Mem. Fac. Fish., Kagoshima Univ.
Vol. 28 pp. 9~16 (1979)

Carotenoids in Sea Mussel, *Mytilus edilus*

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Abstract

In order to study the distribution of the carotenoids in aquatic animals, the carotenoids in mussel, *Mytilus edilus* were separated and identified by using column chromatography. The existence of β-carotene (10.1 %), lutein (5.2 %), zeaxanthin (10.9 %), isomytiloxanthin (34.7 %) and mytiloxanthin (26.6 %) in mussel was confirmed, and the compound produced from isomytiloxanthin by saponification was presumed to be 3′-hydroxy-7′, 8′-ε, β-carotene-3-one (isomytiloxanthone) by MS.

Caroteoids are responsible for the yellow, orange or red color in shellfish, but there are many non-carotenoid pigments in them. The carotenoids in shellfish are interesting from a point of view of pigmentation and food chain.

Carotenoids in shellfish were investigated by several workers[1]. Shimizu et al.[2-6] studied the carotenoids in five kinds of bivalves and revealed the existence of β-carotene and lutein in these shellfish, and that carotenoids also have seasonal variation in relative abundance.

Nishibori[7] isolated alloxanthin (pectenoxanthin) from three kinds of shellfish. Scheer[8] investigated the carotenoids of large sea mussel, *Mytilus californianus*, and the existence of zeaxanthin, pectenoxanthin and fucoxanthin was confirmed, but he could not clarify the structure of mytiloxanthin.

In 1973, Khare et al.[9] isolated mytiloxanthin and its isomer, isomytiloxanthin from mussel, *Mytilus edilus* and clarified its structural formula.

The present investigation was undertaken to clarify the relative abundance of the carotenoids in mussel and the compound produed from isomytiloxanthin by saponification.

Materials and Methods

1) Extraction, separation and identification of the carotenoids:

The carotenoid pigments were extracted with acetone in a Waring blender. The carotenoids in the acetone solution were transferred to petroleum ether from acetone by adding water. The petroleum ether solution of the pigments

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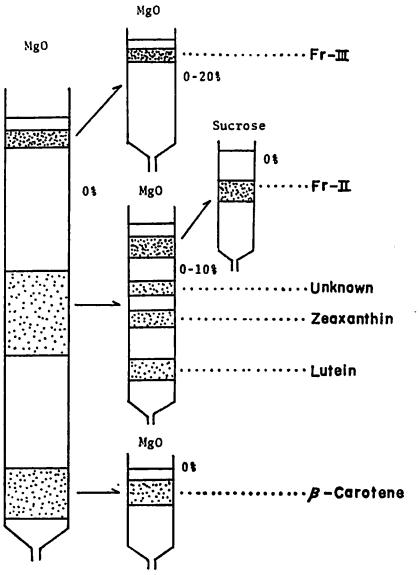


Fig. 1 Chromatographic separation of carotenoids of *Mytilus edulis* on columns of magnesium oxide/hyflosupercel and sucrose. Fractions eluted with acetone in petroleum ether.

was washed with water to remove acetone, dried over anhydrous sodium sulphate and evaporated under vacuum. The pigments were dissolved in a small volume of petroleum ether and chromatographed on a MgO column (MgO: HyfloSupercel=1 : 2), using petroleum ether as developing solvent. Three bands were obtained: Lower band, middle band and upper band as shown in Fig. 1.

β-Carotene: The pigments of lower band were rechromatographed on a MgO column by using petroleum ether as developing solvent. Only one band was obtained. The absorption spectra and the behavior on the column were all in agreement with β-carotene. The Rf value on TLC of this pigment was identical with authentic synthesized β-carotene.

Lutein and Zeaxanthin: The pigments of middle band were rechromatogralphed on a MgO column by using 10 % acetone in petroleum ether as developing solvent. Four bands were obtained.

