Notes

Salmoxanthin, Deepoxysalmoxanthin, and 7,8-Didehydrodeepoxysalmoxanthin from the Salmon *Oncorhynchus keta*

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The absolute configuration of salmoxanthin (5,6-epoxy-5,6-dihydro-β,ϵ-carotene-3,3′,6′-triol) (**1**) first isolated from the salmon *Oncorhynchus keta* was determined to be 3*S*, 5*R*, 6*S*, 3′*S*, 6′*R*. Furthermore, two minor carotenoids, deepoxysalmoxanthin (**2**) and 7,8-didehydrodeepoxysalmoxanthin (**3**), were isolated, and their structures were determined to be (3*R*,3′*S*,6′*R*)-β,ϵ-carotene-3,3′,6′-triol and (3*R*,3′*S*,6′*R*)-7,8-didehydro-β,ϵ-carotene-3,3′,6′-triol by ^{1}H NMR and CD spectroscopy.

Salmoxanthin (**1**) is a characteristic carotenoid in the salmon *Oncorhynchus keta*,[1] and the structure was proposed to be 5,6-epoxy-5,6-dihydro-β,ϵ-carotene-3,3′,6′-triol by chemical and spectroscopic data.[2] This was the same structure as for trollixanthin isolated from *Trollius europaeus* by Karrer et al.[3] However, its stereostructure remained uncertain. In the present investigation, we report the determination of absolute configuration of salmoxanthin **1** and the structural elucidation of two minor carotenoids, deepoxysalmoxanthin (**2**) and 7, 8-didehydrodeepoxysalmoxanthin (**3**), possessing the same 3,6-dihydroxy-ϵ end group as **1** isolated from the salmon *O. keta*.

According to the methods previously reported,[1,2] the Me_2CO extract of the integuments (850 g) of *O. keta* was partitioned between *n*-hexane−Et_2O (1:1) and aqueous NaCl. The organic layer was evaporated to dryness, and the residue was saponified with 10% KOH/MeOH at 37 °C for 12 h. After saponification, the crude carotenoids (10.5 mg) were subjected to column chromatography on Si gel and preparative HPLC on Si gel to yield **1** (2.0 mg), **2** (0.2 mg), and **3** (50 μg).

Salmoxanthin **1** showed absorption maxima at 416, 440, and 470 nm (Et_2O) and a molecular ion peak at *m*/*z* 600.4183 in HREIMS, compatible with the formula $C_{40}H_{56}O_4$. The structure **1** for 5,6-epoxy-5,6-dihydro-β,ϵ-carotene-3,3′,6′-triol, including the geometry of the double bonds and relative stereochemistry of the end groups, was fully characterized by ^{1}H NMR, ^{1}H−^{1}H double quantum filtered COSY (DQF-COSY), and ^{1}H−^{1}H NOESY experiments, as shown in Table 1 and Figure 1. The ^{1}H NMR chemical shifts and coupling constants of the partial structure of the violaxanthin part (H-2 to H-20) of **1** were identical with the corresponding data of all-*E*-violaxanthin.[4,5] All-*E* geometry of the polyene chain (H-7 to H-7′) was confirmed by NOEs data as shown in Figure 1. Furthermore, 3′,6′-*cis* configuration of the 3′,6′-dihydroxy-ϵ end group was deduced by NOESY experiments. NOEs between CH_3-16′ and H-3′ and between CH_3-16′ and H-7′ indicated that CH_3-16′, H-3′, and H-7′ were orientated on the same side of the 3′,6′-dihydroxy-ϵ end group of **1**. Therefore the relative stereochemistry of the two hydroxy groups at C-3′ and C-6′ was established as *cis*.

Table 1. ^{1}H NMR Data (δ mult, *J* in Hz) of Salmoxanthin (**1**) and Deepoxysalmoxanthin (**2**) in $CDCl_3$

