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Carotenoids and their metabolism in the goldfish *Carassius auratus* (Hibuna)

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Abstract

A total of 18 known carotenoids and three new carotenoids, (3*S*, 4*R*, 3′*S*, 6′*R*)-4-hydroxylutein, (3*S*, 3′*S*, 4′*R*)-4-keto-4′-hydroxydiatoxanthin, and (3*S*, 3′*S*, 4′*R*)-4-keto-4′-hydroxyalloxanthin were isolated from the goldfish *Carassius auratus*. On the basis of structural consideration of the isolated carotenoids and the result of our previously reported feeding experiment of lutein A and (3*R*, 3′*R*)-zeaxanthin to goldfish, the present authors proposed the possible metabolic pathways from lutein A, lutein B, (3*R*, 3′*R*)-zeaxanthin, diatoxanthin and alloxanthin to fritschiellaxanthin, α-doradexanthin, (3*S*, 3′*S*)-astaxanthin, (3*S*, 3′*S*)-7,8-didehydroastaxanthin, and (3*S*, 3′*S*)-7,8,7′,8′-tetradehydroastaxanthin, respectively.

Keywords: Carotenoid; Freshwater fish; Cypriniformes; Goldfish; *Carassius auratus*; Oxidative metabolism; Enzymatic hydrolysis; Lipase

1. Introduction

In the course of our investigation on carotenoids of goldfish, we have already reported the isolation of 13 carotenoids as follows, β-cryptoxanthin, lutein A, lutein B (3′-epilutein), zeaxanthin, diatoxanthin, alloxanthin (cynthiaxanthin), 4-hydroxylutein (β, β-carotene-3, 4, 3′-triol), 4-hydroxyzeaxanthin (β, β-carotene-3, 4, 3′-triol), 4, 4′-dihydroxyzeaxanthin (β, β-carotene-3, 4, 3′, 4′-tetrol), α-doradexanthin (4-ketolutein), β-doradexanthin (4-ketozeaxanthin), idoxanthin, and astaxanthin [16]. However, the stereochemistry of these carotenoids still remains undefined. In the case of esterified carotenoids with 3-hydroxy-4-keto-β-end group (e.g. astaxanthin, α-doradexanthin, and β-doradexanthin), traditional saponification (alkaline hydrolysis) gives the corresponding 2, 3-didehydro-3-hydroxy-4-keto derivatives as a result of oxidation. Therefore, at the present study, enzymatic hydrolysis with lipase has been undertaken to isolate such unstable carotenoids in alkaline medium as intact free form. This paper describes further investigation on the stereochemical characterization of the formerly reported carotenoids and the newly isolated carotenoids from goldfish. Furthermore, on the basis of stereostructural consideration of the isolated carotenoids and the previously reported results of the feeding experiment with lutein A and (3*R*, 3′*R*)-zeaxanthin [16], here we report possible metabolic pathways of the carotenoids in goldfish.

2. Material and methods

2.1. Biological material

Goldfish *Carassius auratus* (hibuna) (480 specimens, 880 g) were purchased at a local fish hatchery in Yamatokoriyama city, Nara Prefecture, Japan and their red colored integuments (skin and fin) were striped off from the fresh samples and collected.

2.2. Extraction and isolation of carotenoids

Extraction and isolation procedure was summarized in Fig. 1. The carotenoids were extracted with acetone from the integuments until exhaustion. They were then transferred to *n*-hexane-ether (1:1) by the addition of

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distilled water. The upper layer was washed with water and dried over Na_2SO_4. The extracted solution was concentrated under reduced pressure in N_2 below 40°C. The resulting red residue submitted to column chromatography (CC) on silica gel to give four fractions (fractions 1, 2, 3, and 4) eluted with 5–10, 10–15, 20, and 20–30% ether/*n*-hexane, successively (Fig. 1). Fraction 1 was saponified with 10% KOH/MeOH at 37°C for 3 h, and the unsaponifiable portion was re-chromatographed on silica gel to give four fractions (fractions 1–1, 1–2, 1–3, and 1–4) eluted with 5, 10–20, 20–30, and 40% acetone/*n*-hexane, successively. Fractions 2–4 obtained from the first column chromatography were hydrolyzed with lipase because of the unstable esterified carotenoids in alkaline medium. An acetone-0.05 *M* Tris-HCl buffer (1:99) solution (100 ml) of the carotenoid esters (0.5 mg) was incubated with lipase OF-360 (activity 360 000 units g^{-1}; Meito Sangyo, Nagoya, Japan) (2 g) in N_2 with stirring at 37°C for 24 h. The reaction mixture was extracted three times with 200 ml of *n*-hexane-ether (1:1) [13]. After hydrolysis, each fraction was submitted to further purification by high performance liquid chromatography (HPLC).

2.3. High performance liquid chromatography systems

Separation of Fr. 1–1, 1–2, 1–3, and 1–4 (Fig. 1) were carried out using a Sumichiral OA-2000 column (300 × 8.0 mm ID) at a flow rate of 2.0 ml min^{-1}. System 1; mobile phase: *n*-hexane/CH_2Cl_2/EtOH (52:12:0.3). System 2; mobile phase: *n*-hexane/CH_2Cl_2/EtOH (48:16:0.6). System 3; mobile phase: *n*-hexane/CH_2Cl_2/EtOH (48:16:1.5). System 4; mobile phase: *n*-hexane/ CH_2Cl_2/EtOH (48:16:3.0). Separation of Fr. 2 (Fig. 1) was carried out on the systems 5 and 2. System 5; column: Cosmosil 5SL (250 × 8.0 mm ID); mobile phase: *n*-hexane/EtOAc/CH_2Cl_2/EtOH (70: 20: 40: 2). Flow-rate: 2.0 ml min^{-1}. Separation of Fr. 3 (Fig. 1) was carried out on systems 6 and 2; System 6; column: Chemcosorb 5CN (300 × 4.0 mm ID); mobile phase: *n*-hexane/isopropyl acetate/2-propanol/*N*, *N*-di-iso-propylethylamine (140: 14:0.5:0.1). Flow-rate: 0.7 ml min^{-1}. Separation of Fr. 4 (Fig. 1) was carried out on the system 7. System 7; column: Sumichiral OA-2000; mobile phase: *n*-hexane/ CH_2Cl_2/ EtOH (48:16:2.0). Flow-rate: 2.0 ml min^{-1}.

