A Cytotoxic Carotenoid from the Marine Sponge *Prianos osiros*

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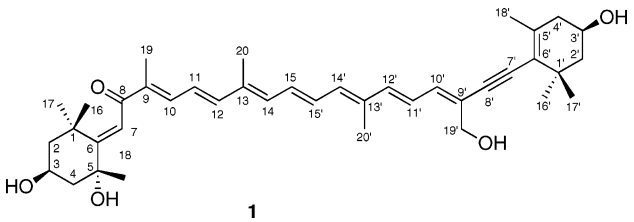
Investigations of the marine sponge *Prianos osiros*, collected in Pohnpei, gave a new cytotoxic acetylenic carotenoid, (3*R*,3′*R*,5*S*)-3,3′,5,19′-tetrahydroxy-7′,8′-didehydro-γ,ϵ-carotene-8-one. The absolute configuration of this carotenoid was solved by interpretation of IR, MS, and 2D NMR spectra and application of the modified Mosher's method. Compound **1** is cytotoxic toward cultured human colon tumor cells, HCT 116 (IC_{50} 4.38 μg/mL).

Marine organisms produce a wide range of carotenoids.[1] The majority of these C_{40} compounds are highly functionalized and may contain double or triple carbon−carbon bonds, sulfated alcohols, or modifications at the trimethylcyclohexene rings.[2−7] Occasionally, allenes are found in conjugation with the polyene chain.[2,8−10] Highly oxidized carotenoids may also contain epoxide, ketone, and ester groups.[6,7,11] Herein we report the identification of a new cytotoxic carotenoid, **1**, obtained from the marine sponge *Prianos osiros*, collected in Pohnpei. A literature search revealed that a number of different compounds have previously been isolated from the *Prianos* spp. including kauluamine,[12] prianosins,[13−15] and norsesterterpene peroxides,[16] but not carotenoids despite the suggestion of their presence; nonpolar extracts from this organism are highly pigmented. Carotenoid **1** is noteworthy for its cytotoxicity to cultured human colon tumor cells (HCT-116; IC_{50} 4.38 μg/mL).



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A frozen specimen of *Prianos osiros* (01-18-156) was lyophilized and extracted twice with methanol. Combined extracts were concentrated and partitioned between solvents in order of increasing polarity: hexane, $CHCl_3$, *n*-butanol, and water. The $CHCl_3$ partition was separated by silica chromatography, and the fraction showing ^{1}H NMR signals of interest was further purified by reversed-phase HPLC on a C_{18} column. Compound **1** was isolated as a dark red solid (4.7 mg, 0.006% of dry weight). The molecular mass, $M = 614$, was determined by ESIMS from the $[M + Na]^+$, $[M - OH]^+$, and $[2M + Na]^+$ ions and the formula, $C_{40}H_{54}O_5$, established by HRFABMS (*m/z* 614.3950, $[M]^+$). The presence of 40 distinct signals in the ^{13}C NMR spectrum concurred with a unique signal for each carbon in the formula $C_{40}H_{54}O_5$ and an index of 14 double-bond equivalents. The ^{13}C NMR spectrum (Table 1) showed unique signals at δ 200.3 (s), 95.4 (s), and 92.3 (s) corresponding to keto- and acetylenic groups, respectively. The UV spectrum exhibited highly red-shifted absorption bands (λ_{max} 460 nm, log ϵ 4.7) that indicated a molecule with 10 to 11 conjugated double bonds and suggested a carotenoid. The FTIR spectrum confirmed a carbon−carbon triple bond (ν 2167 cm^{-1}) and vinyl C−H stretching (ν 3033 cm^{-1}) and revealed the presence of OH (ν 3388 cm^{-1} broad).

Table 1. NMR Data for Compound **1** (400 MHz, $CDCl_3$)

