Pergamon

0031-9422(95)00570-6

Phytochemistry, Vol. 41, No. 2, pp. 483–488, 1996

0031-9422/96 $15.00 + 0.00

DISTRIBUTION OF POLAR LIPIDS IN SOME MARINE, BRACKISH AND FRESHWATER GREEN MACROPHYTES

VALERY M. DEMBITSKY* and OLGA A. ROZENTSVET†

Laboratory of Natural Products, Department of Organic Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

(*Received in revised form 5 June 1995*)

Key Word Index—Green marine; brackish; freshwater macrophytes; phospholipids; DGTS; chemotaxonomy.

Abstract—Diacylglyceryltrimethylhomoserines (DGTS) and phospholipid composition has been studied in 13 marine, three brackish and 16 freshwater species of green macrophytes. DGTS was detected in all marine and brackish algae, and in four freshwater algae. DGTS was not found in any marine or freshwater grasses. It was also demonstrated that the most distinctive feature of the polar lipids of the green macrophytic species examined was the combined production of three polar lipids, DGTS, phosphatidylcholine and phosphatidylglycerol.

INTRODUCTION

Green macrophytes are the dominant flora of all seas and lakes. They play an important role in marine, brackish and freshwater ecosystems, providing food, shelter and physical support for many crustaceans, fish and epiphytic organisms [1].

Betaine lipids, such as diacylglycaryltrimethyl homoserines (DGTS) and diacylglycerylhydroxymethyl-β-alanine (DGTA), constitute a new group of complex ether-linked glycerolipids and are widely distributed in lower plants and algae, but relatively little is known of their biochemistry [2–4]. Polar lipids, including phospholipids and DGTS, covering a considerable percentage in the total lipids, carrying out well-defined physiological and structural functions and being the most conservative lipid components doubtlessy, reflect the evolutional hierarchy of the Plant Kingdom, in particular, among green macrophytes [2, 5].

Environmental factors may alter surface, membrane or storage lipids and their metabolism, though it is not always that these different aspects are differentiated [6–8]. The information presently existing about phospholipids [5, 9, 10] and other organic compounds [11, 12], for instance, sterols [13, 14], carotenoids [15, 16], terpenoids [17, 18], hydrocarbons [19], organic acids [20] and fatty acids [21], allows the use of them as chemical markers for chemotaxonomy.

Quantitative screening of phospholipids and DGTS contained mainly in marine green macrophytes carried out in recent years, has provided data for chemotaxonomic and statistical processing. The present paper deals with a detailed study of the distribution DGTS and other polar lipids among 32 green macrophytes species and their chemotaxonomic significance.

*Author to whom correspondence should be addressed.
†Present address: Institute of Ecology, Togliatti 445003, Russia.

RESULTS AND DISCUSSION

DGTS and phospholipid composition of 11 seaweeds and two marine grasses, three algal species collected in brackish waters, and 16 freshwater green macrophytes species is listed in Table 1. DGTS occurred in all seaweeds, but was not detected in *Posidania oceanica* and *Zostera marina* seagrasses. In brackish species, DGTS varied from 1.4 to 16.8%. Only four fresh-water algal species contained DGTS. Cardiolipin (DPG) was found mainly in the species which did not contain DGTS.

Phospholipid [5, 22, 23] and DGTS compositions of green macrophytes have been reported previously [24–26] and indicated that DGTS is widely distributed in marine species but is not found in all marine and freshwater aquatic plants. Total lipid, neutral, glyco- and polar lipid contents in each examined species are also given in Table 1. Also added to Table 1 are some marine green macrophytic species that had been examined earlier [22, 24]. Special attention was paid to the presence or absence of DGTS and/or phosphatidylcholine (PC). Results of analyses are given for six separate groups: group I – Siphonophyceae, that contained both DGTS and PC; group II – Ulotrichophyceae, that contained only DGTS; group III – including marine grasses only (groups I–III, marine species); group IV – brackish species; group V – freshwater algae (freshwater species belonging to different orders: *D. arnoldioides* and *Draparnaldiella baicalensis*, order Chaetophorales; *Tetraspora*

