The Carotenoids of Algae: Pigments from some Cryptomonads, a Heterokont and some Rhodophyceae

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SUMMARY

The carotenoids of two Cryptophytes, one heterokont and five Rhodophytes have been examined. The main pigments present are, in *Cryptomonas* sp., α -carotene and diatoxanthin; in *Hemiselmis virescens*, α -carotene, and diatoxanthin; in *Vischeria* sp., β -carotene and three unidentified xanthophylls; in *Antithamnion plumula* and *Nemalion multifidum*, β -carotene, lutein and neoxanthin; in *Erythrotrichia carnea*, *Rhodorus marinus* and *Polysiphonia fastigiata*, β -carotene, zeaxanthin and lutein.

INTRODUCTION

The carotenoids of algae have always attracted biochemists because of their diversity as compared with those present in the leaves of higher plants (see Goodwin, 1952, 1961; Strain, 1958). During the past few years interest has been greatly stimulated because of the isolation for the first time of a number of unicellular algae in pure culture and because of the development of suitable media in which they could be cultivated in the laboratory on a comparatively large scale. Reports have appeared on the carotenoids of the Cryptomonad *Cryptomonas ovata* (Haxo & Fork, 1959) and of a number of Chrysophyceae, *Ochromonas danica* and *Prymnesium parvum* (Allen, Goodwin & Phagpolngarm, 1960), *Isochrysis galbana* (Dales, 1960; Jeffrey, 1961), *Pseudopedinella* sp., *Phaeaster* type *Pavolva gyrans*, *Phaeocystis pouchetti*, *Chrysochromulina ericina*, *Dicrateria inomata* and *Hymenomonas* sp. (Dales, 1960) and *Sphaleromantis* sp. (Jeffrey, 1961). Strain (1958) has described the pigments in a large number of Rhodophytes. The present paper reports our findings on two further Cryptomonads, *Hemiselmis virescens* and *Cryptomonas* sp., the heterokont *Vischeria* sp. and on several previously unexamined Rhodophytes: *Nemalion multifidum*, *Antithamnion plumula*, *Erythrotrichia carnea*, *Rhodorus marinus*, and *Polysiphonia fastigiata*.

METHODS

Cultivation. Cultures of *Vischeria* sp. were grown in the medium described in Table 1, at 18–20° and illuminated by 'cool white' fluorescent tubes (175 f.c.). The Cryptomonads were grown under conditions described by Allen, Dougherty & McLaughlin (1959). Three species of red algae (*Nemalion multifidum*, *Antithamnion plumula* and *Erythrotrichia carnea*) were collected on the west coast of Sweden and have since been cultivated in 100 ml. Pyrex flasks of an artificial sea water, ASP 6 (Provasoli, McLaughlin & Droop, 1957). Diatoms and other small algae were removed by washing and careful manipulations under microscope, and by transfers

Table 1. *Medium for Vischeria* sp.

Peptone (Difco) (0.1 %) containing the macroelements and microelements indicated (2 ml. of micronutrient mix/l. medium).

Macronutrients		Micronutrients	
	M		mg./l.
NaNO ₃	0.003	B(H ₃ BO ₃)	0.5
CaCl ₂	0.00022	Mn(MnSO ₄ ·H ₂ O)	0.5
MgSO ₄	0.00062	Zn(ZnSO ₄ ·7H ₂ O)	0.005
NaCl	0.00043	Cu(CuSO ₄ ·5H ₂ O)	0.02
K ₂ HPO ₄	0.00044	Mo(MoO) 85 %	0.01
KH ₂ PO ₄	0.0018	V(NH ₄ VO ₃)	0.01
		Fe(NaFeEDTA)*	4.0

* Diethylenetriaminopentaacetate.

to new flasks. After repeated treatments with antibiotics and fungicides *N. multifidum* was obtained free from bacteria and yeasts (Fries, 1961). The cultures of *A. plumula* and *E. carnea*, however, were not completely freed from bacterial or yeast contaminants. *Nemalion multifidum* was cultivated under a bank of five fluorescent tubes (Philips) consisting of two reflector-backed TL 55, one TL 5, and one TL 17. The light intensity was about 800 lux. The temperature was about 20°. *Antithamnion plumula* was cultivated at 10° under the combined irradiation of an incandescent filament lamp, Luma 500 W., and a mercury-vapour lamp, 265 W., in a light intensity of 700 lux. *Erythrotrichia carnea* was cultivated under the conditions described by Fries (1960). After an incubation time of about 6 weeks the algae were removed from the flasks and freeze-dried immediately without washing. *Rhodospirillum rubrum* was obtained in axenic culture from Dr G. Giraud; it was collected at Banyuls (Giraud, 1958) and cultivated in artificial sea water ASP 6 at 20° F. (Fries, 1960) and under the light conditions described above for *N. multifidum*.

