Isolation of a New Carotenoid Pigment from an Undescribed Gorgonian of the Genus *Muricella*

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Muricellaxanthin, a novel carotenoid pigment has been isolated by activity-guided separation from an undescribed gorgonian of the genus *Muricella* collected from Jaeju Island. Structure of this compound has been determined by a combination of spectral methods. Stereochemistry has been defined by interpretation of nOe data and comparison of CD data with related compounds. Muricellaxanthin exhibited potent lethality against brine-shrimp larvae.

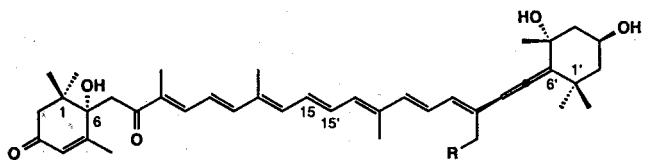
Introduction

Marine animals are widely recognized as very prolific sources of both biologically-active and structurally-unique secondary metabolites.[1,2] Benthic colonial invertebrates including coelenterates, sponges, and tunicates are the most frequently investigated marine animals for the chemical and biomedical purposes. Consequently, most of leading compounds on the development of new drugs from marine sources have been derived from these animals.[3,4] However, chemical investigation of these organisms has been mainly focussed on the tropic and sub-tropic ones while organisms habitating temperate and cold waters have attracted much less attention. Recently we have isolated several novel bioactive compounds from the benthic colonial invertebrates of the Korean and Antarctic shallow waters.[5~9] As a part of our continuous search for novel metabolites, we collected the dark-red gorgonian (sea whip, phylum Cnidaria, order Gorgonacea) *Muricella* sp. off the shore of Jaeju Island. The organic crude extract exhibited moderate toxicity (LC_{50} 125 ppm) against brine-shrimp larvae. Bioactivity-guided silica vacuum flash chromatography followed by C_{18} reversed-phase HPLC has yielded a red pigment. Herein we report the structure and bioactivity of muricellaxanthin, a novel carotenoid pigment.

Results and Discussion

Muricellaxanthin (1) was isolated as a red amorphous solid. The molecular formula of $C_{42}H_{56}O_7$ was deduced by a combination of HRFABMS and ^{13}C NMR spectrometry. The total carbon count and the presence of several carbon signals in the olefinic region (δ 150-110) in the ^{13}C NMR spectrum revealed that **1** was a carotenoid. A moderately strong absorption band at 1940 cm^{-1} in the IR spectrum indicated the presence of an allenic moiety. In addition, absorption bands at 3400, 1745, and 1670 cm^{-1} were interpreted as hydroxyl, ester, and unsaturated ketone functionalities, respectively.

The structure of **1** was determined by a combination of ^1H COSY, HMQC, and HMBC experiments. All of the proton-bearing carbons and their protons were precisely matched by the HMQC experiment (Table 1). A long-range coupling between the protons at δ 5.83 (1H, brs) and 1.90 (3H, brs)



1. R = OAc
2. R = H

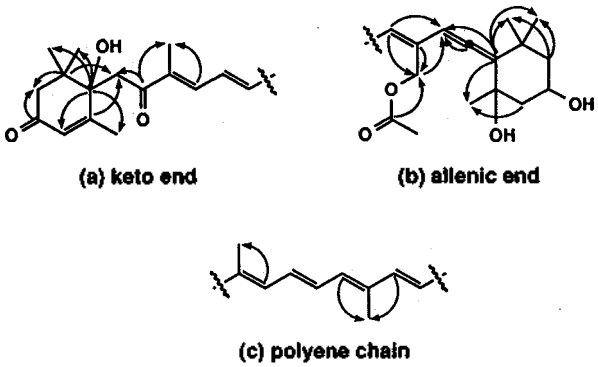
was interpreted as an allylic coupling. This interpretation was confirmed by HMBC correlations between the protons at δ 1.90 and the carbons at δ 167.93 and 125.97. Severe differentiation of the chemical shifts between the olefinic carbons revealed that an electron-withdrawing group, *i.e.* carbonyl group was attached to the double bond. The environment around this α,β-unsaturated carbonyl group was determined by long-range couplings of the protons at δ 2.47, 2.33, 1.08, and 1.05 with adjacent carbons (Table 1). Thus a 4-hydroxy-3,5,5-trimethyl-2-cyclohexenone moiety was determined (Figure 1). Similarly the presence of an α,β,γ,δ-unsaturated carbonyl group was determined by a combination of 2-D NMR experiments. Connection of the unsaturated carbonyl with cyclohexenone system was determined by long-range couplings of the methylene protons at δ 3.05 and 2.93 with carbons at δ 203.47, 167.93, 78.54, and 42.02. Thus, a partial structure containing carbonyl groups was determined (Figure 1a).