Table 1. Composition of polar lipids of same green macrophytes (mol. %)

Species	DGTS	PC	PE	PS	PG	DPG	PI	PA	TL	PL	GL	NL	Collection	Ref.
Marine species														
Seaweeds														
Group I														
Siphonophyceae														
Acetabularia mediterranea	40.9	1.4	15.7	6.6	30.4	—	5.0	tr	3.3	20.0	36.9	43.1	Mediterranean sea	
Bryopsis hypnoidea	11.8	23.7	9.4	4.0	35.5	—	12.8	—	2.8	19.1	n.d.	n.d.	Black Sea	[24]
Bryopsis plumosa	14.2	24.8	12.3	5.2	32.2	—	11.3	—	1.9	14.3	n.d.	n.d.	Black Sea	[24]
Caulerpa prolifera	27.4	5.4	17.5	8.1	34.6	—	7.0	tr	3.2	19.3	39.7	41.0	Mediterranean Sea	
Chaetomorpha capillaris	25.8	20.6	9.6	7.2	29.3	—	7.5	tr	2.6	11.1	36.2	52.7	Mediterranean Sea	
Ch. crassa	6.3	17.9	6.9	6.4	32.1	6.0	17.2	7.2	1.8	3.8	n.d.	n.d.	Black Sea	[24]
Ch. linum	22.1	17.2	10.4	9.0	33.2	—	8.1	—	3.1	14.2	37.4	48.4	Mediterranean Sea	
Cladophora prolifera	31.0	21.1	9.3	4.3	29.1	tr	5.2	tr	2.6	17.2	35.3	47.6	Mediterranean Sea	
Cl. sericea	6.5	21.3	8.8	7.9	46.8	2.1	6.6	—	2.0	16.7	n.d.	n.d.	Black Sea	[24]
Cl. vagabunda	5.2	23.8	7.5	6.5	47.0		8.0	2.0	2.2	18.4	n.d.	n.d.	Black Sea	[24]
Codium vermilara	12.3	30.2	19.6	6.2	26.3	—	5.4	—	2.9	19.3	31.0	49.7	Mediterranean Sea	
Rhizoclonium implezum	6.6	25.3	5.9	10.0	41.6	3.6	10.3	1.3	1.8	7.3	n.d.	n.d.	Black Sea	[24]
Group II														
Ulotrichophyceae														
Endocladia viridis	41.9	—	12.9	10.1	23.5	—	4.3	—	2.9	14.5	n.d.	n.d.	Black Sea	[24]
Enteromorpha compressa	47.3	—	19.5	3.3	25.6	—	6.5	—	1.7	10.1	30.6	59.3	Mediterranean Sea	
En. linza	52.5		15.2	5.7	20.1	—	10.2	3.8	1.6	12.2	32.8	55.0	Mediterranean Sea	
En. linza	39.6	—	11.2	8.9	26.0	—	12.2	4.0	1.2	6.9	n.d.	n.d.	Black Sea	[24]
En. prolifera	36.0	—	10.4	9.7	28.0	—	6.3	—	0.8	8.4	n.d.	n.d.	Black Sea	[24]
Halimeda tuna	40.1	tr	6.9	5.9	40.8		7.0	—	2.7	18.4	38.2	43.4	Mediterranean Sea	
Ulotrix flacca	38.2	—	14.3	13.6	22.1	—	9.3	2.2	1.6	4.8	n.d.	n.d.	Black Sea	[24]
Ulotrix zonata	46.9	tr	13.7	4.1	29.8	tr	5.5	tr	2.2	10.4	40.0	49.6	Mediterranean Sea	
Ulva lactuca	69.6	—	7.1	2.2	20.1	—	1.0	tr	2.8	9.9	41.3	48.8	Mediterranean Sea	
Ulva rigida	51.0	—	10.3	8.3	25.0	—	5.4	—	2.6	8.4	n.d.	n.d.	Black Sea	[24]
Ulva sp.	56.5	—	9.6	7.7	21.3	—	4.9	—	2.1	9.1	n.d.	n.d.	Black Sea	[24]
Urospora penicilliformis	42.0	—	11.9	9.3	25.4	—	7.2	4.2	2.3	6.9	n.d.	n.d.	Black Sea	[24]
Grasses														
Group III														
Posidonia oceanica		41.9	23.1	1.9	17.7	2.9	12.8	tr	5.3	26.4	32.6	41.0	Mediterranean Sea	
Phyllospadix iwatensis	—	42.7	22.4	1.5	16.6	3.3	13.7	tr	10.4	13.1	n.d.	n.d.	Sea of Japan	[22]
Zostera asiatica	—	41.8	24.3	2.6	15.8	3.4	12.1	tr	4.3	25.3	n.d.	n.d.	Sea of Japan	[22]
Zostera marina	—	45.5	24.0	2.6	13.7	2.6	11.3	tr	8.1	19.9	29.9	50.2	Mediterranean Sea	
Zostera marina	—	43.8	23.8	2.2	14.6	3.0	12.6	tr	5.0	29.0	n.d.	n.d.	Sea of Japan	[22]
Zostera marina		43.1	23.9	6.9	13.9	tr	12.2	—	4.3	24.3	n.d.	n.d.	Black Sea	[24]
Zostera nana	—	45.1	24.3	4.8	15.5	tr	10.3	—	3.9	27.8	n.d.	n.d.	Black Sea	[24]

