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Identification of unusual fatty acids of four alpine plant species from the Pamirs

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Abstract

Fatty acid composition and structure in total lipids from the green above-ground parts of four alpine plants, *Oxygraphis glacialis*, *Primula macrophylla*, *Rhodiola pamiroalaica*, and *Swertia marginata*, were established by GC and GC–MS. A total of 55 fatty acids was detected, and 48 of them were identified. Ubiquitous palmitate, linoleate, and linolenate predominated in the lipids accounting for about 72–90% of the total fatty acids. At the same time, the latter contained numerous species, which were unusual for higher plants and included saturated odd-numbered *n*-acids (six C_{15}–C_{25} species, 0.26–1.40%), saturated even-numbered very-long-chain *n*-acids (six C_{20}–C_{30} species, 1.00–2.49%), *iso*-acids (nine C_{15}–C_{26} species, 0.64–1.53%), *anteiso*-acids (four C_{15}–C_{20} species, 0.08–1.57%), certain uncommon mono- and dienoic acids, as well as 16:3ω3, 18:3ω6, and 18:4ω3 acids that are absent from the most higher plants. Nine fatty acids were found here for the first time in higher plants and two may be new to science. The evidence on the unusual fatty acids is discussed with respect to their distribution in living organisms, pathways of biosynthesis, and chemotaxonomic role.
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Keywords: *Oxygraphis glacialis*; *Primula macrophylla*; *Rhodiola pamiroalaica*; *Swertia marginata*; Branched-chain fatty acids; Odd-numbered fatty acids; Very-long-chain fatty acids; Stearidonic acid; γ-Linolenic acid; Chemotaxonomy

1. Introduction

At high altitudes, alpine plants necessarily become adapted to a very harsh habitat involving the combined action of such extreme environmental stress agents as a very short growing season (no more than 2.5–3 months) during the annual cycle, drastic day vs. night changes in ambient temperature, a highly increased luminosity, an enhanced contribution from UV radiation, a predominance of poor soils, etc. Such adaptations might cause considerable shifts in the membrane lipid pattern and, in particular, in their fatty acid (FA) composition (Hadley, 1985). Nonetheless, this issue remains little investigated (Dorne et al., 1986). Therefore, we have studied the fatty acid composition of lipids of whole above-ground organs of four plant species from the Pamirs, all of which are characteristic of the Asian alpine habitats.

2. Results and discussion

2.1. Fatty acid structure

Most of FAs were tentatively identified by comparing equivalent chain-length (ECL) and fractional

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chain-length (FCL) values of their methyl esters (MEs) with the ECLs found earlier by GC on the WCOT columns coated with Carbowax 20M (Table 1) and/or by using FAME standards of known composition. Two FAs, Nos. 20 and 21, were identified only by MS of their picolinyl esters (PEs), and 7 minor FA species remained non-identified. Six of them, X_1–X_6, were characterized by similar FCL values ranging from 0.83 to 0.88 and therefore could be suggested to constitute a separate homologous series, while the No. 32 acid did not belong to this series.

All FAPEs had mass spectra with distinctive M^+ ions and the abundant peaks containing the pyridine ring at m/z = 92, 108, 151, and 164, one of which usually being the base peak. In addition, the identification of both even- and odd-numbered *n*-saturated esters with **m** = 12–30 (Table 1), where **m** is the number of carbon atoms in their aliphatic chains, was confirmed by the presence, in their spectra, of a regular series of fragment ions at $m/z = 164 + \mathbf{m}' \times 14$, where $\mathbf{m}'$ ranged from 1 to $(\mathbf{m} - 4)$. The identification of the Nos. 27, 40, 44, and 46 acids, for which FAPE spectra were unavailable, was supported by the spectra of their MEs containing, respectively, $[M - 43]^+$ ions at m/z = 269, 297, 311, and 325; and $[M - 99]^+$ ions at m/z = 213, 241, 255, and 269; as well as the ions at m/z = 74, 87, 143, and 199. All these ions were characteristic of the FAME mass spectra (Christie, 1989).

The saturated *iso*-FAPE (Nos. 3, 7, 12, 18, 29, 42, 47, and 52) mass spectra were identical to those of the respective *n*-FAPEs with the same **m** as regards the positions of both their M^+ ions and the fragment ions at $m/z = 164 + \mathbf{m}' \times 14$ with the exception of the absence of the ions at $m/z = 164 + (\mathbf{m} - 5) \times 14$, which brought about the appearance, in these spectra, of diagnostic gaps of 28 amu, from $m/z = 164 + (\mathbf{m} - 6) \times 14$ to $164 + (\mathbf{m} - 4) \times 14$ indicating a Me-branch point at the $(\mathbf{m} - 2)$th carbon atom. The corresponding *anteiso*-FAPE (Nos. 4, 14, 19, and 31) mass spectra differed from the preceding ones only in the fact that the 28-amu gap was shifted by 14 amu toward a carbonyl group and so was located between $m/z = 164 + (\mathbf{m} - 7) \times 14$ and $164 + (\mathbf{m} - 5) \times 14$ pointing to a methyl branch at the $(\mathbf{m} - 3)$th carbon atom. Finally, the mass spectrum of the No. 20 acid PE was identical to that of oleic acid (18:1ω9) PE (see below) with the exception of the presence of a 28-amu diagnostic gap between the ions at m/z = 330 and 358, which indicated a methyl branch at C-16. Moreover, in the GC mobility of their MEs, oleic acid exceeded the No. 20 acid by 0.47 ECL units, as was also the case for 15:0 and *iso*-15:0, 16:0 and *iso*-16:0, etc., respectively. So, the No. 20 acid was identified as *iso*-18:1ω8 acid.

The structure of most other monoenoic FAPEs, whose molecular ion was 2 amu lower than that for the corresponding saturated FAPEs, was established from the location (m/z values), in their mass spectra, of 40- and 26-amu diagnostic gaps, as well as a characteristic 14-amu doublet of prominent ions on the distal side of the double bond (Christie, 1989). For the No. 9 FAPE, they were at m/z = 192–232, 206–232, and 246–260, respectively; for the Nos. 10, 16, 23, and 35 FAPEs, at 220–260, 234–260, and 274–288; and for the Nos. 11 and 24 FAPEs, at 248–288, 262–288, and 302–316. The double-bond position in the No. 6 FAPE (15:1ω?) could not be found from its spectrum, and the Nos. 36 and 37 FAs were tentatively identified only on the basis of ECL values of their MEs.

