New Marine Diterpenoids from the Okinawan Soft Coral *Clavularia koellikeri*

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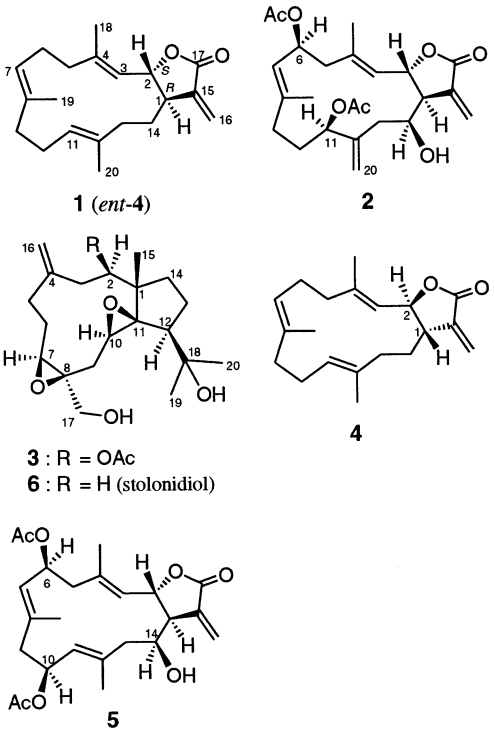
Chemical investigation of the Okinawan soft coral *Clavularia koellikeri* resulted in the isolation of two new cembrane diterpenoids (**1** and **2**) and one new dollabelane diterpenoid, **3**. Their structures were determined on the basis of the results of spectroscopic analysis. Compounds **1** and **3** were examined for in vitro growth-inhibition effects toward tumor cells.

The Okinawan soft coral of the genus *Clavularia* produces a number of structurally unique natural products with various bioactivities. For example, *Clavularia viridis* produces antitumor prostanoids, clavulones,[1,2] and related compounds,[3−5] and *C. koellikeri* contains cytotoxic diterpenoids, kericembranolides.[6] Recently, we reported the isolation and structural determination of seven new cembrane-type diterpenoids from *C. koellikeri*.[7] Further investigation focused on biologically active natural products from *C. koellikeri* and resulted in the isolation of three new diterpenoids, **1**−**3**, as minor components. The structures of **1** and **2** possessing a cembrane skeleton and that of **3** having a dollabelane skeleton were elucidated on the basis of spectroscopic analysis. This paper describes the isolation, structural determination, and bioactivity of these diterpenoids.

Results and Discussion

The MeOH extract of *C. koellikeri*, collected on the coral reef of Ishigaki Island (Okinawa Prefecture, Japan), was partitioned between EtOAc and H_2O to afford an EtOAc-soluble portion. The EtOAc-soluble portion was chromatographed on a silica gel column by elution with hexane, hexane−EtOAc (from 9:1 to 1:2), EtOAc, and MeOH, in turn, to afford eight fractions. Compounds **1** (0.012% yield based on MeOH extract) from the third fraction, **2** (0.0003%) from the sixth fraction, and **3** (0.0057%) from the sixth and seventh fractions were isolated by repeated purification using flash column chromatography, medium-pressure liquid chromatography (MPLC), and HPLC.

The molecular formula of compound **1** was found to be $C_{20}H_{28}O_2$ by HREIMS. All carbons appeared in the ^{13}C NMR spectrum of **1** (Supporting Information). The DEPT spectrum showed three methyls, six sp^3 methylenes, two sp^3 methines involving one oxymethine, one sp^2 methylene, three sp^2 methines, and five sp^2 quaternary carbons involving one carbonyl. These data suggested the presence of three trisubstituted olefins and one exomethylene. The IR (1765, 1667 cm^{-1}) and UV (219 nm, ϵ 3300) absorptions of **1** suggested the presence of an α-methylene-γ-lactone. The presence of the α-methylene-γ-lactone system in **1** was



supported by the following NMR signals: δ_H 5.56 (1H, d, J = 2.3 Hz, H-16a) and 6.22 (1H, d, J = 2.3 Hz, H-16b) ppm in the ^1H NMR spectrum (Supporting Information) and δ_C 121.4 (CH_2, C-16), 140.8 (C, C-15), and 170.5 (C, C-17) ppm in the ^{13}C NMR spectrum. The ^1H NMR spectrum of **1** also disclosed three olefinic protons at δ 4.89 (1H, br t, J = 6.1 Hz, H-7), 4.97 (1H, br dd, J = 5.2, 6.5 Hz, H-11), and 5.06 (1H, br dd, J = 1.1, 9.7 Hz, H-3) ppm, one oxymethine proton in the γ-lactone system at δ 4.87 (1H, dd, J = 4.0, 9.7 Hz, H-2), and three methyl groups connected with olefins at δ 1.57 (3H, br s, H-19), 1.60 (3H, br s, H-20), and 1.71 (3H, d, J = 1.1 Hz, H-18) ppm. These spectroscopic findings, together with the analysis of 2D NMR spectra, suggested compound **1** to be a 14-membered cembrane-type diterpenoid having the structure **1**.