The absorption spectra and behavior on the column of the lowest band were in agreement with pure lutein. The Rf value on TLC of this carotenoid was identical with authentic lutein obtained from marygold.

The absorption spectra of the lower band and its behavior on the column were in agreement with pure zeaxanthin. The Rf value on TLC of this pigment was identical with authentic synthesized zeaxanthin.

Fr-II. The carotenoid of upper band was purified on sugar column and only one band was obtained. It was difficult to crystallize this pigment from n-hexane-ethyl ether. This carotenoid could not be identified from the absor-

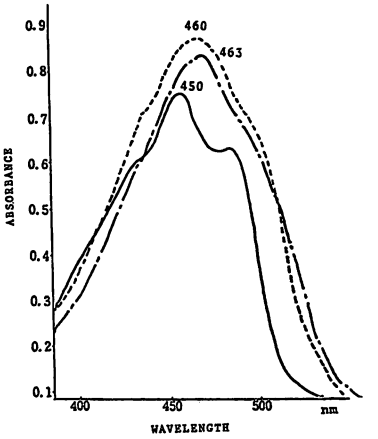


Fig. 2 Absorption spectra of Fr-II and its saponified product.
———, Fr-II in petroleum ether
- - - -, saponified product in petroleum ether
— - —, saponified product in ethyl alcohol

ption spectra and the behavior on the column (Fig. 2). Therefore mass spectrum of the precipitate of this carotenoid was taken.

Mass spectrum shows the presence of peak at M^+ (598), M-92, M-106, M-191, M-197. The mass spectrum and the change of absorption spectra before and after saponification showed this carotenoid to be identical with isomytiloxanthin. Fr-III. The pigment of upper band was rechromatographed on a MgO column by using 20% acetone in petroleum ether and only one band was obtained.

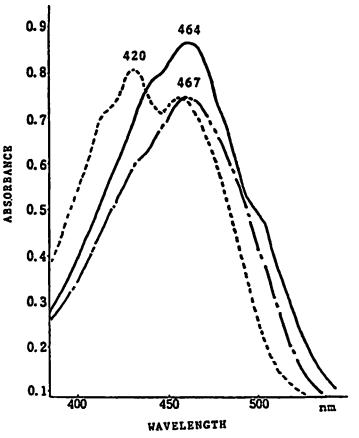


Fig. 3 Absorption spectra of Er-III.
———, in petroleum ether
— - —, in ethyl alcohol
- - - -, after reduction

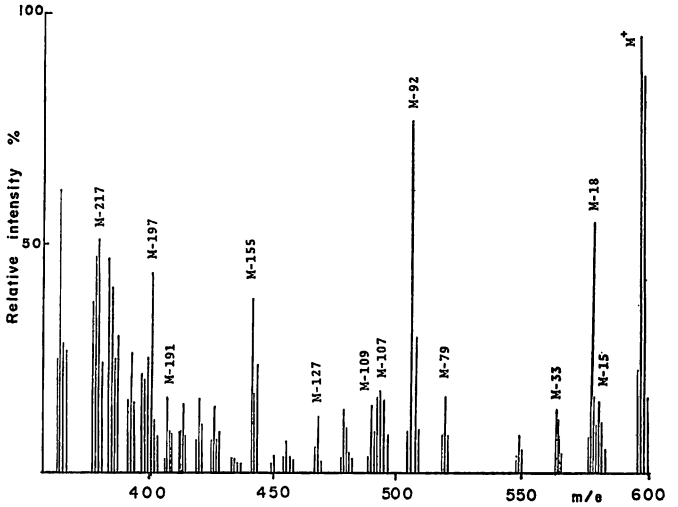


Fig. 4 Mass spectrum of Fr-III

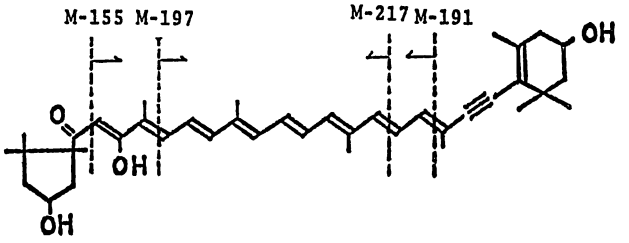


Fig. 5 Schematic representation of the possible ways of formation of the main fragments on the mass spectrum of Fr-III

The isolated pigment was crystallized from n-hexane-ethyl ether.

