アスタキサンチンの生合成 IV

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The Biosynthesis of Astaxanthin—IV. The Carotenoids in the Prawn, *Penaeus japonicus Bate* (Part 1)

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1. Besides astaxanthin, the presence of β-carotene, echinenone, canthaxanthin, dihydroxy-pirardixanthin ester, phoenicoxanthin was confirmed in prawn.

2. A possible metabolic pathway from β-carotene to astaxanthin is proposed, and its sequence ist hus suggested: β-carotene→echinenone→canthaxanthin→phoenicoxanthin→astaxanthin.

It is generally accepted that in animals astaxanthin is formed by the oxidation of carotenoids in food. The carotenoids in crustaceans have been investigated by many researchers:

SØRENSEN[1] studied the carotenoids of fresh water crustaceans, *Halopdium gibberum*, *Heterocope saliens*, and *Gammarus pulex* and first proposed that these animals can oxidize β-carotene or its derivatives in food to astaxanthin.

GOODWIN *et al.*[2] investigated the pigments in marine crustacea and isolated astaxanthin, and β-carotene but did not isolate an astaxanthin ester.

GILCHRIST and GREEN[3] found β-carotene, γ-carotene, a carotene oxide, esterified astaxanthin, and algae carotenoids in *Artemia salina.*

CORNWELL *et al.*[4] isolated β-carotene, lutein, and astaxanthin in *Carvernicolus Crayfish.*

THOMMEN *et al.*[5] studied carotenoids in *Daphnia* and isolated β-carotene, echinenone, and canthaxanthin but did not isolate an astaxanthin. They showed that those animals oxidized β-carotene to astaxanthin via echinenone.

BODEA *et al.*[6,7] confirmed the existence of astaxanthin, β-carotene, hydroxy echinenone, crustaxanthin, and three unidentified pigments in *Crustacea Copepoda Calanoida* and they observed a close biochemical correlation between astaxanthin and three minor carotenoids.

DAVIES *et al.*[8], KRINSKY[9], CZYGAN[10] and HATA *et al.*[11] investigated the carotenoids in *Artemia salina* and have shown that *Artemia* is capable of converting β-carotene into echinenone and that echinenone is converted into canthaxanthin.

The pigments of three variants of the marine isopod *Idotea montereyensis* were in-

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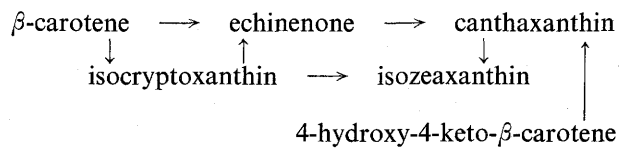
vestigated by LEE[12,13], who isolated β-carotene, echinenone, canthaxanthin, 4-hydroxy-4′-keto-β-carotene, lutein, and lutein ester, but no astaxanthin. The metabolic pathway from β-carotene to canthaxanthin he suggested is as follows: β-carotene→echinenone→4-hydroxy-4′-keto-β-caroten→canthaxanthin. He also isolated β-carotene, isocryptoxanthin, echinenone, 4-hydroxy-4′-keto-β-carotene, canthaxanthin, isozeaxanthin and lutein, but no astaxanthin, in another isopod species, *Idothea granulosa*. He therefore proposed a metabolic pathway for conversion of β-carotene to canthaxanthin in this animal as follows: β-carotene→isocryptoxanthin→echinenone→4-hydroxy-4′-keto-β-carotene→canthaxanthin.

GILCHRIST and LEE[14] isolated β-carotene, δ-carotene, echinenone, isocryptoxanthin, canthaxanthin, lutein, zeaxanthin, lutein-5,8-epoxide, astaxanthin, and 4-hydroxy-4′-keto-β-carotene. A metabolic pathway in this animal was proposed as follows: β-carotene→isocryptoxanthin→echinenone→canthaxanthin→astaxanthin.

