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THE PIGMENTS OF THE PRASINOPHYCEAE AND RELATED ORGANISMS

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Abstract—The pigment compositions of some members of the Prasinophyceae and of related genera have been determined in a semi-quantitative manner. Most members display a relatively normal chlorophycean pattern (*Platymonas chuii, P. tetrathele, P. subcordiformis, P. striata*; *Pyramimonas obovata, P. urceolata, P. grossii*; *Heteromastix* sp. (*B*148), *H. rotunda, H.* sp. (*P*397); *Pedinomonas minor*; *Prasinocladus marinus, P. lubricus, P.* sp. (*P*371); *Mesostigma viride*; *Monomastix minuta*; *Haematococcus* sp.; *Spermatozopsis exsultans*). The other organisms fall into three different groups. Firstly, *Asteromonas propulsa*, whose pigments differ from the normal chlorophycean pattern only in the additional possession of xanthophyll K1. Secondly, a group (*Heteromastix longifilis, H.* sp. (*P*198). *Pyramimonas amylifera, Pachysphaera* sp. (*P*339) and *Pterosperma* sp. (*P*302)) which differs from the normal chlorophycean carotenoid pattern in lacking lutein and possessing xanthophylls K1 and K2. Thirdly, a group (*Micromonas pusilla, M. squamata, Micromonas* sp. (*P*265) and *Nephroselmis gilva*) which shows a carotenoid pigment pattern differing considerably from the normal chlorophycean type. It should be noted that these three unusual groups (with the exception of *Asteromonas propulsa*) all display an accumulation of magnesium 2,4-divinylphaeoporphyrin a_5 monomethyl ester in addition to chlorophylls *a* and *b*. The second unusual pigment group possesses genera which contain species showing a normal chlorophycean-type pigment pattern. The significance of these results is discussed.

INTRODUCTION

THE PRASINOPHYCEAE (in which term the author includes the Loxophyceae sensu Christensen)[1] are a rather ill-defined group of green monads. These organisms may possess one, two or four flagella; the latter are the most common. Cell walls are absent, but the cell membrane is sometimes covered with scales or a theca. The flagella are covered with scales (Prasinophyceae) or are naked (Loxophyceae).[2] The pigment composition of some of these organisms has already been described.[3-8] All members studied have been found to possess chlorophylls *a* and *b*. In some monads, additionally, small amounts of magnesium 2,4-divinylphaeoporphyrin a_5 monomethyl ester are present. Those phytoflagellates lacking the latter show a relatively normal chlorophycean-type carotenoid composition, whereas those possessing it contain novel carotenoid types and can be divided into two groups based upon carotenoid composition.[3-7]

The first group (*Micromonas* and *Nephroselmis*) contain a new keto-xanthophyll (xanthophyll K) as the main carotenoid, together with β-carotene, micronone, violaxanthin, neoxanthin and lesser amounts of other carotenoids.[4,6,7] Xanthophyll K is converted to the less

[1] T. CHRISTENSEN, in *Botanik* (edited by T. W. BOCHER, M. LANGE and T. SØRENSEN), Bd. 2 (Systematisk Botanik), Nr. 2, p. 128, Munksgaard, Copenhagen (1966).
[2] L. S. PETERFI and I. MANTON, *Brit. Phycol. Bull. Brit.* **3**, 423 (1968).
[3] T. R. RICKETTS, *Phytochem.* **5**, 223 (1966).
[4] T. R. RICKETTS, *Phytochem.* **5**, 571 (1966).
[5] T. R. RICKETTS, *Phytochem.* **6**, 19 (1967).
[6] T. R. RICKETTS, *Phytochem.* **6**, 669 (1967).
[7] T. R. RICKETTS, *Phytochem.* **6**, 1375 (1967).
[8] J. P. RILEY and T. R. S. WILSON, *J. Marine Biol. Assoc. U.K.* **47**, 351 (1967).

polar micronone[4] upon saponification. This group (except *M. pusilla*) display a single layer of body and flagellar scales.

