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Mechanism of the Interconversion of Plant Carotenoids into Fish Carotenoids···III*

Carotenoids in the Yellow-golden Carp, *Cyprinus carpio Linne*

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Abstract

The carotenoids in the yellow golden carp were extracted, purified on the columns, characterized by absorption spectra, the behavior on the columns, absorption maxima of the reduction products and co-chromatography with authentic samples.

In the internal organs of the yellow golden carp, the existence of canthaxanthin, lutein and zeaxanthin was confirmed. In the integuments lutein ester, zeaxanthin ester, α-doradexanthin ester and astaxanthin ester were found. It was clarified that in the integuments of the yellow golden carp, the contents of lutein ester were three times of that in the red carp, and the contents of astaxanthin were about one half of that in the latter.

The metabolic pathway in the yellow golden carp was proposed as follows :

Lutein ester → α-Doradexanthin ester → Astaxanthin ester, It was clarified that the yellow golden carp belongs to the RED CARP-FORM from the point of view of the "Biosynthesis of Astaxanthin" in aquatic animals.

The carotenoids in the gold fish, *Carassius auratus* [1], Benibuna, *Carassius auratus* [2], the prawn, *Penaeus japonicus Bate* [3,4], the sea bream, *Chrysophrys major Temminck* and *Schlegel* [5,6], the fancy red carp, *Cyprinus carpio Linne* [7,8], the lobster, *Panulirus japonicus* [9,10], and the crab, *Portunus trituberculatus* [11,12], have intensively been studied. By having fed β-carotene-15, 15′-$^3H_2$ to the gold fish [13], the fancy red carp [8], the sea bream [14], sea bream, the prawn [13], the lobster [10], and the crab [12], it was clarified that the prawn, the the lobster and the crab could convert β-carotene to astaxanthin through the steps of echinenone, canthaxanthin and 3-hydroxy-canthaxanthin, but the sea bream, the red sea bream, the gold fish and the fancy red carp could not convert β-carotene to astaxanthin [8,13,15].

In the carotenoids of the gold fish [1], Benibuna [2], the red carp [14], and the fancy red carp [16], a new keto carotenoid, 3-hydroxy-3′, 4′-diketo-α-carotene was found, its structure was clarified and the name, α-doradecin was proposed for the new keto compound and α-doradexanthin for the estreified carotenoid or mono keto compound [1], It was confirmed that lutein was converted to astaxanthin via α-doradexanthin in the gold fish and the red carp, by having fed, ^{14}C-lutein [17,18].

The present investigation was undertaken to clarify the constituents of the carotenoids in the yellow-golden carp and to confirm the metabolic pathway from plant carotenoids into fish

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carotenoids. In the internal organs of the yellow-golden carp, canthaxanthin, lutein and zeaxanthin were found, and in the integuments the existence of lutein, zeaxanthin, α-doradexanthin and astaxanthin was confirmed. It is very interesting to note that in the integuments of the yellow-golden carp the contents of lutein were three times of that in the red carp and the contents of astaxanthin in the former were about one-half of the latter. It was proposed that lutein would be converted to astaxanthin via α-doradexanthin ester and the yellow-golden carp belongs to the Red Carp-Form on the basis that astaxanthin is biosynthesized [12],

Materials and Methods

Fresh yellow-golden carp (length : about 24 cm) were purchased at a local fish hatchery.

I. The carotenoids in the internal organs of the yellow-golden carp : The internal organs of the yellow-golden carp were collected and extracted with acetone in a Waring blender until no further pigments could be obtained. The separate solutions of the pigments were combined. The acetone solutions of the pigments were transferred to petroleum ether by the addition of water. The petroleum ether solutions of the pigments were washed with water to remove the trace of acetone, concentrated under vacuum and dried over anhydrous sodium sulfate.

The petroleum ether solutions of the pigments 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 it over night [1, 16], The saponified pigments were chromatographed on a Microcel-C column by using 3% acetone in petroleum ether as the developing solvent. Two bands were obtained : Fr-I (lower band, orange) and Fr-II (upper band, orange).

