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DISTRIBUTION OF CHLOROSULPHOLIPIDS IN ALGAE

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Abstract—About 30 species of algae, chosen from a wide range of algal classes and orders, have been examined to see whether the chlorosulpholipids are widely distributed in this division of the plant kingdom. Most of the fresh-water species examined contained chlorosulpholipids, although only *Ochromonas danica* and *O. malhamensis* contained large quantities. The chlorosulpholipids of *O. malhamensis* were predominantly of the tetracosane series, the parent compound of which was tetracosane-1,14-diol disulphate. No chlorosulpholipids were detected in any of the marine species examined.

INTRODUCTION

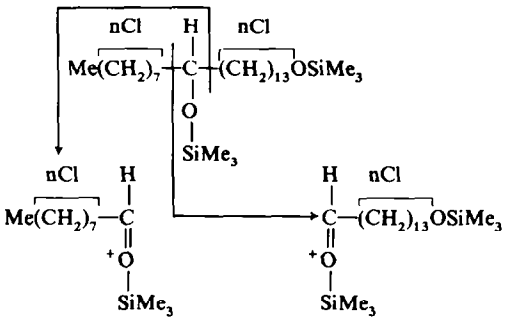
Chlorosulpholipids were discovered in the alga *Ochromonas danica* (Chrysophyta, Chrysophyceae) [1, 2] where they constitute 15% of the lipids and 3% of the dry weight of heterotrophically-grown, stationary phase cells [2]. They have also been shown to occur in the closely related, *Ochromonas malhamensis* [3, 4], in three species of Xanthophyceae, *Tribonema aequale* [5], *Botrydium granulatum* [6] and *Monodus subterraneus* [6] and in two species of Chlorophyta, *Elakatothrix viridis* and *Zygnema* [6]. However an early indication that they were present in higher plants [7] has been negated and shown to be due to contaminating algae [3].

The chlorosulpholipids of *O. danica* have been investigated most intensively to date [3, 4]. In this alga two series of chlorosulpholipids are present, one based on *N*-docosane-1,4-diol disulphate and the other, much less abundant, based on *N*-tetracosane-1,15-diol disulphate. The 13-chloro, 11,15-dichloro-, 2,2,11,13,15-pentachloro- and 2,2,11,13,15,16-hexachloro derivatives of *N*-docosane-1,14-diol disulphate have been identified along with several partly characterized tri- and tetrachloro derivatives. The 14-chloro-, 2,12,14,16,17-pentachloro- and 2,2,12,14,16,17-hexachloro derivatives of *N*-tetracosane-1,15-diol disulphate have also been detected along with a partly characterized trichloro derivative.

In the present work we report the results of a survey of 30 species of algae, chosen from a wide range of classes and orders, for the presence of chlorosulpholipids.

RESULTS AND DISCUSSION

The putative chlorosulpholipid fraction from each alga was extracted, solvolysed and the resulting mixture of chlorosulpholipid diols silylated. The TMSi-diols were analysed by GLC and GC–MS. Initial identification was made by comparison of the R_t (relative to cholestane) of the GLC peaks with those of the TMSi-diols of the *O. danica* chlorosulpholipids. The percentage composition of the chlorosulpholipid mixture was calculated from GLC peak areas. Final identification was, in most cases, made by examining the MS fragmentation pattern and comparing it with that of the equivalent *O. danica* TMSi-diol. The MS of chlorosulpholipid TMSi-diols typically exhibit three diagnostic fragment ions, an M – Me ion (the M^+ ion is rarely seen and when it is present it is of low intensity), a distal fragment ion (DF) and a proximal fragment ion (PF). The DF and PF ions arise by the fragmentations shown in Fig. 1 for chlorosulpholipids of the docosane series. The DF ion (C-22 → C-14-OTMSi) is very abundant and is frequency the base peak. The PF ion (C-1-OTMSi → C-14-OTMSi) is much less abundant and sometimes cannot be seen in the higher chlorinated species; its degree of chlorination may then be elucidated by the characteristic *m/e* values of the 'PF minus *n*HCl' fragmentations. The following fragment ions are also frequently seen, 'DF minus *n*HCl', M – HCl, M – [Me + HCl], M – [TMSiCl], M – [Me + TMSiCl]. The presence of the latter two ions in a MS has been inter preted [2] as indicating that the Cl and TMSi groups lost as TMSiCl were on adjacent carbon atoms. Ions containing Cl atoms occur in clusters with major peaks at intervals of two *m/e* units, due to the occurrence of ^{35}Cl and ^{37}Cl with natural abundances of 75.53 and 24.47% respectively; the shape and complexity of the peak cluster



Distal fragment Proximal fragment

Fig. 1. Principal mass spectral fragmentation of chlorosulpholipid diol TMSi-ethers of the docosane series.

