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LIPIDS OF THE MARINE RED ALGAE, *CHONDRUS CRISPUS* AND *POLYSIPHONIA LANOSA*

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Key Word Index—*Chondrus crispus*; *Polysiphonia lanosa*; Rhodophyceae; red algae; lipids; fatty acids.

Abstract—The marine red algae, *Chondrus crispus* and *Polysiphonia lanosa*, were harvested from the wild and their lipids isolated, characterized and quantified. Double banding of monoglycosyldiacylglycerol, diglycosyldiacylglycerol and sulphoquinovosyldiacylglycerol was observed on TLC, probably caused by the presence of different molecular species. The sulphur analogue of phosphatidylcholine was provisionally identified in both algae. Over 20 fatty acids were observed in each alga, with the main ones being palmitic, oleic, arachidonic and eicosapentaenoic acids. The fatty acid compositions of the acyl lipids were determined. *trans*-Δ^3-Hexadecenoic acid was found exclusively in phosphatidylglycerol. The three glycosylglycerides (monoglycosyl-, diglycosyl- and sulphoquinovosyldiacylglycerol) were major components, with phosphatidylglycerol the main phosphoglyceride in both algae.

INTRODUCTION*

Marine algae are a very large, yet poorly studied part of the plant kingdom. They show a remarkable morphological diversity, ranging from unicellular, flagellate green swimming cells to the giant brown kelps of Antartica which can reach up to 70 m in length. Very little is known about their lipid composition and metabolism. The literature has been summarized up to 1979 in terms of the fatty acids and lipids of marine algae and how the environment influences their endogenous levels [1]. More recent work on brown algae [2–5] and on red algae [6–11] has helped to increase this data base.

The Rhodophyceae or red algae constitute a large group of mostly macroscopic marine plants which are probably the oldest division of eukaryotic photosynthetic organisms as indicated by their chloroplast structure and ancient fossil history [12]. They typically have high levels of palmitic, oleic, arachidonic and eicosapentaenoic fatty acids together with minor amounts of many other acids [1, 13]. The 22C and 24C acids, if present, are only found in trace amounts. There have been no comprehensive studies on the acyl lipids of these algae, unlike higher plants where a large amount of work has been done [c.f. 14, 15], although the fatty acid compositions of monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and 'polar lipids' from *Corallina officinalis*, *Ceramium rubrum* and *Chondrus crispus* have been examined [13]. The presence of MGDG, DGDG, sulphoquinovosyldiacylglycerol (SQDG), phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), diphosphatidylglycerol (DPG) and triacylglycerol (TAG) have been reported in *Porphyridium purpureum* [9, 16]. The fatty acid compositions of some of the lipids from *P. purpureum* and *Porphyra yezoensis* have also been analysed [6–8, 10] while a study of *Phycodrys sinuosa* showed the main lipids to be MGDG, DGDG, SQDG, PC, PG, TAG and non-esterified fatty acids [1].

The determination of algal lipid composition in a given species is an essential foundation on which further study of subjects such as lipid metabolism, subcellular lipid distribution and the effect of environmental factors can be based. This paper provides such a background and describes the isolation, characterisation and quantification of the lipids and fatty acids from two species of marine red algae, *Chondrus crispus* and *Polysiphonia lanosa*.

RESULTS AND DISCUSSION

Prior to determination of the lipid composition of *C. crispus* and *P. lanosa*, a suitable extraction method giving efficient and reproducible lipid recovery had to be established. Five methods described in the Experimental were tested and the total amount of acyl lipid (estimated by fatty acid analysis) in each was found. Because of the known activity of endogenous lipases in plant tissue, some of the methods contained an extraction stage including *iso*-propanol [see 15]. However, a comparison of the acyl lipid patterns for the various methods did not reveal any evidence of lipase-catalysed lipid breakdown (data not shown). A comparison of the fatty acid distribution and total fatty acid extracted from *P. lanosa* by each of these methods is given in Table 1. *Chondrus crispus* showed similar results although method 5 was less efficient for this alga. With the exception of method 3, which showed a significant loss of polyenoates (especially eicosapentaenoate), the methods were reasonably comparable with regard to the pattern of acids extracted. As method 2 gave

*Abbreviations: MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; SQDG, sulphoquinovosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PA, phosphatidic acid; DPG, diphosphatidylglycerol; TAG, triacylglycerol; ANSA, 8-anilino-1-naphthalene sulphonic acid.