The mass spectra of dienoic FAPEs were characterized by M^+, which was 4 amu lower compared to the respective saturated FAPEs, as well as by a definite location (m/z values) of two 40-amu gaps. For the No. 13 FAPE, the gaps were seen at m/z = 206–246 and 246–286, respectively; for the Nos. 21 and 25 FAPEs, at 220–260 and 260–300; and for the No. 38 FAPE, at 248–288 and 288–328. The MEs of 17:2ω5 and *iso*-18:1ω8 acids did not separate from each other, and therefore only their total content in *Primula macrophylla* D. Don (Primulaceae) lipids was given in Table 1. However, the PEs of these FAs did separate making it possible to produce their mass spectra.

The structure of CH_2-interrupted all-*cis* trienoic FAPEs (Nos. 17, 28, and 41; **m** = 16, 18, and 20, respectively), where M^+ decrement (see above) amounted to 6 amu, was established from the locations, in their mass spectra, of (1) three 40-amu gaps between the ions at $m/z = G$, $G + 40$, $G + 80$, and $G + 120$, where $G = 14\mathbf{m} - 32$, and (2) 26-amu gaps between those at m/z = H and $G + 40$; $H + 40$ and $G + 80$; as well as $H + 80$ and $G + 120$, where $H = 14\mathbf{m} - 18$. Moreover, in all of these spectra, there was a particularly prominent ion at $m/z = [M - 69]^+$ produced by a mechanism involving formation of a conjugated triene (Harvey, 1984). The occurrence of this ion made it possible to conclude that the middle double bond in the trienoic FAs was located between the $(\mathbf{m} - 6)$th and $(\mathbf{m} - 5)$th carbon atoms. It is seen that these FAs differing from each other only in the number of methylene groups at the carboxyl end of the chain constituted a homologous series. The No. 26 FA from *P. macrophylla* was tentatively identified on the basis of ECL value of its ME. In addition, nearly the same ECL (18.94) was found in our work for the ME of the authentic γ-linolenic (18:3ω6) acid from evening primrose (*Oenothera biennis* L.) seed oil.

Finally, the No. 30 FAPE mass spectrum with a M^+ decrement of 8 amu was characterized by four 40-amu gaps, which were located at m/z = 178 to 218 to 258 to 298 to 338, as well as by 26-amu gaps between 192 and 218, 232 and 258, 272 and 298, 312 and 338. Although, in accordance with earlier evidence (Harvey, 1984), some of these ions in the lower mass range were

Table 1
Fatty acid molecular species composition (wt%) of total lipids of above-ground parts of four alpine plant species

No.	ECL of FAME		M^+ of FAPE	Structure[b]	*S. marginata*	*O. glacialis*	*R. pamiroalaica*	*P. macrophylla*
	Found	Literature[a]						
1	12.00	12.00	291	12:0	0.07	0.07	0.10	<0.01
2	14.00	14.00	319	14:0	0.41	0.62	2.49	1.26
3	14.52	14.52	333	*iso*-15:0 (13-Me-14:0)		<0.01		
4	14.67	14.68	333	*ai*-15:0 (12-Me-14:0)				0.10
5	15.00	15.00	333	15:0	0.09	0.22	0.07	0.21
6	15.23		331	15:1ω?		0.08	0.06	
7	15.51		347	*iso*-16:0 (14-Me-15:0)	0.13			0.19
8	16.00	16.00	347	16:0	12.02	14.10	11.42	9.85
9	16.19	16.18	345	16:1ω9	0.10	0.07	0.15	
10	16.23	16.25	345	16:1ω7	0.21	0.12	0.06	0.10
11	16.37	16.37	345	16:1ω5				0.67
12	16.50	16.51	361	*iso*-17:0 (15-Me-16:0)	1.07	1.04	0.64	0.56
13	16.63		343	16:2ω5		0.12		
14	16.68	16.68	361	*ai*-17:0 (14-Me-16:0)	0.06		0.04	1.47
15	17.00	17.00	361	17:0	0.08	0.31	0.13	1.19
16	17.19	17.19	359	17:1ω8			0.07	0.16
17	17.21	17.22	341	16:3ω3	0.22	8.05		
18	17.54		375	*iso*-18:0 (16-Me-17:0)	0.26			0.31
19	17.70			*ai*-18:0 (15-Me-17:0)	0.08			
20	17.71		373	*iso*-18:1ω8 (16-Me-17:1ω8)				0.18
21	17.71		357	17:2ω5				
22	18.00	18.00	375	18:0	0.95	0.97	3.22	1.06
23	18.18	18.16	373	18:1ω9	4.37	0.90	2.11	0.95
24	18.26	18.23	373	18:1ω7	0.06	0.35	0.08	0.25
25	18.64	18.64	371	18:2ω6	15.47	11.00	8.52	7.32
26	18.93	18.85		18:3ω6				0.76
27	19.00	19.00	312[c]	19:0	0.03			
28	19.28	19.26	369	18:3ω3	62.21	58.50	67.87	55.37
29	19.51			*iso*-20:0 (18-Me-19:0)		0.07		
30	19.52	19.50	367	18:4ω3				17.04
31	19.62			*ai*- 20:0 (17-Me-19:0)		0.09	0.04	
32	19.80			?	0.04			
33	19.88			X_1	0.05	0.07	0.05	
34	20.00	20.00	403	20:0	0.77	1.50	0.78	0.71
35	20.10	20.08	401	20:1ω11	0.05		0.02	
36	20.18	20.14		20:1ω9	0.03	0.12		
37	20.24	20.22		20:1ω7		0.05	0.05	
38	20.66	20.63	399	20:2ω6	0.02	0.07		
39	20.86			X_2	0.02			
40	21.00	21.00	340[c]	21:0	0.01			
41	21.01	20.95	397	20:3ω3	0.09	0.13	0.10	
42	21.54		431	*iso*-22:0 (20-Me-21:0)	0.05			
43	21.87			X_3	0.04	0.05	0.04	
44	22.00	22.00	354[c]	22:0	0.34	0.29	0.35	0.13
45	22.83			X_4		0.45		
46	23.00	23.00	368[c]	23:0	0.04	0.09	0.06	
47	23.48		459	*iso*-24:0 (22-Me-23:0)	0.01			
48	23.88			X_5	0.06		0.04	
49	24.00	24.00	459	24:0	0.22	0.50	0.76	0.10
50	24.84			X_6	0.02		0.04	
51	25.00	25.00		25:0	0.01		0.04	
52	25.50		487	*iso*-26:0 (24-Me-25:0)	0.01			
53	26.00	26.00	487	26:0	0.14		0.18	0.06
54	28.00	28.00	515	28:0	0.09		0.24	
55	30.00	30.00	543	30:0			0.18	

[a] Data from Christie (1989); Makarenko et al. (1999, 2003); Ratnayake et al. (1989).
[b] *ai*-, *anteiso*-.
[c] M^+ of FAME.

less prominent than those at the high mass end of the spectrum, they were still adequate to reliably fix the position of the double bonds. Furthermore, an intense $[M - 69]^+$ ion at $m/z = 298$ (see above) indicated that the penultimate double bond of the No. 30 FA was located between C-12 and C-13. As a result, this FA was identified as stearidonic (18:4ω3) acid.

