New Acetylenic Carotenoid 6'-Epimonadoxanthin from the Rosary Goby *Gymnogobius castaneus*

Takashi Maoka

Abstract: Carotenoids of the rosary goby *Gymnogobius castaneus* (juzukakehaze in Japanese) were investigated. A new acetylenic carotenoid, (3R,3'R,6'S)-7,8-didehydro-β,ε-carotene-3,3'-diol (1), named 6'-epimonadoxanthin, was isolated along with 18 known carotenoids. The structure of 6'-epimonadoxanthin was determined by UV-VIS, ESI-TOF MS, 1H-NMR, and CD spectral data. The metabolic pathway from diatoxanthin to 6'-epimonadoxanthin is also discussed.

Key words: carotenoid, 6'-epimonadoxanthin, rosary goby, *Gymnogobius castaneus*

1 INTRODUCTION

Gobies are small fish of less than 10 cm in length that belong to the family Gobiidae (Order Perciformes), and inhabit brackish and freshwater environments. Tunaxanthin, lutein, zeaxanthin, alloxanthin, diatoxanthin, and tri-hydroxy-β-carotene have been reported as characteristic carotenoids in gobies by Matsuno et al. 

Furthermore, Tsushima et al. isolated the novel syn-epoxy carotenoids diadinoxanthin B and antheraxanthin B, and an acetylenic tri-hydroxy carotenoid, gobiusxanthin, from the freshwater goby *Rhinogobius brunneus*.

The rosary goby *Gymnogobius castaneus* (juzukakehaze in Japanese) is a small goby (4–6 cm body length) that inhabits the middle stretches of rivers, lakes, and lagoons in North Eastern Japan. Its tail fin is bright orange in color. This goby is edible and is consumed boiled in soy sauce or fried.

In this study, carotenoids of *G. castaneus* were investigated. As a result, a new acetylenic carotenoid, 6'-epimonadoxanthin (1), was isolated along with 18 known carotenoids. This report describes the isolation and structural elucidation of this new carotenoid from the rosary goby *G. castaneus*. Furthermore, the metabolic pathway from diatoxanthin to this new carotenoid (1) is discussed.

2 EXPERIMENTALS

2.1 Apparatus

UV-Visible (UV-VIS) absorption spectra were recorded with a Hitachi U-2001 spectrophotometer (Hitachi Field Navigator, Tokyo, Japan) in diethyl ether (Et2O). LC/MS analysis of carotenoids was performed using a Waters Xevo Q2S Q Tof mass spectrometer (Waters Corporation, Milford, CT, USA) equipped with an Acquity UPLC system. Electrospray ionization (ESI) time-of-flight (TOF) MS spectra were acquired by scanning from m/z 100 to 1,500 with a capillary voltage of 3.2 kV, cone voltage of 40 eV, and source temperature of 120°C. Nitrogen was used as a nebulizing gas at a flow rate of 30 L/h. MS/MS spectra were measured using a Q-TOF MS/MS instrument with argon as a collision gas at a collision energy of 30 V and used a photodiode array detector (PDA) to record UV-VIS absorption spectra from 200 to 600 nm. Acquity 1.7 μm BEH UPLC C18 column (Waters Corporation, Milford, CT, USA) and MeOH are used as stationary phase with a mobile phase at a flow rate of 0.4 mL/min for the HPLC system. 1H-NMR (500 MHz) spectrum was measured with a Varian Unity Inova 500 spectrometer (Varian Corporation, Palo Alto, California USA) in CDCl3, with TMS as an internal standard. Because of small amount of carotenoid sample (about 30 μg), 1H-NMR was measured using a SHIGEMI micro tube (sample solution volume 200 μL) (Shigemi Co., Ltd. Hachioji, Tokyo, Japan). The CD spectrum was recorded in Et2O at room temperature with a Jasco J-5000 spectropolarimeter (JASCO Corporation, Hachioji, Tokyo, Japan).

