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THE CAROTENOIDS OF THE PHYTOFLAGELLATE, *MICROMONAS PUSILLA*

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Abstract—The carotenoids of the green flagellate, *Micromonas pusilla*, have been investigated. The flagellate contained α-carotene, β-carotene, violaxanthin, luteoxanthin and neoxanthin at approximately 3·4, 10·4, 5·7, 2·3 and 12·6 per cent respectively of the total carotenoids. In addition, unidentified and probably previously undescribed carotenoids, M1, F1, F2B1, F2B2, F5B and F6 were present at approximately 3·4, 45·0, 3·5, 2·5, 5·0 and 5·8 per cent respectively of total carotenoids. Reduction of the principal pigment (F1), for which the name micronone has been proposed, yielded a pigment closely related to neochrome, which was probably dihydroxy-6′ (and/or 5′)-hydro-5,8-epoxy-β-carotene. The name microxanthin has been proposed for this pigment. In micronone one of the hydroxyl groups of microxanthin was replaced by a carbonyl grouping. Fraction M1 was probably a 5,6-monoepoxy-monohydroxy derivative of α-carotene. Fractions F2B1 and F2B2 were possibly dihydroxy-mono-5,8-epoxy xanthophylls or possibly dihydroxy-derivatives of α- and β-zeacarotene, and Fraction F6 possibly a tri-hydroxy-derivative of the latter. Fraction F6 was identical with reduced Fraction F5B. Fraction F5B contained one carbonyl grouping. The taxonomic significance of these findings is discussed.

INTRODUCTION

THE marine planktonic green flagellate *Micromonas pusilla* is one of the smallest flagellates known and is of bacterial dimensions. Its fine structure has been reported in two papers.[1,2] In an investigation into its chlorophyll content a protochlorophyll-like pigment was discovered.[3] The organism also showed a number of red xanthophylls on paper chromatography.[3] These results conflicted with the findings of Dr. G. Y. Kennedy, reported by Manton,[1] who found chlorophyll *a*, chlorophyll *b*, α- and β-carotene; and lutein as the only xanthophyll present. Because of this disagreement an investigation into the carotenoid content of this flagellate was carried out, as the carotenoid content of the organism was likely to be of importance in its classification. The classification of this and related organisms is at present in a state of flux.

RESULTS

The carotenoids, isolated by column adsorption chromatography, from *Micromonas pusilla* are shown in Table 1.

The absorption maxima of these pigments untreated, and after hydrochloric acid or borohydride treatment, are shown in Table 2.

The absorption spectra of Fractions M1, F1, F2B1, F5B and F6 are shown in Figs. 1–4. The absorption spectra of F2B2 resembled that of F2B1. Only one absorption spectrum is shown where the spectra of the carotenoid were similar in shape in all the solvents used. The

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[1] I. MANTON, *J. Marine Biol. Ass. U. K.* **38**, 319 (1959).

[2] I. MANTON and M. PARKE, *J. Marine Biol. Ass. U.K.* **39**, 275 (1960).

[3] T. R. RICKETTS, *Phytochem.* **5**, 223 (1966).

absorption spectra of Fraction F1 in chloroform and carbon disulphide were of similar shape to those in ethanol and petroleum ether respectively.

Table 3 presents the M_{50} values, relative polarities (r.p.) and reaction with dilute aqueous hydrochloric acid shown by the carotenoids.

TABLE 1. THE CAROTENOIDS OF *Micromonas pusilla* IN ORDER OF INCREASING ADSORPTION