Checking the structure of **1** {$[\alpha]_D$ +28.9° (*c* 1.25, $CHCl_3$)} in the literature, (−)-*trans*-cembranolide **4** {$[\alpha]_D$ −29.0° (*c* 3.40, $CHCl_3$)} from the Okinawan soft coral *Sinularia mayi*, was found.[8] The total synthesis of the diterpenoid was also reported.[10] The spectral data of **1** were almost identical to those of **4** except for the sign of optical rotation, indicating

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Table 1. ^{13}C and ^{1}H NMR Data of Compounds **2** and **3** in $CDCl_3$ (δ ppm)[a]

2			**3**		
no.	^{13}C	^{1}H	no.	^{13}C	^{1}H
1	46.4 (CH)	2.81 (dd, 2.7, 4.2)	1	49.7 (C)	
2	73.7 (CH)	5.36 (dd, 4.2, 9.3)	2	70.9 (CH)	5.12 (d, 8.7)
3	124.7 (CH)	5.06 (br d, 9.3)	3	37.3 (CH_2)	2.07 (dd, 8.7, 15.8)
4	141.4 (C)				3.10 (d, 15.8)
5	43.2 (CH_2)	2.37 (dd, 8.0, 14.8)	4	144.4 (C)	
		2.56 (dd, 4.5, 14.8)	5	31.4 (CH_2)	2.52 (ddd, 3.0, 12.4, 16.0)
6	69.5 (CH)	5.71 (dt, 4.5, 8.4)			2.84 (br d, 16.0)
7	124.7 (CH)	5.17 (br d, 8.4)	6	24.2 (CH_2)	1.52 (m)
8	139.9 (C)				1.96 (tdd, 3.0, 5.9, 15.1)
9	34.9 (CH_2)	2.16 (td, 4.8, 13.9)	7	58.9 (CH)	3.13 (dd, 3.0, 11.1)
		2.33 (m)	8	62.5 (C)	
10	35.3 (CH_2)	2.34 (m, Hb)	9	25.8 (CH_2)	2.28 (d, 15.6)
		2.57 (m, Ha)			2.58 (dd, 9.1, 15.6)
11	77.6 (CH)	5.10 (dd, 1.6, 8.7)	10	55.7 (CH)	3.51 (d, 9.1)
12	146.5 (C)		11	74.9 (C)	
13	29.7 (CH_2)	1.68 (m)	12	46.8 (CH)	2.37 (br d, 9.9)
		1.74 (m)	13	26.2 (CH_2)	1.50 (m)
14	71.2 (CH)	3.73 (dd, 2.7, 9.8)			2.02 (m)
15	136.4 (C)		14	35.8 (CH_2)	1.48 (m)
					1.65 (m)
16	123.6 (CH_2)	5.65 (d, 2.3, Ha)	15	12.6 (CH_3)	0.79 (3H, s)
		6.34 (d, 2.3, Hb)	16	112.3 (CH_2)	4.74 (br s)
17	169.4 (C)				
18	18.9 (CH_3)	1.92 (3H, d, 1.1)			4.78 (br s)
19	16.7 (CH_3)	1.71 (3H, br s)	17	65.4 (CH_2)	3.58 (br d, 12.4)
20	114.7 (CH_2)	5.22 (br s, Hb)			3.74 (d, 12.4)
		5.29 (br s, Ha)	18	74.6 (C)	
*CH_3*CO	20.9 (CH_3)	2.02 (3H, s)	19	30.1 (CH_3)	1.19 (3H, s)
*CH_3*CO	21.3 (CH_3)	2.05 (3H, s)	20	25.8 (CH_3)	1.29 (3H, s)
CH_3*C*O	170.1 (C)		*CH_3*CO	20.8 (CH_3)	2.04 (3H, s)
CH_3*C*O	170.2 (C)		CH_3*C*O	170.6 (C)	

[a] ^{13}C NMR, 125 MHz; ^{1}H NMR, 500 MHz, J in Hz. Assignments of the ^{13}C and ^{1}H signals were made based on HMQC.

compound **1** to be the antipode of **4**, (+)-*trans*-cembranolide (*ent*-**4**). It is quite interesting to find both enantiomers isolated from the soft corals inhabiting Okinawan reefs.

