Article

Synthesis of (3S,3′S)- and meso-Stereoisomers of Alloxanthin and Determination of Absolute Configuration of Alloxanthin Isolated from Aquatic Animals

Yumiko Yamano 1,*, Takashi Maoka 2 and Akimori Wada 1

1 Kobe Pharmaceutical University, Motoyamakita-machi, Higashinada-ku, Kobe 658-8558, Japan; E-Mail: a-wada@kobepharma-u.ac.jp
2 Research Institute for Production Development, 15 Shimogamo-morimoto-cho, Sakyō-ku, Kyoto 606-0805, Japan; E-Mail: maoka@mbox.kyoto-inet.or.jp

* Author to whom correspondence should be addressed; E-Mail: y-yamano@kobepharma-u.ac.jp; Tel./Fax.: +81-78-441-7562.

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Abstract: In order to determine the absolute configuration of naturally occurring alloxanthin, a HPLC analytical method for three stereoisomers 1a–c was established by using a chiral column. Two authentic samples, (3S,3′S)- and meso-stereoisomers 1b and 1c, were chemically synthesized according to the method previously developed for (3R,3′R)-alloxanthin (1a). Application of this method to various alloxanthin specimens of aquatic animals demonstrated that those isolated from shellfishes, tunicates, and crucian carp are identical with (3R,3′R)-stereoisomer 1a, and unexpectedly those from lake shrimp, catfish, biwa goby, and biwa trout are mixtures of three stereoisomers of 1a–c.

Keywords: carotenoid; alloxanthin; synthesis; chiral HPLC separation; absolute configuration

1. Introduction

Alloxanthin (1) (Figure 1) was first isolated from Cryptomonas algae [1] and its structure was determined to be 7,8,7′,8′-tetreradehydro-β,β-carotene-3,3′-diol by MS, IR and 1H-NMR spectroscopies [2]. Additionally, cynthiaxanthin [3] from the tunicate Cynthia roreezi (Halocynthia roreezi) and pectenoxanthin [4] from giant scallop Pecten maximus were isolated by Japanese scientists.
In 1967, Campbell et al. demonstrated that these two carotenoids were identical with alloxanthin [5]. Therefore, cynthiaxanthin and pectenoxanthin were synonyms of alloxanthin. The absolute configuration of alloxanthin isolated from algae was deduced to be 3R,3′R by X-ray analysis of degradation product of fucoxanthin and in view of biogenetic grounds [6]. Bartlett et al. reported that the ORD spectra of alloxanthin specimens from Cryptomonas algae and tunicate showed an identical shape each other and that both specimens are assumed to have an identical absolute configuration [7].

Since then, alloxanthin was isolated from several aquatic animals, such as shellfishes [8,9], starfishes [10], tunicates [11,12] and freshwater fishes [13,14], etc. These alloxanthin specimens showed similar non-conservative CD with weak negative Cotton effects.

Carotenoids such as astaxanthin, zeaxanthin, lutein, and tunaxanthin in animals are known to exist as a mixture of stereoisomers. Namely, astaxanthin in crustaceans and marine fishes exists as a mixture of three stereoisomers at C3 and C3′-positions [15,16]. Zeaxanthin [17], lutein [18], and tunaxanthin [19] in marine fishes also consist of these stereoisomers. Their absolute configurations were determined by CD spectra and chiral HPLC analyses. Due to its non-conservative CD, absolute configurations of alloxanthin in several origins could not be determined exactly by CD spectra.

In order to determine the absolute configuration of naturally occurring alloxanthin, we synthesized stereoisomers of alloxanthin (1a–c) and established a HPLC analytical method using a chiral column. Applying this method, the absolute configurations of alloxanthin specimens isolated from shellfishes, tunicates and fishes were investigated. Here, we describe these results.
2. Results and Discussion

2.1. Synthesis of (3S,3′S)-Alloxanthin (1b) and meso-Alloxanthin (1c)

We previously reported [20] stereoselective total synthesis of (3R,3′R)-alloxanthin (1a) by use of C_{15}-acetylenic tri-n-butylphosphonium salt 5a (Scheme 1) as a versatile synthon for syntheses of acetylenic carotenoids. This time, (3S,3′S)-alloxanthin (1b) and its meso-stereoisomer 1c were newly synthesized using (3S)-phosphonium salt 5b, which was prepared from 3-epi-actinol 6 [21] in the same procedure [20] as preparation of (3R)-one 5a.