Brackish species													
Algae													
Group IV													
Acrosiphonia sp.	16.8	29.5	12.2	5.1	15.3	—	19.0	2.1	38.2	25.5	40.6	33.9	Caspian Sea
Chara vulgaris	6.9	43.5	20.7	4.2	10.0	tr	13.7	1.0	73.2	27.1	46.3	26.6	Caspian Sea
Nitellopsis obtusa	1.4	45.6	10.4	11.2	4.9	4.0	1.7	1.7	32.5	25.1	39.8	35.1	Caspian Sea
Freshwater species													
Algae													
Group V													
Draparnaldiella arnoldioides	8.4	17.7	13.7	10.7	41.5	—	6.9	1.1	64.1	34.5	38.5	27.0	Lake Baikal
Draparnaldia baicalensis	10.5	8.3	20.4	13.6	40.9	—	6.3	tr	75.7	9.5	66.1	24.4	Lake Baikal
Tetraspora cylindrica	11.1	26.2	17.2	8.9	28.3	—	8.3	—	28.9	16.8	18.1	65.1	Lake Baikal
Ulotrix zonata	13.4	10.6	25.2	14.3	27.4	1.2	7.9	—	17.0	32.6	30.8	36.6	Lake Baikal
Grasses													
Group VI													
Myriophyllum spicatum	—	39.3	14.1	7.3	27.2	6.5	5.0	0.6	56.0	22.3	37.2	40.5	Lake Baikal
Ranunculus sp.	—	35.8	14.7	8.8	24.4	5.8	9.6	0.9	62.0	12.4	34.0	53.6	Lake Baikal
Elodea sp.	—	44.8	15.6	6.1	25.3	tr	8.2	—	57.9	19.0	33.1	47.9	Lake Baikal
El. Nuttalli	—	46.6	19.0	2.5	20.2	tr	11.7	—	73.3	20.4	33.3	46.3	Aquarium
Caltha palustris	—	43.8	26.7	4.1	9.3	3.4	10.0	—	69.7	22.5	32.9	44.6	Vasil'ev Lake
Ceratophyllum submersum	—	44.0	16.5	4.9	14.8	2.6	16.4	—	62.8	26.9	30.4	42.7	Vasil'ev Lake
Ceratopteris thalictoides	—	47.5	23.7	9.1	9.7	4.4	7.4	tr	30.0	29.3	34.5	36.2	Vasil'ev Lake
Pistia stratiotes	—	46.2	26.2	7.7	6.0	5.0	8.6	tr	28.7	22.1	30.6	47.3	Aquarium
Nymphoides alba	—	46.6	25.1	2.3	11.2	5.3	9.6	—	92.0	32.5	16.8	50.7	Aquarium
Vallisneria spiralis	—	46.8	21.1	6.6	13.9	6.1	7.2	—	48.2	20.0	30.9	49.1	Aquarium
V. gigantea	—	42.5	25.9	7.0	12.8	5.2	6.8	—	51.1	19.7	31.6	48.7	Vasil'ev Lake
Utricularia vulgaris	—	47.4	20.4	3.4	11.0	8.8	9.0	—	49.2	23.7	39.6	36.7	Vasil'ev Lake

Values are means ± s.d. (n = 2–4).

