Structures of Carotenoids with 5,6-Dihydro-β-End Groups from the Spindle Shell *Fusinus perplexus*

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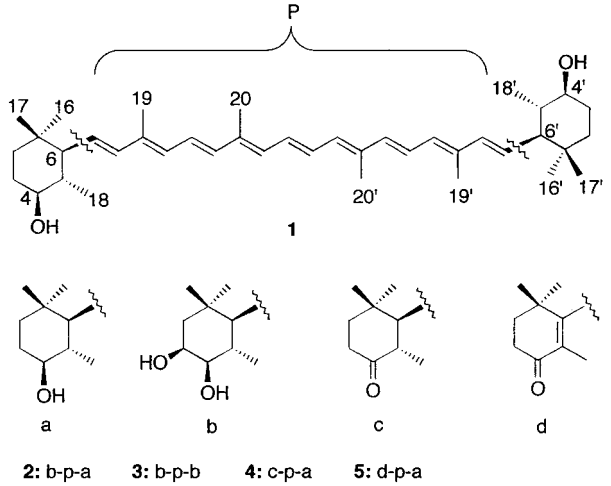
A series of pirardixanthin derivatives, **1**, **2**, **3**, **4**, and **5**, possessing the 5,6-dihydro-β-end group were isolated from the spindle shell *Fusinus perplexus*.[1,2] Their structures and absolute configurations were determined by modern spectroscopic analysis to be (4*S*,5*S*,6*S*,4′*S*,5′*S*,6′*S*)-5,6,5′,6′-tetrahydro-β,β-carotene-4,4′-diol (**1**), (3*S*,4*R*,5S,6*S*,4′*S*,5′*S*,6′*S*)-5,6,5′,6′-tetrahydro-β,β-carotene-3,4,4′-triol (**2**), (3*S*,4*R*,5*S*,6*S*,3′*S*,4′*R*,-5′*S*,6′*S*)-5,6,5′,6′-tetrahydro-β,β-carotene-3,4,3′,4′-tetrol (**3**), (5*S*,6*S*,4′*S*,5′*S*,6′*S*)-4′-hydroxy-5,6,5′,6′-tetrahydro-β,β-caroten-4-one (**4**), and (4′*S*,5′*S*,6′*S*)-4′-hydroxy-5′,6′-dihydro-β,β-caroten-4-one (**5**).

In the course of our comparative biochemical studies of carotenoids in marine animals, the carotenoids of shellfish were investigated. In the previous paper, we reported the isolation of a series of new carotenoids with 5,6-dihydro-β-end groups (pirardixanthin derivatives) from the reddish-orange muscle of the spindle shell *Fusinus perplexus*.[1,2] The present work reports the determination of the absolute configurations of these carotenoids, **1**−**5**.



Results and Discussion

According to the methods previously reported,[1,2] the muscle of *F. perplexus* (1860 g) was extracted with Me_2-CO. Carotenoids were transferred to *n*-hexane−Et2O (1:1) from the Me_2CO solution upon dilution with water. After saponification, the crude carotenoid was subjected successively to chromatography on Si gel and preparative HPLC on Sumichiral OA-2000 to yield **1** (4 mg), **2** (1 mg), **3** (0.4 mg), **4** (0.2 mg), and **5** (0.2 mg).