Canthaxanthin : The pigments of Fr-I (lower band) were rechromatographed on a magnesium oxide column (magnesium oxide : hyflosupercel = 1:2), using 12% acetone in petroleum ether as the developing solvent. Only one band was obtained. Thus purified pigments exhibited absorption maximum at 465 mμ in petroleum ether, was co-chromatographed on a magnesium oxide column with authentic pure canthaxanthin obtained from the prawn [3], and formed a unitary zone. After reduction with sodium borohydride in ethanol, a maxima at 427, 450, 479 mμ. These results show this pigment to be canthaxanthin.

The pigments of Fr-II (upper band) were rechromatographed on a magnesium oxide column (magnesium oxide : hyflosupercel = 1 : 2), using 25% acetone in petroleum ether as the developing solvent. Two bands were obtained : Fr-II-a (lower band, orange) and Fr-II-b (upper band, pink).

Lutein : The pigment of Fr-II-a (lower band, orange) was eluted with acetone from the column. The absorption spectra were identical with those of lutein and the pigement was co-chromatographed on a magnesium oxide column with pure lutein obtained from the gold fish [1], and formed a unitary zone. These results show this pigment to be lutein.

Zeaxanthin : The pigment of Fr-II-b (upper band) was eluted from the column with acetone and transferred to petroleum ether by the addition of water. The pigment thus purified exhibited absorption maxima at 425, 451, 481 mμ, was co-chromatographed on a magnesium oxide column with pure zeaxanthin obtained from Benibuna, *Carassius auratus* [2], and formed a unitary band. These results show this pigment to be zeaxanthin.

II. The carotenoids in the integuments of the yellow-golden carp : Dosal and ventral

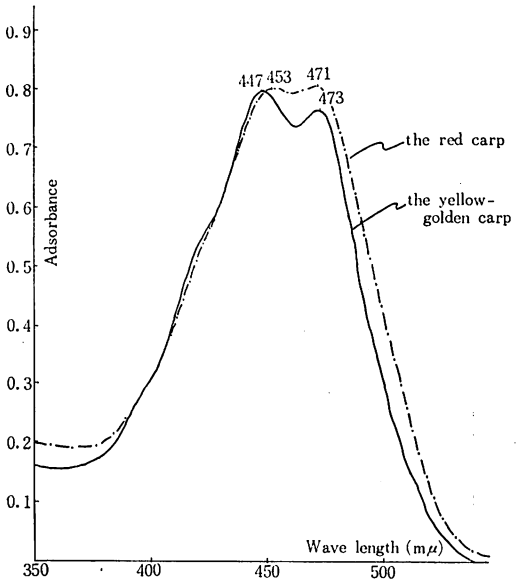

Fig. 1. The absorption spectrum of the crude carotenoids obtained from the yellow-golden carp and the red carp in petroloum ether.

sections of the integuments were collected and extracted exhaustively with acetone until the extract became colorless. The extracted pigment solutions of acetone were combined and transferred to petroleum ether by the addition of water. The petroleum ether solution of the pigments was concentrated under vacuum and dried over anhydrous sodium sulfate (**Fig. 1**)

The petroleum ether solution of the pigments was chromatographed on a Microcel-C column, using 0.3% acetone in petroleum ether as the developing solvent. Three bands were obtained : Fr-I (lower band, orange), Fr-II (middle band, orange) and Fr-III (upper band, red).

The pigments of Fr-I (lower band) were saponified by the above-mentioned method. After saponification the pigments were transferred to petrolum ether by the addition of water. The saponified pigments were rechromatographed on a magnesium oxide column (magnesium oxide : hyflosupercel = 1 : 2), using 25% acetone in petroleum ether as the developing solvent. Two bands were obtained : Fr-I-a (lower band, orange) and Fr-I-b (upper band, pink).

Lutein : The absorption spectra in petroleum ether (λ max 422, 446, 476 mμ) and the behavior on the magnesium oxide column were identical with those of pure lutein obtained from the gold fish [1], The pigment was co-chromatographed on a magnesium oxide column with pure lutein obtained from the fancy red carp [14] and formed a unitary zone. These results show this pigment to be lutein.

Zeaxanthin : The pigment of Fr-I-b (upper band) exhibited absorption maxima at 426, 451 and 477 mμ in petroleum ether. The absorption spectra and the behavior on the column were in agreement with those of pure zeaxanthin. This pigment showed a single zone, when it was co-chromatographed on a magnesium oxide column with a sample of pure zeaxanthin obtained from the red carp [14]. The existence of zeaxanthin was confirmed.