Abbreviations: TL, total lipids, $mg\,g^{-1}$ dry wt; PL; polar lipids; GL, glycolipids; NL, neutral lipids; PL, GL and NL are given as percentages of total lipids; DGTS, diaylglyceryltrimethylhomoserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PG, phosphatidylglycerol; DPG, cardiolipin; PI, phosphatidylinositol; PA, phosphatidic acid; n.d., not detected.

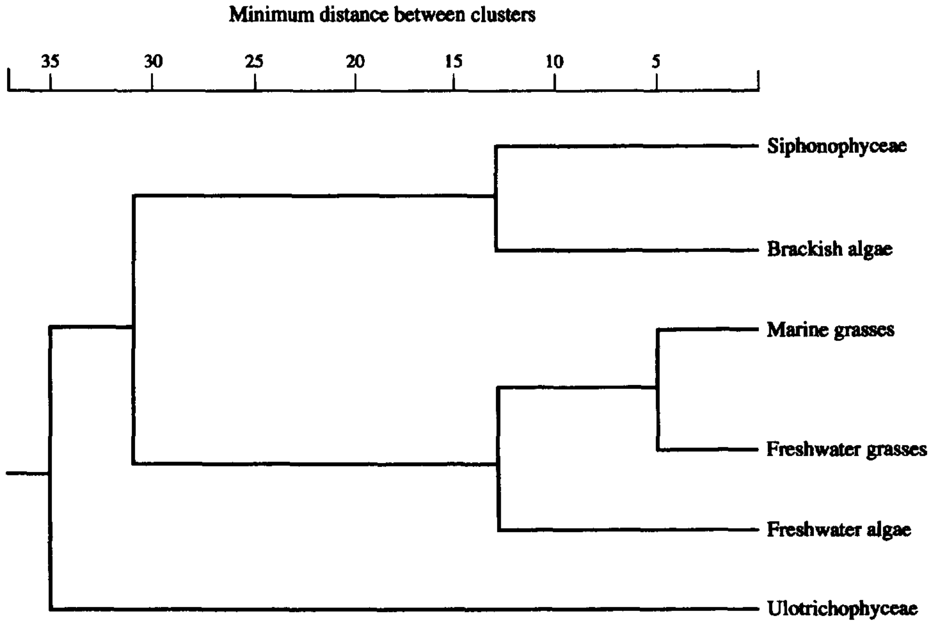

Fig. 1. Phylogenetic tree combining the results of a cluster analysis of the distribution of DGTS and phospholipids among green macrophytes. Dendrogram from cluster analysis (single-linkage method) of polar lipid data (Table 1).

Table 2. Mean values of individual phospholipids and DGTS from 50 green macrophytic species*

	Group I		Group II		Group III		Group IV		Group V		Group VI		Name of exponent		
PL	MV	SD	MV	SD	MV	SD	MV	SD	MV	SD	MV	SD	FC	PDE	MSV
DGTS	17.5	11.7	46.8	9.5	0.0	0.0	8.4	7.8	10.9	2.0	0.0	0.0	177.4	98.0	1330.7
PC	19.4	8.2	0.0	0.0	43.4	1.5	39.5	8.8	15.7	8.1	44.3	3.6	107.2	96.6	744.9
PG	34.8	4.2	25.6	3.5	15.4	1.3	10.1	1.5	34.5	2.7	15.5	2.4	65.3	94.6	380.7
PS	6.8	1.1	7.4	2.1	3.2	0.7	6.8	1.9	11.9	1.4	5.8	1.4	8.4	67.0	24.7
PI	8.4	2.2	6.9	1.9	12.1	0.9	11.5	1.6	7.3	1.2	9.1	1.8	8.0	65.7	32.8
PE	11.1	2.7	11.9	2.2	23.7	0.6	14.4	3.5	19.1	2.8	20.8	2.9	5.1	52.3	21.1
DPG	3.9	1.9	3.0	0.4	2.1	0.2	0.7	0.4	5.3	1.2	0.0	0.0	1.3	8.6	5.1
PA	0.9	0.4	1.2	0.5	0.0	0.0	1.6	0.3	0.3	0.2	0.0	0.0	1.0	0.6	5.6

