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Apocarotenoids from an Association of Two Marine Sponges

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Abstract – Bioactivity-guided fractionation of MeOH extract of an association of two sponges, *Jaspis* sp. and *Poecillastra* sp. resulted in isolation of four apocarotenoids (**1** - **4**). Their structures were determined on the basis of MS and NMR spectroscopic analyses and by direct comparison with those of reported. This is the first report on isolation of these compounds from any sponge. Isolated metabolites were evaluated for cytotoxicity against a small panel of solid human tumor cell lines.
Keywords – Marine sponge, *Jaspis* sp., *Poecillastra* sp., Apocarotenoids, Allene, Cytotoxicity

Introduction

Sponges of the order Astrophorida (subclass Tetractinomorpha) are well known for producing a range of secondary metabolites such as sterols, tetramic acid derivatives, azetidine and acridine alkaloids, glycolipids, macrolides, sesquiterpenes, polyketides, and peptides. Sponges of the genus *Jaspis* are reported to contain many biologically active molecules which include macrolides [jaspisamides (Kobayashi, *et al.*, 1993), jaspamides (Gala, *et al.*, 2007) or jasplakinolide (Crews, *et al.*, 1986)], amino acid derivatives [bengamides (Thale, *et al.*, 2001) and bengazoles (Groweiss, *et al.*, 1999)], and triterpenes (Tang, *et al.*, 2006). Similarly, macrolide lactams [poecillastrins (Takada, *et al.*, 2007)] and sesquiterpene derivatives (Killday, *et al.*, 1993) have been isolated from sponges of the genus *Poecillastra*. Two sponge association of *Jaspis* sp. and *Poecillastra* sp. was known to contain bisimidazoles [wondonins (Shin, *et al.*, 2001)]. As a part of our continuing research aimed at the discovery of biologically active secondary metabolites from marine organisms (Jung, *et al.*; 2007, Bao, *et al.*, 2007; Lee, *et al.*, 2007; Shinde, *et al.*, 2007), we had isolated pectenotoxin II and psammaplins from the 90% MeOH extract of a two sponge association of *Jaspis* sp. and *Poecillastra* sp. by bioactivity-guided fractionation (Jung, *et al.*, 1995; Park, *et al.*, 2003). In continuation of our work on this specimen, we isolated four apocarotenoids from the MeOH extract. In this paper, we describe the isolation, structure elucidation, and cytotoxicity evaluation of **1** - **4** (Fig. 1).

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Experimental

General procedures – Optical rotations were measured with a Jasco P-1020 polarimeter using a 1 dm path length cell. The CD spectra were measured with a Jasco J-715 spectropolarimeter (sensitivity 50 mdeg, resolution 1 nm). The ^{1}H and 2D NMR spectra were recorded at 500 MHz using Varian INOVA 500 spectrometer. ESIMS data were obtained on API-2000 LC/MS/MS spectrometer. Chemical shifts were reported with reference to the respective solvent peaks and residual solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD; δ_H 7.28 and δ_C 77.2 for $CDCl_3$). HPLC was performed on a Gilson 370 pump with a Shodex C18M 10E (preparative, 250 × 10 mm i.d., 5 μm, and 100 Å) column using RI-101 detector.

Animal material – The sponges, collected in November 2002, off the Coast of Jeju Island, South Korea, were frozen immediately after collection and stored at –20 °C until extraction. This specimen was identified as an association of sponges *Jaspis* sp. (order Astrophorida, family Jaspidae) and *Poecillastra* sp. (order Astrophorida, family Pachastrellidae) by Prof. Chung Ja Sim of Hannam University. A voucher specimen was deposited in the Natural History Museum, Hannam University, Daejon, Korea.

Extraction and isolation – The frozen animal material was cut into small pieces and extensively extracted with

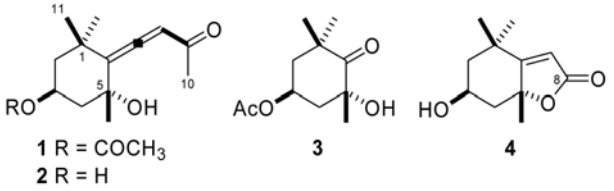

Fig. 1. Structures of compounds **1** - **4**.