no.	δ_C	δ_H[b] [mult., *J* (Hz)]	HMBC (H→C)	COSY[i]
1	38.6 (C)			
2	50.7 (CH_2)	1.42[a]	1, 3, 4, 6	3
		1.73[e] ddd (12.8, 4.4, 2.0)		
3	64.2 (CH)	4.28[c] m		2, 4
4	48.7 (CH_2)	1.53[a]	2, 3, 5, 6	3
		2.22[e] ddd (12.8, 4.4, 1.6)		
5	75.0 (C)			
6	154.1 (C)			
7	124.7 (CH)	6.33 s	1, 5, 6, 8	
8	200.3 (C)			
9	137.8 (C)			
10	143.3 (CH)	7.12 d (9.2)	8, 19	19
11	145.4 (CH)[g]	6.25−6.75		
12	140.3 (CH)[g]	6.25−6.75		
13	136.6 (C)[h]			
14	137.0 (CH)[g]	6.25−6.75		
15	134.5 (CH)[g]	6.25−6.75		
16	30.2 (CH_3)	1.04 s	1, 2, 3, 6, 7, 17	
17	32.0[d] (CH_3)	1.35 s	1, 2, 3, 6, 16	
18	31.5 (CH_3)	1.53 s	4, 5, 6, 7	
19	11.7 (CH_3)	1.94[f] s	8	10
20	13.2 (CH_3)	1.98 s		
1′	36.9 (C)			
2′	46.9 (CH_2)	1.44[a]	3′, 4′	3′
		1.82[e] ddd (12.4, 3.6, 2.4)		
3′	65.1 (CH)	3.98[c] m		2′, 4′
4′	41.8 (CH_2)	2.06[a] ddd (17.6, 9.6, 1.2)	2′, 3′, 5′, 6′, 18′	2′
		2.42[e] ddd (17.6, 5.6, 1.6)		
5′	138.8 (C)			
6′	124.3 (C)			
7′	92.3 (C)			
8′	95.4 (C)			
9′	123.4 (C)			
10′	136.6 (CH)	∼6.5	8′, 9′, 19′	19′
11′	124.1 (CH)[g]	6.25−6.75		
12′	124.0 (CH)[g]	6.25−6.75		
13′	136.5 (C)[h]			
14′	130.8 (CH)[g]	6.25−6.75		
15′	132.5 (CH)[g]	6.25−6.75		
16′	30.9 (CH_3)	1.19 s	1′, 2′, 3′, 6′, 17′	
17′	29.2[d] (CH_3)	1.13 s	1′, 2′, 6′, 16′	
18′	23.0 (CH_3)	1.92 s	5′, 6′, 4′, 7′	
19′	61.2 (CH_2)	4.37 d (6.0)	8′ 9′, 10′	10′
20′	13.1 (CH_3)	1.95[f] s		

[a] Axial H. [b] Overlapped. [c] Assigned by D_2O exchange δ 4.28 (m, 1H, $W_{1/2} \approx 27$ Hz) and δ 3.97 (m, 1H, $W_{1/2} \approx 27$ Hz). [d] Axial Me. [e] Equatorial H. [f−h] Interchangeable. [i]COSY and DQFCOSY results (600 MHz).

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Table 2. Observed NOEs for Compound **1**

irradiated H	observed H, NOE (%)
3	2[c] (0.6), 4[c] (0.9), 17[a] (0.4)
7	10 (1.7), 18 (2.5)
3′	2′[c] (0.9), 4′[c] (1.1), 17′[a] (0.4)
10′	18′ (4.9)
19′	11′[b] (1.4)

[a] Axial Me. [b] H11′ was observed as a doublet of doublets, which must correspond to position 11′. [c] Equatorial H.

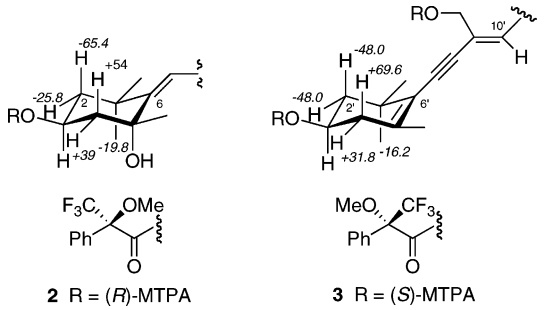

Figure 1. Values for $\Delta\delta$ (italics, in Hz) from Mosher's analysis of **2** and **3** (600 MHz, $CDCl_3$).

The ^{1}H NMR spectrum (Table 1) included multiple overlapped olefinic protons (δ 6−7.1 ppm) of the conjugated system, two broad signals (δ 3.98 m, 1H; 4.28 m, 1H), and one sharp two-proton signal (δ 4.37, d, J = 6 Hz, 2H, coupling to OH) attributed to substitution by two secondary and one primary hydroxyl group, respectively. The identities of these signals were verified by deuterium exchange (addition of D_2O), which reduced the splitting for signals at δ 3.98 and 4.28 and collapsed the doublet at δ 4.37 to a singlet. Analysis of DEPT, gCOSY, gDQCOSY, gHMBC, and gHSQC spectra (Table 1) established the connectivity shown in **1** and linked the conjugated chain to terminal penta- and hexasubstituted cyclohexylidene rings. Although the vicinal couplings of H3 and H3′ were not resolved, deuterium exchange simplified the J splitting of signals H3 and H3′, suggesting large residual splittings on each proton ($W_{1/2} \approx 27$ Hz). The large vicinal couplings and chemical shifts of H3/H3′ are consistent only with axial protons and substitution of C3/C3′ by equatorial hydroxyl groups. Analysis of NOE data (Table 2), obtained by irradiation of protons at positions C3 and C3′, assigned the axial methyl groups C17 (δ 1.19, s, 3H) and C17′ (δ 1.13, s, 3H). Identification of the axial protons at each of the methylene groups C2, C4, C2′, and C4′ was made possible by observation of their larger vicinal couplings (Table 1). The configurations of the vinyl double bonds, in particular C6−C7, C9−C10, and C9′−C10′, and the disposition of substituents about the hexasubstituted cyclohexylidene ring were all verified by NOE experiments (Table 2).

