1–2% of all FAs. Up to 64% of FAs of chain-lengths above C_{18} were found to be represented by a 26:0 acid. The proportion of VLCFAs [76] and 2-hydroxy acids up to C26 was determined in different parts of the cell and in different lipid fractions (Table 6). The major acid is 26:0 even though the 2-OH-26:0 acid makes up 68.3% of all FAs in sphingolipids. The highest amount of VLCFAs was found in phospholipids or glycolipids. VLCFAs also occur in the form of esters with sterols which are known to form an important part of the yeast cell wall [75].

All species of the genera *Rhodotorula* and *Lipomyces* studied so far contain VLCFAs C22–C26. Acids with more than 22 carbon atoms are most often found in *Saccharomyces*, followed by *Rhodotorula and Lipomyces* [70].

Much attention has in the last years been devoted to various mutations of the genes responsible for fatty acid synthesis in yeast. These mutations have been reported to bring about morphological

Table 4
Fatty acids in mushrooms.

Fatty acids	*Armillaria mellea*	*Flammulina velutipes*	*Fomes fomentarius*	*Fomes igniarius*	*Fomitopsis pinicola*	*Ganoderma applanatum*	*Lactarius piperatus*
23:0	1.02	0.51	0.86	4.46	0.32	3.04	0.11
16-25:1	0.43	0.30	2.62	0.80	0.11	1.84	0
25:0	0.89	0.34	0.81	0.99	0.42	1.47	0.13
18-27:1	0	0.28	0	0	0	0	0
27:0	0	0.20	0	0	0	0	0

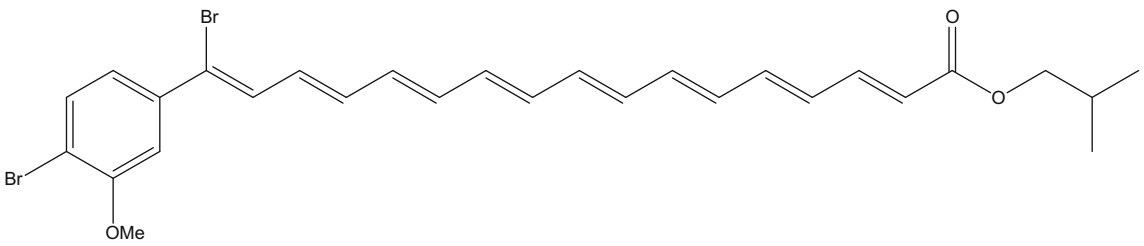


Fig. 4. Structure of xanthomonadin.

Table 6
Relative compositions of VLCFAs in different yeast lipid fractions (%) [76].

Lipids	23:0	25:0
Cells	0.2	2.0
Acid glycolipids	2.4	2.0
Waxes CC[a]	8.8	2.8
Waxes PM[a]	3.8	tr
Sterol esters CC	3.2	1.9
Sterol esters PM	0.4	0.3
Glycolipids	1.2	tr
Sphingolipids	–	0.6
Neutral lipids	3.8	4.0
Neutral glycolipids	–	2
Glycerophosphatides	4.3	1.3

[a] CC = cell content, PM = protoplast membrane.

defects in yeast cells. It is to be regretted that all relevant papers concern only the content of even-numbered VLCFAs [77–82].

Elongation of long-chain fatty acids was investigated in yeast mutants lacking endogenous *de novo* fatty acid synthesis. *In vitro* fatty acid elongation was dependent strictly on the long-chain acyl-CoA primer of 10 or more carbon atoms. The products of *in vitro* fatty acid elongation are fatty acids of 15–17 or 22–26 carbon atoms, depending on whether tridecanoyl-CoA or stearoyl-CoA is used as a primer. *In vitro*, the elongation products are converted in part, by α-oxidation, to their odd-chain-length lower homologues or are hydrolyzed to fatty acids. The *Δfas2* null mutant produces during cultivation on 13:0 + 16:0-supplemented media up to 0.8% of 25:0 [83].

Chain elongation and 2-hydroxylation pathways, which are specific for VLCFAs, have been found in the yeast *Candida utitis*. The chain elongation system acted specifically on fatty acids of chain length C20–C24, especially at a cultivation temperature of 35 °C when the biomass yield was the lowest but the sum of C23–C27 reached ~1/4 of total unsubstituted ^{3}H-acids; there was no detectable elongation of C18 and only trace activity with C19. The product of the elongation enzyme or enzymes was C26 when C20, C22, or C24 was a substrate and was a mixture of C25 and C27 when the substrates were C21 and C23. The enzyme seemed quite specific for the C26 chain length, although there may be some activity for chain lengths C24, C25, and C27 [84].

Morphological analysis of a conditional yeast mutant in acetyl-CoA carboxylase *acc1ts/mtr7*, the rate-limiting enzyme of fatty acid synthesis, suggested that the synthesis of C26 VLCFAs is important for maintaining the structure and function of the nuclear membrane. Lipid analysis of isolated nuclear membranes revealed the presence of a novel C26-substituted PI (phosphatidylinositol). This C26-PI accounts for approx. one percentage of all the PI species, and is present in both the nuclear and the plasma membrane. Remarkably, this C26-PI is the only C26-containing glycerophospholipid that is detectable in wild-type yeast, and the C26-substitution is highly specific for the *sn*-1 position of the glycerol backbone. In contrast to PIs with normal long-chain fatty acids (C16 or C18), the C26-PI greatly reduced the bilayer-to-hexagonal phase transition of liposomes composed of 1,2-dielaidoyl-sn-glycero-3-phosphoethanolamine. The biophysical properties of this lipid are thus consistent with a possible role in stabilizing highly curved membrane domains [85].

Three *fas2* strains of the fission yeast *Schizosaccharomyces pombe* were investigated and found to have missense point mutations at different sites in the gene encoding the α-subunit of fatty acid synthase. Unexpectedly, abnormal phospholipids, phosphatidylcholine and phosphatidylethanolamine, both of which contain a very-long-chain fatty acyl residue (1-melissoyl-2-oleolyl-sn-glycero-3-phosphocholine and 1-melissoyl-2-oleolyl-sn-glycero-3-phosphoethanolamine), accumulated in *fas2* strains in a temperature-sensitive manner. Rescue of the *fas2* strains by addition of palmitate to the medium at restrictive temperature was accompanied by disappearance of these abnormal phospholipids. Accumulation of these lipids in membranes may cause alteration of various cellular functions [86]. These two studies document the unprecedented ability of yeast to synthesize VLCFAs. Though they concern only even chain VLCFAs, they might induce other researchers to search also for odd-numbered VLCFAs in these microorganisms.

The yeast *Candida maltosa* precultivated on liquid *n*-alkanes was found to utilize different solid *n*-alkanes (especially C20–C25). Analysis of cellular fatty acids indicated an assimilation of solid *n*-alkanes via monoterminal oxidation. The resulting fatty acids with substrate chain length were chain-shortened by C2 units down to an optimal range of chain length from C16 to C18 and incorporated into cellular lipids directly or after desaturation. Increased amounts of C17-fatty acids, and traces of C21-fatty acid in the case of cells growing on C23-alkane [87].

8. Lichens and mosses

Lichens are symbiotic communities of a fungus (the mycobiont – an ascomycete or, rarely, basidiomycete) and a green or, more rarely, yellow-green or brown alga or a cyanobacterium (the photobiont) that can produce food for the lichen from sunlight. Some lichens contain both green algae and cyanobacteria. The exact mutual relationships between the photobiont and the mycobiont are not known; they can range from mutually beneficial association to a negative relationship (parasitism). About 17,000 species of lichens are known and new ones are being described every year. With the exception of the free oceans they can be found everywhere - in Antarctica, in mountains at a 7000 m altitude, in deserts or in water. They can grow on all substrates, mostly on stones, rocks, soil, or as epiphytes on trees and shrubs, where the competition by higher plants is small. Many require firm substrates (minerals) because of their slow growth, others (ephemerals) colonize rapidly decaying wood, leaves of tropical woody plants or stone debris. Their competitive advantage is their poikilohydric character – they can withstand large variations in the water content of the thallus. They have no roots and can absorb water through the whole surface.

Examination of fatty acids of eight lichen species belonging to the genus *Cladonia* by GC–MS revealed the presence of VLCFAs, 23:0, 25:1ω8 etc., unusual for lichens [88]. Similar FAs (23:0, 25:1ω8 and 27:1) etc. were found in lichen species from the genus *Parmelia* examined by capillary GC–MS. VLCFAs [89]. Seventy FAs including 23:0 or 25:1ω8 were identified in three lichen species from the order Lecanorales [90]. Likewise, the fatty acids of five lichen species from the subclass Gymnocarpeae, or Discolichenes, growing in the Volga river basin contained odd-numbered VLCFAs up to 23:0 and monoenoic up to 27:1, including 25:1ω8 [91] and so did eight lichen species growing in the Tian Shan Mountains [92]. The tree-growing lichen *Xanthoria parientina* was found to contain odd-numbered VLCFAs up to 23:0 and monoenoic up to 21:1. Hydroxy acids, which were detected only in the glycolipid fraction and whose seasonal dynamics were also studied, included, e.g., i-OH-21:0 and even-numbered acids up to OH-28:1 [93].

Mosses are small, soft plants. They commonly grow close together in clumps or mats in damp or shady locations. They do not have flowers or seeds, and their simple leaves cover the thin wiry stems. There are approximately 12,000 species of mosses classified in the Bryophyta, which are sometimes also taken to include liverworts and hornworts. Apart from the rarely occurring acetylenic acids, the fatty acids from two moss species and one liverwort (*Atrichum angustatum, Brachythecium* sp., and *Marchantia polymorpha*) contained also saturated odd-numbered VLCFAs up to 23:0 and monoenoic up to 27:1 [94].

Endolithic microbial communities inhabiting porous rocks in the cold, dry mountainous regions of Antarctica have been studied extensively as examples of life's adaptations to extreme environments. A suite of VLCFAs (C9–C32) and ai-VLCFAs (ai-C20–ai-C30) were found, along with short-chain i- and ai-FAs, and n-FAs. The relationship between n-VLCFAs (n-C20–n-C32) and ai-VLCFAs suggests that these compounds probably originated from the same group of microorganisms, such as bacteria or endolithic lichens, under moderate pH conditions (pH 3–5). The content of VLCFAs was very high - for instance ai-29:0 acid amounted to 2.67% total FAs [95,96]!

9. Algae, cyanobacteria and photosynthesizing protozoa

Algae and cyanobacteria are simple photosynthesizing organisms traditionally classed among lower plants. Algae can be both unicellular and multicelled forms whose bodies are formed by a thallus. Algae are components of aquatic ecosystems; red algae and sea-weeds provide both a source of food and shelter for different predators. The largest producers of biomass in the seas and oceans are macroalgae and phytoplankton, which is a planktonic community of unicellular photosynthesizing microorganisms inhabiting the aquatic environment of oceans, seas and freshwater lakes and reservoirs. It comprises mainly microalgae and photoautotrophic bacteria and/or protozoa, and is one of the richest sources of PUFAs (e.g. 20:4ω6, 20:5ω3 and 22:6ω3) [20]. The major constituents of phytoplankton are Bacillarophyceae (diatoms), Dinophyceae (dinoflagellates) and Prymnesiophyceae.

The fatty acids found in algae are mostly saturated and monoenoic VLCFAs, accompanied sometimes by hydroxy (methoxy) FAs. Lipids and lipid metabolism in eukaryotic algae, including VLCPUFAs such as arachidonic, eicosapentaenoic and docosahexaenoic acids has been reviewed in great detail in an excellent review by Guschina and Harwood which, however, contains no mention of odd-chain FAs [20].

In 1998, Volkman et al. published a detailed and meticulous review (currently cited almost 250 times) of microalgal biomarkers from different sources. Especially valuable is the information on the occurrence of unusual or distinctive lipids in microalgal classes, cyanobacteria and prochlorophytes [97].

A photosynthetic microbial mat was investigated in a large pond of a Mediterranean saltern (Salins-de-Giraud, Camargue, France) having water salinity from 7% to 15%. Analysis of characteristic biomarkers (e.g., major microbial fatty acids, hydrocarbons, alcohols and alkenones) revealed that cyanobacteria were the major components of the pond, in addition to diatoms and other algae. In addition to many typical bacterial acids the samples contained also odd-numbered VLCFAs such as 23:0 and 25:0, which can originate not from sulfate-reducing, sulfur-oxidizing, anoxygenic phototrophic bacteria and cyanobacteria but from the inputs from higher plants [98].

Saturated odd-numbered FAs such as 23:0, 25:0, and 27:0 have been identified in the marine diatom *Stauroneis amphioxys* [99], the fresh-water protozoan *Euglena gracilis* possesses fatty acids up to C26 (e.g. 23:1, C25), and the fresh-water green alga *Botryococcus braunii* [100] has the unsaturated acids 23:0 and 25:1. Investigation of the FAs composition of colonial green algae of the genus *Botryococcus* from Lake Shira (Siberia) revealed the presence of monounsaturated and very long chain monounsaturated fatty acids, including 25:1 (1.8% of total FA) [101].

Non-hydrolysable macromolecular constituents (algaenans) were isolated from two marine microalgae *Nannochloropsis* sp. and *N. salina*. Flash pyrolysis and chemical degradations allowed for the identification of their chemical structure, which is mainly composed of long-chain (up to C-36) n-alkyl units. The FAs so identified had bimodal characteristics; in contrast to all preceding studies both maxima were odd-numbered, i.e. C17 and C27. However, according to the scheme published in this paper algaenan should contain even-numbered acyl chains and the odd number of carbon atoms in FAs is the result of RuO_4^- effected cleavage [102].

High molecular weight lipids containing odd-numbered VLCFAs (27:0 and 29:0) were isolated from the trilaminar outer wall of *Scenedesmus communis* and *Tetraedron minimum* [103].

Extractable and bound lipids of four species of microalgae from the genus *Nannochloropsis* have been examined and small quantities of compounds identified as C-28–C-34 monohydroxy fatty acids (12-OH-29:0, 14-OH-31:0, and 16-OH-33:0) were detected in both free and bound form. For each carbon chain-length member of the series, a single positional isomer predominated, which was identified as the ω18-isomer from mass spectral fragment ions. The position of the hydroxyl group at ω18 rather than at a constant position relative to the carboxylic acid group indicates that the series results from chain-elongation (or perhaps chain-shortening) of a particular hydroxy fatty acid, rather than hydroxylation of a range of fatty acids. Furthermore, a dihydroxy fatty acid, identified as 16,17-dihydroxytritriacontanoic acids, was also found in the products of acid hydrolysis of the cell residue. The paper provides discussion of possible biosynthetic correlations between these hydroxy and dihydroxy fatty acids and the long-chains diols also produced by these algae [104].

Four unprecedented mid-chain methoxylated fatty acids were identified in the red alga *Schizymenia dubyi* collected in Sicily [105]. By means of gas chromatography–mass spectrometry analyses the methoxylated fatty acids were characterized as 9-methoxypentadecanoic acid, 9-methoxyheptadecanoic acid, 13-methoxy heneicosanoic acid and 15-methoxytricosanoic acid [11]. The biosynthetic scheme 9-MeO-17:0, 13-MeO-21:0, and 15-MeO-23:0 was proposed to be operative in this alga.

Protists of the genus *Emiliania* have long been known to contain lipids featuring some of the longest aliphatic chains. Apart from the dominant ketones they belong also to non-methylene interrupted VLCPUFAs. The marine protozoan *Emiliania huxleyi* was found to contain two unusual acids, 36:2 and 36:3, the nearest lower homologues having 14 fewer carbon atoms [106]. This organism contained also methyl- and ethyl-ketones; however, despite the vast efforts devoted to their analysis none of them was found to contain higher or lower VLCFAs homologues. Ketones were found as C37–C40 homologues of diunsaturated alkenones [107]. Detailed analysis of the PUFAs in this protozoan showed that these compounds do not contain any VLCPUFAs and/or odd-numbered FA [108].

The compositions of non-polar lipids of the coastal haptophyte *Chrysotila lamellosa* grown in batch culture at 10 °C and 20 °C contained, apart from odd- and even-numbered ketones, also methyl and ethyl esters of 36:4, 36:3, and 36:2 acids [107].

Dinoflagellates (Dinophyta) are a group of protozoans, freshwater, brakic as well as marine, that has been traditionally classed among algae. Although some of them are obligate heterotrophs, they are mostly mixotrophic organisms having chloroplasts that were acquired via secondary or tertiary endosymbiosis, and sometimes kleptoplastids. Their cells are "armored", i.e. surrounded by cellulose plate sheaths. They reproduce both sexually and asexually and have complex cell cycles. Many of them are toxic and their evolutionarily interesting features are their nuclei and specific organelles. Lipids and carotenoids are very probably localized in the stigma (the eyespot apparatus); however, no detailed information is this respect is available.

Marine dinoflagellates were found to contain very long and highly unsaturated VLCPUFAs. A series of papers (see below) identified even-numbered VLCPUFAs, which we mention here to point out the variety of biosynthetic processes in these marine protists

and, hopefully, initiate further research in this field, which need not concern solely the identification of odd-numbered VLCPUFAs described in the last paragraph of this section.

Two acids, 28:7ω6 and 28:8ω3, were identified whose proportion was usually in the range of tenths to units of per cent (a maximum of up to 3%) of total FAs. Regretfully, the research in this field has not progressed and important questions concerning, e.g., the biosynthesis and content of these acids in individual lipid classes or their localization in the cell have not been clarified. Yet the research of dinoflagellates could bring interesting results, as exemplified by the paper of Van Pelt et al., who identified 28:8ω3 from the heterotrophic dinoflagellate *Cryptecodinium cohnii* in commercially available oil used as dietary supplement of PUFAs for humans [109].

A short overview of these wonderful organisms and their VLCPUFAs is given below.

A highly cited review of microalgal biomarkers [97] deals in detail with the occurrence of unusual or distinctive lipids in microalgal classes, cyanobacteria and prochlorophytes.

