

Mechanism of the Interconversion of Plant Carotenoids into Fish Carotenoids — I.

Carotenoids in Sea Breams (Part II)

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The function and metabolism of carotenoids in fish has not yet been solved clearly. NICOLA^{1),2)} pointed out that B-carotene was shown to be almost certainly a precursor of astaxanthin in locust eggs. THOMMEN³⁾ reported the results of investigation which indicated that in *Daphnia*, B-carotene can presumably be oxidized to keto forms, echinenone and canthaxanthin. CHICHESTER⁴⁾ noticed that in *Artemia salina* B-carotene can be converted to canthaxanthin via echinenone but lutein, zeaxanthin, isocryptoxanthin and isozeaxanthin cannot be converted to canthaxanthin and they seemed to absorb the zeaxanthin and lutein and deposit them as such in the tissues.

It has commonly considered that fish, with all other animals can not synthesize carotenoids de novo, but they can alter alimentary carotenoids by oxidation and can store the resulted products.

In the previous reports⁵⁾, the contents of astaxanthin in the cultured sea breams at the Ushine Fish Farm, Kagoshima, were only one twentieth of those in the natural sea breams of the same size, caught around the Kagoshima Bay. Therefore the contents of the stomach of natural sea breams were examined, and the half digested *Squilla Oratoria* and other *Crustacea* were found in them. It was confirmed that the most of astaxanthin in natural sea breams were brought about their foods.

In this report, besides astaxanthin, the existence of E-carotene and dihydroxy E-carotene was confirmed in the taraxanthin like fraction of the carotenoids in the natural sea breams caught around the Kagoshima Bay. HIRAO et al.⁶⁾ reported a new xanthophyll from fins of *Thunnus thynnus* which they called tunaxanthin. CROZIER and WILKIE⁷⁾ reported a skin xanthophyll from the green phase of *Apodichthys flavidus*, for which they proposed the structure of 3, 3'-dihydroxy-E-carotene. In spectral conformation and absorption maxima, this material is identical to tunaxanthin.

E-carotene was first isolated by STRAIN⁸⁾ in the green alga, *Bryopsis corticulans*. Recently the existence of this compound was confirmed from *Naricula* and *Bryopsis* by HAXO et al.⁹⁾

It is interesting that the occurrence of E-carotene was confirmed in the sea breams. The precursor of dihydroxy E-carotene would be assumed to be E-carotene in the sea breams. In the other words E-carotene from the foods would be converted to dihydroxy E-carotene

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in the sea breams *de nove*.

Experimental

Total carotenoid pigments were extracted from the red skin of the fresh sea breams caught around the Kagoshima Bay as the usual method¹⁰⁾, and were transferred to petroleum ether. The combined petroleum ether extracts were washed, dried with anhydrous Na_2