Compound **1** showed a molecular ion at *m*/*z* 572.4599, compatible with the formula $C_{40}H_{60}O_2$, and exhibited visible absorption maxima at 414, 437, and 467 nm (Et_2O), indicating the presence of an all *E* nonaene chromophore.[3] ^{13}C NMR showed a total of 20 carbon resonances indicating a symmetrical structure. ^1H NMR data for **1** assigned by DQF-COSY and $^1H-^1H$ decoupling experiments are compiled in Table 1. Overlapped ^1H signals around δ 1.40−1.60 were assigned by $^1H-^1H$ decoupling difference spectra. The proton−proton connectivities of the 4-hydroxy-5,6-dihydro-β-end group, H-2 to H-6 and H-5 to H-18, were elucidated by DQF-COSY. The relative 4,5-trans and 5,6-trans configurations were revealed by the vicinal coupling constants $J_{4,5} = 10$ Hz and $J_{5,6} = 9$ Hz. All *E* geometry of the polyene chain was also confirmed by chemical shift and coupling constant values of olefinic protons and the UV−visible spectrum described above. These relative stereochemistries were further confirmed by NOESY experiments, as shown in Figure 1. The NOESY correlations H-17/H-5, H-17/H-7, and H-18/H-4 were compatible with the relative stereochemistry of the 4-hydroxy-5,6-dihydro-β-end group described above. The absolute configuration at C-4 (4′) in **1** was determined to be *S* by the modified Mosher method.[4,5] The $\Delta\delta$ ($=\delta S - \delta R$) values[5] in the 4-hydroxy-5,6-dihydro-β-end group for the MTPA ester of **1** are shown in Figure 2. The positive $\Delta\delta$ values for the hydrogens oriented on the right side of the MTPA plane and the negative $\Delta\delta$ values for the hydrogens located on the left side of the MTPA plane in the 4-hydroxy-5,6-dihydro-β-end group disclosed the *S*-configuration at C-4. The 5*S*(5′*S*),6*S*(6′*S*)-configurations were assigned as for the 4-hydroxy-5,6-dihydro-β-end group described above. Consequently, the structure of **1** was determined to be (4*S*,5*S*,6*S*,4′*S*,5′*S*,6′*S*)-5,6,5′,6′-tetrahydro-β,β-carotene-4,4′-diol.

Compound **2** showed a molecular ion at *m*/*z* 588.4550, compatible with the formula $C_{40}H_{60}O_3$, and showed visible absorption maxima at 414, 437, and 467 nm (Et_2O), indicating the presence of an all *E* nonaene chromophore.[3] The ^1H NMR data for **2** (Table 1) demonstrated two structural parts. One part involved signals (H-2′ to H-20′) that were identical with signals for **1**. The structure of the remaining part (C-1 to C-20) was elucidated by DQF-COSY and $^1H-^1H$ decoupling experiments. The methine signals at δ 4.01 and 3.17 were assigned to H-3 and H-4, respectively. The coupling constant of 3.5 Hz is consistent with the partial structure of a 3,4-cis glycol in a 5,6-dihydro-β-end group.

The 4,5-trans and 5,6-trans configurations were also deduced by vicinal coupling constants of $J_{4,5} = 10$ Hz and $J_{5,6} = 9.5$ Hz. This stereochemistry was also confirmed by NOESY experiments (Figure 2). NOESY correlations for H-17/H-5, H-17/H-7, H-4/H-18, and H-4/H-3 were compatible with the relative stereochemistry of the 3,4-dihydroxy-5,6-dihydro-β-end group described above. The absolute

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Table 1. ^{1}H NMR Data of **1**, **2**, **3**, **4**, and **5** in $CDCl_3$[a]

