



BETAINE LIPIDS AND ZWITTERIONIC PHOSPHOLIPIDS IN PLANTS AND FUNGI

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Key Word Index—Algae; ferns; fungi; lichens; mosses; plants; evolution; occurrence; betaine; lipids; phospholipids.

Abstract—Of spermatophytes, ferns, mosses, algae, lichens and fungi, 110 species not analysed so far were examined for diacylglyceryl-*N,N,N*-trimethylhomoserine (DGTS), diacylglycerylhydroxymethyl-*N,N,N*-trimethyl- β -alanine (DGTA), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) by TLC by using Dragendorff's and molybdenum-blue reagents for detection. The limit of detection was 0.5 μ g per mg or 0.05 weight % of total lipid. The results reveal that betaine lipids are present exclusively in non-flowering plants, including lichens and fungi and, hence, are produced by autotrophic, as well as heterotrophic organisms. DGTS in small amounts is typical of some Rhodophytes, and in appreciable amounts, of vascular cryptogamic plants and some higher fungi. Estimation of the amounts of DGTS and PC in 29 different species reveal that the total amount of zwitterionic lipids varies considerably amongst organisms. No general correlation could be found for the amounts of DGTS and PC, although Rhodophytes contain traces of DGTS but high amounts of PC. On the basis of these results and the presently available data, the natural distribution of the betaine lipids DGTS, DGTA and diacylglycerylcarboxy-*N*-hydroxymethyl-choline (DGCC) is discussed. In terms of biochemical evolution, the capacity for the formation of DGTS might have been acquired first and, in organisms of the 'DGTS branch', kept until the present time. The formation of DGTA and DGCC might have evolved at a later stage of development in organisms of the 'DGTA-DGCC branch'. © 1997 Elsevier Science Ltd

INTRODUCTION

The lipid composition of flowering plants is dominated by a limited number of fatty acids and polar lipids irrespective of the taxonomic affiliation of the plant [1]. In non-flowering plants comprising vascular cryptogamic plants, mosses, algae, lichens and fungi, in contrast, a large variety of polar lipids are encountered. Our knowledge on both their structure and natural distribution is far from being complete, although the interest in lipids of these so-called lower plants has considerably increased in past years with regard to economic and ecological aspects [2]. Several reviews [3–5] indicate that the betaine lipids represent a prominent class of polar lipids which are widespread but limited to non-flowering plants. Three different compounds are known at the present time, namely diacylglyceryl-*N,N,N*-trimethylhomoserine (DGTS), diacylglycerylhydroxymethyl-*N,N,N*-trimethyl- β -alanine (DGTA) and diacylglycerylcarboxy-*N*-hydroxymethylcholine (DGCC). Their molecular

geometry and charge distribution show some similarity to the ubiquitous phospholipid, phosphatidylcholine (PC). Since in a number of organisms producing betaine lipids, the amount of PC is low or even zero, the question arose whether betaine lipids may substitute for PC as a membrane constituent and/or an intermediate in cellular lipid metabolism [6–10]. Efforts to extend the knowledge on the occurrence of these lipids is also justified for taxonomic reasons, since their presence or absence often reflects the taxonomy and, like DGTA in brown algae, may be used as taxonomic markers [11].

Despite the many data available on the natural distribution of these lipids, several gaps remain. The aim of our work was to extend the search for betaine lipids on additional species in groups of organisms which have not systematically been analysed so far. Examples are especially the red algae and the fungi. In order to decide whether a correlation exists between the amount of PC and that of betaine lipids, the phospholipids PC and phosphatidylethanolamine (PE) have also been considered. Finally, we tried to interpret the presently available data in terms of the possible evolution of pathways responsible for the biosynthesis of betaine lipids.

