

STUDIES ON THE PIGMENTS OF MARINE ANIMALS

VIII. CAROTENOIDS OF SOME SHELLFISHES

KOKICHI NISHIBORI

Notre Dame Seishin University, Okayama

With Plate XXXV, 4 Tables and 10 Text-figures

The foot, gonad and flesh of shellfishes, showing a red, orange and yellow colour, contain some carotenoid pigments. In the gonads and coats of *Pectunculus glycymeris*, glycymerin was found by FABRE and LEDERER (1934). LEDERER also discovered pectenoxanthin from the gonads of *Pecten maximus* (LEDERER, 1934). Mytyloxanthin and zeaxanthin were isolated from the orange coloured flesh of *Mytilus californianus* by SCHEER (1940). Red flesh of *Tugali gigas* contains astaxanthin and a new carotenoid (NISHIBORI, 1957).

Carotenoid pigments in the red-orange foot of *Anadara broughtoni* (Japanese name, Akagai), *Anadara subcrenata* (Japanese name, Sarubō), and in the red-orange ovaries of *Pecten yessoensis* (Japanese name, Hotategai) have been examined and the principal carotenoids in each species were isolated in the crystalline form.

Material and Method

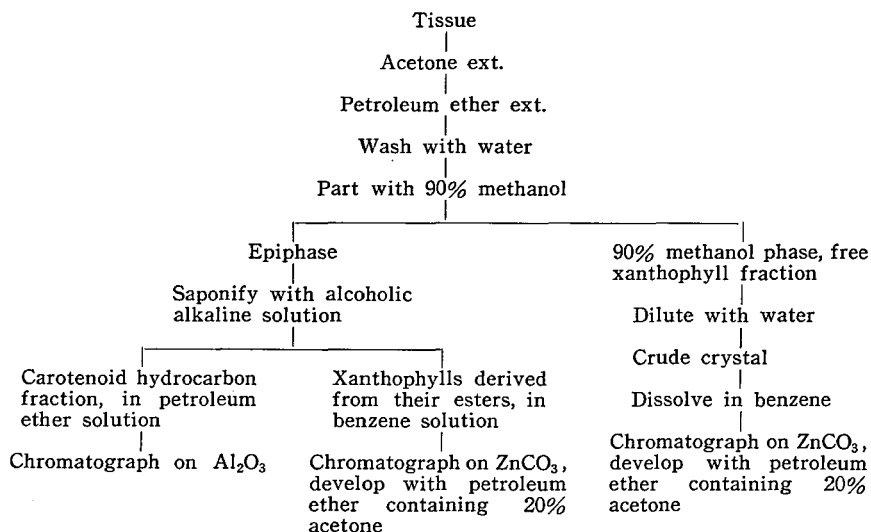
The colour, number and weight of the specimens used in this experiment are summarized in Table 1.

The extraction and separation method of the pigments is shown in Table 2.

Table 1. Colour, number and weight of the specimens.

	<i>Anadara broughtoni</i>	<i>Anadara subcrenata</i>	<i>Pecten yessoensis</i>
Organ	foot	foot	ovary
Colour	red-orange	red-orange	red-orange
Number	64	1120	40
Weight (g)	400	2450	1500

Table 2. Extraction and separation method.



Result

I. *Anadara broughtoni*

An amount of 400 g of the fresh red-orange foot (64 specimens) collected at Fukugawa in Yamaguchi Pref. was used for extraction. Pigments were entirely extracted with acetone and most of the pigment transferred therefrom into the 90% methanol solution forming a deep red colour. The colour of the epiphasic phase was light yellow.

Free xanthophyllic fraction: Red-brown crude crystals (mp. 172°), which were separated when the 90% methanol solution was covered with a trace of petroleum ether and diluted with water, were dissolved in benzene and chromatographed upon the column of zinc carbonate and developed with petroleum ether containing 20% acetone. Three zones were obtained from the top of the column. The upper zone f(I) was a light red-orange in colour and the intermediate zone f(II) was red-orange in colour. The pigment in f(II) was eluted with acetic acid-acetone mixture and the red substance was obtained. CARR-PRICE reaction (+) blue, conc. H₂SO₄ reaction (+) blue, absorption maximum was at 506 m μ in carbon disulphide (Fig. 1). This hypophasic carotenoid has a ketonic property. From the lower deep yellow zone f(III), containing the principal pigment of the specimen, orange-yellow needle-like crystals were obtained and recrystallized from ethanol-water. The yield was about 0.5 mg and mp. was 181° (Plate XXXV, fig. 1). CARR-PRICE and conc. H₂SO₄ reactions were positive, blue and absorption maxima were at ~450, 480, 512 m μ in carbon disulphide, ~435, 463, 492 m μ in chloroform and ~435, 464, 494 m μ in benzene (Fig. 1, Fig. 2). It has a hypophasic property

