New Loliolide Derivatives from the Brown Alga *Undaria pinnatifida*

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Seven carotenoid metabolites including five loliolide derivatives have been isolated from the brown alga *Undaria pinnatifida*, including three new compounds. Structures of these compounds were confirmed by spectroscopic analyses and literature data.

Monoterpenes in the form of loliolides, which are carotenoid metabolites, have been isolated from land plants and marine algae[1] and are well-known to have immunosuppressive, germination inhibitory, and antirepellent activities among others.[2−4] From a component analysis of the brown alga *Undaria pinnatifida* (Harvey) Suringar (Laminariaceae),[5] which is very popular in the Japanese diet,[6] we have isolated three new loliolide derivatives (**1**, **2**, and **3**) as well as four known carotenoid metabolites, loliolide (**4**), isololiolide (**5**), *β*-ionone (**6**),[7] and fucoxanthinone (**7**).[8] It was proven that **1** was a unique loliolide derivative having a hemiacetal group, and **2** and **3** were diastereomers of each other formed by hydration of **4** and **5**.

The alga was soaked in MeOH, and the MeOH extract was partitioned between EtOAc and H_2O. The EtOAc layer was subjected to ODS flash chromatography using a stepwise elution of CH_3CN/H_2O. The 40% CH_3CN fraction was separated by repetitive ODS-HPLC using 20% CH_3-CN to yield three new (**1**−**3**) and four known compounds (**4**−**7**).

New compound **1** has a cyclohexane ring, a loliolide with a composition of $C_{13}H_{20}O_6$, indicated by negative ion HRFABMS at 271.1248 $[M - H]^-$. The ^1H NMR spectrum exhibits characteristic peaks at *δ* 5.28 attributable to an acetal proton and at *δ* 3.14 and 2.65 attributable to two exchangeable protons. The ^{13}C NMR data of **1** reveal the presence of oxygen-bearing carbons resonating at δ_C 214.1, 93.1, 85.6, and 81.3, as shown Table 1.

HMBC correlations between the hydroxy proton signal at *δ* 2.65 and δ_C 81.3, 85.6, and 214.1 were observed, and a correlation between *δ* 5.28 and δ_C 81.3 was also detected. From these results, **1** was identified as a loliolide derivative having hemiacetal and carbonyl groups, as shown Figure 1. The structure was confirmed by the acetylation of **1**, because the hemiacetal proton signal for H-8 was shifted to *δ* 6.17 from *δ* 5.28.

The relative stereochemistry of **1** was determined by NOE measurement. Irradiation of *δ* 2.42 (H-4eq) enhanced the signals at *δ* 5.17 (H-3) and 1.31 (CH_3-5). When *δ* 3.14 (OH-8) and 2.65 (OH-6) were irradiated, the signals of *δ* 1.31 (CH_3-5) and 1.00 (CH_3-1) were enhanced, respectively. Furthermore, irradiation of *δ* 5.28 (H-8) enhanced the other CH_3-1 at *δ* 1.15. Thus, it was proven that H-3, H-4eq, and CH_3-5 were on the same side, and the stereochemistry of **1** was proven to be 3*S**, 5*S**, 6*S**, and 8*R**.

The molecular formula of **2** was established as $C_{11}H_{18}O_4$ by FABMS. The ^1H and ^{13}C NMR spectra were similar to that of known compounds **4** and **5** except for methylene

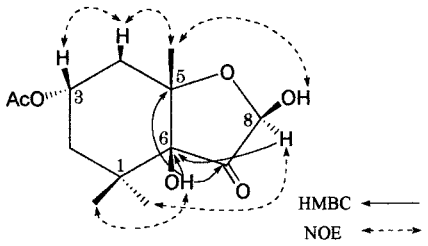

Figure 1. HMBC and NOE correlations of **1**.

