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MONOCYCLIC CROSS-CONJUGATED CAROTENAL FROM AN AEROBIC PHOTOSYNTHETIC BACTERIUM, *ERYTHROBACTER LONGUS*

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Key Word Index—*Erythrobacter longus*, aerobic photosynthetic bacterium, cross-conjugated carotenal, monocyclic carotenoid, (3*R*)-9′-*cis*-3-hydroxy-1′-methoxy-3′,4′-didehydro-1′,2′-dihydro-β,ψ-caroten-19′-al; (3*R*)-1′-methoxy-3′,4′-didehydro-1′,2′-dihydro-β,ψ-caroten-3-ol

Abstract—There were more than 10 kinds of carotenoids in a strictly aerobic photosynthetic bacterium, *Erythrobacter longus* OCh 101. Two novel monocyclic carotenoids among them were isolated and purified. From spectroscopic and chemical evidence, their structures were determined as (3*R*)-9′-*cis*-3-hydroxy-1′-methoxy-3′,4′-didehydro-1′,2′-dihydro-β,ψ-caroten-19′-al and (3*R*)-1′-methoxy-3′,4′-didehydro-1′,2′-dihydro-β,ψ-caroten-3-ol. The cross-conjugated aldehyde group and the tertiary methoxy group have hitherto been confined to the carotenoids of anaerobic photosynthetic bacteria, while the 3-hydroxy-β-ionone group has rarely been found in the carotenoids of anaerobic photosynthetic bacteria.

INTRODUCTION

Bacteria of *Erythrobacter* species synthesize photosynthetic apparatus including bacteriochlorophyll *a* and several carotenoids under highly aerobic conditions, but they can not grow anaerobically even in the light in contrast to typical photosynthetic bacteria [1, 2]. Therefore, their phylogenetic relationship to the anaerobic photosynthetic bacteria is of great interest.

The carotenoids in aerobically grown cells of *Erythrobacter* sp. OCh 114 have been identified [3]. Spheroidenone is the major carotenoid. Small amounts of 2,2′-diketospirilloxanthin and OH-spheroidenone are also found. These keto carotenoids have also been encountered in some species of *Rhodobacter* and *Rhodocyclus*. On the other hand, the carotenoids of *Erythrobacter longus* OCh 101 seem to be quite different from those of *Erythrobacter* sp. OCh 114. Therefore, determination of the structures of the carotenoids is significant for studies on the phylogenesis of *Erythrobacter* species.

In this paper, we report the structures of two novel carotenoids from *E. longus*; a monocyclic cross-conjugated carotenal (**1**) and a monocyclic carotenoid (**3**).

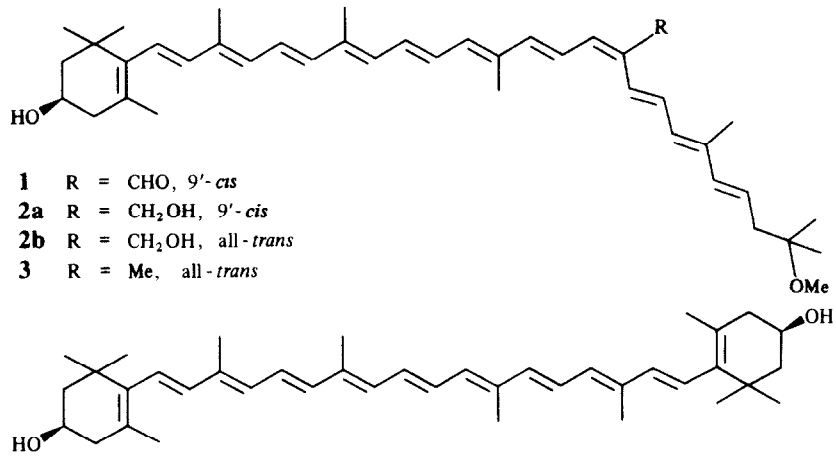
RESULTS AND DISCUSSION

The absorption spectrum of **1** showed only one broad peak at around 510 nm and a very small *cis* peak at around 361 nm in methanol (Fig. 1). Compound **1** was reduced rapidly with sodium borohydride to yield **2** with an accompanying large hypsochromic shift of about 45 nm. These results indicated the presence of a cross-conjugated aldehyde group in **1**, which was confirmed as shown below. The absorption peaks of the major reduction product (**2a**) were at 464 and 493 nm. During illumination of a solution of **2a**, its absorption spectrum was changed gradually. The absorption peaks of the major isomerized product (**2b**) were at 469 and 498 nm (Fig. 1). The value (12) for $\%D_B/D_{II}$ [4] in the absorption spectrum of **2b** was smaller than that for **2a** ($\%D_B/D_{II} = 21$). These results indicated that **2a** and **2b** were the *cis*- and the *trans*-forms, respectively. Consequently, compound **1** was concluded to be the *cis*-form. The *cis*-bond did not seem to be located around the center of the conjugated double bonds, because the *cis*-peaks of **1** and **2a** were very small.