The ultimate qualitative and quantitative composition of total FAs of the alpine plant species lipids (Tables 1 and 2) shows that, as a whole, these species were similar to other green plants in the predominance of ubiquitous FAs, such as palmitic, linoleic, and α-linolenic, in their lipids; together, these FAs accounted for about 72–90% of total FAs. This evidence is consistent with a general view that higher plants contain, for the most part, C_{16}–C_{18} even-numbered *n*-FAs, other FA structures being characteristic of animal and microbial lipids.

At the same time, as demonstrated above, the alpine plant lipids also included numerous unusual FAs, such as very-long-chain (VLC) FAs (C_{20}–C_{30} FAs), *n*-odd-numbered FAs, branched-chain *iso*- and *anteiso*-FAs, hexadecatrienoic (16:3ω3), γ-linolenic (18:3ω6), and stearidonic acids, unidentified X_1–X_6 homologous VLCFAs, etc. The occurrence of all these FAs prompted us to consider certain aspects of FA and lipid biosynthesis in the plants studied here and other organisms.

2.2. Very-long-chain and odd-numbered n-fatty acids

To begin with, even-numbered *n*-VLCFAs comprising ca. 1.0–2.5% of total FAs (Table 2) could be derived from the ester-bound and free VLCFAs of epicuticular waxes (Hamilton, 1995) as well as from some unusual membrane and reserve glycerolipids (Murata et al., 1984; Berry, 1985). Plant VLCFAs are known to be formed in the endoplasmic reticulum of epidermal cells by several elongases via a stepwise malonyl-CoA-dependent C_2-elongation of stearoyl-CoA with NADPH as reductant (Řezanka, 1989), and this might also take place in our alpine plants. It is noteworthy that, except for C_{20}-FAs, no unsaturated VLCFAs were detected; thus, C_{18}-unsaturated FAs very abundant in these plants were little involved in the substrate-specific elongation reactions. It could be assumed that the formation of saturated VLCFAs in the alpine plants was related to a protective function of waxes under extreme environmental conditions (Hamilton, 1995).

The total odd-numbered *n*-FAs were, in their content, always by ca. 1.0–1.5 orders of magnitude lower as compared to the respective even-numbered ones (Table 2). The $C_{<18}$ odd-numbered FAs greatly exceeded in their concentration the odd-numbered VLCFAs, and in *P. macrophylla*, the latter were absent at all (Table 2). Thus, considering the fact that odd-numbered FAs are formed by the chain elongation using propionyl-CoA as a primer (Schneider et al., 1984), it could be suggested that, in alpine plants, this reaction produced mainly $C_{<18}$ FAs. As stated above, odd-numbered FAs are inherent in animal and microbial lipids; for instance, 17:1ω8 acid predominated in the lipids of gastropod molluscs (Kattner et al., 1998), fishes (Addison and Ackman, 1970), and yeast (Zhukov and Vereshchagin, 1970), but up to now, seems not to be identified in higher plants. Other odd-numbered FAs were very rarely found in plants, being present in trace amounts in waxes (Isbell et al., 1996), seed oils (Hilditch and Williams, 1964), and phosphatidylserines (Murata et al., 1984).

2.3. Branched-chain fatty acids

The total FAs included 0.72–2.72% of 13 species of C_{15}–C_{26} *iso*- and *anteiso*-branched-chain FAs, which were mostly present in *Swertia marginata* Schrenk. (Gentianaceae) and *P. macrophylla* (Table 2). In all al-

Table 2
Fatty acid group composition (wt%) of total lipids of above-ground parts of four alpine plant species[a]

FA group	*S. marginata*		*O. glacialis*		*R. pamiroalaica*		*P. macrophylla*		*N*
	%	*n*	%	*n*	%	*n*	%	*n*	
Saturated *n*-even-numbered C ⩽ 18FAs	13.45	4	15.76	4	17.23	4	12.17	4	4
Saturated *n*-odd-numbered $C_{<18}$ FAs	0.17	2	0.53	2	0.20	2	1.40	2	2
Saturated *n*-even-numbered VLCFAs	1.56	5	2.29	3	2.49	6	1.00	4	6
Saturated *n*-odd-numbered VLCFAs	0.09	4	0.09	1	0.10	2			4
iso-FAs	1.53	6	1.11	3	0.64	1	1.15	4	9
anteiso-FAs	0.14	2	0.09	1	0.08	2	1.57	2	4
n-Monoenoic FAs	4.82	6	1.69	7	2.6	8	2.13	5	10
n-Dienoic FAs	15.49	2	11.19	3	8.52	1	7.41	2	4
n-Trienoic FAs	62.52	3	66.68	3	67.97	2	56.13	2	4
n-Tetraenoic FAs							17.04	1	1
X_1–X_6 VLCFAs	0.19	5	0.57	3	0.17	4			6
?	0.04	1							1
Total	100	40	100	30	100	32	100	26	55

[a] *n*, Number of FA molecular species in a given plant species; *N*, number of FA molecular species in all four plant species.

pine plants except for *P. macrophylla*, the *iso*-FAs, in their concentration, exceeded by ca. an order of magnitude the *anteiso* ones (Table 2). As demonstrated recently, the plant branched-chain FAs can be formed via the C_2-elongation (see above) of specific primers (Wagner and Kroumova, 1997). Moreover, having a considerably lower melting point than the respective *n*-FAs, they can increase, just as unsaturated FAs, the fluidity of cell membranes (Annous et al., 1997) and, as a result, the cold-tolerance of living organisms and, incidentally, that of the our alpine species.