position	**1**[a]	**2**[b]
H-2α	1.63 ddd (14.5, 3, 1.5)	1.77 ddd (11.5, 3.5, 1.5)
H-2β	1.25 dd (14.5, 10)	1.48 dd (11.5, 11.5)
H-3	3.91 m	4.00 m
H-4α	2.39 ddd (14.5, 5, 1.5)	2.39 ddd (17, 5, 1.5)
H-4β	1.63 dd (14.5, 8.5)	2.04 dd (17, 10)
H-7	5.88 d (15.5)	6.12[c]
H-8	6.29 d (15.5)	6.12[c]
H-10	6.20 d (11.5)	6.15 d (11.5)
H-11	6.63 dd (15, 11.5)	6.64 dd (15, 11.5)
H-12	6.37 d (15)	6.36 d (15)
H-14	6.26 m	6.25 m
H-15	6.64 m	6.64 m
H-16	0.98 s	1.07 s
H-17	1.15 s	1.07 s
H-18	1.19 s	1.74 s
H-19	1.93 s	1.97 s
H-20	1.97 s	1.97 s
H-2′α	1.81 dd (14, 5.5)	1.81 dd (14, 5.5)
H-2′β	1.66 dd (14, 7)	1.66 dd (14, 7)
H-3′	4.24 m	4.24 m
H-4′	5.64 br. s	5.64 br s
H-7′	5.63 d (15.5)	5.63 d (15.5)
H-8′	6.38 d (15.5)	6.38 d (15.5)
H-10′	6.22 d (11.5)	6.22 d (11.5)
H-11′	6.62 d (15, 11.5)	6.62 d (15, 11.5)
H-12′	6.37 d (15)	6.37 d (15)
H-14′	6.26 m	6.26 m
H-15′	6.64 m	6.64 m
H-16′	0.94 s	0.94 s
H-17′	1.02 s	1.02 s
H-18′	1.68 br. s	1.68 br s
H-19′	1.92 s	1.92 s
H-20′	1.97 s	1.97 s

[a] Chemical shifts were determined at 500 MHz. [b] Chemical shifts were determined at 300 MHz. [c] AB system.

A new carotenoid **2**, named deepoxysalmoxanthin, exhibited a molecular ion peak at *m*/*z* 584.4235 in HREIMS, compatible with the formula $C_{40}H_{56}O_3$ and showed absorption maxima at 421 (sh), 444, and 472 nm (Et_2O). The structure of **2** for β,ϵ-carotene-3,3′,6′-triol (3′,6′-*cis* type) was established by ^{1}H NMR data including ^{1}H−^{1}H decoupling and ^{1}H−^{1}H NOE difference spectra. The ^{1}H NMR data for

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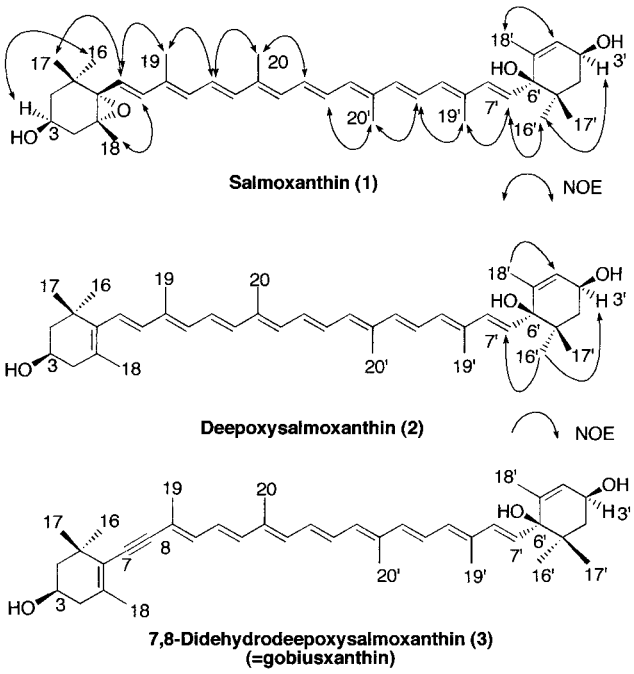

Figure 1. Structures and some key NOE correlations of salmoxanthin (**1**), deepoxysalmoxanthin (**2**), and 7,8-didehydrodeepoxysalmoxanthin (=gobiusxanthin) (**3**).

the partial structures of H-2 to H-20 and H-2′ to H-20′ in **2** were completely in accordance with the corresponding data from zeaxanthin (H-2 to H-20)[4,6] and salmoxanthin (H-2′ to H-20′) (Table 1). Furthermore, the relative stereochemistry of the 3′,6′-dihydroxy-ϵ end group was confirmed by NOE experiments as shown in Figure 1.