Table 1. Principal mass spectral peaks* of the TMSi-diols of those members of the docosane series of chlorosulpholipids so far detected

No. of Cl atoms	0	1	2		3		4		5		6
Position of Cl atoms†	0–0	0–1	0–2	1–1	0–3	1–2	1–3	2–2	1–4	2–3	2–4
MS fragmentation (*m/e* values)											
M^+‡	486	520	554	554	588	588	622	622	656	656	690
M-Me	471	505	539	539	573	573	607	607	641	641	675
M-HCl	—	484	518	518	552	552	586	586	620	620	654
M-[Me + HCl]	—	469	503	503	537	537	571	571	605	605	639
M-TMSiCl	—	412	446	446	480	480	514	514	548	548	582
M-[Me + TMSiCl]	—	397	431	431	465	465	499	499	533	533	567
PF	373	407	441	407	475	441	475	441	509	475	509
PF-HCl	—	371	405	371	439	405	439	405	473	439	473
PF-2HCl	—	—	369	—	403	369	403	369	437	403	437
PF-3HCl	—	—	—	—	367	—	367	--	401	367	401
PF-4HCl	—	—	—	—	—	—	—	—	365	—	365
DF	215	215	215	249	215	249	249	283	249	283	283
DF-HCl	—	—	—	213	—	213	213	247	213	247	247
DF-2HCl	—	—	—	—	—	—	—	211	—	211	211

* The *m/e* value of each peak is that of the member of the cluster which contains only the most abundant isotope of each element present, i.e. ^{35}Cl, ^{12}C, ^{16}O and ^{1}H.

† The position of the Cl atoms in the molecule is indicated by two digits separated by a hyphen, e.g. 1–2, in which the first digit gives the number of Cl atoms distal to the secondary alcoholic oxygen atom and the second digit the number of Cl atoms proximal to it.

‡ M^+ is of low intensity or absent.

PF = proximal fragment, DF = distal fragment (see Fig. 1).

largely depends upon the number of Cl atoms present in the ion and is therefore helpful in confirming the identity of the ion. Unfortunately no fragment ions are obtained which indicate which of the carbon atoms of the chlorosulpholipid TMSi-diol carry the Cl atoms. Therefore, from the MS of the TMSi-diols, it is only possible to identify the chlorosulpholipid in terms of the length of its carbon chain, the number of Cl atoms present and whether the Cl atoms are distal or proximal to the secondary alcoholic oxygen atom. Table 1 gives the *m/e* values of the principal ions in the MS of the TMSi-diol of those members of the docosane series of chlorosulpholipids that have been detected to date.

The results of the survey are summarized in Table 2. Of the 30 species of algae investigated 22 were freshwater and 8 were marine. Evidence of the presence of the docosane series of chlorosulpholipids in all the freshwater algae, except *Euglena gracilis*, was obtained. The presence of members of the tetracosane series was detected only in the two *Ochromonas* spp. In *O. danica* the tetracosane series constitutes up to 5% of the total chlorosulpholipids. In contrast the tetracosane series is predominant in *O. malhamensis*. Moreover, the structures of the chlorosulpholipids of this series are different from those of *O. danica* in that they are based upon tetracosane-1,14-diol disulphate rather than tetracosane-1,15-diol disulphate.

