Novel Marine *di-Z*-Carotenoids: Cucumariaxanthins A, B, and C from the Sea Cucumber *Cucumaria japonica*

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The novel marine carotenoids, cucumariaxanthins A (**1**), B (**2**), and C (**3**), were isolated from the northern sea cucumber *Cucumaria japonica*. Their structures and absolute stereochemistries were determined to be (5*S*,6*S*,5′*S*,6′*S*)-(9*Z*,9′*Z*)-5,6,5′,6′-tetrahydro-β,β-carotene-4,4′-dione for **1**; (5*S*,6*S*,4′*S*,5′*S*,6′*S*)-(9*Z*,9′*Z*)-4′-hydroxy-5,6,5′,6′-tetrahydro-β,β-caroten-4-one for **2**; and (4*S*,5*S*,6*S*,4′*S*,5′*S*,6′*S*)-(9*Z*,9′*Z*)-5,6,5′,6′-tetrahydro-β,β-carotene-4,4′-diol for **3** by extensive spectroscopic analysis and by the modified Mosher's method. Cucumariaxanthin C showed an inhibitory effect on Epstein-Barr virus activation in a short-term in vitro assay.

The carotenoids are among the most widespread of the naturally occurring groups of pigments. Their biological functions and activities as a vitamin A precursor,[1] as a scavenger and/or quencher of active oxygen species,[2] and as an antitumor promoter[3] have been reported. In regard to their antitumor-promoting activity, recent studies indicate that not only β-carotene but also other carotenoids, such as α-carotene, β-cryptoxanthin, lutein, lactucaxanthin, fucoxanthin, and halocynthiaxanthin, may have anticancer and cancer-preventive activity.[3,4]

More than 600 naturally occurring carotenoids are now known, and many novel carotenoids are found in marine animals.[5−7] There are, however, only a few reports on carotenoids in sea cucumbers.[5,6] Matsuno *et al.*[8,9] reported the occurrence of astaxanthin as the major carotenoid in the gonads (both sexes) of the sea cucumbers *Holothuria leucospilota* and *Stichopus japonicus*, and β-carotene, echinenone, canthaxanthin, and zeaxanthin were identified from the gonad of *H. leucospilota* and *S. japonicus*.[9,10] On the other hand, astaxanthin and the esters, canthaxanthin, phoenicoxanthin, and echinenone, were isolated by Bullock and Dawson from the red body wall of *Psolus fabrichii*.[11]

Our research for carotenoids of sea cucumbers, carried out in the course of comparative biochemical studies of carotenoids in marine animals, has led to the isolation of three novel carotenoids from the northern sea cucumber *Cucumaria japonica* Semper (Cucumariidae) belonging to the order Dendrochirotida. These carotenoids were designated as cucumariaxanthins A (**1**), B (**2**), and C (**3**). These same carotenoids were also obtained from the sea cucumbers *Cucumaria echinata* and *Pentacta australis* of the same order Dendrochirotida described above, although they could not be found in *H. leucospilota*, *H. moebi*, *H. pervicax*, or *S. japonicus*, members of the order Aspidochirotida.[12] It is interesting from a comparative biochemical point of view that significant differences in the carotenoid patterns of the two orders were observed.

In this paper, we report the isolation and the structure determination of cucumariaxanthins A (**1**), B (**2**), and C (**3**), and their biological characteristics.

Results and Discussion

General Procedures. Gonads of the sea cucumber *Cucumaria japonica* Semper (18 specimens, 6.5 kg) were extracted with Me_2CO. After transfer to ether−*n*−hexane (1:1) and washing with water, the carotenoids were concentrated under reduced pressure under N_2 below 40 °C. The crude carotenoids (30 mg) were purified by CC on Si gel using increasing percentages of ether in *n*-hexane. Successive purification by HPLC afforded cucumariaxanthins A (**1**, 8 mg), B (**2**, 2 mg), and C (**3**, 0.2 mg).