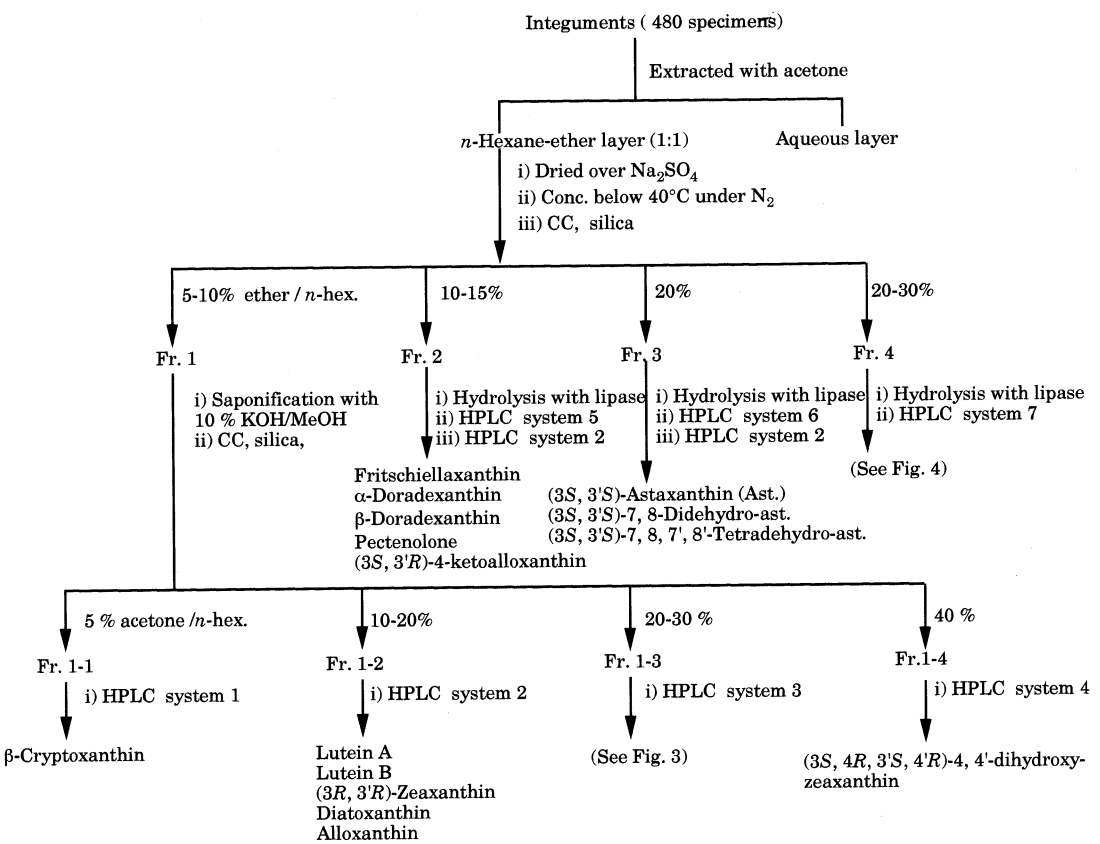
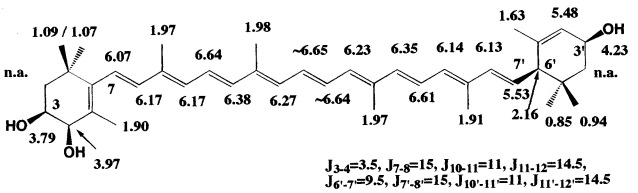
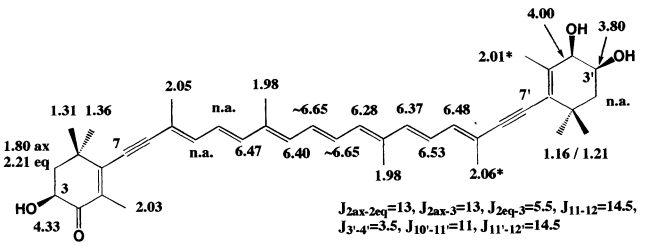


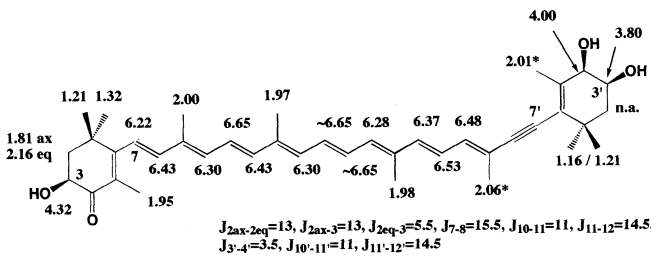
Fig. 1. Isolation of carotenoids from the integuments of goldfish.