The purified pigment has the following characteristics as m.p. 171°C, λ max in petroleum ether 464 mm as shown in Fig. 3. The pigment was reduced by adding sodium borohydride and the absorption spectrum of the reduced pigment was shown in Fig. 3. The mass spectrum shows the presence of peaks at M^+ (598), M-18, M-33, M-79, M-92, M-155, M-191, M-197 and M-217 (Fig. 4). Among these peaks, M-155, M-191, M-197 are characteristic and peak M-155 shows the existence of 3-hydroxy-κ-carotene-4-one end group (Fig. 5)[10].

The NMR spectrum of this pigment shows signals at 0.84 (3H), 1.13 (3H), 1.18 (6H), 1.24 (3H), 1.90 (3H), 1.95 (12H) as shown in Fig. 6[11]. From the absorption spectra, MS and NMR, the structure of this carotenoid was confirmed to be 3, 8, 3′-dihydroxy-7′, 8′-didehydro-k, β-carotene-6-one (mytiloxanthin)

2) The compound produced from isomytiloxanthin by saponification:

Sufficient amount of absolute ethanol was added to the carotenoid of Fr-II for dissolving it complately, then 60% (w/v) aqueous potassium hydroxide was

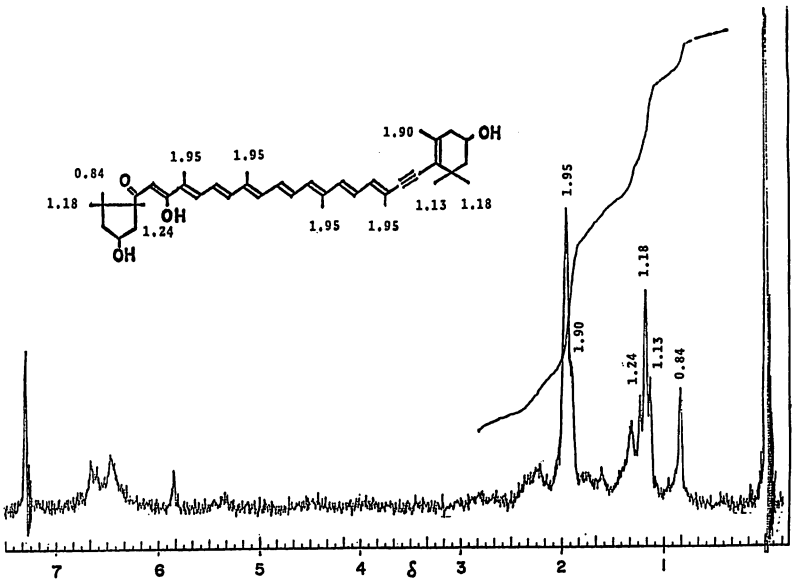


Fig. 6 NMR spectrum of Fr-III

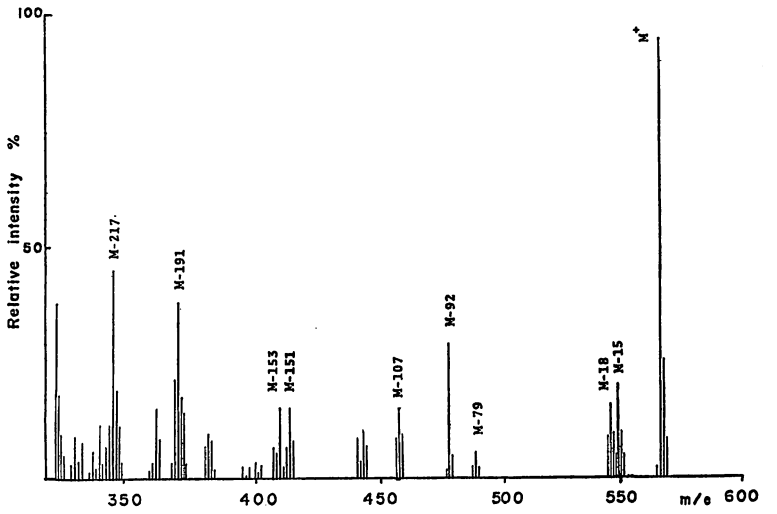
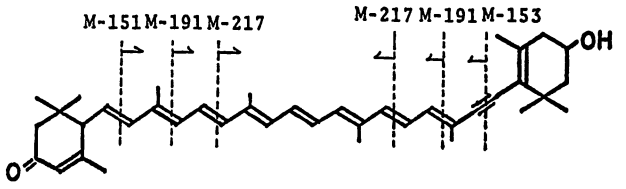


Fig. 7 Mass spectrum fo saponified product of Fr-II

added in the rate of 1 ml of every 10 ml of ethanol solution. The alkali mixture was left overnight in the dark at room temperature. After saponification, the carotenoid in ethanol solution was transferred to petroleum ether by using separating funnel. Then the petroleum ether layer was washed gently with water to remove the alkali.

The petroleum ether solution of the carotenoid was concentrated under vacuum. The carotenoids were separated on a MgO column and several bands



3'-hydroxy-7',8'-didehydro-ε,β-carotene-3-one

(Isomytiloxanthone)