Structure Analysis. Cucumariaxanthin A (**1**) was obtained as deep orange crystals. The presence of an unconjugated >C=O in **1** was indicated by the IR (ν 1714 cm^{-1}) and ^{13}C-NMR (δ 209.4, s) spectra. The molecular formula of **1** was established as $C_{40}H_{56}O_2$ by HREIMS. All 20 carbons were detected in the ^{13}C-NMR, and DEPT experiments established the presence of 28 carbon-bound protons (five methyl groups, two methylene groups, and nine methine groups). These data suggested that **1** had a symmetrical structure, including an unconjugated carbonyl group or groups. The vis spectrum of **1** in Et_2O showed absorption maxima at 407, 430, and 458 nm. An iodine-catalyzed isomerization of **1** gave a product with absorption maxima at 431, 436, and 465 nm. The spectral changes upon isomerization suggested the presence of a nonaene chromophore including *di-Z*-geometry.[13]

In order to elucidate the bond connectivities and the stereochemistry of **1**, DQF-COSY (double-quantum filtered $^1H-^1H$ chemical shift correlation spectroscopy[14,15]), $^{13}C-^1H$ COSY, COSY for long-range coupling detection[16,17] (LR-COSY), spin decoupling difference,[18,19] and NOE difference[20−22] experiments were undertaken in C_6D_6 solution at ambient temperature (24 °C).

The experimental results of **1** are shown in Table 1 and Figures 1 and 2. The DQF-COSY and $^{13}C-^1H$ COSY experiments of **1** elucidated all one-bond $^{13}C-^1H$ connectivities and partial structures representing C18(18′)−C8(8′), C10(10′)−C12(12′), and C2(2′)−C3(3′). Furthermore, the DQF-COSY experiment revealed $^1H-^1H$ long-range couplings (four bonds) between the following proton pairs: H2(2′)−H6(6′), H3(3′)−H5(5′), H16(16′)−H17(17′), and H10(10′)−H19(19′). The decoupling difference experiment of **1**, when the methyl groups at δ 1.88 [H19(19′) and H20(20′)] were decoupled at low r.f. field (applied decoupling field, $\gamma B_2/2\pi$,[23] 4.5

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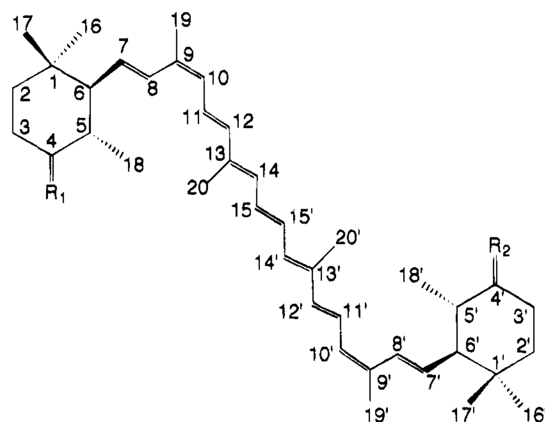
Table 1. ^{13}C- and ^{1}H-NMR Data of Cucumariaxanthins A (**1**), B (**2**), and C (**3**)

