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Odd-numbered very-long-chain polyunsaturated fatty acids from the dinoflagellate *Amphidinium carterae* identified by atmospheric pressure chemical ionization liquid chromatography–mass spectrometry

Tomáš Řezanka [a,*], Linda Nedbalová [b,c], Karel Sigler [a]

[a] Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague, Czech Republic
[b] Charles University in Prague, Faculty of Science, Department of Ecology, Viničná 7, 128 44 Prague 2, Czech Republic
[c] Institute of Botany, Academy of Sciences of the Czech Republic, Dukelská 135, 379 82 Třeboň, Czech Republic

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ABSTRACT

A method is described for the enrichment of odd very-long-chain polyunsaturated fatty acids (VLCPUFAs) by means of RP-HPLC and argentation TLC from total fatty acids of the dinoflagellate *A. carterae* and their identification as picolinyl esters by means of microbore liquid chromatography-mass spectrometry with atmospheric pressure chemical ionization (LC–MS/APCI). The combination of argentation TLC and LC–MS/APCI was used to identify rare and unusual odd VLCPUFAs up to nonacosahexaenoic acid. Two acids, (all*Z*)-nonacosa-11,14,17,20,23-pentaenoic acid (29:5n-6) and (all*Z*)-nonacosa-11,14,17,20,23,26-hexaenoic acid (29:6n-3), were synthesized for the first time to unambiguously confirm their structure. Possible biosynthetic pathways for odd VLCPUFAs are also proposed.

1. Introduction

In nature, very-long-chain polyunsaturated fatty acids (VLCPUFAs, i.e. fatty acids with three and more double bonds and with chain lengths equal to and/or greater than 20 carbons) are found infrequently; for a review of these compounds in animal and plant kingdom see Rezanka (1989). In general, classical, i.e. methylene-interrupted VLCPUFAs can be said to have been found mainly in mammals, with several exceptions (Poulos et al., 1986). Among lower animals and plants, VLCPUFAs have been found in a range of organisms (e.g., *Cladonia* lichens, slime-molds, *Chlorella kessleri,* the marine dinoflagellate *Gymnodinium* sp., algal classes Chlorophyceae and Prasinophyceae) (Rezanka and Podojil, 1984; Dembitsky et al., 1991; Dunstan et al., 1992; Rezanka, 1993, 2002; Mansour et al., 1999a,b, 2003, 2005) and in invertebrates (crustacean *Bathynella natans*) (Rezanka and Dembitsky, 2004).

In the rat, most of the VLCPUFAs are present in sphingomyelin and ceramide and belong to the n-6 series and have an even number of carbon atoms in their chains, but odd-chain VLCPUFAs also occurred in low amounts. They were mostly represented by two tetraenoic acids (27:4n-6 and 29:4n-6) amounting to 0.20% of total FAs. In addition, a series of odd-chains 25:5n-6 to 31:5n-6 was present in amounts from 0.02 to 0.36 of total FAs. The proportion of odd-chain VLCPUFAs can be seen to be one to two orders of magnitude lower than that of even-chain VLCPUFAs of the same chain length; for instance, the amount of 30:5n-6 was 13.4% of total FAs while 31:5n-6 amounted to only 0.21% and 32:5n-6 to 3.9% (Girland et al., 2007).

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 25:3n-6 and 27:3n-6, the series of 23:4n-6 to 31:4n-6, 29:5n-6 and 31:5n-6 (Poulos et al., 1986).

Aveldano et al. (1993) proposed a hypothesis for the biosynthesis of odd-chain VLCPUFAs. They used radioactive precursors, (1-^{14}C-labeled n-6 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 (23:3n-6), 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 speculated that α-oxidation is an important mechanism for the shortening of fatty acids and that odd-chain PUFAs are metabolic intermediates.

Identification of odd-chain VLCPUFAs cannot be performed easily by GC or GC-MS, since the minor n-6 pentaenes with odd-numbered chains tend to coelute with the corresponding hexaenes of

* 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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the n-3 series with even-numbered chains having one carbon less, e.g., 27:5n-6 with 26:6n-3, 29:5n-6 with 28:6n-3, and so on. Something similar occurs between the n-6 tetraenes with odd-chains and the n-3 pentaenes with even chains having one carbon less, e.g., 21:4n-6 with 20:5n-3, and so on. The problem was addressed by resolving the methyl esters according to unsaturation by argentation TLC. Two tetraenoic (25:4n-6 and 27:4n-6) and a series of pentaenoic acids from C25 to C33 were found, all being in the n-6 series; their proportions were minor, about 0.01 to 0.1% of total FAs (Furland et al., 2003).

The occurrence of odd-chain VLCPUFAs, e.g., 23:5n-3, and trace 25:5n-3 has been detected in blubbers of seals caught both in the sea and in freshwater lakes of northern Europe. This finding constitutes an exception since mammals usually possess only odd-chain n-6 VLCPUFAs, not n-3 acids. This finding can be ascribed to the food chain sequence plankton-fish-mammal (Kakela et al., 1995).

Japanese authors (Shimizu et al., 1991; Shirasaka et al., 1995; Zhang et al., 2006) have reported on the identification of odd-chain PUFAs (C17 and C19) in fungi of the genera *Mortierella* and *Saprolegnia* cultivated on odd-chain carbon sources such as n-heptadecane or fatty acids with 13, 15, or 17 carbons. A scheme of the biosynthesis of 17:3n-5 and its successors 19:3n-8, 19:4n-5, and 19:5n-2 has been proposed.