The second group (*Pyramimonas amylifera* and *Heteromastix*) have β-carotene, zeaxanthin, violaxanthin, neoxanthin, xanthophylls K1 and K2 as the main carotenoids, together with smaller amounts of other carotenoids. Xanthophyll K1 has the same spectroscopic and chromatographic properties as siphonein (present in siphonalean green algae), whereas the related xanthophyll K2 is more polar. Saponification of xanthophyll K1 gives xanthophyll K1S, identical in spectroscopic and chromatographic properties to siphonaxanthin, and similarly xanthophyll K2 gives a more polar product upon saponification, xanthophyll K2S.[7] The structures of these pigments will be the subject of later communications. The organisms in this group possess multiple layers of scales upon both body and flagella. There is some overlap of the second group with those Prasinophyceae showing normal chlorophycean-type pigments (*Prasinocladus, Platymonas, Pedimonas*), in that *Pyramimonas obovata* showed normal chlorophycean-type pigments.[7]

The pigments of the available members of the Prasinophyceae (and related groups) have now been further surveyed in order to ascertain the distribution both in genera as yet unexamined and to expand the numbers in the groups already studied.

RESULTS

The R_fs of chlorophylls *a* and *b*, and magnesium 2,4-divinylphaeoporphyrin a_5 monomethyl ester on polyamide-cellulose TLC were 0·58 (range 0·50–0·65), 0·53 (range 0·45–0·59) and 0·00 respectively in the first dimension and 0·14 (range 0·10–0·19), 0·14 (range 0·09–0·18) and 0·00 respectively in the second dimension. The R_fs of authentic samples of xanthophylls, chromatographed on 3MM paper, are given in Table 1. The R_fs of the tentatively identified pigments of the phytoflagellate extracts were generally within ±0·05 of those of the standard. This sort of variability was found using a standard pigment in a number of "identical" repeat chromatograms under the conditions stated.

The approximate quantitative pigment composition of those phytoflagellates possessing chlorophycean-type pigments is shown in Table 2. A pinkish spot running near the main carotene spot on polyamide–cellulose chromatography was detected in the following

TABLE 1. R_fS OF AUTHENTIC XANTHOPHYLLS ON 3MM CHROMATOGRAPHY PAPER AT 20°

Carotenoid	R_fs (×100) in Petrol. ether (60–80°)–$CHCl_3$ (4:1)	R_fs (×100) in Petrol. ether (60–80°)–*n*-PrOH (49:1)
Carotenes	97	97
Micronone	79	82
Lutein	64	73
Zeaxanthin	52	64
Lutein 5,6-epoxide	41	66
Violaxanthin	40	62
Xanthophyll K	22	45
Trollein	4	14
Neoxanthin	4	17
Xanthophyll K1	19	43
Xanthophyll K1S	3	10
Xanthophyll K2	0	11
Xanthophyll K2S	0	0

TABLE 2. THE DISTRIBUTION OF PIGMENTS IN THE PRASINOPHYCEAE AND RELATED PHYTOFLAGELLATES (USING SAPONIFIED EXTRACTS)

Organism	Pigment: Approximate proportion of total carotenoids* — Carotenes	Lutein	Zeaxanthin	Violaxanthin	Neoxanthin	Xanthophyll a	Xanthophyll b	Xanthophyll c	Xanthophyll d	Xanthophyll e	Xanthophyll f	Xanthophyll g	Xanthophyll h	Xanthophyll i	Approximate chlorophyll *a*/*b* ratio†
Platymonas chuii	++	++	±	+	++	∓	∓	±	+						1·0
Platymonas tetrathele	+++	++	±	+	++	∓	±								2·0
Platymonas subcordiformis	+++	++	+	++	+	∓	∓								2·0
Platymonas striata	+++	++	±	+	+	∓	∓								1·0
Pyramimonas obovata	+++	+	+	+	+	±	∓								1·7
Pyramimonas urceolata	+++	++	+	+	+	∓									1·0
Pyramimonas grossii	+++	+	+	+	+	+	±								1·0
Halosphaera russellii (*motile*)	++	±	±	+	+	±	±							+++	0·7
Heteromastix rotunda	+++	++	+	+	+	±	±								1·5
Heteromastix sp. (*P*397)	+++	++	+	+	+	±	∓			±					1·0
Heteromastix sp. (*B*148)	++	++	+	+	+	±	±								1·6
Pedinomonas minor	++++	+++	±	+	±										2·5
Prasinocladus marinus	++	++	+	+	+	±	±								1·0
Prasinocladus lubricus	++	+	±	+	+	±	±						+		0·5
Prasinocladus sp. (*P*371)	++++	++	+	+	+	±									2·0
Mesostigma viride	++	++	+	+	+	±	∓								1·0
Monomastix minuta	++	++	++	++	±	∓	∓								1·5
Haematococcus sp.	++	+++	±	+	+	∓					±	±			1·0
Spermatozopsis exsultans	++	+++	±	+	+	∓	∓								2·8