Fig. 8 Schematic representation of the possible ways of formation of the main fragments on the mass spectrum of saponified product of Er-II

were obtained. The carotenoid of predominant band was purified on a sugar column and only one band was obtained. The crystallines of saponified carotenoid of Fr.-II were obtained from n-hexane-ethyl ether solution.

The characteristics of this carotenoid showed m. p. 138–142°C, λ max in petroleum ether, 460 nm (Fig. 2). Mass spectrum shows the presence of peaks at M^+ (564) M-18, M-92, M-107, M-151, M-153, M-191, M-217 (Fig. 7). The peaks at M-153, M-191, and M-217 show the presence of 3-hydroxy-7, 8-didehydro-β-carotene end group and M-151, M-191, M-217 shows the presence of 3-keto-ε-carotene end group (Fig. 8). From the visible absorption spectrum and MS, the structure of this carotenoid was presumed to be 3′-hydroxy-7′, 8′-didehydro-ε-carotene-3-one and named isomytiloxanthone.

Results and Discussion

The relative abundance of the carotenoids in mussel is shown in Table 1. In general, it is not always easy to detect one kind of carotenoid as a main carotenoid in a certain species, because of seasonal variation as suggested by Simizu et al.[2-6].

In the case of mussel, mytiloxanthin could be considered to be a main ca-

Table 1. Relative abundance of the carotenoids in sea mussel, *Mytilus edilus*.

Carotenoids	Relative abundance (%)
β-Carotene	10.1
Lutein	5.2
Zeaxanthin	10.9
Isomytiloxanthin	34.7
Mytiloxanthin	26.6
Unknown	12.5

rotenoid of this spesies, because mytiloxanthin was detected in every case in this experiment, Khre's and Scheer's experiments.

Therefore, it appears that mussel always has mytiloxanthin as the species specific carotenoid. The most probable precursor of mytiloxanthin is presumed to be fucoxanthin which is widely distributed in planktonic algae. But the possible intermediate as shown in Fig. 9 could not be isolated in this investigation.