Table 1. *Polysiphonia lanosa* lipids extracted using different methods

Fatty acid	Percentage fatty acid composition using method 1	2	3	4	5
14:0	2.8±0.5	0.6±0.3	3.0±1.2	0.6±0.7	3.9±0.7
16:0	31.9±4.3	32.7±1.7	38.1±1.7	27.3±2.9	27.2±1.4
16:1	6.3±2.3	5.6±0.5	7.5±1.4	4.0±1.6	3.4±0.7
16:2	5.1±2.5	1.9±0.6	5.6±3.8	3.1±1.7	2.0±0.5
18:0	1.0±0.7	0.7±0.2	1.0±0.2	1.8±0.2	0.7±0.1
18:1 (*n*-9)	13.0±2.9	11.1±0.7	14.9±1.7	14.8±1.0	10.8±0.2
18:2 (*n*-6)	5.0±0.7	6.9±0.5	4.9±1.6	6.3±1.2	6.5±0.3
18:3	2.3±0.7	2.4±0.5	2.8±1.2	4.1±1.0	2.7±0.3
18:4 (*n*-3)	0.4±0.1	0.2±0.1	0.4±0.3	0.8±0.2	0.2±0.1
20:4 (n-6)	9.3±1.9	10.0±1.0	7.0±0.8	10.3±2.1	10.8±0.2
20:5 (*n*-3)	20.0±1.9	25.9±4.0	12.9±1.0	25.5±3.6	30.3±1.6
Others	2.9±1.6	2.0±1.4	1.9±0.7	1.4±0.5	1.5±0.5
Mass	3.5±1.0	9.8±1.0	6.5±0.5	4.8±0.5	9.2±1.7

Values are means ±s.d. ($n=3$) for material collected in October. Mass in mg fatty acid/g dry wt. 16:1, 16:2 and 18:3 include all isomers. See Experimental for a description of methods 1 to 5.

Fatty acids are shown with the number before the colon representing the carbon chain length and the figure afterwards representing the number of double bonds. Figures in parentheses show the position of the first double bond from the methyl end of the carbon chain.

Table 2. Acyl lipid composition of *Chondrus crispus* and *Polysiphonia lanosa*

Lipid	*Chondrus crispus* μmol lipid /g dry wt	% acyl lipid (wt/wt)	*Polysiphonia lanosa* μmol lipid /g dry wt	% acyl lipid (wt/wt)
MGlyDG1	1.7±0.2	5.6±0.3	1.2±0.2	5.0±0.7
MGlyDG2	3.4±1.7	11.3±4.5	3.0±0.3	12.4±1.3
DGlyDG1	3.7±1.0	14.8±3.3	4.1±0.3	20.5±1.7
DGlyDG2			1.7±1.2	8.5±5.9
SQDG1	1.3±0.3	4.7±1.0	2.8±0.8	12.5±3.8
SQDG2	3.0±0.6	10.8±1.9		
PC+PSC	9.1±1.5	30.1±4.0	4.3±1.2	17.9±5.0
PG	2.4±0.3	7.7±0.9	1.2±0.1	4.9±0.3
PE	0.5±tr	1.5±0.2	0.3±tr	1.2±0.3
PA+DPG	0.4±0.1	1.9±0.5	0.2±0.1	1.1±0.9
TAG	0.9±0.2	3.4±0.3	1.1±0.8	5.2±3.9
FFA	1.8±0.2	2.2±0.2	3.9±0.9	5.8±1.1
EST	0.2±tr	0.6±0.2	0.5±0.2	1.8±0.4
Others	1.7±0.2	5.4±0.7	0.8±0.2	3.2±0.7

Values are means ±s.d. ($n=3$–7) for material collected from January to March. μmol lipid/g dry wt. and % acyl lipid (wt/wt) calculated from fatty acid data by assuming that only 18:0 chains are present and that each lipid has 2 acyl chains expect for TAG (3), DPG (4), FFA (1), EST (1), and Others (1) since these are mostly X lipids which have been assumed to be sphingolipids. tr = trace (<0.05%).

MGlyDG = monoglycosyldiacylglycerol, DGlyDG = diglycosyldiacylglycerol, SQDG = sulphoquinovosyldiacylglycerol, X = unidentified lipids (probably glycosphingolipids), PC = phosphatidylcholine, PSC = phosphatidylsulphocholine, PG = phosphatidylglycerol, PE = phosphatidylethanolamine, PA = phosphatidic acid, DPG = diphosphatidylglycerol, TAG = triacylglycerol, FFA = free (unesterified) fatty acid, EST = esterified sterol. Lipids designated 2 have a slightly higher mobility than the corresponding lipid designated 1.

the best and most reproducible extraction (as measured by total fatty acid recovery), it was chosen for routine use. It should also be mentioned that the lipid quantitations were performed on wild material. We did not take any special steps to remove microbial contamination of the macroalgae apart from extensive washing with sterilized seawater because the relative amounts of microbes were so small. However, for radio-isotope labelling experiments detergent washing was used (see Experimental).

The total material soluble in organic solvent (which included non-acyl lipids and pigments) for *C. crispus* and *P. lanosa* collected in December (means±s.d. where $n=3$) was found to be 59±4 mg/g dry wt and 70±6 mg/g dry wt respectively.

An initial separation into neutral, glycolipid and phospholipid fractions on acid-washed Florisil columns was found to give a better resolution of the component lipids on TLC. Minimal cross-contamination of the fractions was found.

One-dimensional TLC separation of the polar lipids revealed the presence of PC and PG, which appeared to be the major phospholipids, together with small amounts of PE and traces of DPG and phosphatidic acid (PA). No PI or phosphatidylserine was detected in either alga. The PC spot sometimes appeared to partially separate into two bands although both stained appropriately with Dragendorff reagent [17].