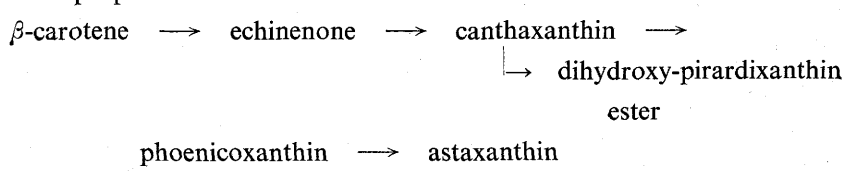
CHICHESTER *et al.*[15,16] recently found that in the Californian strain of *Artemia salina*, the two step conversion of β-carotene into canthaxanthin was the apparent pathway as follows:



HERRING[17] studied the carotenoids in *Daphnia magna* and isolated β-carotene, echinenone, canthaxanthin, one keto-carotenoid (probably 3-hydroxy-4-keto-β-carotene), and astaxanthin. He suggested that the animals were able to form echinenone, canthaxanthin, and astaxanthin from β-carotene.

On the other hand, the carotenoids in prawn, *Penaeus japonicus Bate*, were investigated by MIYAKE *et al.*[18,19], who found the existence of lutein and astaxanthin.

The present investigation was undertaken to determine the pigments and the metabolic pathway from plant carotenoids into astaxanthin in prawn. Besides astaxanthin, the existence of β-carotene, echinenone, canthaxanthin, dihydroxy-pirardixanthin ester, and phoenicoxanthin was confirmed. The following metabolic pathway from β-carotene to astaxanthin is proposed:



Materials and Methods

Fresh wild prawn (length: about 20 cm) were bought at the local fish shop. The

carapaces and the hypodermis were collected and extracted with acetone in a Waring blender until no further pigments could be obtained. The combined acetone solutions were diluted with 2 vol. of water and extracted with petroleum ether. The petroleum ether solution of pigments was repeatedly washed with water to remove acetone.

The petroleum ether extracts were concentrated under vacuum and dried over sodium sulfate. The absorption spectra of the crude carotenoids in petroleum ether is shown in Fig. 1.

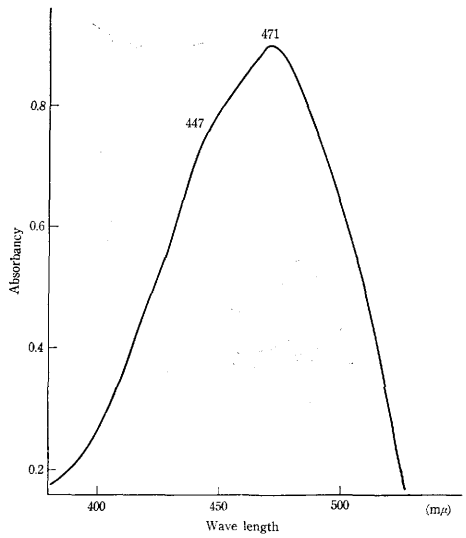


Fig. 1. The absorption spectrum of the crude carotenoids extracted from prawns in petroleum ether.

The petroleum ether solution of pigments was then chromatographed using Microcel-C as adsorbent. Development with petroleum ether resulted in the separation of two bands: (I) yellow and (II) red. Yellow band pigments (Band I) were saponified by dissolving them in 100 ml of absolute ethanol, adding 10 ml of 60% (w/v) aqueous potassium hydroxide solution and leaving the solution overnight at room temperature[20-22]. The saponified pigments were transferred to petroleum ether by adding water, were washed repeatedly with water and dried over anhydrous sodium sulfate.

The saponified pigments thus obtained were rechromatographed on Microcel-C column by using petroleum ether as developing solvent. Two bands of pigments were obtained: (a) orange and (b) orange.

β-Carotene. Lower band: band I-a pigment was rechromatographed on aluminum oxide (grade 2, Wako Pure Chemicals, for chromatographic use) by using petroleum ether as developing solvent. The absorption spectra in petroleum ether and n-hexane were all in agreement with those of β-carotene.