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RESULTS AND DISCUSSION

For the detection of betaine lipids, the most convenient and specific method is the colour reaction with Dragendorff's reagent [12], which is specific for trimethylammonium groups and can therefore also be used for the detection of PC. The colour intensity and thus the sensitivity, could be enhanced by spraying the TLC plates first with Dragendorff's and then with strongly acidic molybdenum-blue reagent [13]. Using a standard solution of DGTS, different amounts of

the lipid were spotted on a plate which after a single development was sprayed with the reagents. The smallest detectable amount was 0.5 µg per spot. If for the analysis, 1 mg of total lipid was loaded on a plate for one dimensional separation, the detection limit was at 0.5 µg mg⁻¹ or 0.05% weight of total lipid. This value also held for DGTA and PC, as confirmed by using the corresponding reference solutions. PE was detected by spraying the plate with molybdenum-blue reagent. The results are summarized in Table 1.

Table 1. Distribution of the betaine lipids, DGTS and DGTA, and the phospholipids, PC and PE, in the plant kingdom. The taxonomic arrangement follows the system of ref. [34]

Division	DGTS	DGTA	PC	PE	Origin
SUBDIVISION					
Class					
Subclass					
Order					
Species					
Procaryota					
CYANOPHYTA					
<i>Synechococcus sp.</i> PCC 6301	—	—	—	—	a
Eucaryota					
Algae					
HETEROKONTOPHYTA					
Bacillariophyceae					
<i>Gomphonema parvulum</i>	—	+	+	—	b
RHODOPHYTA					
Rhodophyceae					
Bangiophycidae					
Bangiales					
<i>Asparagopsis armata</i>	—	—	+	+	h
<i>Porphyra linealis</i>	—	—	+	+	h
Florideophycidae					
Nemalionales					
<i>Gelidium latifolium</i>	—	—	+	—	h, j
<i>Palmaria palmata</i>	—	—	+	+	h
Cryptonemiales					
<i>Corallina officinalis</i>	—	—	+	—	h, l
<i>Dilsea carnosus</i>	—	—	+	+	h
Gigartinales					
<i>Ahnfeltia duplicata</i>	—	—	+	+	h
<i>Calliblephoris jubata</i>	—	—	+	—	h
<i>Chondrus crispus</i>	—	—	+	—	h
<i>Cystocolonium purpureum</i>	—	—	+	—	h
<i>Gracilaria verrucosa</i>	—	—	+	—	h, l
<i>Palotoma marginifera</i>	—	—	+	+	h
<i>Phyllophora pseudoceranoides</i>	+	—	+	+	h
<i>Polyides rotundus</i>	—	—	+	+	g
<i>Rhodophyllis divaricata</i>	—	—	+	+	h
Rhodymeniales					
<i>Lomentaria articulata</i>	+	—	+	—	h
<i>Mastocarpus stellatus</i>	+	—	+	—	h
Ceramiales					
<i>Antithamnion plumula</i>	—	—	+	+	g
<i>Callithamnion tetricum</i>	—	—	+	+	h
<i>Ceramium ciliatum</i>	—	—	+	—	h
<i>Delesseria sanguinea</i>	—	—	+	—	h
<i>Laurentia obtusa</i>	—	—	+	+	h

Table 1—Continued.

