Structure of an Unsaturated Fatty Acid with Unique Vicinal Dimethyl Branches Isolated from the Okinawan Soft Coral of the Genus *Sinularia*

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A new unsaturated fatty acid with unique vicinal dimethyl branches was isolated from the Okinawan soft coral of the genus *Sinularia*. The structure of the compound was determined based on the results of spectroscopic analysis and chemical conversion. The absolute configuration was deduced by applying the Ohrui–Akasaka method.

Key words soft coral; *Sinularia* sp.; unsaturated fatty acid; Ohrui–Akasaka method

Soft corals have been noted as a rich source of structurally unique and biologically active natural products[1,2] such as terpenoids, steroids and prostanoids. During the course of our studies[3—9] on the constituents of Okinawan marine invertebrates, a new unsaturated fatty acid with unique vicinal dimethyl branches, (*E*,*R*)-6,7-dimethylhexadec-7-enoic acid (**1**), was isolated. This paper describes the structural determination of the fatty acid based on the results of spectroscopic analysis and chemical conversion involving the application of the Ohrui–Akasaka method.[10]

Compound **1** was isolated as a colorless oil from the methanol extract of the genus *Sinularia* collected from a coral reef off Ishigaki Island (see Experimental).

The molecular formula of compound **1** was found to be $C_{18}H_{34}O_2$ using the high-resolution electrospray mass spectrum (HR-ESI-MS) and ^{13}C-NMR data. The ^{13}C-NMR spectrum (Table 1) disclosed the signals due to three methyls, eleven sp^3 methylenes, one sp^3 methine, one sp^2 methine, one sp^2 quaternary carbon and one carbonyl carbon. The presence of a carboxylic acid group was suggested by the IR absorptions of 3200—2500 (broad) and 1714 cm^{-1} together with the ^{13}C signal of 180.3 (C) ppm. The ^{1}H-NMR spectrum (Table 1) showed the signals due to one primary methyl, one secondary methyl, one olefinic methyl and one olefinic proton. These spectral data, coupled with two degrees of unsaturation, suggested that compound **1** was an acyclic unsaturated fatty acid with two methyl branches.

Table 1. NMR Data[a] for **1**

Position	**1**	
	δ_C	δ_H
1	180.3 (C)	
2	34.1 (CH_2)	2.33 (2H, t, 7.5)
3	24.8 (CH_2)	1.61 (2H, m)
4	27.1 (CH_2)	1.22 (2H, m)
5	34.5 (CH_2)	1.21 (1H, m)
		1.36 (1H, m)
6	42.6 (CH)	2.06 (1H, sext, 6.9)
7	138.6 (C)	
8	124.5 (CH)	5.12 (1H, t, 6.9)
9	27.7 (CH_2)	1.96 (2H, m)
10—13	29.32 (CH_2)	1.2—1.4 (8H, m)
	29.35 (CH_2)	
	29.54 (CH_2)	
	29.85 (CH_2)	
14	31.9 (CH_2)	1.26 (2H, m)
15	22.7 (CH_2)	1.28 (2H, m)
16	14.1 (CH_3)	0.88 (3H, t, 7.0)
17	19.8 (CH_3)	0.96 (3H, d, 6.9)
18	12.1 (CH_3)	1.48 (3H, s)

a) ^{13}C-NMR: 125 MHz in $CDCl_3$, ^{1}H-NMR: 500 MHz in $CDCl_3$. *J* in Hz. Assignments of ^{13}C and ^{1}H signals were made based on HMQC.

The HMQC analysis revealed the assignment of each direct C–H bond in **1** as summarized in Table 1. The ^{1}H–^{1}H correlations obtained from ^{1}H–^{1}H COSY are shown by the bold lines in Fig. 2 to give four partial structures (**a**—**d**), which were connected by the HMBC correlations as shown by the broken arrows in Fig. 2. The HMBC correlation from H_3-16 to C-14 (CH_2) indicated the presence of a propyl group. The HMBC correlations from H_3-18 to C-7 and C-8 exhibited the presence of a trisubstituted double bond with a methyl group. The presence of another methyl group at the position (C-6) adjacent to the trisubstituted double bond was demonstrated by the HMBC correlations from H_3-18 to C-6 and from H_3-17 to C-7. The structural unit from the carboxylic acid (C-1) to C-5 was indicated by the HMBC correlations from H_2-2 to C-1, and from H_2-3 to C-4 and C-5. The remaining five methylenes should automatically be connected between C-9 and C-14, leading to the gross structure