(3*S*,4*R*,3'*S*,6'*R*)-4'-hydroxylutein (1)



(3*S*,3'*S*,4'*R*)-4-keto-4'-hydroxyalloxanthin (2)



(3*S*,3'*S*,4'*R*)-4-keto-4'-hydroxydiatoxanthin (3)

300 MHz (in $CDCl_3$)
* assigments may be reversed, n.a. : not assigned, $J_{H\text{-}H}$/Hz

Fig. 2. ^{1}H-NMR assignments of new carotenoids (**1**), (**2**), and (**3**).

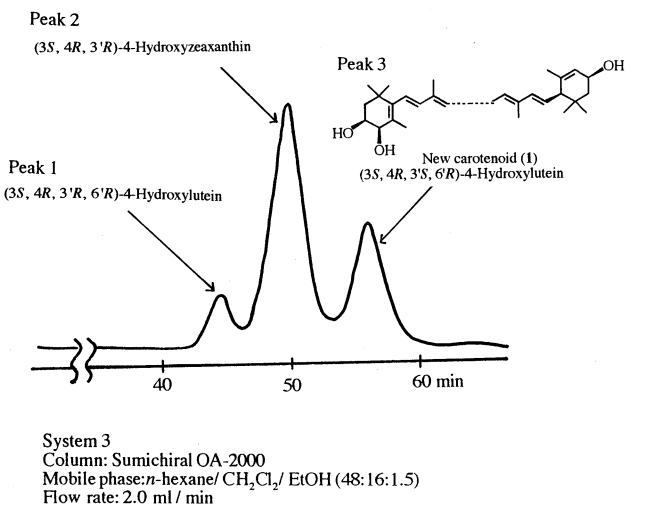


Fig. 3. HPLC chromatogram of Fraction 1–3 obtained from goldfish.

2.4. Identification and structural elucidation of carotenoids isolated from the goldfish

Identification and structural elucidation of each carotenoid was carried out by means of visible absorption spectrum (VIS), electron impact mass (EIMS), circular dichroism (CD), and ^{1}H-nuclear magnetic resonance (NMR) spectroscopy, and by comparing the retention time from HPLC with the value for natural authentic samples or semisynthetical authentic samples, and chemical reactions by our routine procedure [19]. Assignments of ^{1}H-NMR for three new carotenoids, (3*S*, 4*R*, 3′*S*, 6′*R*)-4-hydroxylutein, (3*S*, 3′*S*, 4′*R*)-4-keto-4′-hydroxydiatoxanthin, and (3*S*, 3′*S*, 4′*R*)-4-keto-4′-hydroxyalloxanthin were made by ^{1}H-^{1}H decoupling experiments and by comparing these data with those of known carotenoids possessing the same end group.

2.5. Instruments

VIS in ether was measured with a Shimadzu UV-240 spectrophotometer, while CD spectrum was recorded on a Jasco J-500 C spectropolarimeter in ether/isopentane/EtOH (5:5:2). ^{1}H-NMR spectrum was recorded on a Varian-XL-300 NMR spectrometer (300 MHz) using $CDCl_3$ as solvent. EIMS was measured with a Hitachi M-80 mass spectrometer.

2.6. Authentic samples

The authentic carotenoids were prepared from the following materials: fritschiellaxanthin (from crab *Sesarma haematocheir*) [17], β-doradexanthin (from spiny lobster) [14], pectenolone, (3*S*, 3′*R*)-4-ketoalloxanthin, (3*S*, 3′*S*)-7,8-didehydroastaxanthin, and (3*S*, 3′*S*)-7,8,7′,8′-tetradehydroastaxanthin (from starfish) [12]. The other authentic samples used were from our carotenoid collections [20].

3. Results

3.1. Carotenoids in the goldfish

The amount and percent composition of individual carotenoids in the integuments of goldfish *C. auratus* was shown in Table 1. A total of 18 known carotenoids and three new carotenoids, (3*S*, 4*R*, 3′*S*, 6′*R*)-4-hydroxylutein (**1**), (3*S*, 3′*S*, 4′*R*)-4-keto-4′-hydroxyalloxanthin (**2**), and (3*S*, 3′*S*, 4′*R*)-4-keto-4′-hydroxydiatoxanthin (**3**) were isolated in the present investigation. A total of the 13 out of the 18 known carotenoids, β-cryptoxanthin, lutein A, lutein B, (3*R*, 3′*R*)-zeaxanthin, diatoxanthin, alloxanthin, α-doradexanthin, β-doradexanthin, (3*S*, 4*R*, 3′*R*, 6′*R*)-4-hydroxylutein, (3*S*, 4*R*, 3′*R*)-4-hydroxyzeaxanthin, (3*S*, 3′*S*, 4′*R*)-idoxanthin, (3*S*, 3′*S*)-

Table 1
The amount and percent composition of individual carotenoids in the integuments of goldfish *C. auratus* (Hibuna)

Total contents (mg/100 g body weight)	1.65
Composition (%)	
β-Cryptoxanthin	1.0
Lutein A [(3*R*, 3′*R*, 6′*R*)-lutein]	0.8
Lutein B [(3*R*, 3′*S*, 6′*R*)-lutein, 3′-epilutein]	1.9
(3*R*, 3′*R*)-Zeaxanthin	2.4
Diatoxanthin	1.8
Alloxanthin (cynthiaxanthin)	2.0
Fritschiellaxanthin (4-ketolutein A)	9.2
α-Doradexanthin (4-ketolutein B)	18.1
β-Doradexanthin [(3*S*, 3′*R*)-4-ketozeaxanthin, adonixanthin]	2.4
Pectenolone [(3*S*, 3′*R*)-4-ketodiatoxanthin]	3.4
(3*S*, 3′*R*)-4-Ketoalloxanthin	3.0
(3*S*, 4*R*, 3′*R*, 6′*R*)-4-Hydroxylutein	+
(3*S*, 4*R*, 3′*S*, 6′*R*)-4-Hydroxylutein (**1**)	0.5
(3*S*, 4*R*, 3′*R*)-4-Hydroxyzeaxanthin	1.0
(3*S*, 3′*S*, 4′*R*)-4-Keto-4′-hydroxyalloxanthin (**2**)	4.5
(3*S*, 3′*S*, 4′*R*)-4-Keto-4′-hydroxydiatoxanthin (**3**)	3.0
(3*S*, 3′*S*, 4′*R*)-Idoxanthin	1.5
(3*S*, 3′*S*)-Astaxanthin	36.0
(3*S*, 3′*S*)-7, 8-Didehydroastaxanthin	2.9
(3*S*, 3′*S*)-7, 8, 7′, 8′-Tetradehydroastaxanthin	1.0
(3*S*, 4*R*, 3′*S*, 4′*R*)-4, 4′-Dihydroxyzeaxanthin	0.4
Unidentified carotenoids	3.2