Two *Gymnodinium* species and several other dinoflagellate species were found to contain unusual octacosapolyenoic FAs, namely 28:7ω6 and 28:8ω3 at levels up to 2.2% of total FAs [110]. Potential biosynthetic precursors of the C28 family, all of them even-numbered, were identified and a considerable attention has been devoted to their cultivation. Analyses of cellular contents of different species revealed VLCPUFAs such as 24:5ω6, 24:6ω3, 26:7ω3, 28:7ω6, and 28:8ω3. The dinoflagellate species *Amphidinium carterae* and *Heterocapsa niei* contained very low proportions (up to 0.4%) of the VLCPUFAs, i.e. 28:7ω6 and 28:8ω3. *Pavlova pinguis* also contained 28:8ω3. All species examined contained low proportions of 24:6ω3, with most species also containing traces of 24:5ω6. In addition, the cryptomonads, dinoflagellates and *P. pinguis* contained very low proportions of a C26 PUFAs tentatively identified as 26:7ω3. Unfortunately, the relevant studies ([111] and others – see below) reported no odd-numbered FAs. It is not clear whether this was due to a low and undetectable concentration of these FAs or to insufficient attention being paid to their detection.

The three studies mentioned below represent lists of genera and species in which VLCPUFAs were disclosed, and should serve as an inspiration for further research.

Small amounts of novel very-long-chain highly unsaturated C28 fatty acids were identified in five species of marine dinoflagellates (*Scrippsiella* sp. *Symbiodinium microadriaticum*, *Gymnodinium* sp., *Gymnodinium sanguineum*, and *Fragilidium* sp.) [112].

Eight species of ichthyotoxic marine gymnodinioid dinoflagellates (*Karenia*, *Karlodinium*, and *Takayama*) contained, apart from the common dinoflagellate PUFAs, also VLCPUFAs (28:8ω3 and 28:7ω6) in low levels (<1%). Like in other studies, no odd-numbered FAs were identified [113]. Investigation of the fatty acid and sterol composition of the marine dinoflagellate *Karenia brevis* showed again only even-numbered FA, i.e. the C28 family (28:8ω3 and 28:7ω6) acids [114]. Within the frames of using PUFAs as a dietary supplement, the effects of growth phase on the lipid composition in batch cultures of *Gymnodinium* sp. were monitored over 43 days of culturing. The study showed that the content of 28:8ω3 decreased in time [115].

In conclusion we would like to mention a phenomenon that has as yet not been sufficiently elucidated, i.e. the presence of hydrocarbons in algae. More details will be given in the next chapter on plant waxes. Still, it appears clear that polyene hydrocarbons are derived from PUFAs.

Microalgae are known to contain polyunsaturated alkenes [97]. The most common 21:6 and/or 21:5 are formed by decarboxylation of appropriate PUFAs [116]. A variety of longer chain polyenes has been reported [117] and the structures have been fully elucidated. For example, the heptaenes isolated and identified from the marine diatom *Rhizosolenia setigera* were found to possess six methylene interrupted (*Z*)-double bonds starting at C-3 and an additional terminal or ω2 (*Z*)-double bond. Structural and stable carbon isotopic evidence suggests that these polyenes are biosynthesized by chain elongation of the 22:6ω3 fatty acid, followed by decarboxylation and introduction of double bonds at specific positions [117].

In a 1983 paper describing the content of FAs in green algae of the genera *Chlorella* and *Scenedesmus* we identified, in addition to a manifold excess of even-carbon number VLCFAs, also odd-numbered saturated VLCFAs from 21:0 to 29:0. Their content was always many times lower that that of neighboring even-numbered FAs; for instance, the content of 26:0 was 0.83%, 27:0 0.18% and 28:0 0.73% total FAs. These data show that, although odd-numbered FAs are present in lower amounts, a careful interpretation of mass spectra permits their identification [118].

In another paper concerned with very long chain fatty acids of the green alga *Chlorella kessleri* the fraction of total FAs was selectively enriched with VLCFAs longer than C28 by using RP-HPLC; the removal of all shorter-chain FAs resulted in an increase of the proportions of both even- and odd-numbered VLCFAs. This method is very simple and consists of two successive identical analyses. In the first step the column was injected with a standard (28:0), in the second with the natural sample. After a time necessary for elution of 28:0 all compounds from the natural sample were eluted from the column and further analyzed. This procedure gave an about 100-fold enrichment – the content of 30:1, which was 0.6% total FAs in the preceding study, increased after the enrichment to some 60% total FAs. We thus succeeded in identifying many minor FAs that were not detected in the first paper, *e.g.* the odd-numbered 29:1 (0.063), 31:1 (0.039), 33:1 (0.018), and 35:1 (0.020). The values in brackets are per cent proportions relative to the total FAs, i.e. FAs above C10 [119].

In these two papers the VLCFAs were identified in total lipids. To clarify to which lipid class they belonged we isolated wax esters in heterotrophically cultivated *C. kessleri*. By means of capillary gas chromatography–mass spectrometry, we found that wax esters are composed of VLCFAs up to dotriacontanoic acid and alcohols up to 13-eicosenol. Odd-numbered saturated FAs included 25:0, 27:0, and 29:0, monoenoic acids (25:1, 27:1, and 29:1) were found to have an unusual position of the double bond, namely at the ω18 position.

In a 2002 study we returned to the isolation and identification of VLCFAs from *C. kessleri*, making use of the instrumentation and analytical techniques that have considerably advanced in the meantime. The use of liquid chromatography–mass spectrometry with atmospheric pressure chemical ionization (LC–MS with APCI) allowed us, after preparative reversed phase HPLC of hundred-milligram amounts, to identify unusual VLCFAs up to C47, both saturated and monounsaturated, with two positional isomers (ω9 and ω26). The content of odd-numbered acids is shown in Table 7. The combination of preparative RP-HPLC and microbore LC–MS thus extends the analytical possibilities of both methods and contributes to the acquisition of new information on biological materials [120].

Two of our recent studies dealt with the identification of very-long-chain polyunsaturated fatty acids from *A. carterae* by liquid chromatography–mass spectroscopy with atmospheric pressure chemical ionization (LC–MS/APCI). In the first one [38], the combination of Ag^+-TLC and LC–MS/APCI was used to identify unusual VLCPUFAs up to 36:8ω3 acid but, surprisingly, no odd-numbered FAs were found. In a sequel study [121] we therefore performed another cultivation and isolation targeting especially odd-numbered FA. These FAs were found to be present, though in a 10-fold lower concentration. With the use of Ag^+-TLC and LC–MS/APCI in a single ion monitoring mode for picolinyl esters of VLCPUFAs the

Table 7
Fatty acid composition in *C. kessleri*.

Peak no.	Acid	% of total FA[a]	% of ω-9[b]	% of ω-26[b]
1	29:1	6.3	100	0
2	29:0	9.9		
5	31:1	3.9	–	–
6	31:0	1.3		
9	33:1	1.8	40	60
10	33:0	1.1		
13	35:1	2.0	40	60
14	35:0	1.1		
17	37:1	1.8	30	70
18	37:0	0.5		
21	39:1	1.9	20	80
22	39:0	0.4		
25	41:0	0.2		
27	43:1	0.7	10	90
28	43:0	0.1		
30	45:1	0.4	0	100
32	47:1	0.2	0	100

[a] $\times 10^{-2}$ of total FAs.
[b] Based on abundance of appropriate diagnostic ions from mass spectra.

detection limit was 0.0001% of total FAs. The method revealed the presence of several tens of odd-chain VLCPUFAs and enabled us to advance the hypothesis of odd carbon chain VLCPUFAs biosynthesis illustrated in the scheme below (Fig. 5).

10. Sponges

The approximately more than 5000 living sponge species are classified in the phylum Porifera [122,123], which is composed of three distinct groups: the Hexactinellida (glass sponges) [124], the *Demospongiae* (with a horny skeleton) [125], and the *Calcarea* (calcareous sponges). The class *Demospongiae* is by far the most diverse sponge group. More than 90% of all known living sponge species are demosponges. Sponges are the most primitive multicellular animals consisting of masses of cells embedded in a gelatinous matrix. The matrix is bound together by minute, spine-like structures of calcium or silica called spicules, and spongy organic fibers called spongin. Sponges are sessile organisms with no bilateral symmetry, no clear anterior/posterior axis and limited differentiation of tissues into organs.

Sponge reproduction is by gemmules, and gemmule characters are much used in identification at the genus and species level. Molecular systematics of sponges has been reported using 18S rRNA, 28S rRNA and Hsp70 allozyme divergence [126]. The authors concluded that whatever the genes, the phylogenies obtained suggest that Porifera could be paraphyletic and that the phylogenetic relationships of most of the families and orders of the *Demospongiae* have to be reassessed. Furthermore, sponge classification needs to be supported by chemotaxonomic criteria, in particular regarding FA. Recently, a comprehensive taxonomy was published, which provides state-of-the-art information on the subject [123].

Sponges contain many new metabolites, including lipids, in particular glycolipids and phospholipids [127–131]. Sponge lipids are one of the richest sources of unusual FA. Sponges are very ancient animals which possess unique biomembranes with special structural features, in particular phospholipid FAs and sterols, since sterol-phospholipid interactions are assumed to play a major role in cell membranes [127]. It has been known for some time that these membranes contain a high proportion of unusual fatty acids [127,132–134].

Sponges filter large volumes of water through their pores, capturing for food tiny particles including microorganisms, which contain particular FAs as useful biomarkers. Because of this nutrition mode sponges contain a wide variety of microbial symbionts; a single species may often contain many different symbionts [135–138]. Some bacterial symbionts appear to have evolved with sponges since Precambrian times, and symbiosis has continued because masking capsules have obscured the symbionts from sponge phagocytic cells. The role of different symbionts is obscure. Symbiont bacteria, cyanobacteria and/or algal species are capable of metabolizing a wide range of organic compounds, including fatty acids, thereby adding to the nutrition of the sponge/symbiont system [135]. Symbiont types can occupy up to 40% of the sponge tissue volume, and play an important role in sponge ecology and biotransformation of the organic compounds. Since 1950, over

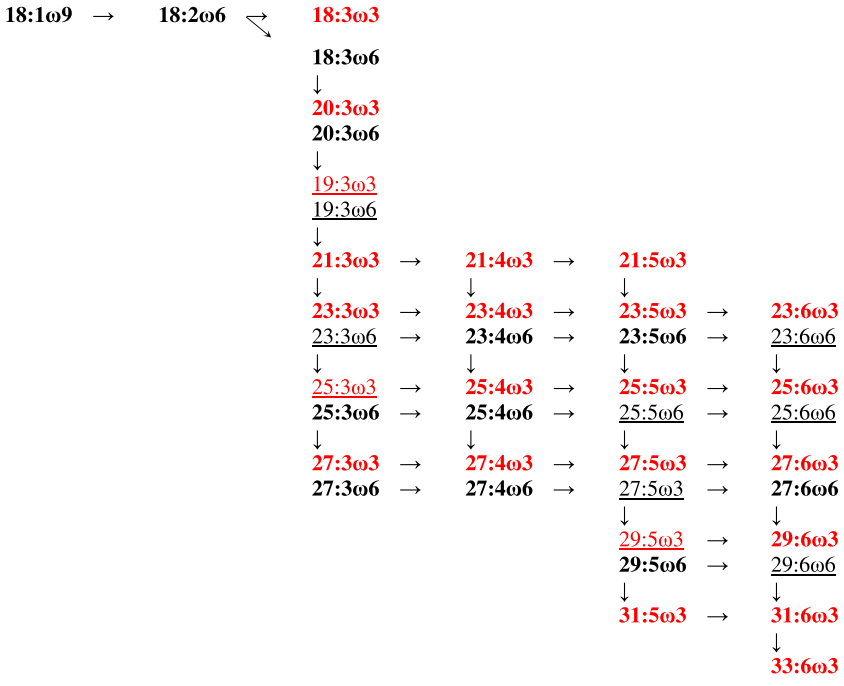


Fig. 5. Hypothesis of odd-numbered VLCPUFA biosynthesis in *Amphidinium carterae*. Black – ω6 isomers, red – ω3 isomers, bold – isomers found in the alga, underlined – isomers not found.

5000 papers have been published reporting the structures of many thousands of metabolites unique especially to marine sponge species ([139] and the preceding review of this series) some of which show high biological activity. Sponge lipids differ markedly in their chemical and biochemical properties from lipids obtained from other living organisms [11,127,140]. Two classes of sponges, *Demospongiae* and *Calcarea*, contain VLCFAs [132].

Investigation of the fatty acid profiles of 55 species of Porifera has confirmed the occurrence of high percentages of both long chain fatty acids (C24–C30) and polyunsaturated acids. These features of the fatty acid profile in conjunction with the content of branched chain acids and the dominance of particular acids in different species allow some systematic discussion. The *Dictyoceratida*, *Clathriidae*, *Halichondrida*, Homoscleromorpha and *Calcarea* are distinguished by aspects of their fatty acid profiles. The diversity in number and type of sponge fatty acids exceeds that of any other phylum. Environmentally induced variation in fatty acid content is such that percentage compositions alone have little taxonomic informational value [132].

The fatty acid content of 30 species of Porifera, including samples of *Hexactinellida* and *Lithistida* for which no fatty acid data previously existed, has been examined. As mentioned above, sponges are unique among animal phyla in the diversity of fatty acids with generally high levels of long chain fatty acids (LCFAs; C24–30, high unsaturation (mainly polyunsaturation), high incidence of branched and odd chain fatty acids. Further, peculiarities in proportions of individual acids of particular chain lengths distinguish the phylum. Seasonal and geographical influences on components of the fatty acid profile limit the extent to which this information can be utilized in a chemotaxonomic sense [124].

Twenty-nine specimens of calcareous sponges (class *Calcarea*, phylum Porifera), covering thirteen representative species of the families *Soleneiscidae*, *Leucaltidae*, *Levinellidae*, *Leucettidae*, *Clathrinidae*, *Sycettidae*, *Grantiidae*, *Jenkinidae*, and *Heteropiidae* were analyzed for their fatty acids. Save for several exceptions, the authors found no VLCFAs such as demospongic acids [141].

It has been reported that both silicious freshwater *Spongilla lacustris* [142] and marine *Spheciospongia vesparia* and *Suberites compata* [143] sponges contain significant amounts of free VLCFAs. Many publications reported the presence of unusual, branched, halogenated and other fatty acids in marine sponge species [7,11,127,133]. Further investigation of different sponge species showed that these organisms contain a number of new, unusual and rare fatty acids.

Major sponge phospholipid FAs include very low amounts of the usual methylene-interrupted PUFA, or none at all, but high levels of VLCFAs (odd-numbered FAs from C23 to C31, representing up to one third of all FAs); these are the so-called demospongic acids. Sponges (Demospongia) are a source of novel FA structures, especially unusual long-chain Δ5,9 FAs with no counterpart in the terrestrial world, with sometimes a third double bond, with possible chain branching or a bromine atom [131,144,145]. Several tens of FAs were identified in each set of sponge phospholipids studied. Some sponges contain more than ten Δ5,9 FAs in their phospholipids and, in several cases, one of them accounted for more than 50% of the total phospholipid FA mixture [145,146]. Such NMI (non-methylene interrupted) FAs are shown in Fig. 9. It is generally admitted that Δ5,9 demospongic acids are biosynthesized by *Demospongiae* through short-chain unsaturated FA, mainly from exogenous Δ9-16:1 [127].

10.1. Saturated FAs

A simple screening was used to determine the proportion of VLCFAs in subclasses of the class *Demospongiae* [147] and others [132,148]. Extraction of total lipids, isolation of acids and GC after

Table 8
Fatty acid compositions of the Porifera. The distribution of individual fatty acid chain-lengths in sponges of the classes *Demospongiae*, *Calcarea* and *Hexactinellida*.