With the exception of *anteiso*-20:0 acid, only the even-numbered *iso*-FAs included branched-chain VLCFAs, and all of them except for *iso*-20:0 acid were observed only in *S. marginata* (Table 1); trace amounts of such VLCFAs were detected in the wax from *Cereus peruvianus* plants (Dembitsky and Řezanka, 1996). At the same time, total FAs in the *sn*-2 position of phospholipids from the petals and chlorophyll-deficient (but not green) leaves of *Antirrhinum majus* and *Nicotiana tabacum* plants contained considerable quantities of *iso*-12:0, -14:0, -16:0, -18:0, and -22:0 acids (Radunz, 1987). No unsaturated even-numbered *iso*-FAs, such as *iso*-18:1ω8 acid from *P. macrophylla*, were found in these plants; however, this acid was recently identified in a marine bacterium (Carballeira et al., 2000). As yet, there is no direct evidence on the pathway of even-numbered *iso*-FA synthesis in higher plants. Meanwhile, in other organisms, they were formed via elongation of a primer, the CoA-derivative of 2-methyl-propionic (isobutyric) acid; this acid was produced by transamination of L-valine, followed by irreversible oxidative decarboxylation of the resulting α-oxo intermediate (Sen Gupta, 1972).

The odd-numbered *iso*-FAs (almost exclusively *iso*-17:0 acid) usually exceeded in their content the even-numbered ones (Table 1). At the same time, these FAs were totally absent in *A. majus* phospholipids (see above), which were very rich in the even-numbered *iso*-FAs (Radunz, 1987), and as yet, to the best of our knowledge, there was no evidence on both the presence and the mechanism of biosynthesis of the odd-numbered *iso*-FAs in higher plants. They are common in other organisms. For instance, in *Tetrahymena pyriformis*, a ciliate protist, *iso*-13:0, -15:0, -17:0, and -19:0 acids comprised as a whole 40% of total saturates, and they were shown to be formed by elongation of 3-methyl-butanoyl-(isovaleryl-)CoA derived from L-leucine via transamination and decarboxylation (see above) (Conner et al., 1974).

All alpine plants except for *P. macrophylla* included two even-numbered species of *anteiso*-FAs, viz., *anteiso*-18:0 and -20:0 (Table 1). In higher plants, an even-numbered *anteiso*-FA was previously found only once, when *anteiso*-22:0 acid was identified in the *A. majus* phospholipids mentioned above (Radunz, 1987). As for other organisms, *anteiso*-16:0 acid occurred in cyanobacteria (Gugger et al., 2002) and *Botrytis cinerea* (Cooper et al., 2000), as well as in adipose tissue and wool grease of lambs (Smith et al., 1979; Nakamura et al., 1994), while this grease also contained *anteiso*-18:0 and -20:0 acids (Moldovan et al., 2002). As yet, there is no evidence on the mechanism of biosynthesis of even-numbered *anteiso*-FAs. On the basis of their structure, it might be suggested that they were formed by elongation of a derivative of 3-methyl-pentanoic (*iso*-hexanoic) acid. However, neither this acid (Markley, 1960), nor a corresponding branched-chain amino acid, i.e., α-amino-4-methyl-hexanoic acid, have been found in nature up to now (Harris and Sokatch, 1988).

Odd-numbered *anteiso*-FAs occurred in all alpine species except for *Oxygraphis glacialis* (Fisch.) Bunge (Ranunculaceae), and *P. macrophylla* was characterized by the greatest content of 14-Me-16:0 acid (Table 1). Earlier, 12-Me-14:0, 14-Me-16:0, and 16-Me-18:0 acids were found in *A. majus* and *N. tabacum* (Radunz, 1987), while 14-Me-16:0 acid was also identified in seed lipids of Pinaceae and *Ginkgo biloba*, a group and a species that have evolved independently for about 300 million years (Wolff et al., 1997). In organisms other than higher plants, where the odd-numbered *anteiso*-FAs are more common, they were formed via elongation of the CoA thioester of (+)-2-methyl-butanoic (α-methyl-butyric) acid derived from L-isoleucine as outlined above (Sen Gupta, 1972). In plants, this mechanism has not been studied in detail as yet, but in *Brassica oleracea*, exogenous ^{14}C-labelled α-Me-butyric acid and L-isoleucine were shown to be selectively incorporated into the C_{17}–C_{27} *anteiso*-FAs (Kolattukudy, 1980).

2.4. Mono- and dienoic fatty acids

As regards the unusual monoenoic FAs, the 16:1ω5 acid was previously detected, sometimes in considerable amounts, in *Geruina avellana* (Proteaceae) seed oil (Hilditch and Williams, 1964), Meliaceae plants (Kleiman and Payne-Wahl, 1984), red algae (Miralles et al., 1990), Glomales fungi (Fontaine et al., 1998), fruit pulp lipids of several species (Yamamoto et al., 1990), and borage oil (Wretensjö et al., 1990), as well as in the mitochondria of etiolated *Elymus sibiricus* and *Zea mays* seedlings (Makarenko et al., 2003), while a specific FAω5-desaturase catalysing its formation from 16:0 acid was isolated from the garden geranium (Mumma et al., 2002). The 20:1ω1 acid was up to now found only in marine-animal lipids (Hilditch and Williams, 1964), while the 16:1ω9 and 20:1ω7 acids were so far identified, in trace amounts, solely in cod liver oil and pig testis lipids (Christie, 1989), and the pathways of their biosynthesis in plants remain unknown. At the same time, other monoenoic FAs, such as 16:1ω7, its C_2-elongation

product 18:1ω7 acid, and 20:1ω9 acid formed via the C_2-elongation of 18:1ω9 acid, are wide-spread in higher plants and so could not be considered as unusual.

Moreover, alpine species contained three minor dienoic FAs (Table 1). The 20:2ω6 acid was found in *S. marginata* and *O. glacialis* (Table 1), and the latter case was consistent with its presence, in noticeable amounts, in the seed oils of several other genera of Ranunculaceae (Aitzetmüller et al., 1997). Traces of 20:2ω6 acid were present in the vacuoles of several higher-plant roots (Makarenko et al., 1999), the mitochondria of *E. sibiricus* and *Z. mays* seedlings (Makarenko et al., 2003), and pig testis (Christie, 1989). Meanwhile, an intense biosynthesis of [^{2}H]-labelled 20:2ω6 acid, presumably via the elongation of [^{2}H]-18:2ω6 acid, was demonstrated in a fungus *Neurospora crassa* mutant supplemented for growth with [^{2}H]-16:0 acid (Stafford et al., 1998); a direct conversion of 18:2ω6 acid into the 20:2ω6 acid also took place in the yeast cells transformed with the genes of specific polyunsaturated FA elongases from a marine alga *Isochrysis galbana* (Qi et al., 2002).