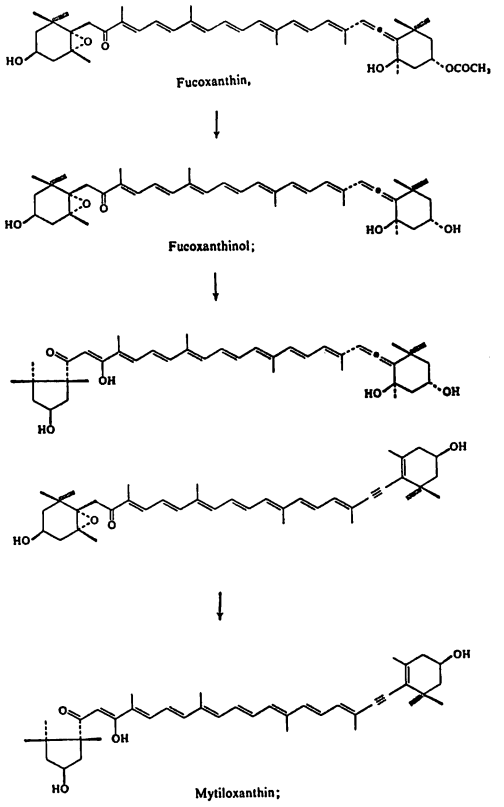


Fig. 9 Possible metabolic pathway and intermediate from fucoxanthin to mytiloxanthin.

The interesting biochemical correration observed by Scheer[8] was the gradual disappearance of mytiloxanthin during starvation and the replacement by zeaxanthin.

From the viewpoint of food chain, biosynthesis and conversion of mytiloxanthin are very interesting and the subjects should be solved in the future.

Acknowledgemnts

The authors acknowledge with the generousity of Oh-hara Fisheries Co. Ltd

(Mr. Ei-ichi OH-HARA) for the gift of sea mussel.

References

1) D. L. FOX: Animal Biochromes and Structural Colours (Second ed. Univ. of California Press, Berkeley, California) 1976.
2) T. SHIMIZU and K. UCHIDA : Carotenoids in bivalves-I, Carotenoids in short-neck clam. *Bull. Jap. Soc. Sci. Fish.*, **34**, 154-158, 1968.
3) T. SHIMIZU and R. MONMA: Carotenoids in bivalves-II, Carotenoids in hard clam. *Bull. Jap. Soc. Sci. Fish.*, **34**, 159-162, 1968.
4) T. SHIMIZU and Y. OHTA: Carotenoids in bivalves-III, Carotenoids in corb shell. *Bull. Jap. Soc. Sci. Fish.*, **34**, 210-213, 1968.
5) T. SHIMIZU and T. NARAHARA: Carotenoids in bivalves-IV, Carotenoids in arkshell., *Bull. Jap. Soc. Sci. Fish.*, **34**, 503-506, 1968.
6) T. SHIMIZU and A. ODA : Carotenoids in bivalves-V, Carotenoids in pecten., *Bull. Jap. Soc. Sci. Fish.*, **34**, 627-632, 1968.
7) K. NISHIBORI: Studies on the pigments of marine animals-VII, Carotenoid of some shellfishes., *Publ. Seto Mar. Biol. Lab.* VII(2) 95-105, 1960.
8) B. T. SCHEER: Some features of the metabolism of the carotenoid pigments of the California sea mussel (*Mytilus californianus*). *J. Biol. Chem.*, **136**, 275-299, 1940.
9) A. KHARE, G. P. MASS and B. C. L. WEEDON : Mytiloxanthin and Isomytiloxanthin, two novel acetylenic carotenoids., *Tetrahedron letters*, **40**, 3921-3924, 1973.
10) Y. TANAKA: Comparative biochemical studies on carotenoids in aquatic animals. *Mem. Fac. Fish., Kagoshima Univ.* **Vol 27**, No. 2, 355-422, 1978.
11) T. W. GOODWIN: Chemistry and Biochemistry of plant pigments (Academic Press, London). 150-175, 1976.