Species	C23	C25	C27	C29	Sum
Demospongiae					
Acanthella sp.	+	+	+	–	+
Aciculties pulchra	0.6	3.4	+	3.5	7.5
Adocia parietaloides	5.0	+	2.0	1.0	8.0
Adocia venustina	3.8	2.8	2.2	–	8.8
Ancorina alata	4.1	4.4	0.8	0.5	9.8
Ancorina sp.[A]	+	2.7	+	–	2.7
Ancorina sp.[B]	+	7.1	+	+	7.1
Ancorina sp.[C]	+	+	1.6	+	1.6
Ancorina stalagmoides	17.9	–	–	–	17.9
Anomoianthella popeae	1.5	0.5	4.0	2.0	8.0
Anthosignella varians	+	1.0	–	–	1.0
Aplysilla rosea[a]	+	1.1	1.1	–	2.2
Aplysilla rosea[b]	1.2	4.9	11.3	–	17.4
Aplysia pennunculata	+	+	1.4	+	1.4
Aplysia sp.	+	+	+	+	+
Axinella australiensis	1.0	1.0	1.0	–	3.0
Axinella polycapella	–	2.0	15.0	–	17.0
Biemna sp.	+	+	+	+	+
Cacospongia sp.[A]	+	2.4	+	–	2.4
Cacospongia sp.[B]	+	9.6	+	–	9.6
Callyspongia diffusa	2.5	0.5	1.0	–	4.0
Callyspongia latituba	0.7	0.5	+	–	1.2
Callyspongia ramosa	1.2	0.5	1.2	–	2.9
Chondrilla australiensis	+	+	+	+	+
Chondrilla nucula	–	+	–	+	+
Chondrilla sp.	1.0	2.5	–	–	3.5
Cinachyra sp.	1.3	1.1	5.8	+	8.2
Clathria sp.	6.0	+	–	–	6.0
Cliona celata	+	4.0	1.0	–	5.0
Crella incrustans	7.2	4.5	+	–	11.7
Dendrilla rosea[A]	1.0	1.0	–	–	2.0
Dendrilla rosea[B]	+	1.7	+	–	1.7
Desmacella dendyi	3.0	3.0	–	–	6.0
Dysidea camera	+	11.0	+	–	11.0
Echinodictyum cancellatum	2.0	3.0	–	–	5.0
Epipolasis novaezelandiae	3.5	28.7	+	–	32.2
Geodia rex	2.0	1.6	+	–	3.6
Halicondria moorei[c]	2.4	3.0	1.8	–	7.2
Halichondria panicea	+	2.0	tr	–	2.0
Haliclona oculata	–	+	3.0	–	3.0
Hymeniacidon hauraki	1.0	2.0	–	–	3.0
Hymeniacidon perleve	1.0	3.5	4.7	+	9.2
Iophon laevistylus	+	+	3.2	–	3.2
Iophon minor[A]	2.0	–	–	–	2.0
Iophon minor[B]	3.0	1.0	–	–	4.0
Iophon proximum	3.0	–	–	–	3.0
Iotrochota birotulata	–	5.0	1.0	–	6.0
Ircinia novaezelandiae	+	0.8	+	–	0.8
Isociella incrustans	+	3.1	+	–	3.1
Isociella sp.	6.0	+	–	–	6.0
Isodyctya deichmannae	–	2.0	–	–	2.0
Latrunculia brevis	+	+	+	–	+
Leiosella sp.	8.8	13.5	+	–	22.3
Lissodendoryx sp.	+	3.0	+	–	3.0
Microciona prolifera	1.0	2.0	+	–	3.0
Monosyringia mortenseni	+	3.5	+	–	3.5
Mycale fibrexilis	+	1.0	–	–	1.0
Ophlitaspongia nov. sp.[A]	2.2	4.9	+	–	7.1
Ophlitaspongia nov. sp.[B]	2.3	6.7	+	–	9.0
Orina petrocalyx[d]	+	1.9	+	3.0	4.9
Orina petrocalyx[c]	+	1.5	3.1	2.6	7.2
Pachastrella sp.	+	+	8.1	+	8.1
Pararhaphoxya sp.	+	7.3	+	–	7.3
Penares tylotaster[A]	8.0	1.0	–	–	9.0
Penares tylotaster[B]	+	+	5.6	+	5.6
Petrosia australis[f]	+	1.8	1.9	+	3.7
Petrosia australis[g]	+	+	+	+	0.0
Petrosia australis[h]	4.7	+	+	+	4.7
Petrosia hebes	+	2.2	14.2	+	16.4
Pleroma sp.	1.8	7.5	–	–	9.3
Phakellia dendyi	3.0	0.5	–	–	3.5
Phakellia flabellata	4.1	6.7	+	–	10.8
Plakina monolopha	+	+	–	–	+

(*continued on next page*)

Table 8 (*continued*)

Species	C23	C25	C27	C29	Sum
Polymastia fusca	+	2.7	+	–	2.7
Polymastia granulosa[A,i]	1.1	2.6	+	–	3.7
Polymastia granulosa[B,j]	+	1.5	3.9	–	5.4
Rasailia sp.	2.8	1.2	1.6	+	5.6
Raspailia topsenti	1.7	2.2	1.4	–	5.3
Siphonochalina sp.	3.0	2.0	–	–	5.0
Solymastia sp.	4.5	1.5	+	–	6.0
Spheciospongia resparia	–	–	–	–	–
Spirastrella purpurea	+	1.8	–	–	1.8
Spirastrella sp.	2.0	6.0	1.4	–	9.4
Spirastrella vagabunda	+	3.9	2.1	–	6.0
Spongia sp.	1.0	25.0	–	–	26.0
Spongilla lacustric	+	6.0	+	–	6.0
Stellata conulosa	+	8.1	+	+	8.1
Stellata crater	3.0	10.6	–	–	13.6
Stellata grubii	–	2.0	5.0	–	7.0
Stellata maori	7.0	1.5	–	–	8.5
Suberites compacta	–	–	–	–	–
Tedania ignis	+	4.0	1.0	–	5.0
Tethya aurantium	1.6	3.9	4.5	–	10.0
Tethya ingalli	+	1.4	+	–	1.4
Tethya nov. sp.	+	+	+	–	+
Theonella sp.	+	+	–	–	+
Xestospongia exigua	–	1.0	–	–	1.0
Xestospongia halichondroides	–	1.0	6.0	–	7.0
Xestospongia sp.[A]	5.0	1.5	4.5	–	11.0
Xestospongia sp.[B]	+	1.7	6.5	+	8.2
Xytopsene sigmatum	–	1.0	+	–	1.0
Calcarea					
Clathrina sp.	1.0	0.5	.	.	1.5
Leucettusa lancifer	+	+	+	–	+
Sycon sp.[A]	4.0	2.0	–	–	6.0
Sycon sp.[B]	7.0	3.2	–	–	10.2
Sycon sp.[C]	3.0	2.7	+	–	5.7
Sycon sp.[D]	7.0	4.0	–	–	11.0
Sycon sp.	+	+	–	–	+
Hexactinellida					
Rossella ijimae	+	0.7	1.9	0.5	3.1
Sympectella rowi	+	+	4.2	+	4.2

+Indicates trace amounts, <0.5%, of a fatty acid.
[a] Collected from the North Island, New Zealand.
[b] Collected from the South Island, New Zealand.
[c] Values were averaged for 15 samples over 16 months.
[d] Different samples of the same species collected from the same or a similar area at approximately the same time of year.
[f,g,h] Different samples of the same species collected from the same or a similar area at approximately the same time of year. *P. australis*; [f]represents a thin crust of the sponge encrusting *E. novaezelandiae.* Both *P. australis*; [g,h]signify a distinct sponge.
[i] Summer collection.
[j] Winter collection.
[A–D] Species collected from a different area in a different year.

initial hydrogenation were performed on more than 100 species. FAs with C23–C29 made up zero to one third (*Epipolasis novaezelandiae*) of all FAs (Table 8). The highest content of different odd chain VLCFa was found in the following sponges: C23-*Ancorina stalagmoides* 17.9%, C-25-*E. novaezelandiae* 28.7%, C27-*Axinella polycapella* 15.0%, and C29-*Aciculties pulchra* 3.5%.

Sponges were also found to contain branched saturated iso- and anteiso-acids with a total number of carbon atoms from C14 to C29; among them C15–C20 acids were the most widespread. Rare acids, i-23:0, i-25:0, and ai-25:0, were found in the Caribbean sponge *Cribrochalina vasculum* [149]. Iso and anteiso VLCFAs with 27 and 29 carbon atoms were found in phospholipids of the sponge *Petrosia pellasarca* [150]. Long chain FAs of iso and anteiso structures are probably synthesized by the sponge itself through the elongation of iso and anteiso predecessors of bacterial origin with shorter hydrocarbon chains. The ratio of iso to anteiso FAs in sponges was very high, obviously due to the fact that a significant part of their biomass was compounded by bacterial symbionts. Thus, in the sponge *Aplysina fistularis*, the content of iso and anteiso FAs (sum of saturated and also unsaturated) reached one third of total FAs [151].

Lipids of sponges contain also a significant number of saturated FAs with midbranching of their carbon chain. For example, in the lipid composition of the Mediterranean sponges *Agelas oroides* and *Astrosclera willeyana*, two various monomethyl interrupted odd VLCFAs were revealed in which methyl group is bonded to C14–C18 in Me-22:0 and Me-24:0, respectively, (3–4% of FAs total content) [152]. The 20-Me-26:0 acid was revealed in the lipids of the *Verongia aerophoba* sponge [153] while 17- and 18-Me-24:0, and 18-Me-26:0 acids were found in the phospholipids of sponges *Cinachyrella alloclada* and *C. kukenthali* from the Red Sea [146]. In our opinion, these longer monomethyl-interrupted FAs may be synthesized by sponges themselves by the elongation of appropriate bacterial predecessors. Sponges from the Baikal Lake contained more odd-numbered 23:0 and 27:0, otherwise the relative proportions of FAs were the same as in samples from other locations. This indicates that there may be no difference between marine and fresh-water sponges [154]. Five FAs not hitherto found in nature were identified in two *Cinachyrella* sponges from the Saudi Arabian Red Sea, namely 17-methyl-24:0, 18-methyl-24:0, 18-methyl-25:0, 18-methyl-26:0 and 18,24-dimethyl-26:0 acids [146].

The FAs of the "living fossil" stromatoporoid *A. willeyana* (Great Barrier Reef) and the demosponge *A. oroides* (Mediterranean Sea) were found to contain large amounts of branched carboxylic acids such as Me-20:0, i-21:0, ai-21:0, 21:0, Me-22:0, i-23:0, ai-23:0, Me-24:0, and 5,9-25:2. These compounds include terminally branched carboxylic acids (iso-/anteiso-) and abundant mid-chain branched carboxylic acids which are characterized by an intriguing variety of structural isomers. Methyl branching points were generally observed between the ω5- and ω9-positions. These complex isomeric mixtures apparently derive from symbiotic bacteria living exclusively in demosponges. As a working hypothesis, it was suggested that their bacterial source organisms have been widespread in the geological past, and are found "inherited" in the protective environment of distinctive sponge hosts in recent marine ecosystems. Furthermore, both sponges contain abundant linear, long-chain demospongic acids [152].

A rare FA with the cyclopropane group, 19,20-methylene-26:0, was found in the marine sponge *Calyx niceaensis* [155]. The content of cyclopropane acids was usually less than 1% of the total FAs.

Polymethyl-branched VLCFAs were not found in sponges, only conventional multi-branched FAs up to C20 being identified.

10.2. Monoenoic FAs

Sponges are specified by a great diversity of monoenoic acids with more than 20 carbon atoms; more than ten such FAs have a linear structure, from 23 to 29 atoms in the acyl chain (Table 9).

Monoenoic FAs with mid-branching of their carbon chain are also found in sponges. The branched VLCFA monoenes 2-Me-17-24:1 and 2-Me-17-26:1 were revealed in the sponge *Halichondria*

Table 9
Monoenoic odd-numbered (C23-C27) unbranched fatty acids detected in sponges.

FA	Content, %	Sponge	References
4-23:1	tr	*Calyx niceaensis*	[155]
16-23:1	0.7	*Amphimedon compressa*	[156]
17-23:1	2.4	*Microciona prolifera*	[157]
18-23:1	0.3	*Amphimedon compressa*	[156]
9-25:1	0.1	*Dysidea fragilis*	[158]
16-25:1	0.9	*Pseudaxinella* aff. *lunaecharta*	[145]
18-25:1	1.0	*Cinachyrella* aff. *schulzei*	[159]
19-25:1	0.7	*Amphimedon compressa*	[156]
19-27:1	n/a	*Petrosia* sp.	[144]
20-27:1	0.5	*Cinachyrella* aff. *schulzei*	[159]

Table 10
Content of branched fatty acids (%) in phospholipids of the marine sponge *Strongylophora durissima* [161].

Acid	Total PL	PI + PS	PC	PG	PE
br19-25:0	0.5	0.5	–	0.7	0.9
br19-27:1	19.3	24.7	30.6	9.9	11.3
br19-27:0	7.5	5.3	4.1	7.7	13.0
br19,21-29:0	2.9	3.9	–	3.0	4.5
br20-29:0	1.9	2.5	–	2.2	2.7

panacea [160]; 19-Me-5-26:1 and 19-Me-5-28:1, in the sponge *Strongylophora durissima* [161]; and 7-Me-8-26:1 in the sponge *Desmapsama anchorata* [162].

Methylene-interrupted dienes such as 16,19-25:2 were detected in sponges of different genera [163].

Nonmethylene-interrupted dienoic FAs of various structures are much more characteristic for marine sponges. 5,9-FAs with unbranched carbon chain, and branched iso and anteiso structures, and FAs with midbranched chains occurred among them most often. 5,9-FAs with trans (*E*) configuration of double bonds were also found in sponges. For example, the sponge *Plakortis halichondroides* contains FAs of iso-structures, 19-Me-5E,9E-20:2 and 21-Me-5E,9E-22:2 and of anteiso-structures, 19-Me-5E,9E-21:2 and 20-Me-5E,9E-22:2 [164].

A total of 56 acids were identified by GC and GC–MS in the fatty-acid composition of total lipids from the Okhotsk sea marine sponge *Forcepia uschakowi*. The main monoenoic acid was 15-Me-24:1(6.1%), which was observed for the first time in sponge lipids. Polyunsaturated acids represented 64.1% of the total FAs of *F. uschakowi*. Of these, the principal ones were non-methylene odd-numbered acids 5,9-25:2 (2.3%), 5,9,18-25:3 (0.4%), 5,9-27:2 (0.7%), 5,9,20-27:3 (0.3%).

The data obtained by Dasgupta et al. [161] in their study of mostly branched VLCFAs from the marine sponge *S. durissima* are summarized below (Table 10).

10.3. Dienoic and polyenoic FAs

In some species of sponges, the polyenoic FAs are represented exclusively by dienes; thus, the ratio of dienes can reach 6–12% of the total FA, e.g., in the sponges *Agelas dispar*, *Anthosigmella varians*, and *Spheciospongia vesparium* [165]. However, most sponge species are specified by polyunsaturated FAs with a greater number of double bonds. All polyenoic FAs found in sponges are divided into two greater groups by the location of their double bonds in the carbon chain: methylene- and nonmethylene-interrupted. The widespread natural methylene-interrupted polyenoic FAs of ω3 and ω6 series mostly have carbon chains from C16 to C22. It is believed that sponges, as well as most heterotrophic organisms, have a limited ability for the biosynthesis of such acids and receive them by nutrition. Methylene-interrupted trienes and higher polyenes such as 21:5ω3 were detected in sponges of different genera [166].

All nonmethylene-interrupted polyenes with unbranched carbon chain that were found in sponges have Δ5,9-bonds (Table 11). However, the diversity of nonmethylene-interrupted dienes in sponges is not limited to Δ5,9 structures only.

Nonmethylene-interrupted dienoic FAs of various structures are much more characteristic for marine sponges. 5,9-FAs with unbranched carbon chain, and branched iso and anteiso structures, and FAs with midbranched chains occurred among them the most often (Table 12). 5,9-FAs with trans configuration of double bonds are also found in sponges.

For example, the sponge *P. halichondroides* contains FAs of iso-structures, 19-Me-5*E*,9*E*-20:2 and 21-Me-5*E*,9*E*-22:2 and of anteiso structures, 19-Me-5*E*,9*E*-21:2 and 20-Me-5*E*,9*E*-22:2 [164].

Table 11
Trienoic nonmethylene-interrupted fatty acids found in sponges.

FA	Content,%	Sponge	References
5,9,17-25:3	0.1	*Dysidea fragilis*	[158]
5,9,19-25:3	0.2	*Dysidea fragilis*	[158]
5,9,19-27:3	0.1	*Microciona prolifera*	[167]
5,9,20-27:3	27.5	*Halichondria panicea*	[168]
5,9,23-29:3	15.6	*Amphymedon viridi*	[169]
5,9,24-31:3	0.6	*Haliclona cinerea*	[170]

Table 12
The content of Δ5,9-dienoic fatty acids in sponges (% from the FAs total).

5,9-FA	Linear	*Iso-*	*Anteiso-*	22-Me
23:2	–	11.2 [164]	1.4 [164]	–
25:2	6.7 [171]	19.5 [174]	30.0 [174]	–
27:2	18.4 [172]	8.4 [172]	18.4 [172]	–
29:2	0.2 [173]	0.8 [175]	1.8 [175]	0.1 [11]
31:2	0.1 [11]	–	–	–

The group of Djerassi [151,172] separated individual types of phospholipids and determined the content of acids in each type in the spongal genera *Aplysia* and *Petrosia*. In the genus *Aplysia*, they contain 22-Me-5,9-28:2 and 5,9,23-29:3 acids. A very high content of the former acid, i.e. 20.0% and 17.8%, was found in PS and PE, respectively. In *Petrosia*, odd chain VLCFAs were found in PI and PS, in which the proportion of, e.g., ai-5,9-27:2 exceeded one quarter of all fatty acids.

Specialized spherulous lectin-producing cells of the sponge *A. fistularis* obtained by centrifugation were extracted and the total lipids separated by column chromatography to obtain different fractions. VLCPUFAs were found only in the fraction of total lipids and in PC, in which 5,9,23-29:3 was among the major constituents [176].

VLCFAs from the whole sponge tissue of *Pseudaxinyssa* sp. contain 5,9-27:2 acid which accounts for more than 6% total acids. Its concentration is equal to the concentration of even-numbered acids such as 5,9-28:2 or 5,9,21-28:3 [177].

Studies of the fatty acids of the main phospholipids of the marine sponge *Microciona prolifera* [178] revealed the presence of odd-numbered acids, mostly br-23:0, 17-23:1, 23:0, 5,9-25:2. Their proportions depended on the type of the phospholipid from which they were isolated.

The FAs present in the phospholipids of the sponge *Tedania ignis* included no unusual FAs, only those typical for sponges (23:0, 1.3%; 24:0, 9.0%; 5,9-25:2, 3.1% and 25:0, 1.5%) [179]. GC and GC–MS study of the fatty acid composition of total lipids from the marine sponge *Tedania dirhaphis* from the Sea of Okhotsk revealed 50 acids. Odd-numbered 5,9-27:2 had a content of 0.7%. The main FA in lipids from *T. dirhaphis* was 5,9,21-28:3, the relative content of which reached 63.3%! [180].

A high percentage of demospongic acids such as 5,9-25:2 (11%) has recently been isolated from freshwater sponges *Heterorotula multidentata* and *Spongilla alba*, Lake Shinji, Shimane prefecture, Japan, and the sponge *Ephydatia fluviatilis* (32% in PS) from Lake Lagunita, Stanford, USA [154,181].

Marine sponge *Halichondria panicea* inhabiting Peter the Great Bay of the Sea of Japan was found to contain odd-numbered fatty acids, predominantly 21:5ω3, *br*-25:1, 5,9-25:2), br-27:1, br-27:2, 5,9-27:2, and 5,9,20-27:3 [182]. *H. panicea* from the coast of south Hokkaido, Japan, was found to possess a high content of VLCFAs, see Table 13 below. The fatty acids were concentrated by Ag^+- and RP-TLC. The proportion of 5,9,20-27:3 (25.4%) in total fatty acids was much higher than had been observed for other sponges. The contents of acids considerably differed from those

Table 13
Contents of the demospongic acids in lipids of the sponge *Halichondria panicea* (wt% of total fatty acids) [182].

Lipid class	5,9,20-27:3
TL	27.5
NL	16.9
GL	19.3
PL	27.0
PS	36.5
PE	27.3
PC	12.6

Table 14
FAs found in the marine Caribbean sponges *A. viridis* and *D. anchorata* [169].