	cucumariaxanthin A (**1**)		cucumariaxanthin B (**2**)	cucumariaxanthin C (**3**)	
carbon no.	δ ^{13}C (mult)[a]	δ ^{1}H (mult, J, Hz)[b]	δ ^{1}H (mult, J, Hz)[b]	δ ^{13}C (mult)[a]	δ ^{1}H (mult, J, Hz)[b]
1, 1′	33.8, s			33.8, s	
2, 2′	41.1, t	1.32 (m)	1.32 (m), (ax) 1.12 (ddd, 13.7, 13.5,3.9) (eq) 1.30 (ddd, 13.7,3.5,3.5)	39.6, t	(ax) 1.12 (ddd, 13.7, 13.5,3.9) (eq) 1.30 (ddd, 13.7,3.5,3.5)
3, 3′	38.2, t	(ax) 2.10 (ddd, 13.5, 11.5,5.0) (eq) 2.21 (dt, 13.5, 3.8)	(ax) 2.10 (ddd, 13.5,11.5,5.0) (ax) 1.41 (dddd, 13.5,12.5,9.8,3.5) (eq) 2.21 (dt, 13.5, 3.8), (eq) 1.62 (dddd, 12.5,4.6,3.5,3.5)	31.7, t	(ax) 1.41 (dddd, 13.5, 12.5,9.8,3.5) (eq) 1.62 (dddd, 12.5,4.6,3.5,3.5)
4, 4′	209.4, s		2.90 (ddd, 10.2,9.8,4.6)	76.0, d	2.90 (ddd, 10.2,9.8,4.6)
5, 5′	44.4, d	1.96 (dq, 11.8, 6.5)	1.96 (dq, 11.8, 6.5), 1.31 (ddq, 10.2, 10.2,6.0)	39.8, d	1.31 (ddq, 10.2, 10.2,6.0)
6, 6′	59.4, d	1.61 (dd, 11.8, 9.8)	1.61 (dd, 11.8, 9.8), 1.40 (dd, 10.2, 9.8)	57.3, d	1.40 (dd, 10.2, 9.8)
7, 7′	131.3, d	5.34 (dd, 15.3, 9.8)	5.34 (dd, 15.3, 9.8), 5.45 (dd, 15.4, 9.8)	132.8, d	5.45 (dd, 15.4, 9.8)
8, 8′	129.9, d	6.70 (d, 15.3)	6.70 (d, 15.3), 6.82 (d, 15.4)	129.6, d	6.82 (d, 15.4)
9, 9′	133.4, s			133.9, s	
10, 10′	130.5, d	6.15 (d, 11.4)	6.15 (d, 11.4), 6.15 (d, 11.9)	129.8, d	6.15 (d, 11.9)
11, 11′	124.0, d	6.99 (dd, 14.8, 11.4)	6.99 (dd, 14.8, 11.4), 7.05 (dd, 14.7, 11.9)	124.2, d	7.05 (dd, 14.7, 11.9)
12, 12′	137.7, d	6.44 (d, 14.8)	6.44 (d, 14.8), 6.43 (d, 14.7)	137.3, d	6.43 (d, 14.7)
13, 13′	136.4			136.4, s	
14, 14′	133.3, d	6.34 (d, 7.8)	6.34 (d, 7.8), 6.31 (d, 8.0)	133.1, d	6.31 (d, 8.0)
15, 15′	130.6, d	6.66 (dm, 7.8)	6.66 (dm, 7.8), 6.64 (dm, 8.0)	130.5, d	6.64 (dm, 8.0)
16, 16′	19.8, q	0.72 (s)	0.72 (s), 0.80 (s)	20.6, q	0.80 (s)
17, 17′	30.1, q	0.78 (s)	0.78 (s), 0.83 (s)	31.2, q	0.83 (s)
18, 18′	13.4, q	1.08 (d, 6.6)	1.08 (d, 6.6), 1.01 (d, 6.0)	17.3, q	1.01 (d, 6.0)
19, 19′	21.2, q	1.88 (s)	1.88 (s), 1.91 (s)	21.3, q	1.91 (s)
20, 20′	12.9, q	1.88 (s)	1.88 (s), 1.85 (s)	12.9, q	1.85 (s)

[a] Measured at 75.4 MHz in C_6D_6. [b] Measured at 300 MHz in C_6D_6.



Cucumariaxanthin A (**1**): R_1= O; R_2= O
Cucumariaxanthin B (**2**): R_1= O; R_2= β-OH, α-H
Cucumariaxanthin C (**3**): R_1= β-OH, α-H; R_2= β-OH, α-H
Diol (**4**): R_1= α-OH, β-H; R_2= β-OH, α-H

Figure 1. Structures and numbering of cucumariaxanthins.