*Correspondence to: Takashi Maoka, Research Institute for Production Development, 15 Shimogamo-morimoto-cho, Sakyu-ku, Kyoto 606-0805, JAPAN
E-mail: maoka@mbox.kyoto-inet.or.jp
Accepted September 2, 2018 (received for review August 3, 2018)
Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
http://www.jstage.jst.go.jp/browse/jos/ http://mc.manuscriptcentral.com/jocs
Preparative HPLC was performed with a Hitachi L-6000 HPLC intelligent pump and Hitachi L-4250 UV-VIS detector (Hitachi Field Navigator, Tokyo, Japan) set at 450 nm. The column used were 250 × 4.6 mm i.d., 5 µm Cosmosil 5SIL-II and Cosmosil 5C18-II (Nacalai Tesque, Kyoto, Japan).

2.2 Animal Material
The rosary goby G. castaneus, collected in Aomori prefecture at October 2014, were purchased from a local fish market.

2.3 Extraction, Isolation, and Identification of Carotenoids
One kg of fresh fish (whole body) was extracted with acetone at room temperature. The acetone extract was partitioned with n-hexane/EtOAc (1:1) and water. The n-hexane/EtOAc (1:1) layer was evaporated to dryness. The residue contained large amount of lipids and carotenoids were existed as fatty acid esterified form \(^{14}\). Thus, the residue was saponified with 5% KOH/MeOH (10 mL) at room temperature for 2 h. Then, carotenoid was extracted with n-hexane/EtOAc (1:1) and washed with water. After evaporating of extract solution, individual carotenoids were separated by according to the methods described by Tsushima et al.\(^3\).

The reddish residue was subjected to silica gel 60 (Nacalai Tesque, Kyoto, Japan) column chromatography (200 × 17 mm). The fraction (Fr.) eluted with EtOAc/n-hexane (1:9 v/v, Fr. 1) was subjected to preparative HPLC on Cosmosil 5C18-II with a methanol at a flow rate of 2.0 mL/min to yield \(\alpha\)-carotene (retention time; rt. 34.0 min, yield 1 µg) and \(\beta\)-carotene (rt. 35.5 min, yield 35 µg). The fraction eluted with EtOAc/n-hexane (5:5 v/v, Fr. 2) was subjected to preparative HPLC on Cosmosil 5C18-II with a methanol at a flow rate of 2.0 mL/min to yield canthaxanthin (rt. 11.6 min, yield 12 µg), \(\alpha\)-cryptoxanthin (rt. 17.0 min, yield 30 µg), \(\beta\)-cryptoxanthin (rt. 18.5 min, yield 55 µg), and echinenone (rt. 27.9 min, yield 10 µg). The fraction eluted with EtOAc/n-hexane (8:2 v/v, Fr. 3) was subjected to preparative HPLC on Cosmosil 5SIL-II with acetone/ n-hexane (25:75 v/v) to yield 3'-hydroxy-echinenone (rt. 10.5 min, yield 10 µg), and astacene (rt. 20.0 min, yield 50 µg). The fraction eluted with acetone/n-hexane (1:1 v/v) was subjected to preparative HPLC on Cosmosil 5 SIL-II with acetone/n-hexane (2:8 v/v) to yield tunaxanthins (rt. 14.0 min), luteins (rt. 14.6 min), zeaxanthin (rt. 15.5 min, yield 50 µg), a new carotenoid (rt. 16.5 min, yield 30 µg), diatoxanthin (rt. 17.0 min, yield 70 µg), and allozeaxanthin (rt. 18.8 min, yield 90 µg). Tunaxanthins fraction was further separated by HPLC on Cosmosil 5C18-II with methanol to yield (3R,6S,3'R,6'S)-tunaxanthin (rt. 15.2 min, yield 20 µg), (3S,6S,3'R,6'S)-tunaxanthin (rt. 16.2 min, yield 18 µg), and (3S,6S,3'S,6'S)-tunaxanthin (rt. 16.8 min, yield 7 µg). Luteins fraction was further separated by HPLC on Cosmosil 5C18-II with methanol to yield (3R,3'R,6'S)-lutein (rt. 13.2 min, yield 30 µg) and (3R,3'S,6'S)-lutein (rt. 16.2 min, yield 18 µg). The fraction eluted with acetone was subjected to preparative HPLC on Cosmosil 5 SIL-II with acetone/n-hexane (3:7, v/v) to yield \(\beta\)-carotene-3,4,3'-trioi (rt. 20.5 min, yield 20 µg) and pectenol (rt. 22.5 min, yield 30 µg). Identification of individual carotenoids were accomplished by UV-VIS, ESI TOF MS, and comparison of retention time with authentic samples. Furthermore, (3S,6S,3'R,6'S)-tunaxanthin, (3S,6R,3'R,6'S)-tunaxanthin, (3R,3'S,6'S)-lutein, (3R,3'R,6'S)-lutein, zeaxanthin, diatoxanthin, allozeaxanthin, and pectenol were analyzed by \(^1H\)-NMR and CD spectral data\(^6\).

