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Comparative Biochemistry of Carotenoids in Algae—III*

On Carotenoids in *Hypnea charoides***

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Abstract

To clarify the components of the carotenoids of *Hypnea charoides*, the carotenoids wete extracted and separated carotene fraction from xanthophyll fraction by partition coefficients according to the method of PETRACEK and ZECHMEISTER[11] Carotene fraction was submitted to the thin layer chromatography using magnesium oxide as adsorbent and 2. 5% acetone in ptroleum ether as developing solvent. Xanthophyll fraction was also submitted to the thin layer chromatography using microcel-C as adsorbent and 15% acetone in petroleum ether as developing solvent.

In *Hypnea charoides* the major carotenoids were α-carotene, β-carotene and lurein. The existence of phytoene was confirmed and also the existence of ε-carotene, prolycopene like and taraxanthin like compounds was identified. Neoxanthin having epoxide was present in very little amounts.

The occurrence of carotenoids as integral parts of the photosynthetic apparatus has led to speculation on their role in photosynthesis. Carotenoids are reported to have a number of functions in photosynthetic and phototactic organisms. Carotenoids play a role in the transport of oxygen and also protect the cell from photodynamic destruction[1),2),3)]. By absorbing light in the region where absorption by chlorophyll is low and transferring this energy to chlorophyll, chlorophyll incease the capacity of a plant or microorganism to gather light for photosynthesis[4)].

The present work was undertaken in order to clarify the carotenoids in *Hypnea charoides*, also to find the carotenoids which can be easily converted to astaxanthin in fishes[5)] and to find biosynthesis of carotenoids in algae.

On carotenoids in red algae, CARTER[6)], HEILBRON[7)] and LYTHGOE[8)] have reported that the predominating carotenoids were β-carotene and lutein. On the other hand, STRAIN[9)] has isolated α-carotene and zeaxanthin, besides β-carotene and lutein. The principle carotenoids in *Porphyra tenera* K. have been reported to be β-carotene which accounts for 60% of those present and little amounts of lutein, α-carotene, zeaxanthin and unconfirmed xanthophyll[10)].

The carotenoids of *Hypnea charoides* have been separated by adsorption chromatography and further characterized by their absorption spectra and behavior on partitioning between petroleum ether and aqueous methanol.

The principal carotenoids of *Hypnea charoides* are α-carotene, β-carotene and lutein,

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and little amounts of ε-carotene, prolycopene like compound and taraxanthin like compound are present.

phytoene like compound and neoxanthin having epoxide are present.

Experimental Procedure

The alga *Hypnea charoides* was collected at the Kagoshima Bay and extracted exhaustively with methanol and acetone. The deeply colored extract was diluted with water and the pigments transferred to petroleum ether in a separatory funnel. This solution was evaporated under reduced pressure, leaving an oil. For saponification 50 m*l*. of a solution of 6% potassium hydroxide in methanol were added, and the pigments were left at room temperature for 8 1/2 hours. The solution was then diluted to 50% methanol by adding the addition of water, and the carotenoids were extracted into petroleum ether, leaving the chlorophyll in the hypophase. The petroleum ether solutipn was washed throughly with water, dried over anhydrous sodium sulfate, and evaporated to an oil under reduced pressure.

partition tests between petroleum ether and aqueous methanol methanol were done according to the method of PETRCEK and ZECHMEISTER[11]. Just prior to use, each solvent phase was saturated with the other. After equilibration by shaking, the polyene hydrocarbones such as carotene were found in the upper phase "epiphasic" in contrast to the xanthophylls, that are hypophasic.

The isolation and identification of carotene fraction by the thin layer chromatography.

The specified amounts of magnesium oxide : hyflosupercel (1 : 2) were blended while dry and 25 g. of this material were mixed with 35 cc. of distilled water in a 250 m*l*. beaker. The mixture was then spread on glass plate (8 × 25 cm.). It is necessary to obtain a smooth surface for ease of detection of bands. The chromatoplates were then dried in an oven at 95°C. for three hours. The plates were cooled very slowly in it and kept in the desiccator.