Partition fraction	Initial separation	Colour adsorbed	Method of 2nd separation	Fractions	Colour adsorbed	Identification	Approx. percentage of total carotenoids
Light petrol fraction	1	Yellow	C	P1	Yellow	α-Carotene	3·4
				P2	Orange-yellow	β-Carotene	10·4
95% methanolic fraction	1	Yellow	B	M1	Yellow	Unidentified M1	3·4
				M2	Yellow	Unidentified	Trace
				M3	Red	Unidentified	Trace
90% methanolic fraction	1	Orange	B	F1	Orange		41·5
	2	Yellow	B	F2A	Orange	F1	3·5
				F2B*	Yellow	Unidentified F2B1	3·5
						Unidentified F2B2	2·5
				F2C	Scarlet-red	Unidentified F2C	0·4
	3	Yellow					
	4	Yellow	B	F4A	Yellow	Violaxanthin	5·7
				F4B		Luteoxanthin	2·3
	5	Orange	A	F5A	Yellow	F4	Trace
				F5B†	Orange-red	Unidentified F5B	5·0
	6	Greenish-yellow		F6		Unidentified F6	5·8
	7	Yellow	B	F7A	Yellow	Neoxanthin	12·6
				F7B	Yellow	Unidentified	Trace

The initial separation of the partition fractions was by chromatography (Method A). Chromatography was carried out: (A) icing sugar eluted with light petrol (b.p. 40–60°) containing increasing concentrations of acetone; (B) magnesium oxide: Celite Hyflo Supercel 1:1 (w/w) eluted as in (A); (C) as in (B) but eluted with hexane containing 2% acetone.

* This fraction was further subfractionated by chromatography by method A.

† This fraction was further purified by chromatography as in (B), but eluting with petroleum ether (b.p. 40–60°) containing increasing concentrations of methanol.

Paper chromatography of acetone extracts of a number of other phytoflagellates, using the method of Jeffrey,[4] also showed the presence of red xanthophylls. These were demonstrated in *Micromonas squamata* (Plymouth No. 290); *Heteromastix longifilis* (Plymouth No. 58); *Heteromastix* sp. (Plymouth No. 198) and *Pyramimonas amylifera* (Plymouth No. 246). The results have been reported previously.[3] These flagellates all contained a protochlorophyll-like pigment.[3] Preliminary work suggests that these red pigments are identical with Fractions F1 and/or F5B reported in *Micromonas pusilla.*

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

TABLE 2. THE ABSORPTION MAXIMA OF THE CAROTENOIDS FROM *Micromonas pusilla* IN VARIOUS SOLVENTS

Fraction	Absorption maxima (nm)				
	Light petrol	Chloroform	Ethanol	95% ethanol	Carbon disulphide
α-Carotene (P1)	(420),[*] 444, 473[†]	(427), 455, 485			
β-Carotene (P2)	(427), 449, 478[†]				
Unidentified M1	421, 445, 473	(427), 453, 482		(420), 444, 472	
M1 after HCl treatment[‡]				422, 448	
Unidentified F1	(417), 440, 467	454	445	447	(442), 470, 500
F1 after borohydride reduction	375, 394, 418, 444	(382), 404, 427, 455	(375), 396, 420, 447	(375), 396, 419, 446	(392), 420, 444, 474
Unidentified F2B1	(402), 425, 451	(410), 437, 463		(405), 428, 453	
Unidentified F2B2	(400), 423, 448	(408), 436, 459		427, 450	
Unidentified F2C	468, 500				
Violaxanthin (F4A)	(390), 415, 438, 468	(397), 425, 450, 481	(392), 417, 441, 470		
F4A after HCl[‡]			380, 402, 426		
Luteoxanthin (F4B)	(375), 401, 422, 448	(385), 410, 433, 458	403, 425, 450		
F4B after HCl[‡]			380, 402, 426		
Unidentified F5B	(430), 458, 484	473	465		
F5B after borohydride reduction	(402), 424, 450	(385), (408), 436, 461			
Unidentified F6	(403), 425, 451	(410), 436, 461	(406), 427, 453	(405), 427, 453	
Neoxanthin (F7A)	411, 434, 465		(390), 413, 437, 465		
F7A after HCl[‡] (neochrome)			(375), 398, 421, 449		(395), 421, 446, 476

[*] Brackets indicate the approximate position of a point of inflexion.

[†] In hexane, α-carotene gives the same maxima in light petrol (b.p. 40–60°).