Table 2. Distribution of chlorosulpholipids in algae

Algal species	Wet wt(g) of cells extracted	Chlorosulpholipids (C_{22} series only)† No Cl‡	Mono-Cl‡	Di-Cl‡	Tri-Cl‡	Tetra-Cl‡	Penta-Cl‡	Hexa-Cl‡
Chlamydomonas simples (l, a, F, w)¶	23.3	0–0 (100)§	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Elakatothrix viridis (l, a, F, x)	12.2	0–0 (9.2)	0–1 (22.3)	0–2* & 1–1 (16.5)	1–2 (5.3)	1–3 & 2–2 (6.2)	2–3 (27.5)	2–4 (12.5)
Chlorella pyrenoidosa (l, b, F, x)	35.3	0–0 (100)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Scenedesmus quadricauda (l, a, F, x)	6.5	0–0 (100)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ulothrix subtillissima (l, c, F, x)	8.6	0–0 (52.5)	Pr (23.0)	Pr (9.4)	Pr (2.3)	Pr (3.1)	Pr (9.4)	Pr (0.4)
Enteromorpha (l, d, M)	19	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Stigeoclonium tenue (l, e, F, x)	11.9	0–0 (9.3)	0–1 (17.4)	0–2* & 1–1 (11.4)	Pr (4.2)	Pr (4.2)	1–4 & 2–3* (9.9)	2–4 (43.5)
Oedogonium cardiacum (l, f, F, x)	8.9	0–0 (100)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Zygnema sp. (2, g, F, x)	23·4	0–0 (27.6)	0–1 (26.1)	0–2* & 1–1 (30.1)	Pr (3.0)	Pr (2.6)	2–3 (8.5)	Pr (2.1)
Cosmarium botrytis (2, h, F, w)	45	0–0 (96.4)	0–1 (3.6)	n.d.	n.d.	n.d.	n.d.	n.d.

Table 2.—*continued*

Algal species	Wet wt(g) of cells extracted	Chlorosulpholipids (C_{22} series only)† No Cl‡	Mono-Cl‡	Di-Cl‡	Tri-Cl‡	Tetra-Cl‡	Penta-Cl‡	Hexa-Cl‡
Euglena gracilis (3, F, x) (Photoauto-)	21·6	b.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Euglena gracilis (3, F, x) (Photohetero-)	18.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ochromonas danica (4, i, F, w)	12	0–0 (7.1)	0–1 (24.5)	0–2* & 1–1 (12.6)	1–2 (6.7)	1–3 & 2–2 (6.5)	1–4 & 2–3* (13.7)	2–4 (25.8)
Ochromonas malhamensis (4, i, F, w)	8.4	0–0	0–1	n.d.	n.d.	n.d.	n.d.	n.d.
Monodus subterraneous (5, j, F, w)	12·1	0–0 (6.5)	0–1 (20.2)	0–2* & 1–1 (15.5)	1–2 (5.2)	1–3 & 2–2 (7.7)	2–3 (18.9)	2–4 (26.0)
Tribonema aequale (5, k, F, w)	8.6	0–0 (8.2)	0–1 (26.5)	0–2* & 1–1 (19.1)	0–3 & 1–2* (7.3)	1–3 & 2–2* (6.4)	2–3 (8.9)	2–4 (23.3)
Botrydium granulatum (5, l, F, w)	18.2	0–0 (8.5)	0–1 (26.8)	0–2* & 1–1 (37.1)	1–2 (10.4)	Pr (3.5)	Pr (4.0)	Pr (9.6)
Fucus serratus (6, m, M)	130	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fucus vesiculosus (6, m, M)	139	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fucus spiralis (6, m, M)	120	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Porphyra umbilicalis (7, n, M)	25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Porphyridium cruentum (7, o, F, z)	40	0–0 (33.1)	0–1 (22.0)	0–2* & 1–1 (11.0)	Pr (2.2)	Pr (1.1)	Pr (8.·)0	2–4 (16.5)
Porphyridium aerugineum (7, o, F, z)	4.5	0–0 (33.3)	0–1 (25.0)	0–2* & 1–1 (27.3)	Pr (7.0)	Pr (1.5)	Pr (5.3)	Pr (0.7)
Rhodymena palmata (7, p, M)	12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Callithamnion sp. (7, q, M, z)	20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Polysiphonia sp. (7, q, M)	28	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cyanidium caldarium (7, F, w)	130	0–0 (93.6)	0–1 (3.5)	Pr (1.9)	n.d.	n.d.	Pr (0.5)	Pr (0.5)
Aphanocapsa 6714 (8, r, F, y)	20	0–0 (78.9)	0–1 (9.7)	0–2* & 1–1 (7.3)	1–2 (2.3)	Pr (1.2)	Pr (0.4)	2–4 (0.2)
Chlorogleopsis fritschii (8, r, F, y)	40	0–0 (12.1)	0–1 (21.5)	0–2* & 1–1 (14.6)	1–2 (3.2)	Pr (4.9)	Pr (22.4)	2–4 (21.4)
Nostoc strain Mac (8, s, F, y)	40	0–0 (6.0)	0–1 (16.8)	0–2* & 1–1 (49.2)	1–2 (5.4)	1–3 & 2–2* (8.9)	2–3 (7.5)	2–4 (6.2)
Cyanophora paradoxa (8?, F, z)	13	0–0	0–1	n.d.	n.d.	n.d.	n.d.	n.d.