Scheme 1. Synthesis of C_{15}-acetylenic tri-n-butylphosphonium salts 5a and 5b.

![Scheme 1](image_url)

Reagents: (a) (i) TMSCl, Et_3N, DMAP, (ii) TMSC≡CH, "BuLi, then aq. KOH, (iii) Ac_2O, pyridine, (iv) CuSO_4, xylene, reflux (Dean-Stark), (v) LiAlH_4; (b) TESCl, Et_3N, DMAP, (c) (i) vinyl bromide 6, Pd(PPh_3)_4, CuI, BHT, 'Pr_2NH, (ii) MsCl, LiCl, γ-collidine, (iii) P^tBu_3, Et_3N.

Compound 6 was converted into terminal alkyne 3b via the addition of lithium acetylide in 72% yield over six steps. The high enantiomeric purity of 3b (99% ee) was confirmed by HPLC analysis [CHIRALPAK AY-H; Daicel, 2-PrOH–n-hexane (5:95)]. Compound 3b was then transformed into the phosphonium salt 5b via Sonogashira cross-coupling of the triethylsilyl (TES)-protected terminal alkyne 4b with vinylbromide 6 in 59% over four steps.

Wittig condensation of C_{10}-dialdehyde 7 with excess amount of (3S)-phosphonium salt 5b in the presence of sodium methoxide in dichloromethane at room temperature and subsequent desilylation stereoselectively provided (3S,3′S)-alloxanthin (1b) (Scheme 2). On the other hand, meso-alloxanthin (1c) was synthesized via condensation between (3S)-phosphonium salt 5b and (3R)-C_{25}-acetylenic apocarotenal 8, which was prepared by Wittig reaction of C_{10}-dialdehyde 7 with (3R)-phosphonium salt 5a in the presence of sodium methoxide in dichloromethane at 0 °C.
Scheme 2. Synthesis of three stereoisomers of alloxanthin (1a–c).

CD spectrum of (3S,3’S)-alloxanthin (1b) showed an antisymmetrical curve having weak Cotton effects to that of previously synthesized [20] (3R,3’R)-alloxanthin (1a) as shown in Figure 2.

Figure 2. CD spectra in Et₂O–isopentane–EtOH (5:5:2) of synthesized (3R,3’R)-alloxanthin (1a) and (3S,3’S)-alloxanthin (1b).

2.2. Determination of Absolute Configuration of Alloxanthin Isolated from Aquatic Animals by HPLC

In order to determine the absolute configuration of naturally occurring alloxanthin, a HPLC analytical method for three stereoisomers 1a–c was investigated. As a result, three synthetic stereoisomers of alloxanthin can be separated using a chiral column (CHIRALPAK AD-H; Daicel) as shown in Figure 3.

Next, alloxanthin specimens isolated from scallop Mizuhopecten yessoensis, oyster Crassostrea gigas, pacific pearl oyster Pinctada margaritifera, freshwater bivalve Unio douglasiae, tunicate Halocynthia roretzi, and crucian carp Carassius auratus grandoculis were subjected to the HPLC method to find that these consist of only (3R,3’R)-stereoisomer 1a. On the other hand, alloxanthin specimens isolated from lake shrimp Palaemon paucidens, catfish Silurus asotus, biwa goby Gymnogobius isaza, and biwa trout Oncorhynchus masou rhodurus consisted of three stereoisomers 1a–c (Table 1).
Figure 3. HPLC elution profile of a mixture of three stereoisomers of alloxanthin (1a–c).