Division					
SUBDIVISION					
Class					
Subclass					
Order					
Species	DGTS	DGTA	PC	PE	Origin
<i>Membranoptera alata</i>	+	—	+	—	h
<i>Phycodrys rubens</i>	+	—	+	—	h
<i>Plumaria elegans</i>	—	—	+	+	g
<i>Rhodomela confervoides</i>	—	—	+	+	g, l
Fungi					
EUMYCOTA					
Zygomycetes					
<i>Rhizopus nigricans</i>	—	—	+	+	c
Ascomycetes					
Endomycetidae					
Endomycetales					
<i>Saccharomyces cerevisiae</i>	—	—	+	+	d
Ascomycetidae					
Heliotales					
<i>Neobulgaria pura</i>	—	—	+	+	e
Xylariales					
<i>Xylaria hypoxylon</i>	+	—	+	+	e
Basidiomycetes					
Heterobasidiomycetidae					
Tremellales					
<i>Pseudohydnum gelatinosum</i>	+	—	+	+	e
<i>Tremiscus hellvelloides</i>	—	—	+	+	e
Dacrymycetales					
<i>Calocera viscosa</i>	+	—	+	+	e
Homobasidiomycetidae					
Poriales (Aphylophorales)					
<i>Hyphoderma praetermissum</i>	+	—	+	+	d
Thelephorales					
<i>Sarcodon imbricatus</i>	—	—	+	+	e
Cantharellales					
<i>Cantharellus cibarius</i>	+	—	+	+	e
<i>C. lutescens</i>	+	—	+	+	e
<i>Ramaria stricta</i>	—	—	+	+	e, j
Polyporales					
<i>Postia caesia</i>	+	—	+	+	e
Agaricales					
<i>Hygrophorus eburneus</i>	+	—	+	+	e
<i>Laccaria amethystina</i>	+	—	+	+	e
<i>Lyophyllum loricatum</i>	+	—	+	+	e
<i>Lepista nuda</i>	—	—	+	+	e
<i>Armillaria mellea</i>	+	—	+	+	e
<i>Micromphale perforans</i>	+	—	+	+	e
<i>Amanita muscaria</i>	—	—	+	+	e
<i>A. phalloides</i>	+	—	+	+	e
<i>A. rubescens</i>	+	—	+	+	e
<i>Agaricus hortensis</i>	—	—	+	+	e
<i>Coprinus comatus</i>	—	—	+	+	e, j
<i>Agrocybe praecox</i>	—	—	+	+	e
<i>Pholiota lenta</i>	—	—	+	+	e, j
<i>Stropharia aeruginosa</i>	—	—	+	+	e, j
<i>Crepidotus cesatii</i> var. <i>cesatii</i>	—	—	+	+	e
<i>C. applanatus</i>	—	—	+	+	e
<i>Cortinarius odorifer</i>	+	—	+	+	e
<i>Galerina sideroides</i>	—	—	+	+	d
<i>Inocybe fraudans</i>	—	—	+	+	e

Table 1—Continued.

Division					
SUBDIVISION					
Class					
Subclass					
Order					
Species	DGTS	DGTA	PC	PE	Origin
Russulales					
<i>Russula mairei mairei</i>	—	—	+	+	e
<i>Lactarius deterrimus</i>	—	—	+	+	e
Boletales					
<i>Boletus edulis</i>	+	—	+	+	e, n
<i>Gyrodon lividus</i>	+	—	+	+	e
<i>Xerocomus badius</i>	+	—	+	+	e
<i>Paxillus atrotomentosus</i>	+	—	+	+	e
Lycoperdales					
<i>Lycoperdon echinatum</i>	—	—	+	+	e
<i>Vascellum pratense</i>	—	—	+	+	e
Geastrales					
<i>Geastrum triplex</i>	—	—	+	+	e
Phallales					
<i>Phallus impudicus</i>	—	—	+	+	e
Deuteromycetes					
<i>Alternaria brassicicola</i>	—	—	+	+	c
<i>A. tenuissima</i>	—	—	+	+	c
<i>Fusarium sp.</i>	+	—	+	+	c
<i>Sclerotium rolfsii</i>	—	—	+	+	c
<i>Stemphylium sp.</i>	—	—	+	+	c
Lichenes					
Ascolichenes					
Lecornales					
<i>Cladonia fimbriata</i>	+	—	+	+	e, j
<i>Parmelia saxatilis</i>	+	—	+	—	e
<i>Peltigera canina</i>	+	—	+	+	e, j
<i>P. collina</i>	+	—	+	+	e
<i>P. horizontalis</i>	+	—	+	+	e
Embryophyta					
BRYOPHYTA					
Anthoceropsida					
Anthocerotales					
<i>Anthoceros sp. Lorbeer</i>	+	—	+	+	i
Marchantiopsida					
Marchantiidae					
Marchantiales					
<i>Concepalum conicum</i>	+	—	+	+	e
<i>Marchantia polymorpha</i>	+	—	+	+	e, j
Bryopsida (musci)					
Sphagnidae					
<i>Sphagnum pallustre</i>	+	—	+	+	e
Bryidae					
Polytrichales					
<i>Polytrichum commune</i>	+	—	+	+	e, k
Dicranales					
<i>Dicranella heteromolla</i>	+	—	+	+	e
Fissidentales					
<i>Fissidens toxifolius</i>	+	—	+	+	e
Grimmiales					
<i>Grimmia pulvinata</i>	+	—	+	+	e
<i>Bartramia halleriana</i>	+	—	+	+	e
Bryales					
<i>Mnium cuspidatum</i>	+	—	+	+	e
<i>M. undulatum</i>	+	—	+	+	e

Table 1—Continued.

