The Biosynthesis of Astaxanthin—XI.* The Carotenoids in the Lobster, *Panulirus japonicus*

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(Received December 11, 1972)

1. The existence of canthaxanthin, 4-hydroxy-echinenone, 3-hydroxy-canthaxanthin and astaxanthin in the carapaces of the lobster, *Panulirus japonicus* was confirmed.

2. β -Carotene, β -zeacarotene, echinenone, isocryptoxanthin and astaxanthin were found in the internal organs.

3. It was proposed that in the lobster, dietary β -carotene is converted to astaxanthin through the steps of isocryptoxanthin, echinenone, 4-hydroxy-echinenone, canthaxanthin and 3-hydroxy-canthaxanthin as follows:

β -carotene $\rightarrow$ isocryptoxanthin $\rightarrow$ echinenone $\rightarrow$ 4-hydroxy-echinenone $\rightarrow$
canthaxanthin $\rightarrow$ 3-hydroxy-canthaxanthin $\rightarrow$ astaxanthin

Astacin was first isolated from the lobster shells¹⁾, and was later recognized as a β -carotene-tetra-ketones by KARRER and coworkers²⁻⁵⁾. KUHN and Sørensen^{6,7)} showed that eggs of the lobster did not contain esterified astaxanthin, but free astaxanthin and established the constitution of astaxanthin. STERN *et al.*⁸⁾ found that ovoverdin, the blue pigment in lobster carapaces, was astaxanthin-protein complex. Ovoverdin was stable between pH 4.0-8.0, had an isoelectric point at pH 7.0, exhibited absorption bands at 640 and 670 $m\mu$, and had one molecule of astaxanthin linked to one molecule of protein. GOODWIN *et al.*⁹⁾ confirmed that esterified astaxanthin occurred in the hypodermis of the lobster, and that unesterified pigment occurred in the eggs of the lobster. They also confirmed that there were no quantitative changes in the astaxanthin levels during embryogenesis or immediately after hatching. Shortly before hatching, however, the chromoprotein, ovoverdin is disrupted and astaxanthin liberated. KUHN *et al.*¹⁰⁾ isolated a blue chromoprotein from the lobster shells, whose prosthetic group was astaxanthin, the native chromoprotein was further purified and divided into α -crustacianin and β -crustacianin.

The present investigation was undertaken to determine the carotenoid pigments in the lobster and to propose the metabolic pathway from plant carotenoids into astaxanthin

* The previous report—X. The carotenoids in the red carp, *Cyprinus carpio* Linne and the inter-conversion of β -carotene-15,15'-³H₂ into their body astaxanthin: *Int. J. Biochem.*, 3, 569-573, 1972.

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in them. In the carapaces of the lobsters and in their internal organs, the existence of canthaxanthin, 4-hydroxy-echinenone, 3-hydroxy-canthaxanthin, β -carotene, isocryptoxanthin, β -zeacarotene, echinenone and astaxanthin was confirmed, and the metabolic pathway from β -carotene to astaxanthin was proposed as follows:

Materials and Methods

Fresh and wild lobsters (length: about 25 cm) were purchased at a local fish shop.

a. **The carotenoids in the carapaces of the lobsters:** The carapaces of the lobsters were collected and extracted with acetone in a Waring blender until no further pigments were obtained. The separate acetone solutions of the pigments were combined and transferred to petroleum ether by the addition of water. The petroleum ether solution of the pigments was repeatedly washed with water to remove the acetone, concentrated under vacuum and dried over anhydrous sodium sulfate.

The petroleum ether solution of the pigments was first chromatographed on a Microcel-C column, using petroleum ether as the developing solvent, and two bands were obtained: Band-I (yellow, lower band) and Band-II (red, upper band). The pigments of lower band (Band-I) were saponified by dissolving them in 50 ml of absolute ethanol, adding 5 ml of 60 percent (W/V) aqueous potassium hydroxide solution, and leaving them overnight¹¹⁻¹³. The saponified pigments were rechromatographed on a Microcel-C column by using 10% acetone in petroleum ether as the developing solvent. Two unidentified pigments were obtained. The pigments of Band-II (red, upper band) were saponified by the above mentioned method. The saponified pigments were rechromatographed on Microcel-C, using 3% acetone in petroleum ether as the developing solvent. Two bands were obtained: Band-II-A (lower band, orange) and Band-II-B (upper band, red).

