Studies on Pigments of Marine Animals—V.*

Carotenoids from the Red Muscle and the Viscera of Giant Shield Shell, *Tugali gigas*

Kokichi NISHIBORI

(Notre Dame Seishin College, Okayama)

Many investigations deal with the distribution of carotenoids in molluscs, and carotene, xanthophyll, pectenoxanthin, glycymelin, mythyloxanthin, zeaxanthin and hopkinsiaxanthin have been reported.¹⁾²⁾ The presence of astaxanthin in molluscus, however, is not conclusive excepting a few cases.

Two carotenoids, astacene and a carotenoid provisionally named "Tugali-carotenoid-X" were isolated from the red muscle of giant shield shell, *Tugali gigas*. Moreover, a carotenoid, which appears to be identical with astacene, has been separated from the viscera of this shellfish.

Experimental

1. EXTRACTION OF THE PIGMENTS

Five hundred and twenty six g. of the minced fresh muscle (40 individuals) were covered with acetone and the pigments were extracted with the same solvent at room temperature and transferred into petroleum ether by dilution of the extract with water. The petroleum ether solution was washed with water and dried over anhydrous Na_2SO_4 . After partition between epiphasic and hypophasic (A) layers by shaking with 90 % methanol solution, 2N-KOH methanol solution was added to the epiphasic layer and saponified overnight in a dark place at room temperature. By dilution with a small amount of water, the red alkaline aqueous methanol phase (B) containing red precipitates, was separated from upper petroleum ether phase (C). The petroleum ether phase (C) was washed with water and dried over anhydrous Na_2SO_4 .

FRACTION (A)

The deep red 90 % methanol solution was covered with a layer of petroleum ether and then diluted carefully with an equal volume of water, when most of the pigments were separated as glistening long leaflets at the boundary of the two layers by standing for 6 days in an ice-room. The yield was about 1.5 mg.

This pigment was crystallized from pyridine-water as orange coloured long leaflets. After recrystallization, m.p. gives 178-9°. Pure pigment formed a single zone by chromatographic adsorption on CaCO_3 with ether development. CARR-PRICE reaction (+) blue, conc. H_2SO_4 reaction (+) blue, HCl-reaction (-). On partition test, this pigment is found in lower layer. Absorption maxima are as follows;

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* This work has been carried out at Hokkaido University.

Fig. 1. Absorption spectra of (a) tugali-carotenoid-X (in chloroform) and (b) astacene (in pyridine) from the muscle of *T. gigas*.

crystals were washed with hot water and with petroleum ether. The yield was about 4.2 mg. After recrystallization from pyridine, the pigment crystallized as black-violet sickle-shaped needles melting at 226°. CARR-PRICE reaction (+) violet-blue, absorption maximum 496 mμ in pyridine, 508 mμ in carbon disulfide. On partition between petroleum ether and 90 % methanol, the pigment is found in the lower layer. Neither the fall of m. p. nor the displacement in spectrum was observed by mixed examination with astacene (m. p. 228°, absorption maximum 510 mμ in CS₂, prepared from crustacea*). This pigment is, therefore, astacene which has been derived from astaxanthin esters in the muscle.

FRACTION (C)

Orange red petroleum ether solution (90 % methanol soluble pigment free) was washed with water and dried over anhydrous Na₂SO₄, then it was chromatographed on alumina (WAKO-active alumina, heated for 1 hour; 2.8 cm×20 cm) and developed with petroleum ether-acetone mixture (1:1). The fractions obtained, were summarized in Table 1. No crystalline pigment has been obtained from these fractions which showed positive CARR-PRICE

Table 1. Absorption maxima of chromatographic adsorbed zones of fraction C

No.†	Colour of the adsorbed zones	Absorption maxima (in CHCl ₃)
1a	Blood red	~480 mμ.
1b	Blood red	~478 mμ.
1c	Orange red	~435, 462, ~484 mμ.
2	Orange	~435, 459, ~485 mμ.
3	Orange	~435, 461, ~490 mμ.
4	Orange red	~435, 460, ~485 mμ.

† Numbered in order of decreasing adsorptive power.

* *Pandalus sp.*

430-5, 461, 490 mμ (in chloroform)
 ——— 479, 510 mμ (in carbon disulfide)
 430-5, 451, 480 mμ (in ethanol)

The crystals were too minute to further study. It is named "Tugali-carotenoid-X" provisionally.

FRACTION (B)

Saponified red alkaline aqueous methanol solution was allowed to stand for a short time after the dilution with water and red precipitates were separated, which were covered with a layer of petroleum ether. Black-violet crystals were precipitated with oily impurities, which were removed by repeated washing with 70 % methanol, after acidification with 50 % acetic acid. The

and conc. H₂SO₄ reactions.

2. A CAROTENOID FROM THE VISCERA OF *Tugali gigas*

The viscera of the shellfish were covered with acetone and the pigments were extracted with the same solvent at room temperature and then transferred into petroleum ether and parted into epiphasic and hypophasic layers as usual.

From the hypophasic layer which contains the greater part of the red pig-

Fig. 2. Absorption spectra of *Tugali gigas* viscera pigments.

- a) epiphasic carotenoid (in chloroform).
- b) hypophasic carotenoid (in chloroform).
- c) astacene (in carbon disulfide).

noids, astacene and a hypophasic carotenoid provisionally named "Tugali-carotenoid-X", have been isolated. It seemed that the astacene was derived from astaxanthin esters in the red muscle and the "Tugali-carotenoid-X" existed at the free state.

A carotenoid which appears to be identical with astacene has also been separated from the viscera of this shellfish.

It has been confirmed, therefore, that astaxanthin is present in molluscs.

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References

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ment, astaxanthin-like crystals (m.p. 205–8°, violet-red plates, CARR-PRICE reaction (+), blue-violet) were separated after the dilution with water in the usual manner.

After the alkaline treatment and acidification with 50 % acetic acid, a crystalline pigment (m.p. 216–8°, absorption maximum 508 mμ in CS₂, violet sickle-shaped needle) has been isolated. From the properties of this pigment, it seems that this crystalline pigment is identical with astacene.

Summary

Pigments of the red muscle of *Tugali gigas* have been examined and two carote-