The absorption spectrum of **3** showed absorption peaks at 468 and 499 nm which were similar to those of **2b**. The absorption peaks indicated that the number of conjugated double bonds was 11 in both **2b** and **3**. Furthermore, both were estimated to be monocyclic carotenoids from the values of %III/II [4] which were 35 and 44 for **2b** and **3**, respectively.

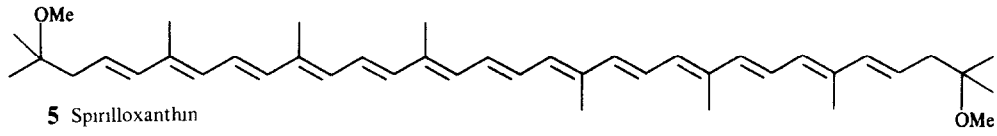
The FDMS revealed a M_r of 596 (compatible with $C_{41}H_{56}O_3$) for **1**. One carbonyl group was present, as shown by the fact that the M_r increased by 2 mass units in the reduction products (**2**). The presence of one primary or secondary hydroxyl group was indicated by the formation of monoacetyl and monosilyl derivatives. In addition, if **1** was assumed to be a C_{40}-skeletal carotenoid, the difference between the molecular weight and the functional groups suggested that **1** contained one methoxy group. The M_r of **3** was 582 (compatible with $C_{41}H_{58}O_2$). In a similar way, the presence of one primary or secondary hydroxyl group and one methoxy group in **3** was indicated.

Assignments of the ^{1}H NMR spectra of these carotenoids were made by comparison with those of zeaxanthin [(3*R*,3′*R*)-β,β-carotene-3,3′-diol, **4**] and spirilloxanthin [1,1′-dimethoxy-3,4,3′,4′-tetrahydro-ψ,ψ-carotene, **5**] from *Chromatium vinosum* (Table 1). The spectrum of **3** indicated that its two end groups were different. One end



1 R = CHO, 9′-*cis*
2a R = CH_2OH, 9′-*cis*
2b R = CH_2OH, all-*trans*
3 R = Me, all-*trans*

4 Zeaxanthin



5 Spirilloxanthin

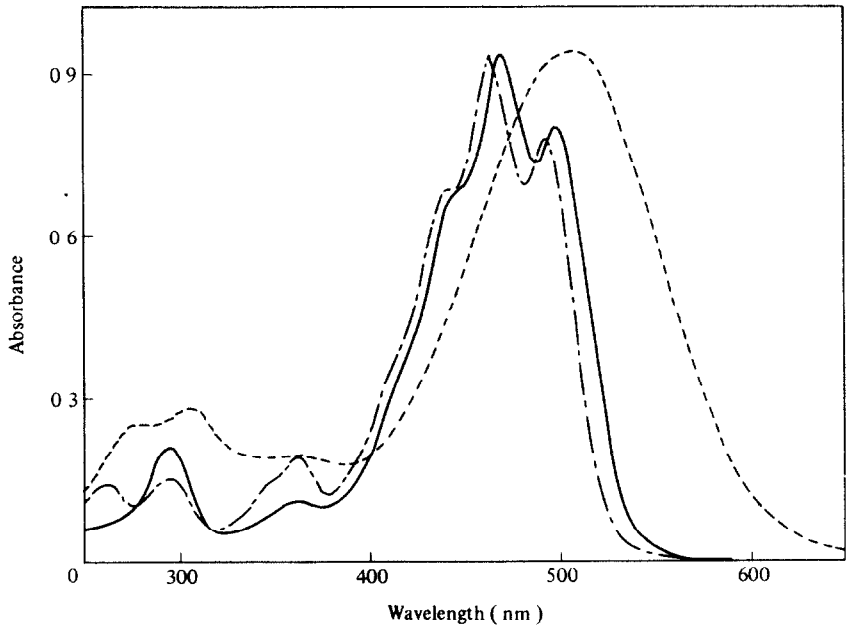


Fig 1 Absorption spectra of **1** (-----), **2a** (— · —) and **2b** (——) in methanol measured immediately after elution from the HPLC system equipped with a photodiodo array detector