Canthaxanthin: The pigments of Band-II-A (lower band) were rechromatographed on a Microcel-C column, using 5% acetone in petroleum ether as the developing solvent. One band was obtained. The absorption spectrum indicated a single absorption maximum at 465 m μ in petroleum ether, and after reduction in ethanol, a maxima at 427, 451, 479 m μ . These results were all identical with those of pure canthaxanthin obtained from the prawn, *Penaeus japonicus* Bate¹⁴. The pigments of upper band (Band-II-B) were rechromatographed on a sugar column by using 0.8% acetone in petroleum ether. Three bands were obtained: Band-II-B-a (lower band, orange), Band-II-B-b (middle band, pink) and Band-II-B-c (upper band, pink).

4-Hydroxy-echinenone: The pigments of Band-II-B-a (lower band) were rechromatographed on a Microcel-C column, using 10% acetone in petroleum ether as the

developing solvent. Thus purified pigment exhibited as single absorption maximum at 454 $m\mu$ in petroleum ether. After reduction with sodium borohydride in ethanol, the pigment showed a β -carotene-like absorption spectrum which was identical with that of isozeaxanthin formed by reducing canthaxanthin¹⁵). These reduction products showed a single spot on mixed thin-layer chromatograms. The behavior on the column, absorption maxima and the similarity of the reduction products of this pigment with those of reduced canthaxanthin indicate to be 4-hydroxy-4'-keto- β -carotene¹⁶).

3-Keto-canthaxanthin (Phoeniconone): The pigments of Band-II-B-b (middle band, pink) were rechromatographed on a sugar column by using 0.2% acetone in petroleum ether as the developing solvent. The absorption spectrum showed a single absorption maximum at 469 $m\mu$, and after reduction with sodium borohydride in ethanol, *maxima at* 426, 451, 479 $m\mu$. These results were identical with those of pure phoeniconone¹⁷) obtained from the prawn, *Penaeus japonicus* Bate¹⁴).

Astacin: The pigments of Band-II-B-c (upper band, pink) were repurified on a sugar column, using 1.5% acetone in petroleum ether as the developing solvent. Thus purified pigments showed a single absorption maximum at 474 $m\mu$ in petroleum ether. These results were identical with those of pure astacin obtained from sea bream^{18,19}).

b. The carotenoids in the internal organs of the lobsters: The internal organs of the lobsters were collected and extracted with acetone in a Waring blender. Acetone solution of the pigments was transferred to petroleum ether by the addition of water. Petroleum ether solution of the pigments was concentrated under vacuum to an oil. The pigments were dissolved in about 10 ml of petroleum ether for chromatography on a Microcel-C column. The column was developed with petroleum ether. Two bands were obtained: Fr-I (lower band, yellow) and Fr-II (upper band, red). The pigments of Fr-I (lower band) were saponified by the above mentioned method. The saponified pigments were rechromatographed on an aluminum oxide column (activity grade II), using 2% acetone in petroleum ether as the developing solvent and three bands were obtained: Fr-I-a (lower band, orange), Fr-I-b (middle band, orange) and Fr-I-c (upper band, orange).

The pigments of Fr-I-a (lower band) were repurified on an aluminum oxide column, using petroleum ether as the developing solvent, and two bands were obtained: Fr-I-a-1 (lower band) and Fr-I-a-2 (upper band).

β -carotene: The pigments of Fr-I-a-1 (lower band) were rechromatographed on an aluminum oxide column by using petroleum ether as the developing solvent. The pigment was identified by the behavior on the column, the shape and position of the absorption maxima as β -carotene (Table 2).

β -zeacarotene: The pigments of Fr-I-a-2 (upper band) were repurified on an aluminum oxide column by using 0.5% acetone in petroleum ether as the developing solvent. The behavior on the column, the shape and the position of the absorption maxima were

all identical with those of pure β -zeaxanthin¹⁸⁾.

Echinenone: The pigments of Fr-I-b (middle band) were rechromatographed on a magnesium oxide column (magnesium oxide: Hyflo Supercel=1:2), using 3% acetone in petroleum ether as the developing solvent. Only one band was obtained. The absorption spectrum indicates a single absorption maximum at 456 m μ in petroleum ether, and after reduction with sodium borohydride in ethanol, a maxima at 425, 451, 479 m μ . These results were all in agreement with those of pure echinenone obtained from the prawn, *Penaeus japonicus* Bate¹⁶⁾.

