Sinulariadiolide, a Novel Marine Norditerpenoid from Okinawan Soft Coral of the Genus, *Sinularia*

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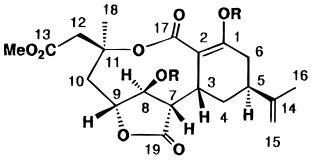
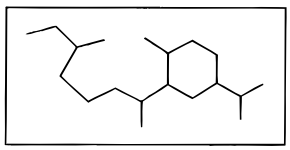
A new marine norditerpenoid, sinulariadiolide, was isolated from Okinawan soft coral of the genus, *Sinularia*. Its structure was determined by spectroscopic analysis and chemical reaction. This compound is structurally characterized by a new carbon skeleton and two lactonic moieties, five-membered and nine-membered, the latter being conjugated to a 2-hydroxylated carbon−carbon double bond. A possible biogenesis of sinulariadiolide is briefly discussed.

Soft corals are rich sources of structurally unique and biologically active diterpenoids.[1] In a previous paper[2] we reported the isolation and structure of yonarolide, a norditerpenoid possessing a novel tricyclo[7.5.0.0^{3,7}]-tetradecane skeleton, from Okinawan soft coral of the genus, *Sinularia*. Further research on related diterpenoids of this soft coral led to the isolation of a novel norditerpenoid, sinulariadiolide (**1**). This compound is characterized by a new carbon skeleton and two lactonic moieties, the nine-membered one of which constitutes a β-hydroxy-α,β-unsaturated ester group. Structural elucidation was made based on spectroscopic analysis and chemical reaction. A possible biogenesis of sinulariadiolide (**1**) is discussed.

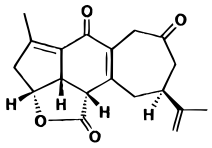
Wet specimens of the soft coral (2.4 kg), collected from the coral reef of Ishigaki Island (Okinawa, Japan), were extracted with methanol. The extract was partitioned between ethyl acetate and water. Repeated chromatographic separation of the ethyl acetate soluble portion (17.2 g) gave sinulariadiolide (**1**) (21 mg, 0.012% yield based on the ethyl acetate soluble portion) along with known norditerpenoids, **2**,[2] **3**,[3] and **4**.[4]



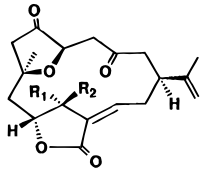
1 R = H sinulariadiolide
5 R = Ac



new carbon skeleton of **1**



2 yonarolide

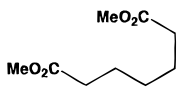


3 R_1 = H, R_2 = OH
4 R_1 = OH, R_2 = H

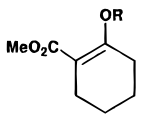
The molecular formula, $C_{20}H_{26}O_8$, was determined by HREIMS; found 394.1653, calcd 394.1628. The IR spectrum of **1** showed absorptions due to hydroxyl (3468 cm^{-1}), carbonyl (1741 cm^{-1}), and conjugated carbonyl (1654, 1617 cm^{-1}) groups. A conjugated system was confirmed by the UV spectrum; λ_{max} (EtOH) 259 nm (ϵ 9500). All 20 carbons appeared in the ^{13}C NMR spectrum (Table 1). DEPT indicated three methyls, four sp^3 methylenes, one sp^2 methylene, five sp^3 methines, one sp^3 quaternary carbon, and six sp^2 quaternary carbons. Based on chemical shifts, the ketonic carbonyl carbon was considered absent and all carbonyl groups were attributable to ester groups.

A secondary hydroxyl group in **1** was demonstrated by chemical reaction. Acetylation of **1** with acetic anhydride in pyridine gave diacetate **5** [δ_H 2.11 (3H, s), 2.20 (3H, s)]. The signal of δ 4.59 ppm (1H, br s) in the ^{1}H NMR spectrum of **1** shifted to a lower field of δ 5.30 (1H, br s) in **5** owing to the acetylation shift.

