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A high level of dihomogammalinolenic acid in brown alga *Sargassum pallidum* (Turn.)

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Abstract

A high content (12.8–17.7% of total fatty acids) of an eicosatrienoic acid was found in the brown alga, *Sargassum pallidum*. The chemical structure of the acid as 8,11,14-eicosatrienoic (dihomo-γ-linolenic) acid was confirmed by physicochemical methods.

Keywords: *Sargassum pallidum*; Phaeophyta; Brown algae; Chemotaxonomy; Fatty acids; Dihomo-γ-linolenic acid; 8,11,14-Eicosatrienoic acid

1. Introduction

In the course of the investigation of fatty acid compositions of marine macroalgae, we found that samples of the alga *Sargassum pallidum* collected in Vitiaz Bay (Sea of Japan) contained a fatty acid which accounted for more than 10% of the total fatty acids. According to chromatographic characteristics on capillary columns of different polarity, this acid was tentatively identified as dihomo-γ-linolenic (DGLA).

This is noteworthy because the concentration of DGLA in the brown algae including another representative of the genus *Sargassum*, *S. miyabei*, was reported to represent less than 1% (Khotimchenko & Svetashev, 1983). The latter DGLA content is in agreement with data obtained by other authors for brown algae (Ackman & McLachlan, 1977; Ackman, 1981; Arao & Yamada, 1989; Dembitsky, Rozentsvet, & Pechenkina, 1990). Seven species of *Sargassum* collected in different parts of the Pacific and Indian Oceans were investigated in our laboratory and all of them, excluding *S. pallidum*, were low in DGLA (Khotimchenko, 1991).

DGLA has attracted increased attention as a precursor of prostaglandin E_1 (Chapkin & Coble, 1991) and a number of eicosanoids of high physiological activity (Chapkin & Coble, 1991; Pacey & Bently, 1992; Iversen, Fogh, Bojesen, & Kragballe, 1991). Accordingly, the problem of adequate sources for DGLA is of current interest.

In this paper, we present data on the fatty acid composition of *S. pallidum* and provide conclusive evidence that eicosatrienoic acid from *S. pallidum* is DGLA.

2. Results and discussion

The most prominent feature of the fatty acid composition of *S. pallidum* is the presence of one component, which was tentatively identified as DGLA. Separation of the fatty acid methyl esters (FAMEs) of *S. pallidum* according to unsaturation by $AgNO_3$ silica gel TLC showed that this acid was concentrated in the zone of trienoic FAMEs. Catalytic hydrogenation of this acid produced *n*-eicosanoic acid (20:0). Column chromatography on silica gel, impregnated with $AgNO_3$, provided effective separation of trienoic fatty acids from other fatty acids. This fraction consisted of mainly 20:3 with 18:3(*n*-6) and 18:3(*n*-3), as additional components. The 20:3 fatty acid was isolated by means of HPLC on a reverse-phase column with a purity higher than 99%.

Mass spectrometry of the FAME of this acid showed a $[M]^+$ m/z 320, which corresponds to methyl eicosatrienoate. The UV spectrum shaved no conjugated bond absorbances and the IR spectrum lacked absorption bands characteristic of *trans*-ethylenic (960–980 cm^{-1}) and acetylenic (2150 cm^{-1}) bonds.

The position of the double bonds closest to the ester group and to the methyl end of 20:3 was determined by oxidative ozonolysis followed by GC of the products. The products resulting from the oxidative scission of the ethylenic bonds and subsequent esterification of the fragments should be dimethyl dicarboxylic (DMC) and

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methyl monocarboxylic (MMC) acids (Sebedio & Ackman, 1978). The ozonolysis products of 20:3 showed two peaks on GC. Their retention times agreed with that of DMC8 and MMC6. These results indicated that the double bond position closest to the ester group of 20:3 is located between C-8 and C-9 (Δ8) and that closest to the methyl end, between C-6 and C-7 (Δ14).

Conclusive evidence of the structure of the 20:3 fatty acid was provided by the results of mass spectrometry of its pyrrolidine derivative. This yielded a series of ions *m*/*z* 70, 98, 113, 126, 140, 154, 168, 182, 194, 208, 222, 234, 248, 262, 274, 288 and a $[M]^+$ at *m*/*z* 359. The presence of intervals of 12 mu between the most intensive peaks of clusters of fragments for C-7–C-8 (*m*/*z* 182 and 194), C-10–C-11 (*m*/*z* 222 and 234) and C-13–C-14 (*m*/*z* 262 and 274) indicated the positions of double bonds at C-8, C-11 and C-14, respectively. These data proved that the structure of the acid isolated from *S. pallidum* was 20:3Δ8,11,14.

Samples of *S. pallidum*, collected in different seasons during three years in different bights of Peter the Great Bay (Sea of Japan) had similar fatty acid compositions. The typical distribution of the fatty acids in *S. pallidum* collected in October at a water temperature of 10°C is shown in the Table 1. The content of DGLA in the samples analysed varied from 12.8 to 17.7% of the total fatty acids and was not related to season and place of collection (data not shown). One more peculiarity of this alga is the predominance of fatty acids of the *n*-6 series over those of the *n*-3 series. The high content of 20:3(*n*-6) can be proposed as chemotaxonomic feature of *S. pallidum*. The reasons for the unusually high concentration of DGLA are now under investigation.

