Carotenoid Distribution in Certain Naturally Occurring Algae and in some Artificially Induced Mutants of *Chlorella pyrenoidosa*

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SUMMARY

The carotenoids of pure cultures of two members of the Class Chrysophyceae were examined; the main pigments in *Ochromonas danica* and *Prymnesium parvum* are fucoxanthin and β -carotene. The main carotenoids of *Cyanidium caldarium* are β -carotene and zeaxanthin; these observations support the view that this alga represents a transitional form between the cryptomonads and the green algae. The distribution of polyenes and carotenoids in a number of ultra-violet induced mutants of *Chlorella pyrenoidosa* is described.

INTRODUCTION

The general nature of the carotenoid pigments in many classes of algae is now reasonably well established (see Goodwin, 1958*a*; 1959*a, b*; 1960). However, little is known of the carotenoids in the class Chrysophyceae, the only report available being that of Heilbron (1942) in which he reported β -carotene, lutein (3,8-dihydroxy- α -carotene) and fucoxanthin (structure unknown) in a mixed culture of *Apistonema carteri*, *Thallochrysis litoralis* and *Gloeschrysis maritima*. The reasons for this dearth of information have been: (a) the difficulty in isolating pure strains of Chrysophyceae; (b) the difficulty in devising culture media on which pure strains could be cultivated in bulk in the laboratory. These difficulties have now been overcome and the first part of this paper is concerned with the nature of the carotenoids present in pure strains of *Ochromonas danica* and *Prymnesium parvum*.

The second part of the paper is devoted to a study of the carotenoids in the unique alga *Cyanidium caldarium*; taxonomically this organism can be considered to be a green alga (Chlorophyceae), although it is known to produce phycocyanins and only chlorophyll *a* (M. B. Allen, unpublished observations), which would suggest a close relationship with the Cyanophyceae (blue-green algae). However, as will be seen, its carotenoids are characteristic of neither the Chlorophyceae nor the Cyanophyceae.

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The final section of the paper deals with the carotenoid distribution in some ultra-violet induced mutants of *Chlorella pyrenoidosa*. Similar investigations on X-ray induced mutants of *C. vulgaris* have been carried out by Claes (1954).

Parallel studies on the distribution of chlorophylls in these algae will be reported elsewhere.

METHODS

Organisms and methods of cultivation

Cultures of *Ochromonas danica* and *Prymnesium parvum* were obtained from Dr J. J. A. McLaughlin. *O. danica* was grown in the defined medium shown in Table 1. *P. parvum* was grown in medium 'DC' of Provasoli, McLaughlin & Droop (1957). Both organisms were grown at 18–20° under approximately 300 f.c. of 'cool white' fluorescent light.

Table 1. *Defined medium for Ochromonas danica*

With either 0.5 % glucose added or 5 % CO₂ in air bubbled through the medium. The inorganic micronutrients are based on the A 4 and B 7 solutions of Arnon (1938).

Macronutrients		Micronutrients	
	(M)		(mg./l.)
MgSO ₄	0.001	Thiamin	10
KNO ₃	0.005	Biotin	2
(NH ₄) ₂ SO ₄	0.005	Fe	4
CaCl ₂	0.0005	Mn	0.5
KH ₂ PO ₄	0.002	B	0.5
		Zn	0.05
		Cu	0.02
		Mo	0.01
		V	0.01

Mutants of *Chlorella pyrenoidosa* (Emerson strain; culture no. C-1. 1.10 in the Kaiser Foundation Research Institute culture collection) were obtained by ultra-violet irradiation with a Hanovia UV lamp. On plating the irradiated cultures, a large number of pigment mutants were found. With one exception (G 34) those investigated in the present work were originally of interest because their chlorophyll synthesis is disturbed so that they produce only chlorophyll *a* (Allen, 1958). The mutants were grown in a medium containing: MgSO₄, 0.001 M; KNO₃, 0.01 M; NaCl, 0.004 M; CaCl₂, 0.0005 M; K₂HPO₄, 0.002 M; glucose 0.5 %; inorganic micronutrients shown in Table 1. Mutants G-41 and G-44 were grown in dim light (100 f.c.); mutant G-34 was grown in the dark, as it is photosensitive.