Polysiphonia fastigiata was collected at Aberystwyth.

Extraction, separation and identification of pigments. Full details of our general procedures have already been reported (Allen *et al.* 1960). In addition thin-layer chromatography with the apparatus designed by Stahl (1962) was used in part of this work.

RESULTS

Carotenoid in Cryptomonads

Cryptomonas sp. strain 35. The carotenoids were chromatographed on a mixture of 3 + 1 (w/w) ZnCO_3 + celite and the column developed with light petroleum containing increasing concentrations of ether. Three main carotenoids were obtained (Table 2). Fraction 1 percolated through the column slowly with light petroleum as eluant, but ran through quickly with light petroleum containing 2% (v/v) diethyl ether. It was purified on activated alumina (Grade H) and exhibited the chromatographic and spectral properties of α -carotene. This was confirmed by co-chromatography on ZnCO_3 + celite (3 + 1 by wt.) with an authentic sample of α -carotene prepared from carrots. No separation was obtained. No β -carotene was detected, although a very faint band, with an indeterminate absorption spectrum, closely followed α -carotene on the column.

Table 2. *The carotenoids present in Cryptomonas sp. strain 35*

Pigment extract chromatographed on ZnCO_3 celite (3 + 1, by wt.); fractions listed in order of increasing absorptive power.

Fraction	Description	Absorption maxima ($m\mu$) in light petroleum (b.p. 60–80°)	Identification	Concn. (mg./g. dry wt.)	% of total pigment
1	Yellow	447.5, 477.5	α -Carotene	0.28	13.5
2	Orange	430*, 451, 482	Diatoxanthin	1.08	73.5
3	Pale yellow	449, 478	Unknown	0.25	13.0
Total				1.61	—

* Inflexion.

Table 3. *The carotenoids present in Hemiselmis virescens*

Pigment extract chromatographed on ZnCO_3 + celite (3 + 1, by wt.); fractions listed in order of increasing absorptive power.

Fraction	Description	Absorption maxima ($m\mu$) in light petroleum (b.p. 60–80°)	Identification	Concn. (mg./g. dry wt.)	% of total pigment
1	Yellow	447.5*, 477.5,	α -Carotene	0.076	15.9
2	Yellow	425, 447.5, 477.5	(?)	0.108	22.5
3	Yellow-orange	430*, 452, 483	Diatoxanthin	0.160	33.0
4	Bright red†	430*, 450, 480	Unknown	0.140	28.6
Total				0.484	—

* Inflexion.

† Maxima in ethanol.

Fraction 2 closely resembled diatoxanthin first described in diatoms by Strain, Manning & Hardin (1944) and reported recently in *Prymnesium parvum* (Allen *et al.* 1960). The pigment most closely resembling diatoxanthin is zeaxanthin. Fraction 2 was, however, easily separated from an authentic specimen of zeaxanthin,

prepared from yellow maize, on a ZnCO_3 + celite column. Furthermore, the absorption maxima of samples obtained at different times were consistently slightly higher than those of zeaxanthin (448–449, 478 $m\mu$, in light petroleum). Fraction 3 was very tightly absorbed on the ZnCO_3 + celite column, and was eluted only with diethyl ether containing a trace of ethanol. It was not identified.

Hemiselmis virescens. The four pigments obtained from this organism (Droop's strain) are described in Table 3. Fraction 1 passed slowly through the column with light petroleum and was shown to be α -carotene by co-chromatography with an authentic sample from carrots on ZnCO_3 + celite (1 + 1) and on activated alumina.

Fraction 2 had the spectral properties of lutein. That this was not lutein was demonstrated by co-chromatography on a ZnCO_3 + celite column with an authentic sample of lutein isolated from grass; complete separation was obtained.

Fraction 3, the main fraction, resembled diatoxanthin in its properties; on co-chromatography with the corresponding pigment from the *Cryptomonas* species examined in the previous experiment, no separation was obtained. Fraction 4, which formed a very tightly bound red zone at the top of the column, could only be eluted with ethanol. It was unidentified.