Table 1
Fatty acid composition (% of total fatty acids) of algae *Sargassum pallidum* collected in October in different years

Fatty acid	Sample 1	Sample 2
14:0	2.9	2.7
15:0	0.2	–
16:0	9.9	10.8
16:1(*n*-7)	4.4	3.2
16:2(*n*-4)	0.8	0.4
18:0	0.4	0.2
18:1(*n*-9)	4.5	7.0
18:2(*n*-6)	25.4	27.5
18:3(*n*-6)	3.5	2.6
18:3(*n*-3)	4.7	4.3
18:4(*n*-3)	3.6	3.9
20:1(*n*-11)	0.6	0.8
20:1(*n*-9)	0.9	–
20:2(*n*-6)	0.2	1.0
20:3(*n*-6)	16.5	14.5
20:4(*n*-6)	15.5	16.9
20:4(*n*-3)	0.6	0.7
20:5(*n*-3)	2.0	2.2
22:1(*n*-11)	0.8	0.6
Other[a]	1.7	0.7
Total	99.1	100
Sum (*n*-3)	11.1	11.2
Sum (*n*-6)	61.1	62.5
(*n*-3)/(*n*-6)	0.18	0.18
Saturated	13.9	14.0
Monoenoic	11.7	11.7
Polyenoic	73.5	74.3

[a] Others include some minor fatty acids (<0.3% each) not listed.

It is recognized that DGLA is widespread in nature but only as a minor component of fatty acids (Shimizu, Akimoto, Kawashima, Shinmen, & Yamada, 1989). However, in several organisms, certain tissues and lipid classes, DGLA is a major acid. DGLA was abundant in lipids of some reef-building corals (more than 10% of the total fatty acids) (Latyshev, Naumenko, Svetashev, & Latypov, 1991). In the microalga, *Ochromonas danica*, DGLA made up 5% of the total fatty acids (Nichols & Appleby, 1969). Several fungi belonging to the genus *Mortirella* also accumulated a significant amount of DGLA (Shimizu, Kawashima, Shinmen, Akimoto, & Yamada, 1988).

In recent years, DGLA has attracted attention as a biologically active substance (Chapkin & Coble, 1991; Iversen et al., 1991; Pacey & Bently, 1992). Therefore the high content of this acid in a widespread marine alga can be both of scientific and practical interest.

3. Experimental

Samples of *S. pallidum* were collected at different times of the year at 0.5–1.0 m depth. The H_2O temperature varied from 0 to 23°C. Only phyloids without epiphytes were taken for analysis. Fresh algae were thoroughly cleaned before extraction. The species was identified by Dr. Yu.E. Petrov (Botanical Institute, Russian Academy of Sciences, St. Petersburg).

3.1. Lipid extraction and fatty acid analysis

Lipids were extracted with $CHCl_3$–MeOH (1:2) (Bligh & Dyer, 1959). Fatty acids were converted to Me esters using 1% Na in MeOH, followed by 5% HCl in MeOH (Carreau & Dubacq, 1978) and purified by silica gel TLC using benzene. The resulting FAMEs were analyzed by FID-GC with fused silica capillary columns (30 m × 0.25 mm), coated with Supelcowax 10 and SPB-5; column temperatures were 210 and 220°C, respectively. The carrier gas was He, split 1:30. Individual peaks of FAMEs were identified by comparing R_t data with those of authentic standards and using ECL measurements (Kramer, Fouchard, & Jenkins, 1985). In addition, argentation TLC was used and FAMEs sepd by prep. $AgNO_3$ TLC by developing with hexane–Et_2O–HOAc (94:4:3) (Dudley & Anderson, 1975) and frs analyzed by GC.

3.2. Isolation of 20:3 fatty acid

Free fatty acids were isolated from fresh algae after saponification with KOH in EtOH and esterified by heating with 1% H_2SO_4 in MeOH (Ackman & McLachlan, 1977). FAMEs were purified by CC on silica gel eluting with hexane and hexane–Et_2O (19:1) and then separated by CC on silica gel impregnated with 10% $AgNO_3$ (Teshima, Kanazava, & Tokiwa, 1978). Frs (10 ml) were collected and checked by $AgNO_3$–silica gel TLC in hexane–Et_2O–HOAc (94:3:3) (Dudley & Anderson, 1975). Detection was performed with 10% H_2SO_4 in MeOH and subsequent heating at 180°C. Trienoic components were eluted with hexane–Et_2O–HOAc (19:1:0.1). Frs containing trienoic fatty acids were combined, evapd and checked for purity by GC. Further purification was carried out by HPLC on a μ-Bondapak C18 column (7.8×300 mm, 10 μm particles, Waters Associates) in 96% EtOH–H_2O (9:1) at a flow rate of solvent 2.5 ml min^{-1} with monitoring at 214 nm.

3.3. Structural determination of 20:3 fatty acid

Catalytic hydrogenation of 20:3 Me ester was carried out in MeOH over PtO_2 at room temperature. Oxidative ozonolysis of the ester in BF_3–MeOH was performed as described in Sebedio and Ackman (1978). Products of ozonolysis were analyzed by GC on a capillary column ($25\ m \times 0.2$ mm) coated with FFAP. The column was held at 80° for 5 min, programmed at 20°C min^{-1} to 180°C and then held at 180°C. Fatty acid pyrrolidides were prepd by heating the Me esters in pyrrolidine with HOAc at 100°C for 30 min (Andersson, Christie, & Holman, 1975). Pyrrolidides were extracted and purified by TLC using $CHCl_3$–Me_2CO (9:1) and analyzed by GC and MS. EI MS were recorded using a direct inlet system at 100–160°C at 70 eV.

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