* ++++ = 40–50 per cent; +++ = 30–39 per cent; ++ = 20–29 per cent; + = 10–19 per cent; ± = 4–9 per cent; ∓ = <4 per cent. The R_fs of unidentified carotenoids on 3MM paper in petrol. ether (60–80°)–$CHCl_3$ (4:1) and in petrol. ether–*n*-PrOH (49:1) respectively were: xanthophyll a, 0,0; xanthophyll b, 0·20, 0·45; xanthophyll c, 0·82, 0·97; xanthophyll d, 0·70, 0·95; xanthophyll e, 0·35, 0·62; xanthophyll f, 0·72, 0·71; xanthophyll g, 0·43, 0·70; xanthophyll h, 0·14, 0·24; xanthophyll i, 0·55, 0·52 respectively.

† In none of these organisms could magnesium 2,4-divinylphaeoporphyrin a_5 monomethyl ester be demonstrated before saponification.

TABLE 3. THE DISTRIBUTION OF PIGMENTS IN THE PRASINOPHYCEAE AND RELATED PHYTOFLAGELLATES*

Organism	Pigment: Approximate proportion of total carotenoids† — Carotenes	Zeaxanthin	Violaxanthin	Neoxanthin	Xanthophyll K1S	Xanthophyll K2S	Xanthophyll b	Xanthophyll j	Approximate chlorophyll *a*/*b* ratio‡
Heteromastix longifilis	++	±	±	++	++	+			2·5
Heteromastix sp. (*P*198)	++	++	±	+	++	+		∓	2·0
Pyramimonas amylifera	+++	+	±	+	+++	+			1·0
Pachysphaera sp. (*P*339)	+	±	±	±	+++	+++	±		0·5
Pterosperma sp. (*P*302)	+	+	±	+	+++	++	∓		2·0

* Some *Heteromastix*, *Pachysphaera*, *Pterosperma*, *Pyramimonas* species, using saponified extracts.

† Same units as Table 2. The R_fs of unidentified carotenoids were: xanthophyll b, 0·20, 0·45; xanthophyll j, 0·62, 0·73. (Chromatography as in Table 2.)

‡ All these organisms displayed magnesium 2,4-divinylphaeoporphyrin a_5 monomethyl ester content in unsaponified extracts.

TABLE 4. THE DISTRIBUTION OF PIGMENTS IN THE PRASINOPHYCEAE AND RELATED ORGANISMS*

Organism	Pigment: Approximate proportion of total carotenoids† — Carotenes	Micronone	Violaxanthin	Xanthophyll K	Neoxanthin	Xanthophyll F6C	Xanthophyll a	Xanthophyll b	Xanthophyll j	Xanthophyll k	Xanthophyll l	Xanthophyll m	Approximate chlorophyll *a*/*b* ratio‡
Micromonas pusilla	++	+++	±	∓	+	+	∓	±			±	∓	1·3
Micromonas squamata	+++	++	+	±	+	+					+		3·0
Micromonas sp. (*P*265)	++	++	+	+	+	+	±		±		±		1·0
Nephroselmis gilva	++	++	±		+	+	±	+		±			0·5

* *Micromonas* and *Nephroselmis*, saponified pigments.

† Same units as Table 2. The R_fs of the unidentified carotenoids on 3MM paper were: xanthophyll F6C, 0·22, 0·20; xanthophyll a, 0,0; xanthophyll b, 0·20, 0·45; xanthophyll j, 0·62, 0·73; xanthophyll k, 0·71. 0·87; xanthophyll l, 0·57, 0·67; xanthophyllm, 0·04, 0·07 respectively. Development as in Table 2. Xanthophyll F6C was identical with xanthophyll F6C from *Micromonas squamata*.[6]

‡ All these organisms displayed magnesium 2,4-divinylphaeoporphyrin a_5 monomethyl ester content in unsaponified extracts.

organisms: *Platymonas chuii, P. striata* and *P. tetrathele*; *Prasinocladus marinus, P. lubricus* and *Prasinocladus* sp. (*P*371); *Pyramimonas urceolata, P. grossii* and *P. obovata*; *Asteromonas propulsa*; *Pedinomonas minor*; *Haematococcus* sp; *Heteromastix* sp. (*B*148); *Spermatozopsis exsultans*. The R_fs corresponded with those of γ-carotene and/or lycopene. *Halosphaera russellii* showed an atypical pigment pattern.