Compound **2** was shown to have the molecular formula $C_{24}H_{32}O_7$ by the combination analysis of HREIMS and NMR spectra (Table 1). ^{13}C NMR and DEPT analyses showed four methyls, four sp^3 methylenes, five sp^3 methines, two sp^2 methylenes, two sp^2 methines, and seven sp^2 quaternary carbons. The IR spectrum of **2** suggested the presence of acetate (1731, 1232 cm^{-1}) and hydroxyl (3478 cm^{-1}) functionalities. The IR and UV spectra showed absorptions due to an α-methylene-γ-lactone [ν_{max} 1766, 1667 cm^{-1}, λ_{max} 220 nm (ϵ 3000)], and the ^{1}H NMR also showed the presence of this moiety [δ 5.65 (d, J = 2.3 Hz), 6.34 (d, J = 2.3 Hz) ppm]. ^{1}H and ^{13}C NMR spectra of **2** showed similarities to those of kericembrenolides, particularly compound **5**.[7] However, one exomethylene group newly appeared in **2** instead of a trisubstituted olefin in **5**. The ^{1}H NMR spectrum of **2** demonstrated two broad singlet signals due to olefinic methyl protons and two three-proton singlet signals at δ 2.02 and 2.05 ppm, owing to the two acetoxyl moieties. The signals at δ 3.73 (dd, J = 2.7, 9.8 Hz) ppm, assigned as the proton bearing the secondary hydroxyl group, and at δ 5.10 (dd, J = 1.6, 8.7 Hz) and 5.71 (dt, J = 4.5, 8.4 Hz) ppm, each bearing a secondary acetoxyl group, were also observed in the ^{1}H NMR spectrum. After assignment of each direct C−H bonding based on HMQC data, the partial structures **a**, **b**, and **c** were established by $^{1}H-^{1}H$ COSY analysis as shown in Figure 1.

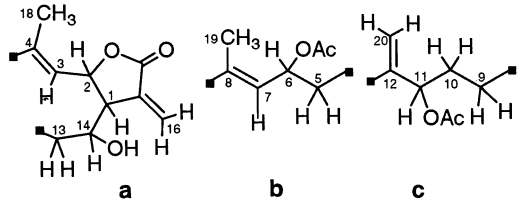


Figure 1. Partial structures for **2** based on $^{1}H-^{1}H$ COSY.

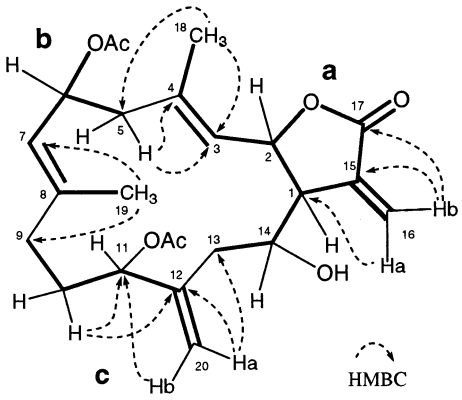


Figure 2. HMBC for **2**.

The partial structures were reasonably connected to each other by following HMBC correlations. The connection between the segments **a** and **b** via C-4 and C-5 was disclosed by the correlations from H-5 to C-3 and C-4 and from H-18 to C-5 (Figure 2). The connection between **b** and **c** via C-8 and C-9 was revealed by the correlations from H-19 to C-9, and that between **a** and **c** via C-12 and C-13 was done by that from H-20 to C-13. These analyses showed the planar structure of **2** to be as depicted in Figure 2.

The *E*-configuration of the two trisubstituted olefins was determined by ^{13}C NMR data [δ 16.7 (C-19) and 18.9 (C-18) ppm][11] and NOE correlations between H-2 and H-18 (olefinic methyl group at C-4) and between H-6 and H-19 (olefinic methyl group at C-8, Figure 3). The relative

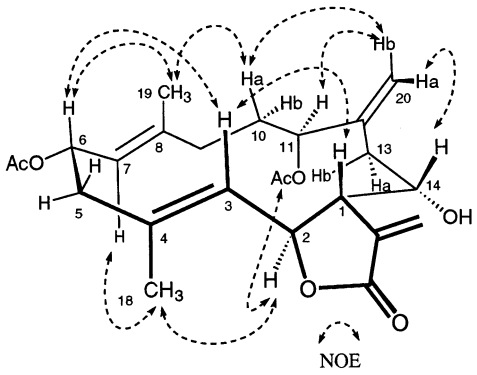


Figure 3. NOE correlations for **2**.