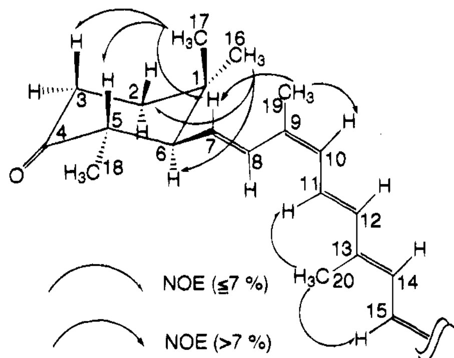


Figure 2. NOE data for cumumariaxanthin A (**1**).

Hz), clarified the existence of long-range couplings between the following proton pairs of H19(19′)−H10-(10′), H20(20′)−H12(12′), and H20(20′)−H14(14′). Then, cross-peak in the LR-COSY (τ^9 = 0.25 s, not shown) spectrum of **1** was observed between H8(8′) and H10-(10′) in the vinyl signal region. On the basis of the results of the through-bond connectivities mentioned above, ^{1}H- and ^{13}C-NMR signals in **1** were assigned unambiguously except for C1(1′), C4(4′), C9(9′), C13(13′) (Table 1). Assignments of the quaternary carbon signals of **1** were based on comparison with those of (9*Z*)-violaxanthin.[24] The connections of C16(16′) and C17-(17′) to C1(1′) were determined on the basis of the NOEs between H16(16′), H17(17′), H6(6′), and H2(2′) as described below. Thus, the carbonyl carbon should be connected with the remaining carbons of C3(3′) and C5-(5′). The ^{1}H and ^{13}C assignments and the bond connectivities for **1** are shown in Table 1 and Figure 1.

The relative stereochemistry of **1** in C_6D_6 solution was established by NOE experiments. As shown in Figure 2, irradiation of the methyl group resonances at δ 1.88 [H19(19′) and H20(20′)] produced NOE enhancements for the signals of H11(11′) (6.5%), H15(15′) (8.0%), H10-(10′) (8.5%), and H17(17′) (13.5%). The large NOE observed at H10(10′) clarified the geometry between the methyl group [H19(19′)] and H10(10′) to be in *Z* relationship. These NOE results also indicated that the distances between the methyl group [H20(20′)] and H15-(15′), H11(11′) and between the methyl group [H19(19′)] and H7(7′) was very short. Then, irradiation of the methyl resonance at δ 0.78 [H17(17′)] produced NOEs for H3(3′)ax (5.0%) at δ 2.10 H5(5′) (7.0%), and H7(7′) (8.0%). In contrast, irradiation of the methyl resonance at δ 0.72 [H16(16′)] produced NOEs for H2(2′) (5.0%) and H6(6′) (7.5%). These results indicated that the methyl group at δ 0.72 [H16(16′)] was spatially close to H2(2′) and also to H6(6′), while the methyl group [H17-(17′)] was spatially close to H3(3′)ax, H5(5′), and H7-(7′). Consequently, the end group in **1** had a chair conformation so that the methyl group [H17, (17′)], H3-(3′)ax at δ 2.01, and H5(5′) and 1,3-diaxial relationship with each other. This result was also supported by the magnitudes of the vicinal-coupling constants[25] for H6-(6′)−H5(5′) (11.8 Hz), H3(3′)ax−H2(2′) (11.5, 5.0 Hz), and H3(3′)eq−H2(2′) (3.9, 3.9 Hz) and by the very small (or no) NOE between methyl group [H17(17′)] and H2-(2′). Therefore, on the basis of the NOE results dis-

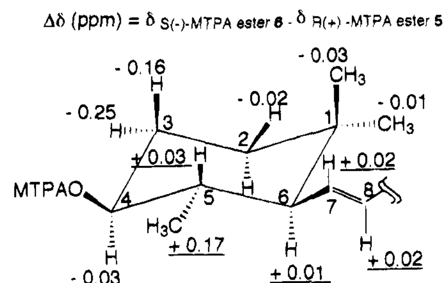


Figure 3. Δδ values obtained for the MTPA esters of cucumariaxanthin C (**3**).

cussed above, the relative stereochemistry for **1** was established to be (9*Z*,9′*Z*)-5,6,5′,6′-tetrahydro-β,β-carotene-4,4′-dione, as shown in Figure 2.