by partition between petroleum ether and 90% methanol but does not possess a ketonic property.

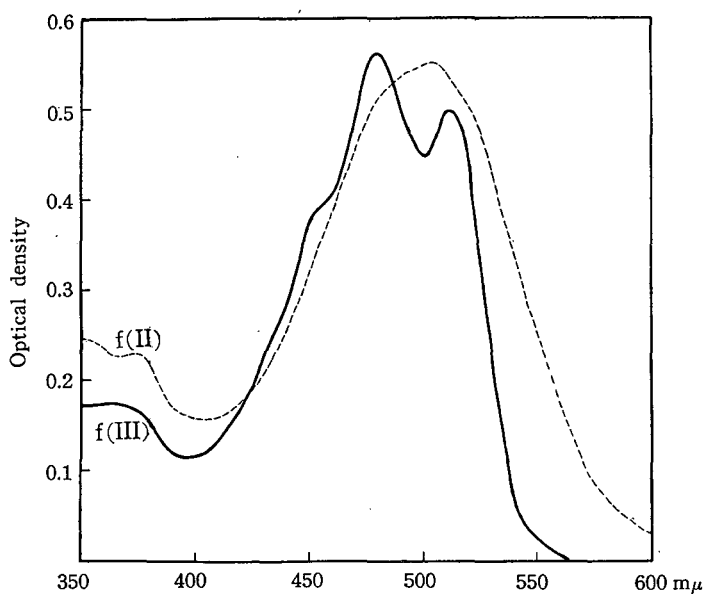


Fig. 1. Absorption spectra of free xanthophyll fraction of *Anadara broughtoni*; free xanthophyll of f(II) and f(III) (mp. 181°) in carbon disulphide.

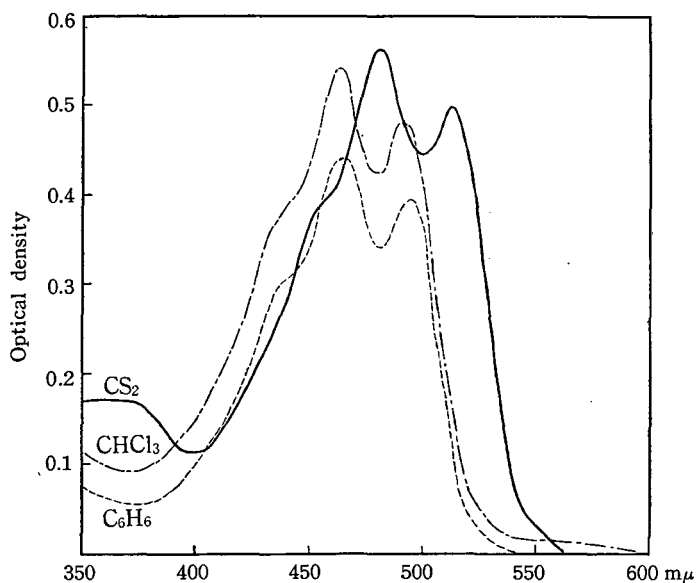


Fig. 2. Absorption spectra of the principal crystalline carotenoid, mp. 181° in *Anadara broughtoni* (in various solvents).

Esterified xanthophyll fraction: The carotenoid of this fraction in benzene was chromatographed upon the column of zinc carbonate and developed with petroleum ether containing 20% acetone. Two zones appeared; the upper red zone f(I) contains a red carotenoid which is eluted with acetic acid-acetone. CARR-PRICE reaction, violet-blue, conc. H_2SO_4 reaction, blue, absorption maxima are at $\sim 445, 478, 505 m\mu$ in carbon disulphide (Fig. 3). It possesses a hypophasic property. The pigment which was eluted from the lower orange zone f(II), showed the absorption maxima at $\sim 445, 481, 510 m\mu$ in carbon disulphide (Fig. 3). CARR-PRICE and conc. H_2SO_4 reactions (+) blue.