	1[b]	**2**[b]	**3**[b]	**4**[c]	**5**[c]
H-2 ax	1.30 ddd (14.5, 10, 3.5)	1.43 dd (15, 3)	1.43 dd (15, 3)	n.a.	1.85 t (6.5)
eq	1.56 ddd (14.5, 3.5, 3.5)	1.84 dd (15, 3)	1.84 dd (15, 3)	n.a.	1.85 t (6.5)
H-3 ax	1.50 dddd (13.5, 10, 10, 3.5)	4.01 ddd (3.5, 3, 3)	4.01 ddd (3.5, 3, 3)	n.a.	2.15 t (6.5)
eq	1.79 dddd (13.5, 3.5, 3.5, 3.5)			n.a.	2.15 t (6.5)
H-4	3.14 ddd (10, 10, 3.5)	3.17 dd (10, 3.5)	3.17 dd (10, 3.5)		
H-5	1.41 ddq (10, 9, 6.5)	1.84 ddq (10, 9.5, 6.5)	1.84 ddq (10, 9.5, 6.5)	n.a.	
H-6	1.48 dd (10, 9)	1.53 dd (9.5, 9.5)	1.53 dd (9.5, 9.5)	n.a.	
H-7	5.40 dd (15.5, 9)	5.45 dd (15.5, 9.5)	5.45 dd (15.5, 9.5)	5.45 dd (15.5, 9.5)	6.24 d (15.5)
H-8	6.05 d (15.5)	6.02 d (15.5)	6.02 d (15.5)	6.07 d (15.5)	6.37 d (15.5)
H-10	6.12 d (11)	6.11 d (11)	6.11 d (11)	6.14 d (11)	6.28 d (11)
H-11	6.63 dd (15, 11)	6.62 dd (15, 11)	6.62 dd (15, 11)	6.62 dd (15, 11)	6.66 dd (15, 11)
H-12	6.34 d (15)	6.35 d (15)	6.35 d (15)	6.36 d (15)	6.43 d (15)
H-14	6.24 m*	6.24 m*	6.24 m*	6.24 m*	6.31 m*
H-15	6.63 m*	6.63 m*	6.63 m*	6.63 m*	6.66 m*
H-16	0.82 s	0.81 s	0.81 s	0.91 s	1.20 s
H-17	0.88 s	1.06 s	1.06 s	1.06 or 1.12 s	1.20 s
H-18	0.91 d (6.5)	0.93 d (6.5)	0.93 d (6.5)	0.94 d (6.5)	1.88 s
H-19	1.93 s	1.93 s	1.93 s	1.93 s	2.00 s
H-20	1.97 s	1.97 s	1.97 s	1.97 s	1.99 s
H-2′ax	1.30 ddd (14.5, 10, 3.5)	1.30 ddd (14.5, 10, 3.5)	1.43 dd (15, 3)	1.30 ddd (14.5, 10, 3.5)	1.30 ddd (14.5, 10, 3.5)
eq	1.56 ddd (14.5, 3.5, 3.5)	1.56 ddd (14.5, 3.5, 3.5)	1.84 dd (15, 3)	1.56 ddd (14.5, 3.5, 3.5)	1.56 ddd (14.5, 3.5, 3.5)
H-3′ax	1.50 dddd (13.5, 10, 10, 3.5)	1.50 dddd (13.5, 10, 10, 3.5)	4.01 ddd (3.5, 3, 3)	1.50 dddd (13.5, 10, 10, 3.5)	1.50 dddd (13.5, 10, 10, 3.5)
eq	1.79 dddd (13.5, 3.5, 3.5, 3.5)	1.79 dddd (13.5, 3.5, 3.5, 3.5)	1.79 dddd (13.5, 4.5, 3.5, 3.5)	1.79 dddd (13.5, 3.5, 3.5, 3.5)	1.79 dddd (13.5, 3.5, 3.5, 3.5)
H-4′	3.14 ddd (10, 10, 3.5)	3.14 ddd (10, 10, 3.5)	3.17 dd (10, 3.5)	3.14 ddd (10, 10, 3.5)	3.14 ddd (10, 10, 3.5)
H-5′	1.41 ddq (10, 9, 6.5)	1.41 ddq (10, 9, 6.5)	1.84 ddq (10, 9.5, 6.5)	1.41 ddq (10, 9, 6.5)	1.41 ddq (10, 9, 6.5)
H-6′	1.48 dd (10, 9)	1.48 dd (10, 9)	1.53 dd (9.5, 9.5)	1.48 dd (10, 9)	1.48 dd (10, 9)
H-7′	5.40 dd (15.5, 9)	5.40 dd (15.5, 9)	5.45 dd (15.5, 9.5)	5.40 dd (15.5, 9)	5.40 dd (15.5, 9)
H-8′	6.05 d (15.5)	6.05 d (15.5)	6.02 d (15.5)	6.05 d (15.5)	6.05 d (15.5)
H-10′	6.12 d (11)	6.12 d (11)	6.11 d (11)	6.12 d (11)	6.12 d (11)
H-11′	6.63 dd (15, 11)	6.63 dd (15, 11)	6.62 dd (15, 11)	6.63 dd (15, 11)	6.63 dd (15, 11)
H-12′	6.34 d (15)	6.34 d (15)	6.35 d (15)	6.34 d (15)	6.34 d (15)
H-14′	6.24 m*	6.24 m*	6.24 m*	6.24 m*	6.24 m*
H-15′	6.63 m*	6.63 m*	6.63 m*	6.63 m*	6.63 m*
H-16′	0.82 s	0.82 s	0.81 s	0.82 s	0.82 s
H-17′	0.88 s	0.88 s	1.15 s	0.88 s	0.88 s
H-18′	0.91 d (6.5)	0.91 d (6.5)	0.93 d (6.5)	0.91 d (6.5)	0.91 d (6.5)
H-19′	1.93 s	1.93 s	1.93 s	1.93 s	1.93 s
H-20′	1.97 s	1.97 s	1.97 s	1.97 s	1.97 s