configurations between C-1 and C-2 were established by the coupling constants from the ^{1}H NMR data ($J_{1,2} = 4.2$ Hz).[7] The relative configurations of the chiral centers at C-6, C-11, and C-14 were indicated from the following NOE analysis. NOE correlations between H-1 and H-3, H-2 and H-18, H-18 and H-7, H-3 and H-6, and H-6 and H-19 exhibited a conformation from C-1 to C-8 as depicted in Figure 3. This conformation was supported by the large ^{1}H coupling constants of $J = 9.3$ Hz between H-2 and H-3 and of $J = 8.4$ Hz between H-6 and H-7 as well as the small coupling constant of $J = 4.5$ Hz between H-5 and H-6, which bisects almost equally the methylene protons at C-5. The NOE correlation between H-3 and H-6 thus indicated the 6*S** configuration. Furthermore, NOE correlations between H-19 and H-10a, and H-10a and H-20b, of the exomethylene at C-20 demonstrated these protons to orient in the same direction in a conformation as depicted in Figure 3. Therefore the NOE correlation between H-20a of the exomethylene at C-20 and H-14 demonstrated these protons to orient in the same side, indicating the 14*R** configuration. The conformation between C-13 and C-14, as depicted in Figure 3, was supported by the small coupling constant of $J = 2.7$ Hz between H-13a and H-14; the dihedral angle of these protons is near 90°. Finally the NOE correlations between H-20b and H-11, and between the acetoxyl methyl protons at C-11[12] and H-2, identified the 11*R** configuration. The absolute configuration for **2** is currently under investigation.

Compound **3**, named 2-acetoxystolonidiol, was found to have the molecular formula $C_{22}H_{34}O_6$ by analysis of HREIMS and NMR data (Table 1). ^{13}C NMR and DEPT analyses showed four methyls, seven sp^3 methylenes, four sp^3 methines, four sp^3 quaternary carbons, one sp^2 methylene, and two sp^2 quaternary carbons. The ^{13}C and ^{1}H NMR spectra of **3** were completely different from those of **1** and **2**, however quite similar to those of the cytotoxic dollabelane diterpenoid, stolonidiol (**6**), initially isolated from the Okinawan soft coral of the genus *Clavularia*[13,14] and also found in this study. Compared with the ^{13}C and ^{1}H NMR spectra of **6**, additional signals at δ_C 170.6 (C), 70.9 (CH), and 20.8 (CH_3) ppm and δ_H 5.12 (d, $J = 8.7$ Hz) and 2.04 (3H, s) ppm due to a secondary acetate moiety at C-2 were observed in those of **3**. The IR data suggested the presence of an acetate moiety (1736, 1230 cm^{-1}) and a hydroxyl group (3443 cm^{-1}). Two-dimensional (2D) NMR analysis disclosed the gross structure of **3** to be a 2-acetoxyl analogue of **6**, as shown in Figure 4.

The relative configurations of seven chiral centers in **3** were deduced by NOE analysis and comparison of the NMR data with those of stolonidiol, whose stereochemistry was established by X-ray crystallographic analysis.[13,14] As shown in Figure 5, the NOE correlations between H-7

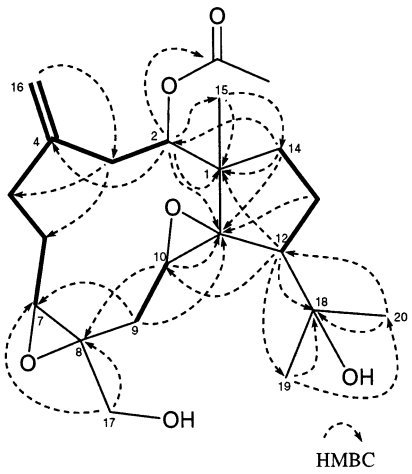


Figure 4. HMBC for **3**.

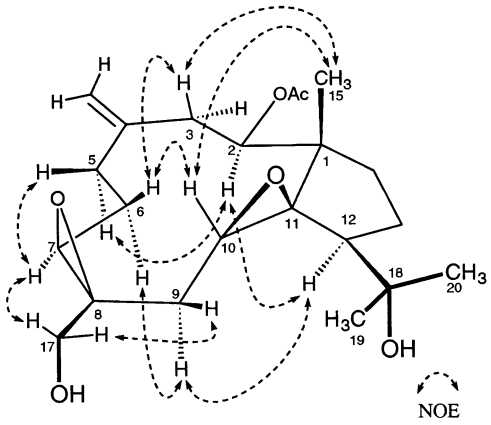


Figure 5. NOE correlations for **3**.

(epoxide) and H-17 (hydroxymethyl) indicated that the proton at C-7 and the hydroxymethyl group at C-8 were located on the same side. Similarly the NOE correlations between H-10 (epoxide) and H-15 (angular methyl group at C-1) indicated the relationship between the H-10 and the carbon−carbon bond from C-11 to C-1. The NOE correlations between H-15 and H-3β, H-3β and H-6β, and H-6β and H-10 as well as the above-mentioned correlation between H-10 and H-15 showed that these protons are oriented on the same side. On the other hand, NOEs between H-2 and H-12, H-12 and H-9α, and H-9α and H-6α indicated these protons to orient in the opposite direction, demonstrating the relative configurations for the chiral centers of C-2 and C-12. These findings strongly suggested the relative stereochemistry for the seven chiral centers in **3** as shown in Figure 5. This was supported by comparison of ^{13}C NMR data of **3** with those of stolonidiol: the ^{13}C chemical shifts from C-5 to C-20 in **3** were found to be almost the same as those for stolonidiol,[13,14] indicating the same relative configurations at C-7, C-8, C-10, C-11, and C-12.