The carotene fraction was placed as a narrow band near the bottom of the plate by using of capillary. The plate was then placed in the container which contained 2.5% acetone in petroleum ether as developing solvent. After development[12], five bands were obtained (Fig. 1).

ε-carotene

ε-carotene was eluted from the thin layer chromatography with ethyl ether. It was identified by the feature, shape and position of the absorption maxima[13] (Table1, Fig. 2).

α-carotene

α-carotene was extracted with acetone, and the absorption maxima are given in Fig. 3, and Table2. This pigment was spectrally indistinguishable from the sample of α-carotene obtained from the carrots, and the two pigments could not be separated in a mixed chromatography on a column of magnesium oxide-hyflosupercel (2 : 1).

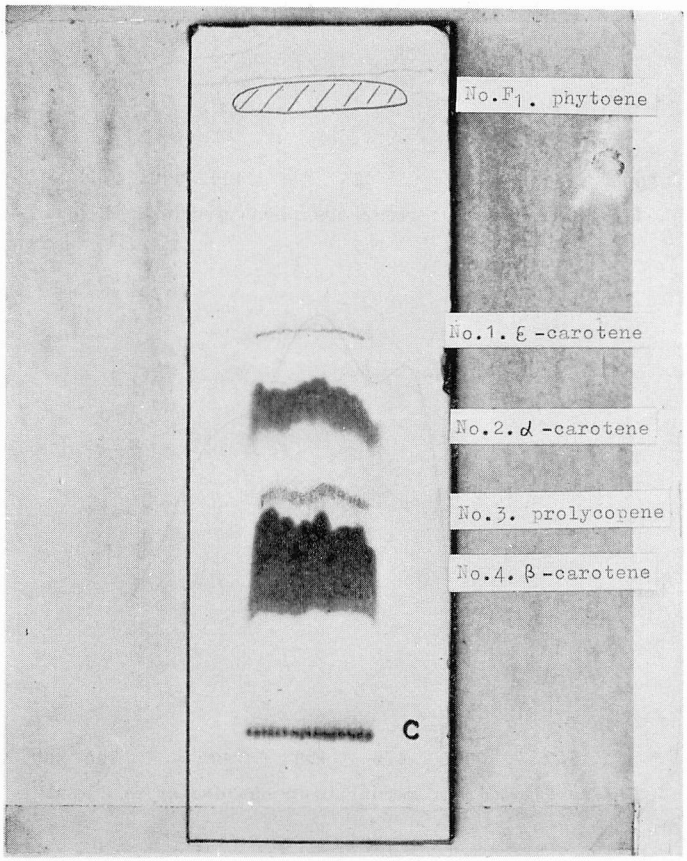


Fig. 1, The separation of the carotene fraction in *Hypnes charoides* by the thin layer chromatography.

Adsorbent : magnesium oxide : hyflosupercel (1 : 2). Developing solvent : 2. 5% acetone in petroleum ether.

Table 1. Spectral characteristics of No. 1 band.

λ max. / Solvent / No. of band	λ max. (mμ) in
	n-Hexane
1	418, 442, 472
ε-Carotene	418, 442, 471

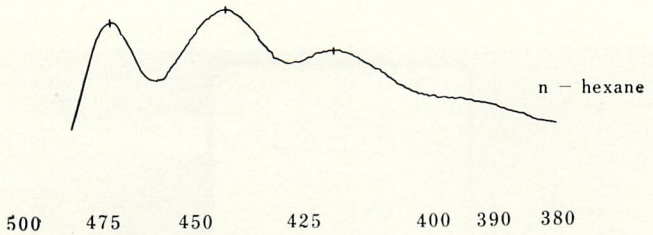


Fig. 2. Characteristic spectral absorption curves of No. 1 band.