Another study dealing with the biosynthesis of odd-chain VLCPUFAs (Nakano et al., 2000) demonstrated that rat liver cells converted exogenous C19 odd-chain-polyunsaturated fatty acids into the corresponding C21- and C23-PUFAs, e.g., from 19:3n-8 to 21:3n-8, 21:4n-8, 23:3n-8, and 23:4n-8; from 19:4n-5 to 21:4n-5, 21:5n-5, 23:4n-5, and 23:5n-5; and from 19:5n-2 to 21:5n-2, 21:6n-2, 23:5n-2, and 23:6n-2. It presumed that these C19 PUFAs were converted through a route mimicking that of docosahexaenoic acid (22:6n-3) from eicosapentaenoic acid (20:5n-3).

The VLCPUFAs from trienes to hexaenes were found in the lipids of *Amphidinium carterae* to contain a series of minor odd-chain homologues. By separating these acids first by degree of unsaturation (by Ag^+-TLC) and then by chain length by (RP-HPLC), we were able to recognize unusual odd-chain polyenoic fatty acids of diverse chain lengths. Their identity was confirmed by APCI mass spectra and also by comparison with synthesized standards. On reverse phase columns, the odd-chain PUFAs of dinoflagellate conformed to the chromatographical behavior expected of a complete homologous series, covering the whole range of chain lengths from 21 to 31 carbon atoms.

2. Results and discussion

As described in our previous paper on fatty acids present in the dinoflagellate *A. carterae* (Rezanka et al., 2008), the VLCPUFAs that have so far been identified are solely even-chain ones, up to C36 (36:7n-6 and 36:8n-3). According to the literature odd-chain VLCPUFAs are contained in the lipids isolated from different sources in amounts one to two orders of magnitude lower than even-chain FAs; we therefore attempted to enrich their fraction by using the previously described and tested method (Van Pelt et al., 1999; Rezanka and Sigler, 2007; Rezanka et al., 2008).

LC–MS/APCI analysis proved a superior method for the identification of PUFA chain length and degree of unsaturation compared to GC–MS. The correlation of retention time with unsaturation for the chain series demonstrated the powerful ability of the LC–MS/APCI system to directly identify PUFAs from the presence of the protonated molecular ion. Close correlations based on both chain length and degrees of unsaturation were demonstrated for the series of PUFAs including VLCPUFAs. Any potential ambiguity for fatty acids of the same molecular weight (e.g., 27:1 and 28:8) is removed by such observation of retention factor (Ag^+-TLC), retention time (RP-HPLC), and fragmentation in mass spectrum. By both methods we succeeded in identifying two series of odd-chain VLCPUFAs. As with even chain VLCPUFA, odd-chain acids were found to include both n-6 and n-3 series. Since their amounts are one to two orders of magnitude lower than even-chain VPCPUFA, their identification was performed using the SIM (single ion monitoring) mode of protonated molecular ions, see Fig. 1.

Ionization and detection of VLCPUFA ions by LC–MS/APCI delivered a significant benefit in detection capability when compared to other detection techniques. The selectivity of SIM gives a significantly lower detection limit due to the lack of chemical interferences at protonated molecular ion compared to total ion current. In SIM mode the detection limit for picolinyl esters of VLCPUFAs was 0.0001% of total FAs. The capability of VLCPUFAs identification afforded by the Ag^+-TLC and LC–MS/APCI system is visibly demonstrated in the co-elution of 27:4n-6 and 21:1n-9. While the total ion chromatogram indicates a broad peak centered at 2494 s, the two separate components (27:4n-6 and 21:1n-9) are clearly resolved by individual mass chromatograms (Fig. 2). Unfortunately, no further information on double bond positions is available from the direct LC–MS/APCI approach and picolinyl esters were therefore used for distinguishing them.

As seen in Table 1, only the odd tetraenoic, pentaenoic and hexaenoic acids were identified. If odd VLCPUFAs with 7 and 8 double bonds are present, their proportion in total FAs is below the detection limit. The absence of heptaenoic and octaenoic acids is comparable with the absence of, e.g., hexaenoic acids in mammalian materials, in which even-chain FAs were identified up to hexaenoic while odd-chain FAs only up to pentaenoic (Poulos et al., 1986; Aveldano et al., 1993). On the basis of our results we cannot determine whether this is due to a ‘‘faulty‘‘ metabolism or simply to their minor proportions that could not be detected, even though our detection range spanned 6 orders of magnitude.

To confirm the structure of odd-chain VLCPUFAs, two of the minor FAs were prepared for the first time by total synthesis using methods described in our previous paper (Rezanka et al., 2008). Briefly, retrosynthetic analysis revealed a suitably protected 11-dodecynol as an ideal starting material. Thus, commercially available 10-bromo-decanol was protected by treatment with 3,4-dihydro-2*H*-pyran in the presence of catalytic amounts of pyridinium p-toluene sulfonate to afford tetrahydropyranyl acetal in 80% isolated yield. Reaction of this acetal with lithium acetylide ethylenediamine complex gave 79% of **3** (Ettmayer et al., 2004) that was allowed to react with all-(*Z*)-1-bromoheptadeca-2,5,8,11,14-pentaene (**4**) or (all-*Z*)-1- bromoheptadeca-2,5,8,11-tetraene (**5**) which were synthesized as previously described (Rezanka et al., 2008) in the presence EtMgBr solution and copper bromide to yield the 2′-((all-*Z*)-nonacosa-14,17,20,23,26-pentaen-11-ynyloxy)tetrahydro-2*H*-pyran (**6**) or 2′-((all-*Z*)-nonacosa-14,17,20,23-tetraen-11-ynyloxy)tetrahydro-2*H*-pyran (**7**), respectively. Our previously described methods, i.e. partial reduction, hydrolysis and oxidation, were used to prepare from these two compounds (all-*Z*)-nonacosa-11,14,17,20,23,26-hexaenoic (**1**) and (all-*Z*)-nonacosa-11,14,17,20,23-pentaenoic (**2**) acids, respectively.