Extraction, separation and identification of pigments

The harvested cultures were freeze-dried, sealed under nitrogen and sent air mail from Berkeley to Liverpool. It was shown in a previous investigation that this transportation has no ill effects on the carotenoids in dried algal cells (Goodwin & Gross, 1958). The dried organisms were weighed and then ground with acid-washed silver sand under acetone in a small mortar. The acetone extract was filtered through a sintered glass crucible (P × 4) and the process continued until the final acetone extract was colourless. The combined acetone extracts were then transferred to a

separating funnel and an equal volume of diethyl ether (freshly distilled over reduced iron to remove peroxides) added; this was followed by adding water slowly until two layers formed. All the pigment was present in the upper ethereal layer, which was washed several times with successive small amounts of water until free from acetone. The ether was removed *in vacuo* and the residue was either chromatographed directly or saponified before chromatography. Except in cases where fucoxanthin was present, the extracts were saponified before chromatography; this removed chlorophylls and lipids which might interfere with the development of chromatograms. Fucoxanthin is the only known carotenoid which is destroyed by alkali, so when this pigment was present the saponification step was omitted.

Saponification of the extracts was carried out by the standard method in use in this laboratory (Goodwin, 1955). The extracts were generally dissolved directly in 2 ml. light petroleum (b.p. 40–60°) ready for chromatography. When the extracts contained large amounts of fucoxanthin or other polyhydroxylated carotenoids (which are insoluble in light petroleum) they were first dissolved in 2–5 drops of ether and then diluted to an appropriate volume with light petroleum. A number of different adsorbents have been used to separate the pigments. Icing sugar (confectioner's sugar, Tate and Lyle Ltd.) was ground in a mortar and activated by heating in an oven at 80° for 6 hr. and then cooling in a desiccator; this adsorbent was used to separate xanthophylls. Alumina was used either activated (Grade 'O', Peter Spence and Sons Ltd., Widnes, Lancashire) or after deactivation according to the method of Goodwin & Srisukh (1949). Activated alumina was used for separating the partly saturated polyene hydrocarbons phytoene and phytofluene from the more unsaturated compounds such as ζ -carotene, neurosporene and β -carotene. Both phytoene and phytofluene are colourless; the latter was located on the column by its characteristic green fluorescence under ultraviolet irradiation. There is, however, no way easily to detect phytoene and it was obtained by collecting all the eluate running before phytofluene in 10 ml. fractions and bulking those fractions which exhibited the characteristic absorption peak of phytoene at 286 m μ (Goodwin, 1952*a*, 1955). Deactivated alumina was used for the rapid separation of β -carotene from oxygen-containing carotenoids.

Zinc carbonate + celite (4:1, w/w) was used extensively for the separation and purification of xanthophylls, and magnesium oxide + celite (4:1, w/w) for the purification of β -carotene. Both were activated by heating to 180°, and stored in the same way as icing sugar.

Alumina columns were prepared by using a slurry, whereas all other adsorbents were packed dry (Goodwin, 1955). The chromatograms were developed by the method of gradient elution. The solvent used in all cases was light petroleum (40–60°) containing progressively increasing amounts of acetone; the apparatus used was very similar to that described by Lederer & Lederer (1957); the original solvent was light petroleum (b.p. 40–60°) and the final solvent light petroleum containing 10% (v/v) acetone. Xanthophylls are separated much more clearly, with little tailing, when gradient elution is used.

The individual pigments were identified (*a*) by their position on the adsorption column relative to other known pigments, (*b*) by the shape of their absorption curves and the position of the absorption maxima in various solvents, (*c*) by mixed chromatography with an authentic specimen (Goodwin, 1955). The quantitative

determination of the various pigment fractions was carried out as described previously (Goodwin, 1955) using the $E_{1\%}^{1\text{cm}}$ values for each pigment quoted by Goodwin (1955). When the $E_{1\%}^{1\text{cm}}$ value of a pigment was unknown, it was assumed to be the same as that of β -carotene, i.e. 2500.

Partition tests were also applied to the isolated pigments. To carry out these tests a solution of the pigment in light petroleum was shaken with an equal volume of 90 or 95 % (v/v) methanol in water in a small test tube (ignition tube). In both tests hydrocarbons remain in the upper layer (epiphase). When 95 % methanol in water was the lower phase then all xanthophylls are hypophasic; with 90 % methanol in water the xanthophylls containing one oxygen function remain with the hydrocarbons in the epiphase whilst the remaining xanthophylls are hypophasic (Goodwin, 1955).

The pigment fractions were examined spectrophotometrically by using either a Unicam SP. 500 or SP. 600 instrument.

