2,3′-Dihydroxycanthaxanthin, a New Carotenoid with a 2-Hydroxy-4-oxo-β-end Group from the Hermit Crab, *Paralithodes brevipes*

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A new carotenoid with a 2-hydroxy-4-oxo-β-end group was isolated from the hermit crab, *Paralithodes brevipes*, as a minor component. Its structure was determined to be 2,3′-dihydroxy-β,β-carotene-4,4′-dione (1) by spectral data and the compound was named 2,3′-dihydroxycanthaxanthin. Chiral resolution of 1 by HPLC using a chiral column provided two stereoisomers, 1a and 1b. The 3′*R* and 3′*S* chirality were determined for 1a and 1b, respectively, by CD spectra.

Key words crab; *Paralithodes brevipes*; carotenoid; 2,3′-dihydroxycanthaxanthin

Marine animals contain various carotenoids of structural variety,[1] some of which exhibit antioxidative,[2] anti-tumor, and anti-carcinogenic activities.[3—5] This prompted us to search for new carotenoids from marine animals such as crabs.

Hermit crabs, *Paralithodes brevipes* (Hanasakigani in Japanese), inhabit the coast of north Hokkaido, the Sea of Okhotsk and the Bering Sea, and are one of the most important species of edible crabs in Hokkaido. Concerning the carotenoids of *P. brevipes*, in 1976, Harashima *et al.*[6] reported the isolation of papilioerythrinone and astaxanthin from the carapace. In 1988, Matsuno and Maoka[7] reported the detailed carotenoid composition, including the stereochemistry of astaxanthin and related carotenoids in *P. brevipes*, and determined the absolute configuration of papilioerythrinone. They also reported the presence of an unidentified polar xanthophyll in *P. brevipes*. In the course of our carotenoid study in marine animals, we recently isolated this unidentified polar xanthophyll from the carapace of *P. brevipes* and determined its structure using spectroscopic data. This paper reports the isolation and structural elucidation of this new carotenoid (**1**).

The Me_2CO extract of the carapace of *P. brevipes* was chromatographed on silica gel using an increasing percentage of Et_2O in hexane and Me_2CO. The fraction eluted with Me_2CO was subjected to HPLC on silica gel with Me_2CO–hexane (6 : 4) and then on ODS with $CHCl_3$–MeCN (1 : 9) to yield **1** (0.8 mg).

Compound **1** showed absorption maximum at 470 nm in Et_2O, suggesting the presence of an astaxanthin-type chromophore.[8] The molecular formula of **1** was determined to be $C_{40}H_{52}O_4$ by HR-FAB-MS. Positive ion FAB-MS/MS showed the characteristic products ions at *m/z* 578 $[M-H_2O]^{+\cdot}$, 504 $[M-92]^{+\cdot}$, 443, and 377 which were almost the same as for astaxanthin (**2**). The ^{1}H- and ^{13}C-NMR data, assigned by 2D NMR experiments, are shown in Experimental. The presence of two secondary hydroxy groups in **1** was revealed by two oxymethins signals at δ 3.90 (H-2) and 4.33 (H-3′) and two hydroxy proton signals at 3.65 (OH-2) and 3.68 (OH-3′). Furthermore, the presence of two carbonyl groups in **1** was confirmed by two carbon signals at δ 198.1 (C-4) and 200.4 (C-4′). The ^{1}H-NMR data for **1** indicated the presence of a 2-hydroxy-4-oxo-β-end group,[9,10] 3-hydroxy-4-oxo-β-end group,[11] and a polyene part.[11] The ^{1}H–^{1}H connectivities of H-2 to H-3, H-2′ to H-3′, and olefinic protons were confirmed by COSY experiments. The all-*E* geometry of the polyene part was confirmed by NOESY data. Therefore, the structure of **1** was determined to be 2,3′-dihydroxy-β,β-carotene-4,4′-dione and the compound was named 2,3′-dihydroxycanthaxanthin. The ^{13}C-NMR of **1**, which was assigned by HSQC and HMBC experiments, was in agreement with this structure. This compound has two chiral centers at C-2 and C-3′. In general, hydroxy-keto-carotenoids such as astaxanthin (**2**) in crustaceans are presented as mixtures of optical isomers.[1,12,13] Therefore, it was assumed that **1** also was presented as a mixture of optical isomers. Chiral resolution of **1** by HPLC using a chiral column, Sumichiral OA-2000,[13] provided two stereoisomers, **1a** and **1b**, which showed mirror image CD spectra. Compound **1a** showed the same signs of Cotton effects in the CD spectrum as (3*R*,3*R*′)-astaxanthin.[14] On the other hand, **1b** showed the same signs of Cotton effects as (3*S*,3*S*′)-astaxanthin.[14] It has been reported that carotenoid having a 3-hydroxy-4-oxo-β-end group exhibits strong Cotton effects in the CD spectrum,[14] while carotenoid having a 2-hydroxy-4-oxo-β-end group has a very weak cotton effect in the CD spectrum.[9,10] It was therefore assumed that the CD spectrum of **1a** and **1b** reflected the chirality at C-3′; thus, the chirality of **1a** and **1b** was suggested to be 3′*R* and 3′*S*, respectively. Nevertheless, the chirality at C-2 in **1a** and **1b** could not be determined because of the small available amount of sample available. It was assumed that both compounds **1a** and **1b** were presented as a mixture of 2*R* and 2*S* optical isomers, because it was reported that carotenoids possessing a 2-hydroxy-β-end group, such as β-caroten-2-ol (**3**) and 2-hydroxy-echinenone (**4**) in crustaceans presented as a mixture of 2*R* and 2*S* optical isomers.[15,16]