As already described by Rezanka et al. (2008), the APCI mass spectra of two peaks had characteristic ions consistent with synthesized (all-*Z*)-nonacosa-11,14,17,20,23,26-hexaenoic (**1**) and (all-*Z*)-nonacosa-11,14,17,20,23-pentaenoic (**2**) picolinyl esters, see Figs. 4 and 5. Picolinyl esters have prominent ions at m/z = 92, 108, 151 (the McLafferty ion) and 164, which are all fragments about the pyridine ring. Furthermore, the pseudomolecular ion $[M + 54]^+$ was also characteristic, but it arises by the addition of acetonitrile from the mobile phase. The molecular ion is easily distinguished and it is always odd-numbered, because of the presence of the nitrogen atom, but most other ions are even-numbered. From the molecular ion, there is the loss of a methyl group,

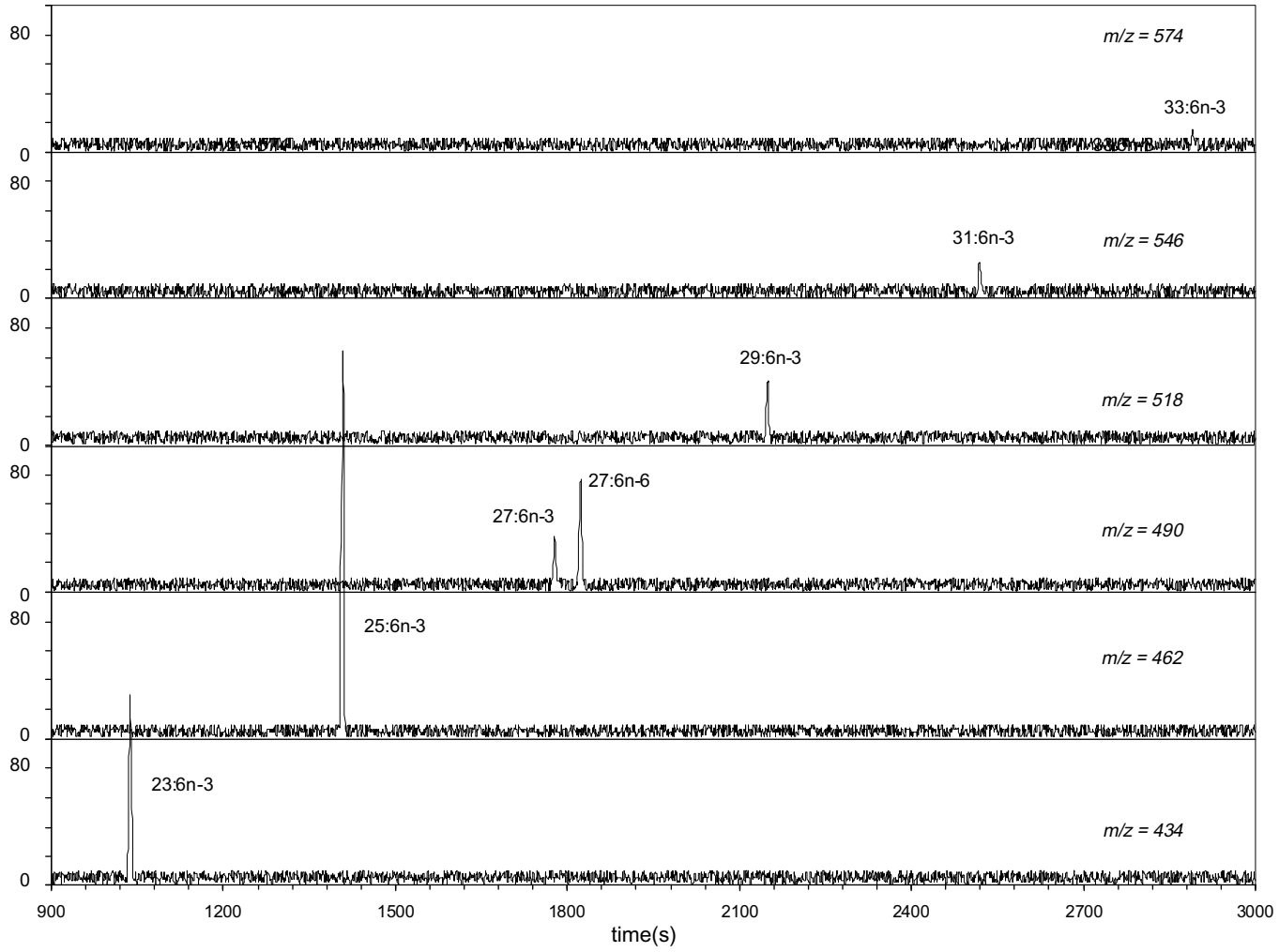

Fig. 1. LC–MS/APCI SIM chromatograms of FAMEs from total FAs of *A. carterae* after Ag^+-TLC; fraction with six double bonds is shown. The SIM traces depict molecular ion masses, see *m/z* values.

followed by a series of ions 14 Da apart for the loss of successive methylene groups. When a double bond is reached, there is a gap of 26 Da. This gap can sometimes be difficult to locate precisely, and a fragmentation at the adjacent methylene group on the carboxyl side giving a gap of 40 is often easier to locate, especially with polyenes, see below. The greater complexity of the mass spectra of PUFA picolinyl esters can make interpretation rather difficult.