Dihydroxy-pirardixanthin. Lower band: Band I-b pigment was run again on aluminum oxide column (grade III), using 15% acetone in petroleum ether as developing solvent. The absorption spectra and the behavior on the column were in agreement with those of dihydroxy-pirardixanthin first obtained from Hydra by KRINSKY *et al.*[23] (Fig. 2). Red band pigments (Band II) were saponified by the above mentioned method. The saponified pigments were rechromatographed on Microcel-C by using 10% acetone in petroleum ether and divided into two fractions, Fr-A and Fr-B.

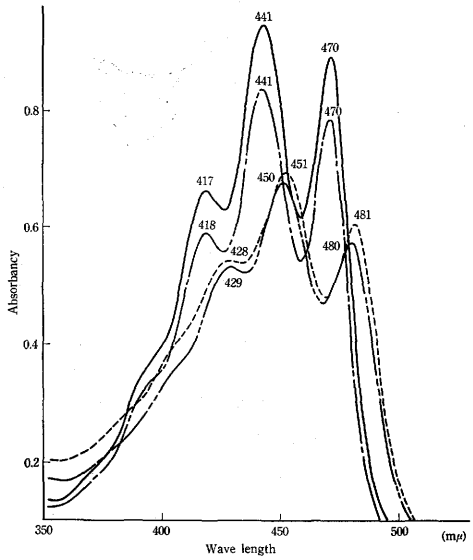


Fig. 2. The absorption spectra of dihydroxypirardixanthin isolated from prawns.
——— in petroleum ether. (417, 441, 470, mμ).
— — — in n-hexane. (418, 441, 470, mμ).
------ in chloroform (428, 451, 481, mμ).
— — — — in benzene (429, 450, 480, mμ).

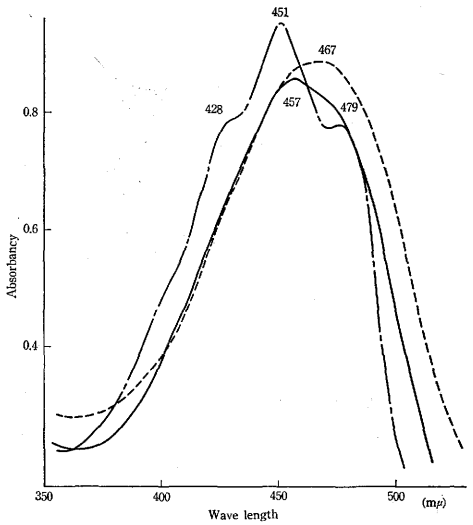


Fig. 3. The absorption spectra of echinenone obtained from prawns.
Solid curve: in petroleum ether.
Dotted curve: in 95% ethanol.
Dashed curve: product of the borohydride reduction of echinenone in 95% ethanol.

Fraction A pigments, which were eluted out from the column, were rechromatographed on Microcel-C using 6% acetone in petroleum ehter, and two bands were obtained.

Echinenone: Lower band pigments of Fraction A was repurifed on Microcel-C. The absorption spectrum indicates a single absorption maximum in petroleum ether and suggests a keto group in conjugation with the double bond system, after reduction, $\lambda_{max.}$ 428, 451, 479 mμ in ethanol (Fig. 3). Those results were all in agreement with that of known echinenone[24].

Canthaxanthin: Upper band pigment of Fraction A was rechromato-

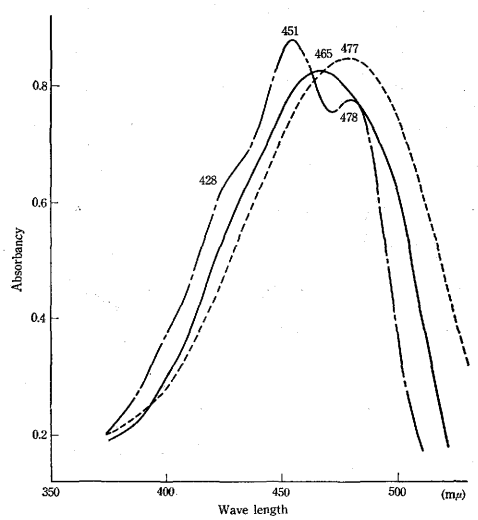


Fig. 4. The absorption spectra of canthaxanthin isolated from Prawns.
Solid curve: in petroleum ether.
Dotted curve: in 95% ethanol.
Dashed curve: product of the borohydride reduction of canthaxanthin in 95% ethanol.