Table 3 shows the approximate composition of one non-chlorophycean-type pigment group in the Prasinophyceae, whilst Table 4 shows the pigments present in the second group. The pink spot running with the same R_f as lycopene was not detected in any organisms in these two groups. Table 5 shows the pigments of *Asteromonas propulsa*. Table 6 shows the combined results of these and earlier surveys.

TABLE 5. THE DISTRIBUTION OF PIGMENTS IN THE PRASINOPHYCEAE AND RELATED ORGANISMS*

	Pigment							
	Approximate proportions of total carotenoids†							
Organism	Carotenes	Lutein	Zeaxanthin	Violaxanthin	Neoxanthin	Xanthophyll K1S	Xanthophyll b	Approximate chlorophyll *a*/*b* ratio‡
Asteromonas propulsa	+++	+	+	+	+	+	±	0·7

* *Asteromonas propulsa*, saponified extracts.

† Same units and R_fs as Table 2.

‡ No magnesium 2,4-divinylphaeoporphyrin a_5 monomethyl ester could be demonstrated in this organism using unsaponified extracts.

TABLE 6. THE GENERA OF ORGANISMS EXAMINED AND THE TYPE OF PIGMENT COMPOSITION OBTAINED

Genus	No. of species examined	Results in Table No.*
Platymonas	4	2
Halosphaera (motile phase)	1	2
Pedinomonas	1	2
Prasinocladus	3	2
Mesostigma	1	2
Monomastix	1	2
Haematococcus	1	2
Spermatozopsis	1	2
Pyramimonas	4	2 + 3
Heteromastix	5	2 + 3
Pachysphaera	1	3
Pterosperma	1	3
Micromonas	3	4
Nephroselmis	1	4
Asteromonas	1	5

* Table 2 = chlorophycean-type pattern; Table 3 = xanthophylls K1 + K2 type; Table 4 = xanthophyll K type; Table 5 = chlorophycean-type + xanthophyll K1.

DISCUSSION

The validity of the methods used is supported by a comparison of the results obtained for those organisms which had previously been studied in a much more intensive manner.[3-7]

The investigation, whilst often not including many organisms in some taxa, indicates that the majority of the Prasinophyceae (and related organisms) possess relatively normal chlorophycean-type pigment patterns. Three organisms, in addition to those described, also fit into this category. These are *Platymonas maculata*,[9] *Halosphaera minor*[8] and *Pedinomonas tuberculata*.[5] Some genera contain both species showing the chlorophycean-type pigment pattern as well as a different type of pigment pattern. Others show only a different non-chlorophycean-type of pigment pattern. The single species of *Asteromonas* examined showed a normal chlorophycean pattern plus xanthophyll K1.

Because extensive investigations have shown that algae in major taxa possess more or less identical pigment compositions within each taxon,[10] it appears probable that the pigment pattern is a relatively primitive feature in an evolutionary sense and that marked variations do not commonly occur within each major taxon. It follows that the *Micromonas* and *Nephroselmis* groups should probably be placed in the same separate taxon (possibly at the class or order level) and that *Heteromastix* (included by some workers[1,11] with these organisms in the Nephroselmidaceae) should not be included in this group.

The position as regards the genera *Heteromastix* and *Pyramimonas* is difficult to assess, since these organisms vary intragenerically in pigment pattern. Those organisms which have been studied in each genus show similar fine structural details.[12-15] It is impossible to assess, without further evidence, whether these organisms reflect converging evolutionary pathways as far as cell structure is concerned or divergence in pigments from the "more primitive" chlorophycean-type pigment stock. Another possibility is that the pigment pattern may reflect a step in the evolutionary pathway to the normal chlorophycean pigment pattern. The latter two possibilities seem the more probable because both carotenoid and chlorophyll biosynthesis are different from that of the normal chlorophycean stock and are obviously linked in some way, as shown by the concomitant presence of magnesium 2,4-divinylphaeoporphyrin a_5 monomethyl ester and unusual carotenoids.

The presence of magnesium 2,4-divinylphaeoporphyrin a_5 monomethyl ester in all the genera (except *Asteromonas*) possessing unusual carotenoids (Table 6) may indicate that they are related in some way. Another peculiarity shown by some of these organisms is that the chlorophyll *a*/*b* ratio is less than one. The organisms shown in Table 3 (*Heteromastix longifilis* etc.) display carotenoid patterns which differ from the normal chlorophycean-type only in the absence of lutein and in the presence of xanthophylls K1 and K2. It is possible that xanthophylls K1 and K2 are precursors or derivatives (in an evolutionary sense) of lutein.