[^{32}P]-Labelling confirmed the presence of PC, PG, PE, PA and DPG together with some very minor phospholipids (not detected by the phosphate stain) in the diglycosyldiacylglycerol, monoglycosyldiacylglycerol and X (unidentified lipid) regions. No attempt was made at structural determination for these lipids.

The major glycolipids appeared to be monoglycosyldiacylglycerol, diglycosyldiacylglycerol and SQDG (Table 2) along with some minor glycolipids X1, X2 and X3. The monoglycosyldiacylglycerol resolved into two bands in both algae, while diglycosyldiacylglycerol and SQDG were often seen to partially resolve into double bands. The *C. crispus* diglycosyldiacylglycerol and *P. lanosa* SQDG double bands did not normally resolve well on one-dimensional TLC so are given as single bands in the tables. The faster moving species on TLC were designated 2.

Since the double bands stained the same as the authentic standards (SQDG bands stained red with α-naphthol reagent whereas the other glycolipids stained purple) and the TLC mobilities remained very similar in a variety of solvent systems, this raised the possibility that the double banding was due to the presence of different molecular species or sugar moieties which slightly altered lipid mobility. Support for the latter came from the discovery of double banding of monoglycosyldiacylglycerol in over 18 genera of cyanobacteria (although not in any of the brown, green or red algae examined [18]. This was caused by the presence of β-monoglucosyldiacylglycerol (also found in rice bran [19]) together with the more usual MGDG (lower mobility).

GLC analysis of the sugars in these glycolipids revealed galactose as the major component of both monoglycosyldiacylglycerol and diglycosyldiacylglycerol. Monoglycosyldiacylglycerol also contained *ca* 30% glucose (plus 5% mannose in the faster moving band) while diglycosyldiacylglycerol contained *ca* 10% glucose. However, as the sugar ratios of the double bands for each glycolipid were relatively similar, the separation of the monoglycosyldiacylglycerol and diglycosyldiacylglycerol double bands was probably not due to different sugar residues. In contrast, differing degrees of acyl chain saturation may have caused the double-banding. Thus, the more unsaturated molecular species always appeared to run faster (see Tables 3 and 4 for fatty acyl composition of the double bands) as we have found for other lipids on prepared TLC plates and has been reported for *Porphyra yezoensis* MGDG [10].

The band corresponding to SQDG was separated and the lipid examined further. Analysis of the sugar content confirmed that the acid stable sulphoquinovose was the major constituent but small amounts of galactose, glucose and mannose were also found by GLC. In addition, a small percentage of the total ^{35}S-radioactivity (from a [^{35}S]-sulphate labelling experiment) was labile to acid and alkaline hydrolysis providing evidence for a sulphate group in addition to the sulphonate group of SQDG. Double banding of SQDG was usually found on the TLC plates, with both bands staining a similar red colour with α-naphthol reagent. It is possible that one of the bands represented sulphated glycolipid. Double banding of SQDG has been found in the diatom *Nitzschia alba* [20] although the cause was not established. A range of novel glycolipids have also been found in brown algae, containing different sugar residues which in some cases were also sulphated or phosphorylated [21–23], so the presence of some of these rather more unusual glycolipids in red algae was not totally unexpected.

[^{35}S]-Labelling also detected four other minor sulpholipids, besides the double SQDG band, in *C. crispus* (Fig. 1). These were found in the region of diglycosyldiacylglycerol, monoglycosyldiacylglycerol, PC and PG. The diglycosyldiacylglycerol, monoglycosyldiacylglycerol and PG bands were only weakly labelled, although this appeared to vary at different times of the year. Labelling of *P. lanosa* lipids was more variable with only the two SQDG bands and a lipid in the monoglycosyldia-

Table 3. Fatty acids of the major *Chondrus crispus* acyl lipids

	Fatty acid composition (% total acids)															
Acyl lipid	14:0	16:0	16:1 (*n*-7)	16:2	18:0	18:1 (*n*-9)	18:2 (*n*-6)	18:3 (*n*-6)	18:3 (*n*-3)	18:4 (*n*-3)	20:2 (*n*-6)	20:3 (*n*-6)	20:4 (*n*-6)	20:5 (*n*-3)	Others	S.I.
MGlyDGl	0.3	25.7	0.4	nd	5.0	11.3	2.5	0.2	8.6	4.0	0.2	0.1	11.0	24.7	5.3	2.3
MGlyDG2	0.1	3.7	0.1	0.1	0.6	2.5	0.7	0.1	0.1	0.7	0.1	nd	22.9	67.6	0.4	4.4
DGlyDG	0.5	31.2	1.0	0.2	1.2	10.8	1.7	0.1	1.2	0.6	0.2	0.1	11.1	38.3	0.7	2.6
SQDGl	9.7	39.8	0.5	tr	1.6	4.9	1.4	0.4	1.4	0.2	0.3	nd	23.1	15.1	0.4	1.9
SQDG2	3.9	43.5	0.5	tr	0.7	2.2	0.7	0.2	1.6	0.5	0.2	nd	15.4	29.7	0.5	2.2
PC + PSC	0.3	11.0	0.9	0.1	1.1	5.4	1.5	2.2	0.3	tr	0.3	0.3	55.1	19.5	2.0	3.4
PG	0.2	25.1	*10.9	0.2	0.8	7.5	0.8	0.6	3.6	0.4	0.2	0.3	23.3	25.1	1.0	2.6
PE	1.2	17.5	5.3	0.1	3.9	16.6	4.5	2.8	3.0	nd	0.8	1.3	23.7	10.5	8.8	2.1
TAG	0.9	15.3	13.3	nd	3.0	12.5	3.0	0.8	1.3	0.7	0.6	0.5	27.4	17.6	2.8	2.4
FFA	3.6	33.6	1.3	1.8	16.4	18.6	2.2	1.1	0.5	nd	1.1	nd	3.4	4.7	11.7	0.7