2.4 Quantitative Analysis of Carotenoids
The total carotenoid content and the amount of carotenoids eluted by column chromatography were calculated using the extinction coefficient of \(E_{20\text{nm}}^{\%}=2,500\) at \(\lambda_{\text{max}}\)\(^1\). In the HPLC analysis, the relative amounts of individual carotenoids were calculated from the peak area detected at 450 nm.

2.4.1 6'-Epimonadoxanthin

Yield: 30 µg. UV-VIS: 423, 445, 474 nm. ESI TOF MS: m/z 556.4130 (M + Na\(^+\), calcld. for C\(_{30}\)H\(_{42}\)O\(_{2}\)Na, 589.4014). \(^1H\)-NMR δ (CD\(_{3}\)OD): 8.86 (3H, s, CH\(_{-16}\)), 0.94 (3H, s, CH\(_{-17}\)), 1.15 (3H, s, CH\(_{-18}\)), 2.03 (3H, s, CH\(_{-19}\)), 1.91 (3H, s, CH\(_{-20}\)), 1.93 (3H, s, CH\(_{-18}\)), 1.96 (3H, s, CH\(_{-20}\)), 1.97 (3H, s, CH\(_{-20}\)), 2.01 (3H, s, CH\(_{-19}\)), 2.07 (1H, dd, J = 18, 10 Hz, H-4B), 2.16 (1H, d, J = 10 Hz, H-6\(^\prime\)), 2.43 (1H, dd, J = 18, 5, 1.5 Hz, H-4a\(^\prime\)), 3.99 (1H, m, H-3\(^\prime\)), 4.23 (1H, m, H-3\(^\prime\)), 5.48 (1H, brs, H-4\(^\prime\)), 5.54 (1H, dd, J = 15, 9 Hz, H-7\(^\prime\)), 6.13, (d, J = 15.5 Hz, H-8\(^\prime\)), 6.14 (1H, d, J = 11 Hz, H-10\(^\prime\)), 6.25 (1H, d, J = 11 Hz, H-14\(^\prime\)), 6.29 (1H, d, J = 11 Hz, H-14), 6.34 (1H, d, J = 15 Hz, H-12\(^\prime\)), 6.35 (1H, d, J = 15 Hz, H-12), 6.46 (1H, d, J = 11 Hz, H-10), 6.51 (1H, dd, J = 15, 11 Hz, H-11), 6.61 (1H, dd, J = 15, 11 Hz, H-11\(^\prime\)), 6.64 (2H, overlapped, H-15, 15'). \(^1\)H-COSY correlations; H-2-H-3-H-2-H-3-H-2, CH\(_{-19}-\)H-10'-H-11'-H-12, H-6'-H-7'-H-8', and H-2'-H3' -H-4', CD: nm (Δε): 230 (0), 245 (-6.0), 265 (-7.0), 290 (0), 328 (-3.0), 350 (0).

3 RESULTS AND DISCUSSION

3.1 Carotenoids of the rosary goby G. castaneus

The 18 known carotenoids and a new carotenoid (Fig. 1 and Table 1) were found in the rosary goby G. castaneus. As in other gobies\(^1,2\), tunaxanthins, luteins, diatoxanthin, and allozeaxanthin were found to be major components.
New Acetylenic Carotenoid 6'-Epimonadoxanthin from the Rosary Goby Gymnogobius castaneus