The configurations of the three stereocenters in **1**, located at opposite termini of the molecule, were conveniently derived by application of the modified Mosher's method[17] in combination with NOE data. Derivatization of **1** with (*R*)- and (*S*)-MTPA, respectively, gave the corresponding tri-MTPA esters **2** and **3**. Assignment of the ^{1}H NMR spectra of **2** and **3** was assisted by gCOSY and ROESY (600 MHz). Examination of the anisotropic shifts ($\Delta\delta$'s, 600 MHz, Figure 1, units in Hz) allowed assignment of the absolute configurations of two chiral centers using Mosher's method according to the standard configuration model.[17] The secondary hydroxyl groups at C3 and C3′ in **1** had *S* and *R* configurations, respectively (note the reversal of CIP priorities). The presence of an MTPA group at the primary hydroxyl group (C19′) in each of **2** and **3** was not considered an impediment to interpretation of the anisotropic shifts at the secondary alcohol esters (C3, C3′) since the nearest stereocenter containing another MTPA group (C3′) was effectively insulated by the intervening cyclohexylideneyne group (C3′−C9−C19′). Since the relative configuration of C5 was known from the NOE data (see above), the complete assignment of **1** is (3*S*,3′*R*,5*S*).

Compound **1** is one of the few carotenoids exhibiting cytotoxicity toward cultured cancer cells (HCT-116 colon tumor cells, IC_{50} 4.38 μg/mL).[18] Conversely, other carotenoids, such as β-carotene and astaxanthin, have been implicated in cytoprotection of normal cells against transformation after challenge with chemical mutagens.[19]

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a 400 MHz Varian Unity NMR spectrometer. NMR spectra for the Mosher's ester derivatives were recorded on a Bruker 600 MHz DRX-600 equipped with a 5 mm cryoprobe. Chemical shifts were referenced to $CHCl_3$ (δ_H = 7.24) and $CDCl_3$ (δ_C = 77.0). IR spectra were recorded from thin films deposited on NaCl plates on a Mattson Galaxy 3000 FTIR. UV spectra were recorded on a Hewlett-Packard 8452A. ESIMS was measured using a Finnigan LCQ Deca mass spectrometer. CD spectra were measured using a JASCO 810 spectrometer. Flash chromatography was carried out on silica gel (40−63 μm, EM Science).

Animal Material. The sponge *Prianos osiros* (01-18-156) was collected in September 2001 by hand using scuba (−18 m) in Pohnpei, Federated States of Micronesia. Sponge material was frozen immediately after collection and stored at −20 °C until use. Voucher samples are archived in the Department of Chemistry, UC Davis.

Extraction and Isolation. A frozen sample of the sponge (325.2 g) was lyophilized (79.1 g dry weight) and extracted with MeOH (800 mL) for 3 h at 4 °C. A second extraction with MeOH (800 mL) was carried out overnight at 4 °C. The combined extracts were concentrated under reduced pressure to 200 mL and diluted with H_2O (20 mL) before partitioning with hexane (200 mL). The aqueous MeOH phase was separated and diluted with H_2O to a final concentration of 40% v/v before partitioning with $CHCl_3$ (220 mL). After separation, *n*-BuOH (20 mL) was added to the aqueous layer and concentrated to remove the MeOH. The residual aqueous phase was then extracted twice with an equal volume of *n*-BuOH (120 mL). The $CHCl_3$-soluble fraction was evaporated to dryness (750 mg) and separated by flash chromatography (silica gel) with elution by a solvent gradient (0 to 100% MeOH in $CHCl_3$). The fourth colored fraction (26.0 mg) was purified by reversed-phase HPLC (Dynamax, 5 μm, C_{18} column, 10 × 250 mm, 25:75 H_2O/MeOH) to give pure **1** as a red solid (4.7 mg, 0.006% of dry weight).

