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ON THE PIGMENTS OF THE CHRYSOPHYCEAE

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(Text-figs. 1–3)

The colour of the chromoplasts in the Chrysophyceae varies from a greenish yellow to a golden brown. Klebs (1892) described this colour as being due to a special pigment which he called 'chrysochrom'. Gaidukov (1900) made an acetone extract of a dense bloom of *Chromulina rosanoffii* (Woronin, 1880) Bütschli, filtered from the tanks in the cold glasshouses of the Botanic Gardens in St Petersburg. He found a water soluble pigment which he called 'phycochrysin' together with 'chrysochlorophyll' and 'chrysoxanthophyll'. Most authors have since questioned the existence of special pigments in the Chrysophyceae (Fritsch, 1937; Smith, 1938). Carter, Heilbron & Lythgoe (1939) analysed extracts of an incrustation of *Apistonema carteri*, *Thallochrysis litoralis* and *Gleochrysis maritima* (identified by F. E. Fritsch) found on the chalk cliffs at Folkestone, and identified β-carotene, fucoxanthin and lutein, using calcium carbonate as adsorbent in their analysis. Heilbron (1942) later drew attention to the fact that these pigments are present in the Bacillariophyceae but not in the Xanthophyceae, throwing doubt on Pascher's view of the close relationship of all three groups. We now know that it is not lutein but diadinoxanthin that is present in the Bacillariophyceae (Strain, Manning & Hardin, 1944). Seybold, Egle & Hülsbruch (1941) then showed that the chlorophyll present in *Hydrurus foetidus* and *Chromulina rosanoffii* was chlorophyll *a*, apparently without other chlorophylls. Seybold (1941) has commented further on the singular occurrence and implications of chlorophyll *a* alone, and this has been recently discussed by Allen (1958). In recent years our knowledge of the pigments of the algae has been greatly advanced by Strain and his colleagues (Strain, 1958) but, while the pigments of the Xanthophyceae and Bacillariophyceae have been examined with the improved techniques now available (Pace, 1941; Strain, Manning & Hardin, 1943, 1944; Strain, 1958), those of the Chrysophyceae have not.

This paper does not represent a complete account of the pigments occurring in these organisms, but results presented here are of interest in view of the relationship of the Chrysophyceae with other algae.

METHOD AND MATERIAL

The pigments of eight species have been analysed. Unialgal cultures were grown for some weeks until a density sufficient to give good extracts was obtained. In most instances about 4–6 l. of culture after 6 weeks of (winter) growth was adequate. The organisms were centrifuged, treated with distilled water to extract any water-soluble pigments, recentrifuged and then extracted with acetone. The acetone extract was taken into light petroleum (B.P. 40–60°) and separated into epiphasic and hypophasic fractions with 90% methanol, the methanolic layer being twice extracted with light petroleum, and the petrol layer similarly extracted with 90% methanol. The hypophasic pigments were driven into diethyl ether by addition of water. Both phases were evaporated to dryness and redissolved in light petroleum. The epiphase was chromatographed on activated alumina or magnesium oxide, the column being developed by addition of acetone to the light petroleum. The hypophase was chromatographed on sugar (Tate and Lyle icing sugar containing 1½% calcium phosphate) and the column developed by dropwise addition of *n*-propanol to the light petroleum. The bands in each case were eluted and portions evaporated and redissolved in hexane and carbon disulphide (epiphasic) or ethanol and carbon disulphide (hypophasic) for examination in a Unicam S.P. 500 spectrophotometer. The chlorophylls were examined in light petroleum.

The following species from the Plymouth cultures have been examined:

	Plymouth no.
Pseudopedinella sp.	167
Phaeaster-type with haptonema	168
Pavlova gyrans Butcher	93
Isochrysis galbana Parke	I
Phaeocystis pouchetii-motile *Prymnesium* stage	147
Chrysochromulina ericina P. & M.	25
Dicrateria inornata Parke	B
Hymenomonas sp.	156

In addition, extracts of the diatom *Phaeodactylum tricornutum* Bohlin and of *Dunaliella tertiolecta* Butcher, a chlorophycean, were also analysed in the same manner to provide specimens of other xanthophylls for running mixed chromatograms with those of the Chrysophyceae.

RESULTS

Cultures of *Isochrysis galbana* were analysed on five or six different occasions and this species has been studied in more detail than the others.

In every species all the pigment was extractable by acetone and was soluble in light petroleum; no water-soluble pigments were found either on the preliminary treatment with distilled water, or when the hypophase was re-extracted with diethyl ether.