RESULTS

Carotenoids in Ochromonas danica and Prymnesium parvum (Chrysophyceae)

Ochromonas danica. Preliminary tests indicated the presence of fucoxanthin, so the step involving saponification had to be omitted. On chromatography on icing sugar, three main carotenoids were obtained (Table 2; the chlorophyll bands were ignored). Fraction A, which passed straight through the column with only light petroleum as developer, was purified on deactivated alumina and its identity with β -carotene was confirmed by co-chromatography with an authentic crystalline specimen (synthetic); no separation was obtained. The general properties of fraction B, which was developed with light petroleum containing 3 % (v/v) acetone, including its destruction by alkali, suggested that it was fucoxanthin. This was confirmed by comparing it with an authentic specimen isolated from *Laminaria saccharina* (Phaeophyceae). Both pigments had the same absorption spectrum and could not be separated on co-chromatography on either icing sugar or a ZnCO_3 + celite mixture. Fraction C which was firmly adsorbed on the column and was present only in small amounts was not identified. Quantitative determinations of the pigment fractions (Table 2) showed that fucoxanthin was the predominating pigment and that the total amount of pigment present was more than that recorded for various Phaeophyceae (see Goodwin, 1952*b*).

Prymnesium parvum. The pigment distribution in this alga was very similar to that observed in *Ochromonas danica*, but minor differences did exist. Fraction A

Table 2. *The carotenoids present in Ochromonas danica*

Pigment extract chromatographed on icing sugar; fractions listed in order of increasing adsorptive power.

Fraction	Description	Absorption maxima (m μ) in light petroleum	Identification	Concn. (mg./g. dry wt.)	% of total pigment
A	Yellow	448, 475	β -Carotene	0.725	17.0
B	Orange	448, 475	Fucoxanthin	3.160	73.8
C	Deep yellow	445	Unknown	0.395	9.2
			Total	4.28	

(Table 3) on purification on deactivated alumina separated into two zones; the lower yellow zone was shown to be β -carotene. The other zone was not unequivocally identified; it exhibited absorption maxima (light petroleum) at 424, 445 and 470 $m\mu$ and was probably a *cis* isomer of β -carotene. Fraction B showed properties very similar to that of zeaxanthin except that its absorption maxima in light petroleum were slightly higher; however, on co-chromatography with an authentic specimen of zeaxanthin prepared from maize the two fractions could be separated. Pigment B was therefore in all probability diatoxanthin, a pigment very similar to zeaxanthin found in diatoms by Strain, Manning & Hardin (1944). Diatoxanthin according to Strain *et al.* has absorption maxima at slightly higher wavelengths than has zeaxanthin and, as just stated, this was also true for pigment B. A comparison of the absorption maxima of the three pigments is given in Table 4. Pigments C and D were present only in trace amounts and could not be unequivocally identified with any known pigment. Fraction E was proved to be fucoxanthin using the same criteria as those described for *O. danica*, and fraction F appeared to be the same pigment as fraction C (Table 3) from *O. danica*. The total pigment concentration was over twice as great as in *O. danica*, but fucoxanthin still represented about 75% of the total pigments present.

Table 3. *The carotenoids present in Prymnesium parvum*

Pigment extract chromatographed on icing sugar; fractions listed in order of increasing adsorptive power.

Fraction	Description	Absorption maxima ($m\mu$) light petroleum	Identification	Concn. (mg./g. dry wt.)	% of total pigment
A	Yellow	—	β -carotene + a <i>cis</i> - isomer	0.70	7.4
B	Orange-yellow	450, 480	Diatoxanthin	0.65	6.1
C	Orange-yellow	420, 445-7	Unknown	0.28	2.7
D	Yellow	425, 450	Unknown	0.52	4.8
E	Orange	448, 475	Fucoxanthin	7.91	74.8
F	Yellow	445	Unknown—same as fraction C (Table 1)	0.51	4.7
Total				10.57	

Table 4. *A comparison of the absorption maxima ($m\mu$) of zeaxanthin, diatoxanthin and pigment B (Table 2)*

	Solvent.	
	Light petroleum (b.p. 40-60)	Ethanol
Zeaxanthin (ex maize; present work)	448, 476-478	450, 478
Diatoxanthin (Strain <i>et al.</i> 1944)	—	453, 478
Pigment B	450, 480	452, 481

Carotenoids in Cyanidium caldarium

The carotenoids present in *Cyanidium caldarium* were found to be β -carotene, zeaxanthin and lutein, with β -carotene predominating (Table 5). β -Carotene was identified in the usual way, whilst the identity of zeaxanthin was confirmed by

co-chromatography with authentic zeaxanthin on two adsorbents: icing sugar and ZnCO_3 + celite (4:1); no separation was obtained in either case. Fraction C was similarly identified by comparison with an authentic specimen of lutein isolated from grass. The total pigment concentration is very much less than in the two members of Chrysophyceae examined.