group of **3** (Table 1, unprimed number) was identical with that of zeaxanthin **(4)**, and the other end group (Table 1, primed number) was also identical with that of spirilloxanthin **(5)** One end group of both **1** and **2** (Table 1, unprimed number) was also identical with that of zeaxanthin **(4)**, but the other end group (Table 1, primed number) was different in part from that of spirilloxanthin **(5)**. In **2**, the signal for an in-chain methyl group at C-19′ or C-20′ disappeared, and the new signal due to a primary hydroxyl group appeared at δ 4 42 [5] In **1**, one of the in-chain methyl groups disappeared, and the new signal at δ9.54 (*d*, $J = 1.9$ Hz) indicated the presence of an aldehyde group and the *cis*-configuration [5–7]. The in-chain methyl group at C-18′ was shifted to a lower field,

Table 1. ^{1}H NMR spectra of **1**, its reduction products (**2**) and **3** from *E. longus*, zeaxanthin (**4**), and spirilloxanthin (**5**) from *C vinosum* dissolved in $CDCl_3$ (chemical shifts, δ)

Protons	1	2	3	4*	5†
H_3-16	1.07 *s*	1.08 *s*	1.07 *s*	(1.07 *s*)	
H_3-17	1.07 *s*	1.08 *s*	1.07 *s*	(1.07 *s*)	
H_3-18	1.74 *s*	1.74 *s*	1.74 *s*	(1.74 *s*)	
H_3-19	1.98 *s*	1.97 *s*	1.97 *s*	(1.97 *s*)	
H_3-20	1.98 *s*	1.98 *s*	1.98 *s*	(1.97 *s*)	
H-2, ax	1.47 *t*	1.48 *t*	1.48 *t*	(1.48)	
H-2, eq	1.77 *m*	1.78 *m*	1.78 *m*	(1.77)	
H-4, ax	2.05 *m*	2.05 *dd*	2.05 *m*	(2.04)	
H-4, eq	2.38 *m*	2.39 *dd*	2.39 *dd*	(2.39)	
H-3, ax	3.98 *m*	4.00 *m*	3.99 *m*	(4.00 *m*)	
HO-3	1.36 *d* 4.8 Hz	1.36 *d* 4.9 Hz	1.36 *d* 4.8 Hz	(1.34 *d*) (5 Hz)	
H_3-16′	1.16 *s*	1.16 *s*	1.16 *s*		1.16 *s* (1.13 *s*)
H_3-17′	1.16 *s*	1.16 *s*	1.16 *s*		1.16 *s* (1.13 *s*)
H_3-18′	2.01 *s*	1.94 *s*	1.93 *s*		1.93 *s* (1.90 *s*)
H_3-19′			1.97 *s*		1.98 *s* (1.96 *s*)
H_3-20′	2.03 *s*	1.99 *s*	1.98 *s*		1.99 *s* (1.96 *s*)
H_2-2′	2.34 *d* 7.6 Hz	2.33 *d* 7.4 Hz	2.34 *d* 7.3 Hz		2.32 *d* (2.31 *d*) 7.4 Hz (6.5 Hz)
Me-O-1′	3.24 *s*	3.24 *s*	3.23 *s*		3.23 *s* (3.21 *s*)
H-19′, CHO	9.54 *d* 1.9 Hz				
H_2-19′, CH_2OH		4.42 *d* 6.0 Hz			

*Parentheses from [20].
†Parentheses from [6]

but the methyl group at C-20 was not shifted. It was concluded that in **1**, the in-chain methyl group at C-19′ was replaced by an aldehyde group, and the signals of both neighbouring in-chain methyl groups at C-18′ and C-20′ were shifted to a lower field.