astaxanthin, and (3*S*, 4*R*, 3′*S*, 4′*R*)-4, 4′-dihydroxyzeaxanthin were previously reported in the goldfish *C. auratus* [16], but the stereochemical characterization of these carotenoids remained to be solved. Furthermore, the following five carotenoids having 3-hydroxy-4-keto-β-end groups, fritschiellaxanthin, pectenolone, (3*S*, 3′*R*)-4-ketoalloxanthin, (3*S*, 3′)-7,8-didehydroastaxanthin, and (3*S*, 3′*S*)-7,8,7′,8′-tetradehydroastaxanthin were newly isolated by enzymatic hydrolysis at the present study.

3.2. Identification of known carotenoids

Fritschiellaxanthin, α-doradexanthin, β-doradexanthin, pectenolone, (3*S*, 3′*R*)-4-ketoalloxanthin, (3*S*, 3′*S*, 4′*R*)-idoxanthin, (3S, 3′S)-astaxanthin, (3*S*, 3′*S*)-7,8-didehydroastaxanthin, and (3*S*, 3′*S*)-7,8,7′,8′-tetradehydroastaxanthin possessing 3-hydroxy-4-keto-β-end groups were isolated as free forms by enzymatic hydrolysis and were characterized their chiralities by CD and chiral HPLC.

3.2.1. α-Doradexanthin and β-doradexanthin

These two carotenoids were isolated from Fr. 2 (Fig. 1) by HPLC. The structure of α-doradexanthin and β-doradexanthin were fully characterized by the EIMS, ^{1}H-NMR and CD spectral data and assigned to be (3*S*, 3′*S*, 6′*R*)-3,3′-dihydroxy-β,ε-caroten-4-one (4-ketolutein B) [2,15], and (3*S*, 3'*R*)-3,3'-dihydroxy-β,β-caroten-4-one (adonixanthin) [22], respectively.

3.2.2. Fritschiellaxanthin, pectenolone and (3S, 3′R)-4-ketoalloxanthin

These three carotenoids were isolated from Fr. 2 (Fig. 1) by HPLC and were identified by VIS, EIMS and CD spectral data [8,11,12,17] and direct comparison with authentic samples on HPLC. Fritschiellaxanthin [17], pectenolone [8] and (3*S*, 3′*R*)-4-ketoalloxanthin [11,12] correspond to 4-keto derivatives of lutein A, diatoxanthin and alloxanthin, respectively.

3.2.3. (3S, 3′S)-7,8-Didehydroastaxanthin, and (3S, 3′S)-7,8,7′,8′-tetradehydroastaxanthin

These two carotenoids were isolated from Fr. 3 (Fig. 1) by HPLC and were identified on the basis of VIS and EIMS spectral data [1]. (3*S*, 3′*S*) chirality of both compounds was confirmed by CD spectral data [1] and direct comparison with authentic samples on HPLC using a chiral column.

3.2.4. (3S, 3′S, 4′R)-Idoxanthin

This carotenoid (Peak 2) was obtained from Fr. 4 (Fig. 1) by HPLC as shown in Fig. 4. Planer structure of idoxanthin was characterized by VIS and EIMS spectral data [18]. (3*S*, 3′*S*, 4′*R*) chirality was proposed by CD spectrum [18] and comparison with semisynthetic stereoisomers of idoxanthin prepared by $NaBH_4$ reduction of (3*S*, 3′*S*)-astaxanthin on HPLC using a chiral column.

3.2.5. (3S, 4R, 3′R, 6′R)-4-Hydroxylutein, and (3S, 4R, 3′R)-4-hydroxyzeaxanthin

These two carotenoids were isolated from Fr. 1–3 (Fig. 1) by HPLC as shown in Fig. 3. (3*S*, 4*R*, 3′*R*,

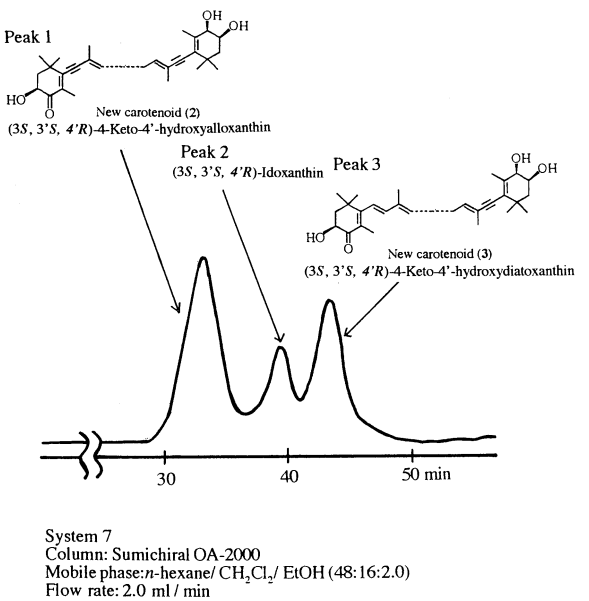


Fig. 4. HPLC chromatogram of Fraction 4 obtained from goldfish.