proton signals at *δ* 2.97 and 2.39 and oxygen-bearing carbon and methylene carbon peaks at δ_C 81.5 and 41.3. HMBC correlations were identified between the methylene signals at *δ* 2.97 and 2.39 (H-7) and carbon signals at δ_C 173.7 (C-8), 81.5 (C-6), and 88.4 (C-5). This result suggests that **2** was the hydration product of **4** or **5**. Although a small amount of **3** was also isolated, it was identified as a diastereomer of **2** by the ^1H NMR (Table 1) and MS spectra. The stereochemistry of **2** was determined on the basis of coupling constants and NOE measurement. The coupling constants of H-3 in **2** appear to be triple triplets of J = 11.6 and 4.1 Hz, indicating that the H-3 occupies an axial position according to the typical ax−ax and ax−eq coupling constants. Irradiation at *δ* 3.90 (H-3) enhanced the signals at *δ* 1.54 (CH_3-5) and 1.09 (CH_3-1). It is also clear from the coupling constants that H-3 in **3** is in the equatorial position. This supports the fact that two methyl groups at C-1 and C-5 of **3** were more deshielded than that of **2** because of 1,3-diaxial interactions between OH and the methyl groups.[9] As these coupling constants and NOE results for **2** and **3** are similar to those for **5** and **4**, respectively, both were related by the same stereostructures. Thus, it is thought that OH-6 in **2** has the *R**-configuration, and **3** has the *S**-configuration, as hydration reaction occurred from the opposite side of the methyl groups at C-1 and C-5 due to steric hindrance.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Shimadzu FT IR-470. NMR spectra were measured on a JEOL JNM-ECP500 operating at 500 MHz for ^1H and 125 MHz for ^{13}C, respectively, and the solvent was $CDCl_3$. The mass spectra were obtained with a JEOL SX-102 mass spectrometer. The optical rotation was determined in $CHCl_3$ on a Perkin-Elmer 341 digital polarimer. HPLC was performed on a Shimadzu LC-10 apparatus equipped with a UV detector using a GL Science column (Inertsil prep-ODS, 20.0 × 250 mm or 10.0 × 250 mm). The solvents were distilled prior to use.

Plant Material. *Undaria pinnatifida* (Harvey) Suringar (Laminariaceae), which was harvested off in April 2000, was

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Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1**−**5** in $CDCl_3$[a]

	1			**2**			**3**		**4** (loliolide)		**5** (isololiolide)	
	δC	δH	*J*(Hz)	δC	δH	*J*(Hz)	δH	*J*(Hz)	δH	*J*(Hz)	δH	*J*(Hz)
1	37.8			36.9								
2	40.4	1.56(dd)	14.2, 11.0	46.5	1.47(dd)	12.4, 12.4	1.67(dd)	14.7, 3.7	1.53(dd)	14.7, 3.7	1.33(dd)	12.1, 12.1
		1.60[b]			1.85(ddd)	13.4, 4.0, 2.8	1.78(ddd)	14.9, 4.4, 2.3	1.97(ddd)	14.5, 3.0, 2.3	2.03(ddd)	12.9, 4.4, 2.2
3	66.9	5.17(m)		63.7	3.90(tt)	11.6, 4.1	4.22 (quintet)	4.6	4.33 (quintet)	3.4	4.13(tt)	11.6, 4.5
4	39.2	1.60[b]		47.0	1.56(dd)	12.4, 12.4	1.94(dd)	14.7, 3.7	1.78(dd)	13.5, 3.7	1.51(dd)	11.7, 11.5
		2.42(dd)	14.2, 4.6		2.41(ddd)	13.1, 4.0, 2.8	2.21(ddd)	10.4, 4.6, 2.3	2.46(ddd)	14.0, 3.2, 2.3	2.53(ddd)	11.5, 4.0, 2.2
5	85.6			88.4								
6	81.3			81.5								
7	214.1			41.3	2.39(d)	17.4	2.50(d)	17.4	5.69(s)		5.71(s)	
					2.97(d)	17.4	2.77(d)	17.4				
8	93.1	5.28(d)	4.6	173.7								
9	25.5	1.15(s)		27.0	1.03(s)		1.24(s)		1.47(s)		1.26(s)	
10	25.6	1.00(s)		23.0	1.09(s)		1.03(s)		1.27(s)		1.31(s)	
11	27.4	1.31(s)		22.8	1.54(s)		1.64(s)		1.78(s)		1.58(s)	
12	170.5											
13	21.3	2.04(s)										
6-OH		2.65(s)										
8-OH		3.14(d)	4.6									

[a] ^{1}H and ^{13}C NMR were measured at 500 and 125 MHz. [b] These peaks were unclear because of overlapping.