Division	DGTS	DGTA	PC	PE	Origin
SUBDIVISION					
Class					
Subclass					
Order					
Species					
Hypnanae					
<i>Ctenidium molluscum</i>	+	—	+	+	e
<i>Hypnum cupressiforme</i>	+	—	+	+	e
<i>Pleurozium schreberi</i>	+	—	+	+	e, k, m
<i>Thuidium tamariscinum</i>	+	—	+	+	e
PTERIDOPHYTA					
Psilotopsida					
Psilotales					
<i>Psilotum triquetrum</i>	—	—	+	+	f
Lycopodiopsida					
Lycopodiales					
<i>Huperzia selago</i>	+	—	+	+	e
<i>Lycopodium annotinum</i>	+	—	+	+	e, k
Selaginellales					
<i>Selaginella kraussiana</i>	+	—	+	+	f
<i>S. palessens</i>	+	—	+	+	f
<i>S. selaginoides</i>	+	—	+	+	e
<i>S. uncinata</i>	+	—	+	+	f, m
<i>S. willdenowii</i>	+	—	+	+	f
Equisetopsida					
Equisetales					
<i>Equisetum arvense</i>	+	—	+	+	e, m
<i>E. scirpoides</i>	+	—	+	+	e
<i>E. silvaticum</i>	+	—	+	+	e
Pteridopsida					
Ophioglossales					
<i>Ophioglossum vulgare</i>	+	—	+	+	f
Polypodiales					
<i>Polypodium vulgare</i>	+	—	+	+	e
<i>Platyserium alaicorne</i>	+	—	+	+	f
Pteridales					
<i>Adiantum hispidulum</i>	+	—	+	+	f
Aspidiales					
<i>Asplenium viride</i>	+	—	+	+	e
<i>Dryopteris dilatata</i>	+	—	+	+	e
<i>Phyllitis scolopendrium</i>	+	—	+	+	e
Blechnales					
<i>Blechnum spicant</i>	+	—	+	+	e, k
Salviniales					
<i>Azolla filiculoides</i>	+	—	+	+	f
SPERMATOPHYTA					
GYMNOSPERMAE					
CONIFEROPHYTINA					
Pinopsida					
Pinidae (coniferae)					
Pinales					
<i>Agatis robusta</i>	—	—	+	+	f
<i>Araucaria curriharia</i>	—	—	+	+	f
<i>A. heterophylla</i>	—	—	+	+	f
<i>Cupressus dapreziana</i>	—	—	+	+	f
<i>Calocedrus formosana</i>	—	—	+	+	f
<i>Pinus mugo</i>	—	—	+	+	f
<i>Podocarpus nivalis</i>	—	—	+	+	f
<i>Sequoiadendron giganteum</i>	—	—	+	+	f
Taxidae					
<i>Taxus baccata</i>	—	—	+	+	f

Table 1—Continued.

Division	DGTS	DGTA	PC	PE	Origin
SUBDIVISION					
Class					
Subclass					
Order					
Species					
CYCADOPHYTINA					
Cycadopsida					
Cycadales					
<i>Cycas tharwanara</i>	—	—	+	+	f
<i>Macrozamia communis</i>	—	—	+	+	f
Gnetopsida					
Gnetales					
<i>Ephedra gerardiana</i>	—	—	+	+	f, m
<i>Gnetum</i>	—	—	+	+	f
MAGNOLIOPHYTINA					
Dicotyledonae					
<i>Magnolia denudata</i>	—	—	+	+	f

a, Prof. E. K. Pistorius, University of Bielefeld; b, G. Pohnert, University of Bonn; c, Prof. M. Sancholle, University of Lille; d, Dr B. Senn, University of Lausanne; e, Bern (field collection); f, Bern, Botanical Garden; g, Helgoland (field collection); h, Roscoff, French Brittany (field collection); i, Dr H. Kubin, University of Trebon; j-m: also analysed by j, Dembitsky [3], k, Eichenberger [5], l, Kato *et al.* [16], m, Sato and Furuya [17], n, Vaskovsky *et al.* [35]. + = > 0.05 wt%, — = < 0.05 wt %.