Results are means of 3–7 samples collected from January to March. S.I. = saturation index (average number of double bonds per acyl chain), tr = trace (< 0.05%), nd = not detected, * = *trans*-Δ^3-hexadecenoic acid (16:1 *trans n*-13). Hexadecatetraenoic acid (16:4*n*-3) was present in small amounts especially in MGlyDG2. See Tables 1 and 5 for fatty acid and lipid abbreviations.

Table 4. Fatty acids of the major *Polysiphonia lanosa* acyl lipids

	Fatty acid composition (% total acids)															
Acyl lipids	14:0	16:0	16:1 (*n*-7)	16:2	18:0	18:1 (*n*-9)	18:2 (*n*-6)	18:3 (*n*-6)	18:3 (*n*-3)	18:4 (*n*-3)	20:2 (*n*-6)	20:3 (*n*-6)	20:4 (*n*-6)	20:5 (*n*-3)	Others	S.I.
MGlyDGl	0.2	27.4	3.4	2.6	0.8	4.3	3.9	0.5	1.0	1.9	0.7	1.0	4.9	46.5	0.9	2.9
MGlyDG2	tr	2.0	0.3	0.1	0.3	1.0	0.9	0.1	tr	0.2	0.4	0.4	13.4	80.3	0.6	4.6
DGlyDG1	1.1	30.4	7.4	0.6	0.5	9.8	5.3	0.3	0.3	0.4	0.5	0.9	4.7	37.4	0.4	2.4
DGlyDG2	0.1	24.2	4.9	0.2	1.8	4.7	2.7	1.4	0.3	1.7	1.0	0.5	6.9	47.8	1.8	3.0
SQDG	3.5	28.5	10.8	1.3	0.7	15.2	3.4	0.5	0.3	0.5	0.9	0.2	6.3	27.2	0.7	2.0
PC + PSC	0.2	4.5	1.5	0.2	1.2	19.0	2.7	2.5	tr	0.8	2.1	2.3	16.8	45.2	1.0	3.4
PG	1.7	15.4	*12.1	2.0	6.8	17.1	0.8	2.9	1.8	7.4	5.8	0.9	2.4	19.0	3.9	2.0
PE	3.0	6.8	8.9	2.7	1.1	21.6	0.9	6.2	nd	9.6	13.7	5.4	3.3	12.3	4.5	2.2
TAG	0.5	11.5	8.9	0.3	2.8	14.0	5.7	0.1	1.4	4.4	nd	2.2	12.0	34.6	1.6	2.9
FFA	3.3	30.1	4.2	0.5	13.1	31.3	2.9	0.6	0.7	4.7	0.6	2.0	0.7	1.8	3.5	0.9

Results are means of 3–7 samples collected from January to March. See Table 3 for explanations.

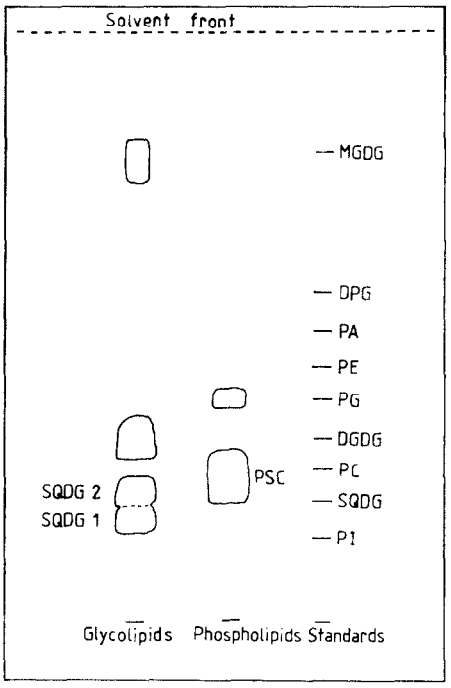

Fig. 1. Autoradiography of [^{35}S]-labelled lipids from *Chondrus crispus* after separation by one-dimensional TLC. *Chondrus crispus* glycolipid and phospholipid fractions obtained by separation on an acid-washed Florisil column, were run on one-dimensional TLC (silica gel 60 plate) in chloroform–methanol–acetic acid–water (170:30:20:7 by vol.) at 25°. The dried TLC plate was exposed to X-ray film for one week. The relative mobilities of authentic standards are indicated. Unlabelled lipids from *C. crispus* are not shown. Three [^{35}S]-labelled lipids were characterized as SQDGl, SQDG2 and phosphatidylsulphocholine. Three other sulpholipids in the region of DGDG, MGDG and PG remained unidentified.

cylglycerol region being labelled consistently, although only weakly for the latter. Labelling of material obtained in January detected three further sulpholipids in the PC, PA and DPG regions although the latter two were very faint.