[‡] 0·01 ml aq. N HCl added per 2 ml ethanolic carotenoid solution (0·005 N final concentration).

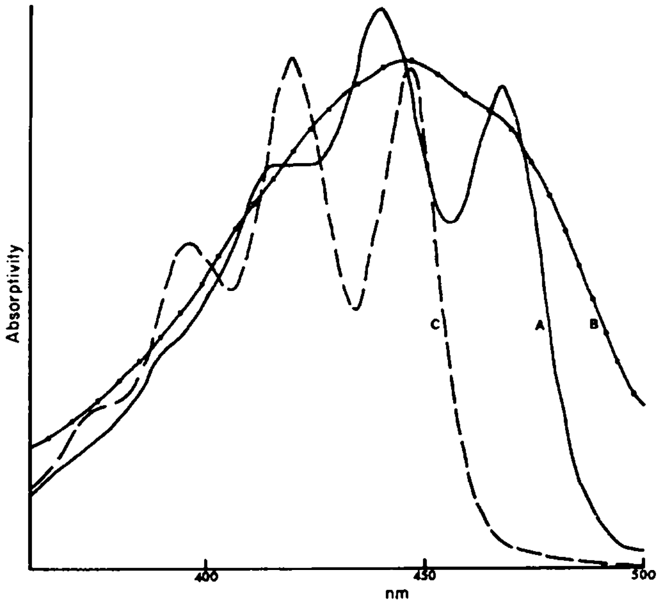

FIG. 1. ABSORPTION SPECTRA OF FRACTION F1.

A = Fraction F1 in light petrol, b.p. 40–60°;
B = Fraction F1 in ethanol;
C = Fraction F1, after borohydride reduction, in ethanol.

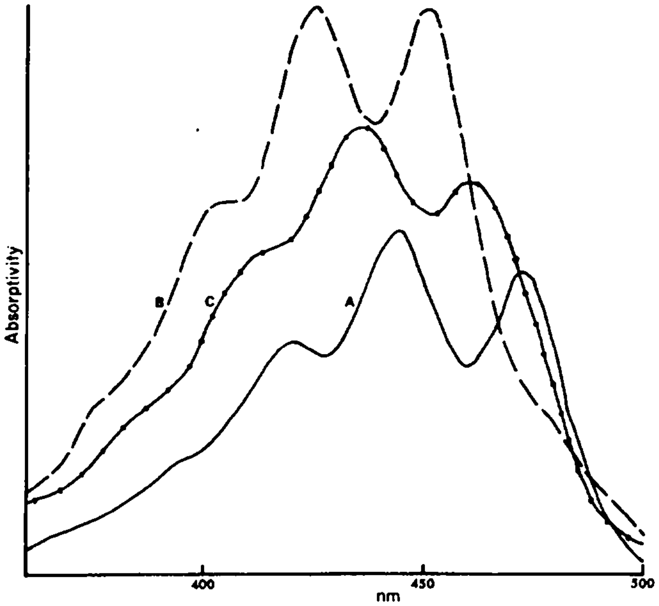

FIG. 2. ABSORPTION SPECTRA OF FRACTIONS M1, F2B1 AND F6.

A = Fraction M1 in light petrol, b.p. 40–60°;
B = Fraction F2B1 in light petrol, b.p. 40–60°;
C = Fraction F6 in chloroform.