Fatty acid[a]	*Amphimedon viridis*	*Desmapsamma anchorata*
7-21:1	0.1	–
11-21:1	0.1	–
13-21:1	0.1	–
15-21:1	0.1	–
i-21:0	–	0.2
ai-21:0	–	0.2
21:0	1.0	2.2
i-23:0	–	1.5
16-23:1	0.2	0.1
17-23:1	0.2	–
18-23:1	0.1	–
23:0	0.2	1.0
5,9-25:2	1.2	0.9
9-25:1	–	0.1
17-25:1	0.2	–
18-25:1	0.1	0.1
19-25:1	1.2	–
25:0	–	1.8
20-27:1	0.1	–
21-27:1	0.1	–
5,9,23-29:3	15.6	–

[a] % of total acids?

previously found for *H. panicea*, see the paragraph above, which may be due to seasonal changes in the species composition of organisms consumed by the sponge. The changes in the content of 27:3 probably are not species-specific and depend on the individual features of the organism. All three demospongic acids (C26, C27 and C28) were abundantly found in PS and PE [168].

Lipids and phospholipids (both plasmalogen and alkyl forms) of the freshwater sponge *Lubomirskia baicalensis* and the sponge's gammarid parasite *Brandtia (Spinacanthus) parasitica* were found to contain some 200 FAs including several tens of odd-numbered FAs. An interesting finding is that some of these fatty acids were found in lipids of the amphipod parasite, indicating that the parasite consumes them with its food and incorporates them into its own structures [163].

Three freshwater sponges, *Ephydatia syriaca*, *Nudospongilla* sp., and *Cortispongilla barroisi* were found to contain 185 conventional FAs, out of which five were new multibranched polyunsaturated fatty acids. These 185 FAs included also odd-numbered VLCFAs that are commonly found in sponges, such as saturated FAs up to 31:0, two positional isomers of monoenoic acids (15-, 17.25:1) or i-, ai- and 5,9-27:2, and also 5,9,18- or 5,9,20-27:3 [183].

Unusual FAs were found in the marine Caribbean sponges *Amphimedon viridis* and *D. anchorata* [169]. A total of 62 acids were identified that included, in addition to common ones, also less conventional acids such as the major, second most abundant 5,9,23-29:3 responsible for 15.6% total FA (Table 14). Many positional isomers of monoenoic acids were also found.

The phospholipids of the Caribbean sponge *Pseudospongosorites suberitoides* contain, in addition to novel cyclopropane fatty acids with a length up to C20, also commonly encountered VLCFAs such as 5,9-i-27:2 and 5,9-ai-27:2. This sponge is interesting in that it lacks usual even-numbered demospongic acids and those longer than C24 include only odd-numbered ones. The reasons for this unusual phenomenon have not been explained although around 66% of the total FAs contained odd carbon chains [184].

Rare fatty acids (5,9-i-25:2 and 5,9-ai-25:2, 9-25:1), the predominant constituents of the free fatty acid fraction from the lipids of the sponge *Geodinella robusta* (Sea of Okhotsk), were isolated and identified [174]. Mixtures of both acids showed cytotoxic activity against mouse Ehrlich carcinoma cells and a hemolytic effect on mouse erythrocytes. The study further identified common FAs such as 25:2, 5,9-i-27:2, and 5,9-ai-27:2.

New unsaturated long-chain fatty acids were identified in the phospholipids from the Axinellida sponges *Trikentrion ioeve* and *Pseudaxinella* cf. *Lunaecharta* [145]. They included "classical" sponge acids such as the positional isomers 25:2 (Δ5, Δ16), or demospongic acids 5,9-i-25:2, 5,9-i-27:2, 5,9-ai-27:2, 5,9-i-29:2, 5,9-ai-29:2, and 5,9-29:2.

The fatty acid composition of phospholipids from the Senegalese sponge *C. alloclada* [185] featured, apart from demospongic acids common in sponges, also odd-numbered acids such as 5,9-i-27:2 or 5,9-27:2. Odd-numbered saturated acids, whether straight-chain or branched, were also identified (ai-27:0, 27:0). Odd-numbered 5,9-27:2 [186] or 29:2 [187] were also found in other marine sponges such as *Spheciospongia confoederata* or *Pseudaxinyssa* sp., respectively, other odd carbon chain acids such as 5Z,9Z-25:2, 2-OH-23:0, 25:1, 2-OH-25:0, and 5Z,9Z-27:2 were identified in the Caribbean sponge *Callyspongia fallax* [188]. From the steryl ester and phospholipid fractions of the marine sponge *Agelas conifera* were isolated FAs with odd number of carbon atoms. The proportion of the odd-numbered 5,9-25:2 was 13.6% of total phospholipid FAs [189].

The composition of Δ5 (Δ4) unsaturated medium- and long-chain fatty acids (FAs) of the freshwater sponge *Baicalospongia bacilifera* was selectively determined by means of an analytical iodolactonization reaction and the structures of FAs were identified by gas chromatography–mass spectrometry (GC–MS), spectrophotometry and chemical degradation. The study demonstrated the presence of 25:2 (1.5%) and 25:3 (2.2%) [190].

The fatty acid composition of the lipids from *Chondrosia reniformis* was investigated and the 57 acids so identified included, among others, 17-Me-5,9-24:2, 5,9-25:2, 21-Me-5,9-26:2, 5,9,?-27:3, 5,9,23-27:3, 5,9-27:2, and 5,9-29:2 [191].

Fatty acid composition of two species of Japanese freshwater sponges *H. multidentata* and *S. alba* featured common demospongic acids including 5,9-25:2 [171].

The phospholipid fatty acid composition of the Caribbean sponge *Erylus goffrilleri* comprised a total of 70 fatty acids with chain lengths between 13 and 29 carbons. Methyl-branched fatty acids predominated, suggesting the presence of a considerable number of bacterial symbionts. Novel fatty acids with carbon chains up to C19 were described and the branched fatty acids 5,9-i-27 and 5,9-ai-27:2, and 5,9-27:2 and 5,9-29:2, were also identified [192].

[1-^{14}C]-labeled *i*-, *ai*-15:0, and 16:0 FAs were used for the study of biosynthetic pathways of *i*-5,9-27:2 and *ai*-5,9-27:2 in the sponge *Jaspis stellifera* [193]. It was shown that long-chain *iso* and *anteiso* FAs were synthesized from short-chain *iso* and *anteiso* predecessors by the pattern *i*-15: 0 → *i*-5,9-27:2; *ai*-15:0 → *ai*-5,9-27:2.

Long-chain FAs with midbranching of hydrocarbon chain were synthesized in a similar way. It was established that 10-Me-16:0 was used as a predecessor in the biosynthesis of the 22-Me-5,9-28:2 acid in the sponge *A. fistularis* [194].

10.4. 2-Substituted FAs

α-Methoxylated fatty acids have only been identified in the phospholipids of sponges. These α-methoxylated fatty acids share the common molecular properties of possessing the *R* configuration at the chiral center, and are basically phospholipid bound. The first naturally occurring α-methoxylated fatty acids were found in phosphatidylethanolamines and phosphatidylserines from the Caribbean sponge *Higginsia tethyoides*, which contained saturated, monounsaturated, and diunsaturated α-methoxylated fatty acids with chain-lengths between 19 and 28 carbon atoms [195,196]. All of these α-methoxylated fatty acids were straight-chain fatty acids and the double bond positions in the monounsaturated fatty acids were encountered in either $\Delta^{17,\ 18,\ 19}$ or ^{21} such as the odd-numbered 2-OMe-21:0, 2-OMe-16-23:1, 2-OMe-23:0, 2-OMe-18-25:1, and 2-OMe-20-27:1. *H. tethyoides* also contains a small quantity of unusual 2-methoxy acids in addition to the common demospongic acids such as 2-MeO-23:0 (0.6%), 2-MeO-18-25:1 (0.5%), 2-MeO-16-23:1 (tr) and 2-MeO-20-25:1 (tr) [196].

Apart from the common demospongic FAs including odd-chain ones, saturated 2-methoxylated fatty acids with chains shorter than C20 were identified in the phospholipids from the Caribbean sponge *C. fallax* [188].

The phospholipids of the sponge *Polymastia gleneni* contain C22-C30 2-acetoxy fatty acids (2-OAc-23:0, 13.9%; 2-OAc-25:0, 16.5%; 2-OAc-27:0, 0.6%; and 2-OAc-29:0, 1.1%), the content of the rest of the constituents was less than 1% [197]. Their structures were determined by means of capillary GC–MS with EI (electron impact) and CI (chemical ionization). Examination of fractionated sponge tissue shows that VLCFAs occur in high proportions in cell membranes.

The Caribbean sponge *Smenospongia aurea* revealed the presence of branched α-hydroxy fatty acids: 2-OH-21:0, 2-OH-i-23:0, 2-OH-23:0, 2-OH-i-25:0, 2-OH-ai-25:0, and 2-OH-25:0. These α-hydroxy fatty acids were associated with phosphatidylethanolamine. It was also shown that sponges such as *S. aurea* are able to synthesize unusual very long chain branched α-hydroxy fatty acids and introduce them into its phospholipids.The sponges *Aplysina lacunosa* and *A. fistularis* also contained considerable amounts of α-hydroxy fatty acids [198].

Chemical examination of the marine sponge *Iotrochota baculifera* of the Indian Ocean furnished a sphingolipid having as the major acid 2-OH-25:0 [199].

Phospholipid fatty acid composition was studied in two Senegalese sponges of the family Suberitidae. More than fifty acids were identified in either *Pseudosuberites* sp. or *Suberites massa*. A series of 2-hydroxy long-chain fatty acids (C22 to C27) accounted for almost 50% of the total acid mixture of *Pseudosuberites* sp., including 2-OH-25:0 (9.2%) and the unusual 2-OH-27:0 not yet reported in any sponge. In addition, other odd-numbered acids common in sponges were identified (5,9-25:2, 5,9-27:2) [200].

The saturated hydroxy acid 2-OH-23:0 (11.5%) was found in the sponge *Amphimedon compressa* from the Caribbean Sea [201] and -methoxylated branched-chain fatty acids were identified in the phospholipids of *A. complanata* [202]. In the sponge *Pseudosuberites* sp., sampled at the coast of Senegal, the content of VLCFAs 2-OH C22-C27 reached 50% from the FAs total; short-chain 2-hydroxy acids were also present [203].

10.5. Brominated FAs

Brominated FAs from marine invertebrates have also been reviewed [71,204]. Recently, a comprehensive review of natural halogenated FAs included those from marine algae and invertebrates [140]. One of the first papers on this topic described brominated long chain fatty acids, including 6-Br-5,9-25:2 from the phospholipids of the tropical marine sponge *Amphimedon terpenensis* [205]. *Cinachyrella* sponges from the Red Sea also contained three 6-brominated acids with the new 6-bromo-5,9-nonacosadienoic [146]. 6-Br-5,9,24-27:3 Acid has been isolated from the sponge *Xestospongia* sp. [206,207]. It should be noted that the 6-bromo-5,9-heptacosadienoic acid displayed moderate activity against murine leukemia L1210 cells and against human carcinoma KB cells.

The very long-chain fatty acids, among them also an odd-numbered 6-Br-5,9-25:2 or 5,9-25:2, were identified in the phospholipids (mainly phosphatidylethanolamine) of the sponge *Agelas* sp. [208]. Unusual brominated 6-Br-5,9-27:2 of linear structure was found in *Petrosia* sp. [144].

Two novel long chain fatty acids, (5Z,9Z)-6-bromo-25-methyl-5,9-hexacosadienoic and (5Z,9Z)-6-bromo-24-methyl-5,9-hexacosadienoic acids were found in the phospholipids of *Petrosia ficiformis* and *P. hebes*. Their structures were elucidated with the help of CI-EI/MS and a homogeneous hydrogenation catalyst [209].

Boreal sponges of the order Halichondrida are rich sources of brominated lipid fatty acids. The comparison of lipid compositions of halichondrid *Demospongiae* from boreal and warmer waters indicates an accumulation of brominated fatty acids in sponges from colder settings. Moreover, the spatial distribution of brominated fatty acids in the tissue of one widely distributed sponge of the North-East Atlantic (*Phakellia ventilabrum*) hints at a function related to membrane fluidity and permeability rather than to defense against predation. However, brominated fatty acids are diagnostic for the presence of bromoperoxidases in sponges and may therefore be potentially useful as markers in a chemical screening for secondary metabolites of pharmacological interest. Unusual odd-numbered demospongic acids were identified, whether with a straight chain or iso-anteiso chain; the following may serve as examples: 6-Br-5,9-i-23:2, 6-Br-5,9-ai-23:2, 6-Br-5,9-i-25:2 (7.1% of total FAs!), 6-Br-5,9-ai-25:2, 6-Br-5,9-25:2, 6-Br-5,9-27:2, and 6-Br-5,9,?-29:3. Positional isomers of monoenoic acids, e.g. 15-27:1 and 17-27:1, were also found [210].

Analysis of the phospholipid fatty acid composition of the tropical marine sponge *Amphimedon terpenensis* (family Niphatidae, order Haplosclerida) revealed the presence of some novel brominated fatty acids, among others also 6-bromo-5,9-pentacosadienoic acid [205]. The sponge has been shown to possess unusual lipids, including unusual fatty acids. The biosynthetic origin of these fatty acids is of interest as the sponge supports a significant population of eubacterial and cyanobacterial symbionts. Three brominated fatty acids (e.g. odd-chain 5,9-6-Br-25:2 accounting for 19.0% total FA), together with phytanic acid, were isolated from both ectosomoal (superficial) and choanosomal (internal) regions of the sponge. Analysis of extracts prepared from sponge/symbiont cells, partitioned by density gradient centrifugation, indicated that the three brominated fatty acids were associated with sponge cells only. The brominated fatty acids were shown to be associated with the phosphatidylserine and phosphatidylethanolamine fractions that are commonly present in marine sponge lipids. This species is unusual in having C25 fatty acids as the major constituent [211].

The phospholipids from the New Caledonian sponge *Cinachyrella aff.schulzei* Keller contained more than 60 fatty acids including odd-numbered FAs such as 5,9-i-25:2, i- and ai-5,9-27:2, 5,9-i-29:2, or Br-5,9-27:2 and rare 16-tricosenoic, 18-pentacosenoic and 21-octacosenoic acids [159].

A series of 18-brominated C23 acetylenic acids and their ethyl esters (Fig. 6) have been found in the Australian sponge *Phakelia carduus*. They have shown positive reaction against the gram-positive bacteria *Staphyloccocus aureus* and *Micrococcus* sp. [212].

10.6. Different FAs

Investigation of glycolipids from the Caribbean sponges allowed the isolation of plakosides A (Fig. 7) and B from *Plakortis simplex*

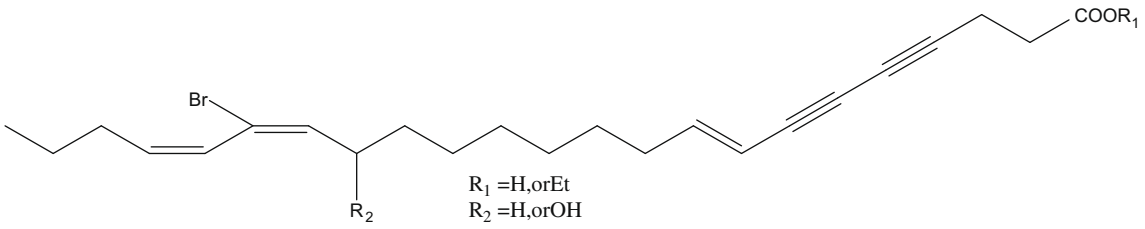


Fig. 6. Structure of four 18-brominated C23 acetylenic acids found in *Phakelia carduus* [212].

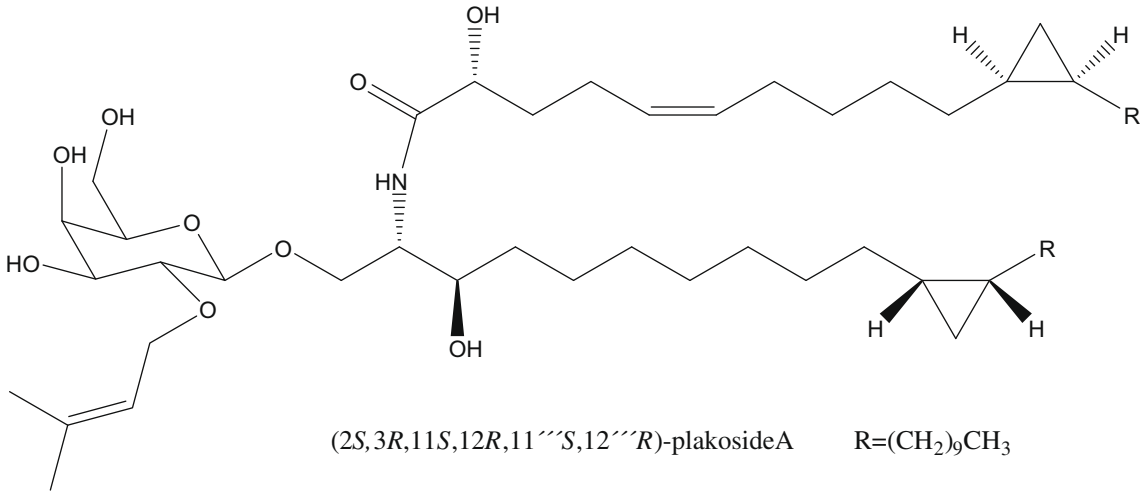


Fig. 7. Structure of plakoside A.