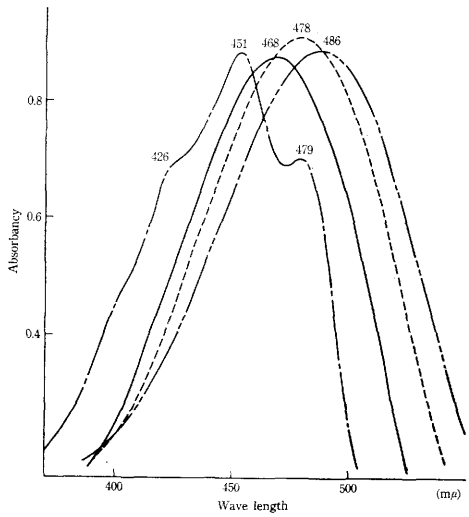


Fig. 5. The absorption spectra of phoeniconone isolated from prawns.
———— in petroleum ether.
— — — — in chloroform.
------ in 95% ethanol.
———— product of the borohydride reduction in 95% ethanol.

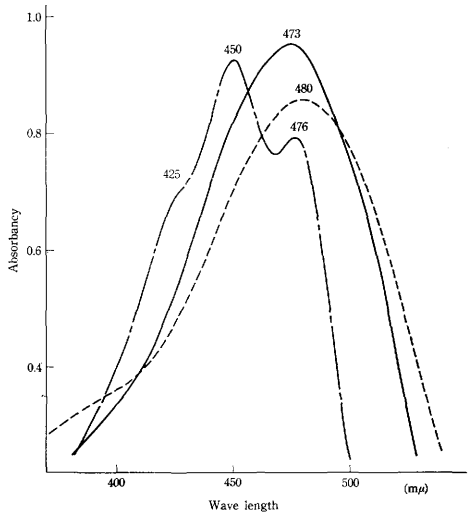


Fig. 6. The absorption spectra of astacin isolated from prawns.
Solid curve: in petroleum ether.
Dotted curve: in 95% ethanol.
Dashed curve: produet of the borohydride reduction in 95% ethanol.

graphed on Microcel-C using 8% acetone in petroleum ether, as developing solvent. The purified pigment exhibited absorption maxima at 465 mμ in petroleum ether and, after reduction with sodium borohydride, $\lambda_{max.}$ 428, 451, 478 mμ (Fig. 4). These values were identical with those of canthaxanthin[25].

Fraction B pigments absorbed on Microcel-C column, were eluted out with 10% acetic acid in ethyl ether. Pigments thus obtained were rechromatographed on dried, powered sugar, using 1.5% acetone in petroleum ether. Two bands were obtained.

Phoeniconone: Lower band pigments were eluted out with acetone and transferred to petroleum ether by addition of water, $\lambda_{max.}$ 468 mμ in petroleum ether, 486 mμ in chloroform and, after reduction with sodium borohydride in ethanol, $\lambda_{max.}$ 426, 451, 479 mμ (Fig. 5). Those results were identical with that of phoeniconone obtained from *Flamingo* by Fox[26].

Astacin: Upper band pigments were extracted with acetone from the sugar column and transferred to petroleum ether by adding water, $\lambda_{max.}$ 473 mμ in petroleum ether, and, after reduction with sodium borohydride in ethanol, $\lambda_{max.}$ 425, 450, 476 μm (Fig. 6). Those values were all in agreement with that of astacin obtained from goldfish[27].