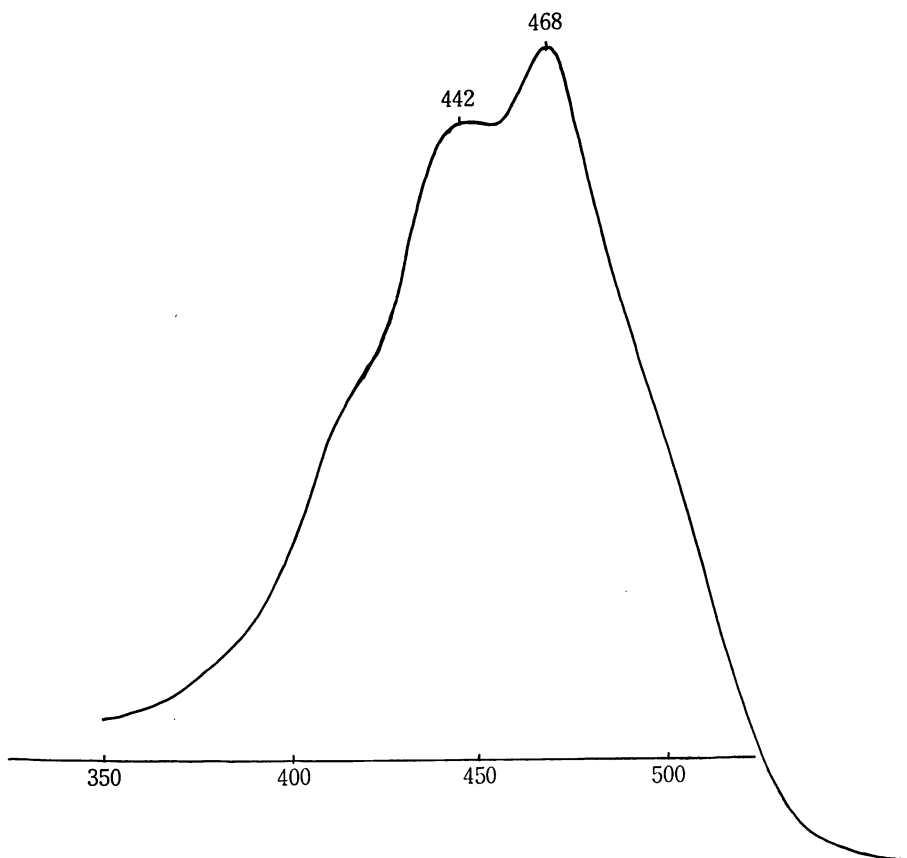


Fig. 1. Absorption spectrum of the crude carotenoids of the sea breams in petroleum ether.

SO_4 , and evaporated to a small volume under reduced pressure. The crude extracts of the red skin of the sea breams produced the band shown in Fig. 1.

The carotenoid mixtures were fractionated on the column containing Al_2O_3 (grade II) using petroleum ether as the developing solvent. (Fig 2), for saponification, 50ml. of a solution of 6% potassium hydroxide in methanol was added to Fraction I (yellow fraction) and the pigments were left at room temperature. The solution was then diluted to 50% methanol by the addition of water and the carotenoids were extracted into petroleum ether. The combined petroleum ether extracts were washed, dried with anhydrous Na_2SO_4 . The

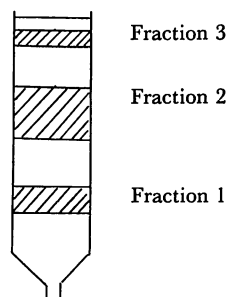


Fig. 2. Carotenoids from whole extracts of sea breams as they appears on alumina when developed with petroleum ether.

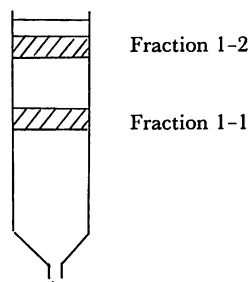


Fig. 3. Separation of carotenoids of Fraction 1 on alumina after saponification when developed with 2-6% acetone in petroleum ether.

carotenoids mixtures were separated chromatographically on the columns of aluminum oxide again using 2-6% acetone in petroleum ether as the developing solvent. Two carotenoid pigments were separated. These were: E-carotene and 3,3'-dihydroxy E-carotene (Fig. 3).