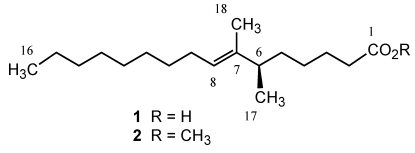

Fig. 1. Structure of New Fatty Acid **1**

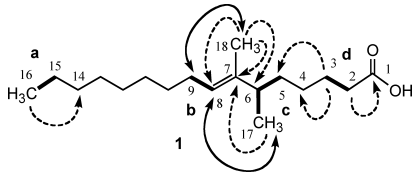

Fig. 2. Gross Structure, ^{1}H–^{1}H Correlations (Bold Lines), Key HMBC Correlations (Broken Arrows) and Key NOE Correlations (Real Arrows) of **1**

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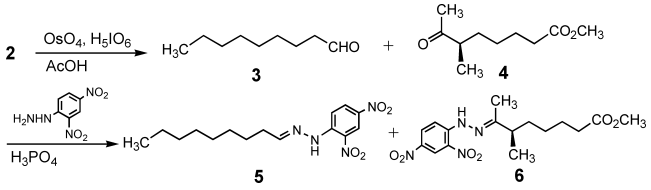
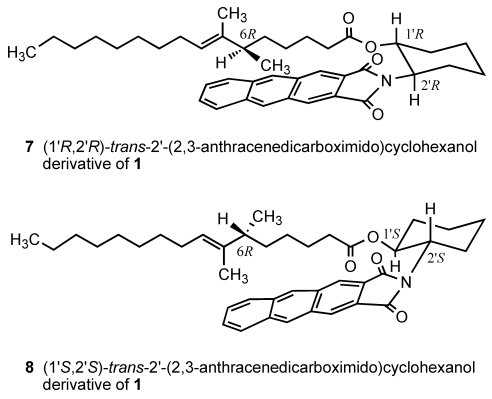


Chart 1. Chemical Conversion of **1**



7 (1′*R*,2′*R*)-*trans*-2′-(2,3-anthracenedicarboximido)cyclohexanol derivative of **1**

8 (1′*S*,2′*S*)-*trans*-2′-(2,3-anthracenedicarboximido)cyclohexanol derivative of **1**

Fig. 3. Structures of *RR*- and *SS*-2Acyclo-OH of **1**

for **1**.

The position of the trisubstituted double bond in **1** was confirmed by the following chemical conversion (Chart 1). Compound **1** was converted to methyl ester **2**, which was treated with osmium oxide (VIII) and periodic acid hydrate at room temperature to give aldehyde **3** and ketoester **4**. Without purification of the products, the mixture was treated with 2,4-dinitrophenylhydrazine to give corresponding 2,4-dinitrophenylhydrazones **5** and **6**. The structures of **5** ($C_{15}H_{22}N_4O_4$) and **6** ($C_{16}H_{22}N_4O_6$) were elucidated by the MS and NMR data. Thus the presence of a trisubstituted double bond between C-7 and C-8 in **1** was established.