In Cyanobacteria, neither betaine lipids, PC nor PE could be detected. This is in accordance with published data [14]. Within prokaryotes, betaine lipids have exclusively been detected in *Rhodobacter sphaeroides*, which synthesizes DGTS under phosphate-limiting conditions [15]. This indicates that in these organisms, the biosynthetic pathway leading to DGTS, although not normally operating, may be induced under certain growth conditions.

In the only species analysed from the Bacillariophyceae, DGTA, as well as PC, were present, while DGTS and PE were not detected. It should be mentioned that in three other species of the same group, PC has also been found, but that in one species, DGCC was present, while DGTS and DGTA were always absent [16]. DGCC in turn, seems to be typical of all the members of the Haptophyceae [16]. Few of them, in addition, contain PC or DGTA [16] or DGTS (Eichenberger, unpublished results). Thus, the lipid pattern of these groups seems to be quite variable.

The Rhodophyta were examined more extensively, because only a few analyses had been carried out in this group. DGTS could be detected in small amounts only in five out of 26 species. This indicates that at least in some red algal species, the biosynthetic pathway for betaine lipids is operating, although in no case did these lipids contribute substantially to the polar lipid pattern in these plants. It should be noted that the major part of betaine-lipid producing red algae belong to the orders Gigartinales, Rhodymeniales and Ceramiales, all of which are part of the subclass Florideophycidae. In the other three orders, in contrast, neither DGTS nor DGTA has been encountered. These results are in accordance with data

obtained from the four species analysed earlier by other authors [3, 16, 17]. All the red algal species analysed here, contain large amounts of PC but rather small quantities of PE. A low PE content of red algae has also been reported by Dembitsky and Rozentsvet [18] and by Khotimchenko *et al.* [19].

Fungi are another group of organisms which have not been systemically examined for betaine lipids. In our experiments, DGTS was detected in 19 out of 42 species. This lipid is produced by members of Ascomycetes, as well as of the Basidiomycetes. A clear correlation, however, between the presence of DGTS and the taxonomic affiliation cannot be recognized, because in all the orders examined, both DGTS-positive and DGTS-negative species were found. It should be noted, however, that DGTS could be detected in all the species of the order Boletales, while in members of the Russulales, Lycoperdales, Geastrales and Phallales, this lipid could not be detected. In addition, all the fungi analysed contained both PC and PE. These results are in keeping with those of Dembitsky [3], who found DGTS in all the members of the Boletales, but not in those of the Russulales. In the group of Deuteromycetes, DGTS was detected in one species only. From these results it may be concluded that DGTS (but not DGTA) is widespread among, but not a regular constituent of, fungi. Within the Basidiomycetes, many of which are edible mushrooms, DGTS exists in edible, as well as poisonous species, and may definitely not be used as a marker for distinguishing between the two groups.

In lichens, DGTS, as well as PC and PE, were present, confirming the results obtained with 36 different species all of which contained DGTS [3].

Since lichens are symbiotic associations of a mycobiont (fungus) and a phycobiont (green or/and blue-green alga), the question arose, whether only one or both organisms contain DGTS. In lichens containing a green alga as a phycobiont, the alga most probably contributes to the DGTS content, since Chlorophyta in most cases contain this lipid, while many fungal species do not. Lichens containing a cyanobacterium as the only phycobiont have not been analysed.

The analysis of 15 different mosses and 20 ferns most of which have not been analysed before, confirmed the already established rule that all Bryophytes and Pteridophytes produce DGTS, as well as PC and PE. The only exception is *Psilotum* from which DGTS is absent, as reported earlier [17].