The absolute configuration of **3** was determined on the basis of modified Mosher's method.[15,16] To apply this method for **3**, the secondary acetate at C-2 was converted to 2NMA esters **8** and **9** via **7** (Scheme 1). Compound **3** was converted to a primary methoxymethyl (MOM) ether and then treated with K_2CO_3 in methanol to obtain **7** in good yield. Esterification using (±)-2NMA followed by separation of diastereomers with HPLC gave esters **8** and **9**. After measuring ^{1}H NMR and CD spectra of compounds **8** and **9**, the $\delta\Delta$ value of each proton was calculated and summarized in Figure 6, indicating the 2*S* configuration.

Table 2. IC_{50} Values of Compound **1** against Cultured Tumor Cells (μg/mL)

cell	IC_{50}	cell	IC_{50}	cell	IC_{50}
Breast cancer		*Stomach cancer*		*Ovarian cancer*	
HBC-4	1.62	St-4	3.90	OVCAR-3	1.14
BSY-1	1.35	MKN1	1.20	OVCAR-4	2.25
HBC-5	2.13	MKN7	0.96	OVCAR-5	2.31
MCF-7	0.90	MKN28	0.96	OVCAR-8	0.93
MDA-MB-231	4.50	MKN45	3.00	SK-OV-3	6.60
Lung cancer		MKN74	0.81	*Brain cancer*	
NCI-H23	3.60	*Colon cancer*		U251	4.20
NCI-H226	1.14	HCC2998	1.29	SF-268	3.00
NCI-H522	0.66	KM-12	1.32	SF-295	3.90
NCI-H460	3.30	HT-29	1.47	SF-539	1.56
A549	1.26	HCT-15	2.01	SNB-75	3.30
DMS273	1.11	HCT-116	1.17	SNB-78	1.59
DMS114	3.60	*Renal cancer*		*Prostate gland cancer*	
Melanoma		RXF-631L	4.80	DU-145	3.30
LOX-IMVI	0.72	ACHN	3.00	PC-3	1.11

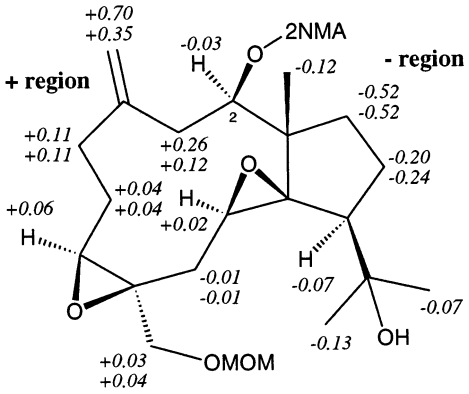
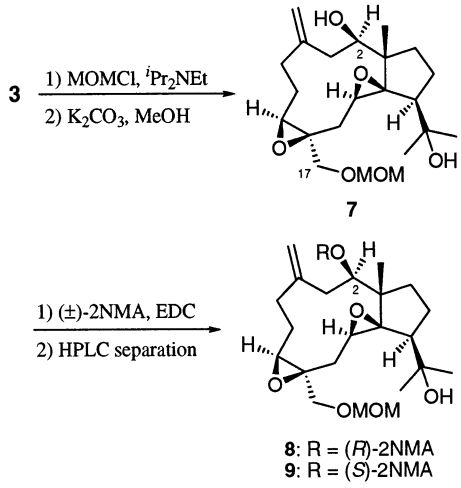


Figure 6. $\delta\Delta$ values (ppm) for 2-NMA esters of **3**.

Scheme 1



These findings concluded the absolute configuration of **3** to be assigned as 1*S*, 2*R*, 7*S*, 8*R*, 10*R*, 11*R*, and 12*S*.