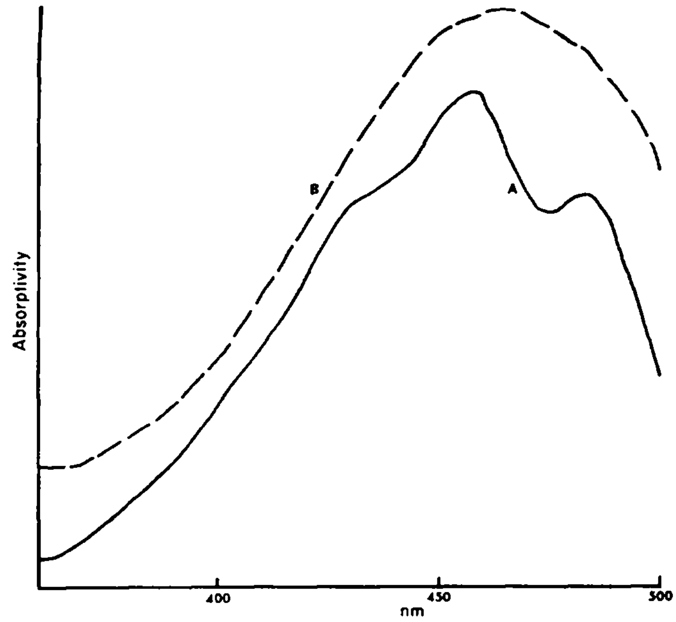

FIG. 3. ABSORPTION SPECTRA OF FRACTION F5B.

A = Fraction F5B in light petrol, b.p. 40–60°;
B = Fraction F5B in ethanol.

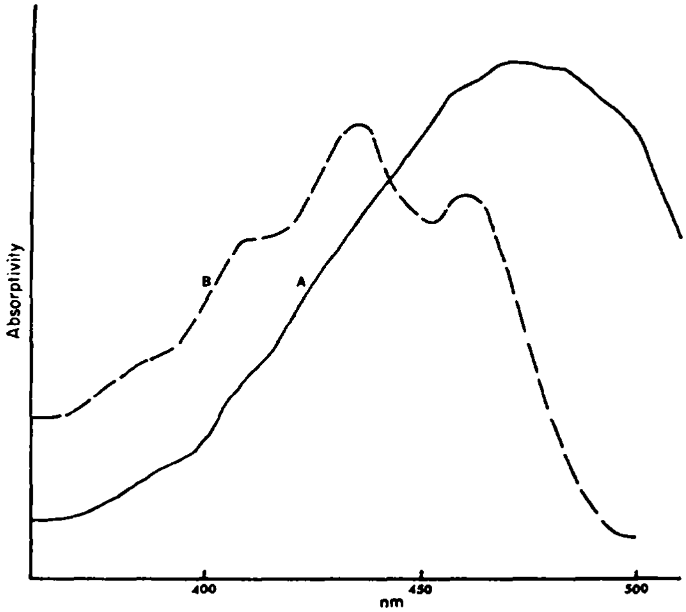

FIG. 4. ABSORPTION SPECTRA OF FRACTION F5B.

A = Fraction F5B in chloroform;
B = Fraction F5B, after borohydride reduction, in chloroform.

TABLE 3. THE M_{50} VALUES, RELATIVE POLARITIES AND REACTIONS WITH DILUTE HCl* OF THE XANTHOPHYLLS OF *Micromonas pusilla*

Fraction	M_{50} value (%)	Relative polarity	λ shift after dilute HCl	Structure indicated
M1	96·5	1·34	+	5,6-Monoepoxide
F1	78·2	2·03	nil	
F1 reduced	70·9	2·31	nil	Monoketone in F1
F2B1	80·1	1·96	nil	
F2B2	78·5	2·02	nil	
F4A	65·6	2·51	+	5,6-Diepoxide
F4B	66·1	2·49	+	5,6-Monoepoxide
F5B	66·3	2·48	nil	
F5B reduced	59·3	2·74	nil	Monoketone in F5B
F6	60·7	2·69	nil	
F7A		roughly 3	+	5,6-Monoepoxide

* As described in Table 2.

DISCUSSION

(a) *Identity of the Carotenoids*

The named carotenoids were identified by their chromatographic behaviour, by the shape and position of absorption maxima in various solvents[5, 6] and, where applicable, their reaction with dilute aqueous hydrochloric acid and their relative polarities (r.p.). It is to be noted that the maxima at 403 nm (in ethanol) for luteoxanthin is higher than published value of about 395 nm.[7] This may well be due to some trace contamination with auroxanthin.