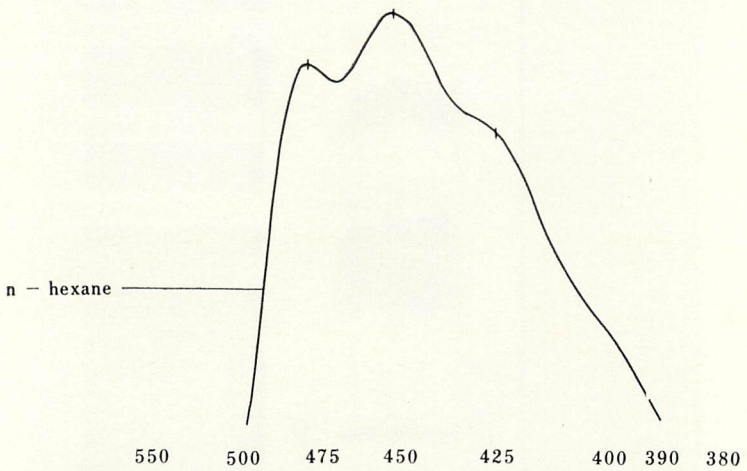


Fig. 3. Characteristic spectral absorption curves of No. 2 band.

Table 2. Spectral characteristics of No. 2 band.

λ max. / Solvent / No. of band	λ max. (mμ) in n-Hexane
2	420, 445, 475
Pure α-carotene	420, 445, 475

Prolycopene like compound

Prolycopene like compound on the plate was collected and extracted with ethyl ether and the characteristic spectral absorption curves are given in Fig. 4 and Table 3. The absorption maximum in n-hexane well agreed with the known value of pure proly copene.[13)]

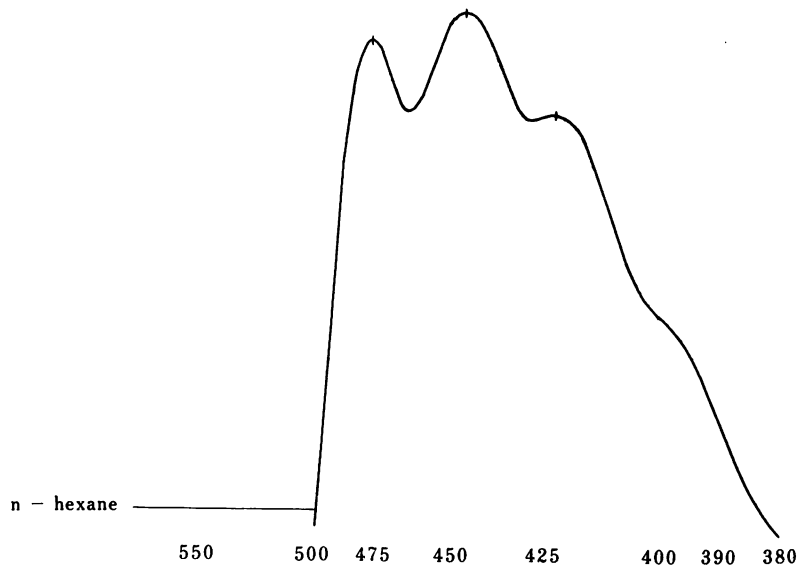


Fig. 4. Characteristic spectral absorption curves of No. 3 band.

Table 3. Spectral characteristics of No. 3 band.

λ max. / Solvent / No. of band	λ max. (mμ) in n-Hexane
3	433, 470
Pure prolycopene	443. 5, 470

β-carotene

β-carotene was eluted with ethyl ether and the characteristic spectral absorption curves are given in Fig. 5 and Table 4[14]. The pure β-carotene isolated from carrots was compared with this carotene, No separation could be achieved in a mixed chromatogram on magnesium oxide column.

Phytoene like compound

Phytoene like compound was colorless and fluorescent, the absorption maximum in n-hexane was given in Fig. 6 and Table 5. The pure phytoene obtained from the carrots was compared with the sample, the two pigments could not be separated in a mixed chromatography on a column of alumina.

Fig. 5. Characteristic spectral absorption curves of No. 4 band.

Table 4. Spectral characteristics of No. 4 band.