Compound **1** was examined for growth-inhibition activities in vitro toward human cancer cells, evaluated in the Japanese Foundation for Cancer Research 39 cell line assay,[17] and the results are summarized in Table 2. Compound **1** inhibited the proliferation of NCI-H522 (lung cancer) strongly with an IC_{50} of 0.66 μg/mL, and those of LOX-IMVI (melanoma) and MKN74 (stomach cancer) with IC_{50}'s of 0.72 and 0.81 μg/mL, respectively. The pattern of differential growth inhibition for **1** was evaluated by the Compare Program and was revealed not to be correlated with that shown by any other compound including the currently used anticancer drugs; the correlation coefficient value was less than 0.5. This indicates that **1** may have a new mode of action. Compound **3** showed moderate cytotoxic activity against human colorectal adenocarcinoma cells (DLD-1) with an IC_{50} of 5.0 μg/mL.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-370 automatic polarimeter. IR spectra were recorded with a Perkin-Elmer FT-IR 1600 spectrophotometer, and UV spectra with a JASCO V-520 spectrophotometer. All NMR spectra were recorded with a Bruker DRX-500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) and DPX-400 (^{1}H, 400 MHz) and a Varian Gemini-300 (^{1}H, 300 MHz) in $CDCl_3$. ^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC spectra were measured by a Bruker DRX-500 using standard Bruker pulse sequences. Chemical shifts are given on a δ (ppm) scale with $CHCl_3$ (^{1}H, 7.26 ppm; ^{13}C, 77.0 ppm) as the internal standard. Mass spectra were taken with a Micromass Auto Spec spectrometer. Column chromatography was carried out on Merck silica gel 60 (70−230 mesh), and flash column chromatography was performed on Merck silica gel 60 (230−400 mesh). Medium-pressure liquid chromatography (MPLC) was carried out with a KHLC-201-43 (Kusano) apparatus using a CIG prepack column (silica gel, CPS-HS-221-05, for normal phase and ODS silica gel, CPO-HS-221-20, for reversed phase). HPLC was conducted with a YMC-Pack SIL-06 column (silica gel, SH-043-5-06, normal phase) and a YMC-Pack ODS-AM column (ODS silica gel, SH-343-5AM, reversed phase).

Animal Material. The soft coral *Clavularia koellikeri* (order Stolonifera, family Clavularidae) was collected from a coral reef of Ishigaki Island, Okinawa Prefecture, Japan, in November 1997, at a depth of 1−2 m. A voucher specimen (No. IR-SC-97-1) is on deposit at Tokyo University of Pharmacy and Life Science, Tokyo, Japan.

Extraction and Isolation. Wet specimens (1.87 kg) were immersed in MeOH (3 × 3 L). After filtration the combined extracts were concentrated under reduced pressure. The MeOH extract (164 g) was partitioned between EtOAc and H_2O, and then the aqueous layer was extracted with *n*-BuOH. Each layer was concentrated under reduced pressure to obtain, in turn, EtOAc- (27.8 g), *n*-BuOH- (12.5 g), and H_2O-soluble (72.6 g) portions. An aliquot of the EtOAc-soluble portion (11.0 g) was chromatographed on a silica gel column (500 g). Stepwise elution with hexane (300 mL), hexane−EtOAc (9:1, 4:1, 2:1, 1:1, and 1:2, each 300 mL), EtOAc (300 mL), and MeOH (600 mL) afforded eight fractions. The repeated separation and purification of the third fraction (1.39 g) [eluted with hexane−EtOAc (4:1)] by using flash silica gel column chromatography, MPLC (normal and reverse phase), and HPLC (normal phase) gave **1** (78.4 mg). The sixth fraction (0.58 g) [eluted with hexane−EtOAc (1:1)] was subjected to silica gel column chromatography, and further purification by MPLC (normal and reverse phase) and HPLC (normal phase) afforded compounds **2** (1.9 mg) and **3** (14.0 mg) along with known

diterpenoids, kericembrenolides[4,5] and stolonidiol.[10] The seventh fraction (0.14 g) [eluted with EtOAc] was subjected to flash silica gel column chromatography followed by MPLC (normal and reverse phase) and HPLC (normal phase) separation to afford additional **3** (24.5 mg).

Compound 1: colorless, viscous oil: $[\alpha]^{25}_D$ +28.9° (*c* 1.25, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 219 (3.52) nm; IR ν_{max} (dry film) 1765, 1667 cm^{-1}; ^{13}C and ^1H NMR, see Supporting Information; HREIMS *m/z* 300.2114 [calcd for $C_{20}H_{28}O_2$, 300.2089 $(M)^+$].

Compound 2: colorless, viscous oil; $[\alpha]^{25}_D$ +28.4° (*c* 0.19, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 220 (3.48) nm; IR ν_{max} (dry film) 3478, 1766, 1731, 1667, 1232 cm^{-1}; ^{13}C and ^1H NMR, see Table 1; HREIMS *m/z* 312.1729 [calcd for $C_{20}H_{24}O_3$, 312.1725 $(M - 2CH_3CO_2H)^+$].