(3*R*,3′*R*,5*S*)-3,3′,5,19′-Tetrahydroxy-7′,8′-didehydro-γ,ε-carotene-8-one (1): dark red solid; UV (EtOH) λ_{max} 460 nm (log ϵ 4.70), λ 348 nm (log ϵ 3.80), 282 nm (log ϵ 3.98), 200 nm (log ϵ 4.12); CD (CH_3CN) λ 219 ($\Delta\epsilon$ −1.59), 252 (+1.62), 287 (−0.63); IR (solid) ν 3388, 3033, 2167, 1599, 1570 cm^{-1}; ^{1}H and ^{13}C NMR data (400 MHz, $CDCl_3$), see Table 1; ESIMS *m/z* 1251 $[2M + Na]^+$, 637 $[M + Na]^+$, 597 $[M - OH]^+$; HRFABMS *m/z* 614.3950 $[M]^+$, calcd for $C_{40}H_{54}O_5$, 614.397125.

Preparation of the (*R*)- and (*S*)-MPTA Esters 2 and 3. To a solution of **1** (0.6 mg, 0.98 μmol) in dry CH_2Cl_2 (90 μL) was added either (*R*)-(+)- or (*S*)-(−)-α-methoxy-α-trifluoromethylphenylacetic acid (0.9 mg, 3.8 μmol) in dry CH_2Cl_2 (55 μL), then (0.14 mg, 1.1 μmol) of DMAP in dry CH_2Cl_2 (4.8 μL), and finally 1,3-dicyclohexylcarbodiimide (1.2 mg, 5.8 μmol) in dry CH_2Cl_2 (48 μL). The solution was stirred under dry nitrogen in the dark for 7 h, quenched by the addition of H_2O (1 mL), and stirred for an additional 10 min. The reaction mixture was extracted with hexane (4 × 0.5 mL), and the combined extracts were concentrated. The residue was dissolved in 1:19 EtOAc/hexane and purified on a silica gel "pencil

column" using 3:17 EtOAc/hexane. The first dark red band was further purified by normal-phase HPLC (Dynamax, 5 μm, 10 $\times$ 250 mm, 1:4 EtOAc/hexane) to provide pure (*R*)-MPTA ester **2** and (*S*)-MPTA ester **3**, 0.54 mg (44% yield) and 0.66 mg (54% yield), respectively.

(*R*)-MPTA Ester 2: ^{1}H NMR (selected signals, 600 MHz, $CDCl_3$) δ 1.06 (s, 3H), 1.14 (s, 3H), 1.14 (s, 3H), 1.41 (s, 3H), 1.48 (s, 3H), 1.81 (s, 3H), 1.92 (s, 3H), 1.96 (s, 3H), 1.99 (s, 3H), 2.14 (dd, J = 17.4, 10.2 Hz, 1H), 2.27 (dd, J = 9.0, 4.8 Hz, 1H), 2.48 (dd, J = 17.6, 5.1 Hz, 1H), 4.96 (d, J = 12.0 Hz, 1H), 5.14 (d, J = 12.6 Hz, 1H), 5.26 (m, 1H), 5.59 (m, 1H); ESIMS *m/z* 1263.1 $[M + H]^+$, calcd for $C_{70}H_{76}F_9O_{11}$ 1263.5, *m/z* 1285.1 $[M + Na]^+$, calcd for $C_{70}H_{75}F_9NaO_{11}$ 1285.5.

(*S*)-MPTA Ester 3: ^{1}H NMR (selected signals, 600 MHz, $CDCl_3$) δ 1.03 (s, 3H), 1.09 (s, 3H), 1.13 (s, 3H), 1.40 (s, 3H), 1.84 (s, 3H), 1.92 (s, 3H), 1.95 (s, 3H), 1.99 (s, 3H), 2.25 (dd, J = 17.4, 9.0 Hz, 1H), 2.33 (dd, J = 12.6, 4.2 Hz, 1H), 2.53 (dd, J = 18.0, 4.8 Hz, 1H), 4.96 (d, J = 11.4 Hz, 1H), 5.14 (d, J = 12.0 Hz, 1H), 5.26 (m, 1H), 5.58 (m, 1H); ESIMS *m/z* 1263.8 $[M + H]^+$, calcd for $C_{70}H_{76}F_9O_{11}$ 1263.5, *m/z* 1285.1 $[M + Na]^+$, calcd for $C_{70}H_{75}F_9NaO_{11}$ 1285.5.

Cytotoxicity Assay against Human Colorectal Cancer Cells (HCT-116). Compounds were assayed in DMSO (final concentration, 1% v/v) and run against etoposide as positive control (IC_{50} 2.0 μg/mL). HCT-116 cells were incubated in 96-well plates for 72 h before addition of MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt].[20] Cell viabilities and end-points were determined from measurements of the well absorbances of the *soluble* formazan product (λ 490 nm) using a Molecular Devices Spectramax Plus microplate reader.

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