Running the [^{35}S]-labelled lipid from the monoglycosyldiacylglycerol region on two-dimensional TLC caused it to separate from the true monoglycosyldiacylglycerol. It did not stain for sugars, sterol, phosphate, choline or amino groups. Too little material was available for a structural determination, however, its mobility indicated that it may have been a S-containing hydrocarbon such as has been detected in some plants [24, 25].

The [^{35}S]-labelled lipid from the diglycosyldiacylglycerol region released free sulphate after mild acid hydrolysis suggesting that it may have been a sulphated glycosphingolipid (on the basis of its mobility). Again there was too little material for a structural determination.

A number of different TLC solvent systems were used in an attempt to resolve the [^{35}S]-labelled lipid from PC. No complete separation could be achieved, indicating that this lipid had very similar properties to PC. Mild alkali hydrolysis of the PC band followed by separation of the aqueous products revealed two separate choline positive spots, with mobilities corresponding to those obtained from the hydrolysis of authentic PC and phosphatidylsulphocholine standards. The slower choline-positive spot also stained phosphate positive as did that of authentic PC indicating that phosphorylcholine was present. Autoradiography of the hydrolysis products from [^{35}S]-labelled lipid revealed the presence of label in the faster moving choline positive spot which corresponded to methoxysulphocholine, a hydrolysis product of phosphatidylsulphocholine, on TLC [20]. Thus the presence of phosphatidylsulphocholine, with a sulphur atom replacing the nitrogen of the choline moiety, together with PC was provisionally identified in both red algae. This sulphur analogue has been found in a number of diatoms including one where it completely replaced PC [20, 26, 27], in the euglenoid *Euglena gracilis* (very low amounts [26]) and in some crustaceans, usually in low amounts (Kates, M., personal communication). The properties of the two analogues are very similar and their roles appear interchangeable [27].

The two minor glycolipids found in *C. crispus* (X_1 and X_2) and the one in *P. lanosa* (X_3) were analysed further. Staining with α-naphthol and sulphuric acid and resistance to alkaline hydrolysis indicated that they may have been glycosphingolipids. Hydrolysis with 2 M HCl for 48 hr at 125° allowed the release of products which were ninhydrin-positive and had mobilities on TLC similar to sphingosine [39]. Retention distances of the different intact lipids were similar to cerebrosides and ceramide polyhexosides. However, they were present in such small amounts that proper identification was not possible.

Quantification of the individual acyl lipids gave results as shown in Table 2. Quantitative analysis was routinely carried out by GLC of derived fatty acids but the data were confirmed, as appropriate, by phosphate, sterol or sugar determinations. These methods showed good agreement. For example, the relative amounts of phospholipids estimated by fatty acid or phosphate determinations being within 10%. The glycolipids represented about 50% by weight of the total acyl lipid present in both algae, which is comparable to plant photosynthetic tissues [14]. PC (+phosphatidylsulphocholine) and PG were the major phospholipids with only small amounts of PE and traces of PA and DPG. As mentioned above, PC and phosphatidylsulphocholine were never fully resolved and, therefore, are combined in Table 2. The monoglycosyldiacylglycerol content was similar in both algae. *Polysiphonia lanosa* has more diglycosyldiacylglycerol than *C. crispus* but the latter has more PC (+phosphatidylsulphocholine), PG and PE on a dry weight basis.

The PE levels in *C. crispus* and *P. lanosa* were lower than those found in the photosynthetic tissues of most terrestrial plants and much lower than in non-photosynthetic tissues [15]. Similar observations have been made in the red algae, *Porphyridium purpureum* [7, 16] and in *Porphyra yezoensis* [10] where only small amounts of phospholipids other than PC and PG were found. The decreased PC (+phosphatidylsulphocholine) levels in *P. lanosa* during the winter [28] is in contrast to the increased levels of PC and PE found in some higher plants on frost hardening [29].

In plants PG levels are probably most influenced by light availability [30, 31] since this lipid is almost entirely located in the chloroplasts, although minor amounts have been found in other organelles such as the mitochondria [15]. This being the case, increased PG levels

may be expected during the summer with greater light availability for red algal growth. However, *P. lanosa* showed the opposite effect with decreasing levels in the summer [c.f. 28], possibly caused by the influence of other seasonally fluctuating environmental factors such as nutrient availability.

Fatty acids were separated and identified by using GLC on different packed or capilliary columns, argentation-TLC followed by GLC and by GLC-mass spectrometry. Quantification of the total fatty acids from *C. crispus* or *P. lanosa* revealed a pattern similar to that found in other red algae [1] with the major forms being palmitic, oleic, arachidonic and eicosapentaenoic acids which together represented more than 80% of the total fatty acid complement in both algae (Table 5). The exact amount varied slightly depending on the season [c.f. 28] with relatively more palmitate being found when the water temperatures were higher. *Chondrus crispus* appeared to react less to growth temperature than *P. lanosa* (Table 5) perhaps because of its habitat on the shore where it was less prone to dessication. Only small amounts of 22C, 24C and polyenoic 16C acids were detected.