6′*R*)-4-Hydroxylutein (peak 1) was characterized by VIS, EIMS and CD spectral data and direct comparison with authentic sample obtained from chiton [20]. This carotenoid corresponds to 4-hydroxy derivative of lutein A possessing with 3,4-*cis* configuration. (3*S*, 4*R*, 3′*R*)-4-Hydroxyzeaxanthin (peak 2) was also identified by the similar manner described above [20]. This carotenoid also possesses 3,4 *cis* glycol structure of β-end group.

3.2.6. (3S,4R,3′S,4′R)-4,4′-Dihydroxyzeaxanthin

This carotenoid isolated from Fr. 1–4 (Fig. 1) was identified as 4, 4′-dihydroxyzeaxanthin (crustaxanthin) by VIS and EIMS spectral data [21]. (3*S*, 4*R*, 3′*S*, 4′*R*) chirality was proposed by CD spectrum and comparison with semisynthetic stereoisomers of 4,4′-dihydroxyzeaxanthin prepared by $NaBH_4$ reduction of (3*S*, 3′*S*)-astaxanthin on HPLC using a chiral column.

It was assumed that diatoxanthin and alloxanthin type tetrol also existed in goldfish from the viewpoint of carotenoids metabolism, but they could not be determined due to a limited amount of the sample.

*3.3. Structural determination of new carotenoids **1**, **2** and **3***

*3.3.1. (3S, 4R, 3′S, 6′R)-4-Hydroxylutein (**1**)*

The carotenoid (**1**) (50 μg) was isolated from Fr. 1–3 (Fig. 3). Compound **1** (peak 3) showed the same VIS and EIMS spectral data as those of (3*S*, 4*R*, 3′*R*, 6′*R*)-4-hydroxylutein described above (peak 1) (Fig. 3) and also provided a triacetate by acetylation. These properties indicated that **1** was one of stereoisomers of 4-hydroxylutein. The 3, 4-*cis* configuration of β-end group and 3′, 6′-*cis* configuration of ε-end group in **1** were determined by ^{1}H-NMR data as shown in Fig. 2[3]. A coupling constant of 3.5 Hz for the H-3 and H-4 oxymethin protons revealed by the *cis* configuration of 3, 4 glycol in β-end group [3]. Furthermore, the characteristic ^{1}H-NMR signals at H-3′ (δ 4.23) and H-6′ (δ 2.16) indicated the *cis* stereochemistry of hydroxy group at C-3′ and polyene chain at C-6′ in ε-end group [3]. The CD spectrum of **1** [λ_{max} 247 ($\Delta\varepsilon + 3.5$), 285 (-5.0) and 310 nm ($+1.0$)] was quite similar to that of lutein B. This fact indicated that **1** possessed the same absolute configurations at C-3, C-3′ and C-6′ as those of lutein B [15] and the chiralities of **1** was determined to be 3*S*, 3′*S*, 6′*R*. Based on the results of relative stereochemistry of 3, 4 glycol described above, chirality at C-4 was assigned to be *R*. Therefore, the structure of **1** was determined to be (3*S*, 4*R*, 3′*S*, 6′*R*)-4-hydroxylutein and its structure corresponded to 4-hydroxy derivatives of lutein B with 3, 4-*cis* configuration.

*3.3.2. (3S, 3′S, 4′R)-4-Keto-4′-hydroxyalloxanthin (**2**)*

This carotenoid (**2**) was isolated from Fr. 4 (Fig. 1) by HPLC as shown in Fig. 4. Compound **2** (peak 1) (0.3 mg) exhibited a molecular ion at *m*/*z* 594 ($C_{40}H_{50}O_4$) and showed absorption maxima at 466 and (496) nm. Compound **2** also provided a triacetate by acetylation. Reduction of **2** with $NaBH_4$ gave a more polar product *m*/*z* 596 ($C_{40}H_{52}O_4$) with absorption maxma at 426 (sh), 453 and 480 nm indicating the presence of alloxanthin (7, 8, 7′, 8′-tetradehydro-β,β-carotene-3,3′-diol) type chromophore [11]. This VIS spectral changes upon reduction suggested that **2** was corresponding 4-keto derivative of alloxanthin [11]. Of the four oxygen functions in **2**, one was ascribed to conjugated carbonyl group by VIS spectral data described above, and the remaining three were ascribed to secondary hydroxy groups by acetylation and three oxymethin signals observed in ^{1}H-NMR (Fig. 2). The oxymethin signal at δ 4.33 (*d*,*d*) was assigned to be H-3 by comparison with these data with that of 7, 8, 7, 8′-tetradehydroastaxanthin [3]. The remaining two oxymethin signals at δ 3.80 (*m*) and δ 4.00 (*d*, $J = 3.5$ Hz) were assigned to H-3′ and H-4′ by comparison with these data of 4-hydroxyalloxanthin [11]. Furthermore, *cis* configuration of 3′, 4′ glycol was determined by a coupling constant of $J_{3'-4'} = 3.5$ Hz [11]. These spectral data coincided with the structure of 4-keto-4′-hydroxyalloxanthin for **2**. Compound **2** showed the negative CD spectrum [λ_{max} 302 nm ($\Delta\varepsilon - 9.5$)], and suggested the same chiralities at C-3 and C-3′ as those of 4-hydroxyalloxanthin [11]. Therefore, the structure of a new carotenoid **2** has been postulated to be (3*S*, 3′*S*, 4′*R*)-4-keto-4′-hydroxyalloxanthin.