*Fraction M*1. This is a 5,6-monoepoxide. The r.p. indicates that it probably also possesses a nonallylic hydroxyl group. A comparison of its absorption spectra and that of its 5,8-epoxide derivative with 5,6- and 5,8-monoepoxides of α-carotene[5, 6] indicates that M1 is probably a 5,6-monoepoxide monohydroxylated derivative of α-carotene.

*Fraction F*1. The single absorption maxima in ethanol and the change in absorption spectrum and r.p. on borohydride treatment indicate that F1 possesses a carbonyl grouping. These spectral properties are said to be characteristic of a carbonyl group in conjugation with the ethylenic double-bond system.[6] The shift of 5 nm to longer wavelengths of the spectrum in ethanol as compared to that in light petrol also indicates the possession of one conjugated keto group.[8] The r.p. indicates that F1 also probably possesses one nonallylic hydroxyl group and an epoxide group. An ethereal solution gave no colour with conc. HCl.

*Fraction F*1 *reduced.* The absorption spectra of this carotenoid supported the idea of an epoxide structure. Lack of reaction with dil. HCl indicated that the possible monoepoxide structure must have the 5,8 position. An ethereal solution of the pigment gave no colour with conc. HCl. This reaction is said to be rather unreliable for epoxides (see Ref. 5 for discussion). Chrysanthemaxanthin gave no reaction with conc. HCl and pure samples of neoxanthin also did not react, although impure samples did.[5] The reduced pigment showed no change of colour

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[5] T. W. GOODWIN. In *Modern Methods of Plant Analysis.* (Edited by K. PAECH and M. V. TRACEY) Vol. 3, p. 272. Springer-Verlag, Berlin (1955).

[6] B. H. DAVIES. In *Chemistry and Biochemistry of Plant Pigments* (Edited by T. W. GOODWIN), p. 489. Academic Press, New York (1965).

[7] N. I. KRINSKY and R. P. LEVINE, *Plant Physiol.* 39, 680 (1964).

[8] T. W. GOODWIN, D. G. LAND and M. E. SISSINS, *Biochem. J.* 64, 486 (1956).

on treatment with acid chloroform, which indicated that neither it nor the unreduced pigment contained allylic hydroxyl groups.[9] This was supported by the change of 0·28 in r.p. on reduction of F1 instead of the 0·17 change on reduction of an allylic carbonyl group. This interpretation conflicted with the spectral properties of F1 which suggested a conjugated carbonyl group and which would presumably result in an allylic hydroxyl group on reduction. The shape of the absorption curve and its maxima were almost identical with the 5,8-epoxide of neoxanthin[10, 11] [3,3′5′ (or 6′)-trihydroxy-6′ (or 5′)-hydro-5,8-epoxy-β-carotene (neochrome)]. However the r.p. of reduced F1 indicated that it had one nonallylic hydroxyl group less (the reduced carbonyl-group making a total of two hydroxyl groupings). A comparison of the absorption maxima with those of mutatoxanthin (3,3′-dihydroxy-5,8-epoxy-β-carotene), with chrysanthemaxanthin (3,3′-dihydroxy-5,8-epoxy-α-carotene), and with flavoxanthin (a stereoisomer of chrysanthemaxanthin),[6] indicated that they were dissimilar. The loss of a conjugated double bond, such as has been suggested in neoxanthin,[10] leads to a drop in absorption maxima.[6] The results indicated that reduced F1 had probably only nine conjugated double bonds (cf. neoxanthin[10]). It thus appears probable that the reduced pigment F1 was a dihydroxy-6′ (and/or 5′)-hydro-5,8-epoxy-β-carotene, and that in the unreduced pigment F1 one of the hydroxyl groups was replaced by a carbonyl group. The untreated pigment has not, to my knowledge, been previously described and the name *micronone* is suggested for it, and *microxanthin* for the dihydroxy-compound.