The gap of 26 amu between *m/z* = 488 and 462 locates the terminal double bond rather easily, and that between *m/z* = 448 and 422 locates a double bond in position 22 of the picolinyl ester **1**. This pattern is repeated up to the first double bond. The gap of 40 amu for the double bond and an associated methylene group, in this instance from *m/z* = 262 to 302 to 342 to 382 to 422 to 462 to 502, (see Fig. 3), is also very useful.

The structures of other PUFAs, including the synthesized compounds, e.g., (all-*Z*)-nonacosa-11,14,17,20,23-pentaenoic picolinyl ester (**2**), were identified in an analogous manner, as illustrated in Fig. 4.

We also used other methods for the full confirmation of the above structures, i.e. hydrogenation of the compounds from the lower spots after Ag^+-TLC. Only peaks corresponding to saturated straight-chain FAMEs were observed by means of GC-MS. The EI mass spectra of these peaks contained fragment ions characteristic for saturated FAMEs, i.e. *m/z* 74, 87 and weak ions at $[M]^+$, together with diagnostic ions at $[M\text{-}MeO]^+$ and $[M\text{-}C_3H_7]^+$.

Further, the PUFAs were not conjugated, as shown by the UV spectra (see experimental). The double bond stereochemistry was established by FTIR. All double bonds were *Z* because the IR spectrum of the PUFAs exhibited absorption at 723 cm^{-1} and no absorption in the 960–980 cm^{-1} regions (Doumenq et al., 1990).

The presence of odd-chain VLCPUFA in the alga poses several important questions, the most fundamental of them being the manner of their biosynthesis. Saturated or monoenoic odd-numbered acids are formed by *de novo* synthesis through successive additions of two-carbon units to an initial three-carbon unit, or by one-carbon shortening of even-numbered fatty acids by α-oxidation (Diedrich and Henschell, 1990). A completely different situation is found with PUFA, especially with VLCPUFA. They are assumed to be formed either (1) from odd-numbered saturated or monoenoic acids via analogous pathways - "mimic routes" as even-numbered PUFA (Nakano et al., 2000), (2) by α-oxidation of even-numbered PUFA (Aveldano et al., 1993) or (3) α-ketoacid elongation without participation of fatty acid synthase-mediated reactions or -independent thioesterases (Kroumova et al., 1994). Another obvious possibility is a mixed biosynthesis, which involves a marriage of routes 1 and 2. We used the data from our analyses, especially the data in Table 1 and Fig. 5 to suggest a tentative

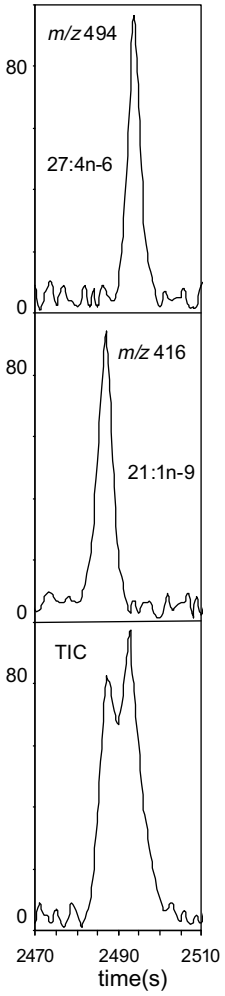

Fig. 2. LC–MS/APCI SIM and TIC chromatograms of total FAMEs from *A. carterae*. Only compounds with retention times between 2470 s and 2510 s are shown.

pathway for the biosynthesis of VLCPUFAs in *A. carterae*. We speculate that α-oxidation is an important mechanism in the shortening of fatty acids and that odd-chain VLCPUFA are normal metabolic products that, however, occur in concentrations an order of magnitude lower than even-numbered ones.

3. Experimental

3.1. Instrumental methods

UV spectra were measured by a Cary 118 (Varian) apparatus in MeOH in the range 200–350 nm. A Perkin–Elmer Model 1310 (PerkinElmer, Norwalk, CT) infrared spectrophotometer was used for scanning infrared spectroscopy of methyl esters as a neat film. NMR spectra were recorded on a Bruker AMX 500 spectrometer (Bruker Analytik, Karlsruhe, Germany) at 500.1 MHz (^{1}H) and 125.7 MHz (^{13}C) in $CDCl_3$. The fatty acids and other compounds were purchased from Sigma-Aldrich (Prague, Czech Republic). GC–MS of a FAME mixture was done on a Finnigan 1020-B in EI mode. The hydrogenated sample was injected onto a 25 m × 0.25 mm × 0.1 μm Ultra-1 capillary column (Supelco, Czech Republic) (injector and detector temperatures of 300 °C) under a temperature program of: 5 min at 50 °C, increasing at 10 °C min^{-1} to 320 °C and 15 min at 320 °C. Helium was the carrier gas at a flow of 0.52 ml min^{-1}. All spectra were scanned within the range *m/z* 50–700.