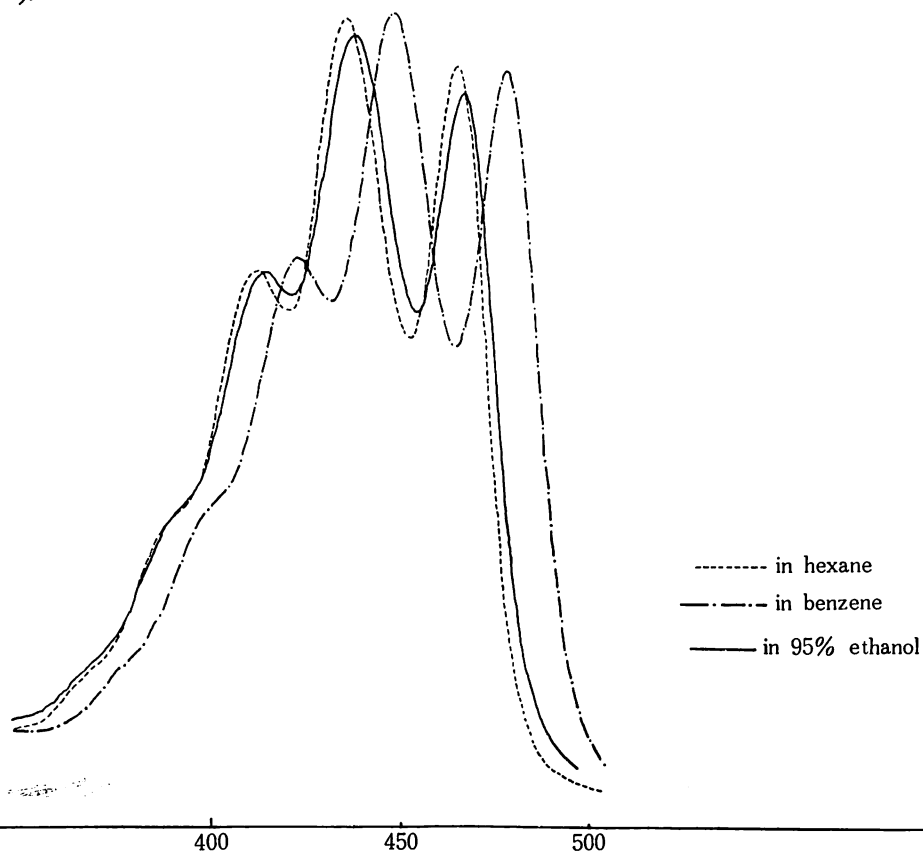


Fig. 4. Absorption spectrum of Fraction 1-1 (E-carotene).

Fraction I-1, E-carotene: E-carotene was eluted out from the columns of aluminum oxide with acetone. It was identified by the feature, shape and position of the absorption maxima and those were agreed with the reference⁹⁾ of E-carotene (Fig. 4).

Table 1. Spectral characteristics of Fraction I-I.

Solvent	λ max.	λ max. (m μ) in		
95% Ethanol	422	444	473	
Benzene	426	455	485	
Hexane	419	441	471	
Petroleum ether	419	441	471	