The presence of another 6-membered ring was also determined by combined spectral methods. The proton COSY experiment readily determined a spin system of $-CH_2-CH(OH)-CH_2-$. Long-range couplings of the methylene carbons at δ 49.43 and 48.96 and an olefinic carbon at δ 118.52 with methyl protons at δ 1.38, 1.34, and 1.09 revealed the presence of a 6-membered ring. The carbon at δ 118.52 and a downfield carbon at δ 201.75 were coupled to a common proton at δ 6.04. The unusual chemical shifts of these carbons and coupling patterns indicated these belong to an allenic system. Couplings of the carbons at δ 132.86 and 59.46 with the olefinic proton at δ 6.04 defined the environment around the allenic group. Downfield chemical shifts (δ 4.91 and 4.75) of the methylene protons and H-C correlations with the acetoxyl carbonyl carbon suggest the acetoxyl group to be atta-

Table 1. NMR Assignments for Muricellaxanthin (**1**)

#	C[a]	H[b]	HMBC[c]	#	C[a]	H[b]	HMBC[c]
1	42.02		1, 2, 16, 17	1′	36.06		16′
2	49.71	2.47 d (18.2)	17	2′	49.43	1.92 m	16′, 17′
		2.33 d (18.2)				1.29 dd (14.4, 12.4)	
3	197.67		2	3′	64.29	4.30 m	
4	125.97	5.83 brs	18	4′	48.96	2.26 brdd (12.9, 4.3)	18′
						1.32 m	
5	167.93		4, 7, 18	5′	73.01		18′
6	78.54		2, 4, 7, 17, 18	6′	118.52		8′, 16′, 17′, 18′
7	38.64	3.05 d (14.4)		7′	201.75		8′
		2.93 d (14.4)					
8	203.47		7, 10, 19	8′	100.83	6.04 s	19′
9	135.06		11, 19	9′	129.64		19′
10	142.11	7.10 brd (11.2)	19	10′	132.86	6.30 d (11.5)	8′, 19′
11	123.25	6.58 dd (14.7, 11.2)		11′	124.66	6.70 m	
12	146.96	6.70 m	10, 11, 20	12′	139.89	6.42 d (14.9)	20′
13	135.65			13′	138.23		
14	137.80	6.46 brd (11.7)	20	14′	133.45	6.31 brd (11.5)	20′
15	130.04	6.66 dd (14.2, 11.7)		15′	133.01	6.77 dd (14.5, 11.5)	
16	23.24	1.08 s	2, 17	16′	29.17	1.34 s	
17	24.86	1.05 s	2, 16	17′	31.89	1.09 s	
18	20.75	1.90 d (1.4)		18′	31.02	1.38 s	
19	11.65	1.95 brs	10	19′	59.46	4.81 d (12.0)	8′
						4.75 d (12.0)	
20	12.97	1.99 brs		20′	12.71	1.99 brs	
				Ac	170.85		19′
					21.08	2.05 s	

[a,b]measured at 125 and 500 MHz, respectively. [c]Numbers of correlated protons. Parameters were optimized for 6 Hz of coupling constants.



(a) keto end (b) allenic end

(c) polyene chain

Figure 1. Partial structures and key HMBC correlations (C→H) of **1**.

ched to this methylene. Thus, a partial structure containing an allenic group was determined (Figure 1b).

The remaining part consisted of four double bonds and two vinyl methyl groups (Figure 1c). Although several long-range H-C couplings were observed by HMBC experiments, precise assignments were not successful because of the severe overlapping of both proton and carbon resonances. Finally this problem was solved by comparison of the ^{13}C NMR data with related compounds.[10] Thus, the structure of muricellaxanthin (**1**) was unambiguously determined as a carotenoid possessing an allenic moiety. Literature survey revealed that muricellaxanthin was closely related with amarouciaxanthin A (**2**) isolated from the Japanese tunicate *Amaroucium pliciferum*.[11] Besides those for the acetoxyl methylene part, comparison of spectral data showed good correlation with the published data for **2**.

Muricellaxanthin contained four asymmetric carbon centers at C-6, -3′, -5′, and allene group. The large coupling constant (w1/2-28 Hz) of the H-3′ proton assigned an axial orientation for this proton. NOESY experiment showed strong correlations of the H-3′ with H-2′β, -4′β, and -16′ protons, while another correlation was found between the H-2′α and H-18′ methyl protons. Therefore, the relative configurations of the C-3′ and -5′ were assigned as 3′*S** and 5′*R**, respectively. However, stereochemistry of the C-6 and allene, two remotely separated asymmetric centers were unable to be determined by NMR methods.