Chondrus crispus had slightly more arachidonate than eicosapentaenoate (quantitatively the most important acids found in this study) whereas other workers [13] found nearly twice as much eicosapentaenoate as arachidonate in this alga. Environmental differences such as temperature and pollution may account for this since the algal collection point at Sully is polluted with heavy metals (raised levels of Cd, Cu, Pb, Mn, Ni, Zn and Fe) which have been shown to reduce polyunsaturated fatty acid formation in another marine alga, *Fucus serratus* [3, 4].

The large number of different fatty acids detected (>20 in each alga) is in contrast to higher plant leaves which generally have a more limited composition [14] with no very long chain (>18C) polyunsaturated fatty acids. The large amounts of 20C polyenoates and low amount of linolenate found in the Rhodophyceae [1] differs from most terrestrial plants (apart from some of the lower orders such as the lichens, mosses and ferns [32–34], where polyenoic 20C acids are rare and linolenate is a major component [15].

Table 5. Changes in the major fatty acid composition of *Chondrus crispus* and *Polysiphonia lanosa* collected in different seasons

Fatty acid	*Polysiphonia lanosa* January (5°)	*Polysiphonia lanosa* August (18°)	*Chondrus crispus* February (5°)	*Chondrus crispus* October (14°)
14:0	2.1±0.2	1.3±0.2	2.4±0.4	1.4±0.8
16:0	15.7±5.9	32.4±1.5	20.9±4.7	26.2±8.5
16:1	6.0±1.2	5.3±0.3	3.4±1.1	0.4±0.3
18:0	1.6±0.3	1.0±0.1	2.9±0.4	1.0±0.4
18:1 (*n*-9)	14.0±1.0	14.3±0.8	8.0±0.7	10.2±1.7
18:2 (*n*-6)	5.4±0.5	5.5±0.3	1.9±0.4	1.0±0.4
18:3 (*n*-6)	0.6±0.1	0.2±0.1	0.5±0.1	0.3±0.1
18:3 (*n*-3)	tr	0.4±0.1	1.5±0.7	0.9±0.4
18:4 (*n*-3)	0.5±tr	0.2±tr	1.1±0.2	0.5±0.1
20:4 (*n*-6)	12.1±2.4	8.7±0.1	27.5±2.7	33.8±4.2
20:5 (*n*-3)	38.8±6.9	28.8±1.2	25.9±3.1	23.9±7.2
Others	3.2±0.5	1.9±0.4	4.0±0.6	0.5±0.2
Mass	19.9±1.6	22.1±2.0	21.4±1.8	15.0±5.8
S.I.	2.8	2.2	2.7	2.7
Samples	5	18	3	3

Values are means ±s.d. Samples = number of samples. Values in parentheses are mean monthly sea temperatures. Mass = mg fatty acid/g dry wt., S.I. = saturation index (average number of double bonds per acyl chain), tr = trace (<0.05%). 16:1 includes all isomers.

It has been suggested that the 20C polyunsaturated fatty acids may participate in chlorophyll stabilization [35], and it is noteworthy that they replace linolenate in the glycolipids of marine algae and lower plants such as ferns. Moreover, eicosapentaenoate and most other *cis* polyunsaturated acids have antibacterial activity [36] and, thus, may be important in protection from microbial attack, especially after wave damage.

Analysis of the fatty acid composition of individual lipids revealed a distribution for the major acyl lipids as shown in Tables 3 and 4. Only small amounts of 16C and 18C polyunsaturated fatty acids were found, leaving arachidonate and eicosapentaenoate as the major polyenoates in every lipid class. These 20C polyenoates, especially eicosapentaenoate, were particularly high in monoglycosyldiacylglycerol, diglycosyldiacylglycerol and PC (+phosphatidylsulphocholine). The highest degree of unsaturation was found in the fastest monoglycosyldiacylglycerol band (monoglycosyldiacylglycerol-2) in both algae, where arachidonate and eicosapentaenoate together represented over 90% by weight of the total fatty acid complement in the winter, although this declined during the warmer summer months [c.f. 28]. A similar observation has been made in *Porphyra yezoensis* [10]. Monoglycosyldiacylglycerol-1 contained much more saturated acids and oleate than monoglycosyldiacylglycerol-2. PC (+phosphatidylsulphocholine) were the next most highly unsaturated lipids with *ca* 65% as arachidonate and eicosapentaenoate. Non-esterified fatty acid was the least unsaturated component. SQDG was the most saturated glycoglycerolipid being particularly rich in palmitate, in agreement with observations for higher plants [c.f. 15, 24]. The most saturated phospholipid was PE (saturation index = 2.1) in *C. crispus* but PG also contained relatively high levels of palmitate. In *P. lanosa* the saturation index was similar for both lipids.

trans-Δ^3-Hexadecenoic acid was found exclusively in PG bringing these red algae into line with the majority of photosynthetic plants so far investigated. This acid has been suggested to be involved in the maintenance of functional light harvesting assemblies and efficient light harvesting [37], although orchids and some plant mutants manage without it [38]. Other aspects of the function of PG and thylakoid lipids in general have been discussed [39].