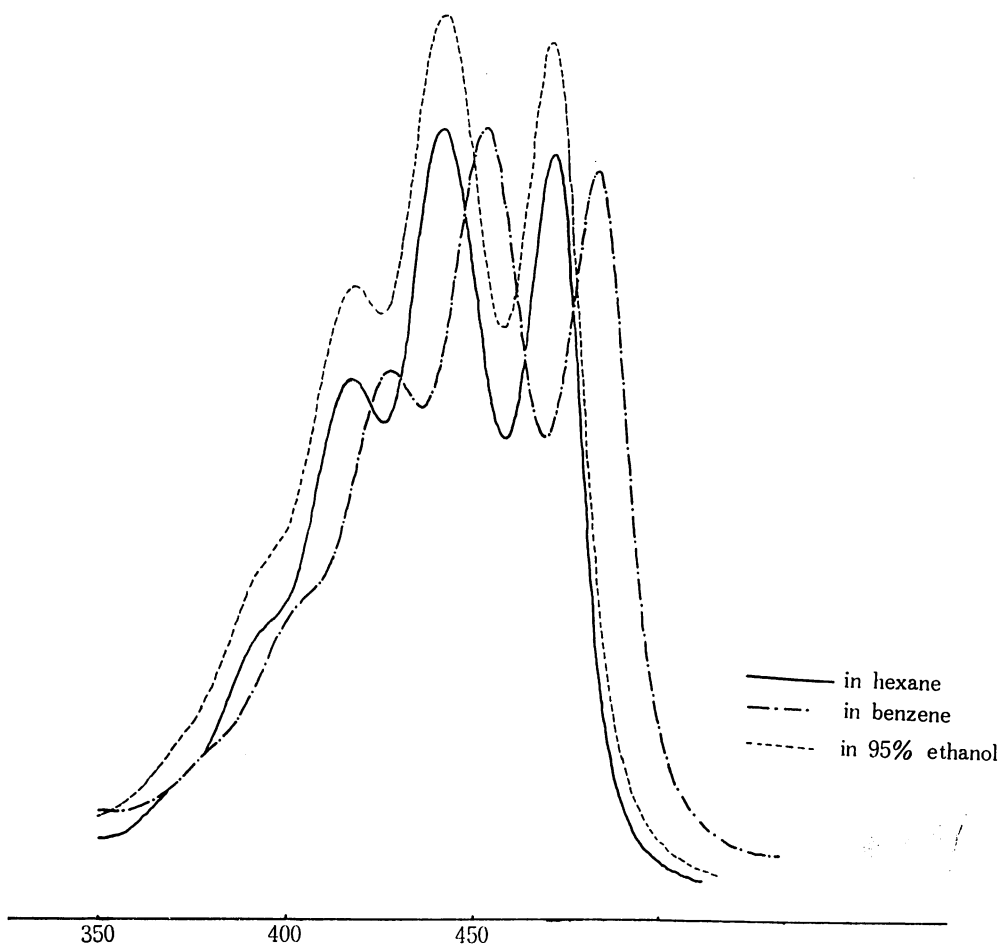


Fig. 5. Absorption spectrum of Fraction 1-2 (3, 3' dihydroxy E-carotene).

Fraction I-2, 3, 3' dihydroxy E-carotene: Fraction I-2 was eluted with acetone. The absorption spectrum is given in Fig. 5. Its absorption spectrum and feature were in

Table 2. Spectral characteristics of Fraction 1-2.

Solvents	λ max.	λ max. (m μ) in		
95% Ethanol	417	439	469	
Benzene	425	450	481	
Hexane	416	439	469	
Petroleum ether	416	438	468	

agreement with those of 3, 3'-dihydroxy E-carotene given by WILKIE et al.⁷⁾.

Fraction II, astaxanthin ester: astaxanthin ester was eluted with acetone and identified by the shape and position of the absorption maxima and in agreement of those reported previously. On reduction with sodium borohydride, a compound with an absorption spectrum typical of B-carotene was obtained, those were in agreement with the data