[a] δ mult (J in Hz). [b]Chemical shifts were determined at 500 MHz. [c]Chemical shifts were determined at 300 MHz, * AA′BB'spin system, n.a. not assigned.

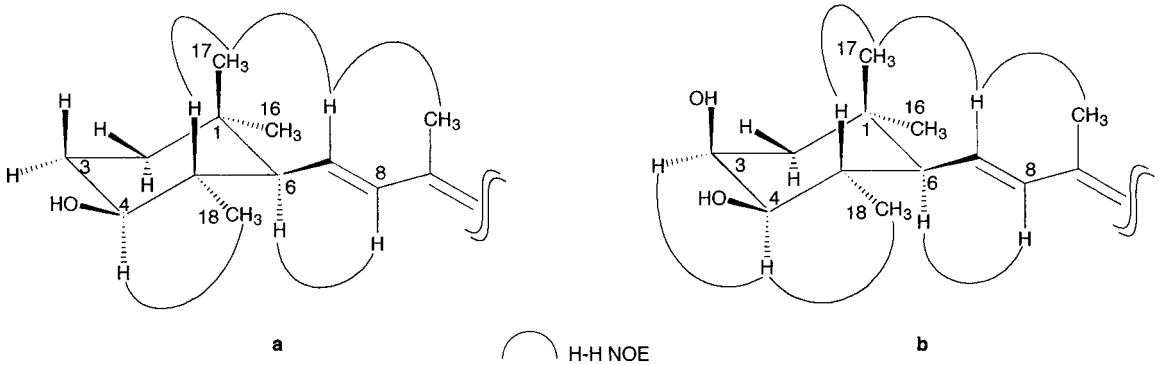

Figure 1. Stereochemistry of the 4-hydroxy-5,6-dihydro-β-end group (a) and the 3,4-dihydroxy-5,6-dihydro-β-end group (b) in **1**, **2**, **3**, **4**, and **5**.

configurations at C-6 and C-6′ of **2** were confirmed by CD spectral data. It is assumed that the CD of carotenoids with saturated β-end groups mainly reflect the chirality of C-6,6′.[6,7] The CD spectrum of **1**, possessing 6*S*,6′*S* chiralities, showed a weak positive Cotton effect at 262 nm. Compound **2** showed a CD spectrum similar to that of **1**, indicating the 6*S*,6′*S* configurations. From these results the structure of **2** was determined as (3*S*,4*R*,5*S*,6*S*,4′*S*,-5′*S*,6′*S*)-5,6,5′,6′-tetrahydro-β,β-carotene-3,4,4′-triol.