Compound 3: colorless, viscous oil; $[\alpha]^{25}_D$ −101.0° (*c* 0.98, $CHCl_3$); IR ν_{max} (dry film) 3443, 1736, 1640, 1230 cm^{-1}; ^{13}C and ^1H NMR, see Table 1; HREIMS *m/z* 358.2140 [calcd for $C_{22}H_{30}O_4$, 358.2144 $(M - 2H_2O)^+$].

Chemical Conversion of 3 to MOM Ether. To a mixture of **3** (6.1 mg, 0.02 mmol) and diisopropylethylamine (0.05 mL, 0.29 mmol) in dichloroethane (0.5 mL) was added choloromethyl methyl ether (MOMCl, 0.02 mL, 0.027 mmol) at room temperature under an argon atmosphere. After the reaction mixture was stirred for 30 min at 50 °C, the mixture was diluted with ether, washed with saturated sodium bicarbonate solution, water, and brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. A yellowish residue was purified by silica gel column chromatography (EtOAc as an eluant) to provide MOM ether (6.9 mg, 100% yield) as a colorless oil.

MOM ether: ^1H NMR (300 MHz, $CDCl_3$, δ ppm) 0.79 (3H, s, H-15), 1.20 (3H, s, H-19), 1.29 (3H, s, H-20), 1.47 (1H, m, H-13a), 1.51 (1H, m, H-14a), 1.51 (1H, m, H-6a), 1.64 (1H, m, H-14b), 1.98 (1H, m, H-6b), 2.03 (1H, m, H-13b), 2.05 (3H, s, H-Ac), 2.06 (1H, dd, *J* = 8.7, 14.9 Hz, H-3a), 2.46 (1H, d, *J* = 15.7 Hz, H-9a), 2.47 (1H, d, *J* = 9.9 Hz, H-12), 2.50 (1H, m, H-5a), 2.58 (1H, dd, *J* = 9.3, 15.7 Hz, H-9b), 2.98 (1H, dd, *J* = 3.0, 11.1 Hz, H-7), 3.10 (1H, br d, *J* = 14.9 Hz, H-3b), 3.35 (3H, s, OMe), 3.50 (1H, d, *J* = 12.5 Hz, H-10), 3.54 (1H, d, *J* = 12.3 Hz, H-17a), 3.67 (1H, d, *J* = 12.3 Hz, H-17b), 3.83 (1H, br d, *J* = 15.7 Hz, H-5b), 4.62 (1H, d, *J* = 6.6 Hz, OCH_2O), 4.64 (1H, d, *J* = 6.6 Hz, OCH_2O), 4.75 (1H, br s, H-16a), 4.78 (1H, br s, H-16b), 5.11 (1H, d, *J* = 8.7 Hz, H-2).

Chemical Conversion of MOM Ether to 7. To a mixture of MOM ether (6.9 mg, 0.02 mmol) in MeOH (1 mL) was added potassium carbonate (5 mg) at room temperature. The reaction mixture was vigorously stirred for 20 h at this temperature, and then a saturated ammonium chloride solution (0.05 mL) was added and concentrated under reduced pressure. An oily residue was purified by silica gel column chromatography (EtOAc as an eluant) followed by HPLC purification [hexane−ether (1:3)] to obtain diol **7** (4.5 mg, 73% yield) as a colorless oil.

Compound 7: $[\alpha]^{25}_D$ −49.7° (*c* 0.30, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, δ ppm) 0.75 (3H, s, H-15), 1.19 (3H, s, H-19), 1.32 (3H, s, H-20), 1.49 (1H, m, H-6a), 1.51 (1H, m, H-14a), 1.53 (1H, m, H-13a), 1.56 (1H, m, H-14b), 1.89 (1H, m, H-6b), 1.93 (1H, dd, *J* = 8.8, 15.3 Hz, H-3a), 2.19 (1H, d, *J* = 12.3 Hz, H-13b), 2.30 (1H, br d, *J* = 9.6 Hz, H-12), 2.42 (1H, d, *J* = 15.6 Hz, H-9a), 2.56 (1H, m, H-5a), 2.57 (1H, dd, *J* = 8.8, 15.6 Hz, H-9b), 2.58 (1H, m, H-5b), 2.93 (1H, br d, *J* = 15.3 Hz, H-3b), 2.99 (1H, dd, *J* = 3.3, 7.4 Hz, H-7), 3.36 (3H, s, OMe), 3.46 (1H, d, *J* = 11.2 Hz, H-17a), 3.52 (1H, d, *J* = 9.1 Hz, H-10), 3.55 (1H, d, *J* = 8.8 Hz, H-2), 3.64 (1H, d, *J* = 11.2 Hz, H-17b), 4.62 (1H, d, *J* = 6.6 Hz, OCH_2O), 4.64 (1H, d, *J* = 6.6 Hz, OCH_2O), 4.87 (1H, br s, H-16a), 4.90 (1H, br s, H-16b); EIMS *m/z* 378 $[M - H_2O]^+$.