Table 4. *The carotenoids present in Vischeria sp.*

Pigment extracts chromatographed on ZnCO_3 + celite (3 + 1, by wt.); fractions listed in order of increasing absorptive power.

Fraction	Description	Absorption maxima ($m\mu$) in light petroleum (b.p. 60–80°)	Identification	Concn. (mg./g. dry wt.)	% of total pigment
1	Yellow	430*, 451, 480	β -Carotene	3.27	64.1
2	Pale yellow	Trace only	Unknown	Trace	—
3	Orange-yellow	422.5, 447, 475	Unknown	0.86	17.0
4	Bright yellow	443, 472	Unknown	0.56	11.0
5	Yellow	425*, 445, 473	Unknown	0.32	7.9
Total				5.01	—

* Inflexion.

Carotenoids in a heterokont

Vischeria sp. Chromatographic separation of the carotenoids of *Vischeria sp.* yielded five components (Table 4). The first band was shown in the usual way to be β -carotene; no trace of α -carotene could be observed. Fraction 2 is a small yellow band which appears to correspond to that observed in other heterokonts by Strain (1958), who states 'the two principal xanthophylls were often preceded by traces of another xanthophyll which formed a light yellow zone'. Fraction 3 was the main xanthophyll fraction; it was easily separated from lutein, which was less strongly absorbed, and was somewhat less strongly absorbed than diatoxanthin; it resembles the 'moderately sorbed xanthophyll' reported by Strain (1958). Fractions 4 and 5 also correspond to unknown xanthophylls reported by Strain. The very high relative concentration of β -carotene should be emphasized.

Carotenoids in Rhodophytes, subclass Florideae

Nemalion multifidum. This alga, which belongs in the order Nemalionales, family Helminthocladiaceae, contained the pigments indicated in Table 5. Rechromatography of fraction 1 on alumina resulted in its separation into two zones; the major pigment was identified as β -carotene and the minor one as α -carotene by co-chromatography with authentic samples. Fraction 3 was identified as lutein by comparison with an authentic sample from grass. The need to identify lutein unequivocally was important, because it has recently been shown that antheraxanthin (5,6-epoxyzeaxanthin), a pigment with an absorption spectrum almost identical with that of lutein, is the main xanthophyll of *Euglena* (Krinsky & Goldsmith, 1960). Fraction 3, which could not be separated from authentic lutein on the

Table 5. *The carotenoids present in Nemalion multifidum*

Pigment extract chromatographed on ZnCO_3 + celite (3 + 1, by wt.); fractions listed in order of increasing absorptive power.

Fraction	Description	Absorption maxima (m μ) in light petroleum (b.p. 60–80°)	Identification
1	Yellow	425*, 446, 478	β -Carotene
2	Pale yellow	442.5, 448, 472	Unknown
3	Yellow-orange	442.5, 446, 475	Lutein
4	Pale yellow	420, 438, 468	Neoxanthin (probably)

α -Carotene also present in small amounts.

* Inflection.

Table 6. *Quantitative distribution of carotenoids in Antithamnion plumula and Nemalion multifidum*

Pigment	<i>A. plumula</i>		<i>N. multifidum</i>	
	mg./g. (dry wt.)	% of total pigment	mg./g. (dry wt.)	% of total pigment
α -Carotene	Traces	—	0.014	1.7
β -Carotene	0.108	14.9	0.103	12.6
Unknown	0.042	5.8	0.050	6.1
Lutein	0.0480	66.5	0.400	49.0
Neoxanthin?	0.092	12.8	0.050	30.6
Total	0.722	—	0.617	—

ZnCO_3 + celite column, was therefore mixed with authentic antheraxanthin prepared from *Euglena gracilis*; the two pigments were easily separated on a ZnCO_3 + celite column. Confirmation was obtained by running a thin-layer chromatogram of the various pigments on a silicic acid + rice starch plate with a mixture of ethyl acetate + light petroleum (85 + 65, by vol.) as developer. Fraction 3 was not separated from lutein, but was separated from antheraxanthin. Fraction 4 closely resembled neoxanthin and was not separated completely from an authentic specimen of neoxanthin, although very slight separation appeared to take place in occasional

samples. We concluded that fraction 4 was probably neoxanthin, but cannot state this categorically. Fraction 2 occurred only in traces and was not identified.