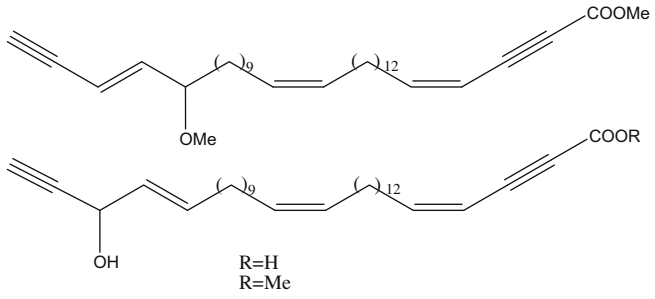
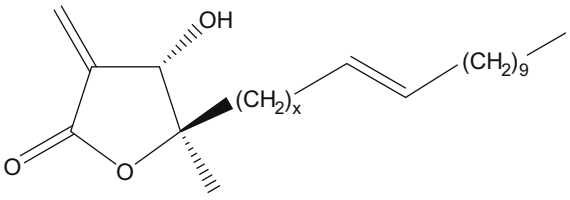


Fig. 8. Structures of acetylenic compounds from the marine sponge *Pellina triangulate* [216].



	x	
1	31	
2	35	
3	31	saturated
4	35	saturated

Fig. 9. Structures of amphiasterins.

[172], and plakosides C and D from *Ectyoplasia ferox* [213] (Fig. 7). These glycolipids are among the most fascinating lipids isolated from marine organisms. They have a unique structure with a prenylated galactose and two cyclopropanyl alkyl chains. All four plakosides had the same α-hydroxylated C22 fatty acid amide with a cyclopropane (i.e. the sum of carbons is 23) at the C11–C12 positions. As the sponges are taxonomically distant from bacteria, it is possible that these unusual glycolipids may be of bacterial origin. Plakosides A and B are immunosuppressants that act through a noncytotoxic mechanism [214,215].

Other unusual compounds (Fig. 8) were isolated from the marine sponge *Pellina triangulate*. Some of these new acetylenic compounds inhibited inosine monophosphate dehydrogenase *in vitro* [216].

Ethanolic extracts from the sponge *Plakortis quasiamphiaster* from South Pacific waters caused a strong inhibition of Kb cancer cells at a concentration of 10 μg/mL; these extracts yielded a group of very unusual metabolites, amphiasterins, which at first glance have nothing in common with fatty acids. However, as seen from the formulas shown below, they are cyclic lactones (Fig. 9) with an odd number of carbon atoms – see amphiasterin A1 and A3 (C49), amphiasterin A2 and A4 (C53), amphiasterin B1 and B4 (C21), B2 (C23), etc. All other amphiasterins (B3-5, C1-4, D1-3, E1) also have odd number of carbon atoms, either C27 or C29 [217].

11. Other invertebrates

A number of authors have paid considerable attention to the occurrence of complex lipids such as, e.g., glycosphingolipids (cerebrosides) in different marine invertebrates. The only odd-numbered acid found in these organisms was 2-OH-23:0. It was identified for instance in a cerebroside mixture obtained from the extract of the starfish *Acanthaster planci* [218] or in the cerebroside reguloside B and two other homologues isolated from the

starfish *Pentaceraster regulus* [219]. Close inspection of chemical and spectroscopic evidence aided in characterizing a new cerebroside (containing the 2-OH-23:0 acid) together with many homologues [220,221] in the sea cucumber *Cucumaria echinata*. Furthermore, a cerebroside containing the 2-OH-23:0 acid, plus numerous homologues, were isolated from the less polar fraction of the MeOH extract of the sea cucumber *Pentacta australis* by preparative reversed phase HPLC [222]. Fractionation of the extract of the sea star *Ophidiaster ophidiamus* afforded new glycosphingolipids, ophidiacerebrosides A-E. Ophidiacerebroside D, which has 2-OH-23:0, showed strong *in vitro* cytotoxicity against 210 leukemia cell lines [223]. A novel glycosphingolipid, temnoside A, has been reported from the MeOH extract of the sea urchin *Temnopleurus toreumaticus*; it contained again the 2-OH-23:0 acid [224].

Novel α-hydroxy fatty acids including odd-numbered 2-OH-14-23:1 and 2-OH-23:0, were identified in the Caribbean urchin *Tripneustes esculentus*. The corresponding non-hydroxylated monounsaturated fatty acids, 7-21:1, 12-21:1, 14-23:1, and 23:0 were also present in *T. esculentus* [225].

Three new glycosphingolipids and a new glycosphingolipid, sulcaceramide, containing again 2-OH-23:0 acid, were isolated from the marine ascidian *Microcosmus sulcatus*, and their structures were elucidated by spectroscopic and chemical analysis. The compounds are the first natural glycosphingolipids whose ceramide contains 2,3-dihydroxy acids including odd-numbered ones: 2,3-diOH-i-21:0, 2,3-diOH-21:0, and 2,3-diOH-23:0 [226,227].

The FAs from PE, PC and PS were studied in samples of digestive glands, gonads and in the total lipid extract of a marine starfish (genus *Henricia*). The alkyl-acyl, alkenyl-acyl and diacyl forms of phospholipids were found; in the PE, more alkyl and alkenyl forms were present, while in PC diacyl ones predominated. The results confirm the known fact that the lipids of their prey (sponges) reach the tissues of the predators (starfish feeding on sponges) [228].

Autotrophic algae serving as food for lower animals can be traced as a direct source of VLCFAs in the fat of sea starfish. Ferguson documented the direct dependence of the presence of FAs in the fat on the nutrition of the starfish *Echinaster* sp. (algae to starfish,%: 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 and 16-26:1 8.5/16.6) [229].

Fatty acid compositions (e.g. saturated acids, i.e. 25:0, 27:0, and 29:0) in the tissues of the clam *Geloina coaxans* collected from Oura mangal, Okinawa, Japan [230]. Many marine invertebrates were found to contain a mixture commonly denoted as C21 PUFA, i.e. a mixture of polyenoic fatty acids with 21 carbon atoms. Some examples would be, e.g., Antarctic pteropods, *Clione imacina* (Order Gymnosomata) and *Clio pyramidata* (order Thecosomata) collected near Elephant Island, South Shetland Islands [231].

Among 10 species of zooplankton collected from the Elephant Island region of the Antarctic Peninsula and from East Antarctica, significant amounts of C21 PUFAs (1.4% and 1.2%) were documented only in *Periphylla periphylla* and *Arctopodema ampla*, respectively [232].

Analysis of the fatty acid compositions in the bivalve molluscs *Mactra chinensis*, *Pandora pulchella*, *Felaniella usta*, and *Megangulus zyonoensis*, and the polychaete *Chaetopterus cautus* in Vostok Bay (Sea of Japan) showed that, although the FA composition of some bivalves was stated to be likely to be indicative of substantial inputs from benthic microalgae and an important role of microbial food chains, the presence of a single odd-numbered VLCFA (21:5ω3) shows that this correlation is not very strong [233].

Seasonal variations in lipid classes and fatty acid composition of triacylglycerols and phospholipids in the digestive gland of the scallop *Pecten maximus* were studied over a period of 16 months. However, the data about the proportion of 21:5ω3, in all cases around 1–2% total FA, are not consistent [234].

Antarctic hyperiids (*Themisto gaudichaudi*, *Hyperia macrocephalus*, and *Primno macropa*) and gammarid amphipods (*Eusirus perdentatus* and *Orshomene rossi*) collected near Elephant Island contained again C21 PUFAs in amounts of about 1% total FAs in all samples. Even-numbered VLCPUFAs were not detected in the 1997 sample but comprised 0.6-2.8% of total fatty acids in almost all 1998 amphipods [235].

The diunsaturated fatty acids 5,9-21:2, 5,9-23:2 and the brominated fatty acid 6-Br-5,9-21:2 were identified for the first time in nature in the phospholipids (mainly phosphatidylethanolamine and phosphatidylserine) of the anemone *Stoichactis helianthus*. These results indicate that Δ5,9 phospholipid fatty acids are not unique to sponges, as previously recognized, but can be found in other marine invertebrates such as an anemone [236].

The lipid, FA, and sterol composition of the New Zealand green lipped mussel (*Perna canaliculus*) and of the Tasmanian blue mussel (*Mytilus edulis*) was described to feature, apart from the common 21:5ω3, also an unusual C23 PUFAs amounting to 0.1–1%, and 23:0 around 0.1% of total FAs [237].

The occurrence of positional isomers of unsaturated fatty acids in triacylglycerols and polar lipids was investigated in the female gonads of a dominant limpet, *Cellana grata* [238]. FAs were fractionated according to the degree of their unsaturation by Ag^+-TLC and their structures were elucidated by GC–MS of picolinyl esters. A total of 125 different FAs ranging from 12 to 24 carbon atoms were identified, among them many odd-numbered acids, e.g., 5,9-21:2, 7,13-21:2, 7,15-21:2, 7,16-21:2, 21:2ω7, 21:2ω6, 21:2, 5,9-23:2, 21:37, and 7,13,16-23:3. These data [238] document that the application of suitable analytical techniques can reveal as yet unidentified compounds even in previously analyzed species (*Cellana grata*, *C. toreuma*) [239]. The dienoic FA was 21:2, which consisted of seven different positional isomers, and was mainly found in the triacylglycerols. Among dienoic FAs isomers, the presence of both the odd-numbered FA 21:2 positional isomers including unusual non-methylene interrupted FA is very rare in living organisms. To the best of our knowledge, this is the first report of tentative identification of all-*cis*-7,13,16-23:3 from living organisms. However, compared with the even-numbered polyenoic FAs occurring in marine gastropods, which include bivalves, limpets, and their gonads, odd-numbered polyenoic FAs with more than 21 carbon atoms, other than 21:5ω3, were not detected due to their very miniscule amounts. Unusual minor nonmethylene-interrupted FAs have been identified in the lipids of gonads from the limpets *Cellana grata* and *Collisella dorsuosa*. Among unsaturated FAs from C18 to C24 identified in this study, odd-numbered dienoic FAs as 7,13-21:2, 7,15-21:2, and 7,16-21:2 were identified in trace amounts (<0.01% of total FA) [240].

Two nudibranch mollusks, *Chromodoris* sp. and *Phyllidia coelestis*, collected from tropical waters of the Northwestern Pacific, were found to contain [241] sixty five FAs including monounsaturated FAs. The main acid (up to 6.2%) was 7-21:1. The nonmethylene-interrupted (NMI) FAs included the 5,9-25:2, iso-5,9-25:2 and a novel isomer 7,13-21:2 (up to 3.9%).

Supercritical fluid extracts of New Zealand green-lipped mussels (*Perna canaliculus*) have been suggested to have a large number of minor FAs. These included the only odd-numbered VLCPUFA, the commonly encountered 21:5ω3 [242].

The occurrence of nonmethylene interrupted FAs in the marine bivalve *Megangulus zyonoensis* was studied by fractionating unsaturated FAs according to the degree of their unsaturation by using Ag^+-TLC and elucidating their structures by GC–MS. Seventy-two unsaturated FAs were found, including the unusual or novel 7,13-21:2, 7,15-21:2, 21:4ω6, 21:4ω5 and 21:5ω3 FAs. This bivalve was extremely rich in the positional isomers of FAs [243].

One of the first cases of isolation of odd-numbered FAs is described below. The search for the source of odd-chain length FAs

identified in the smelt *Osmerus mordax* [244] revealed that the source was the amphipod *Pontoporeia femorata* [245,246]. The odd-numbered FAs so found included 20:1ω8, 20:1ω6, 21:4ω7, 21:4ω5 and 21:5ω2, i.e. all acids that were later gathered under the common name C21 FA. This is a nice example of how suitable analytical techniques affect the outcome of the analysis.

The amphipod *Corophium volutator* has a relatively low total lipid content. Probably due to this fact the amount of 21:5ω3 was found to reach 1–2% in all lipid classes, i.e. triacylglycerols, phospholipids and total lipids [247].

A study of distribution of phospholipids in four Gastropoda species and two Bivalvia species from the Volga river basin recorded major phospholipid classes, phosphatidylethanolamine and phosphatidylcholine. Also, 67 fatty acids including 25 saturated (both iso and anteiso), 24 monoenoic, five dienoic, four trienoic and eight polyenoic compounds were identified by capillary gas chromatography. Relatively high concentrations of nonmethylene-interrupted fatty acids were detected in some species. Odd-numbered FAs were isolated from some of the mollusks in trace concentrations [248].

The fatty acids in the lipids from three species of Holothuroidea obtained near Okinawa have been shown to contain about 1–5% of *cis*-14-tricosenoic acid (23:1ω9) in all samples. The ω9 structure suggested α-oxidation of 24:1ω 9 as the origin [249,250].

Gammarus is an amphipod crustacean genus in the family Gammaridae. It contains about 200 described species, with new ones becoming known at a steady rate. They are the typical freshwater shrimp of Europe, North Asia (Russia) and North America and range widely throughout the Holarctic. A considerable number are also found southwards into the Northern Hemisphere tropics, particularly in Southeast Asia. These interesting crustaceans contain unusual compounds, especially lipids that include also odd-numbered FAs, among them odd-numbered monoenoic FA such as 23:1ω9, other positional isomers of 23:1 and also the widespread 21:5ω3. The 21:5ω3 arrives very probably from the diet, i.e. from plankton serving these invertebrates for nutrition [251–253]. The use of advanced techniques such as liquid chromatography–mass spectrometry with atmospheric pressure chemical ionization (LC–MS with APCI) led to the identification of VLCPUFAs up to 40:7ω6 [254]. A recent study [255] revealed the presence in these crustaceans of all*Z*-4,7,10,13,16,19,22,25,28-tetratriacontanonaenoic acid, i.e. VLCPUFA with the highest degree of unsaturation found so far in nature. However, no odd-numbered FAs were found.

FAs of three invertebrate species - crabs (*Rhithropanopeus harrisii*), crayfish (*Astacus leptodactylus eichwaldi*) and Bivalvia (*Mytilaster lineatus*) were found to contain, apart from tens of even carbon chain FAs, also odd-numbered VLCFAs such as 23:1ω9 or the frequently described 21:5ω3 [256].

A number of unusual fatty acids such as *cis*-11,12-methylene-5-eicosenoate or *cis*-15,16-methylene-5-tetracosenoate and their homologues were identified in the deep-lake invertebrate *Acanthogammarus grewingkii*. These unusual fatty acids may represent a specific feature of animals dwelling in a deep lake or may also be metabolites that are formed in cellular membranes, or be of dietary origin [257].

Furanoid fatty acids are minor but widespread components at low levels in fish, especially in their reproductive tissues. The natural fatty acids found to date can have 16 to 22 carbon atoms. At least fourteen different furanoid fatty acids have now been detected in fish lipids, the most common being 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid together with its homologues, and smaller relative proportions of monomethyl acids, such as 12,15-epoxy-13-methyleicosa-12,14-dienoic acid. In fish, furanoid fatty acids occur in triacylglycerols and cholesterol esters. Similar fatty acids have been found in a variety of yeasts, algae and marine bacteria. Indeed, it is suggested that algae and bacteria via the food chain are the main source of furanoid fatty acids in fish, or in the food chain (fish feed on some invertebrates). Furanoid fatty acids are scavengers of hydroxyl and hydroperoxyl radicals, and there is a suggestion that they may play a part in the cardio-protective effects of dietary fish oils. A study of these acids was performed in invertebrates. Furanoid fatty acids were detected in total lipids from the crab *Rhithropanopeus harrisii*, crayfish *Astacus leptodactylus eichwaldi* and bivalve *Mytilaster lineatus*, collected in the Caspian Sea. The 9-(3,4-dimethyl-5-pentylfuran-2-yl)nonanoic acid (F3), 11-(3,4-dimethyl-5-propylfuran-2-yl)undecanoic acid (F4) and 11-(3,4-dimethyl-5-pentylfuran-2-yl)undecanoic acid (F6) were predominant but the organisms contained also odd-numbered FAs such as 11-(3,4-dimethyl-5-pentylfuran-2-yl)undecanoic acid (F5), 13-(3,4-dimethyl-5-pentylfuran-2-yl)tridecanoic acid (F7) and 15-(3,4-dimethyl-5-pentylfuran-2-yl)pentadecanoic acid (F10) [258].

The major fatty acid components in twelve marine gastropod species from the littoral zone of the Red and Mediterranean Seas, and freshwater Sea of Galilee (*Nassa serata*, *Nassarius albescens*, *Nodilittorina subnodosa*, *Planaxis sulcata, Monodonta turbinata*, *Gibula cineraria*, *Littorina neritoides, Melanoides tuberculata*, *Thedoxus jorduni*, *Pyrgula barroisi* and *Melanopsis praemorsum*) were found to be PUFAs including also the usual minor odd-numbered 21:5ω3. The differences among the PUFAs in these species appear to be due mainly to different environmental conditions, dietary habits and geographic spread [259].

Organisms of the classes Crinoidea and Ophiuroidas were found to contain C24 PUFA; the 24:lω9 acid was minor, whereas the 24:6ω3 one was a major constituent. In the total lipids of *Ophioplocus japonicus*, the content of this VLCPUFA was as much as 9.5% of the total FAs [260].

A long-chain sphingoid base was detected in its monoglycosylceramides from the nematode *Caenorhabditis elegans*. The fatty acids consisted of a series of primarily straight chain, saturated, 2-hydroxylated i-21:0, 21:0, i-23:0, 23:0, i-25:0, 25:0 acids [261].

The occurrence of glycosphingolipids with unique carbohydrate structures in different species of cestode prompted the study of molecular species of the monohexosylceramides in the pseudophyllidean cestode *Spirometra erinacei*. The ceramides were composed of sphinganine and phytosphingosine as sphingoid bases, and of nonhydroxy fatty acids ranging from C16 to C30 and hydroxy stearic acid. The proportion of 27:1 was in the percent range [262].

A study of the fatty acids in the total lipid extract, triacylglycerols, free FAs and polar lipids of the willow leaf beetle *Chrysomela vigintipunctata* (Coleoptera: Chrysomelidae) revealed one hundred and fifteen fatty acids in the total lipids. The mixture comprised compounds with normal and branched chains of 12–30 carbon atoms and zero to six double bonds in different positions in the carbon chain. Both even- and odd-numbered VLCFAs were found, saturated as well as up to 27:1, in amounts below 1% total FAs [263].

12. Insect waxes

The comb waxes of the honeybee species *Apis mellifera*, *A. cerana*, *A. dorsata*, *A. laboriosa*, *A. florea* and *A. andreniformis* have been examined by high-temperature gas chromatography. The analysis revealed tens of different compounds. Odd-numbered long chain alcohols such as C35 were identified in these waxes. On the other hand, though odd-numbered FAs were not among those described, the chromatograms presented in the paper indicate their presence as minor constituents [264,265].