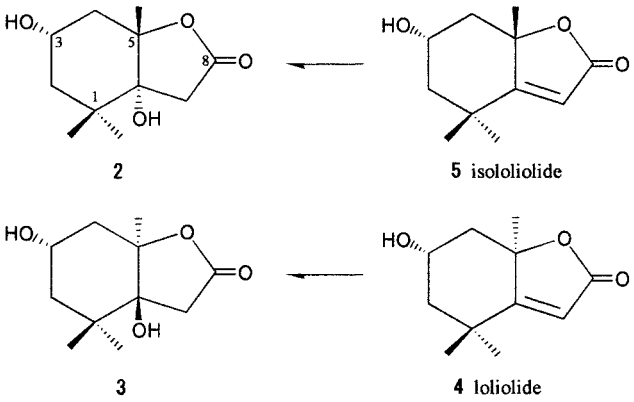

Figure 2. Postulated biosynthesis of **2** and **3**.

obtained at the Miura Peninsula, Kanagawa Prefecture. Dried *Undaria pinnatifida* (Wakame) is a commercial seaweed.

Extraction and Isolation. The sample (972 g) was soaked in MeOH for 1 day, and the solvent was removed under reduced pressure. The residue (116 g) was partitioned between hexane (1.2 L × 3) and water (1.2 L), and the aqueous layer was re-extracted three times with EtOAc (1.2 L). The combined EtOAc extract residue (2.11 g) was subjected to ODS flash chromatography (Wakosil 25C18) using a stepwise elution of CH_3CN/H_2O. A fraction eluted with CH_3CN/H_2O (2:3) was further separated by repetitive ODS-HPLC using CH_3CN/H_2O (1:4) to give **1** (7.5×10^{-5} %), **2** (7.5×10^{-5} %), **3** (2.5×10^{-5} %), **4** (1.4×10^{-3} %), and **5** (5.2×10^{-4} %), **6** (1.2×10^{-4} %), and **7** (3.4×10^{-4} %).

Compound 1: colorless oil; $[\alpha]_D$ +33° (*c* 0.078, $CHCl_3$); IR (KBr disk) 3396, 1770, 1726, 1249, 1020 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRFABMS (matrix, glycerol) *m*/*z* 271.1248 $[M - H]^-$, calcd for $C_{13}H_{19}O_6$, 271.1181).

Compound 2: colorless oil; $[\alpha]_D$ +23° (*c* 0.028, $CHCl_3$); IR (KBr disk) 3353, 3313, 1733, 1627 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; LRFABMS *m*/*z* (relative intensity) 429 $[(2M + H)^+$; 5], 215 $[(M + H)^+$; 26], 197 (21), 179 (12).

Compound 3: colorless oil; $[\alpha]_D$ −64° (*c* 0.013, $CHCl_3$); IR (KBr disk) 3460, 1749, 1631 cm^{-1}; ^{1}H NMR, see Table 1; LRFABMS *m*/*z* (relative intensity) 429 $[(2M + H)^+$; 16], 215 $[(M + H)^+$; 71], 197 (22), 179 (10).

Acetylation of 1. Acetylation of **1** was carried out with acetic anhydride and pyridine by the usual method. The acetylated product was isolated by ODS-HPLC with CH_3CN/H_2O (2:3) in 87% yield: ^{1}H NMR ($CDCl_3$) δ 6.17 (H-8, s), 5.17 (H-3, tt, J = 11.0, 4.6 Hz), 2.62 (OH-6, s), 2.45 (H-4eq, ddd, J = 14.3, 5.0, 1.8 Hz), 2.13 (CH_3COO-8, s), 2.03 (CH_3COO-3, s), 1.64 (H-2eq, ddd, J = 12.5, 4.6, 1.8 Hz), 1.59 (H-2ax, dd, J = 11.2, 10.1 Hz), 1.28 (CH_3-5, s), 1.21 (CH_3-1eq, s), 1.00 (CH_3-1ax, s).

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