We could not find any evidence on the occurrence and pathway of biosynthesis of 17:2ω5 and 16:2ω5 acids in living organisms. The Δ8,11-structure of 16:2ω5 acid was established in this work from the location of two 40-amu diagnostic gaps (see above), and so this acid was different from the 16:2ω4 acid present in the seeds of sunflower mutants (Alvarez-Ortega et al., 1998) and Meliaceae plants (Kleiman and Payne-Wahl, 1984), as well as from the 16:2ω6 acid found in *E. sibiricus* and *Z. mays* mitochondria (Makarenko et al., 2003).

2.5. ω3-Trienoic fatty acids

All higher plants, as regards the composition of ω3-trienoic FAs in their total leaf lipids, are now classified into "18:3 plants" including only 18:3ω3 acid and "16:3 plants", which contain, along with 18:3ω3, at least 2% of 16:3ω3 acid in the total FAs and comprise about 12% of the angiosperm species in the world (Mongrand et al., 1998). Thus, *O. glacialis* is a 16:3 plant, while *S. marginata* with only 0.22% of 16:3ω3 acid, as well as *Rhodiola pamiroalaica* Boriss. (Crassulaceae) and *P. macrophylla*, belong to the 18:3 ones (Table 1). These results obtained here for the first time are consistent with those found earlier, because all other Ranunculales species studied so far are 16:3 plants with 9.8–17.5% of 16:3ω3 acid, whereas all Gentianaceae, Crassulaceae, and Primulaceae species contain only traces (Dorne et al., 1986; Mongrand et al., 1998). Along with some angiosperms, the occurrence of 16:3ω3 acid is characteristic of prokaryotic organisms such as cyanobacteria, as well as of microalgae, mosses, ferns, conifers, and other eucaryotic plant groups of a lower taxonomic position. Thus, the presence of this acid in *O. glacialis* was related to the fact that Ranunculaceae are less advanced from the evolutionary point of view than the other three families just mentioned, which, in the course of evolution, have lost the ability to produce 16:3ω3 acid (Mongrand et al., 1998).

In all 16:3 plants, this acid resides solely in chloroplasts, where it occurs in a specific prokaryotic galactolipid, *sn*-1-18:3ω3,*sn*-2-16:3ω3-monogalactosyldiacylglycerol (18:3,16:3-MGDG), and, to a much lesser extent, in the 18:3,16:3-digalactosyldiacylglycerol, which is formed only via galactosylation of a small part of the 18:3,16:3-MGDG (Roughan, 1987). The prokaryotic (plastidial) pathway of 18:3,16:3-MGDG biosynthesis in chloroplast envelopes involves the in situ galactosylation of 18:1,16:0-diacylglycerol with the formation of 18:1,16:0-MGDG and subsequent desaturation of both FA residues inside the latter to produce 18:3,16:3-MGDG (Demandre et al., 1989). It can be held that the unique features of 16:3ω3 acid are also characteristic of *O. glacialis*.

It is noteworthy that, in contrast to the biosynthesis of 16:3ω3 acid, that of the 18:3ω3 acid is widely known to proceed also outside chloroplasts, where it does not involve MGDG desaturation. Indeed, 18:3ω3 acid and all other major FAs of chloroplasts, except for 16:3ω3 acid (and *trans*-3-hexadecenoic acid), can give rise to the reserve triacylglycerols of oilseeds as well as to the galactose-free polar lipids. Up to now, 16:3ω3-containing triacylglycerols were found only in leaf plastoglobuli, whose formation was induced by senescence of leaves or their treatment with ozone. These triacylglycerols were synthesized in chloroplasts from 16:3ω3 and 18:3ω3 acids released via the catabolism of thylakoid galactolipids; 18:3ω3 acid thus formed was also incorporated into the *sn*-2 position of phosphatidylcholines by acid-exchange reaction (Kaup et al., 2002; Sakaki et al., 1994).

A minor (ca. 0.1%) ω3-trienoic acid, 20:3ω3 acid, was present in all but one alpine species (Table 1). Earlier, it was identified (only from its ECL value) in the mitochondria of etiolated *E. sibiricus* (but not *Z. mays*) seedlings (Makarenko et al., 2003), as well as in herring oil (Hilditch and Williams, 1964). A formation of 20:3ω3 acid via the elongation of ubiquitous 18:3ω3 acid was shown in the *N. crassa* mutant (Stafford et al., 1998), the *I. galbana*-transformed yeast cells (Qi et al., 2002), and the human liver (Leonard et al., 2000). Meanwhile, no evidence on the possibility of its synthesis via an ω3-desaturation of 20:2ω6 acid also present in our alpine plants is available as yet.

2.6. Δ6-Polyunsaturated FAs

In our alpine species, 18:3ω6 and 18:4ω3 acids (the "Δ6-FAs") were present only in *P. macrophylla* (Table 1), and this was consistent with the fact that they were previously found in the lipids of four Himalayan *Primula* species, *P. florindae*, *P. sikkimensis*, *P. alpicola*,

and *P. pulverulenta*, growing at altitudes of between 3800 and 5500 m above sea level (Aitzetmüller and Werner, 1991). Thus, the occurrence of Δ6-FAs can be considered as a chemotaxonomic marker of the genus *Primula*. These FAs are wide-spread in animals, as well as in lower organisms, such as cyanobacteria, algae, fungi, mosses, etc., but an overwhelming majority of higher plants do not produce them. Up to now, the Δ6-FAs were found in ca. 10 families of higher plants including Ranunculaceae (Aitzetmüller and Tsevegsuren, 1994); however, *O. glacialis* belonging to this family was devoid of these FAs (Table 1).

In green somatic tissues of plants, as in *P. macrophylla* (Table 1), both Δ6-FAs occur together in a given lipid class, and there are almost no exceptions to this rule. Until recently, this pattern has been explained on the hypothesis that 18:4ω3 acid is formed via the ω3-desaturation of 18:3ω6 acid produced by the Δ6-desaturation of linoleate (Harwood, 1988). Later, however, it was firmly established that 18:3ω6 and 18:4ω3 acids are synthesized from 18:2ω6 and 18:3ω3 acids, respectively, only under the action of *cis*-Δ6-desaturase, which is highly active in Δ6-FA-containing tissues (Griffiths et al., 1996). The major site of 18:4ω3 acid synthesis in the leaf, at the *sn*-2 position of MGDG, is prokaryotic (Griffiths et al., 1996), as is also the case for the 16:3ω3 acid (see above). However, in contrast to the latter, large amounts of Δ6-FA-containing triacylglycerols, which are nutritionally important, were found in borage, evening primrose, blackcurrant, etc. seed oils (Aitzetmüller and Tsevegsuren, 1994). Moreover, the modern concept of Δ6-FA biosynthesis accounts for the fact that stearidonic acid content greatly exceeded that of γ-linolenic acid in *P. macrophylla* lipids, in which α-linolenic acid concentration was considerably higher than the linoleic acid one (Table 1).