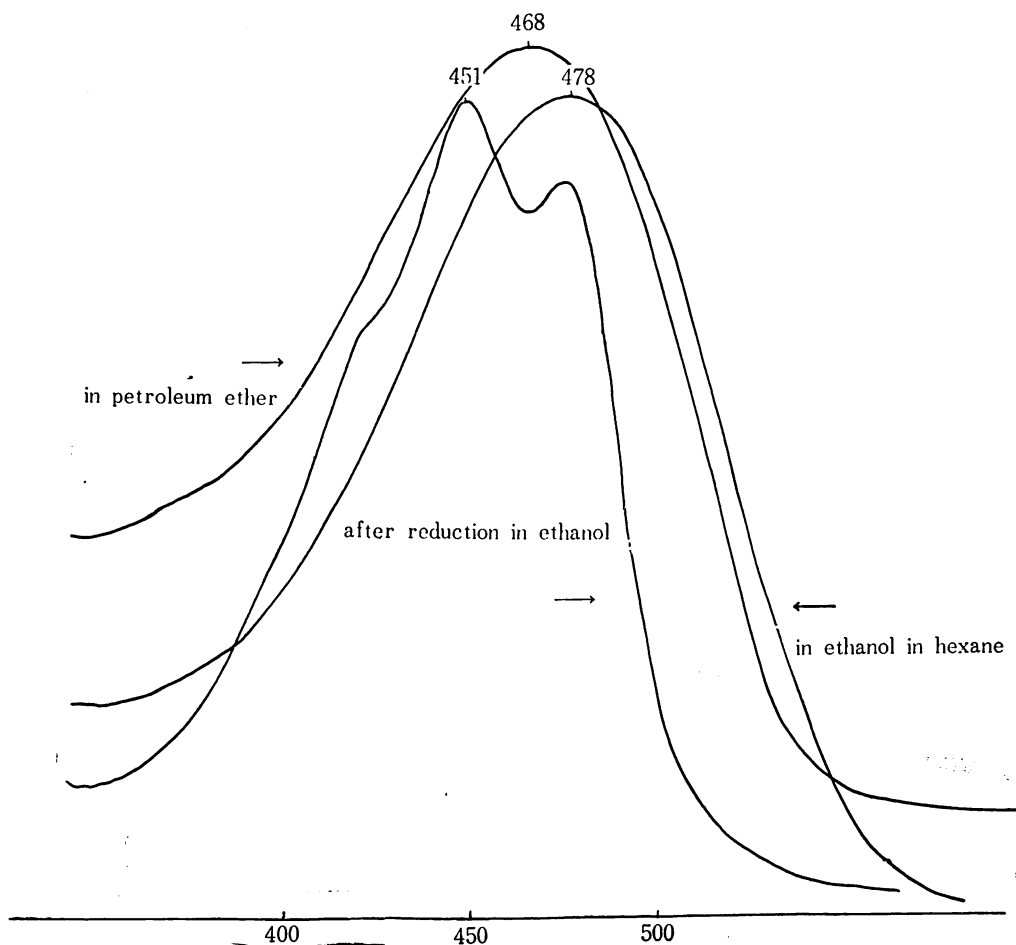


Fig. 6. Absorption spectrum of Fraction II (Astaxanthin ester).

given by GOODWIN¹¹⁾ (Fig. 6).

Fraction III, astacene: astacene was eluted with 10% acetic acid in ethylether. The absorption maxima in petroleum ether shows the symmetrical shape of a keto carotenoids.

Treatment of this compound with sodium borohydride rapidly changes the color from orange to yellow and the absorption spectrum typical to B-carotene was obtained. Those were in agreement with astacene (Fig. 7).