Column: CHIRALPAK AD-H 0.46 × 25 cm (Daicel, Tokyo, Japan); eluent: 2-PrOH–n-hexane (4:96); flow rate: 0.6 mL/min; temperature: 23 °C; detection: 450 nm.

Table 1. Occurrence and percentage composition of alloxanthin stereoisomers in aquatic animals.

| Species             | 3R,3' R 1a | 3S,3'S 1b | meso 1c |
|---------------------|------------|-----------|---------|
| Shellfish           |            |           |         |
| Scallop             | 100        | n.d.      | n.d.    |
| Oyster              | 100        | n.d.      | n.d.    |
| Pacific pearl oyster| 100        | n.d.      | n.d.    |
| Freshwater bivalves | 100        | n.d.      | n.d.    |
| Tunicate            |            |           |         |
| Sea squirt          | 100        | n.d.      | n.d.    |
| Crustacean          |            |           |         |
| Lake shrimp         | 53.7       | 9.6       | 36.7    |
| Fish                |            |           |         |
| Crucian carp        | 100        | n.d.      | n.d.    |
| Biwa goby           | 91.4       | 0.9       | 7.7     |
| Biwa trout          | >99.9      | trace     | trace   |
| Catfish             | 82.9       | 1.5       | 15.6    |

n.d.: not detected.

Previously, one of the authors reported that zeaxanthin in plants, shellfishes, and tunicates consisted of only (3R,3'R)-stereoisomer, whereas zeaxanthin in fishes consisted of three stereoisomers [17]. Similar results were obtained in the case of alloxanthin in aquatic animals. Alloxanthin is de novo synthesized in Chryophyceae and Euglenophyceae microalgae [22]. However, origin of alloxanthin in aquatic animals was remained uncertain. Patrali et al. (1989) [22] and Liaaen-Jensen (1998) [23] reported that alloxanthin in Mytilus edulis might be a terminal metabolite of fucoxanthin through intermediates, halocynthiaxanthin and isomytiloxanthin, based on observation in feeding experiment. However, conversion of isomytiloxanthin into alloxanthin is too complex and there were no direct
evidences for the conversion, especially in aquatic animals. In our experience, isomytiloxanthin has not been isolated from these animals [24]. Shellfishes (bivalves) and tunicates are filter-feeders, which accumulate carotenoids from microalgae. Therefore, alloxanthin in these animals is assumed to originate from Cryptophyceae and Euglenophyceae microalgae, etc. Thus, these alloxanthin specimens consist of only (3R,3′R)-stereoisomer. Crucian carp is omnivorous and feeds not only animal planktons belonging to Cladocera but also microalgae. Therefore, alloxanthin in crucian carp is also assumed to originate from microalgae. On the other hand, alloxanthin in lake shrimp, catfish, biwa goby, and biwa trout exist as a mixture of three stereoisomers. These crustaceans and fishes are carnivorous. Especially, lake shrimp contains a large amount of (3S,3′S)- and meso-alloxanthin (Table 1). Lake shrimp is a one of the major food of catfish and biwa trout. Therefore, (3S,3′S)- and meso-alloxanthin in these fishes might be originated from lake shrimp. However, origin of (3S,3′S)- and meso-alloxanthin in lake shrimp is uncertain.

Catfish is a top predator in Japanese freshwater ecosystems. Catfish ingests astaxanthin from crustaceans whose astaxanthin exists as a mixture of three stereoisomers. Catfish can convert astaxanthin into zeaxanthin [24]. Therefore, zeaxanthin in catfish exists as a mixture of three stereoisomers. Although the origin of stereoisomers of alloxanthin in catfish is uncertain, it might be naturally formed by epimerization of 7,8,7′,8′-tetradehydroastaxanthin originated from crustacean at C3 and C3′-positions and subsequent reduction at C4 and C4′-positions. Further studies are need to reveal the origin of (3S,3′S)- and meso-alloxanthin in crustaceans and fishes.

This is the first report of the occurrence of (3S,3′S) and meso-alloxanthin in nature.