Table 5. *The carotenoids present in Cyanidium caldarium*

Pigment extract chromatographed on icing sugar; fractions listed in order of increasing adsorptive power.

Fraction	Description	Absorption maxima (m μ) light petroleum	Identification	Conc. (mg./g. dry wt.)	% of total pigment
A	Yellow	448, 473	β -Carotene	0.220	54.4
B	Orange-yellow	448, 475	Zeaxanthin	0.129	31.8
C	Yellow	443, 472	Lutein	0.057	13.8
Total				0.406	

Carotenoids in mutants of Chlorella pyrenoidosa

Mutant G. 34. The saponified extracts of this mutant were colourless and thus no carotenoids were present. However, chromatography on activated alumina demonstrated the presence of a colourless polyene, very similar to phytoene. As this compound is neither coloured nor fluorescent, the chromatogram was developed with light petroleum containing 0.2% (v/v) acetone and the eluate collected every 10 ml. A material with an absorption spectrum identical in shape and position of phytoene was obtained. Its distribution in the fractions collected was determined and in order to confirm the identity of this polyene with phytoene, an authentic specimen of phytoene was prepared from carrots, mixed with an equal amount of the mutant polyene and chromatographed as just described. The distribution curves for authentic phytoene and for the mixture were identical with that for the unknown polyene. This clearly demonstrated the identity of the two substances. The concentration of phytoene in the mutant was 1.71 mg./g. dry wt. which is of the same order as that for the total carotenoids in the wild strain of *C. pyrenoidosa* from which the mutant was derived (see Table 9).

Mutant G. 44. When the carotenoids of this mutant were chromatographed on icing sugar it was found that the greater part of the fraction passed rapidly through the column; that is, the main components were hydrocarbon in nature. Chromatography of this fraction on activated alumina yielded the fractions listed in Table 6. Fraction A 1 was identified as phytofluene by the position and shape of its absorption maxima, by its green fluorescence under ultraviolet irradiation and by its failure to separate from an authentic specimen of phytofluene (from carrots) on co-chromatography. Fraction A 2 was further purified on MgO + celite (3:1, w/w) and the major fraction had the same absorption spectrum and adsorptive power as ζ -carotene (Nash & Zscheile, 1945), but complete conformation could not be obtained because no authentic specimen of ζ -carotene was available. The minor component of fraction A 2 was shown to be β -carotene in the usual way. Fractions A 3 and A 4 contained traces of pigments with the same absorption spectrum as ζ -carotene but which were more strongly adsorbed. They were not examined further but they may

have been monohydroxy derivatives of ζ -carotene. It is interesting that although phytofluene is present in the mutant, phytoene is absent.

The xanthophyll fraction (B) was removed from the icing sugar and rechromatographed on ZnCO_3 + celite (4:1, w/w) using gradient elution. Six fractions were obtained, the absorption spectra of which are recorded in Table 7. As the xanthophylls represented only about 15 % of the total pigments and as each fraction was present in approximately equal amounts there was not sufficient material for detailed examination. However, it is highly likely that B 2 is lutein and that B 3 is a di- or poly-hydroxy derivative of ζ -carotene or of a polyene with a chromophoric

Table 6. *The hydrocarbon polyenes present in mutant G. 44 of Chlorella pyrenoidosa*

Pigments separated on MgO + celite mixture; fractions listed in order of increasing adsorptive power.

Fraction	Description	Absorption maxima ($m\mu$) light petroleum	Identification	Concn. (mg./dry wt.)	% of total hydrocarbon polyenes
A 1	Colourless; intense green fluorescence	381, 348, 367	Phytofluene	0.455	16.6
A 2	Yellow	378, 399, 424	ζ -Carotene	2.090	76.0
A 3	Pink	376, 399, 423	Unknown	0.066	2.4
A 4	Yellow	377, 398, 424	Unknown	0.139	5.0
				2.740	

Table 7. *The xanthophylls present in mutant G. 44 of Chlorella pyrenoidosa*

Pigments separated on ZnCO_3 + celite (4:1, w/w) by means of gradient elution; fractions listed in order of increasing adsorptive power.

