A NOVEL PURPLE CAROTENOID, RHODOBACTERIOXANTHIN, FROM *RHODOBACTER CAPSULATUS*

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The structure of a novel purple carotenoid, rhodobacterioxanthin (**1**), isolated from *Rhodobacter capusulatus* was determined to be 13-*cis*-1,1'-dimethoxy-3,4,3',4'-tetradehydro-1,2,1',2'-tetrahydro-φ,φ-caroten-20-al. Compound **1** showed a strong antioxidant effect on lipid peroxidation induced by a free radical and a singlet oxygen.

KEY WORDS *Rhodobacter capsulatus*; rhodobacterioxanthin; carotenoid; antioxidant activity

In the course of our studies on bacterial carotenoids,[1] we isolated a novel purple carotenoid, rhodobacterioxanthin (**1**), from the purple photosynthetic bacterium *Rhodobacter capsulatus*. This paper reports the isolation and structural elucidation of **1** and also deals with the antioxidant activities on lipid peroxidation of **1** and its related carotenoids.

R. capsulatus was cultured in a YPS medium under illumination by daylight fluorescent light at 30℃ for 48 h. The cells (60 g wet weight) were harvested by centrifugation and extracted with acetone. Carotenoids were extracted with hexane-ether (1:1) from the acetone solution by the addition of water. After evaporation of the solvent, the purple-red residue was subjected successively to column chromatography on silica gel and preparative HPLC on ODS[2] to yield **1** (2.0 mg) along with lycopene (20 mg), rhodopin (25 mg), and 2,3-didehydrorhodopin (20 mg).[3]

Rhodobacterioxanthin in ether showed absorption maxima at 331, 389, and 514 nm. High-resolution EI-MS gave a molecular ion peak at m/z 610.4438 compatible with the formula $C_{42}H_{58}O_3$ (calcd. 610.4385). ^{1}H-NMR (Table 1) indicated the presence of nine methyl groups, two methoxy groups, four methylene protons, twenty olefinic protons, and one aldehyde proton in **1**. DQF-COSY and decoupling experiments revealed the following proton-proton connectivities: H-2 to H-4, H-18 to H-8, H-19 to H-12, H-20 to H-12, H-14 to H-20', H-19' to H-12', H-18' to H-8', and H-2' to H-4'. The w-letter long-range coupling between the aldehyde proton at δ 9.56 and H-12 indicated that the aldehyde group was located at C-20.[4] Moreover, the positions of the remaining methyl group at δ 1.16 and the methoxy group at δ 3.24 were confirmed by NOE experiments. NOEs between δ 1.16 [CH_3-16, 17 (16', 17')] to δ 3.24 (OCH_3), and δ 2.33 [H-2(2')] were consistent with the partial structure of the 1-methoxy-1,2-dihydro-φ-carotene moiety.[5] The stereostructure of **1** was determined

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by NOE difference spectra. The NOE between the aldehyde proton (H-20) and H-14 proved to be a Δ-13 *cis* configuration.[4] From the experimental results described above, the structure of **1** was established as 13-*cis*-1,1'-dimethoxy-3,4,3',4'-tetradehydro-1,2,1',2'-tetrahydro-φ,φ- caroten -20-al, as shown in Fig. 1. Generally, carotenoids appear yellow, orange, and red, but **1** is purple. This is the first example of a naturally occurring purple carotenoid.

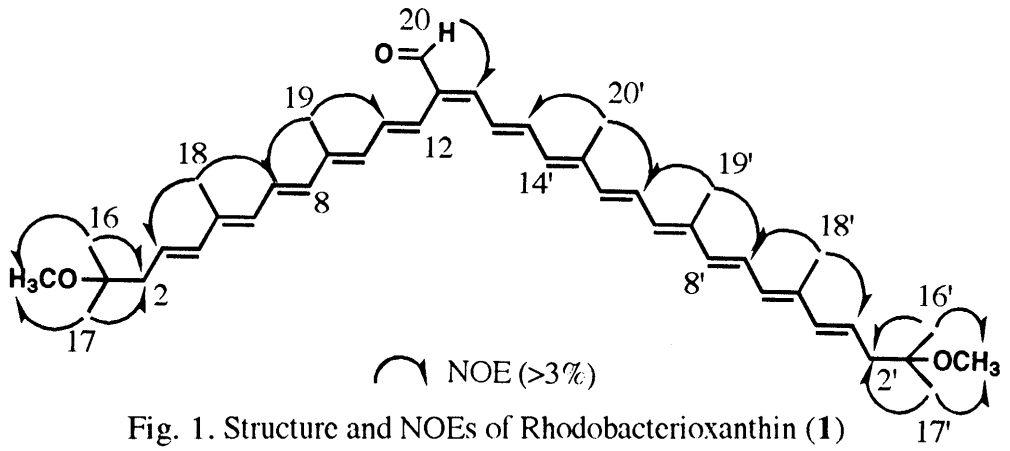

Fig. 1. Structure and NOEs of Rhodobacterioxanthin (**1**)