Fraction F2B1. This was not a 5,6-epoxide. Its r.p. of 1·96 could indicate the presence of two nonallylic hydroxyl groups in the molecule. However its absorption spectra were not in agreement with known dihydroxy-carotenoids. A compound with two allylic hydroxyl groups and one 5,8-epoxide group (r.p. = 2·02) would also roughly satisfy a r.p. of 1·96. The shape of the absorption curve and low wavelengths of the absorption maxima would be consistent with this. The xanthophyll most resembled an unidentified 5,8-monoepoxide reported in *Euglena gracilis* in its spectral properties, but was not as polar.

Fraction F2B2. This showed a r.p. of 2·02 and had no 5,6-epoxide nature. A discussion similar to F2B1 can also be applied to this pigment. Fractions F2B1 and F2B2 might be dihydroxylated α- and β-zeacarotene carotenoids. Their shape and absorption maxima are very similar to α- and β-zeacarotene.

Fraction F2C. This was present in amounts insufficient to do more than determine rather inaccurate absorption curves.

Fraction F5B. This pigment had a r.p. of 2·48 and had no 5,6-epoxide group. In the shape of its absorption spectra it resembled Fraction F1, particularly in the difference in shape between petroleum ether and ethanol or chloroform solution. The increase in relative polarity on borohydride reduction indicated that it contained one carbonyl group. This was supported by the single absorption maximum in ethanol, which also indicated that the carbonyl group was in conjugation with the ethylenic double-bond system. It is possible to account for the r.p. due to functional groups other than the keto-group (1·76) by two allylic hydroxyl groups (r.p. = 1·78).

Fraction F5B reduced. The absorption spectra and lack of reaction with dil. HCl indicate that this pigment contained no 5,6-epoxide group. The absorption spectra of the reduced pigment was identical in shape and position of maxima with Fraction 6, and the relative polarities were also almost identical. The two pigments could not be separated by chromato-

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[9] F. J. Petracek and L. Zechmeister, *J. Am. Chem. Soc.* **78**, 1427 (1956).

[10] T. H. Goldsmith and N. I. Krinsky, *Nature* **188**, 491 (1960).

[11] N. I. Krinsky and T. H. Goldsmith, *Arch. Biochem. Biophys.* **91**, 271 (1960).

graphy by method A. It thus seems probable that the two are identical. The absorption spectra also much resembled Fraction F2B1, but the r.p. of the former was 0·78 greater.

A comparison of the spectral properties of Fraction F5B indicated similarities with other carotenoids but could be distinguished from them by having a different r.p. and a different product of borohydride reduction. Neoperidinin,[12] prepared from peridinin, exhibited a single maximum in ethanol at 464 nm. Little information is available about this pigment, but if it resembles peridinin in its decomposition by alkalies, it cannot be the same as Fraction F5B. It is possible that this pigment is a trihydroxylated derivative of zeacarotene as the absorption spectra of the two are remarkably similar in shape and position of maxima.

Fraction 6. This appeared to be identical with reduced Fraction F5B. It thus did not contain carbonyl or 5,6-epoxide groupings. Its r.p. was 2·69. Treatment with acid chloroform[9] resulted in products showing a single, broad, rather asymmetric peak absorbing at about 455 nm, resembling that of 3,4-dehydro-α-carotene.[13] This finding supported the allylic nature of one or more of the hydroxyl groups of this xanthophyll. Unfortunately insufficient material was available to repeat this observation on Fraction F5B to confirm the allylic nature of any of the hydroxyl groups present in this pigment. The discussion of Fraction F5B (reduced) also applies to Fraction F6.

(*b*) *General Discussion*

The pigment composition of *Micromonas pusilla* is of particular interest as it shows some affinities to the purple photosynthetic bacterium, *Rhodopseudomonas spheroides*, in its possession of a protochlorophyll-like pigment, magnesium 2,4-divinylphaeoporphyrin a_5 monomethyl ester,[3, 14, 15] and also to many algal groups in its carotenoid composition. The majority of its xanthophylls are in a high state of oxidation and show resemblances to fucoxanthin of the diatoms, golden and brown algae, to peridinin of the dinoflagellates, and also to echinenone of the blue-green algae. In its high concentration of epoxide xanthophylls it resembles the euglenoids.