α-Doradecin : The pigment of Fr-II (middle band, orange) indicated a single absorption

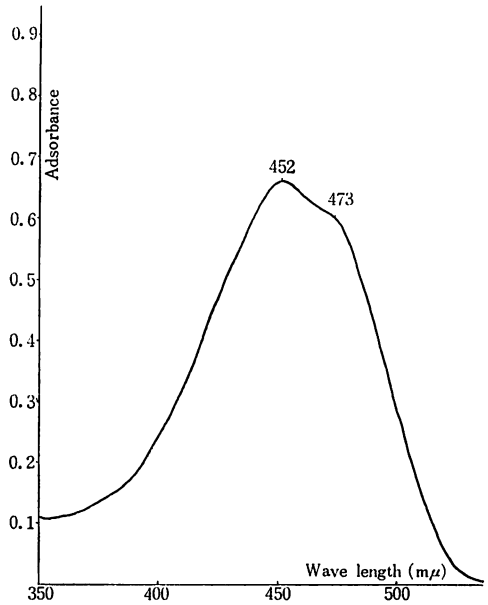

Fig. 2. The absorption spectrum of α-doradexanthin ester obtained from the yellow-golden carp.

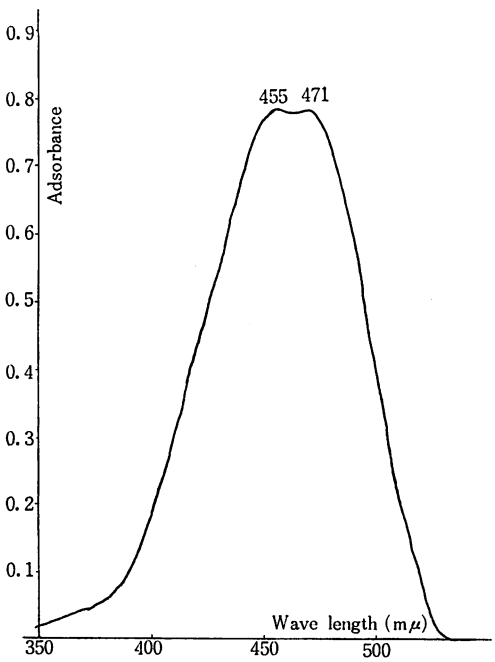

Fig. 3. The absorption spectrum of α-doradecin obtained from the yellow-golden carp in petroleum ether.

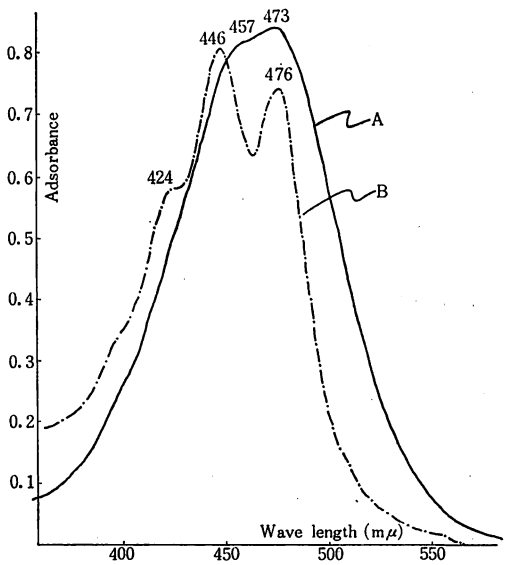

Fig. 4. A. α-Doradecin in ethanol. B. product of the sodium borohydride reduction of α-doradecin.

maximum at 452 mμ in petroleum ether (**Fig. 2**). The absorption spectrum and the behavior on the column were identical with those of α-doradexanthin ester which was first isolated and its name was also proposed by the author [1]. The pigment was saponified by the same method as was reported earlier in the other paper [1]. The saponified pigment was first rechromatographed on a Microcel-C column by using 15% acetone in petroleum ether as the developing solvent in order to remove any contaminating lutein and zeaxanthin. The pigment thus obtained was purified on a sugar column, using 0.5% acetone in petroleum ether as the developing solvent. The pigment thus purified had the following characteristics : λ max = 456, 471 mμ in n-hexane, and after reduction with sodium borohydride in ethanol, λ max = 424, 446, 476 mμ (**Fig. 4**). These values were all in agreement with those of pure α-doradecin, which was first isolated from the gold fish [1], and its name was proposed by the author [1].