Whether the fact that the six species of freshwater organisms studied all show a normal chlorophycean-type pigment pattern has any significance will have to await the results of examination of more species. In general, the results give considerable aid in the systematics

[9] T. R. PARSONS, *J. Fisheries Res. Board Can.* **18**, 1017 (1961).
[10] H. H. STRAIN, in *Biochemistry of Chloroplasts* (edited by T. W. GOODWIN), Vol. 1, pp. 387–406, Academic Press, London (1966).
[11] M. PARKE and P. S. DIXON, *J. Marine Biol. Assoc. U.K.* **48**, 783 (1968).
[12] I. MANTON, K. OATES and M. PARKE, *J. Marine Biol. Assoc. U.K.* **43**, 225 (1963).
[13] E. M. F. SWALE and J. H. BELCHER, *Proc. Linnean Soc. Lond.* **179**, 77 (1968).
[14] I. MANTON, *Proc. Linnean Soc. Lond.* **179**, 147 (1968).
[15] I. MANTON, D. G. RAYNS, H. ETTL and M. PARKE, *J. Marine Biol. Assoc. U.K.* **45**, 241 (1965).

of those organisms showing non-chlorophycean-like pigment patterns, but do not help in the assessment of those organisms possessing chlorophycean-type patterns.

EXPERIMENTAL

Cultures

The sources of the organisms were: The Culture Collection of Algae and Protozoa, Cambridge (indicated by *C* and collection number), Dr. J. H. Belcher, The Windermere Laboratory (indicated by *B* and collection number), and Dr. Mary Parke, The Plymouth Laboratory (indicated by *P* and collection number). I am indebted to Dr. Parke and Dr. Belcher for providing the organisms concerned and for cultural information. Many of the cultures used had been maintained at Nottingham for some time, whereas others were supplied a few weeks before culturing for the experiments. All organisms were grown at 14° with a regimen of 16 hr light (200 lumen/ft² approx.) and 8 hr darkness per 24 hr in 1½ l. volumes. The growth media used is indicated after the organisms listed below. (E = Erdschrieber medium; EB = Erdschrieber medium containing vitamin B_{12} (100 mμg per l.); FW = the bottom of a flask was covered with an approximately 1 mm layer of dried soil and then with 1 l. of Ettl medium (below) and thereafter simmered for 10 min. It was used after standing for 3–4 days (Dr. J. H. Belcher, personal communication). Ettl = medium described in Ricketts[16] for *Pedinomonas*. The monads examined were as follows: *Platymonas chuii* Butcher* (*C* 8/6, E); *Platymonas tetrathele** (*P*272, E); *Platymonas subcordiformis** (*C* 161/1a, E); *Platymonas striata* Butcher* (*P*315, E); *Halosphaera russellii*, motile phase (*P*247, EB); *Pyramimonas amylifera* (*P*246, E); *Pyramimonas obovata* (*P*280, E); *Pyramimonas urceolata* (*P*299, E); *Pyramimonas grossii* (*P*78, EB); *Heteromastix longifilis* (*P*58, E); *Heteromastix* sp. *Roscoff* (*P*397, E); *Heteromastix rotunda* (*P*210, E); *Heteromastix* sp. (*P*198, E); *Heteromastix* sp. (*B*148, FW); *Pedinomonas minor* Korshikov* (Dr. Hans Ettl, Strain 17B,[16] Ettl); *Spermatozopsis exsultans* (*B*, FW); *Prasinocladus marinus* (*P*308, E); *Prasinocladus lubricus* Kuckuck* (*C*163/1, EB); *Prasinocladus* sp.* (*P*371, E); *Mesostigma viride* Lauterborn (*C* 50/1, Ettl); *Monomastix minuta* Skuja (*B*, Ettl); *Nephroselmis gilva* (*P*197, E); *Haematococcus* sp. (Leeds, Ettl); *Asteromonas propulsa* Butcher (*C* 4/1, E); *Pachysphaera* sp. (*P*339, EB); *Pterosperma* sp. (motile, *P*302, EB diluted 1:4 with sea-water); *Micromonas pusilla* (*P*27, E); *Micromonas squamata* (*P*290, E); *Micromonas* sp. (*P*265, E). Those cultures asterisked were axenic, the others were unialgal but bacteria-containing. The cultures were harvested towards the end of the logarithmic phase of growth by centrifugation.