* = Major species.

† = Estimated as TMSi-diols by GLC and GC–MS.

‡ = The achloro-, monochloro- and polychloro- chlorosulpholipid species are designated by two digits separated by a hyphen, e.g. 1–2, in which the first digit gives the number of Cl atoms distal to the secondary alcoholic oxygen atom and the second digit the number of Cl atoms proximal to it.

§ = Figures in parentheses give the percentages of the various chloropholipid species.

¶ Gives the class, order, habitat and source respectively of the algae according to the following key: 1 = Chlorophyceae; 2 = Conjugatophyceae; 3 = Euglenophyceae; 4 = Chrysophyceae; 5 = Xanthophyceae; 6 = Phaeophyceae; 7 = Rhodophyceae; 8 = Cyanophyceae. a = Volvocales; b = Chlorococcales; c = Ulotrichales; d = Ulvales; e = Chaetophorales; f = Oedogoniales; g = Zygnematales; h = Desmidales; i = Chrysomonadales; j = Heterococcales; k = Heterotrichales; l = Heterosiphonales; m = Fucales; n = Bangiales; o = Porphyridiales; p = Rhodomeniales; q = Ceramiales; r = Chorococcales; s = Oscillatoriales; F = freshwater; M = marine; w = obtained from The Culture Centre of Algae and Protozoa, 36, Storey's Way, Cambridge, CB3 ODT; x = gift from Dr. A. K. Jones, Dept. of Botany and Microbiology, U.C.W., Aberystwyth; y = gift form Dr. L. J. Rogers, Dept. of Biochemistry and Agricultural Biochemistry, U.C.W., Aberystwyth; z = gift from Dr. A. J. Smith, Dept. of Biochemistry and Agricultural Biochemistry, U.C.W., Aberystwyth.

Pr = Chlorosulpholipid species detected by GLC but not confirmed unequivocally by GC–MS. n.d. = Not detected.

Table 3. Principal mass spectral peaks* of the TMSi-diols of the tetracosane series of chlorosulpholipids detected in *Ochromonas malhamensis* compared with their equivalents in *Ochromonas danica*

Algal sp.	*O. malhamensis*			*O. danica*		
No. of Cl atoms	0	1	2	0	1	2
Position of Cl atoms†	0–0	0–1	0–2	0–0§	0–1§	0–2‖
MS fragmentation (*m/e* values)						
M^+‡	514	448	582	514	548	582
M-Me	499	533	567	499	533	567
M-HCl	—	512	546	—	512	546
M-[Me + HCl]	—	497	531	—	497	531
M-TMSiCl	—	440	474	—	440	474
M-[Me + TMSiCl]	—	425	459	—	425	459
M-[Me + TMSiCl + HCl]	—	—	423	—	—	423
PF	373	407	441	387	421	455
PF-HCl	—	371	405	—	385	419
PF-2HCl	—	—	369	—	—	383
DF	243	243	243	229	229	229
DF-HCl	—	—	—	—	—	—
DF-2HCl	—	—	—	—	—	—
$(Me)_3Si\text{-}O^+ = CH_2$	103	103	103	103	103	103
$(Me)_3Si^+$	73	73	73	73	73	73

*, †, ‡, PF and DF have the same meanings as in Table 2.
§ = see Ref. [2].
‖ = expected *m/e* values for diCl species based on tetracosane-1.15-diol disulphate yet to be detected in *O. danica* but included for comparison with 0–2 species detected in *O. malhamensis*.