3.2 Structure of the new acetylenic carotenoid (1)

A new acetylenic carotenoid (1) was obtained as a minor component (30 μg from 1 kg of fish, 4.6 % of the total carotenoid amount). This carotenoid exhibited absorption maxima at 423, 445, and 474 nm. The molecular formula of 1 was determined to be C₄₀H₅₄O₂ by ESI-TOF MS data. 1H-NMR signals of 1 indicated the presence of a 7,8-didehydro-3-hydroxy-β-end group (H-2 to H-4, CH₃-16 to CH₃-19), a 3,6-cis-3-hydroxy-ε-end group (H-2, H-6, CH₃-16, CH₃-18), and an all-trans-polyene chain attached by an acetylenic bond at C-7 (H₁₀ to H-15, H-7 to H-15, CH₁₉-19, 20, 19, 20). This assignment was confirmed by 1H-1H COSY. Thus, the structure of this compound was determined to be 7,8-didehydro-β,ε-carotene-3,3-diol with a 3,6-cis configuration (Fig. 2). The absolute configuration of 1 was analyzed by CD spectral data. The CD spectrum of 1 is shown in Fig. 3. The additivity rule of CD was used to determine the chirality of carotenoids with different end groups on both sides of the polyene chain. The CD spectrum of this compound was similar with the additive CD spectra of (3S,3'R,6'S)-alloxanthin and (3R,6S,3'R,6'S)-tunaxanthin, as shown in Fig. 3. Thus, the absolute configuration of this compound was determined as (3R,3'R,6'S). This new carotenoid, (3R,3'R,6'S) - 7,8-didehydro-β,ε-carotene-3,3'-diol (1), corresponded with the 6'-epimer of monadoxanthin[(3R,3'R,6'R) - 7,8-didehydro-β,ε-carotene-3,3'-diol] reported by Pennington et al. Therefore, this carotenoid was named 6'-epimonadoxanthin.

Table 1 Carotenoid content and percentage composition of the rosary goby Gymnogobius castaneus.

| Total Carotenoid | 4.18 μg/g |
|-----------------|-----------|
| Composition     | %         |
| β-Carotene      | 5.8       |
| α-Carotene      | 0.2       |
| Echinone        | 1.9       |
| β-Cryptoxanthin | 8.8       |
| α-Cryptoxanthin | 5.4       |
| Canthaxanthin   | 1.9       |
| 3'-Hydroxy-echinenone | 1.5 |
| Astaxanthin*    | 7.9       |
| (3S,6S,3'S,6'S)-Tunaxanthin | 1.1 |
| (3S,6S,3'R,6'S)-Tunaxanthin | 2.8 |
| (3R,6S,3'R,6'S)-Tunaxanthin | 3.0 |
| (3R,3'S,6'S)-Lutein | 7.8 |
| (3R,3'R,6'S)-Lutein | 4.5 |
| 6'-Epimonadoxanthin (1) | 4.5 |
| Zeaxanthin      | 7.9       |
| Dioxanthin      | 10.6      |
| Alloxanthin     | 14.4      |
| Pectenol        | 4.5       |
| β, β-Carotene-3,4,3'-triol | 3.0 |
| Others          | 2.5       |

*Identified as astacene
3.3 Biosynthesis of 6'-epimonadoxanthin (1) in the rosary goby

It was previously reported that carotenoids with 3-hydroxy-ε-end groups in animals are formed from carotenoids with 3-oxo-ε-end groups via carotenoids with 3-ε-end groups. Namely, the 3-hydroxy-ε-end group in carotenoids is converted to a 3-oxo-ε-end group by oxidation of the hydroxy group at C-3 with transition of the double bond from C5-C6 to C4-C5. Then, the carbonyl group at C3 in the 3-oxo-ε-end group is reduced to a diol. Thus, (3R,3'S,6'S)- and (3R,3'R,6'S)-lutein in fish were formed from zeaxanthin through (3R,6'S)-3-hydroxy-7,8-didehydro-β,ε-caroten-3'-one, as shown in Fig. 4 (right column) [8-11].