3. Conclusion

It is shown that the GC–MS investigation made it possible to detect 55 FAs in alpine plant lipids, and 48 of them were identified. Many of these FAs could be considered as being unusual for higher plants. They included odd-numbered *n*-FAs, branched-chain FAs, certain mono- and dienoic acids, 20:3ω3 acid, as well as 16:3ω3 and Δ6-FAs, which are absent from the great majority of plants. A number of FAs, such as 16:1ω9, 17:1ω8, 20:1ω7, and 20:1ω11, as well as *iso*-15:0, -17:0, and -18:1ω8, were found in higher plants for the first time, while the two species, viz., 16:2ω5 and 17:2ω5, seem to have escaped detection in any living organism up to now; it appears that the still unidentified X_1–X_6 homologous VLCFAs must also fall into the latter category.

At the same time, as demonstrated above, many FAs unusual for our alpine species and other higher plants are quite common for their direct ancestors as well as for other organisms of a lower taxonomic position, such as bacteria, cyanobacteria, fungi, non-green algae, etc., which are unrelated to higher plants. As a whole, the unusual FAs can be regarded as the metabolic relicts remained after the respective FAs were largely lost in the course of plant evolution. Incidentally, this concept does not account for the origin of branched-chain FAs in angiosperms, because their ancestors, the green algae (Chlorophyta), are totally devoid of these FAs (Pohl and Wagner, 1972).

Up to now, plant lipidologists have paid inadequate attention to an accurate identification of ''minor'' or ''trace'' FAs. It can be anticipated that their closer examination in plants, particularly those grown under extreme environmental conditions, will extend our views of composition and metabolism of plant lipids.

4. Experimental

4.1. Plant material and lipid extraction

Green above-ground parts of four plant species, *S. marginata*, *O. glacialis*, *Rh. pamiroalaica*, and *P. macrophylla*, were collected in the Mukor Circus, Pamirs Mountains, Tadjikistan, at the altitude of 4300 m above sea level. Herbarium voucher specimens were deposited in the Herbarium, Department of Botany, Ural University, Ekaterinburg, Russia. Fresh plant material was treated for 30 min with boiling *iso*-PrOH, and total lipids were extracted as described by Belenko et al. (1983).

4.2. Preparation of FAMEs

Total lipids were saponified, the non-saponifiable material was rejected, and the FAs thus obtained were converted into FAMEs as described by Zhukov and Vereshchagin (1970). The FAMEs (ca. 10 mg) were purified by prep. TLC on a 250-μm Kieselgel layer (E.Merck, Darmstadt, Germany) using hexane–Et_2O (9:1) as a mobile phase. The FAME band was detected by spraying the layer with an 0.05% (w/v) solution of 2′,7′-dichlorofluorescein in EtOH and viewing the air-dried plate under UV light at 254 nm. The FAMEs were recovered from the adsorbent by elution with hexane and then separated into saturated, mono-, di-, tri-, and tetraene fractions by argentation chromatography on a solid-phase extraction SPE column using a Bond Elut™ SCX cartridge (Analytichem International, USA) packed with a silica-based benzenesulphonic acid material (Christie, 1989).

4.3. GC analysis of FAMEs

A Carlo Erba Model 4130 gas chromatograph (Carlo Erba, Crawley, UK), which was fitted with a fused-silica WCOT column (25 m × 0.22 mm) coated with Carbowax 20 M^{TM} (Chrompack, UK) and with a split/splitless injection system, was used for separating FAMEs at the injection vol. of 1 μl. H_2 was the carrier gas at 1 ml · min^{-1}; the temp. was maintained at 165 °C, than was raised at 4 °C/min to 195 °C, where it was maintained for a further 20 or 40 min.

To calculate ECLs, the retention time of an *i*th FAME relative to that of a standard (*n*-18:0 acid ME) was found for all FAME components with **m** = 14–26. The values of log (relative retention time) = *F* were used for calculating ECL by equations

$$\mathrm{ECL} = \mathbf{m} + (F_i - F_{\mathbf{m}})(F_{\mathbf{m}+1} - F_{\mathbf{m}})^{-1}$$
$$\text{at } F_{\mathbf{m}} < F_i < F_{\mathbf{m}+1}$$

and/or

$$\mathrm{ECL} = \mathbf{m} + 2(F_i - F_{\mathbf{m}})(F_{\mathbf{m}+2} - F_{\mathbf{m}})^{-1}$$
$$\text{at } F_{\mathbf{m}} < F_i < F_{\mathbf{m}+2},$$

where F_i is the *F* value of an *i*th FAME, $F_{\mathbf{m}}$, $F_{\mathbf{m}+1}$, and $F_{\mathbf{m}+2}$ are the *F* values of the saturated *n*-FAMEs with **m**, **m** + 1, and **m** + 2 carbon atoms in their aliphatic chains, respectively; the ECLs of these esters were assumed to be equal to **m**, **m** + 1, and **m** + 2, respectively.

4.4. GC–MS identification of fatty acids

FAME fractions with a definite degree of unsaturation (see above) were hydrolysed to the free acids, which were then converted into FAPEs as described previously; to remove any residual free FAs, the products were purified by adsorption HPLC on a silica gel column (Christie, 1989). The FAPEs were separated on a fused-silica WCOT column (25 m × 0.2 mm) coated with a cross-linked CP SIL 8CB non-polar phase (Chrompack UK Ltd.). He was the carrier gas, and the oven was programmed from 210 °C (3 min at this temp.) to 230 °C at 1 °C/min and to 270 °C at 2 °C/min. The column outlet was directly connected with the ion source of a Hewlett–Packard 5970 mass selective detector operating at the ionization potential of 70 eV. The temp. of the source was set at 10 °C above the maximum GC column temp. used.

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References

Addison, R.F., Ackman, R.G., 1970. Exceptional occurrence of odd-chain fatty acids in smelt (*Osmerus mordax*). Lipids 5, 554–557.

Aitzetmüller, K., Werner, G., 1991. Stearidonic acid (18:4ω3) in *Primula florindae*. Phytochemistry 30, 4011–4013.