Table 4. Estimation of the total chlorosulpholipids present in selected algae

Algal species	Chlorosulpholipid* mg/g dry wt†	rel. *O. danica*‡	Total lipid§ dry wt	Chlorosulpholipid as a % of total lipid
Ulothrix subtilissima	0.39	1.3	196	0.2
Stigeoclonium tenue	0.16	0.53	159	0.1
Cosmarium botrytis	1.62	5.4	230	0.7
Ochromonas danica	30.0	100	209	14.4
Ochromonas malhamensis	6.6	22	289	2.3
Monodus subterraneus	0.5	1.7	311	0.16
Tribonema aequale	0.4	1.3	87	0.46
Porphyridium aerugineum	1.4	4.7	108	1.3
Cyanidium caldarium	0.03	0.1	25	0.12
Chlorogleopsis fritschii	0.81	2.7	102	0.79
Cyanophora paradoxa	0.03	0.1	34.6	0.23

* Estimated by the method of Kean [35].
† In the calculation of this value the average MW of the chlorosulpholipid mixture was estimated from the composition of the mixture as determined by GLC.
‡ Chlorosulpholipid expressed relative to that of *O. danica* which is taken as 100.
§ Total lipid includes the chlorosulpholipids.

This is clearly indicated by the *m/e* values of the PF and DF ions (see Table 3) which are 14 *m/e* units (equivalent to CH_2) lower and higher respectively than their equivalents from *O. danica*. Only the mono-, di- and non-chlorinated members of this series have so far been detected in *O. malhamensis* (Table 3).

In four of the Chlorophyceae spp. (*Chlamydomonas simplex*, *Chlorella pyrenoidosa*, *Scenedesmus quadricauda* and *Oedogonium cardiacum*) only the non-chlorinated member of the docosane series was detected. Although, technically, this is not a chlorosulpholipid because it does not contain any chlorine atoms, it is usual and convenient to regard it as being one because it is, from a biosynthetic viewpoint, the parent compound of the docosane series [3, 4, 8, 9]. In at least 15 of the freshwater species, evidence for the presence of the full range of the docosine series was obtained and in two others the lesser chlorinated members were detected. *E. gracilis* therefore appears to be the odd one out amongst the freshwater algae examined, for, whether grown photoautotrophically or photoheterotrophically, it did not produce chlorosulpholipids in amounts that we could detect. However GLC of the putative TMSi-diols from *E. gracilis* produced such a complex picture of unidentified peaks that small quantities of the chlorosulpholipid TMSi-diols, if present, could have been totally masked. Therefore we cannot rule out the possibility that *E. gracilis* does produce small quanties of chlorosulpholipids.

In marked contrast to the freshwater algae, none of the 8 marine algae contained detectable levels of chlorosulpholipids. From this we do not conclude that these marine spp. do not contain any chlorosulpholipids but rather that, if they do contain these compounds, the level is at least an order of magnitude lower than that of the lowest freshwater sp. examined. Indeed small quantities of non-chlorinated sulphatides with structures very similar to chlorosulpholipids have been detected in young thalli of three spp. of Fucaceae [10].

Table 4 shows the levels of chlorosulpholipids in 10 of the 15 algae in which the full range of docosane series was detected. Of these algae, 7 spp. had chlorosulpholipid levels in the range 0.16–1.62 mg/g dry wt. *Cyanidium caldarium* had a very low level at 0.03 mg/g dry wt. However the fact that it was grown heterotrophically in the dark could have a bearing on this low level. *Cyanophora paradoxa*, an organism of uncertain taxonomic position but which appears to utilize whole blue-green algae in place of chloroplasts [11], has a similarly low level of chlorosulpholipids. At the other extreme the two *Ochromonas* spp. had very high levels. Indeed *O. danica*, with a chlorosulpholipid level of 30 mg/g dry wt stands out from the other algae of the survey. It is interesting to note that whilst *O. danica* has a high chlorosulpholipid level, it has a very low level of the plant sulphonolipid (6-sulpho-α-D-quinovosopyranosyl-(1 → 1′)-2,3-di-*O*-acyl-D-glycerol) [12]. This contrasts with *F. vesiculosus* [13] and *E. gracilis* [14] which have high levels of the plant sulphonolipid but in which we have failed to detect chlorosulpholipids. Moreover there is a whole range of spp. of Chloro-, Phaeo-, Rhodo- and Cyanophyceae which have been shown to possess easily detectable quantities of the plant sulphonolipid [15–22] yet we have found the chlorosulpholipid level to be low in other spp. from the same classes. There would appear to be a *prima facie*

case for saying that when the plant sulphonolipid in an algal sp. is high, the chlorosulpholipid level will be low and *vice versa*.