The β-hydroxy-α,β-unsaturated ester group, C(OH)═CCOO, in **1** was shown present as follows. The ^{1}H NMR signal at δ 12.0 ppm (1H, s, D_2O exchangeable) in **1** was assignable to the proton of the enolic hydroxyl group hydrogen-bonded to the ester carbonyl group. The presence of the enol group was further supported by the positive iron(III) chloride test of **1**, which showed a brownish color, as well as UV measurement of **1** in basic solution. On adding a drop of 0.1 N sodium hydroxide solution to ethanolic solution of **1**, UV absorption at 259 nm (ϵ 9500) showed a bathocromic shift to 286 nm (ϵ 13300), which shifted back to 259 nm with acidic solution addition. A β-hydroxy-α,β-unsaturated ester group thus appeared to be quite likely present in **1**. Confirmation was made of this by comparison of spectral data of **1** and **5** with those of compounds **7** and **8** prepared from diester **6**.[5]



6



7 R=H
8 R=Ac

The spectral data of enol **7** [IR (film) 1659, 1618 cm^{-1}; UV (EtOH) 257 nm (ϵ 7500), UV (addition of 0.1 N NaOH)

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(1) Faulkner, D. J. *Nat. Prod. Rep.* **1995**, *12*, 223, and previous papers in this series.

(2) Iguchi, K.; Kajiyama, K.; Yamada, Y. *Tetrahedron Lett.* **1995**, *36*, 8807.

(3) Sato, A.; Fenical, W.; Qi-tai, Z.; Clardy, J. *Tetrahedron* **1985**, *41*, 4303.

(4) Bowden, B. F.; Coll, J. C.; Mitchell, S. J.; Mulder, J.; Stokie, G. J. *Aust. J. Chem.* **1978**, *31*, 2049.

(5) Compounds **7** and **8** were known,[6] but their spectral data could not be obtained completely. Thus **7** and **8** were prepared.

(6) Bachmann, W. E.; Fujimoto, G. I.; Wick, L. B. *J. Am. Chem. Soc.* **1950**, *72*, 1995.

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Table 1. NMR Data[a] for Sinulariadiolide (1)

position	^{13}C	^{1}H
1	174.5 (C)	
2	98.3 (C)	
3	36.28 (CH)	2.87 (1H, t, J = 5.5 Hz)
4	37.0 (CH_2)	1.85 (1H, ddd, J = 5.5, 8.4, 13.2 Hz)
		2.04 (1H, ddd, J = 2.9, 6.7, 13.2 Hz)
5	36.20 (CH)	2.65 (1H, m)
6	34.0 (CH_2)	2.26 (1H, dd, J = 6.7, 18.3 Hz)
		2.55 (1H, dd, J = 5.7, 18.3 Hz)
7	58.5 (CH)	2.56 (1H, d, J = 1.8 Hz)
8	83.8 (CH)	4.59 (1H, br s)
9	86.0 (CH)	4.56 (1H, d, J = 6.3 Hz)
10	41.8 (CH_2)	2.28 (1H, dd, J = 6.3, 14.8 Hz, Hα)
		2.62 (1H, br d, J = 14.8 Hz, Hβ)
11	80.8 (C)	
12	45.3 (CH_2)	2.80 (1H, d, J = 15.3 Hz)
		3.09 (1H, d, J = 15.3 Hz)
13	169.8 (C)	
14	146.5 (C)	
15	110.2 (CH_2)	4.68 (1H, s), 4.83 (1H, s)
16	21.4 (CH_3)	1.79 (3H, s)
17	170.1 (C)	
18	26.1 (CH_3)	1.77 (3H, s)
19	176.5 (C)	
OCH_3	51.7 (CH_3)	3.67 (3H, s)
OH		12.0 (1H, s)

[a] 100 MHz in $CDCl_3$ for ^{13}C NMR and 400 MHz in $CDCl_3$ for ^{1}H NMR. Assignments were made based on the HMQC spectrum.

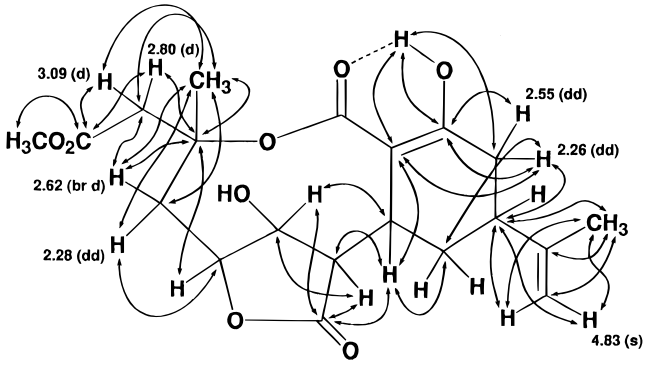

Figure 1. ^{1}H−^{13}C Long-range correlations observed in the HMBC spectrum.