3. Experimental Section

3.1. General

IR spectrum was measured on a Perkin-Elmer FT-IR spectrometer (Perkin-Elmer, Yokohama, Japan), spectrum 100. 1H and 13C NMR spectra were determined on a Varian Gemini-300 superconducting FT-NMR spectrometer (Agilent Technologies, Santa Clara, CA, USA) and the chemical shifts were referenced to tetramethylsilane. Mass spectrum was taken on a Thermo Fisher Scientific Exactive spectrometer (Thermo Fisher Scientific, Bremen, Germany). CD spectra were measured on a Shimadzu-AVIN 62A DS circular dichroism spectrometer (Shimadzu, Kyoto, Japan).

HPLC analyses were performed on Shimadzu-LC-20AT instrument (Shimadzu, Kyoto, Japan) with a photodiode array detector (Waters 996, Tokyo, Japan) and column oven (GL Sciences Model 552, Tokyo, Japan).

NMR assignments are given using the carotenoid number system.

3.2. Synthesis of (3S,3′S)-Allocxanthin (1b) and meso-Alloxanthin (1c)

In the same procedure [20] as preparation of (3R)-phosphonium salt 5a and (3R,3′R)-alloxanthin (1a), (3S)-5b and (3S,3′S)-alloxanthin (1b) were prepared. Spectral data except for optical data of compounds 1b, 3b, 4b and 5b were identical with the corresponding previous reported [20] enantiomers 1a, 3a, 4a and 5a.

(3S,3′S)-Alloxanthin (1b): HRMS (ESI) m/z calcd for C40H53O2 [M + H]⁺ 565.4040, found 565.4038.
Compound 3b: [α]_D^{26} 102.9 (c 1.03, MeOH); HRMS (ESI) m/z calcd for C_{11}H_{17}O [M + H]^+ 165.1274, found 165.1277.

Compound 4b: [α]_D^{23} 68.1 (c 1.00, MeOH); HRMS (ESI) m/z calcd for C_{17}H_{31}OSi [M + H]^+ 279.2139, found 279.2139.

Compound 5b: HRMS (ESI) m/z calcd for C_{33}H_{62}OPSi [M − Cl]^− 533.4302, found 533.4293.

meso-Alloxanthin (1c) was synthesized via condensation between 5b and (3R)-C_{25}-acycyclic apocarotenal 8, which was prepared by Wittig reaction of C_{10}-dialdehyde 7 with 5a as follows.

(2E,4E,6E,8E,10E)-2,7,11-trimethyl-13-[(R)-2,6,6-trimethyl-4-triethylsilyloxy-cyclohex-1-en-1-yl] trideca-2,4,6,8,10-pentaen-12-ynal (8). NaOMe (1 M in MeOH; 1.2 mL, 1.2 mmol) was added to a solution of the (3R)-phosphonium salt 5a (409 mg, 0.73 mmol) and C_{10}-dialdehyde 7 (100 mg, 0.61 mmol) in CH₂Cl₂ (10 mL) at 0 °C. After being stirred at 0 °C for 15 min, the mixture was poured into saturated aq. NH₄Cl and extracted with AcOEt. The extracts were washed with brine, dried over Na₂SO₄ and evaporated to afford a residue, which was purified by flash column chromatography (AcOEt–n-hexane, 1:4) to give the (3R)-C_{25}-acycyclic apocarotenal 8 (165 mg, 57%) as an orange viscous oil: UV-VIS λ_{max} (EtOH)/nm 420; IR ν_{max} (CHCl₃)/cm⁻¹ 2170 (C=C), 1663 (conj. CHO), 1610 and 1599 (split) (C=C), 1552 (C=C); ^1H-NMR (CDCl₃, 300 MHz) δ 0.61 (6H, q, J = 8 Hz), 0.83 (12H), 0.97 (9H, t, J = 8 Hz, CH₃CH₂ × 3), 1.09 and 1.18 (each 3H, s, 1-gem-Me), 1.49 (1H, t, J = 12 Hz, H₆g), 1.74 (1H, ddd, J = 12, 3.5, 2 Hz, H₆a), 1.89 (3H), 1.91 (3H) and 2.03 (6H) (each s, 5-Me, 9-Me, 13-Me and 13’-Me), 2.11 (1H, br dd, J = 17.5, 9.5 Hz, 4-H₇b), 2.30 (1H, br dd, J = 17.5, 5.5 Hz, 4-H₇a), 3.94 (1H, m, 3-H), 6.32 (1H, br d, J = 12 Hz, 14-H), 6.37 (1H, d, J = 15 Hz, 12-H), 6.46 (1H, br d, J = 11.5 Hz, 10-H), 6.66 (1H, dd, J = 15, 11.5 Hz, 11-H), 6.70 (1H, dd, J = 14.5, 11.5 Hz, 15’-H), 6.96 (1H, br d, J = 11.5 Hz, 14’-H), 7.03 (1H, dd, J = 14.5, 12 Hz, 15-H), 9.46 (1H, s, CHO); ^13C-NMR (CDCl₃, 75 MHz) δ 4.82 (C × 3), 6.83 (C × 3), 9.59, 12.96, 18.17, 23.22, 28.61, 30.45, 36.53, 42.11, 47.04, 65.01, 90.10, 98.16, 121.15, 123.84, 126.60, 127.73, 131.75, 134.51, 137.02, 137.07, 137.47, 138.70, 141.26, 148.75, 194.45; HRMS (ESI) m/z calcd for C_{31}H_{47}O_{2}Si (MH)^+ 479.3340, found 479.3347.