Pigments

The pigments were extracted from the packed cell deposits as described in Ricketts.[7] The saponification procedure used was essentially that of Ricketts[7] but the time of saponification was increased from 5 to 60 min at 40°. This was done to ensure a fair measure of conversion of xanthophyll K to micronone.[7] The pigments were examined chromatographically both before and after saponification.

Chromatography

(a) *Chlorophylls.* Two-dimensional TLC of the unsaponified pigments on polyamide-cellulose was carried out according to the method of Egger and Voigt,[17] developing in the first dimension with petrol. ether (100–120°)–MeOH–MeCOEt, 4:1:1, v/v/v, and on the second dimension (after drying at room temp. in the dark) with H_2O–MeOH–MeCOEt, 1:3:3, v/v/v. This method gave good separation of chlorophylls *a* and *b* from the carotenoids and from magnesium 2,4-divinylphaeoporphyrin a_5 monomethyl ester. A pale yellow-green spot, corresponding to the latter, remained at the origin in both dimensions. It was detected in all the organisms in which it had previously been found and was not demonstrated in those organisms which had given negative results in earlier experiments. Its presence was additionally confirmed in those new organisms which showed pale green spots at the origin after polyamide chromatography using the method of Parsons.[3,18] The separation of the carotenoids into carotenes and xanthophylls was good. The carotenes were poorly separated, although it was easily possible to detect lycopene and/or γ-carotene, when these were present. The separation of the xanthophylls was not ideal, the spots often tending to run into one another. It was thought that this might possibly be due to the use of unsaponified extracts. However, when saponified extracts were used the separation was only a little improved. The carotenoids were therefore identified using a different system (below).

(b) *Carotenoids.* The carotenoids were separated and identified in saponified pigment extracts by ascending two-dimensional paper chromatography on Whatman 3MM chromatography paper developing to a 10 cm solvent front at 20° in each case, with: first dimension, petrol. ether (60–80°) containing 20%, v/v

[16] T. R. Ricketts, *Phytochem.* 4, 725 (1965).
[17] K. Egger and H. Voigt, *Z. Pflanzenphysiol.* 53, 64 (1965).
[18] T. R. Parsons, *J. Marine Res.* 21, 164 (1963).

$CHCl_3$; second dimension, petrol. ether (60–80°) containing 2%, v/v *n*-PrOH (after Jeffrey).[19] The carotenes, which were themselves unseparated, were well separated from the xanthophylls (other than the pigment previously tentatively identified as monohydroxy-5,6-epoxy-α-carotene,[7] which overlaps the carotene spot). There was good separation of the xanthophylls, with reasonably consistent R_fs for the same xanthophyll. For reproducible results in particularly humid weather it is necessary to activate the 3MM chromatography paper by incubation at 65° for 1 hr immediately before use. Comparison of R_fs was made with those of authentic samples in single dimensions, run at the side of the paper. Lutein and zeaxanthin were moderately well separated, although there was slight overlapping. The chromatographic system was not very successful when applied to unsaponified extracts because the chlorophylls tended to overlay the xanthophylls. A comparison of the saponified and unsaponified pigments of those organisms with abnormal chlorophycean-type pigment patterns was made using both the polyamide and paper chromatographic systems.

In all cases attempts were made to give a very rough estimate of the amount of each pigment, by relative colour intensities and size of spot. Checks by comparison of this visual estimate with the results of elution of the pigments and spectrophotometric determinations indicated a good agreement, using the *Platymonas tetrathele* and *Pyramimonas obovata* saponified extracts.

Identification of Pigments

No attempt was made to identify rigorously the pigments present in the phytoflagellates. Identification was based entirely upon R_fs in two dimensions, colour and the positions of the pigments relative to one another, unless stated otherwise. Comparison was made with the R_fs of authentic samples of the named pigments obtained in earlier investigations.[3–7] The identity of xanthophylls K1S and K2S in *Pachysphaera* sp. (*P*339). *Pterosperma* sp. (*P*302) and in *Asteromonas propulsa* was additionally confirmed by separation of the pigments and absorption spectra in EtOH and $CHCl_3$ and by spectra after $NaBH_4$ reduction.[7]

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[19] S. W. JEFFREY, *Biochem. J.* **80**, 336 (1961).