EXPERIMENTAL

Organisms, cultural conditions and harvesting procedures. The sources of the organisms used are given in Table 2. *Enteromorpha* sp., *Fucus* spp., *Polysiphonia* sp., *Porphyra umbilicales* and *Rhodymena palmata* were collected from their littoral habitat near Aberystwyth, washed free of salt water and macrocontaminants, and then stored at −20° until required. The species of Xanthophyceae and freshwater Chlorophyta were grown in continuously aerated, Bold's Basal medium [23] contained in Roux bottles standing upright 15 cm from a double band of 'warm white' fluorescent tubes (4 klx) at a temp. of 20–23°. *Ochromonas danica* was grown heterotrophically in 1 l. batches of the medium of Aaronsón and Baker [24] contained in 2 l. conical flasks shaken (150 rpm) in an illuminated (1.5 klx) gyrotary shaker at 15°. *Ochromonas malhamensis* (Pringsheim 933/1a and 1b) was grown statically and photoheterotrophically (4 klx) at 20–23° in the B_{12}-containing medium of Aaronson and Baker [24]. *Euglena gracilis* was grown photoautotrophically at 20° on the medium of Wolken [25] modified by replacing potassium citrate by the sodium salt and NH_4HCO_3 by $NaHCO_3$ (0.3 g/l.) and NH_4Cl (0.3 g/l.) through which 5% CO_2 (v/v) in air was continuously bubbled (1 l./min). The cultures were illumined by 'warm white' fluorescent tubes (3 klx). *E. gracilis* was also grown photoheterotrophically at 20° on the medium of Wolken [26] in the manner described for *O. danica*. *Cyanophora paradoxa* was grown photoheterotrophically (4 klx) in static culture at 16–17° in the medium of Hoogenhout and Amesz [27]. *Aphanocapsa* 6714 was grown photoautotrophically on the medium of Hughes, Gorham and Zender [28] through which 5% (v/v) CO_2 in air was continuously bubbled (1 l./min). The medium was contained in Roux bottles standing in a narrow perspex tank containing water at 30° which was illuminated on either side by a double bank of 'warm white' fluorescent tubes (4 klx). *Chlorogleopsis fritschii* was grown photoautotrophically on the modified medium 'C' of Kratz and Myers [29] to which 2.5 g/l. $NaHCO_3$ was added as a sterile 10% (w/v) solution after autoclaving and through which 5% (v/v) CO_2 in air was continuously bubbled (1 l./min). The growth conditions were identical to those of *Aphanocapsa* 6714 save that the water in the perspex tank was maintained at 35° and that the contents of each Roux bottle was magnetically stirred. *Nostoc* strain Mac was grown photoautotrophically in the Cg10 medium of Van Baalen [30] modified by doubling the KNO_3 content and adding $KHCO_3$ (0.9 g/l.) and through which 5% (v/v) CO_2 in air was continuously bubbled (1 l./min). The growth conditions were identical to those of *Aphanocapsa* 6714 save that the incubation temp. was 35°. *Cyanidium caldarium* was grown heterotrophically in the dark in a gyrotary shaker (100 rpm; 30°) in the medium of Troxler and Bogorad [31] modified by replacing the $(NH_4)_2SO_4$ content by an equivalent quantity of NH_4Cl. *Porphyridium aerugineum* was grown photoautotrophically in the medium of Hoogenhout and Amesz [27] under the same conditions as those described for the Xanthophyceae and freshwater Chlorophyta. *Porphyridium cruentum* was grown photoautotrophically in the medium of Jones *et al.* [32] contained in 10 l. flat-bottomed, bulb-type flasks and through which 5% (v/v) CO_2 in air was continuously bubbled (1 l./min). The flasks were maintained at 25° and illuminated with 'warm white' fluorescent tubes (3 klx). *Callithamnion* sp. was grown in static culture at 15° under subdued illumination (100 lx) from 'warm water' fluorescent tubes on a medium modified from that of Gross [33], consisting of an autoclaved mixture of 0.2 g $NaNO_3$, 0.03 g $NaHPO_4$ and 50 ml of soil water supernatant per l. of filtered sea water. The latter was collected locally, filtered through Whatman No. 1 filter paper and then put through three cycles of heating to 73° and cooling to room temp. with 24 hr intervals between each cycle [34]. The soil water supernatant was prepared by spending 1 kg of air-dried, local soil in 1 l. of deionized water, autoclaving the suspension for 30 min at 1.05 kg/cm² and then filtering while hot firstly through muslin and then through Whatman No. 1 filter paper.