Compound **3** showed a molecular ion at *m*/*z* 604.4471, compatible with the formula $C_{40}H_{60}O_4$, and also exhibited visible absorption maxima at 414, 437, and 467 nm (Et_2O), indicating the presence of an all *E* nonaene chromophore.[3] The ^{1}H NMR data (Table 1) indicated a symmetrical structure for **3**. In the same manner as described above, the planar constitution of **3** was determined to be 5,6,5′,6′-tetrahydro-β,β-carotene-3,4,3′,4′-tetrol by DQF-COSY and ^{1}H−^{1}H decoupling experiments. The relative 3,4 (3′,4′)-cis,

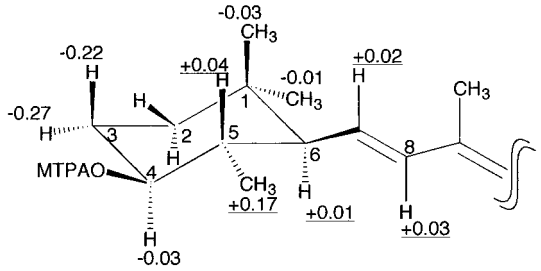

Figure 2. Δδ values (ppm) observed from the (*R*)- and (S)-MTPA ester of **1**.

4,5 (4′,5′)-trans, and 5,6 (5′,6′)-trans configurations were deduced by vicinal coupling constants of H3 (3′), H4 (4′), H5 (5′), and H6 (6′). As for compound **2**, the 6*S*,6′*S* chiralities of **3** were elucidated by CD spectral data. Therefore the structure of **3** was determined to be (3*S*,4*R*,5*S*,6*S*,3′*S*,4′*R*,5′*S*,6′*S*)-5,6,5′,6′-tetrahydro-β,β-carotene-3,4,3′,4′-tetrol.

Compound **4** showed a molecular ion at *m*/*z* 570.4423, compatible with the formula $C_{40}H_{58}O_2$, and also exhibited visible absorption maxima at 414, 437, and 467 nm (Et_2O), indicating the presence of an all *E* nonaene chromophore.[3] The IR spectrum of **4** exhibited a strong absorption at 1714 cm^{-1}, consistent with a saturated carbonyl group. The ^1H NMR data of **4** (Table 1) was compatible with two structural parts. Signals corresponding to H-2′ to H-20′ were completely identical with those of **1**. The structure of the remaining 4-keto-5,6-dihydro-β-end group was characterized by $^1H-^1H$ decoupling experiments. One of the $NaBH_4$ reduction products of **4** showed UV−visible, EIMS, ^1H NMR, and CD spectra identical with those of **1**. Therefore the structure of **4** was elucidated to be (5*S*,6*S*,4′*S*,5′*S*,6′*S*)-4′-hydroxy-5,6,5′,6′-tetrahydro-β,β-caroten-4-one.

Compound **5** showed a molecular ion at *m*/*z* 568.4273, compatible with the formula $C_{40}H_{56}O_2$, and also exhibited visible absorption maxima at 451 and 472 nm (Et_2O). The ^1H NMR data assigned by $^1H-^1H$ decoupling experiments for **5** indicated the presence of the partial structures of canthaxanthin (H-2 to H-20)[8] and compound **1** (H-2′ to H-20′) described above (Table 1). Therefore the structure of **5** was postulated to be 4′-hydroxy-5′,6′-dihydro-β,β-caroten-4-one. The CD spectrum of **5** showed a weak positive Cotton effect at 288 nm, suggesting 6′*S* chirality. Therefore, the structure of **5** was assigned to be (4′*S*,5′*S*,6′*S*)-4′-hydroxy-5′,6′-dihydro-β,β-caroten-4-one.

Compounds **1**, **2**, **3**, **4**, and **5** are all carotenoids possessing a 5,6-dihydro-β-end group. Pirardixanthin derivatives possessing a 5,6-dihydro-β-end group were first isolated as reductive metabolic products of canthaxanthin from the hydra *Hydra pirardi*, which feeds on the crustacean *Artemia salina*, having canthaxanthin as the principal carotenoid.[9] The planar structures of dihydroxypirardixanthin and ketohydroxypirardixanthin were tentatively assumed to be 5,6,5′,6′-tetrahydro-β,β-carotene-4,4′-diol and 4′-hydroxy-5,6,5′,6′-tetrahydro-β,β-caroten-4-one, respectively, based only on UV−vis data, thin-layer chromatography, and chemical reactions ($NaBH_4$ reduction, acid chloroform test, and acetylation).[9] In the present study, compounds **1** and **4** isolated from the spindle shell corresponded to dihydroxypirardixanthin and ketohydroxypirardixanthin isolated from *H. pirardi*, respectively. Compounds **1**, **4**, and **5** are assumed to be reductive metabolites of canthaxanthin,[1,2] and compounds **2** and **3** are assumed to be reductive metabolites of (3*S*)-phoenicoxanthin and (3*S*,3′*S*)-astaxanthin, respectively.[2] On the other hand, the sulfate of **4** has been reported from the brittle star *Ophioderma longicaudum*, but its absolute configulation has not yet been determined.[10]