Similarly, 6'-epimonadoxanthin (1) was speculated to be formed from diatoxanthin through (3R,6'S)-3-hydroxy-7,8-didehydro-β,ε-caroten-3'-one, as shown in Fig. 4 (left column). (3R,6'S)-3-Hydroxy-7,8-didehydro-β,ε-caroten-3'-one, which has not been reported yet in nature, was considered to be a possible key intermediate of this metabolic conversion. This compound has a 3-oxo-ε-end group. It is well known that carotenoids with 3-oxo-ε-end groups are very unstable in alkaline medium [12]. Therefore, this compound was decomposed during the saponification procedure and was not detected in the rosary goby in the present investigation. Furthermore, the corresponding 3'-epimer of 1, (3R,3'S,6'S)-7,8-didehydro-β,ε-carotene-3,3'-diol (2), may have been formed through this metabolic pathway, as shown in Fig. 4 (left column). Indeed, a very minor carotenoid (HPLC retention time; 16.8 min) with absorption maxima at 423, 445, and 474 nm, and molecular formula C_{40}H_{54}O_{2} was observed. This carotenoid may have been (3R,3'S,6'S)-7,8-didehydro-β,ε-carotene-3,3'-diol (2). However, because of the available sample amount, further structural characterization of 2 was not possible.

4 Conclusion

Diatoxanthin, which originated from diatom, is widely distributed among freshwater fish [1-3,11]. However, there are a few reports on the metabolites of diatoxanthin in freshwater fish [1]. In the present investigation, a new metabolite of diatoxanthin, named 6'-epimonadoxanthin (1), was isolated from the rosary goby G. castaneus.

Acknowledgements

The author thanks Associate Professor Takahiko Mukai, Graduate School of Regional Studies, Gifu University, for identification of the rosary goby G. castaneus.
Fig. 4  Possible metabolic pathways of diatoxanthin to 6'-epimonadoxanthin (1) (left column) and zeaxanthin to (3R,3'S,6'S)- and (3R,3'R,6'S)-lutein (right column) in the rosary goby Gymnogobius castaneus.

References
1) Matsuno, T.; Higashi, E.; T. Akita, T. Carotenoid pigments in gobies and five related fishes. Bull. Jap. Soc. Fish. 39, 159-163 (1973).
2) Matsuno, T.; Katsuyama, M. Comparative biochemical studies of carotenoids in fishes-VI Carotenoids of Japanese sculpia and white gobies. Bull. Jap. Soc. Sci. Fish. 41, 675-679 (1975).
3) Tsushima, M.; Mune, T.; Maoka, T.; Matsuno, T. Isolation of stereoisomeric epoxy carotenoids and new acetylenic carotenoid from the common freshwater goby Rhinogobius brunneus. J. Nat. Prod. 63, 960-964 (2000).
4) Maoka, T. Structural studies of carotenoids in plants, animals, and food products. in Carotenoids Nutrition, Analysis and Technology (Kaczor, A.; Baranska, M. eds.), Wiley Blackwell, UK, pp. 103-129 (2016).
5) Engler, G. NMR Spectroscopy. in Carotenoids Vol. 1B (Britton, G.; Liaaen-Jensen, S.; Pfander, H. eds.), Birkhäuser, Basel, Switzerland, pp. 147-260 (1995).
6) Buchecker, R.; Noack, K. Circular dichroism. in Carotenoids Vol. 1B (Britton, G.; Liaaen-Jensen, S.; Pfander, H. eds.), Birkhäuser, Basel, Switzerland, pp. 63-116 (1995).
7) Pennington, F.C.; Haxo, F.T.; Borch, G.; Liaaen-Jensen, S. Carotenoids of cryptophyceae. Biochem. Syst. Ecol. 13, 215-219 (1985).
8) Nagao, A.; Maoka, T.; Ono, H.; Kotake-Nara, E.; Kobayashi, M.; Tomita, M. A 3-hydroxy α-end group in xanthophylls is preferentially oxidized to a 3-oxo ε-end group in mammals. J. Lipid Res. 56, 449-462 (2015).
9) Maoka, T. Carotenoids in marine animals. Mar. Drugs 9, 278-293 (2011).
10) Matsuno, T.; Hirono, T., Ikuno, Y.; Maoka, T.; Shimizu, M.; T. Komori, T. Isolation of three new carotenoids and proposed metabolic pathways of carotenoids in hen's egg yolk. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 84, 477-481 (1986).
11) Matsuno, T. Aquatic animal carotenoids. Fish. Sci. 67, 771-783 (2001).
12) Schiedt, K.; Liaaen-Jensen, S. Isolation and analysis. in Carotenoids vol. 1A (Britton, G.; Liaaen-Jensen, S.; Pfander, H. eds.), Birkhäuser, Basel, Switzerland, pp. 81-108 (1995).