Table 1
Total VLCPUFAs after Ag^+-TLC; only acids with three and more double bonds are given

Peak no.	FA	%	Peak no.	FA	%
1	21:5n3	0.43	35	28:6n6	3.43
2	23:6n3	0.31	36	23:3n3	0.20
3	20:4n3	1.91	37	30:7n6	1.74
4	22:5n3	8.13	38	25:4n3	0.04
5	20:4n6	12.04	39	27:5n3	0.05
6	24:6n3	5.61	40	25:4n6	0.07
7	22:5n6	3.63	41	29:6n3	0.10
8	26:7n3	1.40	42	24:3n3	1.21
9	28:8n3	15.77	43	26:4n3	0.83
10	21:4n3	0.06	44	24:3n6	0.19
11	23:5n3	0.48	45	28:5n3	1.02
12	25:6n3	0.62	46	26:4n6	1.15
13	23:5n6	0.20	47	32:7n3	1.98
14	22:4n3	1.34	48	32:7n6	1.47
15	24:5n3	2.92	49	27:4n3	0.05
16	26:6n3	1.47	50	25:3n6	0.30
17	24:5n6	1.59	51	27:4n6	0.04
18	28:7n3	1.66	52	31:6n3	0.06
19	26:6n6	0.45	53	29:5n6	0.07
20	21:3n3	0.11	54	28:4n3	1.08
21	28:7n6	7.32	55	26:3n6	1.53
22	23:4n3	0.05	56	28:4n6	0.96
23	25:5n3	0.12	57	34:7n3	2.06
24	23:4n6	0.11	58	34:7n6	1.66
25	27:6n3	0.09	59	27:3n3	0.03
26	25:5n6	0.02	60	36:8n3	0.61
27	27:6n6	0.20	61	27:3n6	0.01
28	22:3n3	2.04	62	31:5n3	0.08
29	24:4n3	0.89	63	33:6n3	tr
30	26:5n3	1.27	64	28:3n3	0.51
31	24:4n6	0.76	65	28:3n6	0.45
32	28:6n3	1.72	66	36:7n3	0.49
33	26:5n6	0.38	67	36:7n6	0.19
34	30:7n3	1.24			

The sum of odd FA is 0.61% of total FA, whereas the sum of n-3 odd FA is 0.45% of total FA, tr - traces, less than 0.01%.

HPLC equipment consisted of a 1090 system, PV5 ternary pump and automatic injector (HP 1090 series, Hewlett Packard, USA) and two Hichrom columns HIRPB-250AM 250 × 2.1 mm ID, 5 μm phase particle, in series. This setup provided us with a high-efficiency column – approximately ~26000 plates/250 mm. A quadrupole mass spectrometer system Navigator (Finnigan MAT, San Jose, CA, USA) was used for the analysis. The instrument was fitted with an atmospheric pressure chemical ionization source (vaporizer temperature 400 °C, capillary heater temperature 220 °C, corona current 5 μA, sheath gas – high-purity nitrogen, pressure 0.38 MPa, and auxiliary gas (also nitrogen) flow rate 15 ml/min. Positively charged ions with *m/z* 50–700 were scanned with a scan time of 0.5 s. The whole HPLC flow (0.37 ml/min) was introduced into the APCI source without any splitting. Fatty acid picolinyl esters were separated using a gradient solvent program with acetonitrile (ACN), dichloromethane (DCM) and propionitrile (EtCN) as follows: initial ACN/EtCN/DCM (60:30:10, vol/vol/vol); linear from 5 min to 50 min ACN/EtCN/DCM 30:40:30, vol/vol/vol); held until 60.5 min; the composition was returned to the initial conditions over 8 min. A peak threshold of 0.3% intensity was applied to the mass spectra. Data acquisition and analyses were performed using PC with MassLab 2.0 for Windows XP applications/operating software.

3.2. Standards, cultivation, isolation and synthesis

Standards of fatty acids were prepared as described below. All solvents were double distilled and degassed before use.

The strain of the dinoflagellate *A. carterae* was obtained from the Culture Collection of Algae and Protozoa in Scotland (strain