Former studies suggested that betaine lipids are absent from flowering plants [17]. It was not clear, however, whether this also holds for the most primitive forms of spermatophytes, which had not systematically been examined. Thus, we chose 14 species belonging to the morphologically least developed groups comprising the Coniferophytina, Cycadophytina and Magnoliophytina. In all of them, however, betaine lipids were absent, strongly suggesting that spermatophytes definitely do not produce this type of membrane lipid, as predicted by former studies [5, 17].

Based on analyses demonstrating that certain algae which produce betaine lipids, the content of PC is low or even zero [20, 21] and *vice versa* [21], a negative correlation between the amounts of betaine lipids and PC was suggested. Such a complementary role would be plausible considering that both type of lipids have a zwitterionic character. In order to obtain evidence for or against such a correlation, the content of both DGTS and PC was estimated in 29 different species of various taxonomic affiliations. Appropriate aliquots of the lipid extracts were separated by one-dimensional TLC and the spots visualized with Dragendorff's followed by molybdenum-blue reagent. Quantification was done by comparing the intensity of the Dragendorff-positive spots with reference spots of the same intensity obtained with known amounts of reference lipids under the same conditions. The results are presented in Fig. 1.

The total amount of the zwitterionic lipids DGTS and PC vary over a broad range from 1 to 22% weight of the total lipid suggesting the tendency that flowering plants contain less than 5% (in the form of PC), while in Rhodophytes, these lipids (mainly PC as well) account for up to 20% of the total. In Pteridophytes, Bryophytes and fungi, almost all of which contain both DGTS and PC, the values vary between 1 and 12%. Within two fungal species, high proportions of DGTS seem to compensate for low amounts of PC and, conversely, in Rhodophytes, high amounts of PC for low amounts of DGTS. The quantity of DGTS, however, is independent of that of PC, indicating that no general quantitative correlation exists between the amounts of the two types of polar lipids.

The occurrence of betaine lipids in plants and fungi may also be considered under chemotaxonomic and phylogenetic aspects. A search within brown algae suggested that the presence or absence of DGTA, which is characteristic of this group of algae, correlates with their taxonomic position at the level of orders [21]. In the order Ectocarpales, the character 'DGTA-positive' is typical of certain genera or even species, since *Ectocarpus fasciculatus* is DGTA-positive, while *E. siliculosus* is DGTA-negative indicating that, in this case, the character 'DGTA' may be used as a reliable taxonomic marker [11]. On the other hand, in green algae of which DGTS is characteristic, this compound is absent from *Chlorella vulgaris* and *C. pyrenoidosa* [17, 22], but present in *C. fusca* [23], suggesting that DGTS is produced only by certain species of the genus *Chlorella*. Interestingly, the DGTS-producing *C. minutissima* [24] has recently been affiliated to the class Eustigmatophyceae [25], indicating some uncertainties in the classification. Betaine lipids might therefore also be used as a taxonomic tool in other parts of the plant kingdom.

The distribution of betaine lipids among organisms suggests a relation between the biosynthetic pathways and the phylogenetic affiliation. Within betaine-lipid producing organisms, two main groups can be distinguished. On the one hand, there are a number of organisms which produce DGTS only and, thus, may represent a 'DGTS branch'. It includes the Chlorophyta (except several species of *Chlorella*) and the phylogenetically related Bryophyta and Pteridophyta, both of which are considered to have evolved from green algae as ancestors and, thus, may have adopted from them the capability to produce DGTS. An exception is the group Psilotopsida consisting of *Psilotum* [17], which lacks DGTS and *Tmesipteris* (not analysed so far), representing the most primitive seedless vascular plants. The absence of DGTS from this group of plants could be explained by the loss of the DGTS pathway either on the level of Psilotopsida themselves or on the level of the extinct group of Psilophytopsida, which are considered to be the ancestors of the Psilotopsida. This in turn could also explain the general absence of betaine lipids from Gymnosperms and Angiosperms, since all the living seed plants are thought to have evolved from the Psilophytopsida [34].