ISOCHRYSIS GALBANA

Epiphase

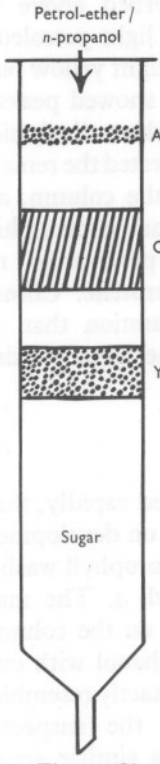
Careful development and slow elution of the bands formed on alumina or magnesium oxide showed that α-, β- and γ-carotenes were present. All the chlorophyll appeared to be chlorophyll *a*, strongly adsorbed above the carotenes and showing maxima at 430, 612 and 660 mμ in light petroleum. The addition of a trace of acetone to the developer caused a light yellow band to separate and move rapidly down the column. This band showed peaks at 445 and 474 mμ in hexane and 477 and 504 mμ in carbon disulphide, characteristic of α-carotene. Further addition of acetone separated the remaining carotene into a main orange band slowly passing down the column, and showing peaks at 451 and 478 mμ in hexane, 485 mμ in carbon disulphide (β-carotene), and a narrower red band above with the main peaks at 461 mμ in hexane and 496 mμ in carbon disluphide, resembling γ-carotene. Chromatography using magnesium oxide effected a better separation than on alumina, when both β-carotene and γ-carotene exhibited the upper maxima characteristic of these compounds.

Hypophase

If extraction and chromatographic analysis were performed rapidly, three yellow or orange bands were obtained on the sugar column on development with *n*-propanol: light petroleum mixtures (Fig. 1). Any chlorophyll washed through first; it always showed the spectrum of chlorophyll *a*. The main orange band resembled fucoxanthin in colour and position on the column, and in having a single absorption maximum (446 mμ) in ethanol with only a slight inflexion near 470 mμ. The suspected fucoxanthin exactly resembled that of *Phaeodactylum*, and a mixed chromatogram of the suspected fucoxanthin from *Isochrysis* and a specimen prepared by a similar separation on icing sugar of the xanthophylls of *Phaeodactylum* did not separate. The spectrum of each was similar (Fig. 2). The lower, yellow band could, from its spectrum, be either lutein or diadinoxanthin. A mixed chromatogram was run with a specimen of lutein prepared from *Dunaliella*, when the two pigments separated again on the column, indicating that the pigment was not lutein. When mixed with diadinoxanthin prepared from *Phaeodactylum*, however, no separation occurred when washed down a sugar column with the appropriate *n*-propanol:light petroleum mixture. The *Isochrysis* pigment also separated when run against violaxanthin from *Dunaliella*.

The two main xanthophylls of *Isochrysis* are, therefore, fucoxanthin (the main pigment) and diadinoxanthin (Fig. 3). The amber band above the fucoxanthin also exhibited a single broad peak in ethanol and was presumably an isomer (neofucoxanthin). If extraction and analysis extended over

a long period, an additional yellow band was sometimes obtained below the diadinoxanthin. This could, from its position on the column, be diatoxanthin, but its absorption spectrum and colour resembled diadinoxanthin and it probably represented an artifact.



	Absorption maxima (mμ)						Pigment
	In ethanol			In carbon disulphide			
Amber-yellow		445			475	504	neofucoxanthin
Orange		446			475	506	fucoxanthin
Yellow	425	445	476	442	474	506	diadinoxanthin

Fig. 1. Chromatographic separation of the hypophasic pigments of *Isochrysis*.

OTHER SPECIES

A similar analysis of the other species produced substantially the same chromatographic picture, though mixed chromatograms have not been run in every case. Chlorophyll *c* was not detected and chlorophyll *a* appeared to be the only chlorophyll present. In the hypophasic fraction of each the two main pigments were fucoxanthin and diadinoxanthin, the fucoxanthin showing maxima in ethanol:

448 mμ	from culture no. 167, 168
447 mμ	25, B
449 mμ	156

These maxima are lower than that found by Strain *et al.* (1944)—453 mμ—but the mixed chromatograms with *Phaeodactylum* fucoxanthin (447 mμ) suggest

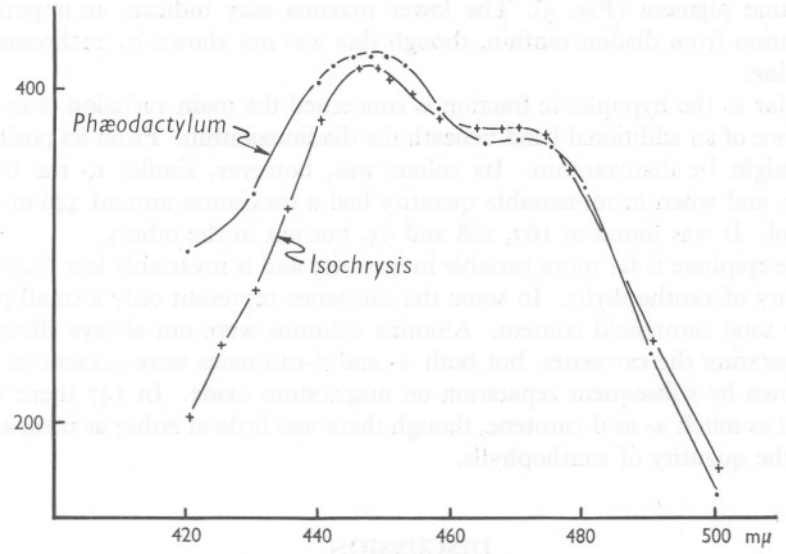


Fig. 2. Absorption spectra of fucoxanthin from *Phaeodactylum* and *Isochrysis* in ethanol.

