The Existence of 3-Hydroxy-ε-carotene (Neothxanthin) in Kiwada, Neothunnus albacora

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The Existence of 3-Hydroxy-ε-carotene (Neothxanthin) in Kiwada, *Neothunnus albacora*

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Abstract

The carotenoids in tuna fish were already investigated and its main carotenoid was isolated as tunaxanthin by HIRAO *et al.* Thereafter, CROZIER determined the structure of tunaxanthin to be 3, 3′-dihydoxy-ε-carotene by partition coefficient.

The present authors isolated tunaxanthin like pigment (neothxanthin) from the fin and integuments of Kiwada, *Neothunus albacora*. From its absorption spectrum and partition coefficient, this carotenoid was presumed to be 3-hydroxy-ε-carotene and an intermediate of tunaxanthin from ε-carotene.

In general, main yellow pigments of tuna fish and mackerel were first isolated by HIRAO *et al*[1] and its structure was elucidated by CROZIER[2], but the metabolic pathway from some carotenoids to tunaxanthin has not yet been clarified.

The present authors isolated 3-hydroxy-ε-carotene from tuna fish, Kiwada, *Neothunnus albacora* and supposed that ε-carotene in an alga[3] would be converted to tunaxanthin through 3-hydroxy-ε-carotene in fish.

Materials and Methods

1) Isolation of neothxanthin and tunaxanthin from tuna fish, Kiwada: The integuments and fins of Kiwada were collected and the carotenoids were extracted with acetone in a Waring blender and the solid materials were separated by filtration. The carotenoid pigments of acetone solution were transferred to petroleum ether by the addition of water. The petroleum ether solution of the crude carotenoids was washed with water to remove trace of acetone, dried over anhydrous sodium sulphate and concentrated to an oil under reduced pressure.

The esterified carotenoids and carotenes were initially saponified by dissolving them in 100 ml of absolute ethyl alcohol, adding 10 ml of 60% (W/V) aqueous potassium hydroxide and leaving it over night under nitrogen at room temperature[4].

The saponified pigments were transferred to petroleum ether with water, dried over anhydrous sodium sulphate and concentrated under vacuum. The purification procedure is shown in **Fig. 1.**

2) Isolation of ε-carotene from *Tethya amamensis*: The sea sponge,

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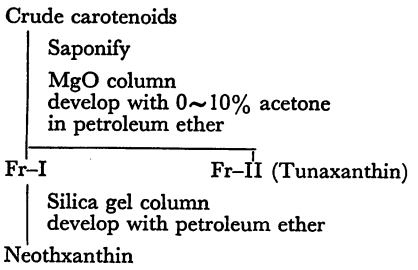

Fig. 1. Isolation of tunaxanthin and neothxanthin from *Neothunnus albacora*

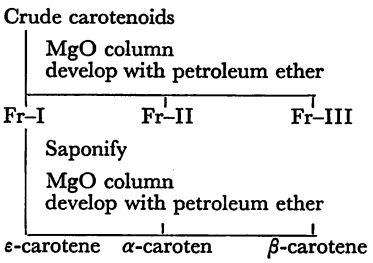

Fig. 2. Isolation of ε-carotene from *Tethya amamensis*.

Tethya amamensis were colleted at Kinko Bay, Kagoshima. The crude carotenoids were extracted, using the same method as already reported in the previous paper[4] and ε-carotene was purified as shown in **Fig. 2.**

3) **Partition coefficients:** The partition of a carotenoid between two immisible solvents such as hexane and 95% methanol is a characteristic that is determined by the presence or absence of certain functional groups. Partition tests between petroleum ether and aqueous methanol were done according to PETRACEK and ZECHMEISTER[5]. Just prior to use, each solvent phase was saturated with the other. The pigment was dissolved in that phase in which it was most readily soluble, and the optical dinsity was recorded. The solution was then shaken with an equal volume of the second solvent phase, and the optical density of the first phase was recorded again.

4) **Methylation and epoxide tests of the carotenoids:** Methylation of the carotenoids was accomplished by adding 5 drops of 2 N hydrochloric acid solution to the pigment dissolved in methanol. The reaction mixture was left at room temperature for 3 hours. Epoxide test was performed by the addition of a few drops of concentrated hydrochloric acid to ethanolic solution of the pigment. The reaction was allowed to proceed for 3 minutes. If the carotenoid has epoxides, the addition of hydrochloric acid results in a shift of absorption spectrum to the shorter wave length.

Results and Discussion

The visible absorption spectra of neothxanthin and tunaxanthin were similar to those of violaxanthin which has two epoxide groups (**Fig. 3**). The epoxide test of neothaxanthin was negative, so the structure of neothxanthin should be similar to those of tunaxanthin or ε-carotene.