Cucumariaxanthin C (**3**), obtained as deep orange crystals, showed characteristic absorption at ν_{max} 3375 cm^{-1} and δ 76.0 in the IR and the ^{13}C-NMR spectra, respectively. The molecular formula of **3** was established as $C_{40}H_{60}O_2$ by using HREIMS. The vis spectrum of **3** in Et_2O showed absorption maxima at 407, 430, and 458 nm, indicating the presence of the same chromophore as in **1**. The ^{13}C-NMR spectrum showed a total of 20 carbon signals, indicating a symmetrical structure. The chemical shifts of the ^{13}C signals of **3** were nearly identical to those of **1**, except for C4(4′), C3(3′), and C5-(5′). The ^{13}C signal in **3** was observed at δ 76.0 compared with 209.4 in **1**, and a characteristic signal was also observed at δ 2.90 in the ^{1}H-NMR spectrum of **3**. These facts suggested that **3** was the 4,4′-diol derivative of **1**. To verify this, sodium borohydride ($NaBH_4$) reduction of **1** in 2-propanol gave two diols in the ratio of 3:2. As expected, cucumariaxanthin C (**3**) was completely identical with the major diol by comparison of physical data, and IR, vis, ^{1}H-NMR, and CD spectra. The structure of the minor diol (**4**) was presumed to be the C-4 epimer of **3** on the basis of MS and ^{1}H-NMR spectra. Therefore, cucumariaxanthin C (**3**) was determined to be (9*Z*,9′*Z*)-5,6,5′,6′-tetrahydro-β,β-carotene-4,4′-diol as shown in Figure 1.

Cucumariaxanthin B (**2**), obtained as deep orange needles, showed characteristic absorption at ν_{max} 3375 and 1714 cm^{-1} in its IR spectrum. The molecular formula of **2** was established as $C_{40}H_{58}O_2$ by using HREIMS. Compound **2** had the same chromophore as **1** and **3** according to the vis spectrum. The ^{1}H-NMR analysis of **2** confirmed the presence of the substructures of both **1** and **3** (Table 1), and $NaBH_4$ reduction of **2** gave the two diols (**3** and **4**). Therefore, the structure of cucumariaxanthin B (**2**) was established as (9*Z*,9′*Z*)-4′-hydroxy-5,6,5′,6′-tetrahydro-β,β-caroten-4-one (Figure 1).

Absolute Configurations. The chemical transformation of **1** and **2** to **3** and their CD data demonstrated the same chirality at C5(5′), C6(6′) in **1**, **2**, and **3** and also the same chirality at C4(4′) including the *trans* configuration between the 4(4′)-OH and 5(5′)-methyl groups in **2** and **3**. Therefore, we applied the modified Mosher's method[26] to **3**. The Δδ values obtained by the method for **3** are shown in Figure 3. On the basis of this result, the *S*-configuration at C4(4′) of **3** was determined. Consequently, **3** exhibited 5(*S*), 5′(*S*), 6(*S*), and 6′(*S*)-configurations.

In conclusion, the novel marine carotenoids, cucumariaxanthins A(**1**), B(**2**), and C(**3**), have been isolated from the northern sea cucumber *Cucumaria japonica*. Their absolute structures were determined to be (5*S*,6*S*,5′*S*,6′*S*)-(9*Z*,9′*Z*)-5,6,5′,6′-tetrahydro-β,β-carotene-4,4′-dione (**1**); (5*S*,6*S*,4′*S*,5′*S*,6′*S*)-(9*Z*,9′*Z*)-4′-hydroxy-5,6,5′,6′-tetrahydro-β,β-caroten-4-one (**2**); and (4*S*,5*S*,6*S*,4′*S*,5′*S*,6′*S*)-(9*Z*,9′*Z*)-5,6,5′,6′-tetrahydro-β,β-carotene-4,4′-diol (**3**). Cucumariaxanthins A (**1**), B(**2**), and C(**3**) are formally derivatives of (9*Z*,9′*Z*)-5,6,5′,6′-tetrahydro-β,β-carotene. The presence of 5,6,5′,6′-tetrahydro-β,β-carotene derivatives has been reported as metabolic products of hydra *Hydra pirardi*[27] fed on canthaxanthin, and the natural occurrence of such tetrahydro-β,β-carotene derivatives was reported from the spindle shell *Fusinus perplexus*,[28] from the prawn *Penaeus japonicus*,[29] and from the brittle star *Ophioderma longicaudum*.[30] But these carotenoids have an (all-*E*)-nonaene chain, while cucumariaxanthins A (**1**), B(**2**), and C(**3**) described in this paper are derivatives of (9*Z*,9′*Z*)-5,6,5′,6′-tetrahydro-β,β-carotene. This is the first example of carotenoids with this particular chromophore in the animal kingdom.