The *E* configuration of the double bond at C-7 was disclosed by the NOE correlations between H-8 and H_3-17 and H_2-9 and H_3-18 (Fig. 2). The absolute stereochemistry of the chiral center at C-6 bearing a methyl group was determined based on the Ohrui–Akasaka method.[10] The method clarifies both the position and absolute configuration of a branched methyl group on a long-chain fatty acid based on the retention times of ester derivatives prepared from a fatty acid and *RR*- and *SS*-*trans*-2-(2,3-anthracenedicarboximido)cyclohexanol in reverse-phase HPLC. When compound **1** has a 6*R* configuration, *RR*-*trans*-2-(2,3-anthracenedicarboximido)cyclohexanol (*RR*-2Acyclo-OH) of **1**, in which the 6*R*-CH_3 is oriented over the plane of anthracene (Fig. 3), is predicted to be eluted from reversed-phase column faster than the corresponding *SS* derivative (*SS*-2Acyclo-OH) of **1**, in which the 6*R*-CH_3 is oriented off the plane of anthracene, based on the theory of the Ohrui–Akasaka method. In fact, the reversed-phase HPLC of the ester derivatives showed that *RR*-2Acyclo-OH derivative **7** was eluted faster than the *SS*-2Acyclo-OH derivative **8**, indicating the *R* configuration at C-6 in **1**. From these findings, compound **1** was assigned to be (*E*,*R*)-6,7-dimethylhexadec-7-enoic acid. Compound **1** is the first natural unsaturated fatty acid with a 2,3-dimethyl-1-propenyl unit. The structure of the dihydro derivative of **1**, 6,7-dimethylhexadecanoic acid, was cited in the literature as a patent[11] describing surfactant properties of mid-chain branched fatty acids, although the compound was not a natural product.

Experimental

General Procedures Optical rotation was measured using a JASCO DIP-370 automatic polarimeter. IR spectra were recorded using a Perkin-Elmer FT-IR PARAGON 1000 spectrophotometer. All NMR spectra were taken using a Bruker DRX-500 (^{1}H; 500 MHz, ^{13}C; 125 MHz) spectrometer. ^{1}H–^{1}H COSY, NOESY, HMQC and HMBC spectra were measured using standard Bruker pulse sequences. Chemical shifts are given on a δ (ppm) scale with $CHCl_3$ (^{1}H; 7.26 ppm) and $CDCl_3$ (^{13}C; 77.0 ppm) as the internal standard. High-resolution ESI mass spectra were taken using a Micromass LCT spectrometer.

Extraction and Isolation Wet specimens (1.6 kg) of the soft coral of the genus *Sinularia*, collected from the coral reef off Ishigaki Island (Okinawa, Japan), were extracted with MeOH (4.0 l, three times). Each MeOH extract was concentrated under reduced pressure. The combined MeOH extract (71.2 g) was partitioned between EtOAc and H_2O. The EtOAc soluble portion (9.4 g) was chromatographed on a silica gel column (100 g) eluted with hexane (600 ml, fraction A), hexane–EtOAc (3 : 1, 600 ml, fraction B), hexane–EtOAc (1 : 1, 600 ml, fraction C), EtOAc (600 ml, fraction D), and MeOH (600 ml, fraction E), to give five fractions. A part (2.0 g) of fraction B (4.5 g) was subjected to flash column chromatography (hexane–EtOAc=9 : 1, 8.5 : 1.5, 8 : 2, 7.5 : 2.5, 7 : 3, 6.5 : 3.5 and EtOAc) to give 13 fractions. The third fraction (580 mg) was further purified by normal-phase (hexane–EtOAc=8 : 2) and reversed-phase HPLC (ODS, MeOH–H_2O=95 : 5, 9 : 1, tetrahydrofuran–H_2O=6 : 4) to give compound **1** (124 mg).

(*E*,*R*)-6,7-Dimethylhexadec-7-enoic Acid (**1**): Colorless oil; $[\alpha]_D^{25}$ −1.0° (*c*=3.86, $CHCl_3$); IR (film) ν_{max} 3200—2500 (broad), 2920, 1714 cm^{-1}; ^{13}C- and ^{1}H-NMR, see Table 1; HR-ESI-MS *m*/*z*: 283.2633 $[M+H]^+$ (Calcd for $C_{18}H_{35}O_2$, 283.2637).

Conversion of 1 to Phenylhydrazones 5 and 6 Compound **1** was methylated to methyl ester **2** by the treatment of **1** with CH_2N_2 in diethyl ether. A small amount of H_5IO_4 (total 34 mg) and 30% AcOH solution (total 50 μl) were added to a mixture of **2** (17 mg) and H_2O–*t*-BuOH (1 : 1, 500 ml). The reaction mixture was stirred at room temperature for 2 h. After the addition of 0.05 M aqueous NaOH solution to pH 6, 2,4-dinitrophenylhydrazine (65 mg) in a 5% aqueous H_3PO_4 solution was added. The reaction mixture was extracted with diethyl ether and the organic layer was washed successively with water twice and brine twice, and was dried over anhydrous $MgSO_4$. After filtration, the filtrate was concentrated under reduced pressure. The residue was chromatographed on a silica gel column. Elution with hexane–EtOAc (9 : 1) gave two fractions. Each fraction was further purified by normal-phase HPLC (hexane–EtOAc=9 : 1) to give compound **5** (3.5 mg) from the first fraction and compound **6** (5.3 mg) from the second fraction.