The likelihood of a much wider occurrence of odd carbon chain VLCFAs in insects is indicated, e.g., by the studies of Nelson et al.

[266,267] who identified, in pupae of the cabbage looper *Trichoplusia ni* (Hubner), for instance 24-methyltetracontan-1-ol and 22,26,34-trimethyloctatriacontan-1-ol as major compounds that originate very probably from the corresponding FAs, 24-methyltetracontanoic and 22,26,34-trimethyloctatriacontanoic.

13. Coal, peat, sediments and soil

Considerable attention has in recent years been devoted to the composition of sediments, whether fossil or current. The most common of these are sediments deposited in marine and lacustrine environments. Part of this research concerns the FAs of coal, peat and soil. VLCFAs are often taken as biomarkers and their analysis and identification is a frequent subject of studies.

Odd-numbered VLCFAs up to C31 (n and ai), together with α,ω-C33 dicarboxylic acids, were identified in peat, lignite, brown coal, bituminous coal, anthracite and graphite The concentrations of acids decreased as coal rank increased. Branched and unsaturated acids disappeared sooner than alkanoic acids. Monoenoic acids were identified only in peat and lignite and are probably thermally degraded at later stages [268]. Soil extracts contain acids up to C30; peat was found to contain acids C14–C30 [269].

In peat-moss, the content of lipid compounds was investigated in relation to the age of the peat layer, which was determined according to the depth from which the sample of peat was removed. The largest amount of VLCFAs was found in the deepest layers, i.e. in depths greater than 20 cm. Many basic types of acids were present: ω-hydroxyalkanoic, dicarboxylic and normal fatty acids. Both even- and odd-chain FAs were detected up to 32:0. The content of 30:0 amounted to 0.7 mg per g dry peat. Milled peat samples from three Finnish peatlands were studied by capillary GC–MS in terms of their extract content and composition of fatty compounds and sterols. The extract yields obtained by CH_2Cl_2-acetone (9:1 v/v) ranged from 49 to 180 mg g^{-1} of dry peat. The wax content of peat extracts varied between 43 and 55 wt%. The acidic part of extracts contained mainly long-chain fatty acids and ω-hydroxy acids with some α,ω-alkanedioic acids. The non-polar lipids included predominantly primary alkanols with smaller amounts of *n*-alkanes and sterols. The extracts of all peat samples were qualitatively similar but the milled peat Al with high heat value (27.1 MJ kg^{-1}) differed from the other peats in extract content and composition. The peat B with enhanced self-heating ability resembled the ordinary milled peats A2 and C in peat technological characteristics and extractive composition. Accordingly, the self-heating ability of milled peat in stockpiles does not correlate directly to the distribution pattern of long-chain fatty compounds and sterols [270].

Peats in Tibet were found to contain long chain components (C21–C35) including odd-numbered FAs from 21:0 to 29:0. They exhibit bimodal distributions with maxima at about C16 and C24 and show an even carbon number predominance [271].

Another study performed on humin and humic acids from peat samples identified free and esterified aliphatic carboxylic acids, again including minor odd-numbered FAs up to 33:0. The chromatographic data given in the study indicate the presence of even longer FA [272].

The distribution of n-fatty acids shows an even/odd carbon atom predominance in peat and lignite samples. The FAs were found to range from C10 to C30. Again, the samples show a bimodal distribution with maxima at C16 and C24. Odd-numbered straight-chain acids were identified up to 29:0 whereas branched fatty acids (iso or anteiso), unsaturated, and OH-FAs longer than 20 carbon atoms were not found [273].

Pyrolysis mass spectrometry and pyrolysis gas chromatography–mass spectrometry were applied to characterize the macromolecular organic matter in the soil humus horizon which is characterized by an accumulation of bound fatty acids. The major fatty acids in this horizon range from C14 to C36 with even over odd carbon predominance. However, the mass spectra reveal that there are also fatty acids with chain length extending from C43 to C53 which show odd over even predominance. A series of long chain C43–C53 fatty acids with odd over even predominance is probably derived from mycobacteria [274].

Traces of odd-numbered VLCFAs (21:1, 23:0, OMe-2-25:0, 25:0, and 29:0) were identified in compost [275]. All these compounds are assumed to have got into the compost from plant lipids [276].

Investigation of soil characteristics of a wild tropical forest from Cote d'Ivoire and the composition of directly extractable soil lipid fraction led to the identification of odd-numbered VLCFAs up to 33:0, with strong general even/odd carbon atom predominance of FAs which could originate from higher plants; however, for instance 23:0 was more abundant than 22:0 (7.8% and 7.9%, respectively). The bimodal distribution of FAs underlines a vegetal origin of part of the fatty acids and a high microbial activity [277].

Acid soil (Plateau de Millevaches, France) yielded odd-numbered VLCFAs up to 31:0, again with even/odd predominance. The samples contained also α,ω-dicarboxylic acids up to C27 [278].

Soil macromolecular lipids identified by pyrolysis and thermochemolysis were revealed to contain VLCFA, the longest odd-numbered identified acid being 31:0. No even/odd predominance was found in α-OH-FAs - thus 2-OH-27:0 had a higher abundance than 2-OH-26:0 and 2-OH-28:0 [279].

Comparatively little attention has been paid to the occurrence of VLCFAs in soil microorganisms [269,280].

Both ω- and β-hydroxy FAs were observed in lipids isolated from Namibian Shelf diatomaceous material [281]. The acids were detected by using capillary column GC–MS after a preliminary fractionation. The study was carried out mainly because the evidence for the presence of gram-negative bacteria in the sediments was sought. Such bacteria contain β-OH-FA, whereas animals have α-OH-FAs in their sphingolipids. The following acids were found: 24:0, 26:0 and OH-24:0.

VLCFA 15-24:1 and saturated acids 23:0–32:0 were detected in samples from mangrove-associated sediments in eastern Australia [282]. The study focused on the influence of the bacterial population on the content of FAs in the samples.

Hydrocarbons, *n*-alkanes, acyclic isoprenoid alkanes, steranes and triterpanes and fatty acids were studied in sediment samples from inland acid hydrothermal environments in Japan to clarify their features and to elucidate their source organisms. Normal alkanes with carbon chain length ranging from nC13 to nC35 and acyclic isoprenoid alkanes iC16, iC18, pristane, phytane and/or squalane were found, together with steranes and triterpanes. The major hydrocarbons were mainly odd-carbon numbered long-chain *n*-alkanes, such as nC29 and nC31. Normal alkanoic acids (nC10–nC34) were detected with the predominance of even-carbon numbers maximizing at nC16 and, nC24, nC26 or nC28, along with iso- and anteiso-branched (i, aC12−i, aC17) and n-alkanoic acids nC16:1 (carbon chain length: number of double bonds), nC18:n, nC20:n and nC22:n. These compounds can be attributed to various source organisms including bacteria, cyanobacteria, microalgae and vascular plants in and around the hydrothermal environments. Low concentrations of hydrocarbons and fatty acids may reflect the low primary productivity of their harsh environment, but the abundances of n-alkenoic acids indicate that fresh organic matter is continuously supplied by microbial activity and vascular plants [283].

Organic matter composition in the sediment of three Brazilian coastal lagoons – District of Macae, Rio de Janeiro (Brazil) was examined for the content of lipids including FA. All samples contained VLCFAs up to 34:0, with even-chain FAs predominating.

Odd-chain FAs up to 29:0 were determined in low amounts for instance in a sample from Imboacica Lagoon sediment total extract (landward, depth interval 3–6 cm) [284].

The fatty acids from five sulfur-rich lacustrine sediments in the Nordlinger Ries in southern Germany were found to comprise several homologous series. Saturated normal monocarboxylic acids (C9–C33) were the dominant series, while isoprenoid acids, iso- and anteiso-acids and monounsaturated acids were minor constituents. Base hydrolysis of kerogen concentrates and extractable polar fractions liberated α,ω-dicarboxylic acids (C7–C28). The bimodal distributions of FAs in the different acid fractions are indicative of both autochthonous and allochthonous origin of the organic matter in these sediments. The major difference between the samples is the greater proportion of higher plant-derived components (>20:0) in some core samples, reflecting the more marginal setting in the former crater lake and closer proximity to higher plant sources. The high even/odd carbon number predominance of the FAs reflects the low maturity of the organic matter in all samples [285].

In addition to a series of di- and tri-methylated fatty acids, as well as saturated and monounsaturated diacids, odd-numbered VLCFAs (23:0 and 25:0) [286] were also identified in polar lipids isolated from environmental subsurface sediment samples from Old Rifle site near Rifle, CO, USA.

Sediments from the Ria Formosa Lagoon in Portugal were again found to contain FAs including odd-numbered ones, arriving from marine, bacterial, terrestrial, and phytoplankton sources [287].

Sediments from the Dead Sea (Israel) were found to contain even-over-odd carbon number predominance of the long-chain fatty acids (C20-C30), the odd-numbered being present as minor constituents up to 29:0. The VLCFAs were assumed to reflect a contribution from terrigenous higher plants [288].

Organic geochemical study on an extremely acid crater lake of an active Kusatsu-Shirane Volcano in Japan has been carried out to clarify the features and sources of organic components in relation to biological activity. A series of FAs ranging from C8 to C32 with a predominance of even-carbon numbers were found in all the samples, along with monoenoic, i- and ai-FAs, 3-OH-FA, and cyclopropyl FAs, all of them up to C20. No PUFAs, however, were found in all the samples. The features of organic components in the lake waters and sediments indicate that they can be attributed to bacteria, e.g. *Thiobacillus thiooxidans*, with some influences of eroded, and/or wind-transported materials containing vascular plant debris from the surroundings of the lake. Low concentrations of fatty acids mainly reflect the paucity of photosynthetic producers and small living biomass in the lake [289].

A series of VLCFAs, branched and also straight chain were identified from some acidic freshwater lakes in Japan. Both even- and odd-numbered FAs were identified, the latter up to C31. The branched compounds had the methyl side-chain at the ω-2 position, i.e., the anteiso. The anteiso compounds are present in the sediments, as well as in suspended material in the lake water. The results indicate that the anteiso compounds are produced within the lake by planktonic microbes and might be useful molecular markers for lake acidity [290].

14. Plant oils

Until recently it was assumed that the occurrence of straight-chain odd-numbered fatty acids in natural lipids was rare. These FAs account for 1-3% of the total FAs in seeds. Recent analytical instrumentation has proved the occurrence of straight-chain odd-numbered FAs as minor constituents in practically every natural fat. What role they actually play in nature's scheme may be eventually established by continued research [291].

Plant-seed oils have, in general, been found to contain fatty acids with a maximum chain-length of C22 (erucic acid) [292]. Exceptions are represented by, for example, the genera *Tropaeolum* [293] and *Ximenia* [294], which were found to contain monoenoic acids 24:1-30:1ω9.

Ximenia sp. is a spiny, deciduous shrub or small tree growing in southern Africa and South America in low altitude areas, woodland and across grassy savannahs, in coastal areas or on riverbanks. The seed oil is edible and used in cooking. However, its principal use is as an emollient, hair oil, conditioner and skin softener, in soap manufacture, and as component of lipsticks and lubricants. The seed fats of *Ximenia caffra*, *X. caffra* var. *natalensis*, and *X. americana* var. *microphylla* were found to contain large proportions of monoenoic acids of the hexacosenoic (ximenic), octacosenoic, and triacontenoic (lumequic) series [294]. *X. americana* was later recorded to contain octadec-11-en-9-ynoic acid, named ximenynic (santalbic) acid as well as icosenoic-triacontenoic acids, all of which belong to the ω-9 series [295,296]. Surprisingly, no identification of monoenoic VLCFAs was done by those authors who studied *Ximenia* seed oil [297–300].

We analyzed VLCFAs from total fatty acids of *Ximenia* oil and performed their identification as picolinyl esters by means of LC–MS/APCI. The method is based on the use of preparative RP-HPLC and subsequent identification of the acids by microbore LC–MS/APCI. The combination of these two techniques was used to identify unusual monounsaturated VLCFAs, both even- and odd-numbered FAs up to 37:1. Four positional isomers, i.e. 5-, 9-, 30-, and 32-37:1, were identified. A combination of modern analytical methods, i.e. semipreparative RP-HPLC and microbore LC–MS was thus shown to extend the analytical possibilities of both methods and contribute to the acquisition of new information about odd-numbered VLCFAs in biological material, see also Table 15 below [37].

Jojoba oil represents one of the most unusual natural sources of VLCFAs, but one which is, however, easily available [301]. This oil, obtained from seeds of *Simmondsia chinensis,* consists of wax esters instead of the more usual triacylglycerols. The wax esters are liquid at ambient temperature, which offers a wide range of practical applications. By using RP-HPLC, various fractions of wax esters having the same number of carbon atoms were separated, the esters were subsequently saponified and the respective chain-lengths and double bond positions were established. Acids up to 26:1ω9 were found to be present in the wax esters, however, in the subspecies producing the high molecular weight oil only.

A number of papers concerned with VLCFAs in plant oils have identified these acids in the oils(s); in fact, during the processing

Table 15
Total odd-numbered FAs from *Ximenia* oil [37].

Acid	Ratio of positional isomers in %[a]				% Total FA
	ω7	ω9	Δ^9	Δ^5	
21:1	7	93	0	0	2.43
23:1	5	95	0	0	0.18
25:1	8	92	0	0	0.32
26:1	10	90	0	0	6.77
27:1	9	91	0	0	0.27
29:1	11	89	0	0	0.096
31:1	14	84	2	0	0.054
33:1[b]	14	80	6	0	0.027
33:0					0.003
35:1[b]	15	58	9	18	0.014
37:1[b]	16	57	8	19	0.012

[a] The ratio of positional isomers, determined solely from the detector response to each one of the compounds and from the mass spectra of the respective picolinyl esters.
[b] Determined only after sample enrichment by semipreparative RP-HPLC.

wax-like lipids are extracted with triacylglycerols from seed surface and transferred into the oils where they accumulate. In 1969, odd-numbered FAs were described in wax esters from sunflower oil tank settlings [302]. Interestingly, the faultless analytical technique enabled the authors to identify odd- and even-numbered saturated FAs from C20 to C30, whereas analyses performed nearly 40 years later did not provide any additional information [303]. Odd-numbered FAs may be found also in canola oil (low erucic acid rapeseed oil), although their presence can be inferred only from the sample chromatogram and was not explicitly mentioned by the authors [304].

Palm oil has also been found to contain long-chain FAs from 20:0 to 32:0, including odd-numbered 23:0, 25:0, and 27:0 in amounts from 0.3% to 1% total FAs [305]. They were isolated by high-vacuum distillation at high temperature; this procedure succeeded in concentrating low-volatile compounds including VLCFAs in the distillation residue.

Also, 17-hexacosenoic, 21-hexacosenoic, and 23-octacosenoic acids were identified in Chilean hazelnut (*Gevuina avellana*) seed oil [306], major 15-tetracosenoic and 17-hexacosenoic acids with minor octacosenoic acid in *Tropaeolum speciosum* [293], 15-tetracosenoic, 17-hexacosenoic, and octacosenoic acids in *T. majus* [307], and triacontenoic acid in *Curupira tefeensis* [308]; 8-dotriacontenoic acid with unusual position of a double bond was identified in the plant *Centella asiatova* [309].

VLCFAs were found in triacylglycerols from the rare wild genus *Coincya*, family Brassicaceae. Its various species and subspecies, for instance *Coincya monensis* subsp. Nevadensis, *C. monensis* subsp. hispida, *C. monensis* subsp. *recurvata* var. Granatensis, and *C. monensis* subsp. *recurvata* var. Johnstonii contained an odd-numbered 21:1 accounting for 0.3–0.6% total FAs [310].

The fatty acids of fresh leaves of different sea grasses such as *Halophila spinulosa* were reported to include, apart from major FAs, also odd-numbered 21:0–27:0 in amounts of 0.1–1.4% total FAs [311].

Minor odd-numbered VLCFAs such as 21:1 (2.20%) and 21:0 (1.20%) and 21:1 (0.90%) were also identified in *Jaborosa araucana* and *Lycium ciliatum* [312], whereas *Crotalaria intermedia* was found to contain odd carbon chain VLCFAs such as 21:0 (0.1%), 23:0 (0.3%), and 25:0 (0.1%) [313].

PUFAs, including VLCPUFAs such as 22:6ω3, have been the subject of many studies, for instance [314–318]. Unfortunately, all these studies completely overlooked the presence of odd-numbered FAs, at least the very likely C21 PUFAs (see the analyses of algae). We are convinced that much more attention should be paid to studying odd-numbered FAs in higher plants.

15. Plant waxes

Surfaces of higher plants and/or their organs are very often covered by a thin layer of waxes that are in fact mixtures of esters of long-chain fatty acids with long-chain fatty alcohols, plus many other classes of compounds that are not the subject of this review. The waxy surface has many different functions, see e.g. [9,10]. The range of chain lengths of the fatty acids is usually from C20 to C30 or more, with a high predominance of even-chain fatty acids. Many waxes were also found to contain odd-numbered FAs, but their concentration is often more than an order of magnitude lower than that of even-numbered ones. The biosynthesis of waxes has recently been described in detail [10]. We present here only examples of plant waxes that are in some way interesting or bring a global view to the whole class of compounds.

VLCFAs in waxes are as a rule bound to a long-chain alcohol; however, an exception to this rule includes waxes on citrus fruits (Table 16).

Table 16
The compositions of VLCFAs (%) from sterol esters on citrus fruits [319].

Fatty acid	Orange[a]	Grapefruit[b]	Lemon[c]	Lime[d]
23:0	0.46	0.47	0.89	0.62
i-25:0	0.20	0.31	0.43	0.86
ai-25:0	0.25	0.25	1.08	1.19
25:0	1.08	1.51	2.79	3.21
i-27:0	0	tr	0.26	0
ai-27:0	tr	0.17	1.09	0.82
27:0	0.20	0.20	0.53	0.61
ai-29:0	tr	tr	0.22	0.17
29:0	tr	tr	0.10	tr

[a] *Citrus cinensis.*
[b] *C. paradisi.*
[c] *C. limon aurantifolia.*
[d] *C. limethioides.*

Other important and economically significant waxes are those found on the surface of seeds that are industrially used for production of plant oils for food industry and for biodiesel.