λ max. / Solvent / No. of band	λ max. (mμ) in n-Hexane
4	425, 452, 481
Pure β-carotene	425, 451, 482

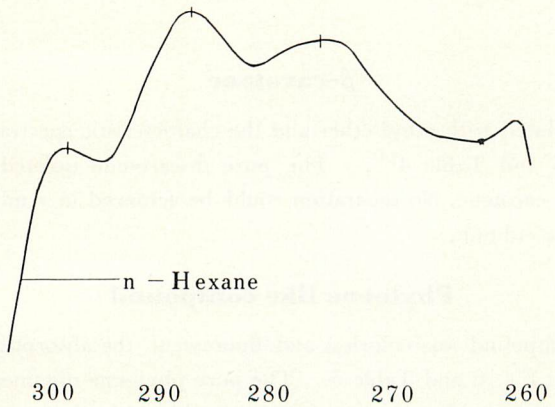


Fig. 6. Characteristic spectral absorption curves of No. F_1 band.

Table 5. Spectral characteristics of No. F_1 band.

λ max. / Solvent / No. of band	max. (mμ) in n-Hexane
F_1	275, 286, 298.5
Pure Phytoene	275, 285, 296

The isolation and identification of xanthophyll fraction by the thin layer chromatography.

The specified amounts of microscel-C were mixed with water and the paste was spread on the glass plate (8 × 25 cm.) and dried at 95°C for 3 hours. The chromatoplate was kept in the desiccator. The sample of the dried xanthophylls was dissolved in acetone

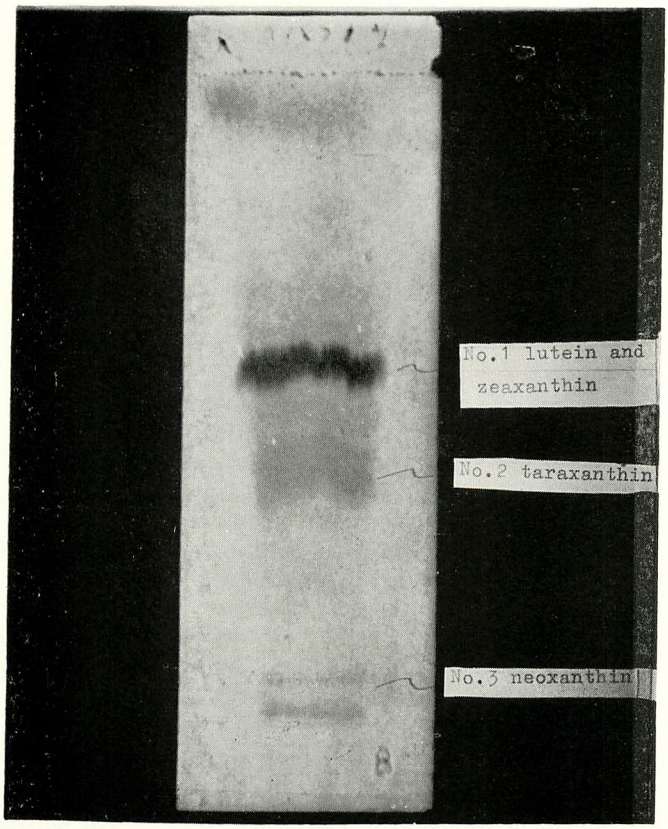


Fig. 7. The separation of the xanthophyll fraction in *Hypnes charoides* by the thin layer chromatography. Adsorbent: microcel-C. Developing solvent: 15% acetone in petroleum ether.

and was placed as a narrow band near the bottom of the thin layer plate. The plate was then placed in the container which contained 15 % acetone in petroleum ether as developing solvent. After development, three bands were obtained (Fig. 7).

The separation of lutein from zeaxanthin in band No. 1.

To separate lutein from zeaxanthin No. 1 band was collected, dissolved in ethyl ether and was submitted to the thin layer chromatography using magnesium oxide-hyflocel (1 : 2) as adsorbent and 25% acetone in petroleum ether as developing solvent. After development, two bands were obtained. (Fig. 8)