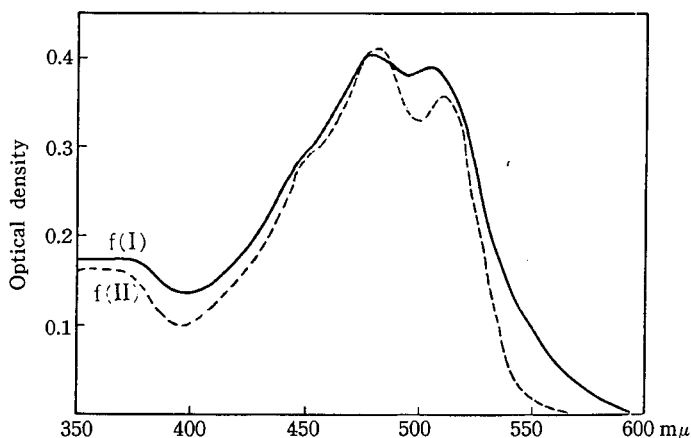


Fig. 3. Absorption spectra of f(I) and f(II) derived from their esters in *Anadara broughtoni* (in carbon disulphide).

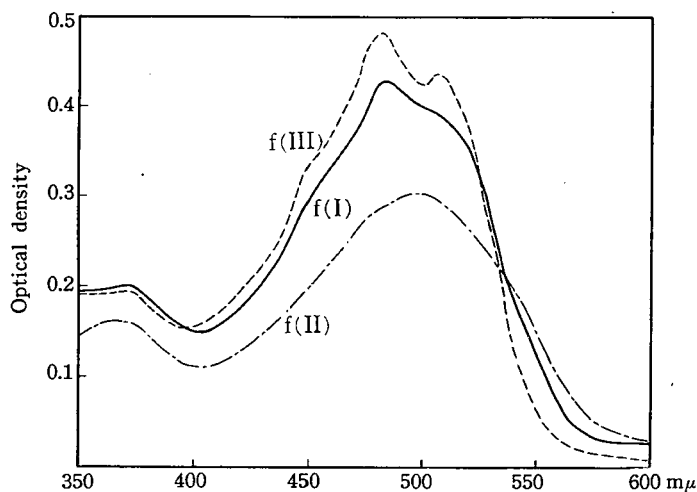


Fig. 4. Absorption spectra of carotenoid hydrocarbon fraction, f(I), f(II) and f(III) in *Anadara broughtoni* (in carbon disulphide).

Carotenoid hydrocarbon fraction: Petroleum ether solution of the pigments of this fraction were chromatographed upon alumina (Merck) and developed with petroleum ether containing 1% acetone. Three zones, yellow zone f(I), orange-yellow zone f(II) and bright yellow zone f(III), were obtained from the top of the column. Carotenoids of each zone were eluted with acetone. The absorption maxima in carbon disulphide were as follows; (Fig. 4). f(I), ~445, 485, ~515 $m\mu$, f(II), 495~500 $m\mu$, f(III), ~450, 481, 507 $m\mu$.

II. *Anadara subcrenata*

Red-orange foot, 2450 g in weight, were used for extraction. The specimens were collected at Sami in Okayama Pref.

Free xanthophyll fraction: The greatest amount of the pigment was found in this fraction. Red-brown crude crystals were precipitated at the boundary between the petroleum ether and dilute 90% methanol.

The yield was about 1.5 mg, mp. 168°. When the crystals were dissolved in benzene, chromatographed upon the column of zinc carbonate, and developed with petroleum ether solution containing acetone (20%), three zones appeared. The pigment in the upper red-orange zone f(I), was eluted with acetic acid-acetone from which the red amorphous substance was separated. The properties of this pigment were as follows; CARR-PRICE reaction, (+) blue, conc. H_2SO_4 reaction, (+) blue, absorption maximum, 500~505 $m\mu$ in carbon disulphide, (Fig. 5), 475~490 $m\mu$ in chloroform. It has a ketonic property. The pigment in the intermediate zone f(II), having an orange colour, was also eluted with acetic acid-acetone and was crystallized in a red needle form from the hot ethanol, mp. 162~