Experimental Section

General Experimental Procedures. The UV−visible (in Et_2O) and CD [in EPA (Et_2O/isopentane/EtOH, 5:5:2)] spectra were recorded at room temperature with a Shimadzu UV-240 spectrophotometer and a JASCO J-500C spectropolarimeter, respectively. The EIMS spectra were recorded using a JEOL JMS-SX 102A QQ or Hitachi M-80 mass spectrometer. The ^1H NMR spectra were measured with JEOL FX-270 (270 MHz), Varian XL-300 (300 MHz), Varian UNITY INOVA 500 (500 MHz), and Bruker DMX 500 (500 MHz) instruments in $CDCl_3$ or CD_2Cl_2 with TMS as an internal standard. DQF-COSY and NOESY (mixing time 1.3 s) were recorded with a Varian UNITY INOVA 500 (500 MHz) without spinning. The ^{13}C NMR and $^{13}C-^1H$ COSY spectra were measured with a Bruker DMX 500 (125 MHz) instrument in CD_2Cl_2. IR spectra (KBr pellets) were recorded using a Shimadzu FT-IR DR-8000 spectrophotometer. HPLC was performed on a Shimadzu LC-6AD instrument with a Shimadzu SPD-6AV spectrometer set at 450 nm. The column used was a Sumichiral OA-2000 (300 × 8.0 mm i.d.).

Animal Material. *F. perplexus* (460 specimens, 11.4 kg) was purchased at the fish market in Yamaguchi Prefecture, Japan, in April 1995. Voucher specimens are deposited at Kyoto Pharmaceutical University (Voucher No. 951).

Isolation of Compounds 1, 2, 3, 4, and 5. The Me_2CO extract of muscle (1860 g) was partitioned between *n*-hexane−Et_2O (1:1) and aqueous NaCl. The organic layer was dried over Na_2SO_4, then evaporated. The red-colored oil was saponified with 10% KOH−MeOH at 37 °C for 12 h, then the unsaponifiable matter was extracted with *n*-hexane−Et_2O (1:1) and washed with water. The extract was dried over Na_2SO_4, then concentrated to dryness. The residue was subjected to column chromatography on Si gel using increasing amounts of Me_2CO in *n*-hexane. Compounds **1** (4.0 mg), **2** (1.0 mg), and **3** (0.4 mg) were eluted with 20, 30, and 50% Me_2CO−*n*-hexane from a Si gel column, respectively, submitted to further purification by HPLC on a Sumichiral OA-2000 column, and eluted with *n*-hexane−CH_2Cl_2−EtOH, 48:16:0.6, 48:16:1.5, and 48:16:3.0, respectively. Compounds **4** (0.2 mg) and **5** (0.2 mg) were eluted with Me_2CO−*n*-hexane (10:90) and were submitted to further purification by HPLC on a Sumichiral OA-2000 column eluting with *n*-hexane−CH_2Cl_2−EtOH (48:16:0.3).