The results of partition coefficient of ε-carotene, neothxanthin and tunaxanthin were shown in **Table 1** and its data of neothaxanthin were compared with those of ε-carotene and tunaxanthin. **Table 2** shows the partition coefficients of some naturally occuring carotenoids determined by PETRACEK and ZECHMEISTER[5] and the

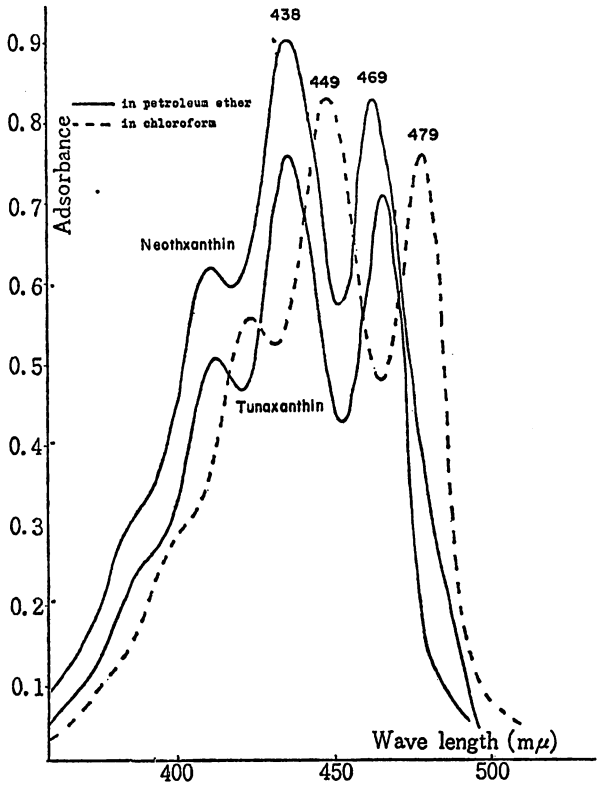

Fig. 3. Absorption spectra of neothxanthin and tunaxanthin.

Table 1. Comparison of experimental data.

Compound	Absorption maxima (Hexane)	Partition ratio	Partition ratio after methylation	Epoxide test
ε-carotene	—, 439, 470	100: 0	100: 0	—
Ref.[3]	—, 439, 470	100: 0	100: 0	—
Tunaxanthin	—, 438, 470	18: 82	87: 13	—
Ref.[3]	—, 436, 466	20: 80	88: 12	—
Neothxanthin	—, 438, 469	84: 16	98: 2	—

structures of those carotenoids are highly dependent on its partition coefficients as shown in its data. From **Table 1** and **Table 2**, the partition ratio of neothxanthin shows that this carotenoid has only one hydroxy group and its structure should be 3-hydroxy-ε-carotene.

This carotenoid would be the intermediate from ε-carotene to tunaxanthin as shown in **Fig. 4.**

Table 2. Partition coefficients of some carotenoids[5].
(Determined in a two-phase system, hexane-95% methanol)

Compound	Functional groups	Partition ratio
Hydrocarbon		
α-Carotene	None	100: 0
β-Carotene	None	100: 0
γ-Carotene	None	100: 0
Lycopene	None	100: 0
Phytofluene	None	100: 0
Alcohol		
4-Hydroxy-α-carotene	One-OH	84: 16
4-Hydroxy-β-carotene (isocryptoxanthin)	One-OH	86: 14
3-Hydroxy-β-carotene (cryptoxanthin)	One-OH	82: 18
3-Hydroxy-γ-carotene (gazaniaxanthin)	One-OH	80: 20
3, 3′-Dihydroxy-α-carotene (lutein)	Two-OH	12: 88
3, 3′-Dihydroxy-β-carotene (zeaxanthin)	Two-OH	11: 89
3, 3′-Dihydroxy-ε-carotene (tunaxanthin)	Two-OH	20: 80
4, 4′-Dihydroxy-β-carotene (isozeaxanthin)	Two-OH	22: 78
Ether		
4-Ethoxy-α-carotene	One-OC_2H_5	99: 1
4-Methoxy3′, 4′-dehydro-β-carotene	One-OCH_3	99: 1
Ester		
Zeaxanthin dipalmitate	Two-$OOCC_{15}H_{31}$	100: 0
4, 4′-Dihydroxy-β-carotene diacetate	Two-$OOCCH_2$	96: 4
Ketone		
4-Keto-α-carotene	One C=O	95: 5
4-Keto-β-carotene (echinenone)	One C=O	93: 7
4-Keto3′, 4′-dehydro-β-carotene	One C=O	92: 8
Keto alcohol		
4-Keto-4-hydroxy-β-carotene	One C=O, one-OH	34: 66

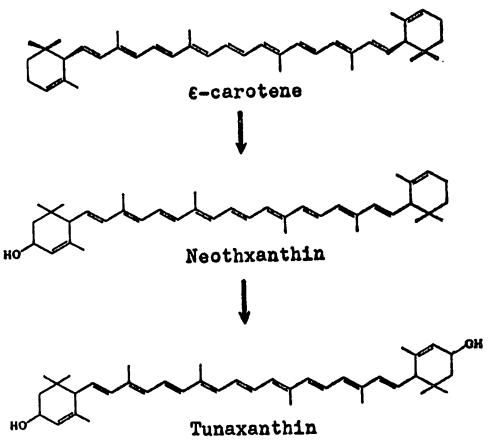

Fig. 4. Possible metabolic pathway from ε-carotene to tunaxanthin.

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