Preparation of meso-alloxanthin (1c). NaOMe (1 M in MeOH; 0.24 mL, 0.24 mmol) was added to a solution of the (3S)-phosphonium salt 5b (113 mg, 0.20 mmol) and (3R)-C_{25}-acycyclic apocarotenal 8 (59 mg, 0.12 mmol) in CH₂Cl₂ (10 mL) at room temperature. After being stirred for further 15 min, the mixture was poured into saturated aq. NH₄Cl and extracted with AcOEt. The extracts were washed with brine, dried over Na₂SO₄ and evaporated to afford a residue, which was purified by flash column chromatography (AcOEt–n-hexane, 1:4) to give the TES-protected condensed product. Subsequently, to a solution of this condensed product in dry THF (5 mL) were added AcOH (1 M in THF; 0.20 mL, 0.20 mmol) and then tetrabutylammonium fluoride (TBAF) (1 M in THF, 0.40 mL, 0.40 mmol). After being stirred at room temperature for 2 h, the mixture was concentrated to give a residue, which was purified by flash column chromatography (AcOEt–n-hexane–MeOH, 50:45:5) to provide meso-alloxanthin (1c) (70 mg, quant.) as red solids. Its spectral data were identical with those of (3R,3’R)-alloxanthin (1a) [20]. HRMS (ESI) m/z calcd for C_{40}H_{53}O_{2} [M + H]^+ 565.4040, found 565.4033.
3.3. Configurational Analysis of Natural Alloxanthin

3.3.1. Animal Materials

Scallop *Mizuhopecten yessoensis* was provided from Hokkaido Research Organization, Abashiri Fisheries Research Institute, Hokkaido, Japan. Oyster *Crassostrea gigas*, and sea squirt *Halocynthia roretzi* were purchased from fisheries market at Kyoto city. Pacific pearl oyster *Pinctada margaritifera* was provided from a pearl aquaculture industry, Ishigaki city, Okinawa Prefecture. Freshwater bivalve *Unio douglasiae*, crucian carp *Carassius auratus grandoculis*, and catfish *Silurus asotus* were purchased from Katata fisheries cooperative, Shiga Prefecture. Biwa trout *Oncorhynchus masou rhodurus* was purchased from Nango Fisheries Center, Shiga Prefecture. Biwa goby *Gymnogobius isaza* and lake shrimp *Palaemon paucidens* were purchased from fisheries market at Maibara city.