(4*S*,5*S*,6*S*,4′*S*,5′*S*,6′*S*)-5,6,5′,6′-Tetrahydro-β,β-carotene-4,4′-diol (1): UV−vis λ_{max} (Et_2O) 414, 437, 467 nm (% III/II = 95); CD (EPA) 223 (Δε −2.2), 235 (0), 262 (+6.0), 315 (0) nm; ^1H NMR ($CDCl_3$, 500 MHz) see Table 1; ^{13}C NMR (CD_2Cl_2, 125 MHz) δ 12.9 (C-20, 20′), 13.2 (C-19, 19′), 17.1 (C-18, 18′), 20.7 (C-17, 17′), 31.1 (C-16, 16′), 31.7 (C-3, 3′), 34.2 (C-1, 1′), 39.7 (C-2, 2′), 40.0 (C-5, 5′), 57.4 (C-6, 6′), 76.7 (C-4, 4′), 125.5 (C-11, 11′), 130.4 (C-15, 15′), 130.6 (C-10, 10′), 131.0 (C-7, 7′), 132.7 (C-14, 14′), 137.4 (C-8, 8′), 135.9 (C-9, 9′), 136.9 (C-13, 13′), 137.4 (C-12, 12′); assignments were made from $^{13}C-^1H$ COSY data; EIMS (70 eV) *m*/*z* 572 [M^+] (100), 554 [$M^+ - 18$] (15), 536 [$M^+ - 36$] (15), 480 [$M^+ - 92$] (45), 466 [$M^+ - 106$] (10); HREIMS *m*/*z* 572.4599 (calcd for $C_{40}H_{60}O_2$, 572.4593).

Preparation of the (*R*)- and (*S*)-MTPA Esters of 1. A solution of (+)-MTPA [α-methoxy-α-(trifluoromethyl)phenylacetyl] chloride (20 mg) in anhydrous pyridine (5 mL) was added to a solution of **1** (2.0 mg) in pyridine (5 mL) at 0 °C. After 60 min at 0 °C, *n*-hexane (20 mL) and H_2O were added. The organic phase was washed five to seven times with H_2O, dried, and evaporated. Purification of the residue by preparative TLC (Me_2CO−*n*-hexane, 3:7) gave the pure (*R*)-MTPA ester (0.8 mg) (**1a**). The use of (−)-MTPA chloride in the same procedure led to 0.8 mg of the (*S*)-MTPA ester (**1b**).

Di-(*R*)-MTPA ester of 1 (1a): UV−vis λ_{max} (Et_2O) 414, 437, 467 nm (% III/II = 95); ^1H NMR ($CDCl_3$) δ 0.65 (6H, d, CH_3 18, 18′), 0.84 (6H, s, CH_3 16, 16′), 0.89 (6H, s, CH_3 17, 17′), 1.58 (2H, dd, H6, 6′), 1.69 (2H, ddq, H5, 5′), 1.70 (2H, dddd, H3, 3′ ax), 1.89 (6H, s, CH_3 19, 19′), 1.96 (6H, s, CH_3 20, 20′),

1.99 (2H, dddd, H3, 3′ eq), 4.66 (2H, ddd, H4, 4′), 5.33 (2H, dd, H7, 7′), 6.05 (2H, d, H8, 8′), 6.11 (2H, d, H10, 10′), 6.24 (2H, d, H14, 14′), 6.34 (2H, d, H12, 12′), 6.60 (2H, dd, H11, 11′), 6.62 (2H, dm, H15, 15′).

Di-(*S*)-MTPA ester of 1 (1b): UV−vis λ_{max} (Et_2O) 414, 437, 467 nm (% III/II = 95); ^1H NMR ($CDCl_3$) δ 0.82 (6H, d, CH_3 18, 18′), 0.83 (6H, s, CH_3 16, 16′), 0.86 (6H, s, CH_3 17, 17′), 1.59 (2H, dd, H6, 6′), 1.48 (2H, dddd, H3, 3′ ax), 1.73 (2H, ddq, H5, 5′), 1.90 (6H, s, CH_3 19, 19′), 1.96 (6H, s, CH_3 20, 20′), 1.72 (2H, dddd, H3, 3′ eq), 4.63 (2H, ddd, H4, 4′), 5.35 (2H, dd, H7, 7′), 6.08 (2H, d, H8, 8′), 6.12 (2H, d, H10, 10′), 6.25 (2H, d, H14, 14′), 6.35 (2H, d, H12, 12′), 6.61 (2H, dd, H11, 11′), 6.62 (2H, dm, H15, 15′).