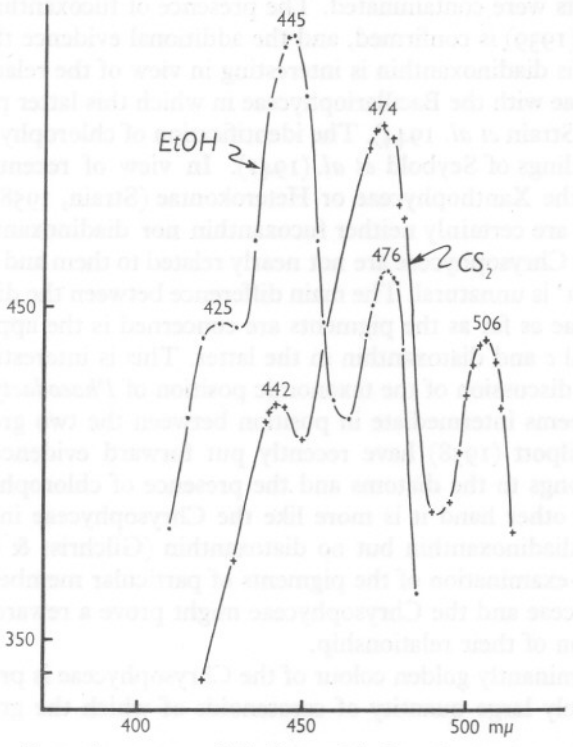


Fig. 3. Absorption spectra of diadinoxanthin from *Isochrysis* in ethanol and carbon disulphide.

the same pigment (Fig. 3). The lower maxima may indicate an imperfect separation from diadinoxanthin, though this was not shown by rechromatographing.

As far as the hypophasic fraction is concerned the main variation is in the presence of an additional band beneath the diadinoxanthin. From its position this might be diatoxanthin. Its colour was, however, similar to the band above, and when in measurable quantity had a maximum around 446 mμ in ethanol. It was found in 167, 168 and 93, but not in the others.

The epiphase is far more variable in quantity and is invariably less than the quantity of xanthophylls. In some the carotenes represent only a small part of the total carotenoid content. Alumina columns were not always effective in separating the carotenes, but both α- and β-carotenes were present in all, as shown by subsequent separation on magnesium oxide. In 147 there was almost as much α- as β-carotene, though there was little of either as compared with the quantity of xanthophylls.

DISCUSSION

No water-soluble pigments have been found and presumably Gaidukov's (1900) extracts were contaminated. The presence of fucoxanthin reported by Carter *et al.* (1939) is confirmed, and the additional evidence that the lutein-like pigment is diadinoxanthin is interesting in view of the relationship of the Chrysophyceae with the Bacillariophyceae in which this latter pigment is also to be found (Strain *et al.* 1944). The identification of chlorophyll *a* alone confirms the findings of Seybold *et al.* (1941). In view of recent work on the pigments of the Xanthophyceae or Heterokontae (Strain, 1958) in which the xanthophylls are certainly neither fucoxanthin nor diadinoxanthin, it would seem that the Chrysophyceae are not nearly related to them and that Pascher's 'Chrysophyta' is unnatural. The main difference between the diatoms and the Chrysophyceae as far as the pigments are concerned is the apparent absence of chlorophyll *c* and diatoxanthin in the latter. This is interesting in relation to the recent discussion of the taxonomic position of *Phaeodactylum* which in some ways seems intermediate in position between the two groups. Lewin, Lewin & Philpott (1958) have recently put forward evidence that *Phaeodactylum* belongs to the diatoms and the presence of chlorophyll *c* confirms this. On the other hand it is more like the Chrysophyceae in having fucoxanthin and diadinoxanthin but no diatoxanthin (Gilchrist & Green, 1960). A detailed re-examination of the pigments of particular members of both the Bacillariophyceae and the Chrysophyceae might prove a rewarding study for the elucidation of their relationship.

The predominantly golden colour of the Chrysophyceae is presumably due to the relatively large quantity of carotenoids of which the greater part are xanthophylls.

I am most grateful to Dr M. Parke for culturing the algae for me at the Plymouth Laboratory, and I also wish to thank Dr B. M. Gilchrist of Bedford College for kindly providing the cultures of *Phaeodactylum* and *Dunaliella*.

SUMMARY

No special pigments have been found in any of the marine Chrysophyceae examined. In all species the only chlorophyll identified was chlorophyll *a*. The carotenes represent a much smaller part of the total carotenoids than the xanthophylls, and the amount varies greatly from one species to another, perhaps accounting for their variable colour. α-Carotene represents a large proportion of the carotenes; γ-carotene was identified only in *Isochrysis galbana*. Fucoxanthin and diadinoxanthin represent the main xanthophylls.

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