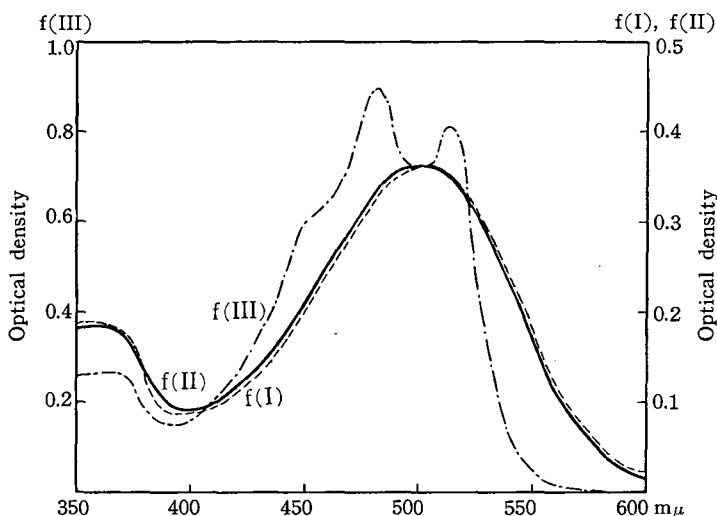


Fig. 5. Absorption spectra of free xanthophyll fraction, f(I), f(II) mp. 162-3° and f(III) mp. 183-4° in *Anadara subcrenata* (in carbon disulphide).

3°. It has a ketonic property showing the absorption maximum at 498~508 $m\mu$ in carbon disulphide (Fig. 5) and 480~490 $m\mu$ in chloroform. CARR-PRICE and conc. H_2SO_4 reactions (+) blue. The lower main zone f(III), having a deep yellow colour, contains the principal carotenoid of this specimen. Orange yellow prisms were obtained and recrystallized from hot ethanol (Plate XXXV, fig. 2), mp. 183~4°. Absorption maxima were at ~450, 482, 512 $m\mu$ in carbon disulphide, ~435, 462, 492 $m\mu$ in chloroform and ~435, 464, 494 $m\mu$ in benzene (Fig. 5, Fig. 6). It showed entirely a hypophasic property. CARR-PRICE and conc. H_2SO_4 reactions were positive, blue.

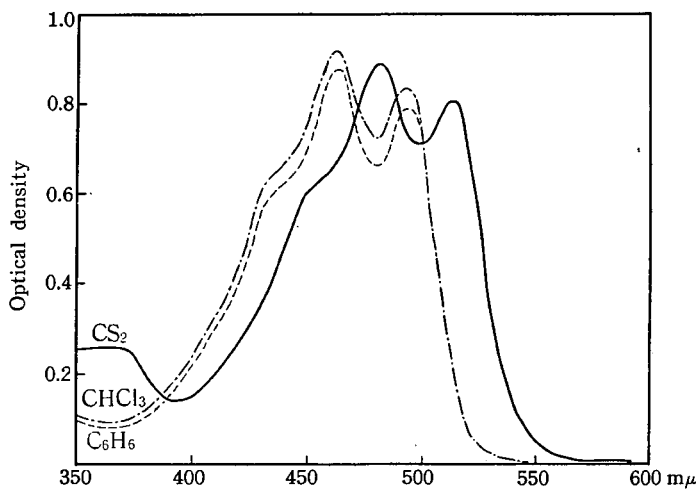


Fig. 6. Absorption spectra of the principal crystalline carotenoid mp. 183-4° in *Anadara subcrenata* (in various solvents).

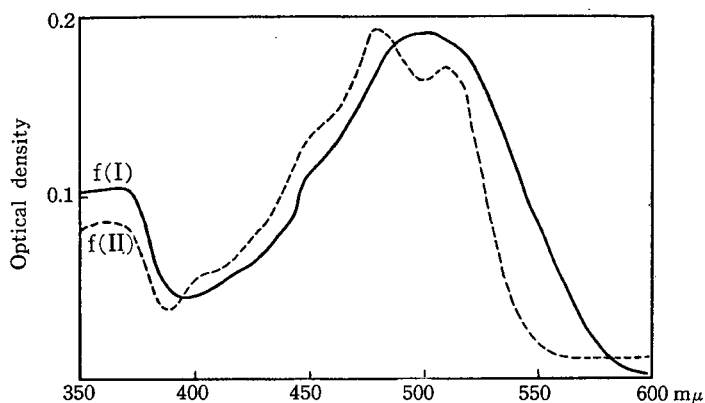


Fig. 7. Absorption spectra of f(I) and f(II) derived from their esters in *Anadara subcrenata* (in carbon disulphide).