The EI mass spectrum of **1** showed characteristic peaks due to the two end groups. The reduced products (**2**) showed peaks corresponding to the elimination of a primary hydroxyl group at *m/z*: 582 $[M-O]^+$ and 562 $[M-2H_2O]^+$ in addition to the peaks due to the end groups. In-chain elimination reactions of carotenoids are known to lead to the formation of [M − 92] (toluene) and [M − 106] (*m*-xylene) ions [8]. The intensity of both ions from **1** was low, and that of the [M − 106] (benzaldehyde) ion was also low, whereas that of the [M − 120] (*m*-tolualdehyde) ion was considerably higher. Similarly, the intensity of the [M − 108] (benzyl alcohol) ion as well as the [M − 92] and the [M − 106] ions from **2** was low, whereas that of the [M − 122] (*m*-methylbenzyl alcohol) ion was considerably higher. The presence of the [M − 120] or the [M − 122] ion indicated that the position of the substitution was not at C-19 nor at C-20. Low intensity of the [M − 106] (*m*-xylene) ion indicated that the position of substitution was not at C-18′ nor at C-20′. Therefore, the position of the aldehyde group of **1** was indicated to be at C-19′.

The CD spectrum of **3** (Fig. 2) was almost compatible with that of all-*trans* rubixanthin [(3*R*)-β,ψ-caroten-3-ol] [9, 10]. The bathochromic displacement of about 10 nm in the CD spectrum of **3** compared to all-*trans* rubixanthin was observed, which was consistent with a similar shift in their absorption spectra (λ_{max} in methanol 293 nm for **3** and 282 nm for all-*trans* rubixanthin). Therefore, the hydroxyl group at C-3 of **3** was indicated to have the same absolute configuration (3*R*) as that of rubixanthin. Similarly, the CD spectrum of **1** (Fig. 2) was almost compatible with that of *cis*-isomers of rubixanthin [9, 10] suggesting that the hydroxyl group at C-3 of **1** had the same absolute configuration (3*R*) as that of *cis*-isomers of rubixanthin. The absolute configuration was unchanged during the reduction, since the CD spectrum of **2a** was compatible with that of **1** (Fig. 2).

The absorption, the ^{1}H NMR and the CD spectra of **1** indicated the *cis*-configuration of this carotenoid. Substitution of the in-chain methyl group for the aldehyde group is known to cause preference for *cis*-configuration of the neighbouring double bond [5, 7, 11]. Therefore, **1** was concluded to have the 9′-*cis*-configuration.

From the spectroscopic and chemical evidence, compound **1** was concluded to be a monocyclic carotenoid, which took a *cis*-configuration and had a cross-conjugated aldehyde group and a tertiary methoxy group.

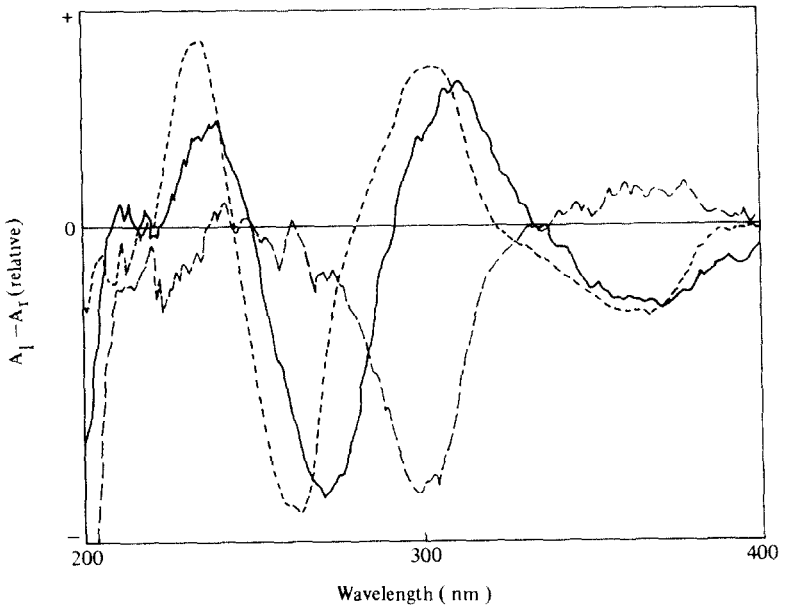


Fig. 2 CD spectra of **1** (- ——), **2a** (-----) and **3** (——) in EPA

The structure thus determined was (3*R*)-9′-*cis*-3-hydroxy-1′-methoxy-3′,4′-didehydro-1′,2′-dihydro-β,ψ-caroten-19′-al Similarly, that of **3** was also determined to be (3*R*)-1′-methoxy-3′,4′-didehydro-1′,2′-dihydro-β,ψ-caroten-3-ol