The relative configurations of these centers and the absolute stereochemistry of the whole molecule were determined by comparison of CD data with those of amarouciaxanthin A (**2**). CD measurement of muricellaxanthin in EPA (Et_2O-isopentane-EtOH, v/v 5 : 5 : 2) gave extrema at 243 ($\Delta\varepsilon$ −32.2) and 272 nm ($\Delta\varepsilon$ +7.2) while **2** exhibited extrema at 245 ($\Delta\varepsilon$ −44.9) and 270 nm ($\Delta\varepsilon$ +16.7). The close simila-

rity of CD data indicated the same stereochemistry for these compounds. Thus, muricellaxanthin (**1**) was determined as (6*S*,3′*S*,5′*R*,6′*R*)-19′-acetoxy-6,3′,5′-trihydroxy-4,5,6′,7′-tetradehydro-7,8,5′,6′-tetrahydro-β,β-carotene-3,8-dione.

Modified carotenoids including amarouciaxanthins and halociaxanthin have been reported to exhibit cytotoxicity against cancer cell-lines.[11] In our measurement of bioactivity, muricellaxanthin (**1**) exhibited significant toxicity against brine-shrimp larvae (LC_{50} 0.16 ppm).

Experimental

General. Nmr spectra were recorded in $CDCl_3$ solutions on a Varian Unity-500 spectrometer. Proton and carbon nmr spectra were measured at 500 and 125 MHz, respectively. All chemical shifts were recorded with respect to internal Me_4Si. IR spectrum was recorded on a Mattson GALAXY spectrophotometer. UV-visible spectrum was obtained in methanol using a Milton-Roy spectrophotometer. Mass measurement was provided by the Mass Spectrometry Facility, Department of Chemistry, University of California, Riverside. The optical rotations were measured on a JASCO digital polarimeter with a 5-cm microcell. CD measurement was made on a JASCO polarimeter using a 0.01 cm cell. Melting point was measured on a Fisher-Jones Apparatus and is reported uncorrected. All solvents used were spectral grade or were distilled from glass prior to use.

Collection and identification of specimens. *Muricella* sp. (sample number 92J-16) was collected by hand using SCUBA at 20-25 m depth in July, 1992 along the shore of Seoguipo, Jaeju Island. Morphological characters of the specimens were very similar with those of another *Muricella* sp. collected at the same time.[7] However, these specimens differ in their characters as follows: tip of twigs shown oblong owing to the lateral arrangements of polyps and the shape of axis, the spindles with complex tubercles up to 0.56×0.14 mm long in the outer layer of coenenchymes, lots of smaller calices situated among larger ones. In addition, silica TLC analysis of the crude extracts showed different patterns between the two chemotypes. The voucher specimens under the code name 92J-16 are on deposit in the octocorallian collection, Natural History Museum, Ewha Womans University, under the curatorship of J.-I. S.

Extraction and isolation. The collected specimens were immediately frozen by dry ice and sent to the laboratory. The animals (2.5 kg) were defrosted and repeatedly extracted with CH_2Cl_2 (3 L×2) and dried under vacuum to yield dark brown syrup (9.23 g). The crude extract was separated by silica vacuum flash chromatography ($60PF_{254}$) by using sequential mixtures of *n*-hexane and EtOAc as eluents. The bioactive fraction (80% EtOAc in hexane) was dried under vacuum and subjected to semi-preparative C_{18} reversed-phase HPLC (YMC ODS column, 1 cm×25 cm, 10% H_2O in MeOH) to yield 16.2 mg of muricellaxanthin (0.65×10^{-3}% of wet animals).

Muricellaxanthin (1). red amorphous solid, mp 86-87°; $[\alpha]_D$ −8.9 (c 0.3, MeOH); HRFABMS: $[M+H]^+$ obsd 673.4086, $C_{42}H_{57}O_7$ requires 673.4104 (+2.7 ppm); IR (KBr) 3400, 2920, 1930, 1745, 1670, 1600, 1520, 1360, 1220, 1050, and 980 cm^{-1}; UV-visible (MeOH) λ_{max} 232, 269, 338, and 453 nm; CD (EPA) 243 ($\Delta\varepsilon$ −32.2) and 272 nm ($\Delta\varepsilon$ +7.2); ^1H and ^{13}C NMR, see Table 1.

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