Short Paper

Isolation and Identification of Rhodoxanthin from the Fish, *Tilapia nilotica**1

In the course of our studies about the carotenoids in fishes, an interesting retro carotenoid, rhodoxanthin (1), was isolated as purplish black crystals from the fish, *Tilapia nilotica*, as a major carotenoid (43% of the total carotenoids) (Table 1).

Table 1. Percentage of individual carotenoids in the integuments of *Tilapia nilotica*

β -carotene	0.5
cryptoxanthin	0.3
tunaxanthin	7.4
lutein	6.1
zeaxanthin	22.9
diatoxanthin	11.9
cynthiaxanthin	6.9
triol and tetrol	trace
rhodoxanthin	42.8
astacene	} 1.2
doradecin

Extraction from the integuments (660g) of *Tilapia nilotica* (eleven specimens, 6,750g) was followed as the usual manner¹⁾. Resulted unsaponifiable matter was chromatographed on MgO: celite 545 (1:1) column eluting with increasing acetone in petroleum ether (p.e.) (β -carotene, cryptoxanthin, tunaxanthin, lutein and zeaxanthin), successively increasing MeOH in acetone (diatoxanthin, cynthiaxanthin, triol, tetrol and a keto carotenoid) and 5% AcOH in MeOH (astacene and doradecin). The fractionated keto carotenoid was rechromatographed on neutral alumina (Woelm, activity grade I) eluting with 30~35% acetone in p.e. and crystallized from p.e., yield 5.8 mg, m.p. 211~212°, C₄₀H₅₀O₂ (M⁺ 562).

The isolated purplish black pigment had R_f 0.55 (silicagel G, 30% acetone in p.e.); λ_{max} in p.e. at (458), 481, (510) nm, in C₆H₆ at (473), 497, (525) nm and in 95% EtOH at 497 nm; MS: M⁺ 562 (100%), fragment ions at m/e 470 (38%, M-92), 456 (67%, M-106), 425 (13%, M-137), 412 (11%, M-150), 399 (11%, M-163), 373 (9%, M-190), 359 (18%, M-203); IR (KBr): 1660 (C=O, conj.), 1575 (C=C, conj.),

1538, 973 and 950 (—CH=CH—, trans) cm⁻¹; NMR (chemical shifts are given in δ , solvent: CDCl₃): 1.25 and 1.38 (C-1, C-1' methyls, 12H), 1.99, 2.00 and 2.01 (methyls in polyene, 12H), 2.15 and 2.29 (C-5, C-5' methyls, 6H), 2.33 and 2.39 (C-2, C-2' methylenes, 4H), 5.93 (C-4, C-4' methyns, 2H), 6.00~6.90 (olefinic protons in polyene, 14H) ppm.

These spectral data were quite similar to those of rhodoxanthin reported by Mayer²⁾, Foppen³⁾ and Cyronak⁴⁾. Then this keto carotenoid was reduced with NaBH₄ in EtOH, and resulting reduction product gave a diacetate and was found to have two secondary allylic hydroxyl groups. This reduction product had λ_{max} in p.e. at 441, 466, 495 nm, in C₆H₆ at 455, 480, 510 nm and in 95% EtOH at 445, 468, 497 nm. These data were just the same with those of eschscholtzanthin (2) described by Cyronak⁴⁾ and Strain⁵⁾. Therefore it seems to be quite all right to consider that the keto carotenoid is identical with rhodoxanthin (1). The mixed fusion with authentic sample of rhodoxanthin (1) isolated from berries of Japanese yew, *Taxus cuspidata* (Ichii in Japanese), did not show any depression of melting point.

Accordingly the authors concluded that the isolated keto carotenoid is rhodoxanthin. Isolation of rhodoxanthin from *Tilapia nilotica* is the first example from animal kingdom. It has hitherto been isolated only from plant and fungus^{3,4,6,7)}. Zeaxanthin was another major carotenoid next to rhodoxanthin and no other retro carotenoid has been found in the integuments of *Tilapia nilotica*. Therefore, the authors regarded zeaxanthin as a possible precursor of rhodoxanthin, but little is known so far about the biosynthetic pathway of such a retro carotenoid as rhodoxanthin. At the present time the fact that an unusual retro carotenoid, rhodoxanthin, has been isolated from animal kingdom as a major carotenoid is interesting from biosynthetic and comparative biochemical points of view.

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Masaaki KATSUYAMA*2 and Takao MATSUNO*2

Kyoto College of Pharmacy, Yamashina, Kyoto 607, Japan.

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*1 テラピアよりロドキサントンの分離, 同定.

*2 勝山政明・松野隆男: 京都薬科大学.