Because there has been so little analysis of marine algal lipids it is not possible to make many comparisons with other published results. However, a few comments can be made. The high degree of monoglycosyldiacylglycerol unsaturation found in *C. crispus* and *P. lanosa* is typical for this lipid in algae [1, 11, 13] and higher plants [15]. It is the most highly unsaturated lipid in the majority of plant systems (an exception being DGDG from *Porphyridium purpureum* which is more unsaturated [8]). The high unsaturation of monoglycosyldiacylglycerol may have significance with regard to the physical properties of the lipid and its function in the chloroplast thylakoid [39]. In general, the fatty acid compositions we obtained for

monoglycosyl- and diglycosyldiacylglycerol agreed very well with those previously reported for *C. crispus* [13].

The PC (+ phosphatidylsulphocholine) unsaturation in both *C. crispus* and *P. lanosa* is somewhat higher than the PC unsaturation found in the unicellular alga *Porphyridium purpureum* [8]. In the latter species, PC was found to contain more palmitate and less 20C polyenoates, although it was still one of the most highly unsaturated lipids. However, arachidonate and eicosapentaenoate together still represented about 50% of the fatty acid in PC from *Porphyridium cruentum* [11] and *Porphyridium purpureum* [8] and, moreover, the latter was cultured at 25°, a temperature which would be expected to increase the relative saturation of endogenous lipids. In the brown alga *F. serratus* arachidonate and eicosapentaenoate represented about 80% of the complement in PC [4]. Bearing in mind the differences in growth temperatures for the algae used in the present work and *P. purpureum* cultured in the laboratory [6–8], the fatty acyl compositions of PE and PG are quite similar in the two studies.

In conclusion *C. crispus* and *P. lanosa* show a complex lipid pattern with most of the structures found in higher plants, together with a range of structural analogues such as the glycolipids containing different sugar moieties and phosphatidylsulphocholine. Further work is required to fully identify and characterize the minor lipids present and to see if other multicellular red algae have similarly complex lipid patterns. However one thing is clear, even though red algae may be the direct descendants of the oldest eukaryotic cells, their lipid and fatty acid compositions are at least as complex, if not more so, than 'higher' terrestrial plants.

EXPERIMENTAL

Fatty acid standards were obtained from Nu-Check Prep. Inc. (Elysian, MN 56028, U.S.A.) and lipid standards from Sigma London Chem. Co. Ltd (Poole, BH17 7NH, U.K.) or isolated from leaf tissue [15].

Chondrus crispus and *Polysiphonia lanosa* (an obligate epiphyte on the brown alga *Ascophyllum nodosum*) were harvested at Sully (G. R. ST167673) on the South Wales coast at low tide. Algae were kept in tanks of filtered seawater at 4° under illumination of *ca* 200 $\mu Em^{-2} sec^{-1}$. (white fluorescent tubes) with a photo-period corresponding to prevailing day length and aerated by a compressed air line. They were washed thoroughly with sterilized seawater and analysed for lipid composition within 2 days of collection.

Extraction of lipids. The following methods were tested as to their suitability: (1) 5 ml boiling *iso*-PrOH was added to weighed, fresh tissue in a sealable tube, sealed and heated at 70° for 30 min to inactive any lipid degrading enzymes [see 15]. After cooling the tissue was ground up with a small amount of acid-washed sand (40–100 mesh) using a mortar and pestle. The homogenate was transferred to a screw-capped tube to which was added 5 ml $CHCl_3$, 2 ml H_2O and 2.5 ml 2 M KCl in 0.5 M K-Pi buffer (pH 7.4) (as [40] except *iso*-PrOH replaced MeOH). The solution was well mixed and then left to separate into a lower organic phase containing the lipids and an upper aq. phase. The aq. phase was removed and the organic phase washed with 4 ml synthetic upper phase (made using the same proportions of components as above but without the tissue). The organic phase was filtered through glass wool and the particulate fraction washed with 3 ml $CHCl_3$–MeOH (2:1). The washings were added to the organic phase and then taken to dryness under a stream of nitrogen ready for transmethylation and GC analysis.

(2) As in method 1 except the tissue was ground up in cold *iso*-PrOH before heating at 70° for 30 min. Once the solution had cooled, the other components were added to extract the lipid.

(3) Tissues were ground with acid-washed sand in 7.5 ml $CHCl_3$–MeOH (1:2). The soln was left at room temp. for 1 hr in tube. 2.5 ml $CHCl_3$, 2 ml H_2O and 2.5 ml 2 M KCl in 0.5 M K-Pi buffer (pH 7.4) were added and the solution mixed well and left to separate into an organic lower and aqueous upper phase. Then as in method (1).

(4) Tissues were ground with acid-washed sand in 2 ml MeOH. 4 ml $CHCl_3$ were added with further grinding. The solution was then filtered and the residue ground in 6 ml $CHCl_3$–MeOH (2:1). The solution was filtered again and the residues washed with 4 ml $CHCl_3$ and 2 ml MeOH. The filtrates and washings were combined and 0.88% KCl added to give a ratio of organics to KCl solution of 4:1. The soln was mixed well and allowed to separate. The upper aq. phase was removed and the lower phase washed with MeOH–H_2O (1:1). The organic phase was taken to dryness under N_2 before prepn of fatty acid methyl esters for GC.