Esterified xanthophyll fraction: After chromatographic adsorption upon zinc carbonate from benzene solution and development with petroleum ether containing acetone (20%), two fractions were obtained. From the upper red zone f(I), an amorphous red substance was eluted with acetic acid-acetone which showed the absorption maxima at ~ 445 , ~ 502 $m\mu$ in carbon disulphide (Fig. 7). It has a ketonic property. CARR-PRICE reaction (+) blue. The pigment in the lower orange zone f(II), was eluted with neutral acetone. It possessed a hypophasic property and the absorption maxima at ~ 450 , 478, 508 $m\mu$ in carbon disulphide (Fig. 7). CARR-PRICE reaction, (+) blue.

Carotenoid hydrocarbon fraction: Three zones were obtained when the petroleum ether solution of the pigment in this fraction was chromatographed upon alumina and developed with petroleum ether containing 4% acetone. Absorption maxima of the pigment of each zone in carbon disulphide were as follows (Fig. 8).

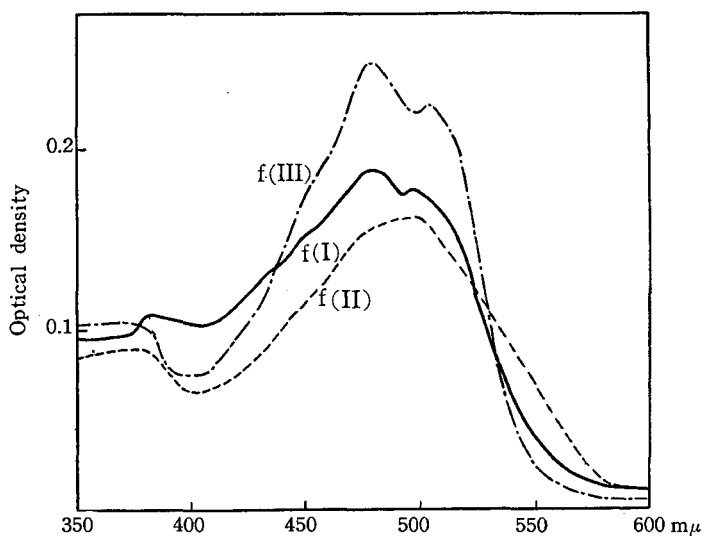


Fig. 8. Absorption spectra of carotenoid hydrocarbon fraction, f(I), f(II) and f(III) in *Anadara subcrenata* (in carbon disulphide).

Light orange zone	f(I) (the upper)	(~ 435), (~ 450), 480, 496 $m\mu$
Light pink zone	f(II) (the intermediate)	~ 475 , ~ 500 $m\mu$
Deep orange zone	f(III) (the lower)	~ 450 , 480, 502 \sim 506 $m\mu$

CARR-PRICE reaction of each carotenoid was blue.

III. *Pecten yessoensis*

An amount of 1500 g of ovaries from 40 specimens which were obtained at a fish market in Sapporo City was used for extraction. The red-orange pigment in acetone extract was transferred therefrom into petroleum ether and then into 90% methanol solution. When the 90% methanol solution of the pigment was

diluted with water after the addition of a layer of petroleum ether, the orange-red crystals appeared at the boundary of the two layers. After washing with petroleum ether, the crystals were recrystallized from aqueous pyridine into orange prisms which melted at 181~182° (Plate XXXV, fig. 3). The yield was about 1.5 mg. It forms a single zone when it is chromatographed upon the column of calcium carbonate from petroleum ether solution containing a trace of pyridine and developed with petroleum ether-acetone mixture. Absorption maxima were at ~434, 465, 496 m μ in benzene (Fig. 9). It possessed a hypophasic property, CARR-PRICE reaction (+), blue and conc. H₂SO₄ reaction (+), blue.