The reason for the conflict of xanthophyll results with those of Dr. G. Y. Kennedy, reported by Manton,[1] is not known. The identity of the organism (in unialgal culture) used in the present study, was confirmed by electron-microscopic observation which showed the typical hair-point flagellum. It had been observed that old stored pigment preparations sometimes gave the 5,8-epoxide derivatives rather than the 5,6-epoxides reported in this study, but the main xanthophyll F1 was relatively stable. The latter bears some resemblance in absorption spectrum to lutein in light petrol solution, but the maxima are at somewhat lower wavelengths.

Micromonas pusilla has a high proportion of epoxide containing xanthophylls (at least 60–70 per cent of the total carotenoids). In this it resembles *Euglena gracilis*.[10] It also possesses a high (51·0 per cent) concentration of carbonyl group containing carotenoids. In its possession of neoxanthin and violaxanthin it resembles *Chlorella pyrenoidosa*[16] and *Chlamydomonas reinhardi*,[7] while differing from them in other ways. The ratio of β-carotene to α-carotene is 3·1, which resembles the results obtained for *Chlorella pyrenoidosa*,[16] but not those obtained for *Chlamydomonas reinhardi* (ratio 10·4).[7] Pigments with similar absorption

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[12] H. H. STRAIN, W. M. MANNING and G. HARDIN, *Biol. Bull. Marine Biol. Lab.* **86**, 169 (1944).

[13] G. KARMAKER and L. ZECHMEISTER, *J. Am. Chem. Soc.* **77**, 55 (1955).

[14] O. T. G. JONES, *Biochem. J.* **86**, 429 (1963).

[15] O. T. G. JONES, *Biochem. J.* **89**, 182 (1963).

[16] M. B. ALLEN, T. W. GOODWIN and S. PHAGPOLNGARM, *J. Gen. Microbiol.* **23**, 93 (1960).

spectra (but different maxima) to Fractions F1 and F5B have been described in two other genera of green algae. Siphonoxanthin, present in several species of *Caulerpa*, showed absorption maxima at 455 nm in ethanol and at 452 and 480 nm in light petrol, whilst red forms of *Protosiphon* contained two unidentified ketonic carotenoids.[17] The former pigments presumably have a ketonic nature.

Since *Micromonas pusilla* contains both chlorophylls *a* and *b*, it is usually included among the Chlorophyta though not necessarily among Chlorophyceae. Thus in the classification used by Parke and Dixon[18] (modified from Christensen[19]) *Micromonas pusilla* and the other flagellates which have been shown to share an apparently similar and distinctive carotenoid composition (Ricketts[3] and the present communication) have been placed in the class Prasinophyceae. Preliminary results for *Pedinomonas minor*, an organism of disputed taxonomic position[20] but which might be placed in the Prasinophyceae, indicated that its carotenoid composition was quite different from that of *Micromonas pusilla* and that it was therefore not very closely related to the latter.

It is also possible to speculate, though probably rather prematurely, that these organisms in the Prasinophyceae (and possibly the Prasinophyceae as a whole) may occupy a position at a branching point in protistan evolution (cf. Goodwin[21]).

EXPERIMENTAL

Cultures

Micromonas pusilla (Plymouth No. 27) was kindly provided by Dr. Mary Parke, of the Plymouth Laboratory, as a unialgal, bacteria-containing culture. It was grown at 24° with 16 hr illumination (200 lm/ft^2) and 8 hr darkness per day. Its identity was confirmed by examining shadowed, osmium tetroxide fixed, direct electron-microscopic preparations in a Siemens Elmiskop 1.

The cells were harvested and the pigments extracted with 90% aq. acetone as described in Ricketts.[3] The possible effect of the bacterial content of the culture was also discussed in this paper. Six litres of culture yielded considerably less than 1 g wet weight of cells.