Aitzetmüller, K., Tsevegsuren, B., 1994. Occurrence of gamma-linolenic acid in Ranunculaceae seed oils. J. Plant Physiol. 143, 578–580.

Aitzetmüller, K., Werner, G., Ivanov, S.A., 1997. Seed oils of *Nigella* species and of closely related genera. OLC-Oleagineux Corps Gras Lipides 4, 385–388.

Alvarez-Ortega, R., Martinez-Force, E., Garces, R., 1998. Enzymatic characterization of high palmitic sunflower mutants. In: Sanchez, J., Cerda-Olmedo, E., Martinez-Force, E. (Eds.), Advances in Plant Lipid Research. Universidad de Sevilla, Seville, pp. 131–133.

Annous, B.A., Becker, L.A., Bayles, D.O., Labeda, D.P., Wilkinson, B.J., 1997. Critical role of anteiso-$C_{15:0}$ fatty acid in the growth of *Listeria monocytogenes* at low temperatures. Appl. Environ. Microbiol. 63, 3887–3894.

Belenko, E.L., Datunashvili, E.N., Tsydendambaev, V.D., Vereshchagin, A.G., 1983. Fatty acid composition of lipids of the grape berry. Sov. Plant Physiol. 30, 879–885.

Berry, S., 1985. Long chain fatty acids (C20 to C24) in winged bean (*Psophocarpus tetragonolobus*) oil. Nutrition Reports International 31, 265–272.

Carballeira, N.M., Pagan, M., Shalabi, F., Nechev, J.T., Lahtchev, K., Ivanova, A., Stefanov, K., 2000. Two novel iso-branched octadecenoic acids from a *Micrococcus* species. J. Nat. Prod. 63, 1573–1575.

Christie, W.W., 1989. Gas Chromatography and Lipids. The Oily Press, Ayr, Scotland.

Conner, R.L., Koo, K.E., Landrey, J.R., 1974. Isovaleric acid as a precursor of odd numbered iso fatty acids in *Tetrahymena*. Lipids 9, 554–559.

Cooper, L.L.D., Oliver, J.E., De Vilbiss, E.D., Doss, R.P., 2000. Lipid composition of the extracellular matrix of *Botrytis cinerea* germlings. Phytochemistry 53, 293–298.

Demandre, Ch., Serghini-Caid, H., Justin, A.M., Mazliak, P., 1989. Fatty acid desaturation in some plants of horticultural interest. In: Biacs, P.A., Gruiz, K., Kremmer, T. (Eds.), Biological Role of Plant Lipids. Akademiai Kiado, Budapest, pp. 3–12.

Dembitsky, V.M., Řezanka, T., 1996. Molecular species of wax esters in *Cereus peruvianus*. Phytochemistry 42, 1075–1080.

Dorne, A.J., Cadel, G., Douce, R., 1986. Polar lipid composition of leaves from nine typical alpine species. Phytochemistry 25, 65–68.

Fontaine, J., Grandmougin-Ferjani, A., Hartmann, M.A., Sancholle, M., 1998. Is the vesicular arbuscular mycorrhizal fungus *Glomus intraradices* able to synthesize its own lipids?. In: Sanchez, J., Cerda-Olmedo, E., Martinez-Force, E. (Eds.), Advances in Plant Lipid Research. Universidad de Sevilla, Seville, pp. 602–605.

Griffiths, G., Brechany, E.Y., Christie, W.W., Stymne, S., Stobart, A.K., 1996. Distribution and biosynthesis of stearidonic acid in leaves of *Borago officinalis*. Phytochemistry 43, 381–386.

Gugger, M., Lyra, C., Suominen, I., Tsitko, I., Humbert, J.F., Salkinoja-Salonen, M.S., Sivonen, K., 2002. Cellular fatty acids as chemotaxonomic markers of the genera *Anabaena*, *Aphanizomenon*, *Mycrocystis*, *Nostoc* and *Planktothrix* (Cyanobacteria). Int. J. Syst. Evol. Microbiol. 52, 1007–1015.

Hadley, N.F., 1985. The Adaptive Role of Lipids in Biological Systems. Wiley and Sons, New York.

Hamilton, R.J., 1995. Waxes: Chemistry, Molecular Biology and Functions. The Oily Press, Dundee, Scotland.

Harris, R.A., Sokatch, J.R., 1988. Branched-chain amino acids. In: Abelson, G.N., Simon, M.I. (Eds.), Methods in Enzymology, vol. 166. Academic Press, New York, pp. 1–534.

Harvey, D.J., 1984. Picolinyl derivatives for the structural determination of fatty acids by mass spectrometry. Biomed. Mass Spectrometry 11, 340–347.

Harwood, J.L., 1988. Fatty acid metabolism. In: Briggs, W.R., Jones, R.L., Walbot, V. (Eds.), Ann. Rev. Plant Physiol. Plant Mol. Biol., vol. 39. Ann. Rev. Inc., Palo Alto, CA, pp. 101–138.

Hilditch, T.P., Williams, P.N., 1964. The Chemical Constitution of Natural Fats. Wiley and Sons, New York.

Isbell, T.A., Carlson, K.D., Abbott, T.P., Phillips, B.S., Erhan, S.M., Kleiman, R., 1996. Isolation and characterization of wax esters in meadowfoam oil. Ind. Crops Prod. 5, 239–243.

Kattner, G., Hagen, W., Graeve, M., Albers, C., 1998. Exceptional lipids and fatty acids in the pteropod *Clione limacina* (Gastropoda) from both polar oceans. Marine Chem. 61, 219–228.

Kaup, M.T., Froese, C.D., Thompson, J.E., 2002. A role for diacylglycerol acyltransferase during leaf senescence. Plant Physiol. 129, 1616–1626.

Kleiman, R., Payne-Wahl, K.L., 1984. Fatty acid composition of seed oils of the Meliaceae, including one genus rich in *cis*-vaccenic acid. J. Am. Oil Chem. Soc. 61, 1836–1838.

Kolattukudy, P.E., 1980. Cutin, suberin, and waxes. In: Stumpf, P.K. (Ed.), The Biochemistry of Plants. A Comprehensive Treatise, Lipids: Structure and Function, vol. 4. Academic Press, New York, pp. 571–645.

Leonard, A.E., Bobik, E.G., Dorado, J., Kroeger, P.E., Chuang, L.T., Thurmond, J.M., Paker-Barnes, J.M., Das, T., Huang, Y.S., Mukerji, P., 2000. Cloning of a human cDNA encoding a novel enzyme involved in the elongation of long-chain polyunsaturated fatty acids. Biochem. J. 350, 765–770.