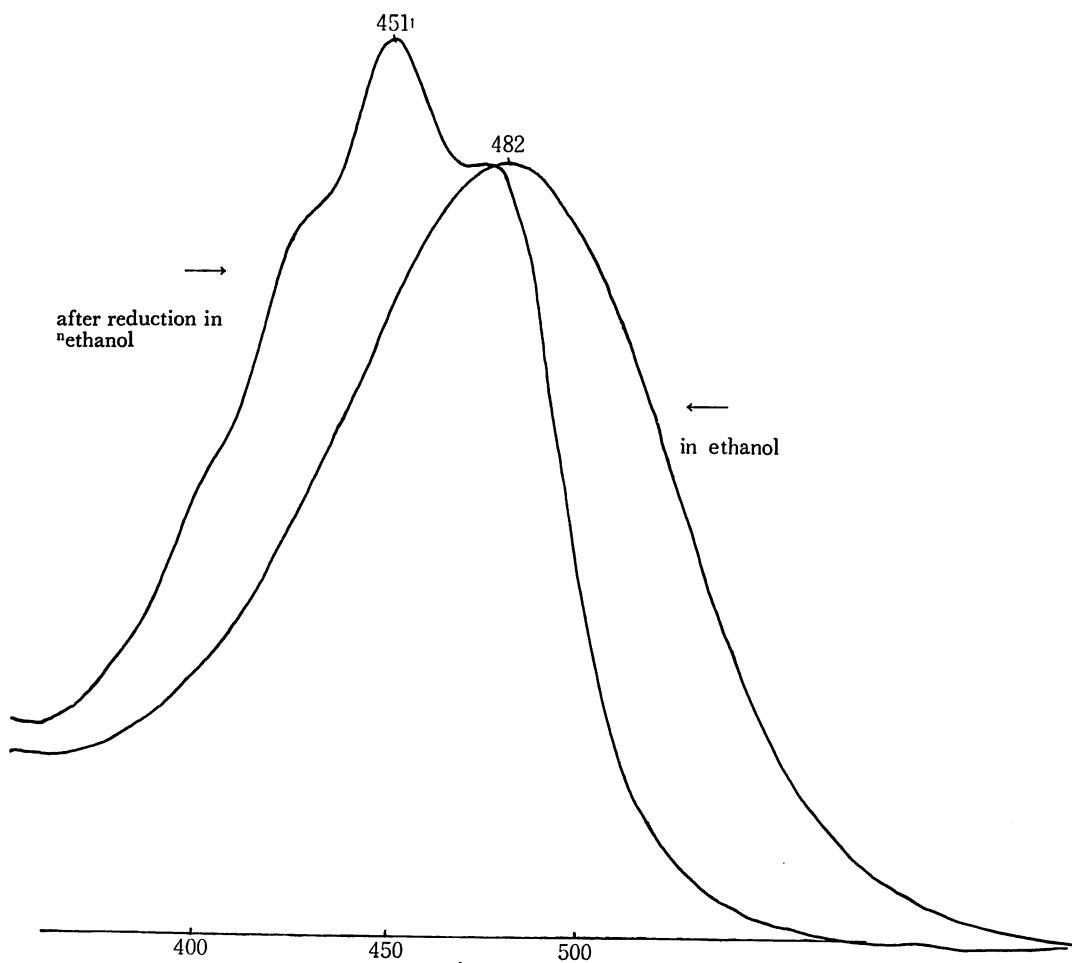
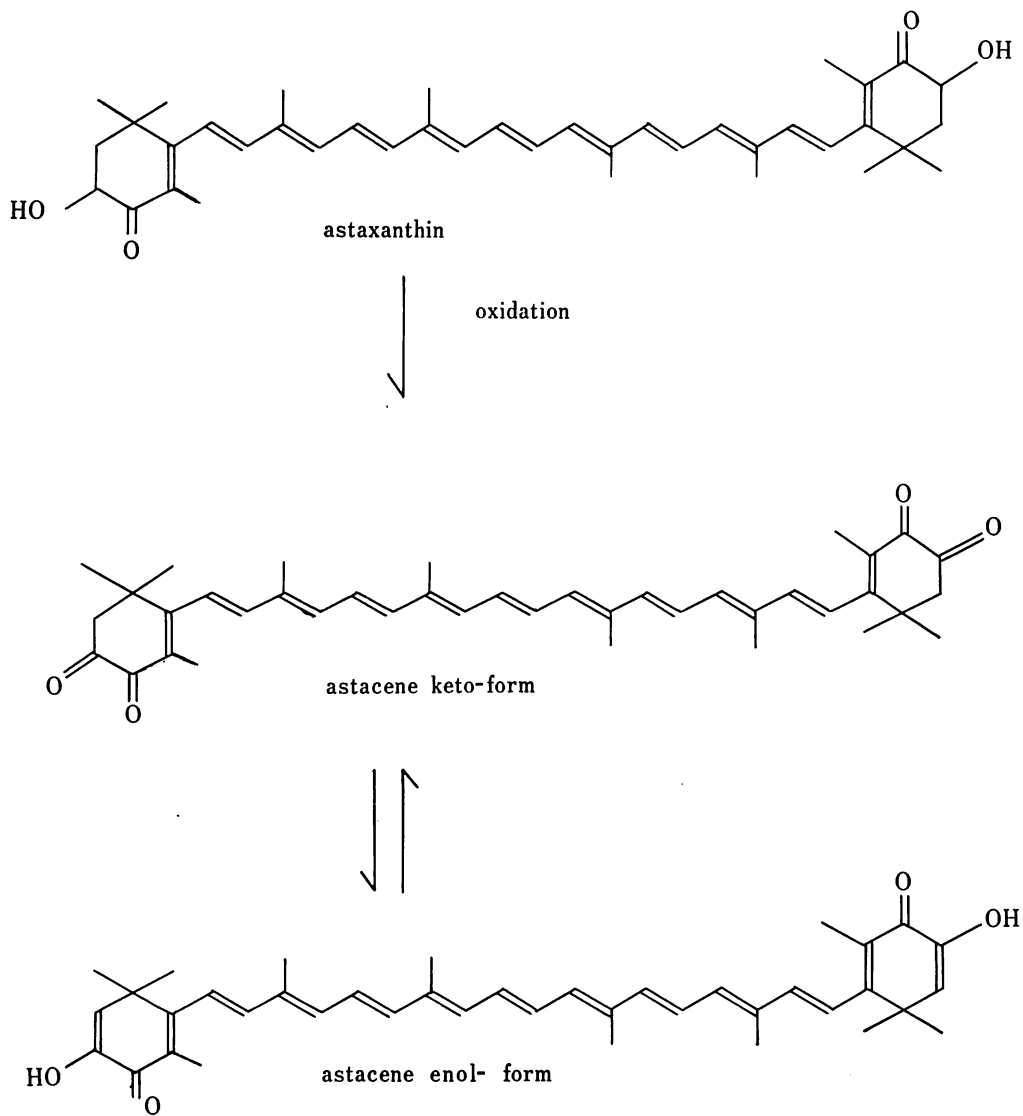