*3.3.3. (3S, 3′S, 4′R)-4-Keto-4′-hydroxydiatoxanthin (**3**)*

This carotenoid (**3**) was isolated from Fr. 4 (Fig. 1) by HPLC as shown in Fig. 4. Compound **3** (peak 3) (0.2 mg) exhibited a molecular ion at *m*/*z* 596 ($C_{40}H_{52}O_4$) and showed absorption maxima at 454–473 nm. As the similar manner described above, the constitution of **3** was established to be 4-keto-4′-hydroxydiatoxanthin by acetylation, reduction with $NaBH_4$ and ^{1}H-NMR experiments. The ^{1}H-NMR indicated the presence of astaxanthin end group [3] and 4-hydroxyalloxanthin end group [11] in **3** as shown in Fig. 2. The *cis* stereochemistry of 3′, 4′ hydroxy groups was also determined by a coupling constant of $J_{3'-4'} = 3.5$ Hz [11]. The absolute configuration for **3** was postulated to be 3*S*, 3′*S*, 4′*R* by CD spectrum [λ_{max} 234 ($\Delta\varepsilon - 3.5$), 263 ($+7.0$), and 302 nm (-12.0)], and ^{1}H-NMR data by comparing with those of pectenolone (4-ketodiatoxanthin) [8] and compound **2** (Fig. 2). Therefore the structure of a new carotenoid **3** has been postulated to be (3*S*, 3′*S*, 4′*R*)-4-keto-4′-hydroxydiatoxanthin.

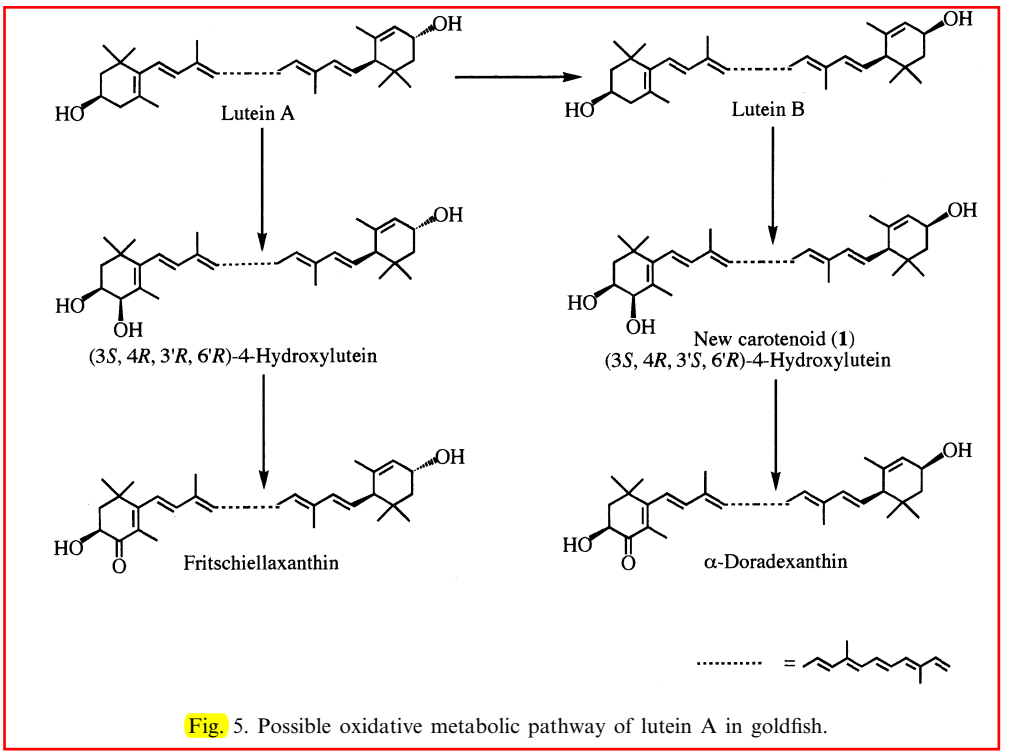


Fig. 5. Possible oxidative metabolic pathway of lutein A in goldfish.

4. Discussion

In 1963, Hirao [7] reported the presence of lutein and astaxanthin-like red pigment from goldfish fed on lutein. The astaxanthin-like pigment in the integuments of goldfish was shown to be esterified α-doradexanthin, isolated as α-doradecin after saponification by Katayama in 1970 [10]. On the basis of structural consideration of the carotenoids isolated, Katayama et al. [10] reported that the principal red pigment of goldfish astaxanthin was formed from lutein via α-doradexanthin (4-ketolutein) and β-doradexanthin (4-ketozeaxanthin) by oxidation and isomerization (α-ionone ring → β-ionone ring). Experimental proof for the conversion of lutein to astaxanthin was obtained from the result of feeding [^{14}C] lutein to the common goldfish of the yellow variety by Hsu et al. [9].