DGTS also appears in the fungal kingdom which have traditionally been grouped with plants, although there is no direct evolutionary connection with plants. As a consequence, it is plausible that lichens consisting of a fungus and a green alga normally containing DGTS, regularly produce this lipid. It is interesting to note that in the Euglenophyta, betaine lipids have not been found so far. This is in accordance with the present view that these organisms evolved from a kinetoplastid ancestor [26], which has been invaded by a basal green alga, presumably showing a strong relationship to a *Chlorella*-like cell [27]. Indeed, in our experiments, betaine lipids were detected neither in

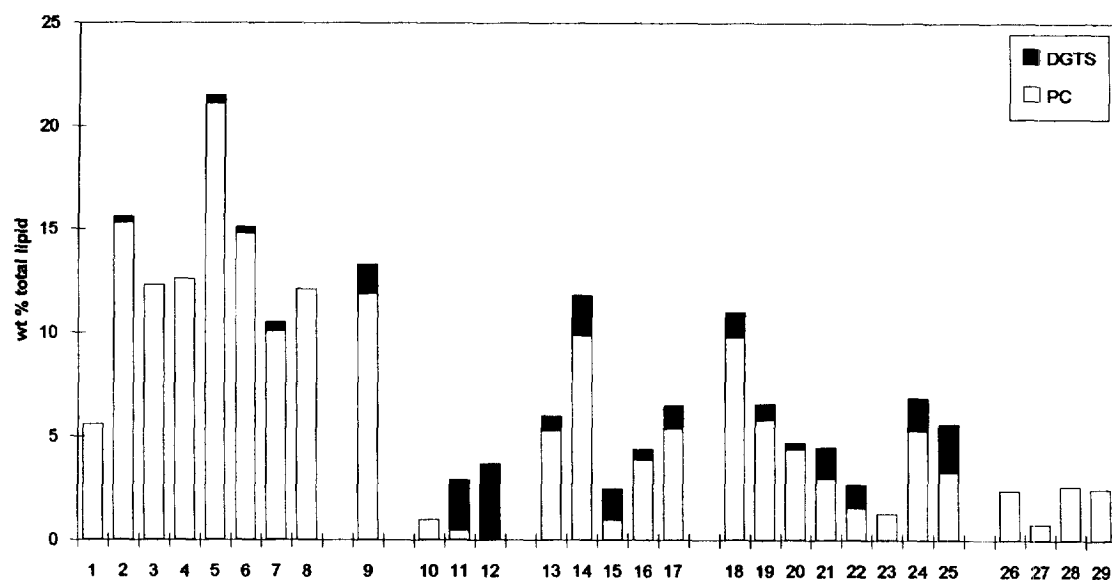


Fig. 1. DGTS and PC content (weight % of total lipid) in different plants and fungi. Values are means of 3 determinations.

Rhodophyta

1. *Asparagopsis armata*
2. *Lomentaria articulata*
3. *Corallina officinalis*
4. *Gelidium latifolium*
5. *Mastocarpus stellatus*
6. *Membranoptera alata*
7. *Phyllophora pseudoceranoides*
8. *Palotoma marginifera*

Lichens

9. *Peltigera canina*

Fungi

10. *Amanita muscaria*
11. *Amanita phalloides*
12. *Postia caesia*
- Bryophyta
13. *Concephalum conicum*
14. *Ctenidium molluscum*
15. *Dicranella heteromolla*
16. *Marchantia polymorpha*
17. *Thuidium tamariscinum*

Pteridophyta

18. *Azolla filiculoides*
19. *Equisetum scirpoides*
20. *Equisetum silvaticum*
21. *Huperzia selago*
22. *Lycopodium annotinum*
23. *Psilotum triquetrum*
24. *Selaginella palessens*
25. *Selaginella willdenowii*

Spermatophyta

26. *Cycas tarwanara*
27. *Magnolia denudata*
28. *Pinus mugo*
29. *Taxus baccata*

the kinetoplasidian organism, *Trypanosoma brucei* (Eichenberger, unpublished results), nor in several species of *Chlorella*.