*Calculated by cluster analysis [31–33].

Abbreviations: MV, Mean value of each polar lipid in group; SD, Standart deflection of each polar lipid in group; FC, Fisher criterion, an appreciation of the effect distinction of the each polar lipid; PDE, individual polar lipid distinction effect expressed by percentage; MSV, individual polar lipid distinction effect expressed by mean square value.

cylindrica, order Tetrasporales; and *Ulotrix zonata*, order Ulotrichales) containing both DGTS and PC, and group VI – freshwater grasses, not containing DGTS.

The green macrophytes of these six groups have their own characteristic set of polar lipids (Table 1). Thus, the main polar lipid class in the family Ulotrichophyceae is DGTS which varied from 36% in *Enteromorpha prolifera* to 69.6% in *Ulva lactuca*. Another characteristic feature of this family is the absent of PC; it is likely that DGTS sometimes "plays the part" of PC. PC notably appears in the family Siphonophyceae where its content varies from 1.4% in *Acetabularia mediterranea* to 30.2% in *Codium vermilara*.

Seven higher plant marine grasses which were collected in different seas have differing phospholipid composition from those of groups I and II in that they lack DGTS, while the PC content reaches over 40%. Freshwater grasses have a similar phospholipid composition (group VI). Such phospholipid composition is characteristic of the majority of higher plants irrespective of habitat.

Fig. 1 shows the combined dendrogram for 50 green macrophytic species, in the space described by eight features (polar lipids). The Euclidean distance between the six groups changes from 5.4 to 36.5. It is the latter groups that form a dispersion complex (Table 2).

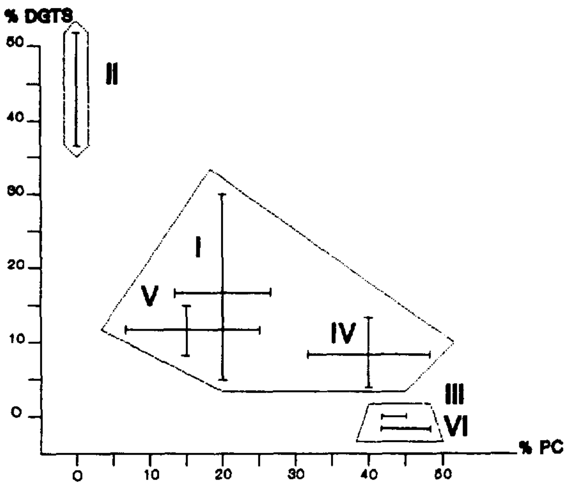

Fig. 2. Relationship of DGTS and PC among six groups of green macrophytes. Group III and IV, combined marine and freshwater grasses (not containing nor producing DGTS). Group I, IV and V, combined marine (Siphonophyceae), brackish and freshwater algae (containing PC and DGTS) and group II including seaweeds only (Ulotrichophyceae). Standard deflection data of DGTS and PC in each group are taken from Table 2.

Phospholipids and DGTS provide the basis for the distinction between species in the dispersion analysis. Each group has a specific polar lipid contributing to the differentiation of species in the cluster grouping (Table 2). The major distinctive effect is produced by DGTS, PC and phosphatidylglycerol (PG). Distinctive effects produced by individual polar lipid classes found in green macrophytes (for all marine, brackish and freshwater species) form the following pattern: DGTS → PC → PG → phosphatidylserine (PS) → phosphatidylinositol (PI) → phosphatidylethanolamine (PE). For minor components and the components of rate occurrence, the distinctive effect is considerably lower than that for the above polar lipids. As an illustration, Fig. 2 shows the relationship between DGTS and PC among six groups of green macrophytes. Groups III, IV combine marine and freshwater grasses. Groups I, IV and V combine marine (Siphonophyceae) and freshwater algae and group II includes seaweeds only (Ulotrichophyceae).