On the other hand, Hata and Hata [5,6] proposed metabolic pathway from zeaxanthin to astaxanthin through β-doradexanthin from the experimental result of feeding [^{14}C] zeaxanthin to goldfish (Wakin, common goldfish, Japanese goldfish), and they found α-doradexanthin as principal metabolic product after feeding [^{14}C] lutein. The results obtained by the two groups of Katayama et al. [10] and Hsu et al. [9], and the other group of Hata and Hata [5,6] were clearly different. In order to determine which carotenoid is metabolized to astaxanthin, test diet containing either lutein or zeaxanthin were fed to goldfish (Orandashishigashira) by Matsuno et al. [16]. In the integument of goldfish fed on zeaxanthin, amount of zeaxanthin (25.8 times of control group), 4-hydroxyzeaxanthin (6.6), β-doradexanthin (5.0), and astaxanthin (3.1) increased significantly. By contrast, when lutein was fed, there was an obvious increase in lutein A (44.9), lutein B (3′-epilutein) (31.8), 4-hydroxylutein (7.6), and α-doradexanthin (9.5), the amount of astaxanthin decreased. Furthermore, β-doradexanthin was not detected. The absence of β-doradexanthin and the decrease in the astaxanthin content in goldfish fed on lutein A suggests that goldfish is not able to convert the ε-end group of carotenoids to β-end group by oxidative metabolism. From the result described above, Matsuno et al. [16] proposed possible metabolic pathways of lutein A and zeaxanthin as follows: Lutein A → Lutein B → 4-hydroxylutein → α-doradexanthin. Zeaxanthin → 4-hydroxyzeaxanthin → β-doradexanthin → astaxanthin. These result are compatible with the pathways proposed by Hata and Hata in 1972 [6], and contradict the pathway proposed by Katayama et al. in 1970 [10] and Hsu et al. in 1972 [9]. Furthermore, Hata and Hata [4] reported the ability of the goldfish to convert 3-hydroxy-β-end group into 3-hydroxy-4-keto-β-end group by the feeding experiment with alloxanthin (cynthiaxanthin).

On the basis of stereostructural consideration of the isolated carotenoids and the previously reported results of the feeding experiment by Matsuno et al. [16], at the present study, we proposed the five possible oxidative metabolic pathways from lutein A, lutein B, (3*R*, 3′*R*)-zeaxanthin, diatoxanthin and alloxanthin to fritschiellaxanthin, α-doradexanthin, (3*S*, 3′*S*)-astaxanthin, (3*S*, 3′*S*)-7,8-didehydroastaxanthin, and (3*S*, 3′*S*)-7,8,7′,8′-

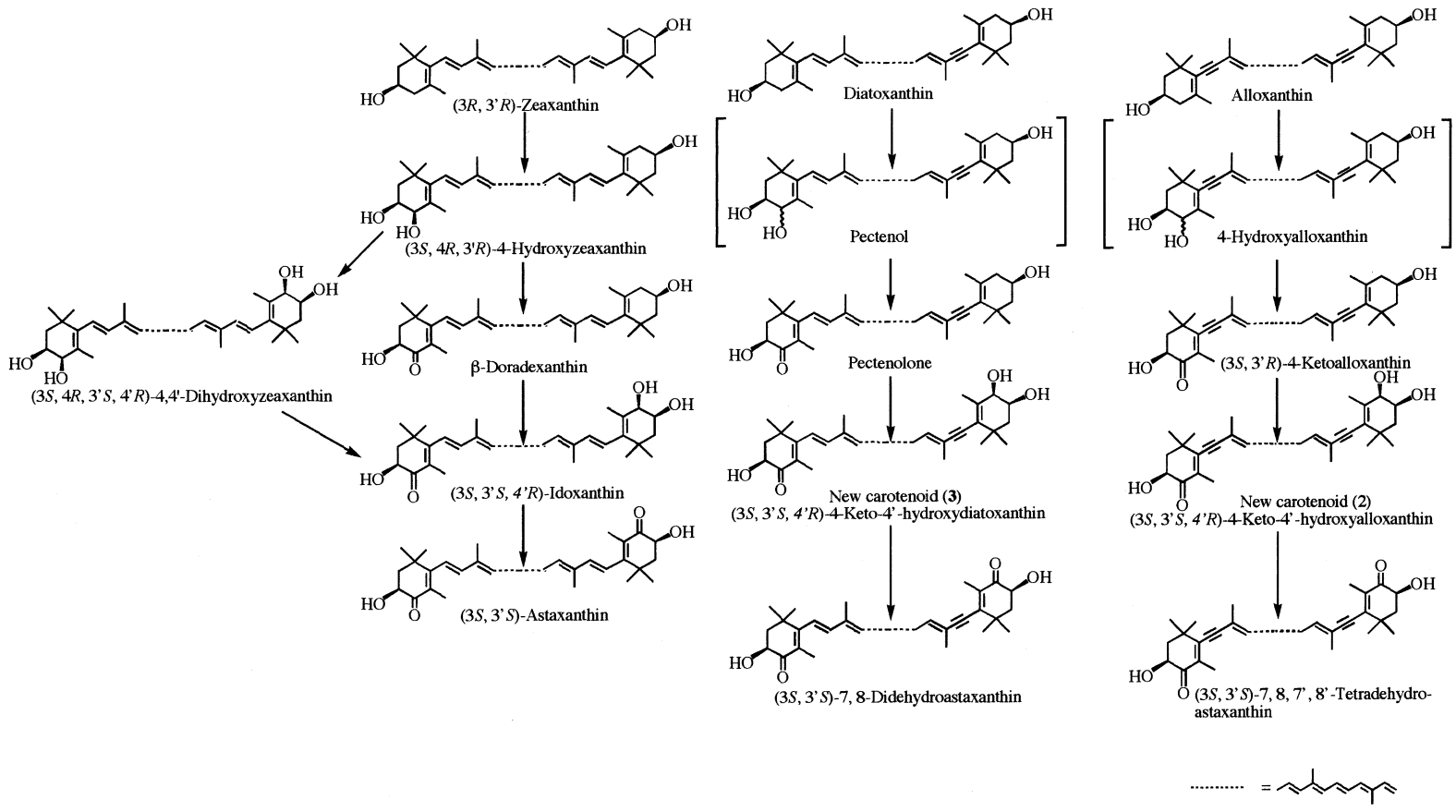


Fig. 6. Possible oxidative metabolic pathways of (3*R*, 3′*R*)-zeaxanthin, diatoxanthin, alloxanthin in goldfish.

tetradehydroastaxanthin, respectively, as shown in Figs. 5 and 6. From the results of the present experiments, it might be assumed that goldfish can oxidize C-4 of 3-hydroxy-β-end group and epimerize hydroxy at C-3 of ε-end group. These metabolic pathways (goldfish type) described above might be typical principal metabolic pathways of carotenoids in the freshwater fishes in order Cypriniformes.