Antithamnion plumula. The pigments in this alga, which belongs in the order Ceramiales, family Ceramiaceae, are the same as those found in *Nemalion multifidum* and will not be described in detail. They do, however, differ quantitatively as indicated in Table 6.

Polysiphonia fastigiata. The pigments in this alga, a member of the order Ceramiales, family Rhodomelaceae, were a comparatively simple mixture of β -carotene, zeaxanthin and lutein (Table 7); these were identified by comparison with authentic pigments. It is significant that zeaxanthin predominates over lutein.

Table 7. *The carotenoids present in Polysiphonia fastigiata*

Pigment extract chromatographed on ZnCO_3 +celite; fractions listed in order of increasing absorptive power.

Fraction	Description	Absorption maxima ($m\mu$) in light petroleum (b.p. 60–80°)	Identification	Concn. (mg./g. dry wt.)	% of total pigment
1	Yellow	430*, 452, 481	β -Carotene	0.024	27.3
2	Orange-yellow	430*, 451, 480	Zeaxanthin	0.040	55.4
3	Yellow	423, 445, 472.5	Lutein	0.024	27.3
			Total	0.088	—

* Inflexion.

Table 8. *The carotenoids present in Erythrotrichium carnea*

Pigment extract chromatographed on ZnCO_3 +celite (3+1, by wt.); fractions listed in order of increasing absorptive power.

Fractions	Description	Absorption maxima ($m\mu$) in light petroleum (b.p. 60–80°)	Identification	Concn. (mg./g. dry wt.)	% of total pigment
1	Yellow	430*, 452, 481	β -Carotene	0.058	29.0
2	Orange-yellow	430*, 451, 480	Zeaxanthin	0.081	40.5
3	Yellow	423, 445, 472.5	(?) Lutein	0.061	30.5
			Total	0.200	—

* Inflexion.

Carotenoids in Rhodophytes, subclass Bangioideae

Erythrotrichia carnea. Only one very small sample (49 mg./dry wt.) of this alga, which belongs to the order Bangiales, was available. Considerable difficulty was encountered in extracting the carotenoids; they were released slowly with absolute methanol; no extraction occurred with acetone or absolute ethanol. A comparatively low concentration of pigment was present (0.2 mg./g. dry wt. compared with, for example, 0.8 mg./g. in *Nemalion multifidum*). All the separations reported here were done on 40 μ g. of pigment. A small column was used (15 $\times$ 0.8 cm.) and the absorption spectra were measured in microcells containing 0.3 ml. instead of 3–4 ml. of solution; the separation obtained is recorded in Table 8. Fractions 1,

and 2 were clearly identified by co-chromatography with authentic samples of β -carotene and zeaxanthin, respectively. As far as could be judged with the small amounts available, no α -carotene was present. Fraction 3 was similar in adsorptive power to lutein, but its absorption maxima were at slightly lower wavelengths. Unfortunately, it was not possible to carry out a mixed chromatogram with authentic lutein, but we feel that C is probably lutein which had been isomerized during experimental manipulation.

Rhodosorus marinus. The pigment separation obtained with this alga, a member of the order Porphyridiales, is given in Table 9. By the usual criteria Fractions 1, 2 and 3 were identified as β -carotene, zeaxanthin and lutein, respectively. There was no indication of the presence of α -carotene and there is more than ten times more zeaxanthin present than lutein.

Table 9. *The carotenoids present in Rhodosorus marinus*

Pigment extract chromatographed on ZnCO_3 + celite (8+1, by wt.); fractions listed in order of increasing absorptive power.

Fractions	Description	Absorption maxima ($m\mu$) in light petroleum (b.p. 60–80°)	Identification	Concn. (mg./g. dry wt.)	% of total pigment
1	Yellow	430*, 452, 480	β -Carotene	0.086	21.0
2	Orange-yellow	430*, 452, 481	Zeaxanthin	0.291	70.9
3	Yellow-orange	427.5, 447.5, 477.5	Lutein	0.024	8.1
			Total	0.401	—

* Inflexion.