The structure of 2,3′-dihydroxy-β,β-carotene-4,4′-dione corresponded to the proposed structure of tilefishxanthin III, isolated from the integuments of red tilefish *Branchiostegus japonicus*, by Asahara *et al.*[17] However, the structure of tilefishxanthin III was postulated by chromatographic and visible spectral properties only.[17] Subsequently, Tsushima and Matsuno revised the structure of tilefishxanthin III to 3,3′-dihydroxy-β,ε-caroten-4-one using modern spectral analysis.[18]

In general, animals do not synthesize carotenoids *de novo* and those found in animals are either directly accumulated

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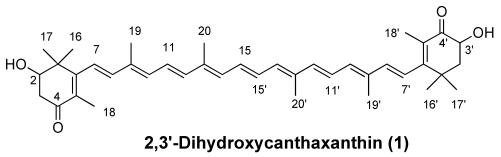

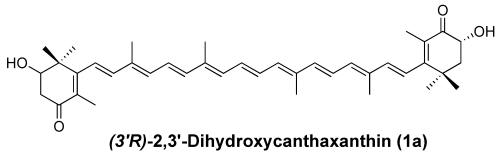

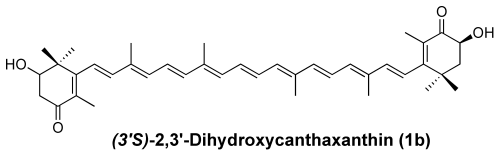

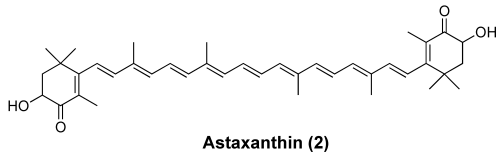

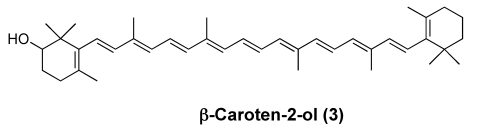

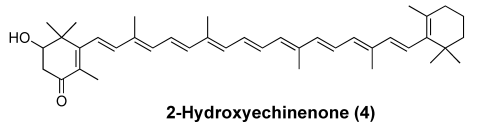

from food or partly modified through metabolic reactions.[19] It was reported that the food sources of hermit crabs are algae, small shellfish and small crustaceans such as cladoceran and isopod.[20] It seems probable that 2,3′-dihydroxycanthaxanthin (**1**) is an oxidative metabolite of *β*-caroten-2-ol (**3**) and/or 2-hydroxy-echinenone (**4**), originating from dietary cladoceran[9] and isopod[16,21] in the food chain.

Experimental

General Experimental Procedures The CD spectra were recorded in Et_2O at room temperature with a JASCO J-720 WI spectropolarimeter. The UV–vis spectra were recorded with a Shimadzu U-2001 spectrophotometer in Et_2O. The ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) spectra were measured in 0.2 ml of $CDCl_3$ solution using a SHIGEMI tube™ (Shigemi Co., Ltd., Tokyo, Japan) with a Varian UNITY *INOVA* 500 spectrometer in $CDCl_3$ with TMS as an internal standard. The positive ion FAB-MS and FAB-MS/MS spectra were recorded using a JEOL JMS-HX/HX 110A four-sector tandem mass spectrometer with *m*-nirobenzyl alcohol (*m*-NBA) as a matrix according to the method previously described.[22] HPLC was performed on a Shimadzu LC-6AD with a Shimadzu SPD-6AV spectrophotometer set at 450 nm.

Animal Material The hermit crab, *P. brevipes*, was purchased at a local fish market in Hokkaido, December.

Extraction and Isolation of Carotenoids The Me_2CO extract of the carapace of *P. brevipes* (1500 g) was partitioned between Et_2O and aqueous NaCl. The organic layer was dried over Na_2SO_4 and then concentrated to dryness. The residue was subjected to silica gel column chromatography using an increasing percentage of Et_2O in *n*-hexane and Me_2CO. The fraction eluted with Me_2CO was subjected to a series of HPLCs on silica gel with Me_2CO–*n*-hexane (6 : 4) and then on ODS with $CHCl_3$–MeCN (1 : 9) to yield **1** (0.8 mg).

In the present investigation, the following known carotenoids were also isolated and identified; *β*-carotene, echinenone, canthaxanthin, adonirubin, adonixanthin, papilioerythrinone, fritschiellaxanthin, astaxanthin, 7,8-didehydroastaxanthin, 7,8,7′,8′-tetradehydroastaxanthin, isocryptoxanthin, lutein, zeaxanthin, diatoxanthin, and alloxanthin.