Esterification of 7 with 2NMA. To a mixture of **7** (4.0 mg, 0.013 mmol), (±)-2NMA (3.6 mg, 0.016 mmol), and DMAP (1.6 mg, 0.013 mmol) in dichloromethane (0.5 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 12.5 mg, 0.013 mmol) at room temperature under an argon atmosphere. After stirring for 1 h at room temperature, the mixture was diluted with ether, washed with 10% sodium citrate solution, saturated sodium bicarbonate solution, water, and brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude products were separated by silica gel column chromatography (EtOAc as an eluant), and the diastereomeric mixture of 2NMA esters was purified by HPLC [reversed phase, H_2O−MeOH (1:4)] to afford esters **8** (1.0 mg, 18% yield) with (*R*)-2NMA and **9** (1.6 mg, 28% yield) with (*S*)-2NMA.

Compound 8: colorless oil; ^1H NMR (500 MHz, $CDCl_3$, δ ppm) 0.64 (3H, s, H-15), 0.89 (1H, m, H-14a), 1.02 (1H, m, H-14b), 1.10 (3H, s, H-19), 1.18 (1H, m, H-13a), 1.23 (3H, s, H-20), 1.43 (1H, m, H-6a), 1.79 (1H, m, H-13b), 1.97 (1H, m, H-6b), 2.06 (1H, dd, *J* = 8.7, 15.8 Hz, H-3a), 2.27 (1H, d, *J* = 9.9 Hz, H-12), 2.42 (1H, d, *J* = 15.6 Hz, H-9a), 2.50 (1H, m, H-5a), 2.53 (1H, dd, *J* = 9.5, 15.6 Hz, H-9b), 2.90 (1H, br d, *J* = 14.2 Hz, H-5b), 2.96 (1H, dd, *J* = 2.8, 10.9 Hz, H-7), 3.13 (1H, br d, *J* = 15.8 Hz, H-3b), 3.33 (3H, s, OMe), 3.42 (3H, s, OMe), 3.43 (1H, d, *J* = 11.3 Hz, H-17a), 3.48 (1H, d, *J* = 9.5 Hz, H-10), 3.66 (1H, d, *J* = 11.3 Hz, H-17b), 4.61 (1H, d, *J* = 6.6 Hz, OCH_2O), 4.63 (1H, d, *J* = 6.6 Hz, OCH_2O), 4.68 (1H, br s, H-16a), 4.75 (1H, br s, H-16b), 4.88 (1H, s, H-2NMA), 5.14 (1H, d, *J* = 8.7 Hz, H-2), 7.49−7.55 (3H, m, H-Nap.), 7.83−7.91 (4H, m, H-Nap.).

Compound 9: colorless oil; ^1H NMR (500 MHz, $CDCl_3$, δ ppm) 0.76 (3H, s, H-15), 1.17 (3H, s, H-19), 1.26 (3H, s, H-20), 1.39 (1H, m, H-6a), 1.41 (1H, m, H-14a), 1.42 (1H, m, H-13a), 1.54 (1H, dd, *J* = 6.3, 12.1 Hz, H-14b), 1.80 (1H, dd, *J* = 8.8, 15.9 Hz, H-3a), 1.93 (1H, m, H-6b), 1.99 (1H, dd, *J* = 6.3, 10.0 Hz, H-13b), 2.34 (1H, d, *J* = 10.6 Hz, H-12), 2.39 (1H, br t, *J* = 3.4 Hz, H-5a), 2.43 (1H, d, *J* = 15.6 Hz, H-9a), 2.54 (1H, dd, *J* = 9.2, 15.6 Hz, H-9b), 2.79 (1H, br d, *J* = 16.3 Hz, H-5b), 2.90 (1H, dd, *J* = 2.8, 10.9 Hz, H-7), 3.01 (1H, br d, *J* = 15.9 Hz, H-3b), 3.33 (3H, s, OMe), 3.40 (1H, d, *J* = 11.2 Hz, H-17a), 3.44 (3H, s, OMe), 3.46 (1H, d, *J* = 9.2 Hz, H-10), 3.62 (1H, d, *J* = 11.2 Hz, H-17b), 3.98 (1H, br s, H-16a), 4.40 (1H, br s, H-16b), 4.60 (1H, d, *J* = 6.6 Hz, OCH_2O), 4.61 (1H, d, *J* = 6.6 Hz, OCH_2O), 4.91 (1H, s, H-2NMA), 5.17 (1H, d, *J* = 8.6 Hz, H-2), 7.48−7.52 (3H, m, H-Nap.) 7.81−7.87 (4H, m, H-Nap.).

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Supporting Information Available: ^1H and ^{13}C NMR data for compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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