DISCUSSION

Cryptomonads. The *Cryptomonas* sp. which was examined in this work is very similar to *Cryptomonas ovata* examined by Haxo & Fork (1959). In both cases, the major carotene is α -carotene, with no β -carotene. The major xanthophyll is diatoxanthin in *Cryptomonas* sp. and in *C. ovata* 'the principal xanthophyll corresponded closely to zeaxanthin; however, identity with diatoxanthin was not precluded' (Haxo & Fork, 1959). Furthermore, the xanthophyll pigments preponderate in both species, the ratio xanthophylls to carotenes being 6.4:1 and 2.4:1 for *Cryptomonas* sp. and *C. ovata*, respectively. In neither species was lutein, fucoxanthin or peridinin observed. The situation in *Hemiselmis virescens* is very similar; diatoxanthin is the main xanthophyll, and α -carotene is the main carotene; β -carotene could not be detected.

Heterokonts (Xanthophyceae). Strain (1958) studied a number of heterokonts including *Vischeria stellata*. The present results on our *Vischeria* sp. are in general agreement with his in that β -carotene is the main carotene present and three or four unidentified pigments are present in the xanthophyll fraction. Lutein was not identified in any specimen. This compares with the observation of Jamikorn (1954) that lutein was present in *Tribonema aequale*, *Heterococcus fuoreresis*, and *Ophio-cytium majus*. A unique feature of our strain of *Vischeria* sp. is that β -carotene predominates over the xanthophylls.

The Rhodophytes. To facilitate more detailed discussion of the red algae the general classification of the Bangioideae and Florideae is given in Table 10. *Rhodossorus marinus* (order Porphyridiales) is like another member of the same order, *Porphyridium cruentum* studied by Strain (1958) in synthesizing zeaxanthin and lutein but not α -carotene. *Erythrotrichia carnea*, a member of another order, Bangiales, in the subclass Bangioideae, qualitatively resembles *R. marinus*; the two algae differ quantitatively, however, in that the amount of zeaxanthin relative to that of lutein is very much greater in *R. marinus* (9:1) than in *E. carnea* (1:8:1). *Erythrotrichia carnea* is generally similar to the two Bangiales, *Porphyra naiadum* and *P. perforata*, studied by Strain (1958) in synthesizing zeaxanthin and lutein; however, *P. perforata* also synthesized α -carotene, which was not observed in *E. carnea* or *P. naiadum*.

Table 10. *The general classification of the Bangioideae and Florideae (Rhodophyta)*

Subclass I, Bangioideae	
Order 1, Porphyridiales	<i>Rhodossorus</i> <i>Porphyridium</i>
Order 2, Goniotrichales	<i>Goniotrichum</i>
Order 3, Bangiales	<i>Erythrotrichia</i> <i>Porphyra</i>
Subclass II, Florideae	
Order Nemalionales	
Fam. Chantransiaceae (<i>Rhodochorton</i>)	
Fam. Helminthocladiaceae (<i>Nemalion</i> , <i>Cumagloea</i>)	
Fam. Chaetangiaceae (<i>Gloiphloea</i> , <i>Galaxaura</i>)	
Order Ceramiales	
Fam. Ceramiaceae (<i>Antithamnion</i>)	
Fam. Rhodomelaceae (<i>Polysiphonia</i>)	

Nemalion multifidum (order Nemalionales, family Helminthocladiaceae) differs from *Cumagloea andersonii* and *Galaxaura* sp. (same family; Strain, 1958) in that it does not synthesize zeaxanthin. Furthermore, *N. multifidum* synthesizes a pigment similar to neoxanthin; Strain (1958) never reported neoxanthin in any of a large number of Rhodophytes which he examined. *Polysiphonia fastigiata* (order Nemalionales, family Rhodomelaceae) is very similar to three other *Polysiphonia* species examined by Strain (1958), viz. *P. aquamara*, *P. californica* and *P. collinsii*. In all cases α -carotene was not detected and zeaxanthin preponderated over lutein; indeed rather surprisingly, no lutein was found by Strain in *P. collinsii*.

The observations recorded here on five Rhodophytes which had not previously been examined confirm the general conclusion of Strain (1958), who examined a very large number of red algae, that the main qualitative variations in the carotenoid pigment systems of the Rhodophytes, irrespective of the order to which they belong, are the presence or absence of zeaxanthin and the presence or absence of α -carotene. The further demonstration of the existence of α -carotene and β -carotene derivatives together in Rhodophytes adds weight to the conclusion that on evidence from caro-

tenoid studies the Rhodophytes occupy a central position in the evolutionary pattern of the algae (Goodwin, 1962).

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