All the algal species grown in the laboratory were harvested at the late logarithmic stage of culture growth. This was determined photometrically in the case of unicellular algae using a Klett–Summerson colorimeter fitted with a Kodak 88A filter. The growth of filamentous algal cultures was determined by dry weight measurements.

Unicellular algae which had been grown in small volumes were harvested by centrifugation at 12000 *g* for 30 min; however the two *Ochromonas* spp. which do not have cell walls and are rather fragile were sedimented at 6000 *g*. Larger cultures of unicellular algae were harvested by continuous flow centrifugation. Filamentous algae were harvested by filtration of the culture through a glass sinter (porosity 3).

Algae which could not be extracted immediately after harvesting were stored at −20° in the dark.

Extraction procedures. The larger seaweeds (*Fucus* spp., *P. umbilicales*) which had been stored at −20° were thawed, cut into small pieces, homogenized in MeOH and then filtered through a glass sinter (porosity 3). The residue was then ground in a mortar and pestle 3 × with hot MeOH and acid-washed silver sand, filtering after each. All the MeOH extracts were then bulked. All the other algae, with the exception of *P. cruentum* and the Cyanophyta, were subjected to two cycles of freezing and thawing and were then suspended in MeOH for 24 hr. The suspension was then filtered through a glass sinter (porosity 3). The cell debris was then washed 2 × with MeOH and all the MeOH extracts bulked. This procedure was sufficient for the complete extraction of chlorosulpholipids from the *Ochromonas* spp. but for the others it was necessary to grind the cell debris 2 × with MeOH and acid-washed silver sand, filtering after each. Sometimes further grinding with hot MeOH was required. All the MeOH extracts were then bulked. In the case of *P. cruentum* and the Cyanophyta the frozen cells were thawed and suspended in deionized H_2O (1:2, v/v). The suspension was slowly added to Me_2CO at −20° with stirring until the Me_2CO concn. reached 80% (v/v) after which it was allowed to stand for 1 hr at 4° with stirring. It was then filtered through Whatman No. 1 filter paper and the residue washed with Me_2CO until the filtrate was clear. The Me_2CO extracts were then bulked. The Me_2CO was removed by fractional distillation and the residual water by lyophilization. The residue was then dissolved in MeOH.

After reducing the MeOH extracts of the algae to an easily handled vol. by rotary evapn at 40° under red. pres., 2 vol. of $CHCl_3$ were added. The resulting soln was then extracted with 0.3 vol of 1% (w/v) KCl 3×. The aq. extracts were bulked, washed with $CHCl_3$, and reduced to 30% of their original vol. by rotary evapn. The resulting extract was then extracted with 2 vol. *n*-BuOH 3 ×. The *n*-BuOH extracts were bulked and reduced to dryness by rotary evapn. The resulting residue was then extracted 3 × with MeOH–$CHCl_3$ (1:1) and the bulked extracts filtered through a glass sinter (porosity 3) and reduced to dryness by rotary evapn. The chlorosulpholipids were separated from this residue by TLC on Si gel using $CHCl_3$–MeOH–H_2O (10:5:1) for development. The chlorosulpholipid zone (R_f 0.35–0.5) was extracted from the Si gel with MeOH–$CHCl_3$–H_2O (7:3:1). In the case of some of the algae in the survey, an aliquot of the

resulting purified chlorosulpholipid mixture was removed for the alkyl sulphate estimation of Kean [35]. This assay was calibrated against the *O. danica* chlorosulpholipid mixture, for which an average MW was calculated from the composition as determined by GLC. In all the algae of the survey, the purified chlorosulpholipid mixture was analysed by solvolysing the chlorosulpholipids to corresponding diols [36], silylating them and subjecting the resulting mixture of TMSi-chlorosulpholipid diols to GLC and GC–MS. GLC was carried out on a 150 × 0.4 cm 1% SE-30 column programmed to 5 min isothermal at 210° followed by a linear increase of 5°/min up to a nominal 265° and then held. The carrier gas flow was O_2-free N_2 (GLC) and He (GC–MS) at 40 ml/min. GC–MS was carried out on a Pye 104 gas chromatograph linked via a single-stage silicone rubber membrane [37] to an AEI MS-30 mass spectrometer. Low resolution MS were obtained with an electron energy of 24 eV, an emission current of 30 μA and a source temp. of 250°.

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