The presence of tertiary methoxy group has hitherto been confined to the carotenoids of Rhodospirillaceae, Chromatiaceae and Chlorobiaceae [12–14] The cross-conjugated aldehyde group has been found only in lycopenal, rhodopinal and their derivatives from Rhodospirillaceae and Chromatiaceae [12–14] However, these carotenals are acyclic and the position of the aldehyde group is at C-20 instead of C-19′ in the carotenal **(1)** of *E. longus*

Monocyclic carotenoids have been found in Chlorobiaceae and Chloroflexaceae, but rarely in Rhodospirillaceae or Chromatiaceae [12, 14] In *Rhodomicrobium vannielii*, the presence of 1′-methoxy-3′,4′-didehydro-1′,2′-dihydro-β,ψ-carotene was indicated, but the structure was determined only by the mass spectrum [15] Also in *Thiocystis gelatinosa*, the presence of 1′-methoxy-1′,2′-dihydro-β,ψ-caroten-4-one was indicated only by the mass and the IR spectra [16]

The (3*R*)-3-hydroxy-β-ionone group has been found in β-cryptoxanthin [(3*R*)-β,β-caroten-3-ol] and zeaxanthin **(4)**, which are widely distributed in green plants. However, this group has rarely been found in the carotenoids of anaerobic photosynthetic bacteria [14, 17]. The presence of β-cryptoxanthine was reported in *R vannielii* based on the mass spectrum [15] On the other hand, carotenoids containing the 3-hydroxy-β-ionone group have been found in some species of aerobic bacteria [14] Zeaxanthin **(4)** is the major carotenoid of *Flavobacterium* strains R1519 and R1560 [18] *Corynebacterium autotrophicum* contains zeaxanthin **(4)** and its rhamnosides [19]

In conclusion, both **1** and **3** are novel monocyclic carotenoids. Monocyclic carotenoids have rarely been found in anaerobic photosynthetic bacteria On the other hand, both carotenoids also contain characteristic groups of the carotenoids in anaerobic photosynthetic bacteria The structures of **1** and **3** indicate that **3** is a precursor of **1** Determination of the structures of other carotenoids in *E longus* is under way

EXPERIMENTAL

Biological material. Erythrobacter longus OCh 101 (IFO No 14126) was a gift from Dr T Shiba (Otsuchi Marine Research Center, Ocean Research Institute, The University of Tokyo) Culture conditions of the bacterium have been described previously [2]

Isolation Carotenoids were extracted from wet cells with $CHCl_3$–MeOH (1 2), evapd, and dissolved in $CHCl_3$ More than 10 peaks due to carotenoids were detected by an HPLC system described below The carotenoid mixture was submitted to silica gel 60 (Merck) column chromatography and eluted with $CHCl_3$ Polar carotenoids and bacteriochlorophyll *a* remained on the column The $CHCl_3$ fraction was evapd, dissolved in *n*-hexane, and again submitted to silica gel 60 column chromatography eluted successively with *n*-hexane and $CHCl_3$ Non-polar carotenoids were eluted with *n*-hexane An orange carotenoid **(3)** was eluted with *n*-hexane–$CHCl_3$ (3 2), and then a purple carotenoid **(1)** was eluted with *n*-hexane–$CHCl_3$ (2 3) Purification by CC was repeated once more Each carotenoid thus obtained gave a single peak on the HPLC system, and a single spot on silica gel high-performance TLC (Merck) developed with CH_2Cl_2–EtOAc (3 1)

Compound **1** was reduced with $NaBH_4$, and a major product **(2a)** was purified by silica gel high-performance TLC Illumination of a solution of **2a** was performed by a fluorescence lamp for a few hours, and the major product **(2b)** could be separated from **2a** only by the HPLC system

Spectral analysis Absorption spectra were obtained by a continuous monitoring HPLC system equipped with a photodiode array detector, MCPD-350 PC System II (Otsuka Electronics, 230–800 nm, 1 4 nm resolution, 1 sec interval) The HPLC system consisted of a pump 6000A and an injector UK6 (Waters) A prepacked column of a Radial-PAK μ Bondapak C_{18} cartridge (100 × 8 mm) installed in a Z-MODULE™ radial

compression separation system was used analytically using MeOH as a solvent (2.0 ml/min). Molecular ions of the carotenoids and their derivatives were measured by an FDMS using carbon emitter at about 20 mA. Fragmentation spectra were measured by an EIMS using an in-beam pipette. The temperature of the inlet port was 190° and that of the ion source was 185°. The ionization voltage was 20 eV. ^{1}H NMR spectra were measured by a Bruker WM400 spectrometer (400 MHz) in $CDCl_3$ at 25°. CD spectra were measured by a JASCO J-500 spectrometer in EPA soln (Et_2O–*i*-pentane–EtOH, 5·5·2) at 25°.