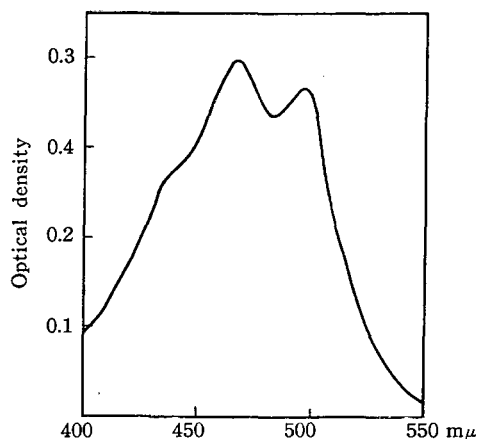


Fig. 9. Absorption spectrum of pectenoxanthin in the ovaries of *Pecten yessoensis* (in benzene).

Discussion

Carotenoid in foot: The principal pigment in the foot of two species of the shellfishes, *Anadara broughtoni* and *Anadara subcrenata* is free xanthophyllic carotenoid and the amount of esterified xanthophyll and carotenoid hydrocarbon is poor. The absorption maxima of the carotenoids obtained are summarized in Table 3.

Table 3. Absorption maxima of carotenoids in the foot of shellfishes *Anadara broughtoni* and *Anadara subcrenata* (in carbon disulphide).

		<i>Anadara broughtoni</i>			<i>Anadara subcrenata</i>	
		mp. (°C)	absorp. max. (m μ)		mp. (°C)	absorp. max. (m μ)
Free xanthophyllic fraction	f(I)	—	—		—	500~505
	f(II)	—	506		162~3	498~508
	f(III)	181	~450, 480, 512		183~4	~450, 482, 512
Esterified xanthophyll fraction	f(I)	—	~445, 478, 505		—	~445, ~520
	f(II)	—	~445, 481, 510		—	~450, 478, 508
Carotenoid hydrocarbon fraction	f(I)	—	~445, 485, ~515		—	~435, ~450, 480, 496
	f(II)	—	495~500		—	~475, ~500
	f(III)	—	~450, 481, 507		—	~450, 480, 502~6

The properties of the principal crystalline carotenoid in the foot of two species of the sea shell are very similar to each other (Table 4).

Table 4. Properties of the principal carotenoid in the foot of *Anadara broughtoni* and *Anadara subcrenata*.

	<i>Anadara broughtoni</i>	<i>Anadara subcrenata</i>
Melting point	181°	183~184°
Crystalline form	orange needles	orange prisms
Absorption maxima (m μ)	CS ₂ , ~450, 480, 512 CHCl ₃ , ~435, 463, 492 C ₆ H ₆ , ~435, 464, 494	~450, 482, 512 ~435, 462, 492 ~435, 464, 494
Partition test	hypophasic	hypophasic
CARR-PRICE reagent	blue	blue
conc. H ₂ SO ₄	blue	blue
HCl reaction	negative	negative

When the mixed benzene solution of the two crystals, mp. 181° of *A. broughtoni* and mp. 183~4° of *A. subcrenata*, was chromatographed upon the column of zinc carbonate and developed with petroleum ether containing 20% acetone, a single zone appeared. The absorption curve of both two crystals was fairly agreeable (Fig. 10). These results show that the two crystalline carotenoids are identical

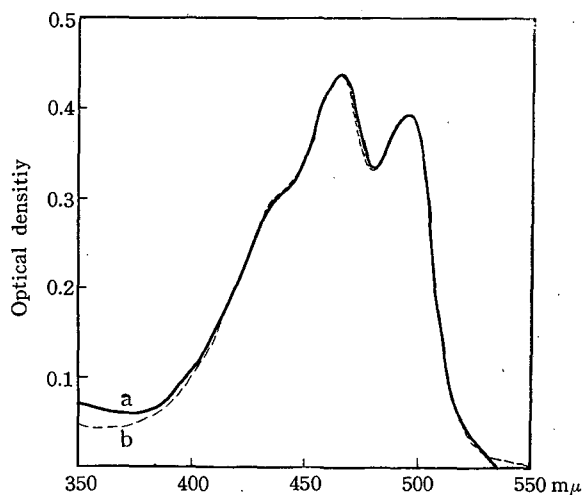


Fig. 10. Absorption spectra of the principal crystalline carotenoid in the foot of sea shell;
(a) ...mp. 181° carotenoid in *Anadara broughtoni*
(b) ...mp. 183~184° carotenoid in *Anadara subcrenata*
(in benzene).

with each other. This carotenoid seems to be almost identical with pectenoxanthin, but unfortunately direct comparison with pectenoxanthin did not carry out because of the lack of the crystals.