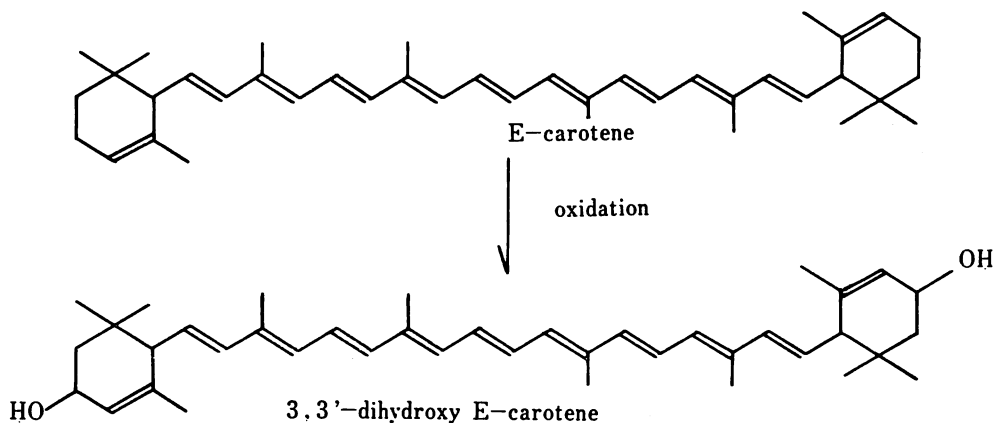
Fig. 7. Absorption spectrum of Fraction III (Astacene).

Discussion

The existence of astaxanthin ester and astacene was already reported in the previous report⁵⁾, Astaxanthin is usually converted to astacene artificially during extraction or saponification.



The occurrence of E-carotene in algae has been reviewed by CHAPMAN and HAXO⁹. E-carotene would come from the foods taken by sea breams and would be converted in the sea breams to 3, 3'-dihydroxy E-carotene de nove.



Those distribution and dietary relation are the subject of further work.

results

Besides astaxanthin and astaxanthin ester, the existence of E-carotene and dihydroxy E-carotene was confirmed in the traxanthin like yellow fraction of the carotenoids in the natural sea breams and E-carotene from the foods would be converted to 3, 3'-dihydroxy E-carotene de novo. These dietary relations are the subjects of further work.

References

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