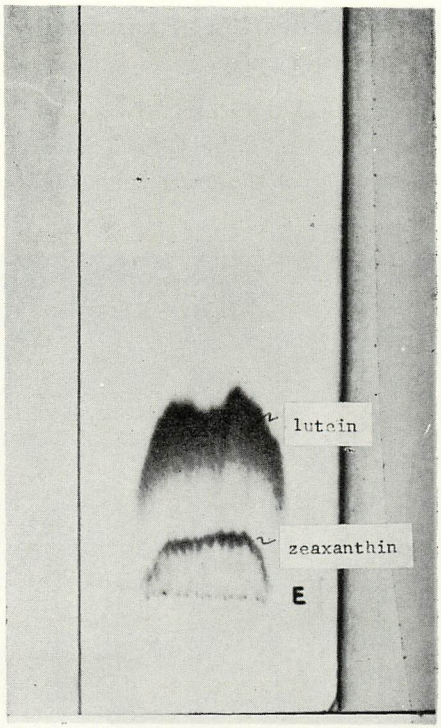


Fig. 8. The separtion of lutein and zeaxanthin mixture by thin layer chromatography.
Adsorvent : magnesium oxide : hyflo-supercel (1 : 2).
Developing solvent : 25% acetone in petroleum ether.

Lutein

Lutein was eluted with ethyl ether and was the major components of the xanthophylls. It was identified by the feature the shape and the position of the absorption maxima[14] (Fig. 9, Table 6). The pure lutein obtained from spinach was co-chromatographed with this sample on the column of magnesium oxide-celeit (1 : 2). No separation could be observed.

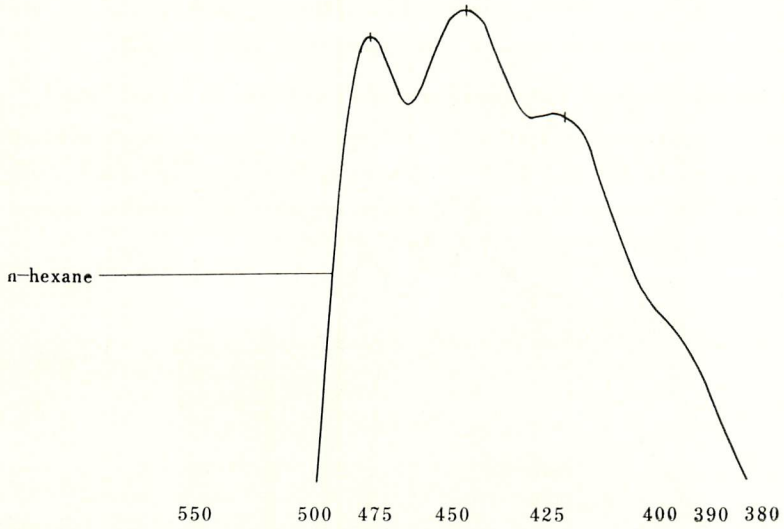


Fig. 9. Characteristic spectral absorption of No. 1 band.

Table 6. Spectral characteristics of No. 1 band.

λ max. / Solvent / No. of band	max. (mμ) in n-Hexane
1	422, 447, 476
Pure lutein	420, 447, 477

Zeaxanthin

No. 2 band was eluted with ethyl ether. Its amount was so little that it was confirmed by means of the behavior of chromatography and its color.

Taraxanthin

Taraxanthin has no epoxide so when a few drops of hydrochloric acid acid solution was added to the ethereal solution of taraxanthin, blue color was not revealed. The absorption maxima are given in Fig. 10 and Table 7.

Neoxanthin

Neoxanthin having monoepoxide was tightly adsorded on the thin layer, from which it