MeOH at room temperature. The MeOH extract showed toxicity against brine shrimp larvae (LD_{50} 43 μg/mL). The MeOH extract was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 layer was further partitioned between 90% aqueous MeOH and *n*-hexane. The aqueous MeOH fraction was selected for further separation on the basis of its toxicity in brine shrimp lethality assay (Meyer, *et al.*, 1982) and subjected to a reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å 500/400 mesh), eluting with gradient solvent system of 50 to 100% MeOH/H_2O to yield nineteen fractions (DCM1-DCM19). Bioactive fractions (DCM1 and DCM5) were selected for further separation because of their potent activity (LD_{50} ~ 20 μg/mL) in the brine shrimp lethality assay and were subjected to repeated reversed-phase chromatographic separation to afford four apocarotenoids. Compound **1** was obtained by purification of fraction DCM5 by repeated reversed-phase HPLC chromatographic separation. Fraction DCM1 was subjected to MPLC on Combiflash Retrieve using Redisep C-18 column, eluting with gradient solvent system of 0 to 70 % MeOH/H_2O to yield ten subfractions. Subfractions, DCM1-4 and DCM1-5 were subjected to reversed-phase HPLC on Shodex C18M 10E (preparative, 250 × 10 mm i.d., 5 μm, and 100 Å) column to obtain compounds **2** - **4**. The isolated compounds were identified by interpretation of their 1D and 2D NMR and MS data, and by direct comparison with those of reported.

Compound 1 – Colorless oil; $[\alpha]^{26}_D$ –25.8° (*c* 0.1, $CHCl_3$); CD (*c* 1 × 10^4 M, MeOH) Δε (nm) –20.21 (207), +17.23 (230), –15.42 (257); ^{1}H NMR (CD_3OD, 500 MHz) δ 5.84 (1H, s, H-8), 5.36 (1H, tt, *J* = 11.5, 4.0 Hz, H-3), 2.22 (1H, ddd, *J* = 13.0, 4.5, 2.5 Hz, H-4a), 2.19 (3H, s, H-10), 2.01 (3H, s, H-15), 1.99 (1H, dd, *J* = 13.0, 4.0, 2.0 Hz, H-2b), 1.55 (1H, dd, *J* = 13.0, 12.5 Hz, H-2a), 1.45 (1H, dd, *J* = 12.5, 12.0 Hz, H-4b), 1.41 (3H, s, H-11), 1.38 (3H, s, H-13), 1.15 (3H, s, H-12); ^{13}C NMR (CD_3OD, based on HSQC and HMBC experiments, 500 MHz) δ 212.0 (C-7), 201.0 (C-9), 171.5 (C-14), 120.0 (C-6), 101.0 (C-8), 72.0 (C-5), 69.0 (C-3), 45.8 (C-2, C-4), 37.0 (C-1), 31.6 (C-12), 30.2 (C-13), 29.0 (C-11), 26.4 (C-10), 21.0 (C-15); LRESIMS *m/z* 289 $[M + Na]^+$.

Compound 2 – Colorlesss oil; $[\alpha]^{27}_D$ –8.0° (*c* 0.05, $CHCl_3$); CD (*c* 1 × 10^4 M, MeOH) Δε (nm) –43.62 (207), +34.67 (231), –24.17 (259); ^{1}H NMR (CD_3OD, 500 MHz) δ 5.81 (1H, s, H-8), 4.20 (1H, tt, *J* = 11.0, 3.5 Hz, H-3), 2.20 (1H, dd, *J* = 12.5, 4.5, 2.5 Hz, H-2a), 2.18 (3H, s, H-10), 1.91 (1H, ddd, *J* = 12.5, 4.0, 2.0 Hz, H-4b), 1.40 (1H, m, H-2b), 1.33 (1H, dd, *J* = 13.0, 12.5 Hz, H-4a), 1.37 (6H, s, H-13, H-11), 1.14 (3H, s, H-12); ^{13}C NMR (CD_3OD, based on HSQC and HMBC experiments, 500 MHz) δ 212.0 (C-7), 201.0 (C-9), 120.0 (C-6), 101.0 (C-8), 72.0 (C-5), 64.4 (C-3), 49.5 (C-2), 45.5 (C-4), 37.0 (C-1), 31.8 (C-12), 29.6 (C-11, C-13), 26.0 (C-10); LRESIMS *m/z* 247 $[M + Na]^+$.