Another minor carotenoid **3**, named 7,8-didehydrodeepoxysalmoxanthin, exhibited a molecular ion peak at *m*/*z* 582.4080 in HREIMS, compatible with the formula $C_{40}H_{54}O_3$ and showed absorption maxima at 420, 445, and 475 nm (Et_2O). EIMS data suggested that **3** was a didehydro derivatives of **2**. The ^1H NMR data of **3** indicated the presence of the partial structure of alloxanthin (H-2 to H-20)[4,7] and salmoxanthin (H-2′ to H-20′). Therefore the structure of **3** was identical with gobiusxanthin, 7,8-didehydro-β,ϵ-carotene-3,3′,6′-triol, which was first isolated in nature from the common freshwater goby *Rhinogobius brunneus*.[8]

The absolute configurations of **1**, **2**, and **3** were deduced by CD spectral data. The CD spectrum of **2** was quite similar to that of lutein D [(3*R*,3′*S*,6′*S*)-β,ϵ-carotene-3,3′-diol],[9,10] having the same chromophore as **2** (Figure 2). This indicated that **2** has the same chirality at C-3, C-3′, and C-6′ as lutein D. Therefore the absolute configuration of **2** was deduced to be 3*R*, 3′*S*, 6′*R*.

Salmoxanthin **1** has two end groups, one is a 3-hydroxy-5,6-epoxy end group and the other a 3,6-dihydroxy-ϵ end group. From the results of deepoxysalmoxanthin described above, it is assumed that substitution at C-6′ (OH-6′) has no influence on both the sign of the Cotton effects and the general shape of the spectrum. Therefore, according to the additivity rule of CD spectra,[11] the CD spectrum of **1** corresponded to that of the calculated CD of 1/2 (3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*S*)-violaxanthin[11,12] (having a 3-hydroxy-5,6-epoxy end group) and 1/2 (3*S*,6*S*,3′*S*,6′*S*)-tunaxanthin (tunaxanthin A)[11,13] (having a 3-hydroxy-ϵ end group), as shown in Figure 3. From the results described above, it seemed that **1** has the same chiralities at C-3, C-5, and C-6 as (3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*S*)-violaxanthin, and at C-3′ and

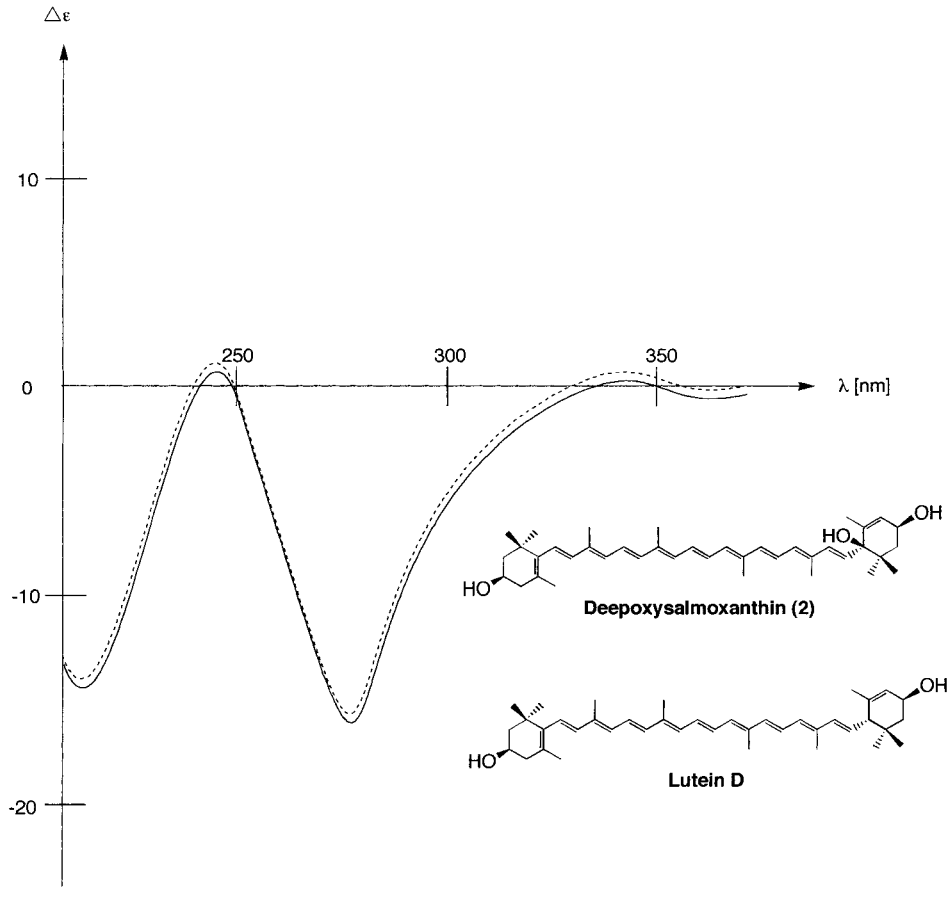

Figure 2. CD spectra of deepoxysalmoxanthin (**2**) (—) and lutein D (···) (in EPA) at 20 °C.