The betaine lipids, DGTS and DGTA, represent a prominent group of polar lipids and have been detected in most cryptogamic plants [2–4] but they have not been detected in seed plants (either angiosperms or gymnosperms) [4]. According to published data [2–4], we can make the assumption that DGTS is a more ancient membrane lipid that was substituted by PC during the process of the evolution of green plants. However, green algae (all marine, brackish and freshwater species), primitive nonvascular plants, much as Bryophytes [27, 28] and vascular plants like Pteridophytes [29], have kept the DGTS biosynthesis pathway. Higher plants probably lost the DGTS biosynthesis pathway in the course of their evolution. The apparent reciprocity of the abundance of DGTS and PC among different species in lower plants and algae as described recently [2–4], suggest that DGTS and PC are similar in their chemical and physical properties [30] and, thus, are probably interchangeable with each other in their roles within the cell. DGTS has a higher phase transition temperature than PC if they have the same acyl groups (for instance, 18:0–18:0): 59.0 and 54.2°, respectively [30]. This suggests that the intermolecular interactions of DGTS molecules are slightly different from those of PC molecules.

Our results demostrate that most the characteristic features of the Chlorophyta are the dominance of DGTS among phospholipids mainly in seaweeds and freshwater algae. Freshwater algae grown in brackish water have less DGTS than PC. Marine and freshwater grasses do not contain DGTS nor do all terrestrial higher plants. Since many DGTS-containing algae do not contain PC, betaine lipids are suggested to be the substitutes for zwitterionic phospholipids possibly providing ecological advantages to these plants. (For instance, for *U. zonata*, the marine species contained DGTS with only a trace of PC but the freshwater species had 13.4% of DGTS and 10.6% of PC). Attempts to extend the survey and to correlate the lipid composition with other taxonomically important criteria will be necessary.

EXPERIMENTAL

Plant material. All marine macrophytes were collected from October 1993 to May 1994 on the Palestine coast of the Mediterranean (from Ashkelon to Akko). Brackish species were sampled during September 1991 at a site located 70 km south of Krasnovodsk on the western coast of the Caspian Sea. Seven freshwater species were collected in August 1992 in Lake Baikal (Western-South Siberia, Russia); five freshwater species sampled in the Vasil'ev Lake near Togliatti (Middle Volga area, Russia) from May to September 1992; four freshwater species were grown in the aquarium and samples from October to December 1992 (Institute of Ecology, Togliatti).

Lipid analysis. Freshly collected macrophytes were thoroughly cleansed of extraneous matter and lipids extracted after boiling in *iso*-PrOH for 2–3 min. Extraction of lipids, their analysis, quantification and identification were carried out as described elsewhere [23, 24].

Cluster analysis. We used the uniting and polythetic procedure of *Q*-analysis (classification of objects in the space of signs) with minimization of internal group dispersion of objects (standard package of programmes STATISTICA for IBM-PC; by its logic the closest to this method is the classification technique [31–32]. Cluster analysis was performed with the use one-factor dispersion analysis [33].

Acknowledgements—The authors thanks Dr I. Dor (Enivronmental Department, Hebrew University) for assistance in identification of marine macrophytic species. The technical assistance in the collection and classification of freshwater and brackish algae and freshwater grasses of Mrs Yulya Mazepova and Mrs Lyubov Taranova is gratefully acknowledged (Institute of Ecology, Togliatti, Russia).