3.3.2. Isolation of Alloxanthin from Aquatic Animals

According to our routine methods, carotenoid was extracted with acetone from animal tissue. The extract was partitioned between Et$_2$O–$n$-hexane (1:1) and water in separating funnel. The organic phase was evaporated and saponified with 5% KOH/MeOH at room temperature for 2 h. Then, unsaponifiable compounds were extracted with Et$_2$O–$n$-hexane (1:1, v/v) from the reaction mixture by addition of water. The organic layer was dried over Na$_2$SO$_4$ and evaporated. The residue was subjected to silica gel column chromatography increasing percentage of Et$_2$O in $n$-hexane. The fraction eluted with Et$_2$O was subjected to HPLC on silica gel with acetone–$n$-hexane (3:7) to afford alloxanthin. Purity of alloxanthin was checked by UV-Vis, $^1$H-NMR, and MS spectral data. Then alloxanthin obtained from aquatic animals was subject to configurational analysis using a chiral column described above.

4. Conclusions

In conclusion, we synthesized stereoisomers of alloxanthin (1a–c) and established a HPLC analytical method using a chiral column to identify them for naturally occurring alloxanthin. Application of this method to various alloxanthin specimens of aquatic animals demonstrated that those isolated from shellfishes, tunicates, and crucian carp are identical with (3R,3′R)-stereoisomer 1a, and unexpectedly those from lake shrimp, catfish, biwa goby, and biwa trout are mixtures of three stereoisomers of 1a–c. This is the first report of the occurrence of (3S,3′S) and meso-alloxanthin in nature. The analytical method can be a powerful tool to identify stereoisomers of alloxanthin in nature in a straightforward manner.

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Author Contributions

Basic idea of the research was proposed by three authors collaboratively. The synthetic and analytical experiments were designed and performed by Y. Yamano. The isolation of natural products was designed and carried out by T. Maoka.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Haxo, F.T.; Fork, D.C. Photosynthetically active accessory pigments of cryptomonads. Nature 1959, 184, 1051–1052.
2. Mallams, A.K.; Waight, E.S.; Weedon, B.C.L.; Chapman, D.J.; Haxo, F.T.; Goodwin, T.W.; Thomas, B.M. A new class of carotenoids. Chem. Commun. 1967, 301–302; doi:10.1039/c19670000301.
3. Tsuchiya, Y.; Suzuki, Y. Biochemical studies of the ascidian, Cynthia rorezi variety drasche. IV Carotenoids in test. Tohoku J. Agric Res. 1959, 10, 397–407.
4. Nishibori, K. Pigments of marine animlas—VIII. Carotenoids of some shellfish. Publ. Seto Mar. Biol. Lab. 1960, 8, 317–326.
5. Campbell, S.A.; Mallams, A.K.; Waight, E.S.; Weedon, B.C.L. Pectenoxanthin, cynthiaxanthin, and a new acetylenic carotenoid, pectenolone. Chem. Commun. 1967, 941–942; doi:10.1039/c29670000941.
6. DeVille, T.E.; Hursthouse, M.B.; Russell, S.W.; Weedon, B.C.L. Absolute configuration of carotenoids. Chem. Commun. 1969, 1311–1312; doi:10.1039/c29690001311.
7. Bartlett, L.; Klyne, W.; Mose, W.P.; Scopes, P.M.; Galasko, G.; Mallams, A.K.; Weedon, B.C.L.; Szabolcs, J.; Toth, G. Optical rotatory dispersion of carotenoids. J. Chem. Soc. Perkin Trans. 1 1969, 2527–2544; doi:10.1039/j39590002527.
8. Hertzberg, S.; Partali, V.; Liaaen-Jensen, S. Animal carotenoids. 32. Carotenoids of Mytilus edulis (edible mussel). Acta Chem. Scand. 1988, B42, 495–503.
9. Maoka, T.; Matsuno, T. Carotenoids of shellfishes—IX. Isolation and structural elucidation of three new acetylenic carotenoids from the Japanese sea mussel Mytilus coruscus. Nippon Suisan Gakkaishi 1988, 54, 1443–1447.
10. Maoka, T.; Tsushima, M.; Matsuno, T. New acetylenic carotenoids from the starfishes Asterina pectinifera and Asterias amurensis. Comp. Biochem. Physiol. 1989, 93B, 829–834.
11. Matsuno, T.; Ookubo, M.; Nishizawa, T.; Shimizu, I. Carotenoids of sea squirts. I. New marine carotenoids, halocynthiaxanthin and mytiloxanthinone from Halocynthia roretzi. Chem. Pharm. Bull. 1984, 32, 4309–4315.
12. Ookubo, M.; Matsuno, T. Carotenoids of sea squirts—II. Comparative biochemical studies of carotenoids in sea squirts. Comp. Biochem. Physiol. 1985, 81B, 137–141.
13. Matsuno, T.; Maoka, T.; Ikuno, Y. Comparative biochemical studies of carotenoids in fish—XXVII. Carotenoids in the eggs of three species of Cyprinidae. *Comp. Biochem. Physiol.* **1986**, *83B*, 335–337.