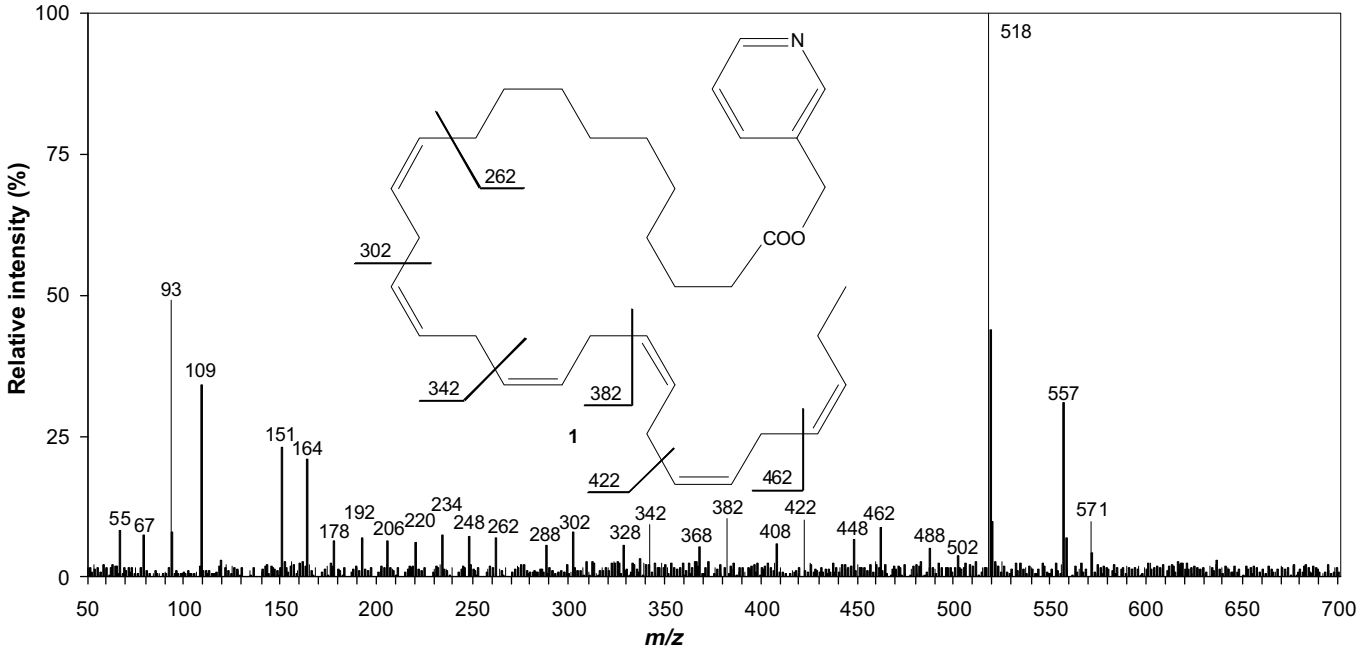

Fig. 3. Mass spectrum of picolinyl ester of 29:6n-3 acid. For explanation of values see the text.

CCAP 1102/3, isolated from a ditch in Essex, England). The alga was cultivated in liquid K minimum medium for marine dinoflagellates (Keller et al., 1987) at 10 °C under continuous light provided by cool white fluorescent lights. After 5 weeks, the culture was harvested by centrifugation at 1500 rpm for 10 min.

The cells of *A. carterae* (195 mg) were extracted with 3 × 30 ml of $CHCl_3$–MeOH (2:1), yielding total lipids (6.8 mg), which were dissolved in toluene (1 ml) and 1% sulfuric acid in methanol (2 ml) was added. The mixture was left overnight in a stoppered tube at 50 °C, then water (5 ml) containing sodium chloride (5%) was added and the esters (2.17 mg) were extracted with hexane (2 × 5 ml). The hexane layer was washed with water (4 ml) containing potassium bicarbonate (2%), dried over anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure.

Ag^+-TLC. The FAMEs were fractionated by Ag^+-TLC using petroleum ether–diethyl ether–acetic acid (90:8:2, v/v) for the first separation and (70:28:2, v/v) for the second separation. Detection was done with 0.2% 2,7-dichlorofluorescein (in ethanol) under UV lamp at 366 nm. The zone of silica gel corresponding to PUFA methyl

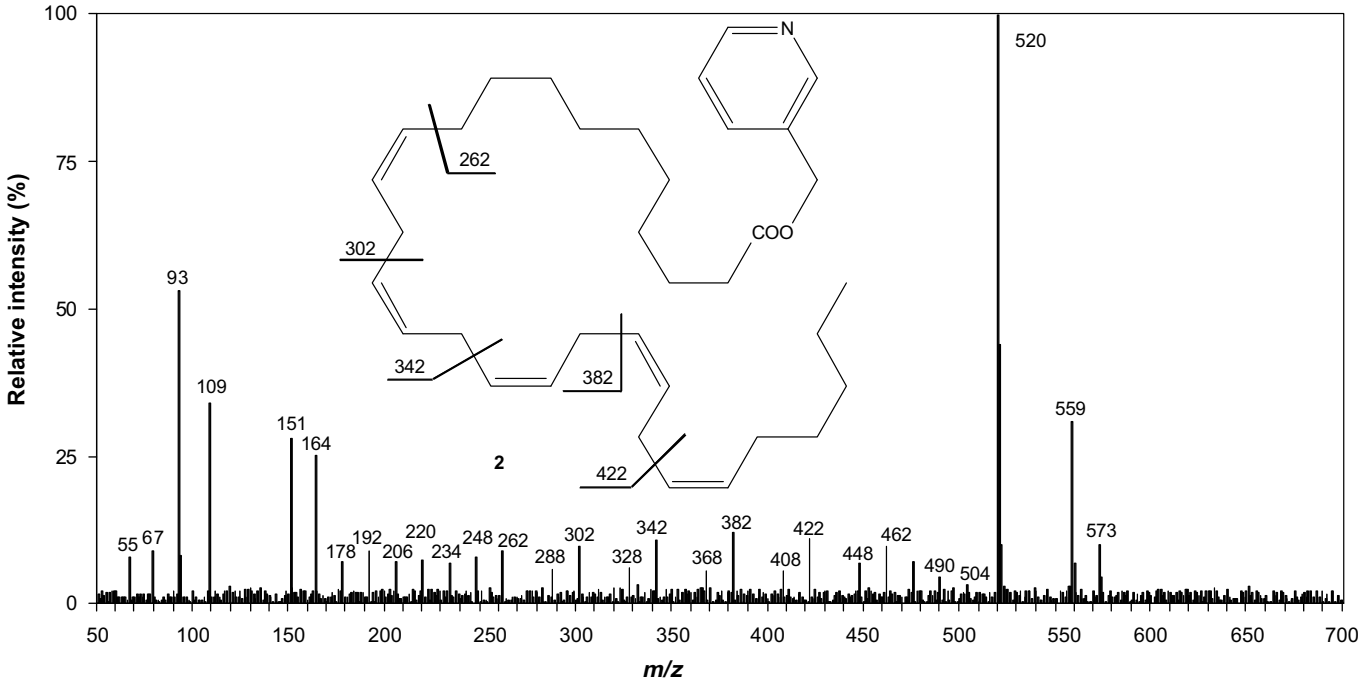

Fig. 4. Mass spectrum of picolinyl ester of 29:5n-6 acid. For explanation of values see the text.