Table 2. Inhibitory Effects of Treatment with Cucumariaxanthins A, B, and C on TPA-Induced EBV-EA Activation

sample	Concentration[a]			
	1×10^3	5×10^2	1×10^2	1×10
β-carotene	2.5[b] (70)[c]	25.0 (>80)	89.4 (>80)	100.0 (>80)
cucumariaxanthin A	13.7 (70)	45.1 (>80)	87.9 (>80)	100.0 (>80)
cucumariaxanthin B	11.5 (70)	33.0 (>80)	75.7 (>80)	100.0 (>80)
cucumariaxanthin C	0.0 (70)	0.0 (70)	48.6 (>80)	92.5 (>80)

[a] Mol ratio/TPA (20 ng = 32 pmol/mL). [b] Values represent percentages relative to the positive control value (100%). [c] Values in parentheses are the viability percentages of Raji cells.

Biological Activities. As a screening study for antitumor promoters, cucumariaxanthins A (**1**), B(**2**), and C(**3**) were examined for their inhibitory effects on the Epstein–Barr virus activation activity of 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in raji cells (Table 2). The results showed that cucumariaxanthin C (**3**) exhibited the strongest inhibitory activity, superior to β-carotene.

Experimental Section

General Method. Silica gel (type 60; Merck) was used for CC (0.063–0.2 mm). HPLC was carried out on a Shimadzu LC-6A instrument with an ODS column Shim-pack PREP-ODS (250 × 20.0 mm i.d., 15 μm). IR and vis spectra were recorded using a Shimadzu IR-27G spectrophotometer in KBr pellet and a Shimadzu UV-240 spectrophotometer in ether solution, respectively. CD spectra were recorded on a JASCO J-500 C spectropolarimeter in ether solution at 20 °C. MS were recorded on a JEOL SX 102A mass spectrometer with a direct inlet system of ionization energy of 70 eV at 180–210 °C. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75.4 MHz) spectra were recorded on a Varian XL-300 spectrometer in C_6D_6 or $CDCl_3$ with TMS as internal standard. DQF-COSY, ^{13}C-^{1}H COSY, LR-COSY, and NOE difference experiments were performed on the same spectrometer, using standard Varian pulse sequences and software version 6.3 Å and/or 6.1E.

Animal Material. *Cucumaria japonica* Semper (18 specimens, 6.5 kg) were collected from Uchiura Bay, Hokkaido, Japan, in March 1989. They were identified

by Dr. Matsuyama of the Muroran Branch of Hokkaido Hakodate Fisheries Experimental Station. A voucher specimen is deposited at the Pharmaceutical Sciences of Natural Resources, Kyoto Pharmaceutical University.

Isolation of Cucumariaxanthins A (1), B (2) and C (3). The gonad (370 g) was separated from the living sea cucumbers, and the carotenoids were immediately extracted with Me_2CO at room temperature. After transfer to ether−*n*-hexane (1:1) by addition of water, the extracted solution was concentrated under reduced pressure in N_2 below 40 °C. The crude carotenoids (30 mg) were purified by CC of Si gel (600 g, 450 × 70.0 mm i.d.) using an increasing percentage of ether in *n*-hexane and by HPLC. Cucumariaxanthin A (**1**, 8.0 mg) was eluted with ether−*n*-hexane (20:80) from a Si gel column and was submitted to further purification by HPLC on an ODS column eluting with CH_2Cl_2−CH_3CN (5:95). Cucumariaxanthin B (**2**, 2 mg) and cucumariaxanthin C (**3**, 0.2 mg) were eluted with ether−*n*-hexane (50:50) and ether from a Si gel column, respectively, and were submitted to further purification by HPLC on an ODS column eluting with CH_2Cl_2−CH_3CN (10:90).