Carotenoid in ovary: The properties of the crystalline carotenoid in the orange coloured ovaries of *Pecten yessoensis*, such as mp., absorption maxima, indicate that this carotenoid is identical with pectenoxanthin (mp. 182°, absorption maxima, 434, 464, 496 m μ in benzene).

Summary

Carotenoids in the red-orange coloured foot of the shellfishes, *Anadara broughtoni* and *Anadara subcrenata*, and in the red-orange ovaries of *Pecten yessoensis* have been examined and the principal crystalline carotenoids were isolated in each species. The principal carotenoids of *A. broughtoni* (mp. 181°) and *A. subcrenata* (mp. 183~184°) are identical with each other and seem to be almost identical with pectenoxanthin. The ovary carotenoid of *Pecten yessoensis* (mp. 181~182°) is identical with pectenoxanthin.

Acknowledgment

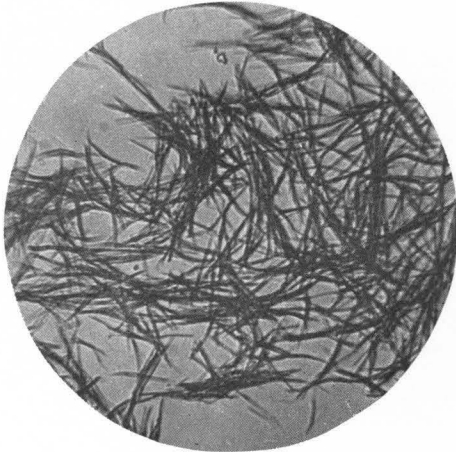
The author wishes to express his deep gratitude to Professor Y. NAKAMURA and Professor T. SAITO of Hokkaido University, for their encouragement and guidance. The author is also grateful to Dr. H. UTINOMI of Seto Marine Biological Laboratory of Kyoto University who gave the opportunity for publication.

REFERENCES

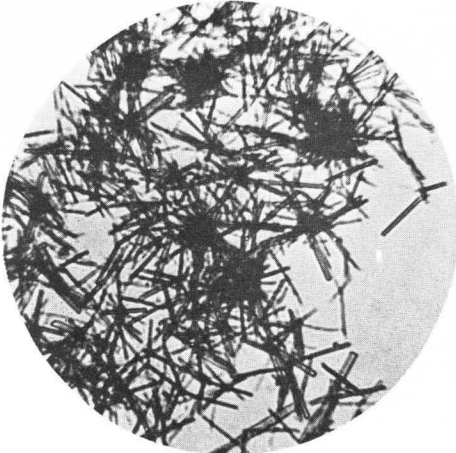
- FABRE, R. et LEDERER, E. 1934. Contributions à l'étude des lipochromes des animaux. Bull. Soc. Chim. biol., Paris, **16**: 105.
- LEDERER, E. 1934. Pigment found in the mollusk *Pecten maximus*. Compt. rend. Soc. biol., **116**: 150-153.
- NISHIBORI, K. 1957. Studies on pigments of marine animals V. Carotenoids from the red muscle and the viscera of giant shield shell, *Tugali gigas*. Bull. Jap. Soc. Sci. Fish., **22**: 715-717.
- SCHEER, B. T. 1940. Some features of the metabolism of the carotenoid pigments of the California sea mussel (*Mytilus californianus*). J. Biol. Chem., **136**: 275-99.

EXPLANATION OF PLATE XXXV

- Fig. 1. Principal crystalline carotenoid, mp. 181°, isolated from the foot of *Anadara broughtoni*.
- Fig. 2. Principal crystalline carotenoid, mp. 183~184°, isolated from the foot of *Anadara subcrenata*.
- Fig. 3. Pectenoxanthin, mp. 181°, isolated from the ovaries of *Pecten yessoensis*.



1



2



3