Isocryptoxanthin: The pigments of Fr-I-c (upper band) were repurified on a magnesium oxide column (magnesium oxide: Hyflo Supercel=1:2), using 5% acetone in petroleum ether as the developing solvent. Only one band was obtained. The pigment has λ max. 425, 449, 476 m μ in petroleum ether and forms a unitary zone in co-chromatogram with pure isocryptoxanthin obtained from the crab, *Portunus trituberculatus*²¹⁾.

Astacin: The pigments of Fr-II (upper band) were saponified by the above mentioned method. The saponified pigments were rechromatographed on a Microcel-C column, using 10% acetone in petroleum ether as the developing solvent. Only one band was obtained. The pigments thus obtained were repurified on a sugar column by using 1.5% acetone in petroleum ether as the developing solvent. The pigments thus purified had a maximum at 474 m μ in petroleum ether, after reduction with sodium borohydride in ethanol, the absorption spectra had a maximum at 427, 451, 477 m μ . These results were all identical with those of pure astacin obtained from gold fish¹²⁾.

Results and Discussions

The carotenoid pigments in the carapaces of the lobsters are listed in **Table 1** in the order which they were eluted from the columns and the relative amounts of each pigment are given as a percentage of the total. The carotenoid pigments of the internal organs are also listed in **Table 2** in the order which they were eluted from the columns. In their internal organs, the existence of β -carotene, β -zeacarotene, echinenone, isocryptoxanthin and astaxanthin was confirmed, and in their carapaces, canthaxanthin, 4-hydroxy-echinenone, 3-hydroxy-canthaxanthin and astaxanthin were found. In the series of this thesis^{14,22)}, it was clarified that in the prawn, β -carotene was converted to their body astaxanthin through the steps of echinenone, canthaxanthin and 3-hydroxy-canthaxanthin by feeding the standard diet containing β -carotene-15,15'-³H₂ dissolved in plant oil. It is very interesting to note that in the internal organs of the prawn, the existence of β -carotene, echinenone, canthaxanthin, 3-hydroxy-canthaxanthin and astaxanthin was confirmed, and 3-hydroxy-canthaxanthin and astaxanthin were found in their carapaces¹⁴⁾, but in the lobster and the crab, *Portunus trituberculatus*²¹⁾, only β -carotene, echinenone and astaxanthin were found in their internal organs, and in their

carapaces, the existence of 4-hydroxy-echinenone, canthaxanthin, 3-hydroxy-canthaxanthin and astaxanthin was confirmed. These results suggest that in the prawn, β -carotene in the diet is incorporated into their body astaxanthin via echinenone, canthaxanthin and 3-hydroxy-canthaxanthin in their internal organs, but in the lobster and the crab, only

Table 1. Spectral characteristics and relative abundance of the carotenoids in the carapaces of the lobster, *Panulirus japonicus*.

Compounds	Relative abundance %	λ max. m μ in		
		Petroleum ether	Ethanol	after reduction with sodium borohydride
Canthaxanthin	4.0	465	475	427, 451, 479
4-Hydroxy-echinenone	trace	454	462	428, 450, 478
3-Keto-canthaxanthin	4.0	469	478	426, 451, 479
Astacin	91.0	474	480	426, 451, 478

Table 2. Spectral characteristics and relative abundance of the carotenoids in the internal organs of the lobster, *Panulirus japonicus*.

Compounds	Relative abundance %	λ max. m μ in		
		Petroleum ether	Ethanol	after reduction with sodium borohydride
β -Carotene	3.0	426, 450, 478	427, 450, 475	—
β -Zeacarotene	1.0	407, 428, 455	408, 427, 456	—
Echinenone	5.0	456	459	425, 451, 479
Isocryptoxanthin	2.0	425, 449, 476	427, 450, 477	—
Astacin	89.0	474	480	427, 451, 477

echinenone might be synthesized from β -carotene in their internal organs via isocryptoxanthin, and the echinenone would be then converted to their body astaxanthin through the steps of 4-hydroxy-echinenone, canthaxanthin and 3-hydroxy-canthaxanthin in the cells of the other parts.

Acknowledgement

The authors acknowledge with gratitude the generosity of F. Hoffman La Roche Ltd., Basle, Switzerland for gift of authentic carotenoids.

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