Cucumariaxanthin A (1): R_f = 0.63 on Si gel G with Me_2CO−*n*-hexane (30:70); deep orange needles; IR (KBr) 1714 cm^{-1}; vis (Et_2O) λ_{max} 407 nm (log ϵ 4.95), 430 (5.15), 458 (5.11); CD (Et_2O) λ_{ext} 290 nm ($\Delta\epsilon$ + 7.1), 257 (0), 212 (−9.6), 200 (−11.8); HREIMS *m/z* 568.4308, calcd for $C_{40}H_{56}O_2$ 568.4339; ^1H-NMR (C_6D_6, 300 MHz) and ^{13}C-NMR (C_6D_6, 74.5 MHz) see Table 1.

Cucumariaxanthin B (2): R_f = 0.53 on Si gel G with Me_2CO−*n*-hexane (30:70); deep orange needles; IR (KBr) 3375, 1714 cm^{-1}; vis (Et_2O) λ_{max} 407 nm (log ϵ 4.95), 430 (5.15), 458 (5.11); CD (Et_2O) λ_{ext} 322 nm ($\Delta\epsilon$ −0.7), 318 (0), 290 (+3.1), 254 (0), 228 (−8.1); HREIMS *m/z* 570.4444, calcd for $C_{40}H_{58}O_2$ 570.4451; ^1H-NMR (C_6D_6, 300 MHz) see Table 1.

Cucumariaxanthi C (3): R_f = 0.43 on Si gel G with Me_2CO−*n*-hexane (30:70); deep orange needles; IR (KBr) 3375 cm^{-1}; vis (Et_2O) λ_{max} 407 nm (log ϵ 4.95), 430 (5.15), 458 (5.11); CD (Et_2O) λ_{ext} 322 nm ($\Delta\epsilon$ −2.1), 278 (0), 262 (+3.6), 244 (0), 228 (−8.7), 218 (0), 208 (+10.7); HREIMS *m/z* 572.4612, calcd for $C_{40}H_{60}O_2$ 572.4631; ^1H-NMR (C_6D_6, 300 MHz) and ^{13}C-NMR (C_6D_6, 74.5 MHz) see Table 1.

Reduction of Cucumariaxanthin A. To cucumariaxanthin A (8.0 mg) in 2-propanol (8 mL) at room temperature was added an excess of a solution of $NaBH_4$ in 2-propanol. After 20 min at room temperature, the reaction mixture was diluted with H_2O and extracted with ether−*n*-hexane (1:1), which 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 (4.0 mg) was identical with the natural cucumariaxanthin C (**3**) judged by direct comparison of vis, MS, IR, CD, and ^1H-NMR spectra.