During seed pressing, whether cold or especially hot the seed triacylglycerols function in fact as a solvent with which the waxes are extracted into the plant oil in which they may form a haze or turbidity, especially at lower temperatures. This haze presents no health hazard but lowers the esthetic value of the product and thereby also its market value. In biodiesel the haze raises the congealing point of the fuel. Because wax esters cause turbidity when undewaxed seed oils are chilled, the winterization process is used to remove them from such oils. Analyzing these minor components is useful in checking dewaxing and winterization processes which are necessitated by the demand for high-clarity.

The saponifiable matter of vegetable oils contains wax esters, i.e. esters of long chain fatty acids with long chain alcohols, in concentrations from 0 to 3%. These components have an influence on the characteristic appearance of cold pressed, extracted or refined oils. In edible oils, for instance, the concentration of wax esters allows detection of adulteration and admixture of oils of inferior quality.

Odd-numbered VLCPUFAs have been found in plant oils quite a long time ago - an example is the 1969 paper reporting their presence in sunflower oil (from 14:0 to 34:0) [302]. Another edible seed oil is the meadowfoam seed oil extracted from the seeds of *Limnanthes alba*. The seeds contain 20–30% oil and it contains over 98% long chain fatty acids. Apart from the dominant even-numbered FAs up to 32:0 it contains also odd-numbered FAs from wax in lower amounts, e.g. 21:0 (0.9%), 23:0 (1.8%), 25:0 (2%), 27:0 (1.2%) and 29:0 (0.7%) of total FAs [320]. Another oil that has been found to contain VLCFAs from wax is transesterified palm oil. It was found to contain methyl esters up to 32:0. An elegant method, viz. a short-path distillation, was used to increase the very low concentration of VLCFAs in the sample. It achieved a 100-fold enrichment of VLCFAs in the distillation residue. In the VLCFAs, both even and odd carbon numbers accounted for 0.26 wt%, with 23:0, 25:0 and 27:0 being the major odd-numbered ones present in the residue [305].

A rather unexpected occurrence of wax was documented by studies of the leaf cuticular waxes from potato (*Solanum tuberosum*) varieties, which revealed odd-numbered VLCFAs 23:0 and 25:0 [321].

Total lipids from the green above-ground parts of four alpine plants *Oxygraphis glacialis, Primula macrophylla, Rhodiola pamiroalaica*, and *Swertia marginata* were also found to contain VLCFAs. The total odd-numbered FAs were, in their content, always by ca. 1.0–1.5 orders of magnitude lower as compared to the respective even-numbered ones. A total of 10 VLCFAs were found including six unidentified compounds that could be 17:2ω5 homologs [322].

The free fatty acid fraction of pollen of the elder (*Sambucus nigra*) was found to contain a number of VLCFAs including a large homologous series of ω9 monoenoic acids (from C22 to C34). The per cent proportion of odd-numbered VLCFAs was surprisingly high; for instance, 31:1ω9 accounted for 6.09% of total free fatty acids. Such high content of odd-chain VLCPUFAs has not been found even in large producers such as marine sponges, which are one of the richest sources of odd-numbered FAs [323].

A number of studies focused on the wax from sugar cane (*Saccharum officinarum*). It was found to be a mixture of VLCFAs from 24:0 to 36:0 [324–326]. This mixture has important pharmacological effects such as cholesterol-lowering as well as antiplatelet and antithrombotic effects. It was found to contain odd-numbered VLCFAs from 25:0 to 35:0 in about 10-fold lower concentrations than the even-numbered acids (for instance 1.22% of 34:0, 0.07% of 35:0, 0.44% of 36:0). VLCFAs from total fatty acids of sugar cane wax were identified as picolinyl esters by means of LC–MS/APCI. The method is based on the use of preparative reversed phase HPLC of 100 mg amounts and their subsequent identification by microbore APCI LC–MS. This high enrichment of a natural samples with VLCFAs enabled the identification of odd-numbered FAs up to 49:0, one of the longest FAs found in the nature. Though present in a minor amount, 0.047% total fatty acids, it could be identified from mass spectrum and retention characteristics. The combination of these two techniques was used to identify unusual saturated VLCFAs up to C50 [36].

Salt marsh plants and surface sediment samples along a transect in the intertidal flat area of Mont–Saint–Michel Bay were analyzed for fatty acids and sterols. The presence of lipid markers of halophytes in the surface layers of the sediment confirms the export of organic matter from the salt marsh to the intertidal flat. The major fatty acids present in the halophylic bacteria were investigated. The polyunsaturated fatty acids 18:2ω6 and 18:3ω3 were the major fraction in *Salicornia europea* (only minor amounts of 25:0 and 27:0 were documented), whereas long-chain fatty acids (VLCFA; from 24:0 to 32:0) contributed just 4%. Conversely, VLCFAs were present in high concentrations in *Spartina anglica* (47%, most of which were even-numbered acids, only one odd-numbered acid, 25:0 being detected). Polyunsaturated 18:2ω6 and 18:3ω3 and VLCFAs contributed similar proportions in *Halimione portulacoides* (38% and 30%, respectively), the odd-numbered VLCFAs being identified up to C29 [327].

Alkyl esters were detected in the wax from *Cereus peruvianus* and their individual molecular species up to C62 were identified by means of capillary GC–MS. The wax esters were composed of fatty acids up to 30:0 and alcohols up to i-34-0. The odd-numbered VLCFAs were up to 27:0 and ai-29:0. More than 600 isomers of the wax esters were identified [328,329].

16. Fish oils

Analyses of fish oils often include determination of iodine value, i.e. the mass of iodine in grams that is consumed by 100 g of a chemical substance (in this case oil). It is used for determining the amount of unsaturation contained in fatty acids and thus the quality of the oil. Some determinations performed in past decades were found to deviate from theoretical calculations based on GC determination of FAs. This led to the finding that fish oils have to contain other, as yet unidentified PUFAs, i.e. VLCPUFAs.

Routine analyses of fish oils by GC usually end with 22:6ω3 and/or 24:1 [330]. VLCPUFAs have been given more attention in respect to their health importance [12]. The few studies that have been carried out on fish oils include edible species. Though there are dozens of studies of the FA composition of fish and fish oils which include VLCPUFAs up to C23, only Baltic herring (*Clupea harengus*) [331,332], and the flatfish (*Hippoglossoides dubius*) [333,334] have been analyzed for longer-chain VLCPUFAs. Therefore, the VLCPUFAs in fish oil profiles are still not usually reported but this group of FAs at a level of 1% would contribute to a higher iodine value [335].

All fish consume fatty acids originally of algal origin, or modified from algal fatty acids. Finally, odd-number chain lengths, such as 21–29 carbon atoms, and even longer chain lengths, such of as much as 30 carbon atoms, may also occur in fish oils.

Depending on fish species, season, nutrition and the locality where the fish were caught, two main acids are found in fish oil in different proportions. These are eicosapentaenoic acid (20:5ω3) and docosahexaenoic acid (22:6ω3), the two major long-chain ω3 polyunsaturated fatty acids which are essential for many functions in the humans.

Commercial fish oil concentrate was found to contain 28:8ω3 but not its precursors or odd-numbered FAs [109]. Kallio et al. did not find any odd-numbered VLCFAs [336] while in subsequent studies they identified them. Two odd carbon chain acids were also found, i.e. the widespread 21:5ω3, whose structure was unambiguously documented [337], and a less common 21:4ω6.

A US patent describes at least 75% (by weight) enrichment of 20:5ω3 and 22:6ω3 in total fatty acids in fish oil. The level of 21:5ω3 rises from 0.9% to 1.6%. The effects of this acid obtained from fish oil concentrate are discussed in the section about mammals [338].

The structure of 21:5ω3 isolated from many marine animals (copepods, euphausiids, shrimps, molluscs, fishes, but also marine turtles, seals, dolphins, and large whales) (Table 17) was unambiguously determined 30 years ago as all-*Z*-heneicosa-6,9,12,15,18-pentaenoic acid by using total synthesis and mass spectra of its pyrrolidides [339].

21:5ω3 was identified in the lancelets (subphylum Cephalochordata, traditionally known as amphioxus) which are a group of primitive small fish-like marine animals. The species in which it was found was *Branchiostoma belcheri tsingtaoense* caught in the Yellow Sea, China [340].

Several species of fish, including, e.g., striped mullet (*Mugil cephalus*) were found to contain as a minor FA 21:4ω6 acid that accounted for up to 1% total FAs. The analyses clearly show that the FAs composition is affected by the type of nutrition since *M. cephalus* exhibited the greatest degree of intraspecific variation in FAs composition but was readily distinguished from all other species by unusually high median levels of FAs with an odd number of carbons, especially 13:0, 15:0, 15:1*n*−8, 17:2*n*−7, 17:2*n*−5, 17:3*n*−5, and 19:4*n*−5 [341].

Odd-numbered fatty acids in marine oils are normally found to total 1–4% of the total FAs. This is most reliably indicated by gas–liquid chromatography of hydrogenated ester samples, since minor amounts of unsaturated odd-numbered fatty acids may be overlooked or confused with acids of the predominant even-numbered

Table 17
Occurrence of 21:5ω3 in marine organisms.

Source of 21:5ω3	% of total FA
Natural particulate matter	0.1–0.5
Diatom (*Skeletonema costatum*)	0.2–0.4
Copepods (*Calanus* + *Centropages* sp.)	0.1–0.2
Copepods (*Temora longicornia*)	<0.1
Euphausid (*Meganyctiphanes norvegica*)	0.2–0.7
Euphausid (*Euphausia* sp.)	0.1–1.1
Herring oil (*Clupea harengus*)	0.1–0.3
Sturgeon oil (*Acipenser oxyrhynchus*)	0.19
Mackerel oil (*Scomber scrombus*)	0.2–0.6
Fin whale blubber (*Balaenopterus physalus*)	0.1–0.9
Dolphin milk (*Tursiops truncatus*)	0.2

chain lengths. An exception to these typical compositions is the oil of the striped mullet *M. cephalus*, which has been found to contain up to 25% saturated, mono-unsaturated and odd-numbered PUFAs [342].

However, the same fish, when cropped at another shore or in another year (Tunis gulf 2000–2001 or Ionian Sea, western Greece, 2007 – both places are in the Mediterranean Sea, not in the ocean) were found to contain no odd-numbered FAs [343,344]. The authors discuss several examples of variation in the content of odd-numbered FAs and propose several theories of their origin but they concentrate only on C15 and C17 acids and do not mention VLCFAs.

The fatty acid composition of smelt, *O. mordax*, was examined in several areas in Eastern Canada. Smelt from Jeddore Harbour, N.S., were unusual in having odd-numbered FAs, predominantly 21:5. This acid was similar in structure to odd-chain acids occurring in other marine lipids, notably mullet, and was distributed among all body and gonad lipids [244]. The presence of odd-numbered FAs in *O. mordax* was found to originate in the food (mostly the amphipods *P. femorata*) – see the section about invertebrates [245].

The component fatty acids of lipids from four species of flatfish (flathead flounder *H. dubius*, scalyeye plaice *Acanthopsetta nadeshnyi*, pointhead flounder *Cleisthenes pinetorum* herzensteini, and Korean flounder *Glyptocephalus stelleri*) caught in the Sea of Japan and Funka Bay, Hokkaido were examined using open-tubular gas–liquid chromatography. About sixty fatty acids were observed. 21:5ω3 Acid was present as an unusual fatty acid in amounts around 0.5% total FAs [333].

Five species of carp important in the diet of many people in India were examined and 21:5ω3 was found in amounts below 1% of total FAs in muscle and liver [345].

Rodriguez et al. [346] examined whether odd-numbered PUFAs can help elucidate PUFAs formation in trout hepatocytes. For this purpose, eight different odd-chain length substrates (19:1ω9, 19:2ω6, 19:3ω3, 21:2ω6, 21:3ω6, 21:4ω6, 21:3ω3, and 21:5ω3) were synthesized and presented to hepatocytes, and the cell fatty acid composition was examined for various products. The analysis of these products showed that all precursors were elongated and at the same time desaturated.

The biological properties of 21:5ω3 present in small amounts in fish oils were compared with even-numbered PUFAs. 21:5ω3 is incorporated into phospholipids and into triacylglycerol in cell cultures to a similar extent as even-numbered PUFAs. 21:5ω3 is a stronger inhibitor of the conversion of α-linoleic acid and dihomo-γ-linolenic acid to arachidonic acid in hepatoma cells than are even-numbered PUFAs. Fish oil that served as a rat diet was found to contain 21:5ω3 (see the section about mammals) [347].

Redfish (*Sebastes* sp.) oil was found to contain, apart from VLCPUFAs, also multibranched FAs such as 2,3,7,11,15-pentamethylhexadecanoic acid amounting to 0.43% total FAs. This acid is present in at least two diastereoisomers, as shown by the split peaks in Fig. 1 of the paper [348].

17. Vertebrates

Among vertebrates, VLCFAs have so far been identified only in fish and mammals although birds, especially those living in water, were also found to possess odd-numbered FAs, mostly branched- and short-chain fatty acids [9,33].

Based on the content and type of VLCFAs this section was divided into two subchapters. The first of them describes mostly saturated and monoenoic FAs that occur predominantly on the surface (skin, hairs, and glands producing wax-like substances such as sebaceous, meibomian and Harderian glands). The other subchapter concerns eye retina, brain, liver, sperm, and testis, in which VLPUFAs predominate.

17.1. FAs in vertebrate body surface structures and secretions

Great attention has been paid to the composition of the lipids, mostly of FAs, secreted by mammalian wax glands. The most frequently studied secretion of all is wool wax, which is a common component of pharmacopoeia and, therefore, is produced on an industrial scale. Excellent reviews [10,14] cover the present knowledge of its content of FA. Since 1874, when the first FA was isolated, until now, 180 different fatty acids have been identified [349,350], though the major attention in the last two decades was devoted to the content of individual lipid classes, not to FAs [351–353].

Still, two recent papers [354,355] have reported on the identification of odd-numbered VLCFAs. One of them describes, apart from other waxes, also the analysis of lanolin, which was found to contain fatty acids in addition to other aliphatic compounds. Every compound class consists of normal-, iso- and anteiso-branched chain isomers. The separation of all molecular species, in the range of carbon atom numbers C11–C29 was achieved on a low polar capillary column. Compound identification has been made by interpretation of mass spectra as well as on the basis of retention parameters [354].

Thermally assisted hydrolysis and methylation in combination with GC/MS was applied to the analysis of natural waxes (bleached beeswax, lanolin, yellow carnauba wax). The identification revealed more than ninety compounds present in the wax samples, i.e. fatty acids, hydroxy fatty acids, fatty alcohols, diols and sterols. Fatty acids were found to include both even-numbered FAs, predominantly i- and n-VLCFAs up to C32, and odd-numbered ai- and n-FAs up to C31 [355].

Products secreted by other mammals, mostly laboratory animals such as rats, were also found to contain VLCFAs, odd-numbered FAs up to C27 (ai-23:0, 23:0, ai-25:0, 25:0, and C27) being identified more than 40 years ago.(18,671/164,294) Also monkey skin was found to contain VLCFAs, see below. Table 18 [356].

Studies performed on rats *in vivo* and in human skin fibroblasts in culture concerned the metabolism of 22-methyl [23,23,23-$^{2}H_{3}$]tricosanoic acid [357]. The authors were unable to detect odd-chain breakdown products of lignoceric acid and demonstrate that VLCFAs are poor substrates for the α-oxidation system in cultured skin fibroblasts.

Glands such as sebaceous, meibomian and Harderian glands were found to produce the same kinds of FAs as those produced on skin and hairs. Their functions were reviewed in several reviews [358,359].

Lipids of the Harderian ophthalmic gland were separated and wax ester and polar lipids (phosphatidylethanolamine and phosphatidylcholine) were detected as the main lipids in rats while

Table 18
Composition of VLCFAs from the skin of monkey [356].

Fatty acid	Sterol ester	Wax ester IIa	Wax ester IIb
i-23:0	8.29	5.23	1.51
ai-23:0	6.85	1.62	0.50
23:0	0.11	0.09	0.01
i-25:0	2.85	2.16	0.78
ai-25:0	1.73	0.95	0.17
25:0	0.08	0.08	0.02
i-27:0	1.23	0.87	0.09
ai-27:0	1.01	0.61	0.06
27:0	0.03	0.02	–
i-28:0	0.30	0.23	–
i-29:0	0.54	0.43	–
ai-29:0	0.42	0.28	–
ai-31:0	0.29	0.24	–
i-33:0	0.14	0.13	–
ai-33:0	0.16	0.13	–

glyceryl ether diester and both polar lipids were the main lipids in mice. Predominant odd-numbered VLCFAs were saturated ones up to 29:0 and monoenoic acids up to 18-27:1 [360].

Human meibomian gland secretions were analyzed for the presence of cholesteryl esters using HPLC–MS/APCI. There were at least 20 different cholesteryl esters species detected, whose fatty acid residues ranged from C18:1 to C34:1. Apart from the major even-numbered FAs the analysis revealed also odd-numbered FAs, i.e. 21:0-29:0. One of the potential reasons for their presence in the gland may be that cholesteryl esters serve as a depot for very long chain saturated and monounsaturated fatty acids in human meibum [361].

Another species in which fatty acids were studied was golden hamster. The Harderian gland of golden hamsters excretes alkyldiacylglycerol, the fatty acid and alkyl compositions of which differ between males and females. A low amount of i-21:0 and ai-21:0 was found only in females [362]. Fatty acids in the Harderian gland of a male consisted of straight chain saturated acids ranging from C12 to C22. Both even- and odd-numbered acids were observed, indicating that acetyl- and propionyl-CoAs were equally used as primers in the fatty acid synthesis. Large amounts of iso- and ante-iso-branched fatty acids were detected in a female. These findings suggested that, in the female gland, isobutyryl-, isovaleryl-, and 2-methylbutyryl-CoAs were used as primers in addition to acetyl- and propionyl-CoAs in fatty acid synthesis. These results suggest that the sexual differences in the lipids from the Harderian gland of the golden hamster are determined at the step of fatty acid synthesis depending on the available precursors in the male and female glands [363].