Fraction	Description	Absorption maxima ($m\mu$) light petroleum	Identification	Concn. (mg./g. dry wt.)	% of total carotenoids
B 1	Yellow	374, 395, 418	Unknown	0.008	0.2
B 2	Orange	421, 443, 470	Lutein	0.064	2.0
B 3	Yellow	374, 397, 423	Hydroxy derivative of ζ -carotene or auroxanthin	0.180	5.5
B 4	Yellow	397, 415, 438	Unknown	0.114	3.3
B 5	Yellow	432, 460	Unknown	0.077	2.3
Total				0.443	13.3

system very similar to that of ζ -carotene. This appears to be the first time that such pigments have been reported. B 1, which is only present in traces, may be a hydroxylated derivative of ζ -carotene or a *cis* isomer of the corresponding ζ -carotene derivative (B 3). B 4 might be auroxanthin or a hydroxylated ζ -carotene, but it was not investigated further, and B 5, which also could not be immediately identified, might be a degradation product.

Mutant G. 41. The pigments detected in this mutant are summarized in Table 8. The general distribution was very similar to that observed in the wild strain and the individual pigments were identified in the usual manner. There are, however, two

minor differences: (a) α -carotene is not present in the mutant; (b) a mixture which could not be successfully separated and identified was different from the unknown fractions in the wild strains.

Table 8. *The carotenoids present in mutant G. 41 of Chlorella pyrenoidosa*

Xanthophylls separated on icing sugar; hydrocarbons on activated alumina; in both cases pigments were eluted with light petroleum containing increasing concentrations of acetone; pigments in order of increasing adsorptive power.

Fraction	Description	Absorption maxima (m μ) light petroleum	Identification	Concn. (mg./g. dry wt.)	% of total carotenoids
A	Yellow	425, 448, 473	β -Carotene	0.513	20.3
B 1	Orange	443, 472	Lutein	0.926	36.7
B 2	Yellow	Mixture	Unknown	0.690	26.4
B 3	Yellow	418, 440, 468	Violaxanthin	0.354	14.0
B 4	Yellow	415, 437, 468	Neoxanthin	0.039	1.6
Total				2.422	

Carotenoids present in Chlorella pyrenoidosa wild-type

In order to compare the pigments in the mutants with those of the parent strain the pigments present in *Chlorella pyrenoidosa* (wild-type) were examined; the results are summarized in Table 9. It will be seen that the main pigments are very similar to those reported in other green algae (Goodwin, 1952*b*, 1955). It is interesting, however, that α -carotene is present, although it is absent from most strains of *C. vulgaris* (Goodwin, 1955).

Table 9. *The carotenoids present in Chlorella pyrenoidosa wild-type*

Separation as described in Table 8 for mutant G 41 of *C. pyrenoidosa*.

Fraction	Description	Absorption maxima (m μ) light petroleum	Identification	Concn. (mg./g. dry wt.)	% of total carotenoids
A 1	Yellow	420, 445, 472	α -Carotene	0.09	3.6
A 2	Orange-yellow	425, 448, 473	β -Carotene	0.33	13.5
B 1	Yellow	442, 470	Lutein	1.11	54.8
B 2	Yellow	415, 438, 466	Violaxanthin	0.22	9.0
B 3	Pale yellow	399, 422, 443	Unknown	0.10	4.2
B 4	Deep yellow	415, 440, 467	Unknown	0.15	6.1
B 5	Yellow	417, 435, 463	Neoxanthin	0.21	8.8
Total				2.21	

DISCUSSION

The Chrysophyceae. The present work on pure cultures of *Ochromonas danica* and *Prymnesium parvum* confirms the earlier work of Heilbron (1942) who found β -carotene and fucoxanthin in a mixed culture of *Apistonema carteri*, *Thallochrysis litoralis* and *Gloeschrysis maritima*; it also demonstrates that fucoxanthin is by far the predominant pigment, representing some 75 % of the total carotenoids present.

Thus qualitatively the carotenoid distribution in these two examples of the class Chrysophyceae resembles very closely that found in the Phaeophyceae. The total amounts of pigment produced by the two algae vary considerably (Tables 2 and 3), but in any case the concentration in these two unicellular Chrysophyceae is much greater than that in the colonial Phaeophyceae for which values ranging from 0.36 to 1.9 mg./g. dry wt. have been recorded (Seybold & Egle, 1938). Indeed *P. parvum* (10.57 mg./g. dry wt.) now becomes, to the best of our knowledge, the richest known algal source of carotenoids, the previous highest value being *c.* 7.0 mg./g. dry wt. recorded for *Euglena gracilis* v. *bacillaris* (Goodwin & Jamikorn, 1954). The diatoms represent the other main class of algae which synthesize large amounts of fucoxanthin, and a relationship between the Chrysophyceae and this group is further indicated by the existence in *P. parvum* of diatoxanthin, a pigment closely related to zeaxanthin and previously considered unique to diatoms (Goodwin, 1952*b*).

