Short Paper

 β,β -Carotene Triol and Tetrol from the Integument of Three Species of Freshwater Fish Belonging to Siluriformes and Salmoniformes^{*1}Miyuki Tsushima,^{*2} Yoshihiro Ikuno, and Takao Matsuno

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In the previous paper,¹⁾ the authors reported the occurrence of β,β -carotene triol [= (3*S*, 4*R*, 3'*R*)-4-hydroxyzeaxanthin] and β,β -carotene tetrol [= (3*S*, 4*R*, 3'*S*, 4'*R*)-4,4'-dihydroxyzeaxanthin] in freshwater fish of the order Cypriniformes. We now report on the triol and tetrol obtained from freshwater fish belonging to Siluriformes and Salmoniformes except Cypriniformes.

The Folktail bullhead *Pseudobagrus fulvidraco* (Siluriformes) (7 specimens, average weight 0.19 kg) was collected from Lake Biwa, Shiga Prefecture, Japan, in December 1988. The coho salmon *Oncorhynchus kisutch* (1 specimen, weight 2.55 kg), and the dog salmon (chum salmon) *Oncorhynchus keta* (2 specimens, average weight 1.88 kg) (Salmoniformes) were purchased from a fish market in Kyoto city, in September 1998. According to the methods previously reported,¹⁾ β,β -carotene triol and tetrol from the integument of these freshwater fish were investigated.

One of the two β,β -carotene triols obtained from the integument of folktail bullhead, coho salmon, and dog salmon was identified as (3*S*, 4*R*, 3'*R*)- β,β -carotene-3,4,3'-triol [= (3*S*, 4*R*, 3'*R*)-4-hydroxyzeaxanthin] (1) by the VIS spectrum, EIMS, and ¹H NMR spectrum.¹⁾ The

other compound (2) also showed the same VIS [$\lambda_{\max}$ 425 (sh), 449, and 476 nm] and EIMS (*m/z* 584) spectral data as those of 1. The ¹H NMR spectral data [δ 1.074 (6H, *s*), 1.078 (3H, *s*), 1.094 (3H, *s*), 1.737 (3H, *s*), 1.813 (3H, *s*), 3.79 (1H, *m*), 3.94 (1H, *d*, *J* = 7.5 Hz), and 4.00 (1H, *m*)] of 2 were almost identical with those of 1, excepting a coupling constant of *J*₃₋₄ = 7.5 Hz in 2 instead of *J*₃₋₄ = 3.5 Hz in 1. These data indicated the 3,4-*trans* configuration of β -end group. Therefore we assumed that compound 2 was (3*S*, 4*S*, 3'*R*)- β,β -carotene-3,4,3'-triol [= (3*S*, 4*S*, 3'*R*)-4-hydroxyzeaxanthin]. In order to confirm the validity of the proposed structure of 2, (3*S*, 4*S*, 3'*R*)-4-hydroxyzeaxanthin was prepared by NaBH₄ reduction of (3*S*, 3'*R*)-4-ketozeaxanthin. As was expected, compound 2 was proved to be completely identical with the semisynthetic (3*S*, 4*S*, 3'*R*)-4-hydroxyzeaxanthin by the comparison of physicochemical data. Therefore the structure of carotenoid 2 was determined to be (3*S*, 4*S*, 3'*R*)- β,β -carotene-3,4,3'-triol [= (3*S*, 4*S*, 3'*R*)-4-hydroxyzeaxanthin]. β,β -Carotene triol previously isolated from the freshwater fish gudgeon (Cypriniformes) was almost solely 1,¹⁾ while β,β -carotene triols from folktail bullhead (Siluriformes), coho salmon and dog salmon (Salmoniformes) were mixtures of

Fig. 1. Suggested reductive metabolic pathway of (3*S*, 3'*S*)-astaxanthin to (3*R*, 3'*R*)-zeaxanthin in folktail bullhead, coho salmon, and dog salmon.

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two diastereomers of β,β -carotene-3,4,3'-triol, namely, **1** and **2**. The percent composition of **1** and **2** of the total carotenoids in folktail bullhead, coho salmon and dog salmon were 3.0% and 1.2%, 2.2% and 2.5%, 3.2% and 2.0%, respectively. Contrary to the case of Cypriniformes fish, zeaxanthin fraction obtained from these fish described above consisted of (3*R*, 3'*R*)-, (3*R*, 3'*S*; *meso*)-, and (3*S*, 3'*S*)-zeaxanthin. Accordingly, these are considered to be reductive metabolic products of (3*R*, 3'*R*)-, (3*R*, 3'*S*; *meso*)-, and (3*S*, 3'*S*)-astaxanthin.²⁾ In the integuments of these fish, (3*S*, 3'*S*)-astaxanthin and (3*R*, 3'*R*)-zeaxanthin were found to be major components of three stereoisomers of astaxanthin and zeaxanthin, respectively. Therefore **1** and **2** are assumed to be intermediates in the reductive metabolic pathway from (3*S*, 3'*S*)-astaxanthin to (3*R*, 3'*R*)-zeaxanthin via (3*S*, 3'*R*)-4-ketozeaxanthin (β -doradexanthin) as shown in Fig. 1.

β,β -Carotene tetrol previously isolated from gudgeon was mainly (3*S*, 4*R*, 3'*S*, 4'*R*)- β,β -carotene-3,4,3',4'-tetrol [(3*S*, 4*R*, 3'*S*, 4'*R*)-4,4'-dihydroxyzeaxanthin] (**3**).¹⁾ β,β -Carotene tetrol fraction from coho salmon (0.8% of the total carotenoids) and dog salmon (1.0%)

were found to contain mainly the same compound **3**. Trace amounts of diastereomers of this tetrol, (3*S*, 4*S*, 3'*S*, 4'*R*)- β,β -carotene-3,4,3',4'-tetrol [(3*S*, 4*S*, 3'*S*, 4'*R*)-4,4'-dihydroxyzeaxanthin] (**4**), and (3*S*, 4*S*, 3'*S*, 4'*S*)- β,β -carotene-3,4,3',4'-tetrol [(3*S*, 4*S*, 3'*S*, 4'*S*)-4,4'-dihydroxyzeaxanthin] (**5**), were also isolated and identified by co-HPLC with the authentic samples. β,β -Carotene tetrol could not be detected from folktail bullhead. The fact that the triols (**1** and **2**) and tetrols (**3**, **4**, and **5**), intermediates in the reductive metabolic pathway from astaxanthin to zeaxanthin as shown in Fig. 1, were not always found in our investigation, may depend on the reaction kinetics, the tissue and species, and the physiological state of fish.²⁾

References

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