Compound 3 – Colorless oil; $[\alpha]^{27}_D$ –1.4° (*c* 0.05, $CHCl_3$); ^{1}H NMR (CD_3OD, 500 MHz) δ 5.46 (1H, tt, *J* = 11.0, 4.0 Hz, H-3), 2.29 (1H, ddd, *J* = 12.5, 4.0, 2.0 Hz, H-2a), 2.08 (1H, ddd, *J* = 12.5, 4.0, 2.0 Hz, H-4a), 2.01 (3H, s, H-11), 1.77 (1H, dd, *J* = 12.5, 11.0 Hz, H-2b), 1.69 (1H, dd, *J* = 12.0, 11.0 Hz, H-4b), 1.36 (3H, s, H-7), 1.24 (3H, s, H-9), 1.08 (3H, s, H-8); ^{13}C NMR (CD_3OD, based on HMBC experiment, 500 MHz) δ 214.5 (C-6), 75.6 (C-5), 69.8 (C-3), 46.5 (C-2), 44.0 (C-4), 28.0 (C-1); LRESIMS *m/z* 237 $[M + Na]^+$.

Compound 4 – Pale yellow oil; $[\alpha]^{27}_D$ –45.6° (*c* 0.1, $CHCl_3$); ^{1}H NMR (CD_3OD, 500 MHz) δ 5.74 (1H, s, H-7), 4.20 (1H, tt, *J* = 11.0, 3.5 Hz, H-3), 2.40 (1H, dt, *J* = 14.0, 2.0, Hz, H-4a), 1.98 (1H, dt, *J* = 15.0, 3.0 Hz, H-2a), 1.72 (1H, m, H-2b), 1.52 (1H, dd, *J* = 14.0, 3.0 Hz, H-4b), 1.75 (3H, s, H-11), 1.45 (3H, s, H-9), 1.26 (3H, s, H-10); ^{13}C NMR (CD_3OD, based on HSQC and HMBC experiments, 500 MHz) δ 186.0 (C-6), 174.0 (C-8), 113.0 (C-7), 89.0 (C-5), 67.0 (C-3), 48.7 (C-4), 47.5 (C-2), 37.0 (C-1), 30.4 (C-10), 27.0 (C-11), 26.8 (C-9); LRESIMS *m/z* 195 $[M - H]^-$.

Results and Discussions

Compound **1** was isolated as colorless oil and its molecular formula was established as $C_{15}H_{22}O_4$ on the basis of ^{13}C NMR and MS analysis. The ESIMS spectrum of **1** showed the $[M + Na]^+$ ion at *m/z* 289. The ^{1}H and ^{13}C NMR data showed signals for allene moiety (δ 5.84/101.0 for H-8/C-8, δ 212.0 for C-7, and δ 120.0 for C-6), five methyl groups (δ 2.19/26.4, 2.01/21.0, 1.41/29.0, 1.38/30.2, and 1.15/31.6) and two quarternary carbonyl carbons (δ 201.0/C-9 and 171.5/C-14). The 1D (^{1}H, ^{13}C and DEPT) and 2D (^{1}H-^{1}H COSY, HMBC, and HSQC) NMR data of **1** were identical to those reported for apo-9'-fucoxanthinone, previously isolated from brown algae (Parameswaran, *et al.*, 1996; Kimura and Maki, 2002; Park, *et al.*, 2004; Mori, *et al.*, 2004), and marine

dinoflagellate (Doi, *et al.*, 1995). Its structure and absolute configuration were reported to be established by synthesis and X-ray crystallographic analysis (Hlubucek, *et al.*, 1974). The stereochemistry of **1** was determined to be (3*S*,5*R*,8*R*) on the basis of identical coupling constant values, specific optical rotation (**1**: $[\alpha]^{26}_{D}$ –25.8°, $CHCl_3$; reported: $[\alpha]^{19}_{D}$ –284°, $CHCl_3$), and superimposable CD spectrum [**1**: Δε (nm) –20.21 (207), +17.23 (230), –15.42 (257); reported: Δε (nm) –3.27 (207), +3.31 (230), –3.13 (257)].