Cyanidium caldarium

Cyanidium caldarium is perhaps the most interesting of the recently examined algae. Taxonomically it can be considered a green alga, although its claim to be classed as a blue-green alga would appear strong on purely biochemical grounds when it is realized that it synthesizes phycocyanins and only chlorophyll *a* (both characteristics of blue-green algae). The component carotenoids are, however, not characteristic of either the Cyanophyceae or the Chlorophyceae. They consist of a relatively large proportion of β -carotene (55 % of the total pigments) with zeaxanthin as the main xanthophyll. The high proportion of β -carotene is reminiscent of the blue-green algae (Goodwin, 1958*b*), although *Chlamydomonas reinhardtii* is also reported to contain comparative large amounts of β -carotene (Sager & Zalokar 1958). The only other alga which is known to produce zeaxanthin or a closely related pigment, in comparatively large amounts, is *Cryptomonas ovata*, the first member of the Cryptophyceae to be examined in pure culture (Haxo & Fork, 1959). The sporadic reports of zeaxanthin in some Phaeophyceae have been shown by Liaaen & Sørensen (1956) to be due to the conversion of violaxanthin into zeaxanthin, brought about by drying the algae without all necessary precautions. The carotenoid distribution in *C. caldarium* is not incompatible with the view that it is a transitional form between the cryptomonads and the green algae, because it is possible that the cryptomonads represent a primitive flagellate groups from which the Chlorophyceae have developed. Quantitatively the amount of carotenoids synthesized is rather low (see Goodwin, 1952*b*).

Mutant strains of *Chlorella pyrenoidosa*

It is interesting to compare the pigments produced by various mutants of *Chlorella pyrenoidosa* with those in the mutant strains of *C. vulgaris* previously described by Claes (1954) (summarized in Table 10). In the first place *C. pyrenoidosa* mutants G. 34 and *C. vulgaris* mutant 5/871 are very similar in that they produce only phytoene; furthermore, in G. 34 the amounts produced are approximately the same as the total amount of carotenoid synthesized by the parent strains. Both are light-sensitive in the presence of oxygen. *C. pyrenoidosa* G. 44 and *C. vulgaris* 5/515 and 9a all accumulate phytofluene and ζ -carotene, but unlike Claes's mutants, G. 44 does not synthesize phytoene. Furthermore, in G. 44 xanthophyll production was

Table 10. *Polyene distribution in mutants of Chlorella pyrenoidosa and C. vulgaris*

+ = present; - = absent.

	<i>C. pyrenoidosa</i>				<i>C. vulgaris</i>				
	Wild-type*	G 34	G 41	G 44†	Wild-type	5/871	5/515	5/520‡	9a§
Phytoene	-	+	-	-	-	+	+	+	+
Phytofluene	-	-	-	+	-	-	+	+	+
β -Carotene	+	-	+	-	+	-	-	-	-
ζ -Carotene	-	-	-	+	-	-	+	+	+
Protetrahydrolycopene	-	-	-	-	-	-	-	+	-
Prolycopene	-	-	-	-	-	-	-	+	-
Lutein	+	-	+	+	+	-	-	-	+
Violaxanthin	+	-	+	-	+	-	-	-	+
Neoxanthin	+	-	+	-	+	-	-	-	+

* Also synthesizes α -carotene.† Also synthesizes a hydroxylated ζ -carotene.

‡ These pigments are formed in the dark; normal plastid pigments are produced in the light.

§ An unidentified carotene is also present.

not completely inhibited (compare mutant 5/515), but it was decreased in amount and qualitatively altered (compare mutant 9a); only about 20% of the total pigments were xanthophylls and they appeared to be mainly either hydroxylated derivatives of ζ -carotene or compounds closely related to auroxanthin. It was not possible to decide between these alternatives. Our mutant G. 41 is interesting in that its ability to synthesize carotenoids has undergone little qualitative or quantitative change; its chlorophyll-synthesizing system has, however, been profoundly affected in that it does not synthesize chlorophyll *b* (Allen, 1958).

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