283 nm (ϵ 7500); ^{1}H NMR ($CDCl_3$) δ 12.1 ppm (1H, s, OH); ^{13}C NMR ($CDCl_3$) δ 97.6 (C), 173.0 (C) ppm] were quite similar to those of **1**. The spectral data of enol acetate **8** [IR (film) 1769, 1721, 1661 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 117.4 (C), 156.0 (C) ppm] were quite similar to those of **5**.

The partial structure of 1-hydroxy-5-isopropenyl-1-cyclohexene moiety involving the above enol group (from C-1 to -6 positions, and C-14, -15, and -16 positions) was elucidated based on ^{1}H−^{13}C long-range correlations observed in HMBC spectrum (Figure 1) and by ^{1}H−^{1}H correlations observed in HOHAHA (TOCSY) spectrum which showed a sequential proton network of H-3 [2.87 (t)], H-4 [1.85 (ddd), 2.04 (ddd)], H-5 [2.65 (m)] and H-6 [2.26 (dd), 2.55 (dd)].

^{1}H−^{13}C long-range correlations observed in the HMBC spectrum and ^{1}H−^{1}H correlations observed in the HOHAHA spectrum also indicated the remaining partial structure (C-3, -7, -8, -9, -10, -11, -12, -13, -18, and -19 positions) involving a β-hydroxybutanolide moiety, which was supported by IR absorptions of 3468, 1770 (sh) cm^{-1}. The HOHAHA spectrum demonstrated a sequential proton network of H-3 [2.87 (t)], H-7 [2.56 (d)], H-8 [4.59 (br s)], H-9 [4.56 (d)], and H-10 [2.28 (dd), 2.62 (dd)]. ^{1}H−^{13}C long-range correlations showed the methylene carbon at C-10 to be connected with the 2-methyl-1-methoxycarbonylmethyl group (C-11, 12, 13 positions), $C(CH_3)CH_2CO_2CH_3$. Low field chemical shift [δ_H 1.77 (3H, s) ppm] of the methyl group (C-18) could be explained by the electron-withdrawing ester group at the carbon atom (C-11) bearing this methyl group.

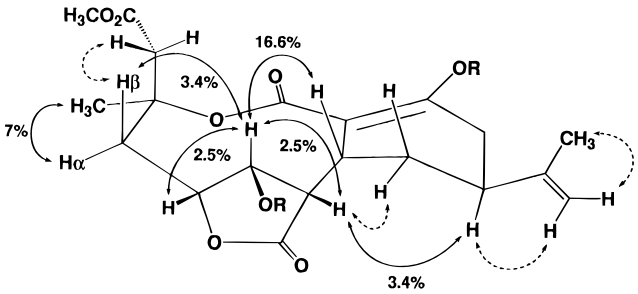

Figure 2. Relative stereochemistry and NOE correlations of **1** (R = H, NOE shown by dotted-line arrows) and **5** (R = Ac, NOE shown by full-line arrows).

In consideration of these findings and the degree of unsaturation (7) of **1**, the carbon at C-11 may reasonably be concluded to be connected with the ester oxygen of the above β-hydroxy-α,β-unsaturated ester group, to make a nine-membered lactonic moiety. Thus, the gross structure of sinulariadiolide was assigned as **1**.

The relative stereochemistry of the six chiral centers in **1** was determined from NOE correlations and coupling constants. NOE correlations between H-3 and H-8 (16.6%), H-7 and H-8 (2.5%), and H-8 and H-9 (2.5%) in diacetate **5** indicated the stereochemistry at C-3, -7, -8, and -9 positions to be that shown in Figure 2, which is supported by the small coupling constants of protons at these positions, J (H-3 and H-7) = 0 Hz, J (H-7 and H-8) = 1.8 Hz, J (H-8 and H-9) = 0 Hz. The stereochemistry at the C-5 position was determined by NOE correlation of H-5 with H-7 (3.4%) observed in **5**. NOE correlations between H-8 and H-10β (3.4%), H-10α and CH_3 at C-11 (7%), and H-10β and H-12 (observed in the NOESY of **1**) could be explained only in the case of the stereochemistry with the partial conformation from C-8 to C-12 shown in Figure 2. This partial conformation is supported by the small coupling constant between H-9 and H-10β (about 0 Hz) owing to the dihedral angle between these protons (near 90°). Absolute stereochemistry was not determined, but was assumed to be the same as that of **3** or **4**, each present with **1** in the soft coral of the genus *Sinularia*, in consideration of the following biogenesis.