References

[1] Bernhard K, Kienzle F, Mayer H, Müller RK. Synthese von optisch aktiven, natürlichen carotinoiden und strukturell verwandten naturprodukten. VIII. Synthese von (3S, 3′S)-7,8,7′,8′-tetradehydroastaxanthin und (3S, 3′S)-7,8-didehydroastaxanthin (Asterinsäure). Helv Chim Acta 1980;63:1473–90.

[2] Buchecker R, Eugster CH, Weber A. Absolute konfiguration von α-doradexanthin und von fritschiellaxanthin einem neuen carotenoid aus Fritschiella tuberosa IYEMG. Helv Chim Acta 1978;61:1962–8.

[3] Englert G. NMR Spectroscopy. In: Britton G, Liaaen-Jensen S, Pfander H, editors. Carotenoids, vol. 1B, Basel: Birkhäuser, 1995, p. 147–260.

[4] Hata M, Hata M. Carotenoid pigments in goldfish (*Carassius auratus* L.). III. Metabolism of ingested Cynthiaxanthin. Tohoku J Agric Res 1970;21:183–8.

[5] Hata M, Hata M. Carotenoid pigments in goldfish. IV. Carotenoid metabolism. Nippon Suisan Gakkaishi 1972;38:331–8.

[6] Hata M, Hata M. Carotenoid pigments in goldfish. V. Conversion of zeaxanthin to astaxanthin. Nippon Suisan Gakkaishi 1972;38:339–43.

[7] Hirao S, Kikuchi R, Taguchi H. Carotenoid pigment in fish. I. Carotenoids in goldfish and fancy coloured carp. Nippon Suisan Gakkaishi 1963;29:371–81.

[8] Hiraoka K, Matsuno T, Ito M, Tsukida K, Shichida Y, Yoshizawa T. Absolute configuration of pectenolone. Nippon Suisan Gakkaishi 1982;48:215–7.

[9] Hsu WJ, Rodriguez DB. Chichester CO. The biosynthesis of astaxanthin. VI. The conversion of [^{14}C] lutein and [^{14}C] β-carotene in goldfish. Int J Biochem 1972;3:333–8.

[10] Katayama T, Yokoyama H, Chichester CO. The biosynthesis of astaxanthin. I. The structure of α-doradexanthin and β-doradexanthin. Int J Biochem 1970;1:438–44.

[11] Maoka T, Matsuno T. Isolation and structural elucidation of three new acetylenic carotenoids from the Japanese sea mussel *Mytilus coruscus*. Nippon Suisan Gakkaishi 1988;54:1443–7.

[12] Maoka T, Tsushima M, Matsuno T. New acetylenic carotenoids from the starfishes *Asterina pectinifera* and *Asterias amurensis*. Comp Biochem Physiol 1989;93B:829–34.

[13] Matsuno T. Structure and characterization of carotenoids from various habitats and natural sources. In: Packer L, editor. Methods in Enzymology, vol. 213. San Diego, CA: Academic Press, 1992, p. 22–31.

[14] Matsuno T, Kusumoto T, Watanabe T, Ishihara Y. Carotenoid pigments of Spiny lobster. Nippon Suisan Gakkaishi 1973; 39:43–50.

[15] Matsuno T, Maoka T. The carotenoids of crab *Paralithodes brevipes* (Hanasakigani in Japanese). Nippon Suisan Gakkaishi 1988;54:1437–42.

[16] Matsuno T, Matsutaka H, Nagata S. Metabolism of lutein and zeaxanthin to ketocarotenoids in goldfish, *Carassius auratus*. Nippon Suisan Gakkaishi 1981;47:605–11.

[17] Matsuno T, Ookubo M. The first isolation and identification of fritschiellaxanthin from a Crab *Sesarma haematocheir* (Akategani in Japanese). Nippon Suisan Gakkaishi 1982;48:653–9.

[18] Matsuno T, Sakaguchi S. Absolute configuration of idoxanthin. Nippon Suisan Gakkaishi 1983;49:1475.

[19] Matsuno T, Tsushima M. Comparative biochemical studies of carotenoids in sea cucumbers. Comp Biochem Physiol 1995; 111B:597–605.

[20] Tsushima M, Maoka T, Matsuno T. Comparative biochemical studies of carotenoids in marine invertebrates. The first positive identification of ε,ε-carotene derivatives and isolation of two new carotenoids from chitons. Comp Biochem Physiol 1989;93B: 665–71.

[21] Tsushima M, Matsuno T. Occurrence of (3*S*,4*R*,3′*R*)-β,β-carotene-3,4,3′-triol and (3*S*,4*R*,3′*S*,4′*R*)-β,β-carotene-3,4,3′,4′-tetrol in the Grudgeon *Pseudogobio esocinus* (cypriniformes). Nippon Suisan Gakkaishi 1999;65:500–1.

[22] Yokoyama A, Izumida H, Miki W. Production of astaxanthin and 4-ketozeaxanthin by the marine bacterium *Agrobacterium aurantiacum*. Biosci Biotech Biochem 1994;58:1842–4.