On the other hand, there are a number of algae producing either DGTA or DGCC and therefore representing the 'DGTA-DGCC branch'. DGTA in combination with DGTS is found in Cryptophytes [28, 29] and in the Chrysophyceae [30], which are part of the Heterokontophyta. Within this division, two other patterns are encountered. In Eustigmatophyceae, DGTS only appears [31], while Phaeophyceae exclusively produce DGTA [11]. Based on these findings one may speculate that the pathway leading to DGTS was the first to appear during evolution and that the capacity of producing DGTA has very likely been acquired in a later stage of development. This is in keeping with the fact that Rhodophytes, which are regarded as a very old taxon [32], in some instances have the ability to synthesize DGTS in small amounts, and with the observation that DGTS acts as the biochemical precursor of DGTA in the Chrysophyceae [8] and Phaeophyceae [33]. The co-existence of DGTS and DTGA in many algae could therefore be explained by a partial conversion of DGTS to DGTA. In

Phaeophyceae which produce DGTA only, the conversion is complete. The regulatory factors which govern the extent of conversion and, hence, the ratio of the two betaine lipids, are not known. In Haptophyta, as well as in Dinophyta and some Bacillariophyceae, DGCC is produced as a third type of betaine lipid, which structurally differs from both DGTS and DGTA by containing an acetal-linked instead of an ether-linked polar group [16]. Some of these organisms, in addition, contain DGTS or DGTA. The biosynthetic route to DGCC is not known yet, nor is there any evidence for a link between the pathways leading to DGTS (or DGTA) and DGCC.

EXPERIMENTAL

Plant material. For most species, field-collected material after careful cleaning was used. For *Synechococcus*, *Gomphonema* and *Rhizopus*, freeze-dried material from laboratory cultures was used.

Lipid analysis. Fresh material (2–5 g) was extracted with at least $\times 10$ the wt of MeOH containing 0.5% butyl hydroxytoluene as an antioxidant. To remove non-lipid constituents, extracts were evapd to dryness

and to the residue Et₂O and satd NaCl soln were added. This led to a two-phase system with the lipids dissolved in the Et₂O phase, which was dried under N₂. Lipids (1 mg) were spotted on silica gel plates 60F₂₅₄ (Merck, 1.05715) and sepd with CHCl₃-MeOH-H₂O (65:25:4) (solvent I) in the 1st dimension and with CHCl₃-MeOH-*iso*PrNH₂-conc. NH₃ (65:35:0.5:5) (solvent II) in the 2nd dimension. Spots were visualized under UV 254 nm and 366 nm after spraying with 2',7'-dichlorofluorescein (DCF) (0.05% in EtOH). For the detection of betaine lipids, the plate was sprayed next with Dragendorff's reagent [12]. DGTS, DGTA and PC gave an orange colour which was intensified by spraying the plate thereafter with molybdenum-blue reagent [13], which made the phospholipids appear as blue spots. In order to determine the smallest detectable amount of DGTS, a reference soln of DGTS was used. Different quantities in the range of 0.1 µg to 10 µg were spotted on a silica gel plate which was developed in solvent I and then sprayed with Dragendorff's and molybdenum-blue reagents. For obtaining a clearly visible spot, 0.5 µg DGTS was needed. The same value also holds true for DGTA, as found by using the corresponding ref. solns.

Estimation of DGTS and PC. Of the lipid extract to be analysed, two different aliquots were spotted on a silica gel plate. In addition, four different but known amounts of DGTS were loaded on the same plate which subsequently was developed in solvent I. After covering the lower half of the chromatogram with a glass plate, the upper half was sprayed with Dragendorff's and molybdenum-blue reagents. The amount of DGTS in the lipid extract was estimated by finding the spots of the same colour intensity and size obtained in the analytical and the ref. lanes. For the determination of PC, the lower half of the plate was developed in Me₂CO-toluene-MeOH-H₂O (16:5.5:4.5:2) and sprayed with DCF. In the spot containing PC, phosphorus was determined colourimetrically according to ref. [36].

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