Table 1. ^{1}H-NMR (δ ppm in $CDCl_3$, 300 MHz) Data for **1**

H-2	2.33	(2H, d, 7.5)[a]	H-2'	2.33	(2H, d, 7.5)
H-3	5.75	(1H, d, t, 15.0, 7.5)	H-3'	5.75	(1H, d, t, 15.0, 7.5)
H-4	6.17	(1H, d, 15.0)	H-4'	6.17	(1H, d, 15.0)
H-6	6.13	(1H, d, 11.5)	H-6'	6.13	(1H, d, 11.5)
H-7	6.68	(1H, d, d, 15.0, 11.5)	H-7'	6.68	(1H, d, d, 15.0, 11.5)
H-8	6.37	(1H, d, 15.0)	H-8'	6.37	(1H, d, 15.0)
H-10	6.22	(1H, d, 11.5)	H-10'	6.26	(1H, d, 11.5)
H-11	7.79	(1H, dd, 15.0, 11.5)	H-11'	6.82	(1H, dd, 15.0, 11.5)
H-12	6.55	(1H, dd, 15.0, 2.0)	H-12'	6.42	(1H, d, 15.0)
H-14	6.82	(1H, d, 12.0)	H-14'	6.35	(1H, d, 12.0)
H-15	6.89	(1H, t-like, 12.0, 12.0)	H-15'	7.09	(1H, t-like, 12.0, 12.0)
H-16	1.16	(3H, s)	H-16'	1.16	(3H, s)
H-17	1.16	(3H, s)	H-17'	1.16	(3H, s)
H-18	1.94	(3H, s)	H-18'	1.94	(3H, s)
H-19	2.04	(3H, s)	H-19'	2.02	(3H, s)
H-20	9.56	(1H, d, 2.0)	H-20'	2.06	(3H, s)
OCH_3	3.24	(3H, s)	OCH_3	3.24	(3H, s)

[a] The coupling constants (J) in parentheses are shown in Hz. s: singlet; d: doublet; t: triplet.

The antioxidant activities on lipid peroxidation induced by 2,2'-azobis(2,4-dimethylvaleronitrile) (AMVN)[6] and the singlet oxygen generated by the photochemical reaction with methylene blue[6] of **1** were examined by the methyl linolate peroxidation method[7]. Rhodobacterioxanthin (**1**) showed

almost the same inhibitory effect on lipid peroxidation induced by a free radical and a singlet oxygen as lycopene, which is known to be the most efficent carotenoid for singlet oxygen quenching,[8] as shown in Fig. 2.

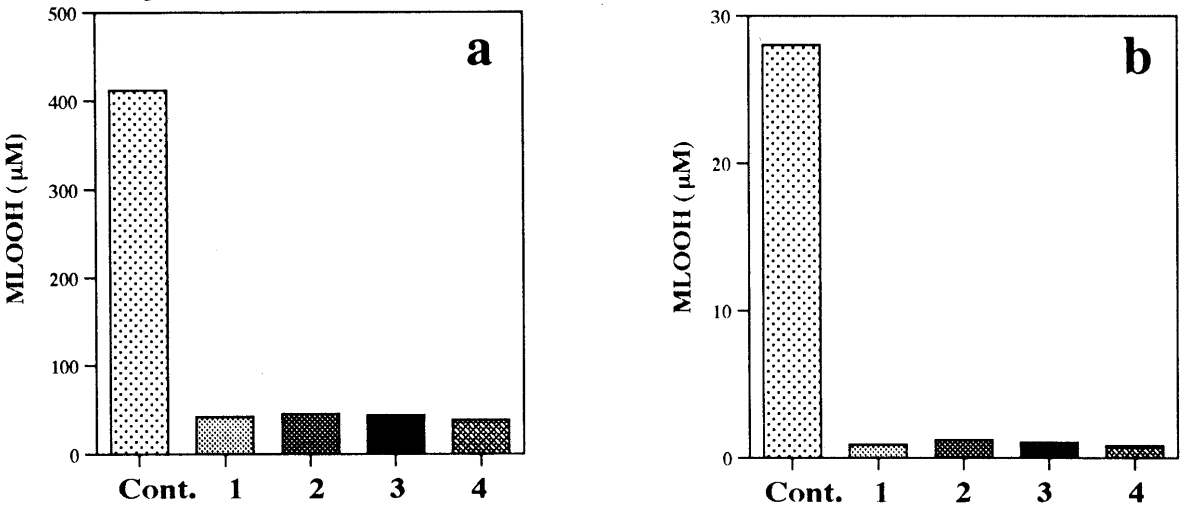

Fig 2. Inhibitory Effects on Lipid Peroxidation Induced by (a) AMVN[6] and (b) Singlet Oxygen[6]
Cont: control; 1: rhodobacterioxanthin; 2: lycopene; 3: rhodopin; 4: 2,3-didehydrorhodopin.

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2) Compound **1** was eluted with hexane-ether (6:4) from a silica gel 60 (Merck) column and was further purified with preparative HPLC on a Shim-pack ODS (15 μm, 20 mm i.d. X 250 mm) using CH_3CN:CH_2Cl_2 (8:2).

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6) Antioxidant assays. (a) AMVN oxidation: 0.1 ml of 2 mM of each carotenoid in EtOH (final conc: 167 μM) was added to 1 ml of 0.1 M of methyl linolate (ML) in hexane-2-propanol (1:1, v/v). Oxidation was then initiated by adding 0.1 ml of 100 mM of AMVN in hexane and the mixture wa incubated under continuous shaking under air at 37℃ for 60 min. The oxidation products, methyl linolate hydroperoxides (MLOOH), were determined by HPLC. (b) Singlet oxygen oxidation: 0.1 ml of 2 mM of each carotenoid in EtOH (final conc: 49 μM) was added to 2 ml of 0.1 M of ML in hexane-2-propanol (1:1, v/v). After addition of 2 ml of 0.1 mM of methylene blue in EtOH, the solution was illuminated by a 27 W daylight lamp at 37℃ for 60 min. MLOOHs were determined by HPLC. The HPLC conditions and procedures for analysis of MLOOHs were described in by Terao.[7]

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