Sinulariadiolide (**1**) appeared to be biosynthesized from **3** by the following reactions. The cyclohexenic ring of **1** is formed by a Michael-type attack of the anion of the active methylene at C-4 adjacent to the ketonic C-3 position to the C-13 position of α,β-unsatulated lactone in **3**. Oxidative cleavage of the bond between C-5 and C-6 in **3** gives the nine-membered lactone and ester moiety at C-13 in **1**.

Experimental Section

^{1}H NMR (400 and 500 MHz) and ^{13}C NMR (100 and 125 MHz) spectra were recorded in $CDCl_3$ solution. ^{1}H Chemical shifts are given in ppm based on $CHCl_3$ (7.26 ppm). ^{13}C Chemical shifts are given in ppm based on the solvent used (77.1 ppm for $CDCl_3$). Numbers of attached protons for ^{13}C signals were determined by DEPT experiments.

Extraction and Isolation. Wet specimens of soft coral of the genus *Sinularia* (2.4 kg), collected from the coral reef of Ishigaki Island (Okinawa, Japan) in 1991, were extracted with MeOH at room temperature. The MeOH extract was partitioned between EtOAc and H_2O to give an EtOAc soluble portion (17.2 g), which was chromatographed on a silica gel column to give fraction 1 eluted with hexane−EtOAc = 10:1 (1 L), fraction 2 eluted with hexane−EtOAc = 1:1 (1 L), and fraction 3 eluted with EtOAc (1 L) and then MeOH (2 L). Fraction 3 (7.5 g) was subjected to repeated flash chromatography to give sinulariadiolide (**1**) (21 mg), yonarolide (**2**)[2] (13 mg), **3** (72 mg),[3] and **4** (348 mg).[4] **1**: colorless powder; $[\alpha]_D$ +91.1° (*c* 0.30, $CHCl_3$); IR (film) 3468, 1770 (sh), 1741, 1654, 1617, 1219 cm^{-1}; UV (EtOH) 259 nm (ϵ 9500); UV (EtOH + 0.1 N NaOH) 286 nm (ϵ 13300); ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) see Table 1; EIMS *m/z* 394 (M^+); HREIMS M^+ *m/z* obsd 394.1653, $C_{20}H_{26}O_8$ requires 394.1628.

Acetylation of Sinulariadiolide (1). To a solution of sinulariadiolide (**1**) (3.8 mg, 0.0096 mmol) in pyridine (0.1 mL) was added acetic anhydride (0.1 mL, 1.06 mmol), and the mixture was stirred at room temperature for 24 h. Following removal of the solvent under reduced pressure, the residue obtained was purified by silica gel column chromatography (hexane−EtOAc = 3:1 as an eluent) to give diacetate **5** (3.3 mg). **5**: colorless oil; $[\alpha]_D$ +64.1° (*c* 0.39, $CHCl_3$); IR (film) 1771, 1738, 1667, 1241 cm^{-1}; ^1H NMR (400 MHz) δ 1.69 (3H, s), 1.75 (3H, s), 2.11 (3H, s), 2.20 (3H, s), 2.36 (1H, dd, J = 5.4, 18.7 Hz), 2.52 (1H, d, J = 1.7 Hz), 2.68 (1H, d, J = 15.1 Hz), 2.73 (1H, m), 2.83 (1H, d, J = 15.9 Hz), 3.20 (1H, d, J = 15.9 Hz), 3.24 (1H, br s), 3.68 (3H, s), 4.61 (1H, d, J = 6.2 Hz), 4.80 (2H, s), 5.30 (1H, br s); ^{13}C NMR (100 MHz) δ 20.7 (CH_3), 20.9 (CH_3), 21.0 (CH_3), 26.1 (CH_3), 35.0 (CH_2), 36.0 (CH), 36.4 (CH), 39.5 (CH_2), 41.6 (CH_2), 44.4 (CH_2), 51.8 (CH_3), 55.9 (CH), 79.5 (C), 82.3 (CH), 86.6 (CH), 110.2 (CH_2), 116.2 (C), 147.0 (C), 159.3 (C), 161.0 (C), 168.3 (C), 170.0 (C), 170.9 (C), 175.4 (C); EIMS *m/z* 478 (M^+); HREIMS M^+ *m/z* obsd 478.1807, $C_{24}H_{30}O_{10}$ requires 478.1839.

Supporting Information Available: Copies of the ^1H NMR, ^{13}C NMR, and HOHAHA spectra of **1** and **5** (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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