REFERENCES

1. Harrison, P. G. and Mann, K. H. (1975) *J. Fish Res. Bd. Can.* **32**, 615.
2. Dembitsky, V. M. (1995) *Prog. Lipid Res.* **34**, in press.
3. Eichenberger, W. (1993) *Planta Physiol. Biochem.* **31**, 213.
4. Sato, N. (1992) *Bot. Mag. Tokyo* **105**, 185.
5. Khotimchenko, S. V., Klochkova, N. G. and Vaskovsky, V. E. (1990) *Biochem. Syst. Ecol.* **18**, 93.
6. Harwood, J. L. (1984) *Dev. Plant Biol.* **9**, 543.
7. Harwood, J. L. (1989) *CRC Crit. Revs. Plant. Sci.* **8**, 1.
8. Harwood, J. L. and Jones, A. L. (1989) *Adv. Bot. Res.* **16**, 1.
9. Dembitsky, V. M. (1989) in *Fats for the Future.* (Cambie, R. C., ed), Chapt. **12**, p. 173. Van Nostrand Reinhol/Avi, London.
10. Dembitsky, V. M. (1991) *Biol. Sci. (Moscow Univ. Press)* **2**, 5.
11. Smith, P. M. (1976) *The Chemotaxonomy of Plants.* Elsevier, New York.
12. Harborne, J. B. and Turner, B. L. (1984) *Plant Chemo-systematics.* Academic Press, London.
13. Goodwin, T. W. (1975) in *Algal Physiology and Biochemistry* (Stewart, W. D. O., ed.), p. 266. Blackwell, Oxford.
14. Patterson, G. W. (1992) in *Physiology and Biochemistry of Sterols* (Patterson, G. W. and Nes, W. D., eds), p. 118. American Oil Chem. Soc., Champain.
15. Liaaen-Jensen, S. (1988) *Biochem. Syst. Ecol.* **16**, 589.
16. Liaaen-Jensen, S. (1989) *Pure and Appl. Chem.* **61**, 369.
17. Sakurai, A. and Fujioka, S. (1993) *Plant Growth Regul.* **13**, 147.
18. Valls, R., Piovetti, L. and Praud, A. (1993) *Hydrobiologia* **260/261**, 549.
19. Nevensel, J. C. (1989) in *Marine Biogenic Lipids: Fats and Oils.* (Ackman, R. G., Ed), Vol. 1, p. 3. CRC Press, Inc. Boca Raton, Florida.
20. Skult, H. (1984) *Ann. Bot. Fennici* **21**, 117.
21. Pohl, P. and Zurheide, F. (1979) in *Marine Algae in Pharmaceutical Science.* (Hoppe, H. A., Levring, T. and Tanaka, Y., eds), p. 473. Walter de Gruyter, Berlin-New York.
22. Khotimchenko, S. V. (1993) *Phytochemistry* **33**, 369.
23. Dembitsky, V. M., Rozentsvet, O. A., Zhuikova, V. S., Vasilenko, R. F. and Kashin, A. G. (1992) *Phytochemistry* **31**, 3259.
24. Dembitsky, V. M. and Rozentsvet, O. A. (1989) *Phytochemistry* **28**, 3341.
25. Sato, N. and Furuya, M. (1985) *Plant Sci.* **38**, 81.
26. Dembitsky, V. M., Rezanka, T. and Rozentsvet, O. A. (1993) *Phytochemistry* **33**, 1015.
27. Dembitsky, V. M. (1993) *Prog. Lipid Res.* **32**, 281.
28. Dembitsky, V. M. and Rezanka, T. (1995) *Biochem. Syst. Ecol.* **23**, 71.
29. Sato, N. and Furuya, M. (1984) *Phytochemistry* **23**, 1625.
30. Sato, N. and Murata, N. (1991) *Biochim. Biophys. Acta* **1082**, 108.
31. Sneath, P. H. A. and Sokal, R. R. (1973) *Numerical Taxonomy – The Principles and Practice of Numerical Classification.* W. H. Freeman & Co., San Francisco.
32. Romesburg, H. C. (1984) *Cluster Analysis for Researches.* Lifetime Learning Publ., Belmont, California.
33. Rozenberg, G. S. and Dolotovsky, I. M. (1988) *Biol Sci.* (USSR) **9**, 105.