2,3′-Dihydroxy-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-carotene-4,4′-dione (1) by spectral data and the compound was named 2,3′-dihydroxy-4-oxo-β-end group. This compound was presented as a mixture of optical isomers, 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′-dihydroxy-4-oxo-β-end group

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

Hermit crabs, *Paralithodes brevipes* (Hanasaki-gi 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. reported the isolation of papilioerythrinone and astaxanthin from the carapace. In 1988, Matsuno and Maoka 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 Me2CO extract of the carapace of *P. brevipes* was chromatographed on silica gel using an increasing percentage of Et2O in hexane and Me2CO. The fraction eluted with Me2CO was subjected to HPLC on silica gel with Me2CO–Et2O, suggesting the presence of an astaxanthin-type carotenoid. By this method, we isolated and determined its structure using spectroscopic data.

### Key words
- crab
- *Paralithodes brevipes*
- carotenoid
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The Me2CO extract of the carapace of *P. brevipes* was chromatographed on silica gel using an increasing percentage of Et2O in hexane and Me2CO. The fraction eluted with Me2CO was subjected to HPLC on silica gel with Me2CO–hexane (6:4) and then on ODS with CHCl3–MeCN (1:9) to yield 1 (0.8 mg).

**Compound 1** showed absorption maximum at 470 nm in EtOH, suggesting the presence of an astaxanthin-type chromophore. The molecular formula of 1 was determined to be C39H50O6 by HR-FAB-MS. Positive ion FAB-MS/MS showed the characteristic product ions at m/z 578 [M+H]+, 504 [M−92]+, 443, and 377 which were almost the same as for astaxanthin (2). The 1H- and 13C-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). Furthermore, the presence of two hydroxy proton signals at 3.65 (OH-2) and 3.68 (OH-3). The 1H-NMR data for 1 indicated the presence of a 2-hydroxy-4-oxo-β-end group, 3-hydroxy-4-oxo-β-end group, and a polyene part. The 1H−H connectivities of H-2 to H-3, H-2′ to H-3′, and olefinic protons were confirmed by COSY experiments. The all-δ geometry of the polynene part was confirmed by NOESY data. Therefore, the structure of 1 was determined to be 2,3′-dihydroxy-β-carotene-4,4′-dione and the compound was named 2,3′-dihydroxy-4-oxo-β-end group. It has been reported that carotenoid having a 3-hydroxy-4-oxo-β-end group has a very weak Cotton effect in the CD spectrum. 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, 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 (3R,3′S)-astaxanthin. On the other hand, 1b showed the same signs of Cotton effects as (3S,3′S)-astaxanthin. It has been reported that carotenoid having a 3-hydroxy-4-oxo-β-end group exhibits strong Cotton effects in the CD spectrum, while carotenoid having a 2-hydroxy-4-oxo-β-end group has a very weak cotton effect in the CD spectrum. 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 2R and 2S optical isomers, because it was reported that carotenoids possessing a 2-hydroxy-β-end group, such as β-carotene-2-ol (3) and 2-hydroxy-echinenone (4) in crustaceans presented as a mixture of 2R and 2S optical isomers. The structure of 2,3′-dihydroxy-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. However, the structure of tilefishxanthin III was postulated by chromatographic and visible spectral properties only. Subsequently, Tsushima and Matsuno revised the structure of tilefishxanthin III to 3,3′-dihydroxy-β,β-caroten-4-one using modern spectral analysis. In general, animals do not synthesize carotenoids de novo and those found in animals are either directly accumulated
It was reported that the food sources of hermit crabs are algae, small shellfish and small crustaceans such as cladoceran and isopod. It seems probable that 2,3'-dihydroxycarotenaxanthin (1) is an oxidative metabolite of β-caroten-2-ol (3) and/or 2-hydroxy-echinenone (4), originating from dietary cladoceran and isopod in the food chain.

**Experimental**

**General Experimental Procedures** The CD spectra were recorded in EtOH at room temperature with a JASCO J-720 W spectropolarimeter. The UV–vis spectra were recorded with a Shimadzu U-2001 spectrophotometer in EtO. The 1H- (500 MHz) and 13C-NMR (125 MHz) spectra were measured in 0.2 ml of CDCl3 solution using a SHIGEMI tubeTM (Shigemi Co., Ltd., Tokyo, Japan) with a Varian UNITY 500 spectrometer in CDCl3 with TMS as an internal standard. The positive ion FAB-MS and HR-FAB-MS spectra were recorded using a JEOL JMS-HX/HX 110A four-sector tandem mass spectrometer with FAB-MS/MS spectra were recorded using a JEOL JMS-700 spectrometer set at 450 nm. 1H-NMR (CDCl3, 500 MHz) and 13C-NMR (CDCl3, 125 MHz) were measured using a JEOL JNM-LA 500 spectrometer. 

**Extraction and Isolation of Carotenoids** The Me2CO extract of the carapace of *P. brevipes* (1500 g) was partitioned between Et2O and aqueous NaCl. The organic layer was dried over Na2SO4 and then concentrated to dryness. The residue was subjected to silica gel column chromatography using an increasing percentage of Et2O in n-hexane and Me2CO. The fraction eluted with Me2CO was subjected to a series of HPLCs on silica gel with Me2CO–n-hexane (6:4) and then on ODS with CHCl3–MeCN (1:9) to yield 1 (0.8 mg).

2,3'-Dihydroxycarotenaxanthin (1): Reddish solid. UV–vis λmax (EtOH) 470 nm. 1H-NMR (CDCl3, 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 = 14Hz, H-2'), 1.89 (3H, s, H-18), 1.95 (3H, s, H-18'), 1.99 (4H, s, H-19, 20, 20'), 2.00 (3H, s, H-19'), 2.16 (1H, dd, J = 14, 6 Hz, H-2'), 2.62 (1H, dd, J = 17, 9 Hz, H-3'), 2.80 (1H, dd, J = 17, 4 Hz, H-3'), 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'), 6.62 (2H, dd, J = 15.5, 11.5 Hz, H-11, 11'), 6.68 (2H, m, H-15, 15'). 13C-NMR (CDCl3, 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-17), 37.0 (C-1'), 42.6 (C-3), 45.3 (C-2'), 62.9 (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), 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, 3-3', 3-7, H-7, 17-3-3', 7-7', H-18/H-8, H-2/H-3, 3-19', 19'-19', H-11, 20/H-11 and H-15, H-16/H-2', 3-3', H-3' and 7', H-3'/H-2', H-2'/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, C-6, H-17/C-1, C-4, C-5, C-6, 18/C-4, C-5 and C-6, 19/C-4, C-5 and C-6, 20/C-4, 20/C-5, H-2/C-3, C-9/C-19 and C-19, 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-19/C-8', C-9' and C-19', H-20/C-12', C-13' and C-14'. HR-FAB-MS m/z 596.3871 (C40H52O4, Calcd for 596.3865). FAB-MS/MS m/z 578 [M–H2O]±, 504 [M–2H]±, 443, and 377.

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**References**