(3R)-9′-*cis*-3-*Hydroxy*-1′-*methoxy*-3′,4′-*didehydro*-1′,2′-*dihydro*-β,ψ-*caroten*-19′-*al* **(1).** The absorption spectrum showed λ_{max}^{MeOH} nm: 510, 361, 306, 280, $\%D_B/D_{II} = 21$ [4] (Fig. 1). The CD spectrum in EPA is shown in Fig 2. FDMS *m/z* 596 $[M]^+$ ($C_{41}H_{56}O_3$); EIMS *m/z* (rel int.): 596 $[M]^+$ (77), 578 $[M-H_2O]^+$ (33), 564 $[M-HOMe]^+$ (13), 523 $[M-C(Me)_2OMe]^+$ (7), 504 $[M-92]^+$ (4), 490 $[M-106]^+$ (4), 476 $[M-120]^+$ (100), 424 $[M-172]^+$ (10). Acetylation gave a monoacetyl derivative (FDMS *m/z*: 638 $[M]^+$). Trimethylsilylation gave a monosilyl derivative (FDMS *m/z*. 668 $[M]^+$). The ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) is shown in Table 1.

(3R)-1′-*Methoxy*-3′,4′-*didehydro*-1′,2′-*dihydro*-β,ψ-*caroten*-3,19′-*diol* **(2).** The *cis*-form **(2a)** had λ_{max}^{MeOH} nm. 493, 464, (440), 363, 293, 261, %III/II = 37, $\%D_B/D_{II} = 21$, and the *trans*-form **(2b)** had λ_{max}^{MeOH} nm 498, 469, (440), 363, 294, %III/II = 35, $\%D_B/D_{II} = 12$ (Fig 1). The CD spectrum is shown in Fig 2. FDMS *m/z* 598 $[M]^+$ ($C_{41}H_{58}O_3$); EIMS *m/z* (rel int.). 598 $[M]^+$ (43), 582 $[M-O]^+$ (9), 580 $[M-H_2O]^+$ (31), 566 $[M-HOMe]^+$ (7), 562 $[M-2H_2O]^+$ (24), 525 $[M-C(Me)_2OMe]^+$ (3), 506 $[M-92]^+$ (5), 492 $[M-106]^+$ (3), 490 $[M-108]^+$ (5), 476 $[M-122]^+$ (100), 424 $[M-174]^+$ (5). Acetylation gave a diacetyl derivative (FDMS *m/z* 682 $[M]^+$). Trimethylsilylation gave a disilyl derivative (FDMS *m/z*: 742 $[M]^+$). The ^{1}H NMR spectrum is shown in Table 1.

(3R)-1′-*Methoxy*-3′,4′-*didehydro*-1′,2′-*dihydro*-β,ψ-*caroten*-3-*ol* **(3).** The absorption spectrum showed λ_{max}^{MeOH} nm: 499, 468, (440), 361, 293, %III/II = 46, $\%D_B/D_{II} = 17$. The CD spectrum is shown in Fig. 2. FDMS *m/z* 582 $[M]^+$ ($C_{41}H_{58}O_2$). Acetylation gave a monoacetyl derivative (FDMS *m/z*: 624 $[M]^+$). Trimethylsilylation gave a monosilyl derivative (FDMS *m/z* 654 $[M]^+$). The ^{1}H NMR spectrum is shown in Table 1.

1,1′-*Dimethoxy*-3,4,3′,4′-*tetrahydro*-ψ,ψ-*carotene* **(5).** Spirilloxanthin **(5)** was purified from *C vinosum*. The absorption spectrum showed λ_{max}^{MeOH} nm 524, 491, 466, 384, 315, %III/II = 44, $\%D_B/D_{II} = 15$. FDMS *m/z*. 596 $[M]^+$ ($C_{42}H_{60}O_2$). Trimethylsilylation gave no silyl derivative (FDMS *m/z*: 596 $[M]^+$). The ^{1}H NMR spectrum is shown in Table 1.

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