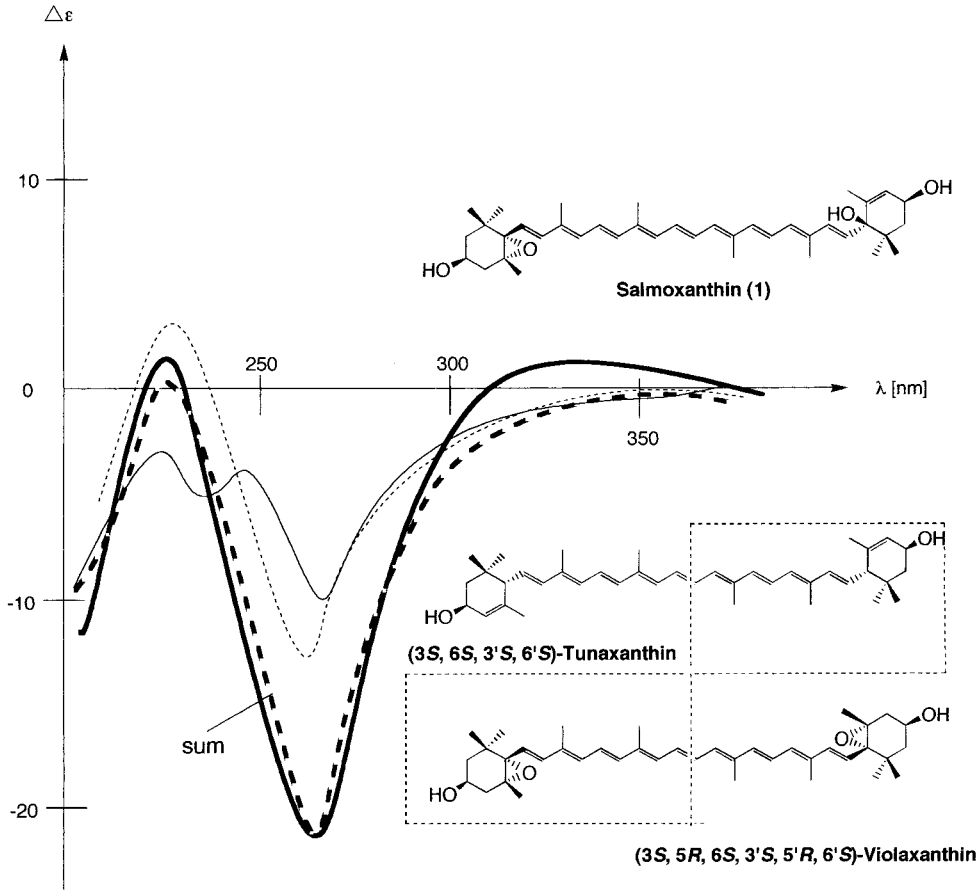

Figure 3. CD spectra of (3*S*,6*S*,3′*S*,6′*S*)-tunaxanthin (1/2 intensity) (—), (3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*S*)-violaxanthin (1/2 intensity) (···), sum of both spectra (– – –), and spectrum of salmoxanthin (**1**) (bold line) (in EPA) at 20 °C.

C-6′ as tunaxanthin A. Therefore the absolute configuration of **1** was postulated to be 3*S*, 5*R*, 6*S*, 3′*S*, 6′*R*.

In a manner similar to that described above, the absolute configuration of **3** was postulated to be 3*R*, 3′*S*, 6′*R*. The CD spectrum of **3** was in good agreement with that calculated spectra of 1/2 (3*R*,3′*R*)-alloxanthin and 1/2 tunaxanthin A. Furthermore, the CD spectrum of **3** was quite similar to that of gobiusxanthin.[8]

Experimental Section

General Experimental Procedures. Visible spectra were recorded on a Shimadzu UV-240 spectrophotometer. Concentrations were calculated using $E^{1\%}_{1cm} = 2500$ at λ_{max} (in Et_2O). EIMS were recorded on a JEOL GCMATE (BU20) mass spectrometer with a direct inlet system with ionization energy of 70 eV. CD spectra were recorded on a Jasco J-500 C spectropolarimeter in EPA [Et_2O−isopentane−EtOH (5:5:2)] solution at 20 °C. The ^{1}H NMR spectra were measured with a Varian XL-300 (300 MHz) and a Varian UNITY INOVA 500 (500 MHz) instrument in $CDCl_3$ with TMS as internal standard. All two-dimensional spectra were recorded with a Varian UNITY INOVA 500 without spinning. DQF-COSY and NOESY were acquired using the standard Varian pulse programs. HPLC was performed on a Shimadzu LC-6AD instrument supplied with a Shimadzu SPD-6AV spectrometer set at 450 nm. The column used was a normal-phase (Cosmosil 5SL, 250 × 8.0 mm i.d.) with a mobile phase of *n*-hexane−Me_2CO−EtOAc (5:2:1).