(5) Tissues were ground with acid-washed sand in 3 ml MeOH. Most of the MeOH was poured off before grinding with a further 2 ml MeOH. The fractions were combined and 10 ml $CHCl_3$ and 4 ml H_2O added. After shaking, 2 drops 1 M HCl were added to the upper aq. phase and the soln allowed to separate. Then filtration of the lower phase and washing of the particulate fraction as in method 1.

Method 2 was used as the standard procedure (see Results and Discussion). The organic phase containing the lipids was stored under nitrogen until required.

Lipid separation. Total lipids were initially separated into neutral, glyco- and phospholipid fractions on an acid-washed Florosil column (60–100 mesh) by sequential elution with 15 column vol. $CHCl_3$, 35 vol. Me_2CO and 15 vol. MeOH [41]. The fractions were sepd into their component lipids by 1D TLC on ready-prepared silica gel 60 plates (E. Merck, Darmstadt, F.R.G.) with petrol (b.r. 60–80°C)–Et_2O–HOAc (40:10:1, at 4°) for neutral lipids and $CHCl_3$–MeOH–HOAc–H_2O (170:30:20:7, at 25°) for polar lipids. 2D TLC was carried out at room temp. using $CHCl_3$–MeOH–28% NH_3 (65:35:7) in the first dimension and Me_2CO–C_6H_6–H_2O (90:30:7) in the second. The lipids were visualized by spraying with 0.2% methanolic 8-anilino-1-naphthalene sulphonic acid (ANSA) and viewing under UV or by exposure to I_2 vapour.

Routine identification was by reference to authentic lipid standards. Individual lipids were identified by the use of specific spray reagents [17, 41], acid and alkaline degradation [17] and autoradiography after labelling with [^{32}P] and [^{35}S].

Isotope labelling. Tissue samples of about 100 mg fr. wt were washed with 0.5% Triton X-100 in seawater to remove surface microbial contamination [c.f. 2], blotted dry and weighed. *P. lanosa* was excised with a small amount of its *A. nodosum* host as this gave a better uptake of label, probably by minimising damage to the epiphyte. The tissue was then incubated in 3 ml artificial seawater ASP-6 ($MgCl_2$ replaced $MgSO_4$ for [^{35}S]-labelling and glycerophosphate omitted for [^{32}P]-labelling [42]), with 2 μCi [^{32}P]-orthophosphate or [^{35}S]-sulphate in a sealed tube, under about 200 $\mu Em^{-2} sec^{-1}$ illumination (warm white fluorescent tubes), at 15°, for 24 hr. After labelling, the tissue was washed and blotted dry. The *P. lanosa* was removed from the piece of *A. nodosum* and weighed before lipid extraction.

Radioactive samples were counted in a scintillant of PCS (Amersham International, Amersham, Bucks)–xylene (2:1, v/v) using an Intertechnique SL4000 scintillation counter. Quench corrections were made using the external standard method.

Gas-liquid chromatography. Fatty acid methyl esters (FAMEs)

were prepared by transmethylation of lipid samples with 2.5% H_2SO_4 in dry MeOH at 70° for 2 hr, allowing to cool, adding 5 ml 5% NaCl and extracting with 3 × 3 ml redistilled petrol (bp 60–80°). Before transmethylation 20 μg pentadecanoic acid, heneicosanoic acid or tripentadecanoin was added as an int. standard to enable quantification. Before the total FAME samples could be run on the GLC, the pigments and other non-FAME components had to be separated, to prevent interference, by running on a 1D TLC plate (silica gel G) in petroleum (bp 60–80°): Et_2O: HOAc (45:5:1) at 4°. The FAME band, detected with ANSA, was extracted with 3 × 3 ml redistilled petrol. Care was taken throughout to avoid oxidative losses of polyunsaturated fatty acids by including 0.01% BHT in solvents and by working under N_2. No evidence of oxidative products was seen.

After extraction, aliquots of the FAMEs were separated with Perkin-Elmer F33 or 8310 GLC on 2 m × 5 mm (i.d.) glass columns packed with polar EGSS-X or EGSS-Y (15% on Chromasorb W-AW 100/120 mesh at 190° and flow rate of 40 ml/min) or with non-polar SE-30 (3% on Supelcoport 80/100 mesh at 150–230° with a ramp rate of 2°/min and a flow rate of 45 ml/min). Individual fatty acids were routinely identified by comparison of their retention times with those for authentic standards. The major acids were fully identified for chain length and double bond position by comparative GC on the different columns, by argentation TLC [41], by hydrogenation followed by GC [41] and by GC-MS using a Varian CH5D mass spectrometer on line to a Varian 620i computer.

Lipid quantification. Phospholipids were quantified by inorganic phosphate assay following hydrolysis [41], glycolipids with phenol–H_2SO_4 [44] and sterols by the method of ref. [45].

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