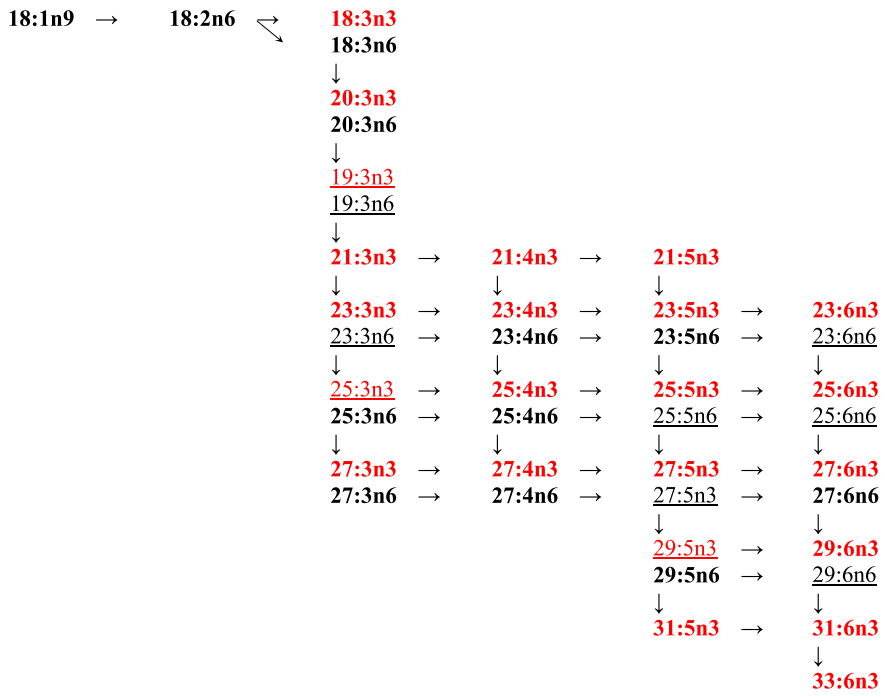

Fig. 5. Hypothesis of odd VLCPUFAs biosynthesis. Black – n-6 isomers, red – n-3 isomers, bold – isomers found in the alga, underlined – isomers not found. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

esters was scraped off and FAMEs were extracted from the silica gel using hexane–diethyl ether (9:1). The FAMEs were washed with saturated NaCl and 2 M NH_3 to remove Ag^+ and dichlorofluorescein, respectively. The structure of FAMEs (1.42 mg) was confirmed by LC–MS as described above.

Picolinyl esters of PUFA. A solution of potassium tert-butoxide in tetrahydrofuran (0.5 ml, 1.0 M) was added to nicotinyl alcohol (1 ml). After mixing, the appropriate fatty acid methyl esters (~0.5 mg) in dry dichloromethane (1 ml) were added, and the mixture was held at 40 °C for 30 min in a closed vial. After cooling to room temperature, water and hexane were added, and the organic phase was collected, dried over anhydrous sodium sulfate, and evaporated.

Hydrogenation of PUFAs was carried out in 1 ml of methanol with catalytic amounts of PtO_2. FAMEs were extracted with *n*-hexane and subjected to GC–MS.

3.3. *(all-Z)-Nonacosa-11,14,17,20,23,26-hexaenoic acid (**1**)*

To an EtMgBr solution (4 ml of a 2 M solution in THF, 8.0 mmol) at 0 °C was added dropwise a solution of **5** (149 mg, 0.56 mmol) in dry THF (2.5 ml). The reaction mixture was warmed up to room temperature over 10 min and then heated under reflux for 10 min (Heitz et al., 1989). After cooling at 0 °C, copper bromide (114 mg, 0.8 mmol) was added, followed by dropwise addition of bromide **6** (173 mg, 0.56 mmol) in dry THF (2.5 ml). The resulting reaction mixture was heated under reflux for 12 h, then cooled to room temperature, quenched by addition of water, and filtered. The reaction mixture was extracted with ether, and the combined organic extracts were washed with brine, dried, and concentrated. Purification of the crude product 2′-((all-*Z*)-nonacosa-14,17,20,23,26-pentaen-11-ynyloxy)tetrahydro-2*H*-pyran on silica gel (hexane-diethylether 9:1) gave 100 mg (36%) of a colorless oil, HREIMS *m/z* calcd. for 494.4124 $C_{34}H_{54}O_2$ $[M]^+$, found 494.4121.

P-2 nickel was prepared via $NaBH_4$ reduction of $Ni(OAc)_2 \cdot 4H_2O$ (74 mg, 0.3 mmol) in absolute ethanol (5 ml). The flask was purged with hydrogen, and ethylenediamine (50 µl, 45 mg, 0.75 mmol) was added followed by a derivative of pyran (99 mg, 0.20 mmol) in absolute ethanol (0.5 ml). Hydrogen uptake was quantitative in 4 h. The reaction mixture was filtered, diluted with water, and extracted with diethylether. The combined ether extracts were washed with water, cold 1 M HCl, brine, dried, and concentrated to give of crude product as brown colored oil. Chromatography on preparative TLC plates, using diethylether–hexane (15:85), gave 87 mg (88%) of polyene 2′-((all-*Z*)-nonacosa-11,14,17,20,23,26-hexaenyloxy)tetrahydro-2*H*-pyran as pale yellow colored oil. HREIMS *m/z* calcd. for 496.4280 $C_{34}H_{56}O_2$ $[M]^+$, found 496.4276.