Makarenko, S.P., Konenkina, T.A., Salyaev, R.K., 1999. Fatty acid composition of lipids from the vacuoles of higher plants. Russian J. Plant Physiol. 46, 643–647.

Makarenko, S.P., Konstantinov, Y.M., Khotimchenko, S.V., Konenkina, T.A., Arziev, A.Sh., 2003. Fatty acid composition of mitochondrial membrane lipids in cultivated (*Zea mays*) and wild (*Elymus sibiricus*) grasses. Russian J. Plant Physiol. 50, 548–553.

Markley, K.S., 1960. Fatty Acids, Their Chemistry, Properties, Production and Uses. Part I. Interscience Publishers, New York.

Miralles, J., Aknin, M., Micouin, L., Gaydou, E.M., Kornprobst, J.M., 1990. Cyclopentyl and ω5 monounsaturated fatty acids from red algae of the Solieriaceae. Phytochemistry 29, 2161–2163.

Moldovan, Z., Jover, E., Bayona, J.M., 2002. Gas chromatographic and mass spectrometric methods for the characterization of long-chain fatty acids. Application to wool wax extracts. Anal. Chim. Acta 465, 359–378.

Mongrand, S., Bessoule, J.J., Cabantous, F., Cassagne, C., 1998. The $C_{16:3}/C_{18:3}$ fatty acid balance in photosynthetic tissues from 468 plant species. Phytochemistry 49, 1049–1064.

Mumma, R.O., Craig, R., Grazzini, R.A., Schultz, D.J., 2002. Plant trichoms, anacardic acids, mite resistance and fatty acid desaturase. In: Murata, N. (Ed.), 15th International Symposium on Plant Lipids. Programs and Abstracts. Okazaki Conference Center, Okazaki, Japan, p. 54.

Murata, N., Sato, N., Takahashi, N., 1984. Very-long-chain saturated fatty acids in phosphatidylserine from higher plant tissues. Biochim. Biophys. Acta 795, 147–150.

Nakamura, S., Nishimura, Y., Inagaki, K., Miwa, N., 1994. Inhibition of tumor cell growth by low-boiling-point saturated fatty acids isolated by molecular distillation and reversed-phase liquid chromatography of hydrolysates of uncytotoxic wool grease secreted from sheep sebaceous gland. Cancer Biochem. Biophys. 14, 113–121.

Pohl, P., Wagner, H., 1972. Fettsäuren im Pflanzen- und Tierreich (eine Übersicht) I: Gesättigte und cis-ungesättigte Fettsäuren. Fette, Seifen, Anstrichm. 74, 424–435.

Qi, B., Beaudoin, F., Fraser, T., Stobart, A.K., Napier, J.A., Lazarus, C.M., 2002. Identification of a cDNA encoding a novel C_{18}-Δ^9 polyunsaturated fatty acids specific elongating activity from the docosahexaenoic acid producing microalga, *Isochrysis galbana*. In: Murata, N. (Ed.), 15th International Symposium on Plant Lipids. Programs and Abstracts. Okazaki Conference Center, Okazaki, Japan, p. 198.

Radunz, 1987. On the functions of methyl-branched chain fatty acids in phospholipids of cell membranes of higher plants. In: Stumpf, P.K., Mudd, J.B., Nes, W.D. (Eds.), The Metabolism, Structure, and Function of Plant Lipids. Plenum Press, New York and London, pp. 197–200.

Ratnayake, W.M.N., Olsson, B., Ackman, R.G., 1989. Novel branched-chain fatty acids in certain fish oils. Lipids 24, 630–637.

Řezanka, T., 1989. Very-long-chain fatty acids from the animal and plant kingdoms. Progr. Lipid Res. 28, 147–187.

Roughan, G., 1987. The control of fatty acid compositions of plant glycerolipids. In: Stumpf, P.K., Mudd, J.B., Nes, W.D. (Eds.), The Metabolism, Structure, and Function of Plant Lipids. Plenum Press, New York and London, pp. 247–254.

Sakaki, T., Tanaka, K., Yamada, M., 1994. General metabolic changes in leaf lipids in response to ozone. Plant Cell Physiol. 35, 53–62.

Schneider, T., Borkowski, L., Betz, 1984. Rotenone-sensitive wax-monoester synthesis in anaerobic *Euglena gracilis*. In: Siegenthaler, P.-A., Eichenberger, W. (Eds.), Structure, Function and Metabolism of Plant Lipids. Elsevier Science Publishers, Amsterdam, pp. 577–580.

Sen Gupta, A.K., 1972. Recent advances in the chemistry and biochemistry of methyl branched fatty acids. Fette, Seifen Anstrichm. 74, 693–705.

Smith, A., Colder, A.G., Lough, A.K., Duncan, W.R.H., 1979. Identification of methyl-branched fatty acids from the triacylglycerols of subcutaneous adipose tissue of lambs. Lipids 14, 953–960.

Stafford, A., McKeon, T.A., Goodrich-Tanrikulu, M., 1998. Conversion of palmitate to unsaturated fatty acids differs in a *Neurospora crassa* mutant with impaired fatty acid synthase activity. Lipids 33, 303–306.

Wagner, G.J., Kroumova, A.B., 1997. A novel pathway for the biosynthesis of straight and branched, odd- and even-length medium-chain fatty acids in plants. In: Williams, J.P., Khan, M.U., Lem, N.W. (Eds.), Physiology, Biochemistry and Molecular Biology of Plant Lipids. Kluwer Academic Publishers, Dordrecht, Holland, pp. 42–44.

Wolff, R.L., Christie, W.W., Coakley, D., 1997. Unusual occurrence of 14-methylhexadecanoic acid in Pinaceae seed oils among plants. Lipids 32, 971–973.

Wretensjö, I., Svensson, L., Christie, W.W., 1990. Gas chromatographic-mass spectrometric identification of the fatty acids in borage oil using the picolinyl ester derivatives. J. Chromatogr. 521, 89–97.

Yamamoto, K., Shibahara, A., Sakuma, A., Nakayama, T., Kajimoto, G., 1990. Occurrence of *n*-5 monounsaturated fatty acids in jujube pulp lipids. Lipids 25, 602–605.

Zhukov, A.V., Vereshchagin, A.G., 1970. Heptadecenoic acid as an internal standard in the gas chromatographic weight determination of fatty acids. J. Chromatogr. 51, 155–166.