Semisynthetic Diol, Diol **4**: R_f = 0.40 on Si gel G with Me_2CO−*n*-hexane (30:70); deep orange needles (2.7 mg); IR (KBr) 3375 cm^{-1}; vis (Et_2O) λ_{max} 407 nm (log ϵ 4.95), 430 (5.15), 458 (5.11); CD (Et_2O) λ_{ext} 322 nm ($\Delta\epsilon$ −2.1), 278 (0), 262 (+3.6), 244 (0), 228 (−8.7), 218 (0), 208 (+10.7); HREIMS: *m/z* 572.4594; calcd for $C_{40}H_{60}O_2$ 572.4631; ^1H-NMR (C_6D_6) δ 0.80 (3H, s, CH_3 16′), 0.81 (3H, s, CH_3 16 or 17), 0.83 (3H, s CH_3 17′), 0.94 (3H, s, CH_3 17 or 16), 0.90 (3H, d, J = 6.0 Hz, CH_3 18), 1.01 (3H, d, J = 6.0 Hz, CH_3 18′), 1.82 (3H, s, CH_3 20), 1.85 (3H, s, CH_3 20′), 1.91 (3H, s, CH_3 19′), 1.93 (3H, s, CH_3 19), 2.90 (1H, ddd, J = 10.2, 9.8, 4.6 Hz, H4′ax), 3.49 (1H, q, J = 3.5 Hz, H4eq), 5.50 (1H, dd, J = 15.4, 9.8 Hz, H7), 5.45 (1H, dd, J = 15.4, 9.8 Hz, H7′), 6.15 (2H, d, J = 11.9 Hz, H10, 10′), 6.31 (2H, d, J = 8.0 Hz, H14, 14′), 6.42 (1H, d, J = 14.7 Hz, H12), 6.43 (1H, d, J = 14.7 Hz, H12′), 6.64 (2H, dm, H15, 15′), 6.93 (1H, d, J = 15.4 Hz, H8), 6.82 (1H, d, J = 15.4 Hz, H8′), 7.05 (1H, dd, J = 14.7, 11.9 Hz, H11, 11′).

Reduction of Cucumariaxanthin B. Reduction of cucumariaxanthin B by the procedure described above gave the two diols **3** and **4**.

Preparation of the (*R*)- and (*S*)-MTPA Ester of Cucumariaxanthin C. A solution of (+)-MTPA [α-methoxy-α-(trifluoromethyl)phenylacetyl]chloride (20 mg) in anhydrous pyridine (5 mL) was added to a solution of **3** (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) (**5**). The use of (−)-MTPA chloride in the same procedure led to 0.8 mg of the (S)-MTPA ester (**6**).

Cucumariaxanthin C *di-R*-MTPA ester (5): vis (ether) λ_{max} 407, 430, 458 nm; ^1H-NMR ($CDCl_3$) δ 0.67 (6H, d, CH_3 18, 18′), 0.87 (6H, s, CH_3 16, 16′), 0.90 (6H, s, CH_3 17, 17′), 1.44 (2H, ddd, H2, 2′eq), 1.65 (1H, dddd, H3, 3′ax), 1.66 (2H, dd, H6, 6′), 1.72 (2H, ddq, H5, 5′), 1.89 (6H, s, CH_3 19, 19′), 1.98 (6H, s, CH_3 20, 20′), 2.00 (2H, dddd, H3, 3′eq), 4.68 (2H, ddd, H4, 4′), 5.37 (2H, dd, H7, 7′), 6.00 (2H, d, H10, 10′), 6.24 (2H, d, H14, 14′), 6.28 (2H, d, H12, 12′), 6.57 (2H, d, H8, 8′), 6.63 (2H, dm, H15, 15′), 6.71 (2H, dd, H11, 11′).

Cucumariaxanthin C *di-S*-MTPA ester (6): vis (ether) λ_{max} 407, 430, 458 nm; ^1H-NMR ($CDCl_3$) δ 0.84 (6H, d, CH_3 18, 18′), 0.87 (6H, s, CH_3 16, 16′), 0.87 (6H, s, CH_3 17, 17′), 1.42 (2H, ddd, H2, 2′eq), 1.49 (1H, dddd, H3, 3′ax), 1.67 (2H, dd, H6, 6′), 1.75 (2H, ddq, H5, 5′), 1.75 (2H, dddd, H3, 3′eq), 1.90 (6H, s, CH_3 19, 19′), 1.98 (6H, s, CH_3 20, 20′), 4.65 (2H, ddd, H4, 4′), 5.39 (2H, dd, H7, 7′), 6.02 (2H, d, H10, 10′), 6.24 (2H, d, H14, 14′), 6.28 (2H, d, H12, 12′), 6.59 (2H, d, H8, 8′), 6.63 (2H, dm, H15, 15′), 6.72 (2H, dd, H11, 11′).

Biological Activities. The Epstein-Barr virus activation assay was carried out according to the method described previously.[4]

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