A solution of polyene THP ether (85 mg, 0.17 mmol) and PPTS (pyridinium *p*-toluenesulfonate) (3 mg, 0.012 mmol) in ethanol (2 ml) was stirred at 45 °C (bath temperature) for 10 h (Miyashita et al., 1977). The solvent was evaporated *in vacuo*, and the residue was chromatographed on preparative TLC, using hexane–diethylether (3:1) to afford pure alcohol ((all-*Z*)-nonacosa-11,14,17,20,23,26-hexaen-1-ol) (69 mg, 98%). HREIMS *m/z* calcd. for 412.3705 $C_{29}H_{48}O$ $[M]^+$, found 412.3701.

To a solution of polyene alcohol (66 mg, 0.16 mmol) in 5 ml acetone was added under stirring at −2 °C in 30 min a solution of $Na_2Cr_2O_7 \cdot 2H_2O$ (48 mg, 0.16 mmol) in 50 µl conc. sulfuric acid and 0.5 ml water (Kunau, 1971). The mixture was stirred for another 2 h at 0 °C and, subsequently, poured into 3 ml of ice water. After the acetone had been completely evaporated under reduced pressure, the aqueous phase was thoroughly extracted with ether. The ether was washed neutral with water, dried, and evaporated, leaving a crude product. After purification by TLC on silica gel (hexane–diethylether, 2:1) the yield was 32.7 mg (48%) of acid **1** as colorless oil, purity 99% (GLC as methyl ester). IR (neat) 3550–2550 (br OH), 1710 (C=O) cm^{-1}; ^1H NMR δ 0.88 (*t*, 3 H, CH_3), 1.22–1.34 (br*m*, 12 H, CH_2), 1.70 (*t*, 2 H, CH_2CH_2COOH), 2.08 (*m*, 2H,=CHCH_2CH$_2$), 2.03 (*m*, 2 H, CH_3CH_2CH=), 2.37 (*t*, 2H, CH_2COOH), 2.82 (br*m*, 10H,=CHCH_2CH=), 5.36 (*m*, 12 H, C*H* =), 10.5 (br*s*, 1 H, COO*H*). ^{13}C NMR δ 14.3 (*q*, C-29); 20.7 (*t*, C-28), 24.5 (*t*, C-3), 25.0-32.0 (*t*, 7xC, C-4–C-10), 25.4–26.0 (*t*, 5x=CH–CH_2–CH=), 33.7 (*t*, C-2), 127.0–129.1 (*d*, 10xC), 129.8 (*d*, C-11), 132.3 (*d*, C-27), 179.4 (*s*, C-1, COOH); EIMS of methyl ester m/z

440 (M^+, 0.1), 408 (M-32, 0.9), 264 α-ion (4), 241 (5), 215 (5), 201 (7), 187 (15), 145 (19), 131 (22), 119 (36), 108 ω-ion (48), 105 (45), 91 (89), 79 (100), 67 (61), 55 (37); HREIMS *m/z* calcd. for 426.3498 $C_{29}H_{46}O_2$ $[M]^+$, found 426.3494.

3.4. (all-Z)-Nonacosa-11,14,17,20,23-pentaenoic acid (**2**)

This compound, prepared from the THP ether (5) (149 mg, 0.56 mmol) and bromide (7) (174 mg, 0.56 mmol) following multi-step synthesis as described for the synthesis of the acid **1**, was obtained (after purification by TLC on silica gel–hexane-diethyl-ether, 2:1) as colorless oil (28.7 mg, 12%); purity 98% (GLC as methyl ester). IR (neat) 3550–550 (br OH), 1710 (C=O) cm^{-1}; ^1H NMR δ 0.88 (*t*, 3H, CH_3), 1.20–1.33 (br*m*, 18H, CH_2), 1.71 (*t*, 2H, CH_2CH_2COOH), 2.10 (*m*, 4H,=$CHCH_2CH_2$), 2.36 (*t*, 2H, CH_2COOH), 2.83 (br*m*, 8H,=$CHCH_2CH$=), 5.37 (*m*, 10H, CH=), 10.4 (br*s*, 1H, COO*H*); ^{13}C NMR δ 14.2 (*q*, C-29); 22.6 (*t*, C-28), 24.6 (*t*, C-3), 25.2-31.8 (*t*, 10xC, C-4–C-10, C-25–C-27), 25.4–25.8 (*t*, 4x=CH–CH_2–CH=), 33.5 (*t*, C-2), 126.9–128.7 (*d*, 8xC), 129.1 (*d*, C-11), 131.9 (*d*, C-24), 179.6 (*s*, C-1, COOH); EIMS of methyl ester *m/z* 442 (M^+, 0.1), 410 (M-32, 0.7), 264 α-ion (5), 241 (6), 215 (7), 159 (14), 150 ω-ion (25), 145 (23), 119 (34), 105 (51), 91 (88), 79 (100), 67 (62), 55 (29); HREIMS *m/z* calcd. for 428.3654 $C_{29}H_{48}O_2$ $[M]^+$, found 428.3651.

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