Pigments

The pigments were extracted from the 90% aq. acetone solution into diethyl ether after dilution with water, washed several times with water, dehydrated with anhydrous sodium sulphate, and evaporated to dryness at room temperature under nitrogen. The pigment was then dissolved in ethanol and saponified as described in Goodwin.[5] The resulting ethereal carotenoid solution was evaporated to dryness under nitrogen. The dried deposit was dissolved in light petrol (b.p. 40–60°), aided by the addition of the minimum quantity of diethyl ether necessary for solution. This solution was then extracted, first with 90% aq. methanol, and later 95% aq. methanol, as described in Goodwin,[5] to yield three fractions

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[17] H. H. STRAIN. In *Manual of Phycology* (Edited by G. M. SMITH), p. 243. Chronica Botanica, Waltham, Mass. (1951).

[18] M. PARKE and P. S. DIXON, *J. Marine Biol. Assoc. U. K.* **44**, 499 (1964).

[19] T. CHRISTENSEN. In *Botanik* (Edited by T. W. BOCHER, M. LANGE and T. SØRENSEN), Bd. 2 (Systematisk Botanik), Nr. 2, 1. Munksgaard, Copenhagen (1962).

[20] H. ETTL and I. MANTON, *Nova Hedwigia* **8**, 421 (1964).

[21] T. W. GOODWIN (Ed.), *Chemistry and Biochemistry of Plant Pigments*, p. 127. Academic Press, New York (1965).

named the 90% methanolic, the 95% methanolic, and the light petrol fraction. The carotenoids were then isolated from these fractions by chromatography as described in Table 1.

Chromatography

(A) Icing sugar (British Sugar Corporation) eluted with light petrol (b.p. 40–60°) containing increasing concentrations of acetone. All reagents used in this study were of analytical quality, where available, and obtained from British Drug Houses Ltd., unless specified otherwise.

(B) Magnesium oxide: Celite Hyflo Supercel 1:1 (w/w) eluted as in (A). The magnesium oxide "for chromatography" was obtained from British Drug Houses Ltd. and the Hyflo Supercel from Koch-Light Laboratories Ltd.

(C) Magnesium oxide: Hyflo Supercel 1:1 (w/w) eluted with hexane containing 2% acetone.[22]

The larger scale chromatography was carried out on 1·4 × 10 cm adsorbent columns. The smaller scale chromatography used 4 × 50 mm adsorbent columns in Pasteur pipettes. Elution was speeded with the aid of positive pressure applied to the top of the columns. In all operations exposure to light was reduced to a minimum. Evaporations of pigments were carried out as rapidly as possible under a stream of nitrogen at slightly above room temperature.

Relative polarities and M_{50} values were determined as described in Krinsky[23] except that light petrol b.p. 40–60°, was used instead of b.p. 30–60°. This seemed to give similar relative polarities. Optical densities in these assays were determined at three wavelengths, which gave supporting evidence for the purity of the fractions.

Ethanolic solutions of carotenoids were treated with dil. HCl, at a final concentration of 0·005 N, for the detection of 5,6-epoxide groups.[7] The reaction was followed spectrophotometrically to completion before determination of absorption spectra. All absorption spectra were determined using a Unicam SP600 spectrophotometer.

Borohydride reduction was carried out as described in Krinsky.[23]

The quantitative determination of the approximate pigment composition was arrived at by assuming $E^{1\%}_{1\text{cm.}}$ values of 2500 for the unidentified carotenoids. The investigations were hampered, in most cases, by the small amounts of the pigments available for analysis.

Acknowledgements—I would like to thank the Science Research Council for financial support. I am indebted to Professor I. Manton, F.R.S., for discussions on the taxonomy of these flagellates.

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[22] L. ZECHMEISTER and L. V. CHOLNOKY, *Principles and Practice of Chromatography* (Translated by A. L. BACHARACH and F. A. ROBINSON). Chapman & Hall, London (1943).

[23] N. I. KRINSKY, *Anal. Biochem.* **6**, 293 (1963).