Compound **2** was isolated as colorless oil with molecular formula $C_{13}H_{20}O_3$, which was determined on the basis of ^{13}C NMR and MS analysis. The $[M + Na]^+$ ion was observed at *m/z* 247 in the ESIMS spectrum of **2**. The ^1H and ^{13}C NMR data were identical with **1** except the absence of acetyl group signals, which was supported by upfield shifted signal of H-3 at δ 4.20 (**1**: δ_H 5.36). Hence, compound **2** was identified as deacetyl derivative of **1** by comparison of its spectral data with those reported. This is the first report on isolation of grasshopper ketone (**2**) from marine source, which was previously isolated from grasshopper *Romalea microptera* (Meinwald, *et al.*, 1968) and several terrestrial plants (Hashimoto, *et al.*, 1991; Iijima, *et al.*, 2003; DellaGreca, *et al.*, 2004; Jiang, *et al.*, 2006). Identical chemical shifts and coupling constants suggested the same stereochemistry as **1**, which was further supported by optical rotation data (**2**: $[\alpha]^{27}_{D}$ –8.0°, $CHCl_3$; reported: $[\alpha]^{25}_{D}$ –32.0°, $CHCl_3$) and superimposable CD spectrum [**2**: Δε (nm) –43.62 (207), +34.67 (231), –24.17 (259); reported: Δε (nm) –1.83 (211), +2.98 (229), –3.43 (255)].

Compound **3** was isolated as colorless oil. In the ESIMS spectrum, $[M + Na]^+$ ion was observed at *m/z* 237, suggesting molecular formula to be $C_{11}H_{18}O_4$, which was supported by ^{13}C NMR data. The ^1H NMR spectrum showed three methyl singlets (δ_H 1.36, 1.24, 1.08), downfield methine proton (δ 5.46/H-3), which suggested that **3** also possess similar skeleton with **1** (absence of allene moiety, presence of ketone signal at δ 214.5/C-6). The stereochemistry was deduced to be identical to that of similar compounds on the basis of comparable ^1H NMR spectral and optical rotation (**3**: $[\alpha]^{27}_{D}$ –1.4°, $CHCl_3$) data. Thus, its structure was identified as (2*R*,4*S*)-4-acetoxy-2-hydroxy-2,6,6-trimethylcyclohexanone, which was previously isolated from the brown alga *Padina tetrastromatics* (Parameswaran, *et al.*, 1996).

Compound **4** was isolated as light yellow oil. Its molecular formula was established as $C_{11}H_{16}O_3$ on the basis of ^{13}C NMR and MS analysis. The ESIMS spectrum of **1** showed the $[M - H]^-$ ion at *m/z* 195. Compound **4** was identified as loliolide by comparison of its spectral and optical rotation data with those reported (**4**: $[\alpha]^{27}_{D}$ –45.6°, $CHCl_3$; reported: $[\alpha]^{22}_{D}$ –100.5°, $CHCl_3$), which was previously isolated from several terrestrial plants (Takasaki, *et al.*, 1999), algae (Kimura and Maki, 2002), and sea hare (Petit, *et al.*, 1980).

The cytotoxicity of the compounds **1**, **2**, and **4** against A-549, SK-OV-3, SK-MEL-2, XF-498, and HCT-15 solid tumor cell lines was studied employing routine method (Mansoor, *et al.*, 2005). These compounds were found to be inactive (ED_{50} > 10 μg/mL) to all cell lines in the panel.

Apocarotenoids isolated in present study have previously been reported from terrestrial plants, marine algae, dinoflagellates, or sea hares. It was suggested that these metabolites are formed by an oxidative degradation of carotenoids during isolation or dietary metabolism. To the best of our knowledge, there has been no report on these four apocarotenoids from any marine sponge. It may be assumed that the original source of these apocarotenoids in sponges might be sponge-associated dinoflagellates or microorganisms. This work is of importance in context of ecological relationship between sponges and other marine organisms and further study may throw light on the marine ecological system.

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