2,3′-Dihydroxycanthaxanthin (**1**): Reddish solid. UV–vis λ_{max} (Et_2O) 470 nm. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.21 (6H, s, H-17, 17′), 1.25 (3H, s, H-16), 1.32 (3H, s, H-16′), 1.81 (1H, t, J=14 Hz, H-2′$_{ax}$), 1.89 (3H, s, H-18), 1.95 (3H, s, H-18′), 1.99 (9H, s, H-19, 20, 20′), 2.00 (3H, s, H-19′), 2.16 (1H, dd, J=14, 6 Hz, H-2′$_{eq}$), 2.62 (1H, dd, J=17, 9 Hz, H-3$_{ax}$), 2.80 (1H, dd, J=17, 4 Hz, H-3$_{eq}$), 3.68 (1H, d, J=1.8 Hz, OH-3′), 3.90 (1H, dd, J=9, 4 Hz, H-2), 4.33 (1H, dd, J=14, 6 Hz, H-3′), 6.22 (1H, d, J=16 Hz, H-7′), 6.23 (1H, d, J=16 Hz, H-7), 6.30 (4H, overlapped, H-10, 10′, 14, 14′), 6.37 (1H, d, J=16 Hz, H-8), 6.43 (1H, d, J=16 Hz, H-8′), 6.44 (1H, d, J=15.5 Hz, H-12), 6.45 (1H, d, J=15.5 Hz, H-12′), 6.66 (2H, dd, J=15.5, 11.5 Hz, H-11, 11′), 6.68 (2H, m, H-15, 15′). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 12.6 (C-19, 19′), 12.8 (C-20, 20′); 13.9 (C-18′), 14.1 (C-18), 26.1 (C-16′), 27.2 (C-16), 29.4 (C-17), 30.7 (C-17′), 32.7 (C-1), 37.0 (C-1′), 42.6 (C-3), 45.3 (C-2′), 69.2 (C-3′), 74.2 (C-2), 123.2 (C-7′), 123.6 (C-7), 124.5 (C-11, 11′), 126.7 (C-5′), 130.2 (C-5), 130.7 (C-15, 15′), 133.7 (C-14), 133.4 (C-14′), 134.5 (C-9), 134.7 (C-9′), 135.2 (C-10′), 135.4 (C-10), 136.6 (C-13), 136.8 (C-13′), 139.5 (C-12), 139.7 (C-12′), 141.7 (C-8), 142.4 (C-8′), 162.0 (C-6), 162.3 (C-6′), 198.1 (C-4), 200.4 (C-4′). Key NOESY correlations H-16/H-2, H-3$_{eq}$ and H-7, H-17/H-3$_{ax}$ and H-7, H-18/H-8, H-2/H-3$_{eq}$, H-19/H-7 and H-11, H-20/H-11 and H-15, H-16′/H-2′$_{eq}$, H-3′ and H-7′, H-17′/H-2′$_{ax}$ and H-7′, H-2′$_{eq}$/H-3′, H-18′/H-8′, H-19′/H-7′ and H-11′, H-20′/H-11′ and H-15′. Key HMBC correlations H-16/C-1, C-2 and C-6, H-17/C-1, C-2 and C-6, H-18/C-4, C-5 and C-6, H-19/C-8, C-9 and C-19, H-20/C-12, C-13 and C-14, H-16′/C-1′, C-2′ and C-6′, H-17′/C-1′, C-2′ and C-6′, H-18′/C-4′, C-5′ and C-6′, H-2′/C-3′, H-19′/C-8′, C-9′ and C-19′, H-20′/C-12′, C-13′ and C-14′. HR-FAB-MS *m/z* 596.3871 ($C_{40}H_{52}O_4$, Calcd for 596.3865). FAB-MS/MS *m/z* 578 $[M-H_2O]^+$, 504 $[M-92]^+$, 443, and 377.

Chiral Resolution of **1**: Chiral HPLC was performed by Sumichiral OA-2000 (300×8 ID) with *n*-hexane–$CHCl_3$–EtOH (48 : 16 : 4) at a flow rate of 2.0 ml/min. The ratio of stereoisomers of **1a** : **1b** were 7 : 3.

(3′*R*)-2,3′-Dihydroxycanthaxanthin (**1a**): Reddish solid. Retention time (t_R) 50.7 min. CD (Et_2O) λ nm ($\Delta\varepsilon$) 226 (−4.0), 230 (0), 246 (5.6), 253 (0), 272 (−6.0), 282 (0), 315 (12.0), 346 (0), 375 (−2.5).

(3′*S*)-2,3′-Dihydroxycanthaxanthin (**1b**): Reddish solid. t_R 52.5 min; CD (Et_2O) λ nm ($\Delta\varepsilon$) 226 (4.0), 230 (0), 246 (−5.6), 253 (0), 272 (6.0), 282 (0), 315 (−12.0), 346 (0), 375 (2.5).

Acknowledgments We whish to thank Dr. H.Yasui, Kyoto Pharmaceutical University for measurement of CD spectra.

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