Animal Materials. Dog salmon (chum salmon) *Oncorhynchus keta* (5 specimens, 9.0 kg) were caught at Kuzuryu River, Fukui Prefecture, in September 1995.

Extraction and Isolation of Carotenoids. The Me_2CO extract of the integuments (850 g) of *O. keta* was partitioned between *n*-hexane−Et_2O (1:1) and aqueous NaCl. The organic layer was dried over Na_2SO_4 and was evaporated to dryness. The residue was saponified with 10% KOH/MeOH at 37 °C for 12 h, then unsaponifiable matters were extracted with *n*-hexane−Et_2O (1:1) and washed with water. The extract solution was dried over Na_2SO_4, then concentrated to dryness. The residue was subjected to column chromatography on Si gel using an increasing percentage of Me_2CO in *n*-hexane. Compound **1** (2.0 mg, 31% of total carotenoids) and compound **2** (0.2 mg, 3%) were eluted with Me_2CO−*n*-hexane (3:7) from a Si gel column and were further purified by HPLC on Si gel with *n*-hexane−Me_2CO−EtOAc (5:2:1). Compound **3** (50 μg, 0.8%) was eluted with Me_2CO−*n*-hexane (2:7) from a Si gel column and was further purified by HPLC on Si gel with *n*-hexane−Me_2CO−EtOAc (5:2:1).

The following additional carotenoids were identified from *O. keta*: β-carotene (0.5% of the total carotenoid), β-echinenone (0.6%), canthaxanthin (0.4%), β-cryptoxanthin (0.7%), (3*S*,5*R*,6*S*,3′*R*)- and (3*S*,5*S*,6*R*,3′*R*)-antheraxanthin (10.0%),[8] zeaxanthin (24.6%), (3*S*,4*R*,3′*R*)- and (3*S*,4*S*,3′*R*)-β,β-carotene-3,4,3′-triol (2.0%),[14] (3*S*,4*R*,3′*S*,4′*R*)-β,β-carotene-3,4,3′,4′-tetrol (1.0%),[14] astaxanthin (obtained as astacene, 20.0%), and 4-ketozeaxanthin (obtained as α-doradecin, 5.0%). The ratio of the stereoisomers of zeaxanthin, analyzed by chiral HPLC, were as follows: 3*R*,3′*R*:3*R*,3′*S*:3*S*,3′*S* (7:1:2).

Salmoxanthin (1): UV−vis λ_{max} (Et_2O) 416, 440, and 470 nm; CD see Figure 3; ^{1}H NMR ($CDCl_3$, 500 MHz) see Table 1; NOESY correlations, CH_3-16 to H-2α, CH_3-16 to H-3, CH_3-17 to H-7, CH_3-17 to H-2β, CH_3-18 to H-4β, CH_3-18 to H-8, H-3 to H-2α, H-3 to H-4α, CH_3-19 to H-7, CH_3-19 to H-11, CH_3-20 to H-11, CH_3-20 to H-15, CH_3-16′ to H-2′α, CH_3-16′ to H-3′, CH_3-16′ to H-7′, CH_3-17′ to H-2′β, CH_3-18′ to H-4′, H-3′ to H-2′α, CH_3-19′ to H-7′, CH_3-19′ to H-11′, CH_3-20′ to H-11′, and CH_3-20′ to H-15′; EIMS (70 eV) *m*/*z* 600 $[M]^+$ (53), and 582 $[M^+ - 18]$ (10); HREIMS *m*/*z* 600.4183 (calcd for $C_{40}H_{56}O_4$, 600.4178).

Deepoxysalmoxanthin (2): UV−vis λ_{max} (Et_2O) 420 (sh),

444, and 473 nm; CD see Figure 2; ^{1}H NMR ($CDCl_3$, 300 MHz) see Table 1; NOE correlations (by NOE difference spectra), CH_3-16′ to H-2′α, CH_3-16′ to H-3′, CH_3-16′ to H-7′, CH_3-18′ to H-4′; EIMS (70 eV) *m*/*z* 584 $[M]^+$ (45), and 566 $[M^+ - 18]$ (5); HREIMS *m*/*z* 584.4235 (calcd for $C_{40}H_{56}O_3$, 584.4230).

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