Five papers by Harvey [364–368] identified odd-numbered saturated FAs up to C25 or C27, respectively. This author was among the first to make use of GC–MS of derivatives containing nitrogen atom in the molecule, i.e. derivatives such as picolinyl esters for FAs.

The VLCFAs from Meibomian glands of steer [369] mouse [367] rat and human [368] are detailed in Tables 19 and 20.

The creamy white skin-surface biofilm, vernix caseosa, which covers the skin of the fetus and the newborn, is known for its multiple biological functions. It contains odd- as well as even-numbered VLCFAs; noteworthy is the presence of i-, ai- and-27:0, which is not a common combination of acids in a ceramide [370].

17.2. FAs in vertebrate body organs

Though published 15 years ago, the extensive and detailed review of VLCFAs written by Poulos [12] is still an unsurpassed source of information about these acids in higher animals.

Quantitation of fatty acyl-coenzymes A in mammalian cells was done by using liquid chromatography–electrospray ionization tandem mass spectrometry [371].

Even when rats were fed fish oil containing 21:5ω3, the presence of this VLCPUFA was not observed among the molecular species of phosphatidylethanolamines, nor in phosphatidylcholines from rat adipocyte plasma membranes [347]; on the other hand, it was documented in triacylglycerols and free fatty acids [372]. Another study of the incorporation of 21:5ω3 present in small amounts in fish oils showed that the acid is a poor substrate for prostaglandin H synthase and for 5-lipoxygenase, but it inactivates prostaglandin H synthase as rapidly as do 20:4ω6, 20:5ω3 and 22:6ω3. 21:5ω3 inhibits thromboxane synthesis in isolated platelets as efficiently as 20:5ω3. 20:5ω3, 21:5ω3, and 22:6ω3 are all weak inducers of acyl-CoA oxidase in hepatoma cells. Therefore, since fish oils contain only small amounts of 21:5ω3, it is unlikely that this fatty acid is of particular significance for the biological effects of these oils, possibly with the exception that it is a strong inhibitor of 20:4ω6 synthesis [373]. Earlier studies by Tsuji et al. [374] had indicated an opposite situation.

Rat liver was shown to convert exogenous C19 odd-chain PUFAs into the corresponding C21- and C23-PUFAs (21:3ω8, 21:4ω8, 23:3ω8, and 23:4ω8 (from 19:3ω8); 21:4ω5, 21:5ω5, 23:4ω5, and 23:5ω5 (from 19:4ω5); 21:5ω2, 21:6ω2, 23:5ω2, and 23:6ω2 (from 19:5ω2)). It was presumed that these C19 PUFAs were metabolized through the same route as docosahexaenoic acid (22:6ω6) is biosynthesised from eicosapentaenoic acid (20:5ω3). In addition, changes of fatty acid composition of cellular lipids in rat liver cells were examined, using 19:4ω and several fatty acid desaturation inhibitors. Curcumin related compounds, curcumin, capsaicinoids, isoeugenol, and gallic acid esters caused great changes of fatty acid composition of cellular lipids based on inhibition of the Δ6 desaturation of C24-PUFAs in rat liver cells [375,376].

Weanling male Wistar rats were fed semipurified diets containing 5% (w/w) of different dietary fats (almond oil, borage oil, butter, canola oil, cocoa butter, coconut oil, cod liver oil, herring oil, Lorenzo's oil, menhaden oil, olive oil, peanut oil, evening primrose oil, safflower oil, vegetable oil shortening, and soybean oil). Although no odd-numbered 23:0 acid was identified in any of the fed oils, it was present in amounts from 2.7% to 8.5% total sphingomyelin fatty acids [377].

Most of the VLCPUFAs present in ceramide and sphingomyelin of rat testis belonged to the ω6 series and had an even number of carbon atoms in their chains, although odd-chain VLCPUFAs also occurred, see below. The following acids were identified: 21:0, 23:0, 23:1, 25:4ω6, 25:5ω6, 27:4ω6, 27:5ω6, 29:4ω6, 29:5ω6, and 31:5ω6. The content of 31:5ω6 was 0.36% and 0.21% (sphingomyelin and/or ceramide, respectively) of the total fatty acids from rat testis and spermatozoa [378].

Analysis of the testicular lipid classes with VLCPUFAs, cholesterol esters and total triacylglycerols in rat and mouse testis showed that the VLCPUFAs predominating in these lipids were a series of ω6 pentaenes and tetraenes with 24–32 carbons, including small amounts of odd-chain PUFA, as were 21:4ω6, 23:3ω6,

Table 19
Composition of VLCFAs from meibomian glands (in % of total FA).

Fatty acid	Mouse	Rat	Human
i-23:0	0.07	0.077	0.145
ai-23:0	0.58	0.197	0.638
i-25:0	0.04	0.220	0.140
ai-25:0	2.18	1.099	1.920
i-27:0	–	0.064	0.047
ai-27:0	1.05	0.304	1.567
29:0	0.23	–	–

Table 20
Composition of monoenoic fatty acids in meibomian gland of steer [369].

Fatty acid	% of total FA	Fatty acid	% of total FA
13-ai-23:1	0.018	13-ai-27:l	0.020
11-ai-23:1	0.026	11-ai-27:1	0.284
9-ai-23:1	0.156	9-ai-27:1	0.036
17-23:1	0.018	21-ai-29:1	0.006
15-23:1	0.261	19-ai-29:1	0.013
13-23:1	0.021	17-ai-29:1	0.009
15-ai-25:1	0.055	15-ai-29:1	0.011
13-ai-25:1	0.060	13-ai-29:1	0.019
11-ai-25:1	0.150	11-ai-29:1	0.030
9-ai-25:1	0.235	9-ai-29:1	0.012
19-25:1	0.075	25-29:1	0.002
17-25:1	0.390	23-29:1	0.030
15-25:1	0.035	21-29:1	0.068
17-ai-27:l	0.032	25-31:1	0.013
15-ai-27:1	0.028	23-31:1	0.087

23:4ω6, 23:5ω6, 25:4ω6, 25:5ω6, 27:4ω6, 27:5ω6, 29:4ω, 29:5ω6, 31:4ω6, 31:5ω6, and 33:5ω6. It should be noted that this longest-chain odd-numbered VLCPUFAs accounts for 0.1% total FAs [379].

Aveldano et al. [380] proposed a hypothesis for the biosynthesis of odd-chain VLCPUFAs in rat seminiferous tubules. They used radioactive precursors, ($^{1\text{-}14}$C-labeled ω tetraenoic fatty acids (20:4, 24:4, and 32:4) and [U-^{14}C]acetate), and determined the presence of both major even-chain FAs and minor odd-chain acids, i.e. one trienoic (23:3ω), three tetraenoic (C_{23}–C_{27}) and four pentaenoic (C_{23}–C_{29}) acids. The chromatogram shown in this study indicates the probable presence of two higher homologues, i.e. 31:5 and 33:5. The authors discuss various possible pathways for the biosynthesis including α-oxidation of even-chain VLCPUFAs. They speculate that α-oxidation is an important mechanism for the shortening of fatty acids and that odd chain PUFAs are metabolic intermediates.

The relative proportions (% of total fatty acids) of odd-chain (15:0–29:0) and long-chain (22:0-30:0) saturated fatty acids in phospholipids of biotin-deficient rat lymphocytes were significantly increased as compared with biotin-supplemented rats, and the ratio of unsaturated fatty acids to saturated fatty acids in the former was significantly decreased mainly due to the reduced composition of polyunsaturated fatty acids in the ω3, ω6, and ω9 pathway. The ratio of *cis*-vaccenic acid to palmitoleic acid in biotin-deficient rats was significantly lower than that in control rats, and was thought to be another important, but previously unreported indicator of biotin deficiency. These changes imply that the elongation and desaturation of unsaturated fatty acids are depressed in lymphocytes of biotin-deficient rats, and may contribute to the associated immunological dysfunction in biotin deficiency through abnormal prostaglandin metabolism and/or cell membrane functions.

VLCPUFAs have been shown to be localized in unusual molecular species of sphingomyelin in the testes and spermatozoa of the ram, bull, rat, and boar and in the spermatozoa of man. Rat and boar testes and spermatozoa contained principally ω6 polyenoic VLCFAs with three to five double bonds and even- and odd-carbon chain lengths up to 34. In the rat and boar testicular sphingomyelin were identified odd-numbered VLCPUFAs such as 31:4ω6 and 31:5ω6, and sphingomyelin-bound VLCPUFAs from bull and boar testes were 23:1, 25:1, 25:0, 27:1, 29:4ω6, 31:4ω6 [381].

Even though fatty acids with even numbers of carbon atoms predominated, a significant amount of odd-numbered fatty acids was detected in boar spermatozoa. These included 25:3ω6 and 27:3ω6, the series of 23:4ω6 to 31:4ω6, 29:5ω6 and 31:5ω6 [382].

The occurrence of odd-chain VLCPUFAs, e.g. 21:5ω3, 23:5ω3, and trace 25:5 have been detected in blubbers of seals caught both in the sea and in fresh-water lakes of northern Europe. This finding constitutes an exception since mammals usually possess only odd-chain ω3 VLCPUFAs, not ω6 acids. This finding can be ascribed to the food chain sequence plankton-fish-mammal [383].

In the lipids of total human brain tissue, the carbon chain length distribution and the double bond positional isomer composition of the monoenoic fatty acids have been determined using GC/MS. The odd chain length long-chain fatty acids consist of ω8 and ω10 isomers, whereas the odd chain length very long-chain (more than 22 carbon) fatty acids are ω7 and ω9 isomers, e.g. 21:1ω8 (0.14), 23:1ω7 (0.10), 23:1ω8 (0.10), 25:1ω7 (0.36), 25:1ω9 (0.84), 27:1ω7 (0.52) (the values in brackets are% of total FAs) [384].

White brain matter and active plaque tissue from adrenoleukodystrophy patients were analyzed for lipid class and fatty acid compositions and the results compared with white matter from normal brain. In the different classes of lipids, predominantly in cholesteryl esters, phosphatidylethanolamines, sphingomyelins, sulfatides, cerebrosides, etc. were two homologous series, i.e. odd-numbered saturated and monounsaturated FAs from C23 to C29. The adrenoleukodystrophy brain tissue was shown to be active in chain-elongating even-numbered saturated and monounsaturated fatty acids, and also in chain-shortening the even-numbered, chain-elongated products of both series, presumably by microsomal α-oxidation, to produce odd-numbered saturated and monounsaturated VLCFAs [385].

Blood cell and plasma lipid classes and their fatty acids were analyzed in a child with X-linked adrenoleukodystrophy (ALD) and three VLCFAs (21:0, 23:0, 23:1, 25:0, and 25:1) were identified in minor amounts. However, the differences in their contents between the control, the ALD patient, and his mother were only marginal [386].

Human umbilical vein endothelial cells were studied to identify FAs in cellular glycerolipids. The cells were incubated for 2–72 h in a medium supplemented with 0.9–2.6 μM [^{14}C] fatty acid. Chain length markedly influenced the extent of elongation of 5,8,11,14-tetraenoates (18:4 > 19:4 > 20:4 > 21:4) [387].

Several novel PUFAs that contain either an oxygen or sulfur atom in the β-position present in human umbilical vein were found to exhibit more selective antiinflammatory properties than their natural PUFAs counterparts. Though these acids are only synthetic and have not been found in nature, we would like to make note of them because they can be used as a potential new class of pharmaceuticals for treating inflammatory diseases, including atherosclerosis. One of them, β-oxa-23:4ω6, unlike natural PUFAs, lacked the ability to stimulate oxygen radical production in neutrophils but caused marked inhibition of agonist-induced upregulation of leukocyte adhesion to cultured human umbilical vein endothelial cells and E-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 expression. In addition, β-oxa-23:4ω6 inhibited acute and chronic inflammatory responses in mice as well as the upregulation of adhesion molecule expression in arterial endothelium. This action of β-oxa-23:4ω6 required a functional 12- but not 5-lipoxygenase or cyclooxygenases, consistent with its metabolism via the 12-lipoxygenase pathway. Whereas β-oxa-23:4ω6 did not affect the activation of mitogen-activated protein kinases by tumor necrosis factor, activation of the IκB kinase/nuclear factor κB pathway was selectively inhibited [388].

A review [389] has been published about VLCFAs in diagnosis, pathogenesis, and therapy of peroxisomal disorders. The incorporation of [$^{2-14}$C] acetate into the lipids of normal and peroxisome-deficient (Zellweger's syndrome) skin fibroblasts showed that triacylglycerols and cholesteryl esters in Zellweger's syndrome cells contain increased levels of labeled saturated and monounsaturated VLCFAs, among others also 25:0 and 25:1. These data suggest that peroxisomes are involved in the chain shortening of the saturated and monounsaturated VLCFA. Unfortunately, no further data are available as according to the authors small amounts of label were recovered in odd carbon chain length fatty acids, and these are referred to as "Other fatty acids." Also, FAs amounting to less than 0.1% were neglected and are indicated by a dash [390].

A novel method using GC–MS was developed for the screening of peroxisomal disorders. In addition to the commonly studied proportions of odd-numbered VLCFAs such as 22:0, 24:0, and 26:0, we used for the purpose the 25:0/22:0 FAs ratio with great success. This ratio was 0.024 in controls while in patients with X-linked adrenoleukodystrophy it was 0.099 and in patients with Zellweger syndrome it was even 0.322. This example documents how advanced analytical methods, combined with the knowledge of odd-numbered FA, can be conveniently used for the diagnostics of serious disorders [391].

The method for measuring the ratios of concentrations of VLCFAs in plasma in patients with adrenoleukodystrophy, Zellweger syndrome, and infantile Refsum's disease involves ultraviolet detection of *p*-bromophenacyl derivatives of fatty acids. It was

validated by comparison with a GC–MS. It identified also odd-chain saturated FAs such as 21:0, 23:0 and 25:0 [392].

Odd-numbered VLCFAs up to 27:0 were identified in a patient with infantile Refsum's disease. Though odd chain FAs have not been explicitly mentioned in the relevant study and their role has not been specified, they clearly play an important part in this disorder [393].

Propionic acidemia is one of the most common disorders of organic acid metabolism, often present during the neonatal period with life-threatening episodes of severe metabolic acidosis and hyperammonemia. The disease is caused by deficiency of propionyl-CoA carboxylase. As a result of this enzyme defect, the catabolism of propionyl-CoA is impeded, leading to an increased intracellular equilibrium concentration of propionyl-CoA in cells. Propionyl-CoA can replace acetyl-CoA as "primer" for de novo long-chain fatty acid synthesis, leading to increased formation of fatty acids with an uneven number of carbon atoms [394,395].

Identification of odd-chain VLCPUFAs cannot be performed easily by GC or GC–MS, since the minor ω6 pentaenes with odd-numbered chains tend to coelute with the corresponding hexaenes of the ω3 series with even-numbered chains having one carbon less, e.g., 27:5ω6 with 26:6ω3, 29:5ω6 with 28:6ω3, and so on. Something similar occurs between the ω6 tetraenes with odd chains and the ω3 pentaenes with even chains having one carbon less, e.g., 21:4ω6 with 20:5ω3, and so on. The problem was addressed by resolving the methyl esters according to unsaturation by argentation TLC. Two tetraenoic (25:4ω6 and 27:4ω6) and a series of pentaenoic acids from C25 to C33 were found, all being in the ω6 series; their proportions were minor, about 0.01–0.1% of total FAs [379].

18. Future perspectives

VLCFAs and in particular those with odd chain-lengths, are a very interesting group of compounds whose chemical properties and potential biological activities are only now being more extensively studied thanks to the recent advances in analytical methods and instrumentation. They are fascinating in terms of their variegated structures (see, for instance, the acetylenic compounds from the marine sponge *P. triangulate*). They are also parts of very interesting and still scantily described compounds such as plakosides. As seen in this review, the biological activities of these compounds and their component odd-number (and other) VLCFAs, though as yet sparsely studied, include, e.g., antigenic properties of mycobacterial glycolipids, antibiotic effects of polyhydroxylated bacterial polyenes, membrane-stabilizing effects of yeast phospholipids, cytotoxic and hemolytic effects of lipids from sponges, effects of brominated FAs on membrane fluidity, their anti-carcinogenic and antibacterial action, noncytotoxic immunosuppressing action of glycolipids from marine sponges, scavenging of hydroxyl and hydroperoxyl radicals by furanoid FAs from fish oils, cholesterol lowering and antithrombic effects of waxes from some plants, or antiinflammatory action of synthetic PUFA containing oxygen or sulphur atom in the β-position.

The standard first choice method for the analysis of VLCFAs, not only odd-numbered, is GC–MS, provided, of course, that the operator does not use such procedure as that described in a paper (better left uncited): "to quantify the FA composition of ceramide/glucosylceramide, a mixture of odd-chain FA (C15–C25) was added to each sample as internal standard." This approach may suit the analysis of a sample that contains both saturated or monoenoic VLCFAs but a high-sensitivity, though more expensive, LC–MS technique is more convenient when analyzing VLCPUFAs. In this method, we foresee the further development of VCLFAs analysis and its use in the study of metabolism of recently examined organisms.

Due to the commercial availability of LC–MS/APCI systems we expect an increase of sophisticated APCI applications in LC–MS in the coming years. While the high purchase price and operating cost seem to be the main limitations in the use of LC–MS/APCI systems, the robustness and sensitivity of analysis, the possibility of simultaneous evaluation of the content of chemical compounds, estimation of their chemical structure and determination of molecular mass make it a technique of an extraordinary value.

This review is the first to summarize the current knowledge on VLCFAs. Based on authors' own experience in the field, it summarizes the information on their occurrence, identification and physiological properties, and refers to other relevant sources as regards their biosynthesis. We hope that the current advances in analytical techniques, increasing availability and decreasing costs of GC–MS and LC–MS instrumentation will in the coming years boost our knowledge of odd-numbered VLCFAs.

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