Astacin : The pigment of Fr-III (upper band, red) was saponified by using the above-mentioned method. The saponified pigment was transferred to petroleum ether by the addition of water. The petroleum ether solution of the pigment was concentrated under vacuum and dried over anhydrous sodium sulfate. The pigment thus obtained was rechromatographed on a Microcel-C column by using 10% acetone in petroleum ether in order to separate any contaminating α-doradecin, lutein and zeaxanthin. The band was cut from the column, eluted with 10% acetic acid in ethyl ether. The pigment was rechromatographed on a powdered sugar column, using 0.5% acetone in petroleum ether as the developing solvent. Only one band was obtained. The pigment showed an absorption maximum at 473 mμ, and after reduction with sodium borohydride in ethanol showed maxima at 427, 450, and 476 mμ. These results were all identical with pure astacin obtained from the gold fish [1].

Results and Discussions

The carotenoid pigments in the internal organs of the yellow-golden carp are listed in **Table 1** in the order which they were eluted from the column and the relative amounts of each pigment are given as a percentage of the total. The carotenoid pigments of the integuments are also listed in **Table 2** together with those of the red carp as reference.

In the internal organs of the yellow-golden carp, canthaxanthin, lutein ester and zeaxanthin ester were found, and in the integuments the existence of lutein ester, α-doradexanthin ester, zeaxanthin ester and astaxanthin ester was confirmed.

Table 1. The spectral characteristics and relative abundances of the carotenoids in the internal organs of the red carp and yellow-golden carp.

Pigments	Spectral characteristics			relative abundances (%)	
	λ max (mμ) in petroleum ether	λ max (mμ) in ethanol after reduction	λ max (mμ) in chloroform	in the red carp	in the yellow-golden carp
Canthaxanthin	465	425, 451, 476		25	23
Lutein	425, 446, 476		433, 458, 487	60	61
Zeaxanthin	429, 451, 480		434, 461, 490	15	16

Table 2. The spectral characteristics and relative abundances of the carotenoids in the integuments of the red carp and yellow-golden carp.

Pigments	Spectral characteristics			relative abundances (%)	
	λ max (mμ) in petroleum ether	λ max (mμ) in ethanol after reduction	λ max (mμ) in chloroform	in the red carp	in the yellow-golden carp
Lutein	422, 446, 475		432, 459, 487	15	45
Zeaxanthin	427, 451, 481		433, 461, 490	5	5
α-Doradecin	455, 471	424, 446, 476		30	20
Astacin	473	423, 451, 480		50	30

It was clarified that the yellow-golden carp did not metabolize lutein to astaxanthin ester in their internal organs but they could convert it in the cells of the other part as the gold fish and the red carp did [1, 14]. The following metabolic pathway from lutein ester to astaxanthin ester via α-doradexanthin ester was proposed (**Fig. 5**).

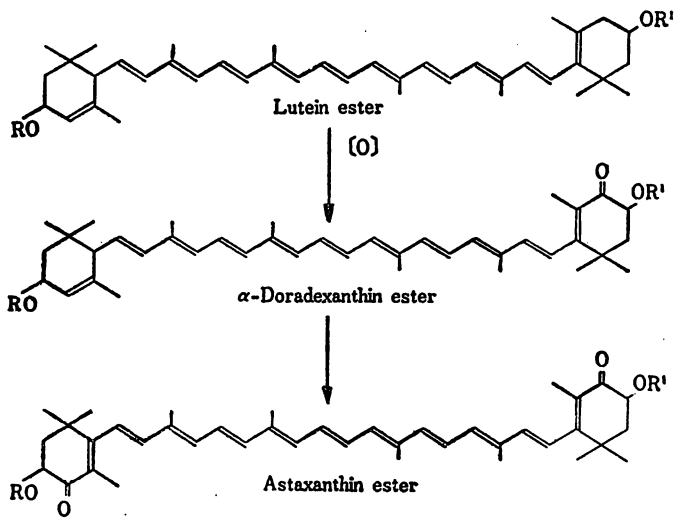

Fig. 5. The metabolic pathway from plant carotenoid to fish carotenoid (astaxanthin),

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