Compound **5**: Yellow amorphous solids; ^{1}H-NMR (300 MHz, $CDCl_3$), δ ppm: 0.89 (3H, s), 1.24—1.38 (10H, m), 1.60 (2H, m), 2.42 (2H, dt, *J*=5.4, 7.3 Hz), 7.53 (1H, t, *J*=5.4 Hz), 7.92 (1H, d, *J*=9.6 Hz), 8.29 (1H, dd, *J*=2.6, 9.6 Hz), 9.12 (1H, d, *J*=2.6 Hz), 11.02 (1H, s, NH); ^{13}C-NMR (75 MHz, $CDCl_3$), δ ppm: 14.1 (CH_3), 22.6 (CH_2), 26.3 (CH_2), 29.2 (CH_2), 29.3 (CH_2), 29.7 (CH_2), 31.8 (CH_2), 32.5 (CH_2), 116.5 (CH), 123.5 (CH), 128.8 (C), 130.0 (CH), 137.7 (C), 145.1 (C), 152.7 (CH); HR-ESI-MS *m*/*z*: 323.1721 $[M+H]^+$ (Calcd for $C_{15}H_{23}N_4O_4$, 323.1719).

Compound **6**: Yellow amorphous solids; ^{1}H-NMR (300 MHz, $CDCl_3$), δ ppm: 1.17 (3H, d, *J*=6.9 Hz), 1.32 (2H, m), 1.48 (1H, m), 1.64 (1H, m), 2.42 (2H, dt, *J*=5.4, 7.3 Hz), 7.53 (1H, t, *J*=5.4 Hz), 7.92 (1H, d, *J*=9.6 Hz), 8.29 (1H, dd, *J*=2.6, 9.6 Hz), 9.12 (1H, d, *J*=2.6 Hz), 11.02 (1H, s, NH); ^{13}C-NMR (75 MHz, $CDCl_3$), δ ppm: 13.5 (CH_3), 17.9 (CH_3), 24.9 (CH_2), 26.9 (CH_2), 33.6 (CH_2), 33.8 (CH_2), 42.4 (CH_2), 51.5 (OCH_3), 116.5 (CH), 123.5 (CH), 129.1 (C), 130.0 (CH), 137.7 (C), 145.3 (C), 161.3 (C), 173.6 (CO); HR-ESI-MS *m*/*z*: 367.1637 $[M+H]^+$ (Calcd for $C_{16}H_{23}N_4O_6$, 367.1608).

Conversion of 1 to *RR*-2Acyclo-OH Derivative 7 and *SS*-2Acyclo-OH Derivative 8 Compound **1** (1.3 mg) was reacted with *RR*-*trans*-2-(2,3-anthracenedicarboximido)cyclohexanol (*RR*-2Acyclo-OH, 1.6 mg) in toluene–CH_3CN (1 : 1, 0.5 ml) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 2.1 mg) and 4-dimethylaminopyridine (DMAP, 1.3 mg) at 40 °C for 13 h. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in $CHCl_3$–MeOH (1 : 1). The solution was passed through a silica gel short column, and the eluate was concentrated under reduced pressure. The residue

was separated by normal phase HPLC eluted with $CHCl_3$–MeOH (9 : 1) to obtain the *RR*-2Acyclo-OH derivative **7** (2.2 mg). The *SS*-2Acyclo-OH derivative **8** (3.8 mg) was also prepared by the reaction of compound **1** (2.4 mg) with *SS*-2Acyclo-OH (3.0 mg) in the presence of EDC (3.8 mg) and DMAP (2.4 mg) in toluene–CH_3CN (1 : 1, 0.5 ml) at 40 °C for 13 h.

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