Results and Discussion

The existence of β-carotene, echinenone, canthaxanthin, dihydroxy-pirardixanthin,

phoeniconone, and astacin was confirmed. The following metabolic pathway from β-carotene to astaxanthin was proposed:

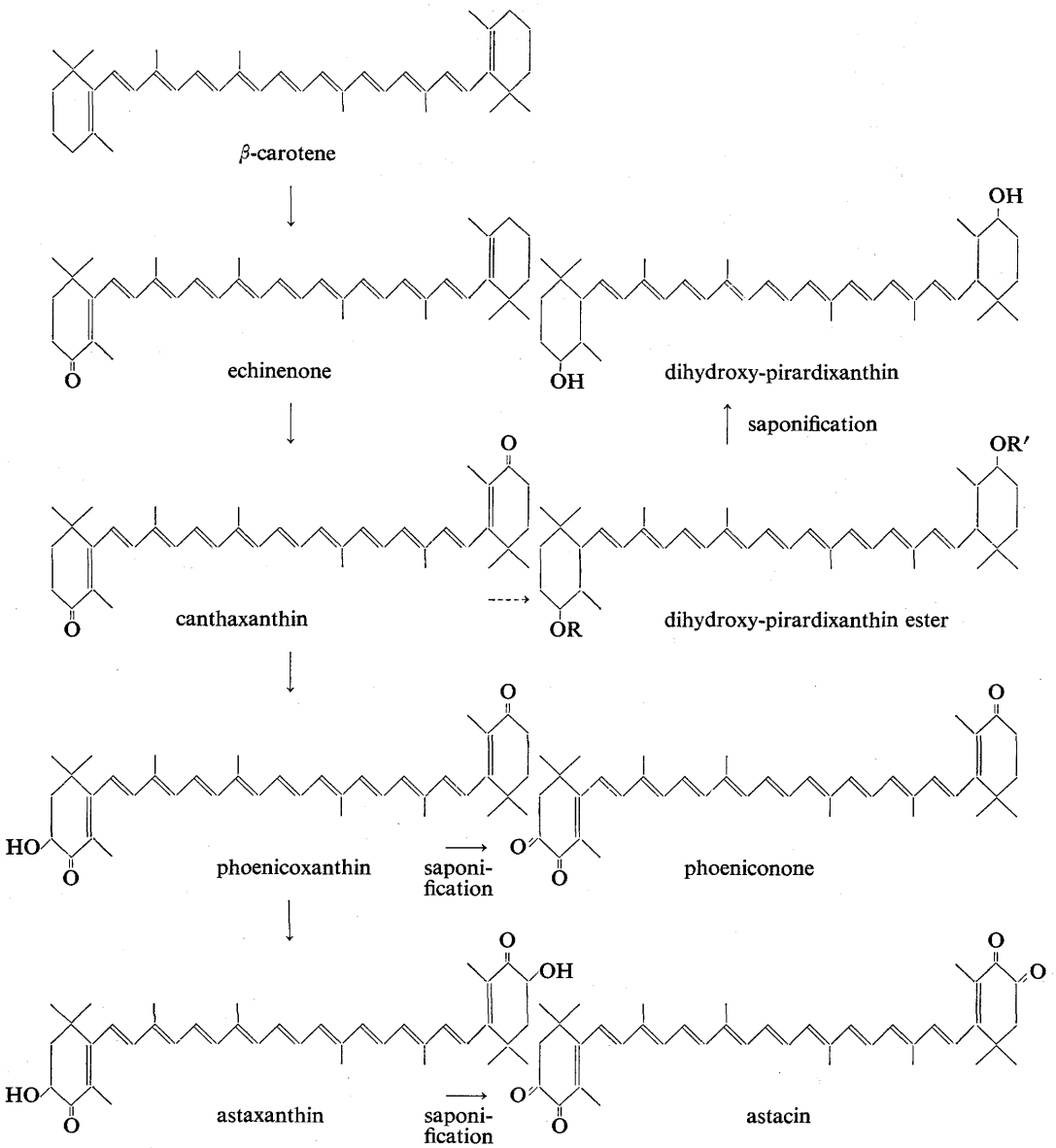


Fig. 7. The metabolic pathway from plant carotenoid, β-carotene, to fish carotenoid, astaxanthin in prawn.

The quantitative observation on the pigmentation of prawn by feeding pure β-carotene, echinenone, and canthaxanthin will be presented in a following paper (part II).

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