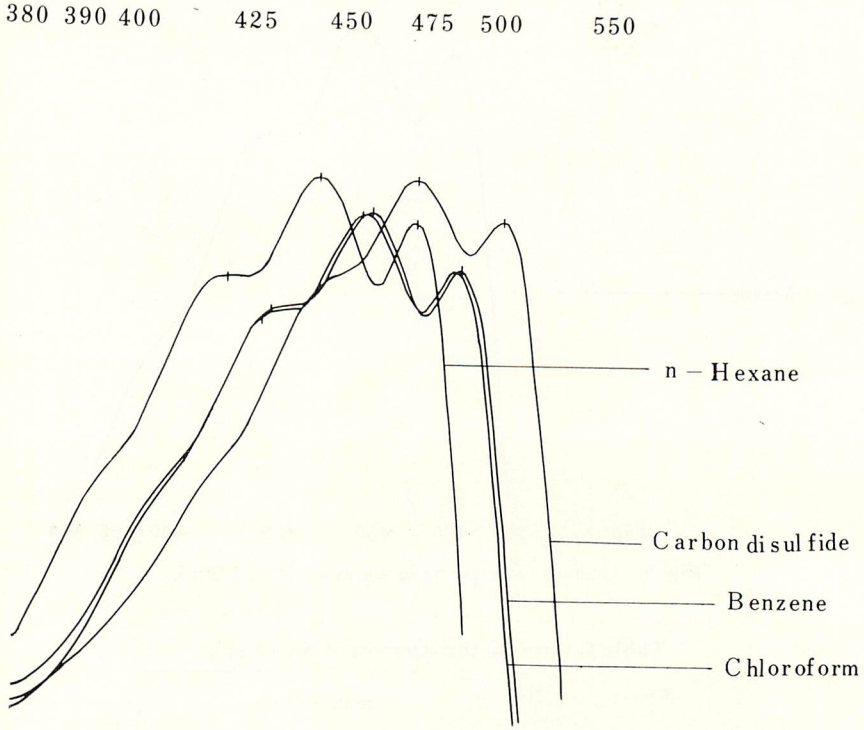


Fig. 10. Characteristic spectral absorption curves of No. 2 band.

Table 7. Spectral characteristics of No. 2 band.

λ max. / Solvent / No. of band	λ max. (mμ) in			
	n-Hexane	Carbon disulfide	Chloroform	Benzene
2	419, 443, 472	443, 471, 502	428, 455, 485	430, 457, 487
Pure taraxanthin	443, 472	441, 469, 501		

was eluted with ethyl ether. However it was present in very little amounts.

The relative abundance of the carotenoids in *Hypnea chroides* is given in in Table 8.

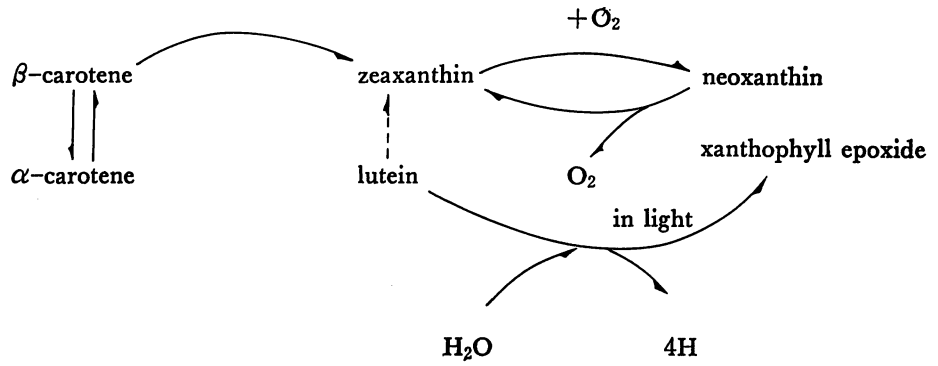
Discussion

It has been suggested that phytoene is an intemediate in the biosynthesis of carotenoids. The auther has confirmed that 2-C^{14}-mevalonic acid served as a substrate in the biosynthesis of carotenoids in Codium, which was reported in the previous part of this series.

Table 8. Relative abundance of the carotenoids in *Hypnea charoides*.

Compounds	Relative abundance
Phytoene	2.0 %
ε-Carotene	0.6
α-Carotene	25.1
Prolycopene	2.2
β-Carotene	41.2
Lutein	25.9
Taraxanthin	2.8
Zeaxanthin	trace
Neoxanthin	trace

It is interesting that the existence of phytoene was confirmed. The structure of neoxanthin is unknown, though it has three hyrooxy groups and one epoxide. It was confirmed that under strong light and anaerobiosis, lutein increased while neoxanthin decreased. Aerobic dark conditions reversed the reaction by using spinach by Chichester et al[15] and Godnev[16].



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