(3*S*,4*R*,5*S*,6*S*,4′*S*,5′*S*,6′*S*)-5,6,5′,6′-Tetrahydro-*β*,*β*-carotene-3,4,4′-triol (2): UV−vis λ_{max} (Et_2O) 414, 437, 467 nm (% III/II = 95); CD (EPA) 223 ($\Delta\epsilon$ −3.1), 240 (0), 262 (+4.7), 300 (0) nm; ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; EIMS (70 eV) *m*/*z* 588 [M^+] (100), 570 [$M^+ - 18$] (16), 552 [$M^+ - 36$] (10), 534 [$M^+ - 54$] (4), 496 [$M^+ - 92$] (30), 482 [$M^+ - 106$] (12); HREIMS *m*/*z* 588.4550 (calcd for $C_{40}H_{60}O_3$, 588.4542).

(3*S*,4*R*,5*S*,6*S*,3′*S*,4′*R*,5′*S*,6′*S*)-5,6,5′,6′-Tetrahydro-*β*,*β*-carotene-3,4,3′,4′-tetrol (3): UV−vis λ_{max} (Et_2O) 414, 437, 467 nm (% III/II = 95); CD (EPA) 220 ($\Delta\epsilon$ −1.0), 238 (0), 263 (+4.0), 320 (0) nm; ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; EIMS (70 eV) *m*/*z* 604 [M^+] (100), 586 [$M^+ - 18$] (16), 568 [$M^+ - 36$] (11), 550 [$M^+ - 54$] (7), 532 [$M^+ - 72$] (4), 512 [$M^+ - 92$] (31), 498 [$M^+ - 106$] (13), 470 [$M^+ - 134$] (22); HREIMS *m*/*z* 604.4471 (calcd for $C_{40}H_{60}O_4$, 604.4491).

(5*S*,6*S*,4′*S*,5′*S*,6′*S*)-4′-Hydroxy-5,6,5′,6′-tetrahydro-*β*,*β*-caroten-4-one (4): UV−vis λ_{max} (Et_2O) 414, 437, 467 nm (% III/II = 95); CD (EPA) 210 ($\Delta\epsilon$ 0), 222 (−4.0), 240 (0), 263 (−5.5), 270 (0), 292 (+4.8), 320 (0) nm; ^1H NMR ($CDCl_3$, 300 MHz), see Table 1; EIMS (70 eV) *m*/*z* 570 [M^+] (100), 552 [$M^+ - 18$] (12), 478 [$M^+ - 92$] (32), 464 [$M^+ - 106$] (12); HREIMS *m*/*z* 570.4423 (calcd for $C_{40}H_{58}O_2$, 570.4437).

Reduction of 4: To **4** (0.2 mg) in MeOH (2 mL) at room temperature was added an excess of a solution of $NaBH_4$ in MeOH. After 20 min at room temperature, the reaction mixture was diluted with H_2O and extracted with ether−*n*-hexane (1:1). The extract was washed with H_2O, dried, and evaporated. Purification of the product by preparative TLC (C_6H_6−EtOAc, 7:3) gave two diols in the ratio of 3:2.

The major diol (0.1 mg) was identical with compound **1** as judged by direct comparison of UV−vis, EIMS, CD, and ^1H NMR spectra.

(4′*S*,5′*S*,6′*S*)-4′-Hydroxy-5′,6′-dihydro-*β*,*β*-caroten-4-one (5): UV−vis λ_{max} (Et_2O) 414 (sh), 451, 472 nm (% III/II = 5); CD (EPA) 232 ($\Delta\epsilon$ 0), 248 (−3.1), 255 (0), 288 (+3.5), 320 (0), 348 (−1.0) nm; ^1H NMR ($CDCl_3$, 300 MHz), see Table 1; EIMS (70 eV) *m*/*z* 568 [M^+] (100), 550 [$M^+ - 18$] (14), 476 [$M^+ - 92$] (29), 462 [$M^+ - 106$] (11); HREIMS *m*/*z* 568.4273 (calcd for $C_{40}H_{56}O_2$, 568.4280).

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