14. Maoka, T.; Akiomoto, N. Structures of minor carotenoids from the Japanese common catfish, *Silurus asotus*. *Chem. Phram. Bull.* **2011**, *59*, 140–145.

15. Ronneberg, H.; Renstrom, B.; Aareskjold, K.; Liaaen-Jensen, S.; Vecchi, M.; Leuenberger, F.J.; Müller, R.K.; Mayer, H. Naturally occurrence of enantiomeric and *meso*-astaxanthin I. Ex lobster eggs (*Homarus gammarus*). *Helv. Chim. Acta* **1980**, *63*, 711–715.

16. Matsuno, T.; Maoka, T.; Katsuyama, M.; Ookubo, M.; Katagiri, K.; Jimura, H. The occurrence of enantiomeric and *meso*-astaxanthin in aquatic animals. *Nippon Suisan Gakkaishi* **1984**, *50*, 1589–1592.

17. Maoka, T.; Arai, A.; Shimizu, M.; Matsuno, T. The first isolation of enantiomeric and *meso*-zeaxanthin in nature. *Comp. Biochem. Physiol.* **1986**, *83B*, 121–124.

18. Matsuno, T.; Maoka, T.; Katsuyama, M.; Hirono, T.; Ikuno, Y.; Shimizu, M.; Komori, T. Comparative biochemical studies of carotenoids in fishes—XXIX. Isolation of new luteins, lutein F and lutein G from marine fishes. *Comp. Biochem. Physiol.* **1986**, *85B*, 77–80.

19. Ikuno, Y.; Shimizu, M.; Koshino, Y.; Maoka, T.; Matsuno, T. Comparative biochemical studies of carotenoids in fishes—XXVII. Stereochemical investigation of carotenoids from yellow-tail rockfish *Sebastes flavidus*. *Nippon Suisan Gakkaishi* **1985**, *51*, 2033–2035.

20. Yamano, Y.; Chary, V.M.; Wada, A. Stereoselective total synthesis of the acetylenic carotenoids alloxanthin and triophaxanthin. *Org. Biomol. Chem.* **2012**, *10*, 4103–4108.

21. Leuenberger, H.G.W.; Boguth, W.; Widmer, E.; Zell, R. Synthesis of optically active natural carotenoids and structurally related compounds. I. Synthesis of the chiral key compound (4R,6R)-4-hydroxy-2,2,6-trimethylcyclohexanone. *Helv. Chim. Acta* **1976**, *59*, 1832–1849.

22. Partali, V.; Tangen, K.; Liaaen-Jensen, S. Carotenoids in food chain studies. III. Resorption and metabolic transformation of carotenoids in *Mytilus edulis* (edible mussel). *Comp. Biochem. Physiol.* **1989**, *92B*, 239–246.

23. Liaaen-Jensen, S. Carotenoids in food chain. In *Carotenoids Volume 3: Biosynthesis and Metabolism*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser: Basel, Switzerland, 1998; pp. 359–371.

24. Maoka, T. Carotenoids in marine animals. *Mar. Drugs* **2011**, *9*, 278–293.

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